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Effect of *Albizia lebbeck* Linn. for Memory Enhancing Activity in Stressed Rats

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Abstract: *Objectives: The present study investigates the potential neuroprotective effects of Albizia lebbeck Linn. on cognitive impairment in a rat model of stress-induced Alzheimer's disease.*
Methods: Alzheimer was experimentally induced in sprague dawley female rats using a chronic restraint stress protocol, where animals were immobilized for 06 hours daily over a 28-day period. The cognitive effects of orally administered Albizia lebbeck Linn. methanolic extract were assessed. Both low and high doses of the authenticated herbal extract were evaluated for their anti-Alzheimer's efficacy using standard behavioral paradigms, including the Conditioned Avoidance Response (CAR), Elevated Plus Maze (EPM), and Morris Water Maze (MWM) tests.
Key Findings: The results of this study demonstrated that oral administration of Albizia lebbeck Linn. extract significantly ($P < 0.01$) reduced escape latency in the Conditioned Avoidance Response (CAR) test and decreased transfer latency in the Elevated Plus Maze (EPM), compared to the negative control group. Additionally, in the Morris Water Maze (MWM) test, treated animals exhibited a marked increase in retention time and a significant reduction in escape latency, indicating improved spatial learning and memory. These behavioral outcomes suggest a notable cognitive enhancement in the extract-treated, stress-induced dementia model.
Conclusions: In summary, Albizia lebbeck Linn. extract effectively ameliorated cognitive deficits and memory impairment in a rat model of stress-induced dementia, highlighting its potential as a therapeutic agent for the management of Alzheimer's disease...

Keywords: Alzheimer, ***Albizia lebbeck* Linn.,** stress, memory, cognitive function, morris water maze (MWM), elevated plus maze (EPM)

I. INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that primarily affects the cerebral cortex, leading to widespread neuronal loss. It is recognized as the most common cause of dementia, clinically characterized by a gradual decline in cognitive abilities, memory impairment and loss of independence in performing daily activities. AD is considered a multifactorial condition with complex pathophysiology. Among the prominent theories proposed to explain its development are the cholinergic hypothesis, which implicates the degeneration of cholinergic neurons and the amyloid hypothesis, which centers on the accumulation of β-amyloid plaques in the brain, (Breijyeh &Karaman 2020).

A hallmark of Alzheimer's disease pathology is the presence of extracellular plaques composed of insoluble β-amyloid peptides (Aβ) and intracellular neurofibrillary tangles (NFTs) formed by hyperphosphorylated tau (P-tau) proteins within neuronal cytoplasm. These neuropathological features contribute significantly to synaptic dysfunction and neuronal death. It is estimated that approximately 70% of the risk for developing AD is genetically determined, highlighting the strong heritable component of the disease, (Silva et. al., 2019).

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II. MATERIAL AND METHOD

Materials

Animals: Healthy sprague dawley female rats of 08-12 weeks,weigh in the range of 200-250 gm (±20).

Apparatus:Cook's pole climbing apparatus, cotton, desiccator; **Glass wares:** test tube, beaker, funnel, measuring cylinder, etc.; heating mantles, needle, soxhlet apparatus, syringe**,** etc.

Chemicals: Methanol, ether, distilled water, etc.

Equipments: Elevated plus maze (EPM), Morris water maze (MWM)

Instrument: Digital weighing balance, etc.

***Plant material: Albizia lebbeck*Linn.** leaves

Figure 01: *Albizia lebbeck* Linn. tree

Figure 02: *Albizia lebbeck* Linn. leaves

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Animals

Healthy female sprague-dawley rats, aged 08 to 12 weeks and weighing between 200 and 250 grams, were selected for the experimental study. The animals were kept in standard laboratory cages made of polyethylene with wire mesh lids and bedding of clean husk. They were housed under controlled environmental conditions following institutional guidelines for animal care and use. The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) under the approval number 650/PO/Re/S-2002/2022/CPCSEA/20.

