

Research Article

Isoquercetin Could Protect Against Ovariectomy-Induced Neuronal Changes in Rats

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Abstract. Menopause occurs gradually and is characterized by increased susceptibility to developing mood disorders. Several studies have suggested that oxidative stress is implicated in the subsequent mood changes. It has been reported that quercetin glycosides may be effective due to their antioxidant abilities. The present work aimed to find out whether quercetin able to act against ovariectomy consequences and possible underlying mechanism(s). Animals were randomly divided into six groups of eight rats each. Group (1) Control group (2) Sham operated group (3) Ovariectomized (OVX) group (4) OVX-Isoquercetin-treated (10 mg/kg, i.p., dissolved in a DMSO/saline solution) group (5) OVX-estrogen-treated (subcutaneous implant of pellets (Innovative Research of America, Toledo, OH) containing 17 β -estradiol (1.5 mg/8 wk) group (6) OVX-Isoquercetin-estrogen treated group. The treatments were initiated two week after both ovariectomy and sham operations and continued for four consecutive weeks. Tested parameters: oxidative stress markers (MDA, GSH, SOD), inflammatory cytokines (TNF- α , IL-6) and brain monoamines (dopamine, nor epinephrine, 5-HT). Results: Isoquercetin either alone or in combination with estrogen can improve: oxidative stress markers (MDA, GSH, SOD), inflammatory cytokines (TNF- α , IL-6) and brain monoamines (dopamine, nor epinephrine, 5-HT). Combination of both isoquercetin & estrogen gives the best results in most of the tested parameters especially in normalizing IL-6 level. Concerning serotonin estrogen was as good as the combined drugs. Conclusion: Isoquercetin has an additive role with estrogen to maintain healthy brain tissue for production of normal monoamine levels.

Keywords: Isoquercetin, estrogen, Antioxidant, antiinflammatory, Brain monoamines.

1. Introduction

Sex hormones have been implicated in neurite outgrowth, synaptogenesis, dendritic branching, myelination and other important mechanisms of neural plasticity. The evidence from animal experiments and human studies reporting interactions between sex hormones and the dominant neurotransmitters, such as serotonin, dopamine and norepinephrine [1]. The trophic effects of ovarian hormones emerge early in brain development and remain throughout adolescence [2] and adulthood [3]. Many of these actions occur in brain regions involved in learning [4] and memory [5], emotion [6], motivation [7], motor control [8], and cognition [9]. During

menopause, women may exhibit numerous symptoms, which include irregular cycle, vasomotor symptoms, dysphoric mood symptoms, insomnia [10, 11].

During menopause, several psychological changes have been shown to act as pro-oxidant, but the association between the psychological status that modify the quality of life and oxidative stress in postmenopausal women is still unclear [45]. Ovarian steroids, mainly estradiol and progesterone, affect brain regions involved in the modulation of mood and behavior [12], and fluctuations in ovarian hormone secretion modify brain neurochemistry [1]. Moreover, the emotional vulnerability windows that occur throughout women's lives

are correlated with reproductive periods marked by considerable hormonal fluctuations, such as menstruation, pregnancy, postpartum period and perimenopause, thus indicating the pivotal role of sex steroids in the control of affective disorders [13]. In menopause period, there is a significant decline on levels of estrogens [14]. Estrogens, of which 17β -estradiol is a principle representative, are hormones belonging to a group of steroid derivatives of cholesterol. Estrogens are synthesized from androgens, primarily in the ovaries, and, to a lesser extent, in the placenta, testes, and adrenal cortex. They regulate growth, development, metabolism, sexual functions, and reproduction, etc. In addition, estrogens have a certain impact on the redox state of cells showing both pro- and anti oxidative properties [15]. Isoquercetin is a natural flavonoid found abundantly in almost all edible vegetables and fruits [16]. There is growing body of evidence showing that quercetin has great therapeutic potential in the prevention and treatment of different chronic diseases, including neurodegenerative and cardiovascular diseases, as well as cancer [11, 17, 18]. It has been shown that quercetin exerts health beneficial effects in a number of cellular and animal models, as well as in humans, through modulating the signaling pathways and gene expression involved in these processes [19]. Consequently, intake of a quercetin-rich diet is supported and is positively correlated with health promotion [17]. Because the role of isoquercetin in prevention against ovariectomy induced mood changes is not fully investigated, the present work aimed to find out whether isoquercetin either alone or as an add on therapy to estrogen can improve these changes.

