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Apple Cider Vinegar Modulates Serum Lipid Profile, Erythrocyte, Kidney, and Liver Membrane Oxidative Stress in Ovariectomized Mice Fed High Cholesterol

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Abstract The purpose of this study was to investigate the potentially beneficial effects of apple cider vinegar (ACV) supplementation on serum triglycerides, total cholesterol, liver and kidney membrane lipid peroxidation, and antioxidant levels in ovariectomized (OVX) mice fed high cholesterol. Four groups of ten female mice were treated as follows: Group I received no treatment and was used as control. Group II was OVX mice. Group III received ACV intragastrically (0.6 % of feed), and group IV was OVX and was treated with ACV as described for group III. The treatment was continued for 28 days, during which the mice were fed a high-cholesterol diet. The lipid peroxidation levels in erythrocyte, liver and kidney, triglycerides, total, and VLDL cholesterol levels in serum were higher in the OVX group than in groups III and IV. The levels of vitamin E in liver, the kidney and erythrocyte glutathione peroxidase (GSH-Px), and erythrocyte-reduced glutathione (GSH) were decreased in group II. The GSH-Px, vitamin C, E, and β-carotene, and the erythrocyte GSH and GSH-Px values were higher in kidney of groups III and IV, but in liver the vitamin E and β-carotene concentrations were decreased. In conclusion, ACV induced a protective effect against erythrocyte, kidney, and liver oxidative injury, and lowered the serum lipid levels in mice fed high cholesterol, suggesting that it possesses oxidative stress scavenging effects, inhibits lipid peroxidation, and increases the levels of antioxidant enzymes and vitamin.

Keywords Oxidative stress · Glutathione peroxidase · Ovariectomize · Hypercholesterolemia · Antioxidant vitamins · Liver

Abbreviations

ACV	Apple cider vinegar
GSH	Glutathione
GSH-Px	Glutathione peroxidase
HDL	High-density lipoprotein
i.p.	Intraperitoneal
LDL	Low-density lipoprotein
OVX	Ovariectomized
ROS	Reactive oxygen species
VLDL	Very low-density lipoprotein

Introduction

Diseases of liver and kidney abnormalities constitute a serious health problem in menopause (Poli 1993). These diseases are usually treated with drugs, dietary or metabolic changes, and vaccines. Liver disease in menopause also induces lipid profile abnormalities because of its important role in lipid metabolism (Luotola et al. 1986; Ulaş and Cay 2011). In this regard, finding new, safe, and effective food supplements has become an important line of research.

Oxidative stress is the result of an imbalance between the rates of free radical production and elimination via endogenous antioxidant mechanisms such as the enzymes glutathione peroxidase (GSH-Px) and catalase as well as

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low molecular weight reductants like α-tocopherol, glutathione (GSH), and ascorbate (Kovacic and Somanathan 2008). This imbalance can be initiated by numerous factors including acidosis, transition metals, nitric oxide, LDL-oxidation, and uncouplers of mitochondrial electron transport (Nazıroğlu and Bransch 2006; Espino et al. 2012).

The major roles of GSH-Px are to catalyze the reduction of hydrogen peroxide to water (Kovacic and Somanathan 2008) and the removal of organic hydroperoxides (Nazıroğlu 2009). GSH is a peptide involved in maintaining oxidant homeostasis and in the cellular detoxification of reactive oxygen species (ROS) in tissues including brain, kidney, and liver (Halliwell 2006). Vitamin E is the most important lipid antioxidant in cells. In addition to its role as free radical scavenger, vitamin C also transforms vitamin E to its active form (Frei et al. 1989).

It has been reported that a number of phenolic compound extracted from fruit can protect against oxidative stress-induced liver and kidney injury because of their antioxidant properties (Yang et al. 2010; Nazıroğlu et al. 2011a, b). This has promoted a substantial increase in the use of supplemental dietary phenols and alternative therapy to treat menopause-induced liver and kidney diseases in women and animals in favor of hormone replacement treatment (Nazıroğlu et al. 2004a, b; Kireev et al. 2010; Nazıroğlu et al. 2011a, b).

