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Research Article
Protective Effects of Trolox on Ketamine-Induced Memory Impairments and Morphological Changes in the Brain

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Abstract

Background and Objective: Ketamine has demonstrated potential in treating various neuropsychiatric disorders at low doses. However, its memory-impairing and neurotoxic effects and abuse potential present limitations to its use. This study aimed to investigate ketamine's effects on memory functions, brain morphology and lipid peroxidation in a time- and dose-dependent manner and the protective effects of Trolox. **Materials and Methods:** Forty-eight male BALB/c mice were administered low and high doses of ketamine (10, 30 mg/kg/day, respectively) sub-chronically and chronically (7 and 21 days, respectively). A subgroup also received Trolox (20 mg/kg/day) for 21 days combined with high-dose ketamine. Following the drug administrations, behavioral tests were performed, including a modified elevated plus maze, a novel object recognition and a passive avoidance test. In the brain, malondialdehyde levels and morphology were examined. The results were analyzed using a one-way analysis of variance followed by a *post hoc* Tukey's test. **Results:** Chronic high-dose ketamine impaired spatial, emotional and recognition memory. Subchronic high-dose ketamine did not affect emotional and recognition memory but did impair spatial memory. Low-dose ketamine did not produce impairments. Malondialdehyde levels were elevated and morphological changes were evident in the chronic high-dose ketamine-applied group. These alterations were attenuated with Trolox. **Conclusion:** The memory-impairing and neurotoxic effects of ketamine are linked to increased oxidative stress. Antioxidant molecules like Trolox can be practical against the toxicity of ketamine.

Key words: Ketamine, memory impairment, neurodegeneration, oxidative stress, malondialdehyde, Trolox, neuroprotection

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Ketamine, an N-Methyl-D-Aspartate (NMDA) receptor antagonist drug, has shown therapeutic promise in treating depression, bipolar disorder, anxiety spectrum disorders, substance use disorders, eating disorders and pain management[1,2]. Despite its potential for treating addiction, it presents a paradox as it also has abuse potential[3]. The therapeutic applications and abuse potential created the need to investigate its adverse effects on memory encoding processes and neuronal morphology. In both clinical and preclinical studies, chronic ketamine use has been associated with memory deficits. It impacts verbal/visual memory, executive functions, spatial recognition and reference memory and other forms of memory[4-7]. This association led to the utilization of ketamine, along with several other NMDA receptor antagonists, to induce memory impairments for modeling dementia and the cognitive side effects of schizophrenia[8,9]. Exposure to ketamine has been associated with structural modifications within the brain. Research indicates ketamine causes diminished dendritic growth, decreased dendritic branches and reduced spine density[10]. Furthermore, it decreases the number of neurons[11], disrupts mitochondrial membranes[12], elevates apoptotic markers and diminishes protective proteins[13]. These effects are related to the applied dose and duration of exposure; higher doses and prolonged use are associated with more severe toxicity[10,14].

Ketamine increases oxidative stress, which is considered crucial to its memory-impairing and neurotoxic effects[11]. Elevated oxidative stress can cause cellular damage and contribute to various neurodegenerative pathological processes[15]. Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid) is a water-soluble analog of vitamin E and a potent antioxidant. It has shown the potential to provide neuroprotection by reducing oxidative damage[16] and may help reduce ketamine-induced alterations.

To understand the time- and dose-dependent effects of ketamine, memory functions (recognition, spatial, emotional), oxidative stress and the morphology of neuronal structures following subchronic and chronic administrations of two doses of ketamine were evaluated in mice. The protective effects of an antioxidant molecule, Trolox in a high-dose chronic ketamine-applied subgroup were also examined.

MATERIALS AND METHODS

Study area: This research project was conducted from January, 2022 to December, 2023 in the Medical Pharmacology Psychopharmacology Laboratory, Dicle University, Turkey.

Animals: Forty-eight male BALB/c mice (DUSAM, Diyarbakir, Turkey) weighing 30-40 g were used in the experiment and separated into six groups. The mice were housed in standard laboratory conditions with a 12 hrs light/dark cycle (lights on at 07:00 am) at a temperature of 22±2°C. They were housed 8 per cage and had access to water and food pellets *ad libitum*. The experiments were conducted between 9:00 and 15:00 and each animal was tested only once.

Ethical consideration: The procedures conducted on the animals were done by the ethical standards defined by the European Community Council Directive (24 November, 1986). Ethical approval was received from the Dicle University Animal Experiments Ethics Committee (No:2015/55).

Drug applications and experimental procedure: A racemic ketamine solution (Ketalar, Pfizer, USA) was used in two different doses (10 and 30 mg/kg/day) for different durations (1 week as subchronic and 3 weeks as chronic). Trolox (20 mg/kg; Sigma, St. Louis, Missouri , USA) was used alone or in combination with high-dose ketamine (30 mg/kg) and chronically applied (3 weeks). Both drugs were dissolved in saline and administered intraperitoneal injection at 10 mL/kg of body weight daily between 9 and 10 am. The drug dosages were chosen based on previous studies in the literature[17-19]. Behavioral tests (novel object recognition, modified elevated plus maze and passive avoidance) were conducted at the end of the injection protocol.

Following behavioral tests, mice were sacrificed by cervical decapitation. Brain tissues were taken and stored at -80°C or in a 10% neutral buffered formaldehyde solution for subsequent biochemical and histological analyses. All experiments were conducted in a semi-soundproof, dimly lit room, except the mEPM test, which was performed in a well-lit environment.

