MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

Effect of methylglyoxal on Parkinson's disease pathophysiology in the rotenone model

Yekta CULPAN[1], Lara OZDEN[2], Yakup GOZDERESI[2], Beril KOCAK[2], Zeynep Hazal BALTACI[2], Ayberk DENIZLI[2], Betul KARADEMIR YILMAZ[3], Rezzan GULHAN[1]

[1] Department of Medical Pharmacology, School of Medicine, Marmara University, Istanbul, Turkey
[2] School of Medicine, Marmara University, Istanbul, Turkey
[3] Department of Medical Biochemistry, School of Medicine, Marmara University, Istanbul, Turkey

Corresponding Author: Yekta CULPAN
E-mail: yculpan@yahoo.com.tr

Submitted: 22.12.2023 **Accepted:** 05.01.2024

ABSTRACT

Objective: Type 2 diabetes mellitus patients have been reported to have a higher incidence of Parkinson's disease. This study aimed to explore the effect of advanced glycation end products precursor methylglyoxal (MGO) on the pathophysiology of Parkinson's disease in a rotenone model.

Materials and Methods: Adult female Wistar rats (n=42) were divided into four groups. Rotenone toxicity was assessed by daily weight measurements and mortality rates. Effect of MGO on blood glucose was evaluated. Locomotor activity, rearing, and rotarod tests were performed to evaluate motor functions, and for neurodegeneration, tyrosine hydroxylase immunoreactivity in the striatum and substantia nigra regions was assessed.

Results: The mortality rate was 9% in the rotenone-applied rats. The mean weight, locomotor activity, rearing activity, and longest time spent on a rotarod were lower in the MGO+Rotenone group than in the Control group. Tyrosine hydroxylase immunoreactivity in the striatum rostral to the anterior commissure in the MGO+Rotenone group was lower than that in the Control and MGO groups. The number of tyrosine hydroxylase positive cells in the substantia nigra pars compacta was comparable among the groups.

Conclusion: When nigrostriatal degeneration was triggered, MGO was found to worsen motor dysfunction and increase damage to dopaminergic neuron projections.

Keywords: Parkinson's disease, Type 2 diabetes mellitus, Methylglyoxal, Rotenone, Tyrosine hydroxylase, Locomotor activity

1. INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease. The prevalence of PD is reported to be 1-2‰ in the general population and >1% in the population over 65 years of age [1, 2]. As the incidence of the disease increases with age, PD is becoming an even more important health problem in our aging world.

Parkinson's disease is characterized by the loss of dopaminergic neurons, particularly in the substantia nigra pars compacta (SNpc). Loss of dopaminergic neurons causes a marked reduction in dopamine in the nigrostriatal dopaminergic terminals from the SNpc to the striatum and is the main cause of the classical motor symptoms of PD, such as bradykinesia, tremor, postural instability, and rigidity [3].

Although, the molecular mechanisms have not been fully elucidated, the pathogenesis of PD is generally thought to be associated with oxidative stress, mitochondrial disorders, endoplasmic reticulum stress (ERS), genetic factors, excitotoxicity, impaired vesicular transport, protein processing disorders, and loss of synapses in dopaminergic neurons [4, 5]. Misfolding, aggregation, and toxicity of the alpha-synuclein protein encoded by the alpha-synuclein gene (SNCA) is one of the most important points in the pathophysiology of PD [6]. Precipitated alpha-synucleins contribute to the increase in misfolded proteins, ERS, and thus to the development of PD by disrupting protein homeostasis [7].

Among the risk factors for PD are advanced age, genetic factors, exposure to pesticides and heavy metals, traumatic brain injury, excessive consumption of dairy products, and type 2 diabetes mellitus (T2DM) [8, 9].

The association between PD and T2DM has started to receive attention as a result of the high incidence of PD in T2DM patients

How to cite this article: Culpan Y, Ozden L, Gozderesi Y, et al. Effect of methylglyoxal on Parkinson's disease pathophysiology in the rotenone model. Marmara Med J 2024: 37(2):166-177. doi: 10.5472/marumj.1480086

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1480086
Marmara Med J 2024;37(2): 166-177

in recent studies [10]. In the meantime, studies have shown that PD treatment is less effective and that PD progression is more severe in patients with T2DM [11].

Type 2 diabetes mellitus is characterized by progressive loss of insulin secretion from pancreatic beta cells, relative insulin deficiency, and hyperglycemia in the background of insulin resistance [12]. Recent studies indicate that β-cell dysfunction and insulin resistance, which are the two most important parameters of T2DM, may be caused by oxidative stress [13]. The association of T2DM with oxidative stress is partly related to the overproduction of reactive oxygen species (ROS). Advanced glycation end products (AGEs) have drawn attention among the sources of ROS identified in diabetic conditions. AGEs are harmful compounds that occur more often, especially under hyperglycemic conditions, and are thought to be involved in the pathology of T2DM and the development of complications [14]. Methylglyoxal (MGO) is a highly reactive AGE precursor that occurs during the glycation process and is involved in the pathology of diabetes [15]. The amount of MGO in the serum of T2DM patients was higher than that of healthy individuals [16].

The molecular cause of this remarkable association between T2DM and PD is currently being investigated. MGO may play a role in this relationship. One of the target proteins of MGO is the alpha-synuclein protein involved in PD pathology [17]. Furthermore, MGO is structurally similar to 3,4-dihydroxyphenylacetaldehyde, which has been shown to increase the risk of developing PD [18, 19]. Finally, MGO induces ERS, and ERS is one of the common and early findings in PD [7].

In our study, we aimed to investigate the effect of long-term MGO administration on the pathophysiology of PD in a rotenone model in rats. For this purpose, the effect of MGO both alone and in the presence of a predisposing condition to PD was investigated.

2. MATERIALS and METHODS

Animals

Forty-two adult female Wistar albino rats (2-3 months old, 203-274 g weight) were used in the experiments. Animals were housed two animals per plexiglass cage under a 12-hour light/dark cycle at 22±2°C and had free access to standard rat chow and tap water. All rats were purchased from Istanbul University Aziz Sancar Institute of Experimental Medicine and the experiments were started after a one-week adaptation period.

All experimental procedures were approved by the Marmara University Ethical Committee for Experimental Animals (96.2021mar).

Experimental design

First, the rats were divided into MGO or control (water) administration groups. Then, the groups were further divided into rotenone or its vehicle (2% dimethyl sulfoxide, 98% Miglyol 812 N) subgroups. Consequently, a total of four groups were formed: Control (water+vehicle of rotenone, n=10), MGO (MGO+vehicle of rotenone, n=10), Rotenone (water+rotenone, n=11), and MGO+Rotenone (n=11). MGO and water were administered orally, rotenone and its vehicle were administered subcutaneously.

Rats were treated with MGO (100 mg/kg/day) or water for 8 weeks (55 days, per oral). Before the rotenone administration, two weeks of MGO were administrated to mimic the AGE effect of T2DM. The rotenone (1.5 mg/kg/day) or its vehicle was started at day-16 and applied for 40 days while MGO or water was administrated concurrently. Behavioral experiments were carried out on day 56, the day after the last treatment. Then, all rats were sacrificed on day 56 under diethyl ether (Merck, Darmstadt, Germany, 100921) anesthesia, and their brains were removed for immunohistochemistry (Figure 1).