2. Martial and Methods

2.1. Animals. All animal care & experimental procedures complied with the guidelines of the Ethical Committee of Faculty of medicine, Benha University, Egypt. Sixty virgin female Sprague-Dawley rats (8 weeks old; 170–200 g) were obtained from El-Nile Company for Pharmaceutical and Chemical industries, Cairo, Egypt. The animals were housed in controlled environmental conditions $22 (\pm 3^{\circ}\text{C})$, relative humidity (30–60%), light and dark cycle 12 and 12 hours.

2.2. Drugs. Isoquercetin, Estrogen were purchased from Sigma chemicals (St. Louis, MO, USA). Ketamine hydrochloride was purchased as vials (Rotexmedica, Trittau, Germany). DMSO, chloramine-T, p-dimethylaminobenzaldehyde (Ehlich reagent), hydroxyproline, 1,1,3,3 tetraethoxypropane and thiobarbituric acid were purchased from Sigma Chemical Co. (St Louis, MO, USA). n-Butanol and trichloroacetic acid were purchased from El-Nasr Chemical Co., Cairo, Egypt. All other chemicals used were of highest grade commercially available.

2.3. Experimental design. Animals were randomly divided into six groups of eight rats each. Group (1) Control group (2) Sham operated group (3) Ovariectomized (OVX) group (4) OVX-Isoquercetin-treated (10 mM/kg, i.p., dissolved in a DMSO/saline solution) [20] group (5) OVX-estrogen-treated (subcutaneous implant of pellets (Innovative Research of America, Toledo, OH) containing 17β -estradiol (1.5 mg/8 wk) [21]; group (6) OVX-Isoquercetin-estrogen treated group. The treatments were initiated two week after both ovariectomy and sham operations and continued for four consecutive weeks.

2.4. Methods

2.4.1. Ovariectomy. Female rats were caged for one week to acclimatize before beginning the experiments. Thereafter, Rats were anaesthetized using ketamine hydrochloride (100 mg/kg, i.p.). A bilateral incision (1.0–1.5 cm) was made through the skin and muscle layers. The ovaries were exposed and surgically removed on ice and the skin and muscles were sutured under aseptic conditions [22]. The same technique was done for sham operated group without removing the ovaries. Upon recovery from anesthesia, rats were individually housed in stainless steel ventilated cages for one week to recover from surgery. Animals were then housed in groups of 8/ each and left for two weeks before starting treatments. This period is usually enough for the cessation of the estrous cycle [23].

2.4.2. Determination of oxidative stress markers. As described above, the supernatant obtained by centrifugation of the 20% homogenate was used for the assessment of oxidative stress markers. Lipid peroxidation was determined by estimating the level of thiobarbituric acid reactive substances measured as malondialdehyde (MDA), according to the method of Mihara [24]. MDA is a decomposition product of the process of lipid peroxidation; thus, it is used as an indicator of this process. Briefly, 0.5 mL of the supernatant was added to 2.5 mL of 20% trichloroacetic acid and 1.0 mL of 0.6% thiobarbituric acid; then the mixture was heated for 20 min in a boiling water bath. After cooling, 4 mL of n-butanol was added with shaking. The alcohol layer was then separated by [centrifugation at $500 \times g$ for 10 min, and the absorbance was measured at 535 nm. The results were expressed as nmol of MDA per g of wet tissue using 1,1,3,3-tetraethoxypropane as standard. Reduced GSH was measured using a commercial kit (Biodiagnostic, Cairo, Egypt). In addition, the antioxidant enzymes level, such as SOD was measured by kit supplier protocol (Biosystems, Agappe) and the values were expressed as U/mg protein. The protein contents were determined by the Bradford method [25].

