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POTENTIAL MEMORY ENHANCING EFFECT OF *CASSIA OCCIDENTALIS* LINN. IN ANIMAL MODEL

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ABSTRACT

The current study investigates the therapeutic potential of ***Cassia occidentalis* Linn.** extract in the management of Alzheimer's disease (AD). Experimental animals were randomly divided into five groups. AD was induced in rats of Groups II, III, IV and V using a restraint stress model, wherein animals were subjected to physical restraint with saline bottles for 06 continuous hours daily over a period of 28 days. Group I served as the normal control and received only saline. Group III was administered the standard drug Rivastigmine (2 mg/kg, intraperitoneally) (Onor et. al., 2007) for 28 days. Groups IV and V received ***Cassia occidentalis* Linn.** methanolic extract orally at doses of 200 mg/kg (low) and 400 mg/kg (high), respectively. Cognitive performance and memory impairment were assessed using the Conditioned Avoidance Response (CAR), Elevated Plus Maze (EPM) and Morris Water Maze (MWM) tests at the end of the experimental protocol. Findings revealed that both low and high doses of Cassia occidentalis Linn. extract significantly ($p < 0.01$) reduced escape latency in the CAR test and transfer latency in the EPM test, when compared to the negative control group (Group II). Additionally, MWM results indicated a notable increase in retention time and a reduction in escape latency in extract-treated groups, demonstrating improved spatial learning and memory. These results suggest that ***Cassia occidentalis* Linn.** methanolic extract effectively ameliorates cognitive deficits and memory impairment in restraint stress-induced Alzheimer's rat models.

KEYWORDS: Alzheimer, stress, ***Cassia occcidentalis* Linn.,** dementia, memory, elevated plus maze (EPM), morris water maze (MWM).

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder primarily marked by significant memory impairment that disrupts daily social and occupational functioning. It represents the most prevalent form of dementia, (Agarwal et. al., 2013). Multiple pathophysiological mechanisms have been implicated in its onset and progression, including the accumulation of amyloid-beta (Aβ) peptides within brain tissues, cytoskeletal abnormalities resulting from the hyperphosphorylation of microtubule-associated tau protein, as well as genetic predisposition, advancing age and environmental influences, (De-Paula et. al., 2012).

Dementia ranks among the foremost causes of disability and dependency on a global scale. Current estimates suggest that over 50 million individuals are affected by this condition, with Alzheimer's disease (AD) accounting for approximately 60–70% of all dementia cases. As the global population continues to age, the number of dementia cases is projected to rise significantly, potentially reaching 131.5 million by the year 2050—particularly impacting low- and middle-income nations. According to the World Health Organization (WHO), the global economic burden of dementia was estimated at approximately USD $818 billion in 2015—equivalent to 01.1% of the worldwide gross domestic product (GDP). This financial impact ranged from 0.2% of GDP in low- and middle-income countries to 1.4% in high-income countries. Given its extensive social, economic and healthcare implications, dementia is recognized as a global public health priority, (Contador et. al., 2024).

MATERIAL AND METHOD

Materials

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

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

Chemicals: Ether, methanol, etc.

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

Instrument: Digital weighing balance, etc.

Plant material: Cassia occcidentalis **Linn.** leaves.

Figure 01: ***Cassia occcidentalis*** **Linn. Leaves.**

Animals

Healthy female sprague dawley rats, aged between 08 and 12 weeks and weighing 200–250 grams, were used for the study. The animals were maintained in standard laboratory conditions, housed in polyethylene cages fitted with wire mesh tops and lined with husk bedding. Environmental parameters such as temperature, humidity and light-dark cycles were regulated as per standard guidelines. All animal handling and experimental protocols were conducted in compliance with ethical norms and were approved by the Institutional Animal Ethics Committee (IAEC) under protocol number 650/PO/Re/S-2002/2022/CPCSEA/20.

METHODS

Preparation of extract

The extraction was carried out using the soxhlet extraction method, a widely recognized and standardized technique for solid–liquid extraction across pharmaceutical, environmental and food science applications. Due to its efficiency and reproducibility, soxhlet extraction remains the preferred protocol for isolating bioactive constituents, particularly oils and phytochemicals, from plant materials. This method is frequently referenced as a benchmark in analytical laboratories, (Patel et. al., 2019).

Collection, Identification, and Authentication

The leaves of ***Cassia occidentalis*** **Linn.** were collected in October 2019 from the Bembla Dam region, located near Babhulgaon in Yavatmal District, Maharashtra, India (BHARAT). The collection site lies approximately 30 kilometers 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-B/2019.

Mature, healthy leaves of the selected plant species were harvested and rinsed multiple times—specifically four washes—with clean water to ensure removal of dust, microbes and other surface impurities. The leaves were then subjected to shade drying to preserve sensitive phytochemical constituents that may degrade under direct heat. Once dried thoroughly, the plant material was ground into a coarse or fine powder using suitable pulverization techniques.

