

Research Paper: Spirulina Ameliorates Oxidative Damage and Inflammation in Rotenone-Induced Neurotoxicity in Male Mice



Marwa E.A. EL-Shamarka¹, Ahmed M.S. Hussein², Ola N. Sayed³, Eman S Said^{4,5}, Marwa A. Mwaheb⁶

1. Department of Narcotics, Ergogenic Aids and poison, National Research Center, Egypt.

2. Department of Food Technology, National Research Centre, Cairo, Egypt.

3. Department of Chemistry- Biochemistry divisions, Faculty of Science, Fayoum University, Egypt.

4. Department of Clinical pharmacology, Faculty of Medicine, Fayoum University, Egypt

5. Department of Pharmacology and toxicology, Faculty of pharmacy, Qassim University, Buraydah 52571, Saudi Arabia.

6. Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Fayoum University, Egypt.



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ABSTRACT

Background: Rotenone, a chemical compound produced naturally by leguminous plants, has conventionally been used as a pesticide by blocking the uptake of oxygen by body cells. Our study aimed to investigate the effect of spirulina on oxidative damage, inflammation, and neurotoxicity in male mice treated by rotenone.

Methods: The experimental animals were divided into 5 groups. Group (I) served as control that received Dimethyl Sulfoxide (DMSO); Group (II) mice treated with rotenone (1.5 mg/kg, s.c.3 times per week); Group (III) mice received rotenone/L-dopa (25 mg/kg, P.O. daily); Group (IV) and Group (V) mice were treated with rotenone/spirulina (200 and 400 mg/kg, P.O. daily) respectively for two weeks.

Results: Rotenone-treated mice indicated impaired motor coordination and activity in wire hanging, wood walking, open field, and star tests. Furthermore, rotenone treatment caused elevation in striatal levels of Malondialdehyde (MDA), Nitric Oxide (NO), Tumor Necrosis Factor (TNF- α), Interleukin -1 beta (IL-1 β), and caspase 3 and decrement in Bcl-2; dopamine and Glutathione (GSH) levels. Moreover, severe neuronal degeneration, striatal DNA fragmentation, and increased striatal 8-OHdG levels and MTH1 expression in the rotenone group. Additionally, spirulina treatment prevented rotenone-induced motor deficits striatal DNA fragmentation and demonstrated good restoration of the substantial neurons with reservation of the typical dark appearance. Besides, rotenone-induced biochemical changes were ameliorated by spirulina treatment as dopamine, Bcl-2, and GSH levels were increased, and striatal MDA, TNF- α , IL-1 β , and caspase 3 levels were decreased.

Conclusion: Natural products like spirulina could reverse rotenone-induced neurotoxicity in male mice due to their anti-inflammatory and antioxidant properties.

* Corresponding Author:

Marwa Ali Mwaheb, PhD.

Address: Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Fayoum University, Fayoum, Egypt.

Tel: +20 (100) 6267354

E-mail: marwa.mwaheb@yahoo.com

1. Introduction

Parkinson's Disease (PD) is the second most progressive neurodegenerative disease after Alzheimer's Disease (AD). It is described by bradykinesia, unbending nature, constipation, rapid-eye-movement, resting tremor, the disorder in sleep behavior, muscular rigidity, and postural instability [1, 2]. The principal neurotic attribute of PD is the dynamic degeneration of dopaminergic neurons in the substantia nigra standards compacta, the loss of dopamine in the striatum, and the nearness of intracytoplasmic considerations in enduring neurons, known as Lewy bodies [3].

Rotenone is an auxiliary metabolite item with bug spray angles originating from Leguminosae gathering plants *Derris* spp. and *Lonchocarpus* spp. Lipophilic exacerbates that crosses organic obstruction because of its hydrophobicity, allowing high ingestion and take-up in the cerebrum. As far back as its first use in the USA, a fish toxicant has included allure among fish ranchers in numerous nations. This is because of its adequacy and transient continuance in the lake water [4, 5]. It is a neurotoxicant and pays little heed to be broadly dispersed in the cerebrum. Rotenone can cause selective neurodegeneration in explicit locales and strong paralysis [6, 7].

Spirulina platensis (Nordest) Geitler or *Arthrospira platensis* (Nordest). The comment is a multicellular filamentous cyanobacterium of blue-green growth from the Oscillatoraceae family presenting high protein substance, alongside high measures of essential unsaturated fats and amino acids, minerals, nutrients, and other flavonoids that exhibited antioxidant and anticancer activities [8, 9]. It is increasing more consideration by therapeutic researchers as a nutraceutical and a wellspring of potential pharmaceuticals.

