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ASSESSMENT OF NEUROPROTECTION BY ETHANOLIC EXTRACT OF ALSTONIA SCHOLARIS IN A CHLORPROMAZINE-TRIGGERED PARKINSONIAN MODEL

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Abstract: Parkinson's disease is a progressive neurodegenerative disorder characterized by dopaminergic neuronal loss, motor dysfunction, and cognitive impairment, largely associated with oxidative stress and neurotransmitter imbalance. The present study was designed to evaluate the neuroprotective potential of the ethanolic extract of Alstonia scholaris in a chlorpromazine-induced Parkinsonism model in experimental animals. Animals were divided into five groups: normal control, chlorpromazine-treated negative control, standard treated group receiving Levodopa (10 mg/kg), and two test groups treated with ethanolic extract of Alstonia scholaris (200 and 400 mg/kg) along with chlorpromazine (3 mg/kg). Behavioral assessments were performed using Morris Water Maze, Elevated Plus Maze, and Y-maze tests to evaluate learning, memory, and exploratory activity. Biochemical parameters including malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and acetylcholinesterase (AChE) were estimated to assess oxidative stress and cholinergic function. Chlorpromazine administration significantly impaired cognitive performance and induced oxidative stress, as evidenced by increased MDA and AChE levels and decreased antioxidant enzyme activities. Treatment with the ethanolic extract of Alstonia scholaris produced a dose-dependent improvement in behavioral and biochemical parameters. The higher dose (400 mg/kg) showed effects comparable to the standard drug, indicating significant neuroprotective activity. The results suggest that Alstonia scholaris possesses potent antioxidant and neuroprotective properties, which may contribute to its beneficial effects against chlorpromazine-induced Parkinsonian symptoms. Further studies are required to isolate active constituents and elucidate the precise mechanisms underlying its neuroprotective action.

Keywords: Parkinsonism; Chlorpromazine, Alstonia scholaris, Neuroprotection, Dopaminergic neurons, Cognitive impairment, Levodopa

INTRODUCTION

Parkinson's Disease is a progressive neurodegenerative disorder characterized by the gradual loss of dopaminergic neurons in the substantia nigra pars compacta of the brain, leading to a deficiency of dopamine in the basal ganglia. Dopamine is a crucial neurotransmitter involved in the regulation of voluntary movement, coordination, and motor control. The disease primarily affects older adults and is recognized as the second most common neurodegenerative disorder after Alzheimer's Disease. Clinically, Parkinson's disease is characterized by cardinal motor symptoms including resting tremor, bradykinesia, muscular rigidity, and postural instability. In addition to motor impairments, patients may experience a variety of non-motor symptoms such as cognitive decline, depression, anxiety, sleep disturbances, autonomic dysfunction, constipation, and olfactory deficits. The pathological hallmarks of Parkinson's disease include degeneration of nigrostriatal dopaminergic neurons and the presence of Lewy bodies;

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intracellular protein aggregates composed primarily of alpha-synuclein. Although the exact etiology remains unclear, genetic mutations, environmental toxins, oxidative stress, mitochondrial dysfunction, neuroinflammation, and abnormal protein aggregation have been implicated in disease development and progression. Current therapeutic strategies, including dopaminergic medications and surgical interventions such as deep brain stimulation, mainly provide symptomatic relief rather than a cure. Therefore, considerable research is focused on identifying neuroprotective agents, including herbal medicines and natural products, that may slow neuronal degeneration and improve the quality of life of affected individuals [1].

Definition of Parkinson's Disease (PD)

Parkinson's Disease is a chronic, progressive neurodegenerative disorder of the central nervous system characterized by the degeneration of dopamine-producing neurons in the substantia nigra of the brain, resulting in dopamine deficiency and impaired motor function. It is clinically manifested by resting tremor, bradykinesia, muscular rigidity, and postural instability, along with various non-motor symptoms such as cognitive impairment, sleep disturbances, and autonomic dysfunction. Parkinson's disease is the second most common neurodegenerative disorder after Alzheimer's Disease.

