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Synergistic Effects of Syndopa and Ethanol Extract of Areca Catechu in Rotenone-Induced Parkinson's Mouse Model

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Abstract

Background: Parkinson's disease (PD), the second most prevalent neurodegenerative condition after Alzheimer's disease, profoundly affects the extrapyramidal motor functions. It is characterized by the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc) and reduced dopamine neurotransmitter levels in the brainstem and striatal regions. This impacts cerebral neural systems vital for motor activity. Oxidative stress and brain tissue degradation are significant contributors to PD's pathogenesis.

Objective: This study aimed to evaluate the neuroprotective potential of Areca catechu, both as a standalone treatment and in synergy with Syndopa, in a rotenone-induced PD mouse model.

Methods: Mature Swiss albino male mice (25–30 g) were grouped into five sets (n=5 each). Group 1 (Control) received 1% CMC orally for 21 days. Groups 2 to 5 were administered Rotenone (2.5 mg/kg, i.v.) over the same duration. Group 3 received a high Syndopa dose (15 mg/kg b.w.), while Group 4 was treated with Areca catechu alone (400 mg/kg b.w.). Group 5 was administered a combination of Areca catechu (400 mg/kg b.w.) and a low dose of Syndopa (7.5 mg/kg b.w.). On day 22, mice were dissected for histopathological (Nissl-stained brain sections) and histochemical studies (immunohistochemical staining of α-Synuclein). Blood samples were extracted for assessing biochemical markers, including lipid peroxidation and total antioxidant capacity.

Results: The combined therapy of Areca catechu (400 mg/kg) and Syndopa (7.5 mg/kg) demonstrated superior neuroprotective outcomes, as evidenced by biochemical analyses, histology, and α-synuclein immunohistochemistry.

Conclusion: The co-administration of Areca catechu (400 mg/kg b.w.) and Syndopa (7.5 mg/kg b.w.) effectively mitigated the rotenone-induced PD symptoms in mice, underscoring the potential of this therapeutic strategy in PD management.

Key-words: Parkinson's Disease (PD), Rotenone-induced Parkinsonism, Areca catechu Neuroprotection, Syndopa Efficacy, α-Synuclein Immunohistochemistry, Oxidative Stress Therapeutics.

Introduction

The most prevalent progressive, multifactorial neurodegenerative condition is Parkinson's disease (PD). It primarily affects elderly adults and has a complicated aetiology 1. Motor dysfunctions are linked to Parkinson's disease (PD), which is pathophysiologically characterised by the loss of midbrain dopaminergic neurons in the substantia nigra (SN). Dopamine (DA), a neurotransmitter, is important for several processes including movement, motivation, memory, reinforcement, motor abilities, and others. Cardinal symptoms of Parkinson's disease (PD), including as stiffness, bradykinesia (akinesia), and postural instability, are caused by decreased or altered neurotransmission as a result of dopaminergic neurons' loss 2. Parkinson's disease significantly lowers a person's quality of life when it strikes. Parkinson's disease (PD) is more common as people age, yet only 4.2% of individuals who are affected are diagnosed before the age of 50. Tremors, akinesia, muscle rigidity, and postural instability are PD symptoms 3. Although PD does not consider itself to be a fatal disorder, it may somewhat reduce life expectancy. People frequently claim that "Parkinson's disease rather than disease" causes their deaths. Cancer, heart disease, and stroke are age-related disorders that frequently result in patient death. The most common cause of death in Parkinson's patients, however, is pneumonia since the condition makes it

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difficult for patients to swallow, increasing their risk of aspirating food or liquids into their lungs and developing aspiration pneumonia. 4

Because they are vulnerable to harmful caregiving factors like increased daily care needs, detrimental effects on social interactions, detrimental effects on family functioning, and adverse effects on quality of life due to progressive neurodegeneration, Parkinson's disease places a significant burden on the patient's family and carers 5. Hereditary Parkinsonism diseases with PD-like clinical symptoms are sometimes referred to as having Parkinson's disease 6. Despite the fact that this goes against the notion that Parkinson's disease is an idiopathic sickness. The terms "familial Parkinson's disease" and "sporadic Parkinson's disease" can be used to distinguish between the disease's hereditary and truly idiopathic variants 7. Parkinson's disease has no recognised cause, and it also has no known treatment. Despite ongoing research in these areas, there is presently no accepted method to treat or prevent PD. Research has advanced significantly, and there is a very good likelihood of identifying the causes, whether genetic or environmental, and comprehending the specific effects of these factors on brain function. 8

Although there is no known cure for Parkinson's disease, the majority of patients can live happy, fulfilling lives by identifying their particular symptoms and selecting the best course of treatment. Since the beginning of time, numerous herbal medicine formulations have been used in ancient Indian ethnotherapeutics to treat PD, and the same has been shown. According to reports, the body becomes used to these medications when they are used frequently, and some of them have also been shown to have extremely harmful adverse effects on different biological systems. The PD has long been referred to in Ayurveda as "Kampa Vata" (Kampa=Shaking), one of the 80 various types of vata nanatmaj vyadhi (vataja disease). The ancient physician Charak, who wrote the Charak Samhita, refers to Kampa Vata as Vepathu. He asserted that the signs of vepathu that were most closely related to one another were tremor (kampa), rigidity (sthambha), and bradykinesia (chestasanga). The condition's etiological elements, signs and symptoms, various treatment techniques, and complications were all fully described by numerous other researchers in their papers. 9

