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Rotenone Toxicity in Male and Female Albino Rats in the Route of Parkinson's Disease Modeling

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ABSTRACT

Background: Rotenone is commonly used for Parkinson's disease (PD) experimental models based on selective neurodegeneration of midbrain dopaminergic neurons and motor dysfunctions. Meanwhile, rotenone is a toxic compound that causes high mortality, requiring a more significant number of experimental animals. Differences between male and female species in response to rotenone toxicity were reported, stating higher sensitivity in males than in females. In spite of the suggested various doses of rotenone, it is essential to regulate its dosage, especially when male and female species are involved in the experiments. The present study aimed to determine the optimal dose and duration of chronic rotenone injections in rats to achieve a relatively lower mortality rate.

Methods: Male and female albino rats were treated with moderate (2 mg/kg/day) and low (0.3-0.5 mg/kg/day) rotenone at different regimens. The brain (substantia nigra, striatum) and spinal cord were analyzed for neurodegeneration using H&E staining. The body weight and mortality of rats were monitored on a daily basis.

Results: Comparative studies indicated that low doses of systemic rotenone injections were less toxic to females than to male rats. Female rats were more sensitive to chronic rotenone exposures, indicated by aggressive and anxious behavior. Nonetheless, the lower mortality rate in female than that in male rats suggested distinct physiological mechanisms to play a role in reduced rotenone toxicity in female rats.

Conclusion: These observations should be considered when male and female rats are involved in PD modeling. The diverse responses to neurotoxin are essential to provide a valid platform for further treatment schemes and clinical outcomes.

Keywords: Parkinson's disease, Rotenone, Female rats, Survival rate, Brain, Spinal cord

Introduction

Parkinson's disease (PD) is the most common neurodegenerative movement disorder seen across the globe in various ethnic groups. This condition is prevalent among old-age populations, with a higher rate in developed countries [*1*]. The complex pathophysiology of sporadic PD includes early premotor and late motor dysfunctions. The leading cause of this condition is progressive neurochemical alterations and degeneration in select brain nuclei in the central and peripheral nervous system [*2*]. The motor dysfunctions include tremors, rigidity, bradykinesia, and postural instability. These disorders are associated with CNS damage and loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) [*3*].

This condition leads to a reduction in the dopaminergic neurotransmission in the caudate nucleus of the corpus striatum [*3*]. Premotor dysfunctions (autonomic, sensory, psychiatric) appear in the early stages of the disease in the extranigral areas, spinal cord distinct nuclei, and the ganglia in the autonomic and peripheral nervous systems [*2*]. Progressive neurodegenerative processes in the central nervous system (CNS) at various disease stages are associated with the formation of Lewy bodies in neurons. These Lewy bodies contain aggregates of misfolded α-synuclein molecules [*2*, *4*].

Numerous epidemiological observations and large meta-analyses indicate the greater susceptibility of males to the development of PD compared to females. Approximately twice as many men as women are at risk of developing PD [*5*]. Environmental and social risk factors may affect men versus women differently due to genetic, hormonal, and other variables. The epidemiology of sporadic PD reveals frequent

occupational exposure to toxicants and a higher incidence of PD in men [6]. Numerous human and animal studies have provided evidence of gender-based differences in the onset and progression of PD, which may be associated with genetic predisposition and age. Other variables include preclinical and clinical manifestations of motor and non-motor symptoms, behavioral and sleep disorders, dyskinesia, depression, and even the response to therapeutic management. The above-mentioned variables suggest that different pathological mechanisms may be involved in the development of PD in men versus women [7, 8].

A multifaceted etiology of non-familiar sporadic PD suggests that aging, traumatic brain injury, infections, neurotoxic drugs, environmental factors, specific immune responses, and interactions with extrinsic and intrinsic factors may trigger the disease onset in genetically predisposed individuals [9]. Epidemiological evidence provides a linkage between the increased risk of developing PD in humans and rotenone contamination. This natural compound is used as a broad-spectrum insecticide and pesticide in agriculture [10].

