Hepatoprotective Effect of Vitamin K2 on Cyclophosphamide-induced Liver Toxicity in Male Mice

[*1]Nusieba A. Mohammed Ibrahim, [2]Yahya Saber E. Mansour, [3]Hayder S. Ali Hussein, [4]Marwa M. Mohammed Bader and [5]Somia M. Abdalla Elsheikhi

[1,2]Department of Pharmacology and Toxicology, Faculty of Pharmacy
Omar Al-Mukhtar University, Albayda, Libya
nusieba.ibrahim@omu.edu.ly and yahya.saber@omu.edu.ly

[3]Department of Pharmacology and Toxicology, Faculty of Pharmacy
University of Mosul, Mosul, Iraq
hayder.hussein24@gmail.com

[4,5]Department of Pharmaceutical Chemistry, Faculty of Pharmacy
Omar Al-Mukhtar University, Albayda, Libya
marwa.mousa@omu.edu.ly and sumia9387@yahoo.com

Abstract

The current study was designed to evaluate the hepatoprotective effect of Vitamin K2 (VK2) against cyclophosphamide-induced liver toxicity in male mice. Sixty (60) healthy mature male mice were used in this experimental study and were randomly divided into four groups (1-4) of fifteen (15) mice per group. Group 1 (control group): mice were injected with 0.5 ml/day of normal saline intraperitoneally (i.p.) for six consecutive days. Group 2 (VK2 treated group): mice were received 50 µg/kg/day of VK2 orally for six days. Group 3: (CP treated group): mice were injected intraperitoneally with a single dose of 100 mg/kg CP on the sixth day of experiment. Group 4 (VK2-CP treated group): mice were received 50 µg/kg/day of VK2 orally for six days and a single dose of 100 mg/kg CP was injected intraperitoneally on the sixth day of experiment. On the day seventh of the experiment, the blood was collected for serum estimation of hepatic marker enzymes levels such as alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate transaminase (AST). After the blood sampling, all mice were killed, the livers were removed, and the histological studies were conducted. The results showed that the injection of CP significantly (P<0.05) increased serum levels of ALT, ALP, and AST compared to the control group. Concomitant treatment of VK2 and CP significantly (P<0.05) decreased serum levels of ALT, ALP, and AST compared to the CP treated group. CP treated group showed severe congestion of portal vein with severe vacuolar hepatopathy, while VK2-CP treated group showed mild congestion of portal vein with mild vacuolar hepatopathy. The current study suggested that the VK2 has chemoprotective effect against CP-induced hepatotoxicity. Therefore, VK2 could be a potent candidate to use as a supplement agent against hepatotoxicity of CP for the patients undergoing chemotherapy.

Keywords: Vitamin K2 (VK2), Cyclophosphamide (CP), Liver, Toxicity, Mice, Hepatic Marker Enzymes, and Histological Studies.

1. Introduction

Cancer has become the most common cause of death in the world in recent years. Chemotherapy is one of the commonest and most frequent methods used for the treatment of cancer. Chemotherapy affects both cancerous cells and healthy cells. Chemotherapeutic agents are generally toxic to healthy cells and can cause critical side effects such as myelosuppression [1]. Therefore, new effective ways must be developed to minimize the toxic side effects of chemotherapeutic agents without losing their chemotherapeutic effectiveness. Various clinical studies have suggested that a combination of chemotherapeutic and chemoprotective agents can be helpful in reducing the systemic toxicity of chemotherapeutic drugs [2,3].