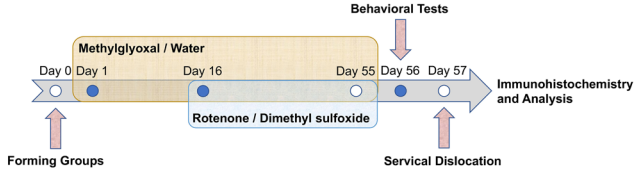

Figure 1. *Timeline of the experimental design. (Blue dots represent days of blood glucose measurement)*

Drugs

Methylglyoxal (Sigma-Aldrich, St. Louis, Missouri, United States of America, M0252) was supplied as a 40% solution in water. To be able to apply 100 mg/kg/day MGO to animals orally, a volume of 213 µL/kg of MGO solution was administered by an automatic pipette (Socorex Acura Manuel, Ecublens, Switzerland, 825). Water (213 µL/kg) administration was similar to MGO administration.

Rotenone (Sigma-Aldrich, St. Louis, Missouri, United States of America, R8875) was prepared daily as a 50x (75 mg/mL) solution in 100% dimethyl sulfoxide (DMSO) (Supelco, St. Louis, Missouri, United States of America, 1.02952). Then, it was diluted with the medium-chain triglyceride Miglyol 812 N (IOI oleochemical, Pulau Pinang, Malaysia) to obtain a rotenone concentration of 1.5 mg/mL (98% Miglyol 812 N, 2% DMSO). The solutions were protected from light. The possibility of precipitation was eliminated by vortexing before each application, and 1.5 mg/kg/day (1 ml/kg) rotenone was administered subcutaneously [20].

A vehicle solution of rotenone was prepared with DMSO and Miglyol 812 N. DMSO was diluted in Miglyol 812 N to a 2% concentration (98% Miglyol 812 N, 2% DMSO). Then, the solution was applied as 1 ml/kg subcutaneously.

Animals were weighed every morning during the experiment, and solutions were applied according to their daily weight. To prevent rotenone-related deaths, the injections were skipped in

http://doi.org/10.5472/marumj.1480086
Marmara Med J 2024;37(2): 166-177

the animals that lost more than 15% of their initial weight until they recovered their weight back.

Behavioral tests and blood glucose measurement

Behavioral experiments were carried out between 9:00 a.m. and 12:00 p.m. The experiments were started after the animals were acclimated to the room for 30 minutes.

Locomotor activity

Locomotor activity test allows a comprehensive study of locomotor and behavioral activity levels in experimental animal models of PD [21]. The test was performed after an acclimation period to the locomotor activity cage. Animals were kept in the room for thirty minutes before the behavioral tests began. The test was performed under moderate illumination and the researcher observed the experiment away from the locomotor activity cage. The rats were placed in a 40x40x40 cm locomotor activity cage (Commat, ACT 508, Ankara, Türkiye), and all movements were recorded for 5 minutes. Before the experiment with the next animal, the locomotor activity cage was cleaned with 70% ethanol (Sigma-Aldrich, St. Louis, Missouri, United States of America, V001229), followed by distilled water, and allowed to dry. Data (distance traveled, % of ambulatory and stereotypical movement time, % of resting time, vertical movement time) were collected with the activity measurement program (Activity metering software, version 2.1, Commat, Ankara, Türkiye) and saved as Microsoft Office Excel (Microsoft Corporation, Redmond, WA, USA) output.

Rearing activity

Rats were placed in a clear plexiglass cylinder (height=30 cm, diameter=20 cm) and recorded for 2 minutes by a video camera. The number of contacts of the forelimbs to the cylinder wall was evaluated [22]. For the behavior to be considered rearing, the animal must raise its forelimb above shoulder level and touch one or both forelimbs against the cylinder wall. Before another rearing, the rat must pull its forelimbs off the cylinder wall and touch the table surface.

Rotarod test

The rotarod test is used to measure motor coordination in mice and rats and to detect motor impairment in experimental PD models[21]. Before the test, the rats were placed on the rod twice daily for three days to allow for acclimatization. The test was performed using a 5 cm diameter rotor (Northel, Bursa, Türkiye). On the experiment day, the rats were placed on the rotating bar, which was accelerating at a constant acceleration, reaching a speed of 40 rotations per minute after 300 seconds. The time spent on the rod was recorded. The test was repeated three times at intervals on the same day. The data were analyzed to determine the average time of the three tests and the longest time spent on the rod.

Blood glucose measurement

Blood glucose was measured between 12:00 – 12:30 p.m. on the 1st, 16th, and 56th days of the experimental protocol (Figure 1). Before the measurement, the tails of the rats were cleaned with 70% ethanol solution and allowed to dry. The lateral vein of the tail was punctured with the help of an insulin injector. The first drop of blood was wiped with cotton, and the second drop of blood was analyzed on a glucometer (Roche, Accu-Chek Performa Nano, Basel, Switzerland) with an appropriate strip (Roche, Accu-Chek Performa, Basel, Switzerland).

Immunohistochemistry

Tissue preparation

The brains were removed after cervical dislocation under diethyl ether anesthesia and placed in a 4% paraformaldehyde (AFG Bioscience, 668011, Illinois, United States of America) solution prepared with 0.1 M phosphate-buffered saline (PBS) (Sigma-Aldrich, St. Louis, Missouri, United States of America, P4417) for immunohistochemistry analyses [23]. The brains were kept in 4% paraformaldehyde solution for 3 days. Then, they were placed into 15% and 30% sucrose (Sigma-Aldrich, St. Louis, Missouri, United States of America, S8501) solutions prepared with 0.1 M PBS, one day apart and were allowed to sink to the bottom in 30% PBS-sucrose solution at 4°C. Then, the brains were treated with methylbutane (Sigma-Aldrich, St. Louis, Missouri, United States of America, 277258), brought to – 30°C with the help of dry ice for 3 minutes, and raised to – 80°C until sectioning. Serial coronal sections (40 µm thick) were taken by a freezing microtome (Microm, Witney, United Kingdom, HM450) from the level of bregma 3.70 mm to the level of bregma – 6.72 mm according to the stereotaxic rat brain atlas [24]. Free floating sections were stored in antifreeze solution (phosphate solution containing 30% glycerol and 30% ethylene glycol) at 4°C.

Immunohistochemical labeling of tyrosine hydroxylase

Sections were rinsed with Tris-buffered saline (TBS) three times, then kept in Tris-EDTA solution at 80°C for 30 minutes and then at room temperature for 20 minutes for possible antigen retrieval treatment. Rinsed sections were kept in a mixture of 3% H_2O_2 and 10% methanol in TBS for 30 min to quench endogenous peroxidase activity and rinsed twice with TBS and once with TBS-Triton X (TBS-T). Nonspecific binding sites were blocked by incubation in TBS-T containing 5% (w/v) normal serum (Vector Laboratories, S2000, RRID: AB_2336617, California, United States of America). Sections were incubated with a tyrosine hydroxylase (TH) antibody (Merck, MAB5280, RRID: AB_2201526, 1:3000, Darmstadt, Germany) containing 1% (w/v) bovine serum albumin/TBS-T for a whole day at room temperature. The next day, the sections were rinsed with TBS-T three times and incubated with biotinylated secondary antibody (Vector Laboratories, BA-2001, RRID: AB_2336180, 1:200, California, United States of America) for 1 hour, rinsed with TBS-T, and incubated in avidin-biotin-peroxidase solution (Vectastain, PK-6100, RRID: AB_2336819, California, United

http://doi.org/10.5472/marumj.1480086
Marmara Med J 2024;37(2): 166-177

States of America) for 1 hour. Finally, the staining was visualized using 2% 3,3′-diaminobenzidine in TBS solution containing 0.01% H_2O_2.