Several studies demonstrated spirulina to apply defensive impact in treating neurodegenerative issues (Parkinsonism & AD issue). Stromberg's study additionally proposes a defensive reaction by upgrading the recovery of striatal dopamine Tyrosine Hydroxylase (TH) positive strands and TH positive neurons in the substantia nigra standards compacta in trial rodent initiated Parkinsonism after bolstering with spirulina [10]. Spirulina strongly impacts hippocampus neural ancestor cells against lipopolysaccharide, which incites intense fundamental incendiary [11].

The current study aimed to evaluate the antioxidant and anti-inflammatory activities of *Spirulina* against neurotoxicity of rotenone in adult male mice.

2. Material and Methods

Rotenone was obtained from Sigma (St Louis, MO, USA) and dissolved in Dimethyl Sulfoxide (DMSO) then given to mice of dose 1.5 mg/kg/ s.c., three times per week for 2 weeks [12]. Carbidopa-Levodopa (in a dose 25mg/kg/day) and L-dopa was given orally for 14 consecutive days [13].

Preparing *Spirulina fusiform*

Fifty grams of spirulina powder was soaked in 1 L of ultra-pure water and shaken continuously for 24 h at room temperature. The mixture was centrifuged, and the supernatant was filtered to remove the cell debris. The sample was freeze-dried and stored at 4 °C for the experiments. The freeze-dried extract was suspended in distilled water and given via oral gavage daily (200 and 400 mg/kg) for 14 days [14].

Determining proximate composition, mineral contents, and amino acids of *Spirulina*

Moisture, crude fiber, ash, carbohydrate, protein, and fat of *Spirulina* were determined according to AOAC [15]. Amino acids were determined in the Central Service Unit, National Research Centre, Cairo, Egypt using LC3000 Amino Acid Analyzer. Individual elements (Selenium, Calcium, Phosphorus, Magnesium, and Sodium) were determined using Atomic Absorption Spectrophotometers (Perkin-Elmer 3300) according to the method of AOAC [16].

Experimental animals

Adult fifty male albino mice weighing 120±10 g were kept in the laboratory under constant temperature conditions (24±2°C) at a minimum one week before and through the experimental work. Moreover, they were maintained on a standard diet and water ad-libitum. The animals were maintained by the guidelines prescribed by the faculty of Science, and the study was approved by the Animal Ethics Committee of the National Research Centre, Egypt. The experimental mice were being divided into 5 groups (n=8/group). Group I (DMSO) served as control; groups II to V were treated with rotenone (1.5 mg/kg, s.c. 3 times per week) [12], either alone or in combination with L-dopa (25 mg/kg, orally) [13] or spirulina (200 and 400 mg/kg, orally) [17], respectively for two

weeks. Then the mice were sacrificed after behavioral studies were performed and their brains were dissected. The striatum of each brain was stored at -80 till the estimation of PD-related markers.

Behavioral tests

Horizontal bar (wire hanging) test

The examined mice were hanged by their forelimbs into a steel rod (25 cm long, 0.2 cm in diameter), 0.25 m above the bench to evaluate their motor strength, and the time for each mouse could hang suspended from the rod was recorded for three trials with a cut-off time of 180 s [18].

Wood walking test

Mice were made for walking over a wooden stick (\approx 1 m in length, 1 cm in width), and the time each mouse spent to reach the end was recorded to assess their motor coordination [19].

Stair test

To assess skilled reaching, mice were placed at the bottom of a stair (30 cm in length) placed at an angle of 55° above the bench, and the latency to climb the stair was recorded for each mouse [20].

Biochemical analysis

Dopamine determination assay

Dopamine (DA) level was measured by ELISA assay (eBioscience, San Diego, CA) in striatum tissues homogenate according to Sperk [21].

Apoptotic and anti-apoptotic biomarkers assays

The ELISA method was used to quantitatively determine Caspase3 and Bcl2 assays in tissue homogenate according to the method described by Saunders et al. [22] and Paxinos and Franklin [23].

Anti-Inflammatory biomarkers assay

TNF- α and IL-1 β levels were determined by ELISA assay using Biosource International and Camarillo, California, USA Kits. (Europe S.A. Belgium and Pierce Biotechnology, Inc.), according to the procedure reported by Brouckaert et al. and Allan et al. [24, 25], respectively.

Oxidative Stress and antioxidant Biomarkers assays

Both MDA and Nitric oxide levels were evaluated in striatum homogenate as oxidative stress biomarkers in all treated groups according to Ohkawa et al. [26] and Montgomery and Dymock [27], respectively. Reduced glutathione level was measured according to the method described by Beutler and associates [28].