Behavioral studies

Novel object recognition (NOR) test: Recognition memory functions were evaluated using a NOR test following a previously employed protocol[20]. The test was done using an open-field apparatus-a black-colored square with high walls (40×40×20 cm, MAY OP-M). The NOR test is based on rodents' preference for novel objects over familiar ones. The test was conducted in three trials: Habituation, acquisition and retention. During the habituation trial, mice were individually placed in the apparatus and allowed to explore freely for 5 min. About 30 min later, the acquisition trial was conducted. In this trial, two identical objects were placed on one side of the square; each mouse was given 5 min to explore.

One hour after the acquisition trial, the retention trial was conducted; one familiar object was replaced with a novel one. The exploration behavior of the mice is defined as facing one of the objects or touching them with their nose. The results were analyzed using Ethovision XT 11 software. The recognition memory function was calculated using the ratio index (RI). It is calculated by the time spent exploring the novel object divided by the time spent exploring both objects multiplied by 100. A higher RI value suggests healthy memory and a lower value indicates impairment.

Modified elevated plus-maze (mEPM) test: The spatial long-term memory functions were evaluated using a mEPM test apparatus (MAY EPM01-M) following a previously utilized protocol[21]. The apparatus contains two open (29×5 cm) and two closed (29×5×15 cm) arms facing each other, connected by a central platform (5×5 cm). The maze was elevated 40 cm above the ground. And the room was illuminated. The test was conducted in two trials: Acquisition (day 1) and retention (day 2). In the acquisition trial, each mouse was placed at the end of an open arm facing away from the center. The transfer latencies to enter either closed arm were recorded. Upon entering, the mice were allowed to explore for 10 sec freely and then were returned to their home cage. Mice that did not enter either closed arm within 90 sec were excluded from the experiment. The retention trial (day 2) was performed 24 hrs after the acquisition trial. The same procedure was executed and the transfer latencies to enter closed arms were recorded. Higher values indicated disrupted spatial memory, while lower values meant intact spatial memory.

Passive avoidance (PA) test: Emotional memory functions were evaluated using a light-dark PA test apparatus (MAY-PA 1014-M) based on a previously utilized protocol[21]. This test is based on two basic rodent behaviors: They prefer to stay in dark places and avoid places associated with an aversive event. The apparatus had one illuminated (2000 lux) and one dark compartment (22×21×22 cm each) connected by an automatic door (7×7 cm). The apparatus had an electrifiable grid floor to shock the animals' paws. The test was conducted in two trials: Acquisition (day 1) and retention (day 2). Each mouse was individually placed in the illuminated compartment during the acquisition trial. After acclimating for 60 sec, the door was opened. The door was closed upon entering the dark compartment and an electric shock (0.25 mA, 1 sec) was delivered. After 30 sec, the mice were returned to their home cage. Mice that did not enter the dark compartment within 300 sec were excluded from the experiments. About 24 hrs after the acquisition trial, a retention trial was performed: The same procedures were applied, but no electric shock was given. Transfer latencies to enter the dark compartment were recorded. A longer transfer latency indicated healthy emotional memory, while shorter values indicated impairment.

Malondialdehyde level measurements: Brain tissue malondialdehyde (MDA) levels were assessed to measure lipid peroxidation using the thiobarbituric acid (TBA) method[22]. Tissues were homogenized in a glass tube using a homogenizer (Fisher Scientific FB50) with 4.5 mL of 5% and 0.5 mL of 10% (w/v) trichloroacetic acid (TCA). The homogenates were centrifuged at 4500 rpm for 15 min (Thermo MicroCL 17R). Then, 1 mL supernatant was mixed with 1 mL of 0.67% TBA solution in another glass tube. The mixture was heated in a water bath at 100°C for 10 min. After cooling, the absorbance levels were measured at 532 nm using a spectrophotometer (UV-1205 Shimadzu). The absorbance values were then converted using a molar extinction coefficient and the results were expressed as nanomoles per milligram of tissue.

Tissue processing and routine staining protocol: Tissue samples fixed in formaldehyde for 24 hrs were washed under tap water, dehydrated in increasing alcohol concentrations, cleared in xylene and embedded in paraffin blocks. Sections of 5 µm thickness were obtained from the paraffin blocks using a rotary microtome (Thermo Shandon Finesse ME+, Runcorn, Cambridge, UK) on adhesive slides for microscopic examination. The tissue sections were either stained with Hematoxylin and Eosin (H&E) for pathological observations or with Luxol fast blue for myelin investigation. For H&E staining, the sections were deparaffinized in two series of xylene, rehydrated through decreasing alcohol concentrations and then placed in distilled water. The H&E stained samples were immersed in Hematoxylin for 8 min, washed under tap water and stained in Eosin for 3 min. Then, they were rinsed in alcohol, immersed in two series of xylene and mounted with entellan. Luxol fast blue staining was performed using a ready-to-use kit following the manufacturer's directions (Bio-Optica, MI, Italy). All stained sections were examined under a camera-equipped light microscope (Zeiss AXIO, Carl Zeiss Microscopy GmbH, Göttingen, Germany) and representative micrographs were captured[23].

Statistical analysis: A One-way Analysis of Variance (ANOVA) followed by a *post hoc* Tukey's test was used to analyze the results. The data were expressed as Mean±Standard Error of the Mean (SEM). Statistical significance was considered if the $p<0.05$.

RESULTS

Novel object recognition test: The ratio index (RI) values were lower in the KET30.21 group compared to the VEH group ($p<0.05$, as illustrated in Fig. 1).

