Raloxifene and Tamoxifen Reduce PARP Activity, Cytokine and Oxidative Stress Levels in the Brain and Blood of Ovariectomized Rats

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Abstract It is well known that 17β-estradiol (E2) has an antioxidant role on neurological systems in the brain. Raloxifene (RLX) and tamoxifen (TMX) are selective estrogen receptor modulators. An E2 deficiency stimulates mitochondrial functions for promoting apoptosis and increasing reactive oxygen species (ROS) production. However, RLX and TMX may reduce the mitochondrial ROS production via their antioxidant properties in the brain and erythrocytes of ovariectomized (OVX) rats. We aimed to investigate the effects of E2, RLX, and TMX on oxidative stress, apoptosis, and cytokine production in the brain and erythrocytes of OVX rats.

Forty female rats were divided into five groups. The first group was used as a control group. The second group was the OVX group. The third, fourth, and fifth groups were OVX + E2, OVX + TMX, and OVX + RLX groups, respectively. E2, TMX, and RLX were given subcutaneously to the OVX + E2 and OVX + TMX, OVX + RLX groups for 14 days after the ovariectomy respectively.

While brain and erythrocyte lipid peroxidation levels were high in the OVX group, they were low in the OVX + E2, OVX + RLX, and OVX + TMX groups. OVX + E2, OVX + RLX, and OVX + TMX treatments increased the lowered glutathione peroxidase activity in erythrocytes and the brain and reduced glutathione and vitamin E concentrations in the brain. β-carotene and vitamin A concentrations in the brain and TNF-α and interleukin (IL)-1β levels in the plasma of the five groups were not significantly changed by the treatments. However, increased plasma IL-4 levels and Western blot results for brain poly (ADP-ribose) polymerase (PARP) in the OVX groups were decreased by E2, TMX, and RLX treatments, although proapoptotic procaspase 3 and 9 activities were increased by the treatments.

In conclusion, we observed that E2, RLX, and TMX administrations were beneficial on oxidative stress, inflammation, and PARP levels in the serum and brain of OVX rats by modulating antioxidant systems, DNA damage, and cytokine production.

Keywords Brain · Cytokine · Apoptosis · Oxidative stress · Selective estrogen receptor modulators

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Abbreviations

E2	17β-estradiol
GSH	Reduced glutathione
GSH-Px	Glutathione peroxidase
LP	Lipid peroxidation
MDA	Malondialdehyde
PARP	Poly (ADP-ribose) polymerase
PARP	Poly (ADP-ribose) polymerase
PUFAs	Polyunsaturated fatty acids
RLX	Raloxifene
ROS	Reactive oxygen species
SERMs	Selective estrogen receptor modulators
SOD	Superoxide dismutase
TMX	Tamoxifen

Introduction

Brains and erythrocytes may be vulnerable to the auto-oxidation of hemoglobin and polyunsaturated fatty acids (PUFAs). These actions may be induced by menopause or may occur in ovariectomized (OVX) experimental animals due to their poor enzymatic antioxidant defenses but a high PUFA content and rich oxygen consumption (Nazıroğlu et al. 2004; Halliwell 2006; Nazıroğlu et al. 2014). Reactive oxygen species (ROS) cause injury to cells and intracellular membranes resulting in lipid peroxidation and may lead to cellular destruction and subsequently, cell death (Halliwell, 2006). In order to scavenge ROS, various antioxidant defense systems exist in the brain and erythrocytes. Glutathione peroxidase (GSH-Px) is responsible for the reduction of hydro and organic peroxides in the presence of reduced glutathione (GSH) (Nazıroglu 2009). Neurons and the brain contain rich GSH content, and it maintains thiol redox balance in the cells (Schweizer et al. 2004). Vitamin E (α-tocopherol) is the most important antioxidant in the lipid structure of neurons. Vitamin E acts to protect cells against the effects of free radicals, which are potentially damaging by-products of the body's metabolism (Halliwell 2006; Nazıroğlu 2007). Vitamin A and β-carotene are scavengers of singlet oxygen radicals (Halliwell 2006). Therefore, ROS can be indirectly evaluated by measurement of various antioxidants such as GSH-Px, GSH, vitamin A, vitamin E, and β-carotene.

In the postmenopausal period, it is well known that the decrease in ovarian hormones induces oxidative stress and apoptosis in many tissues, including the brain (Nazıroğlu et al. 2004). Postmenopausal depletion of endogenous estrogens may contribute to tissue injury and apoptosis via induced oxidative stress (Dilek et al. 2010; Lamas et al. 2015). It has been shown that a reduction in 17β-estradiol (E2) leads to an increase in oxidative stress in the body, which is dependent on the concentration and chemical structure of this hormone (Moreira et al. 2007; Dilek et al. 2010; Moreira et al. 2011). Furthermore, it has been shown that postmenopausal women, compared with premenopausal women, have higher serum concentrations of oxidative stress markers including oxidized GSH and lipid peroxidation (Doshi and Agarwal 2013). The E2 hormone regulates a remarkably large spectrum of brain functions, including learning, memory, emotions, and affective states, as well as motor coordination (Doshi and Agarwal 2013).

