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Effects of Omega-7 on Oxidative Stress in Doxorubicin-Treated Cardiac Tissue

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Abstract

Doxorubicin is considered one of the most effective anticancer drugs; however, its use is limited due to the cytotoxic effects mediated by the generation of reactive oxygen species. Omega-7 is a polyunsaturated fatty acid with antioxidant properties.

The aim was to evaluate a possible protective effect of omega-7 against doxorubicin-induced oxidative stress in cardiac tissue in male rats.

Twenty-eight male rats were divided into 4 groups (7 for each group). **Group 1 (negative control):** healthy animals received normal saline orally as the vehicle for eight successive days and were sacrificed on day 9. **Group 2 (positive control):** Animals that received a single dose of doxorubicin hydrochloride (IP 15mg/kg) and were sacrificed the next day. **Group 3:** The animal administered omega-7 orally at a dose 100 mg/kg/day for eight days followed by single dose of doxorubicin IP (15mg/kg) on day 9. The animals were sacrificed on day 10. **Group 4:** The animal administered omega-7 orally at a dose 300 mg/kg/day for eight days followed by single dose of doxorubicin IP (15mg/kg) on day 9. The animals were sacrificed on day 10. Homogenized heart tissue was prepared to measure reactive oxygen species, superoxide dismutase, glutathione peroxidase, catalase, and malondialdehyde.

In the present study, reactive oxygen species were significantly decreased in the omega-7 treated group when compared to the positive control group *($p<0.05$)*. Superoxide dismutase showed a non-significant difference in the omega-7 treated group in compared to the positive control group *($p>0.05$)*. Catalase, and glutathione peroxidase were significantly increased in the omega-7 treated groups when compared to the positive control group *($p<0.05$)*. malondialdehyde were significantly decreased in the omega-7 treated group when compared to the positive control group *($p<0.05$)*.

This *in vivo* enzymatic study provides a piece of evidence for the possible effect of omega-7 in the attenuation of cardiac toxicity in doxorubicin-treated patients.

Long-term trials may show more obvious effects of omega-7, determine the effect of omega-7 on cardiac biomarkers, and investigate the anti-inflammatory effect of omega-7.

Keywords: Doxorubicin, cardiotoxicity, omega-7, reactive oxygen species, superoxide dismutase.

التأثير الوقائي لأوميغا ٧ ضد السمية القلبية التي يسببها الدوكسوروبيسين في ذكور الجرذان

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الخلاصة

يعتبر دواء الدوكسورباسين أحد أكثر الأدوية المضادة للسرطان فعالية ، ومع ذلك فإن استخدامه محدود بسبب تأثيره السام على القلب بوساطة توليد أنواع الأكسجين التفاعلية. أوميغا ٧ ، أحد مضادات الأكسدة ، يلعب دورا في حماية القلب.كان الهدف هو تقييم التأثير الوقائي المحتمل لأوميغا ٧ ضد الإجهاد التأكسدي الناجم عن دوكسوروبيسين في أنسجة القلب في ذكور الجرذان . تم تقسيم ثمانية وعشرين ذكور جرذان إلى ٤ مجموعات (٧ لكل مجموعة). المجموعة ١ (التحكم السلبي): تلقت الحيوانات السليمة محلول ملحي عن طريق الفم لمدة ثمانية أيام متتالية وتم التضحية بها في اليوم التاسع. المجموعة ٢ (التحكم الإيجابي): الحيوانات التي تلقت جرعة واحدة من دوكسوروبيسين حمض الهيدروكلوريك (15 مجم / كجم). ضحى في اليوم التالي. المجموعة ٣: تم اعطاء أوميغا ٧ عن طريق الفم بجرعة ١٠٠ مجم / كجم / يوم لمدة ثمانية أيام. في اليوم التاسع ، تم إعطاء دوكسوروبيسين IP (١٥ مجم / كجم). تم التضحية به في اليوم العاشر. المجموعة ٤: تم إعطاء أوميغا ٧ عن طريق الفم بجرعة ٣٠٠ مجم / كجم / يوم لمدة ثمانية أيام. في اليوم التاسع ، تم إعطاء دوكسوروبيسين IP (١٥ مجم / كجم) وتم التضحية به في اليوم العاشر.في هذه الدراسة ، انخفضت أنواع الأكسجين التفاعلية بشكل معنوي في المجموعة المعالجة بأوميغا ٧ بالمقارنة مع مجموعة التحكم الإيجابية ($P <$ ٠,٠٥). أظهر السوبراوكساید ديسميوتيز اختلافًا غير معنوي في المجموعة المعالجة بالأوميغا ٧ مقارنة بمجموعة التحكم الإيجابية ($p <$ ٠,٠٥). تمت زيادة الكاتلاز ، والجلوتاثيون بيروكسيديز بشكل كبير في المجموعات المعالجة بأوميغا ٧ عند مقارنتها بمجموعة التحكم الإيجابية ($P <$ ٠,٠٥). انخفض المالوندايالادهليد بشكل معنوي في المجموعة المعالجة بأوميغا ٧ بالمقارنة مع مجموعة التحكم الإيجابية ($P <$ ٠,٠٥)