2.4.3. Estimation of nitrite content in the brain. The nitrite levels of brain tissue were determined using the Greiss reagent to measure the nitric oxide production during stress condition. The absorbance was measured at 542 nm using a spectrophotometer and the nitrite concentration was expressed in mg/g of tissue.

2.4.4. Determinations of inflammatory mediators. Levels of pro-inflammatory cytokines, including TNF- α , and IL-6, were measured in serum using ELISA kits provided by R & D systems (Minneapolis, USA) and the values are expressed as pg/mg protein.

2.4.5. Evaluation of brain monoamines. Monoamine neurotransmitters NE, DA and 5-HT in brain tissues were estimated by HPLC coupled with electrochemical detector [26]. In brief, the brain tissues (100 mg) were homogenized in an ice-cold solution of 0.4 M perchloric acid containing 5mM sodium bisulfite and 0.04 mM EDTA for avoiding oxidation and then centrifuged at 30,000 x g for 15 min at 4°C. The resulting supernatant (10 ml) was chromatographed on a C18 RP column using waters 1465 HPLC equipped with electrochemical detector. The mobile phase consisted of 17.6% methanol (v/v) and 82.4% distilled water containing 0.0876 mM EDTA disodium, 1.512 mM triethylamine, 9 mM DL-10-camphor sulfonic acid, 20 mM Na₂HPO₄.12H₂O and 15 mM citrate at a flow rate of 0.7 ml/min. NE, DA and 5-HT were identified and quantified by comparing their retention times and peak areas to those of standards (prepared as 1 mg/ml stock solutions in 0.4 M perchloric acid) and the concentrations were expressed in ng/g tissue.

3. Statistical Analysis

The obtained data were presented as mean \pm SEM (n = 10). Statistical analysis was performed by One Way Analysis of Variance (ANOVA) followed by Tuckey Kramer post hoc test using computer software program Graph Pad Prism 4 (La Jolla, CA, USA). Values at $P \leq 0.05$ was considered statistically significant.

4. Results

Ovariectomized rats showed significant decrease in GSH tissue level compared to control group, while isoquercetin treated group caused significant increase in GSH tissue level compared to ovariectomized group. Estrogen treated group showed significant increase in GSH tissue level compared to ovariectomized group & significant decrease compared to isoquercetin treated group. Isoquercetin + estrogen treated group caused the same significance as isoquercetin treated group alone. Both groups are better than the group that treated with estrogen alone. Ovariectomized rats showed significant decrease in MDA tissue level compared to control

group, while isoquercetin, estrogen treated and combined groups caused the same significant decrease in MDA tissue level compared to ovariectomized group. Ovariectomized rats showed significant increase in SOD tissue activity compared to control group, while isoquercetin treated group caused significant decrease in SOD tissue activity compared to ovariectomized group (normalize). Estrogen treated group showed significant decrease in SOD tissue activity compared to ovariectomized group & significant decrease compared to isoquercetin treated group. Isoquercetin + estrogen treated group caused the same significance as isoquercetin treated group alone (normalize). Both groups are better than the group that treated with estrogen alone.

Ovariectomized rats showed significant increase in tissue level of TNF- α compared to control group, while isoquercetin treated group caused significant decrease in tissue level of TNF- α compared to ovariectomized group. Estrogen treated group showed significant decrease in tissue level of TNF- α compared to ovariectomized group and isoquercetin + estrogen treated group caused significant decrease in tissue level of TNF- α compared to ovariectomized, isoquercetin treated and estrogen treated groups. Here estrogen is good, isoquercetin is better and combination is the best (Figure 1).

Ovariectomized rats showed significant increase in serum levels of IL-6 compared to control group, while isoquercetin treated group caused significant decrease in tissue level of IL-6 compared to ovariectomized group. Estrogen treated group showed significant decrease in tissue level of IL-6 compared to ovariectomized group and significant increase in tissue level of IL-6 compared to isoquercetin treated group. Isoquercetin + estrogen treated group caused normalization of serum IL-6 with significant decrease in tissue level of IL-6 compared to isoquercetin treated and estrogen treated groups. Here estrogen is good, isoquercetin is better and combination is the best (Figure 2).

