Risperidone and ethyl pyruvate have protective effects against ketamine-induced cognitive impairments in mice

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Abstract. – OBJECTIVE: Ketamine, an N-methyl D-aspartic acid receptor antagonist drug, is reported to produce memory disruptions. The aim of this study was to investigate the protective effects of ethyl pyruvate (EP), a pyruvic acid derivative, and risperidone, an atypical antipsychotic drug, against ketamine-induced cognitive disturbances.

MATERIALS AND METHODS: A passive-avoidance test, a novel object recognition test, and a modified elevated plus maze test were used to assess memory functions. Hippocampal malondialdehyde (MDA) levels were measured to determine the oxidation levels.

RESULTS: Ketamine applications produced memory deficits in all tests and insignificantly increased MDA levels, which were alleviated by risperidone, EP, and combination treatments.

CONCLUSIONS: Increased oxidative stress and neurotransmission imbalance can be responsible for ketamine-induced memory disruptions. With its antioxidant effects, EP may be helpful to reduce cognitive impairments related to schizophrenia either alone or in combination with antipsychotics.

Key Words:
Ethyl pyruvate, Risperidone, Ketamine, Schizophrenia, Cognitive symptoms.

Introduction

The popularity of N-methyl D-aspartic acid (NMDA) receptor antagonizing drugs has been growing over the past few decades. Ketamine, an NMDA receptor antagonist, has beneficial therapeutic effects in treatment-resistant depression, anxiety, and chronic and severe pain[1,2]. Memantine, another NMDA receptor antagonist, is currently prescribed in dementia treatment[3]. Ketamine has evolved into a highly abused substance with its hallucinogenic and euphoric effects[4]. Recently, esketamine, a ketamine enantiomer, has been approved by Food and Drug Administration (FDA) in treatment-resistant depression and major depressive disorder with acute suicidal ideation[5]. Increasing NMDA antagonist drug use rates have emerged the necessity to investigate their acute and long-term effects.

Main concerns regarding the long-term use of ketamine and esketamine remain unaltered, primarily linked to possible neurodegenerative effects, along with its potential for substance abuse[6]. Ketamine-induced neurodegeneration practically limits its widespread use, while its abuse potential reduces its safety[7].

NMDA receptors play essential roles in long-term potentiation, long-term depression, and synaptic plasticity, representing their fundamental role in memory processes[8-10]. Antagonizing these receptors with specific pharmacological agents produces cognitive disturbances resembling schizophrenia[11].

Risperidone is an atypical antipsychotic drug reported to reduce the cognitive symptoms of schizophrenia[12]. Neurotransmission dysregulation is a significant cause of memory disruptions[13], and risperidone is believed to protect memory by regulating dopaminergic and serotonergic transmission[14,15]. However, the mechanisms underlying the protective effects of risperidone are not yet entirely clear.

Continuous administration of ketamine is reported to increase oxidative stress[16]. Increased oxidation of biomolecules leads to apoptosis and even results in neuronal death[17]. Therefore, evaluation of oxidation levels is crucial to understanding the effects of ketamine on memory processes.

Ethyl pyruvate (EP), a derivative of pyruvic acid, functions as an endogenous antioxidant in cells and is reported to have antioxidant, antiapoptotic, and antiinflammatory effects in various preclinical studies[18-20].

The present study aimed to investigate the protective effects of risperidone and EP on ket-

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amine-induced cognitive impairments in mice. A passive avoidance test, a novel object recognition test and a modified elevated plus-maze test were used to evaluate memory functions. After the behavioral test, brain hippocampi tissues were isolated to measure malondialdehyde levels as an indicator of lipid peroxidation.

Materials and Methods

Animals

56 male BALB/c mice (35-45 gr) taken from local animal colony facility (DUSAM, Dicle University, Diyarbakir, Turkey) were separated into 7 groups, each comprised of 7-9 mice. Mice were kept in cages in the laboratory for two weeks before the experiments in standard laboratory conditions (21±1.5°C, 12 h light/dark cycle, light onset at 8.00 pm). The mice were supplied ad libitum access to food pellets and water. Ethics permission was acquired from Dicle University Animal Ethics Committee (DUHADEK, Number: 2019-02). All procedures concerning the test subjects were conducted in accordance with the European Community Council Directive of 24 November 1986. All subjects were naive to the experiments and tested individually. All experiments were conducted within 9-14 a.m. in a semi-soundproof and dimly lit room (approximately 100 lux).