Areca catechu, often known as the areca palm, is a kind of palm that is typically produced for its seeds in India, Malaysia, Taiwan, and many other nations. Millions of people who live along the east coast of Africa and the western Pacific chew betel nuts, which are ranked fourth behind nicotine, ethanol, and caffeine. Due to its fruit, Areca catechu, a member of the Arecaceae family, is incorrectly known as a betel tree. The arecanut is usually eaten with a betel leaf, a member of the Piperaceae family. This medium-sized, beautiful palm tree has a trunk diameter of 20 to 30 cm and grows straight to a height of 20 metres. The 1.5–2 m long, pinnate leaves have a dense number of leaflets. For commercial purposes, Areca catechu is planted for its significant seed yield, the Areca nut. Alkaloids such as arecaine and arecoline are found in the seed. The betel nut's active components are made up of about nine alkaloids, the most prevalent of which is arecoline, a strong cholinergic agonist that can penetrate the blood-brain barrier (BBB) and has a variety of parasympathetic effects. When administered for 10 days, Areca catechu ethanol extract has been found to have antidepressant activity in earlier studies 10. Areca nut is mostly composed of polyphenols, fat polysaccharides, fibre, and protein. In addition, these nuts contain the alkaloids arecoline (0.1–0.7%) as well as arecadine, guvacoline, and guvacine in trace levels. Vagbhata (in the 4th century AD) listed the medicinal characteristics as being useful against leucoderma, leprosy, cough, fits, worms anaemia, and obesity. Recent research has demonstrated the pharmacological benefits of areca catechu , including their hypoglycemic effect, mitotic activity, anthelminthic activity, cholinomimetic action, and anti-epileptic activity. However, numerous studies have also linked areca nut to the development of cancer.11

A dopa decarboxylase inhibitor called carbidopa is combined with levodopa to treat the symptoms of idiopathic Parkinson disease and other illnesses that have parkinsonian symptoms (Syndopa- Levodopa+ Carbidopa). N-amino-alpha-methyl-3-hydroxy-L tyrosine monohydrate is the chemical name for carbidopa. Due to its chemical composition, it is able to effectively inhibit aromatic amino acid decarboxylase (DDC), and it does not penetrate the blood-brain barrier. Carbidopa is always provided alongside levodopa due to its action. The combined therapy is used to lessen nausea and vomiting brought on by levodopa. Carbidopa prevents levodopa's peripheral conversion to dopamine and the decarboxylation of oxitriptan (5-HT) into serotonin by aromatic L-amino acid decarboxylase when combined with levodopa. Levodopa and oxitriptan are thereby transported to the central nervous system in greater quantities. Levodopa's bioavailability is increased by carbidopa's capacity to prevent the GI tract from metabolising levodopa. Additional circulating levodopa units have been demonstrated to temporarily improve symptoms by increasing the efficiency of the remaining dopaminergic neurons.

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Before being used as a treatment in humans, any novel chemical must first be tested on rats or mice to determine its efficacy, safety, and side effects. The only way to do this is to test the novel chemical on animals. In this context, preclinical research is conducted to evaluate the safety and efficacy data from animal models that warrant doing the study in humans. Finding a medication that might supplement LD would be beneficial since it would allow for LD dose decrease while eliminating the different motor and nonmotor symptoms that can occasionally go along with it. The presynaptic molecule -syn seems to aggregate into harmful oligomers and fibrils as one of the mechanisms causing neurodegeneration. Therefore, finding a remedy that would bind to α-syn and either dissolve hazardous aggregates or stop them from developing would be helpful. This skill might help PD sufferers with their symptoms. Overall, the PD therapy that is currently on the market is insufficient for treating the condition. The current study examined the antioxidant capability of Areca catechu alone or in conjunction with Syndopa in a mouse model of rotenone-induced PD symptoms.

Materials and methods:

2.1Experimental Animals:

Male Swiss albino mice weighing 25–30 g at ten weeks old were purchased from the Mass Biotech Laboratory in Chennai, India. Before beginning research, animals were acclimated to the laboratory environment for 7 days in vented polypropylene cages. They were fed a regular pellet meal and given access to unlimited amounts of water while being kept in ambient settings with a natural light/dark cycle at 25± 2°C and 40–60% relative humidity. All animal experimentation was conducted in accordance with institutional animal ethics committee approval and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (reference number: (IAEC-SU/CLAR/RD/007/2023) guidelines.

2.2Ethical Approval:

The research proposal was sanctioned by the of Saveetha Medical College, Chennai (IAEC-SU/CLAR/RD/007/2023) andanimal experiments were conducted as specified by the guidelines of Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA, New Delhi, India).

2.3Chemicals

The main chemicals used in this study were procured from the following source: rotenone, TCI (Portland); olive oil, SYNDOPA from SIMATS (Tamil Nadu, India); dimethyl sulfoxide (DMSO), Pure Chemicals; from HiMedia (Mumbai, India). The Areca catechu sample was authenticated by Central Ayurveda Research Institute (NABL Accreditation), Arumbakkam, Chennai, Tamil Nadu, India. All other chemicals and reagents used were ofanalytical grade.

2.4Preparation of rotenone

In 1 mL of 100% DMSO, 50 mg of rotenone were dissolved. To create the stock solution, 0.2 mL from this solution was mixed with 19.8 mL of olive oil.

Preparation of Areca catechu:

500g of coarsely ground Areca catechu were used to make ethanol using the Soxhlet apparatus method. The solvent was decanted and distilled in a hot water bath after 72 hours. After being concentrated, the extract was run over a silica gel (100–200 mesh) chromatography column.This procedure resulted in an ethanolic extract of Areca catechu (yield: 47g).

