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ARTICLE

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An experimental study in male Wister rats: Impact of AILE in preventing ketamine induced spatial memory impairment in MWM and RAM

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ABSTRACT: ***Background:*** Memory impairment involves a decline in cognitive functions such as memory, thinking, behavior, and the ability to perform daily tasks. ***Objective:*** This study aimed to evaluate the effects of AILE on ketamine-induced memory impairment in male Wistar rats in Morris Water Maze (MWM) and Radial Arm Maze (RAM) tests. ***Methods:*** This experimental study was carried out in the Department of Physiology, BSMMU. The rats were divided into three groups: Group 1 (G1) normal memory, Group 2 (G2), memory impaired, Group 3 (G3) experimental. Each group was further divided into subgroups based on memory performance tests using the RAM and MWM. Data were expressed as mean±SEM and analyzed using SPSS (version 16). ANOVA with Bonferroni post hoc tests and Student's paired t-tests were applied, with $p \leq 0.05$ considered statistically significant. ***Results:*** Ketamine-treated rats demonstrated significantly increased working memory errors ($p \leq 0.001$) and reference memory errors ($p \leq 0.001$) in the RAM, along with delayed escape latency ($p \leq 0.001$) and fewer target crossings ($p \leq 0.001$) in the MWM compared to normal rats. Pretreatment with AILE significantly reduced working memory errors ($p \leq 0.001$) and reference memory errors ($p \leq 0.001$) in the RAM and improved escape latency ($p \leq 0.001$) and target crossings ($p \leq 0.001$) in the MWM relative to ketamine-treated rats. Notably, memory performance variables in AILE-pretreated rats were comparable to normal rats, except for a significantly higher frequency of target crossings ($p \leq 0.05$) in the MWM. **Conclusion:** The results suggested that ketamine significantly impairs spatial learning and memory, as indicated by increased errors in the RAM and poorer performance in the MWM.

Keywords: Animal Models, Memory Impairment, Ketamine, Working Memory, Reference Memory.

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INTRODUCTION

Recent studies in psychology, physiology, and anatomy suggest that memory is becoming increasingly specialized. Cognitive processes are an essential part of our daily activities, with memory being a key component in learning. Various types of memory play a crucial role in both formal and informal learning environments [1]. It encompasses the brain's ability to retain and retrieve

information. Memory impairment, often a hallmark of dementia, has become a pressing global challenge. According to the WHO, an estimated 57.6 million people worldwide had dementia in 2021, with a higher prevalence in low- and middle-income countries (LMICs) [2]. However, compared to high-income countries (HICs), data on dementia in LMICs, including Bangladesh, remains limited and often incomplete. The national prevalence of dementia in Bangladesh is significant, with nearly one in twelve individuals aged 60 and above affected. Key risk factors contribute to its occurrence in the older population. Projections indicate that by 2051, the number of dementia cases will nearly double, reaching approximately 3.4 million [3].

Memory can be categorized based on storage duration and the nature of stored information [4]. Based on storage duration, memory is classified into short-term, intermediate, and long-term memory [5]. Short-term memory, often termed "working memory," is a temporary system for holding and manipulating information needed for cognitive tasks like reasoning and comprehension [6]. Conversely, long-term memory, also known as "reference memory," encompasses declarative memory for spatial and contextual information. It plays a crucial role in remembering consistent procedures and environmental rules, often resistant to interference and reinforced by repetition [7]. Mechanistically, working memory relies on persistent neural firing, while reference memory involves long-term synaptic changes such as long-term potentiation (LTP) [8]. The N-methyl-D-aspartate (NMDA) receptor, an ionotropic glutamate receptor, is pivotal in memory processes. Its activation by glutamate and glycine facilitates calcium and sodium influx into neurons, essential for synaptic plasticity [9] However, blocking NMDA receptors impairs learning and memory by inhibiting LTP [10]. Ketamine, a non-competitive NMDA receptor antagonist, is widely used in memory impairment models. At sub-anesthetic doses (e.g., 15 mg/kg), ketamine impairs working and reference memory acquisition and retrieval without inducing anesthesia [11]. To evaluate memory impairment, various experimental tools have been employed, including the RAM and MWM. These tests assess spatial working and reference memory using the animal's ability to navigate based on environmental cues [12]. The RAM evaluates the geographical relationship between the maze and external cues, while the MWM relies on spatial markers to locate a hidden platform, both of which are strongly correlated with hippocampal synaptic plasticity and NMDA receptor function.