Cyclophosphamide (CP) is one of the most commonly and widely used drugs for cancer chemotherapy. It was approved in 1959 by the US Food and Drug Administration (FDA) [4]. CP is an alkylating agent nitrogen mustard with anticancer and immunosuppressant properties, commonly used for the treatment of various types of cancer such as acute and chronic leukemia, multiple myeloma, lymphomas, and solid tumors [4] and also in the treatment of autoimmune diseases such as nephrotic syndrome [5]. Despite its numerous clinical applications, the usage of CP is generally limited due to its undesirable toxic adverse effects including hepatotoxicity, which limit its usefulness [6,7]. CP-induced hepatotoxicity has been characterized by altered liver function marker enzymes and distortion of liver architecture [7]. CP is metabolized by hepatic microsomal cytochrome P450 enzymes into two active metabolites: phosphoramide mustard (PAM) and acrolein (ACR) [8]. While CP's immunosuppressive and antineoplastic effects are related with PAM, ACR is responsible for its undesirable toxicity [9]. ACR, a toxic metabolite of CP, generates highly reactive oxygen species (ROS) such as superoxide anion (O^{-}_{2}), hydroxyl radical ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2) during its oxidative metabolism and depresses the antioxidant defense mechanisms in the liver [10]. Various studies showed that antioxidant intake can control the reaction of chemotherapy and also minimize and/or stop the toxic adverse side effects of chemotherapeutic drugs [11,12]. Thus, prescribing of antioxidant agents during chemotherapy in order to reduce oxidative stress due to CP seems necessary. Vitamin K2 (VK2 or menaquinone-7) is a fat-soluble bioactive vitamin, which is produced by *Bacillus subtilis natto* [13]. VK2 has both long half-life and good bioavailability [13]. Several recent trials have suggested a main role of vitamin K2 to be as an antioxidant [14,15,16]. VK2 has an antioxidative activity of 10 to 100 times more than any other well-known radical scavengers, such as alpha-tocopherol and ubiquinone by which it protects cells, tissues, and organs from the oxidative damage of ROS [17]. Because VK2 possesses excellent antioxidative activities and free radical-scavenging properties, there is a likely possibility that VK2 may act as a prophylactic against hepatoxicity induced by CP. Therefore, the aim of this current study was to determine if VK2 has hepatoprotective effect against CP-induced liver toxicity in male mice. Histological examination of liver tissues was also used to determine any morphological influence of VK2 on CP toxicity.

2. Materials and Methods

2.1 Drugs and Chemicals

Cyclophosphamide (500 mg single dose vial) was acquired commercially from SANDOZ Pharmaceuticals, Austria, while VK2 (100 µg, tablets) was purchased from HerbalLeaf Pharmaceuticals, India. All other chemicals and solvents used for this study were of the highest purity and analytical grade.

2.2 Experimental Animals and Design

Sixty (60) healthy mature male mice (3 months old, weighing 20±5 g) were used in this experimental study. They were housed under standard laboratory conditions (12:12 hr light/dark cycles, humidity 30-60%, and temperature 23 ± 2 °C). Mice were fed with standard food pellets and fresh drinking water via *ad libitum* throughout the experiment period. Animal experiments were conducted according to the guidelines of the Institutional Animal Ethics Committee (Approval No. Ethics/Research/OMU/2020-45).

In this experimental study, sixty (60) healthy mature male mice were randomly divided into four (4) groups (n=15 per group) as follows. Group 1 (control group): mice were injected with 0.5 ml/day of normal saline intraperitoneally (i.p.) for six consecutive days. Group 2 (VK2 treated group): mice were received 50 µg/kg/day of VK2 orally via oral gavage feeding needles for six days. Group 3: (CP treated group): mice were injected intraperitoneally with a single dose of 100 mg/kg CP on the sixth day of experiment. Group 4 (VK2-CP treated group): mice were received 50 µg/kg/day of VK2 orally via oral gavage feeding needles for six days and a single dose of 100 mg/kg CP was injected intraperitoneally on the sixth day of experiment. On the day seventh of the experiment, the mice were intraperitoneally anesthetized with diethyl ether and the blood was collected for serum estimation of hepatic marker enzymes activities. After the blood sampling, all mice were killed by an overdose of ether, the liver tissues were removed for further analysis, and the histopathological examinations were conducted.

2.3 Serum Hepatic Marker Enzymes Activities Estimation

Serum samples were used for the biochemical estimation of ALT, ALP, and AST hepatic marker enzymes activities by KENZA BIOLABO AUTOMATIC BIOCHEMISTRY ANALYZER. The results were expressed as units/liter (IU/L).

2.4 Histopathological Examination

Liver of each mouse was removed and used for a histopathological examination according to the routine method [18]. Tissue samples were fixed in 10% buffered formaldehyde solution for 24h and embedded in paraffin blocks. Slides were prepared (5 µm thick), stained with hematoxylin-eosin (H&E), and analyzed for pathology using light microscopy.

2.5 Statistical Analysis

All values were presented as mean ± SD and were analyzed using statistical package SPSS (version 23, Chicago, USA) by one-way ANOVA and LSD test. A *p*-value less than 0.05 was considered statistically significant.

3. Results

3.1 Biochemical Findings

As can be seen from Table 1, the results showed that the injection of CP significantly (P<0.05) increased serum levels of ALT, ALP, and AST compared to the control group. Concomitant treatment of VK2 and CP significantly (P<0.05) decreased serum ALT, ALP, and AST activities compared to the CP treated group.

Table 1: Serum ALT, ALP, and AST Levels in the different experimental groups (IU/L).

