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AIM AND SCOPES

Cell Membranes and Free Radical Research is a print and online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate cellular function, and the control or alteration of these processes by the action of receptors, neurotransmitters, second messengers, cation, anions, drugs or disease.

The journal has been publishing three volumes in a year.

Areas of particular interest are four topics. They are;

A- Ion Channels (Na^+ - K^+ Channels, Cl^- channels, Ca^{2+} channels, ADP-Ribose and metabolism of NAD^+, Patch-Clamp applications)

B- Oxidative Stress (Antioxidant vitamins, antioxidant enzymes, metabolism of nitric oxide, oxidative stress, biophysics, biochemistry and physiology of free oxygen radicals

C- Interaction Between Oxidative Stress and Ion Channels

(Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and NAD^+ on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels)

D- Gene and Oxidative Stress (Gene abnormalities. Interaction between gene and free radicals. Gene anomalies and iron. Role of radiation and cancer on gene polymorphism)

READERSHIP

Biophysics
Biochemistry
Biology
Biomedical Engineering
Pharmacology
Physiology
Genetics
Cardiology
Neurology
Oncology
Psychiatry
Neuroscience

Keywords

Ion channels, cell biochemistry, biophysics, calcium signaling, cellular function, cellular physiology, metabolism, apoptosis, lipid peroxidation, nitric oxide synthase, ageing, antioxidants, neuropathy.

Capparis ovata modulates ovariectomize induced-oxidative toxicity in brain, kidney and liver of aged mice

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List of abbreviations

C. ovata, Capparis ovata
GSH, reduced glutathione
GSH-Px, glutathione peroxidase
MDA, malondialdehyde
PUFAs, polyunsaturated fatty acids
ROS, reactive oxygen species

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Abstract

Oxidative stress is a critical way of damage in various physiological stress-induced disorders including age and menopause. *Capparis ovata (C. ovata)* may useful treatment of ovariectomize in old women because it contains flavonoids which demonstrated as an antioxidant. The study in overiectomized mice, as a model of menopausal status, of the effects of *C. ovata* on lipid peroxidation (LP) and antioxidant levels in aged mice.

Forty female aged mice (12 months old) were randomly divided into four groups. First group was used as control although second group was *C. ovata* group. Third group was ovariectomized group. *C. ovata* was given to ovariectomize-induced mice constituting the fourth groups for 28 days via gastric gavage. End of the experiment, brain cortex, kidney and liver samples were taken from all groups.

The LP levels in the brain cortex, kidney and liver in ovariectomized group were higher than in control and *C. ovata* groups whereas they were decreased by *C. ovata* administration. Vitamin E concentrations in brain cortex and liver, β-carotene concentration in liver were decreased by the ovariectomize exposure although they were increased in the ovariectomized group by the *C. ovata* administration. The vitamin A, vitamin C, glutathione peroxidase and glutathione values in the brain cortex, liver and kidney did not change in the four groups by ovariectomize or *C. ovata* treatments.

In conclusion, the experimental ovariectomize is associated with elevated oxidative stress although treatment with the *C. ovata* induced protective effects on the oxidative stress in the aged ovariectomized mice.

Keywords

C. ovata; menopause; antioxidant; oxidative stress; vitamin E.

Introduction

Oxidative stress is defined as an imbalance between higher cellular levels of reactive oxygen species (ROS) e.g. superoxide and hydroxyl radicals and cellular antioxidant defense (Kovacic and Somanathan 2008). Generation of ROS is ubiquitous since ROS are generated during aerobic metabolism i.e., mitochondrial and aged protein oxidations. Kidney (Ozkaya et al., 2011, Ulas and Cay 2011), liver (Kireev et al., 2010, Unal et al., 2011) and the brain (Shrilatha and Muralidhara 2007, Roriz-Filho et al., 2009) may be vulnerable to oxidative stress induced by aging and menopause and become exposed to ROS continuously generated via the auto-oxidation of polyunsaturated fatty acids (PUFAs). Because of their high rate of oxygen consumption, high content of PUFAs and poor enzymatic antioxidant defence, the brain exhibit increased vulnerability to aging and menopause-induced oxidative stress (Roriz-Filho et al., 2009). Lipid peroxidation causes injury to cells and intracellular membranes and may lead to cell destruction and subsequently cell death defense (Kovacic and Somanathan 2008, Baquer et al., 2009). In order to scavenge ROS, various defense systems namely enzymatic and non enzymatic antioxidants exist in the brain cortex, kidney and liver.