Despite advancements in pharmacological treatments for memory impairment, existing drugs such as cholinesterase inhibitors and memantine often exhibit limited long-term efficacy and significant adverse effects, creating a need for alternative therapies [13]. Non-pharmacological approaches, including exercise, mental stimulation, and enriched diets, have shown promise [14]. Among potential alternatives, AILE has gained attention for its wide-ranging medicinal properties, including antioxidant, anti-inflammatory, and neuroprotective effects. Previous studies have shown that AILE reverses working and reference memory deficits in animal models of memory impairment, likely through its antioxidant activity, as evidenced by decreased malondialdehyde (MDA) levels and increased superoxide dismutase (SOD) levels [15,16]. While numerous studies have explored AILE's therapeutic potential, limited research has investigated its effects on memory and the role of NMDA receptors in this process. Previous findings suggest that AILE may mitigate memory deficits associated with neurodegenerative conditions like Alzheimer's disease through its antioxidant properties. However, the precise mechanisms remain underexplored, particularly their interaction with NMDA receptor-mediated pathways. This study aims to address this gap by evaluating the effects of AILE (300 mg/kg/day) on ketamine-induced memory impairment in male Wistar rats and examining the role of NMDA receptors in its action. The findings could provide valuable insights into the therapeutic potential of AILE as a novel intervention for memory impairment.

OBJECTIVE

The objective of the study is to evaluate the role of AILE in preventing ketamine-induced spatial memory impairment in Wistar rats, using the MWM and RAM tests.

METHODS

Study Design and Location

This experimental study was conducted from March 2020 to February 2021 in the Memory Laboratory, Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh.

Study Population and Sample Size

Fifty-four male Wistar rats weighing 200 ± 50 g were used, sourced from the central animal house, BSMMU. The sample size was calculated using a formula based on effect size, with 18 rats allocated to each treatment group and 10:8 rats assigned to both RAM: MWM test per group.

Experimental Design

Rats were divided into three groups, with further subdivisions based on the memory performance tests:

Table 1: Experimental Design of the Study Group

Group	Treatment	Number of Rats
Group1(Normal Memory) G1	Normal saline (5 ml/kg) for 26 days, RAM test	10
	Normal saline (5 ml/kg) for 26 days, MWM test	8
Group2(Memory impaired) G2	Ketamine (15 mg/kg, i.p.) during acquisition phase, RAM test	10
	Ketamine (15 mg/kg, i.p.) during working memory test and acquisition phase, MWM test	8
Group 3 (Experimental) G3	AILE (300 mg/kg/day) orally for 26 days + Ketamine (15 mg/kg, i.p.), RAM test	10
	AILE (300 mg/kg/day) orally for 26 days + Ketamine (15 mg/kg, i.p.) during specified phases, MWM test	8

Drugs and Supplementation

Azadirachta indica Leaf Extract (AILE): 300 mg/kg, orally [15]
Ketamine: 15 mg/kg, intraperitoneally.[17]
Normal saline: 5 ml/kg, orally.

Extraction of AILE

The fresh leaves of *Azadirachta* indica extract collected from Bangladesh Agricultural University (BAU), Mymenshing and identified by an expert taxonomist. Fresh green leaves of *A.Indica* were washed and diseased/dried leaves were discarded. The clean leaves were shade-dried for 3 days. The dried leaves were crushed and soaked in double distilled water in a 1:4 ratio for 3 days. The mixture was then filtered using Whatman No.1 filter paper. The filtrate was heat-evaporated to remove water and concentrate the extract. The concentrated extract was stored in a refrigerator until use. It was filtered and the filtrate was concentrated over a water bath to obtain solidified extract.

Study Procedure for RAM and MWM Tests

General Procedure

A total of 54 rats were randomly assigned for both memories performing test. Each experimental group (normal saline, ketamine, and AILE + ketamine) underwent either test. Rats were acclimatized to the experimental setup for seven days. The tests were conducted in three distinct phases to assess working and reference memory performance: habituation, acquisition, and retention (for RAM) or probe trial (for MWM).

Radial Arm Maze Test

Apparatus

The RAM consisted of a central octagonal platform (42 cm diameter) with eight equidistant arms (60 cm long, 17 cm wide, 25 cm high). Each arm contained a recessed food cup (2 cm deep, 3 cm diameter) located 4 cm from its end. Transparent plexiglass guillotine doors separated each arm from the central platform and were remotely operated via a pulley system to control access. The maze was situated in a well-lit room containing extra-maze visual cues, such as shelves, a desktop, an air conditioner, and an almirah, aiding spatial orientation [18,19].

Procedure

Test was performed according to the methods of previous researches [18,19]. Each trial began 30 minutes after the rats received their assigned treatment (normal

saline, ketamine, or AILE + ketamine).

Habituation Phase (6 Days: Days 16–21)

Days 16–17: Paired rats explored the maze for 10 minutes with food scattered across the platform and arms.

Days 18–19: Individual access to the maze was provided, with food baited only in the cups at the ends of all eight arms.

Days 20–21: Four randomly selected arms were baited, and all eight doors remained open for free exploration.