Numerous routes of rotenone exposure have been used to induce Parkinsonian-like conditions in rodents. These include subcutaneous and intraperitoneal injections, stereotaxic infusions, intravenous, intranasal, and oral administrations [11-17]. Systematic subcutaneous administration of rotenone to rodents (Sprague-Dawley and Lewis rats, mice) reproduces behavioral, anatomical, neurochemical, and neuropathological features of PD. The pathological alterations are discernible by progressive striatal dopaminergic neuron loss and denervation in caudate putamen's reduced tyrosine hydroxylase (TH) immunoreactivity in the SNpc, and L-DOPA-responsive Parkinsonian-like locomotor abnormalities [11, 12, 18, 19]. The neurotoxicity of rotenone is explained by its capability to cross biological membranes, particularly the midbrain dopaminergic neurons, where it inhibits complex I (NADH dehydrogenase-ubiquinone oxidoreductase) enzyme activity in the mitochondrial respiratory chain. The subsequent elevation of ROS and RNS culminates in mitochondrial dysfunction and oxidative stress that mediates the neurotoxic environment surrounding vulnerable dopaminergic neurons [20]. This model provides a valuable platform to study the mechanisms of neurodegeneration and screening of potential neuroprotective agents in PD. In spite of reported different doses and regimens of rotenone administration, the mortality rate in rodents caused by rotenone toxicity is often neglected, which is an obstacle in modeling routes for PD.

Aim of the Study: The present study aimed to establish the optimal dose and duration of chronic rotenone injections to albino rats to achieve a relatively reduced mortality rate in male and female animals. These steps are essential to establish a valid model for evidenced-based treatments and clinical outcomes. Further, we investigated the effects of rotenone in male and female rats' brains, i.e., substantia nigra, striatum, and spinal cord, based on histological examinations.

Materials and Methods

Animals: Four groups of albino rats (total n = 42) were used in these experiments, including middle-aged male rats (n = 10; 11 months; ~230 g), adult male rats (n = 12; 6 months; ~200 g), adult female rats (n = 20; 6 months: ~200-230 g), and control rats (n = 5 in each group). Rats in respective groups were treated with moderate (2 mg/kg/day) and low (0.3-0.5 mg/kg/day) doses of rotenone (intraperitoneal or subcutaneous injections) at different regimens: Groups I, II, III – every alternative day, Group IV – 2 days apart. Rotenone (Sigma, St. Louis, MO, USA) was dissolved in 1:1 (v/v) dimethylsulfoxide (DMSO; Sigma) and polyethylene glycol (PEG-300; Sigma). Control animals received vehicles (DMSO/PEG-300 1:1) on scheduled days. Animal care, regular monitoring for general health conditions and weight, and experimental protocols were approved by the Animal Care and Use Committee at the H. Buniatian Institute of Biochemistry, NAS RA (IRB 0001621; IORG0009782). Rats were decapitated under anesthesia with Nembutal (40-50 mg/kg), then the brain and spinal cord were dissected on ice and stored at -32°C for further analyses. Rats with severe signs of illness induced by rotenone were immediately euthanized ahead of time.

Histological Examinations: Neurodegenerative changes in the substantia nigra, striatum, and cervical spinal cord of the rat brain tissue were evaluated by histological examinations, using sections stained with H&E. Paraffin wax sections were made based on the routine lab protocols. Dissected whole brain and spinal cord tissue samples (cervical) were fixed in 4-5% formalin (24-48 h, 4ºC), washed with 0.1 M phosphate buffer (pH=7.2), gradually dehydrated in 70%, 80%, and 96% ethanol, washed in water, and prepared as paraffin blocks. Then, the blocks were sectioned into (10-15 μm) slices using a microtome (Semi-Auto Rotary Paraffin Microtome 202A). The slices were air-dried, the paraffin removed with xylene (1 and 2), then slices were rehydrated with ethanol (96%, 80%, 70%), washed in distilled water, and air-dried. The slide samples were stained with H&E following the previously established method [21]. Briefly, histological Specimens were fixed in 96% ethanol for 10 min, dehydrated in ethanol (96%, 75%), washed in distilled water, consecutively stained with hematoxylin (5 min), washed in tap water, then stained with 1% and eosin (1 min). Afterward, they were washed in tap water, dehydrated in ethanol (75%, 96%), cleared in xylene twice, and mounted on slides with DPX (Cat. # 06522; Sigma-Aldrich; Merck KGaA). All steps were

performed at room temperature. Preparations were examined under an illuminated light digital microscope (SWIFT Motic Europe M 10; Motic Europe, S.L.U.) at 4x, 10x, 40x, and 100x magnifications. The images were formatted using Corel DRAW X8 (version 18) software (Alludo, Ottawa, Canada).

