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Effect of AILE on Working and Reference Memory of Ketamine Induced Memory Impaired Male Wistar Rats in Radial Arm Maze (RAM)

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ABSTRACT

Background: Memory impairment involves a decline in memory, cognition, behavior and daily functioning. Conventional treatments often fall short due to the complex mechanisms underlying memory loss, diminishing effectiveness over time and having significant side effects. **Objective:** The study aimed to evaluate the effects of AILE on spatial working and reference memory in male Wistar rats with ketamine-induced memory impairment. Ketamine, a known NMDA receptor antagonist, was used to induce cognitive deficits, which were assessed using the Radial Arm Maze (RAM). **Methods:** This experimental study was conducted in the Department of Physiology at Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, using 30 male Wistar rats (200±50 g body weight) sourced from the university's central animal house. The rats were divided into three groups: Group 1 (G1) normal memory, Group 2 (G2) memory impaired, Group 3 (G3) experimental, respectively. Ethical approval for this research was obtained from the Institutional Review Board (IRB) of BSMMU. Data were analyzed using ANOVA, Bonferroni post hoc tests, and Student's paired t-test with significance set at $p \leq 0.05$. **Results:** In the RAM test, ketamine-treated rats exhibited a significant increase in both working memory errors and reference memory errors ($p \leq 0.001$), indicating substantial memory impairment. However, rats treated with AILE showed a significant reduction in both working memory errors and reference memory errors ($p \leq 0.001$) compared to the ketamine-only group. These results suggest that AILE effectively mitigates ketamine-induced cognitive deficits, improving both working and reference memory performance in the RAM. **Conclusion:** AILE demonstrated significant neuroprotective effects against ketamine-induced memory impairment, likely through modulation of NMDA receptor function, reduction of oxidative stress, and inhibition of apoptotic pathways.

Keywords: *Azadirachta Indica*, Memory Impairment, NMDA Receptor, Ketamine, Radial Arm Maze.

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INTRODUCTION

Memory is a fundamental cognitive function essential for learning and adapting behavior, involving the processes of encoding, storing, and retrieving information. Its impairment is often linked to neurological conditions such as dementia, which presents

a growing global health challenge. Approximately 46.8 million people worldwide are affected by dementia, with projections suggesting an increase to 74.7 million by 2030 and 131.5 million by 2050, nearly doubling every 20 years [1]. Among individuals aged 60 and older, the prevalence of dementia stands at about 4.8% and continues to rise [2].

Memory can be categorized by duration and content. In terms of time, it is classified into short-term, intermediate, and long-term memory [3]. Short-term memory, often called "working memory," involves the temporary holding and manipulation of information needed for immediate tasks [4]. In contrast, long-term memory, or "reference memory," includes stable, declarative information about environments and repetitive events [5]. Neurophysiological studies suggest that working memory relies on sustained neural activity, while reference memory involves enduring synaptic changes [6]. The NMDA receptor plays a critical role in memory by supporting synaptic plasticity necessary for learning. Disruptions in NMDA receptor function, such as those caused by ketamine—a non-competitive NMDA receptor antagonist—can lead to memory impairments [7]. Studies show that ketamine at sub-anesthetic doses, like 15 mg/kg, can disrupt spatial working and reference memory in animals [8]. Commonly, memory impairments are assessed using tasks such as the Radial Arm Maze (RAM) and the Morris Water Maze, which evaluate an animal's ability to navigate spatial cues [9].

Azadirachta indica, or neem, has a long history in traditional medicine, yet its specific effects on memory are less explored. Some studies indicate that it can improve cognitive function in certain contexts. For instance, it has been shown to reverse memory impairments in rats with chronic cerebral hypoperfusion [10]. More recently, the aqueous leaf extract of *Azadirachta indica* (AILE) demonstrated significant enhancements in spatial working and reference memory in rat models of Alzheimer's disease; These cognitive benefits are thought to be linked to AILE's antioxidant properties, which include reducing malondialdehyde (MDA)—a marker of oxidative stress—and increasing superoxide dismutase (SOD) activity [11]. However, the current body of research on AILE's cognitive effects is insufficient to make definitive conclusions. Notably, there is limited information regarding the role of NMDA receptors in AILE's potential protective effects against memory impairment. This gap is significant, as understanding how AILE might influence NMDA receptor activity could clarify its mechanisms in enhancing cognitive function, particularly under conditions of disrupted synaptic signaling. To address these gaps, the present study will investigate the impact of AILE, administered at a dosage of 300 mg/kg/day, on ketamine-induced memory impairment in male Wistar rats. A key focus will be the exploration of the NMDA receptor's role in mediating AILE's cognitive benefits. This research aims to expand current knowledge on herbal interventions for memory disorders, potentially offering new insights into treatments for conditions linked to NMDA receptor dysfunction.

OBJECTIVE

This study aims to investigate the effects of AILE on ketamine-induced spatial memory impairment in male Wistar rats, specifically examining its influence on working and reference memory through the RAM.

METHODS

Study Design

Total 30 rats were divided into three groups: Group 1 (G1) normal memory (oral normal saline treated, 5ml/kg/day for 26 days), Group 2 (G2) memory impaired (intraperitoneal ketamine treated, 15mg/kg/day during 5 days of acquisition phases), Group 3 (G3) experimental (oral AILE treated, 300mg/kg/day for 26 days and intraperitoneal ketamine, 15mg/kg/day during 5 days of acquisition phase).

Study Settings

The study was conducted in the Memory Laboratory within the Department of Physiology at Bangabandhu Sheikh Mujib Medical University (BSMMU), located in Dhaka, Bangladesh. This facility provided a controlled environment suitable for precise and reproducible experimental procedures involving animal models.

Time Period

The research was carried out over a one-year period, from March 2020 to February 2021. This duration encompassed all phases of the study, including preparatory activities such as animal acclimatization, experimental procedures, and data analysis. The extended timeline ensured that each phase of the study, from initial training to memory assessment, was systematically conducted.