Tamoxifen (TMX) and raloxifene (RLX) are non-steroidal selective estrogen receptor modulators (SERMs) that have been used in the treatments of estrogen-dependent breast cancers and protection of bone (Jordan 2003). E2-positive neurons interact with TMX because TMX can easily penetrate the blood-brain barrier (Jordan 2003). SERMs, including TMX, act as estrogen agonists on selected targets (bone and brain) while being estrogen antagonists in the breasts and uterus

(Jordan 2003; Doshi and Agarwal 2013). Recent studies suggest that RLX has a neuroprotective action in the central nervous system and demonstrates a pharmacological profile similar to that of E2 both in OVX rats and postmenopausal women (Yaffe et al. 2001; Moreira et al. 2005; Moreira et al. 2007). Most degenerative diseases are consequences of excessive levels of oxidation and apoptosis through increases in mitochondrial depolarization and Ca^{2+} entry (Nazıroğlu 2011). Mitochondrial-dependent pathways, such as superoxide radical production and apoptotic pathways, are regulated by E2, TMX, and RLX (Yang et al. 2004; Moreira et al. 2005; Moreira et al. 2007; Moreira et al. 2011); however, there are also conflicting results on this subject (Albukhari et al. 2009).

The benefits of E2, TMX, and RLX on mitochondria and Ca^{2+} entry into the brain and neurons are well known (Moreira et al. 2005; Moreira et al. 2007). However, their effects on lipid peroxidation, antioxidants, inflammation, and caspase activities in the brain and blood are still not well understood. Hence, we aimed to evaluate whether there is a protective effect of E2, TMX, and RLX on oxidative stress, enzymatic antioxidants, and apoptosis status in an OVX-rat menopause model.

Materials and Methods

Animals

Forty female Wistar albino rats weighing 170 ± 10 g and aged 8–12 weeks were used for the experimental procedures. All of the rats were housed under standard conditions of light (12 h of daylight/12 h of darkness) and temperature (22 ± 2 °C). Animals were housed in individual plastic cages with bedding. Standard rat food and tap water were available ad libitum for the duration of the experiments. The experimental protocol of the study was approved by the Ethical Committee of the Medical Faculty of Suleyman Demirel University (SDU). 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 SDU.

Experimental Design

The 40 rats were randomly divided into five groups with eight rats per group as follows:

Group I. Control group: A placebo (0.1 ml dimethyl sulfoxide [DMSO] + 0.9 ml physiological saline [0.9 NaCl w/v]) was subcutaneously administrated to the group for 14 days.

Group II. Ovariectomized group (OVX): OXV was induced and DMSO was subcutaneously supplemented for 14 days (Dilek et al. 2010).

Group III. Ovariectomized + Estrogen group (OVX + E2): Animals received subcutaneous E2 (80 µg/kg/day) for

14 consecutive days after OVX treatment (Kramer and Bellinger 2013).

Group IV. Ovariectomized + Tamoxifen group (OVX + TMX): Animals were OVX and TMX (1 mg/kg/day) was subcutaneously given for 14 consecutive days.

Group V. Ovariectomized + Raloxifene group (OVX + RLX): Animals received subcutaneous RLX (1 mg/kg/day) for 14 consecutive days after OVX treatment (Huang et al. 2007).

Bilateral ovariectomies were performed in all groups except for the control group as previously described (Dilek et al. 2010). E2, TMX, and RLX (Cayman Chemical Inc., Istanbul, Turkey) were dissolved in DMSO (0.1 ml).

Anesthesia and Blood Collection and Preparation of Blood Samples

After 12 h of last E2, TMX, and RLX dose administrations, all rats were anesthetized with a cocktail of ketamine hydrochloride (80 mg/kg) and xylazine (10 mg/kg) administered i.p. before sacrifice of each rat and removal of blood samples (4−6 ml).

Blood samples were separated into plasma and erythrocytes by centrifugation at $1000 \times g$ for 15 min at +4 °C. The erythrocyte samples were washed three times in cold isotonic saline (0.9 %, v/w), then hemolyzed with a nine-fold volume of phosphate buffer (50 mM, pH 7.4). After addition of butylhydroxytoluol (4 μl per ml), hemolyzed erythrocytes and plasma samples were stored at −85 °C (WUF-80 Wisd laboratory Inc., China) for <1 months pending measurement of vitamin assays. The hemolyzed erythrocytes and plasma samples were used immediately for lipid peroxidation and enzymatic activity.

