



Effect of Two Doses of Vitamin K2 (Menaquinone-7) on Doxorubicin - Induced Cardiotoxicity in Rats

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ABSTRACT

The objective of this study was to evaluate the impact two doses of Menaquinones-7 (MK-7) on doxorubicin- induced cardio toxicity in rats. Sixty adult rats of both sexes were used in this study; the animals were enrolled into six groups of 10 animals each. Group I: negative control (D.W treated); Group II: MK-7 16 µg/kg; Group III: MK-7 48 µg/kg; Group IV: positive control (doxorubicin 15 mg/kg); Group V: MK-7 16 µg/kg + doxorubicin 15 mg/kg; Group VI: MK-7 48 µg/kg + doxorubicin 15 mg/kg. At day twelve of the study, blood was collected for serum preparation for the estimation of LDH, CK-MB and AST. The heart of each animal was excised for homogenate preparation and estimation of MDA, TAOS and caspase-3, and histological examination. MK-7 significantly ($p < 0.05$) decrease serum AST, MDA, TAOS and caspase-3 content in heart tissue homogenate and there was an improvement in the histopathological lesions of the heart in group V and group VI compared to group IV. However, MK-7 had no significant ($p > 0.05$) effect regarding LDH or CK-MB. In conclusion, MK-7 may have protective effect against doxorubicin- induced cardio toxicity in rats.

Keywords: Menaquinone-7, doxorubicin, cardio toxicity, apoptosis, oxidative stress, rats.

INTRODUCTION

Cancer is growing disease due to a gradually increase in the population growth rate and ageing; and different type of chemotherapeutic agent was used for cancer treatment.¹ Doxorubicin is an anthracycline still widely used in modern cancer treatments for different type of malignancy² despite the advent of targeted therapy.³ However, its beneficial effect was limited by its adverse effect on heart⁴, kidney⁵, liver⁶ and other organs^{7,8} with its main toxicity on heart and liver. Ewer and Lippman *et al.* (2014) classified cardio toxicity of chemotherapy into 2 types. Type 1 which is irreversible due to myocyte injury, and Type 2 which is reversible with cessation of drug and not associated with ultra structural abnormalities. Anthracyclines are supposed to cause Type 1 cardiotoxicity.⁹

Several mechanisms involved in doxorubicin cardio toxicity including induction of oxidative stress⁸ activation of apoptosis⁷, intra cellular calcium dysregulation¹⁰ and other.^{11,12} Various potential protectants were evaluated for their possible protective effect against doxorubicin toxicity.^{3,6,7,8,13}

Fat-soluble vitamin K is an essential micronutrient¹⁴ for which there is two forms: phylloquinone (vitamin K1; VK1) and the menaquinones (vitamin K2; VK2; MK-n). Menaquinones differ in side chains of varying length. In this study we used menaquinone-7 (MK-7) because of its long half-life and good bioavailability¹⁵; it is produced by bacteria and is present in fermented foods such as cheese or sauerkraut.¹⁶ Several authors demonstrated the beneficial protective role of long chain vitamin k against

cardiovascular and bone diseases.¹⁷⁻¹⁹ The aim of our research was to evaluate the impact of MK-7 on doxorubicin- induced cardio toxicity in rats.

MATERIALS 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

Doxorubicin as doxorubicin hydrochloride (50 mg vial) was purchased from Pfizer, Italy. MK-7 (MenaQ7 capsule 180 µg) was purchased from Omicron Pharmaceuticals, Norway.

Experimental protocol

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

Group I: Animals were received 0.5 ml of distilled water as intraperitoneal (IP) doses. This group served as a negative control.

Group II: Animals were received MK-7 (16 µg/kg b.w./day) orally by oral gavage for 11 successive days.



Group III: Animals were administered MK-7 (48 µg/kg b.w./day) orally by oral gavage for 11 successive days.

Group IV: Animals were injected with single dose of doxorubicin (15 mg/kg b.w.) intraperitoneally. This group served as a positive control.

