The effect of Marjoram and Cocoa on oxidative stress caused by ovariectomy in rats

Marwa Ezz El-Din Ibrahim† & Tasneem Sobhy Fahmy*  

Abstract

It has known that postmenopausal life affects oxidative status for bodies. Ovariectomy in rats was the same effect on postmenopausal for several organs. In the present study, it can be hypothesized that dietary high in antioxidant such as marjoram and cocoa could protect against weakness and oxidative stress following ovariectomy in rats. Thirty-five female albino rats, ten 3-month old weighing (180 ± 10 g) were used. The first main group (n=7) was fed on the basal diet (+ve control). The second main group (n = 22) was subject to ovariectomy surgery to induced oxidative stress. Then rats were divided into 4 subgroups (7 rats each). Subgroup 1 was fed on basal diet (+ve control). Subgroup 2, 3 were fed on the basal diet and supplemented with dried marjoram, cocoa at the level of 10%, respectively for three month. Subgroup 4 were fed on the basal diet and supplemented with mixed (5% Marjoram+5% Cocoa) for three month. Oxidative status was evaluated by SOD, Catalase, GSH, and MDA in Liver, Heart and Kidney tissues. The marjoram-treated rats result showed significantly P<0.05 increased level of antioxidant enzymes in liver heart and kidney tissues compared to positive control. The results of antioxidant enzyme for kidney tissues showed significant increased for SOD, CAT and GSH for group treated cocoa 10% compared to positive control. Also, It could be notice that cocoa 10% group and group treated with mixed (cocoa 5% + marjoram 5%) showed significantly P<0.05 highest level of SOD, CAT and GSH in liver and
heart tissue. Also, the presented work showed MDA status decreased significantly in heart, liver, kidney tissues for all treated groups compared to (+ve control). Finally, consumption of coca and marjoram has a protective role against oxidative stress caused by ovariectomy surgery, it is suggested to use these components especially cocoa through postmenopausal life.

Key Words: antioxidant enzyme, Ovariectomized, postmenopausal, Super Oxide Dismutase, Catalase, Glutathione, malonyldialdehyde, rats.
Introduction

Oxidative stress has identified as an imbalance between oxidative and antioxidative status that increased reactive oxygen species production which initiates lipid peroxidation. Antioxidant protect system prevents molecular and cellular damage by reducing free radicals (Halliwell, 2007; Gunay et al., 2011; Morrone et al., 2005). The evaluate of antioxidant enzyme activities are useful indicator of the antioxidant status in most mammals (Serin et al., 2008; Halliwell, 2012; Kozlik et al., 2015; Tang et al., 2016). Many studies on the evaluation of oxidative/antioxidative status in women and female rodents after ovariectomy have been studied by (Kankofer et al., 2007; Serin et al., 2008; Gunay et al., 2011; Szczubial et al., 2015).

It has known that postmenopausal life affects oxidative status and causes metabolic diseases such as osteoporosis and cardiovascular diseases (Gurdol et al., 1997; Kankofer et al., 2007; Castelao et al., 2008; Yang et al., 2014; Tang et al., 2016). Similarly, ovariectomy in rats has long-term effects on several organs such as liver, intestines and myocardium due to deficiency of ovarian hormones, particularly estrogens following the surgery (Morrone et al., 2015; Tang et al., 2016; Barp et al., 2017; Gomez et al., 2007; Murphy, 2011). Estrogens have demonstrated to defense the liver and intestines from oxidative damage due to its antioxidative properties (Sener et al., 2008). Kim et
also postulated that estrogen deficiency may develop cytokine production in peripheral blood mononuclear cells and increase interleukin-6 (IL-6) concentrations associated with oxidative stress after menopause in women.