Storage: The resulting powdered material of leaves was stored in airtight glass containers under hygienic conditions and used for extraction as needed, based on batch requirements.

Extraction Process

A sufficient quantity of the powdered plant material was placed into a cellulose thimble, which is designed to retain solids while permitting the passage of liquids

(Redfern et al., 2014). This setup was used in a soxhlet apparatus to carry out a continuous solid–liquid extraction process. The thimble was inserted into the extractor and an organic solvent was heated to initiate boiling. The resulting vapors ascended into the condenser, where they were cooled and converted back to liquid form. This liquid accumulated in the extraction chamber, gradually immersing the plant powder. Once the chamber reached a specific level, the solvent carrying the dissolved phytochemicals siphoned back into the boiling flask. This cycle continued automatically until exhaustive extraction was achieved, (Patel et. al., 2019).

Post Extraction Process

After completion of the soxhlet extraction, the resulting extract was subjected to evaporation on a water bath at a controlled temperature of 40º C - 45º C to concentrate and dry the material. The dried extract was carefully collected and stored in a tightly sealed glass container and kept under refrigerated conditions. Before administration, the extract was appropriately diluted with a suitable solvent and given to the animals based on the required dosage schedule.

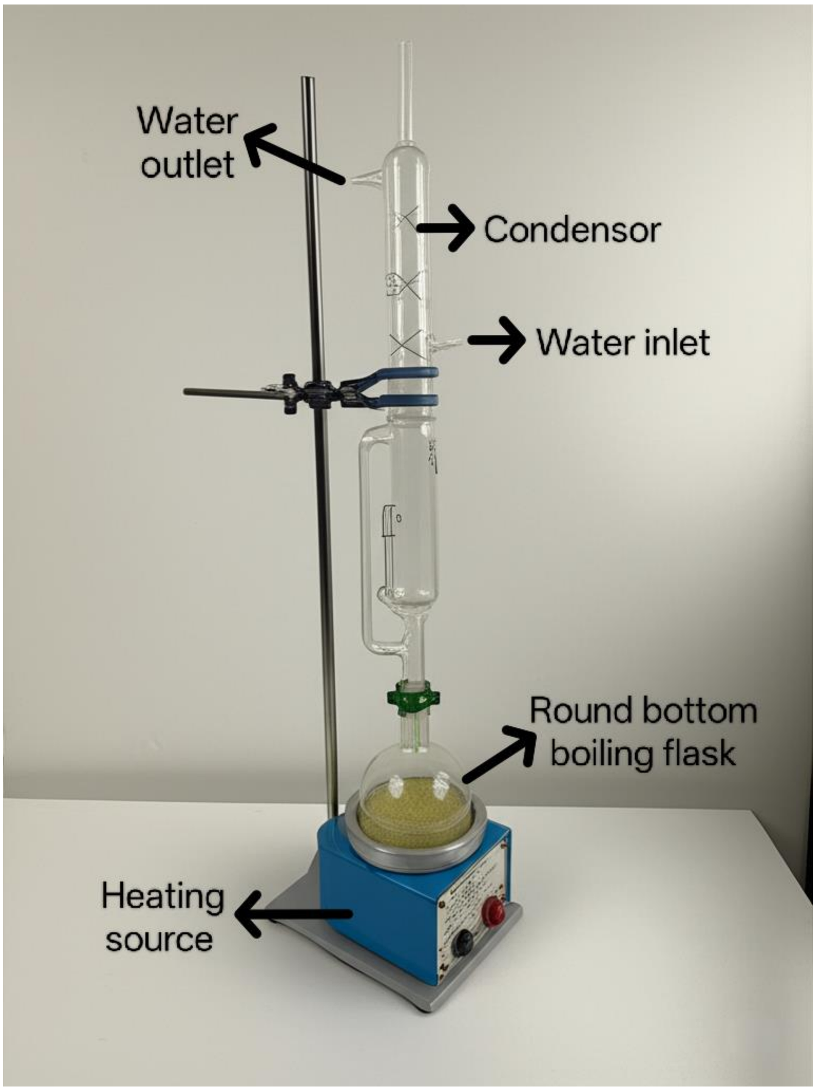

Figure 02: Soxhlet apparatus.

Drugs and Dose

Rivastigmine at a dose of 02 mg/kg (intraperitoneally) was used as the standard reference drug and prepared by dilution in distilled water. For the test groups, two concentrations of ***Cassia occidentalis* Linn.** methanolic leaves extract were prepared: a low dose (200 mg/kg) and a high dose (400 mg/kg), both dissolved in distilled water. All drug solutions were freshly prepared on the day of experimentation and administered orally. Dosages were determined individually based on the body weight of each rat in their respective experimental groups.