Drugs and Treatment

Ketamine was purchased from Merck Co. (Darmstadt, Germany), risperidone and EP were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The drugs were dissolved in saline and administered intraperitoneally. Ketamine (25 mg/kg), risperidone (0.2 mg/kg), and EP (50 mg/kg) were administered for two weeks before the experiments, and the vehicle group was given saline in a volume of 10 ml/kg. The drug doses were defined based on previous behavioural studies[21,22].

Passive Avoidance Test

A one-trial, step-down, light-dark passive avoidance (PA) test apparatus (MAY-PA 1014-M) was used to measure the effects of the drugs on emotional learning and memory based on contextual fear conditioning. In this test, the animals learn to evade a particular place linked with an aversive experience. After putting the mice in the light compartment, the passing time to the dark compartment of the mice was considered as step-through latencies. If the mice did not pass in 300 seconds, the experiment ended afterward, and the test subject was excluded from the experiment.

The apparatus consisted of two compartments (22×21×22 cm). The illumined white compartment was connected to a dark compartment with an automatically operated flat-box door at floor level. The ground of the apparatus was decorated with an electrifiable grid floor. In the acquisition trial (day 1), the mice were put in the light compartment, and the door was opened after 30 seconds. When the mice entered the dark compartment, the door was automatically shut, and an inescapable electrical shock was given to the animal's paws (0.5 mA, 3 s). The mice then returned to their cages, and the grid floor was rinsed thoroughly between each test to prevent affecting the following mice with the olfactory cues. A retention trial (day 2) was conducted a day following the acquisition trial, and step-through latencies of the mice to enter the dark compartment were determined. Step-through latencies in the retention trial are considered as a ratio of emotional learning. Lower step-through latencies mean a disrupted memory, while higher latencies represent an improved memory[23].

Novel Object Recognition Test

According to the previously described protocol, the novel object recognition (NOR) test was conducted[24]. Non-spatial declarative memory performances of the mice were analyzed using the NOR test. All tests were recorded with a video camera, positioned centrally over the open field (OF) test apparatus (40x40x35 cm). The NOR test has consisted of 3 sessions: habituation, training, and retention. The mice were gently put in the OF test apparatus in the habituation session without any objects placed. They were waited to explore freely for 5 min. 30 min after the habituation session, a training session was performed. Two identical objects (two cubes) were placed on the OF test apparatus in a symmetrical position 10 cm beside the side walls. The mice were put facing away from the objects and were allowed to explore freely for 5 mins. The mice directing their nose to objects or touching them is considered as exploration behaviour. 60 minutes after the training session, the mice were put again to the apparatus for the testing session (retention) and were allowed freely to explore one familiar (a cube) and a novel (a sphere) object for 5 mins. The exploration times of the familiar and novel objects in the training and testing sessions were measured. The ratio index (RI) was calculated. RI was determined by novel object exploration time (NT) relative to the total object exploration time (TT), multiplied by 100 [RI=NT/(NT+TT)*100]. Higher RI values indicate healthy memory, and lower values indicate a null preference.

Modified Elevated Plus-Maze Test

Spatial long-term memory was measured using the modified elevated plus-maze test (mEPM). This experiment is based upon the avoidance behaviour of rodents from open spaces and heights, preference for the enclosed and protected areas of the maze. The maze consisted of two open arms (29 cm long×5 cm wide) enclosed by a plexiglass edge (1 cm) to avoid falls and two enclosed arms (29 cm long×5 cm wide, 15 cm high walls). Two open and two closed arms were stationed on the opposite sides and were united by a square-shaped centrepiece (5×5 cm). The maze was completely coloured black and 50 cm high above the floor. In the acquisition session (day 1), each mouse was individually placed at the distal edge of an open arm facing away from the centrepiece. The time passes for mice to enter either closed arms (with all four paws) were recorded and considered transfer latencies. If the mouse did not enter one of the closed arms in 90 s, it is excluded from the experiment. After entrance to either closed arms, the mice were allowed to move freely regardless of closed or open arms for 10 secs. Then, the mice were returned to their home cage. 24 h after the acquisition trial, a retention trial was conducted. The mice were placed on the same distal edge of the open arm, and the transfer latencies were calculated[25]. Higher transfer latencies represent a disrupted memory, while lower values indicate a better memory.

