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Potential Effects of Pomegranate on Lipid Peroxidation and Pro-inflammatory Changes in Daunorubicin-induced Cardiotoxicity in Rats

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ABSTRACT

Background: Daunorubicin-induced acute cardiotoxicity caused by oxidative stress and free radical formation. Pomegranate possessed a significant *in vitro* free radical scavenging activity. Therefore, the aim of this study was estimations of the role of pomegranate effects in daunorubicin-induced cardiotoxicity.

Methods: A total of 21 Sprague male rats were allocated into three groups, seven animals in each group. Group A: Control group received distilled water. Group B: Treated group with daunorubicin 20 mg/kg via intraperitoneal injection daily for the 12th day for total cumulative dose of 240 mg/kg. Group C: Pretreatment group with pomegranate 25 mg/kg for 6 days orally, then daunorubicin 20 mg/kg administrated concomitantly for the next 6 days with a cumulative dose of 120 mg/kg. Cardiac troponin I ([cTn I] pg/ml), malondialdehyde (MDA) (ng/ml), interleukin 17 (IL-17 pg/ml), and cardiac lactate dehydrogenase (LDH) (pm/ml), all these biomarkers were used to measure the severity of cardiotoxicity.

Results: Daunorubicin at a dose of 20 mg/kg lead to pronounced cardiac damage that reflected on through elevations of serum cTn and serum LDH levels significantly $P < 0.01$, it induced lipid peroxidation during cardiotoxicity that reflected through an elevation in the serum MDA significantly $P < 0.01$, moreover, daunorubicin induces pro-inflammatory changes in cardiotoxicity; it raises the IL-17 serum level significantly $P < 0.01$ as compared with control. Pomegranate pretreatment demonstrated a significant cardioprotection from daunorubicin-induced cardiotoxicity; it attenuated the cardiac damage through reduction of cTn, LDH, MDA, and serum IL-17 level significantly $P < 0.01$ as compared with daunorubicin-treated group.

Conclusions: Pomegranate demonstrated significant cardioprotection in daunorubicin-induced cardiotoxicity through reduction of oxidative stress, lipid peroxidation, pro-inflammatory, and cardiac injury biomarkers.

Keywords: Cardiotoxicity, daunorubicin, pomegranate

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INTRODUCTION

Daunorubicin is an anthracycline antibiotic that mainly used as an antineoplastic agent against various tumor types; its value was limited due to their perilous side effects like cardiotoxicity.[1]

Daunorubicin-induced acute cardiotoxicity is caused by oxidative stress and free radical formation that induced by a high daunorubicin dose more than 15 mg/kg, which provoking oxidative myocardial injury. Furthermore, daunorubicin is converted by intracellular endogenous enzymes into semiquinone that generating a free radical.[2] The heart is more vulnerable to oxidative damage due to less antioxidant enzyme activity (superoxide dismutase, glutathione, and catalase enzymes) consequently, accumulated free radicals leading to lipid peroxidation, mitochondrial membrane damage, injury of nucleic acid, and destruction of myocardium endoplasmic reticulum.[3] Moreover, the daunorubicin-induced release of cytokine-like tumor necrosis factor and interleukin 17 (IL-17) that lead to dilated cardiomyopathy and ventricular dysfunction.[4]

Daunorubicin-semiquinone radical binds iron and form complex called anthracycline-iron free radical complex, which reduces oxygen to superoxide that converted into oxygen and hydrogen peroxide, all of these free radicals lead to oxidative stress and lipid peroxidation in daunorubicin-induced cardiotoxicity.[5]

Thus, antioxidant agents and medicinal plants demonstrated a cardioprotective effect on daunorubicin-induced acute cardiotoxicity, one of these medicinal plants named pomegranate (punica grantum) which is belongs to the family of Punicaceae, there are two species in this family punica granatum and punica protopunica, the second one found only in socotra (Indian ocean Island). The most significant and beneficial effects of pomegranate are related to their active constituents such as punicic acid, anthocyanins, flavones, and flavonols.[6]

Pomegranate possessed a significant *in vitro* free radical scavenging activity and is being studied for its potential biological property in human being; numerous studies demonstrated a reliable anti-oxidant effect of pomegranate in attenuation of risk factors of ischemic heart disease through inhibitions of low-density lipoprotein oxidation, foam cell formation, and macrophage oxidation.[7,8]

Furthermore, pomegranate is regarding as angiotensin converting enzymes inhibitor that inhibits (ACEIs), thus, it is regarded as a natural ACEI in reduction of blood pressure and affording a cardioprotective effect.[9]

Vascular and cardiac protection effects of pomegranate may be produced through enhancement of natural anti-oxidant system or act as direct anti-oxidant.[10]

Therefore, the aim of this study was estimations the role of pomegranate effects on the oxidative stress, lipid peroxidation, and pro-inflammatory changes in daunorubicin-induced cardiotoxicity.

