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Unravelling the Role of Lipoxin A4 in Rotenone Induced Parkinson's Disease in Experimental Male Albino Rats

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- Parkinson's disease
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- PPAR gamma receptor
- TNFα
- Elevated T maze test.

Abstract

Thesecond most prevalent age-related neurodegenerative illness is Parkinson's disease (PD). The purpose of our study was to examine how lipoxin A4 contributes to rotenone-induced Parkinson's disease in male albino experimental rats.Three equal groups of thirty male rats were created: Control I, Rotenone-treated Group II, and Lipoxin A4-treated Group III. Finally, tests of rats' locomotor activity, forced swimming, and elevated T maze were performed.A colorimetric technique was utilized to assess the biomarkers of oxidative stress (MDA and GPX). Furthermore, measurements were made of the inflammatory markers TNFα, IL6, and INOS.In addition,PPAR gamma receptor was measured. Group III that received lipoxin exhibited a significant improvement in motor tests, a drop in inflammatory markers (TNFα, IL6, and INOS) and oxidative stress markers (MDA), and a significant rise in antioxidant GPX levels. Additionally, our results indicated a significant increase in PPAR gamma receptor.Therefore, Lipoxin A4 can be considered to be used as a treatment for parkinsonism.

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Introduction

About 3% of adults 65 and older have Parkinson's disease (PD), the second most prevalent neurodegenerative illness that is strongly correlated with age. It involves increasing and advanced sickness, as well as various multi-factors. In PD patients, it results in motor system dysfunction, including bradykinesia, stiffness, and resting tremors. Damage to dopaminergic (DA) neurons at the substantia nigra pars compacta (SNpc) is the cause of these symptoms (**1).**Non-motor symptoms of the condition include sleep abnormalities, autonomic nervous system dysfunction, neuropsychiatric issues, visual impairments, cognitive impairment, and gastrointestinal disorders(**2)**.

The etiology of dopaminergic neuronal loss is not fully understood; nonetheless, evidence indicates a relationship among mitochondrial dysfunction, neuropathological mechanisms, neuronal inflammation, and compromised proteasomal protein clearance. However, this disease is being well-characterized, the diagnosis is only confirmed by the post-mortem autopsy, and the actual trigger of the disease is not obvious**(3).**

Lipids play a significant role in regulating various metabolic processes and the expression of transcription factors, influencing nervous system function, insulin sensitivity, and both the oxidation and synthesis of fatty acids. Multiple studies have showed the polyunsaturated fatty acids (PUFAs) anti-inflammatory effects and their probable use in treatment of neurodegenerative diseases, as lateral sclerosis, Huntington's disease, multiple sclerosis, dementia, Parkinson's disease and Alzheimer's disease(**4)**.

Originating from omega 6, an arachidonic acid derivative produced by the lipoxygenase enzyme, lipoxins (LXs) have a number of pro- and anti-inflammatory properties and are classified based on the hydroxyl group location at the molecule structure. Small quantities of LXA4 and LXB4 are found in physiological conditions. However, their levels are increased in pathological conditions in order to reduce inflammation **(5).**

A number of researches on acute renal injury, gynecological inflammation, lung disease, gastrointestinal injury, and peritoneal inflammation have found that synthetic lipoxins, such as LXA4, have a significant anti-inflammatory contribution. Several studies have demonstrated that lipoxin has a significant impact on the control of disorders of the central nervous system, including Alzheimer's and cerebrovascular diseases**(6).**

It has been acknowledged that a malfunction in the resolution phase causes persistent inflammation, which in turn causes tissue necrosis, fibrosis, and permanent damage. Recent research has shown that persistent inflammation can lead to a number of pathological conditions that were previously thought to be inflammatory, including cancer, atherosclerosis, Alzheimer's disease, and cardiovascular illnesses. This highlights the significance of controlling the resolution process **(7).**

Today's treatments for these conditions include corticosteroids and non-steroidal anti-inflammatory drugs, but they have more side effects, including high blood pressure, osteoporosis, elevated blood sugar, bleeding, neurological changes, and in some cases, treatment resistance. Nonetheless, LXs have are potent anti-inflammatory drugs with minimal adverse effects, suggesting that they are predictive biomarkers. We still don't fully comprehend their mechanism or their true purpose**(8).**

Nuclear receptors contain transcription factors called peroxisome proliferator-activated receptors (PPARs), which play a part in the control of oxidative stress, mitochondrial function, inflammation, glucose and lipid metabolism, and wound healing. It has been established that they play a clear part in controlling neurodegenerative diseases and regulating the inflammatory process **(9).**The aim of this work is to study the potential role of LXA4 inparkinson's disease through PPARγ-mediated pathways.

