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Investigation of the pharmacological, behavioral and biochemical effects of boron on rats with rotenone-induced Parkinson's Disease

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ABSTRACT

Parkinson's disease (PD) is a progressive neurodegenerative disorder of the central nervous system**.** In different studies, it has been investigated that boric acid has positive effects on different mechanisms that are important in PD. The aim of our study was to investigate the pharmacological, behavioral and biochemical effects of boric acid on rats with experimental PD with Rotenone. For this purpose, Wistar-albino rats were divided into 6 groups. Only normal saline was applied subcutaneously (s.c) to the first control and sunflower oil to the second control group. Rotenone was administered (s.c) to 4 groups (groups 3-6) at a dose of 2 mg/kg for 21 days. Only rotenone (2mg/kg, s.c) was administered to the third group. Boric acid was administered intraperitoneally (i.p.) at 5 mg/kg, 10 mg/kg, and 20 mg/kg to groups 4, 5, and 6, respectively. During the study, behavioral tests were applied to the rats, and then histopathological and biochemical analyzes were performed from the sacrificed tissues. According to the data obtained, a statistically significant difference ($p<0.05$) was observed between the Parkinson's group and the other groups in motor behavior tests, excluding the catalepsy test. Boric acid exhibited dose-dependent antioxidant activity. As a result of the histopathological and immunohistochemical (IHC) examination, a decrease in neuronal degeneration was observed at the increasing doses of boric acid, while gliosis and focal encephalomalacia were rarely encountered. There was a significant increase in tyrosine hydroxylase (TH) immunoreactivity, especially in group 6, with a dose of 20 mg/kg of boric acid. From these results, we conclude that the dose-dependent effect of boric acid may protect the dopaminergic system with antioxidant activity in the pathogenesis of PD. However, the effectiveness of boric acid on PD needs further investigation in a larger, more detailed study using different methods.

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Introduction

Parkinson's disease (PD) is a progressive disease characterized by the degeneration of dopaminergic neurons in the substantia nigra and interruption of the transmission of dopaminergic pathways, resulting in motor symptoms and the formation of Lewy bodies in neurons of the hippocampus and cerebral cortex of the central nervous system (1). The most important marker in the diagnosis of PD is the loss of fifty percent of nigrostriatal dopaminergic neurons and the presence of intracytoplasmic α-synuclein positive Lewy bodies (LC) and dystrophic Lewy neurites, especially in the subcortical nuclei and hippocampus, and less frequently in the cerebral cortex (2). At the onset of symptoms, up to 60-80% of dopaminergic neurons are lost in the Substansia Nigra, while dopamine metabolites such as homovanillic acid, 3,4-dihydroxy phenyl acetic acid (DOPAC), dopamine transporter (DAT) and tyrosine hydroxylase (TH) are at reduced levels in the striatum (3).

Although many pathophysiological hypotheses have been put forward about the pathogenesis of PD in which many mechanisms and aging, genetic and environmental factors play a role, there are two hypotheses that predominate. One of these is protein misfolding and aggregation. The other is mitochondrial dysfunction. The most important of these hypotheses; are oxidative stress damage and mitochondrial dysfunction, neuron death due to excitotoxicity, neuroinflammation, familial/genetic factors and the prion hypothesis (3-7).

Many animal models are used in PD. The most common and valid method today is the models created with toxins. These toxins are rotenone, paraquat, and 6-Hydroxy dopamine (6-OHDA), and 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP) (8). Rotenone is a potent mitochondrial Complex I enzyme inhibitor pesticide, and administration of rotenone causes a uniform Complex I inhibition in the rat brain. It is, therefore, different from MPTP, which is selectively toxic to dopaminergic neurons because it is dependent on the dopamine (DA) transporter and 6-OHDA, which can only be given by local injection. In addition to uniform Complex I inhibition, rotenone causes selective degeneration of the nigrostriatal dopaminergic pathway (9).

Oxidative damage occurs with rotenone, and as a result of chronic systemic exposure, selective oxidative damage occurs especially in dopaminergic brain regions. It has also been reported in in-vitro studies that rotenone causes oxidative stress and eliminates the toxic effects of antioxidant therapy (10). Behaviorally, as a result of exposure to rotenone, symptoms similar to PD occur in

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rats (11).

Boron (B) is a metalloid with semiconductor properties, which is not found alone in nature, but in combination with other elements. The simplest of these compounds are boron oxide (B_2O_3) and boric acid (H_3BO_3, BA). Boric acid is odorless, tasteless, and stable in the air. It is a weak acid in the form of white crystals, which is absorbed quite rapidly and excreted from the body in the urine (12, 13). The World Health Organization (WHO) recommends that the average safe intake level of boron for adults should be 1-13 mg/day, and the upper limit should be 20 mg/day (14). Recent studies show that boron plays a role, especially in the development of the immune system, brain functions, and blood cells, as well as in the prevention and treatment of cancers, reproductive system, and bone diseases (15).

