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RESEARCH ARTICLE

Recuperative effect of estrogen on rotenone-induced experimental model of Parkinson's disease in rats

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Abstract

Parkinson's disease (PD) is described as the loss of dopaminergic neurons located in the substantia nigra (SN) region of the brain and a progressive motor failure. Increased frequency of PD in women, especially after menopause, suggests the effect of estrogen. This view has been supported with empirical studies. Therefore, the effect of estrogen in an experimental model of Parkinson's disease induced by rotenone was investigated. A total of 32 female Wistar Albino rats were randomly assigned to four groups (control group, ovariectomy group, Parkinson's group, Parkinson's + estrogen group). The Parkinson's group received rotenone subcutanously at the dose of 2.5 mg/kg bw, on the 1st, 2nd, 3rd 4th, 6th, 9th, 12th, 15th, 18th, and 21st days animals in the Parkinson's + estrogen group received retonon as in the Parkinson's group and was additionally subcutaneously given estrogen (implant containing 0.5 mg 17 β-estradiol lasting for 21 days). The rats were subjected to rotarod, pole, and swimming tests at the end of the experiment for comparison of their motor activities, and then, histopathological and biochemical analyses were performed on the tissues that were extracted. The rotarod results revealed that Parkinson's group had the shortest time (32.33 ± 3.98 sn) than the groups of control (92.50 ± 12.60 s) ovariectomy (71.42 ± 10.58 s), and Parkinson's + estrogen (71.37 ± 9.26 s). The results of pole disclosed that return and landing time prolonged for Parkinson's group when compared with other groups (return time for control 2.98 ± 0.38 s, ovariectomy 3.02 ± 0.75 s, Parkinson 5.91 ± 0.33 s, Parkinson's + estrogen 3.48 ± 0.42 s and landing time for control 5.30 ± 0.59 s, ovariectomy 5.45 ± 0.73 s, Parkinson 9.80 ± 0.90 s, Parkinson's + estrogen 5.37 ± 1.02 s). Parkinson's group had longest (90.71 ± 12.56 s) swimming time to reach the target when compared with control (33.16 ± 8.68 s), ovariectomy (47.37 ± 12.19 s), and Parkinson's + estrogen (49.82 ± 5.78 s). Histopathological examination indicated a significant difference in tyrosine hydroxylase-stained cells (dopaminergic neurons and dopamine) between the Parkinson's + estrogen group and the Parkinson's group. The biochemical analyses of Caspas-3 activation in SN and striatum (STR) was significantly different between the Parkinson's + estrogen group and the Parkinson's group, but this difference was not observed in STR while evaluating Bcl-2. The results of this study suggested that estrogen may have a recuperative effect on PD.

Keywords Parkinson's disease · Estrogen · Caspase-3 · Bcl-2 · Rotenone

Introduction

In the first report of Parkinson's disease (PD), Dr. James Parkinson described the disease as "involuntary tremor with less muscle strength, even when supported" (Parkinson 1817). PD is a widespread neurodegenerative disorder with age being the major risk factor. The disease affects 0.5–1% of the population between the ages of 65 and 69, while it affects 1–3% of the population over the age of 80 (De Lau and Breteler 2006; Toulouse and Sullivan 2008). There is a digressive loss of dopamine producing neurons in PD in the substantia nigra (SN) pars compacta, resulting in exhaustion of dopamine in striatum (STR). This causes emergence of abnormal dopamine deficiency and extrapyramidal function (Loonam et al. 2003; Centonze et al. 2004) and uncontrolled motor behavioral disorders, postural imbalance, stiffness (Lotharius and Brundin 2002), and bradykinesia (Recchia et al. 2004).

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Experimental studies have demonstrated that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and other mitochondrial function inhibitors (pesticides, rotenone) produce clinical outcomes resembling to PD, including behavioral deficits perceived in PD, nigrostriatal degeneration, and protein aggregation (Vila et al. 2000; Alam and Schmidt 2002; Greenamyre et al. 2003; Sathiya et al. 2013). Rotenone is lipophilic and reported to easily cross the blood brain barrier. Rotenone blocks the complex-I unit of the electron transport chain through accumulation in mitochondria (Dauer and Przedborski 2003; Blandini and Armentero 2012). Intracranial administration of rotenone in rats was demonstrated to result in motor dysfunction and reduce tyrosine hydroxylase (TH) immunoreactivity throughout the nigrostriatal pathway (Carriere et al. 2014).

