Ameliorative Effect of Wheat Germ Oil in Potassium bromate Induced Biochemical and Histopathological Changes in Male Rat's Heart

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ABSTRACT

Potassium bromate (BRO) is a toxic substance that is used as a food additive in food. Wheat germ oil (WO) can improve lipid metabolism and reduce oxidative stress. Attributable to its high level of polyunsaturated fatty acids and vitamin E is a portion of good nutritious food. The current work was aimed to investigate the possible action of WO on the damage effects of Potassium bromate in heart tissue. Adult male rats (n=40) were equally divided into 5 groups; Control (Cont. (-)), BRO (Cont. (+)), groups 3, 4 and 5 orally given WO in doses of 150,300 and 450 mg/kg respectively. The results showed that oral gavage of wheat germ oil at three hundred and four hundred and fifty mg/kg.b.wt., two-intoxicated rats with BRO for four weeks significantly reduced cholesterol, triglycerides, lipid profile, and serum inflammatory cytokines against the Cont. (+) group. The histopathological examination of heart tissue confirmed these results. The current study indicates that oral administration of WO induces potent cardioprotective in rats intoxicated by BRO, this effect could be explained by WO antioxidant and anti-inflammatory properties.

Key Words: *Potassium bromate, wheat germ oil, rats, lipid profile, inflammatory cytokines.*

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INTRODUCTION

Oxidative stress is a state in which oxidation exceeds body defense antioxidant systems. It is the result of an imbalance between the development of reactive oxygen species (ROS) and the antioxidant defenses of the body against them [1]. Oxidative stress results from oxygen-using metabolic reactions that disrupt the equilibrium state of pro-oxidant/antioxidant reactions in living organisms [2]. Reactive oxygen species (ROS) can oxidize proteins, lipids, and nucleic acids under these conditions, resulting in cell death or transformation [3].
Hence, if ROS are not successfully scavenged by cellular components, they cause illnesses, and can interact in a cytotoxic manner with biological systems [4].

Oxidative stress has a great effect in cardiovascular illnesses [5]. Over the past few decades, oxidative stress's role in processes of cardiovascular disease such as ischemia-reperfusion injury, atherogenesis, and cardiac remodeling had been known [6].
Potassium bromate is widely used as a food additive in the bread-making processes (like a flour improver, strengthening the dough and allowing higher rising) [7]. The BRO is also used in the fish paste and fermented beverages. The risk of potassium bromate assessment studies has shown that it is highly toxic because it causes lipid peroxidation and oxidative damage to DNA [8]. The BRO causes structural changes in the heart muscle of rats and leads to cardiotoxicity [9].
Antioxidants are compounds that inhibit the initiation or

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propagation of oxidizing chain reactions and also can slow or avoid lipid oxidation [10]. Antioxidants fight oxidative stress by neutralizing excess free radicals and preventing them from causing chain reactions that lead to different diseases and premature aging [11].

Globally, there is an increasing concern in natural medicines which are related to the huge number of research studies about pharmacological activities of the bioactive constituents and their potential to manage different diseases [12].

Wheat germ is high in fiber, which had been recommended for patients at risk of heart disease, colon, and diabetes [13]. It is one of α-tocopherol's richest natural sources, the form of tocopherol and has the highest concentration of vitamin E. The WO has been associated with reduced animal plasma cholesterol [14].Also, WO has the potential to reduce oxidative stress and increase the metabolism of lipids [15]. This research focused on assessing WO's protective effect on BRO intoxicated rats.

MATERIAL AND METHODS

Material

Wheat germ oil

Wheat germ oil, used in this study purchased from Abazeer organic store, Jeddah, KSA.

Potassium Bromate

Potassium bromate (BRO) is a bromic acid potassium salt. It purchased from Cayman Chemicals and BioVision Incorporated, USA, as a white powder.

Kits and Chemicals

Cholesterol, triglycerides, lipid profiles and inflammatory cytokines kits purchased from Sigma Aldrich, Germany.

Rats and Diet

Forty male albino rats (200 – 220 g)provided from King Fahd Research Center, KAU. Basal diet ingredients obtained from Baghafar Company for Pharmaceutical and Chemical, Jeddah, KSA.

Ethical Approval

The experimental study was adhering under rules of Canadian ethic upon approval for the biomedical committee, KFMRC, KAU, KSA.

Methods

Basal Diet and Toxicity Induction

Diet had been established as outlined in Reeves *et al.*, (1993). The induction of toxicity induced by intraperitoneal (i.p.) injection with a single dose of potassium bromate 125 mg/kg b.w.t[16].