Alzheimer's Induction

The animals were randomly assigned into five groups. Alzheimer's-like symptoms were experimentally induced in Groups II, III, IV and V using a chronic restraint stress model, while Group I served as the normal control and received saline without exposure to stress.

To induce stress, rats were immobilized daily using plastic restraint devices (e.g., saline bottles) for 06 continuous hours over a span of 28 days. This method effectively simulates cognitive impairment associated with prolonged stress, (Wesam et. al., 2024).
Group II (Negative Control): Exposed to restraint stress without any therapeutic intervention.
Group III: Treated with the standard drug Rivastigmine (02 mg/kg, IP) (Onor et. al., 2007) following stress exposure.
Groups IV and V: Administered ***Cassia occidentalis* Linn.** methanolic extract orally at doses of 200 mg/kg (low) and 400 mg/kg (high), respectively, throughout the study duration.

Conditioned Avoidance Response (CAR)

The Cook’s pole-climbing apparatus was used to assess conditioned avoidance behavior. The setup consisted of a sound-attenuated experimental chamber measuring 25 × 25 × 25 cm, with a floor composed of stainless steel rods arranged as a grid. A vertical pole (2.5 cm in diameter) was suspended from the center of the chamber ceiling through a designated opening. The apparatus was equipped with switches to activate an auditory stimulus (buzzer) and a foot shock (electric current), as illustrated in the figure 03.

Upon activation, the buzzer emitted a sound for a predefined duration, followed by the delivery of a mild electric shock through the stainless steel grid floor. The interval between the auditory cue and the onset of the shock, as well as the intensity and duration of the current, could be precisely adjusted based on the experimental protocol, (Kamila et. al., 2014).

Protocol

The Conditioned Avoidance Response (CAR) test was conducted using Cook’s pole-climbing apparatus. In this paradigm, a mild foot shock was administered following the presentation of a conditioned stimulus, such as a light or tone. During the training phase, rats were conditioned to associate the stimulus with the impending shock, learning to climb the pole to avoid rather than escape the shock after it had begun. This protocol specifically measured the animal’s ability to anticipate and prevent aversive stimuli, rather than respond reactively after shock onset, (Smith et. al., 2004).

Figure 03: Cook’s Pole Climbing Apparatus.

Testing Protocol

The Conditioned Avoidance Response (CAR) was assessed using a modified version of the method described by Fellow and Cook. Each rat was first placed in the Cook’s pole-climbing apparatus and given a 02-minute acclimatization period (as illustrated in Figure 03). Following acclimation, a buzzer served as the conditioned stimulus and was activated for 05 seconds. After this interval, a mild electric shock (0.5 mA) was delivered for 20 seconds through the stainless steel grid floor to motivate the rat to climb the suspended pole and avoid the aversive stimulus.

Rats were screened for responsiveness during the initial trials and only those demonstrating at least one successful escape response on either day one or two were included in the final study. Each rat received 10 trials per day at 1-minute intervals, repeated across 05 consecutive days, (Marawar et. al., 2014).

Elevated Plus Maze (EPM)

The Elevated Plus Maze apparatus, constructed from wood, consisted of two open arms (50 × 10 cm) with low plexiglass borders (1 cm) to prevent accidental falls and two enclosed arms (50 × 10 × 40 cm). The arms were arranged perpendicularly, with open and closed arms positioned opposite to each other. All arms were connected by a central square platform measuring 10 × 10 cm. The entire structure was elevated 50 cm above the ground, as illustrated in the figure 04. To enhance spatial distinction, the open arms and central platform were painted white, whereas the enclosed arms were painted black, (Hlinák and Krejčí, 2002).

Protocol

Spatial learning was assessed using the Elevated Plus Maze (EPM) learning paradigm, (Kulkarni and Reddy, 1998). The key parameter measured was transfer latency, defined as the time taken by the rat to move from an open arm to one of the enclosed arms. This latency served as an index of learning efficiency.

The experiment leveraged the natural aversion of rodents to open and elevated spaces; animals instinctively seek the safety of enclosed arms for protection. After each trial, the maze was thoroughly cleaned with an alcohol–water solution to eliminate residual olfactory cues that could influence behavior.

