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Does *Nigella sativa* oil prevent ketamine induced spatial memory impairment? An experimental study in male Wistar rats

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Abstract

Background: Spatial memory impairment has significant negative influence on both survival and quality of life. *Nigella sativa* oil (NiSO) has been investigated for its potential to reduce memory impairments in various experimental models. **Objectives:** To assess the effects of NiSO on ketamine induced working and reference memory impairment in male Wistar rats. **Methods:** This experimental study was conducted from March 2020 to February 2021 in the Department of Physiology, after obtaining ethical approval from the Institutional Review Board of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. For this purpose, 60 male Wistar rats were divided into normal memory (normal saline 5 ml/kg for 26 days), impaired memory (ketamine 15 mg/ kg during acquisition phase) and experimental (treated with NiSO 1 ml/kg for 26 days and ketamine 15 mg/kg during acquisition phase) groups. All groups underwent Radial arm maze (RAM) and Morris water maze (MWM) tests. Variables were working memory error (WME, re-entry into already visited arm) and reference memory error (RME, entry into non-baited arm) in RAM along with escape latency (EL, the moment of a rat's entrance into the water upto it's arrival at the platform) and target crossing (TC, number of crossing of the quadrant from where the platform was removed) in MWM.

Data were expressed as mean±SEM and statistically analyzed with ANOVA followed by Bonferroni post-hoc test, where p≤0.05 was considered as significant. **Results:** Significantly (p≤0.001) higher WME and RME as well as significantly (p≤0.001) delayed EL and reduced TC were found in memory impaired rats when compared to normal memory rats. However, significantly (p≤0.001) lower WME and RME as well as significantly reduced EL (p≤0.001) and higher frequency of TC (p≤0.001) were observed in experimental rats, when compared to memory impaired rats. Moreover, these variables were almost similar in the experimental rats, in comparison to, those of normal memory rats except significantly (p≤0.01) higher TC in MWM test. **Conclusions:** NiSO prevented working and reference memory impairment as well as enhanced reference memory in rats.

Keywords: Memory impairment, ketamine, working memory error, reference memory error, escape latency, target crossing, *Nigella sativa* oil.

Introduction

Spatial memory impairment is associated with aging, smoking, alcohol consumption, physical inactivity, obesity, social isolation and diseases such as, hypertension, diabetes, depression, cancer, syphilis, HIV, hypo or hyperthyroidism etc.[1,2]

It hampers daily activities, causing affected individuals to abandon leisure activities due to feelings of incompetence. Emotional distress like anxiety, depression, frustration can also occur.[3,4]

Memory impairment is a symptom of dementia, which has a global prevalence of 4.86% among individuals aged 60 and above and increasing day by day .[5] In Bangladesh, 3.6% rural people above 60 years age suffer from dementia.[6]

The spatial memory which is storage and retrieval of information within the brain, is needed to recall locations and object placements.[7] Working memory, a form of short term memory, temporarily stores limited spatial information for immediate access during cognitive tasks. This information is either quickly forgotten or stored as long-term reference memory.[8] Reference memory, in contrast, retains consistent and useful information for several weeks or months, aiding decision-making and spatial navigation with ease.[9]

Many mechanisms have been proposed for this spatial memory formation where different receptors are involved.[8] One of these is NMDA (N-methyl D-aspartate) receptor, an ionotropic glutamate receptor which promotes memory, learning and cognition specially in spatial context.[10-12] In working memory, this receptor is associated with persistent neural activity and in reference memory, with various forms of synaptic plasticity in the CNS including long term potentiation (LTP).[8,13]

Ketamine, a noncompetitive NMDAR antagonist, is reported to impair spatial memory in human abusers[14] and different animal models.[12,15] Ketamine may block NMDA receptors in the prefrontal cortex, disrupting pyramidal neuron activity and impairing working memory. It may also block NMDA receptors on GABAergic interneurons in the cerebral cortex and hippocampus (krystal), leading to excessive glutamate release and AMPA receptor activation.

This triggers Ca^{++} influx, disrupting homeostasis, causing mitochondrial dysfunction, and leading to apoptosis.[16] Moreover, ketamine showed oxidative stress and cholinesterase activity in brain, impairing spatial memory.[17] It has been found that a sub-anesthetic dose of 15 mg/kg body weight (intraperitoneal) does not induce anesthesia or locomotor impairment but impairs acquisition of spatial memory. [12,15]

So far, spatial memory impairment has no definitive curative treatment. Prolonged use of current drugs regime leads to declining efficacy and severe side effects.[18] Consequently, researchers are exploring alternatives, including herbal remedies. *Nigella sativa,* a medicinal herb, showed various beneficial effects. The Prophet Hazrat Muhammad (*sallâ Allâhu ¿alayhi wa-sallam*) stated that the black seed can heal every disease except death (Sahih al-Bukhari 71:591,592).[19] *Nigella sativa* oil (NiSO) was found to attenuate scopolamine-induced memory impairment.[20] It was also found to enhance memory in ethanol induced memory impaired rats,[21] normal rats,[22,23] and healthy human volunteers.[24] Its neuroprotective, cholinergic, and antioxidant properties contribute to its memory-enhancing effects. But as far as we searched, no study was found to explore the role of NMDA receptor in prevention of memory impairment by this herb. On the basis of this background, the present study was done to evaluate the effects of NiSO on Ketamine induced memory impaired male Wistar rats.

