

Effect of Areca Catechu Against Rotenone-Induced Parkinson's Disease in Wistar Albino Rats

Kanchan Bhati ¹, Amit ²

Kharvel Subharti College of Pharmacy(Subharti University) Subhartipuram, NH-58,
Delhi–Haridwar Bypass Road, Meerut, Uttar Pradesh
Delhi Pharmaceutical Sciences and Research University, New Delhi

ABSTRACT

The goal of the current investigation was to determine if *Areca catechu* might protect Wistar albino rats from developing Parkinson's disease (PD) triggered by Rotenone. Globally, the frequency and incidence of PD are concerning developments. In affluent nations, the extension of human life is increasingly accompanied with a rise in neurodegenerative illnesses, the majority of which have merely symptomatic treatments rather than effective therapies. Plant based therapy have piqued the scientific community's attention in recent years. Neuroinflammation, oxidative stress, and apoptosis are presently proposed factors underlying the etiology of Parkinson's disease (PD). Current understanding of the PD pathophysiology has changed with the identification of several gene variants over the last 10 years, which could offer insight into the processes behind the pathogenesis of atypical instances of PD. Many antiparkinsonian medications have been created for the conduct of Parkinson's disease, but unfortunately, their possible adverse effects have caused physicians to seriously worry about their usage. In this direction, we therefore, planned to explore the antiparkinsonian effect of *Areca catechu* against Rotenone-induced PD in Wistar albino rats. Animals were separated into 6 groups: Normal control; Toxic (Rotenone 1.5 mg/kg); *Areca catechu* + Rotenone [I]; *Areca catechu* + Rotenone [II]; *Areca catechu per se*; Levodopa-carbidopa + Rotenone. Dosing was done for 28 days along with a daily dose of Rotenone for 28 days and after last day of dosing, weight of all animals were weighed individually before sacrificing of the animals. On the 29th day, animals were sacrificed and various biochemical parameters pertaining to TBARS, GSH, Catalase, and SOD were estimated in the blood after serum separation by homogenizer at 3000 RPM for 15 min. Apart from biochemical parameters, various neurobehavioral tests including Beam crossing task (BCT), Morris water maze (MWM), Rotarod Test, Forced swim test (FST), and Catalepsy test were also performed to establish the antiparkinsonian effect of *Areca catechu*. In the end, in our study found that *Areca catechu* showed a potent antiparkinsonian effect against Rotenone-induced Parkinson's disease.

Keywords: Parkinson's disease, Rotenone, *Areca catechu*, Neurodegenerative diseases.

INTRODUCTION

Neurodegenerative Diseases:

Degenerative illnesses of the nervous system have enormous economic and public health repercussions for individuals all over the world. Parkinson, Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) are three of the principal neurodegenerative diseases. You can split the word "neurodegenerative" into two parts.

- Neuro refers to the brain.
- Degenerative refers to deterioration or death. One of the worst consequences of improper brain cell communication is a neurodegenerative illness.

Parkinson's Disease, sometimes known as "shaking palsy," is a condition that affects certain parts of the brain that regulate balance, posture, and movement. Because it is a complicated disease with a wide range of symptoms (Bradykinesia, Tremors, Rigid Muscles), not every person with the disorder experiences the same issues.

Grading of disease severity:

There are numerous clinical scales that have been established to evaluate and define the harshness of PD. The Hoehn and Yahr (HY) scale, which was first used in biomedical studies in the 1960s and has since become the industry-standard staging method for PD, is the most well-known example. The initial version included five stages that were identified by symptoms such as bilateral involvement (stage 2) and weakened postural reflexes (stage 3). Later, a modified version was released, including transitional levels between the earlier 5 stages (Marras & Lang, 2004). The HY scale was created before to the development of dopaminergic replacement therapy (DRT), and the authors did not assume that the disease would proceed in stages sequentially. It is only descriptive. The HY scale's signature design for classifying each stage is a little unclear because clinical symptoms and functional impairment might exist in combinations that don't always fit the criteria for that stage. More specific scales have now been devised, frequently validated using the HY scale as the reference, to measure the development of the illness and the impact of treatment (Goetz et al., 2004). The Unified PD Rating Scale (UPDRS), which was formed in the 1980s and later studied by the Movement Disorders Society (MDS), is currently the most popular clinical rating scale. The UPDRS is a multifaceted tool that consists of a clinical assessment (part III) and three survey parts (I, II, and IV); parts I and II of the survey address observations of daily living (both motor and non-motor), part III examines clinical motor signs, and parts IV addresses therapy complications (dyskinesias and motor fluctuations). In the UPDRS, declining scores represent an evolution of symptoms and/or disability.

