# Evaluation of the Genoprotective and Cytoprotective Activity of Vitamin K-7 Against Doxorubicin in Bone Marrow Cells and Spleen Cells of Rats

**Ali Faris Hassan[1], Ruaa Fadhil Abbas[2], Nada Naji Al-Shawi[1], Intesar Tarik Numan[3]**

*[1]Lec. Dr. Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad/Iraq, [2]Dr. Ministry of Health/Iraq, [3]Prof. Dr. College of Pharmacy, Al-Bayan University/Iraq*

## Abstract

Doxorubicinisa chemotherapeutic drug that showed a great treatment potential, unfortunately, itis not-selective to cancer cells and affects many other cells. Vitamin K-7 is available from nutritional sources and dietary supplements,it has been reported that it had many beneficial effects.

**Objective:** Evaluate the protective effect of vitamin K-7 against cytotoxicity and genotoxicity induced by doxorubicin.

**Method:** Seventy-two rats divided equally into two groups, each group divided into 6 subgroups as following **Group I**: received distilled water. **Group II**: receiveda single dose of Doxorubicin (15mg/kg). **Group III**: received Vitamin K-7(16μg/kg) for 11 successive days. **Group IV**: received Vitamin K-7 (48μg/kg) for 11 successive days.**Group V**: received Vitamin K-7(16μg/kg) for 11 consecutive days before a single dose of Doxorubicin 15mg/kg on day11. **Group VI**: received Vitamin K-7 at a dose of 48 μg/kg for 11 consecutive days before a single dose of Doxorubicin 15 mg/kgat day 11.

**Results:** Group II caused significant reduction ($P$<0.05) in mitotic index associated with an elevation in the micronucleus appearance and the total chromosomal aberrations compared to those of control Group I rats; meanwhile, Groups IV and V decreased chromosomal aberration, micronucleus appearance and increase mitotic index compared to Group II rats treated with doxorubicin.

**Conclusion:** Vitamin K-7 has protective effects against genotoxicity and cytotoxicity induced by doxorubicin.

***Keywords:*** *Doxorubicin, Vitamin K-7, micronucleus appearance, mitotic index, chromosomal aberration.*

## Introduction

Doxorubicin (DOX) is an anthracycline drug that showed a great treatment potential, being regarded as one of the highly potent of the Food and Drug Administration-(FDA) approved chemotherapeutic drugs(1). Such a drug can combatrapidly dividing cells and suppress disease progression. Despite its broad therapeutic effectiveness, it has been reported that the major hindering factor of DOX chemotherapy is its significant toxic effect on various organs; furthermore, early clinical assessments on DOX during phase II and III studies showed common side effects of acute vomiting and nausea, gastrointestinal (GI) problems, baldness,and disturbances to the nervous system (often causing hallucinations and light-headedness)(2). Unfortunately, DOX has been reported not to be specifically targeted only cancer cells but, it can also affect the growth of many other cell types in the body. The severity of unwanted effects and their occurrence by DOX may depend on the dosage of such a chemotherapeutic drug and the duration of its use(3).

**Corresponding Author:**
**Ali Faris Hassan**
Lec. Dr. Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad/Iraq
e-mail: ali_1371982@yahoo.com

Moreover, authors have been well documented that DOX had toxic effects mostly on the bone marrow (BM) cells[4], heart[5], liver[6], brain[7], kidney[8] and testis[9].

Vitamins are essential micronutrients that are very important for physical well-being conditions, and vitamin deficiency can have serious health consequences[10]. Vitamin K is a fat-soluble vitamin,which presents in three forms: vitamin K-1 (phylloquinone), vitamin K-2 (menaquinone), and vitamin K-3 (menadione).In general vitamin K plays essential roles in blood coagulation, regulation of calcium metabolism in tissues, cell growth and proliferation, oxidative stress (OS), inflammatory reactions, and hemostasis[11][12].

