

Impact of Two Doses of Vitamin K2 (Menaquinone-7) on Doxorubicin-Induced Hepatotoxicity in Rats

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Abstract

The objective of this study was to evaluate the impact two doses of Menaquinones-7 on 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 (rats administered distilled water); Group II: Menaquinones-7 at a dose of 16 µg/kg; Group III: Menaquinones-7 at a dose of 48 µg/kg; Group IV: positive control (Doxorubicin 15 mg/kg); Group V: Menaquinones-7 at a dose of 16 µg/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, blood was collected for serum preparation for the estimation of alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin (TB). The liver of each animal was excised for histological examination. High dose of MK-7 significantly ($P<0.05$) decreased serum ALT, ALP, and TB and there was an improvement in the histopathological lesions of the liver in group V and group VI compared to group IV. In conclusion, MK-7 may have protective effect against Dox-induced hepatotoxicity in rats.

Keywords: Menaquinone-7, Doxorubicin, Hepatotoxicity, Rats.

تأثير جرعتين من Menaquinone-7 على سمية الكبد المستحثة بواسطة الدوكسوروبيسين في الجرذان.

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الخلاصة

الهدف من هذه الدراسة هو تقييم أثر جرعتين من Menaquinone-7 على سمية الكبد المستحثة بواسطة عقار الدوكسوروبيسين في الجرذان. تم استخدام 60 جرذاً بالغاً من كلا الجنسين حيث قسمت الحيوانات بصورة عشوائية الى ست مجموعات وفي كل مجموعة 10 حيوانات. المجموعة الاولى: مجموعة المراقبة السلبية واعطيت ماء مقطر؛ المجموعة الثانية أعطيت 16 مايكروغرام/كغم من Menaquinone-7؛ المجموعة الثالثة اعطيت 48 مايكروغرام/كغم من Menaquinone-7؛ المجموعة الرابعة: المراقبة الايجابية واعطيت 15 ملغم/كغم من عقار الدوكسوروبيسين؛ المجموعة الخامسة اعطيت 16 مايكروغرام/كغم من Menaquinone-7 قبل اعطاءها 15 ملغم/كغم من عقار الدوكسوروبيسين؛ المجموعة السادسة اعطيت 48 مايكروغرام/كغم من Menaquinone-7 قبل اعطاءها 15 ملغم/كغم من عقار الدوكسوروبيسين. في اليوم 12 من الدراسة، جُمع مصل الدم لغرض قياس مستوى الانزيمات الالانين امينوترانسفيريز ALT والالكالين فوسفاتيز ALP و البيليروبين الكلي TB. اضافة الى ذلك تم أستئصال الكبد لغرض الدراسة النسيجية. بينت النتائج أن الجرعة العالية من Menaquinone-7 خفضت بصورة معنوية ($P<0.05$) مستويات الانزيمات ALT و ALP ومستوى البيليروبين الكلي TB في مصل الدم وهناك تحسن في ضرر نسيج الكبد في المجموعتين الخامسة والسادسة مقارنة بالمجموعة الرابعة. ويمكن الاستنتاج بأن Menaquinone-7 قد يكون له تأثير وقائي ضد سمية الكبد المستحثة بواسطة عقار الدوكسوروبيسين في الجرذان. الكلمات المفتاحية: Menaquinone-7، الدوكسوروبيسين، سمية الكبد، الجرذان.

Introduction

In the complicated world of malignancy treatment, the administration of chemotherapeutic agents deliberately considered to be cytotoxic and causes negative consequences.

The liver is the primary site of metabolism for many drugs, and this liver-drug interaction must be accounted while dosing chemotherapy⁽¹⁾.

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Received: 11 /6/2017

Accepted: 28/8/2017

Doxorubicin (Dox) was approved in 1974 by Food and Drug Administration (FDA); it is an anthracycline still widely used in modern cancer treatments for different type of malignancy^(2, 3) despite the advent of targeted therapy⁽⁴⁾. However, its beneficial effect was limited by its adverse effects on heart⁽⁵⁾, kidney⁽⁶⁾, liver⁽⁷⁾ and other organs^(8, 9) with its main toxicity on heart and liver. Dox has adverse effects on the liver. One reason may be that the liver is the main organ contributed to metabolism of exogenous and endogenous toxins including Dox, leading to toxic metabolites formation. Hence, resulting in hepatotoxicity⁽¹⁰⁾; where, the hepatic damage in this respect was reported to be irreversible in a dose-dependent manner as reported by investigators^(11, 12). Another reason is that Dox may drastically increase lipid oxidation and mitochondrial reactive oxygen species (ROS) content, and decrease liver antioxidant enzymes and mitochondrial function⁽¹³⁾.