Materials and methods:

Duration of study

This experimental study was conducted from March 2020 to February 2021 in the Department of Physiology, after obtaining ethical approval from the Institutional Review Board of Bangladesh Medical University (BMU), Dhaka, Bangladesh (Registration no. BSMMU/2020/8872).

Drugs

Normal saline (Beximco Pharma Limited, Bangladesh), ketamine (ACI Pharmaceuticals Limited, Bangladesh), and di-ethyl ether (MERCK, Germany) were purchased from the local market. *Nigella sativa* oil [according to Pighinelli and Gambetta (2012) [25]] was obtained from a local *'ghani'*.

Procurement and maintenance of animals

A total of 60 male Wistar rats (200 ± 50 gm) were obtained from the central animal house of BMU. They were housed in specially designed plastic cages with four rats per cage under a 12-hour light/dark cycle in the animal research laboratory of Department of Physiology, BSMMU.[26] During acclimatization, they had free access to standard laboratory food[26] and cooled boiled water *ad libitum*. All experiments were conducted according to the þGuidelines for the Animal Experimentation Ethics Committee (AEEC)'of the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b, Bangladesh).[27] To prevent circadian effects, all trials were conducted between 8:00 to 16:00.[28]

Experimental design and dose schedule

Based on treatments, rats were randomly divided into 3 groups (20 rats/group):

- Normal memory: oral normal saline 5 ml/kg[28] for 26 days
- Impaired memory: intraperitoneal ketamine 15 mg/ kg[12] during the acquisition phase of RAM test and working memory test as well as acquisition phase of reference memory test during MWM test
- Experimental: oral NiSO 1 ml/kg[29] for 26 days and intraperitoneal ketamine 15 mg/kg during the same test phases as the impaired memory group.

Further, based on memory performance tests, all groups were divided into RAM and MWM test groups (10 rats/group).

2.5 Spatial memory assessment by Radial arm maze (RAM) test:

Test tools and circumstances

In the experiment, an 8-arm standard radial maze[10, 11, 30] was set up in a well-lit room (Figure 1) with extra maze visual cues, such as, shelves, desktop computers, air conditioners, doorways, etc. . The maze remained in a fixed position. Constructed of plexiglass, it stood 70 cm above the ground and comprised of eight arms (length 60 cm from the center, width 17 cm, height 25 cm) radiating from a central octagonal platform (42 cm diameter). A recessed food cup (2 cm deep × 3 cm across) positioned 4 cm from the end of each arm. Clear plexiglass guillotine doors were used via a pulley system to control access to arms from the central platform.

RAM Test

Tests were done according to previous researches.[10, 11, 31] All 30 rats underwent a 7-day room acclimatization before RAM test. Each rat completed two trials daily (trial 1 and trial 2), spaced three hours apart. In this test, a fasting rat had to search food. To motivate the rats, they were food-deprived (but not water-deprived) for around 10 hours before Trial 1. Trial 1 was initiated 30 minutes after administering the prefixed treatment based on group assignment. The maze was meticulously cleaned with 70% alcohol after every trial in order to reduce any lingering odor. Rats were introduced to the bait (small jilapi pellets) in their cages for three days before habituation began.This test comprised 3 phases (Figure 2A).

(i). Habituation (from day 16 to day 21)

Rats were introduced to the maze for habituation for 6 consecutive days. On day 16, two rats at a time explored the maze for 10 minutes with baits scattered across the floor (platform, 8 arms, and food cups) to lessen each rat's resistance to exploration. Each rat explored individually with the same baiting on day 17. These two days were used to acclimatize the rats procedurally and instrumentally for this test. On day 18 and 19, baits were placed only in the eight food cups. However, on day 20 and 21, randomly selected (by lottery) any 4 arms were baited (Figure 1b). These arms remained fixed for each rat throughout the test but varied between rats. During this period, all gates remained open.

