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Research Article

The Effects of *Dorema aucheri* Hydroalcoholic Extract on Blood Levels of Antioxidant Enzymes (SOD and GPX) and Vitamins (E and C) in vivo

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ABSTRACT

Dorema aucheri is a plant from the Apiaceae family, which has several compounds such as flavonoids and kumarines. Flavonoids have antitumor, anticancer and estrogenic properties. Antiandrogenic properties of kumarines are also known. The aim of this research is to examine the *Dorema aucheri* hydroalcoholic extract effects on SOD and GPX activities and serum concentration of vitamins E and C. Sixty adult male Wistar rats weighing about 200 to 220 g were divided into four groups of fifteen. The control group did not receive any drug. The other three experimental groups including low (100 mg/kg BW), medium (200 mg/kg BW) and maximum (400 mg/kg BW) received *Dorema aucheri* hydroalcoholic extract daily for 28 days orally. After 14 and 28 days all animals in the different groups were weighed and their blood collected, and SOD and GPX activities, and vitamin E and C concentrations were measured by validated standard methods. The results showed that in the short term, SOD and GPX activities, as well as vitamin C concentration increase depending on the dose, but in the long term these parameters decrease proportional to the increase of the dosage. Vitamin E concentration had no significant changes during our study.

Key Words: *Dorema aucheri* hydroalcoholic extract, antioxidant enzymes, antioxidant vitamins

ÖZET

DOREMA AUCHERI HİDROALKOL EKSTRAKTININ İN VİVO KAN ANTİOKSİDAN ENZİMLERİ (SOD VE GPX) VE VİTAMİN (E VE C) DÜZEYLERİ ÜZERİNE ETKİSİ

Dorema aucheri, Apiaceae familyasından flavonoid ve kumarinler gibi çeşitli komponentleri içeren bir bitkidir. Flavonoidler antitümöral, antikanserojenik ve östrojenik özelliklerine sahiptir. Kumarinlerin antiandrojenik özellikleri de bilinmektedir. Bu araştırmanın amacı, *Dorema aucheri* hidroalkolik ekstraktlarının SOD ve GPX aktiviteleri ile E ve C vitaminlerinin serum konsantrasyonları üzerine etkilerini araştırmaktır. 60 adet, 220-220 g ağırlığında ergin Wistar sıçanı 15 adetlik 4 gruba ayrılmıştır. Kontrol grubuna herhangi bir ekstrakt verilmemiştir. Diğer üç deneme grubuna 28 gün boyunca ağız yoluyla, düşük (100 mg/kg CA), orta (200 mg/kg CA) ve yüksek (400 mg/kg CA) düzeyde *Dorema aucheri* hidroalkolik ekstraktı verilmiştir. Tüm gruplarda bulunan hayvanlar 14 ve 28 gün sonra

tartılmış ve alınan kanlarından SOD ve GPX aktiviteleri ile vitamin E ve C konsantrasyonları valide edilmiş standart metodlar ile ölçülmüştür. Elde edilen sonuçlar, kısa vadede SOD ve GPX aktivitelerinin yanısıra vitamin C konsantrasyonunun doza bağlı olarak arttığını, ancak uzun vadede bu parametrelerin doz artışı ile orantılı olarak azaldığını göstermiştir. Çalışma esnasında E vitamini konsantrasyonu düzeylerinde anlamlı değişiklik gözlenmemiştir.

Anahtar Kelimeler: *Dorema aucheri* hydroalkolik ekstraktı, antioksidan enzimler, antioksidan vitaminler

Introduction

Dorema aucheri belonging to the Apiacea family plants is used as food by the local residents of Southern Iran. It is believed that this plant contains medicinal properties and one of the materials that is in this plant is flavonoids (Mokhtari et al., 2008a). Flavonoids constitute a large group of polyphenolic compounds that have antioxidant properties (Azarneuoshan et al., 2010).These compounds are a type of highly effective natural antioxidant that control the blood cholesterol and triglyceride, and are also used specifically in kidney problems (Kamyab Moghadas et al., 2010).