METHODS

This experimental study was done in Department of Clinical Pharmacology, College of Medicine, Al-Mustansiriyia University, Baghdad, Iraq, from May to June 2015, this study was approved by Scientific Juries and Moral Committee of Animal Experimentation. Humane care for animals was done according to the guide to the care and use of laboratory animal.

Study design

A total of 21 Sprague male rats were allocated into three groups, seven animals in each group. The animal body weight was 250–300 g, they were attained from Iraqi National Center, the animals were housed and fed an ordinary chow and distilled water was provided *ad libitum*, then the animals were kept at room temperature and 12-h light-dark cycle. All drugs were purchased from private pharmacy pomegranate extract capsule 250 mg (apeiron, Germany) and daunorubicin 200 mg (rubex vial, santus pharma), then dilution was done with specific solutes to reach the final concentration of 25 mg/kg and 20 mg/kg of pomegranate and daunorubicin, respectively.

- Group A: Control group received distilled water 6 ml/kg orally for 12 days
- Group B: Treated group with daunorubicin 20 mg/kg via intraperitoneal injection daily for 12 days for total cumulative dose of 240 mg/kg
- Group C: Pretreatment group with pomegranate 25 mg/kg for 6 days orally, then daunorubicin 20 mg/kg administrated concomitantly for the next 6 days with cumulative doses of 120 mg/kg.

After 24 hrs of the last dose of drugs, animal decapitated under light anesthesia, and 3 ml of blood sample was obtained. Serum was taken by centrifugation at 3500 rpm for 15 min.

Assessment of cardiac biomarkers

Cardiac troponin I ([cTn I] pg/ml), malondialdehyde (MDA) (ng/ml), IL-17 pg/ml and cardiac lactate dehydrogenase (LDH) (pmol/ml), all of these biomarkers were used to measure the severity of cardiotoxicity, they were estimated by ELISA kit method according to the kit instructions (ELISA kit Catalog NO: e-el-ro566).

Statistical methods

The results were presented as mean ± standard error ANOVA Bonferroni test was used for evaluating the significance of differences regarding $P < 0.01$.

RESULTS

There were 14 rats received daunorubicin with a cumulative dose of 240 mg/kg in daunorubicin-treated group and 120 mg/kg in pretreatment groups as part of the protocol, none of these treated animals with daunorubicin were died through the experimental period.

Daunorubicin at a dose of 20 mg/kg leads to pronounced cardiac damage that reflected on elevation of serum cTn I and serum LDH from 25.09 ± 0.57 pg/ml and 88.74 ± 0.30 pm/ml in control rats to 211.20 ± 0.615 pg/ml and 301.04 ± 0.989 pm/ml, respectively with significance differences value of $P = 0.00001$ for cTn and $P = 0.00009$ for LDH respectively, also; it induced lipid peroxidation during cardiotoxicity that reflected on an elevation in the serum MDA level from 25.25 ± 0.49 ng/ml in control rats to 282.75 ± 2.75 ng/ml in daunorubicin-treated rats significantly $P = 0.00003$. Moreover, daunorubicin induces pro-inflammatory changes in cardiotoxicity; it raises the IL-17 serum levels significantly from 56.245 ± 0.84 pg/ml in control rats to the 231.04 ± 0.707 pg/ml in daunorubicin-treated rats $P = 0.000026$, [Table 1].

Pomegranate pretreatment demonstrated a significant cardioprotection from daunorubicin-induced cardiotoxicity; it attenuated the cardiac damage through reduction of serum cTn I and serum LDH with $P = 0.00012$ and $P = 0.00037$, respectively.

In addition, pomegranate decreases lipid peroxidation significantly through reduction of serum level of MDA level from 282.75 ± 2.75 ng/ml in daunorubicin-treated rats to the 133.175 ± 0.671 ng/ml in pomegranate pretreated rats significantly $P = 0.000103$.

Regarding the pro-inflammatory amelioration in pomegranate, it reduced serum IL-17 levels from 231.04 ± 0.707 pg/ml in daunorubicin treated rats to the 165.85 ± 0.120 pg/ml in pomegranate pretreated rats significantly $P = 0.00021$, [Table 2].

Furthermore, the intergroup differences demonstrated significant differences $P < 0.01$ among daunorubicin and daunorubicin with pomegranate as compared with control. Therefore, cardiac injury, lipid peroxidation, and pro-inflammatory biomarkers were higher in daunorubicin-treated rats and lower in the pomegranate pretreated rats, [Table 3].