Measurement of Lipid Peroxidation

To measure the oxidation levels, we measured malondialdehyde levels of the hippocampi tissue. We used the thiobarbituric acid (TBA) method to assess MDA levels, which is based on the reaction of MDA with TBA. After the behavioural tests, the mice were sacrificed by cervical decapitation for further analyses. Hippocampi were separated to determine the tissue MDA levels. The weighted tissue samples were put in ice-cold 0.5 ml 10%, w/v, trichloroacetic acid (TCA), plus 4.5 ml 5% (w/v) TCA. Next, hippocampi tissues were homogenized (Fisher Scientific Model FB50) and centrifuged (15 mins) at 4500 rpm (model). After centrifugation, supernatant (1 ml) was put into a glass tube with an equivalent volume of 0.6% (w/v) TBA. The mixture was heated and kept at 100°C for 10 minutes. Following cooling, the absorption spectrum at 532 nm was measured with a spectrophotometer (UV-1205 Shimadzu). Outcomes were estimated using the molar extinction coefficient concerning tissue weights, and the results were shown as nmol/gram tissue[26].

Statistical Analysis

SPSS 24 (IBM, Armonk, NY, USA) was used for statistical analysis. A one-way analysis of variance (ANOVA) ensued with a post hoc Tukey test was used if significant differences were detected between test groups. The data are presented as mean values ±SEM. When *p*-values were less than 0.05, the differences between the test groups were considered statistically significant.

Results

Passive Avoidance Test

On the first day (acquisition trial) of the PA test, no significant difference was observed between test groups ($p>0.05$) (Figure 1).

On the second day (retention trial) of the PA test, step-through latencies were lower in the ketamine applied group (KET) compared with the vehicle group (VEH) ($p<0.05$). Ketamine-EP combination (KE), ketamine-risperidone combination (KR), and ketamine-risperidone-EP combination (KRE) groups had higher step-through latencies compared with the KET group ($p<0.05$) (Figure 2).

Novel Object Recognition Test

Ratio index (RI) values were lower in the KET group compared with the VEH group ($p<0.05$) and were slightly increased in KE, KR, and KRE groups compared with the KET group ($p>0.05$) (Figure 3).

Modified Elevated Plus-Maze Test

No significant difference was observed in transfer latencies in the modified elevated plus-maze test (mEPM) first-day trial ($p>0.05$) (Figure 4).

In the mEPM test second-day trial, the KET group had higher transfer latencies compared with the VEH group ($p<0.05$). Latencies were lower in KE, KR, and KRE groups compared with the

