PBR
Pharmaceutical & Biomedical Research

December 2020. Volume 6. Number 4

Original Article:

The Protective Effects of *Origanum vulgare* L. Extract on Genetic Damage of Cyclophosphamide in Mice Blood Lymphocytes Using Micronucleus Test

Mohammad Shokrzadeh[1,2], Emran Habibi[3], Amir Shadbooreštan[2], Aroona Chabra[4], Amirhossein Ahmadi[1*]

1. Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.
2. Department of Toxicology and Pharmacology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.
3. Department of Pharmacognosy, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.
4. Student Research Committee, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.

*** Corresponding Author:**
Amirhossein Ahmadi, PhD.
Address: *Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.*
E-mail: *amirhossein_pharma@yahoo.com*

Article info:
Received: 02 Aug 2019
Accepted: 10 Feb 2020

Keywords:
Origanum vulgare, Antioxidant, Cyclophosphamide, Oxidative stress, Genotoxicity, Micronucleus

ABSTRACT

Background: Despite its various clinical applications, cyclophosphamide (CP), an alkylating chemotherapeutic agent, has demonstrated numerous side effects, including genetic toxicity.

Objectives: This study investigated the protective action of *Origanum vulgare* L., a powerful antioxidant plant, on the genotoxicity of CP in the mice blood lymphocytes.

Methods: The mice were pre-treated orally with different doses of 50, 100, 200, or 400 mg/kg O. vulgare ethanolic extract once a day for 7 consecutive days. One hour after the final dose of O. vulgare, each animal received a single intraperitoneal administration of 200 mg/kg CP. After 24 hours, the preventive effect of O. vulgare was evaluated using an in vitro micronucleus (Mn) test in cytokinesis-blocked lymphocytes, which is a reliable genotoxicity test. All doses of O. vulgare caused significant reductions in the CP-induced Mn formation, which served as an indicator of DNA damage in the peripheral blood lymphocytes.

Results: The total reduction of the Mn in binucleated lymphocytes were 67% and 75% for doses of 200 and 400 mg/kg of O. vulgare, respectively ($P<0.001$). The antioxidant plant demonstrated dose-dependent protective effects against CP-induced Mn formation and genotoxicity in the blood lymphocytes of the mice. O. vulgare can reduce the damage to DNA through its potent antioxidation activity and free radical scavenging properties.

Conclusion: Since it is widely used as a safe herbal medicine for many diseases, O. vulgare could be used to relieve the adverse effects of cyclophosphamide, especially against the genetic damages of normal cells in patients undergoing chemotherapy.

Citation Shokrzadeh M, Habibi E, Shadbooreštan A, Chabra A, Ahmadi A. The Protective Effects of Origanum vulgare L. Extract on Genetic Damage of Cyclophosphamide in Mice Blood Lymphocytes Using Micronucleus Test. Pharmaceutical and Biomedical Research. 2020; 6(4):297-302. http://dx.doi.org/10.18502/pbr.v6i4.5116

doi: http://dx.doi.org/10.18502/pbr.v6i4.5116

Introduction

Cyclophosphamide (CP), a nitrogen mustard alkylating agent, is widely used in the treatment of various human diseases, such as breast cancer, lung cancer [1], systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis [2]. Because of its numerous side effects, including nausea, vomiting, alopecia, mucosal ulceration, pulmonary toxicity, hematopoietic and bone marrow suppression, reproductive toxicity, nephrotoxicity, urotoxicity, cardiotoxicity, and hepatotoxicity, the use of CP is limited [3-7]. Chemically reactive metabolic products of CP cause cytotoxicity that alkylate DNA and proteins leading to the production of cross-linking in DNA [8]. Furthermore, the biotransformation of CP is mediated by the participation of cytochromes P450 mixed-function oxidase to form the highly toxic metabolites, phosphoramide mustard, and acrolein. In this way, CP could eventually produce redundant Reactive Oxygen Species (ROS). These reactive compounds can damage critical macromolecules, such as DNA, causing chronic diseases, including cancer [9].