Parkinson's disease stages: (Lees et al 1983)

The Parkinson's foundation explains the disease's five phases so that we may know how it progresses naturally.

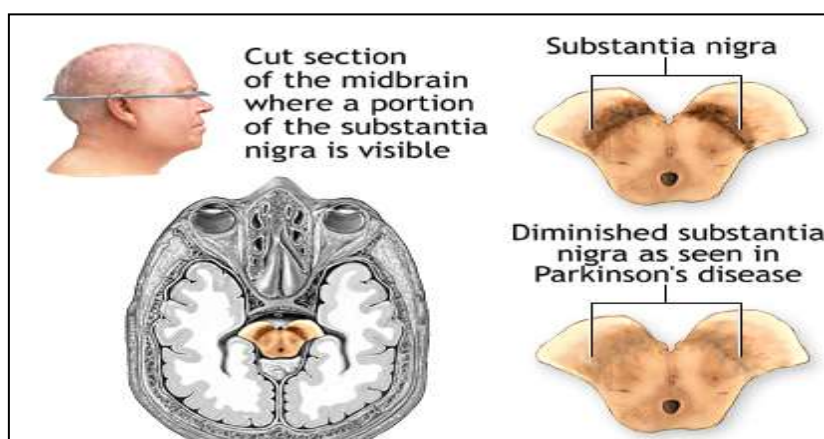
1. STAGE ONE - Symptoms may be present at this stage, but they aren't sufficiently severe to impact ordinary activities or lifestyle. The indications are so slight at this opinion they're often overlooked.
2. STAGE TWO - Compared to stage 1, the symptoms are far more pronounced. Rocking and trembling may be more noticeable, as well as alterations in facial expressions and rigidity.
3. STAGE THREE - On the other hand, your probabilities of trailing your poise and deceleration down your reactions have increased. Overall, your motions sluggish down. This is the reason why stage 3 understands an upsurge in falls.
4. STAGE FOUR - Standing alone is now achievable. Yet, touching about can need the usage of a hiker or another caring of aid.
5. STAGE FIVE - Extensive limb stiffness may also result in freezing while sitting, making it difficult to walk or stand. People at this stage frequently use crutches and are unable to stand upright without falling. Help is required continuously to prevent falls.

Types of Parkinson's disease (Gaweda-Walerych et al., 2008)

Idiopathic PD - Generally recognized as Parkinson's, is the greatest mutual cause of parkinsonism. Idiopathic denotes an uncertain etiology. Tremors, stiffness, and a decreased ability to move are among the most typical signs of idiopathic PD.

Parkinson's with an Early Onset - Early-onset PD, commonly referred to as youth-onset PD, is an uncommon form of the disease that affects people between the ages of 21 and 40 or 50. (studies use varying age cut-offs). Early-onset PD symptoms are comparable to those of idiopathic PD; however, they often worsen more slowly. 4 In spite of this, symptoms often have a more severe psychological and social effect since individuals are in the peak of their careers when they are diagnosed. Early-onset PD is distinguished by the incidence of levodopa-related side indications, such as dyskinesia (uncontrollable motion), and the wearing-off syndrome (in which the drug becomes less effective).

Familial Parkinson's disease - About 10 to 15 percent of instances of PD are thought to be hereditary, according to newly uncovered statistics. 5 These PD instances run in families are brought on by mutations (DNA alterations) in certain genes that are handed down from genetic parents to offspring. Many genes have been associated to PD, and research has shown how gene mutations affect risk and the course of the illness. For instance, autosomal dominant PD has been related to alterations in the SNCA gene.



MATERIAL AND METHODS

The *Areca catechu* nuts were bought at the neighbourhood market, peeled, and sun-dried for a few days. Powdered and fat-free petroleum ether was used to defatify the dried, chopped nuts. Using a Soxhlet apparatus and 400 mL of 70% ethanol from Qualigens Fine Chemicals in Mumbai, India. The residue was hot extracted over five cycles. To create dried extract, this extract was filtered, lyophilized, and concentrated over a water bath. A desiccator was used to keep this extract dry (Quality Traders, Eranakulum, Kerala). The research made use of an extract suspended in 4% gum acacia from Nice Chemicals in Cochin, India (Shanbhag et al 2006).