الخلاصة: تقدم هذه الدراسة الأنزيمية في الجسم الحي دليلاً على التأثير المحتمل لأوميغا ٧ في توهين سمية القلب لدى المرضى المعالجين بدوكسوروبيسين.قد تظهر المسارات طويلة المدى ، والعدد الأكبر من الفئران ، تأثيرات أكثر وضوحًا للأوميغا ٧ ، ودراسة تأثير أوميغا ٧ في الإنسان ، ودراسة تأثير أوميغا ٧ بالاشتراك مع الأدوية المضادة للالتهابات.

الكلمات المفتاحية: دوكسوروبيسين ، سمية القلب ، أوميغا ٧ ، أنواع الأكسجين التفاعلية ، سوبراوكساید دسميوتيز

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Introduction

Doxorubicin (Adriamycin)® is one of the most potent and effective chemotherapeutic agents that treat various cancers such as liver, kidney, breast, stomach, and hematological cancers [1]. Unfortunately, its use has been limited due to its serious and fatal side effects that range from changes in myocardial structure and function to severe cardiomyopathy and congestive heart failure [2]. There are several hypotheses to explain doxorubicin cardiotoxicity, but oxidative damage to cellular components is believed to be a major factor in doxorubicin toxicity [3, 4]. The quinone moiety in the doxorubicin structure participates in reduction-oxidation (REDOX) processes which result in oxidative damage to myocardial tissue [5]. The ROS generation and hence their cytotoxic potential is enhanced by reducing the levels of the antioxidant enzymes including superoxide dismutase (SOD), catalase, and peroxidase during the doxorubicin treatment [6].

Omega-7 is found mainly in cold-water fish and sea buckthorn berry and contains mainly palmitoleic acid (16:1, Cis-9-hexadecenoic acid) and vaccenic acid ((11E)-11-octadecenoic acid) [7]. Omega-7 is considered a nonessential fatty acid in humans as it can be made endogenously [8]. Diets rich in omega-7 fatty acids have been shown to have beneficial health effects, such as increasing levels of HDL cholesterol and lowering levels of LDL cholesterol [9, 10]. Previous studies have shown that omega-7 has various health benefits such as reducing cardiovascular risk and enhancing insulin sensitivity [11]. It also has antioxidant properties that mediate wound healing activity as evidenced by a significant increase in reduced glutathione levels (a major endogenous thiol antioxidant) and reduced production of reactive oxygen species (ROS) in damaged tissue [12]. To date, the efficacy of omega-7 on oxidative stress in cardiac damage of doxorubicin has not been established. Herein, we aim to evaluate the possible protective effects of omega-7 against oxidative stress generated in the cardiac tissue by doxorubicin in male rats through attenuation of the oxidative stress responses.

Materials and Methods

Material

The drugs doxorubicin HCL and omega-7 were used in this study. Doxorubicin was purchased from Pfizer labs, New York, USA, and omega-7 was purchased from Source Naturals, USA.

Animal conditioning

Twenty-eight Wister male rats, weighing 150-250 gm, were kept in polypropylene cages under controlled conditions: regular light/dark cycle at the temperature of 22 ± 2 ℃. The rats were fed commercial pellets and the tap water ad libitum.

Experimental design

The rats were divided into 4 groups including 7 rats in each group

1. Group 1 (negative control): healthy animals received normal saline orally as the vehicle for eight consecutive days. The animals were sacrificed on day 9.

2. Group 2 (positive control): animals that received a single dose of doxorubicin HCl (IP 15mg/kg) (13). The animals sacrificed the next day.

3. Group 3: the animal administered omega-7 orally at a dose 100 mg/kg/day for eight days followed by single dose of doxorubicin IP (15mg/kg) on day 9. The animals were sacrificed on day 10.

4. **Group 4:** the animal administered omega-7 orally at a dose 300 mg/kg/day for eight days followed by single dose of doxorubicin IP (15mg/kg) on day 9. The animals were sacrificed on day 10.