Group V: Animals orally administered MK-7 at a dose of 16 µg/kg b.w. /day and 15 mg/kg of doxorubicin.

Group VI: Animals treated with 48 µg/kg b.w./day MK-7 with 15mg/kg of doxorubicin.

In animals' groups (V and VI), each dose of MK-7 was administered once daily for 11 consecutive days; and at day 11, they received single dose of doxorubicin (15 mg/kg b.w.) 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 blood was collected for serum preparation. After necropsy, heart was excised for homogenate preparation and histological examination.

Estimation of serum biochemical parameters:

The serum samples was used for the estimation of the enzymes activities of lactate dehydrogenase (LDH), creatine kinase (CK-MB) isozyme, and aspartate aminotransferase (AST) by Automated Biochemistry analyzer (KENZA, Biolabo, France).

Estimation of malondialdehyde (MDA), total antioxidant capacity (TAOC) level, and caspase-3 level in heart and tissue homogenate samples

The preparation of heart 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 heart 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 °C. The supernatant was carefully collected and stored at -50 °C until the time for the determination of malondialdehyde (MDA) content, total antioxidant capacity (TAOC) level, and caspase-3 level. Estimation of MDA and TAOC was made by using of the quantitative sandwich ELISA kit (My BioSource, USA). Sandwich-ELISA kit (Elabscience Biotechnology, USA) was utilized for estimation of caspase-3 level in heart homogenate.

Histological Examination

After necropsy, heart was removed and a fragment of the left ventricle was used for a histopathological examination according to the method of Suvarna *et al* (2012) by using paraffin sections technique; the fragments were fixed in 10% formaldehyde solution, embedded in paraffin, segmented, and then stained with haematoxylin/eosin. Morphological examination of the samples was studied using light microscopy.²⁰

Statistical Analysis

Data were expressed as the mean values, mean± standard error of the mean (SEM). 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) by IBM SPSS (statistical package for social sciences) version 23. Differences were considered statistically significant for P-value less than 0.05.

RESULTS AND DISCUSSION

Heart toxicity is the major factor hindering doxorubicin treatment. Doxorubicin cardio toxicity is mediated by several mechanisms for instance, oxidative stress, apoptosis induction and intracellular calcium dysregulation.^{7, 8, 10} Previous studies indicated that there was a strong association between the oxidative stress and the cardiac inflammatory response, comprising the release of pro-inflammatory cytokine following doxorubicin treatment such as TNF-α, which mediate myocardial damage^{21,22} and IL-17 level²³. Moreover, doxorubicin induced a significant elevation in the expression of the apoptotic marker specifically; caspase-3 in kidney tissue and caused initiation of inflammation and oxidative stress.²²

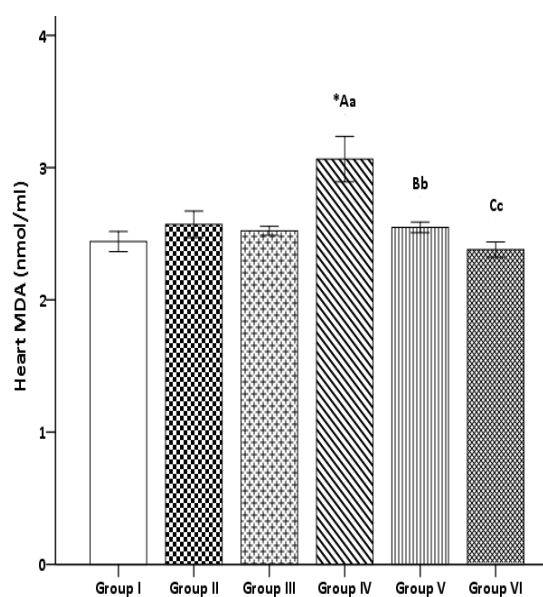
Clearly, the current research revealed cardio toxicity of doxorubicin as demonstrated by markedly significant ($p < 0.05$) increment in serum level of LDH, CK-MB, and AST in positive control group with respect to negative control group as shown in see Table 1. These results are in agreement with many studies.^{12, 24, 25} This rising may be attributed to the recognized cardiac toxic effects of doxorubicin that result in cellular damage and leakage of intracellular enzymes. Furthermore, Doxorubicin administered in a single intraperitoneal dose (15 mg/kg) resulted in oxidative stress via elevation of MDA content (Figure 1) and reduction of TAOC level (Figure 2) in heart tissue homogenate significantly ($p < 0.05$) in the doxorubicin treated rats compared with negative control group; these results coincided with Singh *et al.* (2015).²⁶ The increment in MDA, secondary products during lipid peroxidation, may be attributed to the effects of free radicals, generated as a result of doxorubicin treatment, on NADH- dependent microsomal lipid peroxidation and thus induces a lipid radical chain reaction causing oxidative damage to cell membrane.²⁷ Apoptosis induction after 24 hours of doxorubicin injection was observed by statistically significant ($p < 0.05$) increase in caspase-3 level in heart tissue homogenate in the doxorubicin treated rats compared with negative control group (Figure 3). These results are in line with those of Ueno *et al.* (2006).²⁸ Histopathologically, heart section of positive control group characterized by necrosis of cardiac myocytes, extreme invasion of inflammatory cells primarily neutrophils and penetration of neutrophils in the interstitial tissue was observed (Figure 4 D).