Sample Size and Group Allocation

The sample size and group allocation of this study was as follows:

Group 1 (G1) normal memory

Sample Size: 10 rats

Treatment: Rats in this group received normal saline to serve as a baseline comparison for the study.

Group 2 (G2) memory impaired

Sample Size: 10 rats

Treatment: This group was exposed to ketamine, a substance known to induce memory impairment, providing a model for memory deficits against which the effects of AILE could be assessed.

Group 3 (G3) experimental

Sample Size: 10 rats

Treatment: Rats in this group were given AILE following ketamine-induced memory impairment. This allowed for evaluating the potential therapeutic effects of AILE on memory recovery or protection.

Each group was carefully managed to ensure comparable conditions and accurate measurement of memory performance across different treatments. The even sample distribution provided sufficient statistical power to detect significant differences among the groups in behavioral tests.

Animal Selection

Thirty (30) rats having 200±50 gm body weight was obtained from central animal house of BSMMU, Dhaka. All rats were kept in the rat laboratory of the department of Physiology, BSMMU and were housed in specially built plastic cages with 4 rats per cage under a 12/12-hour light/dark cycle. The ambient room temperature was maintained at around 27° to 28°, corresponding to thermoneutral zone for rodents [12]. All the rats had free access to the standard laboratory food, cooled boiled water *ad libitum*, during acclimatization. To avoid circadian influences, all the experiments was performed at daytime between 08.00 and 16.00.

Study Procedure

Apparatus

The experiment included an 8-arm typical radial arm maze fabricated from transparent plexiglass (figure 1). It was situated 70 centimeters above the ground. The maze had a center octagonal platform with a diameter of 42 cm, encircled by eight uniformly distributed arms. Each arm measured 60 cm in length, 17 cm in width, and 25 cm in height. The arms included recessed food bowls, measuring 2 cm in depth and 3 cm in diameter, positioned 4 cm from the terminus of each arm. Transparent plexiglass guillotine doors partitioned the arms from the central platform, which could be elevated or lowered to regulate access to each arm. The doors were controlled by a pulley system, allowing the researcher to remotely open or close any door. The labyrinth was located in a brightly illuminated room including prominent extramaze visual indicators (e.g., cabinet, shelves, desk, and air conditioner), assisting the rats in spatial navigation [14]. During the trial, the maze was consistently positioned in relation to these exterior cues [15]. The rats conducted the RAM test over a duration of 33 days.

Procedure

The experiment followed established methods [13,14]. A total of 30 rats, with 10 rats per group, underwent training for the RAM test, consisting of three phases: habituation (6 days), acquisition (5 days), and retention (8 days) (Figure 2). The rats were acclimatized to the room for 7 days before training commenced. During each phase, the rats underwent two trials daily, separated by 3 hours. To incentivize the rats to forage for food, they were subjected to a food deprivation period of roughly 10 hours before each trial, while water was accessible ad libitum [16]. The preliminary trial commenced for 30 minutes following the administration of the designated therapy (AILE, ketamine, or saline) in accordance with the rats' group allocation. Subsequent to each experiment, the maze was meticulously sanitized using water and 70% alcohol to eradicate remaining scents.

Habituation/Shaping Phase (6 Days - Day 16 to Day 21)

During the first two days, rats were paired together and given access to the maze with food scattered across the platform and arms. This facilitated acclimatization to the maze environment. From Day 18 to Day 19, each arm's food cup was baited, and the rats were given individual access to the maze. On Days 20 and 21, four arms were randomly selected and baited (via lottery) for the rats to explore. During this phase, the gates of all eight arms were kept open for free exploration.

Acquisition Phase (5 Days - Day 22 to Day 26)

During acquisition, four of the eight arms were baited with "jilapi" in a food cup. Each trial began by placing a rat at the center of the maze with the gates closed. Upon opening all gates, the rat could enter any of the arms. After selecting an arm, the rat consumed the food and exited the arm, whereupon the gate was closed. After a 5-second delay, all gates were reopened for the next trial. The trials lasted 10 minutes, or until the rat had eaten all the food from the four baited arms. After each trial, a 3-hour interval given before the second trial.

Retention Phase (7 Days - Day 27 to Day 33)

Following the acquisition phase, the rats were kept in their home cages for 6 days without further training. Daily doses of their assigned treatment were administered during this period. On Day 33, a retention test was conducted with two trials. The procedure was similar to the acquisition phase, but the 6-day delay tested the rats' ability to retain the learned maze configuration.

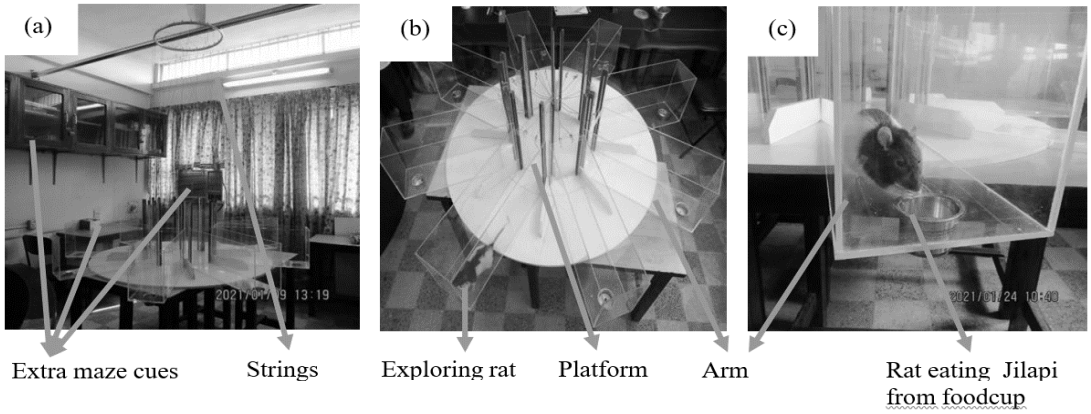

Figure 1: Radial arm maze (a) with extra maze cues (b) at a glance (c) rat eating bait in food cup