The free radical theory of aging states that age related degenerative processes are to a large extent the consequence of damage induced by ROS (Ames 2010). The rate of ROS production per time unit increases with age. A growing body of evidence now suggests that aging involves, in addition, progressive changes in ROS mediated regulatory processes, resulting in altered gene expression (Hayashi et al., 2012). Mitochondria appear to be the major source of the oxidative lesions that accumulate with age and these lesions have been proposed as the major cause of cellular aging and death (Roriz-Filho et al., 2009). Aging shows a pro-oxidative shift in the systemic thiol/disulfide redox state, similar to the shift seen in old age (Uzun et al., 2010). These mitochondrial oxidative stress conditions are typically associated with tissue degeneration (Espino et al., 2011).

Capparis ovata (C. ovata) belongs to the Capparidaceae Family. This green spiny shrub distributes throughout the Mediterranean basin and grows wildly in Turkey. It has been known as a traditional herbal medicine for its diuretic, antihypertensive and tonic properties for centuries (Tlili et al., 2011). Previous chemical studies have reported that they have alkaloids, lipids, polyphenols, flavonoids, and glucosinolates (Conforti et al., 2011). Furthermore, *C. Spinosa* extract was reported to be rich in antioxidants such as α-tocopherol, γ-tocopherol and sitosterol (Matthäus and Ozcan 2005) as well as flavonoids such as kaempferol, rutin, quercetin, and quercetin derivatives (Tlili et al., 2010, Conforti et al., 2011). Recently it was shown that extracts of *C. ovata* have been showed antinociceptive effects in mice (Arslan and Bektaş 2010). The methanol extract of capparis species including *C. ovata* showed a noteworthy antioxidant/free radical scavenging effectiveness in various in vitro models (Matthäus and Ozcan 2005, Tlili et al., 2010, Yang et al., 2010) and this extract has been suggested to treat oxidative stress-based pathological diseases.

In women with normal reproductive function the oestrogenic compounds are secreted as oestrogen in great quantity mainly by ovaries. Oestrogen exerts diverse non reproductive actions on multiple organs, including brain (Abbas and Elsamanoudy 2011). Estrogen deprivation and oxidative stress have been well established as two main factors closely related to the pathological development of neurological disease such as Alzheimer's disease (Hua et al., 2007). The Women's Health Initiative Study reported that hormone replacement does not improve and may actually impair cognitive function in postmenopausal women (Shumaker et al., 2003). *C. Ovata* instead of hormone replacement therapy may improve oxidative stress induced-cognitive function brain function in postmenopausal women.

It has not been studied whether *C. Ovata* in ovariectomized mice modifies alterations in the antioxidant enzyme system and lipid peroxidation of brain cortex, kidney and liver. We aimed to evaluate the effects of *C. Ovata* on oxidative stress and enzymatic antioxidants in ovariectomized- mice menopause model.

Materials and methods

Animals

Forty female Mus musculus mice weighing 35±5 g were used for the experimental procedures. Mice were allowed 1 week to acclimatize to the surroundings before beginning any experimentation. 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. The temperature was maintained at 22±2 ºC. A 12/12 h light/dark cycle was maintained, with lights on at 06.00, unless otherwise noted. Experimental protocol of the study was approved by the ethical committee of the Medical Faculty of Suleyman Demirel University (Protocol Number; 06.07.2010-05). Animals were maintained and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory animals prepared by the Suleyman Demirel University.

Preparation C. Ovata extracts

C. ovata samples were collected around of Isparta, Mediterranean region of Turkey. Ethanol extracts of flowers were prepared by Forestry Industry Engineer Department of Suleyman Demirel University, Isparta, Turkey and they gift to the study. Content of *C. ovata* was analyzed by HPLC in central laboratory of Suleyman Demirel University Phenolic and total flavonoid contents of *C. Ovata* in ethanol were 34,4 and 553,5 mg/ml, respectively.

Experimental groups

Experimental Design four groups as follows:

Control group (n=10): Placebo (physiologic saline) was supplemented to the first group via gastric gavage.