Preparation of Brain Samples

Brain samples were obtained and prepared as previously described (Dilek et al. 2010; Senol et al. 2014; Kahya et al. 2015). The removed tissues of brain cortex samples were washed twice with cold physiological saline. They were held in glass bottles in a deep freeze (−33 °C) for 1 month.

Half of the frozen brain samples were used in Western blot analyses. The remaining brain samples were placed on ice and cut into small pieces using scissors. The tissue samples were homogenized in 5 volumes (1:5, w/v) of ice-cold Tris-HCl buffer (50 mM, pH 7.4) by an ultrasonic homogenization (SONOPULS HD 2070, Bandelin Electronic, Berlin, Germany), and they were centrifuged for 5 min at 3000 rpm. All preparation procedures were performed on ice.

Lipid Peroxidation and Protein Determinations in Erythrocyte and Brain

Lipid peroxidation levels as malondialdehyde (MDA) in the hemolyzed erythrocytes and brain homogenate were measured with the thiobarbituric-acid reaction by the method of Placer et al. (1966). The values of lipid peroxidation in the erythrocyte and brain samples were expressed as μmol/g protein. The protein contents in the hemolyzed erythrocytes and brain homogenate were measured by method of Lowry et al. (1951) with bovine serum albumin as the standard.

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

The GSH contents of the erythrocytes and brain were measured at 412 nm using the method of Sedlak and Lindsay (1968). GSH-Px activities of erythrocytes were measured spectrophotometrically (UV-1800, Shimadzu, Kyoto, Japan) at 37 °C and 412 nm according to the Lawrence and Burk method (1976). GSH-Px activity and GSH level in the erythrocyte and brain samples were expressed as μmol/g protein.

β-carotene, Vitamins A, and Vitamin E Analyses in the Brain Samples

Vitamins A (retinol) and E (α-tocopherol) were determined in the brain samples by a modification of the method described by Desai (1984) and Suzuki and Katoh (1990). Brain samples of 0.25 g 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. Calibrations were performed using standard solutions of all-trans retinol and α-tocopherol in hexane.

The levels of β-carotene in the brain samples were determined according to the method of Suzuki and Katoh (1990). The value of β-carotene in hexane was measured at 453 nm in a spectrophotometer, and pure hexane was used as blank.

Western Blot Analyses

Western blotting was performed using standard procedures. To detect β-actin, poly (ADP-ribose), polymerase (PARP), procaspase 3, and procaspase 9 protein expressions, the frozen cells were homogenized in lysis buffer; the supernatant was

removed and conserved after centrifuge at 16,000g, 20 min. The total protein was assessed using Bradford reagent at 595 nm. Obtained bands were visualized using ECL Western HRP Substrate (Millipore Luminate Forte, USA), and visualition was achieved through X-ray film (GE Healthcare, Amersham Hyperfilm ECL, UK) and normalized against β-actin protein. The data are presented as relative density over the pretreatment level (experimental/control).

Cytokine Determinations in Plasma

Plasma cytokine [TNF-α, interleukin (IL)-1β, and IL-4] levels were measured using a commercial enzyme-linked immunosorbent assay (ELISA), following the manufacturer's instructions as described in previous studies (Senol et al. 2014; Kahya et al. 2015) (Multimode microplate reader, Infinite® 200 PRO series, Männedorf Switzerland). They employ the quantitative sandwich techniques. All ELISA kits were purchased from DRG Inc. (Marburg, Germany). Determinations were made in duplicate, and TNF-α, IL-1β, and IL-4 results are expressed in nano gram (ng) and picogram (pg) per milliliter, respectively.

Statistical Analysis

All results are expressed as means ± standard deviation (SD). Data were analyzed using the SPSS statistical program (version 17.0, software, SPSS. Chicago, IL, USA). Significance in five groups was first checked by ANOVA-Kruskal Wallis test. Then, a paired Mann-Whitney U test was performed in the five groups, and p values of less than 0.05 were regarded as significant.

Results

Lipid Peroxidation Results in the Brains and Erythrocytes

The mean erythrocyte and brain lipid peroxidation values in the five groups are shown in Tables 1 and 2, respectively. The results showed that the lipid peroxidation levels in the brain ($p < 0.001$) and erythrocytes ($p < 0.01$) in the OVX group were significantly higher than in the control group. The E2, TMX, and RLX administrations caused a decrease in lipid peroxidation levels of the brain and erythrocytes ($p < 0.001$) relative to the OVX group. Additionally, lipid peroxidation levels of the brain were reduced more by RLX treatment relative to the OVX + TMX group ($p < 0.001$).