Acquisition Phase (5 Days: Days 22–26)

Four predetermined arms were baited with food. The rat was placed at the maze center, and all gates were opened. Upon entry into an arm, the rat consumed the food, exited the arm, and the gate was closed. After a 5-second delay, all gates were reopened for the next choice.

Trials lasted 10 minutes or until the rat retrieved all baited rewards. Each day, rats underwent two trials, separated by a 3-hour interval.

Retention Phase (7 Days: Days 27–33)

Following acquisition, rats were kept in home cages without maze exposure for six days but continued receiving their assigned treatment.

On Day 33, retention was tested in two trials using the same procedure as in the acquisition phase.

Morris Water Maze Tests

Apparatus

Morris Water Maze [20,21] was a circular pool, 150 cm in diameter and 50 cm high that was filled with water to a depth of 30 cm with 24℃-26℃s water. It was arbitrarily divided into north-west (NW), north-east (NE), south-east (SE) and south- west (SW) quadrants. A black platform of 28 cm height was placed in the center of any quadrant. The whole inner wall of the pool and platform was painted black to avoid visual cue in the pool. The platform was the only escape place from the water. Eight start locations in the pool for rat were labeled as north-west (NW), north-east (NE), south-east (SE), south- west (SW), south (S), north (N), east (E) and west (W). MWM test was conducted in a well-illuminated room which contain numerous extra maze cues such as rack, window, door, shelve, computer, camera, experimenters etc.

Procedure

Test was performed according to the methods of previous researches [20,21]. Eight (8) rats from each group (total 24) were separated for MWM test and were room acclimatized for 7 days. This test was divided into reference memory rest and working memory test. Total duration of MWM test was 33 days. Every day each rat was brought into the memory lab for reference and working memory test. The initial trial was started 30 minutes after administering the prefixed treatment based on their group assignment.

Reference memory test

Habituation Phase (Days 19–21)

Each rat was introduced to the water pool for 3 minutes daily over three consecutive days without the escape platform. This allowed the rats to familiarize themselves with the pool environment.

Acquisition Phase (Days 22–27)

During the acquisition phase, each rat underwent four swimming trials daily for six consecutive days. The platform was submerged 2 cm below the water surface and placed in a specific quadrant, consistent across all trials for each day. The starting locations for each trial were randomized. For the first trial on Day 22, the platform was placed in the NE quadrant, and the rat was released into the pool from the SW quadrant, facing the pool wall. The rat was allowed 60 seconds to locate the platform. If it failed, it was gently guided to the platform and allowed to remain there for 20 seconds before being returned to its cage. A 30-second interval was maintained between trials. Subsequent trials on the same day involved start locations in the SE, S, and W quadrants, respectively. The sequence of start locations changed daily but position of the platform was fixed during the whole procedure.

Probe Trial (Day 28)

Twenty-four hours after the last acquisition trial, the platform was removed from the pool. Each rat was released from a distal starting point and allowed to swim for 60 seconds. The number of crossings over the former platform location was recorded as an indicator of spatial reference memory retention.

Working Memory Test (WMT)

Pretraining

For each rat, previously completed acquisition phase of reference memory test was considered as the pre-training phase of working memory test.

Training and Test

Four (4) days after pre-training, 4 trials per day were done with 30 seconds inter-trial interval for 4 consecutive days. In each trial, the test procedure of acquisition phase of reference memory test was followed. But position of platform was changed each day. Escape latency of all trials will be recorded.

Treatment plan

The following treatment plan were followed in both procedures.

Table 2: Treatment plan of RAM and MWM test

Phase	Duration	Day	RAM		Duration	Day	MWM	
			Treatment	Platform			Treatment	Platform
Room acclimatization	7 days	Days 1–7	No treatment	No baiting	7 days	Days 1–7	No treatment	Without platform
Instrumental acclimatization	8 days	Days 8–15	*Azadirachta indica* leaf extract (AILE) or Normal saline (NS)	No baiting	11 days	Days 8–18	AILE or NS	Without platform
Habituation	6 days	Days 16–21	AILE or NS 16-17 18-19 20-21	Baiting scattered all over maze Baiting in 8 foodcup Baiting in any 4 foodcup (randomly selected).	3 days	Days 19–21	AILE or NS	Without platform
Acquisition (Reference memory)	5 days	Days 22–26	AILE or NS ketamine Three treatment groups: NS, Ketamine, AILE	Baiting in any 4 foodcup	6 days	Days 22–27	AILE or NS + Ketamine	With platform (4 trials/day)
Retention Phase/Probe trial	6 days	Day 27-32	AILE or NS	-	1 day	Day 28	AILE or NS	Without platform

Retention testing/ Working memory training & test	1 day	Days 33	AILE or NS Three treatment groups NS, AILE, Ketamine	Baiting in any 4-food cup	4 days	Days 30–33	AILE or NS + Ketamine	With platform (4 trials/day)

