

# Apple cider vinegar supplementation modulates lipid peroxidation and glutathione peroxidase values in lens of ovariectomized mice

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## List of abbreviations

GSH, glutathione  
GSH-Px, Glutathione peroxidase  
ROS; reactive oxygen species  
OVX, ovariectomized  
i.p., intraperitoneal

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## Abstract

Epidemiological studies reported that increased risk of cataracts and oxidative stress in postmenopausal women although aetiology of the mechanisms has not been clarified. Apple cider vinegar may useful treatment of ovariectomize (OVX)-induced oxidative lens injury via its antioxidant properties. We aimed to investigate effects of apple cider vinegar on lipid peroxidation, glutathione peroxidase (GSH-Px) and reduced glutathione (GSH) values in OVX mice fed high cholesterol.

Thirty-two mice were used in the study and they were divided into four equal groups. First group was used as control. Second group was used OVX. Apple vinegar (0.6% of feed) was intragastrically giving to consisting apple vinegar and OVX+apple vinegar mice for 28 days. All groups except control group were fed cholesterol rich ration (5% cholesterol). After 28 days, lens samples are taken to study lipid profile and antioxidant values.

Lipid peroxidation levels in the lens were higher in OVX group than in control whereas their levels were lower in apple vinegar and OVX+apple vinegar groups than in OVX group. The GSH-Px activities were lower in OVX group than in control although their activities were higher in apple vinegar and OVX+apple vinegar groups than in OVX group. The GSH levels did not change by OVX and apple vinegar administrations.

In conclusion, apple cider vinegar decreased in lens oxidative injury by modulating GSH-Px in mice fed with high cholesterol.

## Keywords

Antioxidant; Glutathione peroxidase; Oxidative stress; Ovariectomize; Lens.

## Introduction

Reactive oxygen species (ROS) are continuously generated in physiological conditions and are effectively controlled/eliminated by intracellular and extracellular antioxidant systems such as the enzymes glutathione peroxidase (GSH-Px) and catalase as well as the low molecular weight reductants such as vitamin E and glutathione (GSH) (Kovacic and Somanathan, 2008; Nazıroğlu, 2012). Oxidative stress has been defined as an imbalance between increased ROS production and inadequate antioxidant defense, a particular state characterized by an overload in oxidants, which may culminate in cellular dysfunction. Oxidative stress causes profound lens injury to a number of intracellular macromolecules such as DNA and proteins in eye (Kovacic and Somanathan, 2008). The GSH-Px catalyzes the reduction of hydrogen peroxide to water (Nazıroğlu, 2009). The GSH-Px can also remove organic hydroperoxides. The GSH is regulator peptide in the maintenance of oxidant homeostasis and the cellular detoxification of ROS in tissues including lens and cornea (Ganea and Harding, 2006). Oxidative stress is widely recognized as one of the major causes of senile cataract. Elevated level of oxidative stress in lenses has been associated with induction of cataract (Nazıroğlu et al. 2004; Simşek et al. 2005).

The menopause transition is a period marked by significant physical and psychological changes associated with cessation of sex hormone secretion. It has been suggested that the increased risk of cataracts for women is due to the reduction in estrogen levels after menopause (Brinton, 2008; Gajjar et al. 2009; Ozcura et al. 2010). The antioxidant role of oestrogen has been attributed to the phenolic ring, which binds to oxidants such as hydrogen peroxide (Kumar et al. 2010). Although the exact mechanism of oestrogen role against oxidative stress is not known oestrogen modulated oxidative stress through supporting antioxidant mechanisms in lens of human (Prokai-Tatrai et al. 2008). However, it is not clear how oestrogen modulates the antioxidative defence mechanisms in response to the oxidative insult, and the precise of oestrogen and antioxidant in the visual system is not known (Gajjar et al. 2009).

Apple cider vinegar is widely used in salad and foods. The main component of vinegar is acetic acid, being present at concentration of 3-5% (Sakakibara et al. 2006). Other constituents of vinegar include polyphenolic compounds, some vitamins, minerals, mineral salt, amino acids and organic acids (Budak et al. 2011; Denis et al. 2013). Hence, the apple vinegar may modulate

OVX-induced oxidative stress in lens. However, scientific database about apple vinegar on antioxidant systems is limited. In recently studies acetic acid has been reported could be beneficial on oxidative stress in lens of diabetic rats (Juskova et al. 2011; Zhang et al. 2011). Hence, apple cider vinegar may modulate oxidative injury in lens of OVX mice.