DNA damage

Serum oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine level, was measured in all prepared sera of treated groups as described by Crawley [23, 29].

Histopathological examination

The brain specimens of control, Parkinson's' model, and different treated groups were fixed in 10% buffered neutral formalin. Formalin-fixed brain specimens were routinely dehydrated by alcohol, cleared in xylol, and finally embedded in paraffin. According to Bancroft and Gamble, paraffin blocks were serially sectioned at 4–5 μ m thickness and stained with H&E stain [30].

Statistical analysis

The collected data were expressed as mean \pm standard error. The statistical significances between means were analyzed using one-way Analysis of Variance (ANOVA) followed by Tukey's multiple range test. $P < 0.05$ was considered statistically significant. Multivariate Analysis of Variance (MANOVA) was performed with a post hoc Bonferroni test in SPSS.

3. Results

Results of the behavioral tests

There was no significant change in the grooming of mice in group II that received rotenone compared to the control group in the open field test. On the other hand, there was a significant decrease ($P \leq 0.001$) in rearing, and ambulation in the rotenone treated group, compared to the control group (62.56% & 65.3%, respectively). The latency period determined a significant increase ($P \leq 0.001$) in the rotenone group compared to the control. There was a significant amelioration of rearing, ambulation, and latency period in groups III-V treated with l-dopa or spirulina combined with rotenone, compared to the rotenone group (Figure 1).

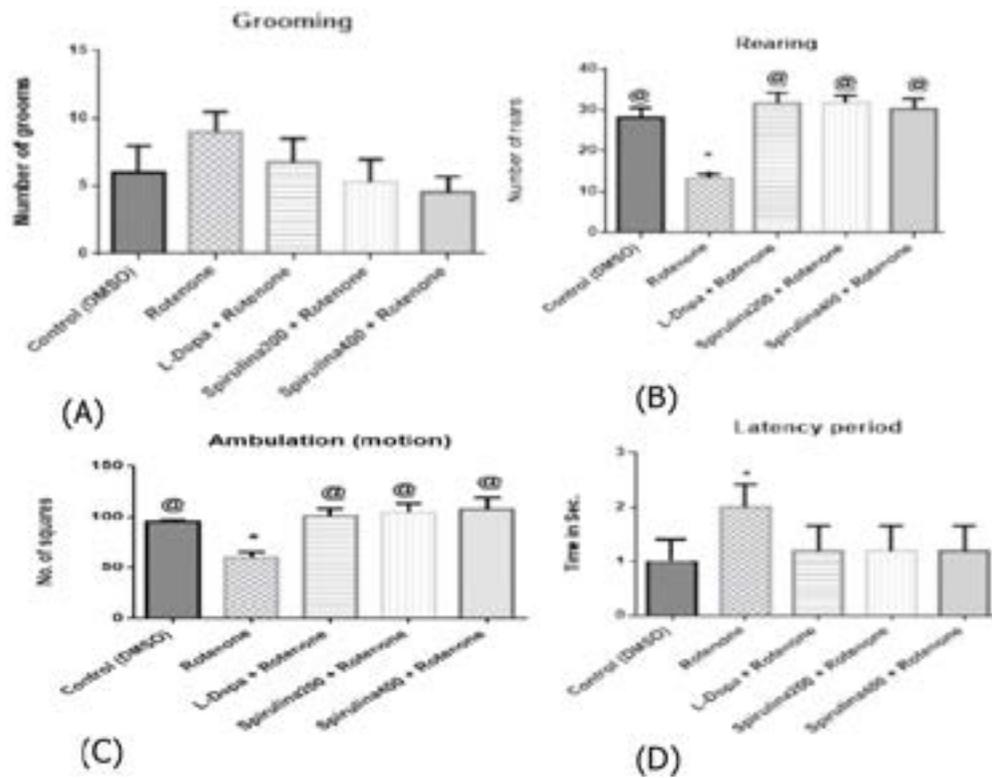


Figure 1. Open field test (grooming A, rearing B, ambulation C, and latency period D) in all treated groups. * Significantly different from control group; @ Significantly different from rotenone group