Ovariectomized group (n=10): Animals in the group were ovariectomized and placebo (physiologic saline) was supplemented to the group via gastric gavage (Dilek et al., 2010).

C. ovata group (n=10): Animals in the group received given *C. Ovata* (100 mg/kg/day) for 28 consecutive days via gastric gavage (Ghule et al., 2007, Arslan and Bektas 2010).

Ovariectomize+ *C. ovata* group (n=10): Animals in the group were ovariectomized and then *C. ovata* (100 mg/kg/day) was given to these animals for 28 consecutive days via gastric gavage.

After 12 hours of last *C. ovata* dose administration all mice were sacrificed and kidney, liver and brain samples were taken.

Anesthesia and preparation of tissue samples

Mice were anesthetized with a cocktail of ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg) administered i.p. before sacrifice of each mice.

The brain was also taken as follows; the cortex was dissected out after the brain was split in the mid-sagittal plane. Following removal of the cortex, brain cortex kidney and liver were dissected from total brain as described in our previous study (Nazıroğlu et al., 2008).

Kidney, liver and brain cortex tissues were washed twice with cold saline solution, placed into glass bottles, labeled and stored in a deep freeze (-33 °C) until processing (maximum 10 hours). After weighing, half of the cortex was placed on ice, cut into small pieces using scissors, and homogenized (2 minutes at 5000 rpm) in 5 volumes (1:5, w/v) of ice-cold Tris-HCl buffer (50 mM, pH 7.4), by using a ultrasonic homogenizer (Bandelin electronic GmbH & Co. Berlin, Germany). All preparation procedures were performed on ice. The homogenate was used for determination of LP and antioxidant levels.

After addition of butylhydroxytoluol (4 μl per ml), kidney, liver and brain cortex homogenate were used for immediate lipid peroxidation levels and enzyme activities. Antioxidant vitamin analyses were performed within 3 months.

Lipid peroxidation determinations

Lipid peroxidation levels in the kidney, liver and brain homogenate were measured with the thiobarbituric-acid reaction by the method of Placer et al (Placer et al., 1966). The values of lipid peroxidation in the kidney, liver and brain homogenate were expressed as μmol/g tissue, respectively.

Reduced glutathione (GSH), glutathione peroxidase (GSH-Px) and protein assay

The GSH content of the kidney, liver and brain homogenate was measured at 412 nm using the method of Sedlak and Lindsay (Sedlak and Lindsay 1968). Absorbances were measured at 412 nm. A standard curve of reduced glutathione was used to calculate GSH levels.

GSH-Px activities of the kidney, liver and brain homogenate were measured spectrophotometrically (UV-1800, Shimadzu, Kyoto, Japan) at 37 ºC and 412 nm according the Lawrence and Burk (Lawrence and Burk 1976). The protein contents in the tissue homogenates were measured by the method of Lowry et al (Lowry et al., 1951). with bovine serum albumin as the standard.

Tissue vitamins A, C and E and β- carotene analyses

Vitamins A (retinol) and E (α-tocopherol) were determined in the kidney, liver and brain samples by a modification of the method described by Desai (Desai 1984) and Suzuki and Katoh (Suziki and Katoh 1990). About 200 μg kidney, liver and brain samples were saponified by the addition of 0.3 ml of 60% (w/v in water) KOH and two ml of 1% (w/v in ethanol) ascorbic acid, followed by heating at 70°C for 30 min. After cooling the samples on ice, 2 ml of water and 1 ml of n-hexane were added and mixed with the samples that were then rested for 10 min to allow phase separation. An aliquot of 0.5 ml of n-hexane extract was taken and vitamin A levels were measured at 325 nm. Then reactants were added and the absorbance value of the hexane extract was measured in a spectrophotometer at 535 nm. Calibration was performed using standard solutions of all-trans retinol and α-tocopherol in hexane.

The levels of β-carotene in the tissue samples were determined by the method of Suzuki and Katoh (1990).

Two milliliters of hexane were mixed with 0.25 g tissue and absorbance of β- carotene in hexane was measured at 453 nm in the spectrophotometer.

Quantification of vitamin C (ascorbic acid) in the the kidney, liver and brain homogenate samples was performed according to the method of Jagota and Dani (Jagota and Dani 1982). The absorbance of the samples was measured spectrophotometrically at 760 nm.