Pathophysiology

The pathophysiology of Parkinson's Disease is characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta of the midbrain, resulting in a marked reduction of dopamine levels in the striatum. Dopamine plays a crucial role in regulating voluntary movement through the basal ganglia circuitry. The loss of dopamine disrupts the balance between excitatory and inhibitory pathways within the basal ganglia, leading to impaired motor control and the characteristic symptoms of Parkinson's disease, including tremor, rigidity, bradykinesia, and postural instability. A major pathological hallmark of Parkinson's disease is the accumulation of misfolded Alpha-synuclein protein within neurons, forming intracellular inclusions known as Lewy bodies. These protein aggregates interfere with normal cellular functions, promote neuronal damage, and contribute to neurodegeneration. Oxidative stress also plays a significant role in disease progression, as excessive production of reactive oxygen species (ROS) causes lipid peroxidation, DNA damage, and protein oxidation, ultimately leading to neuronal death. Mitochondrial dysfunction further exacerbates neuronal injury by impairing ATP production and increasing oxidative stress. Neuroinflammation, mediated by activated microglia and the release of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, contributes to the progressive loss of dopaminergic neurons. Additionally, excitotoxicity caused by excessive glutamate activity results in increased intracellular calcium levels, triggering apoptotic pathways and neuronal damage. Genetic mutations in genes such as SNCA, LRRK2, PINK1, and PARK2, along with environmental factors including pesticide exposure, heavy metals, and aging, may initiate or accelerate these pathological processes. The combined effects of dopamine depletion, protein aggregation, oxidative stress, mitochondrial dysfunction, and neuroinflammation ultimately lead to the progressive neurological and motor impairments observed in Parkinson's disease [2-4].

Monoamine oxidase (MAO-A and MAO-B) involvement in PD

Monoamine oxidases (MAOs) are mitochondrial enzymes responsible for the oxidative deamination of monoamine neurotransmitters such as dopamine, serotonin, and norepinephrine. Two isoforms, MAO-A and MAO-B, are present in the brain and play important roles in the pathogenesis of Parkinson's Disease.

MAO-A

MAO-A is primarily found in catecholaminergic neurons and preferentially metabolizes serotonin, norepinephrine, and dopamine. Although MAO-A contributes to dopamine degradation, its role in Parkinson's disease is less significant than that of MAO-B. Excessive MAO-A activity can increase the production of reactive oxygen species (ROS) during neurotransmitter metabolism, thereby contributing to oxidative stress and neuronal damage.

MAO-B

MAO-B is predominantly localized in astrocytes and glial cells and is the principal enzyme involved in dopamine metabolism in the aging brain. In Parkinson's disease, MAO-B activity is often increased, leading to enhanced breakdown of dopamine

and further depletion of already reduced dopamine levels in the striatum. During dopamine metabolism, MAO-B generates hydrogen peroxide (H_2O_2), a reactive oxygen species that can be converted into highly toxic hydroxyl radicals. These free radicals induce oxidative stress, lipid peroxidation, mitochondrial dysfunction, and neuronal degeneration [5-7].

Role in Parkinson's Disease Pathogenesis

The increased activity of MAO-B contributes to:

- Accelerated dopamine depletion.
- Increased oxidative stress through ROS generation.
- Mitochondrial damage and energy failure.
- Progressive degeneration of dopaminergic neurons in the substantia nigra.
- Worsening of motor symptoms such as tremor, rigidity, and bradykinesia.

Therapeutic Significance

Because MAO-B plays a crucial role in dopamine degradation, MAO-B inhibitors are widely used in Parkinson's disease treatment. Drugs such as:

- Selegiline
- Rasagiline
- Safinamide

inhibit dopamine breakdown, increase dopamine availability in the brain, reduce oxidative stress, and improve motor function. Some studies suggest that MAO-B inhibitors may also exert neuroprotective effects by reducing free radical formation and slowing neuronal degeneration.

Mitochondria involvement in PD

Mitochondrial dysfunction is a central mechanism in the pathogenesis of Parkinson's Disease and plays a critical role in the degeneration of dopaminergic neurons in the substantia nigra. Mitochondria are responsible for cellular energy production through oxidative phosphorylation and are essential for maintaining neuronal survival, calcium homeostasis, and redox balance. Neurons, particularly dopaminergic neurons, have high energy demands and are therefore highly vulnerable to mitochondrial damage. One of the most significant mitochondrial abnormalities observed in Parkinson's disease is the impairment of Complex I (NADH-ubiquinone oxidoreductase) of the electron transport chain. Reduced Complex I activity decreases ATP production and increases the leakage of electrons, resulting in excessive generation of reactive oxygen species (ROS). The accumulation of ROS causes oxidative stress, leading to lipid peroxidation, protein oxidation, DNA damage, and ultimately neuronal death. Mitochondrial dysfunction also disrupts calcium regulation within neurons. Excess intracellular calcium activates various enzymes that damage cellular structures and trigger apoptotic pathways. Furthermore, damaged mitochondria release pro-apoptotic factors such as cytochrome c, which activate caspases and promote programmed cell death of dopaminergic neurons. Several genes associated with familial Parkinson's disease are directly involved in mitochondrial maintenance and quality control. Mutations in PINK1, PARK2 (Parkin), and DJ-1 impair mitophagy, the process by which damaged mitochondria are identified and removed. Failure of mitophagy leads to the accumulation of dysfunctional mitochondria, increased oxidative stress, and progressive neuronal degeneration. Environmental toxins such as MPTP, rotenone, and paraquat can also induce Parkinsonian symptoms by inhibiting mitochondrial Complex I, further supporting the role of mitochondrial dysfunction in disease development [8-10].