2 Materials and Methods:

2.1 Experimental animals

Thirty male albino rats of a local strain were used in this study. The rats, which weigh between 150 and 200 grams, were housed in separate animal cages (10 rats per cage) in the research lab of the physiology department, where they were housed at room temperature (22–25°C) with a 12-hour light-dark cycle and given unlimited access to food and water. The dead rats were changed during the workday.The ethical committee of Tanta University's faculty of medicine approved all procedures under code number (36264PR/6/24).

2.2 Drugs and chemicals:

The supplier of rotenone was Kahira Pharmaceutical Co. in Cairo, Egypt. Sigma-Aldrich Co. (Louis, MO) provided LXA4. These medications were all dissolved in saline.

2.3 Animal groups:

The animals were fasted for 24 hours prior to the induction of Parkinson's disease, but were allowed free access to water. Rats were allocated randomly into three groups, ten rats each, as follows:

I- Control group: For five weeks, the rats in this group were given an intraperitoneal (I.p.) injection of 1 milliliter of normal saline once every day.

II- Rotenone treated group: The rats of this group received(o.5 ml) rotenone (2mg/kg) subcutaneously once a day for 5 weeks **(10).**

III- Lipoxin, Rotenone treated group: The rats of this group receivedsingle dose of lipoxin-A4 (0.1 µg/kg) intraperitoneallyafter 5 weeks of rotenone injection**(11).**

At the end of experimental period all the animals from all groups were subjected for:

2.4 Behavioral tests:

1- Locomotor Activity Test (Open field test).

This test was conducted in accordance with the methodology outlined by (12) in order to evaluate exploratory behavior as an indicator of spontaneous motor activity. The open field test was utilized to assess the rat's locomotor activity.The rats were given fifteen minutes to freely explore the equipment throughout this test. That device had a 60 cm × 60 cm black floor, white 60 cm high walls, and wood covered in impermeable Formica.The lighting in this test room was controlled and quiet. Every rat was positioned in the middle of the open field, and for fifteen minutes, the distance traveled every five minutes was documented.The equipment was cleaned using a 5% ethanol solution following each rat's behavioral assessment.

2- Forced swimming test.

The forced swimming test, which was performed in accordance with the protocol outlined by (13) and focused on the animal's reaction to the danger of drowning, was interpreted as assessing vulnerability to negative mood.This test was based on the finding that animals adopt an immobile posture when placed in a stressful situation from which they have no way of escaping. (i.efloating occurs without any motions other than those required to keep the nose above water)following an initial period of agitation.This behavior is used

to examine depressive-like behavior and hopelessness. Each animal was housed separately in a 40 cm high by 23 cm wide cylinder with water up to 25 cm below the top.Following an initial adaption period of one minute, the immobility time was assessed for five minutes. A measure of an antidepressant-like effect is the decrease in this immobility time.

3- T-maze test: Elevated T-maze (ETM) task.

Rats' learning and memory characteristics were assessed using the ETMaccording to the method described by Conde et al. (1999)**(14).**The ETM featured three equal-sized arms (30 cm x 6 cm) and was constructed of wood. Two opposing arms were perpendicular to one arm, which was 16 cm high and surrounded by walls. The open arms were enclosed by a 0.5 cm high Plexi glass border. The entire piece of equipment was raised 40 centimeters off the ground. On the training day,each rat was positioned at the distal end of the enclosed arm, facing the arm junctionand was permitted to investigatethe enclosed arm. The trial came to an end when the rat either stayed in the enclosed arm for a maximum of 300 seconds or entered one of the open arms with all four paws. Rats were re-exposed to the ETM as many times as necessary to stay in the enclosed arm for 300 seconds (avoidance criterion) during the training session, which was done on a single day.Learning was assessed by counting the number of trials required to meet the avoidance criterion. For every trial, the amount of time the animal spent in the enclosed arm was monitored and noted(avoidancelatency).

