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ORIGINAL RESEARCH ARTICLE

Repeated ketamine doses elevate superoxide dismutase activity in a pharmacological model of schizophrenia-like phenotypes in mice

Yusuf Usman[1]*, Adegbuyi Oladele Aderibigbe[2], and Fatai Adewale Fehintola[2]

[1]Department of Pharmacology, Faculty of Basic Medical Sciences, Federal University of Health Sciences Ila-Orangun, Ila-Orangun, Osun State, Nigeria

[2]Department of Pharmacology, Faculty of Basic Medical Sciences University of Ibadan, Ibadan, Oyo State, Nigeria

Abstract

This study evaluated behavioral phenotypes and superoxide dismutase (SOD) enzymatic activity in a repeated sub-anesthetic dose of ketamine (KET) administered to model schizophrenia in an animal study. The animals were divided into three (3) experimental groups. The KET alone group received sub-anesthetic dose of KET (20 mg/kg) for 14 consecutive days. The control group vehicle (VEH) received distilled water (10 mL/kg) as a VEH, while the KET and risperidone (RISP) group (KET + RISP) received a sub-anesthetic dose of KET (20 mg/kg) alone for 7 consecutive days, followed by RISP (0.5 mg/kg) administered 1-h post-KET treatment from days 8 to 14. All treatments were administered intraperitoneally (i.p.). Twenty-four hours after the last treatment, behavioral phenotypes (locomotor activity and cognition) were assessed using the locomotor activity cage and the elevated plus maze (EPM). Thereafter, SOD enzymatic activity was evaluated in homogenized brain tissue from each mouse using spectrophotometric analysis. Animals that received KET (20 mg/kg i.p) alone showed a significant ($P < 0.05$) increase in movement counts and rearing events in the locomotor activity test. It also prolonged the latency to enter the open arms during the anxiety-induced cognitive assessment in the EPM, compared to animals that received distilled water or those that received KET and RISP. SOD enzymatic activity was significantly elevated in the KET group compared to the VEH and KET + RISP groups. The elevated SOD enzymatic activity may represent a compensatory response to the oxidative stress induced by repeated sub-anesthetic doses of KET.

Keywords: Superoxide dismutase; Ketamine; Schizophrenia; Phenotypes; Mice; Model

***Corresponding author:**
Yusuf Usman
(usman.yusuf@fuhsi.edu.ng)

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1. Introduction

Superoxide dismutase (SOD) is an endogenous antioxidant enzyme that plays a critical role in converting superoxide radicals into less toxic hydrogen peroxide and oxygen molecules, thus protecting cells from oxidative damage.[1] In schizophrenia, altered SOD enzymatic activity has been observed, with studies showing both increased and decreased activities, depending on the stage of the disease and treatment status, with increased levels in first-episode schizophrenic patients.[2] Changes in endogenous

antioxidant enzyme activity are implicated in the pathogenesis of schizophrenia.[3] Increased SOD enzymatic activity has been reported in schizophrenic patients.[4] In severe schizophrenia, changes in SOD enzymatic activity have been experimentally shown to be associated with the general pathology of the disorder and the exacerbation of its symptoms.[2]

Ketamine (KET), an N-Methyl-D-Aspartate (NMDA) receptor antagonist, is commonly used in preclinical research to model schizophrenia in animals. Its sub-anesthetic doses have been shown to induce a variety of schizophrenia-like symptoms, including hyperactivity, cognitive deficits, social withdrawal, anxiety and behavioral changes, which mimic positive, negative, and cognitive symptoms of the disorder as seen in humans.[5] Acute or repeated KET administration has been used to induce schizophrenia-like phenotypes and to study the underlying neurobiological mechanisms in animal studies.[6,7] The drug's action on glutamatergic neurotransmission, specifically its antagonism of NMDA receptors, leads to an increase in dopamine release, which is thought to be the underlying mechanism of some of these effects.[8] The antagonistic effect of KET on NMDA receptors alters cognitive functions, as demonstrated in assessments of working memory using alternation tasks,[9] which could be associated with anxiety overload. In human studies, administration of sub-anesthetic doses of KET in healthy volunteers blocks NMDA receptors, thereby interfering with sensory information processing.[10] A single low dose of KET in the range of 10 – 30 mg/kg induces impaired working memory in a dose-dependent manner; the impaired working memory can be reversed by atypical antipsychotic drugs such as risperidone (RISP).[11] Behavioral paradigms such as hyperactivity and space exploration in the open field test (OFT) are used to assess psychotic-like behaviors in rodents.[7] The simplicity, reproducibility, and versatility of these paradigms make them essential tools for evaluating schizophrenia-like behaviors in animal models. Schizophrenia, a complex psychiatric disorder, is phenotypically characterized by hyperactivity as a positive symptom in rodents.[6] The OFT is particularly effective in evaluating hyperactivity, which is integral to schizophrenia pathophysiology.[12] Behavioral tests, including the elevated plus maze (EPM), have been extensively used to assess anxiety, risk-taking behavior, and cognitive impairments, which are often altered in schizophrenia.[13] The validity of these tests in assessing cognitive impairments lies in their ability to evaluate excessive and chronic anxiety, which may be interpreted in the context of thinking, memory, concentration, and decision-making.[13] Brain cognitive processing is a function of dopaminergic transmission in the prefrontal cortex (PFC) and is critically important for working memory.[8] *In vivo* microdialysis study have shown that acute KET administration impairs working memory, which is accompanied by increased dopamine release in the PFC.[14] In patients with schizophrenia, KET exacerbates both positive and negative symptoms, indicating that NMDA receptor antagonists may act on a neural system already compromised in psychosis.[15]