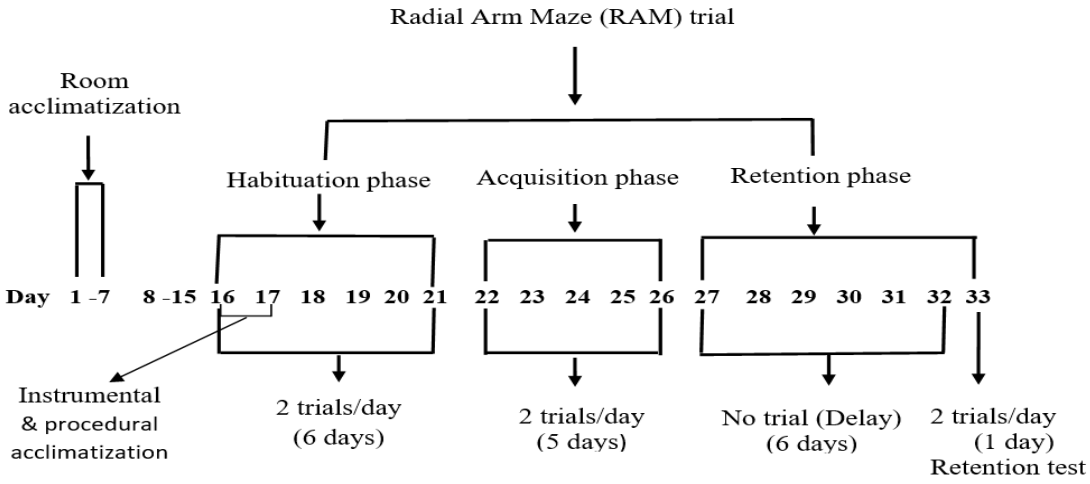

Figure 2: Working Plan in Different Days of Radial Arm Maze (RAM)

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 *Azadirachta 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.

Treatment Plan

The rats underwent a sequence of phases starting with room acclimatization, followed by habituation and acquisition, where they were trained to explore the maze and learn the food locations. During the retention phase, no additional training occurred, and the treatment continued. The final retention testing on Day 33 assessed their memory retention abilities. The following table shows the treatment plan of Radial arm maze test.

Table 1: Treatment plan of Radial arm maze test

Phase	Duration	Days	Treatment	Baiting
Room acclimatization	7 days	Day 1–7	No treatment	No baiting
	8 days	Day 8–15	*Azadirachta indica* leaf extract (AILE) Or Normal saline (NS)	No baiting
Habituation	6 days	Day 16–21	AILE or NS 16-17 18-19 20-21	Baiting scattered all over maze Baiting in 8 food cups Baiting in any 4 food cup (randomly selected).
Acquisition	5 days	Day 22–26	AILE or NS ketamine Three treatment groups: NS, Ketamine, AILE	Baiting in any 4 food cup
Retention Phase	6 days	Day 27-32	AILE or NS	
Retention testing	1 day	Day 33	AILE or NS Three treatment groups NS, AILE, Ketamine	Baiting in any 4 food cup

Data Collection and Analysis

Data were gathered from the RAM test, concentrating on the memory performance of the rats during various phases of the experiment. The principal variables assessed were reference memory errors (RME), indicating the count of initial entries into unbaited arms, and working memory errors (WME), characterized as the number of re-entries into previously visited arms. Errors were documented for each rat during the acquisition and retention stages of the RAM test. The data were presented as mean ± SEM (standard error of the mean) for each treatment group. The data were analyzed with SPSS (version 16.0). A one-way Analysis of Variance (ANOVA) was conducted to evaluate the differences between groups, followed by the Bonferroni post-hoc test for pairwise comparisons. Paired Student's t-tests were employed for within-group comparisons. Statistical significance was defined as $p < 0.05$. The investigation sought to assess the effects of AILE, ketamine, and normal saline on the working and reference memory mistakes in rats, consequently evaluating the treatments' influence on memory retention.

Table 2: Study Variables for Radial Arm Maze Test (RAM) Test

Memory Type	Aspect of Memory	Variable	Unit
Working Memory	Acquisition & Retrieval	WME- Number of re-entry into baited arm	Frequency/minute
Reference Memory	Acquisition & Retrieval	RME-Number of unbaited arms explored first time	Frequency/minute

Ethical Considerations

All experiments in this study adhered to the ethical guidelines set by the Animal Experimentation Ethics Committee (AEEC) of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). The rats were cared for by qualified laboratory staff from the Department of Physiology at BSMMU. All animals were sacrificed after completing the behavioral tests, under deep anesthesia to minimize suffering. The study followed strict ethical standards to ensure the humane treatment of animals throughout the research process. Ethical approval was obtained following a thorough review by the Institutional Review Board (IRB) of BSMMU.