Many natural compounds in plants have potential protective effects on diseases. Medicinal plants derive their potential chemopreventive properties from their chemical components, such as phenolics and flavonoids. It is generally recognized that the biological advantages of these compounds are derived from their antioxidant and free radical scavenging activities [10, 11]. In a 2008 study, we observed that hesperidin, a citrus flavonoid, reduced the genotoxicity induced by CP in the bone marrow cells of mice when were orally given before CP administration [12]. We also demonstrated that *Origanum vulgare* extract protected human blood lymphocytes against genotoxicity induced by internal irradiation. Since *O. vulgare* showed a potent antioxidant and free radical scavenger in the study, it could effectively protect blood lymphocytes from DNA damages and genetic toxicity of internal radiation-induced free radicals and ROS through quenching these free radicals [13].

O. vulgare L. is the only species of the genus Origanum belonging to the Labiatae family that grows wildly in Iran. Because of its powerful antioxidant activities, *O. vulgare* has been used for the treatment of cancer, heart disease, and hypertension [14]. We recently observed that the pretreatment of mice with the aerial parts of *O. vulgare* extract at various doses for 7 days reduced the organ toxicity of mice induced by CP. *O. vulgare* reduced lipid peroxidation, restored the depletion of antioxidant enzymes, and protected cellular destruction in different organs, such as liver and lung [15, 16].

In another study, we showed that *O. vulgare* inhibited the genotoxicity of CP in mice bone marrow, completely normalized the bone marrow cells depression, restored the inhibition of cell proliferation, and protected mice from bone marrow hypocellularity induced by CP [4]. The high antioxidant activity of *O. vulgare* appears to be due to the phenolic OH groups present in flavonoids and phenolic compounds that can donate hydrogen atoms to the proxy radicals formed in the first stage of lipid oxidation [17]. The results of various studies have shown that the antioxidant effects of *O. vulgare* can be due to the presence of dominant phenolic compounds, such as carvacrol and thymol in its essential oil [18]. Thus, the possible protective role of *O. vulgare* extract against CP-induced genotoxicity was determined using the micronucleus (Mn) test in mice blood lymphocytes.

Materials and Methods

Preparation of O. vulgare extract

O. vulgare L. dried aerial parts were obtained from Giah Essence Phyto-Pharmaceutical Co., Golestan, Iran. The plant was identified, authenticated, and extracted by our university pharmacognosist. Three hundred grams of dried plant powder was macerated with 3000 mL of ethanol (75%) for 72 hours. After evaporation of the solvent under reduced vacuum at temperatures below 40° C, an extract was obtained.

Study animals

For this randomized and controlled animal study, male Naval Medical Research Institute (NMRI) mice weighing 25±3 g were prepared from the Pasteur Institute of Iran (Amol branch, Iran). All animals were housed at the university's animal house and kept at a controlled room temperature of 23±2° C under a 12/12 h light/dark cycle. The animals were acclimatized for 1 week before the study and standard laboratory food and water were freely provided. All animals received human assistance according to the criteria established in the "Guide for the care and use of laboratory animals" prepared by the Mazandaran University of Medical Sciences, Sari, Iran. The study protocol was approved by the Research Committee of the University.

Experimental treatment of *O. vulgare*

The mice were divided into 7 groups (n=5 for each group). The mice in the negative control (group 1) received only distilled water (10 mL/kg) orally once a day for 7 days. The positive control mice (group 2) received