Experimental Groups	ALT (IU/L)	ALP (IU/L)	AST (IU/L)
Group 1 (control group)	61.5 ± 1.2	58.3 ± 5.7	59.0 ± 4.8
Group 2 (VK2 treated group)	60.0 ± 1.1	56.6 ± 3.4	57.2 ± 4.9
Group 3 (CP treated group)	91.2 ± 3.0*	87.5 ± 4.5*	89.1 ± 3.2*
Group 4 (VK2-CP treated group)	70.8 ± 2.8**	66.3 ± 5.2**	68.4 ± 3.6**

Data are expressed as mean ± SD. * Significantly different (P<0.05) with respect to the control group using unpaired Student t-test. ** Significantly different (P<0.05) with respect to the CP treated group using unpaired Student t-test.

3.2 Histopathological Findings

The liver sections of control group and VK2 treated group showed normal liver histology (Figs. 1 & 2 respectively). The histological sections of CP treated group showed severe congestion of portal vein with severe vacuolar hepatopathy (Fig. 3), while the liver sections of VK2-CP treated group showed mild congestion of portal vein with mild vacuolar hepatopathy (Fig. 4).

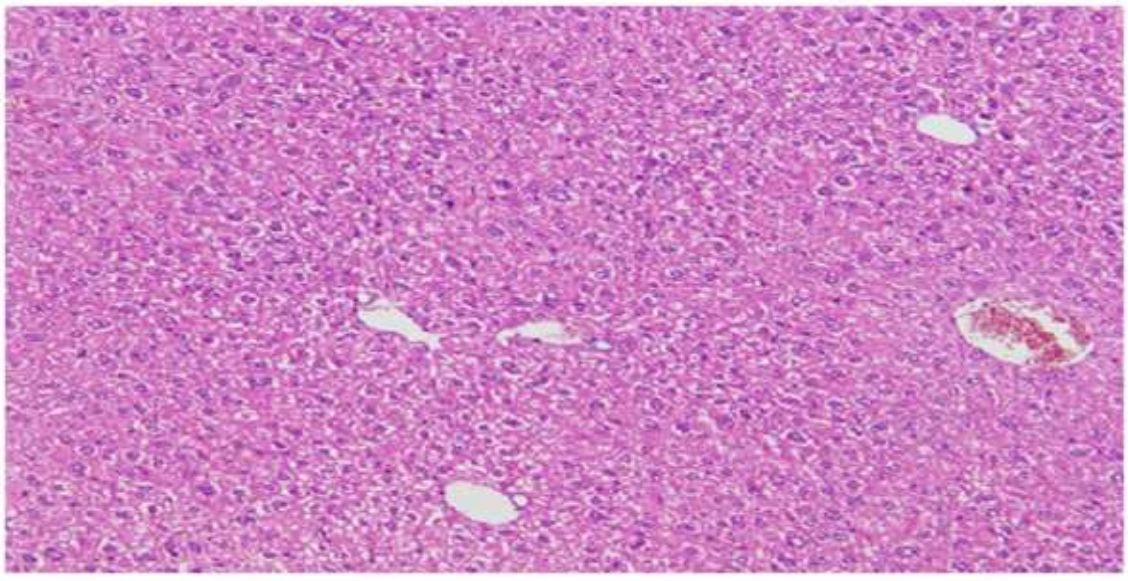

Figure 1: A microscopic image of mouse liver tissue of control group (H&E X 200) showing a normal liver histology.