RESULTS

Working Memory Error (WME)

In the present study, the mean±SEM WME, at day 22 were 2.80±0.36, 3.4±0.70, 1.00±0.21 frequency/trial for trial 1 and 1.70±0.40, 3.80±0.36, 0.50±0.17 frequency/trial for trial 2 in Group 1 (G1) normal memory, Group 2 (G2), memory impaired, Group 3 (G3) experimental, respectively. Similarly, at day 23, these variables were 2.00±0.30, 2.40±0.49, 0.50±0.16 frequency/trial for trial 1 and 1.00±0.26, 2.20±0.44, 0.30±0.15 frequency/trial for trial 2, at day 24, 2.00±0.29, 2.60±0.27, 0.40±0.16 for trial 1 and 1.20±0.25, 1.90±0.31, 0.30±0.15 frequency/trial for trial 2; at day 25, 1.40±0.22, 2.80±0.51, 0.40±0.16 frequency/trial for trial1 and 0.50±0.22, 1.9±0.28, 0.20±0.13 frequency/trial for trial 2; at day 26, 0.80±0.13,2.30±0.45, 0.30±0.15 frequency/trial for trial1 and 0.40±0.22, 2.20±0.36, 0.00±0.00 frequency/trial for trial 2 in G1, G2 and G3 respectively. After an interval of 7 days on the day 33, the mean±SEM WME were 1.40±0.22, 2.60±0.52, 0.40±0.16 frequency/ trial for trial 1 and 0.50±0.22,1.8±0.33, 0.20±0.13 frequency/trial for trial 2 in G1, G2 and G3 respectively. Here, the mean values of this variable were significantly different among all groups in all the trials of all the experimental days. In addition, the mean±SEM WME values of the G2 were significantly higher ($p \leq 0.05$) in comparison to G1 in all the trials of all the days except in trial 1 of day 22, 23, 24 and retention day. Moreover, mean values of this variable in the G3 were significantly ($p \leq 0.05$) lower in comparison to those of G2 in al the trial of all days. Also, there was no significant difference of mean values between G1 and G3 in all the trial of all days except day 22.

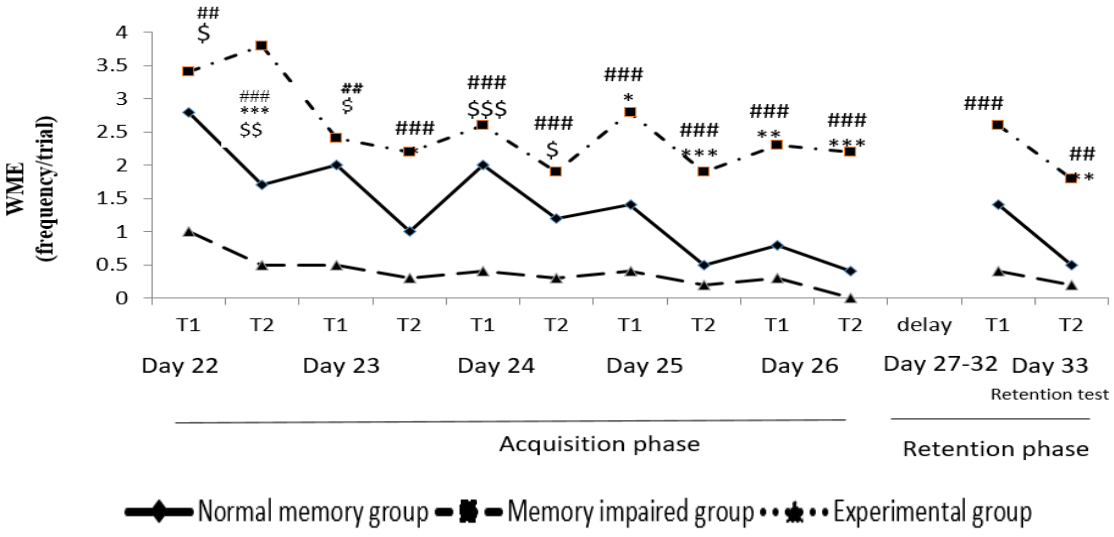

Figure 3: Working Memory Error (WME). Each line represents the mean SEM of trials conducted on 10 rats. T1: average of trial 1 on that day; T2: average of trial 2 on that day. Statistical analysis was conducted using ANOVA (among groups) followed by Bonferroni's post hoc test (between groups); *=normal memory vs. memory impaired; #=memory impaired vs. experimental; Normal memory versus experimental memory; In the analysis of findings, $p \leq 0.05$ was deemed significant; */#/$:$p \leq 0.05$; **/##/$$:$p \leq 0.01$ ***/ ###/$$$: $p < 0.001$; Error bars are eliminated for clarity.

Each line represents the mean SEM of trials conducted on 10 rats. T1: average of trial 1 on that day; T2: average of trial 2 on that day. Statistical analysis was conducted using ANOVA (among groups) followed by Bonferroni's post hoc test (between groups); *=normal memory vs. memory impaired; #=memory impaired vs. experimental; Normal memory versus experimental memory; In the analysis of findings, p≤0.05 was deemed significant; */#/$:p≤0.05; **/##/$$:p≤0.01 ***/ ###/$$$: p < 0.001; Error bars are eliminated for clarity.

WME of T1s and T2s (with 3 hours interval) in acquisition phase of RAM

In the present study, the mean±SEM RME of 5T1s (trial 1) of 5 days of acquisition phase were 1.80±0.33, 2.7±0.19 and 0.52±0.12 frequency/trial in G1,G2 and G3 respectively. However, values of this variable after 3 hours interval that is, the mean±SEM mean WME of 5T2 (trial2) 5 days of acquisition phase were 0.96±0.23, 2.40±0.35 and 0.52±0.12 frequency/trial in G1, G2 and G3 respectively. Here, the mean values of this variable in trial 2 were lower in comparison to trial 1 in all group. However, these differences were statistically significant in group G1 and G3. The present study shows the statistical comparison of different groups (G1,G2 and G3) at two time points (T1 and T2). The p-values are provided for each comparison to assess the significance of differences between the time points within each group. A p-value less than 0.05 indicates a statistically significant difference, with notable results observed for G1 T1 vs G1T2 (p = 0.002) and G3T1 vs G3T2 (p = 0.019), while the comparison between G2T1 and G2T2 shows no significant difference (p = 0.262).