In addition, many in-vivo and in vitro studies have reported that the tube has antioxidant activity (16-19). Since 96% of boron is found in the form of boric acid in organisms, boric acid was preferred as the boron component in our study. It was aimed to investigate the pharmacological/ biochemical, behavioral and immunohistochemical effects of the experimental PD model created in rats to investigate the anti-parkinsonian activation.

Materials and Methods

Subjects

In this study, experimental groups were created from 52 Wistar albino female rats with a body weight between 200-250 g rats housed in rooms at 22 ± 2 °C, fed standard pellets and fresh water ad libitum. The animal care protocol and experimental study was approved by the Animal Care and Use Committee of Van YuzuncuYil University (2020/05-01).

Procedure

The animals to be used in the experiment were randomly assigned to 6 groups, with 6 rats in the control and sunflower oil (solvent) groups and 10 rats in the other drug-administered groups.

1. Control group 1 (C1, n=6): The rats were administered 0.1 mg/kg saline (0.9% NaCl) by the intraperitoneal (IP) route for 21 days.
2. Control group 2 (C2, n=6): The rats were administered 0.1 mg/kg of sunflower oil by the subcutaneous (SC) route for 21 days.
3. Rotenone group (ROT, n=10): The rats were administered 2 mg/kg of Rotenone dissolved in sunflower oil by SC route for 21 days (20).
4. Boric acid 5 mg/kg + Rotenone group (B5+ROT, n=10): 5 mg/kg boric acid (by dissolving in 0.9 NaCl) IP was administered to rats 2 hours before rotenone administration. 2 mg/kg rotenone was dissolved in solvent (sunflower oil) and administered to rats by SC route for 21 days (21).
5. Boric acid 10 mg/kg + Rotenone group (B10+ROT, n=10): 10 mg/kg boric acid (by dissolving in 0.9 NaCl) IP was administered to rats 2 hours before rotenone administration. 2 mg/kg rotenone was dissolved in solvent (sunflower oil) and administered to rats by SC route for 21 days (21).
6. Boric acid 20 mg/kg + Rotenone group (B20+ROT, n=10): 20 mg/kg boric acid (by dissolving in 0.9 NaCl) IP was administered to rats 2 hours before rotenone administration. 2 mg/kg rotenone was dissolved in solvent (sunflower oil) and administered to rats by SC route for 21 days (21).

Behavioral tests were performed at the beginning of the study (on day 0) and 24 hours after the 21st day of drug administration. After all the applications on the rats were finished, they were sacrificed and blood, organ and tissue materials were taken and prepared for analysis.

Behavioral Procedures

Monitoring General Movements

To evaluate the development of Parkinson's, the general movements of rats were evaluated according to the Ludolph movement analysis test scores (22). Before the start of the study, on day 0 and 24 hours after the 21st day of the study, the behavior of each animal was evaluated and a corresponding number was given according to the following criteria, respectively; normal behavior: 0; a general slowdown in displacement due to a slight glitch in the hind legs:1; irregularity in movements and abnormalities in gait:2; paralysis of the hind leg: 3; difficulty in movement due to paralysis in the front and hind legs:4; lying on back: 5.

Locomotor Activity Measurement

The locomotor activity (square crossings) of the rats was measured inside a box as the number of transitions from one square to another. The bottom of the box was divided into six squares of equal size, and the number of squares passed in the setup was evaluated as locomotor activity. All four legs of the animal were completely switched to the other square, and all squares it passed through for a total of 15 minutes were counted (23, 24).

Catalepsy Measurement

To assess the cataleptic behavior, a stick test was measured (25). In the stick test, the rats were placed with their forelegs on a 9 cm high horizontal bar, parallel to the ground, in a semi-standing position, and then the clock was run. As soon as the rat lifted one of its legs from the stick, the timer was stopped and the time was recorded. The longest time the rat should stay on the stick was determined to be 180 seconds (26).

Measurement of Muscle Activity

The muscle activities of the rats were measured with the Rotarod device. First, the speed of the instrument was fixed at 20 rpm and the measurement was made.To familiarize the rats with the device, two preliminary trials were performed. The time that the rats could stay in the Rotarod device was measured. The highest value that rats that did not fall off the Rotarod device could stay in was 120 seconds (26).

Cylinder Test

The cylinder test is a method in which the forelimbs are determined by the number of times they touch the cylinder wall to measure motor asymmetry in rats with Parkinson's. (27). Initially, three mirrors are placed at different angles on the side of a 25 cm diameter glass cylinder ensuring that all sides of the cylinder can be seen during recording. Then, the behavior of the rat placed in the cylinder was observed for 10 minutes, counting how many times the rats touched the cylinder wall with their right forelimbs,

left forelimbs, and both right and left forelimbs at the same time. Situations where the right and left forelimbs were used simultaneously to touch the cylinder wall, were recorded for 35 seconds as separate right and left touches. The results were calculated as the ratio of the number of wall touches with the ipsilateral (right) and contralateral (left) forelimbs to the total number of touches.