Apoptosis is programmed cell death that can occur both physiologically and pathologically (Brill et al. 1999), and has a crucial role in development of PD. Apoptosis is induced in two main pathways: activation of cell surface death receptors (extrinsic pathway) (Ashkenazi and Dixit 1998) or mitochondria (intrinsic pathway) (Green and Reed 1998). The Bcl-2 protein family contains pro-apoptotic and anti-apoptotic proteins that fulfill an important function in regulation of apoptosis, particularly in the upstream of irreversible cellular damage, and play a major role in the intrinsic pathway that proceeds on the mitochondrial level (Gross et al. 1999). In the mammalian system, caspases may be grouped as inflammatory caspases (caspases 1, 4, 5), effector or executive caspases (caspases 3, 6, 7), and initiator caspases (caspases 2, 8, 9, 10) (Cohen 1997). The role of initiator caspases and effector caspases is very important for cell death by both intrinsic and extrinsic apoptosis (Nakagawa et al. 2000).

Epidemiological and clinical findings propose that female sex hormones may affect the inception and severity of Parkinson's disease symptoms. For example, PD is observed 50% more frequent in males (Mayeux et al. 1992), suggesting the possible protective effect of estrogens against the disease (Dluzen and McDermott 2000). It was reported that gender differences due to estrogen levels may have an effect on response to treatment of dopaminergic drugs used in treatment of PD, drug clearance, and blood pressure (Wright et al. 1997).

Therefore, the recuperative effects of estrogen on a rotenone-induced experimental model of PD in rats was disclosed by evaluating motor abilities and biochemical and histopathological traits in this study.

Materials and methods

Experimental design

In this study, a total of 32, 4–6-month-old female Wistar Albino rats weighing 190–250 g were used. The animals were kept at room temperature of approximately 25 °C with ventilation and in a 12-h light-dark cycle until the initiation of the study. All groups were fed ad libitum. The rats, 8 animals in each group, were randomly divided into 4 groups. Control group: No intervention was executed. Ovariectomy group: Only ovariectomy was performed in the rats in this group. Rats were underwent to anesthesia using the combination of ketamine (35–50 mg/kg bw; Ketalar, Pfizer, Turkey) and xylazine (10 mg/kg, bw; Alfazyne %2, Ege-Vet, Turkey) Following anesthesia, the rats were laid down on their back and the operation area was shaved and asepsis was achieved. The abdominal cavity was reached by cutting the skin, muscle layers, and peritoneum through median incision. The suspensory ligaments and vessels of the right and left ovaries were ligated using 3.0 vicryl suture and removed. After this procedure, the peritoneum and muscles were sutured with simple continuous and skin with horizontal U stitches.

A penicillin-based antibiotic (Vetimisin, VETAŞ, Turkey) was used for any post-operation complications. Rats were daily examined for any post operation complications. None of the rats developed complications. Parkinson's group: The Parkinson's model was created by applying subcutaneous rotenone (Samantaray et al. 2007) at a dose of 2.5 mg/kg bw for 10 days (1st, 2nd, 3rd, 4th, 6th, 9th, 12th, 15th, 18th, 21st days) after ovariectomy. Parkinson's + estrogen group: Subcutaneous rotenone (Samantaray et al. 2007) was applied for 10 days (1st, 2nd, 3rd, 4th, 6th, 9th, 12th, 15th, 18th, 21st days) at a dose of 2.5 mg/kg bw after ovariectomy and a total of 0.5 mg of 17 β-estradiol-containing implant whose effect lasted for 21 days was placed subcutaneously after PD model developed (Gillies et al. 2004). Rotenone was applied by dissolving in a dimethyl sulfoxide (DMSO) and polyethylene glycol-300 (PEG-300) solution prepared at a 1/1 ratio (Samantaray et al. 2007).

Randomization

A number was assigned to each selected rat, and the numbered rats were allotted to the groups using a web-based randomization protocol (Suresh 2011).

Assessment of motor activities

At the end of the study, rotarod test was implemented to evaluate the motor activity and balance of the animals. An experimental set-up was formed as instructed by Bohlen et al. (2009). The animals were led to run at 30 rpm on a rotarod assembly for five days. On 6th day, the animals were tested in the rotarod mechanism without any intervention, and the time between the initial run and the fall from the treadmill was recorded.

The pole test was also applied to the animals at the end of formation of the groups to determine bradykinesia. The

technique elaborated by Mann and Chesselet (2015) was used for the pole test, and a metal rod with a diameter of 0.8 cm and a length of 50 cm was utilized. The rod was wrapped with gauze to promote better grasping and prevent animal body damage. The animals were adjusted to hold and walk on the rod for 3 days prior to testing. On the 4th day, the animals were left to the top of the rod with a head-up position, and the downward rotation times and landing times were recorded.