Apple cider vinegar is widely used in salad dressings, marinades, vinaigrettes, food preservatives, chutneys, and other common foods. The main classes of phenols in apples and ACV are flavonoids and polyphenolic compounds (Denis et al. 2013). A number of studies have shown that these foods have a variety of pharmacological functions, including antioxidant (Yang et al. 2010; Denis et al. 2013), antidiabetic (Shishehbor et al. 2008), and cholesterol lowering (Budak et al. 2011) properties, without adverse effects. However, information on the effects of ACV on antioxidant systems is very limited. For example, there are no scientific reports on the use of ACV in menopausal women or ovariectomized animals as a potential source of natural antioxidant phenols.

It is known, however, that the deficiency of ovarian hormones promotes the generation of ROS, resulting in oxidative stress, cell damage, or death (Budak et al. 2011). The beneficial effects of estrogen include reducing total- and low-density cholesterol (LDL) through enhanced LDL receptor binding and clearance (Noh et al. 1999; Sanchez-Rodriguez et al. 2011). Additionally, by reducing hepatic lipase activity, estrogen promotes the formation of larger, less atherogenic LDL (Nazıroğlu et al. 2004a, b; Sanchez-Rodriguez et al. 2011). Hence, the use of ACV instead of cholesterol lowering drug therapy in menopause may improve oxidative stress-induced lipid profile liver and kidney function in ovariectomized mice as a model of postmenopausal women.

The aim of the current study was to investigate protective effects of ACV on ovariectomy-induced erythrocyte, liver, and kidney oxidative damage as well as lipid profile changes in mice fed high cholesterol and its free radical scavenging activity in vivo.

Materials and Methods

Animals

Forty female Swiss mice weighing 36–40 g were used for the experimental procedures. Twenty of them remained intact as control and ACV groups, and the ovaries were removed in the remaining 20 animals in order to constitute groups II and IV. The mice were housed in individual plastic cages with bedding. Standard food and tap water were available ad libitum for the duration of the experiments unless otherwise noted. The temperature was maintained at 22 ± 2 °C. A 12/12 h light/dark cycle was maintained, unless otherwise noted. The ACV was provided as a gift from the Agricultural Faculty of Suleyman Demirel University and it was diluted with water so that it would provide 0.6 % of the animals' daily diet (Budak et al. 2011). This concentration was found to be innocuous to mice in a pre-study in which various concentrations of ACV were tested. Hypercholesterolemia was induced by daily gavage administration of 1 ml/100 g body weight of a cocktail containing 100 g cholesterol, 30 g propylthiouracil, and 100 g cholic acid in 1 liter peanut oil (Vogel and Vogel 1997).

The mice were maintained and used in accordance with the Animal Welfare Act for the Care and Use of Laboratory Animals of Suleyman Demirel University (SDU), and the local ethical committee of the Medical Faculty, SDU, approved the experimental protocol designed for this study (Protocol Number; 2009: 27-08).

Experimental Groups

The mice were randomly divided into four groups of ten that were treated for 28 days, as follows:

Group I was used as control and was fed a cholesterol-rich feed (5 % cholesterol). The only treatment was intragastrical application of physiological saline, as placebo (Dilek et al. 2010).

Group II (OVX), the ovaries were surgically removed. Otherwise, the mice were treated as described for the controls.

Group III (ACV) for which in addition to the high-cholesterol diet, the animals were given 0.6 % apple cider vinegar instead of a placebo.

Group IV (ACV + OVX) in which the animals were ovariectomized and treated with ACV, as described for group III.

Animal Model of Menopause

For removal of the ovaries, the two OVX groups of mice were anesthetized by intraperitoneal administration of a cocktail of ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg) (Kireev et al. 2010; Dilek et al. 2010). Briefly, the surgical procedure for ovariectomy equithesin and two 4-mm incisions were made through the skin and the muscle back walls in parallel with the bodyline. The ovaries were then located, and a silk thread was tightly tied around the oviduct, including the ovarian blood vessels. The wound was sutured with a synthetic absorbable thread. Depocilin was used to prevent of infection. The ovariectomized mice were allowed to fully recover before beginning the study protocol.