Determination of Acetylcholinesterase Activity (AsChE)

Ellman method of activity of acetylcholinesterase enzyme (28) was used. The basis of this method is to measure the yellow anion (2-nitro-5 thiobenzoate) formed during the reaction at pH 8 at 412 nm in the spectrophotometer. Thiocholine ester was used as substrate.

Total Antioxidant Status (TAS) and Total Oxidant Status (TOS) Analysis

TAS and TOS levels were measured by spectrophotometric methods (29, 30). Results are expressed as mmolTrolox® q / L and as mol H2O2 q/L. The ratio of TOS to TAS is defined as the oxidative stress index (OSI) and is a marker of oxidative stress. The OSI value was calculated according to the formula below. OSI (arbitrarynite unit, AU) = [TOS (μmol H2O2 Eq/L)/TAS (μmolTroloxEq/L) × 10] 26.

Immunohistochemical Analysis

Histological Examination

Tissues from animals were fixed in 4% paraformaldehyde for 24 hours. Tissues were embedded in paraffin after histological processing. 5 μm thick sections were taken from paraffin blocks by a microtome. The sections stained hematoxylin-eosin. Sections were then sealed with Entellan (Merk 1.07961.0500, Germany), and viewed under a light microscope (Olympus BX53, Tokyo, Japan). Histopathological examination was evaluated semiquantitatively according to the degree of pathological changes observed in the tissue.

Immunohistochemical Examination

After the tissues were fixed in 4% paraformaldehyde for 24 hours, they were embedded in paraffin blocks following routine histological steps. The sections of 5 μm thickness were taken from paraffin blocks to poly-lysine slides by a microtome. The sections taken were deparaffinized and rehydrated. An immunohistochemical procedure was performed on brain sections using Tyrosine Hydroxylase (anti-TH, Bioss, dilution ratio: 1/200, bs-0016R), Tumor Necrosis Factor-alpha (anti-TNF-alpha, Bioss, dilution ratio: 1/100, bs-2081R) and inducible Nitric Oxide Synthase (anti-Inos, Bioss, dilution ratio: 1/100, bs-2072R) primary antibodies. The samples were covered with entellan and examined under a light microscope (Olympus BX53, Tokyo, Japan).

Statistical Analysis

SPSS (version 20) was used for TAS and TOS OSI analyses. Kruskal-Wallis, a non-parametric analysis method, was preferred for the differences between the groups ($p<0.05$). One-Way ANOVA was used to determine which group caused the difference. A post hoc multiple comparison test (Tukey HSD) was then used. Means with a p-value of 0.05 or less were considered significant relative to each other.

SPSS (version 20) was used for Ludoph movement analysis, locomotor activity, catalepsy and muscle activity measurements and cylinder test statistical analysis. One-Way ANOVA was used to determine which group caused the difference. Afterward, the post hoc multiple comparison test (Tukey HSD) was used. Means with a p-value of 0.05 or less were considered significant when compared with each other.

Descriptive statistics (traits) for the variables studied in the cylinder test analysis were presented as median, mean, standard deviation, minimum and maximum values. The Kruskal-Wallis test was performed to compare the groups. The Wilcoxon test was also used to compare the right and left paws. The statistical significance level was accepted as 5% and SPSS (version 20) program was used for all statistical calculations.

Results

Biochemical Tests Results

TAS levels were significantly decreased in the ROT, ROT+B5 and ROT+B10 groups compared to the C1 group ($p<0.001$). Statistically significant decreases were observed in the ROT and ROT+B5 groups compared to the C2 group, and a significant increase was observed in the ROT+B20 group ($p<0.05$). It was observed that TAS levels increased statistically significantly in all Boron-administered groups compared to the ROT group ($p<0.001$), but the highest increase was observed in the ROT+B20 group (Figure 1).

TOS levels increased significantly in the rotenone ($p<0.001$), ROT+B5 ($p<0.05$) and ROT+B10 ($p<0.05$) groups compared to the C1 group. It was observed that TOS levels were statistically significantly decreased in the ROT+B20 group compared to the ROT group ($p<0.001$, Figure 2).

OSI levels were significantly increased in the ROT ($p<0.001$), ROT +B5 ($p<0.001$) and ROT+B10 ($p<0.05$) groups compared to the C1 group. A statistically significant increase was observed in the ROT ($p<0.001$) and ROT+B5 ($p<0.05$) groups compared to the C2 group. Statistically significant decreases were observed in the ROT+B10 ($p<0.05$) and ROT+B20 ($p<0.001$) groups compared to the ROT group (Figure 3).