Experimental Protocol

After the acclimatization period (one week), rats were separated unsystematically into five groups, 8 rats/ group. The first group kept as a Cont. (-). A single intraperitoneal dose of potassium bromate at a dosage of 125 mg/kg b.wt. was administered into groups of (2-5) to cause toxicity.

On the last day of the experimental period (4 weeks), all rats were fasting for 12 hours,blood was collected for biochemical analysis and then all rats were sacrificed to collect heart for histopathological examination.

Group (2)	Cont. + orally given purified water by gavage + high-fat diet.
Group (3,4 and 5)	WO in doses of 150,300 and 450 mg/kg b.wt./day for 4 Weeks.

Biological Evaluation

Each second day for each group feed intake (FI) was reported during the experimental time, and the animals were weighed twice weekly in all groups. Biological values measured by calculating the percentage of body weight gain (BWG percent) as well as the ratio of feed efficiency (FER) was also calculated [17].

Serum Lipid Levels Assay

Serum lipid levels assessed as described in the protocol provided in ELSA kits obtained from My BioSource, USA.

Serum Proinflammatory Cytokines

Serum interleukin 1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) were measured using ELISA kits from Abbexa, Cambridge, UK.

Histopathological Examination

Heart dipped in neutral formalin (10%) for histopathological examination.

Statistical Analysis

Results analysis by ANOVA one-way analysis of variance, values presented as mean ± SMD for eight rats/ group.

RESULTS

It is clear from Table 1 that means of daily feed intake of Cont. (+) was 17.16 g/day compared to Cont. (-) with a mean of 26.88 g/day. Oral administration of wheat germ oil at three dosage levels 150, 300 and 450 mg/kg b.wt. Increased feed intake as compared to Cont. (+). Feed efficiency ratio in Cont. (+) significantly ($P< 0.05$) decreased when compared to Cont. (-) by 48.48 %. Significant ($P< 0.05$) increases were observed in rats

orally given wheat germ oil in doses of 150, 300 and 450 mg/kg b.wt., as compared to the Cont. (+) by 58.82, 73.53 and 85.29 % respectively as depicted in Table 1.

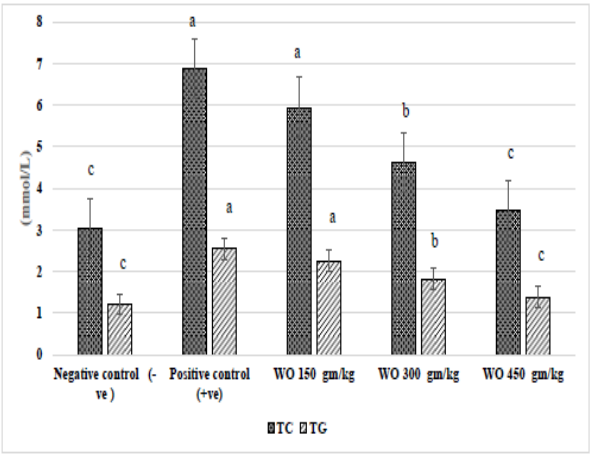
Concerning body weight gain, the results showed that there was a significant decrease in Cont. (+) when compared to Cont. (-).Oral administration of WO in doses of 150, 300 and 450 mg/kg b.wt., caused significant P< 0.05 increases in body weight gain when compared to Cont. (+) as shown in Table 1.

Table 1. Effect of oral administration of wheat germ oil on feed intake (FI), feed efficiency ratio (FER) and body weight gain percent (BWG %) in potassium bromate–intoxicated rats.

Groups	Mean of daily feed intake(g/rat/d)	Feed efficiency ratio(FER)	Bodyweight gain (%)
Cont (-)	26.88	0.66 ± .014[a]	17.36 ± 1.64[a]
Cont (+)	17.16	0.34 ±.013[c]	5.94 ±.39[c]
WO 150 gm/kg	22.46	0.54 ±.014[b]	13.63 ± .98[b]
WO 300 gm/kg	25.38	0.59 ±.011[a]	15.98 ± 1.06[a]
WO 450 gm/kg	26.39	0.63 ±.012[a]	16.87 ± 1.15[a]

-Values presented as mean ±SMD.
-Values with different superscript letters within a column are significantly different at P<0.05.