DISCUSSION

Daunorobicin-induced cardiotoxicity has been widely reviewed, and it has been reported that daunorubicin and other anthracyclines antibiotic cause heart failure, cardiac electrical abnormalities, and cardiomyopathy.[11]

The probable mechanism for proposed cardiotoxic effects of daunorubicin includes myocardial oxidative stress, mitochondrial damage, lipid peroxidation, and dysregulation of adrenergic signaling.[12]

In this study, daunorubicin at a dose of 20 mg/kg significantly leads to a cardiotoxic injury that reflected off an elevation in serum level of cTn I and LDH, cTn I has been accounted as a highly sensitive marker of myocardial injury and can be of value in the detection of anthracycline-induced cardiotoxicity.[13] Therefore, elevation of both LDH and cTn I during daunorubicin chemotherapy pointed out the occurrence of cardiotoxicity as found in this study.

Moreover, daunorubicin induces oxidative stress, free radical formation and finally induction of lipid peroxidation since; cardiac tissues are rich in mitochondria and its metabolism mainly depend on oxidative pathway, thus, intracellular is buildups of daunorubicin provoked mitochondrial free radicals productions which lead to lipid peroxidation that reflected off an increasing in the serum level of MDA.[14] Xiao, 2015 study showed that MDA is a stable end product of lipid peroxidation and regarded as a reliable marker for estimation of free radical induced tissue damage and lipid peroxidation[15] as in daunorubicin-induced cardiotoxicity of present study.

Moreover, the current study demonstrated that daunorubicin induces inflammatory consequences of cardiotoxicity through an increment in the serum level of IL-17.

IL-17 is a pro-inflammatory cytokine linking adaptive and innate immunity, secreted from a subset of CD4 called Th17, also, it is secreted from natural killer cell,[16] it is responsible for the development of dilated cardiomyopathy in viral myocarditis through enhancement of lymphocyte and neutrophil infiltrations, induction of IL-6 and interferon secretions and cyclooxygenase-2 (COX-2) inhibition.[17]

Amador, *et al*. study pointed out that IL-17 blocking antibodies decreased cardiac inflammatory cytokine

Table 1: Effects of daunorubicin on cardiac, pro-inflammatory and lipid peroxidation biomarkers during experimental cardiotoxicity compared with contro

Biomarkers	Control ($n=7$)	Daunorubicin ($n=7$)	95% CI Upper-Lower	*t*	*P* value
MDA ng/ml	25.25±0.49	282.75±2.75*	−230.75-284.24	−34.84	0.00003*
cTnI pg/ml	25.09±0.57	211.20±0.615*	−179.32-192.89	−83.88	0.00001*
LDH pm/ml	88.74±0.30	301.04±0.989*	−202.77-221.8	−77.6409	0.00009*
IL-17 pg/ml	56.245±0.84	231.04±0.707*	−165.87-183.71	−60.173	0.000026*

Data are expressed as mean±SE *$P<0.01$ as compared with control, MDA=Maondialdehyde, cTnI=Cardiac troponin I, LDH=lactate dehydrogenase, IL-17=Interleukin 17

Table 2: Effects of pre-treatments with pomegranate on cardiac, pro-inflammatory and lipid peroxidation biomarkers in daunorubicin-induced cardiotoxicity

Biomarkers	Pomegranate ($n=7$)	Daunorubicin ($n=7$)	95% CI Upper-Lower	t	P value
MDA ng/ml	133.175±0.671*	282.75±2.75	−122.98-176.17	−19.97	0.000103*
cTnI pg/ml	82.815±0.247*	211.20±0.615	−123.02-133.74	−73.21	0.00012*
LDH pm/ml	177.205±0.657*	301.04±0.989	−113.97-133.70	−39.42	0.00037*
IL-17 pg/ml	165.85±0.120*	231.04±0.707	−58.31-72.06	−34.35	0.00021*

Data are expressed as mean±SE *P<0.01 as compared with daunorubicin group. MDA=Maondialdehyde, cTnI=Cardiac troponin I, LDH=Lactate dehydrogenase, IL-17=Interleukin 17

Table 3: Effects of daunorubicin and daunorubicin with pomegranate on cardiac, pro-inflammatory and lipid peroxidation biomarkers during experimental cardiotoxicity compared with control

Biomarkers	Control ($n=7$)	Daunorubicin ($n=7$)	Daunorubicin + Pomegranate ($n=7$)
MDA ng/ml	25.25±0.49	282.75±2.75*	133.175±0.671**
cTnI pg/ml	25.09±0.57	211.20±0.615*	82.815±0.247**
LDH pm/ml	88.74±0.30	301.04±0.989*	177.205±0.657**
IL-17 pg/ml	56.245±0.84	231.04±0.707*	165.85±0.120**