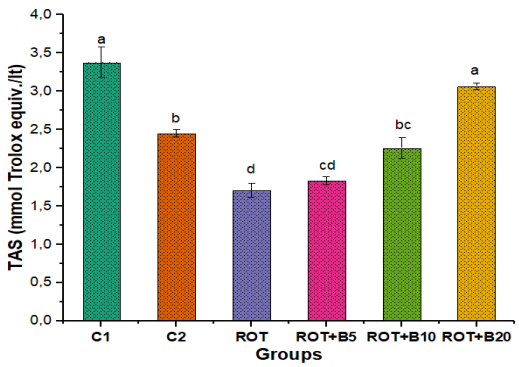

Figure 1. TAS levels (mmol Trolox equivalent/L, C1: Control normal saline, C2: Control sunflower seed oil, ROT: Rotenone, ROT+B5: Rotenone+Boric acid (5mg/kg), ROT+B10: Rotenone+Boric acid (10mg/kg), ROT+B20: Rotenone+Boric acid (20mg/kg)). *a,b,c,d: Different lowercase letters represent statistically significant differences between groups (p<0,05).*

AsChE levels were significantly decreased in the ROT+B10 group compared to the C1 group ($p<0.05$, Figure 4).

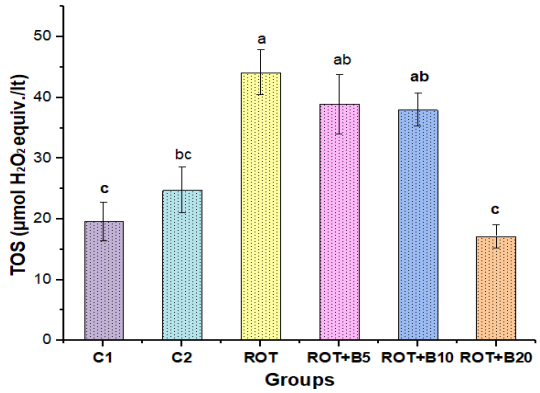

Figure 2. TOS levels (µmol H2O2 equivalent/L, C1: Control normal saline, C2: Control sunflower seed oil, ROT: Rotenone, ROT+B5: Rotenone+Boric acid (5mg/kg), ROT+B10: Rotenone+Boric acid (10mg/kg), ROT+B20: Rotenone+Boric acid (20mg/kg)). *a,b,c,d: Different lowercase letters represent statistically significant differences between groups (p<0,05).*

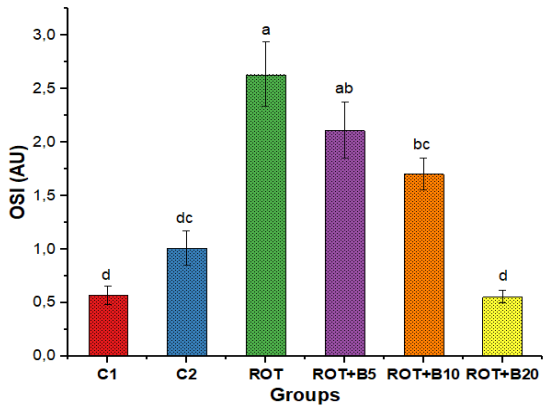

Figure 3. OSI levels (arbitrarynite unite; AU, C1: Control normal saline, C2: Control sunflower seed oil, ROT: Rotenone, ROT+B5: Rotenone+Boric acid (5mg/kg), ROT+B10: Rotenone+Boric acid (10mg/kg), ROT+B20: Rotenone+Boric acid (20mg/kg)). *a,b,c,d: Different lowercase letters represent statistically significant differences between groups (p<0,05).*

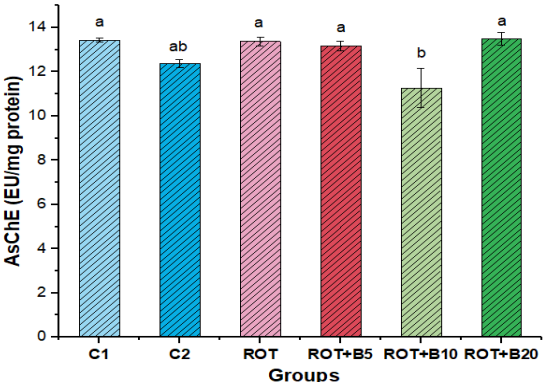

Figure 4. AsChE activity levels (EU/mg protein, C1: Control normal saline, C2: Control sunflower seed oil, ROT: Rotenone, ROT+B5: Rotenone+Boric acid (5mg/kg), ROT+B10: Rotenone+Boric acid (10mg/kg), ROT+B20: Rotenone+Boric acid (20mg/kg)). *a,b,c,d: Different lowercase letters represent statistically significant differences between groups (p<0,05).*

According to the Ludolph motion analysis test, there was a significant increase in the ROT group compared to the C1 and C2 groups (movements were impaired). There was a significant decrease in the movement analysis of the ROT+B10 and ROT+B20 groups compared to the ROT group (movements improved, $p< 0.05$), except for ROT+B5 (Figure 5).