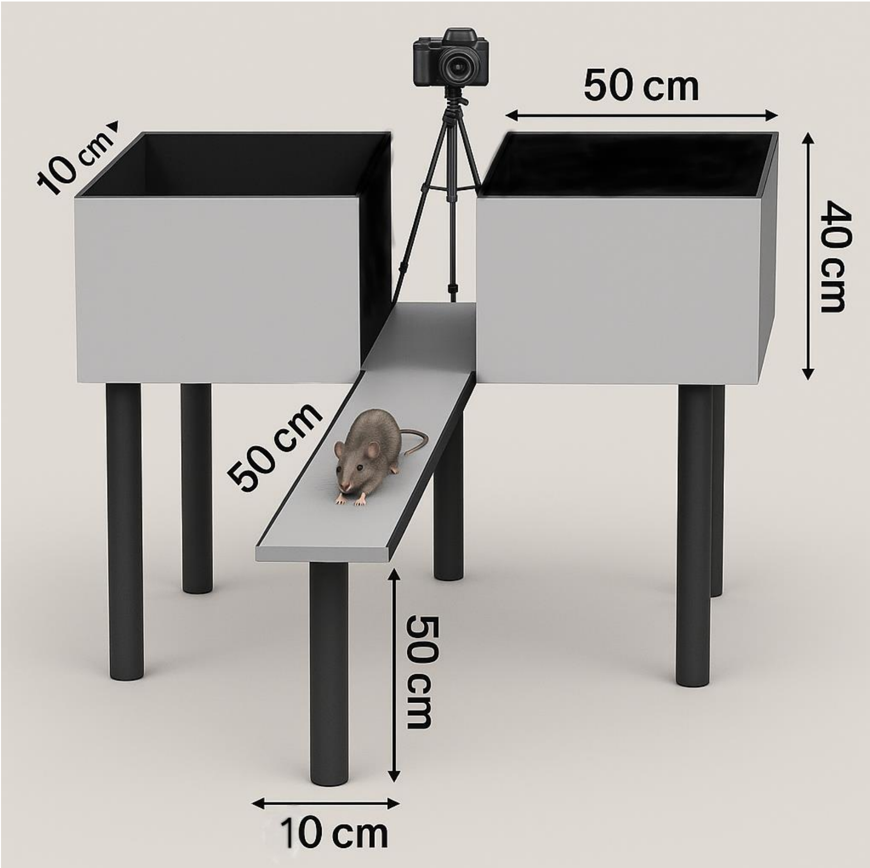

Figure 04: Elevated Plus Maze (EPM).

Testing Protocol

The experimental procedure was adapted from the method of Hlinák and Krejčí, with slight modifications. On Day 01 (acquisition session), each rat was gently placed at the distal end of an open arm of the Elevated Plus Maze, facing away from the central platform. The transfer latency—defined as the time taken to enter one of the enclosed arms—was recorded as an index of learning. Repeated exposure to the open arms during subsequent sessions is known to reduce transfer latency, reflecting acquisition and retention of spatial memory.

An entry into the enclosed arm was considered valid when the rat fully crossed the imaginary boundary between the central platform and the enclosed arm with all four limbs. Animals that failed to enter an enclosed arm within 90 seconds were excluded from the study.

Following entry into an enclosed arm, each rat was allowed to explore the maze freely for a total duration of 02 minutes, after which it was returned to its home cage. A retention session was conducted 24 hours after the acquisition trial, on Days 02, 03, 04 and 05 to assess memory retention. During each retention session, the rat was again placed at the distal end of an open arm, facing away from the central platform and transfer latency was recorded.

All behavioral experiments were carried in a pharmacology research laboratory, under natural lighting conditions, (Hlinák & Krejčí, 2002).

Morris Water Maze (MWM)

The Morris Water Maze apparatus consisted of a large circular black 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 virtually divided into four quadrants: the target quadrant (T), right (R), left (L) and opposite (O) relative to the target, as shown in the figure 05.

Each quadrant was marked with distinct visual cues (symbols) to help the animal orient itself within the pool. A circular black platform (08 cm in diameter) was placed in the target quadrant, submerged 1 cm below the water surface, and remained in a fixed position throughout the experiment. To obscure the platform's location, the water was darkened using a non-toxic black dye. Behavioral data were captured using a video camera mounted directly above the center of the pool and connected to a computer system for tracking and analysis, (Morris, 1984).