Data Collection and Analysis

Data were collected from the RAM and MWM tests to assess spatial working and reference memory. For RAM, Reference Memory Errors (RME), defined as first-time entries into unbaited arms, measured reference memory, while Working Memory Errors (WME), or re-entries into baited arms, assessed working memory. In MWM, Escape Latency (EL), the time taken to locate the hidden platform during acquisition, evaluated spatial learning, and Target Crossings (TC), the frequency of crossing the platform's previous location during the probe trial, indicated memory retention. Data were expressed as mean ± SEM and analyzed using SPSS (version 16). ANOVA, followed by Bonferroni post hoc tests, was used for intergroup comparisons, and Student's paired t-test was applied for within-group analyses. A significance threshold of $p \leq 0.05$ was set to determine meaningful differences in memory performance across experimental conditions.

Ethical Considerations

The study followed the guidelines of the Animal Experimentation Ethics Committee of ICDDR, B. Ethical clearance was obtained from the Institutional Review Board (IRB) of BSMMU. All procedures minimized animal suffering, adhered to humane practices, and ensured compliance with standard protocols.

RESULTS

Radial arm maze

Working memory test

Working memory errors (WME): Acquisition of working memory

WME of normal memory and AILE pretreated experimental rats were significantly decreased from day 22 to day 26 as acquisition was progressing. But in memory impaired rats WME remain almost similar from day 22 to day 26 as they failed to acquire spatial working memory. However, these observations were further evidenced by statistically significant difference in WME between normal memory and memory impaired rats ($p \leq 0.05$; $p \leq 0.01$; $p \leq 0.001$) as well as between memory impaired and experimental rats ($p \leq 0.01$; $p \leq 0.001$). AILE prevented memory impairment which was evidenced by similar WMEs of experimental rats to those of normal memory rats in almost all trials across the days.

However, WME in AILE pretreated experimental rats were significantly lower in almost all trial in first 3 acquisition day (day 22 to day 24) in comparison to those of normal memory rats which indicate that AILE not only prevent ketamine induced memory impairment it is also induces early acquisition of experimental rats than that of normal memory rats. In addition, AILE caused very prompt knowledge acquisition in AILE pretreated experimental rats as evidenced by almost flat graph of trial 2 from 1st acquisition day onward across days. However, these comparisons were statistically non- significant (Figure 1A).

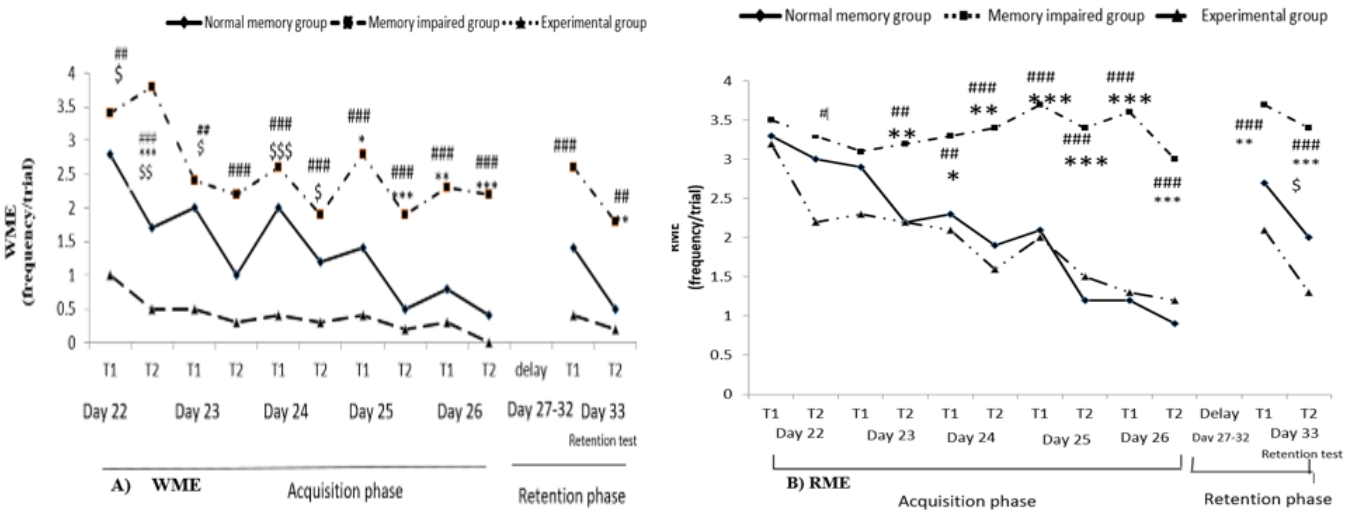
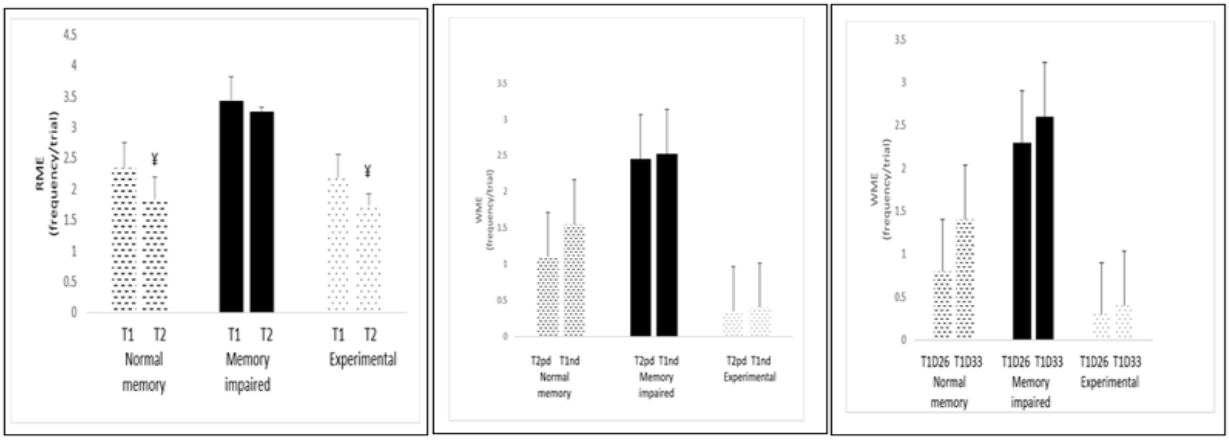