Methods

Preparation of Extract

Extraction of the plant material was performed using the soxhlet apparatus (as shown in figure 03), a conventional and extensively adopted technique for solid–liquid extraction. This method is commonly employed in pharmaceutical and related research fields for its ability to efficiently extract essential compounds from plant sources. Due to its high reliability and standardization, soxhlet extraction is widely regarded as a reference method in numerous laboratory protocols for isolating oils and phytoconstituents, (Patel et. al., 2019).

Collection, Identification and Authentication

The leaves of ***Albizia lebbeck*** **Linn.** were collected in October 2019 from the Old Antargaon region, situated near Near Karalgaon along Dhamangaon road, in Yavatmal District, Maharashtra, India (BHARAT). The collection site islocated approximately 15 kilometers away from Yavatmal city.

Botanical identification and authentication of the plant specimen were carried out at Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola's Vasantrao Naik College of Agricultural Biotechnology, Waghapur Road, Yavatmal. The specimen was authenticated under reference number VNCABT/Ytl/Hort/1591-A/2019.

Fresh and healthy leaves of the aforementioned plant were carefully collected and thoroughly washed four times with clean water to eliminate surface contaminants such as dirt and microbes. The cleaned leaves were then dried in the shade to prevent the degradation of heat-sensitive phytoconstituents. Once completely dried, the leaves were ground into a fine powder using appropriate mechanical methods.

Storage: The powdered material of leaves was stored in an airtight glass container and utilized for extraction as required, on a batch-wise basis.

Extraction Process

Extraction was carried out using an adequate quantity of the powdered plant material, which was packed into a porous cellulose thimble as described by — (Redfern et. al., 2014). The technique followed was a continuous solid–liquid extraction. The thimble, functioning similarly to filter paper, retained the solid content while allowing only the liquid extract to pass through. The loaded thimble was placed inside the extraction chamber of a soxhlet apparatus. An organic solvent was heated to generate vapors, which rose into the condenser and subsequently condensed into liquid form. This condensed solvent repeatedly filled the thimble, enabling continuous extraction of soluble phytoconstituents from the plant material. The cycle was repeated until the complete extraction was achieved, as outlined by— (Patel et. al., 2019).

Post Extraction Process

Following soxhlet extraction, the obtained extract was concentrated and dried using a water bath maintained at a controlled temperature of 40º C - 45º C. The dried extract was then transferred to a clean, airtight glass container and stored under refrigeration conditions to preserve its stability. Prior to administration, the extract was reconstituted in an appropriate solvent and administered to the animals according to their respective dosage regimens.

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Figure 03: SOXHLET APPARATUS

Drugs and Dose

Rivastigmine was administered as the standard drug at a dose of 02 mg/kg via intraperitoneal (IP) injection, using distilled water as the diluent. Methanolic extract of ***Albizia lebbeck*** **Linn.**leaves was prepared in two dose concentrations—200 mg/kg (low dose) and 400 mg/kg (high dose)—by dissolving the extract in distilled water. All solutions were freshly prepared on each experimental day and administered orally. The required dose for each rat was calculated based on its individual body weight to ensure accurate dosing across all experimental groups.

Induction of Alzheimer

The animals were randomly divided into five groups. Alzheimerwas experimentally induced in rats belonging to Groups II, III, IV and V using a chronic restraint stress model, while Group I (normal control) received saline and was not exposed to stress. For the stress protocol, rats were immobilized using plastic restraint devices (e.g., saline bottles) for 06 consecutive hours daily over a 28-day period, a method known to mimic stress-induced cognitive impairment, (Wesam et.al., 2024).

Group II (Negative Control) received restraint stress without any treatment.

Group III was administered the standard drug, Rivastigmine(02 mg/kg, IP), (Onor et. al., 2007)following stress exposure.