Consequences of Mitochondrial Dysfunction in PD

- Reduced ATP production and cellular energy deficiency.
- Increased generation of reactive oxygen species (ROS).
- Oxidative damage to proteins, lipids, and DNA.
- Impaired calcium homeostasis.
- Activation of apoptotic pathways.
- Progressive loss of dopaminergic neurons in the substantia nigra.
- Development of motor and non-motor symptoms of Parkinson's disease.

Role of cellular Calcium on Parkinsonism

Cellular calcium (Ca^{2+}) plays a crucial role in neuronal signaling, neurotransmitter release, gene expression, and cell survival. In Parkinson's Disease, dysregulation of intracellular calcium homeostasis is considered an important factor contributing to the degeneration of dopaminergic neurons in the substantia nigra pars compacta. Dopaminergic neurons of the substantia nigra exhibit autonomous pacemaking activity that

relies heavily on L-type voltage-gated calcium channels (Cav1.3). Continuous calcium influx through these channels places a high metabolic burden on neurons, increasing the demand for ATP and mitochondrial activity. Over time, excessive intracellular calcium accumulation can overwhelm the cell's buffering mechanisms, leading to mitochondrial dysfunction and oxidative stress. Elevated intracellular calcium levels stimulate the production of reactive oxygen species (ROS) and promote the opening of the mitochondrial permeability transition pore, resulting in mitochondrial damage and reduced ATP synthesis. Excess calcium also activates calcium-dependent enzymes such as calpains, phospholipases, and endonucleases, which can degrade cellular proteins, membranes, and DNA. Furthermore, calcium overload triggers apoptotic pathways through the release of cytochrome c from mitochondria, leading to programmed neuronal death. Abnormal calcium signaling is also associated with excitotoxicity. Excessive activation of glutamate receptors, particularly NMDA receptors, allows large amounts of calcium to enter neurons, causing cellular injury and accelerating neurodegeneration. Additionally, aggregation of α-synuclein may disrupt calcium homeostasis, further enhancing neuronal vulnerability [11-13].

Role of Iron in Parkinsonism

Iron is an essential trace element required for numerous biological processes, including oxygen transport, mitochondrial respiration, neurotransmitter synthesis, and cellular metabolism. However, abnormal iron accumulation in the brain, particularly in the substantia nigra, is a characteristic feature of Parkinson's Disease and contributes significantly to its pathogenesis. In Parkinson's disease, excessive iron deposition occurs within dopaminergic neurons and glial cells of the substantia nigra. Elevated iron levels promote the formation of highly reactive hydroxyl radicals through the Fenton reaction, in which ferrous iron (Fe^{2+}) reacts with hydrogen peroxide (H_2O_2) to generate reactive oxygen species (ROS). These free radicals cause oxidative damage to cellular lipids, proteins, DNA, and mitochondria, leading to neuronal dysfunction and death. Iron also interacts with dopamine metabolism. Dopamine can undergo oxidation in the presence of excess iron, producing toxic quinones and additional ROS that further damage dopaminergic neurons. Moreover, iron promotes the aggregation and misfolding of α-synuclein, a protein that forms Lewy bodies, one of the pathological hallmarks of Parkinson's disease. The accumulation of α-synuclein aggregates disrupts normal neuronal function and accelerates neurodegeneration. Mitochondrial dysfunction associated with iron overload further contributes to neuronal injury by impairing ATP production and increasing oxidative stress. Excessive iron can also trigger ferroptosis, an iron-dependent form of programmed cell death characterized by lipid peroxidation and membrane damage [12-15].

Role of neuroinflammation in the pathogenesis of PD

Neuroinflammation is a critical factor in the development and progression of Parkinson's Disease. It is characterized by the chronic activation of the brain's immune system, particularly microglia and astrocytes, which leads to the release of inflammatory mediators that contribute to neuronal injury and death. Increasing evidence suggests that neuroinflammation not only accompanies neuronal degeneration but also actively participates in the pathogenesis of Parkinson's disease. Under normal conditions, microglia serve as resident immune cells of the central nervous system and protect neurons from injury and infection. However, in Parkinson's disease, factors such as α-synuclein aggregation, oxidative stress, mitochondrial dysfunction, environmental toxins, and neuronal damage activate microglia excessively. Activated microglia release pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), nitric oxide (NO), and reactive oxygen species (ROS). These inflammatory mediators create a toxic environment that promotes further neuronal degeneration. The accumulation of misfolded α-synuclein, a major component of Lewy bodies, acts as a potent trigger for microglial activation. Activated astrocytes further amplify the inflammatory response by producing cytokines and chemokines, thereby sustaining chronic inflammation within the substantia nigra. This persistent inflammatory state damages dopaminergic neurons and disrupts neuronal communication. Neuroinflammation is closely linked with oxidative stress and mitochondrial dysfunction. Inflammatory mediators increase ROS production, while damaged mitochondria release signals that further