Evaluation of tyrosine hydroxylase immunostaining

All sections were photographed by a microscope (Zeiss Axio Zoom. V16, Zeiss, Germany) connected to a camera system. The striatum region, which contains the dopaminergic fibers, was assessed for TH immunoreactivity positivity (TH+) using densitometric measurement. The evaluation of the SNpc region, where the dopaminergic cell bodies are located, was evaluated by counting TH+ cell bodies. Densitometric measurement and cell count were made using ImageJ software (V1.53, NIH, USA) and evaluated by using Microsoft Office Excel.

Striatum

The striatum is divided into 3 regions according to the rat brain atlas to analyze the immunoreactivity [24]. For ease of explanation, sections taken from the striatum in between AP: 2.28 mm — 0.00 mm from the bregma were labeled as rostral to the anterior commissure (RAC), from AP: 0.00 mm — – 0.48 mm level of the anterior commissure (LAC), and from AP: – 0.48 mm — 1.08 mm caudal to the anterior commissure (CAC). For the striatum region, a total of 7 photographs were evaluated from the RAC, LAC, and CAC regions at x20 magnification. Because the striatum has a patchy appearance, 6 areas with immunoreactivity and 3 areas without immunoreactivity (background regions) were selected in each photograph for immunoreactivity density assessment. To standardize the values, the average immunoreactive region value was subtracted from the average background region value and corrected for each animal by calculating the immunoreactivity of naive animals. Data are given as averages for the RAC, LAC, and CAC measurements.

SNpc

Sections between AP: – 4.80 mm — – 5.40 mm reference to bregma were named SNpc sections. For each brain, 5 photos per section from 3 sections containing the SNpc region were taken at x40 magnification. Cell bodies were marked and counted with the free-marking option using the ImageJ software program. Data are given as the average number of cells contained in each photograph.

Statistical Analysis

All statistical analyses and graphical representations were performed using GraphPad Prism v8 (GraphPad Prism version 8.0.0 for Mac, GraphPad Software, San Diego, California USA, www.graphpad.com) and Microsoft Office Excel. Continuous data are presented as the mean ± standard error. Frequency and percentage (%) were used to represent categorical variables. The experimental groups were compared with one-way ANOVA, and pairwise comparisons were performed using the post hoc Tukey test. Blood glucose measurements on the 1st, 16th, and 56th days were compared using analysis of variance for repeated measurements (repeated-measures ANOVA). The statistical significance level was accepted as $p<0.05$.

3. RESULTS

Administration of solutions and mortality

All animals in the control (n=10) and MGO+Rotenone (n=11) groups survived. One animal out of 10 from the MGO group died on the 51st day of the experiment, while two animals out of 11 from the Rotenone group died on the 31st and 47th days of the experiment. In our study, mortality in animals treated with rotenone was 9% (2/22).

To prevent rotenone-related deaths, the rotenone injections were skipped in the animals that lost more than 15% of their initial weight until they recovered their weight back while MGO/water administration was continued as long as the animal survived. The rotenone group received rotenone for 35±2 days, and the MGO+Rotenone group received rotenone for 30±3 days.

Weight change

The average daily weights of the groups recorded throughout the experiment are shown in Figure 2. The mean weights of the groups on day 0, day 16 (the beginning of rotenone/vehicle application), and day 55 (the last day of the experiment) were compared both within and between groups. There was a significant weight gain in the control group ($p<0.001$). While there was no significant difference between weights at baseline (238±14 g) and on the 16th day (241±14 g) ($p=0.333$), weight on the 55th day (262±5 g) was significantly higher than that on days 0 and 16 ($p<0.001$, both). The mean weight of the MGO group increased on day 55, but there was no significant difference between the weight at baseline (236±7 g), day 16 (241±7 g), and day 55 (247±13 g) ($p=0.322$). The mean weight of the Rotenone group decreased on day 55 of the experiment, but there was no significant difference between the weight at baseline (234±17 g), on day 16 (237±15 g) and day 55 (226±32 g) ($p=0.194$). The mean weight of the MGO+Rotenone group decreased on day 55 of the experiment, but there was no significant difference between the weight at baseline (241±23 g), day 16 (244±19 g) and day 55 (217±42 g) ($p=0.082$).

When the weight was compared between the groups, there was no significant difference between the weight of the groups at baseline and the 16th day ($p=0.737$; $p=0.822$, respectively), while there was a significant difference between the weight on the 55th day ($p=0.022$). In pairwise comparisons, while there was a significant difference between the weight of the control group and the MGO+Rotenone group ($p=0.023$), there was no significant difference between the MGO group and the Rotenone group with each other and with the other groups ($p>0.05$).

Behavioral tests and blood glucose measurement

Behavioral experiments were performed in the Control group (n=10), the MGO group (n=9), the Rotenone group (n=9) and the MGO+Rotenone group (n=9). In addition to the 3 animals that died, 2 rats in the MGO+Rotenone group were not included in the locomotor activity tests due to their unfavorable general condition.

http://doi.org/10.5472/marumj.1480086
Marmara Med J 2024;37(2): 166-177

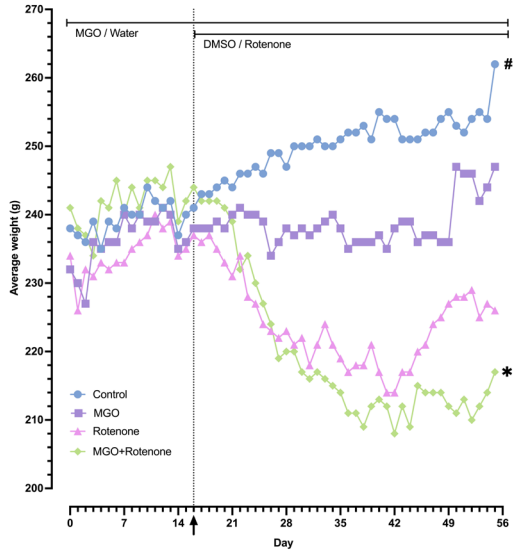

Figure 2. *The average daily weight of the groups recorded throughout the experiment. (The dotted line and arrow indicate the start day of rotenone or DMSO administration. * There was a significant difference between the average weights of the groups on the 55th day (p=0.022). In pairwise comparisons, the average weight of the MGO+Rotenone group was lower than that of the Control group on the 55th day (p=0.023). # There was a significant weight gain in the Control group on the 55th day (262±5 g) than that on days 0 and 16 (p<0.001, both))*