Statistical analysis

All results were expressed as means ± standard deviation (SD). Significant values in four groups were assessed with Mann-Whitney U test. Data was analyzed using the SPSS statistical program (version 9.05 software, SPSS Inc. Chicago, Illinois, USA). P-values of less than 0.05 were regarded as significant.

Results

Lipid peroxidation levels

The mean brain cortex, kidney and liver lipid peroxidation values in the four groups are shown in Figures 1, 2 and 3, respectively. The results showed that the brain cortex, kidney and liver lipid peroxidation levels in the ovariectomize group were significantly ($p<0.001$) higher than in the control group. The *C. ovata* administration caused a decrease in the lipid peroxidation levels of brain cortex, kidney and liver ($p<0.05$) relative to the ovariectomize group.

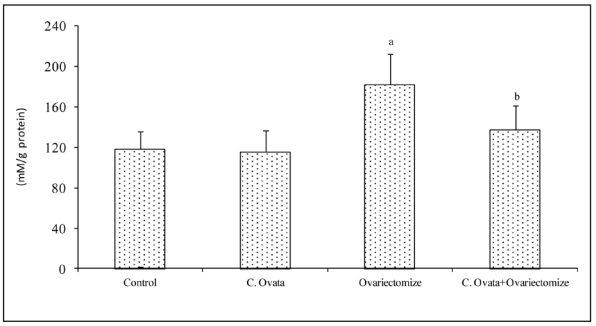

Figure 1. The effects of *C. ovata* on brain lipid peroxidation levels in ovariectomize mice (n=10 and mean ± SD). [a]p<0.001 and versus control group. [b]p<0.05 and versus ovariectomize group.

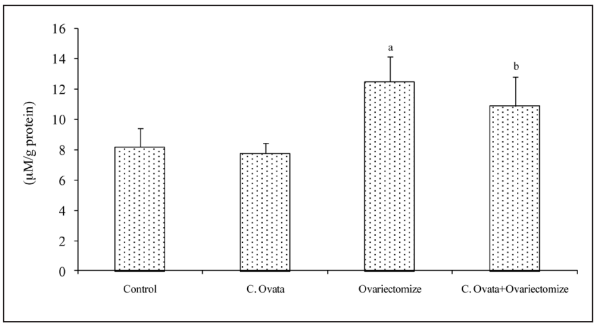

Figure 2. The effects of *C. ovata* on kidney lipid peroxidation levels in ovariectomize mice (n=10 and mean ± SD). [a]p<0.001 and versus control group. [b]p<0.05 and versus ovariectomize group.

GSH and GSH-Px values

The mean GSH levels and GSH-Px activities in the brain cortex, kidney and liver of the four groups are shown in Tables 1, 2 and 3, respectively. The results showed that there was no statistically significant difference in brain cortex , kidney and liver GSH-Px activity among the groups.

Antioxidant vitamin concentrations

The mean vitamin A, vitamin C, vitamin E and β-carotene concentrations in the brain cortex, kidney and liver of the four groups are shown in Tables 1, 2 and 3, respectively. Vitamin E concentrations were significantly ($p<0.05$) lower in ovariectomize group than in control. However, vitamin E concentrations in brain cortex and liver were significantly ($p<0.01$) higher in *C. ovata*+ovariectomize group than in ovariectomize group. The β-carotene concentrations in liver were also significantly ($p<0.05$) lower in ovariectomize group than in control although β-carotene concentrations increased significantly ($p<0.001$) in *C. ovata*+ovariectomize group.

Discussion

We found that lipid peroxidation levels in brain cortex, kidney and liver were increased by experimental menopause, whereas liver vitamin E and β-carotene concentrations and brain cortex vitamin E concentrations decreased. Hence, experimental menopause model in the animals is characterized by increased lipid peroxidation and decreased vitamin E and β-carotene concentrations. Administration of *C. ovata* caused a decrease lipid peroxidation level in brain cortex, kidney and liver although vitamin E in brain cortex and liver, and β-carotene concentrations in liver increased.