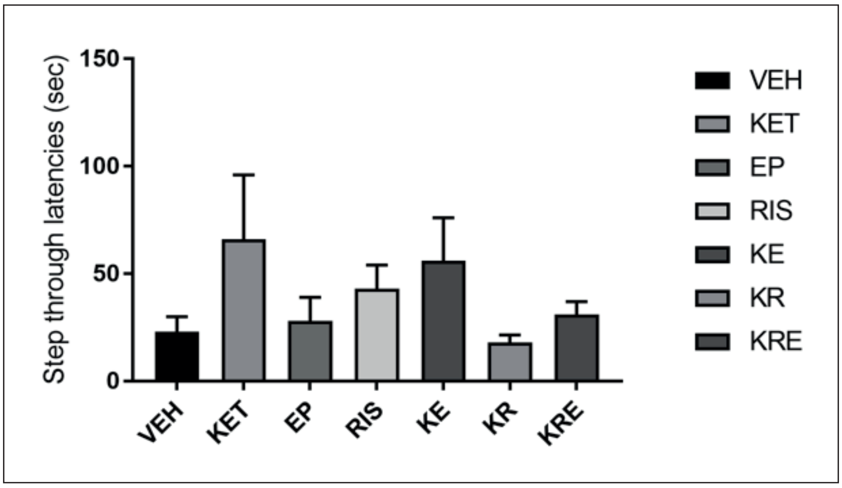

Figure 1. Acquisition trial of the passive avoidance test, step-through latencies. VEH: vehicle, KET: ketamine (25 mg/kg, 14 days), EP: ethyl pyruvate (50 mg/kg, 14 days), RIS: risperidone (0.2 mg/kg, 14 days), KE: ketamine-ethyl pyruvate combination, KR: ketamine-risperidone combination, KRE: ketamine-risperidone-ethyl pyruvate combination. Each column represents the mean ± SEM of 7-9 mice. One-way ANOVA followed by a post hoc Tukey test was used for statistical analysis.

KET group, but the differences were not significant (Figure 5).

Measurement of Tissue Malondialdehyde Levels

Malondialdehyde levels of the hippocampi were higher in the KET group compared with the VEH group, but the differences were insignificant ($p>0.05$). Compared with the KET group, MDA levels were lower in KE, KR, and KRE groups; similarly, the differences were insignificant (Figure 6).

Discussion

The popularity of ketamine has increased over the past few decades after the discovery of its effects on mood and anxiety-related disorders[1]. It is one of the top abused substances due to its hallucinogenic and euphoric effects[6]. Ketamine can mimic positive symptoms of schizophrenia and extended high-dose applications are reported to cause cognitive disruptions[9,11]. Increased employment of the substance has created the need to uncover its pharmacodynamic effects, mechanism of action, and toxicity.

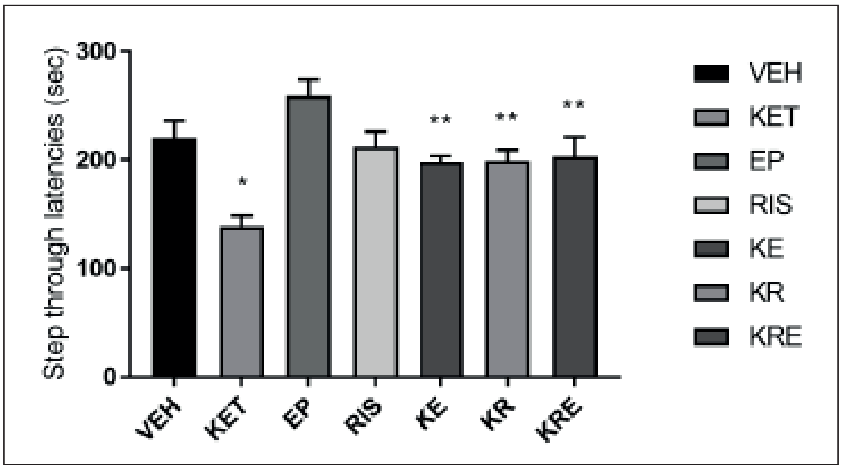

Figure 2. Retention trial of the passive avoidance test, step-through latencies. VEH: vehicle, KET: ketamine (25 mg/kg, 14 days), EP: (50 mg/kg, 14 days), RIS: risperidone (0.2 mg/kg, 14 days), KE: ketamine-ethyl pyruvate combination, KR: ketamine-risperidone combination, KRE: ketamine-risperidone-ethyl pyruvate combination. Each column represents the mean ± SEM of 7-9 mice. $^{*}p<0.05$ *vs*. VEH group, $^{**}p<0.05$ *vs*. KET group. One-way ANOVA followed by a post hoc Tukey test was used for statistical analysis.

