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PHARMACOLOGICAL EVALUATION OF NEUROPROTECTIVE POTENTIAL OF STANDARDIZED EXTRACT OF RUBIA CORDIFOLIA IN EXPERIMENTAL NEURODEGENERATION

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ABSTRACT: This study evaluated the cognitive-enhancing and neuroprotective effects of Rubia cordifolia extract in a rat model of memory impairment induced by Scopolamine (1 mg/kg, i.p.). Animals were treated orally with the extract at doses of 200 and 400 mg/kg and compared with a standard therapy (Donepezil, 3 mg/kg). Cognitive performance was assessed using the Morris Water Maze, Elevated plus Maze, and Y-Maze paradigms to measure spatial learning, memory retention, exploratory behavior, and working memory. Scopolamine administration produced significant deficits across behavioral tasks. Treatment with Rubia cordifolia resulted in marked, dose-dependent improvement in these parameters, with the higher dose showing efficacy comparable to the standard drug. Biochemical evaluation of brain homogenates demonstrated a reduction in acetyl cholinesterase and lipid per oxidation levels, along with restoration of antioxidant defenses indicated by increased superoxide dismutase and reduced glutathione. The findings indicate that the extract enhances cognitive function through modulation of cholinergic activity and mitigation of oxidative stress, supporting its potential role as a natural agent for the management of memory deficits and related neurodegenerative conditions.

Keywords: Rubia cordifolia; Scopolamine; No tropic activity; Cognitive impairment; Memory enhancement; Oxidative stress; Acetyl cholinesterase.

INTRODUCTION

Parkinson's disease [1]

Parkinson's disease (PD) is a neurodegenerative disorder caused by the progressive loss of mesencephalic dopaminergic neurons in the substantia nigra innervating the striatum. It was first described by neurologist James Parkinson in 1817 that he called ''Shaking Palsy'', or ''paralysis agitans''. The causes are unknown although risk factors in the genetic and toxic domain are being discovered. An important path physiological feature in PD is the loss of part of the dopaminergic neurons in the substantia nigra (SN) resulting in a specific disorganisation of the complicated basal ganglia (BG) circuits. The relay functions at the level of the striatum e.g., are out of balance leading to disturbed sub corticocortical interactions. Parkinson's disease (PD) is the second most common neurodegenerative disease, primarily affecting people of ages over 55 years (approximately 1.5% to 2.0%), although young adults and even children can also be affected. Research on the

pathogenesis of PD has rapidly advanced due to the development of animal models. Through the use of these models, the striatal dopamine deficiency could be associated with the motor symptoms of PD, and levodopa (dihydroxyphenylalanine or L-dopa) was first applied to compensate striatal dopamine losses. L-Dopa treatment still remains the standard of PD therapies. Unfortunately, long-time use of L-dopa results in dyskinesia (involuntary movements). Moreover, the specific etiology of PD is still unknown. Thus, the development of animal models is essential for better understanding pathogenesis and progression of PD and testing therapeutic agents for the treatment of PD patients [1-6]

Definition 7

Parkinsonism is a clinical syndrome consisting of four cardinal features: bradykinesia (slowness and poverty of movement), muscular rigidity, resting tremor (which usually abates during voluntary movement), and an impairment of postural balance leading to disturbances of gait and falling.

Path physiology [8-10]