Crucially, the animals' anxiety levels were evaluated throughout the first three trials of the training session (i.e., trials 1, 2, and 3). After a 30-second break between trials, the animals were put back in their original cages. Rats were re-exposed to the enclosed arm in two follow-up trials (i.e., test and retest) 24 hours following training (test session). Memory retrieval was estimated by measuring the amount of time the animal spent in the enclosed arm.The experiments took place in a calm, dimly lighted room with a 3 m × 3 m observer positioned at least 2 m away from the open arms of the apparatus. Every experiment occurred from 13:00 to 17:00. Between rats, a 5% ethanol solution was used to clean the equipment.

2.5 Tissue sample preparation:

The following parameters were measured after the brain tissue was removed and sliced into tiny pieces.

2.6 Real time quantitative PCR estimation forperoxisome proliferator-activated receptor gamma gene) relative gene expression:

The Gene Jet RNA purification kit was used to isolate total RNA from brain tissue samples in accordance with the kit's instructions (Thermo Scientific, # k 0731 USA) (15). A NanoDrop spectrophotometer (NanoDrop Technologies, Inc., Wilmington) was used to measure the concentration and purity of total RNA at the OD260 and OD260/280 ratios, respectively. The samples were subsequently kept at -80 °C. Using Primer 5.0 software, the primer sequences were conceptualized as follows: The forward primer (NCBI GenBank Nucleotide accession # NM_053655.3) was 5′-CATGCTTGTGAAGGATGCAAG-3′. On the other hand, 5′-TTCTGAAACCGACAGTACTGACAT-3′ was the reverse primer. Using the previously established 2-ΔΔCt technique **(16),** the relative expression level of gene was calculated after being normalized to the B-actin housekeeping gene.

2.7 Biochemical analysis of homogenates of brain tissues samples

The cold phosphate buffer (pH 7.4) was used to homogenize the brain tissues. After that, the brain homogenates were put in centrifugation tubes and centrifuged for 20 minutes at 3,000 rpm. Inflammatory indicators such as tumor necrosis factor-α (TNF-α) (Cat# No MBS355371), and interleukin-6 (IL-6) (Cat# No ab290735) were all measured using the resultant supernatants and ELISA kits. Glutathione peroxidase (Cat# No ab252833), the quantity of inducible nitric oxide synthase (iNOS) (Cat# No ab15323), and malondialdehyde (MDA), an oxidation marker (Cat# No NWK-MDA01), were allmeasuredcalorimetrically.

In accordance with infection control and safety protocols, the sacrificed animals will be shipped in a special package with hospital biohazard (17).

Approval code: 3626PR/6/24.

2.8 Statistical analysis:

The mean ± standard deviation was used to illustrate the findings. One-way ANOVA was used to ascertain the study's results, and Tukey's test was used to determine their significance. When p-values were less than five, statistical significance was taken into account. The SPSS program (Version 23.0, IMB, NY) was used for all of the analyses.

3. Results

3.1 Effect of LXA_4 on Locomotor Activity (fig 1A):

Administration of rotenone significantly reduced locomotor activity ($p<0.05$) in comparison to the control group. However, compared to the Rotenone group, LipoxinA4 therapy significantly ($p<0.05$) enhanced locomotor activity. It was found that the control group and the LipoxinA4-treated group did not significantly vary in this assessed parameter

3.2 Effect of LXA_4 on forced swimming test (fig1B):

According to our findings, the rotenone group's immobility time during the forced swimming test was significantly ($p<0.05$) longer than that of the control group. In contrast to the rotenone group, the Lipoxin A4-treated group demonstrated a substantial ($p<0.05$) decrease in immobility time during the forced swimming test. It was found that the control group and the Lipoxin A4-treated group did not significantly vary in this assessed parameter.