Statistical Analyses: The study data were collected and tabulated separately from three independent experiments, and the mean±SEM values were plotted. The statistical significance of differences among the study groups was analyzed using One-way ANOVA. The changes among the groups were considered significant at $P<0.05$.

Results

Rotenone-induced Mortality and Body Weight Assessment: Dosage, rotenone injection schedule, and the induced mortality rate in the rats are indicated in Table 1. The body weights in the control and experimental rats were monitored on a daily basis. The weight of the control animal group (n = 20 rats) was increased by the end of the study, lasting from 20 to 50 days. The changes in the body weights of rotenone-injected rats compared to controls and their survival rates are demonstrated in Figure 1.

In Group I, the middle-aged male rats (n = 10) received a moderate dose of rotenone (2 mg/kg) through intraperitoneal injections every second day. In this group, rotenone caused high mortality (80%) on the first and third days after injections, indicating its high toxicity. By the 20th day of the study, only two rats (n = 2) in Group I survived that had received a total of 18.5 mg/kg rotenone. The body weights of the survived rats decreased steadily after the first five rotenone injections, with the maximum weight loss being 25% on the 10th day compared to the control group. Although there was some weight gain between the 10th and 20th days, by the 20th day, the body weight of the rats was 20% less compared to controls (Figure 1A).

In Group II, the male rats (n = 12) received low rotenone dosage (0.5 mg/kg) through subcutaneous injections on alternate days. By the 30th day of the study, the three survived rats (n = 3) that had received a total of 6.5 mg/kg rotenone were decapitated. The mortality rate in this group was 75%. There were noticeable weight gains in the three rats that survived during the first 15 days of the rotenone injections; then, they exhibited a sharp weight loss in the survived rats by the 30th day; the difference was 13% ($P<0.05$) compared to controls (Figure 1B).

In Group III, the adult female rats (n = 10) received low rotenone dosage at 0.5 mg/kg (subcutaneous) on alternate days. Four rats (n = 4) survived that had received a total of 7 mg/kg of rotenone by 14 injections. They were decapitated on the 35th day of the study. The mortality rate in this group was 60%. Rotenone caused a 37% weight loss ($P<0.05$) in the rats over the 35 days of the study (Figure 1C).

In Group IV, the adult female rats (n = 7) received low rotenone dosage at 0.3-0.4 mg/kg subcutaneously on alternate days. Four out of seven rats (n = 4) survived. They had received a total of 5.7 mg/kg of rotenone over 16 injections and showed a mortality rate of 42.86% ($P<0.05$). They were decapitated on the 50th day. Rotenone injections caused a 28% body weight loss, which remained steady over the 50 days of experiments (Figure 1D).

Comparatively, a high survival rate was observed in the adult female rats after rotenone injections (Group IV, 60%) compared to the male rats in Group II (Group II, 25%). The lowest survival rate (20%) was 20%, observed in the middle-aged male rats (Figure 1E). Between groups III and IV, the female rats that had received low rotenone dosage (0.3-0.4 mg/kg) on alternate days exhibited more beneficial outcomes from the agent and higher survival rates.

Table 1. Rotenone injections to male and female albino rats.

Groups	Control Rats	Experimented Rats	Rotenone Dosage	Mortality	Survival and Cumulative Rotenone Dose
Group I	n = 5	n = 10 middle-aged male 11 months	2 mg/kg (intraperitonea) moderate dose	80% n = 8 died on the 1st and 3rd days	n = 2 received 18.5 mg/kg over 20 days
Group II	n = 5	n = 12 adult male 6 months	0.5 mg/kg (subcutaneous) low dose	75 % n = 9 died	n = 3 received 6.5 mg/kg over 30 days
Group III	n = 5	n = 10 adult female 6 months	0.5 mg/kg (subcutaneous) low dose	60% n = 6 died	n = 4 received 7 mg/kg over 35 days
Group IV	n = 5	n = 10 adult female 6 months	0.3-0.4 mg/kg (subcutaneous) low dose	40% n = 4 died	n = 6 received 5.7 mg/kg over 50 days