Modified-elevated plus-maze test: In the retention trial of the mEPM test, transfer latencies were significantly higher in the KET30.7 and KET30.21 groups compared to the VEH group ($p<0.05$, as illustrated in Fig. 2).

Passive avoidance test: In the PA test retention trial, transfer latencies were lower in the KET30.21 group compared to the VEH group ($p<0.05$, as illustrated in Fig. 3).

Malondialdehyde levels: In the KET30.21 group, brain malondialdehyde (MDA) levels were significantly higher compared to the VEH group ($p<0.01$). Additionally, MDA levels were lower in the KETT group compared to the KET30.21 group ($p<0.05$, as illustrated in Fig. 4).

Histopathological results: The KET10.7 and KET10.21 groups did not exhibit significant morphological changes and were similar to the VEH group (Fig. 5a-c). In the KET30.7 group, pyknotic neuronal and glial cell nuclei were observed, along with dilated vessels and congestion (Fig. 5d). The KET30.21 group showed severe histopathological alterations, including widespread dilation, severe congestion in cerebral vessels and widespread pyknotic nuclei in neurons and glial cells (Fig. 5e).

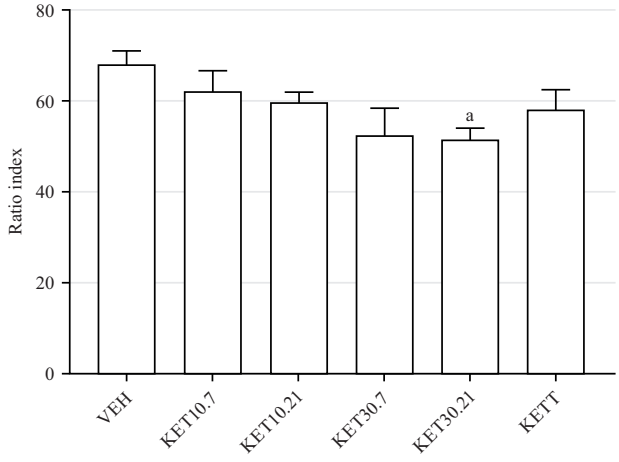

Fig. 1: NOR test ratio index values

VEH: Vehicle, KET10.7: Ketamine (10 mg/kg, 7 days), KET10.21: Ketamine (10 mg/kg, 21 days), KET30.7: Ketamine (30 mg/kg, 7 days), KET30.21: Ketamine (30 mg/kg, 21 days), KETT: Ketamine (30 mg/kg, 21 days) and Trolox (20 mg/kg, 21 days), [a]$p<0.05$, each column represents the Mean±SEM of 7-9 mice and one-way ANOVA followed by a *post hoc* Tukey's test was used for statistical analysis

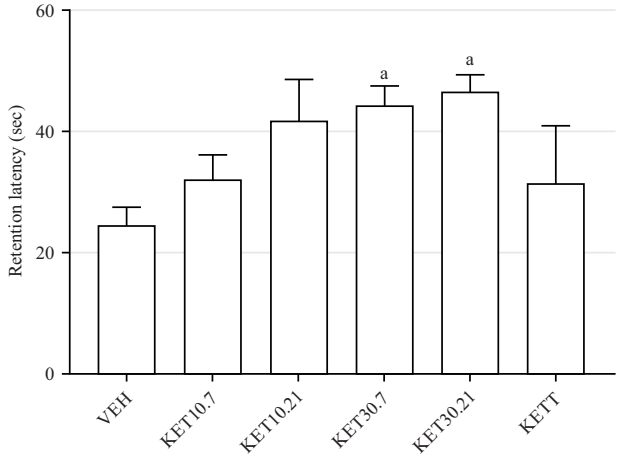

Fig. 2: Transfer latencies in the mEPM test

VEH: Vehicle, KET10.7: Ketamine (10 mg/kg, 7 days), KET10.21: Ketamine (10 mg/kg, 21 days), KET30.7: Ketamine (30 mg/kg, 7 days), KET30.21: Ketamine (30 mg/kg, 21 days), KETT: Ketamine (30 mg/kg, 21 days) and Trolox (20 mg/kg, 21 days), [a]$p<0.05$, each column represents the Mean±SEM of 6-8 mice and one-way ANOVA followed by a *post hoc* Tukey's test was used for statistical analysis

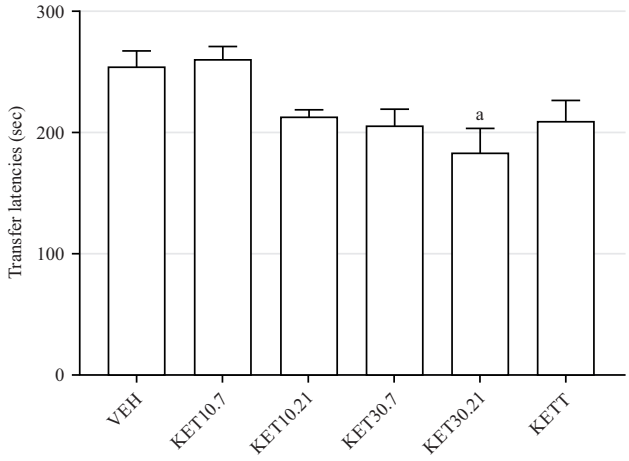

Fig. 3: Transfer latencies in the passive avoidance (PA) test retention trial

VEH: Vehicle, KET10.7: Ketamine (10 mg/kg, 7 days), KET10.21: Ketamine (10 mg/kg, 21 days), KET30.7: Ketamine (30 mg/kg, 7 days), KET30.21: Ketamine (30 mg/kg, 21 days), KETT: Ketamine (30 mg/kg, 21 days) and Trolox (20 mg/kg, 21 days), [a]p<0.05, each column represents the Mean±SEM of 7-9 mice and one-way ANOVA followed by a *post hoc* Tukey's test was used for statistical analysis