Blood and Tissue Samples

All the mice were sacrificed after 12 h of the last ACV administration to obtain the necessary blood and tissue samples. The blood was separated into plasma and erythrocytes by centrifugation at 1,500×*g* for 10 min at +4 °C. The erythrocyte samples were washed three times in cold isotonic saline (0.9 %, v/w), and hemolysis was accomplished by adding a nine-fold volume of 50 mM, pH 7.4 phosphate buffer. The hemolyzed samples were stored at −30 °C for not more than months pending measurement of enzymatic activity.

All preparation procedures were performed on ice. The liver and kidney samples were washed twice with cold saline solution, placed into glass bottles, labeled, and stored in at −33 °C until needed. After weighing, the tissue samples were placed on ice, cut into small pieces with scissors and mixed with five volumes (1:5 w/v) of ice cold 50 mM, pH 7.4 Tris-HCl buffer, and then homogenized for 2 min at 5,000 rpm using a glass Teflon homogenizer (Caliskan Cam Teknik, Ankara, Turkey). The homogenate was immediately used for establishing the extent of lipid peroxidation and antioxidant enzyme levels. The antioxidant vitamin analyses were performed within 3 months.

Lipid Peroxidation Determination

The lipid peroxidation levels were determined by the TBA method of Placer et al. (1966) using a UV-1800 Spectrometer (Schimadzu, Kyoto, Japan). The values are expressed as μmol/gram protein and as μmol/gram hemoglobin (Hb).

Reduced Glutathione (GSH), Glutathione Peroxidase (GSH-Px), and Protein Assays

The GSH content in erythrocyte, kidney, and liver samples was measured by the spectroscopic method of Sedlak and Lindsay (1968), as described in a previous study (Nazıroğlu et al. 2004a). The GSH-Px activity in erythrocyte, kidney, and liver was measured spectrophotometrically at 37 °C according to the method of Lawrence and Burk (1976). The protein contents in the liver and kidney were measured by the method of Lowry et al. (1951) with bovine serum albumin as the standard. Drabkin's reagent was used in the erythrocyte for determination of Hb.

Vitamins A, C, and E and β-carotene Analyses

The levels of vitamins A and E were determined in the kidney and liver samples by a modification of the methods described by Desai (1984) and Suzuki and Katoh (1990) as described in a previous study (Nazıroğlu et al. 2004a). Liver and kidney samples (0.25 g) were saponified by the addition of 0.3 ml KOH (60 % w/v in water) and 2 ml of 1 % (w/v in ethanol) ascorbic acid, followed by heating at 70 °C for 30 min. After cooling on ice, 2 ml of water and 1 ml of n-hexane were added to the samples, mixed, and allowed to separate into phases. An aliquot of 0.5 ml of n-hexane extract was taken, and the vitamin A concentrations were measured at 325 nm. Then, reactants were added and the absorbance value of hexane was measured in a spectrophotometer at 535 nm. Calibration was performed using standard solutions of all-trans retinol and α-tocopherol in hexane.

The concentrations of β-carotene in the kidney and liver samples were determined according to the method of Suzuki and Katoh (1990). 2 ml of hexane was mixed with 0.25 g tissue sample. The concentration of β-carotene in hexane was measured spectrophotometrically at 453 nm.

The quantitative determination of ascorbic acid in the kidney and liver samples was performed according to the method of Jagota and Dani (1982). The absorbance of the samples was measured spectrophotometrically at 760 nm.

Measurement of Triglyceride and Total Cholesterol

Serum triglycerides, total cholesterol, VLDL cholesterol, and element levels were measured in an autoanalyzer (Olympus AV 2700) at the Isparta State Hospital by using standard laboratory techniques (Nazıroğlu et al. 2011a, b).

Statistical Analysis

All results were expressed as mean ± SD. Significant values in the four groups were assessed by the unpaired Mann–Whitney *U* test. Data were analyzed using the SPSS statistical software (v. 17.0, SPSS Inc. Chicago, Illinois, USA). *p* values of less than 0.05 were regarded as significant.