Fat-soluble vitamin K is an essential micronutrient (14) for which there is two forms: phyloquinone (vitamin K1) and the menaquinones (vitamin K2; MK-n). Menaquinones vary in the length and in the degree of saturation at the aliphatic side chains. Menaquinone-7 (MK-7) has long half-life and good bioavailability⁽¹⁵⁾; it is produced by *Bacillus subtilis natto*⁽¹⁶⁾. Several authors demonstrated the beneficial protective role of long chain vitamin k against cardiovascular and bone diseases⁽¹⁷⁻¹⁹⁾.

The aim of this study is to evaluate the impact of two doses of MK-7 on Dox-induced hepatotoxicity 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 hydrochloride (50 mg vial) was purchased from Pfizer, Italy. Menaquinone-7 (MenaQ7 capsule 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 0.5 ml of distilled water (DW) as intraperitoneal (IP) dose. This group served as a negative control.

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

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

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

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

Group VI: Rats were orally administered 48 µg/kg body weight/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 body weight) 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.

Estimation of serum biochemical parameters:

Serum samples were used for the estimation of the enzymes activities of alanine aminotransferase (ALT), and alkaline phosphatase (ALP), in addition to total bilirubin (TB) level by Automated Biochemistry analyzer (KENZA, Biolabo, France).

Histological examination

After necropsy, liver of each rat was removed and used for a histopathological examination according to the routine method (20) utilizing paraffin sections technique; the fragments were fixed in 10% formaldehyde solution, embedded in paraffin, segmented, and then stained with haematoxyline/eosin. Morphological examination of the samples was studied using light microscopy.

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 of two doses of MK-7 on serum liver biomarkers

Table 1 showed that group II rats, which received MK-7 (16 $\mu\text{g}/\text{kg}$ body weight/day) orally by oral gavage for 11 consecutive days and group III rats, which administered MK-7 (48 $\mu\text{g}/\text{kg}$ body weight/day) orally by oral gavage for 11 consecutive days, produced non-significant ($P>0.05$) differences in the serum ALT, ALP enzymes activities and TB with respect to negative control group. Besides,

single intraperitoneal dose of Dox significantly ($P<0.05$) increased the serum activities of ALT, ALP, and TB in comparison with negative control group. Furthermore, in group V rats, which received MK-7 at a dose of 16 $\mu\text{g}/\text{kg}$ body weight/day orally prior to 15 mg/kg of Dox, there were non-significant ($P>0.05$) differences in the intended enzymes compared to positive control group; however, in group VI rats received MK-7 at a dose of 48 $\mu\text{g}/\text{kg}$ body weight/day orally prior to 15 mg/kg of Dox exhibited significant ($P<0.05$) decrease in serum ALT, ALP enzymes activities, and TB compared to positive control group.

Table (1): Effects of various treatments on serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin (TB) in rats.

Group	Serum ALT levels (IU/L)	Serum ALP levels (IU/L)	Serum TB levels (IU/L)
Group I	56.2 \pm 3.3	240.3 \pm 8.528	0.111 \pm 0.01
Group II	62.2 \pm 1.2	276.4 \pm 26.73	0.117 \pm 0.004
Group III	61.1 \pm 1.139	265.2 \pm 30.9	0.115 \pm 0.007
Group IV	93.9 \pm 10.5 ^{*Aa}	390.1 \pm 18.4 ^{*Aa}	0.143 \pm 0.005 ^{*Aa}
Group V	75.1 \pm 6.1 ^{Aa}	343.8 \pm 32.8 ^{Aa}	0.123 \pm 0.008 ^{Aa}
Group VI	66.2 \pm 6.7 ^{Ba}	317.1 \pm 9.09 ^{Ba}	0.115 \pm 0.011 ^{Ba}

- Data are expressed as mean \pm standard error of means (SEM)

- **Group I:** negative control (D.W); **Group II:** MK-7 16 $\mu\text{g}/\text{kg}$; **Group III:** MK-7 48 $\mu\text{g}/\text{kg}$; **Group IV:** positive control (Dox 15 mg/kg); **Group V:** MK-7 16 $\mu\text{g}/\text{kg}$ prior to single IP dose of Dox 15 mg/kg; **Group VI:** MK-7 48 $\mu\text{g}/\text{kg}$ prior to single IP dose of Dox 15 mg/kg.

- * 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) is non-significantly different ($P>0.05$) among groups IV, V and VI utilizing ANOVA and LSD.