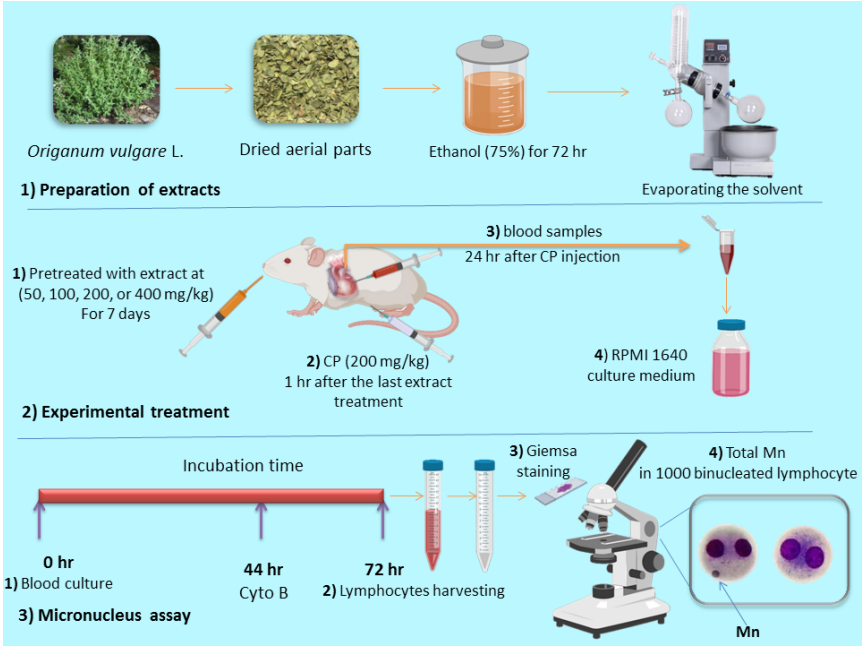


Figure 1. The schematic image of the experiment and each part of the study

distilled water (10 mL/kg) orally once a day for 7 days following a single IP injection of toxic CP (200 mg/kg) in distilled water (10 mL/kg) on the 7th day of the experiment 1 h after the last distilled water administration. In groups 3-6, the mice were treated orally (gavages) with different doses of *O. vulgare* (50, 100, 200, or 400 mg/kg) in distilled water (10 mL/kg) per day for 7 consecutive days, followed by a single IP injection of CP 1 h after the last *O. vulgare* treatment. For the *O. vulgare* control group (group 7), the mice received a high daily dose of *O. vulgare* (400 mg/kg) in distilled water (10 mL/kg) orally for 7 consecutive days, followed by no CP treatment to guarantee the credibility and safety of the *O. vulgare* in the high dosage. Twenty-four hours after the administration of CP in the aforementioned groups, all the mice were anesthetized with petroleum ether. One milliliter of blood was taken from each mouse via cardiac puncture and transferred to the Mn test.

Determination of the Mn

For each mouse blood sample, 0.5 mL aliquots were added in duplicate to 4.5 mL of RPMI, also known as RPMI 1640 or Roswell Park Memorial Institute Medium, culture medium containing glutamine (Invitrogen) (Gibco), consisting of a 20% bovine fetal serum mixture (Sigma), 50 µL/mL of phytoagmagututin (Gibco) and antibiotics (50 IU/mL of penicillin and 50 µg/mL of streptomycin; Gibco). All cultures were incubated at 37±1° C in a humidified atmosphere of 5% carbon dioxide and 95% oxygen. After 44 hours of culturing, a cytochalasin B solution (Sigma; final concentration, 5 µg/mL) was added to the culture medium. After an incubation period of 72 hours, the cells were collected by centrifugation for 8 minutes at 1000 rpm, resuspended in cold 0.075 M potassium chloride, and immediately fixed in a solution of acetic acid and methanol (6:1) three times.

Several drops of the fixed cells were placed on clean microscopic slides, air-dried, and stained in 15% of the Giemsa solution for 20 min. All slides were evaluated using a 100x magnification microscope to determine the frequency of bi-nucleated micronuclei in cytokinesis that was stopped with a well-preserved cytoplasm. The criteria used to evaluate the micronuclei were the following: a diameter between 1/16 and 1/4 of the main nuclei's diameter, the non-refractive nature, the independence of the primary nucleus, and a lack of overlap with the primary nucleus. Besides, to be counted as bi-nucleated cells, the two nuclei of the main cells had to be completely divided. Three slides were prepared for each mouse, and a total of 1000 binucleated cells were observed to determine the frequency of the micronuclei. The schematic picture of the experiment and each part of the study is displayed in Figure 1.

Statistical analysis

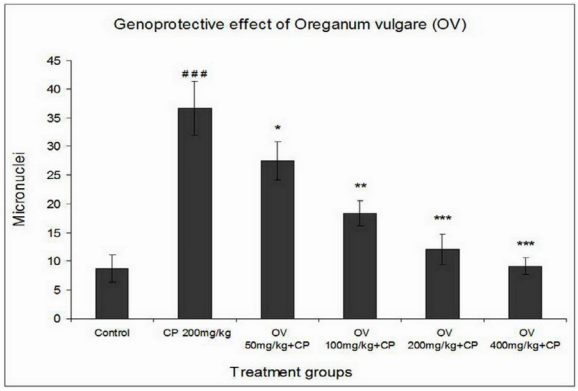
The total number of Mn per 1000 binucleated lymphocytes is represented as the Mean±SD of 5 mice. A 1-way Analysis of Variance (ANOVA) was used followed by the Honestly Significant Difference (HSD) Tukey test to

assess the statistical significance. A value of p less than 0.05 was considered significant.