2.5Experimental Design:

Six mice were placed in each of the seven groups, which were created at random from the mice. One day before the start of the experiments, the groups were formed. The investigation lasted for 21 days (injection and oral dosage). To identify each mouse, a non-toxic permanent marker pen was used to mark its tail. Each animal was weighed separately, and the body weights were noted. There were five groups of six mice each among the mice. CMC was administered to Group 1 (the vehicle control group) at a rate of 0.3 ml per mouse (p.o.) and Group 2 (the Parkinson control group) at a rate of 2.5 mg per kilogramme of body weight (i.p.) per animal (12). PD (rotenone 2.5 mg/kg b.w. -0.2 mg/mouse i.p. + syndopa 15 mg/kg b.w. -0.3 mg/mouse (p.o.)) is the third group receiving standard therapy. Group 4: Test Group I [PD (rotenone 2.5 mg/kg body weight – 0.2 mg/mouse i.p) + Areca catechu ethanolic extract 400 mg/kg body weight – 0.3 mg/mouse (p.o)].Group-5 Test Group-II - Combination Treatment [PD (rotenone 2.5 mg/kg body weight- 0.2 mg/mouse i.p)+ 400 mg/kg body weight of Areca catechu ethanolic extract + 7.5 mg/kg Syndopa- 0.3 mg/mouse (p.o)].For 21 days, the medication was

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taken orally once every day. The animals were given isoflurane anaesthesia on day 22 of the trial. The brain tissues were carefully removed and used for histology and immunohistochemistry research.

2.6Estimation of total antioxidant capacity (TAC):

Thermo Fischer Scientific Company's commercial Bradford assay kit was used to evaluate the protein content of serum samples, and the results of TAC in serum were computed based on the level of total serum protein. An enzymatic reaction that involves the conversion of 3, 5-dichloro-2- hydroxybenzenesulphonate to a coloured product detected colorimetrically at 505 nm determines the residual H2O2 content. TAC in serum is measured in (mmol/L) units.

2.7Measurement of alpha synuclein protein expression and brain histopathology:

By performing α-Synuclein staining in brain samples, an immunohistochemical investigation was conducted to assess the PD marker. Thermo Fischer Scientific Company's HRP/DAB detection IHC kit was applied in accordance with the manufacturer's instructions. After soaking the tissues in 10% formalin, a paraffin block was created. Xylene was used to remove the paraffin, and a series of alcohols was used to dehydrate the tissue slices. The endogenous peroxidase activity was eliminated by incubating the sample in 3% hydrogen peroxide for 30 minutes at room temperature after two rinses in phosphate-buffered saline lasting 5 minutes each. By incubating the sample with normal goat serum (3 drops in 3% BSA in PBS) for 30 minutes, the non-specific binding sites were inhibited. The sections were then immunoreacted with 10 g/mL primary antibodies against alpha-synuclein (Sigma Aldrich Company) overnight at 4°C, after antigen retrieval (100 Citrate Buffer) for 20 min in a home pressure cooker and blocking non-specific binding sites with protein block. After that, the primary antibody (1:500) was incubated for 60 min at room temperature. After being rinsed with PBS, slices were treated for 60 min at room temperature with biotinylated anti serum (goat antiserum to rabbit IgG 1:100 dilution; Cell Path, UK). The slices were then rinsed three times in PBS and incubated in the working streptavidin HRP solution for 60 min at room temperature. The sections were then counterstained, rinsed in water, and incubated with DAB-hydrogen peroxide for 30 minutes and viewed under olympus light microscope. Utilising software, image analysis software was used to quantify the intensity. The substantia nigra pars compacta (SNC) region's Nissl-stained neurons were counted for each rat as part of the Brain Histopathology approach. The neurons were computed using a random selection technique of the microscope while the stained sections were focussed under 40X and 100X objective lenses. The Olympus CX31 light microscope was used to analyse the slides, and image-capture software was used to quantify the results. Using ImageJ software 1.45, images of sections were inspected and cell counting was performed. Within a 1mm2 area, the number of viable cells was counted.

2.8Measurement of LPO:

To quantify LPO in samples, the Lipid Peroxide (LPO) ELISA Kit uses a two-site sandwich ELISA. On a microplate, an antibody that targets mice LPO has been pre-coated. Any LPO that is present is bound by the immobilised antibody after standards and samples are pipetted into the wells. The wells are then filled with HRP-Conjugated LPO detection antibody after any unbound materials have been taken out. A Chromogen solution is added to the wells after a wash to get rid of any unbound HRP reagent, and colour develops in proportion to how much LPO was bound in the first stage. The growth of the colour is halted, and the color's intensity is gauged.

Before beginning the assay method, prepare all the reagents. All Standards and Samples should be added to the microplate in two separate additions. Add a benchmark: Testing sample wells after setting standard wells. 50 mL of the standard's diluted solution should be added to the standard well. As an example: To the testing sample well, add 40 L of the sample diluent. Then pour 10 mL of the sample into the testing sample well; the blank well has no effect. 45 minutes at 37 °C with a plate cover over the top. 5. For a total of five washes, aspirate each well and wash four times, each taking 1-3 minutes.

Using a squirt bottle, manifold dispenser, or autowasher, wash the area by adding 250 mL of Wash buffer to each well. To perform well, fluids must be completely removed at each phase. After the last wash, aspirate or decant any leftover Wash buffer. The plate should be turned over and blotted with fresh paper towels. Except for the blank well, add 50 L of HRP-conjugated detection antibody to each well.Put a plate cover over it. 30 minutes of incubation at 37 °C. Aspiration and washing should be repeated five times as in step. To each well, add 50 mL of chromogen solution A and 50 mL of chromogen solution B. Mix gently, then incubate at 37 °C for 15 minutes. shield against light. Each well needs 50 L of Stop Solution added. The wells' colour need ideally transition from blue to yellow. Gently tap the plate to ensure full mixing if the wells are green or the colour

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change does not seem to be uniform. Within 15 minutes, read the optical density (O.D.) on a microtiter plate at 450 nm.

2.9Statistical Analysis

Mean and SEM are used to express the results. For comparison between the groups, a one-way ANOVA was employed, followed by the Bonferroni correction. P values of 0.05 were considered statistically significant.