In animal models, KET induces behavioral and electrophysiological changes that parallel psychosis-like features observed in humans.[7] Late adolescence and early adulthood stages reflect the developmental periods when schizophrenia symptoms typically emerge in humans.[7,16] Behavioral, biochemical, and cognitive deficits induced at this stage closely mimic human schizophrenia phenotypes, including hyperactivity, sensorimotor gating deficits, and cognitive impairments.[7,16] Studies using pharmacological interventions, such as glutamate receptor antagonists like KET, demonstrate that developmental manipulations during late adolescence or early adulthood effectively reproduce schizophrenia-like behaviors. This allows for the evaluation of therapeutic interventions, such as antipsychotics or antioxidants, during critical neurodevelopmental windows in rodents.[7] Repeated administrations of low doses of KET (10, 20, and 30 mg/kg) have been shown to increase glutamate release in the PFC, thus non-NMDA receptors activation triggers dysregulation of dopaminergic neurotransmission in the PFC, thereby contributing to cognitive impairments.[17] Psychotic-like phenotype induction by KET suggests that endogenous dysfunction or dysregulation of NMDA receptor-mediated transmission may play a role in schizophrenia pathogenesis, potentially in conjunction with altered dopamine transmission. Furthermore, NMDA receptor antagonism affects serotonergic and GABAergic transmission in the PFC.[16] Most animal studies on NMDA receptor blockade focus on single-dose drug administration to induce schizophrenia-like phenotypes and mimic behavioral deficits. However, the effects of prolonged NMDA receptor antagonism in exploring the behavioral and neurobiological abnormalities of schizophrenia remain underexplored.[7]

Chronic exposure to sub-anesthetic doses of KET alters oxidative stress markers, including changes in SOD enzymatic activity, potentially driven by the activation of nicotinamide adenine dinucleotide phosphate oxidase in neurons.[18,19] These changes may also reflect a compensatory response to oxidative damage.[19,20] KET-induced schizophrenia models not only mimic behavioral symptoms but also replicate underlying biochemical abnormalities, such as the dysregulation of the endogenous antioxidant system.[21,22] While SOD is critical

in the pathogenesis of schizophrenia, limited research has explored its association with psychopathological symptoms. Notably, studies have revealed a significant positive correlation between SOD enzymatic activity and symptom severity across the positive, negative, and general psychopathology subscales of the positive and negative syndrome scale in chronic schizophrenia patients.[23] Elevated SOD enzymatic activity has also been linked to impaired cognitive performance, particularly in memory and decision-making tasks, as observed in the Y-Maze and locomotor behavior in an OFT.[24]

Neuroprotective effects of atypical antipsychotics in addressing the underlying pathophysiological mechanisms of the disorder have been reported. These therapeutic effects could be attributed to their role in microglial activation or maintenance of antioxidant mechanisms in the cerebral cortex and hippocampus.[25,26] This study examines the effects of repeated sub-anesthetic doses of KET administration on SOD enzymatic activity and schizophrenia-like behaviors in mice. Hyperactivity was measured in the OFT, while spatial working memory, as an indicator of cognitive dysfunction, was assessed with the EPM. These assessments provide insights into hyperactivity and anxiety-induced poor exploratory performance as representations of positive and cognitive symptoms of schizophrenia in an animal model, respectively.

2. Materials and methods

2.1. Animals

Male and female Swiss mice at postnatal days 56 – 70 (8 – 10 weeks) used for this study were obtained from the Department of Pharmacology and Therapeutics Animal House, Faculty of Basic Medical Sciences, University of Ibadan (Ibadan, Nigeria). They were housed in plastic cages under standard laboratory conditions (12 h light/dark cycle, temperature range of 25 ± 2°C, and relative humidity of 60 ± 5). The mice were provided with a standard diet and water ad libitum. They were acclimatized in the laboratory for 2 weeks before the experimental procedure. The animals were handled according to the institutional-based research ethics committee and National Institutes of Health (NIH) guidelines for the use and care of laboratory animals.

2.2. Drugs and chemicals

KET hydrochloride (Ranbaxy Pharm. Nigeria), RISP (Afrab Chem, Nigeria), carbonate buffer, pH 9.5, 200 mM (G-Biosciences, St Louis, USA), phosphate buffer solution, 1.0 M, pH 7.4 (Sigma-Aldrich, USA), and adrenaline (Sigma-Aldrich, USA) were used in this study. All chemicals and drugs were of analytical and pharmaceutical grades, respectively.