Requirements

Species	: Albino Wistar rats
Age/Weight	: 4-5 weeks old(200-250g wt)
Gender	: Either sex
Number to be used	: 36
No. of days each animal will be housed	: One month
Proposed source of animals	: R. V. northland institute

Wister albino rats weighting 180–260 grams, were purchased from the RV Northland Pharmacy College in Greater Noida, Uttar Pradesh. The animals were fed pellets and allowed unlimited admission to nourishment & water. They were maintained in polypropylene enclosures at a temperature of 21°C with a 12-hour cycle of darkness and light. Six animals were each made comprising each of the six groups from which the animals were separated. The procedure for conducting the experiment complied with the guidelines established by the CPCSEA, New Delhi.

Drugs: Test drug Procured by internet sources from MERLIONS NATURALS approved by FSSAI, 39 Grand Vishala Ind. Estate.

Test drug - Areca nuts.

Standard drug- Levodopa with the carbidopa, procured from local market Dadri.

Study Design:

Animals were separated into 6-groups and the duration of the study was 28 days. The treatment schedule for each group is as follows:

S. No.	Groups	No. of animals	Treatment
I	Normal control	6	Normal saline 0.5 ml/day, p.o. for 28 days
II	Toxic control	6	Rotenone 1.5 mg/kg/day, i.p. for 28 days
III	<i>Areca catechu</i> [I] + Rotenone	6	<i>Areca catechu</i> 100 mg/kg/day, p.o. for 28 days + Rotenone 1.5 mg/kg/day, i.p. for 28 days
IV	<i>Areca catechu</i> [II] + Rotenone	6	<i>Areca catechu</i> 300 mg/kg/day, p.o. for 28 days + Rotenone 1.5 mg/kg/day, i.p. for 28 days
V	<i>Areca catechu per se</i>	6	<i>Areca catechu</i> 300 mg/kg/day, p.o. for 28 days
VI	Levodopa-carbidopa + Rotenone	6	Levodopa-carbidopa (100 + 25 mg/kg), p.o. for 28 days + Rotenone 1.5 mg/kg/day, i.p. for 28 days

METHODS FOR ESTIMATION

a) Thiobarbituric Acid Reactive Substances (TBARS)

Principle

Free revolutionaries play a part during the time spent on lipid peroxidation. A heterogeneous combination of peroxides that separate to frame substances, for example, malondialdehyde, are the fundamental results of such corruption (MDA). The colorimetric response of Thiobarbituric corrosive with MDA is a sensitive method for measuring lipid peroxidation. The assay provides an estimate of the amount of Thiobarbituric acid reactive molecules (TBARS), e.g., MDA. It is additionally alluded to as the TBARS test.

Reagents

- 1) 0.8% TBA solution: 0.8 g of TBA will be disintegrated in refined water and the capacity complete up and around to 100 ml.
- 2) 30% TCA solution: 30 gm of Trichloroacetic acid will be disintegrated in refined aquatic and the capacity made up and about to 100 ml.
- 3) Standard TEP reagent – 0.02 g (± 0.004 g) of TEP (1, 1, 3, 3 – tetra ethoxy propane) will be dissolved in 40% ethanol. A further dilution of 100 ml with purified water of this explanation to 1000 ml will be done. The third dilution of 100ml of the above solution to 500 ml with distilled water will finally be done. This dilution contained 4 μ g of reagents per ml.

Procedure

The buildup of the tissue homogenate 10% was eliminated, and 1 ml of the way of life medium was centrifuged at 10,000 rpm. It was then given 0.5 ml of 30% TCA and 0.5 ml of 0.8 percent TBA. The cylinders were put in a shaking water shower at 80OC for 30 minutes with aluminum foils on top of them. The cylinders will be eliminated following 30 minutes and kept up in the super cold water for 10 minutes. From that point onward, they went through 15-minute centrifugation at 3000 rpm. At 540 nm and room temp., the enhancement's absorbance was estimated against a reasonable clear. 1.0 ml of refined water, 0.5 ml of 30% TCA, and 0.5 ml of 8% TBA made up the clear.

b) Reduced Glutathione (GSH)

Principle

This spectrophotometric technique depends on the Ellman technique, where one mole of 2-nitro-5-mercaptobenzoic acid is formed for every mole of -SH groups that convert 5, 5'-dithiobis-2-nitrobenzoic acid (DNTB). At 412 nm, spectrophotometry reveals the bright yellow color of the nitro mercapto benzoic acid ionic species.