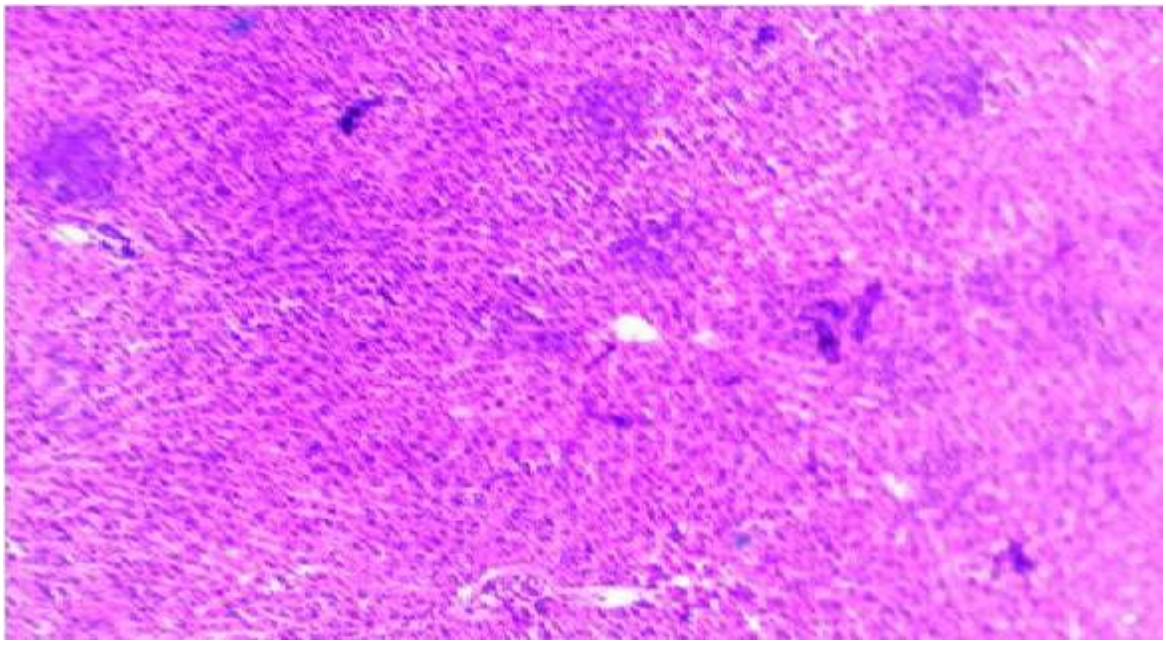

Figure 2: A microscopic image of mouse liver tissue of VK2 treated group (H&E X 200) showing a normal liver histology.

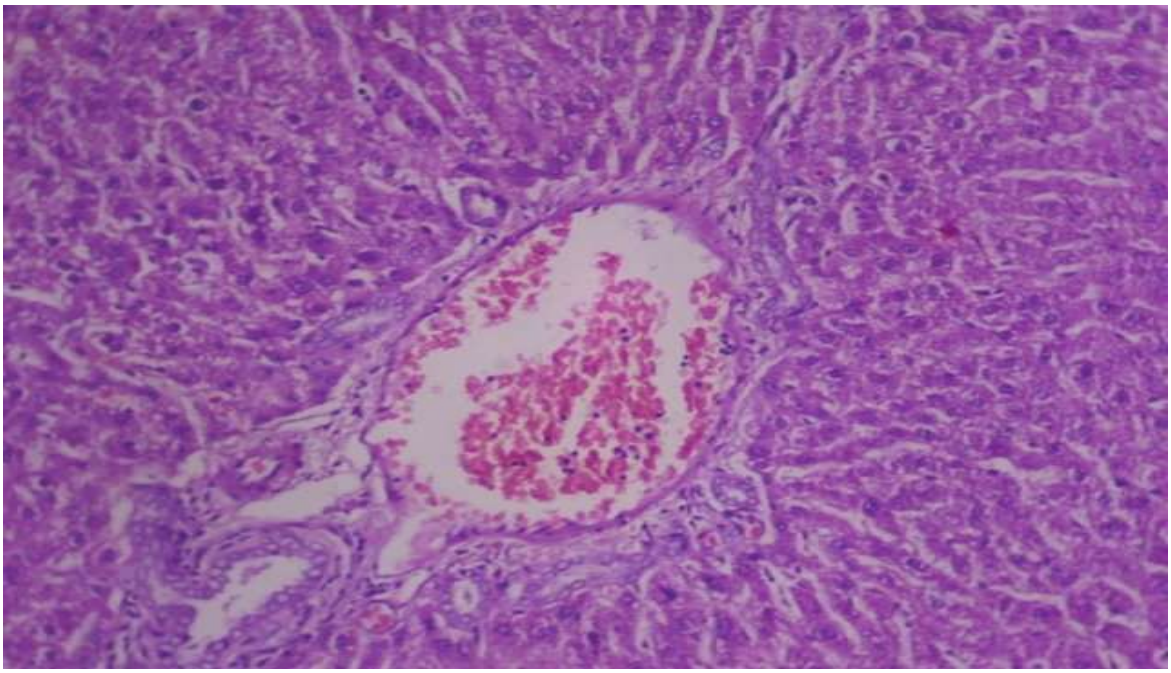

Figure 3: A microscopic image of mouse liver tissue of CP treated group (H&E X 200) showing a severe congestion of portal vein with severe vacuolar hepatopathy.