In the current study, we investigated the role of menopause on lens oxidative stress using OVX mice fed high cholesterol. The ability of apple cider vinegar to modulate the adverse effects of oxidative stress by inhibiting the lipid peroxidation is also elucidated in the current study.

## Material and methods

### Animals

Thirty-two female Swiss Mouse weighing 36-40 g were used for the experimental procedures. Sixteen of them remained intact as control and vinegar groups, and the other of 16 had been ovariectomized for conducting OVX and OVX+vinegar. Animals were housed in individual plastic cages with bedding. Standard mice food and tap water were available ad libitum for the duration of the experiments unless otherwise noted. The temperature was maintained at  $22 \pm 2$  °C. A 12/12 h light/dark cycle was maintained, unless otherwise noted. Experimental protocol of the study was approved by a local ethical committee of the Medical Faculty of Suleyman Demirel University (SDU) by protocol number: (Protocol Number; 2009: 27-08).

### Experimental Design

Thirty-two female Swiss mice were randomly divided into four equal groups as follows:

Group I (n=8). Placebo (physiological saline) were intragastrically given to this group for 28 days.

Group II (n=8). The OVX was induced in the mice

Group III (n=8). Apple Cider Vinegar (0.6% of feed) was intragastrically given to this group for 28 days.

Group IV (n=8), Apple Cider Vinegar (0.6% of feed) was intragastrically given to this group for 28 days after OVX.

All rats were fed by fed cholesterol rich ration (5% cholesterol) for 28 days. In mice, hypercholesterolemia was induced by gavage by daily administration of 1 ml/100 g body weight of a cocktail containing in 1 liter peanut oil: 100 g cholesterol, 30 g propylthiouracil and 100 g cholic acid over a period of 28 days (Vogel and Vogel, 1997). Apple cider vinegar was taken from Agricultural Faculty of Suleyman Demirel University and it was diluted

with water and it was given to the animals 0.6% of diets (Shishehbor et al. 2006).

After 12 hours of last apple vinegar dose administration all mice were sacrificed and lens samples were taken.

### Animal model of menopause: Ovariectomy

Mice were anesthetized with a cocktail of ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg) were administered i.p. before ovariectomization. Antibiotic was administered for prevent of infection. Twenty of 40 anesthetised mice ovariectomized as described in our previous study (Dilek et al. 2010; Naziroğlu et al. 2011).

Anesthesia, preparation of tissue and blood samples

The lens samples were washed twice with cold saline solution, placed into glass bottles, labelled and stored in a deep freeze (-33 °C) until processing. After weighing all tissue, they were placed on ice, cut into small pieces, using scissors, and homogenized (2 minutes at 5000 rpm) in five volumes (1:5, w/v) of ice-cold Tris-HCl buffer (50 mM, pH 7.4), by using a ultrasonic homogenizer. All preparation procedures were performed on ice. The homogenate was used for determination of LP and antioxidant levels. The lens homogenate was used for immediate determination of LP levels and enzyme activities. Antioxidant analyses were performed within 3 months.

### Lens lipid peroxidation determinations

LP levels in the lens homogenate samples were measured with the thiobarbituric-acid reaction by the method of Placer et al. (1966). The values of LP in the lens were expressed as  $\mu\text{mol}/\text{gram}$  protein. Although the method is not specific for LP, measurement of thiobarbituric-acid reaction is an easy and reliable method, which is used as an indicator of LP and ROS activity in biological samples.

Lens reduced glutathione (GSH), glutathione peroxidase (GSH-Px) and protein assay

The GSH content of the erythrocytes was measured at 412 nm using the method of Sedlak and Lindsay (1968). GSH-Px activities of erythrocytes were measured spectrophotometrically at 37°C and 412 nm according to the Lawrence and Burk (1976) method. The protein content in the erythrocyte was measured by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

### Statistical analysis

All results were expressed as means  $\pm$  SD. Significant values in the four groups were assessed with an unpaired Mann-Whitney U test. Data were analyzed using the SPSS statistical program (version 17.0 software, SPSS Inc. Chicago, Illinois, USA). P-values of less than 0.05 were regarded as significant.