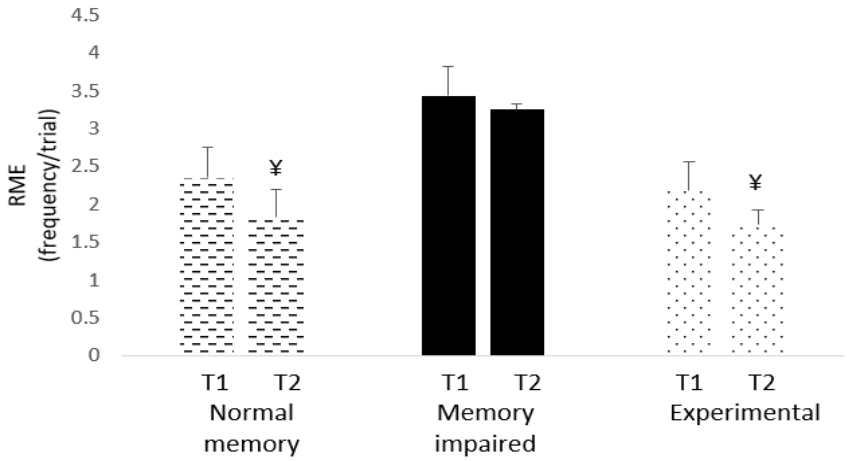

Figure 4: WME after 3 hours interval (Day 22 to Day 26). Each bar symbolizes mean±SEM trial of 10 rats. T1: mean trial1 on that day; T2: mean trial 2 on that day; Statistical analysis was done by Student's paired t test (between trials); p≤0.05 was considered as significant; ¥≤0.05; ¥¥≤0.01.

Each bar symbolizes mean±SEM trial of 10 rats. T1: mean trial1 on that day; T2: mean trial 2 on that day; Statistical analysis was done by Student's paired t test (between trials); p≤0.05 was considered as significant; ¥≤0.05; ¥¥≤0.01.

WME of T2s and T1s (with 21 hours interval) in acquisition phase of RAM

In the present study, mean±SEM mean WME of 4 (four) T2s of previous days (day 22, 23, 24, 25) in acquisition phase were 1.10±0.24, 2.45±0.45, 0.35±0.06 frequency/trial in group G1,G2 and G3 respectively. However, after 21 hours, the mean±SEM mean WME of 4 (four) T1s of 4 next days (Day 23, 24,25, 26) were 1.55±0.28, 2.52±0.131 and 0.40±0.04 frequency/trial in group G1, G2 and G3 respectively. Here, mean values of this variable in trial 1 of next days were higher in comparison to those of T2s of previous day in all groups. However, these differences were statistically non-significant in all groups. The study results shows statistical comparisons between different groups at various time points and phases. The p-values are

provided for each comparison to evaluate the significance of differences observed. The comparisons include G1T2pd vs G1T1nd (p= 0.093), G2T2pd vs G2 T1nd (p = 0.891), and G3T2pd vs G3T1nd (p = 0.495). All comparisons show p-values greater than 0.05, indicating no statistically significant differences between the groups across the specified time points and phases.

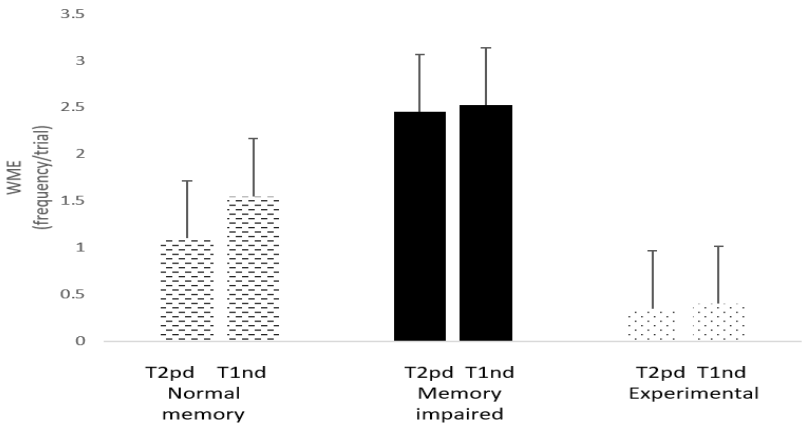

Figure 5: WME after 21 hours interval (Day 22 to Day 26). Each bar symbolizes mean±SEM trial of 10 rats. T2pd: mean working memory error of trial 2 of previous days (Day 22, 23, 24, 25); T1nd mean working memory error of trial 1 of next days (Day 23, 24, 25, 26); Statistical analysis was done by Student's paired t test (between trials); p≤0.05 was considered as significant; ¥≤0.05; ¥¥≤0.01.

Each bar symbolizes mean±SEM trial of 10 rats. 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); Statistical analysis was done by Student's paired t test (between trials); p≤0.05 was considered as significant; ¥≤0.05; ¥¥≤0.01.

WME of T1s of day 26 and day 33 (with 7 days interval) in RAM

In the present study, mean±SEM WME at trial 1 in day 26 were 0.80±0.13, 2.30±0.44 and 0.30±0.15 frequency/trial in G1, G2 and G3 respectively. However, these values after 7 days at trial 1 in day 33 were 1.40±0.22, 2.60±0.52 and0.4±0.16 frequency/trial in group G1, G2 and G3 respectively. Here, the mean values of this variable in day 33 were higher in all groups in comparison to those of day 26. However, these differences were statistically non-significant. This study shows a statistical comparison of groups G1, G2, and G3 at two different time points (D26 vs D33). The p-values for each comparison are provided to assess the significance of the differences between the groups at the specified time points. The comparisons include G1T1D26 vs G1 T1D33 (p = 0.081), G2 T1D26 vs G2 T1D33 (p= 0.726), and G3 T1D33 vs G3 T1D33 (p = 0.591). None of these comparisons reach statistical significance, with all P-values greater than 0.05.