Pathologically, PD is characterized by severe loss of substantia nigra (SN) dopaminergic nAAEURons, visible in brain sections as depigmentation of the substantia nigra in the midbrain. It is estimated that approximately 60% to 70% of the SN dopamine cells are lost by the time a patient first presents for clinical evaluation, diagnosis, and treatment. Neuroimaging reveals a typically asymmetric loss of dopamine terminals in the striatum, which progresses over time leading to further clinical deterioration. Since the early 1980s and the discovery of a potent neurotoxin (MPTP-MPP+), a by-product of illicit drug synthesis, the environment has figured prominently in proposed etiologies for Parkinson's disease. After the original description of this environmental "insult" to the dopamine-producing cells of the substantia nigra, a number of other environmental neurotoxins have been described, which have led to the Parkinson a state. These discoveries have led to the suggestion that Parkinson's disease may arise as a combined consequence of the ongoing aging process coupled with environmental exposure(s) that accelerate the process of nigral cell death. The unusual clustering of individuals who later developed Parkinson's Disease (including Michael J. Fox), in a Canadian recording studio, emphasizes the possible relation of environment to disease development. The third component of the puzzle is the possibility that some individuals may have a predetermined genetic susceptibility to these environmental "insults." While Parkinson's disease has been observed to occur throughout the world and in virtually all ethnic groups, there is a very low incidence among Asians and African patients as opposed to white patients. This observation suggests that genetic factors may possibly have an important role in disease production. Other evidence involves twin studies which initially failed to show a high concordance rate among monozygotic twins but are now being reconsidered in light of new evidence. In addition, family history appears to be a very strong predictor, after age, for development of the disease. Recently, a number of families in Greece and Italy with a very high penetrance of Parkinson's disease were shown to have a mutation on chromosome 4 for the alpha synuclein gene. This is a presynaptic protein of unknown function but with the potential, upon further study of this muted gene, to provide insights into the pathogenesis of this form of autosomal dominant Parkinson's disease. Another gene abnormality on the long arm of chromosome 6 has been identified in patients with a peculiar autosomal recessive form of young onset disease. The protein product of this gene is named Parkin and seems to promote the degradation of certain neuronal proteins. It is closely related to the ubiquitin family of proteins involved in several neurodegenerative disease states. Research continues at a very high level to identify susceptibility genes and shed additional light on the genetics of Parkinson's disease.

Monoamine oxidase (MAO-A and MAO-B) involvement in PD [11-16]

MAO exists as two is forms with different substrate selectivity's and different distributions in brain and between species. In man, dopamine is largely metabolized by MAO-B although it can also be a substrate for MAO-A. However, dopaminergic in the striatum contain relatively little MAO-B but the A-is form is present. Rather MAO-B is found extensively in glial cells and localized to the outer mitochondrial wall. Under normal physiological conditions, dopamine released into the synapse by impulse flow is rapidly 'inactivated' by the high affinity reuptake process that constitutes the dopamine transporter. In PD, the number of

presynaptic terminals in the striatum is extensively depleted and now MAO-B in surrounding glial cells becomes a major focus for dopamine metabolism. This provides a targeted and disease specific mechanism through which dopamine degradation can be inhibited by the use of selective MAO-B inhibitors. Selegiline and rasagiline are selective MAO-B inhibitors that irreversible inhibit the enzyme by the covalent binding of the propargylamine moiety to the active site in the mitochondrial membrane. The long-lasting inhibition of MAO-B is held responsible for the symptomatic improvement in motor symptoms occurring in monotherapy in early PD and as adjunct therapy to L-dopa and/or dopamine agonist treatment in midland late-stage illness. However, it has been implied that both drugs may also alter the rate of progression of PD through the DATATOP study and subsequent investigations of selegiline and the TEMPO and ADAGIO studies of rasagiline. This raises the question of how such effects might be mediated. Initially, the inhibition of the metabolism of dopamine by MAO-B was thought to lower oxidative stress by preventing the formation of toxic oxygen free radical species. But subsequently the emphasis has evolved to support an action of both selegiline and rasagiline in preventing apoptotic processes leading to cell death through effects at the level of mitochondria. Recent interest has centred on rasagiline actions based on the finding of improved clinical scores with early drug use in the ADAGIO study and the preclinical evidence to support such an effect will now be explored in greater detail.

MATERIALS AND METHODS

Collection and authentication of plant material

Rubia Cordifolia a were collected from local area of Ranga Reddy district Hyderabad. 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.

Extraction

The whole plant of Rubia cordifolia is collected, washed thoroughly to remove impurities, and shade-dried until it attains a constant weight. The dried material is then coarsely powdered and used for extraction. A known quantity of the powdered whole plant is placed in a cellulose thimble and subjected to Soxhlet extraction using a suitable solvent such as ethanol or methanol in a 1:4 ratio (drug: solvent). The apparatus is assembled with a round-bottom flask containing the solvent, a Soxhlet chamber with the thimble, and a condenser on top. The system is heated, allowing the solvent to vaporize, condense, and repeatedly pass through the plant material. This continuous cycle enables efficient extraction of phytoconstituents from Rubia cordifolia. The extraction is carried out for about 6–8 hours or until the solvent in the siphon tube becomes colourless, indicating complete exhaustion of the material. After completion, the extract is collected and concentrated by evaporating the solvent using a rotary evaporator or water bath to obtain a semi-solid crude extract, which is then stored in an airtight container for further photochemical and pharmacological evaluation.