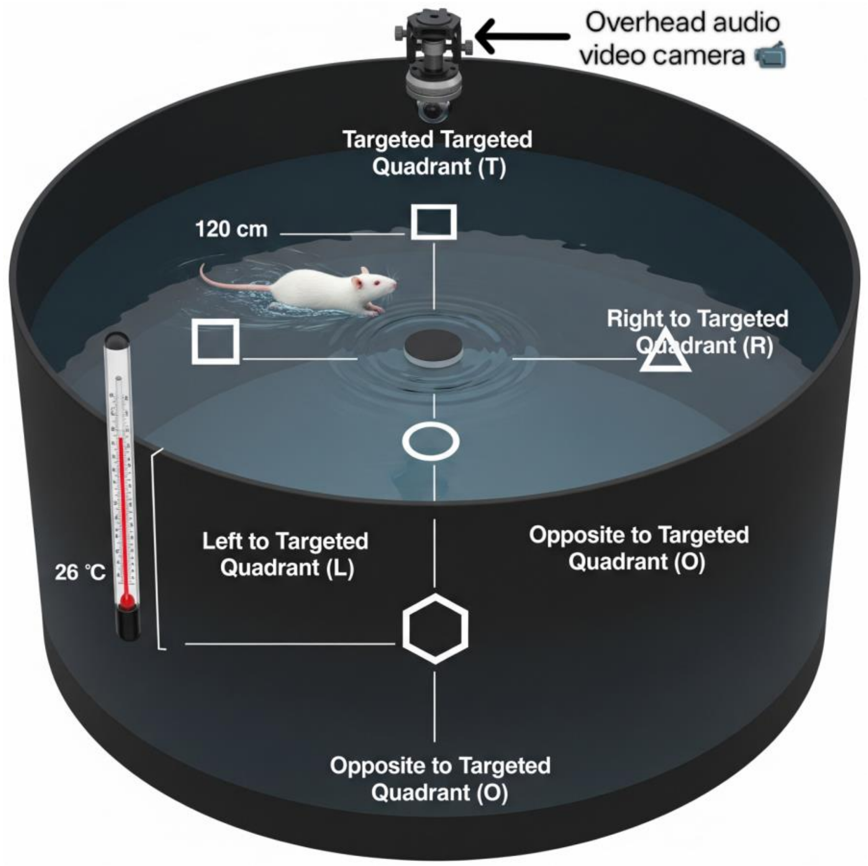

Figure 05: Morris Water Maze (MWM).

Protocol

The Morris Water Maze task was employed to evaluate the spatial learning and memory of rats using distal visual cues positioned around the perimeter of the maze. The testing phase was conducted over four consecutive days, with four trials per day, each separated by a 05-minute interval, (Liu et al., 2002).

To prevent location bias, the release position of each rat was randomly altered for every trial on a given day (Alaei et al., 2007). On Day 01, rats underwent initial training with a visible platform to familiarize them with the task. During the acquisition phase on Days 02, 03 and 04, the platform was submerged below the water surface, requiring the rats to rely on spatial cues to locate it, (Liu et. al., 2002).

The hidden platform version of the Morris Water Maze primarily assesses spatial memory and is particularly sensitive to hippocampal dysfunction, whereas the visible platform variant evaluates non-spatial learning and is more susceptible to disruption by dorsal striatum lesions, (McDonald & White, 1994).

Escape latency—defined as the total time taken by a rat to locate the hidden platform within 02 minutes—was used as a measure of learning performance. Rats that were unable to swim were excluded from the study. In cases where a rat was able to swim but failed to locate the platform within the allotted time, it was gently guided to the platform by the experimenter and allowed to remain there for 01 minute to facilitate learning.

A progressive reduction in escape latency across acquisition trials was interpreted as an indicator of spatial learning. On Day 05, a probe trial was conducted to assess memory retention and cognitive flexibility. In this reversal learning task, the platform was removed and the retention time—the total time spent in the target quadrant over 02 minutes—was recorded, (Liu et. al., 2002).

Acquisition Phase (Training Phase)

Day 01: Each rat was introduced into the pool from the apex of the target quadrant, facing the center. A visible platform was used to train the animal to locate it using external cues. Escape latency (time taken to reach the platform) was recorded. After each trial, the rat was gently dried with a soft towel and returned to its home cage. A total of four trials were conducted per rat with a 05-minute inter-trial interval and the starting position was varied randomly for each trial to minimize positional bias.

Days 02, 03 and 04: The platform was submerged 01 cm below the water surface, making it invisible. The training procedure was repeated as on Day 01, maintaining consistency in trial number, inter-trial intervals and randomization of release points.

Retention Phase (Probe Trial / Reversal Task)

Day 05: The platform was removed from the pool to assess memory retention and spatial learning flexibility. Each rat was released from the apex of the target quadrant and retention time—the total time spent in the quadrant where the platform had previously been located—was recorded over a 02-minute period.

RESULTS

This study evaluated the neuroprotective and cognitive-enhancing effects of ***Cassia occidentalis* Linn.** methanolic extract in a restraint stress-induced Alzheimer's disease rat model. Cognitive function was assessed using three behavioral models: Conditioned Avoidance Response (CAR), Elevated Plus Maze (EPM) and the Morris Water Maze (MWM). The data obtained 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 - CO – LD (***Cassia occidentalis* Linn.** low dose 200 mg/kg orally**)**
Group-V - CO – HD (***Cassia occidentalis* Linn.** high dose 400 mg/kg orally**)**

Table 01: Effect of *Cassia occidentalis* Linn. extract on condition avoidance response.