Reagents required

- EDTA (0.2 M): 22.3g of EDTA will be liquified in 300ml of warm dual distilled water.
- EDTA (0.02 M): 20 ml of overhead solution will be diluted to 200ml of dual distilled water
- DTNB (0.01 M): 99mg of DNTB will be dissolved in 25 ml of absolute methanol.
- Tris buffer (0.4 M, pH 8.9): 24.2 g of Tris buffer will be liquified in 100 ml of dual distilled water, 100 ml of 0.2 M EDTA will be added to it and the capacity of solution will be complete up to 1000ml with dual purified water. The solution pH was attuned to 8.9 with 1 N HCl.
- TCA (50%): 50 g of TCA will be liquified in 100 ml of dual distilled water.

Procedure

Test

A known amount of brain tissue, weighing between 300 to 600 mg, will be homogenised in 5-8 ml of 0.02 M EDTA before being mixed with 4.0 ml of cold distilled water. 1 ml of 50% trichloroacetic acid (TCA) was added after thorough mixing, and the mixture was agitated sporadically for 10 min using a vortex mixer. The mixture was put into centrifuge tubes after 10 minutes, washed with EDTA, and centrifugation at 6000 rpm for fifteen minutes. After centrifugation, 4.0 ml of 0.4 M Tris buffer was added to 2 ml of the supernatant (pH 8.9). After thoroughly blending the whole solution, 0.1 ml of 0.01M DTNB was added. Within 5 minutes after adding DTNB, the absorbance was measured at 412 nm against a control sample.

Blank

The procedure will follow the same steps as for the test with the exception that tissue homogenization was replaced with 0.02 M EDTA.

Standard

The method will be same as for the test expect that standard glutathione solution (50µg/ml) was used in dwelling of tissue homogenate.

Result

Effect of *Areca catechu* against (TBARS)-induced in Parkinsonian rats.

The level of TBARS was found to be knowingly high (**P<0.001) in Rotenone-treated rats. The level of TBARS was significantly decreased when treated with *Areca catechu* 100 and 300 mg/kg (*P<0.05 and ##P<0.01 correspondingly). *Areca catechu* 300 mg/kg and standard drug levodopa-carbidopa treated group also presented a significant reduction in TBARS levels (###P<0.001).

Groups	TBARS nmol of MDA/mg of protein
Control	0.14±0.047
Rotenone	0.73±0.018***
AC 100 + Rotenone	0.57±0.013#
AC 300 + Rotenone	0.37±0.013##
AC 300 per se	0.28±0.009###