Antioxidants protect cells from the damaging effects of free radicals, which are molecules that contain an unshared electron. Free radicals damage cells and may contribute to the development of cardiovascular disease and cancer (Verhagen et al., 2006). Unshared electrons are highly energetic and react rapidly with oxygen to form reactive oxygen species (ROS). The body forms ROS endogenously when it converts food to energy, and it is also exposed to free radicals from environmental exposure, such as cigarette smoke, air pollution, and ultraviolet radiation from the sun. ROS are part of signaling mechanisms among cells. Selenium is an integral component of several selenoproteins including the glutathione peroxidases (GPX), which catalyze the reduction of harmful peroxides (Holben and Smith, 1999). Maintaining an optimum level of selenium and GPX, therefore, is important to protect the host from the development of diseases induced by oxidative damage such as cardiovascular disease (Salonen et al., 1982) and cancer (Combs and Gray, 1998).

Vitamin E is a fat-soluble antioxidant that stops the production of ROS formed when fat undergoes oxidation. Scientists are investigating whether, by limiting free-radical production and possibly through other mechanisms vitamin E might help prevent or delay the chronic diseases associated with free radicals. Vitamin E might also block the formation of carcinogenic nitrosamines formed in the stomach from nitrites in foods and protect against cancer by enhancing immune function (Weitberg and Corvese, 1997).

Dorema aucheri is used more traditionally, the rate of neoplastic diseases in the regions with high consumption of *Dorema aucheri* has been increases recently and it is believed that this increase is related to this plant, for example mammary gland tumor effects have been reported for *Dorema aucheri* in dose 400 mg/kg (Afshoun et al., 2010), and neoplastic effects have been reported for the stem and leaf oil extract of it (Hajipour et al., 2009), but this idea is a discussing subject between researchers, some of them say this plant has antineoplastic effects due to its antioxidant agents. There is less study about antioxidant effects of *Dorema aucheri* (body enzymatic and non-enzymatic antioxidant).

For this purpose, we planned this study to investigate the effects of different doses and consumption periods of *Dorema aucheri* hydroalcoholic extract on superoxide dismutase and glutathione peroxidase activities, and the serum concentration of vitamin E and C.

Materials and Methods

Animals

Blood samples were taken from the heart of sixty adult male Wistar rats weighing approximately 200 to 220 g divided into four groups of fifteen (control group and experimental groups that were include A, B and C groups). The control group did not receive any drug. The other three experimental groups including low (100 mg/kg BW/day) (groups A_1

and A_2 which samplings were done after 14 and 28 days of receiving *Dorema aucheri* hydroalcoholic extract orally, respectively), medium (200 mg/kg BW/day) (groups B_1 and B_2 samplings were done after 14 and 28 days of receiving *Dorema aucheri* hydroalcoholic extract orally, respectively) and maximum (400 mg/kg BW/day) (groups C_1 and C_2 samplings were done after 14 and 28 days of receiving *Dorema aucheri* hydroalcoholic extract orally respectively).

Blood sampling

After 14 and 28 days all animals were weighed and their bloods were collected from the heart vein. Blood sampling were done after 14 days of A,B and C groups were assigned as A_1,B_1 and C_1 groups and blood sampling were done after 28 days of A,B and C groups were assigned as A_2,B_2 and C_2 groups. In control group blood sampling was done after 28 days from the heart vein and was assigned control group .For the determination of haemoglobin, superoxide dismutase (SOD) and glutathione peroxidase (GPX), blood samples were collected into vacutainers containing ethyl-enediamine tetra-acetic acid (EDTA) as an anticoagulant. To determine serum vitamins E and C, blood samples were collected into vacutainers and serum was separated by centrifugation at 750 *g* for 15 min and stored at -20°C until use. The samples with haemolysis were thrown away.

Biochemical analysis

Haemoglobin concentration was measured by Cyanmethaemoglobin method (Jain, 1986). SOD activity was measured by a modified method of iodophenyl nitrophenol phenyltetrazolium chloride (RANSOD kit, catalog no. SD 125; Randox, Ltd. United Kingdom). This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2- (4-Prodiodophenyl)-3- (4- nitrophend)-5-phenyl-tetrazoliumchloride (INT) to form a red formazan dye. The superoxide dismutase activity was then measured by the degree of inhibition of this reaction. One unit of SOD was considered a 50% inhibition of reduction of INT under the condition of the assay. GPX was measured by the method of Paglia and Valentine (1967) (RANSEL kit, catalog no. RS 505; Randox, Ltd. United Kingdom). GPX catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (G-S-S-G) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance was measured at 340 nm. Digestion of serum was performed by a mixture of perchloric and nitric acid (3:7 ratios respectively).Vitamin E and C were measurement by Japanese Shimadzu bA model HPLC set with ultraviolet detector.