Morris maze test was performed to determine the motor activity and learning-memory levels in the animals. For this purpose, a flotation pool (diameter 150 cm, depth 60 cm) was prepared as described by Morris (1984). Triangle, square, and cross marks were placed in the east, west, and north directions, respectively, so that the animals could find directions and learn the location of the platform. A 10- × 20-cm platform was placed on the east side of the pool near the center. The pool was filled up to a depth of 30 cm (27 °C) with water. In order to blur the water and prevent the platform from appearing, some milk powder was added to the water. For flotation testing, the platform was adjusted to remain 2 cm above water for 5 days. The animals were left at the same point, always from the south, and taught to find the platform for 5 days. The rats who could not find the platform in the first minute during the teaching stage were helped in finding the platform and learning its location. On the 6th day, the test was started by submerging the platform 1 cm below the water surface. The rats were released from the same point in the south, and the times of finding the platform without any intervention were recorded for each rat.

Histopathological and biochemical evaluations

At the end of the experiment, all rats were deprived of food overnight, and put out under ketamine/xylazine anesthesia by cervical dislocation in accordance with ethical rules.

The bregma point on the skull was determined and marked for isolation of SN and STR from the brain tissue so as to complete the biochemical and histopathological analyses. Subsequently, according to the rat brain atlas, the anterior − 3.95 to − 6.45 of SN and the anterior − 3.01 to 3.24 of STR were isolated from the marked bregma point of the extracted brain tissue (Papp et al. 2014). The SN and STR specimens extracted in a single lobe of each animal were placed in 10% formaldehyde for histopathological evaluations. A homogenate was prepared with the SN and the STR from the other lobe using a phosphate buffer solution (PBS) for biochemical analysis.

Cell-P program using Olympus bx 53 microscope was used to photograph. TH positive cell count was made using Image J program. The same method was used for STR region; the painted areas were determined as percentage.

The Bcl-2 and Caspase-3 levels in tissues were determined by ELISA kits from the FineTest Company. TH-monoclonal antibody and caspase 3-polyclonal antibody and hematoxylin eosin staining were carried out as histopathological analyses.

Statistical analyses

Analyses of variance for each variable using all morphological, histopathological, and biochemical traits were conducted. The means of all variables for each of the groups were estimated. The mean separations were conducted using Tukey's HSD test. GraphPad 8.1 (San Diego, CA, USA) was used for the statistical analyses. The statistical significance was assessed on the 5% probability level unless otherwise indicated.

The study was initiated after obtaining approval from the Ethics Committee for Animal Experiments at Kafkas University (KAÜ-HADYEK 2017-029).

Results

Motor activities

Rotarod test

The rotarod test results revealed that the mean running times differed significantly among the groups ($P < 0.05$). The mean duration of running was 92.50 ± 12.60 s in the control group, 71.42 ± 10.58 s in the ovariectomy group, 32.33 ± 3.98 s in the Parkinson's group, and 71.37 ± 9.26 s in the Parkinson's + estrogen group. The multiple comparison test indicated that the rats in the Parkinson's group were running on the rotarod device significantly shorter than the other three groups. Nonetheless, the difference among the control, ovariectomy, and Parkinson's + estrogen groups was not statistically significant (Table 1).

Pole test

Pole test results differed significantly among the groups ($P < 0.05$). The mean duration of turning was 2.98 ± 0.38 s in the control group, 3.02 ± 0.75 s in the ovariectomy group, 5.91 ± 0.33 s in the Parkinson's group, and 3.48 ± 0.42 s in the Parkinson's + estrogen group. The mean separation among the groups indicated a significant difference between the Parkinson's group and the other groups ($P < 0.05$). The results of the pole test also indicated that the mean landing time was 5.30 ± 0.59 s in the control group, 5.45 ± 0.73 s in the ovariectomy group, 9.80 ± 0.90 s in the Parkinson's group, and 5.37 ± 1.02 s in the Parkinson's + estrogen group. The mean landing time in the Parkinson's group was significantly higher in comparison to the other groups (Table 1).