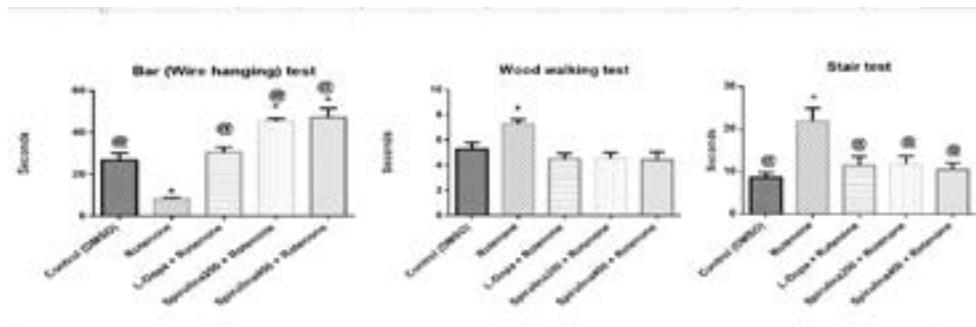


Figure 2. Wire hanging, wood walking, and stair test results in all treated groups. * Significantly different from control group; @ Significantly different from rotenone group

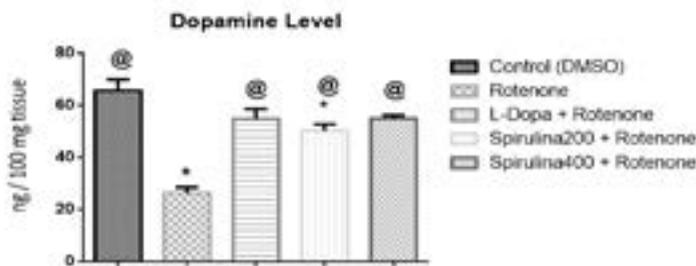


Figure 3. Dopamine level in all treated groups. * Significantly different from control group; @ Significantly different from rotenone group

Our study reported that rotenone-treated mice displayed a shorter hanging time when suspended from a steel rod by 68.95% than the control group. Treatment with spirulina (200 & 400 mg/kg) prolonged the hanging ability by 446% and 466.5%, respectively, versus the rotenone group. The time taken by mice treated with rotenone to traverse a wooden stick was significantly increased by 38% than by control mice. In contrast, treatment with L-dopa and spirulina combined with rotenone almost reversed the rotenone effect in the wood walking test. The examined mice that received rotenone (group II) exhibited an increment in time taken to ascend the stairs by 155%, compared with controls. On the other hand, treatment with L-dopa and spirulina (groups III-V) reversed the effect of rotenone (Figure 2).

Biochemical results

Dopamine content

In the present study, dopamine level was significantly decreased ($P \leq 0.001$) in groups II and IV by 59.9% and 16.6%, respectively, compared with dopamine level in group I. However, there was a significant improvement in dopamine levels in groups III–V, compared with group II (Figure 3).

Apoptotic and anti-apoptotic biomarkers

Treatment with L-dopa and spirulina (groups III-V) attenuated striatal apoptosis induced by rotenone in group II (Figure 4). There was a significant increase ($P \leq 0.001$) in the caspase-3 level in the rotenone group and a significant decrease in Bcl2 level in the same group (476.9% & 52.8% respectively), compared with those of the control (group I).

Anti-Inflammatory biomarkers

From the current study, both TNF- α and IL-1 β levels were significantly increased ($P \leq 0.001$) in the rotenone treated group with 532.7% and 321.8%, respectively, compared with the control group. Treatment with L-dopa and spirulina (200 & 400 mg/kg) reduced the elevated levels of these cytokines; however, it failed to restore the normal level of the control (Figure 5).

Oxidative stress and antioxidant biomarkers

Our data suggested no significant change ($P = 0.421$) in NO level in all treated groups, compared to the controls; while there was a significant increase ($P \leq 0.001$) in MDA level in group II (44.5%), compared to control and a significant improvement of its level in groups III-V, com-

pared with the group II. There was a significant decrease ($P \leq 0.001$) in the level of reduced glutathione by 38.5% in group II that received rotenone, compared to control with an amendment in its level in group III-V compared to group II (Figure 6).

DNA damage

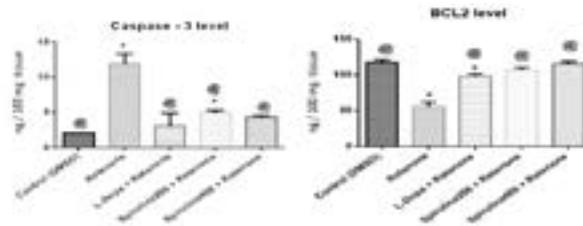
There was a striatal DNA fragmentation, and increased striatal 8-OHdG levels and MTH1 expression in the rotenone treated group. Similarly, oxidative DNA damage in the form of oxidized guanine is retained in the mitochondrial and nuclear DNA of dopamine-producing neurons of the SN in PD (Figure 7).

