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RESEARCH ARTICLE

Hepatoprotective Potentials of Menaquinone - 7 against Doxorubicin Associated Hepatotoxicity

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Abstract

Dox, is still widely used in modern cancer treatments for different type of malignancy despite the advent of targeted therapy. However, its beneficial effect was limited by its toxicity on various organs. The objective of this study was to investigate the hepatoprotective effect of menaquinone-7 against hepatotoxicity induced by doxorubicin in rats. Sixty adult rats of both sexes were used in this study; the animals were randomly enrolled into six groups of 10 animals each. Group I: negative control; Group II: Menaquinones-7 at a dose of 16µg/kg; Group III: Menaquinones-7 at a dose of 48µg/kg; Group IV: positive control (Doxorubicin 15mg/kg); Group V: Menaquinones-7 at a dose of 16µg/kg administered prior to a single dose of Doxorubicin 15 mg/kg; Group VI: Menaquinones-7 at a dose of 48 µg/kg administered prior to a single dose of Doxorubicin 15 mg/kg. On day twelve of the study, the liver of each animal was excised for homogenate preparation and estimation of malondialdehyde, total antioxidant capacity and caspase-3 by ELISA technique as the markers of oxidative stress and apoptosis respectively. High dose of Menaquinones-7 significantly (P<0.05) decreased malondialdehyde content, and increase total antioxidant capacity content in group VI compared to group IV. However, neither group V nor group VI exhibited significant (P>0.05) differences in caspase-3 activity in liver tissue homogenate compared to positive control group. Menaquinones-7 may have hepatoprotective effect against doxorubicin-associated hepatotoxicity in rats.

Keywords: *Menaquinone-7, Doxorubicin, Hepatotoxicity, Rats, Tissue homogenate.*

Introduction

Cancer is the second leading cause of death globally, and was responsible for 8.8 million deaths in 2015[1]. However, mortality rates from cancer have declined over the past 30 years largely because of early detection strategies, improved surgical approaches, as well as advances in cancer therapeutics[2,3].

The family of anthracycline drugs originated in the 1950's with the identification of daunorubicin from the soil bacterium *Streptomyces peucetius*[4]. Agents in this pharmacological group of antineoplastic drugs include doxorubicin (Dox), daunorubicin, epirubicin, and idarubicin. Dox, approved in 1974 by Food and Drug Administration (FDA), is still widely used in modern cancer treatments for different type of malignancy[5, 6] despite the advent of targeted therapy[7]. However, it's beneficial effect was limited by its adverse effect on heart[8], kidney[9], liver[10] and other organs[11, 12] with its main toxicity on heart and liver. Dox seems to accumulate mostly in the liver, most likely due to the organ's role in metabolism[13].

Vitamin K is a fat-soluble vitamin known to activate not only blood coagulation factors, but also tissue-specific extrahepatic vitamin-K-dependent proteins (VKDP) through post-translational modification of γ-carboxylation, which converts glutamate (Glu) residues to γ-carboxy-glutamate (Gla)[14]. The two naturally-occurring forms of vitamin K are phylloquinone (vitamin K1) and menaquinones (MKs; collectively known as vitamin K2)[15] which differ in length and in the degree of saturation at the aliphatic side

chains. Menaquinone-7 (MK-7) has long half-life and good bioavailability[16]. Several authors demonstrated the beneficial protective role of long chain vitamin k against cardiovascular and bone diseases[17-19]. Vitamin K cycle could act as a potent antioxidant[20]. The aim of this study is investigate the hepatoprotective of MK-7 against hepatotoxicity induced by doxorubicin in rats.

Material and Methods

Experimental Animals

Sixty adult albino rats of both sexes, three months old, weighing 160-250gm were used in this study; they were obtained from and maintained in the Animal House of the College of Pharmacy, Baghdad University under conditions of controlled temperature. The animals were fed commercial pellets and tap water *ad libitum* throughout the experiment period. The study was approved by the Scientific- and the Ethical-Committees of the College of Pharmacy/ University of Baghdad.

Drugs

Dox as hydrochloride (50 mg vial) was purchased from Pfizer, Italy. Menaquinone-7 (MenaQ7 pill 180 µg) was purchased from Omicron Pharmaceuticals, Norway.