Steroid hormones, especially estriol and estradiol,

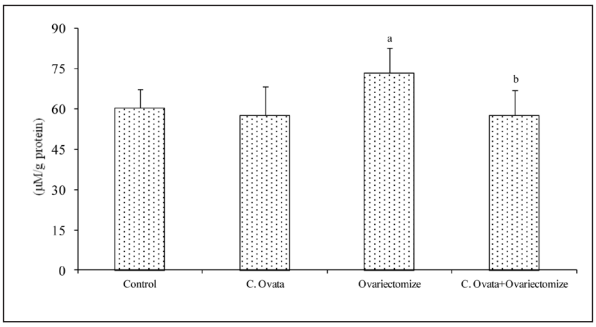

Figure 3. The effects of *C. ovata* on liver lipid peroxidation levels in ovariectomize mice (n=10 and mean ± SD). [a]p<0.001 and versus control group. [b]p<0.05 and versus ovariectomize group.

Table 1. The effects of *C. ovata* on glutathione peroxidase (GSH-Px), reduced glutathione (GSH) and antioxidant vitamin values in brain of ovariectomize mice (mean ± SD).

Parameters	Control (n=10)	Capari (n=10)	Ovariectomize (n=10)	Capari+Ovariectomize (n=10)
GSH (mmol/g protein)	10.79 ± 1.76	12.61 ± 1.38	11.26 ± 2.06	12.57 ± 1.76
GSH-Px (IU/g protein)	76.67 ± 12.74	75.22 ± 6.68	75.29 ± 7.92	71.88 ± 7.82
Vitamin A (mmol/g tissue)	2.70 ± 0.35	2.88 ± 0.36	2.62 ± 0.45	2.74 ± 0.29
Vitamin C (mmol/g tissue)	62.45 ± 12.27	79.49 ± 25.67	74.94 ± 24.65	62.45 ± 25.81
Vitamin E (mmol/g tissue)	12.80 ± 1.07	12.90 ± 1.72	10.10 ± 0.89[a]	11.20 ± 0.66[b]
β-carotene (mmol/g tissue)	1.31 ± 0.22	1.36 ± 0.21	1.27 ± 0.18	1.31 ± 0.20

[a]p<0.05 versus control group.
[b]p<0.05 versus ovariectomize groups.

Table 2. The effects of *C. ovata* on glutathione peroxidase (GSH-Px), reduced glutathione (GSH) and vitamin C values in kidney of ovariectomize mice (mean ± SD).

Parameters	Control (n=10)	Capari (n=10)	Ovariectomize (n=10)	Capari+Ovariectomize (n=10)
GSH (mmol/g protein)	9.12 ± 0.88	9.49 ± 0.86	8.97 ± 0.71	9.60 ± 0.67
GSH-Px (IU/g protein)	14.85 ± 1.95	13.94 ± 1.02	14.33 ± 1.83	14.91 ± 1.99
Vitamin C (mmol/g tissue)	124.9 ± 18.5	126.0 ± 11.1	134.0 ± 11.7	134.0 ± 32.0

[a]p<0.001 versus control group.
[b]p<0.05 versus ovariectomize groups.

Table 3. The effects of *C. ovata* on glutathione peroxidase (GSH-Px), reduced glutathione (GSH) and antioxidant vitamin values in liver of ovariectomize mice (mean ± SD).

Parameters	Control (n=10)	Capari (n=10)	Ovariectomize (n=10)	Capari+Ovariectomize (n=10)
GSH (mmol/g protein)	16.68 ± 1.70	14.53 ± 1.55	15.04 ± 1.82	14.21 ± 1.15
GSH-Px (IU/g protein)	20.37 ± 1.64	19.60 ± 1.93	19.07 ± 1.32	19.14 ± 1.24
Vitamin A (mmol/g tissue)	56.80 ± 0.18	56.90 ± 0.19	56.80 ± 0.18	56.90 ± 0.11
Vitamin C (mmol/g tissue)	155.6 ± 29.8	163.5 ± 35.6	140.8 ± 21.56	143.1 ± 27.4
Vitamin E (mmol/g tissue)	11.10 ± 1.41	11.50 ± 0.63	9.50 ± 0.85[b]	10.60 ± 0.60[c]
β-carotene (mmol/g tissue)	1.61 ± 0.22	1.61 ± 0.27	1.37 ± 0.28[a]	2.21 ± 0.43[d]