Preparation of heart tissue homogenate

All rats were sacrificed by cervical dislocation under diethyl ether anesthesia and heart tissues were isolated and processed for analysis [14]. Briefly, the heart was rapidly excised, and washed with a pre-cooled PBS (pH=7.4, 4℃) to rinse away any residual blood. Then, blotted on filter paper, and chopped into fine pieces. For each rat, tissue homogenate was prepared by adding 0.4 g of the minced tissue and 3.6 ml of PBS (pH=7.4, 4℃) into a tube [15]. Homogenization was then accomplished using a tissue homogenizer (Dyna-Passion® WT130, Success Technic Industries, Selangor, Malaysia) at set 3 for 1 minute at 4℃. Samples were kept on ice throughout all the above-mentioned steps. The resultant suspension was then subjected to a freeze-thaw cycle and centrifuged in a refrigerated centrifuge (HERMLE Labortechnik GmbH, Germany) at 10,000 rpm for 10 minutes at 4℃. The resultant supernatant was immediately collected and stored at −20℃ until the day of analysis when it was used for the estimation of ROS, SOD, GPX, CAT, and MDA levels [15, 16].

Statistical analysis

All results of the study were demonstrated as Mean± Standard deviation (SD) and data input and analysis were examined by statistical package for social sciences program version 24 (SPSS V 24) and a t-test was performed to compare the means of groups; (P *values*<0.05) were regarded as significantly different.

Results and Discussion

Data analysis in Table 1 showed a significant increase in ROS levels in group 2 (positive control) in comparison to group 1 (negative control) *(p<0.05)*. Interestingly, co-administration of omega-7 in group 3 (100mg/kg) and group 4 (300mg/kg) showed a significant reduction in ROS levels in comparison with the group 2 *(p<0.05)*.

Table 1. The Effect of Omega-7 on the level ROS production induced by doxorubicin in rats

	Group 1 (negative control)	Group 2 (positive control) at dose 15mg/kg	Group 3 (omega-7 at dose 100mg/kg)+ doxorubicin	Group 4 (omega-7 at dose 300mg/kg)+ doxorubicin
ROS (nmol/mg protein)	2.1±0.095	4.814±0.229*	3.671±0.188#	2.814±0.207#

Data are expressed as Mean ± STD, n=7.
*Significantly different compared to group I (negative control) (P<0.05).
#Significantly different compared to group II (positive control) (P<0.05).

In Table 2, There was a significant decrease in superoxide dismutase (SOD) levels in group 2 (positive control) in comparison with group 1*(p<0.05)*. The co-administration of omega-7 in group 3 (100mg/kg) and group 4 (300mg/kg) caused non-significantly difference in SOD levels in comparison with the group 2 (positive control) *(p>0.05)*.

The level of glutathione peroxidase (GPX) was significantly decreased in group 2 (positive control) compared with group 1 *(p<0.05)*, although there is no significant change *(p>0.05)* in the level of GPX in group 3 (100mg) in comparison to group 2, the higher dose of omega-7 (300mg/kg) in group 4 showed significant *(p<0.05)* increase in GPX level in comparison with group 2.

There was a significant (p<0.05) decrease in the level of catalase (CAT) enzyme in group 2 (positive control) when compared with group 1. The co-administration of omega-7 in group 3 (100mg) and group 4 (300mg) significantly increased CAT levels *(p<0.05)* in comparison with group 2 (positive control).

Table 2. The effect of different doses of omega-7 on oxidative stress enzymes

Groups	SOD (nmol/mg protein)	GPX (nmol/mg protein)	CAT (nmol/mg protein)
Group 1 (negative control)	1.65 ± 0.0112	1.467± 0.124	0.413± 0.015
Group 2 (positive control) at dose 15mg/kg	0.978 ± 0.227*	0.808± 0.020*	0.21± 0.017*
Group 3 (omega-7 at dose 100mg/kg)+ doxorubicin	1.144± 0.191	1.036± 0.126	0.319± 0.02#
Group 4 (omega-7 at dose 300mg/kg)+ doxorubicin	1.459 ± 0.198	1.214 ± 0.143#	0.383± 0.07#

Data are expressed as Mean ± STD, n=7.
*Significantly different compared to group I (negative control) *(P<0.05)*.
#Significantly different compared to group II (positive control) *(P<0.05)*.

In Table 3, cardiac MDA levels were significantly (p<0.05) elevated in the doxorubicin group in comparison with the normal group. Co-administration of omega -7 (100mg / kg) in group 3 shows a non - significant change (p > 0.05) in comparison with the doxorubicin group, while omega-7 (300mg/kg) in group 4 showed a significant elevation (p<0.05) in comparison with the doxorubicin group.