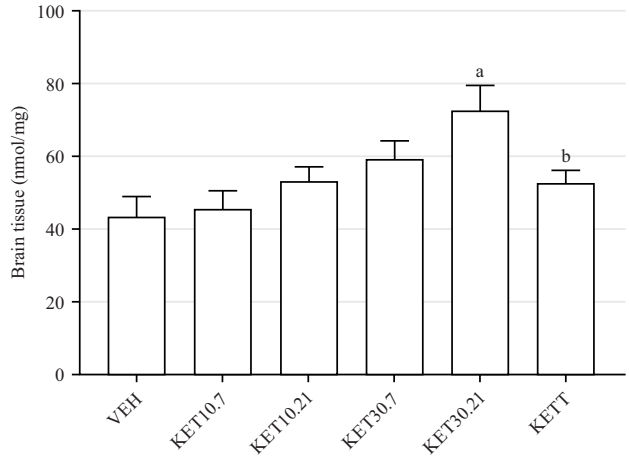

Fig. 4: Malondialdehyde (MDA) levels of the brain (nanomoles per milligram of brain tissue)

VEH: Vehicle, KET10.7: Ketamine (10 mg/kg, 7 days), KET10.21: Ketamine (10 mg/kg, 21 days), KET30.7: Ketamine (30 mg/kg, 7 days), KET30.21: Ketamine (30 mg/kg, 21 days), KETT: Ketamine (30 mg/kg, 21 days) and Trolox (20 mg/kg, 21 days), [a]p<0.01 vs VEH, [b]p<0.05 vs KET30.21, each column represents the Mean±SEM of 7-9 mice and one-way ANOVA followed by a *post hoc* Tukey's test was used for statistical analysis

The KETT group displayed a reduction in pathological changes, particularly cellular pyknosis (Fig. 5f). However, it exhibited perivascular and perineural edema and perivascular cavity gaps, which were absent in the other groups.

Observations from Luxol fast blue stained samples revealed no significant differences in axonal myelination density in the VEH, KET10.7, KET10.21 and KET30.7 groups (Fig. 6a-d, respectively). However, the KET30.21 group exhibited a decrease in myelination (Fig. 6e). However, as indicated by Luxol fast blue positivity, myelination density was similar to the VEH group in Trolox-treated animals (KETT, Fig. 6f). Representative micrographs of histopathological H&E and Luxol fast blue stained samples are provided in Fig. 5-6, respectively.

DISCUSSION

The present study's findings indicate that prolonged exposure to high doses of ketamine leads to significant memory deficits, increased oxidative stress and structural changes in the brain. It's worth noting that the administration of Trolox, an antioxidant, was effective in reducing these toxic effects, providing protection against ketamine-induced memory impairments. These findings highlight the crucial role

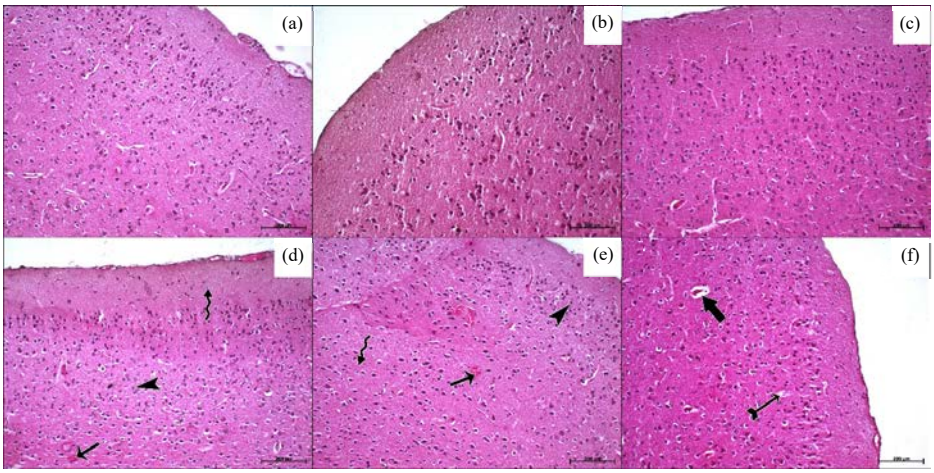

Fig. 5(a-f): Hematoxylin and Eosin (H&E) stained brain sections of the groups, (a) VEH, (b) KET10.7, (c) KET10.21, (d) KET30.7, (e) KET30.21 and (f) KETT

Vascular congestion (arrow), pyknosis in pyramidal neurons (arrowhead) and neuroglia cells (curved arrow), increased perivascular (thick arrow) and perineural (arrow with tail) cavities and edema, Staining: Hematoxylin & Eosin and Bar: 200 µm