activate immune cells, creating a vicious cycle of inflammation and neuronal injury. Chronic neuroinflammation also impairs the blood-brain barrier, allowing peripheral immune cells to infiltrate the brain and exacerbate neuronal damage [16-19].

Current treatments

The current treatment of Parkinson's Disease is primarily focused on symptomatic management, as no definitive cure is currently available. The cornerstone of therapy is dopamine replacement with Levodopa, usually administered in combination with Carbidopa or benserazide to enhance its effectiveness and reduce peripheral side effects. Other commonly used medications include dopamine agonists such as Pramipexole and Ropinirole, MAO-B inhibitors such as Selegiline and Rasagiline, COMT inhibitors such as Entacapone, anticholinergic agents, and amantadine, which help control various motor symptoms and reduce treatment-related complications. In advanced stages of the disease, Deep Brain Stimulation may be employed to improve motor function and reduce medication requirements. Non-pharmacological interventions, including physiotherapy, occupational therapy, speech therapy, nutritional support, and regular exercise, play an essential role in maintaining mobility, balance, and quality of life. Furthermore, ongoing research is exploring neuroprotective strategies such as stem cell therapy, gene therapy, neurotrophic factors, anti-inflammatory agents, antioxidants, and medicinal plants with the aim of slowing disease progression and preserving neuronal function [20].

MATERIALS AND METHODS

Collection and authentication of plant material

Alstonia scholaris a were collected from local area of Ranga Reddy district Hydrabad . was selected for investigation. The plant material was taxonomically identified and authenticated by Dr. Madhava Chetty, Head of Department, Botany, Sri Venkateshwara academia, Tirupathi, Andhra Pradesh.

Animals

Healthy, adult Wistar rats of both sexes (180-220g) were obtained from the Central animal house facility from our institution. The animals were kept in a well-ventilated room and the animals had exposed to 12 hrs day and night cycle with a temperature between 20±3 °C. The animals were housed in large spacious, hygienic polypropylene cages during the course of the experimental period. The animals were fed with water and rat feed adlib tum. All experiments were performed after obtaining prior approval from CCSEA and IAEC. The animals were housed in suitable environmental conditions. Jeeva life sciences The Institutional Animal Ethics Committee approved the protocol of this study.

Method of preparation of plants extract

Alstonia scholaris were cut in to small pieces and powdered carefully by using mechanical blender. The dried coarse powdered crude plant (50g) was transferred to a round bottom flask and in 1;4 ratio soxhlation apparatus was used to extrat. continenously heated for 6-8 hours extract so obtained was filtered then used for drying the extract. Percentage yield of the ethanolic extract was found was used for experiment.

Acute toxicity study [21]

An acute toxicity study is a short-term experimental assessment conducted to evaluate the adverse effects of a substance following a single administration or multiple administrations within a 24-hour period. The study is primarily used to determine the safety profile of a test compound, estimate its median lethal dose (LD_{50}), identify target organs affected by toxicity, and establish appropriate dose levels for further pharmacological and toxicological investigations. Typically performed according to guidelines such as OECD 420, 423, or 425, the test involves administering the substance to healthy laboratory animals, usually rats or mice, and observing them for a period of 14 days. During this observation period, animals are monitored for mortality, behavioral changes, clinical signs of toxicity, body weight variations, and alterations in food and water intake. At the end of the study, a gross pathological examination is conducted to detect any organ abnormalities. Acute toxicity studies are essential in drug development and safety evaluation because they provide preliminary information on the potential hazards associated with a substance and help ensure its safe use in subsequent experimental and clinical studies.