Results

Figure 2 shows a significant difference in the Mn percentage in binucleated lymphocytes of mice treated with CP as compared to the controls (P<0.001). The mice treated with a single dose of CP (200 mg/kg) had an Mn frequency for 1000 binucleated lymphocytes of 36.71±4.63, but the untreated control animals had a frequency of 8.76±2.35. The incidence of micronucleated binucleated lymphocytes in the mice that were pretreated with several doses of *O. vulgare* for 7 consecutive days before the CP administration was significantly lower than in the mice that received CP alone (Figure 2).

The percentage reduction in the total number of micronucleated binucleated cells was 25%, 49.9%, 67%, and 75% for doses of 50, 100, 200, and 400 mg/kg of *O. vulgare*, respectively. The reduction was greater at higher doses of *O. vulgare* at 200 and 400 mg/kg compared to the reduction at doses of 50 and 100 mg/kg. Also, the maximum protective effect was observed at the highest dose of *O. vulgare*, where CP-induced genotoxicity was completely prohibited and the Mn was significantly reduced (P<0.001). In addition, *O. vulgare* at the maximum dose of 400 mg/kg did not induce any additional micronuclei in the mouse blood lymphocytes, indicating that the plant is not genotoxic at the maximum dose and is completely safe.



PBR

Figure 2. In vivo protection of O. vulgare extract at different doses against CP-induced Mn in mouse blood lymphocytes

The frequency of Mn per 1000 lymphocytes is represented as the Mean±SD of five mice.

###P<0.001: control sample compared with animals treated with a single dose of CP (200 mg/kg).

*P<0.05: CP-treated mice compared with OV-pretreated mice (50 mg/kg) before CP administration.

**P<0.01: CP-treated mice compared with OV-pretreated mice (100 mg/kg) before CP administration.

***P<0.001: CP-treated mice compared with OV-pretreated mice (200 or 400 mg/kg) before CP administration.

OV: *O. vulgare*; CP: Cyclophosphamide; Mn: Micronucleus.

Discussion

CP is used for the treatment of a wide variety of malignancies, such as leukemia, lymphoma, breast, lung, prostate, and ovarian cancers [19]. CP is inactive in vivo and in vitro and is metabolized by hepatic microsomal enzymes in the liver. CP can exert its biological activities by metabolites, mainly phosphoramidite mustard [20]. However, alkylating metabolites like acreoline can bind to the many macromolecules, including amino acids, proteins, and peptides, but the most important binding site is the DNA in which the DNA cross-linking and subsequent DNA can occur [21].

In our recent studies, CP administration resulted in the production of ROS, which caused lipid peroxidation and peroxidative damages to vital organs of animals [4, 15, 16]. Cellular and tissue toxicities were observed as the therapeutic dose of CP increased. CP and its metabolites can bind to the DNA, and cause chromosome breaks, Mn formation, and finally cell death [21]. We previously reported CP-induced genetic damages in mouse bone marrow cells [4, 5, 12]. In the current study, DNA damage was evaluated by the Mn test in mice blood lymphocytes. An administration of 200 mg/kg CP caused DNA damage and formed Mn in animals' blood lymphocyte. It was evident that exposure to CP resulted in genotoxicity as compared to untreated control animals.

Many kinds of research have been interested in focusing on the antioxidant activity of natural products to find therapeutic agents. Recently, the search to obtain novel, more effective with fewer side effects agents from natural origin compounds have dramatically increased. These efforts could minimize the toxicity of chemotherapy to normal cells without affecting anticancer efficacy. Thus, the deleterious effects of ROS and other free radicals of CP, which can damage cells and tissues can be inhibited by natural antioxidants.

O. vulgare is a flavorful plant used throughout the world to treat hyperglycemia, leukemia, and respiratory diseases. It is an aromatic plant with a wide distribution throughout Asia, and especially Iran. The major aqueous constituents of oregano are rosmarinic acid, eriocitrin, luteolin-7-oglucoside, apigenin-7-O-glucoside, origanol A and B and ursolic acid [22]. Rosmarinic acid and origanol A and B, the largest constituents of aqueous oregano

extract, have anti-oxidative activities [23, 24]. Previous studies have reported *O. vulgare*'s essential oil antioxidant capacity, and the antioxidant effects have been linked to thymol, carvacrol, δ-terpinene, and p-cymene [25-27].