Groups IV and V were treated with low (200 mg/kg) and high (400 mg/kg) doses, respectively, of ***Albizia lebbeck*****Linn.** methanolic extract, administered orally throughout the experimental period.

All experimental groups were evaluated for cognitive performance using a battery of behavioral tests, including the Conditioned Avoidance Response (CAR), Elevated Plus Maze (EPM) and Morris Water Maze (MWM) to assess learning, memory and spatial navigation abilities.

Conditioned Avoidance Response (CAR)

The CAR test was conducted using a pole climbing apparatus (illustrated in figure 04) housed within a sound-attenuated chamber measuring 25 × 25 × 25 cm. The floor of the chamber consisted of a steel grid capable of delivering

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an electric foot shock and a vertical pole (02.5 cm in diameter) was suspended from the center of the chamber ceiling. Each rat was placed inside the chamber and given a 02-minute acclimatization period. A conditioned stimulus (a tone) was presented for 05 seconds, followed by an unconditioned stimulus—a mild foot shock (0.5 mA) delivered through the grid floor for 20 seconds. The animal's escape behavior was assessed by measuring the latency to climb the pole, which was recorded as an indicator of learned avoidance, (Tripathi et. al., 2021).

Figure 04: COOK'S POLE CLIMBING APPARATUS

Elevated Plus Maze (EPM)

The Elevated Plus Mazeequipment (as shown in figure 05) was employed to assess spatial learning and memory. The apparatus consisted of a plus-shaped structure elevated above the floor, comprising two open arms (50 × 10 cm) and two closed arms (50 × 40 × 10 cm). Each rat was placed at the distal end of an open arm, oriented away from the central junction of the maze. The escape latency, defined as the time taken by the animal to enter either of the closed arms, was recorded as a measure of cognitive performance. Following entry into a closed arm, the rat was allowed to remain in the maze for a total of 02 minutes. Each animal underwent five trials per day, with an inter-trial interval of 05 minutes, (Tripathi et. al., 2021).

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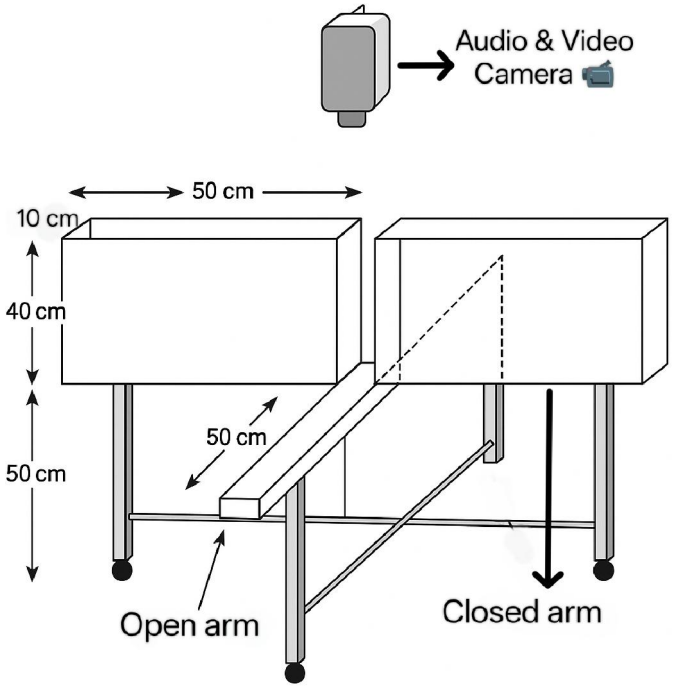

Figure 05: ELEVATED PUS MAZE (Schematic representation)

Morris Water Maze (MWM)

The Morris Water Maze (as shown in figure 06) was employed to evaluate spatial learning and memory in rats. The apparatus consisted of a black circular pool measuring 120 cm in diameter and 50 cm in height, filled with water to a depth of 30 cm and maintained at a temperature of 26 ± 2°C. The pool was conceptually divided into four quadrants: the target quadrant (T), right (R), left (L) and opposite (O) relative to the target. A hidden platform was placed in the target quadrant (T) on Day 01 and remained submerged during testing on Days 02, 03 and 04.