Data are expressed as mean±SE *P<0.01 as compared with control, P value calculating via ANOVA Bonferrony test, **P<0.01 as compared with daunorubicin group. MDA=Maondialdehyde, cTnI=Cardiac troponin I, LDH=Lactate dehydrogenase, IL-17=Interleukin 17

injury via down-regulation of tumor growth factor and up-regulation of cardiac COX-2/prostaglandin E2 pathway which is responsible for cardioprotection and coronary vasodilatations.[18]

These studies correspond with the present findings, which showed that daunorubicin-induced inflammatory changes in cardiotoxicity that reflected on through an elevation in serum level of IL-17 was significantly during daunorubicin-induced cardiotoxicity.

Thus, oxidative stress, lipid peroxidation, inflammatory induction, and direct myocardial damage all were interconnected in provoking and augmenting of cardiotoxicity induced by daunorubicin.

Regarding, the cardioprotection from daunorubicin-induced cardiotoxicity Conway, *et al*. study revealed that a numerous of synthetic drugs are currently used to overcome the cardiotoxicity such as β-blockers, ACEI inhibitors, statins, and cyclosporine,[19] these agents leads to various side effects such as dry coughs with ACEI inhibitors, fatigue with β-blockers, and muscle damage with statins, which are not appearing with the use of herbal medicine, thus the utilize of these supplements had to turn out to be well-liked in latest years.[20]

Therefore, pretreatment with pomegranate significantly attenuates the cardiotoxic effects of daunorubicin through significant reduction of cardiac injury biomarkers, lipid peroxidation, and pro-inflammatory cytokine; this effect may relate to the polyphenolic compounds which have significant biological activity including antioxidant effect.[21]

The antioxidant activity of pomegranate is related to ellagic acid, which has *in vitro* a prominent free radical scavenging activity and potentiates different antioxidant pharmacological and biochemical pathways thus; it may be regarded as a cardioprotective agent but not prevent other toxicity like cisplatin-induced nephrotoxicity in rats.[22]

Moreover, pomegranate contained other antioxidant constituents such as quercetin, gallic acid, cyanide-3-glucose, and myricetin that are potent agents in attenuations the reduction of heart weight/body weight ratio in doxorubicin cardiotoxicity via a protective effect on cardiac myofibrils from free radical oxidative stress.[23,24]

In addition, pomegranate significantly reduced the level of caspase-3 protease which is a marker for apoptosis and DNA fragmentation, thus; pomegranate like other antioxidants prevents daunorubicin-induced apoptosis in experimental cardiotoxicity.[25] However, unfortunately; this marker was not measured in present study.

Furthermore, pomegranate regarded as natural ACEI and through this effect it demonstrated a potential cardioprotective action since; ACEIs have a potent antioxidant activity, free radical scavenging activity, mitochondrial protection, and inhibition of free radical generations, all of these mechanisms may explain the role of ACEI in the prevention of anthracycline-induced cardiotoxicity.[26] Mohan, *et al*. research demonstrated that pomegranate in animal model study, lead to potential cardioprotection from doxorubicin-induced cardiotoxicity through reduction in serum levels of cardiac creatine kinase-MB and LDH with significant elevation in the antioxidant glutathione,[27] which *per se* explain the attenuation in daunorubicin-induced cardiotoxicity in the present study.

Furthermore, pomegranate boosts HDL effect by stimulation of paraoxonase enzyme (PON-1), an enzyme attached to the surface of HDL that show an age-dependent reduction in its activity, therefore, pomegranate revealed a significant cardioprotection regardless of age unlike statins which boost HDL but not PON-1 activity.[28]

In addition, punicic acid of pomegranate led to inhibition of vascular adhesion molecule in cardiac and vascular endothelium, hence pomegranate regarded as anti-inflammatory agent[29] which may explain the reduction effect of pomegranate on IL-17 in the

present study, also, a numerous studies demonstrated the anti-inflammatory property of pomegranate through inhibition of nuclear factor E2-related factor during carcinogenesis which plays an important role in initiation of inflammatory pathways to cardiotoxicity.[30] Finally, co-administration of pomegranate with daunorubicin leads to significant attenuation in the induced cardiotoxicity through reduction of lipid peroxidation, antioxidant activity, and anti-inflammatory effect.

CONCLUSIONS

Pomegranate demonstrated significant cardioprotection in daunorubicin-induced cardiotoxicity through reduction of oxidative stress, lipid peroxidation, pro-inflammatory, and cardiac injury biomarkers.

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Conflicts of interest

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