Ovariectomized rats showed significant increase in nitrite (NO nitric oxide) content in the brain compared to control group, while isoquercetin treated group caused significant decrease in nitrite content in the brain compared to ovariectomized group. Estrogen treated group showed significant decrease in nitrite content in the brain compared to ovariectomized group and isoquercetin + estrogen treated group caused significant decrease in nitrite content in the brain compared to ovariectomized group (Figure 3).

4.1. Effects of isoquercetin and estrogen on dopamine level in ovariectomized rats. Ovariectomized rats showed significant decrease in dopamine level compared to control and sham groups, while isoquercetin treated group caused significant increase in dopamine level compared to ovariectomized group but still at a significant lower level compared to control and sham groups. Estrogen treatment showed significant increase in dopamine level compared to ovariectomized group but still at a significant lower level compared to control

and sham groups. Combination of both isoquercetin + estrogen caused significant increase in dopamine level compared to ovariectomized, isoquercetin treated and estrogen treated groups but still at a significant lower level compared to control and sham groups. Here the combination is the best (Table 2).

4.2. Effects of isoquercetin and or estrogen on nor epinephrine (NE) level in ovariectomized rats. Ovariectomized rats showed significant decrease in dopamine level compared to control and sham groups, while isoquercetin treated group caused significant increase in dopamine level compared to ovariectomized group but still at a significant lower level compared to control and sham groups. Estrogen treatment showed significant increase in dopamine level compared to ovariectomized group but still at a significant lower level compared to control and sham groups. Combination of both isoquercetin + estrogen caused significant

increase in dopamine level compared to ovariectomized, isoquercetin treated and estrogen treated groups but still at a significant lower level compared to control and sham groups. Here the combination is the best (Table 2).

4.3. Effects of isoquercetin and or Estrogen on serotonin level (5-HT) in ovariectomized rats. Ovariectomized rats showed significant decrease in 5-HT level compared to control and sham groups, while isoquercetin treated group caused significant increase in 5-HT level compared to ovariectomized group but still at a significant lower level compared to control and sham groups. Estrogen treatment showed significant increase in 5-HT level compared to ovariectomized and isoquercetin treated groups but still at a significant lower level compared to control and sham groups. Combination of both isoquercetin + estrogen showed the same results as estrogen alone (Table 2).

Table 1: Effects of Isoquercetin and or Estrogen on GSH, MDA levels and SOD activity in ovariectomized rats.

Treatment	Parameter		
	GSH ($\mu\text{mol/g tissue}$)	MDA (nmol/g tissue)	SOD (U/mg protein)
Control	4.85 ± 0.17	20.93 ± 0.69	2.85 ± 0.11
Sham	4.43 ± 0.12	20.88 ± 0.17	2.67 ± 0.09
OVX	$2.8^{a,b} \pm 0.15$	$33.35^{a,b} \pm 1.5$	$3.5^{a,b} \pm 0.08$
OVX + Isoquercitrin	$4.26^c \pm 0.25$	$21.56^c \pm 1.1$	$2.77^c \pm 0.06$
OVX + Estrogen	$3.6^{a,b,c} \pm 0.2$	$22.4^c \pm 0.9$	$3.1^{a,b} \pm 0.05$
OVX + Isoquercitrin + Estrogen	$4.1^c \pm 0.18$	$19.5^c \pm 0.6$	$2.3^c \pm 0.07$

Data presented are means \pm SEM. (n = 8).

aP < 0.05; significantly different from control group.

bP < 0.05; significantly different from OVX group. ANOVA followed by Tukey-Kramer post hoc test.

a: significant difference from control group.

b: significant difference from sham group.

c: significant difference from ovx (ovariectomized) non treated group.

d: significant difference from ovx (ovariectomized) isoquercetin treated group.

e: significant difference from ovx (ovariectomized) estrogen treated group.

Table 2: Effects of isoquercetin and or estrogen on dopamine, norepinephrine and serotonin levels in ovariectomized rats.