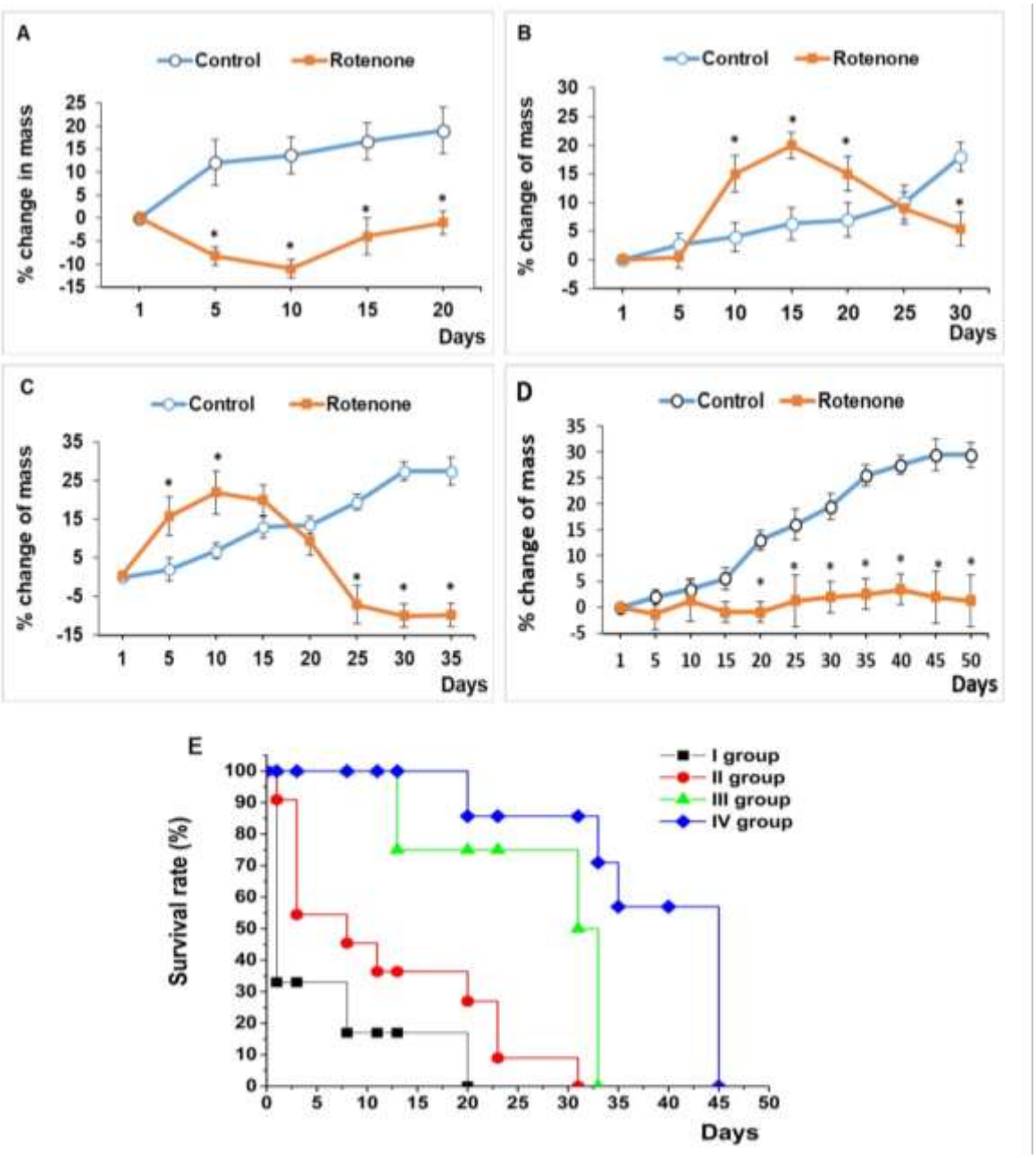

Figure 1. Rotenone-induced changes of body mass in albino rats. Assessment of body mass and changes (%) in the rotenone-injected rats compared to control. (A) middle-aged male rat (n=2), received moderate rotenone dose (2 mg/kg; intraperitoneal); (B) adult male rats (n=3), received low rotenone (0.5 mg/kg; subcutaneous); (C) adult female rats (n=4), received low rotenone dose (0.5 mg/kg; subcutaneous); (D) adult female rats (n=6) received low rotenone dose (0.3-0.4 mg/kg; subcutaneous); *P<0.05 (One-way ANOVA). (E) Survival rate (%) in 4 experimental groups of albino rats: Group I – middle-aged male rats (20%); Group II – adult male rats (25%); Group III – adult female rats (40%); Group IV – adult female rats (60%).