Table 1 Means and std. errors of four groups for motor activities

Groups	Rotarod test	Pole test		Morris maze test
	Running time (s)	Turning time (s)	Landing time (s)	Platform finding time (sec)
Control	92.50 ± 12.60	2.98 ± 0.38	5.30 ± 0.59	33.16 ± 8.68
Ovariectomy	71.42 ± 10.58	3.02 ± 0.75	5.45 ± 0.73	47.37 ± 12.19
Parkinson	32.33 ± 3.98*	5.91 ± 0.33*	9.80 ± 0.90*	90.71 ± 12.56*
Parkinson's + estrogen	71.37 ± 9.26	3.48 ± 0.42	5.37 ± 1.02	49.82 ± 5.78
P value	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$

*Means indicated significantly different based on Tukey's mean separation test ($P < 0.05$, $N = 8$).

Morris maze test

The result of Morris-Mae test also revealed a significant difference among the four groups ($P < 0.05$). The mean platform finding time was 33.16 ± 8.68 s in the control group, 47.37 ± 12.19 s in the ovariectomy group, 90.71 ± 12.56 s in the Parkinson's group, and 49.82 ± 5.78 s in the Parkinson's + estrogen group. A pattern similar to the two previous motor activity tests was evident, where the Parkinson's group was significantly deviated from the other groups (Table 1).

Biochemical findings

Caspase-3 analysis

Caspase-3 analyses were performed in both SN and STR of the brain tissue samples. The overall model was highly significant ($P < 0.05$). The quantitative results indicated that the SN caspase-3 value was 15.34 ± 0.07 ng/ml in the control group, 15.99 ± 0.19 ng/ml in the ovariectomy group, 16.49 ± 0.07 ng/ml in the Parkinson's group, and 15.68 ± 0.14 ng/ml in the Parkinson's + estrogen group. There was a significant difference between the control group and the Parkinson's group, as well as between the control group and the ovariectomy group ($P < 0.001$). Similarly, a significant difference was found between the Parkinson's and Parkinson's + estrogen groups ($P < 0.001$). Nonetheless, the difference between the control and Parkinson's + estrogen groups and the difference between the Parkinson's and ovariectomy groups were not statistically significant (Table 2).

When the results of STR caspase-3 were considered, a pattern analogous to motor activities was deduced in which the mean value for the control group was 15.40 ± 0.15 ng/ml, that of the ovariectomy group was 15.83 ± 0.26 ng/ml, the Parkinson's group was 16.71 ± 0.09 ng/ml, and the Parkinson's + estrogen group was 15.67 ± 0.25 ng/ml. A statistically significant difference was found between the Parkinson's group and the other groups ($P < 0.05$). There was no statistically significant difference among the other three groups, indicating a pattern similar to most of the analyses above (Table 2).

Bcl-2 analysis

Mean SN Bcl-2 level was 0.43 ± 0.06 ng/ml in the control group, 0.34 ± 0.02 ng/ml in the ovariectomy group, 0.11 ± 0.06 ng/ml in the Parkinson's group, and 0.30 ± 0.03 ng/ml in the Parkinson's + estrogen group. A significant difference was evident between the Parkinson's group and the other groups ($P < 0.05$). There was no statistically significant difference among the Parkinson's + estrogen group, control group, and ovariectomy group ($P < 0.05$). A significant difference was deduced from STR Bcl-2 results where the mean STR Bcl-2 value for the control group was 0.55 ± 0.04 ng/ml, the one for the ovariectomy group was 0.52 ± 0.03 ng/ml, that of the Parkinson's group was 0.40 ± 0.02 ng/ml, and the one for the Parkinson's + estrogen group was 0.48 ± 0.02 ng/ml. According to these data, a statistically significant difference was found between the Parkinson's and control groups ($P < 0.05$). There was no statistically significant difference between the other groups (Table 2).

Histopathological findings

No histopathological changes were observed in the substantia nigra regions of the brains of the control animals (Fig. 1a). In the brain of the ovariectomy group, there was a decrease in the number of neurons in the substantia nigra regions (Fig. 1b). Neuronal degeneration in the substantia nigra, central chromatolysis, and neuronophagia were detected in the animals in the Parkinson's group (Fig. 1c). Although neuron degeneration and neuronophagia were observed in the Parkinson's + estrogen group, the number of degenerated neurons was found to be lower in comparison to the Parkinson's group (Fig. 1d). The number of neurons in the substantia nigra region was lower in all other groups, especially in the Parkinson's group in comparison to the control group.