Figure (1) demonstrated the effect of WO on TC and TG levels against BRO intoxicated male rats. High cholesterol diet (cont. (+)) caused a significant (P<0.05) increases in both TC and TG levels as compared with the control negative group. Oral administration of WO in doses of 300 and 450 mg/kg.b.wt., caused significant P< 0.05 decreases in TC and TG levels as compared with the cont. (+) group.



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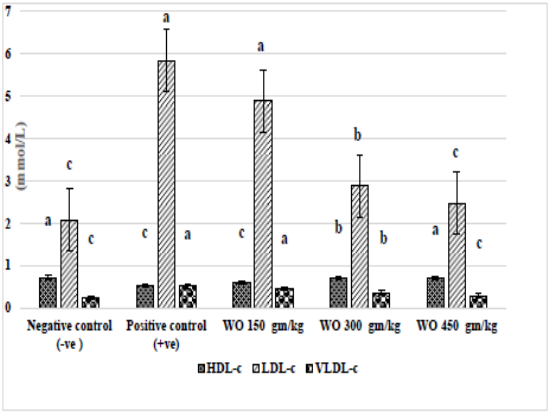
- Values with different superscript letters within a column are significantly different at P<0.05.

Figure 1. Effect of oral administration of wheat germ oil on cholesterol (TC) and triglyceride (TG) levels in potassium bromate–intoxicated rats.

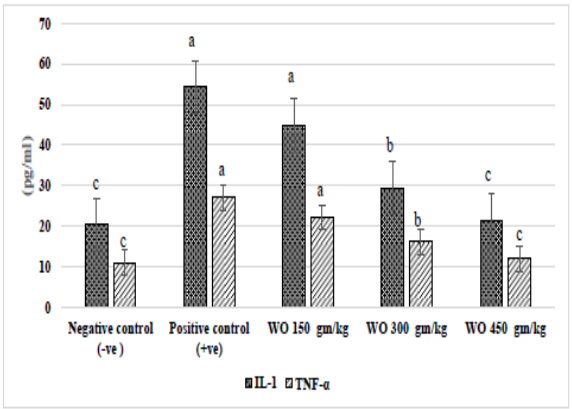
Figure (2) showed the effect of WO on lipid profile in BRO intoxicated male rats, BRO caused a significant decrease in HDL associated with significant (P<0.05) increases in both LDL and VLDL as compared with Cont. (+). Oral administration of the WO in doses of 300 and 450 mg/kg b.wt., caused significant P< 0.05 increase in HDL accompanying with significant decreases in LDL and VLDL levels as compared with the cont. (+) group.



- Values presented as mean ±SMD.
- Values with different superscript letters within a column are significantly different at P<0.05.

Figure 2. Effect of oral administration of wheat germ oil on lipid profile in potassium bromate–intoxicated rats.

The results revealed that cont. (+) the group recorded significant increases (p<0.05) in IL-1 and TNF-α levels comparing with the control negative group. Treatment with WO for 4 weeks at the two doses (300 and 450 mg/kg) induced significant improvement (p<0.05) in IL-1 and TNF-α values compared with cont. (+) group. No significant changes seen in the group of rats orally given 150 mg/kg of WO (Fig 3).



- Values presented as mean ±SMD.
- Values with different superscript letters within a column are significantly different at P<0.05.

Figure 3. Effect of oral administration of wheat germ oil on serum IL-1 and TNF-α levels in potassium bromate–intoxicated rats.

Histopathological Results

Heart sections of normal control rats showed normal branching and anastomosing muscle fibers (Fig. 4.A). Heart of the positive control group showing focal pale areas associated with intercellular hemorrhage compressing the myocardial muscle fibers (Fig. 4. B). While the heart of the intoxicated rat treated with the WO in a dose of 150 mg/kg showing intramuscular edema associated with intramuscular inflammatory cell infiltration (Fig.4.C). Heart of intoxicated rat treated with the WO in a dose of 300mg/kg showing slight myocardial blood vessel congestion (Fig.4. D). Heart Sections of rats received orally 300 mg/kg b.wt. WO showing normal tissue structure (Fig. 4. E).