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.

Pharmacological Evaluation

Animal Toxicity Study

Animal toxicity studies are conducted to evaluate the safety profile of a test substance before pharmacological screening. Healthy adult Wistar albino rats (or mice) of either sex are selected and acclimatized under standard laboratory conditions (temperature 25 ± 2°C, relative humidity 45–55%, 12 h light/dark cycle) with free access to food and water. The animals are fasted overnight prior to dosing. The test extract is administered orally in graded doses according to OECD guidelines (commonly OECD 423 or 425 for acute toxicity). A single dose or stepwise increasing doses of the extract are given, and animals are observed individually for the first 4 hours and periodically for 24 hours, then daily for 14 days for any signs of toxicity such as changes in skin, fur, eyes, behavior, tremors, convulsions, salivation, diarrhea, lethargy,

or mortality. Body weight, food and water intake are also recorded. At the end of the study, animals are sacrificed if required and vital organs may be collected for histopathological examination. The results are used to determine the median lethal dose (LD_{50}) or to confirm a safe dose range for further pharmacological studies.

ANIMAL GROUPING

Group I	Normal Control (Vehicle only)
Group II	Disease Control (Scopolamine-induced) 1 mg/kg i.p.
Group III	Standard Drug (Donepezil) 3 mg/kg p.o.
Group IV	Rubia cordifolia Extract (Low dose) 200 mg/kg p.o.
Group V	Rubia cordifolia Extract (High dose) 400 mg/kg p.o.

Morris Water Maze [17]

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 is measured to assess memory retention.

Elevated Plus Maze [18]

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 [19]

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, and 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) [20]

Acetyl cholinesterase 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) - Lipid Per oxidation (TBARS method) [21]

Lipid per oxidation 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 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) [22]

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) [23]

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.

Results

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

Percentage of yield

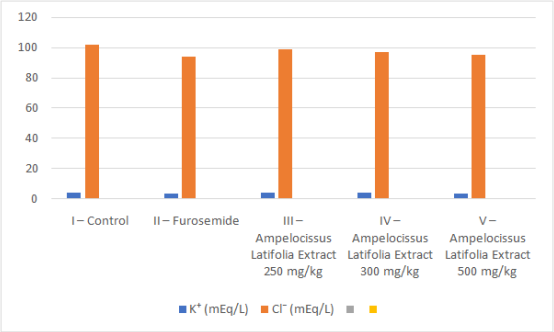
$$Percentage\ of\ yield = \frac{26g}{200g} \times 100 = 13\%$$

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

Morris Water Maze

Group	Treatment	Escape Latency (sec) Day 1	Day 2	Day 3	Probe Trial: Time in Target Quadrant (%)
I Group	Normal Control (Vehicle only)	42.3 ± 2.1	31.5 ± 1.8	22.4 ± 1.5	38.6 ± 2.0
II Group	Disease Control (Scopolamine 1 mg/kg i.p.)	68.7 ± 2.8	62.4 ± 2.5	55.9 ± 2.2	18.3 ± 1.6
IIIGroup	Standard Drug (Donepezil 3 mg/kg p.o.)	55.2 ± 2.4	40.6 ± 2.0	28.7 ± 1.7	32.8 ± 1.9
IV Group	Rubia cordifolia Extract (200 mg/kg p.o.)	60.5 ± 2.6	48.3 ± 2.1	36.9 ± 1.9	27.4 ± 1.7
V Group	Rubia cordifolia Extract (400 mg/kg p.o.)	52.8 ± 2.2	37.9 ± 1.9	25.6 ± 1.6	35.7 ± 2.1