Sr. No	Group	Escape Latency (sec.)
1)	Group-I	0.15±0.01
2)	Group-II	05.51±0.11$^{@}$
3)	Group-III	0.21±0.01**
4)	Group-IV	01.98±0.075**
5)	Group-V	01.5±0.063**

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

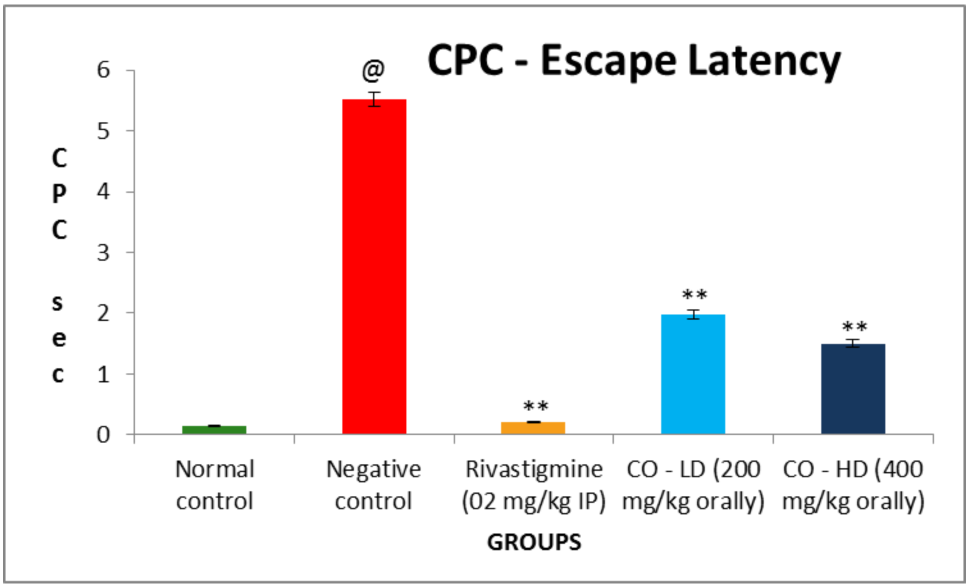

Figure 06: Effect of *Cassia occidentalis* Linn. extract on escape latency in condition avoidance response using cook's pole climbing apparatus in restraint stress induced alzheimer rats.

Conditioned Avoidance Response (CAR)

As shown in table 01 and figure 06, the negative control group (Group II), subjected to chronic restraint stress without treatment, exhibited a significant increase in escape latency (05.51 ± 0.11 seconds) compared to the normal control group (Group I: 0.15 ± 0.01 seconds; p < 0.01). This suggests severe impairment in learning and memory functions due to stress-induced cognitive decline.

Group III, treated with the standard drug Rivastigmine, showed considerable reduction in escape latency (0.21 ± 0.01 seconds; p < 0.01 vs Group II), indicating restored memory performance. Treatment with ***Cassia occidentalis* Linn.** methanolic extract in both low

(Group IV: 01.98 ± 0.075 seconds) and high doses (Group V: 01.5 ± 0.063 seconds) also produced significant reductions in escape latency compared to the negative control. The higher dose demonstrated a greater effect, highlighting the dose-dependent neuroprotective potential of the extract.

Table 02: Effect of *Cassia occidentalis Linn.* extract on elevated plus maze.

Sr. No	Group	Transfer Latency (sec.)
1)	Group-I	07.05 ± 0.10
2)	Group-II	33.41 ± 0.49$^{@}$
3)	Group-III	08.46 ± 0.05**
4)	Group-IV	15.11 ± 0.14**
5)	Group-V	13.81 ± 0.07**

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

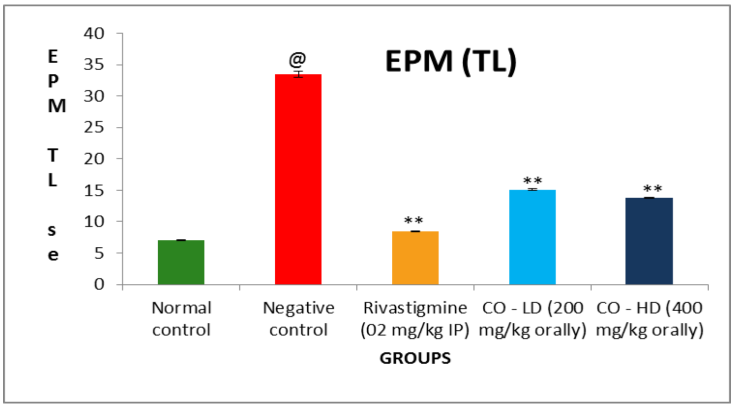

Figure 07: Effect of *Cassia occidentalis* Linn. extract on transfer latency in elevated plus maze equipment in restraint stress induced alzheimer rats.