Based on immunohistochemical staining, the highest neuronal immunoreactivity group was the control group. The TH-

Table 2 Means and std. errors of four groups for biochemical parameters

Groups	Caspase-3 (ng/ml)		Bcl-2 (ng/ml)	
	SN	STR	SN	STR
Control	15.34 ± 0.07a	15.40 ± 0.15a	0.43 ± 0.06a	0.55 ± 0.04a
Ovariectomy	15.99 ± 0.19bc	15.83 ± 0.26a	0.34 ± 0.02a	0.52 ± 0.03ab
Parkinson	16.49 ± 0.07c	16.71 ± 0.09b	0.11 ± 0.06b	0.40 ± 0.02b
Parkinson's + estrogen	15.68 ± 0.14ab	15.67 ± 0.25a	0.30 ± 0.03a	0.48 ± 0.02ab
P value	$P < 0.001$	$P < 0.05$	$P < 0.05$	$P < 0.05$

a,b,c: Means indicated by different letters are significantly different based on Tukey's mean separation test ($P < 0.05$, $N = 8$).

positive immunoreactivity of the SN and STR regions are shown in Figs. 2a, b, c, and d and 3a, b, c, and d, respectively (SN; Fig. 2; STR, Fig. 3).

The average number of neurons showing immunoreactivity in SN was 116 in the control group (Fig. 2a), 42 in the ovariectomy group (Fig. 2b), 35 in the Parkinson's group (Fig. 2c), and 82 in the Parkinson's + estrogen group (Fig. 2d). A significant increase was observed in positive neurons in animals treated with estrogen.

The mean area of the TH-immunoreactive fibrils in the STR region was 49.74% in the control group (Fig. 3a) and 42.41% in the Parkinson's group (Fig. 3c). This rate was 45.44% in the Parkinson's + estrogen group (Fig. 3d) and 46.44% in the ovariectomy group (Fig. 3b; Table 3). In this context, it was found that there was a statistically significant difference between the Parkinson's group and the control group. Both the Parkinson's group and the control group also differed from the other two groups ($P < 0.001$). The most intense caspase-3 activity was observed in the Parkinson's group (Fig. 4c). While the caspase-3 activity was decreased in the Parkinson's + estrogen group (Fig. 4d), no immunoreactivity was detected in the control (Fig. 4a) and ovariectomy groups (Fig. 4b).

Discussion

In this study, we investigated the effects of estrogen on a rotenone-induced PD model. In addition to the apoptosis parameters, the study also evaluated physical (motor activities), histopathological and immunohistochemical parameters, and discerned information regarding the effects of estrogen on PD.

The rotarod test measures motor abilities in rats or mice (Brooks and Dunnett 2009; Borse et al. 2011) and is indicative of motor coordination and balance (Monville et al. 2006). A significant reduction in the falling time in this study indicated the locomotor and motor activity disorder in PD model, and an increase in the falling time of the PD group undergoing

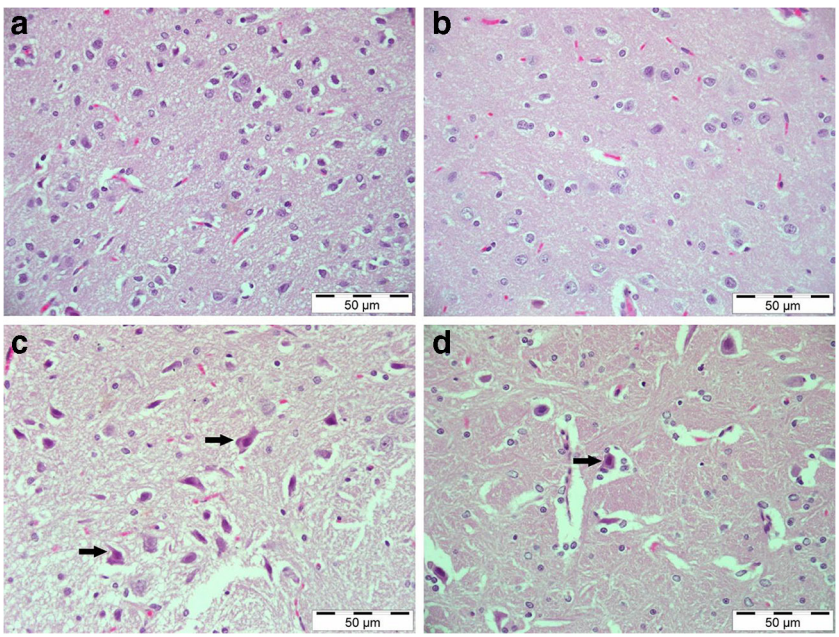

Fig. 1 Hemotoxylin eosin staining in brain tissue of **a** control group, **b** ovariectomy group, **c** Parkinson's group-central cromatolisis (arrow), and **d** Parkinson's + estrogen group-nueronophagy (arrow) (bar = 100 µm)