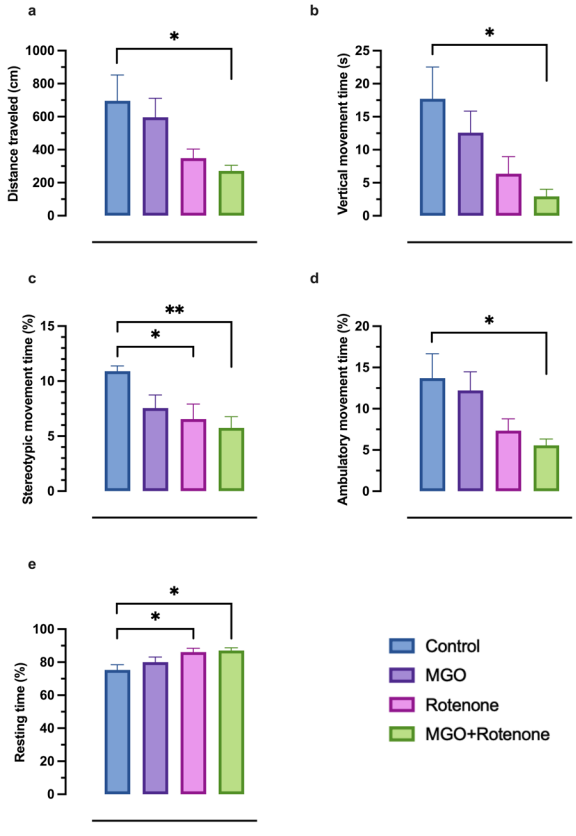

Figure 3. *Comparison of the locomotor activities of the groups. (a) Distance traveled (cm), (b) vertical movement time (s), (c) stereotypic movement time (%), (d) ambulatory movement time (%), (e) resting time (%). (Mean ± standard error of mean was given. * p<0.05, ** p<0.01, one-way ANOVA)*

http://doi.org/10.5472/marumj.1480086
Marmara Med J 2024;37(2): 166-177

Locomotor activity

The distance traveled was significantly different between groups (p=0.023). In pairwise comparisons, the distance traveled by the MGO+Rotenone group (272±33 cm) was significantly lower than that the distance traveled by the Control group (696±157 cm) (p=0.035). There was no significant difference according to the distance traveled between the MGO group (596±115 cm) and the Rotenone group (348±55 cm) and the other groups (p>0.05) (Figure 3a).

The vertical movement time was significantly different between groups (p=0.017). In pairwise comparisons, the vertical movement time of the MGO+Rotenone group (2936±1100 ms) was significantly lower than that of the Control group (17721±4808 ms) (p=0.017). There was no significant difference according to vertical movement time between the MGO group (12580±3265 ms) and the Rotenone group (6382±2583 ms) and the other groups (p>0.05) (Figure 3b).

The percentage of stereotypical movement time was significantly different between groups (p=0.006). In pairwise comparisons, the % of stereotypical movement time in the Control group (10.9%±0.5) was significantly higher than that in the MGO+Rotenone (5.8%±1.0) and Rotenone groups (6.6%±1.4) (p=0.008, p=0.024, respectively). There was no significant difference between the other groups (p>0.05) (Figure 3c).

The percentage of ambulatory movement time was significantly different between groups (p=0.028). In pairwise comparisons, the % ambulatory movement time in the Control group (13.7%±3) was significantly higher than that in the MGO+Rotenone group (5.6%±0.8) (p=0.043). There was no significant difference according to % of ambulatory movement time between the MGO group (12.2%±2.2) and the Rotenone group (7.3%±1.4) and with the other groups (p>0.05) (Figure 3d).

The percentage of resting time was significantly different between groups (p=0.012). In pairwise comparisons, the % of resting time in the MGO+Rotenone group (87.1%±1.7) and the Rotenone group (86.1%±2.3) was significantly higher than that in the Control group (75.3%±3.2) (p=0.018, p=0.035, respectively). There was no significant difference between the other groups (p>0.05) (Figure 3e).

Rearing activity

The rearing activity was significantly different between groups (p=0.002). In pairwise comparisons, the rearing activity of the Control group (10±2) was significantly higher than that of the MGO+Rotenone group (2±1) and the Rotenone group (3±1) (p=0.007, p=0.030, respectively). Additionally, the rearing activity of the MGO group (9 ± 1) was significantly higher than that of the MGO+Rotenone group (p=0.021). There was no significant difference in other pairwise comparisons (p>0.05) (Figure 4).

Rotarod Test

The average time spent on the rod in the three tests was 215±25 s in the Control group, 150±40 s in the MGO group, 155±38 s in the Rotenone group, and 94±31 s in the MGO+Rotenone group. Although, the average time spent on the rod in the three tests was shorter in the MGO+Rotenone group, there was no significant difference between the groups (p=0.098) (Figure 5a).

The longest time spent on the rod was significantly different between groups (p=0.015). In pairwise comparisons, the longest time spent on the rod in the Control group (263±19 s) was significantly longer than that in the MGO+Rotenone group (103±29 s) (p=0.008). There was no significant difference according to the longest time spent on the rod between the MGO group (181±43 s) and the Rotenone group (171±38 s) and with the other groups (p>0.05) (Figure 5b).

Blood glucose measurement

When the blood glucose levels measured on the 1st, 16th and 56th days were compared within the group and between the groups, no significant difference was found in any group (p>0.05).

Immunohistochemistry

The TH immunoreactivities of the striatum and SNpc regions in the coronal sections taken from the experimental groups at x2 magnification are shown in Figure 6a. Additionally, TH immunoreactivities at x20 magnification for the striatum and x40 for the SNpc are shown in Figure 6b.

TH immunoreactivity in the striatum

There was a significant difference in the percentages of RAC TH immunoreactivity of the groups (p=0.019). In pairwise comparisons, the percentage of RAC TH immunoreactivity in the Control group (58.8%±1.8) and MGO group (57.2%±1.5) was significantly higher than that in the MGO+Rotenone group (37.8%±7) (p=0.027, p=0.047, respectively). The percentage of RAC TH immunoreactivity in the Rotenone group (47.6%±6) was not significantly different from that in the other groups (p>0.05). The comparison of the percentages of RAC TH immunoreactivity of the groups is shown in Figure 7a.

When the percentage of LAC TH immunoreactivity of the groups was evaluated, the percentage of the Control group was 55.7%±1.7, 55.6%±1.6 in the MGO group, 48.4%±4.3 in the Rotenone group, and 49.7%±3.9 in the MGO+Rotenone group. There was no significant difference between the percentages of LAC immunoreactivity positivity of the groups (p=0.255). The comparison of the percentages of LAC immunoreactivity positivity of the groups is shown in Figure 7b.

When the percentages of CAC TH immunoreactivity of the groups were evaluated, the percentages of the Control group were 51.5%±2.1 and 49.9%±1.9 in the MGO group, 48.2%±2.6 in the Rotenone group, and 49.2%±1.6 in the MGO+Rotenone group. There was no significant difference between the percentages of groups' CAC immunoreactivity positivity (p=0.720). The comparison of the percentages of CAC immunoreactivity positivity of the groups is shown in Figure 7c.

TH-immunoreactivity in the SNpc

When the number of TH+ cells in the SNpc of the groups was evaluated, the number of cells in the Control group was determined to be 19±1, 18±1 in the MGO group, 17±1 in the Rotenone group and 15±2 in the MGO+Rotenone group. There was no significant difference between the numbers of TH+ cells in the SNpc of the groups (p=0.136). A comparison of the groups in terms of the number of TH+ cells in the SNpc is shown in Figure 7d.