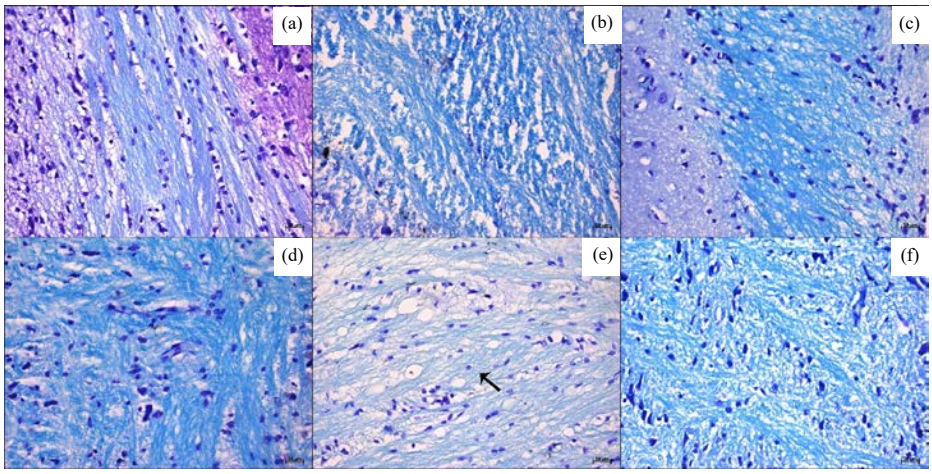

Fig. 6(a-f): Myelination level with considering Luxol fast blue density in the white matter of the groups, (a) VEH, (b) KET10.7, (c) KET10.21, (d) KET30.7, (e) KET30.21 and (f) KETT

Reduced myelination (arrow) in neuronal processes, Staining: Luxol fast blue and Bar: 20 µm

of oxidative stress in ketamine-induced neurotoxicity and cognitive impairments. They were consistent with previous research showing similar cognitive deficits and neurotoxic effects associated with prolonged and high-dose ketamine use[24,25].

The memory-impairing effects of ketamine are stated to be associated with increased oxidative stress and reduced antioxidant defense systems in the hippocampus and prefrontal cortex, which are pivotal regions for learning and memory processes[26,27]. Measuring oxidative stress serves as a practical method to detect neurotoxicity. Antioxidant molecules can defend against disruptions related to increased oxidative stress. Trolox is a water-soluble analog of vitamin E and a potent antioxidant known for its neuroprotective effects[28]. Accordingly, Trolox can protect neuronal integrity and memory functions against ketamine-induced alterations. Malondialdehyde (MDA) is a byproduct of lipid peroxidation. Its levels are elevated in neurodegenerative processes. Recent studies indicate that ketamine increases oxidative stress. In a recent study, 10 mg/kg ketamine administrations for 12 weeks increased MDA levels in the prefrontal cortex and hippocampus[27]. A separate investigation revealed an elevation in brain MDA levels following administering 20 mg/kg of ketamine for 14 days[29]. In the literature, antioxidant molecules

are reported to attenuate ketamine-induced memory impairments[30,31]. In this study, the chronic high-dose ketamine-applied group had elevated MDA levels, while other ketamine-applied groups had no significant alterations. Notably, administering Trolox mitigated MDA levels in the chronic high-dose ketamine-applied group. These findings support that prolonged high-dose ketamine exposure leads to marked oxidative stress in the brain. The protective effect of Trolox emphasizes the extent of increased oxidative stress in chronic ketamine use.

The impact of ketamine on recognition memory has been documented. According to a study, ketamine applications (5 and 10 mg/kg, 5 days) resulted in memory impairments[32]. A recent study reported memory impairments following 20 mg/kg ketamine administrations for 14 days, which were reduced with an antioxidant molecule[30]. Another study reported memory impairments following thrice-a-week 30 mg/kg ketamine applications for a month[33]. In a recent study, acute 15 mg/kg ketamine administration did not impair recognition memory, yet it caused disruptions when given for 10 days[34].

In the present study, recognition memory functions were examined using a NOR test. Low-dose ketamine applications did not affect recognition memory, while high-dose chronic applications caused significant impairments. Trolox alleviated the memory-impairing effects. These findings highlight the dose- and time-dependent nature of ketamine's toxic effects on recognition memory, also emphasizing the vulnerability of this memory type to increased oxidative stress. The neuroprotective effects of Trolox suggest a promising route to alleviate ketamine-induced recognition memory deficits.

Ketamine has been shown to affect spatial memory functions[35-37]. Prolonged high-dose ketamine administrations are linked with disrupted memory[7,38]. A study reported a dose-dependent decline in spatial working-memory functions following different dose ketamine administrations (7.5, 15 or 30 mg/kg) for 8 weeks[39]. Ketamine in a dose of 20 mg/kg for 14 days disrupted memory in a Y-maze test[29]. Interestingly, another study reported no impairments in spatial memory functions with a considerably high dose of ketamine (30 mg/kg) administration for 6 months[18].

Ketamine may have bimodal effects on memory, as evidenced by a study that reported reduced stress-induced spatial memory impairments with a chronically applied tiny dose of ketamine (4 mg/kg). The same dose caused memory impairments in non-stressed animals. These effects were observed in the Morris water maze test, which measures spatial memory, but not in the Barnes maze test, which measures working memory[40]. In current study, spatial memory functions were assessed using an mEPM test. Administration of chronic and subchronic high-dose ketamine resulted in increased time delays in locating the closed arms, indicating disrupted spatial memory. Conversely, low-dose ketamine administrations did not lead to similar disruptions. Besides, Trolox attenuated chronic high-dose ketamine-induced impairments. These results indicate dose-dependent and context-specific effects of ketamine on spatial memory functions. Trolox's protective effects underscore oxidative stress's significance in spatial memory functions.