Results

3.1Effect of AC on LPO in Rotenone-Induced Parkinson mice model:

In groups I to V, the predicted LPO (nmol/L) values were 5.125 0.245, 12.581 0.350, 6.632 0.200, 6.843 0.805, and 6.040 0.209, respectively. The two-site sandwich ELISA test used by the Lipid Peroxide (LPO) ELISA Kit revealed a significant difference in LPO levels across the groups. In the PD mice group, there was a noticeably higher amount of lipid peroxidation than in the control group. The lipid peroxidation levels were decreased in the PD group of mice treated with either Areca catechu ethanolic extract alone or Areca catechu ethanolic extract combined with Syndopa. In particular, a combination of Areca catechu (400 mg/kg) with Syndopa (7.5 mg/kg) produced a greater protective effect to combat lipid peroxidation.

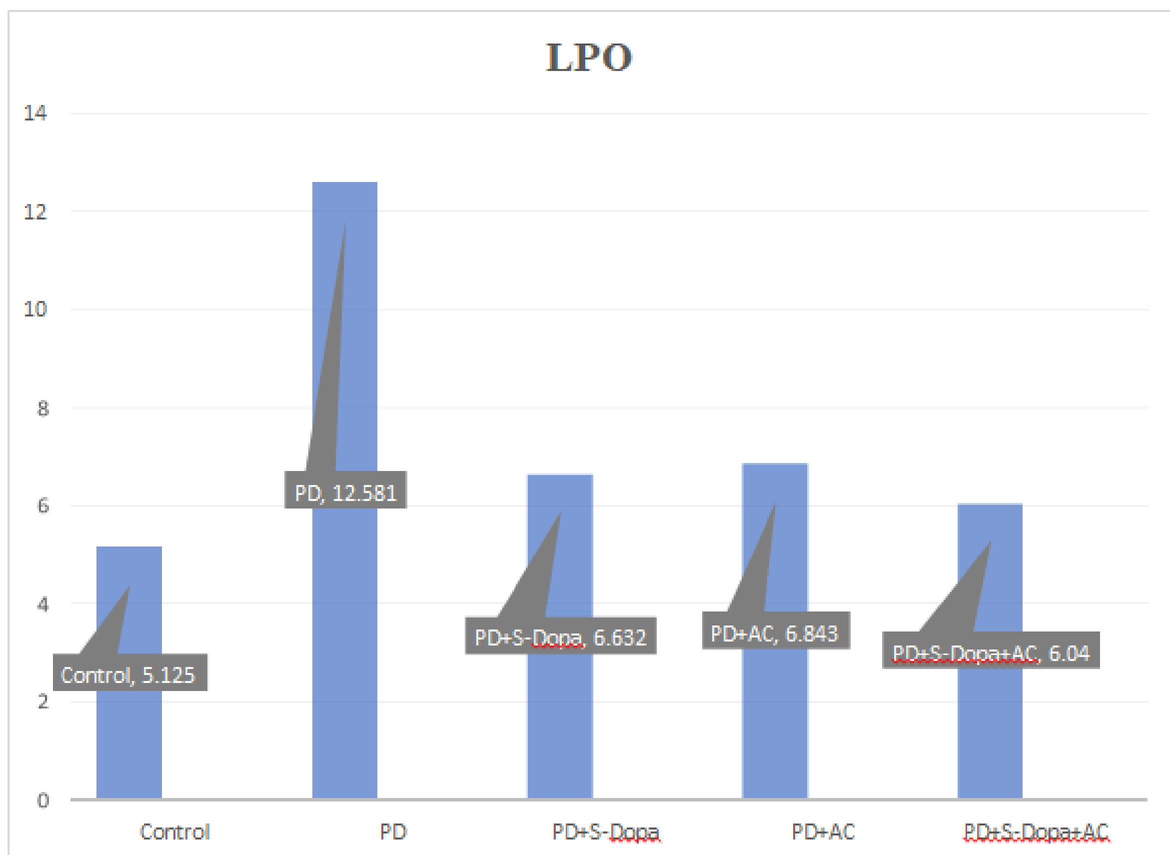

Figure 1: Neuroprotective effect of Areca catechu on rotenone (2.5 mg/kg) induced changes in LPO

3.2Effect of AC on Total anti-oxidant capacity in Rotenone-Induced Parkinson mice model:

The TAC (mmol/L) for groups I through V was calculated to be 594.7, 71.6, 411.1, 1.4, 208.9, and 551.3, respectively. The TAC levels varied significantly amongst the groups (Figure 4B). The TAC significantly improved after receiving the combo medication therapy. In contrast to Group 5, which received treatment with a combination of Areca catechu extract (400 mg/kg), Syndopa (7.5 mg/kg), and Rotenone (2.5 mg), Group 2 (PD) mice showed a respective 85.24% decrease in TAC levels when compared with control, while Group 5 showed a respective 13.4% decrease when compared with control.