GSH and GSH-Px Results in the Brains and Erythrocytes

The mean GSH levels and GSH-Px activities in the erythrocytes and brains of the five groups are shown in Tables 1 and 2,

respectively. The GSH-Px activities in the brains and erythrocytes of the OVX group were significantly lower ($p < 0.001$) than in the control group. Brain GSH concentrations in the OVX group were markedly lower ($p < 0.01$) than in the control group, and there were no significant differences in erythrocyte GSH levels among the groups. The brain GSH concentrations were increased by E2, TMX, and RLX treatments ($p < 0.01$). The brain and erythrocyte GSH-Px activities were increased by the administration of E2, TMX, and RLX ($p < 0.001$). However, the GSH-Px activity of the brain was increased more by the RLX treatment relative to the OVX + TMX group ($p < 0.001$).

Antioxidant Vitamin Concentrations in the Brain

The mean vitamin A, vitamin C, and vitamin E concentrations in brains of the five groups are shown in Tables 1 and 2, respectively. Vitamin E ($p < 0.05$) concentrations in the brain were markedly ($p < 0.01$) lower in the OVX group compared to the control group. Decreased brain vitamin E concentrations were improved by RLX administration ($p < 0.001$); however, these concentrations were not improved by E2 and TMX administrations. There were no significant differences in brain vitamin A and β-carotene concentrations in the five groups.

Results of Cytokines in the Plasma

Figures 1, 2, and 3 show the mean IL-1β, IL-4, and TNF-α levels in the plasma of the five groups, respectively. Mean IL-1β levels in the control, OVX, OVX + E2, OVX + TMX, and OVX + RLX groups were 77, 95, 83, 84, and 85 ng/ml, respectively. Mean IL-4 levels in the control, OVX, OVX + E2, OVX + TMX, and OVX + RLX groups were 62, 69, 61, 60, and 62 pg/ml, respectively. Mean TNF-α levels in the control, OVX, OVX + E2, OVX + TMX, and OVX + RLX groups were 42, 54, 47, 46, and 45 ng/ml, respectively. The IL-4 levels were significantly (p 0.05) increased in the plasma of rats with OVX, and these levels were significantly (p 0.05) decreased with E2, TMX, and RLX treatments. There were no significant differences in plasma IL-1β and TNF-α levels in the five groups.

Results of Procaspase 3, Procaspase 9, and Poly (ADP-ribose) Polymerase (PARP)

Caspase 3 and 9 activities are important in the executioner caspase-activated pathways and mitochondrial apoptotic pathway, respectively (Li et al. 1997). During the apoptotic cascade, cytochrome c released from the mitochondria was associated with procaspase 3 and 9. The procaspases were further processed to caspase 3 and caspase 9 (Li et al. 1997). We assayed procaspase 3 and procaspase 9 activities as indicators of apoptosis (Figs. 4 and 5). The activities of the procaspase 3

Table 1 The effects of 17β-estradiol (E2), tamoxifen (TMX), and raloxifene (RLX) on lipid peroxidation (LP), reduced glutathione (GSH) and glutathione peroxidase (GSH-Px) values in erythrocyte of ovariectomized (OVX) rats (mean ± SD)

Parameters	Control ($n = 8$)	OVX ($n = 8$)	OVX+ E2 ($n = 8$)	OVX + TMX ($n = 8$)	OVX+ RLX ($n = 8$)
LP (μmol/g protein)	25.80 ± 2.17	37.70 ± 4.21[b]	24.10 ± 2.68[d]	25.30 ± 3.73[d]	27.00 ± 2.54[d]
GSH (μmol/g protein)	20.00 ± 1.07	18.90 ± 2.07	21.50 ± 2.02	22.10 ± 2.56	21.00 ± 1.96
GSH-Px (IU/g protein)	32.00 ± 1.80	25.00 ± 2.53[c]	32.60 ± 3.37[d]	31.50 ± 2.32[d]	31.10 ± 1.63[d]

[a] $p < 0.05$, [b] $p < 0.01$, and [c] $p < 0.001$ versus control. [d] $p < 0.001$ versus OVX group

and 9 were significantly ($p < 0.05$) lower in the OVX group compared to the controls, and the results indicated that the conversion rates of procaspase 3 and 9 to caspase 3 and 9 were increased in the brain by induction of OVX. The procaspase activities were also increased in the OVX + E2, OVX + TMX, and OVX + RLX groups as compared to the control group ($p < 0.05$).

PARP is an abundant enzyme present in cells that indicates and signals damage to DNA repair mechanisms (Nazıroğlu 2007). The PARP activities were markedly ($p < 0.05$) higher in the OVX group compared to the controls. The PARP activities were decreased in the OVX + E2, OVX + TMX, and OVX + RLX groups as compared to the OVX group ($p < 0.05$).