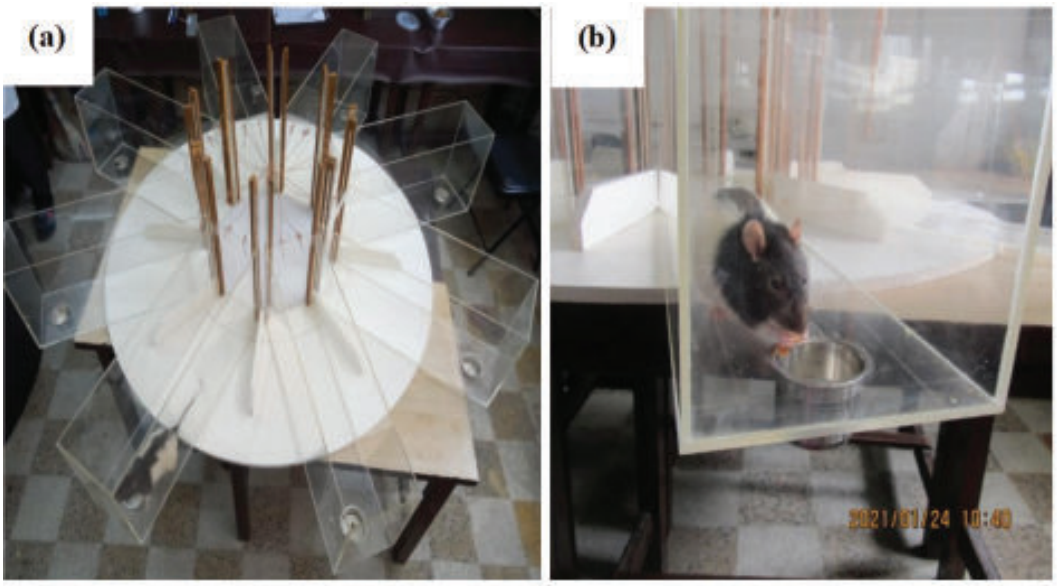

Figure 1: Radial arm maze (a) at a glance (b) rat eating bait in food cup.