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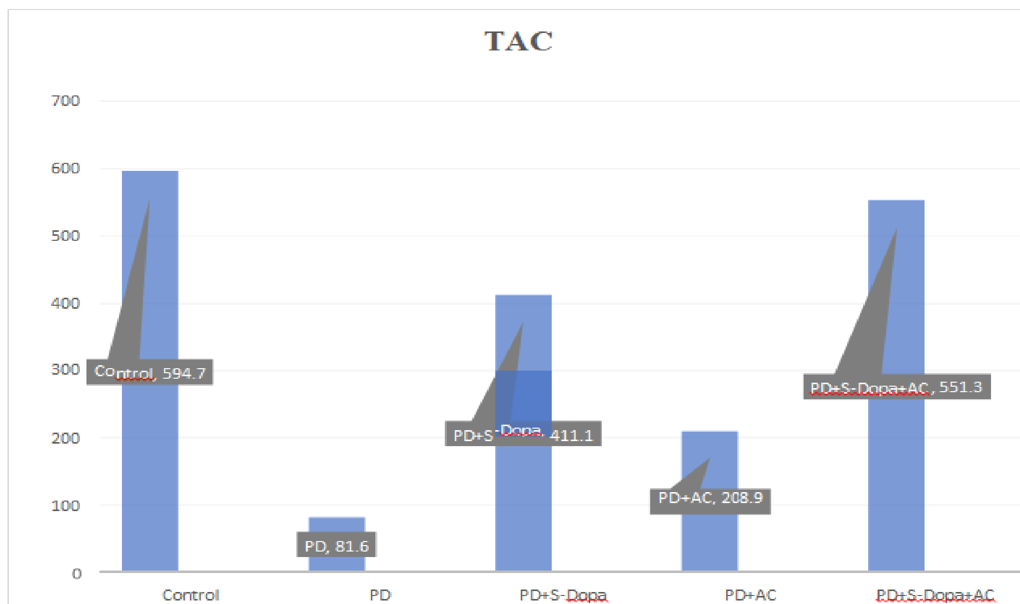

Figure 2: Neuroprotective effect of Areca catechu on rotenone (2.5 mg/kg) induced changes in TAC.

3.3Effect of AC on Immunohistochemical staining of α-Synuclein in Rotenone-Induced Parkinson mice model: A good response was seen in the antibody for α -Synuclein, which was primarily found in injured brain cells. Brains from PD mice had considerably more alpha synuclein than brains from the control group (Figure 4, Slide B vs. Figure 4, Slide A). The levels of α -Synuclein expression were significantly reduced in those that received Areca catechu extract treatment. While the levels of this protein were found to be reduced in the brain samples from the Syndopa treatment group (Figure 4, Slide C), the Areca catechu (400 mg/kg) group, and the In Combination group (Figure 4, Slide E), the levels of this protein were found to be very low, similar to the control group. All of the groups' synuclein expression has been examined. Results were presented and significantly compared to a group that received rotenone.

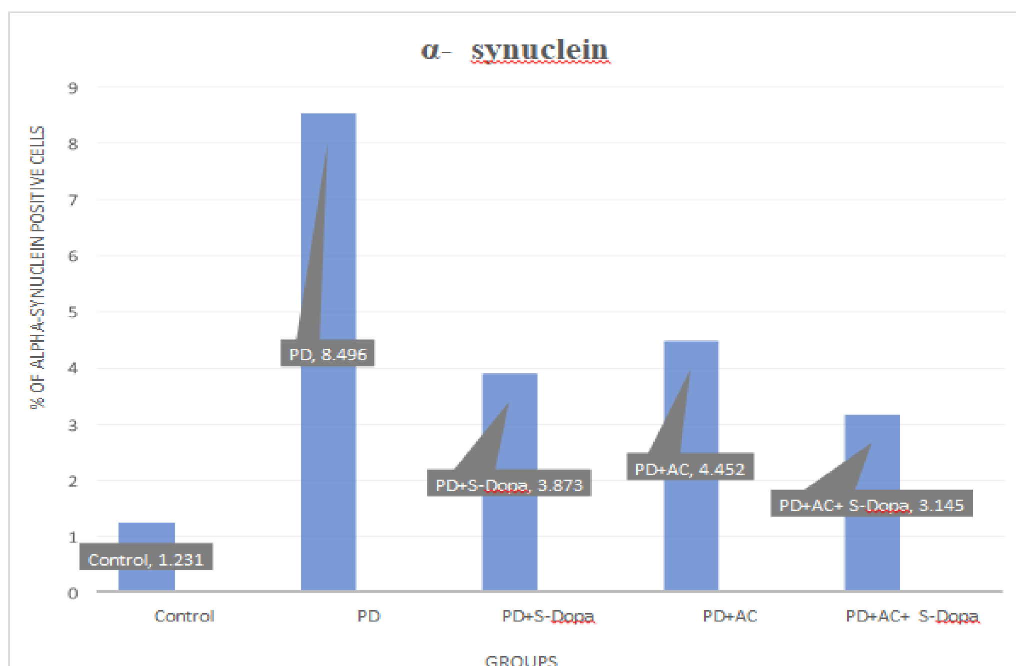

Figure 3: Neuroprotective effect of Areca catechu on rotenone (2.5 mg/kg) induced changes in α-Synuclein.