Neurodegeneration in the Brain and Spinal Cord of Female Rats: Rotenone-induced neurodegeneration in the brain and spinal cord samples were examined microscopically on H&E stained sections. The histological studies indicated that low doses of rotenone injections (0.3-05 mg/kg) over 35-50 days in Groups III and IV, respectively, caused substantial neurodegenerative changes in the brain (SNpc) and spinal cord samples of adult female rats compared to those of the controls. Representative photomicrographs (Figures 2A and 2B) showed numerous stained purplish blue nuclei in dopaminergic neurons of SNpc in the control rats. Conversely, reduced density of dopaminergic neurons was observed in the SNpc of rotenone-injected female rats (Figures 3C and 3D). In the SNpc slides of rotenone-injected rats, the damaged dopaminergic neurons looked smaller than those of the controls, where condensed nuclei were observed surrounded by edematous tissue.

Representative photomicrographs (Figures 2E and 2F) indicated that the ventral horn area and large motor neurons were present in the spinal cord samples of control female rats. In this group, the ventral spinal cord had regular morphological organization, and the edging area between the grey and white matter was distinct (Figures 2E and 2F). In rotenone-injected rats (Figures 2G and 2H), the edging area between the grey and white

matter was fused, and the morphological shapes of the fewer motor neurons were visible in the ventral horn. The morphological shapes of the motor neurons in this group were impaired with the axons and dendrites shortened (Figures 2G and 2H).

The neurotoxic effects of rotenone in the female rat spinal cord were compared between the two different regimens of neurotoxin injections. Representative photomicrographs (Figures 3A and 3B) demonstrate that after 14 injections on alternate days (Total: 7 mg/kg), some morphological alterations occurred in the ventral motor neurons (Figures 3A and 3B). These neurons exhibited purplish-blue stained nuclei. Some of the motor neurons appeared with shortened or disrupted processes, but their overall shapes had been preserved. After 25 rotenone injections (Total: 18.5 mg/kg), the ventral horn area was noticeably affected. The microscopic observations indicated dramatic cell losses and degenerative changes in the ventral horn. In this group, fewer motor neurons were observed with disrupted morphology (Figures 3C and 3D).

Although the chronic injections of rotenone affected the body weights in both male and female rats, less neurotoxicity and reduced mortality rates were documented among the female rats. The low dosage of rotenone induced neurodegenerative changes in the brain and spinal cord cells of adult female rats.

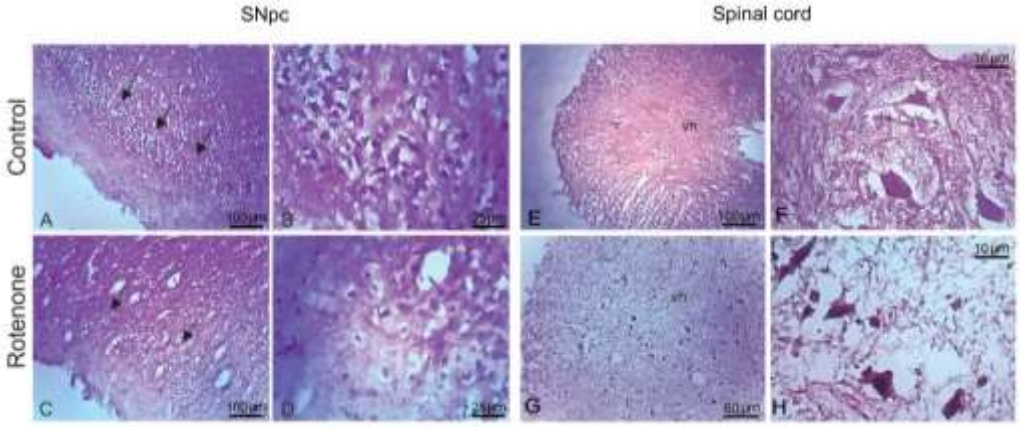

Figure 2. Rotenone-induced neurodegeneration in the rat brain (SNpc) and spinal cord. Neurodegeneration was assessed through H&E staining in brain and spinal cord coronal sections (10-15 µm) of control and rotenone-injected female albino rats. Representative photomicrographs and arrows show numerous stained purplish blue nuclei of dopaminergic neurons in the SNpc of control rats (A, B) and reduced density of dopaminergic neurons in the SNpc of rotenone-injected rats (C, D). Representative photomicrographs and arrows indicate ventral horn area and large motoneurons with axons and processes in the spinal cord of control rats (E, F) and degenerated motoneurons with shortened processes in rotenone-injected rats (G, H). Images are taken at 4x (A, C), 10x (E), 40x (G), and 100x (B, D) magnifications; n≥3.