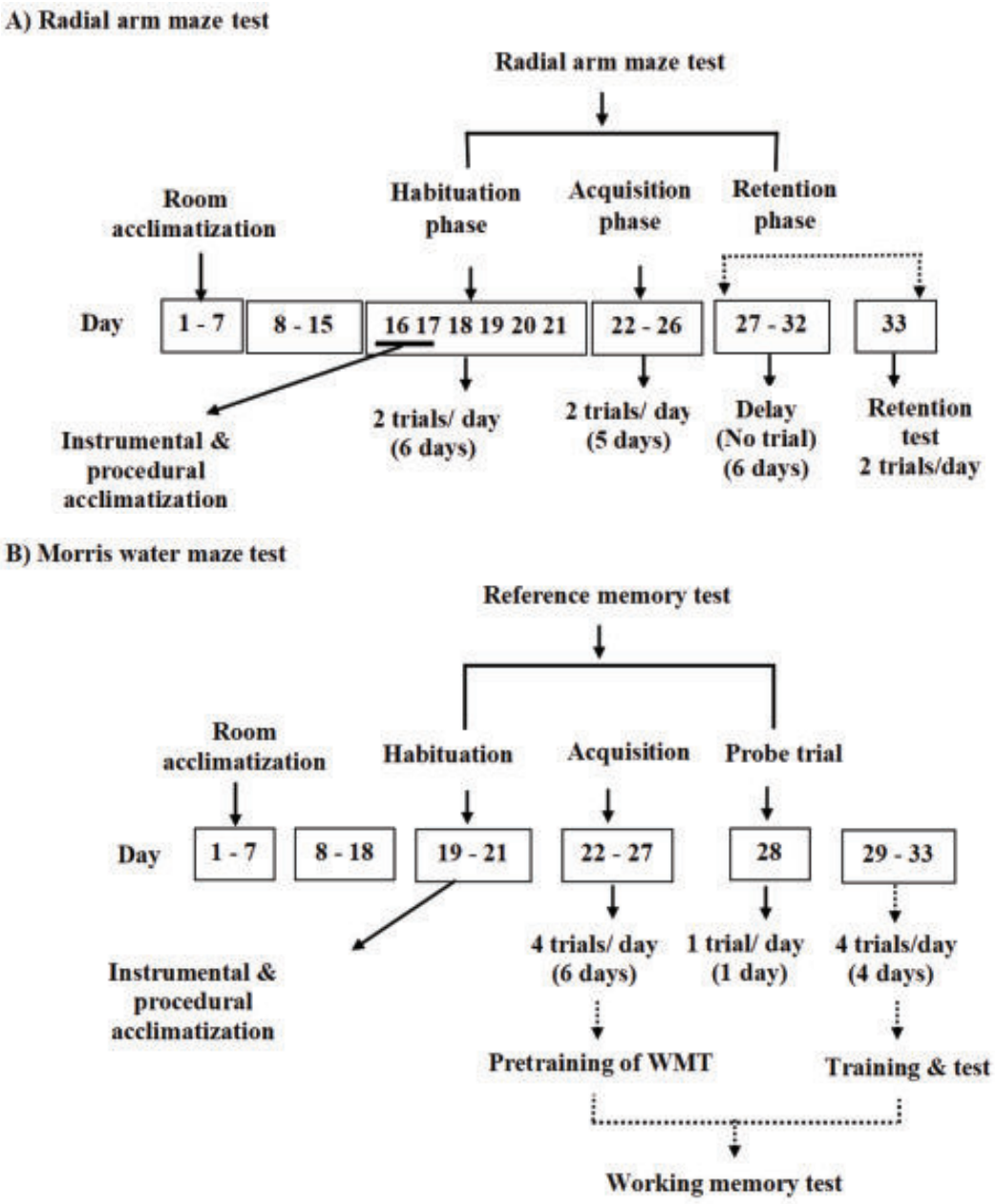

Figure 2: Work plan of A) Radial arm maze test and B) Morris water maze test

(ii). Acquisition phase (from day 22 to day 26)

The performance was tested for 5 days. Randomly chosen any 4 of the 8 arms (previously determined in habituation phase) were baited by 'jilapi' only in food cup. At the start, the rat was placed at the center of the platform with all gates closed. Then all gates were opened at a time. When the rat entered any one arm, other gates remained closed. After exploration, the rat came out and its gate was closed. Just 5 seconds later, all gates were

reopened and the whole process was repeated. Trials lasted a maximum of 10 minutes or until the rat ate all baited jilapis, whichever occurred first.

(iii). Retention phase (from day 27 to day 33)

Seven days after the last acquisition day, a retention test was conducted with two trials in a single session in similar manner as in acquisition phase.

Evaluation of spatial memory

A rat's entry into an arm was counted when all four paws were inside. Working memory error (WME) was calculated by the number of re-entries of a rat in a baited arm and number of first time entries into unbaited arms as reference memory errors (RME).[11, 22]

Spatial memory assessment by Morris water maze (MWM) test:

Test tools and circumstances

The MWM, according to earlier studies,[32, 33] was a water-filled circular pool (150 cm diameter x 50 cm height). To prevent visual cues within the pool, the entire inner wall and platform were painted a non-toxic black color. The pool was placed in a room containing extra maze cues (such as, racks, window, door, shelves, computer, experimenter etc.), for orientation. Eight starting points, north (N), south (S), east (E), west (W), north-east (NE), north-west (NW), south-east (SE) and south-west (SW) were used, dividing the pool into four quadrants. In the centre of the NE quadrant, a black round platform (15 cm in diameter X 28 cm in height) was placed with its top 2 cm below the water's surface to make it invisible from the inside.

Two testing paradigms were applied sequentially, to evaluate reference and working memory (Figure 2B).

Reference memory test:

Rats swam for three minutes without a platform for consecutive 3 days during the instrumental acclimatization and habituation phase. During the acquisition phase, The platform was placed in the NE quadrant. The rats underwent four trials daily for six consecutive days starting from different points in a varying sequence. Each trial lasted 60 seconds or until the rat found and climbed onto the platform. An inter-trial period of 50 seconds (20 seconds on the platform + 30 seconds for self-drying) was maintained.

A stop watch was used to measure the escape latency (EL) (time from the moment of a rat's entrance into the water up to it's arrival at the platform)[34] to evaluate the rats' learning ability. Similar 4 trials were given for all rats for continuous 6 days. On the first trial each day, rats reached the platform by chance, that constituted the information stage and the subsequent trials required matching to the novel position for every day, as platform was fixed for 6 days. The average EL of 1st to 6th acquisition days was measured to assess memory consolidation.

About 24 hours after the last acquisition trial, spatial probe trial was given to assess learning strength/ retrieval. In this trial, the platform was taken out of the pool and rats were allowed to swim *ad libitum* for 60 seconds during which target crossing (TC, the quadrant of MWM from where the platform was removed)[32] was measured.

Working memory test:

About 48 hours after the probe trial, the working memory test was done using a testing paradigm adapted from Sarihi et al. (2000).[32] Here, the 6-day acquisition phase of reference memory test was regarded as pre-training phase. Then, a training and test phase was conducted over 4 consecutive days with 4 trials per day. Everyday, the platform position was changed but kept constant for daily 4 trials. Each rat was released from four different starting points each day which were distant from platform position. On the first trial of each day, rats reached the platform by chance, that constituted the information stage

and the subsequent trials required matching to the novel position for that day as platform was changed every day. The mean EL in training and test phase was recorded as above to assess learning ability .