Studies have reported ketamine-induced emotional memory impairments[41]. In a study, 25 mg/kg ketamine administration for seven days provoked memory impairments. Contrarily and interestingly, anesthetic doses (50 or 100 mg/kg) over the same duration did not produce similar impairments[42]. In another study, different doses of ketamine (5, 10 and 15 mg/kg) were acutely administered: Higher doses (10 and 15 mg/kg) disrupted emotional memory functions, but 5 mg/kg did not produce similar disruptions[43]. These results indicate dose-dependent alterations in ketamine's effects. A recent study reported no significant changes in emotional memory functions after daily and thrice-a-week 30 mg/kg ketamine administrations for a month. Nonetheless, impairment was seen after daily administrations of hydroxynorketamine (30 mg/kg), a ketamine metabolite, while thrice-weekly administrations, interestingly, generated improvements[33]. These results show the bimodal effects of ketamine. In this study, emotional memory functions were assessed using a PA test. Low-dose ketamine did not affect memory, while chronic high-dose applications generated memory deficits. In contrast, subchronic high-dose applications did not produce the same effects. Trolox alleviated the disruptions in the chronic high-dose ketamine-applied group. These findings show the dose-dependent toxicity of ketamine on emotional memory functions.

Research has highlighted notable changes in neuronal structures' morphology and protein expression levels following ketamine exposure[44], particularly in developing neurons[45]. Nuclear condensation, fragmentation of neuronal cells and enlargement of cell bodies are characteristic symptoms associated with ketamine use, indicating neurotoxicity[46]. A recent study reported alterations in dendritic, filopodia-type and mushroom-type spines in neurons following administering two ketamine doses (5 and 10 mg/kg) for 5 days. In different regions of the brain, i.e., CA1-hippocampus, dorsal striatum and ventromedial prefrontal cortex, different effects were observed (an increase or a decrease in spine density) showing the bimodal effect of ketamine on neuroplasticity[32]. Histopathological examinations

in the present study have demonstrated various degenerative effects, particularly with chronic high-dose ketamine use, including reduced myelination, congested and dilated vessels and cellular nuclei pyknosis. These morphological alterations may contribute to cognitive decline induced by ketamine and impact overall brain function. Nevertheless, these effects were mitigated in the group treated with Trolox. These findings highlight the role of oxidative stress in ketamine-induced neurotoxicity and suggest that interventions targeting oxidative damage offer a promising approach to preserving neuronal integrity and preventing cognitive decline.

CONCLUSION

The study provides evidence of the dose- and time-dependent neurotoxic and memory-impairing effects of ketamine, primarily through increased oxidative stress. Chronic high-dose ketamine exposure leads to significant oxidative damage, as indicated by elevated MDA levels, morphological changes and impairments in recognition, spatial and emotional memory. Trolox appears to have a neuroprotective effect, mitigating ketamine-induced memory deficits and oxidative damage. These findings highlight the role of oxidative stress in ketamine-induced neurotoxicity and suggest that antioxidant interventions like Trolox may help maintain cognitive functions and neuronal health. Given the wide range of therapeutic indications and growing abuse of ketamine and its negative impact on brain functions, further research is required to explore strategies to prevent or reverse its harmful effects.

SIGNIFICANCE STATEMENT

This study investigates ketamine-induced cognitive impairments and neurotoxicity by examining the effects of different doses and durations on memory functions, brain morphology and oxidative stress in mice. Notably, it shows the dose- and time-dependent neurotoxic and memory-impairing effects of ketamine and that the antioxidant Trolox can mitigate these harmful effects. These findings improve our knowledge of oxidative stress' part in ketamine's neurotoxic effects and recommended potential strategies to reduce harm, particularly in prolonged and high-dose use.

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REFERENCES

1. Johnston, J.N., B. Kadriu, C. Kraus, I.D. Henter and C.A. Zarate Jr, 2024. Ketamine in neuropsychiatric disorders: An update. Neuropsychopharmacology, 49: 23-40.
2. Erdinc, M., E. Uyar, I. Kelle and H. Akkoc, 2019. Anti-nociceptive effects of low dose ketamine in mice may be mediated by the serotonergic systems. Psychiatry Clin. Psychopharmacol., 29: 252-256.
3. Trujillo, K.A. and S.D. Iñiguez, 2020. Ketamine beyond anesthesia: Antidepressant effects and abuse potential. Behav. Brain Res., Vol. 394. 10.1016/j.bbr.2020.112841.
4. Chan, K.W.S., T.M.C. Lee, A.M.H. Siu, D.P.L. Wong, C.M. Kam, S.K.M. Tsang and C.C.H. Chan, 2013. Effects of chronic ketamine use on frontal and medial temporal cognition. Addict. Behav., 38: 2128-2132.
5. Zhang, C., Y. Xu, B. Zhang, W. Hao and W.K. Tang, 2020. Cognitive impairment in chronic ketamine abusers. Psychiatry Res., Vol. 291. 10.1016/j.psychres.2020.113206.
6. Li, Y., R. Shen, G. Wen, R. Ding and A. Du *et al*., 2017. Effects of ketamine on levels of inflammatory cytokines IL-6, IL-1β, and TNF-α in the hippocampus of mice following acute or chronic administration. Front. Pharmacol., Vol. 8. 10.3389/fphar.2017.00139.
7. Ding, R., Y. Li, A. Du, H. Yu and B. He *et al*., 2016. Changes in hippocampal AMPA receptors and cognitive impairments in chronic ketamine addiction models: Another understanding of ketamine CNS toxicity. Sci. Rep., Vol. 6. 10.1038/srep38771.
8. Franscescon, F., T.E. Müller, K.T. Bertoncello and D.B. Rosemberg, 2020. Neuroprotective role of taurine on MK-801-induced memory impairment and hyperlocomotion in zebrafish. Neurochem. Int., Vol. 135. 10.1016/j.neuint.2020.104710.
9. Santiago, J.E.S., G.R. Roldán and O. Picazo, 2022. Ketamine as a pharmacological tool for the preclinical study of memory deficit in schizophrenia. Behav. Pharmacol., 34: 80-91.
10. Li, X., H. Saiyin, X. Chen, Q. Yu, L. Ma and W. Liang, 2024. Ketamine impairs growth cone and synaptogenesis in human GABAergic projection neurons via GSK-3β and HDAC6 signaling. Mol. Psychiatry, 29: 1647-1659.
11. Zhang, X., Y. Cui, X. Song, X. Jin and X. Sheng *et al*., 2023. Curcumin alleviates ketamine-induced oxidative stress and apoptosis via Nrf2 signaling pathway in rats' cerebral cortex and hippocampus. Environ. Toxicol., 38: 300-311.
12. Zuo, D., Y. Liu, Z. Liu, J. Cui and X. Zhou *et al*., 2018. Alcohol aggravates ketamine-induced behavioral, morphological and neurochemical alterations in adolescent rats: The involvement of CREB-related pathways. Behav. Brain Res., 349: 80-90.
13. Zuo, D., L. Lin, Y. Liu, C. Wang and J. Xu *et al*., 2016. Baicalin attenuates ketamine-induced neurotoxicity in the developing rats: Involvement of PI3K/Akt and CREB/BDNF/Bcl-2 pathways. Neurotoxic. Res., 30: 159-172.