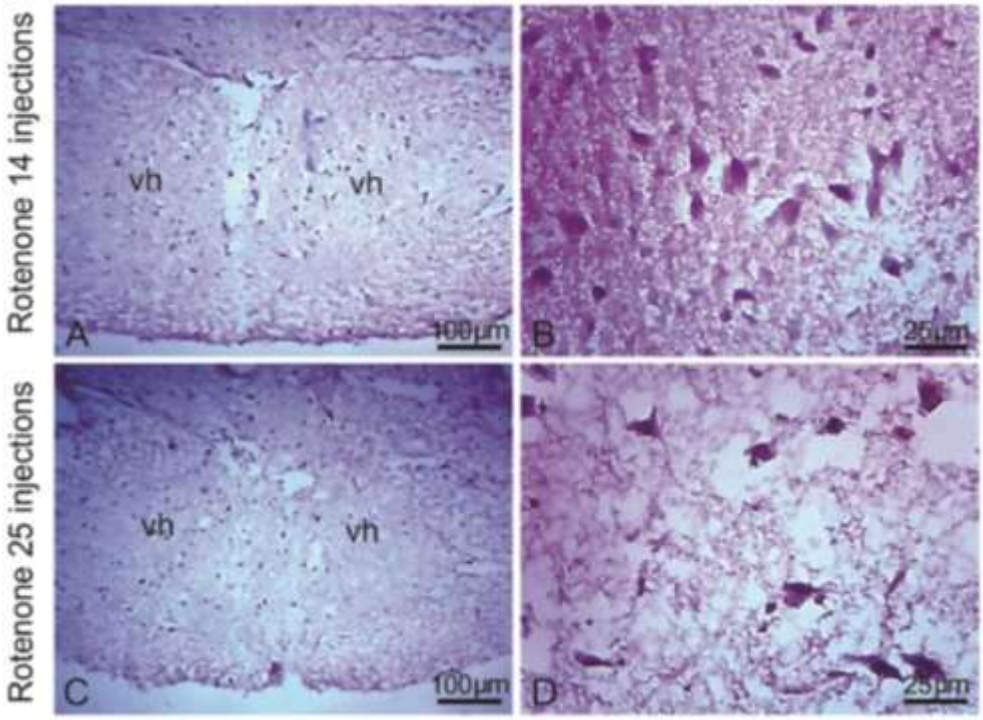

Figure 3. Neurodegeneration in the rat spinal cord after prolonged rotenone exposure. Neurodegeneration was assessed through H&E staining of rat spinal coronal sections (10-15 µm). Representative photomicrographs of ventral horn and motoneurons after 14 (A, B) and 25 injections of rotenone (C, D). Prolonged rotenone exposure (25 injections) led to significant loss and morphological changes in the ventral motoneurons (C, D). Images are taken at 4x (A, C) and 100x (B, D) magnifications; n≥3.

Discussion

The rotenone-induced neurological changes appear to simulate the pathogenesis of PD consistent with complex I inhibition and mitochondrial dysfunction found in humans with PD [22]. The inhibition of complex I followed by alterations in the mitochondrial OxPhos system, acute oxidative stress, and escalation of free radicals generation ($^{\bullet}OH$, $O_2^{\bullet-}$, H_2O_2, $NO^{\bullet}$, $ONOO^-$) have been suggested as the primary events associated with the neurotoxicity of dopaminergic neurons (SNpc, striatum) [11, 23]. The increased generation of hydroxyl groups ($^{\bullet}OH$) was detected in isolated mitochondria from the rats' cerebral cortex and in the substantia nigra and the striata areas ipsilateral to the same side of rotenone infusion [23]. Recent imaging and mass cytometry analyses have revealed a deficiency of essential mitochondrial proteins in the midbrain's dopaminergic neurons of postmortem PD patients compared to control cases. These changes indicate the disturbed regulation of mitochondrial adaptability and mitophagy [24].