Figure 1: Working memory error (WME) and Reference memory error (RME) in different days of Radial arm maze in different groups of rats. Each line symbolizes mean±SEM trials for 10 rats. T1: mean trial 1 on that day; T2: mean trial 2 on that day. Statistical analysis was done by ANOVA (among groups) followed by Bonferoni's post hoq test (between groups); *=normal memory vs memory impaired; #=memory impaired vs experimental; $: normal memory vs experimental; In the interpretation of results p≤0.05 was considered as significant; */#/$:p≤0.05; **/##/$$:p≤0.01 ***/ ###/$$$:p≤0.001; Error bar is omitted for clarity.

Both normal memory and experimental rats stored information for a very short period (3 hours) as evidenced by significantly lower (p≤0.01) WMEs in T2 than T1 in both these groups (Figure 2a). This storage also extended over a period of 21 hours in both these groups as evidenced by similar WMEs in both trials in both groups (Figure 2b). However, these acquisition and storage was not evidenced in ketamine induced memory impaired rats since these rats failed to acquire.

Working memory errors (WME): Retrieval of working memory

After acquisition, a 6-day delay was given before the retention test. Both normal memory and experimental group could remember whatever they acquired 7 days earlier as evidenced by non-significantly higher WMEs in T1 of D33 in comparison to those of T1 of D26. On the contrary, since memory impaired rats failed to acquire, they made similar errors (Figure 2c).



a) WME after 3 hours interval (Day 22 to 26) b) WME after 21 hours interval (Day 22 to 26) c) WME after 7 days interval (Day 26 vs 33)

Figure 2: WMEs with different interval in RAM in different groups of rats. Each bar symbolizes mean±SEM trial of

10 rats. T1: mean trial1 on that day; T2: mean trial 2 on that day; T2pd: mean working memory error of trial 2 of previous days (Day 22, 23, 24, 25); T1nd mean working memory error of trial 1 of next days (Day 23, 24, 25, 26); T1D26: mean trial1 of day 26; T1D33: mean trial 1 of day 33 Statistical analysis was done by Student's paired t test (between trials); p≤0.05 was considered as significant; ¥≤0.05; ¥¥≤0.01

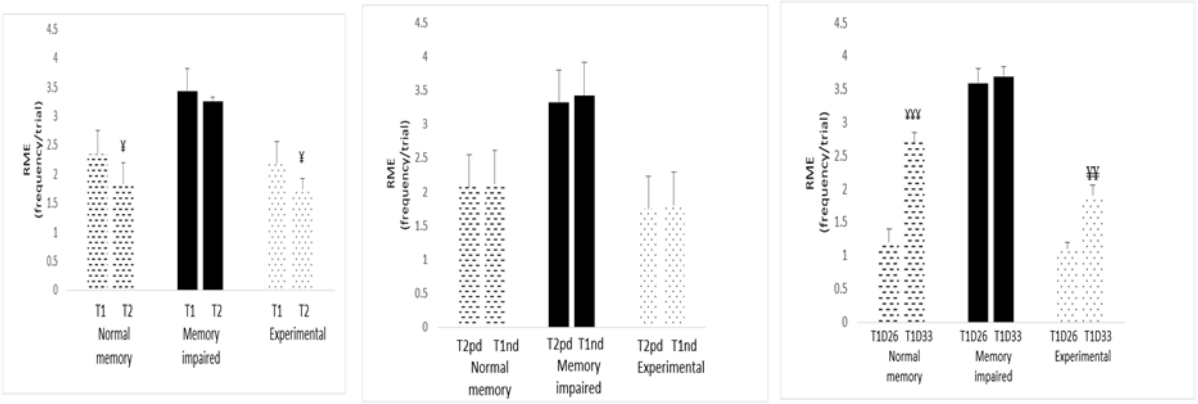
Reference memory test:

Reference memory errors (RMEs): Acquisition of reference memory

RME in normal memory rats were significantly decreased from day 22 to day 26 as acquisition was progressing. But in memory impaired rats RMEs remain almost similar from day 22 to day 26 as they failed to acquire spatial reference memory whereas AILE pretreated experimental rats showed similar decrement in RMEs as that of normal memory rats. However, these observations were further evidenced by statistically significant difference in RME between normal memory and memory impaired rats (p≤0.05; p≤0.01; p≤0.001) as well as between memory impaired and experimental rats (p≤0.05; p≤0.01; p≤0.001) except both trials of 1st acquisition day (Figure 5B). Both normal memory and experimental rats stored information for a very short period of time (3 hours) as evidenced by significantly lower (p≤0.05) RMEs in T2 than T1 in both these groups (Figure 3a). This storage also extended over a period of 21 hours in both these groups as evidenced by similar RMEs in both trials in both groups (Figure 3b). However, these acquisition and storage was not evidenced in ketamine induced memory impaired rats since these rats fail to acquire.

Reference memory errors (RMEs): Retrieval of reference memory

After acquisition, a 6-day delay was given before the retention test. In contrast to working memory, reference memory could not be retrieved by normal memory rats as evidenced by significantly higher RMEs in T1 of D33 in comparison to that of D26. However, experimental group could remember whatever they acquired 7 days earlier as evidenced by non-significantly higher RMEs on T1 of D33 in comparison to those of T1 of D26. On the contrary, since impaired memory rats failed to acquire, they made similar errors (Figure 3c).



a) RME after 3 hours interval (Day 22 to 26)

b) RME after 21 hours interval (Day 22 to 26)

c) RME after 7 days interval (Day 26 vs 33)

Figure 3: Reference memory error (RME) with different interval in Radial arm maze in different groups of rats. Each bar symbolizes mean±SEM trial of 10 rats. T1: mean trial 1 of that day; T2: mean trial 2 of that day; T2pd: mean RME error of trial 2 of previous days (Day 22,23,24,25); T1nd: mean RME of trial 1 of next days (Days 23,24,25,26);T1D26: mean trial 1 of day 26;T1D33: mean trial 1 of day 33; Statistical analysis was done by Student's paired t test (between trials). In the interpretation of results, p≤0.05 was considered as significant. ¥≤0.05; ¥¥≤0.01; ¥¥¥≤0.001.

Morris water maze

Working memory test

Escape latency in training and test: Working memory acquisition and retrieval

Escape latency was gradually decreased from 1st trial to 4th trial in all group of rats in this spatial memory test. However, this decrement was statistically significant

between normal memory and memory impaired rats (p≤0.001) as well as between memory impaired and experimental rats (p≤0.001). AILE prevented memory impairment by ketamine in experimental rats which was almost similar to those of normal memory rats in all trials (Figure 4A).

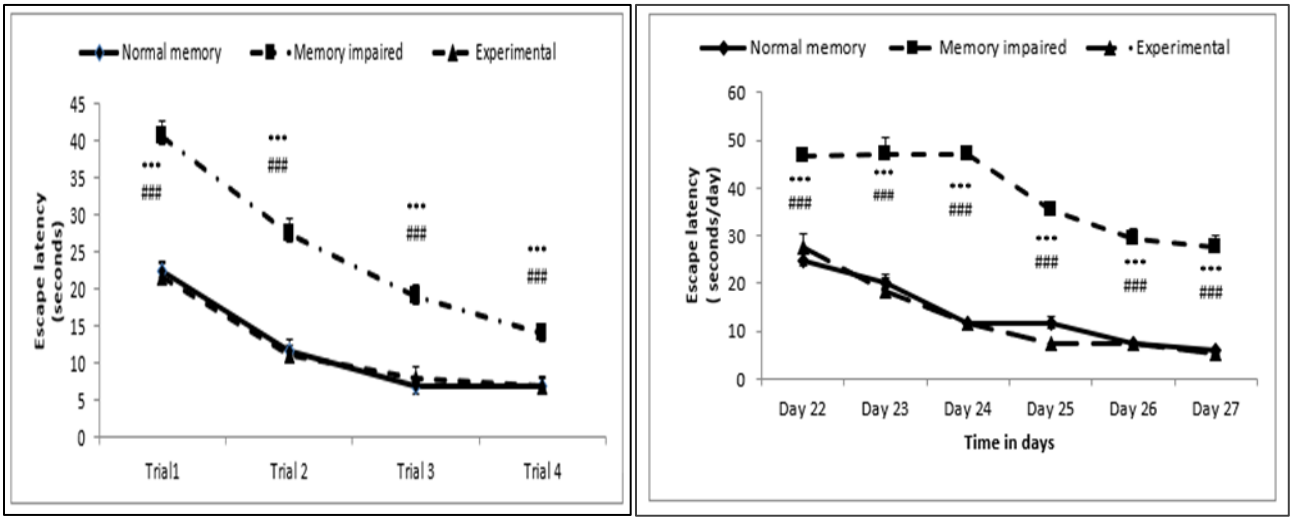
Escape latency in acquisition: Reference memory acquisition