Locomotor activity was evaluated. While the number of square passes of the ROT group decreased significantly compared to the C1 and C2 groups, there was a significant increase in the number of square passes of the ROT+B10 mg/kg and ROT+B20 mg/kg groups compared to the ROT group (Figure 6).

While a significant decrease was observed in the Rotarod device residence time in the ROT group compared to the C1 and C2 groups, the Rotarod residence time of the ROT+B5, ROT+B10 and ROT+B20 groups increased significantly compared to the ROT group ($p < 0.05$, Figure 7).

In the device, the behavior of the animals to lift the right and left forelimbs alone or at the same time was evaluated on the 21st day of the study. The rotenone group showed a significant decrease in the behavior of lifting the right paw alone and simultaneously with both the right and left paws compared to the control groups($p<0.05$, Figure 8).

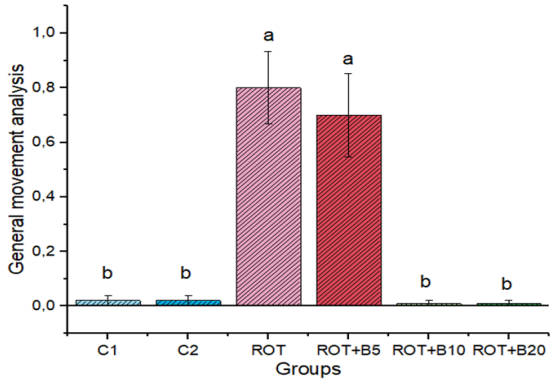

Figure 5. General Movement Analysis (Ludolph Movement Analysis). C1: Control normal saline, C2: Control sunflower seed oil, ROT: Rotenone, ROT+B5: Rotenone+Boric acid (5mg/kg), ROT+B10: Rotenone+Boric acid (10mg/kg), ROT+B20: Rotenone+Boric acid (20mg/kg). *a,b,c,d: Different lowercase letters represent statistically significant differences between groups (p<0,05).*

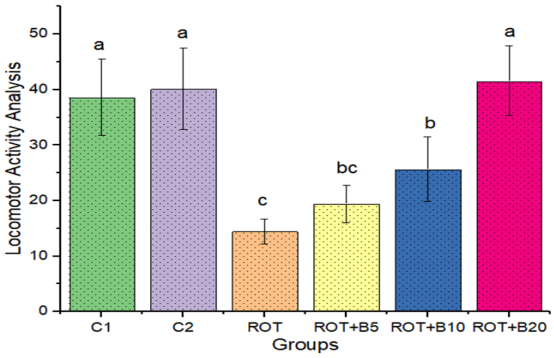

Figure 6. Locomotor Activity Analysis (Square Crossing). C1: Control normal saline, C2: Control sunflower seed oil, ROT: Rotenone, ROT+B5: Rotenone+Boric acid (5mg/kg), ROT+B10: Rotenone+Boric acid (10mg/kg), ROT+B20: Rotenone+Boric acid (20mg/kg). *a,b,c,d: Different lowercase letters represent statistically significant differences between groups (p<0,05).*

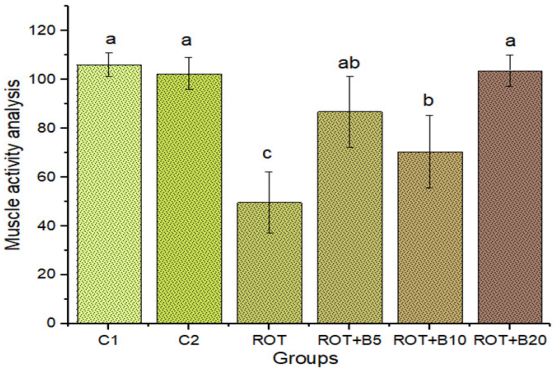

Figure 7. Muscle Activity Analysis (According to the Rotarod analysis test). C1: Control normal saline, C2: Control sunflower seed oil, ROT: Rotenone, ROT+B5: Rotenone+Boric acid (5mg/kg), ROT+B10: Rotenone+Boric acid (10mg/kg) ROT+B20: Rotenone+Boric acid (20mg/kg). *a,b,c,d: Different lowercase letters represent statistically significant differences between groups (p<0,05).*

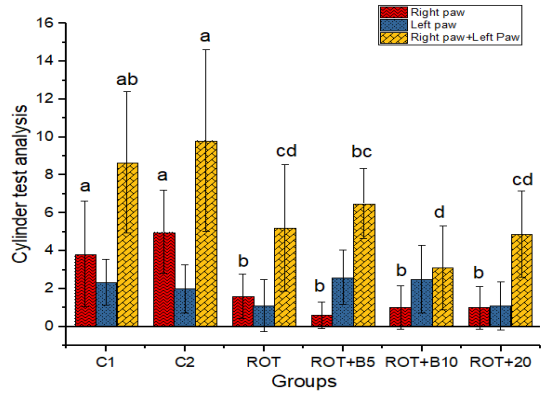

Figure 8. Cylinder Test Analysis. C1: Control normal saline, C2: Control sunflower seed oil, ROT: Rotenone, ROT+B5: Rotenone+Boricacid (5mg/kg), ROT+B10: Rotenone+Boricacid (10mg/kg), ROT+B20: Rotenone+Boric acid (20mg/kg). *a,b,c,d: The lowercase letters in the same column represent statistically significant differences among the groups.*