Statistical analysis

The data were expressed in SI units and analyzed by repeated measurements ANOVA, Duncan, Spearman and T-test using SPSS/PC software (Norusis, 1993). All values were expressed as mean and standard error (SE), and $P<0.05$ was seen as statistically significant.

Results

The mean±SE of vitamins E and C and SOD and GPX in 60 rats in different groups are shown in Table 1. The significant differences between each parameter in different groups are shown in Table 2 to 5. In the all of the groups *Dorema aucheri* in the short term increased the serum concentration of vitamin C and SOD and GPX activities, but in over a long period of use, the serum concentration of vitamin C and activities of SOD and GPX decreased in all groups (Table 1). It should be mentioned that these changes were dependent on dose.

Serum concentration of vitamin E had no significant differences in all groups (Table 3). This study shows that serum concentration of C vitamin and GPX and SOD activities have been most affected in C group compared with two other experimental groups .The highest and the lowest serum concentration of C vitamin ,GPX and SOD activities were observed in C1 and C_2 groups respectively (Table 1).

Compared to control group, there were significant differences between the mean serum

concentrations of C vitamin and SOD activities in B_1, C_1, B_2, C_2 groups, and no significant differences between the mean serum concentrations of C vitamin and SOD activities in A_1 and A_2 groups were observed (Tables 2, 5). Compared to control group, GPX activity hasn't been affected significantly by *Dorema aucheri* hydroalcoholic extract in A_1 and B_1 groups; but there were significant differences between the mean GPX activity in C_1, A_2, B_2 and C_2 groups (Table 4).

There were significant differences between the mean serum concentrations of C vitamin in B_1 group in comparison to A_1 and C_1 groups (Table 2).

Table 1. The mean ± SE of vitamins E and C, also SOD and GPX activities in different groups[a].

Tablo 1. Farklı gruplarda vitamin E ve C ile SOD ve GPX aktivitelerina ait ortalama ± standart hatalar.

Groups		SOD(UI/gHb) Mean ± SE	GPX(UI/gHb) Mean ± SE	Vit E(µmol/L) Mean ± SE	Vit C(µmol/L) Mean ± SE
	Control	1251.830±11.667	32.850±0.465	0.2186±0.003	15.188±0.266
A	**A_1**	1270.49±7.5692	34.97±0.4995	0.2216±0.0745	15.80±0.1792
	A_2	1221.04±7.65	31.91±0.2701	0.2212±0.00341	14.75±0.260
B	**B_1**	1419.64±14.098	45.33±0.1486	0.2161±0.00319	17.699±0.40
	B_2	1163.51±10.095	25.98±0.9944	0.2174±0.0029	12.671±0.03622
C	**C_1**	1479.94±11.5725	51.53±1.305	0.2157±0.00343	19.069±0.1832
	C_2	1146.86±10.4352	24.04±0.8309	0.218±0.00288	11.62±0.3107

[a] control group, groups A_1, B_1 and C_1 received 100, 200, 400mg/kg BW of *Dorema aucher* for 14 days respectively, groups A_2, B_2 and C_2 received 100, 200, 400mg/kg BW of *Dorema aucher* for 28 days respectively.

Table 2 The significant differences between serum concentrations of vitamin C in different groups.

Tablo 2. Farklı gruplardaki vitamin C serum konsantrasyonları arasındaki önemli farklılıklar.

Groups	Control	A_1	B_1	C_1	A_2	B_2	C_2
Control	-						
A_1	1.000	-					
B_1	0.000*	0.000*	-				
C_1	0.000*	0.000*	0.031*	-			
A_2	1.000	0.291	0.000*	0.000*			
B_2	0.000*	0.000*	0.000*	0.000*	0.000*	-	
C_2	0.000*	0.000*	0.000*	0.000*	0.000*	0.288	

Table 3. The significant differences between serum concentrations of vitamin E in different groups.