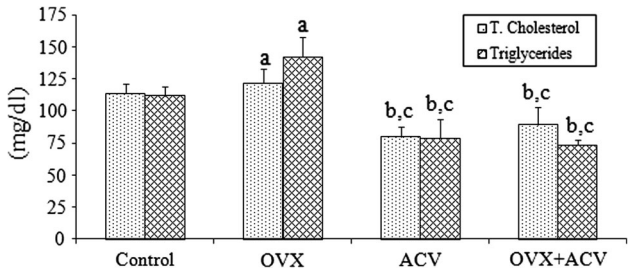

Fig. 1 Effects of apple cider vinegar (ACV) on total cholesterol and triglyceride levels in serum of ovariectomized (OVX) mice (mean ± SD and $n = 10$). [a]$p < 0.05$ and [b]$p < 0.001$ as compared with group control. [c]$p < 0.001$ as compared with OVX group

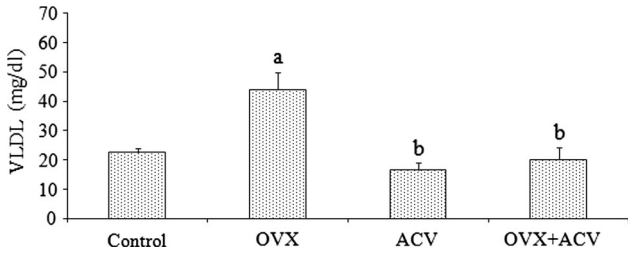

Fig. 2 Effects of apple cider vinegar (ACV) on VLDL cholesterol levels in serum of ovariectomized (OVX) mice (mean ± SD and $n = 10$). [a]$p < 0.001$ $p < 0.001$ versus control. [b]$p < 0.001$ versus OVX group

Results

Serum Element, Total Cholesterol and Triglycerides Levels

Serum total cholesterol, triglycerides, and VLDL cholesterol levels are shown in Figs. 1 and 2, respectively. The total cholesterol levels as in groups I–IV were 113, 121, 79, and 89 mg/dl, respectively. The triglycerides levels in these groups were 111, 142, 78, and 73 mg/dl, respectively. In the same order, the total cholesterol levels were 22, 43, 16, and 20 mg/dl, respectively. The serum triglycerides ($p < 0.05$), total ($p < 0.05$) and VLDL ($p < 0.001$) cholesterol levels were significantly higher in group II relative to controls, but significantly lower in groups III and IV relative to groups I and II ($p < 0.001$). The levels of sodium, potassium, and chlorine in serum samples are shown in Table 1. No significant changes in the levels of these elements were detected in all study groups.

Lipid Peroxidation Results

The lipid peroxidation levels in erythrocytes, liver, and kidney are shown in Tables 2, 3, and 4, respectively. Lipid peroxidation is considered to be one of the principal indicators of OVX-induced oxidative erythrocytes, liver, and kidney injury (Nazıroğlu et al. 2004a, b; Ulaş and Cay 2011; Sanchez-Rodriguez et al. 2011). The lipid peroxidation levels in the erythrocytes ($p < 0.05$), liver ($p < 0.05$), and kidney ($p < 0.01$) were significantly higher in group II than in the controls. However, pretreatment of mice with ACV effectively inhibited OVX-induced lipid peroxidation levels in 1 erythrocytes ($p < 0.05$), liver ($p < 0.05$) and kidney ($p < 0.01$).

GSH and GSH-Px Values in Liver and Kidney

GSH-Px is a strong antioxidant enzyme within enzymatic ROS scavenger systems, while GSH is a non-enzymatic thiol-containing antioxidant against OVX-induced cellular toxicity (Nazıroğlu 2009; Kovacic and Somanathan 2008). The GSH levels and GSH-Px activity in erythrocytes, liver, and kidney are shown in Tables 2, 3, and 4, respectively. The data show that the GSH-Px activity in erythrocytes ($p < 0.05$) and kidney ($p < 0.01$), and GSH levels in erythrocytes ($p < 0.05$) significantly decreased in group II. However, the GSH-Px activity in erythrocytes ($p < 0.05$) and kidney ($p < 0.01$), and the GSH levels in erythrocytes ($p < 0.05$) were higher in groups III and IV relative to group II. The GSH level in liver and kidney and GSH-Px activity in liver did not change in the four groups statistically.