Cocoa bean is loaded with the polyphenols such as quercetin (including its glucoside), clovamide, deoxyclovamide and procyanidin, Epicatechin, (+)-catechin (Campos et al., 2018, Hammerstone et al., 1999). Research indicates that the flavonoids, a class of polyphenols, have antioxidant characteristics with potential health benefits. The specific antioxidants in chocolate (i.e., cocoa flavonoids) include catechin and epicatechin, which are single flavanol molecules structurally similar to the antioxidants found in grapes and tea (Raloff, 2009; Lodhi and Vadner, 2019). Cocoa can substantially increase a person’s energy level, since it contains two stimulating methylxanthines - a significant amount of theobromine and a small amount of caffeine (Keen 2001, Sorond et al., 2008). PEA (phenylethylamine) is a chemical found in cocoa/cacao beans which increases the activity of neurotransmitters (brain chemicals) in certain areas of the brain which control the ability to focus attention and stay alert (Lee et al., 2003, Crew et al., 2008). Cocoa also appears to have anti-aging and anti-inflammatory properties. Cocoa is a good source of the minerals magnesium, sulphur, calcium, iron, zinc, copper, potassium, and manganese; plus some of the B Vitamins. Cocoa enhanced clot prevention afforded by cocoa flavonols (Rein et al., 2000). consumption of cocoa and dark chocolate (DC) has protective effects against cardiovascular diseases, in particular improvement of vascular endothelium function and blood pressure (BP) (Voskoboinik et al., 2019, Allen et al., 2008, Mohan and Deepa, 2008, and Spadafranca et al., 2010).

Marjoram is one of the most popular culinary herbs in the world, which was grown in Egypt over 3,000 years ago and Egypt produces 90% of the world’s supply. It has also been prescribed in the form of a herbal tea (infusion) in folk medicine to treat different illness (Ramadan,
et al., 2014). Sweet marjoram leaves contain acids (carnosic, oleanolic and ursolic acids), cis-sabinene hydrate, flavonoids (diosmetin, luteolin and apigenin), hydrocarbons (P-cymene and c-terpinene), phenolic glycosides (arbutin, methyl arbutin, vitexin, orientin and thymonin), phenolic terpenoids (thymol and carvacrol), tannins, sitosterol and triacontane. Preliminary trials have suggested possible antioxidant properties of the sweet marjoram plant (Ramadan, et al., 2014, Vagi, et al., 2009 and Heo et al., 2004).

Majorana has been uses to treat wide range of infections. It could be related to extensive phytochemical, experimental and clinical investigations. Its active constituents include Monoterpene derivatives, terpenic esters, monoterpenol and sesquiterpenoids. Experimental studies have demonstrated its free radical scavenging, anti-acetyl cholinesterase, insecticidal, synergistic effects, apoptotic, anti-proliferative activity, anti-mutagenic, genotoxic potential, antimicrobial and anti-ulcer activity and it has calming effect on anxiety and depressant activities. As a from all the studies, that researchers done and concluded that Marjoram have be used as functional food for humans by combine with unit operations of food processing for treatment of various ailments. Since herb possesses more than one health beneficial property and there is also a possibility of synergy among them in their action, a herb diet is likely to make life not only more “spicy” but more healthy also (Saxena, et al., 2016).

The aim of this study, it can be hypothesized that dietary high in antioxidant such as marjoram and cocoa could protect against weakness and oxidative stress following ovariectomy. Evaluate the changes in oxidative status markes in liver, heart and kidney tissues in rats by measuring their antioxidant enzymes in tissues.

Material and Methods
Rats and Diet:

Female albino rats of Sprague Dawley strain weighing $180 \pm 10$ g were purchased from the Laboratory Animal Colony, Ministry of Health and Population, Helwan, Egypt. Basal diet constituents were obtained from El-Gomhorya Company, Cairo, Egypt.

Chemicals and fed ingredient

Antioxidant status for liver, kidney and heart kits were purchased from Sigma-Aldrich Co. (St. Louis, Missouri, USA). Cocoa powder was purchased from local market. Also, Marjoram purchased from local market, then powdered to mixed with diet.

Methods:

Preparation of basal diet:
The basal diet (AIN-93M) was prepared according to Reeves et al., 1993. Diet was formulated to meet the recommended nutrients levels for rats.

Induction of ovariectomy in female rats:

Ovariectomy is to be the procedure that gives reliable model of postmenopausal life due to deficiency of ovarian hormones (Morrone et al., 2005). The method was done according to (Lasota and Klonowski, 2004) briefly, Ten $\varpi$-month old female rats were made operation after placing an animal on its ventral surface. Ovariectomy was preceded by a midline dorsal skin incision, $7$ cm long, approximately half way between the middle of the back and the base of the tail. Incisions of the muscles were made bilaterally. After peritoneal cavity was accessed, the ovary was found, surrounded by a variable amount of fat. Ligation of the blood vessels was necessary. The connection between the Fallopian tube and the uterine horn was cut and the ovary moved out. Because of muscle bleeding, its incision required suturing. Three single catgut stitches were placed on the skin.