Vitamin K-7 (menaquinone-7) is the common name for vitamin K-2 [13].Vitamin K-7 is a long-chain menaquinone that is not produced by human tissue [14].Moreover, vitamin K-7 may be producedby phylloquinone (vitamin K-1) in the colon by *Escherichia coli*bacteria[15]. However, these menaquinones synthesized by bacteria in the gut appear to contribute minimally to overall vitamin K status[16,17].Moreover, vitaminK-7 is rapidly becoming popular as a supplement and is available as over the counter, it has also been reported that such type of vitamin K hadbeneficial effects like reducethe risk of osteoporosis, osteoarthritis (OA), and vascular and tissue calcification[18].

Chromosomal aberration it's any change in either the structure of chromosome called or the number of chromosomes[19]. Structural abnormalities examples are deletions, ring chromosomes acentric chromosomes or dicentric chromosome[20].

Mitotic index is a measure for the proliferation status of a cell population and defined as the ratio between the number of cells in mitosis and the total number of cells[21].

## Materials and Method

**Animals and Treatment Protocols:** Seventy-two(72) adult Wistar Albino experimental rats of both sexes, weighing 160-250gm were used in this study; they were obtained from and maintained in the Animal House of the College of Pharmacy,University of Baghdad. The animals were maintained under normal conditions of temperature, humidity and light/dark cycle.

The experimental rats were randomly allocated into six groups, each containing 6 rats as follows: **Group I**: Rats received 0.5ml of distilled water (D.W.) intraperitoneally (IP) injected,this group served as (negative control). **Group II**: Rats were IP injected with a single dose of DOX (15mg/kg BW) (positive control). **Group III**: Animals received MK-7 (16μg/kg BW/day) orally by oral gavagefor 11 successive days. **Group IV**: Animals were administered MK-7 (48μg/kg BW/day) orally byoral gavage for 11 successive days. **Group V**: Rats received MK-7 (16μg/kg BW/day)orally byoral gavage for 11 consecutive days before a single IP dose of DOX 15mg/kg on day11. **Group VI**: Animals treated with MK-7 at a dose of 48 μg/kg orally-administeredonce daily for 11 consecutive days before a single IP dose of DOX 15 mg/kgon day 11.

**Evaluation of the mitotic index and chromosomal aberrations in bone marrow cells and spleen-cells:** After 24 hours of the end of treatment, all rats were IP injected with 1mg/kg colchicine, and then two hours later, the animals were sacrificed by cervical dislocation. The BM sample of each rat was aspirated from the femur bone and spleen cells have been extracted from spleen, as previously reported elsewhere[22].

**Evaluation of the micronucleus appearance in bone marrow(BM) cells:** At the end of treatment, rats were sacrificed by cervical dislocation. Bonemarrow (BM) samples were aspirated from the femur bone by using fetal calf serum and processed using for the evaluation of an appearance of micronucleus[22].

**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 ANOVA test was performed to compare among test groups;and ($P$ values$<0.05$)were regarded as statistically significant.

## Results

Table (1) showed that, Group III rats produced a non-significant difference in mitotic index compared to such index in negative control rats ($P>0.05$) in both BM- and spleen-cells; but at the Group IV rats, the mitotic index was significantly increased ($P<0.05$) compared to negative control rats in both BM- and spleen-cells. Moreover, mitotic index was significantly different when comparison was Group II, Group V and Group VI($P<0.05$) in both BM- and spleen cells. Additionally, There was a significant difference ($P<0.05$) in the mitotic index Group V and Group VI *in* both BM- and spleen-cells.

**Table (1). Effects of various treatments on the incidence of mitotic index in the bone marrow and spleen cells of experimental rats' groups**

| Treatment Groups | Mitotic Index | |
|---|---|---|
| | Bone Marrow Cells | Spleen Cells |
| Distilled water (Negative control) (Group I) | 14.1±3.2 | 12.1±1.8 |
| Doxorubicin (Positive control) 15mg/kg (Group II) | 4.67±1.6$^{*a}$ | 3.1±1.3$^{*a}$ |
| Vitamin K-7 at dose 16μg/kg (Group III) | 13.3±2.2 | 11.6±2.1 |
| Vitamin K-7 at dose 48 μg/kg (Group IV) | 18.6±2.7$^{*}$ | 15.8±1.7$^{*}$ |
| Doxorubicin 15 mg/kg plus Vitamin K-7 at dose 16 μg/kg (Group V) | 7.8±2.3$^{Ab}$ | 6.1±1.4$^{Ab}$ |
| Doxorubicin 15 mg/kg plus Vitamin K-7 at dose 48 μg/kg (Group VI) | 9.1±1.4$^{Bc}$ | 8.5±1.8$^{Bc}$ |

Data are expressed as mean±SD.