Antioxidant Vitamin Concentrations in Liver and Kidney

The concentrations of β-carotene and the levels of vitamins A, C, and E in liver and kidney are shown in Tables 3 and 4, respectively. The vitamin E stores in liver were markedly depleted in group II ($p < 0.05$), and the vitamin E and β-carotene concentrations in liver were significantly depleted in groups III and IV ($p < 0.01$) relative to groups II and I.

Pretreatment of mice with ACV effectively increased the levels of vitamins C ($p < 0.05$), E ($p < 0.05$), and β-carotene ($p < 0.01$) in kidney. The vitamin A concentrations in liver and kidney did not significantly change in any of the four groups.

Discussion

It was observed that the lipid peroxidation values for erythrocytes, liver, and kidney and the serum triglycerides, total and VLDL cholesterol concentrations were increased and that the GSH-Px activity and level of GSH were decreased in ovariectomized mice, proving that the protocol worked as an experimental menopause model. Supplementation with ACV resulted in an increase of the

Table 1 Effects of apple cider vinegar (ACV) supplementation on serum sodium, potassium, and chloride levels in ovariectomized (OVX) mice (mean ± SD)

Parameters	Control ($n = 10$)	OVX ($n = 10$)	ACV ($n = 10$)	OVX + ACV ($n = 10$)
Sodium (mg/dl)	163.2 ± 24.8	145.2 ± 9.2	142.5 ± 7.4	155.5 ± 21.2
Potassium (mg/dl)	4.60 ± 0.89	4.91 ± 0.81	4.70 ± 0.40	4.68 ± 0.59
Chloride (mg/dl)	124.8 ± 16.7	110.2 ± 4.9	107.0 ± 7.8	113.3 ± 14.8

Table 2 Effects of apple cider vinegar (ACV) supplementation on erythrocytes lipid peroxidation (LP), reduced glutathione (GSH), and glutathione peroxidase levels in ovariectomized (OVX) mice (mean ± SD)

Parameters	Control ($n = 10$)	OVX ($n = 10$)	ACV ($n = 10$)	OVX + ACV ($n = 10$)
GSH-Px (IU/g Hb)	13.00 ± 1.49	10.90 ± 3.52[a]	12.80 ± 3.07[b]	12.90 ± 2.90[b]
GSH (μmol/g Hb)	10.8 ± 1.28	9.37 ± 1.55	10.40 ± 1.73	10.20 ± 1.51
LP (μmol/g Hb)	11.80 ± 1.44	14.30 ± 1.31[a]	12.00 ± 1.85[b]	11.70 ± 1.42[b]

[a] $p < 0.05$ versus control group

[b] $p < 0.05$ versus OVX group

Table 3 Effects of apple cider vinegar (ACV) supplementation on liver lipid peroxidation (LP), reduced glutathione (GSH), glutathione peroxidase (GSH-Px), vitamin A, C, E and β-carotene levels in ovariectomized (OVX) mice. (mean ± SD)

Parameters	Control ($n = 10$)	OVX ($n = 10$)	ACV ($n = 10$)	OVX + ACV ($n = 10$)
GSH-Px (IU/g protein)	16.90 ± 1.39	17.70 ± 2.06	16.50 ± 3.32	16.30 ± 3.52
GSH (μmol/g protein)	6.93 ± 0.87	6.56 ± 0.39	7.55 ± 1.31	6.86 ± 1.58
LP (μmol/g protein)	10.80 ± 1.26	13.30 ± 1.59[a]	9.86 ± 1.52[c]	10.70 ± 1.00[c]
Vitamin A (μmol/g tissue)	50.16 ± 0.45	53.89 ± 1.84	49.84 ± 1.17	53.07 ± 0.95
β-carotene (μmol/g tissue)	2.62 ± 0.11	2.35 ± 0.09	2.06 ± 0.39[b,c]	1.84 ± 0.17[b,d]
Vitamin C (μmol/g tissue)	0.68 ± 0.09	0.66 ± 0.14	0.63 ± 0.09	0.69 ± 0.08
Vitamin E (μmol/g tissue)	9.54 ± 0.56	9.10 ± 0.46[a]	7.62 ± 1.80[b,d]	6.08 ± 0.96[b,d]