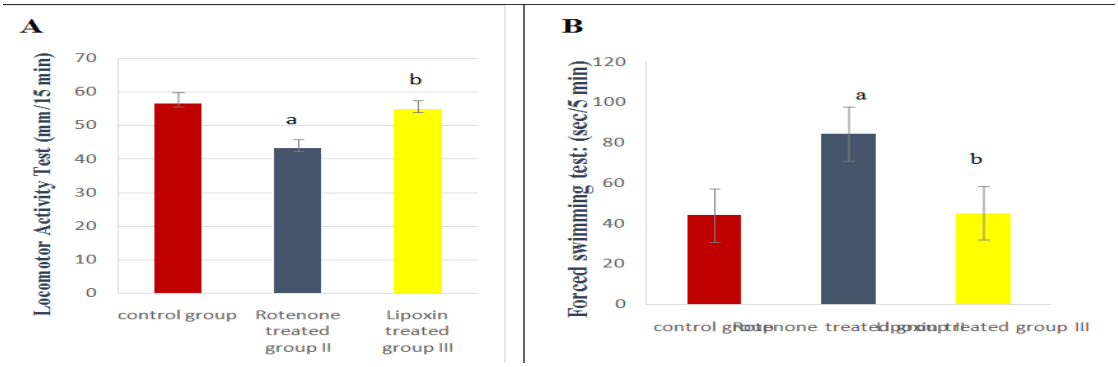

Fig. 1: Graphical representation of effects of Lipoxin A4 on
A) Locomotor Activity (fig 1A)
B) forced swimming test (fig1B)
Data are represented as mean ± SD
a denotes statical significance ($p\leq 0.05$) a shows significance as compared to control group
b shows significance ($p\leq 0.05$) as compared to rotenone treated group

3.3 Effect of LXA_4 on Elevated T maze test (Training session in seconds: Trial 1,2,3 and numbers of trails) (fig.2):

In comparison to the control group, we have shown that rotenone treatment significantly ($p<0.05$) lengthens the duration that animals spend in the enclosed arm during the raised T maze test (Trials 1, 2, 3, and the number of trails). However, when compared to the rotenone group, Lipoxin therapy results in a substantial ($p<0.05$) reduction in the amount of time that animals spend in an enclosed arm during the elevated T maze test (Trials 1, 2, 3, and the number of trails). It was found that the control group and the LipoxinA4-treated group were not significantly different in this assessed parameter.