Many types of research have been shown that the components of aqueous *O. vulgare* extracts, such as ursolic and rosmarinic acids, exert a potent antioxidant effect by scavenging free radicals [28, 29]. In addition, thymol selectively protected human blood lymphocytes against DNA damage induced by bleomycin as an anti-cancer and significantly reduced Mn frequency [30]. Therefore, we evaluated the protective effects of *O. vulgare* extract on CP-induced genotoxicity in mouse blood lymphocytes. The *O. vulgare* extract had dose-dependent protective effects and reduced the frequency of CP-induced Mn in lymphocytes. Administration of 200 and 400 mg/kg of *O. vulgare* for 7 consecutive days before CP injection produced the maximum protection in animal lymphocyte cells and reduced the Mn frequency by approximately 67% and 75%, respectively. These results are consistent with our previous studies. We have recently reported that *O. vulgare* pretreatment mitigated radiation-induced oxidative stress and subsequent DNA damage in human blood lymphocytes [13].

The protective effects of *O. vulgare* on DNA could be explained by its ability to strengthen the antioxidant defense system, eliminate ROS-inducing lipid peroxidation and scavenge free radicals. It seems that the primary mechanism of the protective action of *O. vulgare* is the direct and or indirect interaction with these ROS because a powerful free radical scavenging effect was demonstrated in our previous study [13]. Therefore, it appears that *O. vulgare* scavenged and trapped free radicals induced by CP and, through this mechanism, it could prevent the damage induced by free radicals. Thus, *O. vulgare* may prevent the Mn formation and the DNA damage that could have been induced by free radicals produced by CP in human lymphocytes. The protective effect of *O. vulgare* is likely due to the antioxidative properties of the major containing flavonoids and phenolic compounds.

We show *O. vulgare* has a dose-related protection effect against genotoxicity induced by CP in mouse blood lymphocytes. CP can produce oxidative stress in the cells through the generation of ROS and free radicals led to DNA damages, genotoxicity, and even cell death. The main characteristic of antioxidants is the ability to quench these toxic free radicals. Therefore, *O. vulgare* may reduce CP-induced DNA damage in mice through its antioxidant activity and free radicals scavenging properties. Since *O. vulgare* has been widely used as an additive therapy and herbal medicine for various diseases, it may be a potential candidate for a safe supplement against harmful side effects induced by dangerous chemical agents, particularly for the patients undergoing chemotherapy upon further investigations and clinical trials.

Ethical Considerations

Compliance with ethical guidelines

All ethical principles are considered in this article.

Funding

This work was supported by a grant from the Mazandaran University of Medical Sciences, Sari.

Authors' contributions

All authors contributed in preparing this article

Conflict of interest

The authors declared no conflict of interest.

References

[1] Zhang J, Tian Q, Zhou S-F. Clinical pharmacology of cyclophosphamide and ifosfamide. Curr Drug ther. 2006; 1(1):55-84. [DOI:10.2174/157488506775268515]

[2] Perini P, Calabrese M, Rinaldi L, Gallo P. The safety profile of cyclophosphamide in multiple sclerosis therapy. Expert Opin Drug Saf. 2007; 6(2):183-90. [DOI:10.1517/14740338.6.2.183] [PMID]

[3] Chabra A, Shokrzadeh M, Naghshvar F, Salehi F, Ahmadi A. Melatonin ameliorates oxidative stress and reproductive toxicity induced by cyclophosphamide in male mice. Hum Exp Toxicol. 2013; 33(2):185-95. [DOI:10.1177/0960327113489052] [PMID]

[4] Habibi E, Shokrzadeh M, Ahmadi A, Chabra A, Naghshvar F, Keshavarz-Maleki R. Genoprotective effects of *Origanum vulgare* ethanolic extract against cyclophosphamide-induced genotoxicity in mouse bone marrow cells. Pharm Biol. 2015; 53(1):92-7. [DOI:10.3109/13880209.2014.908399] [PMID]