During each trial, rats were released from varying start points and the escape latency—the time taken to locate the submerged platform within 02 minutes—was recorded. Each rat underwent four trials per day, with a 05-minute inter-trial interval, across four consecutive days. On Day 05, the platform was removed to conduct a probe trial, during which the retention time, defined as the total time spent in the target quadrant (T) over a 02-minute period, was recorded as a measure of memory retention, (Tripathi et. al., 2021).

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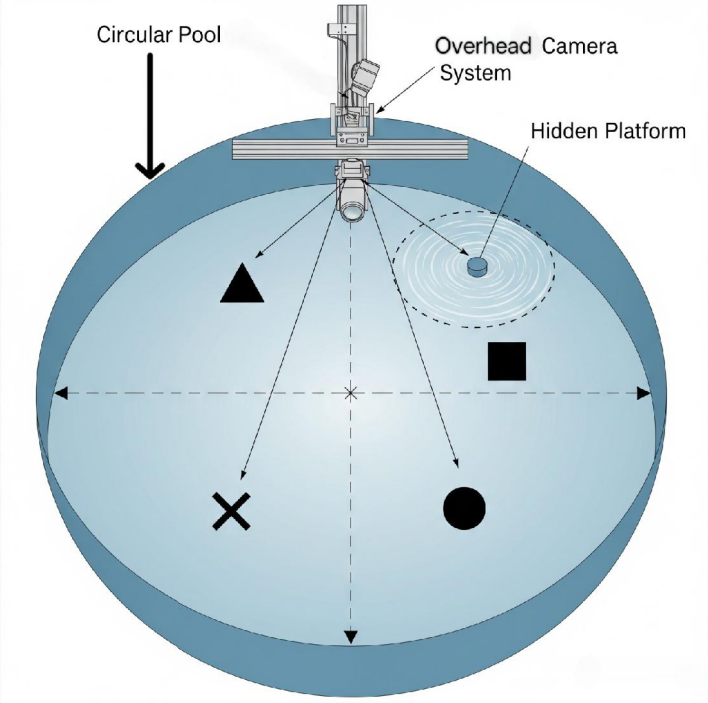

Figure 06: MORRIS WATER MAZE (MWM)

Statistical Analysis

All experimental data were presented as mean ± standard deviation (SD), with a sample size of six animals per group (n = 06). Statistical comparisons among groups were conducted using one-way analysis of variance (ANOVA). Post hoc analysis was performed using Dunnett's test to evaluate differences between the control and treatment groups. A p-value of less than 0.05 was considered statistically significant.

IV. RESULTS

The present study investigated the effect of ***Albizia lebbeck* Linn.** methanolic extract on cognitive performance& memory impairment in a restraint stress-induced Alzheimer's disease model in rats. Behavioral assessment was carried out using three validated models: Conditioned Avoidance Response (CAR), Elevated Plus Maze (EPM) and the Morris Water Maze (MWM). The observations from each test are discussed below.

GROUPS

Group-I - Normal control (Saline)
Group-II - Negative control (Stressed animal)
Group-III - Standard drug – Rivastigmine (02 mg/kg IP)
Group-IV - AL – LD (***Albizia lebbeck*Linn.**low dose 200 mg/kg orally**)**
Group-V - AL – HD (***Albizia lebbeck*Linn.**high dose 400 mg/kg orally**)**

Table 01: Effect of *Albizia lebbeck* Linn. extract on condition avoidance response

Sr. No	Group	Escape Latency (sec.)
	Group-I	0.15±0.01

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	Group-II	05.51±0.11@
	Group-III	0.21±0.01**
	Group-IV	03.21±0.075**
	Group-V	02.21±0.075**