Treatment	Parameter		
	Dopamine (ng/g wet tissue)	Nor epinephrine (ng/g wet tissue)	5-HT (ng/g wet tissue)
Control	610 ± 10.2	78 ± 2.1	121 ± 10.1
Sham	650 ± 11.3	68 ± 3.3	205 ± 8.2
OVX	$390^{a,b} \pm 9.1$	$42^{a,b} \pm 2.3$	$101^{a,b} \pm 9.1$
OVX + Isoquercitrin	$480^{a,b,c} \pm 6.8$	$58^{a,b,c} \pm 4.1$	$135^{a,b,c} \pm 7.6$
OVX + Estrogen	$495^{a,b,c} \pm 12.1$	$56^{a,b,c} \pm 2.8$	$168^{a,b,c,d} \pm 5.8$
OVX + Isoquercitrin + Estrogen	$560^{a,b,c,d,e} \pm 13.4$	$66^{a,b,c,d,e} \pm 4.9$	$178^{a,b,c,d} \pm 3.7$

Data presented are means \pm SEM. (n = 8).

aP < 0.05; significantly different from control group.

bP < 0.05; significantly different from OVX group. ANOVA followed by Tukey-Kramer post hoc test.

a: significant difference from control group.

b: significant difference from sham group.

c: significant difference from ovx (ovariectomized) non treated group.

d: significant difference from ovx (ovariectomized) isoquercetin treated group.

e: significant difference from ovx (ovariectomized) estrogen treated group.

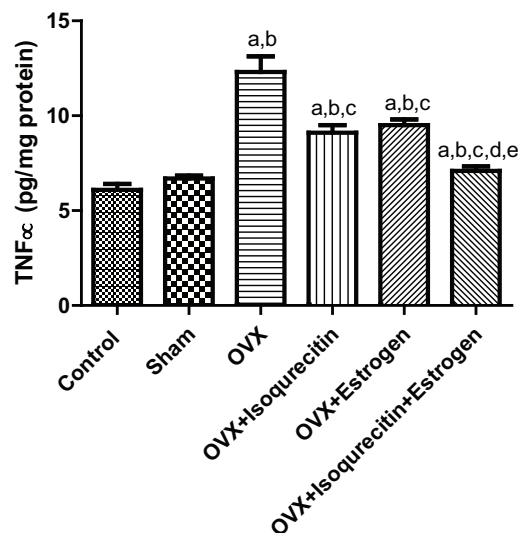


Figure 1: Effects of Isoquercetin and or Estrogen on tissue level of TNF- α in ovarioectomized rats. Data are expressed as the mean \pm SEM. (n = 8). $^{\#}P < 0.05$; significantly different from control group. $^{*}P < 0.05$; significantly different from OVX group. ANOVA followed by Tukey-Kramer post hoc test. a: significant difference from control group. b: significant difference from sham group. c: significant difference from ovx (ovarioectomized) non treated group. d: significant difference from ovx (ovarioectomized) isoquercetin treated group. e: significant difference from ovx (ovarioectomized) estrogen treated group.

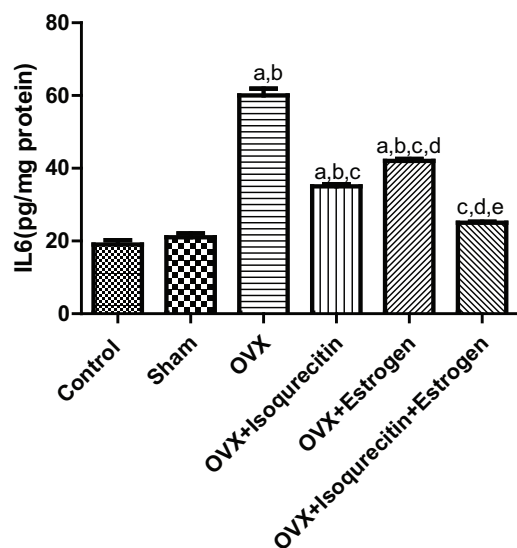


Figure 2: Effects of Isoquercetin and or Estrogen on tissue level of IL-6 in ovarioectomized rats. Data are expressed as the mean \pm SEM. 8 animals were used in each group. $^{\#}P < 0.05$; significantly different from control group. $^{*}P < 0.05$; significantly different from OVX group. ANOVA followed by Tukey-Kramer post hoc test. a: significant difference from control group. b: significant difference from sham group. c: significant difference from ovx (ovarioectomized) non treated group. d: significant difference from ovx (ovarioectomized) isoquercetin treated group. e: significant difference from ovx (ovarioectomized) estrogen treated group.