Experimental animal design

Thirty-five female Ten $\varpi$-month old albino rats were housed in well aerated cages under hygienic conditions and were fed on basal diet
for one week for adaptation. All diets were formulated to cover the nutrient requirements of rats following the recommendations of the American Institute of Nutrition (AIN-93M) (Reeves et al., 1993). After this week the ovariectomy operation were done for all groups rats except negative control group. Rats were divided into five groups of seven animals each as follows:

**Group 1:** (N = 7) fed on Ain-93M and used as a negative control (Negative control).

**Group 2:** Fed on Ain-93M, induction of ovariectomy were made according to the above protocol and used as positive control.

**Group 3:** Induction of ovariectomy were made as above and fed on Ain-93M and mixed with + 10% Marjoram daily, for 12 weeks.

**Group 4:** Induction of ovariectomy were made as above, fed on Ain-93M and mixed with + 10% Cocoa daily, for 12 weeks.

**Group 5:** Induction of ovariectomy were made as above, fed on Ain-93M and mixed with + (5% Marjoram+5% Cocoa) daily, for 12 weeks.

After 3 month treatment, animals were anesthetized and decapitated. Liver, heart and kidney were excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination, dried by blotting with filter paper and weighed. The tissues were then kept in freezer at -70 degree until analysis.

### Liver Homogenate preparation:
Liver were perfused with saline and homogenized in chilled potassium chloride (1.17%) using a homogenizer. The homogenates were centrifuged at 800 g for 5 minutes at 4°C to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500 g for 20 minutes at 4°C to get the post mitochondrial supernatant which was used to assay SOD, CAT, GSH and MDA, HNE activity.

### Heart Homogenate preparation:
Homogenates were prepared on ice in the ratio 4 g tissue for 16 ml of phosphate pH 7.5, containing 1 mM/L Na₂ EDTA, 10 ml of 500 mM/L BHT (butylated hydroxytoluene) in acetonitrile was added to prevent formation of new peroxides during the assay. The homogenates were centrifuged at 2000 minutes at 40 C and frozen at -700 C until analysis.

**Kidney Homogenate preparation:**

Kidney homogenates were obtained by using a tissue homogenator, Ultra Taurax T-25 Polytron, at 4o C. The homogenates (1:10 w/v) were prepared by using a 100 mmol KCl buffer (pH 7.0) containing EDTA 0.3 mM. All homogenates were centrifuged at 600 g for 60 minutes at 4°C and the supernatant was used for biochemical assays.

**Biochemical analyses in liver, heart and kidney tissues:**

Estimation of Super Oxide Dismutase levels SOD Levels in the cell free supernatant was measured by the method of (Kono et al., 1978). Estimation of Catalase activity CAT activity was assayed by the method of (Sinha, 1979). Estimation of Glutathione GSH activity was determined by the procedure of (Carlberg and Mannervik 1985). Estimation of malonyldialdehyde (MDA) was determined spectrophotometrically according to the method by (Ohkawa et al., 1979).
Results and discussion

Table (1): Effect of Marjoram and Cocoa powders on ovariectomy rats on oxidative status in Liver tissue.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>SOD (U/g tissue)</th>
<th>CAT (mmol/g tissue)</th>
<th>GSH (U/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (-ve)</td>
<td>5.21±0.06</td>
<td>106.33±2.02</td>
<td>0.21±0.06</td>
</tr>
<tr>
<td></td>
<td>Control (+ve)</td>
<td>5.24±0.64</td>
<td>108.24±7.39</td>
<td>0.21±0.06</td>
</tr>
<tr>
<td></td>
<td>Marjoram 10%</td>
<td>9.02±1.04</td>
<td>74.00±6.24</td>
<td>3.00±0.22</td>
</tr>
<tr>
<td></td>
<td>Cocoa 10%</td>
<td>1.36±0.31</td>
<td>92.00±2.02</td>
<td>3.00±0.22</td>
</tr>
<tr>
<td></td>
<td>(Cocoa 5% + Marjoram 5%)</td>
<td>4.04±0.43</td>
<td>113.22±2.33</td>
<td>3.00±0.22</td>
</tr>
</tbody>
</table>

Means ± SE with different letters superscripts in the same column are significant at P < 0.05 using one way ANOVA test. n= 8 rats/group.