All values are mean ±SD, @p<0.01 compared with control group, **p<0.01 compared with negative control group

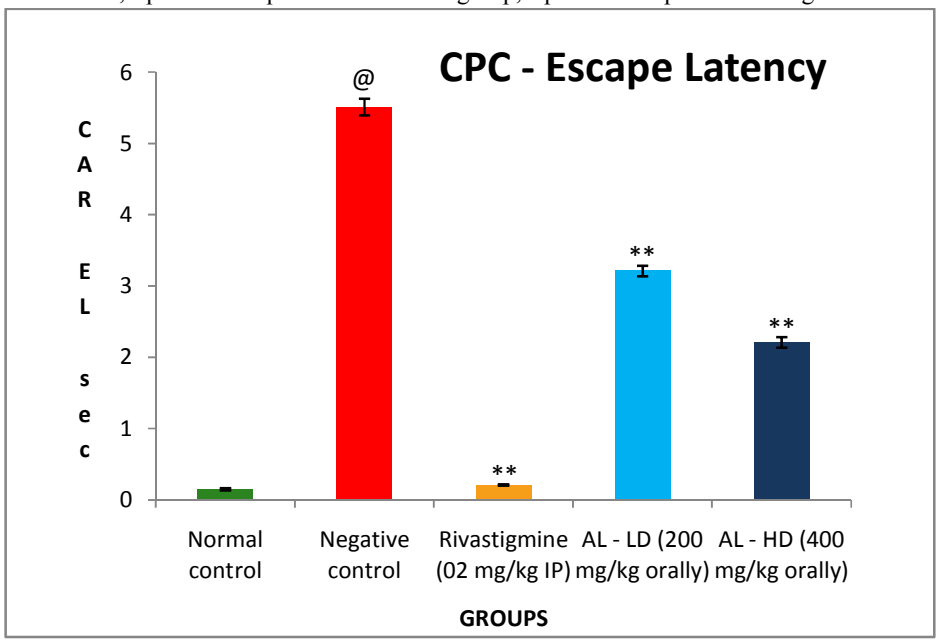

Figure 07: Effect of *Albizia lebbeck* Linn.extract on escape latency in condition avoidance response using cook's pole climbing apparatus in restraint stress induced alzheimer rats

Conditioned Avoidance Response (CAR)

As shown in table 01 and figure 07, rats in Group II (negative control), which were exposed to restraint stress without any treatment, demonstrated a significant increase in escape latency (5.51 ± 0.11 seconds) compared to Group I (normal control), which showed a latency of 0.15 ± 0.01 seconds ($p < 0.01$). This reflects impaired memory and learning ability due to stress-induced neurodegeneration.

Treatment with the standard drug Rivastigmine (Group III) resulted in a substantial decrease in escape latency (0.21 ± 0.01 seconds; $p < 0.01$ vs Group II), indicating notable cognitive improvement. Similarly, administration of ***Albizia lebbeck* Linn.** methanolic extract in low (Group IV) and high (Group V) doses also significantly reduced escape latency (03.21 ± 0.075 seconds and 02.21 ± 0.075 seconds, respectively) compared to the negative control. The high-dose group showed greater improvement, suggesting a dose-dependent neuroprotective effect.