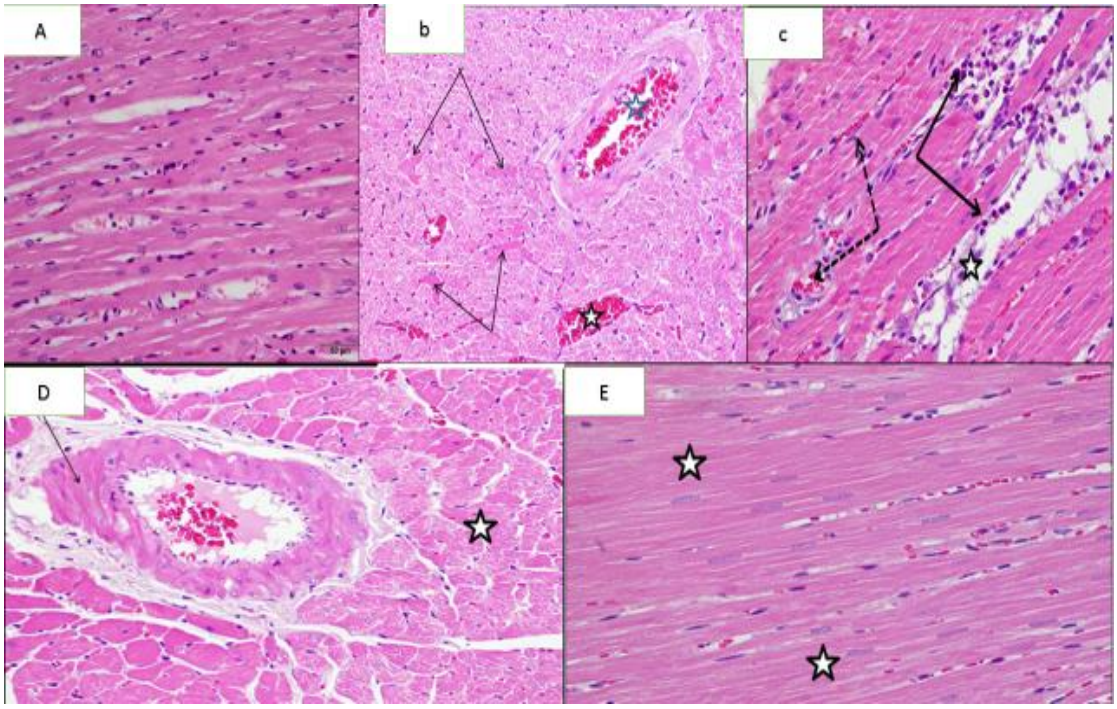


Figure 4: **Photomicrography illustrating H&E-stained sections of the heart in different groups. Heart sections of normal control rats showed normal branching and anastomosing muscle fibers (Fig. 4. A). Heart of the positive control group showing focal pale areas associated with intercellular hemorrhage compressing the myocardial muscle fibers (Fig. 4. B). While the heart of the intoxicated rat treated with the WO in a dose of 150 mg/kg showing intramuscular edema associated with intramuscular inflammatory cell infiltration (Fig. 4.C). Heart of intoxicated rat treated with the WO in a dose of 300mg/kg showing slight myocardial blood vessel congestion (Fig. 4. D). Heart Sections of rats received orally 300 mg/kg b.wt. WO showing normal tissue structure (Fig. 4. E).**

DISCUSSION

By producing reactive oxygen species, potassium bromate induces oxidative stress in human erythrocytes and alters the cellular antioxidant defense system [18]. There are many adverse effects of BRO in laboratory animals as well as in humans; it has nephrotoxic and ototoxic effects [19]. The BRO is a carcinogen that causes tumors in the renal cells, mesotheliomas and follicular tumors of thyroid cells in rats [20]. Active oxygen radicals are highly likely to be involved in these effects leading to damage to DNA [18]. We also noted its potential for platelet toxic effects [21].

Wheat germ oil is an excellent source of vitamin E and polyunsaturated fatty acids [22]. It is one of α-tocopherol's richest natural sources [23]. Wheat germ oil also has the potential to reduce oxidative stress and improve lipid metabolism [24]. Also, it had significantly higher levels of vitamin E in the blood and liver, providing greater protection against antioxidants [14].

Results of the present study showed that there was a reduction in the mean of daily feed intake and a significant reduction in both body weight gain percent (BWG%) and feed efficiency ratio (FER) in the positive control rats as compared to the negative control group. These effects might be attributed to the toxic effects of BRO that induce anorexia with subsequent reduced feed intake and body weight gain via its neurotoxic effect on the central nervous system. Our results were in the same line with Okalie and Ikewuchi, [14] who reported that there was a significant reduction in body weight of rabbits received potassium bromate. However, our results were, in contrast, Watanabe *et al*., [8] who reported that BRO did not affect the body weight, might the differences between our results and the

previous findings attributed to the differences between the animal models and the dose of BRO injection used in both studies.