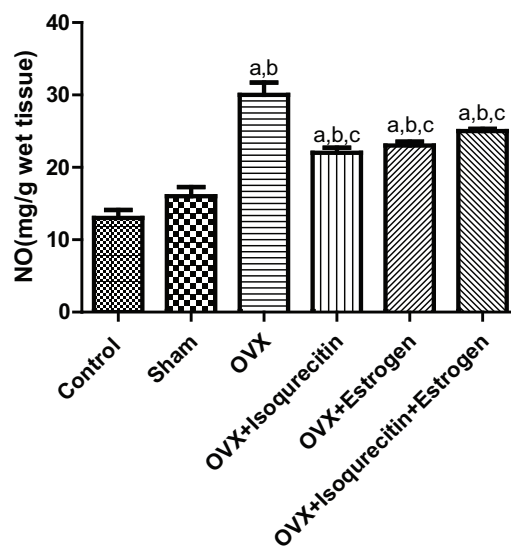


Figure 3: Effects of Isoquercetin and or Estrogen on nitrite content in the brain of ovarioectomized rats. Data are expressed as the mean \pm SEM. 8 animals were used in each group. $^{\#}P < 0.05$; significantly different from control group; $^{*}P < 0.05$; significantly different from OVX group; ANOVA followed by Tukey-Kramer post hoc test. a: significant difference from control group. b: significant difference from sham group. c: significant difference from ovx (ovarioectomized) non treated group. d: significant difference from ovx (ovarioectomized) isoquercetin treated group. e: significant difference from ovx (ovarioectomized) estrogen treated group.

5. Discussion

In the present work ovariectomy simulate menopause in females where the levels of estrogen are markedly reduced with stress mediated abnormalities.

In the current study ovarioectomized animals showed markedly increased level of lipid peroxidation in the brain tissue and superoxide dismutase activity along with decreased GSH level.

This is in accordance with previous data indicated that ovariectomy as well as reduced estrogen levels in females increased oxidative stress in the form of high level of MDA in different body organs and tissues. Amongst these studies the study of [27] who reported that decreased estrogen level led to increased oxidative stress. Also, [28]. Oxidative stress mediated ovariectomy has been reported also to increase the free radical environment that led to depletion of GSH and induce the activity of SOD as natural defense mechanism [29, 30].

This clearly support our data where brain content of GSH was markedly decreased however the activity of SOD and MDA level was increased. Further support of these findings is the study of [31] who found that cases with increased oxidative stress had decreased GSH and GSSG levels.

From this it could be suggested that menopause led to increased production of ROS in the brain with subsequent lipid peroxidation that consumed GSH reservoir and induce SOD activity to buffer against this stress insult. It has been reported that ovariectomy (menopause) usually accompanied by mood changes due to disturbance in the normal pattern of brain monoamines [1, 12].

In the present work ovariectomized animals showed markedly decreased levels of brain monoamines dopamine, serotonin, N.E.

These results are in line with [46] who found that serotonin level is lower in ovariectomized rats. Also, [32] proved that oxidative stress conditions (ovarectomy) led to decreased level of N.E, dopamine.

The interrelation between ovariectomy induced oxidative stress and post menopausal disturbance in brain mono amines was studied in our work.

This is augmented by previous literatures such as [33, 34].

In this study ovariectomy caused significant increase in tissue levels of TNF- α , IL6 and NO.

These data are in agreement with [35, 36] reported that ovariectomy induced high levels of TNF- α and IL6. Also, [37] found that ovariectomy caused significant increase in tissue level of nitric oxide (NO).

In this work the use of isoquercetin, estrogen either alone or in combination to prevent post menopausal brain monoamine disturbances and the underlying mechanisms is one of the few trials in that aspect. So, the effect of isoquercetin administration on brain monoamines(dopamine, norepinephrine, serotonin) as well as oxidative stress parameters (MDA, GSH, SOD) and the implicated mediators including inflammatory cytokine IL 6, TNF- α and nitrite has been studied.