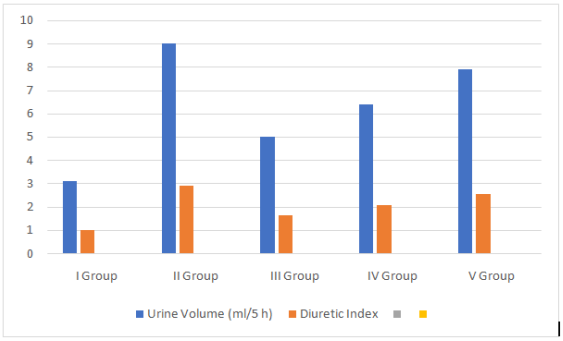
- The scopolamine-induced disease control group showed a significant increase in escape latency and reduced time spent in the target quadrant, indicating impaired learning and memory.
- The standard drug group significantly improved spatial memory performance compared to disease control.
- Both doses of Rubia cordifolia extract showed dose-dependent improvement in learning and memory.
- The high dose (400 mg/kg) demonstrated effects comparable to the standard drug, suggesting potential nootropic activity.



Elevated Plus Maze

Group	Treatment	Time Spent in Open Arms (sec)	Time Spent in Closed Arms (sec)	% Open Arm Entries	Total Entries
Group I	Normal Control (Vehicle only)	95.2 ± 3.4	185.6 ± 4.1	42.8 ± 2.0	12.4 ± 0.8
Group II	Disease Control (Scopolamine 1 mg/kg i.p.)	38.6 ± 2.5	242.3 ± 5.2	18.7 ± 1.5	7.2 ± 0.6
Group III	Standard Drug (Donepezil 3 mg/kg p.o.)	78.4 ± 3.0	202.5 ± 4.3	36.9 ± 1.8	10.8 ± 0.7
Group IV	Rubia cordifolia Extract (200 mg/kg p.o.)	62.1 ± 2.8	220.7 ± 4.6	29.4 ± 1.6	9.3 ± 0.5
Group V	Rubia cordifolia Extract (400 mg/kg p.o.)	84.7 ± 3.2	195.8 ± 4.0	39.6 ± 1.9	11.6 ± 0.8

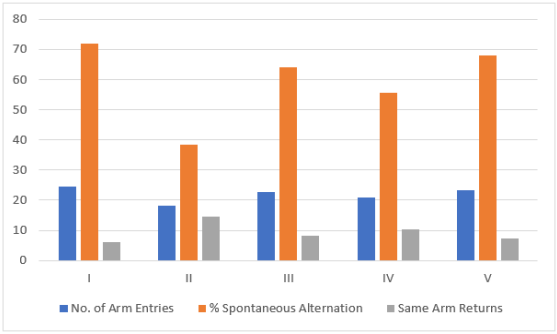
The scopolamine-induced disease control group showed significant anxiety-like behavior, indicated by reduced time and entries in open arms. The standard drug group significantly increased open arm exploration, indicating anxiolytic and cognitive improvement. Rubia cordifolia extract showed dose-dependent improvement in behavior. The 400 mg/kg dose showed effects close to the standard drug, suggesting potential anxiolytic and memory-enhancing activity.



Y-Maze test

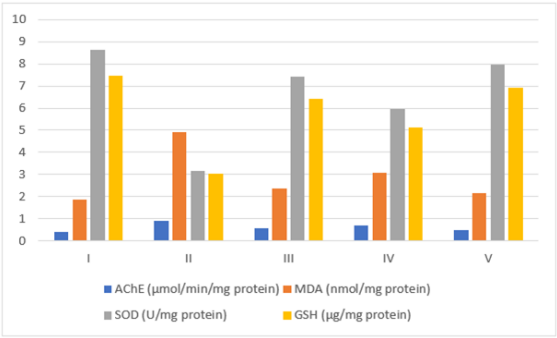
Group	Treatment	No. of Arm Entries	% Spontaneous Alternation	Same Arm Returns
Group I	Normal Control (Vehicle only)	24.6 ± 1.2	71.8 ± 2.4	6.2 ± 0.8
Group II	Disease Control (Scopolamine 1 mg/kg i.p.)	18.3 ± 1.0	38.5 ± 2.1	14.7 ± 1.1
Group III	Standard Drug (Donepezil/ 3 mg/kg p.o.)	22.7 ± 1.1	64.2 ± 2.3	8.1 ± 0.9
Group IV	Rubia cordifolia Extract (200 mg/kg p.o.)	20.9 ± 1.0	55.6 ± 2.0	10.3 ± 0.8
Group V	Rubia cordifolia Extract (400 mg/kg p.o.)	23.4 ± 1.2	67.9 ± 2.5	7.4 ± 0.7