Table 1: Effect of two doses of MK-7 on serum LDH, CK-MB, and AST in rats.

Group	Serum LDH levels (IU/L)	Serum CK-MB levels (IU/L)	Serum AST levels (IU/L)
Group I	889.5±106.1	612.2 ±10.8	164.8 ± 6.2
Group II	782.6±82.3	640.7 ± 63.48	169.7 ± 14.2
Group III	905.1±87.6	578 ±68.2	175.6 ± 12.9
Group IV	1846.7±153.3 ^{*Aa}	825.8 ±29.5 ^{*Aa}	311.9 ± 31.2 ^{*Aa}
Group V	1521.9±123.1 ^{Aa}	825.3 ±34.8 ^{Aa}	215.5 ± 12.7 ^{Bb}
Group VI	1774.8±108.3 ^{Aa}	830.8 ±10.5 ^{Aa}	208.5 ± 6.4 ^{Cc}

- Data are expressed as mean ± standard error of means (SEM).
- No. = Number of rats.
- *= Significantly different ($p < 0.05$) with respect to the negative control group.
- Values with non –identical capital letters superscripts (A, B, and C) are significantly different ($p < 0.05$) with respect to positive control group.
- Values with non –identical small letters superscripts (a, b, and c) are significant differences among group IV, group V, and group VI whenever $p < 0.05$.

**Figure 1:** Bar chart showing MDA contents in heart tissue homogenate in various experimental rats' groups.

- *= Significantly different ($p < 0.05$) with respect to the negative control group.
- Values with non –identical capital letters superscripts (A, B, and C) are significantly different ($p < 0.05$) with respect to positive control group.
- Values with non –identical small letters superscripts (a, b, and c) are significant differences among group IV, group V, and group VI whenever $p < 0.05$.

In the present study, the impact of two doses of MK-7 on doxorubicin- induced cardio toxicity in rats was evaluated. MK-7, both low and high doses, ameliorated serum level of AST, heart content of MDA, TAOC and caspase-3, see Table 1, Figure 1, Figure 2 and Figure 3; and improved histopathological lesions of the heart in group V and group VI compared to positive control group (Figure 4 E and F). However, MK-7 had no significant ($p > 0.05$) effect