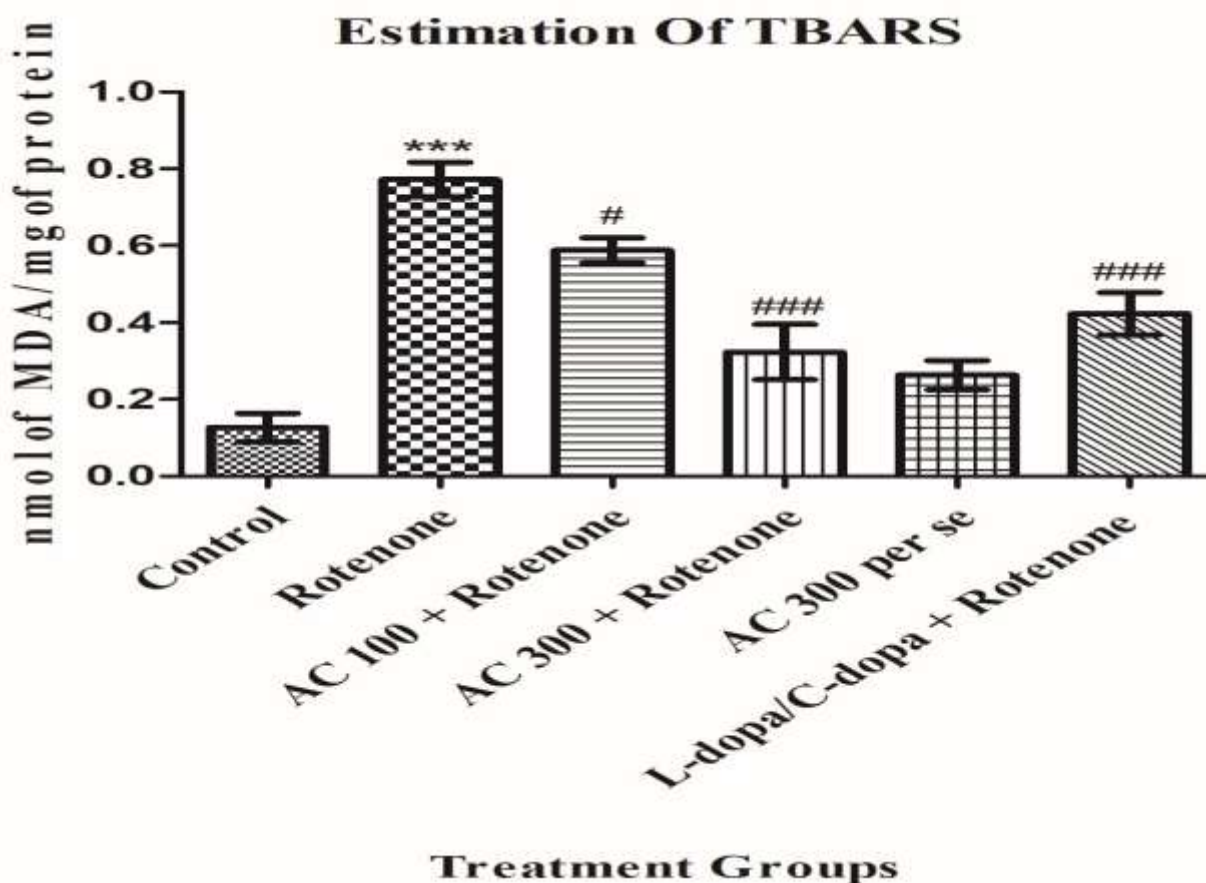


Figure 5: Bar graph showing results of TBARS in various groups

Values are the mean $\pm$ SEM (n=6) showing ***P < 0.05 (Normal control vs Rotenone); #P < 0.05 (AC 100 + Rotenone vs Rotenone); ##P < 0.01 (AC 300 + Rotenone vs Rotenone); ####P<0.001 (AC 300 vs Rotenone) using one way ANOVA with Tukey's comparison test. AC: *Areca catechu*.

Effect of *Areca catechu* against (Catalase)-induced in Parkinsonian rats.

The level of Catalase was found to be significantly high (**P<0.001) in Rotenone-treated rats. The level of Catalase was significantly decreased when treated with *Areca catechu* 100 and 300 mg/kg (^{ns}P > 0.05 and #P<0.51 respectively). *Areca catechu* 300 mg/kg treated group also presented a significant reduction in Catalase levels (##P<0.01).

Groups	Catalase nmol of H2O2/min/mg of protein
Control	5.60 $\pm$ 1.94
Rotenone	1.71 $\pm$ 3.42***
AC 100 + Rotenone	2.44 $\pm$ 4.85###
AC 300 + Rotenone	4.14 $\pm$ 2.45##
AC 300 per se	4.47 $\pm$ 3.01###