Induction of Parkinsonism by MPTP [22]

Parkinsonism can be experimentally induced using 1-Methyl-4-Phenyl-1,2,3,6-T etrahydropyridine (MPTP), a neurotoxin that selectively damages dopaminergic neurons in the substantia nigra. MPTP is administered to mice through intraperitoneal

injections at appropriate doses over a specified period. After crossing the blood-brain barrier, MPTP is converted by monoamine oxidase-B (MAO-B) in astrocytes to its active metabolite, 1-methyl-4-phenylpyridinium (MPP^+). MPP^+ is selectively taken up by dopamine transporters into dopaminergic neurons, where it inhibits mitochondrial complex I, resulting in oxidative stress, ATP depletion, and neuronal death. The loss of nigrostriatal dopaminergic neurons leads to a marked reduction in striatal dopamine levels and produces motor deficits resembling those observed in Parkinson's disease, including bradykinesia, rigidity, tremors, and impaired locomotor activity. This model is widely used for studying the pathophysiology of Parkinson's disease and evaluating the neuroprotective and antiparkinsonian effects of therapeutic agents.

Experimental grouping [23]

Group I Normal Control – Received vehicle (distilled water or 0.5% CMC) only.

Group II Negative Control (Parkinsonian Control) – Received chlorpromazine (3 mg/kg, i.p.) to induce Parkinsonism.

Group III Standard Control – Received chlorpromazine (3 mg/kg, i.p.) + Levodopa (10 mg/kg, p.o.).

Group IV Chlorpromazine + Ethanolic Extract of Alstonia scholaris (200 mg/kg, p.o.).

Group V Chlorpromazine + Ethanolic Extract of Alstonia scholaris (400 mg/kg, p.o.).

Morris Water Maze [24]

Procedure

Healthy adult Wistar rats are acclimatized to the laboratory environment for at least 7 days before testing. The apparatus consists of a circular pool filled with opaque water maintained at 25 ± 1 °C and divided into four imaginary quadrants. A small escape platform is submerged 1–2 cm below the water surface in the center of one quadrant, and distinct visual cues are placed around the room. During the acquisition (training) phase, conducted for 4–5 consecutive days, each rat is subjected to four trials per day from different starting points and allowed 60–120 seconds to locate the hidden platform. If the rat fails to find the platform, it is gently guided to it and allowed to remain there for 15–20 seconds. Escape latency, path length, and swim speed are recorded. Twenty-four hours after the last training session, a probe trial is performed by removing the platform and allowing the rat to swim freely for 60 seconds. The time spent in the target quadrant and the number of crossings over the previous platform location are measured to assess memory retention.

Elevated Plus Maze [24]

Healthy adult Wistar rats are acclimatized to the laboratory environment for at least 7 days prior to testing. The apparatus consists of a plus-shaped maze elevated about 50 cm above the floor, with two open arms and two closed arms of equal dimensions connected by a central platform. On the training day, each rat is placed individually at the end of an open arm facing away from the central platform. The time taken by the rat to move from the open arm into one of the closed arms is recorded as transfer latency (TL), with a cut-off time of 90 seconds. The rat is allowed to explore the maze for an additional 20 seconds and then returned to its home cage. After 24 hours, the retention trial is conducted in the same manner, and the transfer latency is again recorded. A decrease in transfer latency on the retention day compared to the training day indicates improved learning and memory, while an increase suggests memory impairment.

Y-Maze test [25]

Healthy adult Wistar rats are acclimatized to the laboratory for at least 7 days before testing. The Y-maze apparatus consists of three identical arms (labeled A, B, and C) positioned at 120° to each other. Each rat is placed at the end of one arm and allowed to explore the maze freely for 8 minutes. The sequence and number of arm entries are recorded. An entry is considered when all four paws of the rat enter an arm. Spontaneous alternation behavior is calculated as successive entries into all three different arms (e.g., ABC, BCA, CAB). The percentage alternation is determined using the formula:

$$\% \text{ Alternation} = \frac{\text{Number of alternations}}{\text{Total arm entries} - 2} \times 100$$

A higher percentage of spontaneous alternation indicates better working memory, while a reduction suggests memory impairment.

TO ESTIMATE BRAIN BIOCHEMICAL MARKERS

Acetylcholinesterase assay (AChE) [26]

Acetylcholinesterase activity in brain tissue is estimated by Ellman's colorimetric method. A 10% (w/v) brain homogenate is prepared in ice-cold 0.1 M phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm for 15 minutes at 4 °C. The supernatant is used for the assay. In a cuvette, add 2.6 ml of phosphate buffer (pH 8.0), 0.4 ml of brain homogenate, and 0.1 ml of DTNB (5,5′-dithiobis-2-nitrobenzoic acid). After mixing, add 0.02 ml of acetylthiocholine iodide as the substrate to initiate the reaction. The increase in yellow color due to the formation of thionitrobenzoate ions is measured spectrophotometrically at 412 nm for 2 minutes at 30-second intervals. The enzyme activity is expressed as µmoles of acetylthiocholine hydrolyzed per minute per mg of protein.