Statistical analysis

Results were expressed as mean±SEM of the study variables. Statistical tests were done by ANOVA (among groups) followed by Bonferroni post-hoc test (between groups) in SPSS (version 16.0), where $p \leq 0.05$ was considered as statistically significant.

Results:

RAM test

Effect of NiSO on working memory error (WME)

The mean WME in ketamine treated memory-impaired rats was significantly ($p \leq 0.001$; $p \leq 0.01$; $p \leq 0.05$) higher than in normal memory and NiSO pretreated experimental rats during most of acquisition and retention days except at trial 1 on day 22, 23 & 24 and trial 2 on day 24 indicating impaired memory in this group and reduction of memory impairment in NiSO pretreated experimental. Again, the lack of significant WME differences between normal memory and experimental rats on all acquisition and retention days except day 23 ($p \leq 0.05$) and day 24 ($p \leq 0.01$) demonstrated NiSO not only protected against ketamine-induced working memory impairment but also enhanced early acquisition in experimental rats than normal rats (Figure 3A).

When WME was compared between trial 2 and trial 1 during the acquisition phase, both normal and experimental rats showed a significant reduction ($p \leq 0.05$), whereas ketamine-treated rats displayed no change, indicates gradual reduction of memory error in normal memory and experimental and persistent impairment in ketamine (Figure $4A_1$). This suggests NiSO aids in memory recovery and retain learning enhancement. Again, no significant differences were found between the number of errors in trial 1 of the following day and trial 2 of the previous day across all groups (Figure $4B_1$). This suggests no difference in residual memory for a very short period among all group of rats. NiSO did not affect short term residual memory. In addition, the mean WME difference at T1 between days 33 and 26 was non-significant across all groups (Figure $4C_1$), further supporting this finding.

Effect of NiSO on reference memory error (RME)

Higher RME of the ketamine treated memory impaired rats ($p \leq 0.001$) in comparison to normal memory and NiSO pretreated experimental rats during both trials on most acquisition and retention days, except trial 1 and 2 on day 22 & T1 on day 23. A significant ($p \leq 0.001$) difference in RME between normal and experimental rats on day 33 (Figure 3B) suggests that ketamine increased errors while NiSO reversed this effect. These results indicate that NiSO not only prevents ketamine-induced memory loss but also enhanced retention of reference memory beyond normal levels. Furthermore, Lower RME in the normal ($p \leq 0.05$) and NiSO-pretreated groups ($p \leq 0.05$) compared to the ketamine group between trial 1 and trial 2 during the acquisition phase (Figure $4A_2$) suggests that memory errors gradually decreased in normal and experimental rats but remained unchanged in ketamine-treated rats. Additionally, while normal memory rats showed a significantly higher mean RME on day 33 compared to day 26, no significant difference was observed in the experimental and memory-impaired groups, suggesting that NiSO did not affect residual memory (Figure $4C_2$).

MWM test

Working memory performance

Figure 5A showed that Ketamine impaired working memory, as evidenced by significantly increased ($p \leq 0.001$) EL, compared to normal memory rats. However, NiSO improved our rats' learning ability performance, as evidenced by statistically ($p \leq 0.001$) significant differences in mean EL of memory impaired and experimental rats. Strikingly, mean EL between experimental and normal memory across all trials and test days were found statistically non-significant indicating