[a] $p < 0.05$ and [b] $p < 0.01$ versus control group

[c] $p < 0.05$ and [d] $p < 0.01$ versus OVX group

Table 4 Effects of apple cider vinegar (ACV) supplementation on kidney lipid peroxidation (LP), reduced glutathione (GSH), glutathione peroxidase (GSH-Px), vitamin A, C, E and β-carotene levels in ovariectomized (OVX) mice (mean ± SD)

Parameters	Control ($n = 10$)	OVX ($n = 10$)	ACV ($n = 10$)	OVX + ACV ($n = 10$)
GSH-Px (IU/g protein)	14.70 ± 0.83	12.80 ± 1.21[b]	15.90 ± 1.12[a,e]	14.88 ± 1.88[c]
GSH (μmol/g protein)	5.07 ± 0.39	5.26 ± 0.29	5.07 ± 0.47	5.26 ± 0.73
LP (μmol/g protein)	4.60 ± 0.89	6.11 ± 1.05[b]	4.93 ± 0.48[d]	6.34 ± 0.78
Vitamin A (μmol/g tissue)	10.47 ± 1.97	10.37 ± 1.26	12.01 ± 1.38	11.13 ± 1.54
β-Carotene (μmol/g tissue)	0.81 ± 0.07	0.79 ± 0.09	1.06 ± 0.17[d]	1.83 ± 0.13[d]
Vitamin C (μmol/g tissue)	0.35 ± 0.09	0.34 ± 0.09	0.50 ± 0.07[c]	0.50 ± 0.06[c]
Vitamin E (μmol/g tissue)	6.72 ± 0.67	6.17 ± 0.71	7.62 ± 1.80[b]	7.65 ± 0.99[c]

[a] $p < 0.05$ and [b] $p < 0.01$ versus control group

[c] $p < 0.05$, [d] $p < 0.01$ and [e] $p < 0.001$ versus OVX group

antioxidants GSH and GSH-Px and a decrease of the tissues lipid peroxidation and serum lipid profiles.

Lipid peroxidation has been implicated in the pathogenesis of OVX-induced oxidative injury due to menopause-induced estrogen deficiency, which results in cell membrane damage. Sexual hormones play an important role in the progression of liver diseases. The lipid peroxidation level was higher in the OVX mice, revealing a deficient antioxidant defense system. This observation confirms previous studies showing that OVX led to an increase of lipid peroxidation and decrease of GSH-Px activity and antioxidant vitamin levels (Poli et al. 1993; Nazıroğlu et al. 2011a, b; Nazıroğlu et al. 2004a, b; Dilek et al. 2010; Lucas et al. 2006).

It is well known that menopause and removal of ovaries induce kidney and liver oxidative injury (Ulaş and Cay 2011; Barp et al. 2012). Additionally, the present study was also designed to explore the protective effects of ACV as free radical scavenger on OVX-induced oxidative damage to erythrocyte, liver, and kidney tissues. Administration of ACV improved the antioxidant defense system and decreased lipid peroxidation in erythrocytes, kidney, and liver.

Flavonoids and phenolic agents occur widely in many plants. Recently, the role of phenolic compound and flavonoids in the prevention of menopause-induced oxidative stress and disease has gained great interest (Yang et al. 2010; Nazıroğlu et al. 2011a, b; Nikolić et al. 2012). Apple cider vinegar contains high concentrations of polyphenols and flavonoids (Yang et al. 2010; Budak et al. 2011; Denis et al. 2013), which explains its antioxidant properties against oxidative damage to erythrocytes and tissues.