The chemical composition of the Spirulina powder:

The current study reported that Spirulina powder contains a high quantity of protein (60.73%), fat (5.92%), and carbohydrate (15.81%); thus, it makes it supply high calories (359.44 Kcal/100 g). The results of minerals content showed that the Spirulina powder contained high concentrations of Calcium (153.80 mg/100 g), Phosphorus (101.10mg/100 g), and Sodium (762.70mg/100 g). The results of the amino acids profile showed that the Spirulina powder contained high amounts of Tyrosine (89.67mg/g protein), Methionine (71.81 mg/g protein), Leucine (66.72 mg/g protein), and Isoleucine (60.24mg/g protein) (Table 1).

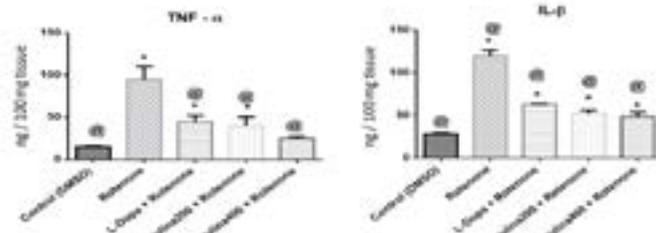
Histopathological examination results

The microscopic examination of different sections of the substantia nigra of control mice presented normal darkly stained substantial neurons (Figure 8A). The striatum of those control mice showed a normal histological appearance of striatal neurons (Figure 9A). While the examination of brain sections of rotenone administered mice revealed marked histological alterations in the substantia nigra and the striatal areas. Regarding the substantia nigra of rotenone administered mice, the examination data indicated marked neuronal degeneration with an apparent loss of the dark melanin pigmentation of those neurons and necrosis of the other neurons; some of them appeared ghost-like without any nuclear structures (Figure 8B). Most mice generated a marked loss of the substantial neurons with the appearance of remnants of necrotic neurons with prominent vacuolar degeneration of other neurons (Figure 8C). However, the striata of those mice revealed widespread neuronal degeneration and shrinkage with marked pericellular and perivascular edema and necrosis, and apoptosis (Figure 9B).



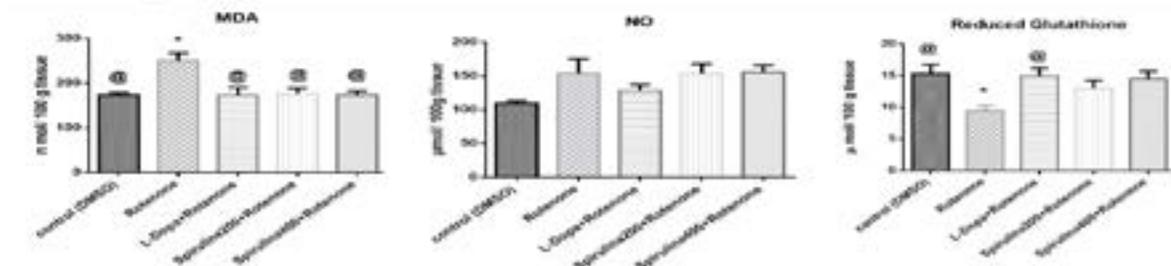
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Figure 4. Both caspase-3 and Bcl2 levels in all treated groups. * Significantly different from control; @ Significantly difference from Rotenone



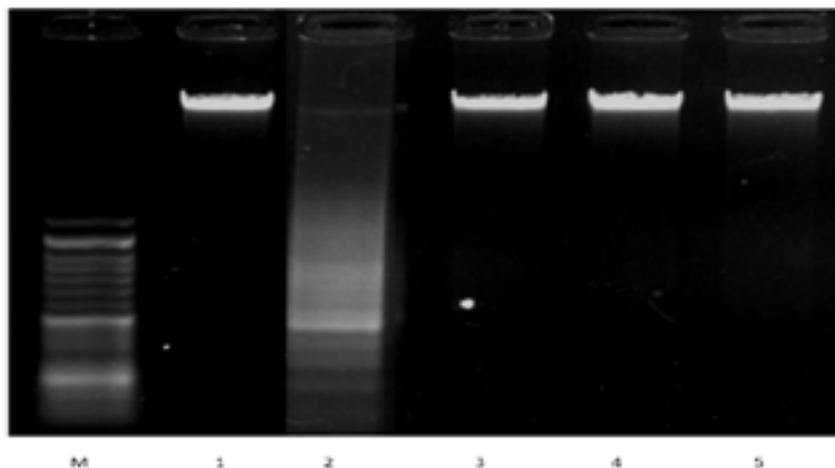
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Figure 5. Anti-Inflammatory biomarkers levels in all treated groups. * Significantly different from control group; @ Significantly difference from rotenone group



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Figure 6. MDA, NO, and GSH levels in all treated groups. * Significantly different from control; @ Significantly difference from Rote



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Figure 7. An agarose gel electrophoresis presenting DNA fragmentation. M: DNA marker with 100 bp. Lane1, 3, 4, &5: DNA without streaks or laddering of groups I, III-V. Lane 2: DNA with marked streaks and laddering (fragmented) of group II.