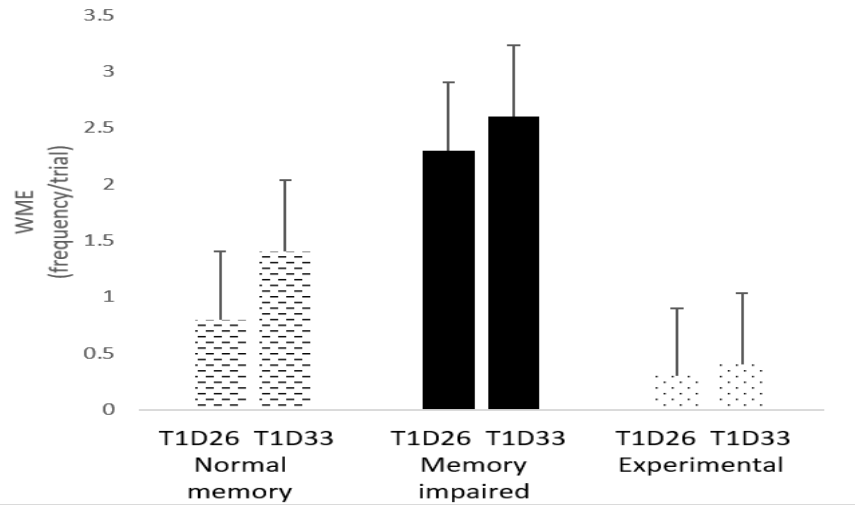

Figure 6: WMEs after 7days interval in RAM in different groups of rats. Each bar symbolizes mean±SEM trial of 10 rats. T1D26: mean trial1 of day 26; T1D33: mean trial 1 of day 33 Statistical analysis was done by Student's paired t test (between trials); p≤0.05 was considered as significant; ¥≤0.05; ¥¥≤0.01.

Each bar symbolizes mean±SEM trial of 10 rats. 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 Error (RME)

In the present study, mean±SEM RME, at day 22 were 3.30±0.30, 3.50±0.22, 3.2±0.24 frequency/trial for trial 1 and 3.00±0.21, 3.30±0.30, 2.20±0.13 frequency/trial for trial 2 in G1, G2 and G3 respectively. Similarly, at day, 23 the values were 2.90±0.18, 3.1±0.18, 2.3±0.36 frequency/ trial for trial 1, and 2.20±0.13, 3.20±0.20, 2.2±0.24 frequency/trial for trial 2; at day 24, 2.30±0.15, 3.30±0.21, 2.10±0.17 frequency/trial for trial 1 and 1.90±0.18, 3.40±0.22, 1.60±0.33 frequency/trial for trial 2; at day 25, 2.10±0.18, 3.70±0.15, 2.00±0.14 frequency/trial for trial 1 and 1.20±0.13, 3.40±0.16, 1.50±0.22 frequency/trial for trial 2; at day 26, 1.20±0.20, 3.60±0.22, 1.3±0.15 frequency/trial for trial 1 and 0.90±1.20,3.00±0.21, 1.20±0.24 frequency/trial for trial 2, in G1, G2 and G3 respectively. After an interval of 7 days on day 33, the mean±SEM RME were 2.70±0.15, 3.70±0.15, 2.10±0.17 frequency/trial for trial 1 and 2.00±0.21, 3.40±0.16, 1.30±0.15 frequency/trial for trial 2, in G1, G2 and G3 respectively. Here, mean values of this variable were significantly different among all groups of in the trials of all days except day 22 and trial 1 of day 23. In addition, the mean±SEM RME of the G2 were significantly (p≤0.05) higher in comparison to the G1 in all the trials 2 of all days except day22 and trial1 of 23. Moreover, mean values of this variable in the G3 were significantly (p≤0.05) lower in comparison to those of G2 in all the trials of all the days except trial 1 of day 22and 23. Moreover, there was significant difference of mean values between G1 and G3 in both trial on day33 of experiment.

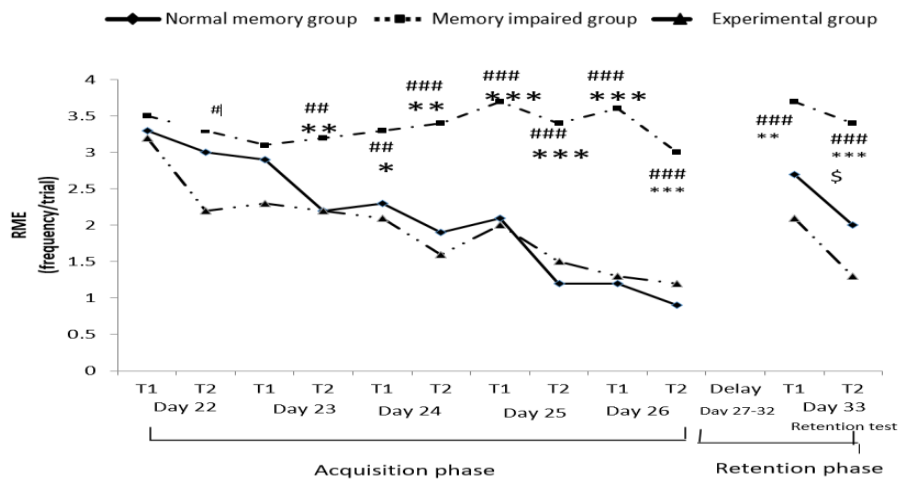

Figure 7: Reference memory error (RME). Each line symbolizes mean±SEM trials for 10 rats. T1: mean trial 1 on that day; T2: mean trial 2 on that day. Statistical analysis was done by ANOVA (among groups) followed by Bonferoni's post hoq test (between groups); *=normal memory vs memory impaired; #=memory 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.

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.

RME of T1s and T2s (with 3-hour interval) in acquisition phase of RAM

In the present study, the mean±SEM RME of 5T1s (trial 1) of 5 days of acquisition phase were 2.36±0.36, 3.44±0.11 and 2.18±0.31 frequency/trial in G1,G2 and G3, respectively. However, values of this variable after 3 hours interval that is, the mean±SEM mean RME of 5T2 (trial2) 5 days of acquisition phase were 1.84±0.37, 3.26±0.07 and 1.74±0.19 frequency/trial in group 1, 2 and 3 respectively. Here, the mean values of the variable in trial 2 were lower in comparison to trial 1 in all group. However, these differences were statistically significant in group 1 and 3.