Malondialdehyde (MDA) [27]

Lipid peroxidation in brain tissue is estimated by measuring malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances (TBARS) method. A 10% (w/v) brain homogenate is prepared in ice-cold 0.1 M phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm for 15 minutes at 4 °C. To 1 ml of the supernatant, add 2 ml of TBA–TCA–HCl reagent. The mixture is heated in a boiling water bath for 15 minutes, then cooled and centrifuged. The pink chromogen formed due to the reaction between MDA and thiobarbituric acid is measured spectrophotometrically at 532 nm. The results are expressed as nmoles of MDA per mg of protein.

Superoxide dismutase (SOD) [28]

Superoxide dismutase activity in brain tissue is estimated by its ability to inhibit the auto-oxidation of epinephrine at alkaline pH. A 10% (w/v) brain homogenate is prepared in ice-cold 0.1 M phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm for 15 minutes at 4 °C. In a cuvette, add 2.5 ml of carbonate buffer (0.05 M, pH 10.2) and 0.1 ml of EDTA, followed by 0.05 ml of the supernatant. The reaction is initiated by adding 0.3 ml of freshly prepared epinephrine solution. The increase in absorbance due to epinephrine auto-oxidation is recorded at 480 nm for 4 minutes. One unit of SOD activity is defined as the amount of enzyme required to inhibit 50% of epinephrine auto-oxidation, and the results are expressed as units per mg of protein.

Catalase (CAT) [29]

Catalase activity in brain tissue is measured by monitoring the decomposition of hydrogen peroxide. A 10% (w/v) brain homogenate is prepared in ice-cold 0.1 M phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm for 15 minutes at 4 °C. In a cuvette, add 1.9 ml of phosphate buffer and 0.1 ml of the supernatant. The reaction is initiated by adding 1.0 ml of hydrogen peroxide solution. The decrease in absorbance due to the breakdown of H_2O_2 is recorded spectrophotometrically at 240 nm for 1 minute. Catalase activity is expressed as µmoles of hydrogen peroxide decomposed per minute per mg of protein.

Glutathione (Gsh) [30]

Reduced glutathione in brain tissue is estimated using Ellman's reagent (DTNB). A 10% (w/v) brain homogenate is prepared in ice-cold 0.1 M phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm for 15 minutes at 4 °C. To 1 ml of the supernatant, add 1 ml of 10% trichloroacetic acid (TCA) to precipitate proteins and centrifuge. Take 0.5 ml of the clear supernatant and add 2 ml of phosphate buffer followed by 0.25 ml of DTNB solution. The yellow color formed is measured spectrophotometrically at 412 nm. The results are expressed as µg of GSH per mg of protein.

Statistical evaluation

All values were represented as mean values ± SEM (standard error of the mean), and the data was analyzed using one way ANOVA followed by a Dunnett's t-test using GraphPad Prism 9. The results were considered statistically significant if $p < 0.05$, $p < 0.01$, or $p < 0.001$.

Results

Percentage of yield

$$Percentage\ of\ yield = \frac{Weight\ of\ crude\ extract}{Weight\ of\ dried\ material} \times 100$$

$$Percentage\ of\ yield = \frac{26g}{200g} \times 100 = 13\%$$

50gms of drug dissolved in 250ml of solvent in 1:4 Ratio

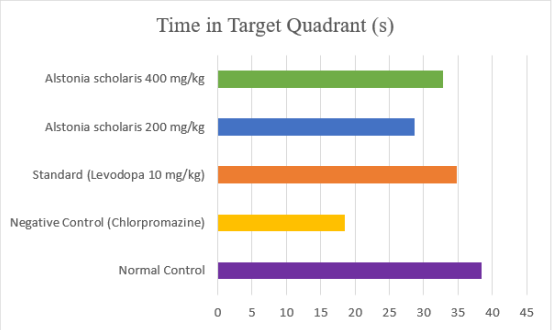
PHYTOCHEMICAL TESTS

Sl. No.	Phytoconstituent	Test Result
1	Alkaloids	–ve (Absent)
2	Glycosides	–ve (Absent)
3	Carbohydrates	–ve (Absent)
4	Proteins	–ve (Absent)
5	Amino acids	+ve (Present)
6	Steroids	–ve (Absent)
7	Flavonoids	+ve (Present)
8	Terpenoids	+ve (Present)

Morris Water Maze Test

Group	Time in Target Quadrant (s)
Normal Control	38.42 ± 1.15
Negative Control (Chlorpromazine)	18.56 ± 0.94
Standard (Levodopa 10 mg/kg)	34.78 ± 1.08***
Alstonia scholaris 200 mg/kg	28.64 ± 1.02**
Alstonia scholaris 400 mg/kg	32.85 ± 1.11***