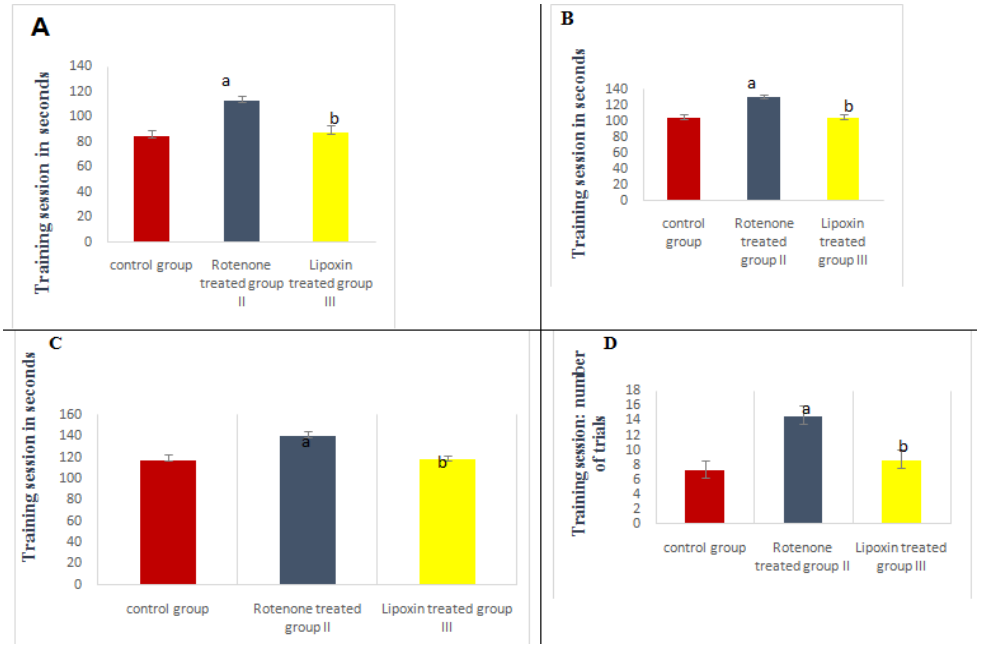

Fig. 2: Graphical representation of effects of Lipoxin A4 on
A) Elevated T maze test (Trial 1)
B) Elevated T maze test (Trial 2)
C) Elevated T maze test (Trial 3)
D) Elevated T maze test (Numbers of trails)
Data are represented as mean ± SD
a denotes statical significance ($p\leq 0.05$) a shows significance as compared to control group
b shows significance ($p\leq 0.05$) as compared to rotenone treated group

3.4 Effect of LXA_4 on Elevated T maze test (Test Session and Re test Session) (fig.3):

Rotenone administration significantly ($p<0.05$) shortens the time animals spend in the enclosed arm during the raised T maze test (Test Session & Retest Session) as compared to the control group. However, when compared to the rotenone group, LipoxinA4 administration significantly ($p<0.05$) increased the amount of time the rats spent in the enclosed arm during the raised T maze test (Test Session & Retest Session). It was found that the control group and the LipoxinA4-treated group did not significantly vary in this assessed parameter.

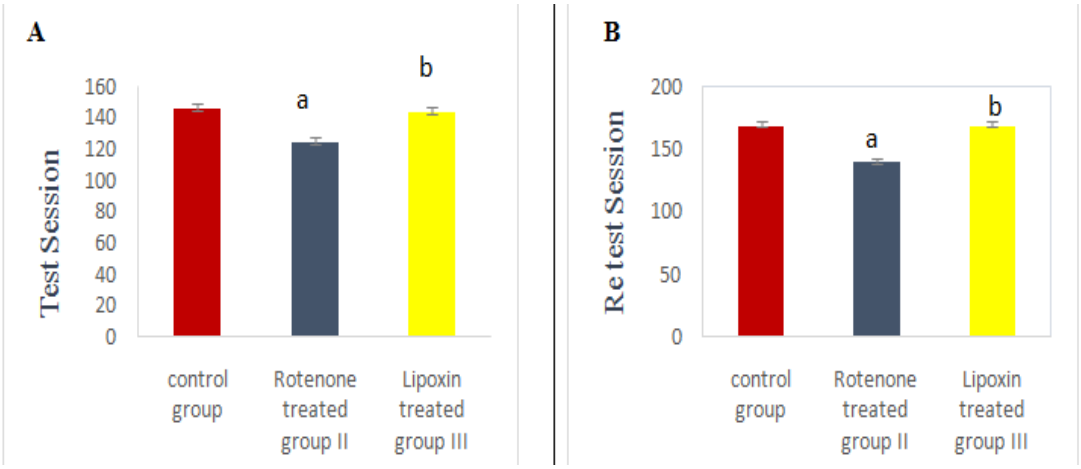

Fig. 3: Graphical representation of effects of Lipoxin A4 on
A) Elevated T maze test (test session)
B) Elevated T maze test (retest session)
Data are represented as mean ± SD
a denotes statical significance ($p \leq 0.05$) a shows significance as compared to control group.
b shows significance ($p \leq 0.05$) as compared to rotenone treated group.

3.5 Effect on oxidative stress markers (fig.4):

We have shown that when compared to the control group, rotenone treatment results in a significant ($p<0.05$) rise in MDA. However, when compared to the Rotenone group, LipoxinA4 therapy significantly ($p<0.05$) reduced MDA. Additionally, it was noted that the LipoxinA4-treated group's measured parameter increased significantly ($p<0.05$) when compared to the control group. However, when compared to the control group, rotenone treatment significantly ($p<0.05$) reduced GPX. However, GPX was considerably ($p<0.05$) higher in the LipoxinA4 group than in the Rotenone group. Additionally, it was noted that the LipoxinA4-treated group's measured parameter decreased significantly ($p<0.05$) when compared to the control group.