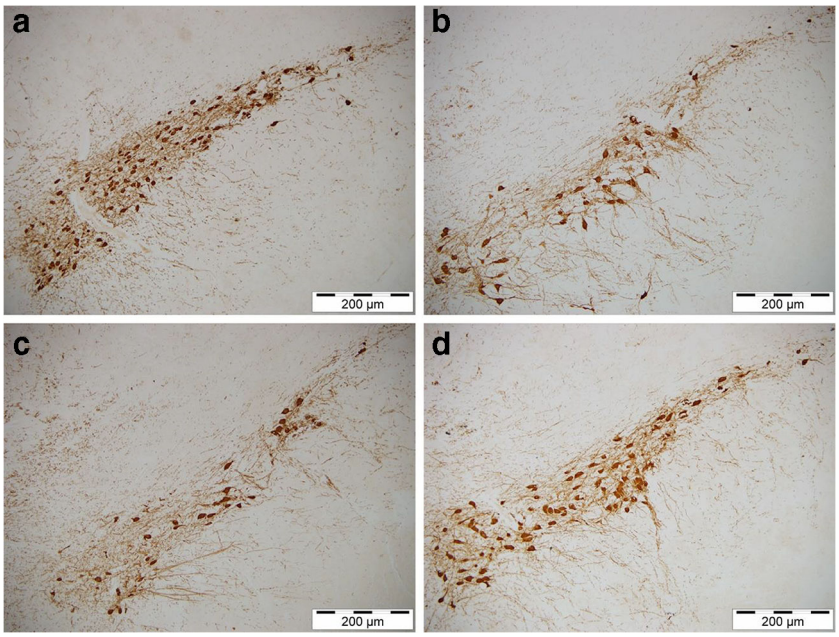

Fig. 2 Immunohistochemical TH (dopaminergic neurons) in SN of **a** control group, **b** ovariectomy group, **c** Parkinson's group, and **d** Parkinson's + estrogen group (bar = 200 μm)

estrogen treatment when compared with the PD group might be due to protective effects of estrogen on this motor activity disorder. Rotarod discrepancies are categorized as "non-dopamine-dependent" motor capacity in Parkinson's disease models, because they can occur before or in the absence of nigrostriatal dopaminergic loss (Magen and Chesselet 2010; Hickey and Chesselet 2011). Thus, the efficacy of estrogen was clearly demonstrated by the rotarod test as reported previously (Rodriguez-Perez et al. 2013; von Wrangel et al. 2015; Nakaso et al. 2016; Zhang et al. 2017).

The pole was previously been used to evaluate basal ganglia-based movement disorders in rats and mice (Ogawa et al. 1985, 1987; Matsuura et al. 1997; Sedelis et al. 2001; Fernagut et al. 2003; Fleming et al. 2004). Here, we detected that bradykinesia in rats with PD was completely improved, and no movement disorder was present in the estrogen-treated group. A similar result was previously reported by Ozsoy et al. (2011).

Flotation test was used in some animal models to assess motor deficits (Schaar et al. 2010). Morris Maze enabled us to test learning and memory impairment. We determined the presence of the protective effect of estrogen on learning and memory by detecting a decrease in the platform finding time among the rats in the PD + estrogen group. The protective