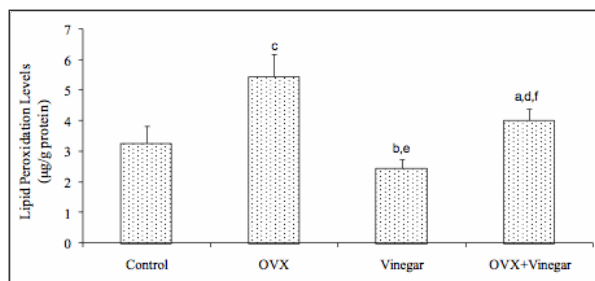
## Result

### Lipid peroxidation results

Lipid peroxidation levels in the lens are shown Figure 1. The lipid peroxidation levels as  $\mu\text{g}/\text{g}$  protein in control, OVX, vinegar and OVX+vinegar groups were 3.26, 5.45, 2.46 and 4.02, respectively. The lipid peroxidation levels were significantly ( $p < 0.001$ ) higher in OVX group than in control group. However, vinegar supplementation decreased the lipid peroxidation levels in the lens samples and the lipid peroxidation levels were significantly lower in vinegar ( $p < 0.001$ ) and OVX+vinegar ( $p < 0.01$ ) groups than in OVX groups.

### GSH levels

The GSH levels in the lens are shown in Figure 2. The GSH levels as  $\mu\text{g}/\text{g}$  protein in control, OVX, vinegar and OVX+vinegar groups were 2.81, 3.54, 3.05 and 2.09, respectively. The GSH levels did not differ in the four groups.

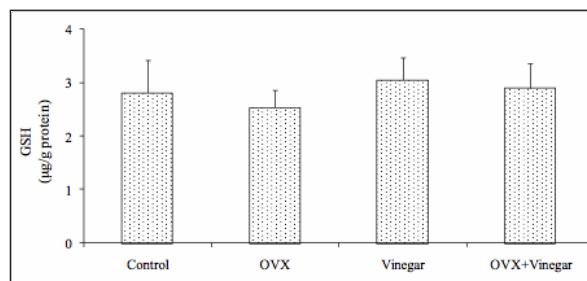


**Figure 1.** Effects of apple vinegar on lipid peroxidation levels in lens of ovariectomized (OVX) mice. (mean $\pm$ SD and n=8).

<sup>a</sup> $p < 0.05$ , <sup>b</sup> $p < 0.01$  and <sup>c</sup> $p < 0.001$  versus group control.

<sup>d</sup> $p < 0.01$  and <sup>e</sup> $p < 0.001$  versus OVX group.

<sup>f</sup> $p < 0.001$  versus vinegar group.



**Figure 2.** Effects of apple vinegar on glutathione (GSH) levels in lens of ovariectomized (OVX) mice. (mean $\pm$ SD and n=8).

### GSH-Px activities

The GSH-Px activities in the lens samples are shown in Figure 3. The GSH-Px levels as IU/g protein in control, OVX, vinegar and OVX+vinegar groups were 12.4, 8.39, 10.80 and 9.48, respectively. The GSH-Px activity was significantly ( $p < 0.01$ ) lower in OVX group than in control group. However, the GSH-Px activity were higher in vinegar ( $p < 0.01$ ) and OVX+vinegar ( $p < 0.05$ ) groups than in OVX groups.

### Discussion

The evidences of the eye as a target organ for gender-specific hormones has been well documented (Leske et al. 2004). The protective effects of estrogens in induction of senile cataracts through over production of ROS have been well documented in human and animal experiments (Simşek et al. 2005; Wagner et al. 2008; Ozcura et al. 2010). Apple cider vinegar contains high amount of antioxidants such as acetic acid and flavonoids. Hence, the apple cider vinegar and acetic acid induced protective role against diabetes-induced oxidative stress in lens animal experiments (Sakakibara et al. 2006; Shishehbor et al. 2008; Juskova et al. 2011; Zhang et al. 2011). This study evaluated protective role of apple cider vinegar on oxidative stress in lens of OVX mice.