[a]p<0.05 and [b]p<0.01 versus control group.
[c]p<0.05 and [d]p<0.001 versus ovariectomize groups.

are natural antioxidants (Mooradian 1993). Kume-kick et al.(Kume-Kick et al., 1996) reported that all female brain areas increased ascorbate loss after gonadectomy,

indicating enhanced oxidative stress. Incubation of primary neuronal cultures with 17β-estradiol showed an increased survival of cells reducing lipid peroxidation (Vedder et al., 1999). Estrogen also exerts diverse nonreproductive actions on multiple organs, including the brain (Wise 2002). And it has been shown that estrogen deprivation is implicated in the pathogenesis of neurodegenerative conditions, such as Alzheimer's disease and cerebral ischemia (Shumaker et al., 2003). These reports provided evidence for the hypothesis that protection against oxidative damage is afforded by ovarian sex hormones. The current study indicated that overiectomization in rats produced an increase in lipid peroxidation levels of brain cortex, kidney and liver samples. Hence, *C. ovata* could be a good alternative to the synthetic estrogens in postmenopause. Our results are in accordance with previous reports of lipid peroxidation increment in brain, erythrocytes and plasma during overiectomization of animals or postmenopausal women (Kume-Kick et al., 1996, Nazıroğlu 2007, Dilek et al., 2010).

Oxidative stress is defined as an imbalance between higher cellular levels of ROS and cellular antioxidant defense systems (Nazıroğlu 2007). Lipid peroxidation levels as MDA is a major oxidative degradation product of membrane unsaturated fatty acid and has been shown to be biologically active with ROS properties (Placer et al., 1966). In the current study, ovariectomize-induced oxidative stress enhanced brain cortex lipid peroxidation levels in the animal system although fat soluble antioxidants (vitamin E, and β-carotene) concentrations decreased. Brain tissue is highly vulnerable ROS, because; (1) it generates very high levels of ROS due to its very high aerobic metabolism and blood perfusion, and it has a relatively poor enzymatic antioxidant defense; (2) it is enriched PUFAs that are preferentially susceptible to oxidative injury; (3) the damaged neuronal DNA in the adult brain cannot be effectively repaired since there is no DNA replication (Ilhan et al., 2005).

Vitamin E (α-tocopherol) has been considered a lipophilic antioxidant in humans and it is important for a normal liver and brain function (Nazıroğlu 2007). Thus vitamin E plays an important role against oxidation. It has been suggested that age-related estrogen loss results in a deficit of the antioxidant protection (Arteaga et al., 1998). Flavonoids are polyphenolic compounds with diverse bioactivities including anti-inflammatory and antioxidant (Conforti et al., 2011). Cappari species have rich alkaloids, lipids, polyphenols, flavonoids, and glucosinolates (Conforti et al., 2011). Furthermore, *Capparis* extracts ware reported to be rich in antioxidants including vitamin E (Matthäus and Ozcan 2005) and β-carotene (Goyal et al., 2009) as well as flavonoids (Confirti et al., 2011). The vitamin E concentrations in brain cortex and liver were higher in the *C. ovata* treatment group than in the ovariectomize group although lipid peroxidation levels were decreased by the supplementations. Taking into consideration the data given here, the observed increased concentrations of brain cortex and liver vitamin E and liver β-carotene in *C. ovata* treatment group indicates an essential role of *C. ovata* administration in normalizing antioxidant vitamin concentration in ovariectomized mice. Result of current study supports this hypothesis that ovariectomize-induced oxidative brain injury coincides with an enhanced oxidative stress in brain cortex. We may also speculate that the antioxidant potential of *C. ovata* is sole reason responsible for the ovariectomize-induced oxidative brain injury.

Conclusion

In conclusion, our blood and brain results in the ovariectomized group are consistent with a generalized antioxidant abnormality in different tissues of ovariectomized animals (Dilek et al., 2010). However, *C. ovata* supplementation prevented ovariectomized-induced oxidative injury through modulation of vitamin E and β-carotene concentrations. Therefore, use of it could be a potential approach in arresting or inhibiting the oxidative toxicity caused by ovariectomized and aging. These data are very encouraging, *C. ovata* may constitute a good alternative to a novel therapeutic strategy in ovariectomized and age mice.

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