EL in normal memory and experimental rats significantly decreased more from day 22 to day 27 as acquisition was progressing. But in memory impaired rats, decrement in EL occurred from day 24 onwards as acquisition phase progressed. However, these observations were further evidenced by statistically significant difference in EL between normal memory and memory impaired rats (p≤0.001) as well as between memory impaired and experimental rats (p≤0.001).

AILE prevented memory impairment by ketamine in experimental group which was almost similar to those of normal memory rats in all trials (Figure 4B).

Target crossings in probe trial: reference memory retrieval

Students unpaired t test demonstrated that ketamine caused reference memory impairment in our memory impaired rats as evidenced by significantly decreased (p≤0.001) target crossings in comparison to those of normal memory rats. However, AILE not only prevented memory impairment by ketamine in experimental rats but also enhanced retrieval of reference memory which was evidenced by significantly higher TC in comparison to those of memory impaired (p≤0.001) and normal memory rats(p≤0.042) (Figure 5).



A **B**

Figure 4: A. Escape latency (EL) in training and test. B. Escape latency in acquisition phase in different trials and different days of Morris water maze test in different groups of rats. Trial 1: mean± SEM of 4 T1s (trial 1) of 8 rats in 4 days of training and test. Trial 2: mean± SEM of 4 T2s (trial 2) of 8 rats in 4 days of training and test training and test. Trial 3: mean± SEM of 4 T3s (trial 3) of 8 rats in 4 days of training and test. Trial 4: mean± SEM of 4 T4s (trial 4) of 8 rats in 4 days of training and test. Each day symbolizes mean ±SEM escape latency of 4 trials in that day of acquisition phase for 8 rats. Statistical analysis was done by ANOVA (among group) followed by Bonferroni's post hoq test (between trial). *: Normal memory vs Memory impaired; #: Memory impaired vs Experimental; $: Normal memory vs Experimental. In the interpretation of results, p≤0.05 was considered as significant. */#/$: p≤0.05;**/##/$$: p≤0.0;, ***/###/$$$: p≤0.001.

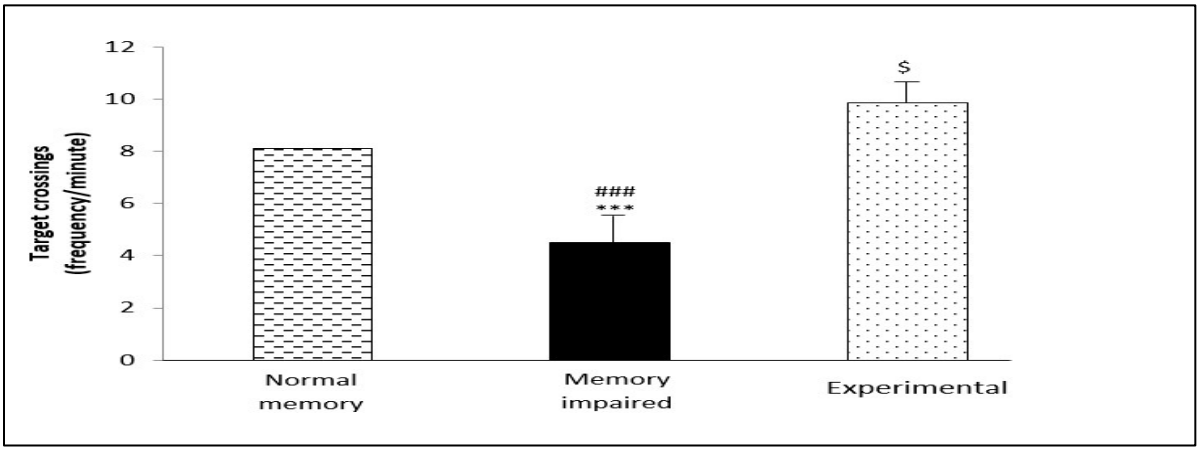


Figure 5: Number of target crossings on probe trial at day 28 of MWM in different groups of rats. Each bar symbolizes number of mean±SEM target crossings for 8 rats. Statistical analysis was done by ANOVA (among groups) followed by Bonferroni's post hoq test (between groups), *: Normal memory vs Memory impaired; #: Memory impaired vs Experimental; $: Normal memory vs Experimental. In the interpretation of results, p≤0.05 was considered as significant.*/#/$:p≤0.05,**/##/$$: p≤0.01, ***/###/$$$: p≤0.001.