Discussion

We found that lipid peroxidation levels in the brain and erythrocytes, as well as brain PARP and plasma IL-4 levels, were increased by OVX induction. However, GSH-Px activity, proapoptotic procaspase 3 and 9 activities, and GSH and vitamin E concentrations were decreased by the induction of OVX. Therefore, OVX-induced estrogen deficiencies were characterized by increased oxidative stress and cytokine production along with decreased antioxidant levels. Lipid peroxidation in the erythrocytes and brain, PARP activities in the brains, and IL-4 levels in the plasma were decreased by E2, TMX, and RLX administrations; however, GSH-Px, procaspase 3 and 9 activities and vitamin E levels in the brain were increased by the treatments. Thus, we have shown that E2, TMX, and RLX treatments modulated the balance of pro- and antioxidants in rats by downregulating the levels of oxidative stress while upregulating the GSH redox system. To the best of our knowledge, the current study is the first to compare the three treatments (E2, TMX, and RLX) with particular reference to their effects on cytokine production, PARP activity, caspase activity, and antioxidant redox systems in OVX-induced oxidative brain injury in rats.

Calcium ion accumulation has been suggested to be a key regulator of cell survival, but these ions can also induce apoptosis in neuronal death (Kumar et al. 2014). Mitochondria are essential for the production of ATP, through oxidative phosphorylation, the regulation of intracellular Ca^{2+} homeostasis, and they are the main generators of ROS (Bejarano et al. 2009; Espino et al. 2010). Depolarization of mitochondria through an overload of

Table 2 The effects of 17β-estradiol (E2), tamoxifen (TMX), and raloxifene (RLX) on lipid peroxidation (LP), reduced glutathione (GSH), glutathione peroxidase (GSH-Px), and antioxidant vitamin concentrations in brain of ovariectomized (OVX) rats (mean ± SD)

Parameters	Control ($n = 8$)	OVX ($n = 8$)	OVX+ E2 ($n = 8$)	OVX + TMX ($n = 8$)	OVX+ RLX ($n = 8$)
LP (μmol/g protein)	22.50 ± 1.71	32.40 ± 4.65[c]	22.40 ± 3.86[f]	21.70 ± 3.56[f]	12.90 ± 1.90[f, h]
GSH (μmol/g protein)	15.40 ± 0.50	13.00 ± 1.56[b]	15.50 ± 1.30[e]	15.40 ± 1.53[e]	15.60 ± 1.40[e]
GSH-Px (IU/g protein)	28.70 ± 1.64	20.80 ± 2.19[c]	27.30 ± 1.98[f]	28.50 ± 1.96[f]	32.70 ± 1.79[f, h]
Vitamin A (μmol/g tissue)	2.73 ± 0.34	2.38 ± 0.36	2.57 ± 0.09	2.45 ± 0.32	2.60 ± 0.23
β-carotene (μmol/g tissue)	1.19 ± 0.10	1.06 ± 0.12	1.12 ± 0.07	1.08 ± 0.09	1.01 ± 0.09
Vitamin E (μmol/g tissue)	10.80 ± 0.66	6.32 ± 0.72[c]	6.42 ± 0.37[c]	6.78 ± 0.34[c]	8.72 ± 0.38[c, f, h]