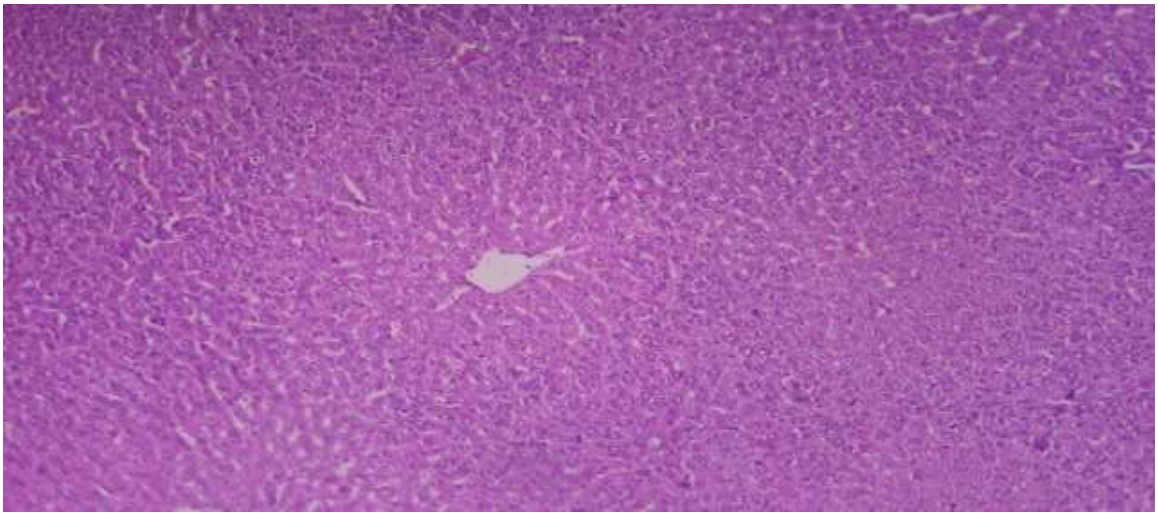

Figure 4: A microscopic image of mouse liver tissue of VK2-CP treated group (H&E X 200) showing a mild congestion of portal vein with mild vacuolar hepatopathy.

4. Discussion

The cellular mechanisms by which CP causes hepatotoxicity are poorly understood; however, several studies have shown that CP treatment is associated with induction of oxidative stress by the generation of free radicals and ROS [19,20]. In the current study, pretreatment with VK2 lowered the levels of serum toxicity enzymes, and the values were comparable with that of the control animals. Also, the histological slides of livers treated with VK2 prior to CP administration proved that VK2 protected the hepatocytes from pathological lesions induced by CP, which showed normal size and shape of liver cells with the normal appearance of nucleolus and nuclear membrane and this comes in agreement with Abd El-aziz et al, (2001) [21]. This suggests the hepatoprotective role of VK2. Previous studies have reported that antioxidants can preserve normal tissues against CP toxicity [22]. Hence, it is important to give an antioxidant before using CP in patients with malignancies or autoimmune diseases. In the current study, a single dose of CP administration (100 mg/kg) to mice resulted in a significant ($P<0.05$) increase in the levels of serum ALT, ALP, and AST. These findings are compatible with other previous studies [23,24,25]. The histopathological studies of this current study also proved that the CP caused damage to the liver, and this was evidenced by the severe congestion of portal vein and signs of degeneration of hepatocytes in the form of swelling cells up to ballooning, vacuolated cytoplasm. This might be due to membrane damaging potential of the CP's metabolites. In the current study, treating of mice with CP (100 mg/kg) single dose i.p, after 6 days of VK2 administration (50 µg/kg/day) resulted in a significantly improved hepatic marker enzymes and histopathological studies compared to the CP treated group. This agrees with Aldahmash and El-Nagar (2016) who studied the protective effect of VK2 against hepatic ischemia-reperfusion injury in rats [26]. The histopathological observations in this current study suggested the possibility of VK2 being able to protect the liver tissues and thus decreasing the serum toxicity enzymes in the blood.

5. Conclusion

Our results obtained in this current study supported the hepatoprotective role of VK2 against CP-induced liver toxicity. VK2 decreased the levels of serum toxicity enzymes (ALT, ALP, and AST) and the histopathological examinations confirmed the chemoprotective effect of VK2 against liver toxicity of CP. From these observations, it is possible to conclude that the pretreatment of VK2 effectively improved hepatotoxicity induced by CP in male mice through its ability to scavenge the oxidative free radicals generated by CP. Therefore, VK2 could be a potent candidate to use as a supplement agent against hepatotoxicity of CP for the patients undergoing chemotherapy.

6. Financial Support and Sponsorship

Nil.

7. Conflicts of Interest

We hereby declare that there are no conflicts of interest regarding the publication of this research study.

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