Oral administrations of wheat germ oil in doses of 150, 300 and 450 mg/kg. b.wt., to potassium bromate–intoxicated rats showed significant increases in FI, BWG% and FER when compared to the positive control group, their effect might be due to the antioxidant nutrient (Vit. E), the content of wheat germ oil, which neutralizes the toxic effect of BRO. The present result agreed with those obtained by Leenhardt*et al*. [14] and Saleh*et al*. [25] who reported that diet supplemented with either carrot or wheat germ oil improved the food consumption, BWG and feed efficiency ratio (FER) in rats injected with benzene.

The current results showed that there were significant changes in the plasma levels of TC, TG, LDL, VLDL, and HDL of cont. (+), when compared with normal control. The WO reversed these changes in a dose-dependent manner as confirmed by histopathological examination of the heart. These results may be due to antioxidant agents, the WO is highly rich in vitamin E and mixed tocopherols [26, 27]. Vitamin E acts as an inhibitor of oxidative processes in body tissues, protecting against oxidation of the unsaturated fat in the body. Yousef*et al*. [28] and Katiyar*et al*. [29] reported that wheat germ oil could reduce oxidative stress, improve lipid metabolism. Moreover, it had significantly higher protective levels of vitamin E in the blood and liver, conferring greater antioxidant protection [14]**.**

The present study revealed a significant increase in serum pro-inflammatory cytokines of BRO intoxicated rats when compared to control rats. Pretreatment with WO significantly reduces both IL-1 and TNF-α, this effect could be due to WO antioxidant activity. The WO thought to be anti-inflammatory because of its high vitamin E content and known as an active natural antioxidant [30]. The WO is rich in tocopherol, considered a strong antioxidant due to its radical scavenging activity, which prevents lipid peroxidation of the cell membrane [31].

CONCLUSION

It can be hypothesized that WO improves lipid parameters, and serum inflammatory cytokineswhile it increases FER and BWG % in BRO intoxicated rats. However, it might be necessary to do more researches on WO to ensure that it will be beneficial in reducing the toxicity effect of BRO.

Conflict of Interest

The author declares that no conflict of interest

Financial Resources of the Study

None.

REFERENCES

[1] Burton GJ, Jauniaux E. Oxidative stress. Best practice & research Clinical obstetrics & gynaecology. 2011 Jun 1;25(3):287-99.

[2] Valko M, Rhodes C, Moncol J, Izakovic MM, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-biological interactions. 2006 Mar 10;160(1):1-40.

[3] Gawlik-Dziki U, Świeca M, Sułkowski M, Dziki D, Baraniak B, Czyż J. Antioxidant and anticancer activities of Chenopodium quinoa leaves extracts–in vitro study. Food and Chemical Toxicology. 2013 Jul 1;57:154-60.

[4] Al-Ogaidi I. Evaluation of the Antioxidant and Anticancer Effects of Biodegradable/Biocompatible Chitosan–Alginate Nanoparticles Loaded with Vitamin C. Internatıonal Journal of Pharmaceutıcal Research and Allıed Scıences. 2018 Jan 1;7(3):189-97.

[5] Dhalla NS, Temsah RM, Netticadan T. Role of oxidative stress in cardiovascular diseases. Journal of hypertension. 2000 Jun 1;18(6):655-73.

[6] Vijay P, Vimukta S. The Role of Natural Antioxidants in Oxidative Stress Induced Diabetes Mellitus. Research Journal of Pharmaceutical Sciences. 2014 Apr 25;3(4):1-6.

[7] Parsons JL, Chipman JK. The role of glutathione in DNA damage by potassium bromate in vitro. Mutagenesis. 2000 Jul 1;15(4):311-6.

[8] Watanabe S, Tajima Y, Yamaguchi T, Fukui T. Potassium bromate-induced hyperuricemia stimulates acute kidney damage and oxidative stress. Journal of health science. 2004;50(6):647-53.

[9] Ballmaier D, Epe B. DNA damage by bromate: mechanism and consequences. Toxicology. 2006 Apr 17;221(2-3):166-71.

[10] Repetto MG, Llesuy SF. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Brazilian journal of medical and biological research. 2002 May;35(5):523-34.

[11] Singh DK, Li L, Porter TD. Policosanol inhibits cholesterol synthesis in hepatoma cells by activation of AMP-kinase. Journal of Pharmacology and Experimental Therapeutics. 2006 Sep 1;318(3):1020-6.

[12] Mansoury M. Evıdence-Based Therapeutıc Actıvıty of Pomegranate and Its Actıve Constituent Ellagıc Acıd. Pharmacophore. 2019 Apr 1;10(1):30-36.