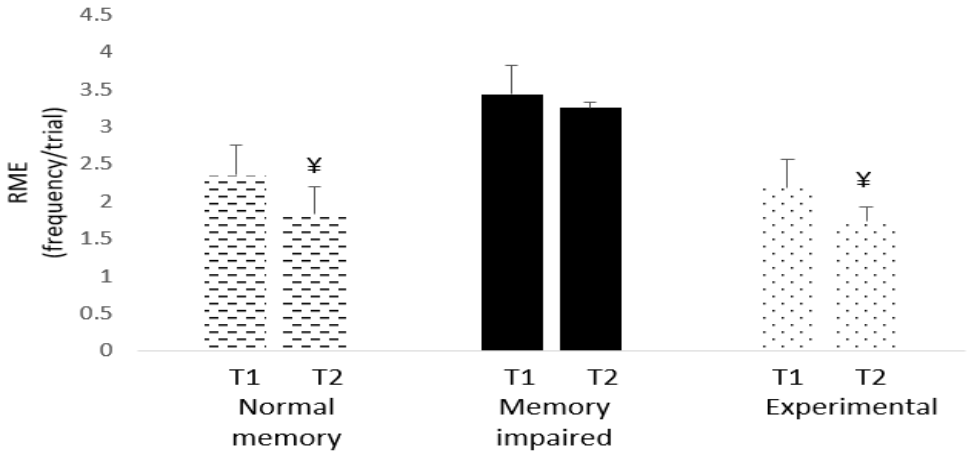

Figure 8: RME after 3 hours interval (Day 22 to Day 26). Each bar symbolizes mean±SEM trial of 10 rats. T1:

mean trial 1 of that day; T2: mean trial 2 of that day; 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.

Each bar symbolizes mean±SEM trial of 10 rats. T1: mean trial 1 of that day; T2: mean trial 2 of that day; 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.

RME of T2s and T1s (with 21-hour interval) in acquisition phase of RAM

In the present study, the mean±SEM mean RME in 4(four) T2s (trial 2 of previous days (day 22, 23, 24, 25) of acquisition phase were 2.07±0.37, 3.32±0.04, 1.75±0.22 frequency/trial in G1, G2 and G3 respectively. However, after 21 hours, the mean±SEM mean RME of 4 (four) T1s (trial 1) of 4 next days (day 23, 24, 25, 26) were 2.12±0.35, 3.42±0.13 and 1.80±0.24 frequency/trial in G1, G2 and G3 respectively. Here, mean values of this variable in trial 1 of next days were higher in comparison to those of T2s of previous day in all groups. However, these differences were statistically non-significant in all groups.

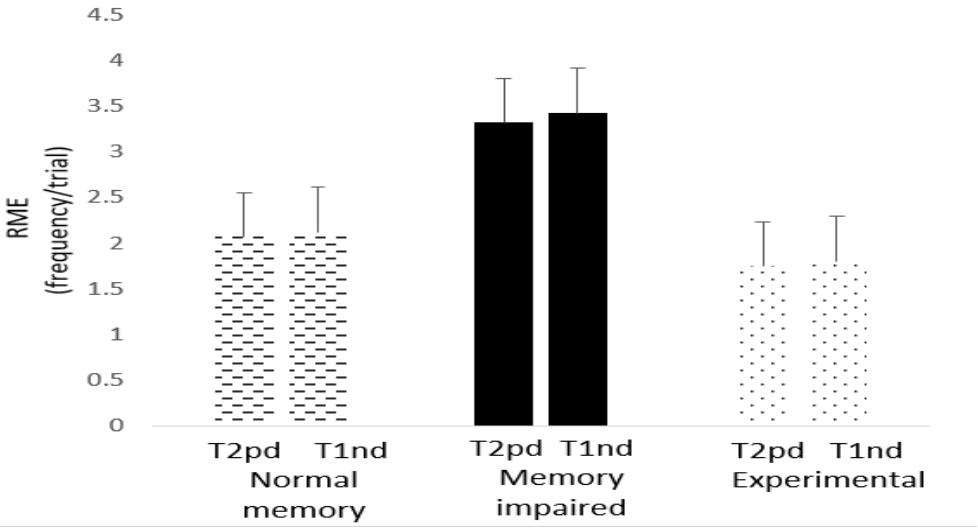

Figure 9: RME after 21 hours interval (Day 22 to Day 26). Each bar symbolizes mean±SEM trial of 10 rats.; T2pd: mean RME error of trial 2 of previous days (Day 22,23,24,25); T1nd: mean RME of trial 1 of next days (Days 23,24,25,26); Statistical analysis was done by Student's paired t test (between trials). In the interpretation of results, p≤0.05 was considered as significant. ¥≤0.05; ¥¥≤0.01; ¥¥¥≤0.001.

Each bar symbolizes mean±SEM trial of 10 rats.; 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); 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. The statistical comparisons between G1, G2, and G3 at two different phases (T2pd vs T1nd). The p-values for each comparison are included to assess the significance of the differences observed. The comparisons include G1 T2pd vs G1 T1nd (p = 0.495), G2 T2pd vs G2 T1nd (p = 0.423), and G3 T2pd vs G3 T1nd (p = 0.495). All comparisons show p-values greater than 0.05, indicating no statistically significant differences between the phases for the respective groups.