Evaluation of Immunohistochemical Analysis

Hematoxylin-Eosin Evaluation

In the histological examination of the cortex and the striatum, the normal neuronal histological structure was observed in the control groups treated with saline and sunflower oil. Neuronal degeneration, gliosis and focal encephalomalacia in many areas were observed in the rotenone group. Similar findings were observed in the Boric acid (5mg) + Rotenone group with the Rotenone group. Neuronal degeneration, gliosis and focal encephalomalacia decreased in the boric acid (10 mg) + Rotenone group. In the boric acid (20 mg) + Rotenone group, neuronal degeneration was decreased, and gliosis and focal encephalomalacia were rarely observed (Figure 9).

Immunohistochemical Analysis

In the immunohistochemical examination in the striatum, TH immunoreactivity was normal in the control groups to which serum physiological and sunflower oil were applied, a significant decrease in TH immunoreactivity was observed in the rotenone group, and a significant increase in TH immunoreactivity was observed in the treatment groups, especially in the boric acid 20 mg group, depending on the increasing dose (Figure 10).

In the immunohistochemical examination in the cortex, TNF-alpha immunoreactivity was normal in the control groups treated with serum physiological and sunflower oil, a significant increase in TNF-alpha immunoreactivity in the rotenone group, and no significant change in TNF-alpha immunoreactivity was detected in the treatment groups at 5 and 10 mg doses of boric acid. A low level of decrease in TNF-alpha immunoreactivity was detected at 20 mg dose of boric acid (Figure 11).

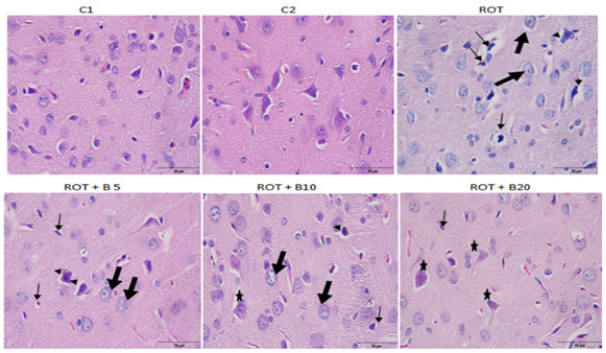

Figure 9. C1: Control (saline / serum physiologic) normal neurons C2: Control (sunflower oil) normal neurons ROT+ B5: Rotenone + Boric Acit (5 mg), ROT+ B10: Rotenone + Boric Acit (10 mg) ROT+ B20: Rotenone +Boric Acit (20 mg, thin arrow: Degeneration of the neurons, thick arrow: Gliosis, arrowhead: Focal encephalomalacia, black star: Normal neurons (Hematoxylin-Eosin, Scale bars: 50 µm).

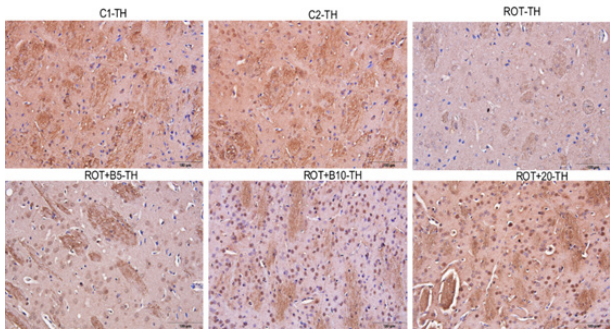

Figure 10. C1 TH: Control (saline / serum physiologic) Tyrosine Hydroxylase, C2 TH: Control (sunflower oil) Tyrosine Hydroxylase, ROT+ B5 TH: Rotenone + Boric Acit (5 mg) Tyrosine Hydroxylase, ROT+ B10 TH: Rotenone + Boric Acit (10 mg) Tyrosine Hydroxylase, ROT+ B20 TH: Rotenone +Boric Acit (20 mg) Tyrosine Hydroxylase (Scale bars: 100 µm).