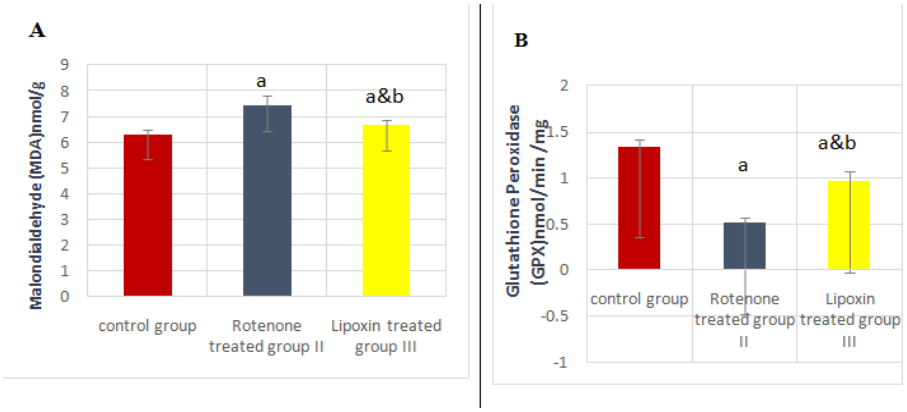

Fig. 4: Graphical representation of effects of Lipoxin A4 on
A) MDA
B) GPX
Data are represented as mean ± SD
a denotes statical significance ($p \leq 0.05$) a shows significance as compared to control group
b shows significance ($p \leq 0.05$) as compared to rotenone treated group.

3.5 Effect of LXA_4 on PPAR gamma receptor (tab.1):

The injection of rotenone significantly ($p<0.05$) reduced the PPAR gamma receptor in comparison to the control group. However, in contrast to the Rotenone group, LipoxinA4 therapy significantly ($p<0.05$) enhanced PPAR gamma receptor. Additionally, it was noted that the LipoxinA4-treated group's measured parameter decreased significantly ($p<0.05$) when compared to the control group.

Table1: Effect of LXA_4 on PPAR gamma receptor.

Parameters	*Control group*	*Rotenne treated group*	*Lipoxin treated group*
PPAR gamma receptor	1 ± 0	0.76± 0.07 a	0.92± 0.04 a&b

Data are given as mean ± *SD*
a shows significance p≤ 0.05 as compared to control group
b shows significance p≤ 0.05 as compared to Rotenone treated group.

3.6 Effect on inflammatory markers (fig.5):

According to our findings, rotenone treatment raised TNFα, IL6, and INOS considerably ($p<0.05$). However, when compared to the Rotenone group, LipoxinA4 administration significantly ($p<0.05$) reduced TNFα, IL6, and INOS. Additionally, it was noted that the control group and the LipoxinA4-treated group had no significant differences in these assessed parameters.