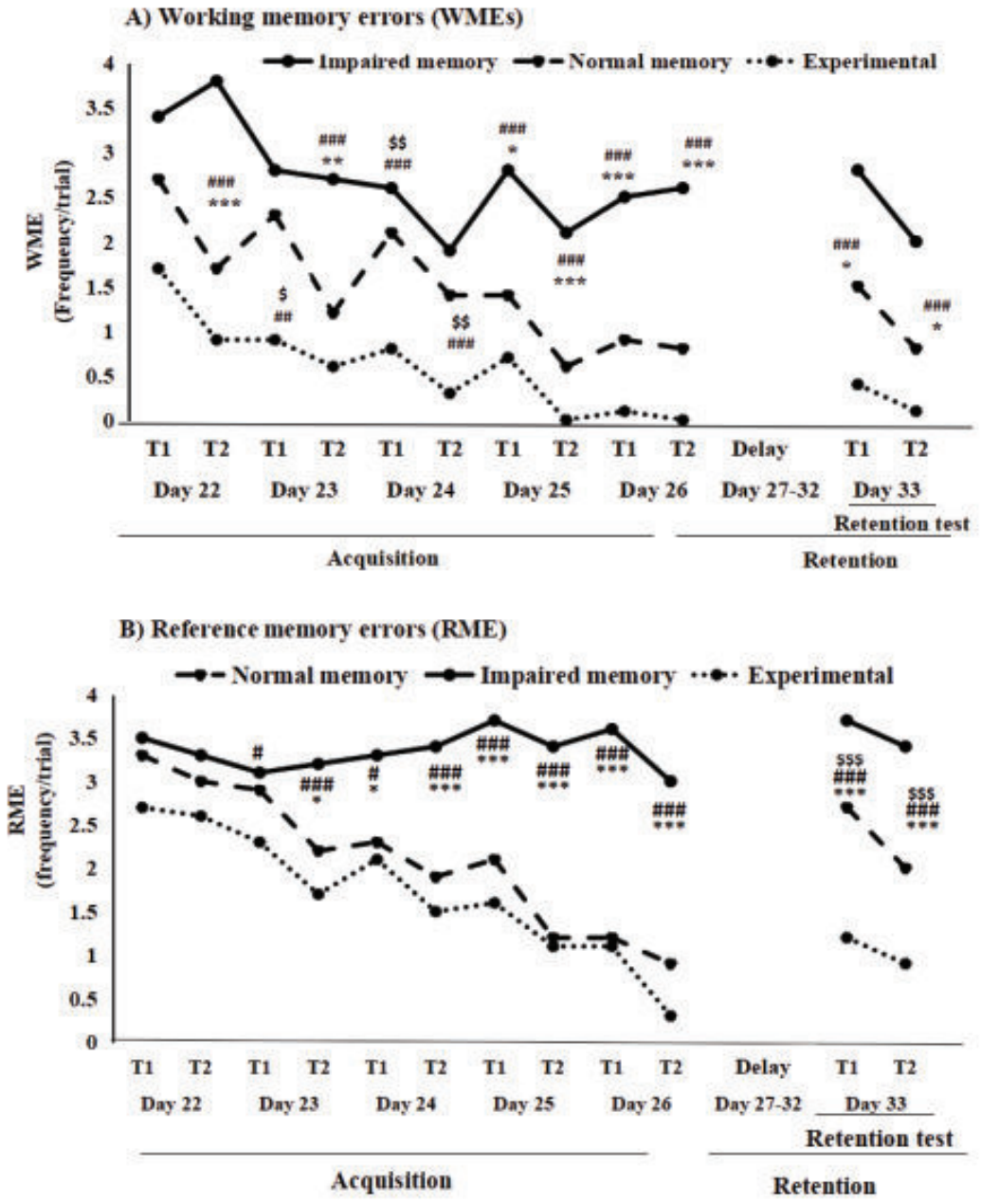

Figure 3: WMEs (Working memory errors) and RMEs (Reference memory errors) in different days of Radial arm maze in different groups of rats. Each line symbolizes mean±SEM trials of 10 rats. T1: mean trial 1 on that day; T2: mean trial 2 on that day. Statistical analysis was done by ANOVA (among groups) followed by Bonferroni Post hoc test (between groups). * = Normal memory vs impaired memory; $ = Normal memory vs Experimental; # = Impaired memory vs Experimental; In the interpretation of results, pd"0.05 was considered as significant; */#/$: pd"0.05; **/##/$$: pd"0.01; ***/###/$$$: pd"0.001; Error bar was omitted for clarity.