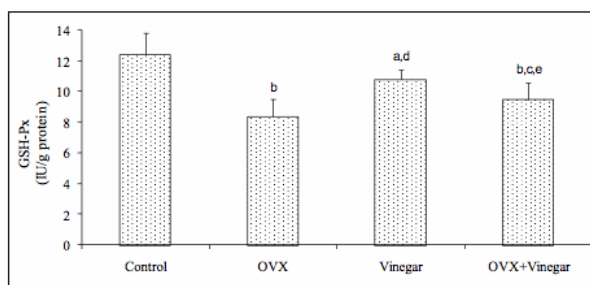
Cataract is a worldwide leading cause blindness and is a multi-factorial eye disease. Oxidative damage resulting from ROS is considered to be a major risk factor in the pathogenesis of cataract (Kovacic and Somanathan, 2008). Elevated levels of oxidative stress have been reported in the aqueous humour of cataract patients, and ROS have been implicated in the cataract formation (Fulgêncio Cunha et al. 2013). Mitochondria in lens are especially sensitive to oxidative stress and the ROS induces oxidative stress production through mitochondrial membrane depolarization (Babizhayev, 2011; Nahomi et al.

2013). It has been reported that oestrogen hormone can protect human lens against oxidative stress by preserving mitochondrial function (Gajjar et al. 2009; Kumar et al. 2010). The lens is equipped with antioxidant enzymes, which can prevent the toxic effects of ROS. Superoxide anion is dismutated by the enzyme, superoxide dismutase, to yield hydrogen peroxide, which is then converted to water by GSH-Px and catalase (Nazıroğlu, 2012). In the current study, we observed that lipid peroxidation levels were higher in OVX group than in control although GSH-Px activities were lower in OVX group than in control. Thus, the lens GSH-Px activities in the OVX rats may be decreased as a result of their action in inhibiting free radicals.

Moreover, the present study was also designed to explore the protective effects of a free radical scavenger, apple vinegar on the OVX-induced oxidative lens injury. In the current study, lipid peroxidation levels OVX-induced lipid peroxidation was decreased by apple cider vinegar supplementations. Flavonoids and phenolic acids have been reported to prevent different eye diseases, at least in part, by inhibiting over production of ROS and apple cider vinegar contains high concentrations of flavonoids (Jia et al. 2011; Rooban et al. 2012). All the data reported above help us to discuss a possible antioxidant role played by apple cider vinegar against OVX-induced oxidative lens and cataract formation. Hence, the OVX-induced ROS production was modulated by via the flavonoids and phenolic acids contents of apple cider vinegar.

The antioxidant enzyme system inherent in the cellular defense system is the most important defense mechanism against ROS. GSH and GSH-Px act as antioxidants, and have preventive effect against extensive production of ROS by OVX and menopause induction. To our knowledge there are no papers on GSH-Px and GSH values in lens of menopausal women and animal. There are some reports on the values in different tissues of menopausal women and animal but results of the studies are conflicting. Turgut et al. (2013) reported that GSH levels were increased in rats by OVX induction although GSH-Px enzymatic activity) did not change. Shafin et al. (2012 reported increase of GSH-Px activity in blood of postmenopausal women. Aksakal et al. (2012) reported decrease of GSH-Px activity in heart of OVX rats. In the results of current study, the lens GSH values (insignificantly) and GSH-Px activities (significantly) in OVX group are decreased by OVX induction and it indicated decrease of the antioxidant through over production of ROS during OVX.

In conclusion, we observed that OVX is associated with increase in lipid peroxidation and reduction of GSH-



**Figure 1.** Effects of apple vinegar on glutathione peroxidase (GSH-Px) activities in lens of ovariectomized (OVX) mice. (mean±SD and n=8).

<sup>a</sup> $p < 0.05$  and <sup>b</sup> $p < 0.01$  versus group control.

<sup>c</sup> $p < 0.05$  and <sup>d</sup> $p < 0.01$  versus OVX group.

<sup>e</sup> $p < 0.05$  versus vinegar group.

Px activity in the lens. The administration of apple cider vinegar to the OVX mice fed high cholesterol is capable of reducing the lipid peroxidation values in lens samples.

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MN formulated the present hypothesis and was responsible for writing the report. MG, MK, İSÖ and CÖ was responsible for analysis of the data. The authors confirm that there is no conflict of interest to declare in the manuscript.

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► Oxidative stress, ovariectomize, hypercholesterolemia

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