Table 02: Effect of *Albizia lebbeck* Linn.extract on elevated plus maze

Sr. No	Group	Transfer Latency (sec.)
	Group-I	07.05 ± 0.10

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	Group-II	33.41 ± 0.49$^{@}$
	Group-III	08.46 ± 0.05**
	Group-IV	21.26 ± 0.08**
	Group-V	17.58 ± 0.11**

All values are mean ±SD, $^{@}$p<0.01 compared with control group, **p<0.01 compared with negative control group

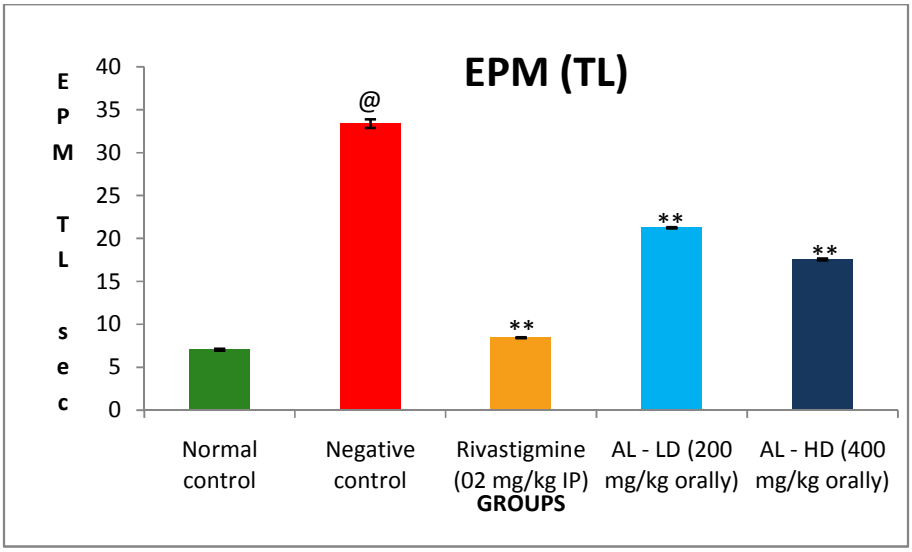

Figure 08: Effect of *Albizia lebbeck* Linn.extracton transfer latency in elevated plus maze equipment in restraint stress induced alzheimer rats

Elevated Plus Maze (EPM)

Table 02 and figure 08 display the data from the EPM test. Group II animals exhibited significantly prolonged transfer latency (33.41 ± 0.49 seconds) compared to Group I (07.05 ± 0.10 seconds; $p < 0.01$), indicating deficits in learning and memory.

Rats treated with Rivastigmine (Group III) showed a marked reduction in latency (08.46 ± 0.05 seconds; $p < 0.01$ vs Group II). Both low and high doses of ***Albizia lebbeck* Linn.** methanolic extract (21.26 ± 0.08 seconds and 17.58 ± 0.11 seconds, respectively) showed statistically significant improvement compared to the negative control, again with stronger effects in the higher dose group.

These results reinforce the plant extract's potential role in restoring spatial memory and learning abilities affected by chronic stress.

Table 03: Effect of *Albizia lebbeck* Linn.extract on morris water maze

Sr. No	Group	Escape Latency (sec.)	Retention time (sec)
	Group-I	08.58±0.43	54.75±0.93
	Group-II	48.16±0.56$^{@}$	18.21±0.19$^{@}$

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	Group-III	12.68±0.13**	49.96±0.71**
	Group-IV	29.63±0.41**	28.18±0.26**
	Group-V	26.18±0.19**	32.01±0.29**

All values are mean ±SD, @p<0.01 compared with control group, **p<0.01 compared with negative control group