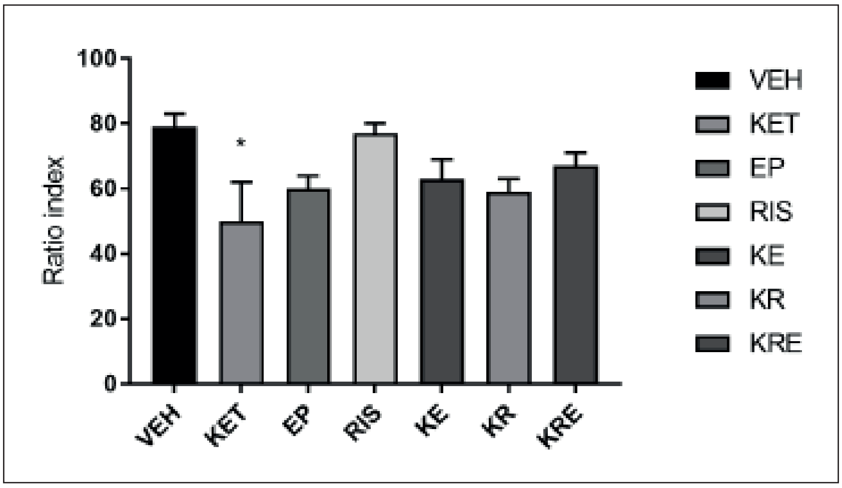

Figure 3. Ratio index values of the novel object recognition test. VEH: vehicle, KET: ketamine (25 mg/kg, 14 days), EP: ethyl pyruvate (50 mg/kg, 14 days), RIS: risperidone (0.2 mg/kg, 14 days), KE: ketamine-ethyl pyruvate combination, KR: ketamine-risperidone combination, KRE: ketamine-risperidone-ethyl pyruvate combination. Each column represents the mean ± SEM of 7-9 mice. $^*p<0.05$ *vs.* VEH group. One-way ANOVA followed by a post hoc Tukey test was used for statistical analysis.

Ketamine is reported to increase serotonergic and dopaminergic transmission by decreasing serotonin and dopamine transporter activity[27]. It also has a direct agonistic affinity to 5-HT2 and D2 receptors[28,29]. Ketamine is reported to activate the mesolimbic dopaminergic system[30]. These interactions may collectively contribute to positive symptoms development. High occupation of dopaminergic receptors and excessive serotonin release is a critical cause for cognitive disruptions[31]. On the other hand, ketamine is reported to increase oxidation, which is believed to have roles in its effects on memory[32]. Risperidone is a second-generation antipsychotic drug. It regulates dopaminergic transmission by antagonizing serotonin 2A (5-HT2A) and dopamine 2 (D2) receptors. Tolerable adverse effects of risperidone made it a preferred option to treat positive and negative symptoms and reduce cognitive disruptions of schizophrenia[33,34]. The protective effect of risperidone on memory represents dopaminergic and serotonergic transmission balance is necessary for the brain to function properly[14,35].

Literature data state that long-term ketamine applications alter brain monoamine levels[27],

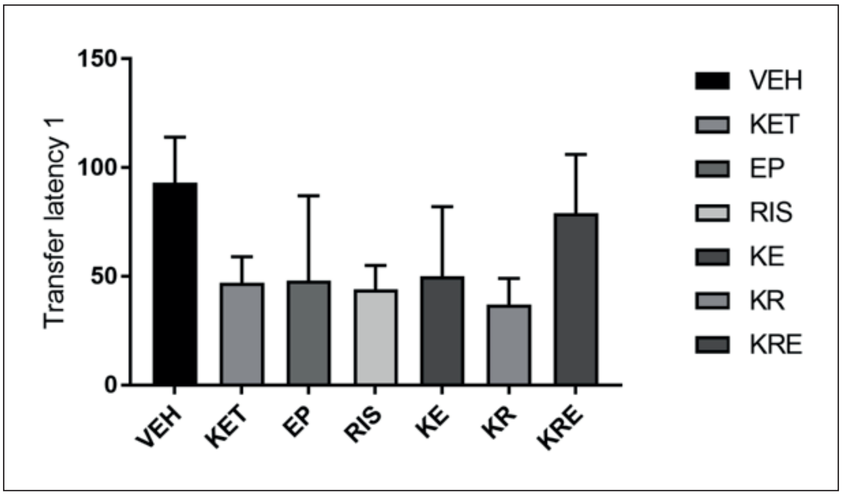

Figure 4. Transfer latencies (day 1) of mice in the modified elevated plus-maze test. VEH: vehicle, KET: ketamine (25 mg/kg, 14 days), EP: ethyl pyruvate (50 mg/kg, 14 days), RIS: risperidone (0.2 mg/kg, 14 days), KE: ketamine-ethyl pyruvate combination, KR: ketamine-risperidone combination, KRE: ketamine-risperidone-ethyl pyruvate combination. Each column represents the mean ± SEM of 5-7 mice. One-way ANOVA followed by a post hoc Tukey test was used for statistical analysis.