Tablo 3. Farklı gruplardaki vitamin E serum konsantrasyonları arasındaki önemli farklılıklar.

Groups	Control	A_1	B_1	C_1	A_2	B_2	C_2
Control	-						
A_1	1.000	-					
B_1	1.000	1.000	-				
C_1	1.000	1.000	1.000	-			
A_2	1.000	1.000	1.000	1.000	-		
B_2	1.000	1.000	1.000	1.000	1.000	-	
C_2	1.000	1.000	1.000	1.000	1.000	1.000	

Table 4. The significant differences between erythrocyte GPX activities in different groups.
Tablo 4. Farklı gruplardaki eritrosit GPX aktiviteleri arasındaki önemli farklılıklar.

Groups	Control	A_1	B_1	C_1	A_2	B_2	C_2
Control	-	-					
A_1	1.000	-					
B_1	1.000	0.000*	-				
C_1	0.000*	0.000*	0.000*				
A_2	0.000*	0.315	0.000*	0.000*	-		
B_2	0.000*	0.000*	0.000*	0.000*	0.000*	-	
C_2	0.000*	0.000*	0.000*	0.000*	0.000*	1.000	

Table 5. The significant differences between erythrocyte SOD activities in different groups.
Tablo 5. Farklı gruplardaki eritrosit SOD aktiviteleri arasındaki önemli farklılıklar.

Groups	Control	A_1	B_1	C_1	A_2	B_2	C_2
Control	-	-					
A_1	1.000	-					
B_1	0.000*	0.000*	-				
C_1	0.000*	0.000*	0.004	-			
A_2	0.952	0.036	0.000*	0.000*	-		
B_2	0.000*	0.000*	0.000*	0.000*	0.007	-	
C_2	0.000*	0.000*	0.000*	0.000*	0.000*	1.000	

Discussion

In this study, in all of groups that received *Dorema aucheri* for 14 days, the serum concentration of vitamin C, SOD and GPX activities increased, and there were significant differences between C vitamin concentration and SOD activities in groups B_1 and C_1 in comparison to control group. In group A_1 this increase was not considerable, so there were no significant differences between the parameter levels in this group in comparison to control group. Also, there were significant differences only between GPX activities in group C_1 in comparison to control group, and although GPX activities in group B_1 and A_1 in comparison to control group showed some increase, there were no significant differences between these groups in comparison to control group. In contrast, in the all of groups that received this plant extract over a 28 day period the serum concentration of vitamin C, SOD and GPX activities decreased proportional to dose, so the higher the dose of *Dorema aucheri*, the greater the decrease. There were significant differences between vitamin C concentration and SOD activity in groups B_2 and C_2 in comparison to control group, but there was no significant difference between serum levels of these parameters in group A_2 in comparison to control group. There were significant differences between serum activities of GPX in all of the groups after 28 days in comparison to control group.

There were significant differences between the mean serum concentration of vitamin C in B_1 group in comparison to A_1 and C_1 groups that shows the vitamin C concentration in short term has been affected by *Dorema aucheri* hydroalcoholic dose and severity.

Shortly, these findings show that the effects of *Dorema aucheri* hydroalcoholic extract on serum concentration of vitamin C and SOD activity are related to dose, but GPX changes are related to dose and term of administration of *Dorema aucheri* hydrialcoholic extract.

These changes in serum levels of vitamin C, SOD and GPX can be due to the fact that *Dorema aucheri* extract has an oxidative stress effect on the body, and in a short time the body

defense system increases antioxidant production and serum antioxidant levels but in the long term these antioxidants are used up more than they are produced, so their serum levels decrease. The findings in this study are the same as Afshoun et al. (2010) have reported, which showed *Dorema aucheri* extract at a dosage of 400mg/kg increases mammary gland tumor size and mortality percent, but decreases the rats weight. However at doses of 200mg/kg the size of mammary gland tumor and mortality percent decreases, while the rats weight increases. Hajipour et al. (2009) also reported neoplastic effects for the stem and leaf oil extract of *Dorema aucheri*.