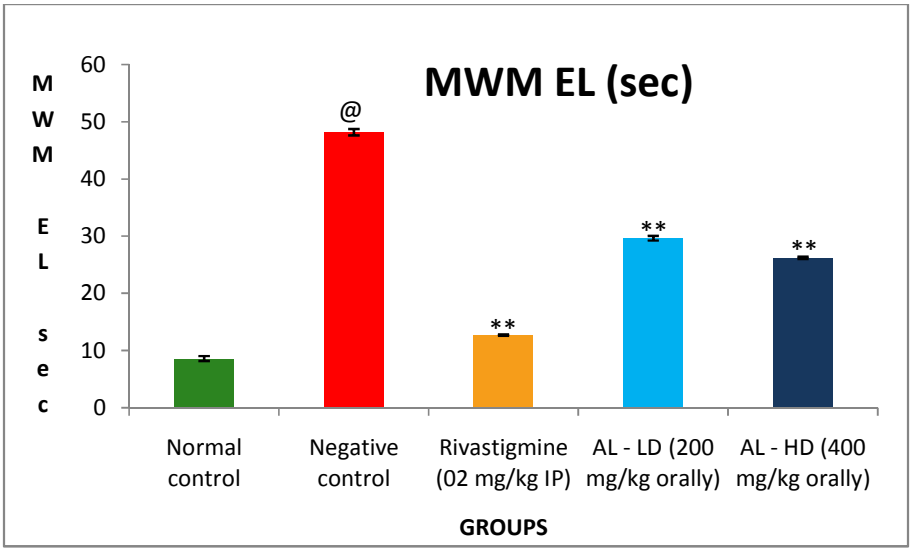

Figure 09: Effect of *Albizia lebbeck* Linn.extract on escape latency in morris water maze equipment in restraint stress induced alzheimer rats

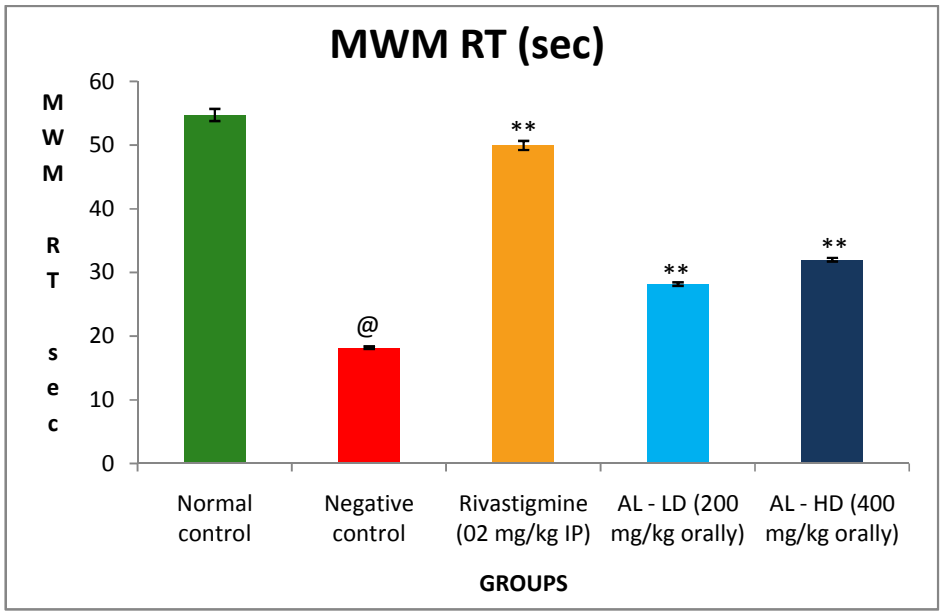

Figure 10: Effect of *Albizia lebbeck* Linn. extracton retention time in morris water maze equipment in restraint stress induced alzheimer rats

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Morris Water Maze (MWM)

The MWM test further supported the cognitive-enhancing effect of ***Albizia lebbeck* Linn.** methanolic extract. According to table 03, figure 09 and figure 10, rats in Group II took significantly longer to locate the hidden platform (escape latency: 48.16 ± 0.56 seconds) and spent less time in the target quadrant (retention time: 18.21 ± 0.19 seconds) compared to Group I (escape latency: 08.58 ± 0.43 seconds; retention time: 54.75 ± 0.93 seconds; $p < 0.01$).