*significantly different compared to distilled water (P<0.05).

- Values with non-identical small letters superscripts (a, b, and c) are considered significantly different when a comparison among group II, group V and group VI(*P*<0.05).
- Values with non-identical capital letters superscripts (A, and B) are considered significantly different when a comparison between group V and group VI (*P*<0.05).

(*P<0.05*).

In table (2), Group III produced a non- significant difference *(P>0.05)* in the micronucleus appearance compared to negative control rats in BM cells; but, when the dose of vitamin K-7 increased to 48μg/kg, the micronucleus appearance was significantly decreased when compared to negative control in BM cells *(P<0.05)*. Moreover, the micronucleus appearance was significantly different when comparison was made among Group II,Group V, and Group VI in BM cells(*P<0.05)*; In addition, there is a significant difference (*P<0.05)*in the micronucleus appearance in BM cells of Group V and Group VI rats.

**Table (2): Effects of various treatments on the incidence of micronucleus appearance in the bone marrow of experimental rats' groups**

| Treatment Groups | Micronucleus Appearance |
|---|---|
| Distilled water (Negative control) (Group I) | 3.8±1.2 |
| Doxorubicin (Positive control) 15mg/kg (Group II) | 31.3±2.1$^{*a}$ |
| Vitamin K-7 at dose 16μg/kg (Group III) | 4.5±2.1 |
| Vitamin K-7 at dose 48 μg/kg (Group IV) | 2.1±1.1$^{*}$ |
| Doxorubicin 15 mg/kg plus Vitamin K-7 at dose 16 μg/kg (Group V) | 24.5±2.1$^{Ab}$ |
| Doxorubicin 15 mg/kg plus Vitamin K-7 at dose 48 μg/kg (Group VI) | 16.1±2.8$^{Bc}$ |

Data are expressed as mean±SD.

*significantly different compared to distilled water (*P*<0.05).

- Values with non-identical small letters superscripts (a, b, and c) are considered significantly different when a comparison among group II, group V and group VI (*P*<0.05).
- Values with non-identical capital letters superscripts (A, and B) are considered significantly different when a comparison between group V and group VI (*P*<0.05).

(*P<0.05*).

In the table (3), Group III and IV produced a non-significance difference *($P<0.05$)* in total chromosomal aberrations compared to negative control rats in both BM- and spleen- cells. Furthermore, total chromosomal aberrations were significantly different (*$P<0.05$*) in BM-and spleen-cells when comparison was made among doxorubicin Group II, Group V, and Group VI. Additionally, there was a significant difference ($P<0.05$) in total chromosomal aberrations in Group V rats compared to the corresponding aberrations in Group VI rats both bone marrow cells and spleen cells.

**Table (3): Effects of various treatments on the incidence of total chromosomal aberrations in bone marrow cells and spleen cells of experimental rats' groups**

| Treatment Groups | Total Chromosomal Aberration | |
|---|---|---|
| | **Bone Marrow Cells** | **Spleen Cells** |
| Distilled water (Negative control) | 0.09±0.05 | 0.07±0.04 |
| Doxorubicin (Positive control) 15mg/kg | $0.34±0.01^{*a}$ | $0.3±0.02^{*a}$ |
| Vitamin K7 at dose 16µg/kg | 0.07±0.02 | 0.06±0.03 |
| Vitamin K7 at dose 48 µg/kg | 0.05±0.01 | 0.04±0.01 |
| Doxorubicin 15 mg/kg plus Vitamin K7 at dose 16 µg/kg | $0.22±0.02^{Ab}$ | $0.193±0.02^{Ab}$ |
| Doxorubicin 15 mg/kg plus Vitamin K7 at dose 48 µg/kg | $0.176±0.03^{Bc}$ | $0.163±0.02^{Bc}$ |

Data are expressed as mean±SD.

*significantly different compared to distilled water ($P<0.05$).