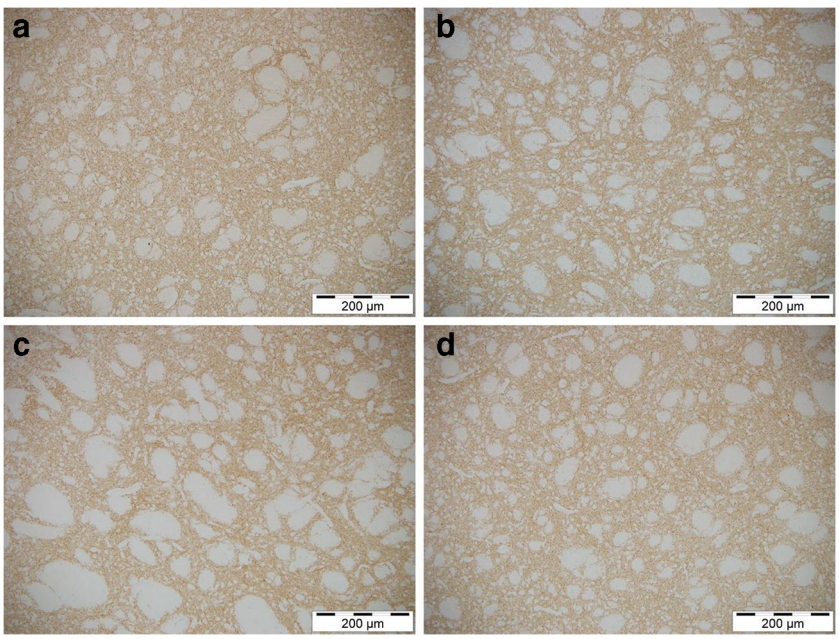

Fig. 3 Immunohistochemical TH (dopamine) in STR of **a** control group, **b** ovariectomy group, **c** Parkinson's group, and **d** Parkinson's + estrogen group (bar = 200 μm)

Table 3 Means and std. errors of four groups for immunohistochemical measurement of dopamine

Groups	The mean area of TH-immunoreactive fibrils in the STR region (%)
Control	49.74 ± 0.64a
Ovariectomy	46.44 ± 0.77b
Parkinson	42.41 ± 0.98c
Parkinson's + estrogen	45.44 ± 0.64b
P value	$P < 0.001$

a,b,c: Means indicated by different letters are significantly different based on Tukey's mean separation test ($P < 0.05$, $N = 8$).

effect of estrogen on learning and memory was previously reported (Campos et al. 2013).

In order for rats to successfully complete the pole, rotarod, and flotation tests, their muscular structures need to work regularly. In this case, motor activity tests are more effective diagnostic methods in dopamine deficiency. Likewise, pathological and biochemical data reveal that these tests provide accurate diagnoses in patients with PD.

PD is reported to be 50% higher in males in comparison to females (Mayeux et al. 1992), suggesting a possible protective effect of estrogen in term of susceptibility to the disease (Dluzen and McDermott 2000). Estrogen replacement therapy was reported to increase the putaminal dopamine active carrier measured by TRODAT SPECT scan in 13 menopausal women with PD (Gardiner et al. 2004). Low-dose estrogens showed slight improvement in motor disability and motor fluctuations in postmenopausal women (Blanchet et al. 1999; Strijks et al. 1999; Tsang et al. 2000; Nicoletti et al. 2007). Both in vivo and in vitro studies have already demonstrated estrogen possess antioxidant and anti-inflammatory properties (Simpkins et al. 2009).

Parkinson's disease result in loss of dopaminergic neurons due to mitochondrial complex-1 dysfunction as was the case in our study where rotenone-induced mitochondrial dysfunction and thus PD. Mitochondria produce the majority of cellular ATP and reactive oxygen species (ROS) through mitochondrial electron respiratory chain. Under oxidative stress, mitochondrial processes play critical role in cellular life or death. Estrogen is proved to protect mitochondrial function by preserving mitochondrial membrane potential in cell culture models. (Dykens et al. 2003; Simpkins et al. 2009) Additionally, estrogen alleviates mitochondria-derived ROS (Wang et al. 2001). Previous studies suggest that estrogen reduces mitochondrial inflammation process, especially with its antioxidant effect. Furthermore, estrogen can inhibit the release of microglial superoxide and phagocytic activity which may shed light on estrogen's anti-inflammatory effect (Bruce-Keller et al. 2000).

We determined that estrogen in female Wistar Albino rats with a rotenone-induced PD model had a significant effect on motor activities and apoptosis that are common in PD. Employing tyrosine hydroxylase immunohistochemical staining, it was deduced that estrogen administration had a protective effect on dopaminergic neurons in SN that vanished in PD. Additionally, we also determined that the reduction of dopamine in the STR in PD was also significantly suppressed by estrogen administration. The amount of dopamine was determined via the density of the stained area in the form of composition. Dopamine and dopaminergic neurons detected by TH immunohistochemical staining are also important in