Experimental Protocol

Rats were randomly allocated into six groups, each containing 10 rats (5 males and 5 females) as follow:

Group I: Rats were received 0.5ml of DW as intraperitoneal (IP) dose. This group served as a negative control.

Group II: Rats were received MK-7 (16µg/kg B.Wt/day) orally by oral gavage for 11 consecutive days.

Group III: Rats were administered MK-7 (48µg/kg B.Wt/day) orally by oral gavage for 11 consecutive days.

Group IV: Rats were intraperitoneally injected with single dose of Dox (15mg/kg B.Wt). This group served as a positive control.

Group V: Rats were orally administered MK-7 at a dose of 16 µg/kg B.Wt/day prior to 15mg/kg of Dox.

Group VI: Rats were orally administered 48µg/kg B.Wt/day prior to 15mg/kg of Dox.

In groups (V and VI) animals, each dose of MK-7 was administered once daily for 11 consecutive days; and at day 11, they received single dose of Dox (15 mg/kg B.Wt) by IP injection. Twenty-four hour after the end of the treatment duration (i.e. at day 12), the animals were euthanized by diethyl ether and after necropsy, the liver of each animal was excised for homogenate preparation and estimation of malondialdehyde (MDA), total antioxidant capacity (TAOC) and caspase-3 by ELISA technique using ELISA system (HUMAN, Germany) as the markers of oxidative stress and apoptosis respectively.

Estimation of malondialdehyde (MDA), Total Antioxidant Capacity (TAOC) Level, and caspase-3 Level in Liver Tissue Homogenate Samples

The preparation of liver homogenates involved removal of excess blood by rinsing in ice-cold phosphate buffer saline (PBS) (pH= 7.4). Then the tissues minced to small pieces and put in 15ml test plastic tube containing chilled PBS solution (pH= 7.4); where, (10mg liver tissue in 100µl PBS). Homogenization was performed by means of tissue and cell lab homogenizer (Success Technic Industries, Malaysia) in icy condition. After that, the homogenates was centrifuged for approximately 15 minutes at 1500×g (or 5000 rpm) at 4 ºC.

The supernatant was carefully collected and stored at -50 ºC until the time for the determination of malondialdehyde (MDA) content, total antioxidant capacity (TAOC) level, and caspase-3 level[21-23]. Estimation of MDA and TAOC was made by using of the quantitative sandwich ELIZA kit (My Bio Source, USA). Sandwich-ELISA kit (Elabscience Biotechnology, USA) was utilized for estimation of caspase-3 level in liver homogenate.

Statistical Analysis

Data were expressed as mean± standard error of the mean (SEM). Unpaired Student t-test was used for testing the significant difference between two groups. The statistical significance of the differences among various groups was determined by one-way analysis of variance (ANOVA) and

Least Significant Difference (LSD) analysis by IBM SPSS (statistical package for social sciences) version 23. Differences were considered statistically significant for *P*-value less than 0.05.

Results

Effects on MDA

Dox administered intraperitoneally (15 mg/kg B.Wt) caused significant ($P < 0.05$) raise in MDA contents in liver homogenate with respect to negative controls. A significant ($P < 0.05$) decline in MDA content was detected in group VI when compared to group IV (Figure 1).

Effects on TAOC

Figure 2 shows that Dox alone (15 mg/kg B. Wt, IP) decreased TAOC content in liver homogenate significantly ($P < 0.05$) compared to negative control group; combination treatment of Dox with MK-7 (48 µg/kg/ B. Wt) raised TAOC significantly ($P < 0.05$) compared to positive control group.