[a] $p < 0.05$, [b] $p < 0.01$ and [c] $p < 0.001$ versus control. [d] $p < 0.05$, [e] $p < 0.01$, and [f] $p < 0.001$ versus OVX group. [g] $p < 0.01$ and [h] $p < 0.001$ versus OVX + TMX
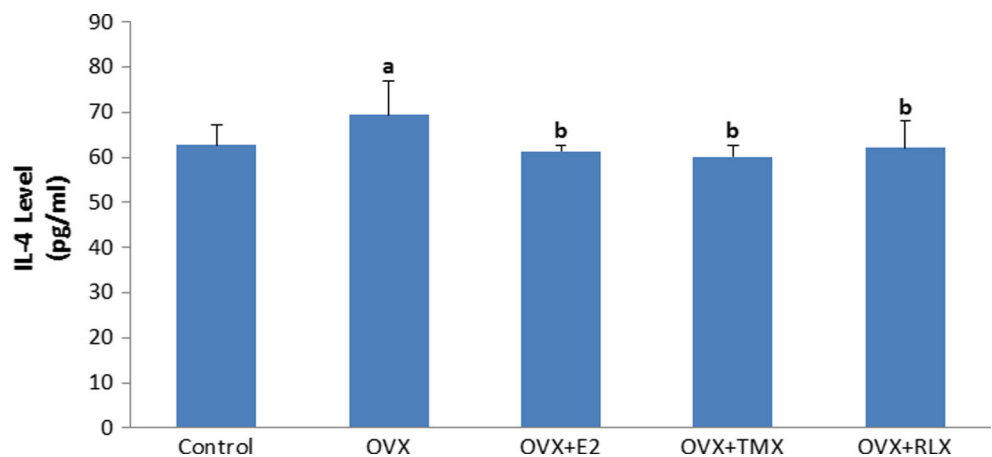
Fig. 1 The effects of 17β-estradiol (E2), tamoxifen (TMX), and raloxifene (RLX) on plasma IL-1β level in ovariectomized (OVX) rats (mean ± SD and $n = 8$)
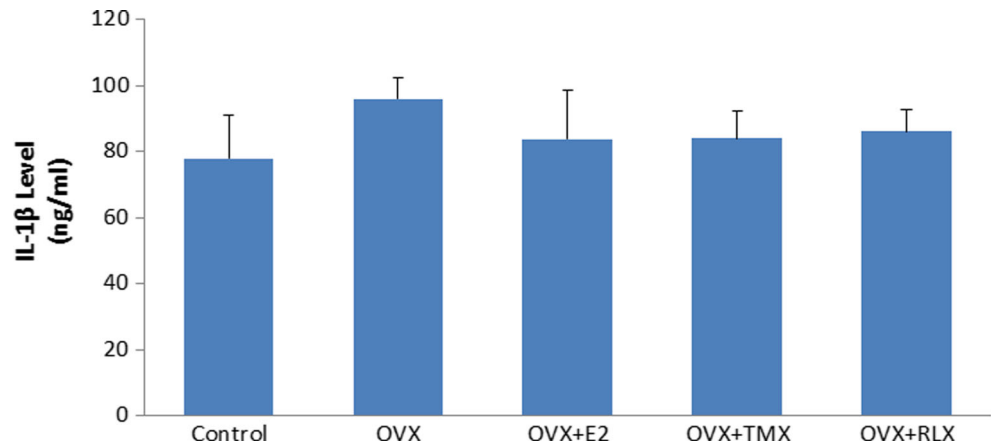

Ca^{2+} entry can induce an apoptotic program by stimulating the release of apoptosis-promoting factors, such as cytochrome c and caspase 3, and by generating ROS due to respiratory chain damage (Li et al. 1997; Uğuz et al. 2009). If mitochondrial membrane depolarization occurs in the neurons, it activates excessive ROS production, caspase 3 and caspase 9 apoptotic pathways (Espino et al. 2010). Accumulating evidence indicates that E2, TMX, and RLX (Yang et al. 2004; Moreira et al. 2005; Moreira et al. 2007; Moreira et al. 2011) reduce apoptosis and oxidative stress through the modulation of mitochondrial functions and Ca^{2+} entry into the brain and neurons (Yu et al. 2004; Altmann et al. 2015). However, they are oxidant and apoptotic through the induction of mitochondrial membrane depolarization in the liver (Albukhari et al. 2009). It has also been reported that the mitochondrial permeability transition induced by the oxidants was reduced by TMX though the inhibition of sulfhydryl group oxidations (Cardoso et al. 2004). However, it is well known that mitochondria in different tissues induce

distinct actions and responses in the treatment of the same agents (Moreira et al. 2007; Albukhari et al. 2009; Moreira et al. 2011), which explains several pathophysiological functions of differential toxicity. In the current study, brain and erythrocyte lipid peroxidation and plasma cytokine (IL-4) levels were increased in the OVX rats, although the brain and erythrocyte GSH-Px, the brain GSH, and vitamin E concentrations were decreased in the OVX rats. However, lipid peroxidation, PARP activity, and cytokine production were decreased by E2, TMX, and RLX treatments through the modulation of mitochondrial permeability transition pore functions, and the GSH and GSH-Px values were increased in OVX rats by the three treatments. The reduced vitamin E concentrations were increased by the RLX treatment. However, erythrocyte GSH and brain vitamin A and β-carotene concentrations did not change between the five groups. Adaptive vitamin antioxidant responses of the brain were accompanied by brain and erythrocyte GSH-Px, GSH, and vitamin E antioxidant downregulations.