In mammalians, the combined actions of various cellular antioxidants are critical for the effective detoxification of free oxygen radicals. Among the cellular antioxidant enzymes, GSH-Px has been extensively studied. It converts hydrogen peroxide to water by using GSH as substrate (Kovacic and Somanathan 2008 Nazıroğlu 2009). Ovariectomized mice showed decreased antioxidant capacity in kidney and erythrocytes, as evidenced by decreased activity of the antioxidant enzymes, which is in agreement with earlier apple and apple juice reports (Poli 1993; Nazıroğlu et al. 2004a, b; Avci et al. 2007; Dilek et al. 2010; Nazıroğlu et al. 2011a, b). Treatment with ACV prevented the reduction of the antioxidant enzyme activity and subsequent oxidative injury to erythrocytes and kidney (Avci et al. 2007; Ulaş and Cay 2011; Yuan et al. 2011).

Vitamin C has been shown to be an important antioxidant, to regenerate vitamin E through redox cycling, and to raise intracellular GSH levels (Frei et al. 1989). As such, vitamin C plays an important role in protein -SH group protection against oxidation. High concentrations of vitamin C have are found in ACV (Denis et al. 2013). The GSH-Px activity, vitamin C, vitamin E, and β-carotene concentrations in kidney, and GSH-Px and GSH level in erythrocytes increased in the ACV-treated groups. These observed increases indicate an important role of ACV in normalizing GSH-Px and antioxidant vitamin concentration in OVX mice.

The vitamin E and total cholesterol concentrations in liver were also decreased in the ACV- and OVX + ACV groups. Vitamin E is an important antioxidant in biological systems (Nazıroğlu 2007). Vitamin E promotes homeostasis in living cells by a mechanism of incorporation into cell membranes or by entering the cells. Liver possesses an α-tocopherol-binding protein specific for vitamin E, facilitating its incorporation into cells and subsequent transfer from liver to cells through cholesterols (Hacquebard and Carpentier 2005; Traber 2007). The property of tocopherol that appears to be related to most manifestations of deficiency is its inhibitory effect on the auto-oxidation of unsaturated fatty acids.

Estrogen deficiency increases generation of free oxygen radicals, which induces oxidative stress and results in cell damage or death (Budak et al. 2011). Beneficial effects of estrogen in liver and blood include reducing cholesterol through enhanced LDL receptor binding and clearance (Noh et al. 1999; Sanchez-Rodriguez et al. 2011). Additionally, by reducing hepatic lipase activity, treatment with this hormone promotes the formation of larger and less atherogenic LDL cholesterol particles (Noh et al. 1999; Nazıroğlu et al. 2004a, b).

In the present study, we were not able to measure all serum lipid values such as HDL and LDL values in mice due to limited amounts of serum. However, we observed that OVX-induced changes of triglycerides, total and VLDL cholesterol are modulated by ACV supplementation. Similarly, Shishehbor et al. reported that ACV improved the serum lipid profile in normal and diabetic rats by decreasing serum triglycerides, LDL and increasing serum HDL-cholesterol (Shishehbor et al. 2008). It was also recently reported that ACV decreased triglyceride and VLDL levels in rats fed high cholesterol when compared to animals on high-cholesterol diets without ACV supplementation (Budak et al. 2011). In agreement with these observations, total cholesterol and triglycerides were lower in the ACV-treated mice fed high cholesterol.

In conclusion, the results presented in this study suggest that ovariectomy is associated with an increase of serum triglycerides, total and VLDL cholesterol and with erythrocyte, liver, and kidney lipid peroxidation and reduction of some antioxidants in tissues and cells. The administration of apple cider vinegar possesses a protective effect against ovariectomy-induced blood, liver, and kidney oxidative injury in mice fed high cholesterol and that such protection may be due to free oxygen radical scavenging effects, reduced lipids and lipid peroxidation, and increased antioxidant enzyme and vitamin levels. The results in blood, liver, and kidney may be of help to physicians and nutritionists in the use of apple cider vinegar as supplement in the treatment of oxidative stress-induced toxicity.

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Conflict of interest The authors declare that there are no conflicts of interest in the current study.

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