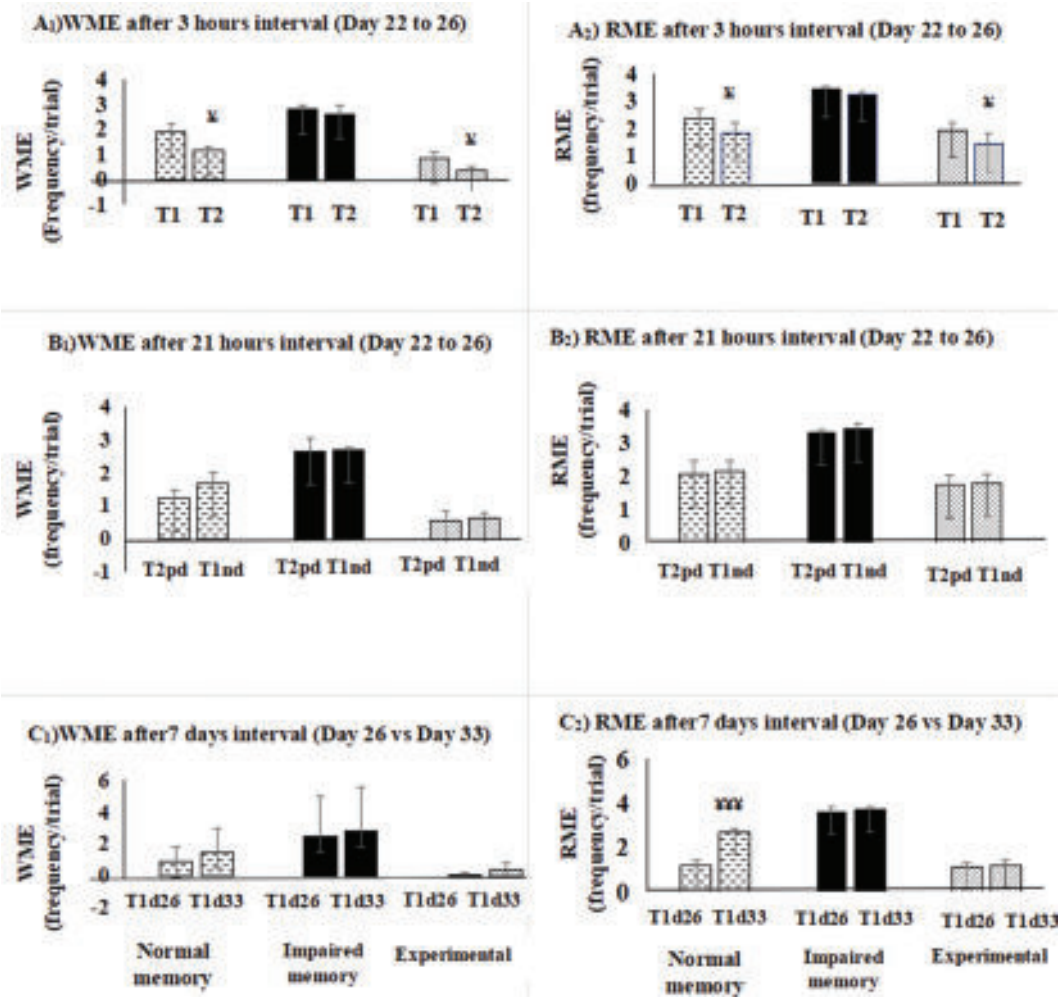

Figure 4: WMEs (Working memory errors) and RMEs (Reference memory errors)with different interval in Radial arm maze in different groups of rats. Each bar symbolizes mean±SEM trial of 10 rats. T1: mean trial 1 on that day; T2: mean trial 2 on that day; T2pd: mean working memory error of trial 2 of previous days (Day 22, 23, 24, 25); T1nd: mean working memory error of trial 1 of next days (Day 23, 24, 25, 26); T1d26: mean trial 1 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; ¥: p≤0.05; ¥¥¥: p≤0.001.

that ketamine impaired the learning ability of working memory which was reversed near to normal by NiSO.

Reference memory performance

Ketamine-treated rats showed significantly (p≤0.001) higher mean EL across all acquisition days compared to normal memory rats. However, NiSO improved our rats' learning ability performance, as evidenced by statistically (p≤0.001) significant differences in mean EL of our memory impaired and NiSO pretreated experimental rats. Strikingly, mean EL between experimental and normal memory on all trials was found statistically non-significant except day 22. These data revealed that ketamine impaired the learning ability of working memory and this impairment was reversed near to normal by NiSO (Figure 5B). However, The significantly higher

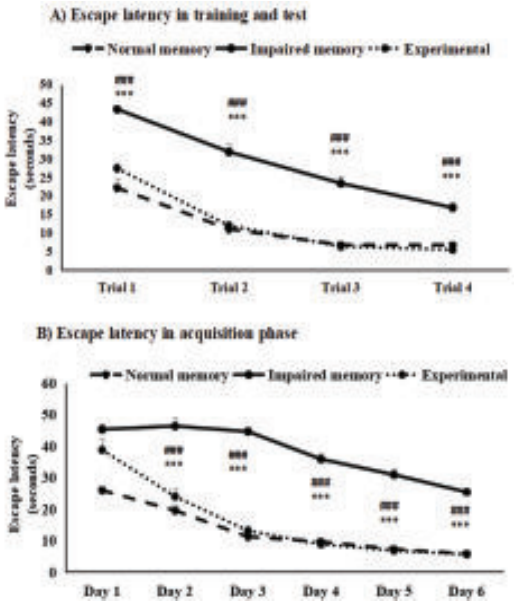

Figure 5: A) Escape latency in training and test, B) Escape latency in acquisition phase in different days of Morris water maze test in different groups of rats. Trial 1: mean±SEM of 4 trial 1s of 10 rats during training and test; Trial 2: mean±SEM of 4 trial 2s of 10 rats during training and test; Trial 3: mean±SEM of 4 trial 3s of 10 rats during training and test; Trial 4: mean±SEM of 4 trial 4s of 10 rats during training and test. Each day symbolizes mean±SEM escape latency of 4 trials in that day of acquisition phase for 10 rats. * = Normal memory vs impaired memory; $ = Normal memory vs Experimental; # = Impaired memory vs Experimental; In the interpretation of results, pd^0.05 was considered as significant; */#/$: pd^0.05; **/##/$$: pd^0.01; ***/###/$$$: pd^0.001.