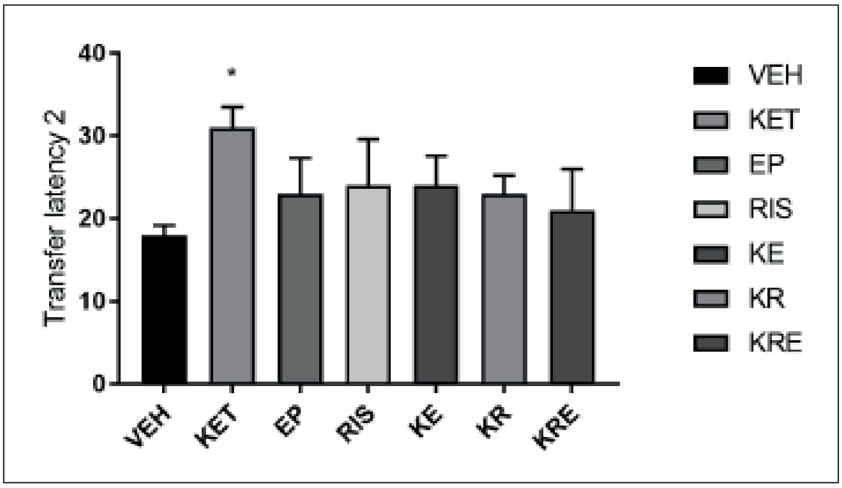

Figure 5. Transfer latencies (day 2) of mice in the modified elevated plus-maze test. VEH: vehicle, KET: ketamine (25 mg/kg, 14 days), EP: ethyl pyruvate (50 mg/kg, 14 days), RIS: Risperidone (0.2 mg/kg, 14 days), KE: ketamine-ethyl pyruvate combination, KR: ketamine-risperidone combination, KRE: ketamine-risperidone-ethyl pyruvate combination. Each column represents the mean ± SEM of 5-7 mice. $^{*}p<0.05$ *vs.* VEH group. One-way ANOVA followed by a post hoc Tukey test was used for statistical analysis.

increase oxidation[32], and induce apoptosis[36] leading to cognitive disturbances[37]. Ethyl pyruvate is a potent antioxidant molecule. It has essential roles in intermediary metabolism. Researchers have shown protective effects of EP in neurodegenerative diseases[38]. Cho et al[39] reported neuronal cell death reductions with EP treatment against kainic acid-induced neurotoxicity. Moro reported beneficial effects of EP following traumatic brain injury with improved oxidative metabolism and reduced inflammation[40]. A study by Ozacmak et al[41] highlighted that EP administration could alleviate memory impairment caused by chronic cerebral hypoperfusion. Another study[42] reported attenuated cognitive decline, microglia activation, and impaired neurogenesis with EP treatment in an experimental sepsis model. A recent study[43] reported improved sensorimotor function and reduced myelin loss with EP treatments against traumatic brain injury.

In this experiment, ketamine-applied groups had lower step-through latencies in the PA test retention trial. The amygdala-dependent emotional memory deficits were reversed by risperidone and EP treatments. Cho et al[39] also reported reduced