Table 1. proximate composition, mineral contents, and amino acids of Spirulina

Chemical Compositions		Values
Amino acids (mg/g)	Threonine	37.66
	Valine	56.25
	Methionine	71.81
	Cystine	17.21
	Isoleucine	60.24
	Leucine	66.72
	Histidine	12.34
	Tyrosine	89.67
	Phenylalanine	14.74
	Lysine	13.59
	Aspartic acid	29.88
	Glutamic acid	14.85
	Serine	10.93
	Glycine	10.45
	Arginine	7.51
Alanine	17.58	
Proline	29.78	
Chemical composition (g/100g) (mean±SD)	Moisture	5.30±0.44
	Crude protein	60.73±1.06
	Fat	5.92±0.22
	Crude fiber	3.22±0.15
	Ash	14.32±0.35
	Total carbohydrates	15.81±0.29
Minerals (mg/100 g)	Selenium	0.005
	Calcium	153.80
	Phosphorus	101.10
	Magnesium	7.90
	Sodium	762.70

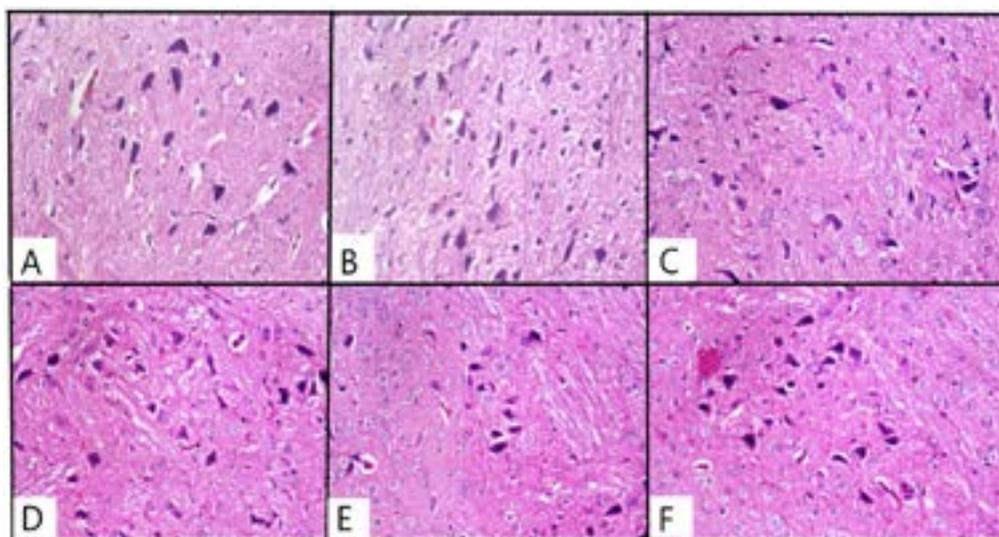


Figure 8. A) A photomicrograph of the Substantia nigra of control mice showing normal darkly stained substantial neurons. B) Substantia nigra of rotenone administrated mouse presenting neuronal degeneration with loss of the melanin dark pigmentation and necrosis of the other neurons, some appearing ghost-like. C) Substantia nigra of rotenone administrated mouse presenting the loss of the substantial neurons, remnants of necrotic neurons, and vacuolar degeneration of the remaining. D) Substantia nigra of rotenone administrated mouse and treated with L-Dopa showing restoration of most of the substantial neurons with mild vacuolar degeneration and scattered necrosis of some neurons, notice loss of the melanin pigment from some of the neurons. E) Substantia nigra of rotenone administrated mouse and treated with 200mg of spirulina, demonstrating the moderate restoration of the substantial neurons with the restoration of the content of melanin pigment and few necrotic cells. F) Substantia nigra of rotenone administrated mouse and treated with 400mg of s Spirulina suggesting a good restoration of the substantial neurons with reservation of the typical dark appearance and only mild vacuolation of a few of them (H&E, X400).