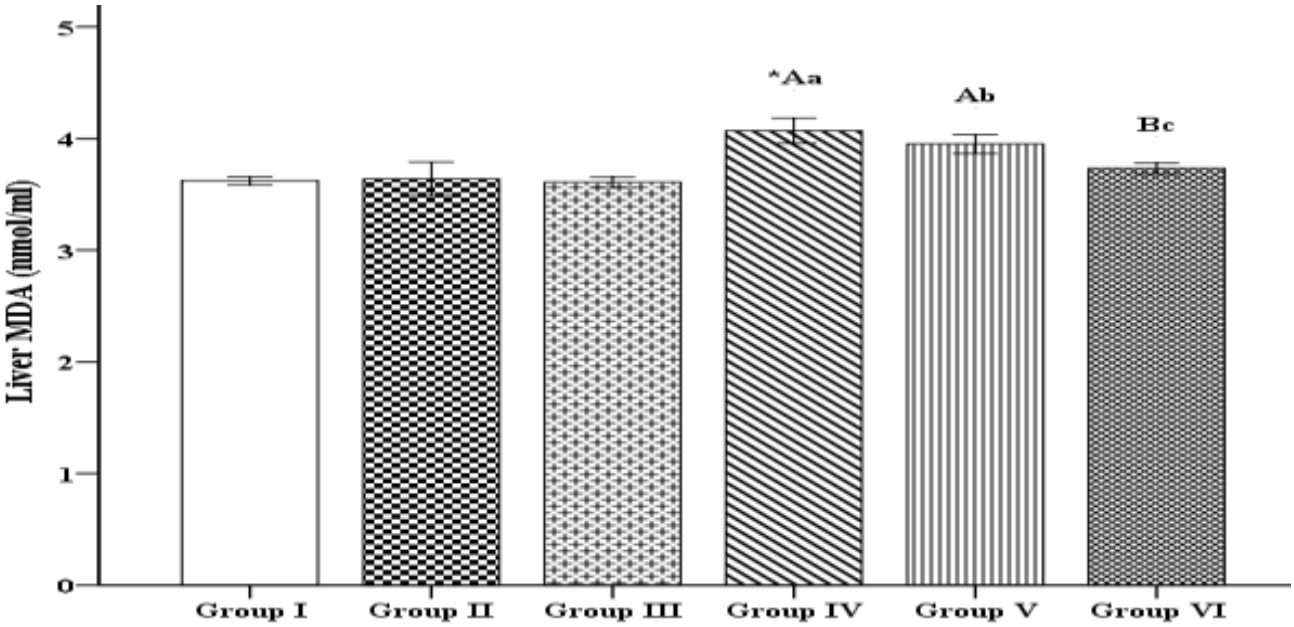

Figure 1: Bar chart showing MDA contents in liver tissue homogenate in various experimental rats' groups. *= Significantly different (*P*<0.05) with respect to the negative control group utilizing unpaired Student t-test. Values with non–identical superscripts capital letters (A and B) are significantly different (*P*<0.05) compared to positive control group utilizing unpaired Student t-test. Values with non–identical superscripts small letters (a, b and c) are significantly different (*P*<0.05) among groups IV, V and VI utilizing ANOVA and LSD.