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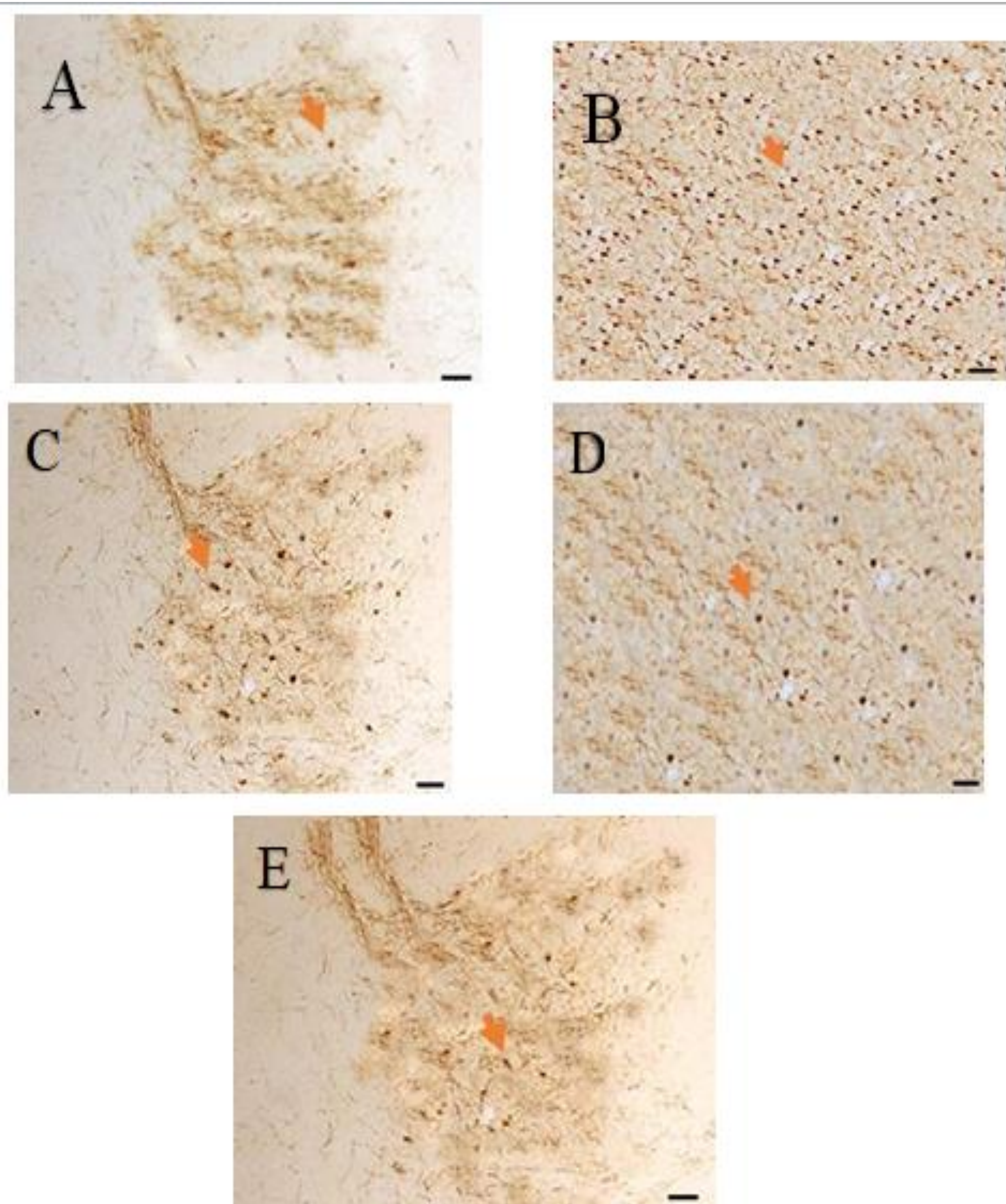

Figure 4. showing Immunohistochemistry Of α-Synuclein Protein In Brain Tissue (100x Magnification)

1. **Control** - showing very less expression of α-Synuclein (arrow mark indicates dark brown color positive expression)
2. **Rotenone** - showing higher intensity of with high positive α-Synuclein expression.
3. **Rotenone + Syndopa (15mg/kg)** - showing less expression of α-Synuclein.
4. **Rotenone + *Areca catechu* (400mg/kg)** - showing mild but less positive expression of α-Synuclein.
5. **Rotenone + *Areca catechu* (400mg/kg) + Syndopa(7.5mg/kg)** - showing very less positive expression of α- Synuclein similar to control.

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3.4Effect of AC on Brain histopathology in rotenone-induced Parkinson mice model:

Figure 5 shows the histological alterations found in the brain tissues of distinct groups. The striatum's regular architectural form could be seen in the brain tissue of the control group (Figure 5, Slide A), which also contained neuronal cells with conspicuous nuclei (small arrows), cytoplasm, and cytoplasm. In the PD group (Figure 5, Slide B), there were more pyknotic nuclei (arrow marks), LB, and signs of neuronal degeneration, including fewer neurons and shrinking cytoplasm. In Figure 5, Slide C, tissue from the Syndopa group, there was reduced evidence of pyknotic nuclei (arrow mark). Less evidence of pyknotic nuclei was seen in the PD + Areca catechu extract (400mg/kg) group (Figure 5, Slide D), while moderate evidence of normal neurons was seen in the LB group. There were more optimally sized cells with well-defined nuclei and less neuronal infiltration (Black arrow) in the brain tissue of the PD + Areca catechu extract (400mg/kg) + Syndopa (7.5mg/kg) group (Figure 5, Slide E), indicating less brain tissue damage.