Group III (Rivastigmine-treated) rats showed improved performance with escape latency of 12.68 ± 0.13 seconds and retention time of 49.96 ± 0.71 seconds ($p < 0.01$ vs Group II). Groups IV and V (treated with ***Albizia lebbeck* Linn.** methanolic extract) also showed significant cognitive improvements. Escape latency was reduced to 29.63 ± 0.41 seconds and 26.18 ± 0.19 seconds, while retention time increased to 28.18 ± 0.26 seconds and 32.01 ± 0.29 seconds, respectively.

These findings highlight the potential of ***Albizia lebbeck*Linn.** in mitigating cognitive impairments and promoting memory retention in stress-induced dementia models.

V. DISCUSSION

This study explored the neuroprotective and cognition-enhancing properties of ***Albizia lebbeck* Linn.** methanolic extract in a rat model of restraint stress-induced Alzheimer's disease. The results demonstrated that both low and high doses of the extract significantly improved behavioral outcomes across all three experimental paradigms—Conditioned Avoidance Response (CAR), Elevated Plus Maze (EPM), and Morris Water Maze (MWM).

In the CAR test, a significant decrease in escape latency was observed in extract-treated animals compared to the negative control group, suggesting enhanced associative learning. This behavioral improvement may be attributed to the potential role of ***Albizia lebbeck* Linn.** in modulating synaptic transmission and neural excitability—both of which are adversely affected in Alzheimer's pathology.

The EPM test further confirmed cognitive improvement, as evidenced by a marked reduction in transfer latency. This suggests that the extract not only improves spatial memory but may also exert anxiolytic-like effects, possibly through the regulation of neurochemical systems such as GABAergic or serotonergic pathways. Notably, the high-dose group showed performance approaching that of the standard drug, Rivastigmine, indicating dose-dependent efficacy.

In the MWM test, both escape latency and retention time improved significantly in extract-treated groups. These findings support the idea that ***Albizia lebbeck* Linn**. enhances hippocampal function, which is vital for spatial learning and memory consolidation. The increased time spent in the target quadrant during the probe trial suggests stronger memory retention and spatial orientation ability in treated animals.

Previous phytochemical investigations of ***Albizia lebbeck* Linn.**have identified bioactive compounds such as saponins, flavonoids and tannins, many of which possess antioxidant, anti-inflammatory, and neuroprotective properties. These constituents may contribute to the observed behavioral improvements by mitigating oxidative stress, reducing neuroinflammation, and enhancing cholinergic function—three key contributors to Alzheimer's disease progression.

Importantly, the restraint stress model used in this study effectively induced cognitive impairment, supporting its validity as a model of stress-exacerbated Alzheimer's pathology. Chronic stress is known to dysregulate the hypothalamic–pituitary–adrenal (HPA) axis and elevate corticosterone levels, leading to neuronal atrophy and impaired memory. The reversal of these effects by ***Albizia lebbeck* Linn.** treatment reinforces the extract's therapeutic potential in stress-related cognitive dysfunction.

VI. CONCLUSION

The findings of this study indicate that ***Albizia lebbeck* Linn.** extract exhibits significant memory-enhancing and neuroprotective activity in a restraint stress-induced rat model of Alzheimer's disease. The extract was effective in improving performance in conditioned avoidance, spatial learning, and memory retention tests, demonstrating both preventive and therapeutic potential.

These results support the traditional use of ***Albizia lebbeck* Linn.** in cognitive disorders and highlight its promise as a plant-based intervention for the management of Alzheimer's disease. Further investigations are warranted to elucidate its underlying mechanisms at the molecular level and to evaluate its long-term safety and efficacy in clinical models.

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Author Contributions

Dr. Deepak Suresh Mohale was responsible for the conceptualization, supervision and final review of the manuscript. Miss Prajakta Kishor Bansod carried out the experimental procedures and contributed to the writing and editing of the manuscript.

Funding

This research was conducted without the support of any external funding.

Conflict of Interest

The authors declare no conflict of interest related to this study.

Ethical Approval

The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) under registration number 650/PO/Re/S-2002/2022/CPCSEA/20.

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