Table 3. The effect of different doses of omega-7 on malondiahdehyde

	Group 1 (negative control)	**Group 2 (positive control) at dose 15mg/kg**	**Group 3 (omega-7 at dose 100mg/kg)+ doxorubicin**	**Group 4 (omega-7 at dose 300mg/kg)+ doxorubicin**
MDA (nmole/mg protein)	0.921± 0.043	1.427± ٠.068*	1.268± 0.138	1.127± 0.080#

Data are expressed as Mean ± STD, n=7.
*****Significantly different compared to group I (negative control) (P<0.05).
#Significantly different compared to group II (positive control) (P<0.05).

Discussion

The high prevalence of cardiomyopathy and heart failure are the main side effect connected to the usage of doxorubicin [17]. Many studies have focused on the doxorubicin-induced apoptosis signaling mechanism, and several reports have suggested that doxorubicin-induced apoptosis plays a significant role in its cytotoxicity, which is connected to the formation of reactive oxygen species (ROS) derived from doxorubicin's redox activation [17, 18].

In the present study administration of doxorubicin to rats in group 2 (positive control) resulted in an oxidative stress response evidenced by the significant elevation of cardiac MDA, and ROS levels and a significant reduction in cardiac content of antioxidant enzymes including SOD, GPX, and CAT in compared to group 1 (negative control). These findings were consistent with earlier research, highlighting the critical role played by oxidative stress in the development of cardiac damage upon exposure to doxorubicin [19, 20]. In addition, pre-treatment with omega-7 in groups 3 and 4 induced a significant decrease in cardiac ROS and MDA levels combined with a significant increase in cardiac GPX, and CAT activities compared to the positive control group.

Importantly, treatment with omega-7 (100mg and 300mg/kg) caused a significant decrease in ROS levels compared with the positive control group. This result is consistence with a prior investigation into the effectiveness of omega-7 in relation to skin ageing [11]. Superoxide dismutase (SOD) is an enzyme that catalyzes the initial step of the anti-oxidation process, which turns excess oxidizing ions into oxygen and hydrogen peroxide, thereby protecting cells from oxidative damage. From the study, SOD levels were significantly decreased in group 2 compared with the negative control group. Moreover, prophylactic treatment with omega-7 in groups 3 and 4 showed a non-significant difference in SOD levels compared with the doxorubicin-treated group, which disagrees with a previous study about the antioxidant effect of omega-7 in H2O2-treated skin cells [21]. Glutathione peroxidase (GPX) is an antioxidant enzyme class that has the ability to scavenge free radicals. This in turn aids in redox balance, intracellular homeostasis, and the prevention of lipid peroxidation. Catalase (CAT) is well known to catalyze the degradation of H2O2 into water and oxygen in an energy-dependent process in the cells exposed to oxidative stress. Interestingly, GPX and CAT levels were significantly decreased in group 2 (positive control) compared with group 1 (negative control). However, treatment with omega-7 (100 and 300mg/kg) caused a significant increase in CAT levels compared with group 2. Although, GPX levels showed a non-significant difference in group 3 (100mg/kg omega-7), the higher dose (300mg/kg omega-7) in group 4 caused a significant increase in GPX levels compared to group 2. Similar findings were found in earlier investigations on other polyunsaturated fatty acids with reference to these two parameters [22]. MDA is a secondary lipid peroxidation product, and it can serve as an indicator of cell membrane damage. In this study, the administration of doxorubicin caused a significant increase in MDA levels. However, co-treatment with omega-7 causes a significant decrease in MDA levels in a dose dependent manner. Although there is no other investigation connecting between the effect of omega-7 and MDA levels, previous studies on omega-3 fatty acids showed a similar finding to our result [23]. A few theories have been suggested to explain the mechanism underlying the beneficial impact of polyunsaturated fatty acids. It might be carried out through the replacement of anthracycline-peroxidized fatty acids in membranes and other lipid-containing structures such as membranes [24, 25], changes in the metabolism of eicosanoids [26], or the restoration of the cytokine network's imbalance [27]. In this investigation, it was found that therapy with omega-7 fatty acids significantly increased antioxidant status.

Conclusion

According to the present finding, omega-7 could play an important role as an endogenous antioxidant and could also be used as a cytoprotective element in chemotherapy and an antioxidant drug in doxorubicin-induced cardiotoxicity.

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Conflicts of Interest

The author declares that there was no conflict of interest.

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Ethics Statements

This study was approved by the scientific and ethical committees of the College of Pharmacy University of Baghdad

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