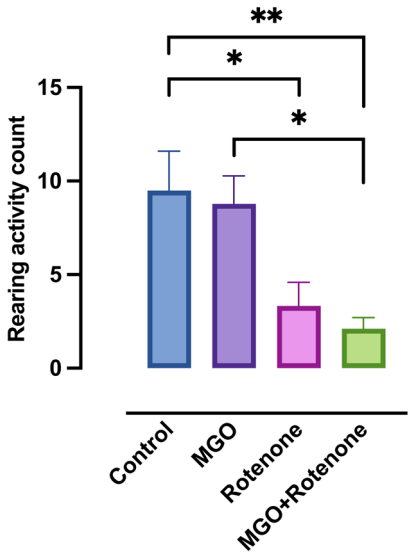

Figure 4. *Comparison of the rearing activity of the groups. (Mean ± standard error of mean was given. * p<0.05, ** p<0.01, one-way ANOVA)*

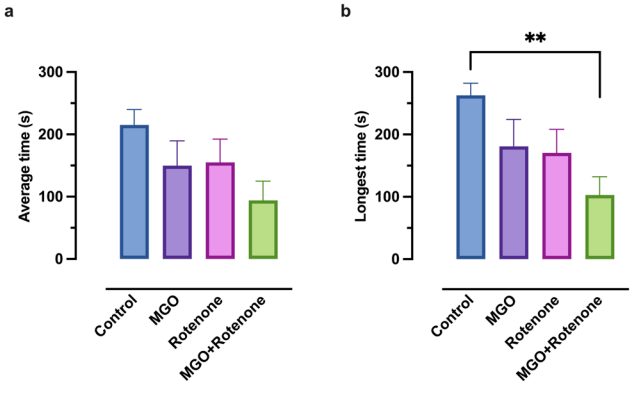

Figure 5. *Comparison of time spent on the rod of the groups in the rotarod test. (a) The average time spent on the rod, (b) the longest time spent on the rod. (Mean ± standard error of mean was given. ** p<0.01, one-way ANOVA)*

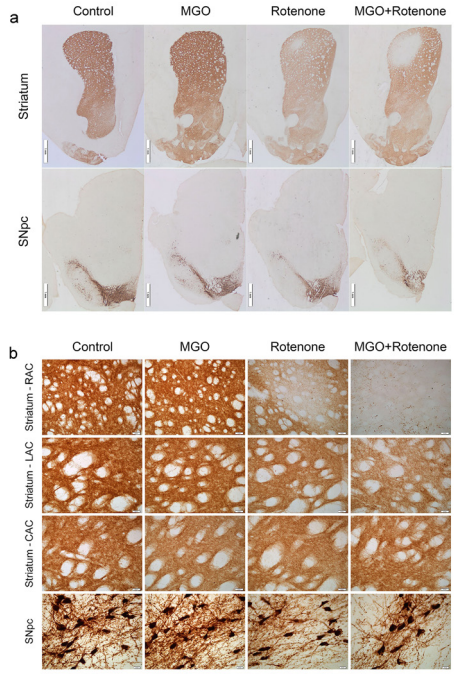

Figure 6. *TH immunoreactivity in the striatum and SNpc in coronal section samples of the groups. (a) Coronal sections at x2 magnification (chart scale= 1 mm), (b) coronal sections at x20 magnification for the striatum (chart scale= 50 µm) and x40 magnification for the SNpc (chart scale= 20 µm).*

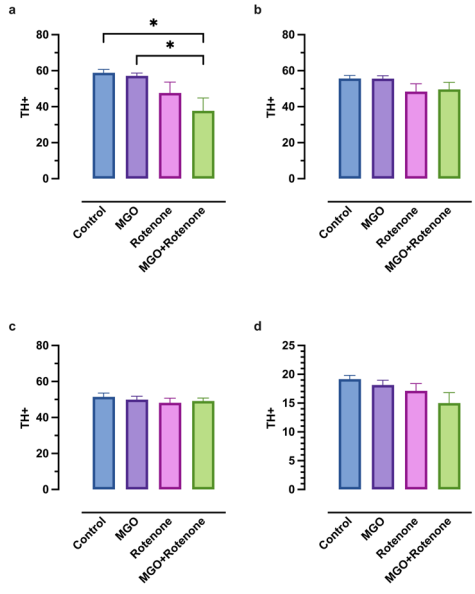

Figure 7. *Comparison of the tyrosine hydroxylase positivity of the groups. (a) Rostral to the anterior commissure, (b) level of the anterior commissure, (c) caudal to the anterior commissure, (d) substantia nigra pars compacta. (Mean ± standard error of mean was given. * p<0.05, one-way ANOVA)*

http://doi.org/10.5472/marumj.1480086
Marmara Med J 2024;37(2): 166-177

4. DISCUSSION

In this study, the effect of the advanced glycation end product precursor MGO on the development of PD pathophysiology was investigated. MGO application in the rotenone-induced PD model aggravated motor disorders and increased striatal dopaminergic damage without any statistically significant change in the number of dopaminergic cells in the SNpc. It also noticeably amplified the weight loss in animal however, no significant effect was observed on blood sugar. The findings of our study indicate that advanced glycation end product precursor MGO may play a role in increased PD pathophysiology in patients with T2DM.

Previous studies reported that rotenone administration, used in modeling PD, causes a decrease in axonal dopamine in the dorsal striatum, and chronic rotenone administration reduces dopamine concentrations in the anterior striatum [25, 26]. In our study, the MGO+Rotenone group had TH+ in the RAC striatum region that was significantly lower than that of the other groups, but there was no significant difference between the groups in the LAC and CAC striatum regions. In other words, the administration of MGO in conjunction with rotenone exacerbated dopaminergic loss in the striatum, but this loss was limited to the dorsolateral region of the anterior striatum.

Degeneration of dopaminergic neurons in the SNpc is thought to be a possible cause of dysfunction in the dorsal striatum, which is the projection target [27, 28]. The dorsal striatum is responsible for action selection and movement control, so dysfunction of this region also contributes to the common symptoms of Parkinson's disease [29, 30]. According to our findings, the deterioration in motor activity observed in the MGO+Rotenone group may be due to the dopaminergic loss observed in the RAC striatum.

In a study conducted on Thy1-aSyn mice, intracerebroventricular administration of MGO did not show any significant difference in TH immunoreactivity in the SNpc between the MGO-treated and nontreated groups [31]. On the other hand, in another study in which MGO injection was applied to the SNpc and striatum in Thy1-aSyn mice, a statistically significant decrease in TH+ neurons was reported in the experimental group compared to the control group [17]. In our study, the number of TH-immunoreactive cells in the SNpc decreased in the MGO+Rotenone group compared to the other groups, but there was no statistically significant difference between the groups. Consistent with our results, a study that established a PD model in mice by intranasal rotenone administration showed significant motor loss, but no dopaminergic loss was observed in the striatum and SNpc [23]. Although, striatal dopaminergic degeneration was observed in our study, the reason why there was no difference in TH immunoreactivity between groups in the SNpc may be that not enough time had passed for the neuron bodies in the SNpc to degenerate. Another possible reason may be that due to the weight loss in the MGO + Rotenone group, they received fewer Rotenone/DMSO injections compared to the other groups for ethical reasons.