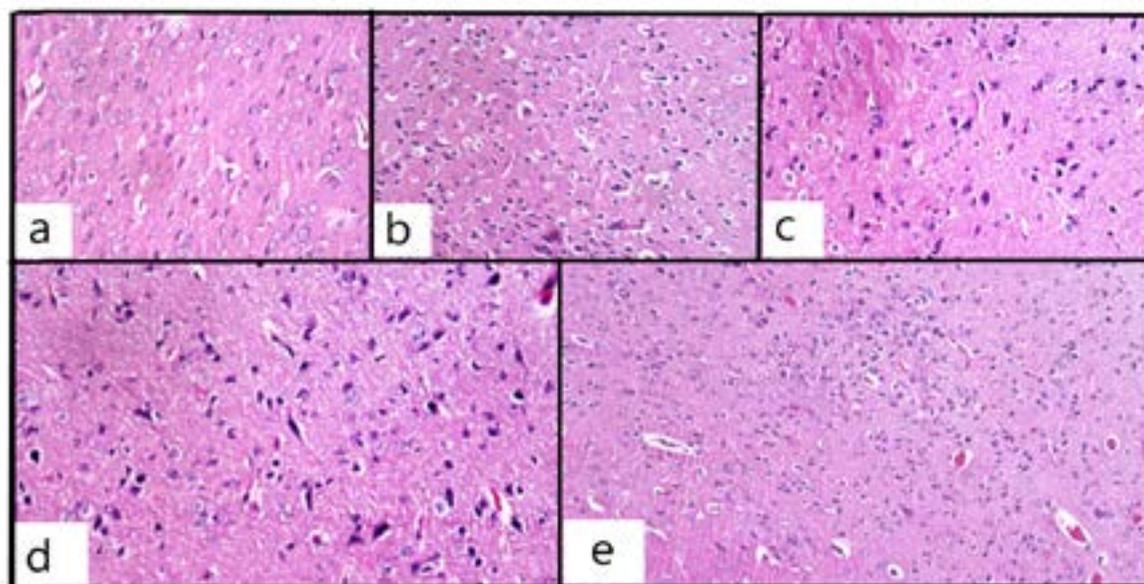


Figure 9. a) A photomicrograph of Striatum of the control mouse shows striatal neurons' normal histological appearance. b) Striatum of rotenone administrated mouse showing widespread neuronal degeneration, shrinkage with pericellular and perivascular edema, as well as necrosis and apoptosis. c) Striatum of rotenone administrated mouse and treated with L-Dopa showing mild perineuronal edema, mild degeneration of some neurons, and scattered neuronophagia. (H&E, X400). d) Striatum of rotenone administrated mouse and treated with 200mg of spirulina showing moderate degenerative changes in the striatal neurons, scattered necrosis, and few neuronophagia. e) Striatum of rotenone administrated mouse and treated with 400mg of Spirulina showing the moderate degree of restoration of the striatal neurons, only a few appearing shrunken with perineuronal edema and few apoptotic cells (H&E, X200).

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Concerning the treated groups, the administration of L-Dopa could restore the observed alterations. On the other hand, the drug administration presented a dose-related restoration of the observed histological alterations. The substantia nigra of rotenone administrated mice and treated with L-Dopa indicated the restoration of most of the substantial neurons with mild vacuolar degeneration and scattered necrosis of some neurons. Mild loss of the melanin pigment from some neurons was also noticed (Figure 8D). The striatum of rotenone administrated mice and treated with L-Dopa demonstrated mild perineuronal edema with mild degeneration of some neurons and scattered neuronophagia (Figure 9C).

The substantia nigra of rotenone administrated mice and treated with 200mg of the drug revealed moderate restoration of the substantial neurons with the restoration of their melanin pigment content and a few scattered necrotic cells (Figure 8E). The striata of those mice presented a moderate degree of degenerative changes of the striatal neurons; some of which appeared shrunken with scattered necrotic neurons and few neuronophagia (Figure 9D).

The substantia nigra of rotenone administrated mice and treated with 400mg of the drug showed good res-

toration of the substantial neurons with reservation of the typical dark appearance and only mild vacuolation of a few of them (Figure 8F). The striatal areas of rotenone administrated mice and treated with 400mg of the drug demonstrated a moderate degree of restoration of the striatal neurons. Only a few appeared shrunken with perineuronal edema and few apoptotic cells (Figure 9E).

4. Discussion

Rotenone administration has been appeared to emulate essential parts of Parkinsonism Issue (PD) pathology, for example, dopaminergic neurodegeneration in the Substantia Nigra (SN). These outcomes in the exhaustion of associated neurotransmitters ultimately result in motor dysfunction [31, 32], oxidative pressure, neuroinflammation, and α - synuclein conglomeration [32].