In the present study, The result of antioxidant enzyme on liver tissue on ovariectomy rats showed Super Oxide Dismutase SOD, Catalase CAT and Glutathione GSH decreased significantly on ovariectomy group without any treated (positive control) compared to other groups. Rats treated with Marjoram showed increased significant level of liver SOD (906.30±5.54, at P<0.05), CAT (74.00±6.24, P<0.05), GSH (3.00±0.22, P<0.05) compared to positive control (table 1). Also, best result showed both groups cocoa 10% and group treated mixed with cocoa 5% + marjoram 5% showed increased significantly at P<0.05 in SOD and CAT compared to all group.
Figure (1): Effect of Marjoram and Cocoa powders on ovariectomy rats on malonyldialdehyde MDA status in Liver tissue.

The result showed that significant decrease in malonyldialdehyde MDA for liver tissue in all treated groups marjoram 10%, cocoa 10% and group mixed with (cocoa 5% + marjoram 5%) P<0.05 (152.24±3.52 b, 92.00±2.02 c and 29.30±4.63 c, P<0.05, respectively) compared to control positive 221.67±6.17 a. The lowest value of MDA was significantly in control negative 64.33±3.17 d, then groups cocoa 10% and group mixed with (cocoa 5% + marjoram 5%). No significant differences between last groups as showed in figure (1).

<table>
<thead>
<tr>
<th>Control (-ve)</th>
<th>Control (+ve)</th>
<th>Marjoram 10%</th>
<th>Cocoa 10%</th>
<th>(Cocoa 5% + Marjoram 5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/ g tissue)</td>
<td>64.33</td>
<td>281.67</td>
<td>152.24</td>
<td>92</td>
</tr>
</tbody>
</table>

MDA (nmol/ g tissue) 64.33 281.67 152.24 92 89.3
Table (2): Effect of Cocoa and Marjoram powders on ovariectomy rats on oxidative status in heart tissue.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>SOD (U/g tissue)</th>
<th>CAT (mmol/g tissue)</th>
<th>GSH (U/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-ve)</td>
<td></td>
<td>4.96±0.10</td>
<td>100.06±1.73</td>
<td>4.97±0.10</td>
</tr>
<tr>
<td>Control (+ve)</td>
<td></td>
<td>0.72±0.07</td>
<td>40.00±2.30</td>
<td>0.72±0.07</td>
</tr>
<tr>
<td>Marjoram 10%</td>
<td></td>
<td>2.60±0.21</td>
<td>119.13±4.22</td>
<td>2.60±0.21</td>
</tr>
<tr>
<td>Cocoa Powder 10%</td>
<td></td>
<td>2.21±0.12</td>
<td>90.00±3.21</td>
<td>2.21±0.12</td>
</tr>
<tr>
<td>(Cocoa 5% + Marjoram 5%)</td>
<td></td>
<td>2.60±0.21</td>
<td>119.13±4.22</td>
<td>2.60±0.21</td>
</tr>
</tbody>
</table>

Means ± SE with different letters superscripts in the same column are significant at P < 0.05 using one way ANOVA test. n= 8 rats/group.

The parameter analyzed in heart tissue showed that all group treated with marjoram 10%, cocoa 10% and mixed them showed significantly increase in antioxidant enzyme compared to positive control. The highest content significantly at P<0.05 in antioxidant enzyme showed in group ovariectomy treated with 10% cocoa powder, level of SOD (9.88±4.10), CAT (119.13±4.22) GSH (2.60±0.21) compared to positive control (4.00±0.50, 40.00±2.30 and 0.72±0.07, respectively) as showed in table (2).
Figure (9): Effect of Marjoram and Cocoa powders on ovariectomy rats on malonyldialdehyde MDA status in heart tissue.