In PD, the decrease in dopamine levels in the striatum leads to a decrease in voluntary motor movement control, bradykinesia and akinesia, which are the classic symptoms of PD [32]. Damage to the nigrostriatal circuit reduces locomotor activity in experimental animal models of PD [33]. In our study, the distance traveled, stereotypic movement and resting time obtained from the locomotor activity test were evaluated together in this context. The distance traveled and stereotypic movement time were significantly lower, and the percentage of resting time was higher in the MGO + Rotenone group than in the Control group, which is a clear indication of a significant decrease in locomotor movement. Although, the stereotypical movement time was lower and the resting time was higher in the Rotenone group than in the Control group, this difference was higher in the MGO+Rotenone group. Similar to our findings, animal studies have shown that locomotor activity decreases in PD groups compared to Control groups [33, 34]. In a study conducted in healthy animals to evaluate the effect of MGO on behavioral parameters, MGO was infused into the cerebral lateral ventricle in a volume of 5 µL at a concentration of 3 µM/µL. In the open field test performed at the 12th hour after MGO injection, a statistically significant decrease in locomotor activity was reported in the experimental group compared to the control group [35]. A single dose of 50 mg/kg/day MGO did not alter locomotor activity in a study looking at the impact of intraperitoneal MGO injection on motor activity in mice, but doses of 80 and 200 mg/kg/day caused a significant reduction in locomotor activity [36]. The reason for the loss of motor activity may be that MGO-derived free radicals and toxins react with dopamine in the brain and reduce the effect of dopamine [37]. MGO has also been reported to act as a partial agonist of $GABA_A$ receptors, and acutely high levels of MGO induce locomotor depression [38].

Another parameter obtained from the locomotor activity test is the ambulatory movement time. It has been shown that a decrease in locomotor activity, including ambulatory movement, occurred in the PD model created by applying 2 mg/kg rotenone subcutaneously to Sprague Dawley rats for 35 days, and this decrease could be partially prevented with antioxidant treatment [39]. In our study, the ambulatory movement time in the MGO+Rotenone group was significantly lower than that in the Control group. This finding indicates that MGO, which causes oxidative stress, increases motor impairment in the rotenone model of PD.

One of the parameters measured by the locomotor activity test is the number and duration of vertical movements. Another test method used to evaluate vertical movement is the rearing test. In animal models of PD, a decrease in the number of vertical movements, vertical movement time, and number of rearings is expected [21]. In a previous study, while no difference was observed between the experimental and control groups in vertical movements in rats receiving 2, 2.5 or 3.5 mg/kg rotenone intravenously for 21 days, a significant decrease in the number of rearings was reported in the experimental group compared to the control group [22]. It has also been shown that rearing behavior is reduced in rotenone models performed

http://doi.org/10.5472/marumj.1480086
Marmara Med J 2024;37(2): 166-177

with different doses and application routes [20, 40]. Our results obtained from the MGO+Rotenone group suggest that MGO decreases the number of rearings and the number and duration of vertical movements that develop due to PD.

The time spent on the rotarod device is shortened in rats and mice with PD [20, 40]. In a study in which the rotarod test was performed to evaluate motor coordination, 1.5 mg/kg rotenone was administered subcutaneously every other day for 11 days, and the time the rotenone group remained on the rod was found to be lower than that of the control group [41]. In the present study, the MGO+Rotenone group had a shorter average time spent on the rod, but this difference was not statistically significant. The high intragroup variation and the limited number of subjects to overcome this variation prevented statistical significance from being reached. When evaluated according to the longest time spent on the rod, the MGO+Rotenone group was able to stay on the rod for a shorter time than the Control group. In a study conducted on Thy1-aSyn mice, intracerebroventricular MGO was administered to the mice, and it was shown that the experimental group was able to stand on the rod less than the control group [31]. Our results demonstrate that MGO contributes to the decline in motor coordination, and they are consistent with findings from the literature.

One of the disadvantages of the PD rotenone model is the high mortality rate with systemic administration [42]. In our study, the mortality rate in animals administered rotenone was 9% (2/22). In the study conducted by Zhang et al., rotenone was administered subcutaneously to rats at a dose of 1.5, 2 or 2.5 mg/kg/day for 5 weeks, and mortality rates were reported as 0%, 6.7% and 46.7%, respectively [20]. In the study conducted by Fleming et al., the mortality rates of rotenone administered subcutaneously at doses of 2, 2.5, 3.5, or 5 mg/kg/day were 33% (4/12), 64% (7/11), 91% (10/11), and 100% (3/3), respectively [22]. In another study conducted in 2017, the mortality rate was reported as 28.5% (12/42) in rats administered 1.5 mg/kg/day rotenone intraperitoneally for 45 days [43]. There could be a number of reasons for the reported variations in death rates, including different animal strains, sexes, and housing circumstances. In our study, animals that lost more than 15% of their basal weight were not given the injection on that particular day to decrease mortality related to rotenone. For the same reason, Miglyol 812 N, which contains medium-chain fatty acids, was used to dilute rotenone, and the amount of DMSO in the application solution was kept at 2%. Additional losses were prevented by applying rotenone subcutaneously at a dosage of 1.5 mg/kg/day. The 9% mortality rate in our study shows that the methods we used in the rotenone model were successful in reducing deaths.

It has been reported that chronic systemic administration of rotenone causes weight loss in animals, and this is related to the systemic toxicity of rotenone as well as weight loss due to PD symptoms [44, 45]. In our study, the average weight of the MGO+Rotenone group was found to be significantly lower than that of the Control group at the end of the experiment. In a study published by Sharma et al., 2 mg/kg/day rotenone was administered subcutaneously to rats for 35 days. In weekly weight monitoring, no weight loss was observed in the first 2 weeks, but gradually increasing weight loss was reported in the 3rd, 4th, and 5th weeks [46]. In another study, 3 mg/kg/day rotenone infusions were given subcutaneously for 28 days. The rotenone groups showed a time-dependent decrease in body weight compared to rats administered vehicle or saline. In the study, regular weight loss was observed in the rotenone group, especially in the first 2 weeks [47]. In our study, low-dose rotenone application was preferred, and weight loss was minimized by skipping the dose in animals with a weight loss of more than 15% compared to their basal weight.

MGO binds to insulin by targeting arginine residues located in the insulin B chain and N-terminus. MGO-modified insulin chain B is heavier than free insulin, resulting in less glucose uptake and utilization [48]. In a study conducted with continuous infusion of MGO at a dose of 60 mg/kg/day for 28 days, a significant increase in fasting blood glucose along with a decrease in fasting plasma insulin levels was reported [49]. While some research indicates that hyperglycemia triggers the production of MGO and that MGO aids in the development of hyperglycemia, other works do not track these effects. Despite impaired glucose tolerance, no significant change in fasting blood glucose was observed in mice administered a low dose (1% v/v) of MGO in drinking water for 2 months with fetal exposure [50]. In a study in which MGO was given to Wistar albino rats at a dose of 50-75 mg/kg/day for 14 weeks, no significant difference was observed in terms of fasting blood glucose between the control group and the group receiving MGO [51]. The lack of apparent difference in blood glucose levels between the groups in our study that received and did not receive MGO may be because the dose of MGO used was insufficient to induce beta cell dysfunction and insulin resistance. Another important point to note is that fasting blood glucose was not measured in our study, and spot blood glucose was measured at 12:30 pm while they had access to feed and water in their cages.