It is critical to promote the antioxidants and neuroprotective activities of naturally occurring spirulina algae to improve their efficacy in experimental studies to elevate their importance to humans [34]. The obtained data suggested that spirulina powder indicated high protein, fat, and carbohydrate contents, which supply high calories and high amounts of calcium, phosphorus, and sodium. Furthermore, spirulina contained some essential amino

acids, such as Tyrosine, Methionine, Leucine, and Iso-leucine. The results of the chemical composition of spirulina are in agreement with those obtained and Morsy and associates [35].

The current study reported exacerbated the mind harm brought about by rotenone in dopaminergic neurons in the striatum. Rotenone causes neuronal cell death through expanded oxidative pressure, a significant pathogenetic system adding to DA-ergic neurodegeneration in human PD. Researchers accordingly demonstrated the expanded arrangement of receptive oxygen metabolite, increased lipid peroxidation items, and protein carbonyls. Social tests showed a particular decline in dopamine consumption and diminished engine coordination with augmented bradykinesia. Our results agreed with data reported by Alzahrani et al. [36] and Farombi et al. [37] exhibited by the hindered locomotor capacity surveyed in two well-recorded social tests, the shaft test and open field tests.

Our results reflected a significant decrease in dopamine level in the rotenone treated group, with amelioration of its level with L-dopa and spirulina high dose. This is similar to the findings of Gu'naydin and colleagues [38]. The biochemical examination affirmed that rotenone-treated mice reduced one-quarter of the striatal dopamine level in the vehicle-treated mice, compared with rotenone treatment with Tribulus Terrestris extract, revealing a significantly improved dopamine level.

Compared with the control, our data showed a significant elevation in both TNF- α and IL-1 β levels in the group treated with rotenone. These findings agreed with Sharma et al. [39] and Wang and associates [40]. The supplementation of mice with a low and high dose of spirulina significantly reduced ($P \leq 0.001$) their levels in group IV and V, compared with group II. Data from the current study indicated a significant increase ($P \leq 0.001$) in MDA and NO levels with a significant decrease in GSH level in group II that received rotenone compared with group I. Similar results were observed by Abdel-Salam et al. [41] and Zhang and associates [42]. This occurred alongside stamped oxidative pressure presented by the expansion in the lipid peroxidation final result MDA and the decline in the cell reinforcement atom GSH in tissues. Rotenone infusion also expanded articulation of the proinflammatory cytokine TNF- α and the apoptotic factor caspase-3 in tissues. While treatment mice with spirulina improved MDA, NO, and GSH, these findings agreed with results obtained by Cho and colleagues [43].

Our study data reported a significant increase ($P \leq 0.001$) in caspase-3 level due to nigrostriatal damage, the loss of substantia nigra TH-ir, neuronal damage, focal gliosis in the cerebral cortex with a significant decrease in Bcl-2 level in group II that injected with rotenone, compared to control these similar with the findings reported by Liu et al. [44] and Jiang et al. [45], with refinement in their levels in groups treated with spirulina.

Microscopic examination of the brain sections of rotenone-treated mice revealed marked histological alterations in both the substantia nigra and the striatal areas. Regarding the SN of rotenone administered mice, the examination suggested marked neuronal degeneration with an apparent loss of the dark melanin pigmentation of those neurons and necrosis of the other neurons. These are similar findings reported by Ngala and associates [46]. Moreover, other research-recorded control sections suggested decreased staining of the substantial neurons with vacuolar degeneration, necrosis, shrinkage, and ghost appearance of most of them [33]. Sections of the standard treatment group showed good restoration of the substantial neurons. Most of them had near to normal appearance, with only a few necrotic cells with scars neuronophagia. On the other hand, there was a significant improvement in the SN and the striatal areas in brains of rotenone-treated mice by Spirulina supplementation in groups IV and V. These results agree with results obtained by Wang et al. [47] and Ange and associates [48].

In the present research, rotenone-induced a significant elevation in MDA, NO, TNF- α , IL- β , and a significant decrease in dopamine, Bcl-2, GSH levels, compared to the controls with remarked change in brain tissue. These results agreed with those of other studies [39-42], improving their levels after treatment with spirulina [40-43].

5. Conclusion

The current study provided oxidative damage in nucleic acid is a crucial risk factor for experimental PD in mice induced by rotenone. The histopathological changes in brain tissues and the biochemical parameters in rotenone-treated mice were improved after spirulina administration. This evidence a protective effect of spirulina against the neurotoxicity of rotenone connects to spirulina's antioxidant and anti-inflammatory effects.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee Of the Medicine college, Fayoum university (Code: 2456633).

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Author's contributions

All authors equally contributed to preparing this article

Conflict of interest

The authors declared no conflict of interest

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