14. Ke, X., Y. Ding, K. Xu, H. He and D. Wang *et al*., 2018. The profile of cognitive impairments in chronic ketamine users. Psychiatry Res., 266: 124-131.
15. Teleanu, D.M., A.G. Niculescu, I.I. Lungu and C.I. Radu *et al*., 2022. An overview of oxidative stress, neuroinflammation, and neurodegenerative diseases. Int. J. Mol. Sci., Vol. 23. 10.3390/ijms23115938.
16. Babahajian, A., A. Sarveazad, F. Golab, G. Vahabzadeh and A. Alizadeh *et al*., 2019. Neuroprotective effects of trolox, human chorionic gonadotropin, and carnosic acid on hippocampal neurodegeneration after ischemiareperfusion injury. Curr. Stem Cell Res. Ther., 14: 177-183.
17. Duan, T.T., J.W. Tan, Q. Yuan, J. Cao, Q.X. Zhou and L. Xu, 2013. Acute ketamine induces hippocampal synaptic depression and spatial memory impairment through dopamine D1/D5 receptors. Psychopharmacology, 228: 451-461.
18. Sun, L., W.P. Lam, Y.W. Wong, L.H. Lam and H.C. Tang *et al*., 2011. Permanent deficits in brain functions caused by long-term ketamine treatment in mice. Hum. Exp. Toxicol., 30: 1287-1296.
19. Sharma, S.S. and R.K. Kaundal, 2007. Neuroprotective effects of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), an antioxidant in middle cerebral artery occlusion induced focal cerebral ischemia in rats. Neurol. Res., 29: 304-309.
20. Akar, F., O. Mutlu, I.K. Celikyurt, E. Bektas and M.H. Tanyeri *et al*., 2014. Effects of zaprinast and rolipram on olfactory and visual memory in the social transmission of food preference and novel object recognition tests in mice. Drug Target Insights, Vol. 8. 10.4137/DTI.S14813.
21. Gumuslu, E., O. Mutlu, I.K. Celikyurt, G. Ulak, F. Akar, F. Erden and M. Ertan, 2016. Exenatide enhances cognitive performance and upregulates neurotrophic factor gene expression levels in diabetic mice. Fundam. Clin. Pharmacol., 30: 376-384.
22. Pompella, A., E. Maellaro, A.F. Casini, M. Ferrali, L. Ciccoli and M. Comporti, 1987. Measurement of lipid peroxidation *in vivo*: A comparison of different procedures. Lipids, 22: 206-211.
23. Seker, U., D.E. Kavak, B.C. Guzel, S.B. Baygeldi and M. Yuksel *et al*., 2022. Targeting soluble guanylate cyclase with Riociguat has potency to alleviate testicular ischaemia reperfusion injury via regulating various cellular pathways. Andrologia, Vol. 54. 10.1111/and.14616.
24. Shibuta, S., T. Morita, J. Kosaka, T. Kamibayashi and Y. Fujino, 2015. Only extra-high dose of ketamine affects L-glutamate-induced intracellular Ca^{2+} elevation and neurotoxicity. Neurosci. Res., 98: 9-16.
25. Sun, L., Q. Li, Q. Li, Y. Zhang and D. Liu *et al*., 2014. Chronic ketamine exposure induces permanent impairment of brain functions in adolescent cynomolgus monkeys. Addict. Biol., 19: 185-194.
26. Mert, D.G., N.H. Turgut, E. Arslanbas, H. Gungor and H. Kara, 2019. The influence of quercetin on recognition memory and brain oxidative damage in a ketamine model of schizophrenia. Psychiatry Clin. Psychopharmacol., 29: 1-7.
27. El Shehaby, D.M., R.I. El-Mahdy, A.M. Ahmed, A. Hosny and N.M. Abd El-Rady, 2020. Neurobehavioral, testicular and erectile impairments of chronic ketamine administration: Pathogenesis and ameliorating effect of N-acetyl cysteine. Reprod. Toxicol., 96: 57-66.
28. Sarveazad, A., A. Babahajian, A. Yari, F. Goudarzi, M. Soleimani and M. Nourani, 2016. Neuroprotective role of trolox in hippocampus after ischemia reperfusion injury in mouse. Int. J. Vitamin Nutr. Res., 86: 229-235.
29. Annafi, O.S., O.M. Aluko, A.T. Eduviere, O. Omorogbe and S. Umukoro, 2017. Probable mechanisms involved in the antipsychotic-like activity of methyl jasmonate in mice. Naunyn-Schmiedeberg's Arch. Pharmacol., 390: 883-892.
30. Farkhakfar, A., S. Hassanpour and M. Zendehdel, 2023. Resveratrol plays neuroprotective role on ketamine-induced schizophrenia-like behaviors and oxidative damage in mice. Neurosci. Lett., Vol. 813. 10.1016/j.neulet.2023.137436.
31. Vasconcelos, G.S., N.C. Ximenes, C.N.S. de Sousa, T. de Queiroz Oliveira and L.L.L. Lima *et al*., 2015. Alpha-lipoic acid alone and combined with clozapine reverses schizophrenia-like symptoms induced by ketamine in mice: Participation of antioxidant, nitrergic and neurotrophic mechanisms. Schizophr. Res., 165: 163-170.
32. Suárez-Santiago, J.E., S. Orozco-Suárez, A. Vega-García, L.Á. Bautista-Orozco and O. Picazo, 2020. Repeated ketamine administration induces recognition memory impairment together with morphological changes in neurons from ventromedial prefrontal cortex, dorsal striatum, and hippocampus. Behav. Pharmacol., 31: 633-640.
33. Riggs, L.M., X. An, E.F.R. Pereira and T.D. Gould, 2021. (*R,S*)-ketamine and (*2R,6R*)-hydroxynorketamine differentially affect memory as a function of dosing frequency. Transl. Psychiatry, Vol. 11. 10.1038/s41398-021-01685-0.
34. Acevedo, J., N.E. Mugarura, A.L. Welter, E.M. Johnson and J.A. Siegel, 2023. The effects of acute and repeated administration of ketamine on memory, behavior, and plasma corticosterone levels in female mice. Neuroscience, 512: 99-109.
35. Huang, L., Y. Liu, W. Jin, X. Ji and Z. Dong, 2012. Ketamine potentiates hippocampal neurodegeneration and persistent learning and memory impairment through the PKCγ-ERK signaling pathway in the developing brain. Brain Res., 1476: 164-171.
36. Ribeiro, P.O., P.C. Rodrigues, A.M. Valentim and L.M. Antunes, 2013. A single intraperitoneal injection of ketamine does not affect spatial working, reference memory or neurodegeneration in adult mice: An animal study. Eur. J. Anaesthesiology, 30: 618-626.