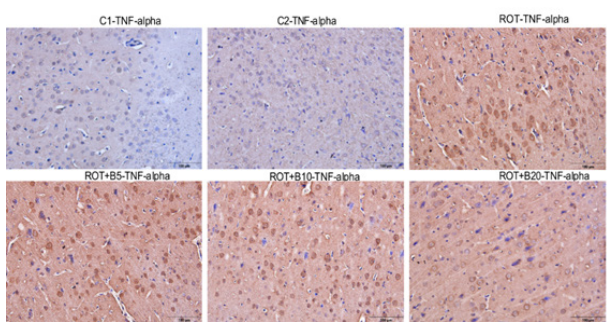

Figure 11. C1 Tumor necrosis factor-alpha (TNF-alpha): Control (saline / serum physiologic) TNF-alpha, C2 TNF-alpha: Control (sunflower oil) TNF-alpha, ROT+ B5 TNF-alpha: Rotenone + Boric Acit (5 mg) TNF-alpha, ROT+ B10 TNF-alpha: Rotenone + Boric Acit (10 mg) TNF-alpha, ROT+ B20 TNF-alpha: Rotenone +Boric Acit (20 mg) TNF-alpha (Scale bars: 100 µm).

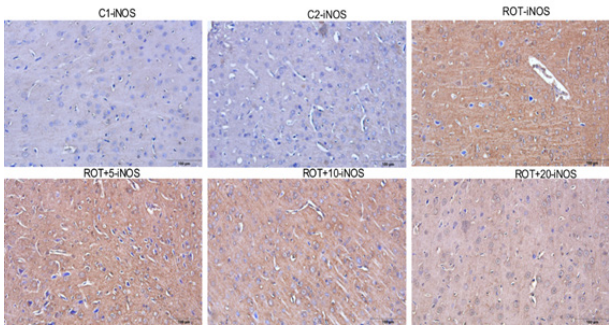

Figure 12. C1 Inducible nitric oxide synthase (iNOS): Control (saline / serum physiologic) iNOS, C2 iNOS: Control (sunflower oil) iNOS, ROT+ B5 iNOS: Rotenone + Boric Acid (5 mg) iNOS, ROT+ B10 iNOS: Rotenone + Boric Acid (10 mg) iNOS, ROT+ B20 iNOS: Rotenone +Boric Acid (20 mg) iNOS (Scale bars: 100 µm).

In the immunohistochemical examination of the cortex, iNOS immunoreactivity was normal in the control groups to which serum physiological and sunflower oil was applied, there was a significant increase in iNOSimmunoreactivity in the rotenone group, and no significant change was found in the iNOSimmunoreactivity in the treatment groups at 5 and 10 mg doses of boric acid. A decrease in iNOSimmunoreactivity was observed at a dose of 20 mg of boric acid (Figure 12).

Discussion

Parkinson's disease is defined as neuronal loss and extensive intracellular protein (alpha (α)-synuclein) accumulation in certain areas of the substantianigra region of the brain, and these two main neuropathologies are considered an important criterion in the diagnosis of Idiopathic Parkinson's disease (31-34).

In Parkinson's disease, neuronal degeneration in certain regions of the brain occurs in specific neuron types, and the loss of dopaminergic neurons is that other midbrain dopaminergic neurons are preserved and limited to the ventrolateral substantianigra region, but this last stage makes the disease even more special. The excessive loss of neurons even at the beginning of the disease indicates that the damage in this region begins before the onset of motor symptoms. Recent clinical studies support this view (31, 32).

Other neuropathology is the accumulation of abnormal aggregates of α-synuclein in the cytoplasm of certain neurons in different brain regions, and Lewy bodies composed largely of accumulated α-synuclein are microscopic bodies that were described many years ago. Following the development of histopathological methods, a wider range of α-synuclein aggregates were identified and Lewy bodies appeared to occur in neurons in the olfactory system and in cholinergic and monoaminergic neurons at the onset of the disease. This progression of α-synuclein aggregates was also present in limbic and neocortical brain regions. (31, 32, 34).

Rotenone, which is used as a broad-spectrum insecticide, herbicide and pesticide, has been reported to induce degeneration of the nigrostriatal dopaminergic system, similar to the histological changes of Parkinson's disease, and also cause behavioral changes when given to rats (11, 35). As an experimental model of Parkinson's Disease, ultrastructural changes were observed in the substantia nigra at doses of 1.5-2.5 mg/kg/day, where the appropriate dose for rats was 2 mg/kg/day, but 2 mg/kg/day rotenone did not reduce the neuropathology of parkinsonism (α-Synuclein Aggregation etc.) and it has the advantage of practical application with its low mortality rate (36, 37).

Subcutaneous rotenone administration resulted in a decrease in the immunoreactivity of TH immunohistochemically together with histological changes (9,38), while TNF-α resulted in an increase in both blood profile and immunoreactivity, and studies also showed an increase in iNOS immunoreactivity (39-41). In our study, as a result of the administration of 2 mg/kg/day of subcutaneous rotenone, a decrease in TH immunoreactivity and an increase in TNF-α and iNOS immunoreactivity were observed in the rotenone group, which is consistent with other studies (38,40,41).