Elevated Plus Maze (EPM)

As presented in table 02 and figure 07, rats in Group II displayed significantly prolonged transfer latency (33.41 ± 0.49 seconds) compared to Group I (07.05 ± 0.10 seconds; p < 0.01), reflecting impairment in spatial learning.

Administration of Rivastigmine (Group III) led to a sharp decrease in transfer latency (08.46 ± 0.05 seconds), which was statistically significant (p < 0.01 vs Group II).

The ***Cassia occidentalis* Linn.** methanolic extract - treated groups also showed marked improvements. Group IV (15.11 ± 0.14 seconds) and Group V (13.81 ± 0.07 seconds) demonstrated significant reductions in latency (p < 0.01 vs Group II), with Group V again showing better performance.

These outcomes indicate that the extract enhanced learning and memory abilities compromised by chronic stress.

Table 03: Effect of *Cassia occidentalis* Linn. extract on morris water maze.

Sr. No	Group	Escape Latency (sec.)	Retention time (sec)
1)	Group-I	08.58±0.43	54.75±0.93
2)	Group-II	48.16±0.56$^{@}$	18.21±0.19$^{@}$
3)	Group-III	12.68±0.13**	49.96±0.71**
4)	Group-IV	23.6±0.26**	34.7±0.41**
5)	Group-V	20.33±0.28**	37.91±0.24**

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

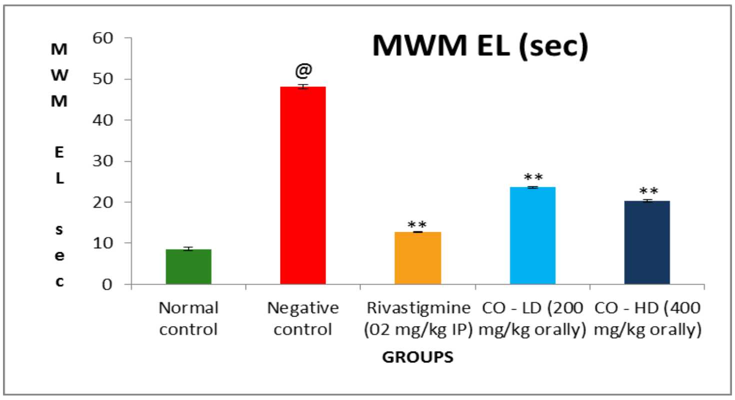

Figure 08: Effect of *Cassia occidentalis* Linn. extract on escape latency in morris water maze equipment in restraint stress induced alzheimer rats.

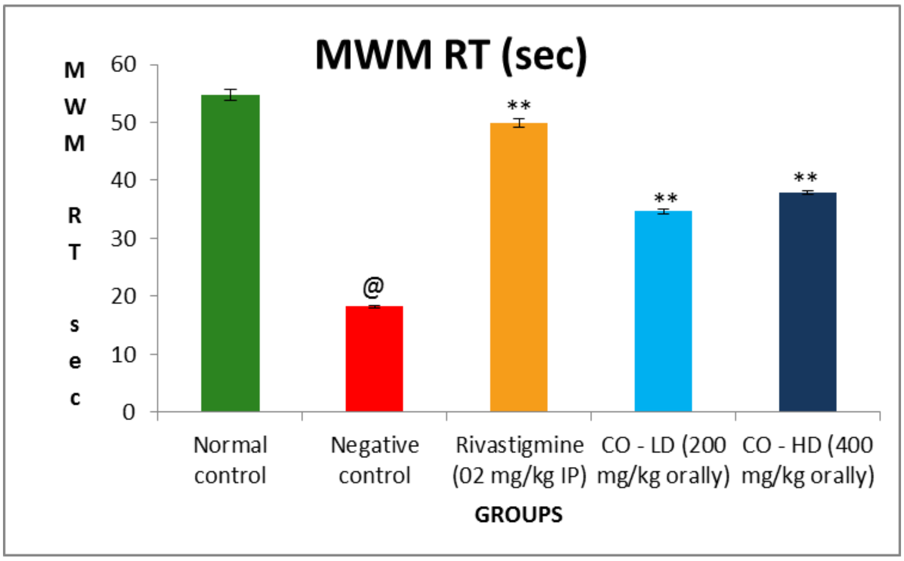

Figure 09: Effect of *Cassia occidentalis* Linn. extract on retention time in morris water maze equipment in restraint stress induced alzheimer rats.

Morris Water Maze (MWM)

Table 03 along with figures 08 and 09 provide the results from the MWM test. In the negative control group (Group II), escape latency was significantly prolonged (48.16 ± 0.56 seconds) and retention time was reduced (18.21 ± 0.19 seconds) compared to the normal control (Group I: 08.58 ± 0.43 seconds for escape latency; 54.75 ± 0.93 seconds for retention time; $p < 0.01$). These changes suggest impaired memory acquisition and poor retention.