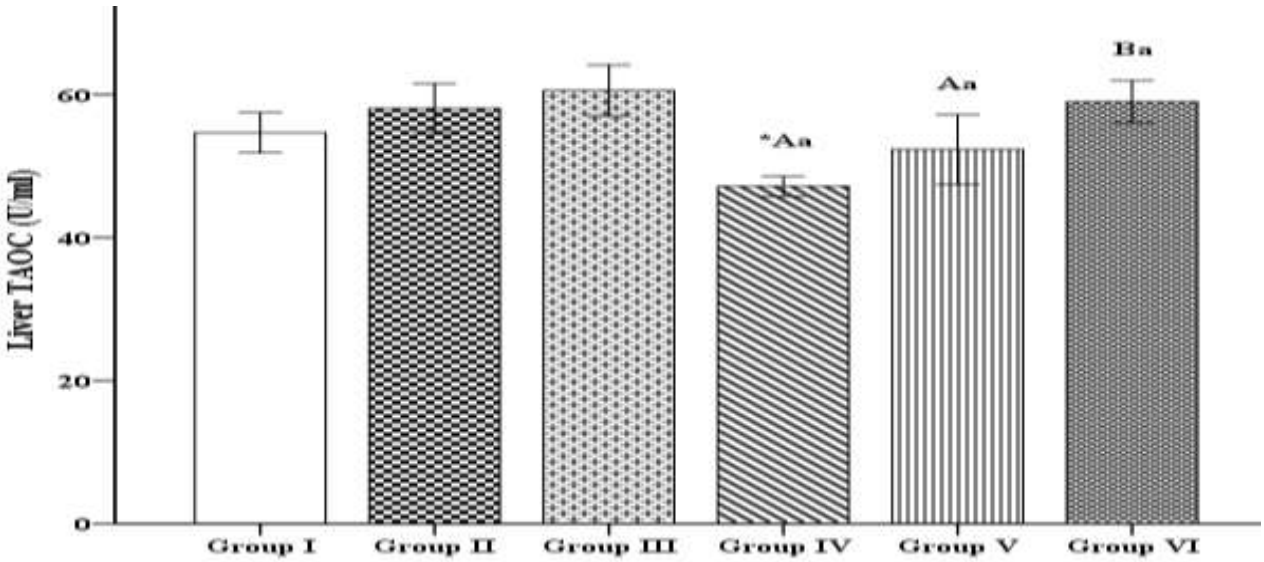

Figure 2: Bar chart showing TAOC contents in liver tissue homogenate in various experimental rats' groups. *= Significantly different (*P*<0.05) with respect to the negative control group utilizing unpaired Student t-test. Values with non–identical superscripts capital letters (A and B) are significantly different (*P*<0.05) compared to positive control group utilizing unpaired Student t-test. Values with an identical superscript small letter (a) are non-significantly different (*P*>0.05) among group IV, V and VI utilizing ANOVA and LSD

Effects on Caspase-3

A significant ($P < 0.05$) elevation in caspase-3 level in liver homogenate was observed as a result of single dose of Dox as compared to negative control group. Group V and group VI, exhibited no significant ($P > 0.05$) differences in caspase-3 in liver homogenate with respect to positive control group (Figure 3).

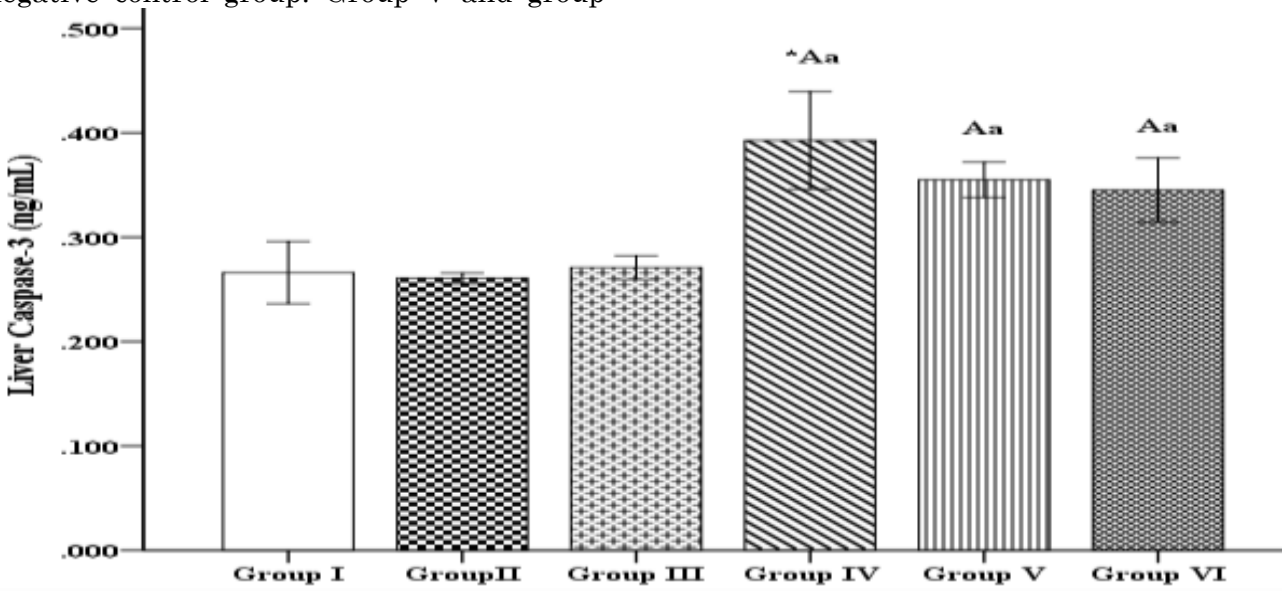

Figure 3: Bar chart showing Caspase-3 level in liver tissue homogenate in various experimental rats' groups. *= Significantly different ($P<0.05$) with respect to the negative control group utilizing unpaired Student t-test. Values with an identical superscript capital letter (A) are non-significantly different ($P>0.05$) compared to positive control group utilizing unpaired Student t-test. Values with an identical superscript small letter (a) are non-significant different ($P>0.05$) among groups IV, V and VI utilizing ANOVA and LSD