[5] Hosseinimehr SJ, Ahmadashrafi S, Naghshvar F, Ahmadi A, Ehasnalavi S, Tanha M. Chemoprotective effects of Zataria multiflora against genotoxicity induced by cyclophosphamide in mice bone marrow cells. Integr Cancer Ther. 2010; 9(2):219-23. [DOI:10.1177/1534735409360361] [PMID]

[6] Mahmoud AM, Hussein OE, Ramadan SA. Amelioration of cyclophosphamide-induced hepatotoxicity by the brown seaweed *Turbenariaornata*. Int J Clin Toxicol. 2013; 1:9-17. [DOI:10.14205/2310-4007.2013.01.01.2]

[7] Shokrzadeh M, Chabra A, Naghshvar F, Ahmadi A. The mitigating effect of *Citrullus colocynthis* (L.) fruit extract against genotoxicity induced by cyclophosphamide in mice bone marrow cells. Sci World J. 2013; 2013:980480. [DOI:10.1155/2013/980480] [PMID] [PMCID]

[8] Hales BF. Comparison of the mutagenicity and teratogenicity of cyclophosphamide and its active metabolites, 4-hydroxycyclophosphamide, phosphoramide mustard, and acrolein. Cancer Res. 1982; 42(8):3016-21. [PMID]

[9] Tiwari AK. Imbalance in antioxidant defence and human diseases: Multiple approach of natural antioxidants therapy. Current Sci. 2001; 81(9):1179-87. [PMID] https://www.jstor.org/stable/24106434

[10] Noroozpour Dailami K, Azadbakht M, Lashgari M, Rashidi Z. Prevention of selenite-induced cataractogenesis by hydroalcholic extract of Echium amoenum: An experimental evaluation of the Iranian traditional eye medication. Pharm Biomed Res. 2015; 1(4):40-7. [DOI:10.18869/acadpub.pbr.1.4.40]

[11] Shokrzadeh M, Azadbakht M, Shakibamanesh H. The hepatoprotective effect of Arnebia euchroma hydro-alcoholic extract against liver toxicity induced by CCl4 in mice. Pharm Biomed Res. 2017; 3(4):23-9. [DOI:10.18502/pbr.v3i4.87]

[12] Ahmadi A, Hosseinimehr SJ, Naghshvar F, Hajir E, Ghahremani M. Chemoprotective effects of hesperidin against genotoxicity induced by cyclophosphamide in mice bone marrow cells. Arch Pharmacal Res. 2008; 31(6):794-7. [DOI:10.1007/s12272-001-1228-z] [PMID]

[13] Arami S, Ahmadi A, Haeri SA. The radioprotective effects of *Origanum vulgare* extract against genotoxicity induced by (131) I in human blood lymphocyte. Cancer Biother Radiopharm. 2013; 28(3):201-6. [DOI:10.1089/cbr.2012.1284] [PMID]

[14] Pirigharnaei M, Zare S, Heidary R, Khara J, EmamaliSabzi R, Kheiry F. The essential oils compositions of Iranian Oregano (*Origanum vulgare* L.) populations in field and provenance from Piranshahr district, West Azarbaijan province, Iran. Avicenna J Phytomed. 2011; 1(2):106-14. [DOI:10.22038/AJP.2011.129]

[15] Habibi E, Shokrzadeh M, Chabra A, Naghshvar F, Keshavarz-Maleki R, Ahmadi A. Protective effects of *Origanum vulgare* ethanol extract against cyclophosphamide-induced liver toxicity in mice. Pharm Biol. 2015; 53(1):10-5. [DOI:10.3109/13880209.2014.908399]

[16] Shokrzadeh M, Ahmadi A, Chabra A, Naghshvar F, Salehi F, Habibi E, et al. An ethanol extract of *Origanum vulgare* attenuates cyclophosphamide-induced pulmonary injury and oxidative lung damage in mice. Pharm Biol. 2014; 52(10):1229-36. [DOI:10.3109/13880209.2013.879908] [PMID]

[17] Lagouri V, Blekas G, Tsimidou M, Kokkini S, Boskou D. Composition and antioxidant activity of essential oils from oregano plants grown wild in Greece. Z Lebensm Unters Forsch. 1993; 197:20-3. [DOI:10.1007/BF01202694]