Treatment with Rivastigmine (Group III) led to significant improvement in both parameters (escape latency: 12.68 ± 0.13 seconds; retention time: 49.96 ± 0.71 seconds; $p < 0.01$ vs Group II). Similarly, treatment with ***Cassia occidentalis* Linn.** methanolic extract resulted in enhanced memory performance. Group IV (escape latency: 23.6 ± 0.26 seconds; retention time: 34.7 ± 0.41 seconds) and Group V (escape latency: 20.33 ± 0.28 seconds; retention time: 37.91 ± 0.24 seconds) both showed statistically significant improvements compared to Group II. These findings suggest that ***Cassia occidentalis* Linn.** methanolic extract improved spatial memory and recall, with the higher dose yielding more prominent results.

Overall, the behavioral results support the therapeutic potential of ***Cassia occidentalis* Linn.** methanolic extract in improving cognitive functions and alleviating memory deficits in stress-induced Alzheimer's rat models.

DISCUSSION

The present study evaluated the neuroprotective and cognitive-enhancing potential of ***Cassia occidentalis* Linn.** methanolic extract in a restraint stress-induced Alzheimer's disease (AD) model in rats. Behavioral assessments were conducted using validated paradigms—Conditioned Avoidance Response (CAR), Elevated Plus Maze (EPM), and Morris Water Maze (MWM)—to determine learning ability, memory retention, and spatial navigation.

In the CAR test, extract-treated groups (IV and V) exhibited a significant reduction in escape latency compared to the negative control (Group II), indicating improved conditioned learning. These findings suggest that ***Cassia occidentalis*** **Linn.** may facilitate associative learning processes possibly by modulating stress-related neurotransmission pathways, such as glutamatergic or cholinergic signaling.

Similarly, the EPM results demonstrated a marked decrease in transfer latency in the extract-treated groups, with the high-dose group (400 mg/kg) showing results comparable to the standard drug Rivastigmine. The reduction in transfer latency reflects improved spatial memory and reduced anxiety-like behavior—two cognitive domains severely impaired in AD. The anxiolytic-like activity of ***Cassia occidentalis*** **Linn.** may be attributed to its known phytoconstituents like flavonoids, alkaloids, and phenolic compounds, which possess antioxidant and anti-inflammatory properties.

MWM findings further supported the efficacy of the extract, as evidenced by shorter escape latencies and prolonged retention times in treated groups. These observations indicate enhanced hippocampal-dependent spatial learning and memory consolidation. Notably, the reversal task conducted on day 05 confirmed the extract's role in facilitating memory retention and cognitive flexibility.

Taken together, the results align with previous literature suggesting the beneficial effects of ***Cassia occidentalis*** **Linn.** in neurodegenerative and oxidative stress-related models. Its action may be mediated through multiple mechanisms, including attenuation of oxidative stress, modulation of the hypothalamic–pituitary–adrenal (HPA) axis, and enhancement of cholinergic neurotransmission—each of which plays a crucial role in the pathophysiology of AD.

The use of restraint stress as an Alzheimer's model in this study is justified by its capacity to mimic the chronic psychological stress known to exacerbate amyloidogenesis, tau hyperphosphorylation, and hippocampal neurodegeneration. The model also closely replicates the behavioral and cognitive deficits observed in early-stage AD, thereby offering translational relevance to human pathology.

CONCLUSION

This study provides compelling evidence that ***Cassia occidentalis*** **Linn.** methanolic extract significantly improves cognitive function and memory performance in a rat model of stress-induced Alzheimer's disease. The extract's efficacy was comparable to that of Rivastigmine, the standard cholinesterase inhibitor, particularly at the higher dose (400 mg/kg). Behavioral outcomes from CAR, EPM, and MWM tests support the extract's potential neuroprotective role.

Given its promising effects, ***Cassia occidentalis*** **Linn.** may serve as a potential candidate for the development of plant-based therapeutic interventions in the management of Alzheimer's disease. However, further studies involving molecular characterization, dose optimization and long-term safety profiling are warranted to fully elucidate its mechanisms and clinical applicability.

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Author Contributions

Dr. Deepak Suresh Mohale conceptualized the study, supervised the research activities and contributed to the final editing of the manuscript. Miss Prajakta Kishor Bansod conducted the experimental work, prepared the initial draft and participated in manuscript revision.

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Conflict of Interest

The authors declare that there is no conflict of interest associated with this research work.

Ethical Approval

All animal experiments were performed in accordance with the ethical standards and were approved by the Institutional Animal Ethics Committee (IAEC) under protocol number 650/PO/Re/S-2002/2022/CPCSEA/20.

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