The chronic inhibition of complex I by rotenone in rodents produces generalized mitochondrial failure, and multisystem degenerations, as well as loss of nigrostriatal dopaminergic neurons and fibers [13]. Moreover, general health issues, hypokinesia, and digestive disorders have been reported in rats as the consequences of systemic rotenone-induced toxicity between the third and fifth days after rotenone administration. However, these were not associated with specific motor deficits in PD cases [25]. Like other parkinsonian neurotoxins, such as 6-hydroxydopamine, 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine (MPTP), paraquat, the rotenone-induced model has limitations commonly related to animal weight loss and mortality.

The present study has revealed discrepancies in rotenone-induced toxicity between the male and female albino rats and the deteriorated health conditions depending on the dosage and given regimen. Moderate and low rotenone dosage (2.0 and 0.5 mg/kg) on alternate days led to substantial weight loss and high mortality rates (80% and 75%) in the middle-aged male rats. On the other hand, a low mortality rate (60%) was observed among the female rats when treated at a low rotenone dosage (0.5 mg/kg). Among all experimental groups, the lowest mortality rate (40%) was observed in adult female rats, receiving the lowest rotenone treatment (0.3-0.5 mg/kg) on alternate days. In spite of the substantial weight loss, the survival rate in adult female rats was higher than that of the middle-aged male rats (Table 1; Groups I and II).

It has been reported earlier that 7-month-old rats were more sensitive to chronic rotenone treatment (2.75-3.0 mg/kg) than 3-month-old ones. This result was associated with the rapid development of motor deficits, causing postural instability, bradykinesia, and rigidity. Histologically, these rats exhibited striatal dopamine depletion, nigrostriatal degeneration, loss of TH-positive neurons, and a relatively low mortality rate (~10%) that occurred immediately after injection [*19*].

In an earlier study [*12*], intraperitoneal administration of low and medium doses of rotenone (1.5 vs. 2.5 mg/ml) to young, 7-week-old Sprague Dawley male rats caused a metabolic deficiency in the nigrostriatal dopaminergic system, dopamine depletion and reduced striatal dopamine level in the striatum, resulting in cataleptic behavior (rigidity, akinesia,) and hypoactivity. However, dose-dependent mortality was not stated in that study. In another work, daily rotenone treatment (2.5 mg/kg) over 60 days caused approximately a 33% mortality rate, occurring after 30 days of administration [25]. There were no significant changes in the rats' body weight during the first 30 days; however, 8–10% weight loss was recorded at the end of the experiment, while a 20% weight loss occurred in the high-dose group (3 mg/kg) [26].

According to the findings of the study that used rotenone in vivo in a PD rat model, the optimal dose was 2-3 mg/kg/day resulted in early striatal dopaminergic denervation and lesions in 2-month-old Sprague Dawley or Lewis male rats. The effects of this treatment in the SNpc varied depending on the duration of the treatment [11]. An estimated rotenone level of 20-30 nM in the brain was enough to partially inhibit the complex I activity (73%) and stimulate ROS generation, causing oxidative damage to proteins, DNA, cytochrome C release to the cytoplasm, increased α-synuclein aggregation, and formation of Lewy bodies like cytoplasmic inclusions in the rat nigral neurons of rotenone-treated rats. However, non-specific acute toxicity, illness, and mortality caused by rotenone became a substantial experimental burden in animals while developing this PD model [26].

High doses of rotenone for short periods have produced cardiovascular toxicity, non-specific brain lesions, and death [*11*]. The highest dosage of rotenone (12 mg/kg) affected the dopamine metabolism in the rat brain, as evidenced by the increase in striatal dopamine metabolites, such as 4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) [27]. In mice, repeated rotenone intraperitoneal injections (5 mg/kg) over four days have stimulated oxidative deamination of dopamine, free radicals generation, and increased hydroxyl radical marker, i.e., 2,3-dihydroxybenzoic acid from sodium salicylate [28]. A dose-dependent decline in the survival rates was observed in adult male Lewis rats infused with rotenone [14]. Low rotenone treatment over more extended periods (similar to chronic human exposure to toxicants) has induced motor anomalies even in animals that do not develop neurological degeneration. This finding suggests a pervasive neurological effect, i.e., moderate mitochondrial dysfunction in vivo [14]. In another

investigation, injections of 2.5 mg/kg of rotenone caused a 46.7% mortality rate in male Wistar rats, compared to only a 6.7 % mortality rate at a 2 mg/kg dose [28]. It has been indicated that daily rotenone injections at lower dosages do not affect body weight even after five weeks of treatment; however, it has been useful in terms of mitochondrial damage, α-synuclein formation, and PD features while causing low mortality rates.