On the other hand, there are limitations in this study. To cope with the problems of weight loss and mortality caused by choosing the rotenone model, rotenone was not administered to rats that lost 15% of their basal weight. One result of this is that the MGO+Rotenone administered group received fewer rotenone injections than the Rotenone group. This may have limited the progression of motor impairment and nigrostriatal degeneration in the MGO+Rotenone group. It is possible that this prevented the difference between the two groups from becoming more evident. Another limitation of the study is that a-synuclein accumulation is not measured in the brain, and blood levels of orally administered MGO could not be measured.

Conclusion

In this study, which examined a possible mechanism of the T2DM-PD relationship through MGO, an advanced glycation end-product precursor, it was determined that the use of MGO together with rotenone exacerbated the motor symptoms of PD and increased the neurodegeneration seen in the disease. To the best of our knowledge, this study is the first to investigate

http://doi.org/10.5472/marumj.1480086
Marmara Med J 2024;37(2): 166-177

the potential effect of long-term MGO administration on PD pathophysiology using the rotenone model of PD. Therefore, we believe that our study will contribute to filling the gap of T2DM-PD relationship in the literature. Although, our study showed the possible contribution of methylglyoxal to the pathophysiology of PD, the mechanism of this effect needs to be investigated in terms of biochemical processes.

Compliance with Ethical Standards

Ethical approval: Approval of the study for all experimental procedures was obtained from The Marmara University Ethical Committee for Experimental Animals (96.2021mar) and performed in line with guidelines.

Financial support: This work was supported by the Marmara University Scientific Research Committee with the thesis project number TTU-2022-10441.

Conflict of interest: The authors have no relevant financial or nonfinancial interests to disclose.

Authors' contributions: All authors contributed to the study's conception and design. YC and RG: Material preparation, data collection, and analysis, YC: Writing the first draft of the manuscript. All authors commented on the first draft of the manuscript. All authors read and approved the final manuscript.

REFERENCES

[1] von Campenhausen S, Bornschein B, Wick R et al. Prevalence and incidence of Parkinson's disease in Europe. Eur Neuropsychopharmacol 2005; 15: 473-90. doi: 10.1016/j.euroneuro.2005.04.007.

[2] Aarsland D, Batzu L, Halliday G M et al. Parkinson disease-associated cognitive impairment. Nat Rev Dis Primers 2021; 7: 47. doi: 10.1038/s41572.021.00280-3.

[3] Dauer W and Przedborski S. Parkinson's disease: mechanisms and models. Neuron 2003; 39: 889-909.

[4] Exner N, Lutz A K, Haass C, Winklhofer K F. Mitochondrial dysfunction in Parkinson's disease: molecular mechanisms and pathophysiological consequences. Embo J 2012; 31: 3038-62. doi: 10.1038/emboj.2012.170.

[5] Ebrahimi-Fakhari D, Wahlster L, and McLean P J. Protein degradation pathways in Parkinson's disease: curse or blessin. Acta Neuropathol 2012; 124: 153-72. doi: 10.1007/s00401.012.1004-6.

[6] Stefanis L. α-Synuclein in Parkinson's disease. Cold Spring Harb Perspect Med 2012; 2: a009399. doi: 10.1101/cshperspect.a009399.

[7] Hetz C, Saxena S. ER stress and the unfolded protein response in neurodegeneration. Nat Rev Neurol 2017; 13: 477-91. doi: 10.1038/nrneurol.2017.99.

[8] Ascherio A, Schwarzschild M A. The epidemiology of Parkinson's disease: risk factors and prevention. Lancet Neurol 2016; 15: 1257-72. doi: 10.1016/s1474-4422(16)30230-7.

[9] De Pablo-Fernandez E, Goldacre R, Pakpoor J, Noyce A J, Warner T T. Association between diabetes and subsequent Parkinson disease: A record-linkage cohort study. Neurology 2018; 91: e139-e142. doi: 10.1212/wnl.000.000.0000005771.

[10] Cereda E, Barichella M, Pedrolli C, et al. Diabetes and risk of Parkinson's disease: a systematic review and meta-analysis. Diabetes Care 2011; 34: 2614-23. doi: 10.2337/dc11-1584.

[11] Sandyk R. The relationship between diabetes mellitus and Parkinson's disease Int J Neurosci 1993; 69: 125-30. doi: 10.3109/002.074.59309003322.

[12] Galicia-Garcia U, Benito-Vicente A, Jebari S, et al. Pathophysiology of Type 2 diabetes mellitus. Int J Mol Sci 2020; 21:6275. doi: 10.3390/ijms21176275.

[13] Henriksen E J, Diamond-Stanic M K, Marchionne E M. Oxidative stress and the etiology of insulin resistance and type 2 diabetes. Free Radic Biol Med 2011; 51: 993-9. doi: 10.1016/j.freeradbiomed.2010.12.005.

[14] Nowotny K, Jung T, Höhn A, Weber D, Grune T. Advanced glycation end products and oxidative stress in type 2 diabetes mellitus. Biomolecules 2015; 5: 194-222. doi: 10.3390/biom5010194.

[15] Dornadula S, Elango B, Balashanmugam P, Palanisamy R, Kunka Mohanram R. Pathophysiological insights of methylglyoxal induced type-2 diabetes. Chem Res Toxicol 2015; 28: 1666-74. doi: 10.1021/acs.chemrestox.5b00171.

[16] Lu J, Randell E, Han Y, Adeli K, Krahn J, Meng Q H. Increased plasma methylglyoxal level, inflammation, and vascular endothelial dysfunction in diabetic nephropathy. Clin Biochem 2011; 44: 307-11. doi: 10.1016/j.clinbiochem.2010.11.004.

[17] Vicente Miranda H, Szego É M, Oliveira L M A, et al. Glycation potentiates α-synuclein-associated neurodegeneration in synucleinopathies. Brain 2017; 140: 1399-419. doi: 10.1093/brain/awx056.

[18] Biosa A, Outeiro T F, Bubacco L, Bisaglia M. Diabetes mellitus as a risk factor for Parkinson's disease: a molecular point of view. Mol Neurobiol 2018; 55: 8754-63. doi: 10.1007/s12035.018.1025-9.

[19] Fitzmaurice A G, Rhodes S L, Lulla A, et al. Aldehyde dehydrogenase inhibition as a pathogenic mechanism in Parkinson disease. Proc Natl Acad Sci U S A 2013; 110: 636-41. doi: 10.1073/pnas.122.039.9110.

[20] Zhang Z N, Zhang J S, Xiang J, et al. Subcutaneous rotenone rat model of Parkinson's disease: Dose exploration study. Brain Res 2017; 1655: 104-13. doi: 10.1016/j.brainres.2016.11.020.

[21] Meredith G E, Kang U J. Behavioral models of Parkinson's disease in rodents: a new look at an old problem. Mov Disord 2006; 21: 1595-606. doi: 10.1002/mds.21010.

[22] Fleming S M, Zhu C, Fernagut P O, et al. Behavioral and immunohistochemical effects of chronic intravenous and subcutaneous infusions of varying doses of rotenone. Exp Neurol 2004; 187: 418-29. doi: 10.1016/j.expneurol.2004.01.023.

[23] Sharma M, Kaur J, Rakshe S, Sharma N, Khunt D, Khairnar A. Intranasal exposure to low-dose rotenone Induced alpha-synuclein accumulation and Parkinson's like symptoms without loss of dopaminergic neurons. Neurotox Res 2022; 40: 215-29. doi: 10.1007/s12640.021.00436-9.

[24] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. Hard cover 6th edition. Elsevier, 2006.