Fig. 2 The effects of 17β-estradiol (E2), tamoxifen (TMX), and raloxifene (RLX) on plasma IL-4 level in ovariectomized (OVX) rats (mean ± SD and $n = 8$). $^{a}p < 0.05$ as compared with group control. $^{b}p < 0.05$ as compared with group OVX

Fig. 3 The effects of 17β-estradiol (E2), tamoxifen (TMX), and raloxifene (RLX) on plasma TNF-α level in ovariectomized (OVX) rats (mean ± SD and $n = 8$)
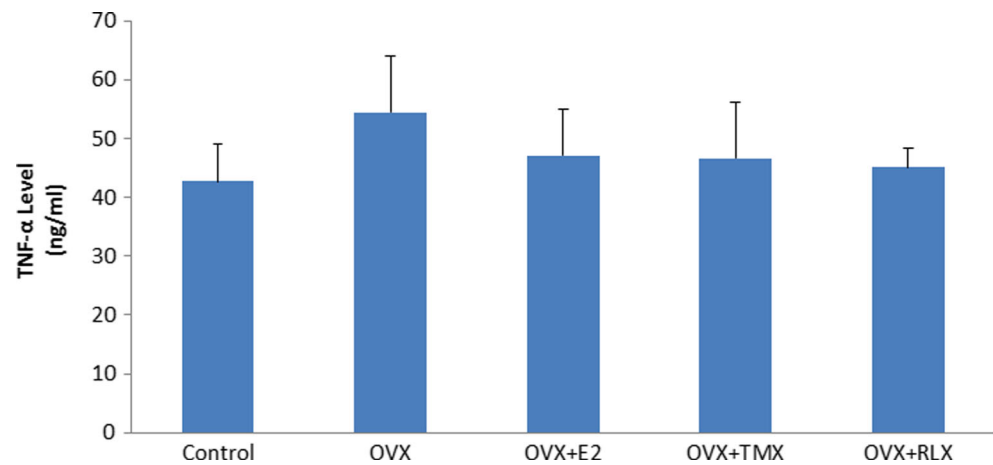

The antioxidant enzyme system inherent in the cellular defense system is the most important defense mechanism against ROS. GSH and GSH-Px act as antioxidants and have a preventive effect against the extensive production of ROS by OVX induction. The mitochondrial permeability transition pore has thiol groups and GSH redox-sensitive sites, which are opened by oxidation (Moreira et al. 2005; Moreira et al. 2007). Several studies have suggested a protective role of E2 and TMX on GSH and GSH-Px values in the brain through a modulation of mitochondrial permeability transition pores (Cardoso et al. 2004; Moreira et al. 2005; Moreira et al. 2007), although conflicting results in human and animal studies have also been presented (Albukhari et al. 2009). In the current study, GSH levels and GSH-Px activity were increased

in the erythrocytes and brains of E2-, TMX-, and RLX-treated rats by inhibiting oxidative stress. Similarly, Moreira et al. (2005) reported that oxidative stress levels in the brains of TMX-treated rats were reduced by supporting antioxidant thiol groups and GSH levels as well as the inhibition of mitochondrial permeability transition pores.

In the current study, PARP activity in the brain increased in the OVX group, while its activity was low in the OVX + E2, OVX + TMX, and OVX + RLX groups. However, procaspase 3 and procaspase 9 activities in the OVX group decreased, and this may have been induced by the conversion of procaspase 3 and 9 to caspase 3 and 9. The procaspase 3 and 9 activities were increased in the OVX groups by E2, TMX, and RLX treatments. The current study provides a mechanistic