[13] Mohamed NE, Anwar MM. Efficacy of wheat germ oil in counteracting of some biochemical hazards induced by sodium nitrate in rats. Isotope and Radiation Research. 2010 Jul 1;42(1):211-27.

[14] Leenhardt F, Fardet A, Lyan B, Gueux E, Rock E, Mazur A, Chanliaud E, Demigné C, Rémésy C. Wheat germ supplementation of a low vitamin E diet in rats affords effective antioxidant protection in tissues. Journal of the American College of Nutrition. 2008 Apr 1;27(2):222-8.

[15] Acid Wheat Germ Oil. Journal of the American Oil Chemists' Society. 2001 Jan 1;78(1):71-76.

[16] Khan N, Sultana S. Abrogation of potassium bromate-induced renal oxidative stress and subsequent cell proliferation response by soy isoflavones in Wistar rats. Toxicology. 2004 Sep 1;201(1-3):173-84.

[17] Chapman DG, Castillo R, Campbell JA. Evaluation of protein in foods: 1. A method for the determination of protein efficiency ratios. Canadian Journal of Biochemistry and Physiology. 1959 May 1;37(5):679-86.

[18] Ahmad MK, Amani S, Mahmood R. Potassium bromate causes cell lysis and induces oxidative stress in human erythrocytes. Environmental toxicology. 2014 Feb;29(2):138-45.

[19] Achukwu PU, Ufelle SA, Ukaejiofo EO, Ejezie FE, Nwachukwu DN, Nwagha UI, Nworie WC, Anyaehie US. The Effect of Potassium Bromate on Some Haematological Parameters of Wistar Rats. Nigerian Journal of Physiological Sciences. 2009;24(1):59-61.

[20] Arai T, Kelly VP, Minowa O, Noda T, Nishimura S. The study using wild-type and Ogg1 knockout mice exposed to potassium bromate shows no tumor induction despite an extensive accumulation of 8-hydroxyguanine in kidney DNA. Toxicology. 2006 Apr 17;221(2-3):179-86.

[21] Jiunn-Jye Chuu CJ, Lin-Shiau SY. The detrimental effects of potassium bromate and thioglycolate on auditory brainstem response of guinea pigs. Chinese Journal of Physiology. 2000;43(2):91-6.

[22] Hussein SA, Abdel-Aal SA, Elghwab AI. Biochemical role of wheat germ oil on biomarkers of oxidative stress and inflammatory response in a rat model of endotoxemia. Benha Vet Med J. 2014;27:157-67.

[23] Mohamed DA, Ismael AI, Ibrahım AR. Studying the anti-inflammatory and biochemical effects of wheat germ oil. Deutsche Lebensmittel-Rundschau. 2005;101(2):66-72.

[24] Sliai AM. Protective Effects of Wheat Germ Oil on Doxorubicin-Induced Hepatotoxicity in Male Mice. Intern. J. Res. Stud. Bios. 2015;3:21-5.

[25] Saleh ZA, Ibrahim KS, Farrag AR, Shaban EE. Effect of carrot and wheat germ oil supplementation on antioxidant status of rats exposed to benzene. Polish Journal of Food and Nutrition Sciences. 2010;60(2).

[26] Sies H, Stahl W. Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. The American journal of clinical nutrition. 1995 Dec 1;62(6):1315S-21S.

[27] Khalifa FK, Khalil FA, Barakat HA, Hassan MM. Protective role of wheat germ and grape seed oils in chlorpyrifos-induced oxidative stress, biochemical and histological alterations in liver of rats. Australian Journal of Basic and Applied Sciences. 2011;5(10):54-66.

[28] Yousef MI, Awad TI, Mohamed EH. Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by Vitamin E. Toxicology. 2006 Oct 29;227(3):240-7.

[29] Katiyar SK, Mantena SK, Meeran SM. Silymarin protects epidermal keratinocytes from ultraviolet radiation-induced apoptosis and DNA damage by nucleotide excision repair mechanism. PloS one. 2011 Jun 22;6(6):e21410.

[30] Paranich VA, Cherevko OI, Frolova NA, Paranich AV. The effect of wheat germ oil on the antioxidant system of animals. Likars' ka sprava. 2000 Mar(2):40-4.

[31] Zhu KX, Lian CX, Guo XN, Peng W, Zhou HM. Antioxidant activities and total phenolic contents of various extracts from defatted wheat germ. Food Chemistry. 2011 Jun 1;126(3):1122-6.