EL in the experimental group compared to normal memory rats on day 22 ($p \leq 0.01$) suggested a delayed learning onset in the experimental group.

In our study, memory impaired rats showed retrieval impairment, as evidenced by significantly ($p \leq 0.001$) lower TC than normal memory rats. NiSO significantly ($p \leq 0.001$) increased TC in experimental rats compared to the impaired memory group. Additionally, the difference between experimental and normal memory rats was significant ($p \leq 0.01$) (Figure 6), indicating that NiSO not only prevented ketamine-induced retrieval impairment but also enhanced learning strength beyond normal levels.

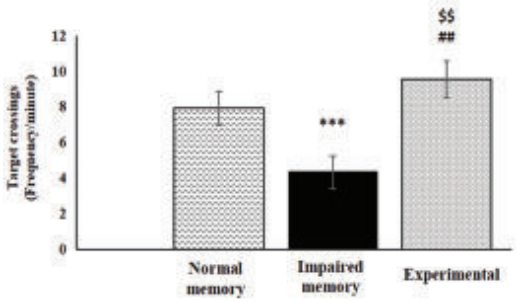

Figure 6: Target crossing in Morris water maze in different group of rats. Each bar symbolizes mean±SEM for 10 rats. * = Normal memory vs impaired memory; $ = Normal memory vs Experimental; # = Impaired memory vs Experimental; In the interpretation of results, $p \leq 0.05$ was considered as significant; */#/$: $p \leq 0.05$; **/##/$$: pd"0.01; ***/###/$$$: $p \leq 0.001$.

Discussion

The study was undertaken to assess the effects of NiSO on memory using ketamine-induced memory-impaired male Wistar rats. The effects of NiSO on working and reference memory acquisition and retrieval were evaluated using RAM and MWM which were commonly used tools. In the RAM test, rats were placed in the center of a maze with arms extending outward. Some arms contained baits, requiring the rats to remember which arms were previously visited and which still contained food. The goal was to make to learn and remember the most optimal paths, minimizing rat's visit to unbaited arms and revisits to previously visited arms. In the MWM test, rats had to locate a hidden platform to escape the water by swimming the shortest distance possible.

In our study, a sub-anesthetic dose of ketamine (15 mg/kg) impaired both working and reference memory in memory impaired group, as evidenced by significantly increased WME and RME in the RAM test and prolonged EL with reduced target crossings in the MWM test, in comparison to those of normal memory rats. Similar observations of increased escape latency were also reported by other authors.[12, 15]

In contrast, NiSO prevented memory impairment in experimental rats, as evidenced by significantly lower WME and RME in the RAM test and decreased escape latency and increased number of target crossings in the MWM test, in comparison to those of memory impaired rats. Similar observation of decreased escape latency was reported by other investigators.[21, 29] Here, NiSO might increase the expression of NR1 subunit of NMDA receptors and PSD-95 (post synaptic density 95) in the hippocampus.[23] PSD-95 might promote surface expression of NMDARs, enhance their channel opening and decrease desensitization of NMDAR responses.[35, 36] Furthermore, both *N. sativa* and its active component, thymoquinone, might prevent upregulation of cyto c gene expression, caspases in CNS,[37] decrease oxidative stress in CNS[20] and decrease acetylcholinesterase activity in the brain.[38] Therefore, either increment of the subunits of NMDA receptors or its regulatory protein or antiapototic proteins / decrement of oxidative stress or acetylcholinesterase / above all might contribute to the prevention of working and reference memory impairment in NiSO-treated rats.

Since ketamine is an NMDA receptor antagonist and NiSO counteracted ketamine-induced impairment, it is apparent that this action involved NMDA receptor.

A noteworthy finding in our study was that NiSO not only prevented memory impairment, but also enhanced reference memory retrieval in experimental rats beyond normal levels. Additionally, NiSO-treated rats showed delayed acquisition in the MWM test compared to normal memory rats. As far our knowledge goes, this was the first data showing delayed acquisition of NiSO pretreated group in the MWM. However, no relevant study was available to support and explain this observation of NiSO.

Conclusion

From this study it might be concluded that *Nigella sativa* oil prevents working and reference memory impairment and enhance reference memory in male Wistar rats.

In this study, manually operated memory performance tests were used. So we recommend computer operated memory performance tests to ascertain our findings.

Conflict of interest

Authors declare no conflict of interest.

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