In the present study MDA status in heart tissue showed, all groups treated with marjoram 10abled, cocoa 1 and mixed them showed significantly decrease MDA (129.8±1.26, 129.6±1.27 and 129.8±1.27, P<0.05, respectively) compared to positive control (209.8±1.21 a).

More highly decrease in MDA was in cocoa powder 10abled compared to positive control and other treated groups figure (9).

Table (10): Effect of Marjoram and Cocoa powders on ovariectomy rats on oxidative status in kidney tissue.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SOD (U/g tissue)</th>
<th>CAT (mmol/g tissue)</th>
<th>GSH (U/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-ve)</td>
<td>129.8±1.26 a</td>
<td>129.6±1.27 b</td>
<td>129.8±1.27 a</td>
</tr>
<tr>
<td>Control (+ve)</td>
<td>129.6±1.27 d</td>
<td>129.6±1.27 d</td>
<td>129.6±1.27 c</td>
</tr>
<tr>
<td>Marjoram 10abled</td>
<td>129.8±1.26 c</td>
<td>129.6±1.27 c</td>
<td>129.8±1.27 c</td>
</tr>
<tr>
<td>Coca Powder 10abled</td>
<td>129.0±1.21 b</td>
<td>129.6±1.27 b</td>
<td>129.6±1.27 b</td>
</tr>
<tr>
<td>(Cocoa 5% + Marjoram 5%)</td>
<td>99.0±1.21 b</td>
<td>99.0±1.21 b</td>
<td>99.0±1.21 b</td>
</tr>
</tbody>
</table>

The results of antioxidant enzyme for kidney tissues showed significant increased for SOD, CAT and GSH for groups treated with marjoram 10abled, cocoa 1 and mixed them compared to positive control. It could be notice that cocoa 10abled group and group treated with mixed (cocoa 5% + marjoram 5%) showed highest level of SOD (129.0±1.21, P<0.05), CAT (129.6±1.27, P<0.05) and GSH (129.6±1.27, P<0.05). As showed in table 3 no significant differences between CAT level for control negative (129.0±1.21 b, P<0.05) and mixed (cocoa 5% + marjoram 5%) group (129.0±1.21 b, P<0.05) table (9).
Figure (4): Effect of Marjoram and Cocoa powders on ovariectomy rats on malonyldialdehyde MDA status in kidney tissue.

In the present study MDA status in kidney tissue showed, all groups treated with marjoram 10%, cocoa 10% and mixed them showed significantly decrease MDA (139.00±4.72 b, 104.00±2.22 d and 127.00±3.60 c, P<0.05, respectively) compared to positive control (296.00±4.52 a, P<0.05). More highly decrease in MDA was in 10% cocoa powder group compared to positive control and other treated groups as showed in figure (4). Also, it is notice that control negative was the lowest significant value (63.00±2.02 e, P<0.05) compared to all groups.

Discussion

It has known that postmenopausal life affects oxidative status for bodies (Yang et al., 2014; Tang et al., 2016). Ovariectomy in rats was the same effect on postmenopausal for several organs (Morrone et al., 2016; Tang et al., 2016; Barp et al., 2016), because estrogens have demonstrated to defense the liver and intestines from oxidative damage due to its antioxidative properties (Sener et al., 2005). Several studies indicated that ovariectomy resulted in antioxidative/oxidative imbalance.
in most mammals (Muthusami et al., 2006; Kankofer et al., 2007; Serin et al., 2008; Günay et al., 2011; Tang et al., 2012).

The our result indicated that decrease in antioxidant enzyme and increase in MDA in liver, heart, kidney tissue for ovariectomy group rat without any treated (control positive). This result it could be related to serous inflammation after ovariectomy may cause oxidative stress in body organs (Cronauer et al., 1999). Also, it has been indicated that ovariectomy causes to alterations in oxidative/antioxidative balance because of anaesthetic agents (Serin et al., 2008; Gunay et al., 2011; Szczubial et al., 2010). The obtained result for positive control was agreement with (Anadol, et al., 2012) which reported that serum MDA concentration significantly increased while SOD and GPx activities decreased on day 1 after ovariectomy surgery.