regarding serum level of LDH or CK-MB. MK-7 is a fat-soluble vitamin produced by *Bacillus subtilisnatto*²⁹ with several bone and cardiovascular beneficial effects.^{18, 19} In this study, MK-7 was utilized because of its long half-life and good bioavailability than MK-4.¹⁵ The results of the current study exhibited the protective effect of MK-7 against doxorubicin- Induced cardio toxicity; this effect could be explained by potent antioxidant capacities when reduced to KH2 (dihydroquinone)³⁰ that in turn may result in attenuation of oxidative stress induced by doxorubicin; also, MK-7 raised TAOC (SOD/CAT /GSH-PX/GSH) content in heart homogenate which in turn may result in enhancement of antioxidant capacity. Beside, many of the positive impacts of MK-7 could be accredited to MK-4 since all vitamin K homologues can be converted to MK-4 *in vivo*.³¹ In addition, MK-4 levels in extra hepatic tissues did not rise after intake of nutritional dose of MK-4, however, MK-7 significantly raised MK-4 in extra hepatic tissues. Thus, MK-7 is a better source for MK-4 *in vivo* than MK-4 itself.³² Several studies reported the antioxidant and anti-inflammatory influence of vitamin K analogues *in vivo* and *in vitro*. Vervoort *et al.* (1997) showed that vitamin K2 was an inhibitor of microsomal lipid peroxidation in rat liver microsomes.³³ Thereafter, Li *et al.* (2003) demonstrated that sub molar concentration of vitamin K1 and MK-4 could potentially preclude oxidative cell death to developing oligodendrocytes and neurons in basal-defined medium without cystine. However, the cytoprotective effect of vitamin K and MK-4 was independent on of their well-known biological role in carboxylation and they were not antioxidants by themselves but has potent antioxidant capacities when metabolized to KH2 (dihydroquinone) in cells.³⁰ Vitamins K analogues could provide a protection for human embryonic kidney cells culture against H₂O₂- induced oxidative stress, and increase cell viability.

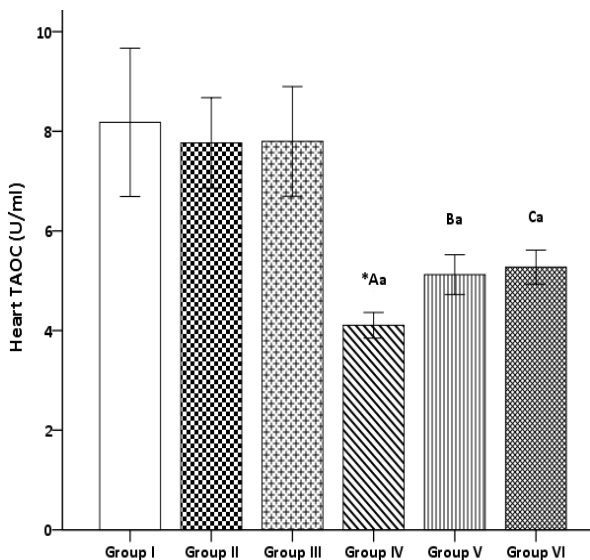


Figure 2: Bar chart showing TAOC content in heart tissue homogenate in various experimental rats' groups.

- *= Significantly different ($p < 0.05$) with respect to the negative control group.
- Values with non –identical capital letters superscripts (A, B, and C) are significantly different ($p < 0.05$) with respect to positive control group.
- Values with non –identical small letters superscripts (a, b, and c) are significant differences among group IV, group V, and group VI whenever $p < 0.05$.

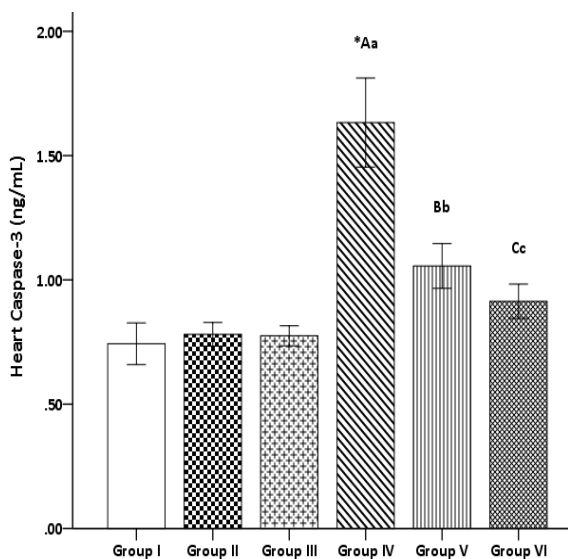


Figure 3: Bar chart showing Caspase-3 level in heart tissue homogenate in various experimental rats' groups.