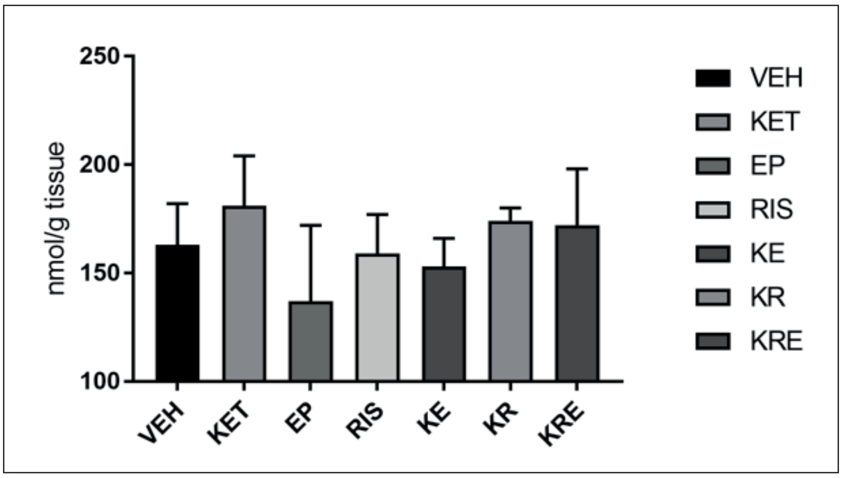

Figure 6. Malondialdehyde levels of the hippocampi tissue. VEH: vehicle, KET: ketamine (25 mg/kg, 14 days), EP: ethyl pyruvate (50 mg/kg, 14 days), RIS: risperidone (0.2 mg/kg, 14 days), KE: ketamine-ethyl pyruvate combination, KR: ketamine-risperidone combination, KRE: ketamine-risperidone-ethyl pyruvate combination. Each column represents the mean ± SEM of 7-9 mice. One-way ANOVA followed by a post hoc Tukey test was used for statistical analysis.

impairment in a passive avoidance test with EP treatment.

Ketamine reduced the NOR test ratio index values indicating short-term recognition memory deficit. Ketamine applied groups spent less time exploring the novel object compared with the vehicle group. Risperidone and EP, alone or in combination, slightly lessened ketamine-induced memory disruptions in the NOR test. Similarly, chronic pyruvate supplementation is reported to increase the exploration behavior of rodents in odour recognition task[44]. Risperidone, on the other hand, is reported to reverse recognition memory deficits induced by post-weaning social isolation[45].

Modified elevated plus maze (mEPM) test was conducted to measure spatial memory functions. In the mEPM test, transfer latencies of ketamine applied groups were increased, indicating memory deficits. Cognitive impairment was reduced with risperidone and EP treatments. Shi et al[46] reported improved spatial memory functions with EP after traumatic brain injury, and Koivisto has shown similar results with chronic pyruvate treatment, both in a morris water maze (MWM) test[44]. Celikyurt reported MK-801-induced memory disruption reductions with risperidone. She has shown improved spatial memory functions using an mEPM and an MWM test[15].

Ketamine is known to increase oxidation in the literature which contributes to neurodegenerative processes[16]. EP is reported to reduce neurodegeneration due to augmented oxidation[47]. In this study, MDA levels were assessed and regarded as an indicator of lipid peroxidation. MDA levels were insignificantly increased with ketamine applications. EP treatments were reversed the ketamine-induced MDA level alterations. Nevertheless, the differences were not found significant.

Alleviation of ketamine-induced cognitive impairments with EP can be linked to reduced oxidation, apoptotic markers, and inflammation, while the protective effects of risperidone may be primarily linked to improved neurotransmission balance.

Conclusions

According to the results of our study, we conclude that ethyl pyruvate can be a promising agent to alleviate ketamine-induced memory disturbances. However, a combination of EP and risperidone was not proven synergistic. With its potent antioxidant effects, EP can be considered an adjunct therapy to antipsychotics that are not as effective as risperidone to relieve cognitive symptoms. EP may be a profitable add-on in schizophrenia treatment to increase patients' comfort who are suffering from cognitive symptoms.

Contributors

Experiments were conducted by Emre Uyar (EU) and Hasan Eriman (HE). Ilker Kelle (IK) designed the study protocol, analyzed the results. The article was written by IK and Meral Erdinc (ME).

Conflict of Interest

The authors declare that there is no conflict of interest.

Funding Statement

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Availability of Data and Materials

The datasets related to this study are available from the corresponding author on reasonable inquiry.

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