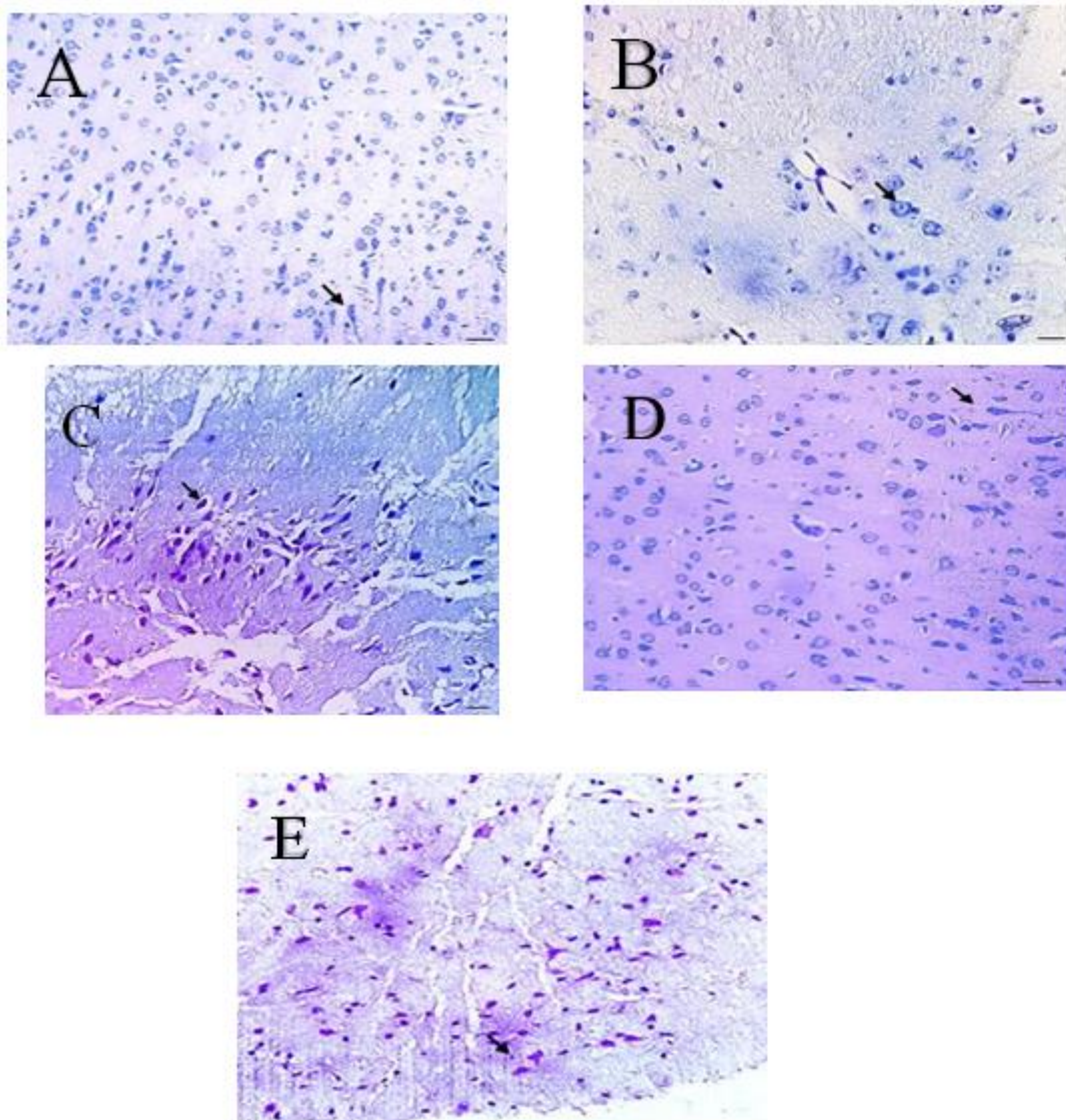

Figure 5: Histology of Nissl-Stained Brain tissues. (100X & 40X

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2 **Control** - normal neuronal cells with prominent nucleus (small arrow),

3 **Rotenone** - higher presence of pyknotic nuclei (arrow mark), LB, neuronal degeneration with decrease in the number of neurons and shrinkage of cytoplasm.

4 **Rotenone + Syndopa (15mg/kg)** - less evidence of pyknotic nuclei (arrow mark).

5 **Rotenone+ *Areca catechu* (400mg/kg)**- less evidence of pyknotic nuclei and LB withmoderate evidence of normal neurons

6 **Rotenone+ *Areca catechu* (400mg/kg) + Syndopa (7.5mg/kg)** - evidence of optimal sizedcells with well-defined nucleus and less neuronal (Black arrow) infiltration indicating less brain tissue damage.

Table 1: Neuroprotective effect of Areca catechu on rotenone induced changes in Lipid Peroxidation, Total anti-oxidant capacity and Alpha Synuclein in PD mice

Table 1: **Neuroprotective effect of *Areca catechu* on rotenone induced changes in Lipid Peroxidation, Total anti-oxidant capacity and Alpha Synuclein in PD mice**

Groups	**Lipid Peroxidation (LPO)**	**Total Anti-oxidant capacity method (TAC)**	**α-Synuclein**
Control	5.125±0.245	594.7±4.7	1.231±0.130
Rotenone	12.581±0.350	81.6±2.1	8.496±0.521
Rotenone + Syndopa(15mg/kg)	6.632 ±0.200	411.1±1.4	3.873±0.945

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Rotenone+ *Areca catechu* extract 400mg/kg	6.843 ±0.805	208.9±1.2	4.452±0.224
Rotenone+ *Areca catechu* extract 400mg/kg+ Syndopa 7.5mg/kg)	6.040 ± 0.209	551.3±3.9	3.145±0.369

Discussion

The results of the current investigation showed that mice given rotenone intraperitoneally for 21 days by that route developed motor impairments and PD pathology. We established that rotenone given intravenously caused a progressive movement disturbance with signs of Parkinson's disease. When given orally to PD mice, Areca catechu's ethanolic extract greatly reduced oxidative stress and its related problems. Importantly, combining Areca catechu with Syndopa treatment produced a greater anti-PD protective effect. There are numerous molecular mechanisms and pathways involved in the aetiology and pathogenesis of Parkinson's disease, even though the precise mechanism is poorly known. Both oxidative stress and the production of ROS play very important vital functions among all 14. The gradual decline of organ and tissue function that occurs with age. The oxidative stress theory of ageing is an evidence-based hypothesis that claims that structural damage and functional losses are caused by the accumulation of harmful oxidative damage to macromolecules (lipids, DNA, and proteins) that is caused by reactive oxygen and nitrogen species (RONS) that are produced throughout the lifespan 15. Aged animals have reportedly experienced oxidative damage to macromolecules like lipids, proteins, and DNA due to ROS and other reactive oxidants 16. Because it plays a part in the chain of events that results in the degeneration of dopaminergic cells, oxidative stress is regarded to be a key factor in dopaminergic neurotoxicity. Environmental factors that cause mitochondrial dysfunction, which is closely related to the production of reactive oxygen species, include neurotoxins, pesticides, insecticides, herbicides, dopamine (DA), and genetic mutations in PD-associated proteins.17