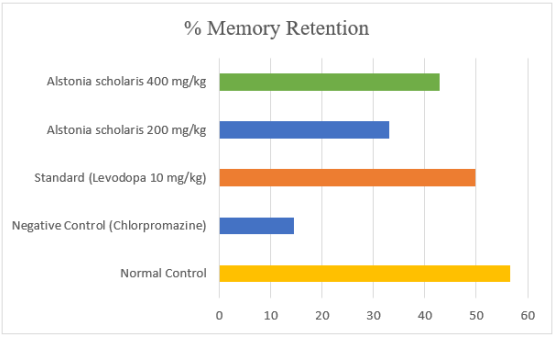
Chlorpromazine administration significantly impaired spatial learning and memory, as evidenced by increased escape latency and reduced time spent in the target quadrant compared with the normal control group. Treatment with levodopa significantly improved cognitive performance. The ethanolic extract of Alstonia scholaris produced a dose-dependent improvement in learning and memory, with the 400 mg/kg dose showing effects comparable to the standard treatment, indicating significant neuroprotective activity against chlorpromazine-induced cognitive deficits.



Elevated Plus Maze

Group	% Memory Retention
Normal Control	56.52 ± 2.14
Negative Control (Chlorpromazine)	14.58 ± 1.26
Standard (Levodopa 10 mg/kg)	49.86 ± 1.92***
Alstonia scholaris 200 mg/kg	33.12 ± 1.68**
Alstonia scholaris 400 mg/kg	42.84 ± 1.81***

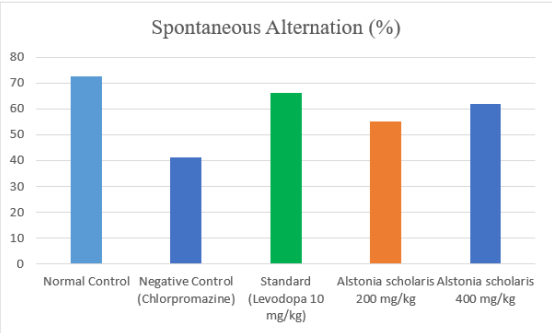
Chlorpromazine-treated animals exhibited a significant increase in transfer latency and a decrease in memory retention compared to the normal control group, indicating impairment of learning and memory functions. Administration of levodopa significantly reduced transfer latency and improved memory retention. Treatment with the ethanolic extract of Alstonia scholaris produced a dose-dependent improvement in cognitive performance, with the 400 mg/kg dose showing a marked reduction in transfer latency and enhanced memory retention, suggesting significant neuroprotective and cognition-enhancing effects against chlorpromazine-induced Parkinsonian deficits.



Y-Maze test

Group	Spontaneous Alternation (%)
Normal Control	72.48 ± 1.62
Negative Control (Chlorpromazine)	41.36 ± 1.28
Standard (Levodopa 10 mg/kg)	66.25 ± 1.54***
Alstonia scholaris 200 mg/kg	55.18 ± 1.41**
Alstonia scholaris 400 mg/kg	61.84 ± 1.49***

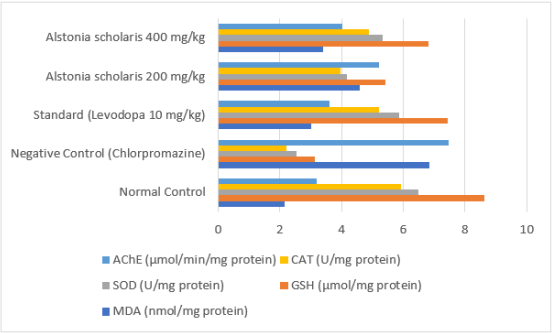
Chlorpromazine administration significantly reduced spontaneous alternation behavior and total arm entries, indicating impairment of working memory and exploratory behavior. Treatment with Levodopa significantly improved spontaneous alternation and exploratory activity. The ethanolic extract of Alstonia scholaris produced a dose-dependent improvement in cognitive performance, with the 400 mg/kg dose showing a significant increase in spontaneous alternation behavior and arm entries, suggesting enhanced working memory and neuroprotective potential against chlorpromazine-induced cognitive deficits.