In the present study marjoram successfully improved the undesirable effects caused by ovariectomy and success in restored almost all variables antioxidant enzyme AST, CAT, GSH and decrease MDA in liver, heart, kidney tissue to near their negative control levels. Also, Combination of cocoa and Marjoram caused a significant modulation of deleterious effect of oxidative stress. The our result was agreement with Saleh et al. (2018) who study the effect marjoram against oxidative stress induced by paracetamol in male albino rats, and mentioned that Marjoram or moringa+ marjoram at dose 250mg/kg/day increase in antioxidant enzyme and decrease in MDA compared to positive control. Marjoram are rich in nutrients, minerals, vitamins and antioxidants which improve the body health in general and can improve the immunity suggesting them as valuable medicinal plants to protect against the deleterious toxic effects (Auwal et al., 2013 and Frank et al., 2014). Consistently, Fakurazi et al. (2011) stated that β-Carotene in Moringa leaves is efficiently converted into vitamin A in the body that has shown significant hepatoprotective effect. Marjoram was well documented to increase globulin and have high antioxidant capacity that could protect from liver, heart and kidney damage (Abd El-Ghany and El-Metwally, 2011).
Fakurazi et al., (2014) stated that certain phenolic compounds in marjoram may induce production of glutathione-S-transferase and other antioxidant enzymes. Additionally, the ability of these phenolic compounds to bind to some minerals as copper and iron can protect against their oxidative effects (Ferguson, 2001). Abd El-Ghany and El-Metwally, (2010) used Marjoram leaves to protect against liver injury induced by carbon tetrachloride due to its high content of antioxidant compounds that are released during toxicity and can protect cells against reactive oxygen species.

In present study, we determined the oxidative status of Liver, Heart and Kidney tissues for ovariectomy rats after prolonged treated of Cocoa powder in rats. Prolonged treated of cocoa and combination with cocoa and marjoram showed decrease accumulation of MDA in heart, liver and kidney tissue, implicated oxidative stress. It has been reported that malondialdehyde is a well-characterized mutagen (Esterbauer et al., 1991) that reacts with deoxyguanosine to form a major endogenous adduct with DNA in human livers.

Increased SOD level, Catalase and GSH level in liver heart and kidney tissues for treated group with Cocoa powder was observed (Table 1, 2 and 3). SOD is the major antioxidant enzyme that provides the body’s first enzymatic step in the defense system against oxidative stress. (Landmesser et al., 2004). Catalase is used by cells to defend against the toxic effects of hydrogen peroxide (Michiels et al., 1994). High intracellular GSH levels promote better survival under such conditions (Kurosawa et al., 2001, Ruzaidi et al., 2003). Increased activity of these enzymes as a result of polyphenol intake has been reported in the literature (Young et al., 2001). It could be reported that increasing enzyme antioxidant in tissues in our result for group cocoa treated was to cocoa have antioxidant properties and contains a number of different compounds such as polyphenols, caffeine, sterols, terpenes, and methylxan-thines. Cocoa has been potential mechanism for beneficial effects (Spadafranca et al., 2001, Mursu et al., 2004). Analikumar et al., (2001)
suggesting an enhanced protection of the liver, heart against oxidative stress situations by these antioxidants.

The our result agreement with Noori, et al., (2009) who examined the oxidative status in terms of lipid peroxidation and antioxidant enzymes in different tissues and found that group rats treated with cocoa at level (1 g/kg b.w.) showed significantly increased level of GSH in liver and heart tissue, Catalase in liver and heart, SOD in liver, and decrease in MDA in liver tissues. Fraga et al., (2009) reported a decrease in serum MDA levels after 15 days of consuming milk chocolate in young healthy adults, while those who ate white chocolate showed higher levels of oxidative stress. Rein et al., (2009) and Wang et al., (2009) both observed an inverse association between different amounts of flavanol-rich dark chocolate and plasma thiobarbituric acid reactive substances (TBARS) concentrations in healthy subjects 8 hours after injection dark chocolate riches in cocoa.

Conclusion

The consumption of cocoa and marjoram have a protective role against oxidative stress caused by ovariectomy in rats and have ability to improvement of almost all evaluated parameters. Therefore, it is suggested to use these components especially cocoa as nutritional habits in diet to protect body organs through postmenopausal life for women.

References