[18] Roofchaee A, Irani M, Ebrahimzadeh MA, Akbari MR. Effect of dietary oregano (*Origanum vulgare* L.) essential oil on growth performance, cecal microflora and serum antioxidant activity of broiler chickens. Afr J Biotechnol. 2011; 10(32):6177-83. https://www.ajol.info/index.php/ajb/article/view/94499

[19] Khan TS, Sundin A, Juhlin C, Wilander E, Oberg K, Eriksson B. Vincristine, cisplatin, teniposide, and cyclophosphamide combination in the treatment of recurrent or metastatic adrenocortical cancer. Med Oncol (Northwood, London, England). 2004; 21:167-77. [DOI:10.1385/MO:21:2:167]

[20] McDonald GB, Slattery JT, Bouvier ME, Ren S, Batchelder AL, Kalhorn TF, et al. Cyclophosphamide metabolism, liver toxicity, and mortality following hematopoietic stem cell transplantation. Blood. 2003; 101(5):2043-8. [DOI:10.1182/blood-2002-06-1860] [PMID]

[21] Murata M, Suzuki T, Midorikawa K, Oikawa S, Kawanishi S. Oxidative DNA damage induced by a hydroperoxide derivative of cyclophosphamide. Free Radic Biol Med. 2004; 37(6):793-802. [DOI:10.1016/j.freeradbiomed.2004.05.009] [PMID]

[22] Sheibani V, Afarinesh M, Hajializadeh Z, Abbasnejad M, Haghpanah T, Arabnezhad R, et al. Evaluation of *Origanum vulgare* L. ssp. viridis leaves extract effect on discrimination learning and LTP induction in the CA1 region of the rat hippocampus. Iran J Basic Med Sci. 2011; 14(2):177-84. [DOI:10.22038/IJBMS.2011.4984]

[23] Kulisic T, Krisko A, Dragovic-Uzelac V, Milos M, Pifat G. The effects of essential oils and aqueous tea infusions of oregano (*Origanum vulgare* L. spp. hirtum), thyme (*Thymus vulgaris* L.) and wild thyme (*Thymus serpyllum* L.) on the copper-induced oxidation of human low-density lipoproteins. Int J Food Sci Nutr. 2007; 58(2):87-93. [DOI:10.1080/09637480601108307] [PMID]

[24] Matsuura H, Chiji H, Asakawa C, Amano M, Yoshihara T, Mizutani J. DPPH radical scavengers from dried leaves of oregano (*Origanum vulgare*). Biosci Biotechnol Biochem. 2003; 67(11):2311-6. [DOI:10.1271/bbb.67.2311] [PMID]

[25] Halici M, Odabasoglu F, Suleyman H, Cakir A, Aslan A, Bayir Y. Effects of water extract of Usnea longissima on antioxidant enzyme activity and mucosal damage caused by indomethacin in rats. Phytomedicine. 2005; 12(9):656-62. [DOI:10.1016/j.phymed.2004.06.021] [PMID]

[26] Odabasoglu F, Aslan A, Cakir A, Suleyman H, Karagoz Y, Halici M, et al. Comparison of antioxidant activity and phenolic content of three lichen species. Phytother Res. 2004; 18(11):938-41. [DOI:10.1002/ptr.1488] [PMID]

[27] Russo A, Bonina F, Acquaviva R, Campisi A, Galvano F, Ragusa N, et al. Red orange extract: Effect on DNA cleavage. J Food Sci. 2006; 67(8):2814-8. [DOI:10.1111/j.1365-2621.2002.tb08821.x]

[28] Di Sotto A, Mazzanti G, Carbone F, Hrelia P, Maffei F. Inhibition by beta-caryophyllene of ethyl methanesulfonate-induced clastogenicity in cultured human lymphocytes. Mutat Res. 2010; 699(1-2):23-8. [DOI:10.1016/j.mrgentox.2010.04.008] [PMID]

[29] Lambert RJ, Skandamis PN, Coote PJ, Nychas GJ. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J Appl Microbiol. 2001; 91(13):453-62. [DOI:10.1046/j.1365-2672.2001.01428.x] [PMID]

[30] Arab H-A, Fathi M, Mortezai E, Hosseinimehr SJ. Chemoprotective effect of thymol against genotoxicity induced by bleomycin in human lymphocytes. Pharm Biomed Res. 2015; 1(1):26-31. [DOI:10.18869/acadpub.pbr.1.1.26]