- Values with non-identical small letters superscripts (a, b, and c) are considered significantly different when a comparison among group II, group V and group VI ($P<0.05$).
- Values with non-identical capital letters superscripts (A, and B) are considered significantly different when a comparison between group V and group VI ($P<0.05$).

(*$P<0.05$*).

## Discussion

In the current study, in Group II rats caused significant reduction ($P<0.05$) in the mitotic index associated with an elevation in the micronucleus appearance in bone marrow cells and elevation in the chromosomal aberrations in both bone marrowand spleen-cells compared to those in negative control; meanwhile,(Group IV) and (Group V), decreased chromosomal aberrations, micronucleus appearance and increased mitotic index compared to those in (Group II) rats. Tables (1, 2, and 3)

A previous study showed that reactive oxygen species (ROS) cause DNA damage in cells[23,24]. Moreover, mitochondria are the site of ROS production due to electrons escaping from electron transport chain and production of superoxide anion ($O_2^{\bullet -}$); moreover, DOX can drive these ROS productions through enzymes within the mitochondria, including the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, cytochrome P-450 reductase, and xanthine oxidase (XO), can convert DOX in the form of quinone to semiquinone[25], which can be regenerated back to its parental quinone readily by reacting with oxygen with the generation of $O_2^{\bullet -}$, which in turn could further be changed to other ROS[26], butvitamin K can alter redox balance in cells[27]. In its reduced form, vitamin K hydroquinone (KH2) can protect phospholipid membranes from peroxidation by ROSuptake[28]. Furthermore, vitamin K inhibitedof 12-lipoxygenaseenzyme which preventsROS formation[29]; alsovitamin K reducesOS by lowering levels of pro-inflammatory factors[30].

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

## References

1. Carvalho C, Santos RX, Cardoso S, et al. Doxorubicin: the good, the bad and the ugly effect. Curr Med Chem. 2009; 16: 3267-3285.
2. Wang B, Ma Y, Kong X, et al. NAD administration decreases doxorubicin-induced liver damage of mice by enhancing antioxidation capacity and decreasing DNA damage. Chem Biol Interact. 2014; 212:65-71.
3. Chatterjee K, Zhang J, Honbo N, et al. Doxorubicin cardiomyopathy.Cardiology 2010; 115: 155-162.
4. Kaustubh B, Vineet G, Salman H, Seetharama D, Sharon A M, Benny B, et al. The opposite effects of Doxorubicin on bone marrow stem cells versus breast cancer stem cells depend on glucosylceramide synthase.Int J Biochem Cell Biol. 2012; 44(11): 1770–1778.
5. Potnuri AG, Kondru SK, Samudrala PK, et al. Prevention of Adriamycin induced cardiotoxicity in rats: A comparative study with subacute angiotensin-converting enzyme inhibitor and nonselective beta-blocker therapy. IJC Metabolic & Endocrine2017; 14:59-64.
6. Sutejo IR and Efendi E. Antioxidant and hepatoprotective activity of garlic chives (Allium tuberosum) ethanolic extract on doxorubicin-induced liver injured rats. Int J Pharm Med Biol Sci. 2017; 6(1): 20-23.
7. Jansen CE, Dodd MJ, Miaskowski CA, et al. Preliminary results of a longitudinal study of changes in cognitive function in breast cancer patients undergoing chemotherapy with doxorubicin and cyclophosphamide. Psychooncology2008; 17: 1189-1195.
8. Kocahan S, Dogan Z, Erdemli E, et al. Protective effect of quercetin against oxidative stress-induced toxicity associated with doxorubicin and cyclophosphamide in rat kidney and liver tissue. Iran J Kidney Dis. 2017; 11(2):124-131.
9. Badkoobeh P, Parivar K, Kalantar SM, et al. Effect of nano-zinc oxide on doxorubicin-induced oxidative stress and sperm disorders in adult male Wistar rats. Iran J Reprod Med. 2013; 11(9): 355-364.
10. Sarah LB. Vitamin K: food composition and dietary intakes.Food Nutr Res. 2012; 56: 10.3402.
11. Gross J, Cho WK, Lezhneva L, Falk J, Krupinska K, Shinozaki K, et al. A plant locus essential for phylloquinone (vitamin K1) biosynthesis originated from a fusion of four eubacterial genes. J Biol Chem.2006;281:17189–96.
12. Suttie JW. Vitamin K. In: Coates PM, Betz JM, Blackman MR, et al, eds. Encyclopedia of Dietary Supplements. 2nd ed. London and New York: Informa Healthcare; 2010:851-60.
13. Kidd P. Vitamins D and K as pleiotropic nutrients: clinical importance to the skeletal and cardiovascular systems and preliminary evidence for synergy. Altern Med Rev. 2010; 15(3):199-222.
14. Schurgers LJ, Teunissen KJ, Hamulyák K, Knapen MH, Vik H, Vermeer C. Vitamin K-containing dietary supplements: comparison of synthetic vitamin K1 and natto-derived menaquinone-7. Blood. 2007;109(8):3279-3283.
15. Vermeer C, Braam L. Role of K vitamins in the regulation of tissue calcification. Journal of Bone and Mineral Metabolism2001; 19 (4): 201–6
16. Weber P.Vitamin K and bone health. Nutrition. 2001;17 (10): 880–7.
17. Ching KC. Dietary intake of menaquinones and risk of cancer incidence and mortality. The American Journal of Clinical Nutrition2010; 92(6): 1533–1534.
18. Schurgers LJ,Teunissen KJF,Hamulyak K,Knapen, MHJ,Vik H,Vermeer C. Vitamin K-containing dietary supplements: Comparison of synthetic vitamin $K_1$and natto-derived menaquinone-7.Blood 2007; 109(8): 3279-3283.
19. Rodriguez E, Sreekantaiah C, Chaganti RSK. Genetic changes in epithelial solid neoplasia. Cancer Res 1994; 54(13):3398-3406.
20. Tomiyama K, Toyokawa H, Nakao A, Nalesnik MA,Stolz D B,Murase N, et al. Multiple Types of Stem Cells in the Kidney: Roles of Bone Marrow (Bm)-Derived Versus Local Stem Cells on Renal Tubular Cell Regeneration After I/R injury. Transplantation 2006;82 (1): 623-624
21. Luong RH, Baer KE, Craft DM, Ettinger SN, Scase TJ, Bergman PJ, Prognostic significance of intra-tumoral microvessel density in canine soft-tissue sarcomas. Vet Pathol 2006; 43: 622–631.