Discussion

Several mechanisms were attributed to Dox mediated cell death including oxidative stress, apoptosis, intracellular calcium dysregulation, topoisomerase II poisoning, DNA adduct formation, and ceramide overproduction[2, 25]. Recently, Gao *et al.* (2016) reported that, Dox could initiate inflammation via markedly increase of inflammatory-related proteins including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1 ß), and interleukin-6 (I IL-6) in the liver[26].

The current study revealed that Dox induced hepatotoxicity, which was evident by significant ($P<0.05$) elevation of MDA content and reduction of TAOC in liver tissue homogenate in the Dox treated rats compared with negative control group. These results are in line with those of Kocahan *et al.* and Raskovic *ET al.*[27, 11]. These findings confirm the well-known hypothesis which state that a major role is played by free radicals in Dox-induce hepatotoxicity.

The semiquinone form of Dox is a toxic short-lived metabolite which interacts with molecular oxygen and initiates a cascade of reactions, producing reactive oxygen species (ROS). ROS generation and lipid peroxidation, and hence increment in MDA level, have been suggested to be responsible for Dox -induced hepatotoxicity[28, 29]. Caspase-3 is a key mediator of apoptosis in mammalian cells[30], significantly ($P< 0.05$) increased in liver tissue homogenate of Dox-treated group.

This result is in agreement with Sliai[31], but it disagrees with the results of Dirks-Naylor AJ *ET al.*[32] who found that acute Dox treatment did not increase caspase-3 activity. In the current study, MK-7 administered at a dose of 48 µg/kg prior to single IP dose of Dox 15 mg/kg B.Wt (group VI), significantly ($P<0.05$) lowered MDA content liver tissue homogenate when compared to group IV. Moreover, combination treatment of Dox with MK-7 (48 µg/kg/ B.Wt) raised TAOC to a significant ($P< 0.05$) level.

However, MK-7 had no significant ($p > 0.05$) effect regarding caspase-3. MK-7 is a fat-dissolvable vitamin produced by *Bacillus subtilis natto*[33] with a multiple bone and cardiovascular advantageous impacts. In this study, hepatoprotective effect of MK-7 could be attributed to potent antioxidant capacities

of MK-7 when reduced to KH2 (dihydroquinone) during vitamin k cycle[34], that in turn may result in diminution of oxidative injury induced by Dox.

It has been accounted for that, a number of the positive effects of MK-7 could be certify to menaquinones-4 (MK-4) where, all vitamin K homologues can be changed over to MK-4 *in vivo*[35]. Numerous studies reported the antioxidant and anti-inflammatory influence of vitamin K analogues *in vivo* and *in vitro*.

Vervoort *et al.* revealed that vitamin K2 was an inhibitor of microsomal lipid peroxidation in rat liver microsomes[20]. Vitamin k1 and MK-7 had the capability to block activation of 12-lipooxygenase (12-LOX) and to inhibit reactive oxygen species (ROS) generation in pre-oligodendrocytes, hence, prevent oxidative cell death[36].

Recent study demonstrated that MK-7 could modulate immune and inflammatory reactions in the dose–response inhibition of TNF-α, interleukin-1 alpha (IL-1α), and IL-1ß gene expression in the cell culture of human monocyte-derived macrophages (hMDMs) *in vitro* after lipopolysaccharide (LPS) stimulation[37]; this effect might make a contribution to attenuation of the liver inflammatory response resulted from Dox treatment. Additionally, MK-7 was found to be able to protect heart against Dox-induced cardiotoxicity via antioxidant and anti-apoptotic effects[38]. In conclusion, MK-7 may have hepatoprotective effect against Dox-associated hepatotoxicity in rats via antioxidant effect as illustrated by its ability to lessen lipid peroxidation product, and enhance antioxidant capacity.

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