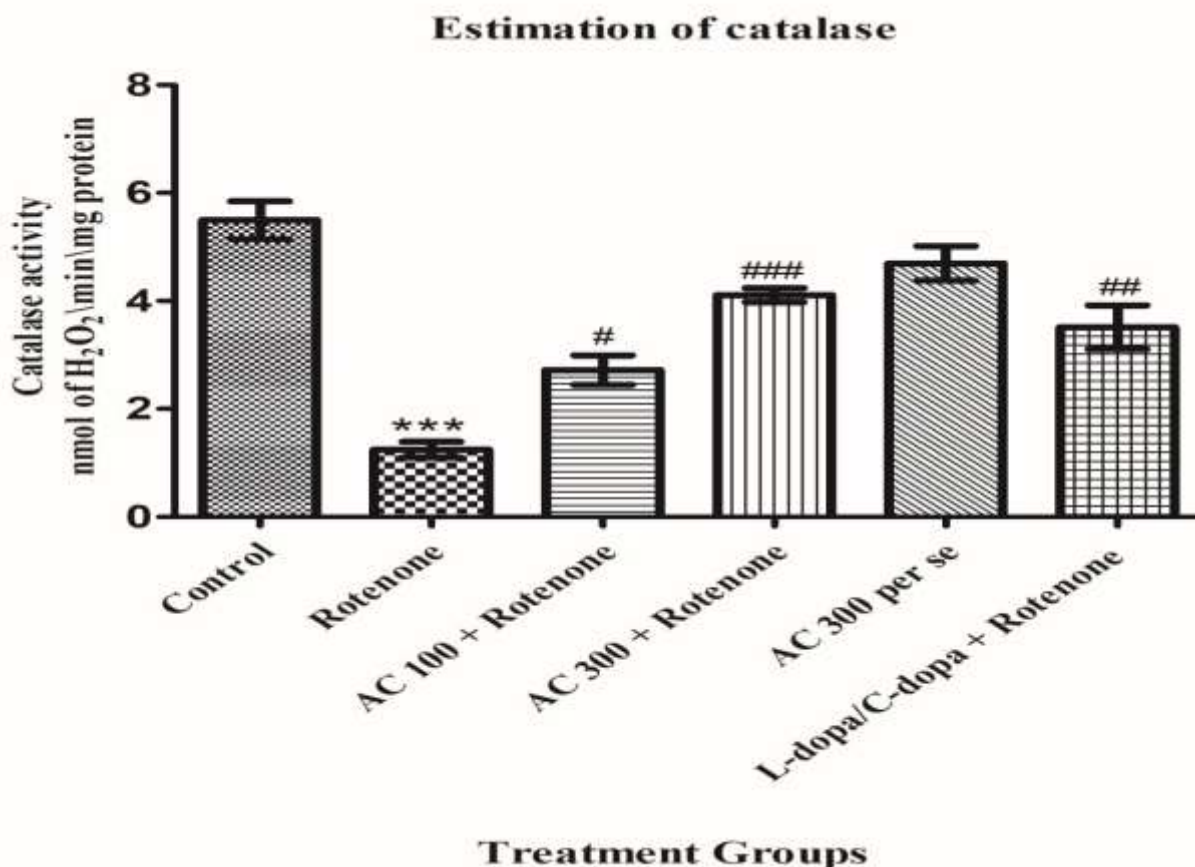


Figure 6: Bar graph showing results of Catalase in various groups.

Values are the mean $\pm$ SEM (n=6) showing ***P < 0.05 (Normal control vs Rotenone); ^{ns}P > 0.05 (AC 100 + Rotenone vs Rotenone); #P < 0.05 (AC 300 + Rotenone vs Rotenone); ###P < 0.01 (AC 300 vs Rotenone) using one way ANOVA with Tukey's comparison test. AC: *Areca catechu*.

DISCUSSION

This study sought to shed light on how *Areca catechu* affected antioxidant markers like TBARS, Catalase, superoxide dismutase (SOD) by Rotenone administered to young rats. Parkinson disease was induced in Wister rats by Rotenone and treated with the dose of test drug for 28 days. Vehicle and *Areca catechu* *per se* were also administered for 28 days for comparative study. *Areca catechu* have anti-inflammatory and ROS scavenging properties. When administered *Areca catechu*, Rotenone-treated rats' performance in the MWM was noticeably worse. In rats, Rotenone generated mitochondrial oxidative damage that could be defended against by *Areca catechu*. Additionally, rats given Rotenone had their cognitive deficiencies reduced by *Areca catechu*. In the MWM test, chronic management of *Areca catechu* (300 mg/kg) therapy enhanced memory performance. However, it was hypothesised that might significantly improve animal memory impairment. *Areca catechu* have significantly increased the level of antioxidant markers such as TBARS, SOD, and catalase that are reduced by the Rotenone toxic drug. When related to the control group, Rotenone significantly decreases the level of TBARS, SOD, and catalase. On the other hand, the toxic drug Rotenone significantly decreased the weight of animals on the 7th, 14th, 21st and 28th day, but *Areca catechu* significantly amplified the heaviness of animals on the 21st and 28th day, when compared to the toxic group. The forced swim test was performed on the 28th day. In the evolution, there was a significant increase (p<0.01) in immobility time in the Rotenone-treated group when compared to the control collection.

CONCLUSION

In conclusion, our work suggests that supplementing with *Areca catechu* can shield rats' serum and brain tissues against oxidative damage brought on by Rotenone. *Areca catechu* have potent antioxidant effects through up and down-regulating brain function. In cases of Rotenone-induced Parkinson disease, *Areca catechu* may also support memory and learning function, antioxidant activity, and potential therapeutic effectiveness. Although the precise link is found between oxidative stress and Parkinson disease, several studies have recommended that oxidative tension might be a major factor in several illnesses, particularly those that affect the neurological system. A conventional pharmacological therapy group might also be added to this model as a constraint for this investigation. As a result, our research has demonstrated that *Areca catechu* can lessen Rotenone-induced learning and memory impairment as well as oxidative damage by taking part in a diversity of biological progressions like antioxidant, anti-apoptotic, and anti-inflammatory impacts on nitric oxide pathways.

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