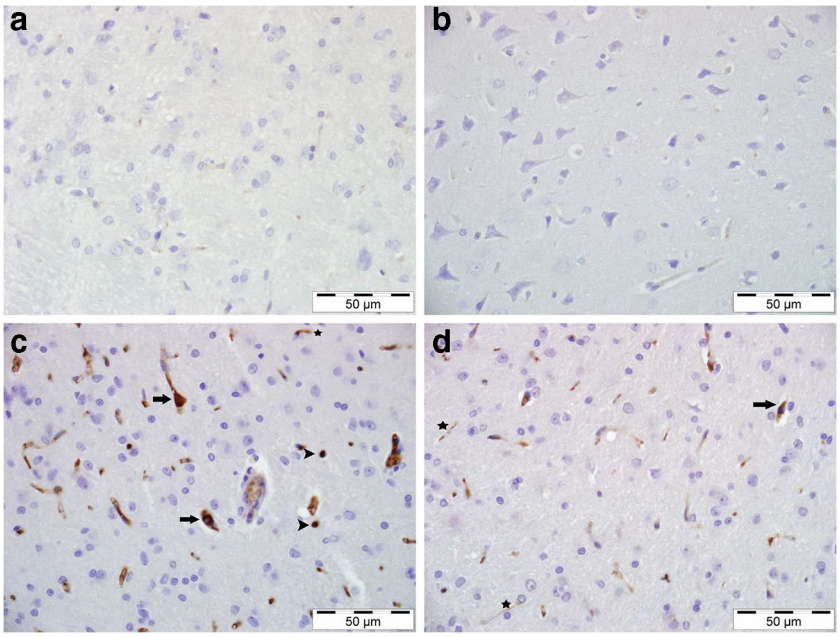

Fig. 4 Immunohistochemical caspase-3 in SN of brain tissue of **a** control group; **b** ovariectomy group; **c** Parkinson's group-neuron (arrow), neuroglial cells (arrow) and neuroglial fibers (star); and **d** Parkinson's + estrogen group-neuron (arrow) and neuroglial extensions (star) (bar = 50 μm)

the diagnosis of PD (Nordström et al. 2015). TH immunohistochemical staining has been used in diagnosis in the same way (Gören 2009; Ozsoy et al. 2011; Di et al. 2012; Lee et al. 2012; Haytural and Tüzün 2013; Rodriguez-Perez et al. 2013; Kim et al. 2014; Nordström et al. 2015; Nakaso et al. 2016; Zhang et al. 2017).

The pathogenesis of PD involves the death of a large number of cells (apoptosis). It is known that both intrinsic and extrinsic pathways are effective in deterioration of dopaminergic neurons. Particularly in models with mitochondrial complex-1 inhibition, the importance of the intrinsic pathway, the mitochondrial pathway, is known to be great. However, it was reported that the extrinsic pathway may be effective on this intrinsic pathway (Da Costa and Checler 2011). In particular, we focused on the mitochondrial pathway, the intrinsic pathway of apoptosis, based on the rotenone-induced mitochondrial complex-1. In this context, we obtained significant results by focusing on the Bcl-2 intrinsic pathway and Caspase-3 parameters which are the last steps of apoptosis.

We also found that when the rotenone group and the control group were compared in terms of apoptosis, Bcl-2 was in agreement with the results reported by Haytural and Tüzün (2013). Nonetheless, the Caspase-3 parameters were not similar to the aforementioned study. However, when the estrogen group and Parkinson's group were compared, Bcl-2 in SN showed an increase in the estrogen group, but no statistically significant increase was observed in STR. Thus, we determined that BCl-2 activity in SN increased with estrogen administration. Another parameter, Caspase-3, showed a statistically significant decrease in both STR and SN when the Parkinson's group was compared with the Parkinson's + estrogen group. The loss of specific dopaminergic neurons by inhibition of the mitochondrial complex-1 with rotenone induction is well documented. In this context, SN-Bcl-2 data in the intrinsic mechanism of the mitochondrial pathway of apoptosis support neuronal loss. The significant difference detected in the Caspase-3 data in STR is congruent with the mechanism. We also observed that caspase-3 immunohistochemically stained the cells in the Parkinson's group, which confirmed the biochemical Caspase-3 results. Similar results were also reported by Samantaray et al. (2007).

It is known that estrogen alters both the functions of presynaptic SN neurons and postsynaptic targets in the striatum, and thus, it has a strong modulating effect on dopaminergic translocation within the basal ganglia (Shulman 2002). Additionally, estrogen is known to have a positive effect on BCL, which is anti-apoptotic (Ünal et al. 2010). In this study, we determined the protective effect of estrogen on cell death by using biochemical and pathological evaluations in PD.

In conclusion, the data gathered in this study may indicate that estrogen might had very important regulatory and recuperative effects on the course and symptoms of the disease in the experimental rat model of PD.

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Contributors MM and HAE developed the study. MM conducted the trial design, data acquisition, data interpretation. MM and HAE contributed to writing of the report. All authors approved the final draft of the manuscript.

Data availability Not applicable.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval The study was initiated after obtaining approval from the Ethics Committee for Animal Experiments at Kafkas University (KAÜ-HADYEK 2017-029).

Consent to participate Not applicable.

Consent to publication Not applicable.

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