RME of T1s of day 26 and day 33 (with 7 days interval) in RAM

In the present study, the mean±SEM RME at trial1 in day 26 were 1.20±0.20, 3.60±0.22 and 1.10±0.10 frequency/trial in G1, G2 and G3 respectively. However, these values, after 7 days interval at trial 1 in day 33 were2.7±0.15, 3.70±0.15 and1.9±0.17 frequency/trial in G1, G2 and G3 respectively. Here, mean values of this variable in day 33 were higher in all groups in comparison to those of day 26. However, this difference was statistically significant in G1 and G3. The statistical comparisons between G1, G2, and G3 at two distinct time

points (D26 vs D33). The P-values indicate the significance of differences between these time points within each group. The comparison between G1 T1D26 and G1 T1D33 shows a statistically significant difference (p= 0.000), as does the comparison between G3 T1D26 and G3 T1D33 (p= 0.003), both indicating strong evidence of differences at these time points. In contrast, the comparison between G2 T1D26 and G2 T1D33 (p = 0.726) does not show a statistically significant difference.

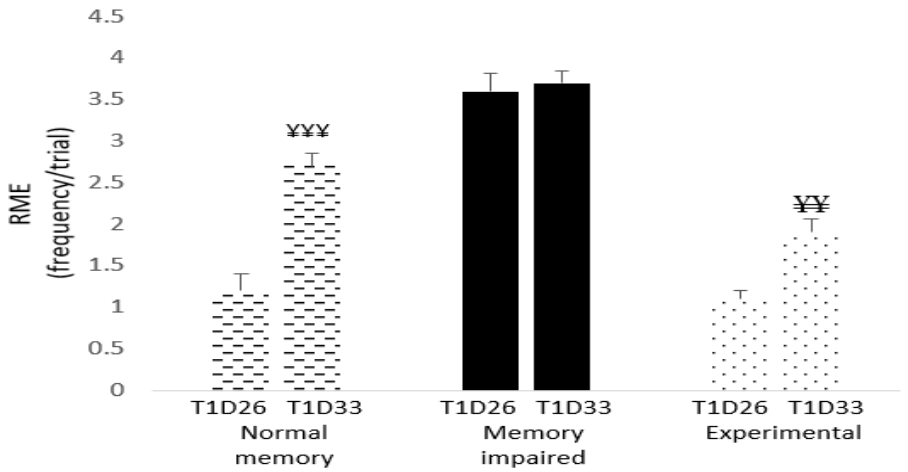

Figure 10: RME after 7days interval in Radial arm maze in different groups of rats. Each bar symbolizes mean±SEM trial of 10 rats. T1D26 Mean trial 1 of day 26; T1D33: mean trial 1 of day 33; Statistical analysis was done by Student's paired t test (between trials). In the interpretation of results, p≤0.05 was considered as significant. ¥≤0.05; ¥¥≤0.01; ¥¥¥≤0.001.

Each bar symbolizes mean±SEM trial of 10 rats. 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.

DISCUSSION

The current study sought to investigate the effects of AILE on working and reference memory in male Wistar rats with ketamine-induced memory impairment, utilizing the RAM as the assessment tool. A sub-anesthetic dose of ketamine (15 mg/kg) was employed to induce memory deficits, which manifested as increased errors in both working and reference memory. These findings align with previous studies that have demonstrated similar cognitive impairments following ketamine administration [17]. The cognitive deficits are likely attributable to the blockade of NMDA receptors in critical regions for memory processing, such as the prefrontal cortex and hippocampus. Consistent with earlier research, the ketamine-treated rats in this study exhibited a significant rise in errors related to working and reference memory in the RAM, suggesting disrupted cognitive processing. Ketamine's antagonistic action on NMDA receptors, particularly within the prefrontal cortex and hippocampus, is known to impair normal synaptic activity and neurotransmission, leading to cognitive deficits. This effect may be due to the inhibition of NMDA receptor-mediated signaling on GABAergic interneurons, which causes a dysregulation of glutamate transmission, resulting in neuronal dysfunction, mitochondrial damage, apoptosis, and increased oxidative stress [18, 19].

In contrast, AILE administration in ketamine-treated rats showed a protective effect on both working and reference memory, as evidenced by a significant reduction in memory errors in RAM. This suggests that AILE can counteract the cognitive impairments induced by ketamine. The neuroprotective effect of AILE may involve modulation of NMDA receptor function, enhancement of antioxidant defenses, and inhibition of apoptotic pathways. Previous literature supports the neuroprotective properties of herbal extracts like AILE [10,11]. The decreased errors in working and reference

memory observed in the RAM suggest that AILE may positively influence NMDA receptor function and enhance neurotransmission. Components of AILE, such as quercetin, are known to increase the expression of NR2A and NR2B subunits of NMDA receptors, potentially facilitating synaptic plasticity and memory retention [20]. Furthermore, AILE's ability to decrease the expression of pro-apoptotic proteins, including cytochrome c and caspase, in the hippocampus supports its role in mitigating the neurotoxic effects of ketamine, which lead to mitochondrial dysfunction and neuronal apoptosis [21]. Additionally, AILE's antioxidant properties may contribute to reducing oxidative stress, a key factor in cognitive decline, thereby improving cognitive function in ketamine-treated rats. The combination of mitigating oxidative stress and enhancing NMDA receptor functionality likely explains the observed improvements in both working and reference memory.

Overall, the study provides strong evidence that AILE exerts a protective effect on both working and reference memory in rats with ketamine-induced cognitive deficits, potentially through a combination of NMDA receptor modulation, reduction of oxidative damage, and suppression of apoptotic pathways.

CONCLUSION

The findings of this study indicate that AILE has a protective effect on both spatial working and reference memory in male Wistar rats with ketamine-induced memory impairment. By reducing memory errors in the Radial Arm Maze, AILE demonstrated its capacity to counteract ketamine's detrimental effects on cognitive function. These results underscore AILE's therapeutic potential in managing cognitive impairments associated with NMDA receptor dysfunction. Further research is recommended to elucidate the precise mechanisms and broader applications of AILE in cognitive impairment.

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