In the present study the use of isoquercetin, estrogen either alone or in combination results in: isoquercetin treated group caused significant increase in GSH tissue level compared to ovariectomized group. Estrogen treated group showed significant increase in GSH tissue level compared to ovariectomized group & significant decrease compared to isoquercetin treated group. Isoquercetin + estrogen treated group caused the same significance as isoquercetin treated group alone. Both groups are better than the group that treated with estrogen alone. Isoquercetin, estrogen treated and combined groups caused the same significant decrease in MDA tissue level compared to ovariectomized group. Isoquercetin treated group caused significant decrease in SOD tissue activity compared to ovariectomized group (normalize). Estrogen treated group showed significant decrease in SOD tissue activity compared to ovariectomized group & significant decrease compared to isoquercetin treated group. Isoquercetin + estrogen treated group caused the same significance as isoquercetin treated group alone (normalize). Both groups are better than the group that treated with estrogen alone.

These data are in line with [38] who found that isoquercetin reorganize and modulate effects of inflammation and oxidative stress. Moreover [39] who reported that isoquercetin reestablish the peripheral cholinergic activity by decreasing oxidative stress events. Hernández et al., (2000) proved that estrogen treatment can reestablish plasma anti oxidant properties. Stepniak et al., (2016) suggests that under physiological conditions estrogen may contribute to protecting the ovary against oxidative damage.

In the current work groups received isoquercetin, estrogen or isoquercetin + estrogen showed low levels of (NO, TNF- α , IL6) if compared to ovariectomized non treated animals. Concerning TNF- α and IL6 estrogen is good, isoquercetin is better and combination is the best.

Combination of both drugs gives the best results in normalizing IL-6 level. These data are augmented by [38] and [40] who reported that isoquercetin reorganize and modulate effects of inflammation and oxidative stress. Hernández et al., (2000) found that estrogen treatment is related to an increase in NO synthesis and/or preventing oxidative stress, then improving endothelial function. Also, Shivers et al., (2015) estradiol significantly decreased pro inflammatory tumor necrosis factor (TNF)- α and IL levels.

The obtained data showed that combination of isoquercetin and estrogen give the best results in buffering the level of dopamine and norepinephrine, while concerning serotonin estrogen alone is as good as being combined with isoquercetin.

Isoquercetin rebalanced the disturbance in brain mono amines either alone or in combination with estrogen so, it is very interesting to note that this effect may be indirect as isoquercetin could inhibit oxidative stress mediated brain inflammation helping estrogen to give better results than being alone. These results are in accordance with [41] who concluded that quercetin can protect against CNS toxicity by reverting dopamine activity. On the other hand [44] suggest that isoquercetin is a weak (but safe) MAO-A inhibitor in the modulation of 5-HT levels in the brain. Moreover [42] indicate that piperine can enhance the antioxidant and anti-inflammatory properties of isoquercetin, and exhibits strong neuroprotective effects against neurotoxicity.

Treatment with estrogen is well established as it was found that externally administered estrogen could improve these changes and produce an extent of rebalance in central monoamines. This was indicated by [43] whose findings provide physiological and anatomical evidence for neuroprotective effects of estrogen. Also, [46] concluded that estradiol may improve perimenopause symptoms by increasing progesterone and boosting serotonin pathway. Further findings showed by [1] revealed that neurotransmitter systems do not work in isolation and sex hormones act on multiple sites, highly intertwined with serotonin, dopamine, GABA and glutamate.

On the basis of the previous findings it could be suggested that isoquercetin act against ovariectomy-induced production

of ROS and prevent neuronal damage as well as inflammation which confers a healthy environment for brain tissue to produce normal levels of monoamines. Conclusively: The use of isoquercetin along with estrogen shed attention to new strategies during the control of mood changes after menopause. This can be due to an additive effect as estrogen directly compensate the female sex hormone level in addition to indirect effect of isoquercetin (antioxidant, antiinflammatory) to maintain healthy brain tissue for production of normal mono amine levels.

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Competing Interests

The author declares no competing interests.

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