37. Hong, Y., S. Meng, S. Wang, T. Liu and J. Liu, 2022. Ulinastatin alleviates repetitive ketamine exposure-evoked cognitive impairment in adolescent mice. Neural Plast., Vol. 2022. 10.1155/2022/6168284.
38. Kingir, E., C. Sevinc and G. Unal, 2023. Chronic oral ketamine prevents anhedonia and alters neuronal activation in the lateral habenula and nucleus accumbens in rats under chronic unpredictable mild stress. Neuropharmacology, Vol. 228. 10.1016/j.neuropharm.2023.109468.
39. Onaolapo, A.Y., O.J. Ayeni, M.O. Ogundeji, A. Ajao, O.J. Onaolapo and A.R. Owolabi, 2019. Subchronic ketamine alters behaviour, metabolic indices and brain morphology in adolescent rats: Involvement of oxidative stress, glutamate toxicity and caspase-3-mediated apoptosis. J. Chem. Neuroanat., 96: 22-33.
40. Trofimiuk, E., P. Wielgat, J.J. Braszko and H. Car, 2019. Stress and ketamine, bimodal influence on cognitive functions. Behav. Brain Res., 360: 354-364.
41. Farahmandfar, M., A. Akbarabadi, A. Bakhtazad and M.R. Zarrindast, 2017. Recovery from ketamine-induced amnesia by blockade of GABA-A receptor in the medial prefrontal cortex of mice. Neuroscience, 344: 48-55.
42. Peng, S., Y. Zhang, H. Wang, B. Ren and J. Zhang, 2011. Anesthetic ketamine counteracts repetitive mechanical stress-induced learning and memory impairment in developing mice. Mol. Biol. Rep., 38: 4347-4351.
43. Farahmandfar, M., A. Bakhtazad, A. Akbarabadi and M.R. Zarrindast, 2016. The influence of dopaminergic system in medial prefrontal cortex on ketamine-induced amnesia in passive avoidance task in mice. Eur. J. Pharmacol., 781: 45-52.
44. Miao, H.C., M. Liu, F. Wu and H.B. Li, 2022. Expression changes of c-Fos and D1R/p-ERK1/2 signal pathways in nucleus accumbens of rats after ketamine abuse. Biochem. Biophys. Res. Commun., 629: 183-188.
45. Farber, N.B. and J.W. Onley, 2003. Drugs of abuse that cause developing neurons to commit suicide. Dev. Brain Res., 147: 37-45.
46. Slikker Jr., W., X. Zou, C.E. Hotchkiss, R.L. Divine and N. Sadovova *et al*., 2007. Ketamine-induced neuronal cell death in the perinatal rhesus monkey. Toxicol. Sci., 98: 145-158.