The scopolamine-treated disease control group showed a marked reduction in spontaneous alternation behavior, indicating impaired short-term spatial working memory. The standard drug significantly restored alternation percentage. Rubia cordifolia extract improved memory performance in a dose-dependent manner. The 400 mg/kg dose produced results close to the standard group, suggesting notable nootropic potential.



Group	Treatment	AChE (µmol/min/mg protein)	MDA (nmol/mg protein)	SOD (U/mg protein)	GSH (µg/mg protein)
Group I	Normal Control (Vehicle only)	0.42 ± 0.03	1.85 ± 0.12	8.62 ± 0.40	7.48 ± 0.36
Group II	Disease Control (Scopolamine 1 mg/kg i.p.)	0.91 ± 0.05	4.92 ± 0.20	3.14 ± 0.28	3.02 ± 0.22
Group III	Standard Drug (Donepezil 3 mg/kg p.o.)	0.55 ± 0.04	2.36 ± 0.15	7.42 ± 0.35	6.41 ± 0.30
Group IV	Rubia cordifolia Extract (200 mg/kg p.o.)	0.68 ± 0.04	3.08 ± 0.18	5.96 ± 0.32	5.12 ± 0.27
Group V	Rubia cordifolia Extract (400 mg/kg p.o.)	0.49 ± 0.03	2.14 ± 0.14	7.98 ± 0.38	6.92 ± 0.33

Scopolamine markedly increased AChE and MDA while decreasing SOD and GSH, confirming cholinergic dysfunction and oxidative stress. The standard drug significantly restored antioxidant status and reduced AChE activity.Rubia cordifolia extract produced dose-dependent neuroprotection.The 400 mg/kg dose nearly normalized biochemical parameters, supporting its antioxidant and anti-AChE (nootropic) potential.



DISCUSSION

The present study demonstrates the nootropic potential of Rubia cordifolia against Scopolamine-induced cognitive impairment, as evidenced by improvements across behavioral and biochemical assessments. Scopolamine-treated animals showed marked deficits in spatial learning, memory retention,

and working memory in the Morris Water Maze, Elevated Plus Maze, and Y-Maze tests, along with increased anxiety-like behavior. Treatment with the extract produced dose-dependent enhancement in performance, with the 400 mg/kg dose showing effects comparable to the standard drug (Donepezil). Biochemical analysis further supported these findings, where the extract significantly reduced elevated acetyl cholinesterase (AChE) and malondialdehyde (MDA) levels while restoring antioxidant enzymes superoxide dismutase (SOD) and reduced glutathione (GSH). These outcomes suggest that the cognitive benefits of Rubia cordifolia may be mediated through enhancement of cholinergic neurotransmission and attenuation of oxidative stress, likely due to its rich phytochemical composition, thereby indicating its promising role as a neuroprotective and memory-enhancing agent.

CONCLUSION

This study establishes that the extract of Rubia cordifolia exhibits noteworthy memory-enhancing and neuroprotective effects against Scopolamine-induced cognitive deficits. Performance in the Morris Water Maze, Elevated Plus Maze, and Y-Maze tasks showed significant recovery of learning, memory, and exploratory behavior in animals treated with the extract, with the 400 mg/kg dose producing outcomes comparable to the standard therapy (Donepezil). Biochemical analyses corroborated these findings by demonstrating reduced acetyl cholinesterase and lipid peroxidation levels alongside improved antioxidant defenses (SOD and GSH). Overall, the results suggest that the cognitive benefits of Rubia cordifolia are mediated through enhancement of cholinergic transmission and mitigation of oxidative stress, highlighting its promise as a natural agent for managing memory impairment and related neurodegenerative conditions.

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