The findings showed that boric acid provided dose-related improvement in the histological examination of the striatum with hematoxylin-eosin, especially in the brain tissue. While a dose-dependent increase was observed in TH enzyme activity, which is the rate-limiting step of dopamine production and an important biomarker in the pathogenesis of PD, a significant increase was observed in TH immunoreactivity, especially in the 6th group, which was administered 20 mg of Boric Acid. The preservation of the activity of the TH enzyme, which has a role in the pathogenesis of PD, has aroused a strong curiosity about the unknown potential for boric acid and has created a strong argument for future research. It has been reported in previous studies that rotenone triggers inflammation with oxidant activity (9, 39, 40). According to our own findings, rotenone caused a significant increase in TNF-α and iNOS, which are important inflammation biomarkers. Again, recent studies (40) report that the progression of Parkinson's disease and anti-inflammatory agents that can show neuroprotective effects can prevent or reduce neurodegeneration by inhibiting inflammatory oxidative enzymes. In our study, although boric acid showed mild anti-inflammatory activity in the groups in which 5, 10 mg/kg was administered, it was shown that neurodegeneration caused by the increase in TNF-alpha and INOS immunoreactivity could be reduced, with a more pronounced neuroprotective effect, especially in the groups administered 20 mg/kg.

In the experimental rotenone model, it is seen that rotenone causes an increase in oxidant capacity and a decrease in antioxidant capacity (20, 40, 42-44). Rotenone provides a role in neuronal loss by causing damage due to oxidative stress by being a specific complex I inhibitor to increase oxidative stress-mediated neuropathology in the pathogenesis of Parkinson's. The fact that rotenone increases oxidative stress in the brain or causes an increase in blood oxidative stress biomarkers in previous studies and in our study is explained by this mechanism (20, 45, 46).

In addition, apoptotic factor changes due to oxidative stress caused by rotenone are seen not only in brain tissue but also in other systems (47-48). The findings of our study show that it improved brain inflammation, immunohistochemical parameters (iNOS and TNF- α) and blood tissue oxidative stress parameters (TAS, TOS), and protected rotenone-induced dopaminergic damage with the local and systemic increase in antioxidant capacity.

Studies with boric acid also reported the oxidant capacity and other proinflammatory biomarkers decreased and total antioxidant capacity increased (13,49-51).

The findings of the study showed that the antioxidant activity of boric acid is dose-dependent and that it can prevent oxidant damage caused by rotenone toxicity with its antioxidant activity. Additionally, it has a protective effect against the loss of dopaminergic neurons due to oxidative stress underlying Parkinson's pathogenesis, and it has a positive health effect against many pathological conditions related to Parkinson's disease and oxidative stress. It has been concluded that it may have prophylactic as well as therapeutic efficacy.

AsChE is mainly found at neuromuscular junctions and cholinergic-type chemical synapses due to its function of terminating synaptic transmission (52). It is thought that acetylcholinesterase plays a role in the apoptosis mechanism in the pathogenesis of the neurotoxin model of PD (53). Abdel-Salam et al. reported that rotenone caused a decrease in AsChE activity (40). AsChE levels in our study were significantly decreased only in the Rotenone+Bor10 group compared to the SF control group. This situation can be interpreted as the result of the individual differences in the animals in the experimental group and the variability of the methods in AsChE determination. It can be suggested that AsChE activity should be examined in rotenone models in comparison with different methods in future studies

PD, is an idiopathic disease of the nervous system, presenting motor and non-motor symptoms. Although it occurs more commonly in the elderly as a chronic and progressive neurodegenerative disease, it can also be seen in younger individuals. Parkinson's patients often have resting tremor, rigidity, bradykinesia, and sluggish posture. In addition, this disease may be associated with neurobehavioral diseases (depression, anxiety), cognitive impairment and autonomic dysfunction (such as orthostasis and hyperhidrosis) (54).

In the general movement analysis, which we evaluated with the Ludolph analysis scale, while there was a significant deterioration in movements in the rotenone group compared to the control groups, these deteriorations showed gradual improvement in movements according to the dose of boron applied. In our study, in which we evaluated locomotor activity with the number of square transitions, the decrease in activity in the rotenone group showed improvements with boron application. In addition, in the analysis where we evaluated the muscle activity with a rotarod, the decrease in the efficiency with rotenone was corrected with boron applications, and an increase in the muscle activity was observed. In the analysis in which we evaluated the motor asymmetry with the cylinder test, the foreleg lifting behavior of the rotenone group showed a significant decrease compared to the control group. This indicates a decrease in motor functions. Many studies, including behavioral and movement disorders induced by rotenone administration, have shown similar results to our findings (39, 55-60).

In conclusion, depending on the dose of Boric acid, It has been shown that it improves motor behaviors, and when examined histopathologically and immunohistochemically, it improves neuronal degeneration, protects the dopaminergic system, and increases antioxidant capacity.

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Conflicts of interest

There are no conflicts of interest.

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