The histopathological examination of rats' liver tissue

Histological examinations of liver sections of the negative control group, group II (rats orally received MK-7 16 $\mu\text{g}/\text{kg}$ for 11 consecutive days), and group III (rats orally received MK-7 48 $\mu\text{g}/\text{kg}$ for 11 consecutive days) showed normal liver section (figure 1 A, B, and C, respectively). Examination of liver section of positive control group (Dox-treated rats) showed severe dilation of the central veins

with severe vacuolar degeneration of hepatocytes with a narrowing or disappearance of sinusoids (figure 1 D). The lesion in group V (MK-7 16 $\mu\text{g}/\text{kg}$ prior to a single dose of Dox 15 mg/kg) revealed mild congestion of central veins with moderate vacuolar degeneration (figure 1 E). Besides, liver section of group VI (MK-7 48 $\mu\text{g}/\text{kg}$ prior to a single dose of Dox 15 mg/kg) exhibited mild vacuolar degeneration (figure 1 F).

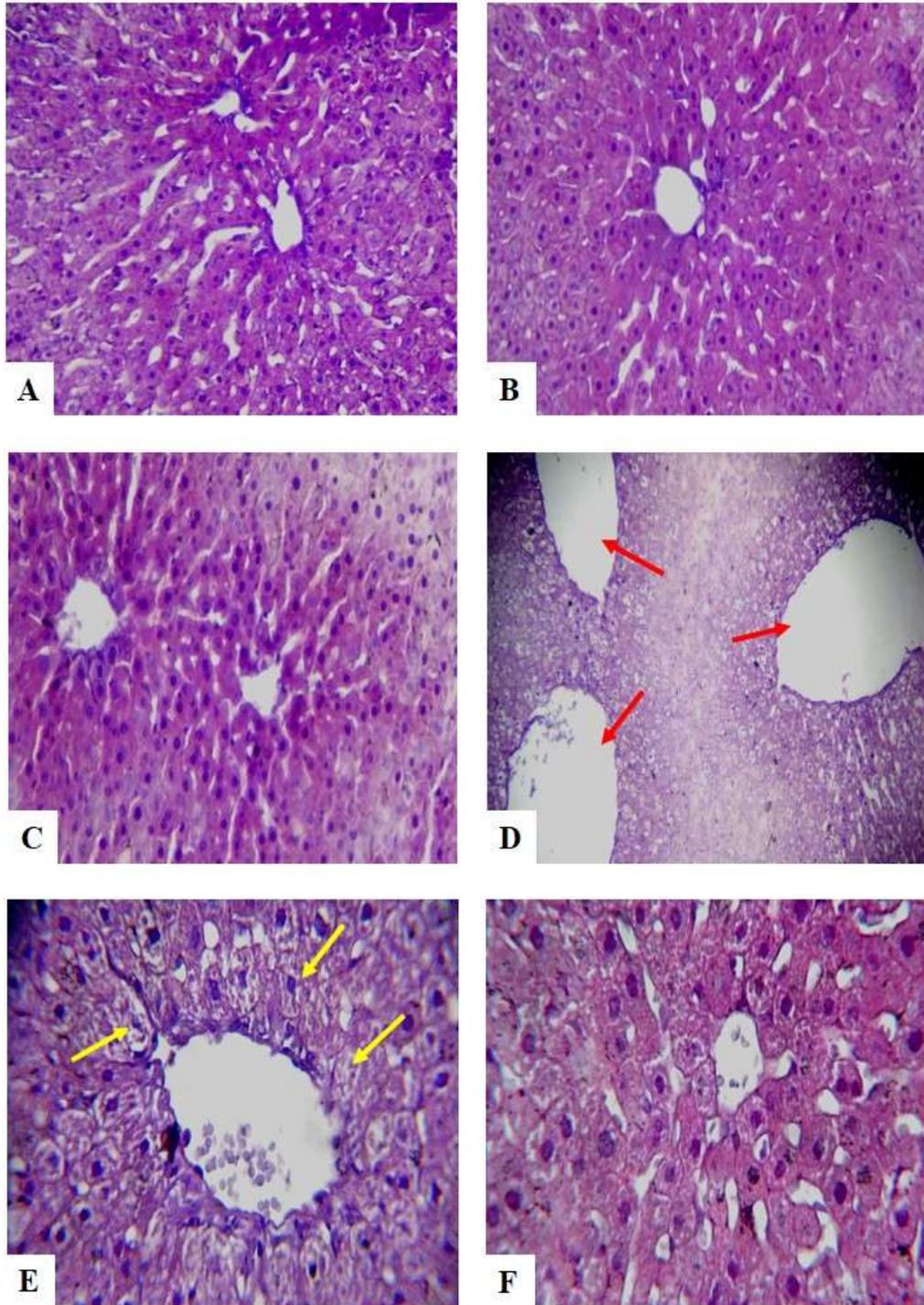


Figure (1): Histopathological section of liver in various experimental rats' groups; (haematoxyline and eosin; X20). A: group I (negative control (D.W)); B: group II (MK-7 16 $\mu\text{g}/\text{kg}$); C: group III (MK-7 48 $\mu\text{g}/\text{kg}$); D: group IV (positive control (Dox 15 mg/kg); E: group V (MK-7 16 $\mu\text{g}/\text{kg}$ prior to a single dose of Dox 15 mg/kg); F: group VI (MK-7 48 $\mu\text{g}/\text{kg}$ prior to a single dose of Dox 15 mg/kg ; X 40). Negative control group, group II, and group III showed normal liver section. Positive control group characterized by severe dilation of the central veins with severe vacuolar degeneration of hepatocytes (red arrow). The lesion in group V revealed mild congestion of central veins with moderate vacuolar degeneration (yellow arrow). Besides, liver section of group VI exhibited mild vacuolar degeneration.

Discussion

Toxicity is the major factor hindering Dox treatment. The mechanisms of Dox mediated cell death include oxidative stress, apoptosis, intracellular calcium dysregulation, topoisomerase II poisoning, DNA adduct formation, and ceramide overproduction^(21, 22). Additionally, Dox initiates inflammation via markedly increase of inflammatory-related proteins including TNF- α , IL-1 β , and IL-6 in the liver⁽²³⁾.

The current study revealed that Dox-induced hepatotoxicity, which was evident by significant ($P < 0.05$) elevation in serum activities of ALT and ALP with respect to negative control group. The results are in agreement with studies of others⁽²⁴⁻²⁶⁾. The elevation of the serum activities of the intended enzymes may be attributed to the recognized hepatotoxic effect of doxorubicin that result in cellular damage and leakage of such enzymes to the extracellular space. Furthermore, there was a significant ($P < 0.05$) increase in serum TB level in animals received single intraperitoneal dose (15 mg/kg body weight) of Dox compared with the negative control group. These results are in line with those of Salman (2013)⁽²⁷⁾. Since the liver is responsible for clearing the blood from bilirubin thus, increasing serum TB level indicated a reduction in the excretory capability of the liver as a consequence of liver injury⁽²⁸⁾.