22. Allen J W, Shuler C F, Menders RW, Olatt SA. A simplified technique for in-vivo analysis of sister chromatid exchange using 5-bromodeoxyuridine tablets, Cytogenet. Cell Genet, 1977; 18: 231-237.
23. Marnett LJ. Oxyradicals and DNA damage. Carcinogenesis2000; 21: 361–370.
24. Halliwell B, Aruoma OI. DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems. FEBS Lett. 1991; 281: 9–19.
25. Schimmel KJ, Richel DJ, van den Brink RB, et al. Cardiotoxicity of cytotoxic drugs. Cancer Treat Rev. 2004; 30(2):181-191.
26. Doroshow JH. Effect of anthracycline antibiotics on oxygen radical formation in rat heart. Cancer Res. 1983; 43(2):460-472.
27. Westhofen P, Watzka M, Marinova M, Hass M, Kirfel G, Müller J, et al. Human vitamin K 2,3-epoxide reductase complex subunit 1-like 1 (VKORC1L1) mediates vitamin K-dependent intracellular antioxidant function. J. Biol. Chem. 2011;286:15085–15094.
28. Mukai K, Itoh S, Morimoto H. Stopped-flow kinetic study of vitamin E regeneration reaction with biological hydroquinones (reduced forms of ubiquinone, vitamin K, and tocopherolquinone) in solution. J. Biol. Chem.1992;267:22277–22281.
29. Li J, Wang H, Rosenberg PA. Vitamin K prevents oxidative cell death by inhibiting activation of 12-lipoxygenase in developing oligodendrocytes. J. Neurosci. Res. 2009;87:1997–2005.
30. Shea MK, Cushman M, Booth SL, Burke GL, Chen H, Kritchevsky SB. Associations between vitamin K status and hemostatic and inflammatory biomarkers in community-dwelling adults. The Multi-Ethnic Study of Atherosclerosis. Thromb. Haemost. 2014;112:438–444.