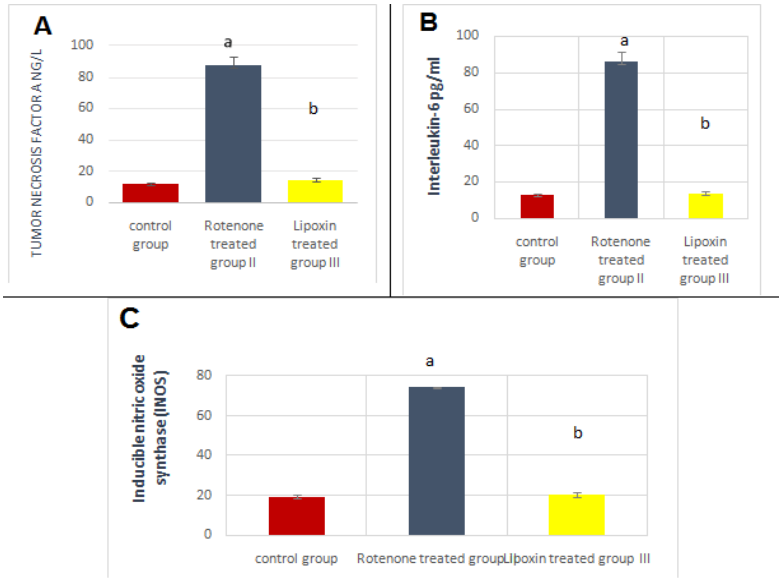

Fig. 5: Graphical representation of effects of Lipoxin A4 on
A) TNFα
B) IL6
C) INOS
Data are represented as mean ± SD
a denotes statical significance (p≤ 0.05) a shows significance as compared to control group
b shows significance (p≤ 0.05) as compared to rotenone treated group

4. Discussion

This study investigated the role of Lipoxin A4 (LXA4) in alleviating the behavioral and biochemical deficits induced by rotenone in a Parkinson's disease (PD) model using male albino rats. The findings indicate that LXA4 significantly

mitigates rotenone's detrimental effects, emphasizing its potential as a neuroprotective agent.

Behavioral analyses revealed significant impairments in motor activity, depressive-like behavior, and anxiety levels in rotenone-treated rats. These results are consistent with rotenone's known ability to disrupt dopaminergic pathways, leading to motor dysfunction and neuropsychiatric manifestations **(1).**

LXA4 treatment effectively restored motor activity, reduced immobility in the forced swimming test, and normalized anxiety-like behaviors in the elevated T-maze test. This aligns with previous studies suggesting that LXA4 can ameliorate neurobehavioral deficits through its anti-inflammatory and antioxidant properties **(5).**

Rotenone administration induced significant oxidative stress, evidenced by elevated malondialdehyde (MDA) levels and reduced glutathione peroxidase (GPX) activity. Additionally, rotenone increased pro-inflammatory markers, including TNFα, IL6, and iNOS, while suppressing PPARγ expression. These findings are consistent with studies demonstrating that rotenone triggers neuroinflammation and oxidative damage, leading to neuronal dysfunction **(3).**

LXA4 treatment reversed these alterations by reducing MDA and inflammatory cytokines while restoring GPX activity and PPARγ levels, highlighting its dual role in oxidative stress reduction and inflammation modulation **(7, 9).**

The observed upregulation of PPARγ by LXA4 is particularly noteworthy, as this receptor is a key regulator of mitochondrial function, redox balance, and inflammation. PPARγ activation has been linked to reduced neuroinflammation and improved neuronal survival in neurodegenerative conditions **(9).**

These findings suggest that LXA4 exerts its neuroprotective effects, at least in part, through PPARγ-mediated pathways, a mechanism supported by previous studies on lipoxins in neurodegenerative diseases **(6).**

The findings underscore the therapeutic potential of LXA4 in PD. Current treatments, such as non-steroidal anti-inflammatory drugs and corticosteroids, often cause significant side effects, including neurological disturbances and metabolic imbalances **(8).**

LXA4 offers a safer alternative, with its ability to target multiple pathological pathways, including oxidative stress and neuroinflammation, making it a promising candidate for further development.

While this study provides valuable insights, it is not without limitations. The short duration of the experiment limits the understanding of LXA4's long-term efficacy and safety. Moreover, the study did not investigate the molecular mechanisms beyond the measured markers. Future research should focus on the chronic effects of LXA4, explore its interactions with existing PD therapies, and evaluate its effects in other neurodegenerative models to validate its therapeutic potential **(4).**

5. Conclusion

In conclusion, LXA4 demonstrated significant efficacy in reversing the behavioral and biochemical alterations induced by rotenone. By targeting inflammation, oxidative stress, and regulatory pathways like PPARγ, LXA4 shows promise as a novel therapeutic agent for PD. These findings warrant further investigation into its potential role in preclinical and clinical settings.

6. Declarations and statements Ethics approval and consent to participate:

We conducted the study protocol according to The Local Committee of Research and Medical Ethics of the Faculty of Medicine, Tanta University.

Availability of data and material:

The corresponding author can provide the datasets used and/or analyzed during the current work upon request.

Competing interests:

The authors declare to have no conflicts of interest.

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