Fig. 4 The effects of 17β-estradiol (E2), tamoxifen (TMX), and raloxifene (RLX) on procaspase 3 and procaspase 9 activities in the brain of ovariectomized (OVX) rats (mean ± SD and $n = 8$). [a] $p < 0.05$ as compared with group control. [b] $p < 0.05$ as compared with group OVX
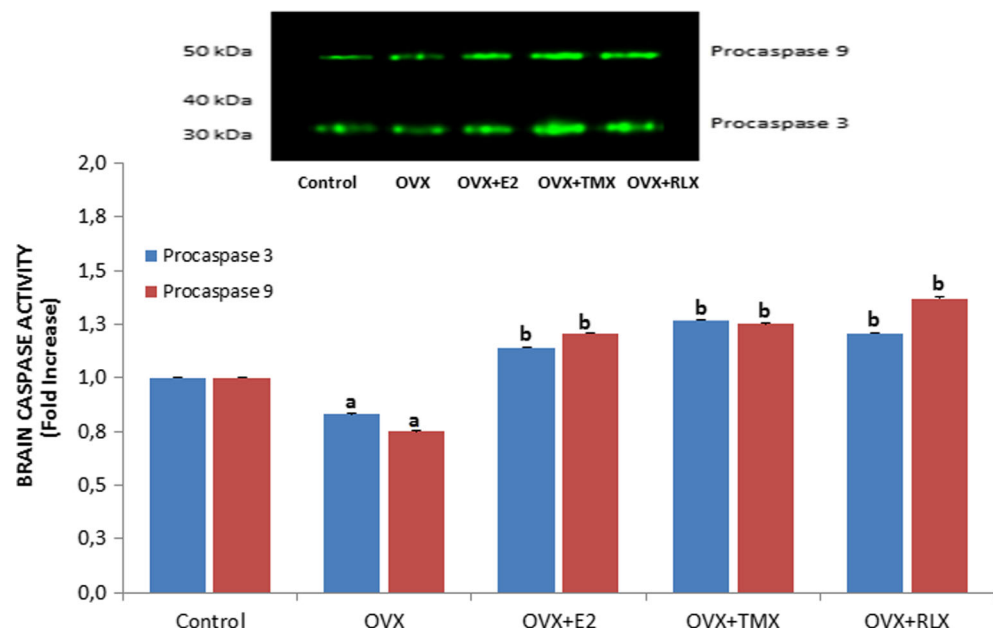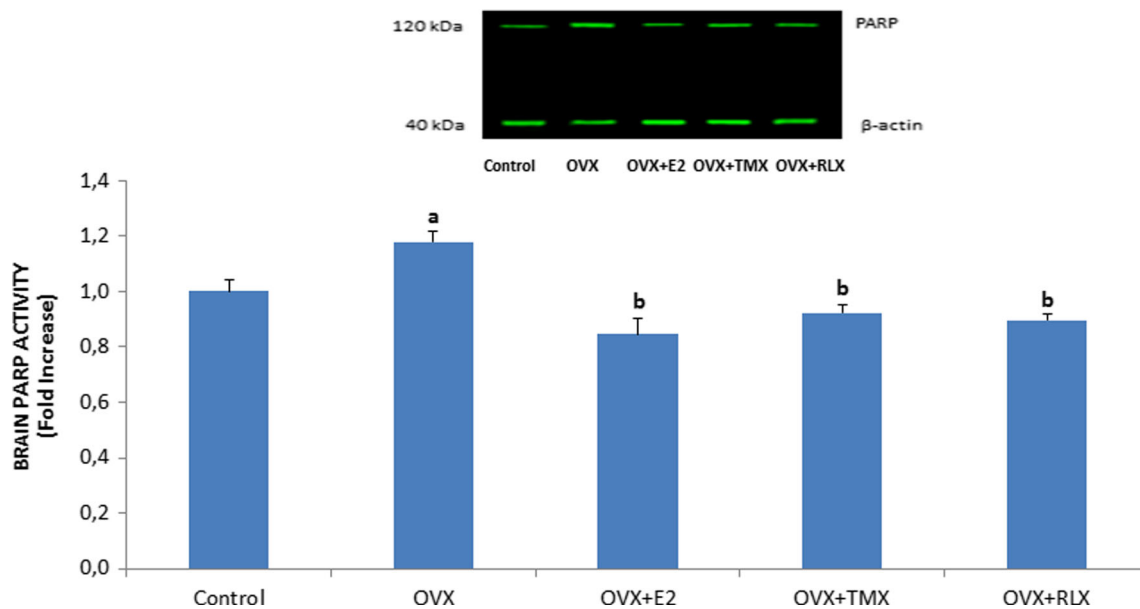
Fig. 5 The effects of 17β-estradiol (E2), tamoxifen (TMX), and raloxifene (RLX) on PARP activity in the brain of ovariectomized (OVX) rats (mean ± SD and $n = 8$). [a]$p < 0.05$ as compared with group control. [b]$p < 0.05$ as compared with group OVX

underpinning for the antioxidant actions of RLX and TMX, and by demonstrating that it reduces lipid peroxidation production in the brain, reduces DNA damage due to excessive ROS production after OVX induction, and decreases proapoptotic caspase 3 and 9 activations. The activation of PARP has been shown to lead to DNA injury, and PARP activity in the brain is markedly increased after OVX induction (Siegel and McCullough 2013). Indeed, previous studies have shown that there is a strong relationship between oxidative stress and PARP activation in the brain after OVX induction (Zhang et al. 2007; Ying and Xiong 2010) and that oxidative stress in neurons can induce PARP activation (Nazıroğlu 2007; Nazıroğlu 2015).

In conclusion, the current results support the idea that the neuroprotective and anti-inflammatory roles of E2, RLX, and TMX are primarily due to their antioxidant-like actions. Therefore, E2, RLX, and TMX can reduce lipid peroxidation, IL-4, and PARP levels in the brain and erythrocytes of rats with OVX by virtue of their inherent antioxidant properties. The antioxidant effects and anti-PARP activities of E2, RLX, and TMX on the brain might be induced by increases in the GSH redox system. Thus, E2, RLX, and TMX treatments may have a beneficial effect in managing oxidative brain injuries as well as their oxidant complications.

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