Gender-related differences in dopaminergic vulnerability to environmental toxicants or Parkinsonian neurotoxins require corrections in the route of PD modeling when both male and female animals are involved [*29*, *30*, *31*]. Comparative studies have shown reduced susceptibility of adult female rats (7-9-month old) to rotenone-induced neurodegeneration compared to age-matched male rats. This result was despite the equal toxin concentration found in the brains of both genders of these animals [31]. Higher dosages of rotenone (3.2 and 3.6 mg/kg) were necessary to stimulate α-synuclein accumulation and to produce 30-33% losses of nigrostriatal neurons and terminals in the substantia nigra of female rats, compared to male rats treated with 2.8 mg/kg of rotenone.

Rotenone has affected the iron metabolism and binding protein transferrin, lysosomal dysfunction, and microglial activation differently, as female rats required higher doses of rotenone for significant transferrin accumulation, increase in lysosomal protein CD68 and microglial neuroinflammatory responses in dopaminergic neurons [31]. Neurosteroid dehydroepiandrosterone (DHEA) and 17β-estradiol treatments in male mice have prevented MPTP-induced striatal dopamine depletion and provided neuroprotection to dopaminergic neurons through implication of estrogen receptors (ERα, ERβ) in the brain [32].

Activation of neuroprotective mechanisms associated with the preservation of iron homeostasis and a-synuclein autophagy without excluding the role of estrogens might elucidate resistance to rotenone toxicity in females [33]. These observations are consistent with frequent occupational exposure to toxicants and a high incidence of PD in men [6]. Human cohort studies have reported that women display less severe PD phenotype, signifying possible impacts of genetic, epigenetic, and sociocultural factors [30]. Animal studies have indicated that reduced androgen levels in male rats provided highly selective protection against the damaging effects of 6-hydroxydopamine on their cognitive functions [34]. Therefore, the neuroprotective role of estrogen for dopaminergic neurons against various types of toxic injuries, without excluding the role of androgens, should be evaluated in future studies [30].

The use of low doses of rotenone for more extended periods is a similar model for chronic environmental human exposure to compounds that ultimately result in PD. The histological findings of the current study indicate that prolonged exposure of female rats to low dosages of rotenone can lead to the degeneration of dopaminergic neurons in the nigrostriatum and the loss or degeneration of spinal motor neurons in the ventral horn. These findings warrant the inclusion of female animals, such as rats or mice, in experimental studies aimed at PD modeling. Such research may elucidate gender-related critical mechanisms of PD pathogenesis, which suggests that female rats could have undergone remodeling of neurological tissue due to PD after using low doses of rotenone over a prolonged period, which might be the cause of higher survival rates among these rats.

Conclusions

The findings of the present study on diverse groups of male and female albino rats indicated that systemic injections of low rotenone doses (0.3-0.5 mg/kg/day) are less toxic compared to medium or high doses (≥2 mg/kg/day), resulting in higher survival rate in both animal genders. Treatment with low rotenone doses for longer periods caused degeneration of dopaminergic neurons in the nigrostriatum and loss or degeneration of motor neurons in the ventral horn of rats' spinal cord. Female rats were generally more resistant to rotenone than males, suggesting distinct physiological mechanisms play a role in lower rotenone toxicity in female rats. The inclusion of female rats or mice in experimental PD modeling studies will help elucidate critical gender-related mechanisms toward Parkinson's disease pathogenesis. The diverse responses to neurotoxin in male and female animals are essential to provide a valid basis for further treatment schemes, study design, and clinical outcomes.

Conflict of Interests

The authors have no conflict of interest to disclose.

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Compliance with Ethical Guidelines

Experimental protocols were approved by the Animal Care and Use Committee at the H. Buniatian Institute of Biochemistry, NAS RA (Ref. Letter N2, 2024, IRB 0001621; IORG0009782).

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Funding acquisition: F. Sarukhanyan, V. Knaryan, A. Hakobyan.

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Supervision: F. Sarukhanyan, V. Knaryan

Visualization: I. Sahakyan, S. Abrahamyan.

Writing – original draft: F. Sarukhanyan, V. Knaryan.

Writing – review & editing: F. Sarukhanyan, V. Knaryan, I. Sahakyan, S. Abrahamyan.

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