- *= Significantly different ($p < 0.05$) with respect to the negative control group.
- Values with non –identical capital letters superscripts (A, B, and C) are significantly different ($p < 0.05$) with respect to positive control group.
- Values with non –identical small letters superscripts (a, b, and c) are significant differences among group IV, group V, and group VI whenever $p < 0.05$.

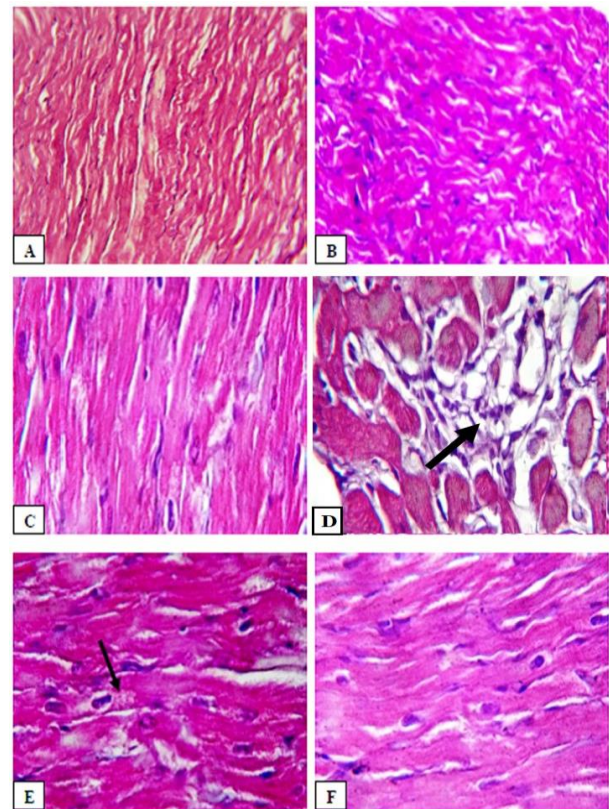


Figure 4: Histopathological section of heart in various experimental rats' groups; haematoxyline and eosin (X 40). A: group I; B: group II; C: group III; D: group IV; E: group V; F: group VI. Heart section of positive control group characterized by necrosis of cardiac myocytes, extreme invasion of inflammatory cells primarily neutrophils and penetration of neutrophils in the interstitial tissue (thick arrow) (D). The lesion in group V mild vacuolation in the cytoplasm of the cardiac muscles (thin arrow) (E). Meanwhile, heart section in group VI showed congestion of blood vessels (F).

Also it was found that vitamin K 2, 3-epoxide reductase complex like 1 (VKORC1L1) was the enzyme that accountable for driving vitamin K-mediated intracellular anti oxidation pathways critical to cell survival.³⁴ Another study reported the capability of vitamin k1 and MK-7 to block activation of 12-lipoxygenase (12-LOX) and to inhibit reactive oxygen species (ROS) generation in preoligodendrocytes, hence, prevent oxidative cell death.³⁵ Another proposed mechanism for the protective effect of MK-7 against doxorubicine-induced cardio toxicity was the suppression of apoptosis through the inhibition of caspase-3, suggesting that MK-7 has an important role against the progression of cardio myopathy due to doxorubicin. Recently, MK-7 was proved to be able to modulate immune and inflammatory reactions in the dose–response inhibition of TNF- α , IL-1 α , and IL-1 β gene expression and protein production in the cell culture of human monocyte-derived macrophages (hMDMs) *in vitro* after lipopolysaccharide (LPS) activation³⁶; this effect could contribute to attenuation of the cardiac inflammatory response resulted from doxorubicin treatment.

CONCLUSION

The findings of the present study revealed that MK-7 may have protective effect against doxorubicin- induced cardio toxicity in rats.

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