In the present study, MK-7 administered at a dose of 48 $\mu\text{g}/\text{kg}$ prior to single IP dose of Dox 15 mg/kg body weight (group VI), significantly ($P < 0.05$) lowered serum level of ALT, ALP, and TB compared to positive control group. Moreover, there were improvement of the histopathological lesions of the liver in -group V rats administered MK-7 at a dose of 16 $\mu\text{g}/\text{kg}$ prior to single IP dose of Dox 15 mg/kg body weight and -group VI rats that received 48 $\mu\text{g}/\text{kg}$ MK-7 prior to single IP dose of Dox 15 mg/kg body weight compared to positive control group. This hepatoprotective effect of MK-7 could be attributed to potent antioxidant capacities of MK-7 when reduced to KH2 (dihydroquinone) during vitamin k cycle⁽²⁹⁾, that in turn may result in attenuation of oxidative stress induced by Dox.

Several beneficial effects of MK-7 on bone and cardiovascular system were elucidated by authors^(18, 19). Moreover, it has been reported that, many of the positive impacts of MK-7 could be accredited to menaquinones-4 (MK-4) where, all vitamin K homologues can be converted to MK-4 *in vivo*⁽³⁰⁾ and many studies

reported the antioxidant and anti-inflammatory influence of vitamin K analogues *in vivo* and *in vitro*; Furthermore, Vervoort *et al.* (1997) showed that vitamin K2 was an inhibitor of microsomal lipid peroxidation in rat liver microsomes⁽³¹⁾. Additionally, Vitamin k1 and MK-7 had the capability to block activation of 12-lipoxygenase (12-LOX) and to inhibit ROS generation in pre-oligodendrocytes, hence, prevent oxidative cell death⁽³²⁾.

In conclusion, MK-7, in a high dose, may have protective effect against Dox-induced hepatotoxicity in rats as demonstrated by liver function evaluation and histological examination. To the best of our knowledge, this is the first study that examines the effects of MK-7 at two doses (16 $\mu\text{g}/\text{kg}$ and 48 $\mu\text{g}/\text{kg}$) each prior to doxorubicin on the liver. Therefore, we did not have a thorough chance to compare the results of this study with other reports.

Acknowledgments

This article was abstracted from Ph.D. thesis submitted to the Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad. The authors gratefully thank the staff of Ministry of Health and Environment, National Center for Drug Control and Research, Baghdad, Iraq. Thanks to lecturer Sulake F. Al-Zubaidi, College of Veterinary Medicine, Al-Qasim Green University, Babylon, Iraq for his valuable assistance.

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