[25] Bao L, Avshalumov M V, Rice M E. Partial mitochondrial inhibition causes striatal dopamine release suppression and medium spiny neuron depolarization via H2O2 elevation, not ATP depletion. J Neurosci 2005; 25: 10029-40. doi: 10.1523/jneurosci.2652-05.2005.

[26] Alam M, Danysz W, Schmidt W J, Dekundy A. Effects of glutamate and alpha2-noradrenergic receptor antagonists on the development of neurotoxicity produced by chronic rotenone in rats. Toxicol Appl Pharmacol 2009; 240: 198-207. doi: 10.1016/j.taap.2009.07.010.

[27] Cenci M A, Francardo V, O'Sullivan S S, Lindgren H S. Rodent models of impulsive-compulsive behaviors in Parkinson's disease: How far have we reached? Neurobiol Dis 2015; 82: 561-73. doi: 10.1016/j.nbd.2015.08.026.

[28] Samii A, Nutt J G, Ransom B R. Parkinson's disease. Lancet 2004; 363: 1783-93. doi: 10.1016/s0140-6736(04)16305-8.

[29] Kravitz A V, Freeze B S, Parker P R, et al. Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry, Nature, 2010; 466: 622-6. doi: 10.1038/nature09159.

[30] Cataldi S, Stanley A T, Miniaci M C, Sulzer D. Interpreting the role of the striatum during multiple phases of motor learning. Febs j, 2022; 289: 2263-2281. doi: 10.1111/febs.15908.

[31] Chegão A, Guarda M, Alexandre B M et al. Glycation modulates glutamatergic signaling and exacerbates Parkinson's disease-like phenotypes, NPJ Parkinsons Dis 2022; 8: 51. doi: 10.1038/s41531.022.00314-x.

[32] Panigrahi B, Martin K A, Li Y, et al. Dopamine is required for the neural representation and control of movement vigor. Cell, 2015; 162: 1418-30. doi: 10.1016/j.cell.2015.08.014.

[33] Taylor T N, Greene J G, Miller G W. Behavioral phenotyping of mouse models of Parkinson's disease. Behav Brain Res 2010; 211: 1-10. doi: 10.1016/j.bbr.2010.03.004.

[34] Su R J, Zhen J L, Wang W, Zhang J L, Zheng Y, Wang X M. Time-course behavioral features are correlated with Parkinson's disease-associated pathology in a 6-hydroxydopamine hemiparkinsonian rat model. Mol Med Rep 2018; 17: 3356-63. doi: 10.3892/mmr.2017.8277.

[35] Lissner L J, Rodrigues L, Wartchow K M, et al. Short-term alterations in behavior and astroglial function after intracerebroventricular infusion of methylglyoxal in rats. Neurochem Res 2021; 46: 183-96. doi: 10.1007/s11064.020.03154-4.

[36] Szczepanik J C, de Almeida G R L, Cunha M P, Dafre A L. Repeated methylglyoxal treatment depletes dopamine in the prefrontal cortex, and causes memory impairment and depressive-like behavior in mice, Neurochem Res 2020; 45: 354-70. doi: 10.1007/s11064.019.02921-2.

[37] Hipkiss A R. On the relationship between energy metabolism, proteostasis, aging and Parkinson's disease: Possible causative role of methylglyoxal and alleviative potential of carnosine. Aging Dis 2017; 8: 334-45. doi: 10.14336/ad.2016.1030.

[38] Distler M G, Plant L D, Sokoloff G, et al. Glyoxalase 1 increases anxiety by reducing GABAA receptor agonist methylglyoxal. J Clin Invest 2012; 122: 2306-15. doi: 10.1172/jci61319.

[39] Nehru B, Verma R, Khanna P, Sharma S K. Behavioral alterations in rotenone model of Parkinson's disease: attenuation by co-treatment of centrophenoxine. Brain Res 2008; 1201: 122-7. doi: 10.1016/j.brainres.2008.01.074.

[40] Palle S, Neerati P. Improved neuroprotective effect of resveratrol nanoparticles as evinced by abrogation of rotenone-induced behavioral deficits and oxidative and mitochondrial dysfunctions in rat model of Parkinson's disease. Naunyn Schmiedebergs Arch Pharmacol 2018; 391: 445-53. doi: 10.1007/s00210.018.1474-8.

[41] Kandil E A, Abdelkader N F, El-Sayeh B M, Saleh S. Imipramine and amitriptyline ameliorate the rotenone model of Parkinson's disease in rats. Neuroscience. 2016; 332: 26-37. doi: 10.1016/j.neuroscience.2016.06.040.

[42] Greenamyre J T, Cannon J R, Drolet R, Mastroberardino P G. Lessons from the rotenone model of Parkinson's disease. Trends Pharmacol Sci 2010; 31: 141-2; author reply 142-3. doi: 10.1016/j.tips.2009.12.006.

[43] Khadrawy Y A, Salem A M, El-Shamy K A, Ahmed E K, Fadl N N, Hosny E N. Neuroprotective and therapeutic effect of caffeine on the rat model of Parkinson's disease induced by rotenone. J Diet Suppl 2017; 14: 553-72. doi: 10.1080/19390.211.2016.1275916.

[44] Greene J G, Noorian A R, Srinivasan S. Delayed gastric emptying and enteric nervous system dysfunction in the rotenone model of Parkinson's disease. Exp Neurol 2009; 218: 154-61. doi: 10.1016/j.expneurol.2009.04.023.

[45] Drolet R E, Cannon J R, Montero L, Greenamyre J T. Chronic rotenone exposure reproduces Parkinson's disease gastrointestinal neuropathology. Neurobiol Dis 2009; 36: 96-102. doi: 10.1016/j.nbd.2009.06.017.

[46] Sharma N, Khurana N, Muthuraman A, Utreja P. Pharmacological evaluation of vanillic acid in rotenone-induced Parkinson's disease rat model. Eur J Pharmacol 2021; 903: 174112. doi: 10.1016/j.ejphar.2021.174112.

[47] Ravenstijn P G, Merlini M, Hameetman M, et al. The exploration of rotenone as a toxin for inducing Parkinson's disease in rats, for application in BBB transport and PK-PD experiments. J Pharmacol Toxicol Methods 2008; 57: 114-30. doi: 10.1016/j.vascn.2007.10.003.

[48] Jia X, Olson D J, Ross A R, Wu L. Structural and functional changes in human insulin induced by methylglyoxal. Faseb J 2006; 20: 1555-7. doi: 10.1096/fj.05-5478fje.

[49] Dhar A, Dhar I, Jiang B, Desai K M, Wu L. Chronic methylglyoxal infusion by minipump causes pancreatic beta-cell dysfunction and induces type 2 diabetes in Sprague-Dawley rats. Diabetes 2011; 60: 899-908. doi: 10.2337/db10-0627.

[50] Ankrah N A, Appiah-Opong R. Toxicity of low levels of methylglyoxal: depletion of blood glutathione and adverse effect on glucose tolerance in mice. Toxicol Lett 1999; 109: 61-7. doi: 10.1016/s0378-4274(99)00114-9.

[51] Matafome P, Santos-Silva D, Crisóstomo J, et al. Methylglyoxal causes structural and functional alterations in adipose tissue independently of obesity. Arch Physiol Biochem 2012; 118: 58-68. doi: 10.3109/13813.455.2012.658065.