The medications that have been used to treat PD patients come with a number of side effects and repercussions after prolonged use. More than 50% of patients with chronic L-Dopa usage have dyskinesia. Tryptophan metabolism is impacted by L-Dopa therapy via the kynurenine pathway. For the purpose of examining Areca catechu's neuroprotective properties in rotenone-induced PD mice, Areca catechu was examined both on its own and in combination with Syndopa. One of the finest rat models for Parkinson's disease (PD) is rotenone, a specific inhibitor of mitochondrial complex I that worsens oxidative stress-mediated neuropathology. 18

One of the primary pathogenic characteristics of Parkinson's disease (PD) is the degradation of the nigrostriatal dopaminergic system, which rotenone causes 19. Rotenone causes motor impairments and selective nigrostriatal neurodegeneration when injected continuously through a jugular vein cannula connected to a subcutaneous osmotic minipump. It also causes cytoplasmic inclusions that are positive for -Synuclein and ubiquitin 20. The considerably increased levels of lipid peroxides, nitric oxide, peroxynitrate, and urea in the group that received only rotenone point to the treatment's impact on oxidative stress and damage connected to oxidative stress, which may involve free radicals and improper brain energy metabolism. The delivery of rotenone caused oxidative stress in the brain, which is consistent with a number of other published research. Malondialdehyde, an end product of lipid peroxidation, was increased, indicating that membrane lipids were being attacked by intracellular reactive oxygen metabolites. In vitro experiments and investigations on mouse brains have demonstrated that rotenone enhances the generation of reactive oxygen species and lipid peroxidation products 21. The increased production of reactive oxygen metabolites and the resulting oxidative stress are thought to be a significant pathogenic factor causing the cell death brought on by rotenone. To investigate its neuroprotective properties in rotenone-induced PD mice, Areca catechu extract was dose-dependently examined both alone and in conjunction with Syndopa. PD mouse groups were treated with Areca catechu extract alone or in combination with Syndopa, and these treatments had an antioxidative effect that reversed changes in lipid peroxidation.

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These outcomes could be attributed to the therapeutic and neuroprotective effects of the medications used. These outcomes might be a result of the antioxidant capabilities of Areca catechu extract 22 and synergistic impact with Syndopa to enhance nerve conduction and cerebral dopamine availability. Through determination of TAC levels, the antioxidant function of Areca catechu extract in PD was also assessed. Reduced TAC levels in PD suggest increased ROS levels. This is consistent with other research that claim oxidative damage contributes to the aberrant neural changes in PD. 23

The levels of TAC were raised by treatment with Areca catechu extract, both alone and in combination with Syndopa.In the current investigation, rats treated with rotenone had reduced TAC levels. According to studies, PD patients may experience oxidative damage to their DNA, proteins, and lipids due to low levels of TAC 24,25. Reduced TAC levels may be a significant factor in the development of oxidative stress in the brain. One of the main elements of PD pathogenesis is oxidative stress, which also includes stress brought on by hydroxyl radicals. Both the groups treated with Areca catechu extract as well as the Areca catechu extract plus Syndopa combination groups significantly reversed the lower levels of TAC in the PD group. However, the effect was more evident in the treatment groups that combined Areca catechu extract with Syndopa, with the largest effect occurring at a dose of 400 mg/kg of Areca catechu extract plus 7.5 mg/kg of Syndopa. All of these results point to oxidative stress and the production of ROS as the cause of the rise in -Synuclein expression. Our results imply that Areca catechu, which functions as an antioxidant, dramatically reduced -Synuclein pathology and safeguarded midbrain cells. However, a combined therapy using Areca catechu in addition to lower doses of Syndopa (7.5mg/kg) of conventional therapy produced superior results.

All these generation of ROS, oxidative stress, increase in α-Synuclein protein expression lead to damage of the brain tissues. Rotenone a pesticide is known to induce neurotoxicity. Studies have linked the neuromuscular coordination in rats that rotenone-induced metabolic alterations and cerebral damage in brain regions cause. After 1, 7, and 14 days of unilateral intranigral treatment of rotenone in adult male Swiss mice, they used Nissl staining to look at the histological changes rotenone-induced in the striatum (STR) and substantia nigra (SN). Nissl staining revealed significant morphological changes in cell area or shape. Additionally, there was evidence of neuronal degeneration in the areas of rotenone- treated rats by CV staining, which included dark staining, shrunken nuclei, and deformed neuronal cells

26. In our study Histology of Nissl stained brain tissues changes observed in our study suggested that there was higher presence of pyknotic nuclei , LB, neuronal degeneration with decrease in the number of neurons and shrinkage of cytoplasm in the PD mice. Also the decreased cerebral infarction area in Areca catechu treated groups and histopathological observations confirmed the above findings. In our study there was evidence of mice treated with combination of Areca catechu (400mg/kg) plus Syndopa (7.5mg/kg) group, brain tissues showed optimal sized cells with well-defined nucleus and less neuronal infiltration indicating less brain tissue damage found the neuroprotective effect of Areca catechu by its antioxidant action. Results of present study concluded the therapeutic as well as neuroprotective activity of Areca catechu (400mg/kg) and Syndopa (7.5mg/kg) combination in rotenone induced PD complications in mice.

Conclusion

In this study, rotenone-induced Parkinsonism in Swiss Albino mice led to diminished Total Antioxidant Capacity (TAC), elevated Lipid Peroxidation (LPO) levels, and heightened α-Synuclein expression, underlining compromised antioxidant defenses and the emergence of PD-like neuropathological changes. Treatment with Areca catechu (400 mg/kg dose), either standalone or combined with Syndopa (7.5 mg/kg), notably mitigated these biochemical and histological disturbances. These interventions not only reduced oxidative stress and α-Synuclein expression in the brain but also showcased pronounced neuroprotection. The collective findings underscore Areca catechu's potential, both individually and synergistically with Syndopa, as an efficacious therapeutic strategy for Parkinson's disease management.

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Conflict Of Interest

The authors declare that they have no conflict of interest.

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