Sadeghi et al. (2007a, b) reported *Dorema aucheri* in doses of 500mg/kg has antihyperlipidemic, hypercholesterolemic and hepatic effects and these characteristics can be due to its antioxidant agents, but these researchers mentioned that this extract given at a dose of 500 mg/kg is toxic for rats.

Selenium and α-tocopherol decrease the risk of suffering to prostatic neoplasm (Clark et al., 1996; Duffield-Lillico et al., 2002; Heinonen et al., 1998). Consumption of selenium, vitamin E and beta carotene together decrease neoplasmic mortality (Blot et al., 1993). Chan et al. (1998) and Knekt et al. (1994) reported the reduction in prostatic, mammalian neoplasms and coronary mortality is dependent on an increase in vitamin E intake (Chan et al., 1998; Knekt et al., 1994).

No significant differences were seen between serum concentration of vitamin E in short (after 14 days) and long (after 28 days) term in any group that received *Dorema aucheri* in comparison to control group. Serum concentrations of vitamin E depend on the liver, which takes up the nutrient after the various forms are absorbed from the small intestine. It seems that, in this study the lack of significant changes in serum E vitamin concentration is due to liver control. The liver preferentially resecretes only alpha-tocopherol via the hepatic alpha-tocopherol transfer protein (Traber et al., 2006); the liver metabolizes and excretes the other vitamin E forms (Traber, 2007). As a result, blood and cellular concentrations of other forms of vitamin E are lower than those of alpha-tocopherol (Dietrichb et al., 2006; Sen et al., 2006). Vitamin E is active as an antioxidant, it is involved in immune function and, as shown primarily by in vitro studies of cells, cell signaling, regulation of gene expression, and other metabolic processes (Traber et al., 2006). The higher intakes of vitamin E from foods and supplements could decrease the risk of colon cancer, especially in women <65 years of age (Lee et al., 2005). The adults who took supplemental vitamin E for 10 years or longer had a reduced risk of death from bladder cancer (Lee et al., 2005).

Extracts of *Dorema aucheri* can significantly reduce pain depending on the dose in acute phase ($P<0.05$). However, in chronic phase, a high dose of the extract can reduce pain; also the highest dose of extract can reduce paw edema in carrageenan test (Mokhtari et al., 2008b).

Generation of free radicals is an integral feature of normal cellular function. Contrary, excessive generation and/or insufficiant removal of free radicals causes destructive and irreversible damage to the cell (Lopaczyski and Zeisel, 2001). Reactive oxygen species (ROS) involving superoxide radical, hydrogen peroxide and hydroxyl radical have a great influence on the normal function of biomolecules like proteins, nucleic acids and cell membrane phospholipids. Free radicals are generated during stepwise reduction of molecular oxygen (Singh et al., 1999). Halliwell and Gutteridge (1999) described various lines of defense to reactive oxygen species in animals. Enzymes with important antioxidant functions include: i) superoxide dismutase (SOD), which catalyses the dismutation of superoxide radical to hydrogen peroxide and water, ii) catalase (CAT), which catalyses the breakdown of hydrogen peroxide to oxygen and water, and iii) glutathione peroxidase (GPX), which facilitates the destruction of both hydrogen peroxide and organic peroxides. Reduced glutathione (GSH), a tri-peptide thiol, is an important antioxidant,

as well as a co-factor for various antioxidant enzymes (Kidd, 1997). SOD is the first line of defense against ROS and is active in catalyzing detoxification of superoxide radical (Gonzales et al., 1984).

Conclusion

The findings of this study showed that *Dorema aucheri* hydroalcoholic extract affected enzymatic and none enzymatic antioxidants. These effects are related to dose and term. In short term this effect is increasing and in long time is decreasing. In both cases greater effects are observed in higher dose. It is necessary to mention that GPX activity in long term is more sensitive to Dorema aucheri consumption. We concluded that Dorema aucheri hydroalcoholic extract in the long term and especially with high dose can be (have) an oxidative stress for the body. On the other hand, its oxidative effects are more than its flavonoid effects, so it is administered to prevent long term consumption of this plant.

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