DISCUSSION

The present study aimed to evaluate the effects of AILE on memory and NMDA receptor activity in

ketamine-induced memory-impaired male Wistar rats. Ketamine (15 mg/kg), at a sub-anaesthetic dose, led to both working and reference memory impairments, as evidenced by significantly increased WME and RME in the RAM, as well as prolonged escape latency and reduced target crossings in the MWM, compared to normal memory rats. These findings align with previous studies, where ketamine is reported to block NMDA receptors on postsynaptic pyramidal neurons in the prefrontal cortex, disrupting persistent neural activity essential for working memory [22,23]. Ketamine may also block NMDA receptors on GABAergic interneurons, leading to disinhibition of glutamatergic pyramidal neurons, causing an excessive release of glutamate and activation of AMPA receptors. This triggers excessive calcium influx through voltage-gated calcium channels, disrupting calcium homeostasis and promoting mitochondrial dysfunction, ultimately leading to cell apoptosis through caspase activation and cytochrome c (cyt c) release [24,25,26]. Additionally, ketamine-induced oxidative stress could further contribute to cognitive impairment [27]. In contrast, AILE treatment significantly ameliorated these deficits, as demonstrated by reduced WME and RME in RAM, and improved performance in MWM, with decreased escape latency and increased target crossings compared to ketamine-only treated rats. This finding is consistent with previous studies reporting similar effects of AILE on memory performance [16]. AILE may exert its protective effects by decreasing caspase and cyt c expression in the hippocampus and reducing oxidative stress in the brain [28]. Furthermore, quercetin, a flavonoid present in AILE, has been shown to enhance the expression of NMDA receptor subunits NR2A and NR2B in mice, suggesting that AILE may improve NMDA receptor function [29]. These mechanisms, including the reduction of pro-apoptotic proteins, alleviation of oxidative stress, and enhancement of NMDA receptor activity, likely contribute to the prevention of memory impairment induced by ketamine. AILE not only prevented memory deficits but also enhanced the retrieval of reference memory after a 24-hour interval, an effect not previously reported for this medicinal herb. While no supporting studies are available to explain this specific outcome, these findings suggest that AILE may also play a role in promoting memory retrieval, offering a novel avenue for future research on its cognitive-enhancing properties.

In this study working and reference memory acquisition improved significantly in AILE-treated rats, showing performance similar to normal memory rats, while ketamine-impaired rats failed to acquire spatial memory. AILE enhanced early acquisition, as evidenced by lower working memory errors (WME) in the first few days of training. Memory retention was preserved in AILE-treated rats, with short-term (3-hour) and long-term

(21-hour) storage of information, unlike ketamine-impaired rats.Reference memory retrieval was better in AILE-treated rats, with significantly higher target crossings in the probe trial compared to both normal and ketamine-impaired rats, suggesting AILE may enhance memory recall. The findings suggest AILE not only protects against ketamine-induced cognitive deficits but also enhances memory acquisition and retrieval, making it a promising candidate for future therapeutic research in memory disorders.

CONCLUSION

The findings indicate that AILE effectively mitigates ketamine-induced spatial learning and memory impairments, suggesting its potential as a neuroprotective or cognitive-enhancing agent. Based on these results, AILE could be explored as a therapeutic option for conditions involving memory deficits, such as schizophrenia or neurodegenerative disorders. The recommendations include,

Clinical Translation: Further studies should explore the safety, optimal dosage, and long-term effects of AILE in preclinical and clinical settings.

Mechanistic Investigations: Research should focus on identifying the molecular and neurobiological mechanisms through which AILE improves cognitive function.

Comparative Studies: AILE's efficacy should be compared with standard cognitive enhancers or neuroprotective agents to assess its relative benefits.

Broader Cognitive Assessments: Future studies should evaluate AILE's impact on other cognitive domains, such as attention and executive function.

Exploring the neurochemical pathways involved in AILE's protective effects against ketamine-induced cognitive deficits.

Investigating whether AILE has potential applications in human cognitive disorders, including Alzheimer's disease, schizophrenia, or drug-induced cognitive impairments.

Conducting longitudinal studies to assess the durability of AILE's effects on cognition.

Evaluating potential side effects or interactions with other pharmacological agents.

These findings open avenues for further research into natural compounds with neuroprotective properties, offering potential therapeutic interventions for cognitive disorders.

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