Biochemical Parameters

Group	MDA (nmol/mg protein)	GSH (µmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	AChE (µmol/min/mg protein)
Normal Control	2.14 ± 0.08	8.62 ± 0.21	6.48 ± 0.17	5.92 ± 0.16	3.18 ± 0.09
Negative Control (Chlorpromazine)	6.85 ± 0.19	3.12 ± 0.11	2.54 ± 0.09	2.21 ± 0.08	7.46 ± 0.18
Standard (Levodopa 10 mg/kg)	3.02 ± 0.10***	7.45 ± 0.18***	5.86 ± 0.15***	5.21 ± 0.14***	3.62 ± 0.10***
Alstonia scholaris 200 mg/kg	4.58 ± 0.14**	5.42 ± 0.16**	4.18 ± 0.12**	3.96 ± 0.11**	5.21 ± 0.13**
Alstonia scholaris 400 mg/kg	3.41 ± 0.11***	6.83 ± 0.17***	5.34 ± 0.14***	4.88 ± 0.13***	4.02 ± 0.11***

Chlorpromazine administration significantly increased oxidative stress as evidenced by elevated malondialdehyde (MDA) levels and increased acetylcholinesterase (AChE) activity, along with a marked decrease in endogenous antioxidant defenses including reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT). Treatment with Levodopa significantly reversed these biochemical alterations, restoring antioxidant status and reducing lipid peroxidation. The ethanolic extract of Alstonia scholaris produced a dose-dependent improvement in biochemical parameters, with the 400 mg/kg dose showing significant antioxidant and neuroprotective effects, suggesting its ability to mitigate chlorpromazine-induced oxidative stress and cholinergic dysfunction.



DISCUSSION

The present study was designed to evaluate the neuroprotective potential of the ethanolic extract of Alstonia scholaris in a chlorpromazine-induced Parkinson's disease model. Chlorpromazine administration produced significant behavioral, cognitive, and biochemical alterations characteristic of Parkinsonism, including impaired learning and memory, reduced exploratory behavior, and increased oxidative stress. These changes are consistent with dopaminergic dysfunction and oxidative damage in the nigrostriatal pathway. Behavioral studies using Morris Water Maze, Elevated Plus Maze, and Y-maze tests revealed significant cognitive deficits in the chlorpromazine-treated group, as evidenced by increased escape latency, reduced spontaneous alternation behavior, and decreased memory retention. These findings indicate impairment in both spatial and working memory, which may be attributed to dopaminergic depletion and cholinergic imbalance in the brain. Biochemical analysis further supported these observations, showing increased lipid peroxidation (MDA) and

acetylcholinesterase (AChE) activity, along with decreased levels of endogenous antioxidants such as glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT). These results suggest that chlorpromazine induces oxidative stress-mediated neuronal damage, which plays a crucial role in Parkinsonian neurodegeneration. Treatment with Levodopa significantly improved behavioral performance and restored antioxidant status, confirming its dopaminergic replacement effect. Importantly, the ethanolic extract of Alstonia scholaris demonstrated a dose-dependent neuroprotective effect, significantly improving learning, memory, and exploratory behavior while also restoring biochemical parameters toward normal levels. The higher dose (400 mg/kg) showed effects comparable to the standard drug, indicating strong neuroprotective potential. The observed effects of Alstonia scholaris may be attributed to its rich phytochemical constituents such as alkaloids, flavonoids, and phenolic compounds, which possess strong antioxidant and free radical scavenging properties. These compounds may help in reducing oxidative stress, preserving neuronal integrity, and modulating neurotransmitter levels, thereby improving cognitive and motor functions. Overall, the results suggest that the ethanolic extract of Alstonia scholaris exhibits significant neuroprotective activity against chlorpromazine-induced Parkinsonism, likely through antioxidant, anti-cholinesterase, and neurochemical modulatory mechanisms. Further studies are required to isolate active constituents and elucidate precise molecular pathways involved in its neuroprotective action.

Conclusion

The present study demonstrates that chlorpromazine effectively induces Parkinsonism-like symptoms in experimental animals, characterized by significant behavioral, cognitive, and biochemical alterations associated with oxidative stress and neurotransmitter imbalance. The ethanolic extract of Alstonia scholaris showed a marked neuroprotective effect against chlorpromazine-induced neurotoxicity. Treatment with the extract significantly improved learning and memory performance in Morris Water Maze, Elevated Plus Maze, and Y-maze tests, along with a restoration of normal exploratory behavior. Biochemical findings further confirmed its protective role by reducing lipid peroxidation (MDA), decreasing acetylcholinesterase (AChE) activity, and enhancing endogenous antioxidant enzymes such as GSH, SOD, and CAT. Among the tested doses, the higher dose (400 mg/kg) exhibited more pronounced neuroprotective effects, comparable to the standard treatment with Levodopa. These findings suggest that the ethanolic extract of Alstonia scholaris possesses significant antioxidant and neuroprotective properties, likely due to its phytochemical constituents. In conclusion, Alstonia scholaris may serve as a potential therapeutic candidate for the management of Parkinson's disease, although further studies are required to isolate active compounds and validate its mechanism of action through advanced molecular investigations and clinical trials.

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