2.3. Dose selection

Doses administered were based on previous studies that demonstrated the induction of schizophrenia-like behaviors and altered oxidative stress in murine models.[6,26] KET (20 mg/kg) was administered to the KET group and RISP (0.5 mg/kg) was given along with KET to the KET + RISP group, and distilled water (10 mL/kg) was administered to the Vehicle (VEH) group as the VEH, based on previously established protocols.[6] KET was diluted in distilled water (VEH) and administered intraperitoneally (i.p.) as previously described.[26] Similarly, RISP was dissolved in the VEH before intraperitoneal (i.p.) administration.[6,26]

2.4. Experimental design

Male and female mice were randomly assigned to one of three groups: control (VEH), KET, or KET and RISP (KET + RISP), with no sex-specific ratio, as previously used.[6] The KET group received sub-anesthetic dose of KET (20 mg/kg, i.p.) alone once daily for 14 consecutive days. The control group (VEH) was given distilled water (10 mL/kg, i.p.) for the duration of the experiment. Animals in the KET and RISP (KET + RISP) group were pre-treated with KET (20 mg/kg, i.p.) from day 1 to day 7 and received RISP (0.5 mg/kg, i.p.) from the day 8 to day 14, 1 h after KET administration.[22]

2.4.1. Behavioral assessments

Twenty-four hours after the last treatment, each mouse was subjected to behavioral tests to assess schizophrenia-like symptoms. Hyperactivity was evaluated using the OFT, while cognitive deficits were evaluated using the EPM, as previously described with some modifications.[25-27] Each animal was tested individually, beginning with the OFT, followed by the EPM.

2.4.2. OFT

Hyperactivity was evaluated in an open field apparatus for 5 min, as previously described,[26] with minor modifications. Briefly, spontaneous locomotor activity was measured in an activity cage (Ugo Basile, Varese, Italy) with dimensions of 39 × 28 × 26 cm, featuring a front-view glass wall. The movement activity of each mouse was automatically recorded by the activity cage for 5 min. Data were presented as the number of horizontal and vertical motor activities measured by the apparatus in response to animal movements, and the number of times each mouse raises the forelimbs (rearing), respectively. The apparatus was thoroughly cleaned with 70% ethanol before introducing subsequent mouse. Before the locomotor activity test, each animal was habituated to the activity cage (Ugo Basile, Varese, Italy) for 10 min daily over 3 consecutive days after chronic KET administration.

2.4.3. EPM

Cognitive function was assessed using an EPM apparatus with 2 open arms (25 × 5 cm) and 2 closed arms (25 × 5 × 20 cm) extending from the cross-center platform (5 × 5 cm). The entire apparatus was elevated to 50 cm above the floor. At the start of the test, each mouse was placed on the cross-center platform facing an open arm. The latency period to enter the open arms was observed and recorded for 5 min using a stopwatch as a paradigm for assessing memory impairment.[13,27] A prolonged delay in entering the open arms was considered an indicator of altered exploratory performance, representing anxiety-induced decision-making deficits in working memory. Before testing, each mouse was exposed to the EPM apparatus for 5 min daily over 3 consecutive days to ensure familiarity.

2.5. Biochemical analysis

Immediately after the behavioral tests, each mouse was sacrificed by cervical dislocation, performed by trained and experienced personnel, in accordance with the institution and national guidelines on animal welfare. Then, the animals were decapitated, and their brain tissues were carefully removed. The whole brain tissue of each animal was homogenized for 60 s in 5 mL of 10% w/v cold phosphate buffer (0.1 M, pH 7.4, 4°C) using a homogenizer. The homogenates were stored in a refrigerator until biochemical analysis was carried out. Subsequently, the homogenates were centrifuged at 10,000 g for 10 min at 4°C. The resulting homogenized supernatant was separated from the pellets for SOD activity assay. The SOD activity in each brain homogenate was determined as described.[28] Briefly, 0.5 mL of the homogenate was diluted with 4.5 mL of distilled water to make a 1 in 10 dilution. Anhydrous sodium bicarbonate (6.897 g) was added to the carbonate buffer solution to achieve a pH of 10.2. An aliquot of 0.2 mL of the diluted sample was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2) and allowed to equilibrate in a spectrophotometer. The reaction was initiated by adding 0.3 mL of freshly prepared 0.3 mM adrenaline (epinephrine) dissolved in distilled water, which was quickly mixed by inversion. The reference cuvette (blank) contained 2.5 mL of carbonate buffer, 0.3 mL of substrate (adrenaline), and 0.2 mL of distilled water. The increase in absorbance at 480 nm was monitored for 60, 120, and 180 s.

Calculation: Increase in absorbance per minute $= \frac{A3\text{-}A0}{2.5}$; [28]

Where A_0 = absorbance after 60 s, A_3 = absorbance after 180 s

$$\%\,\text{inhibition} = 100 - \frac{\text{Increase in absorbance for substrate}}{\text{Increase in absorbance for blank}} \times 100$$

One unit of SOD activity is defined as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline.[28]

2.6. Statistical analysis

The data (behavioral and biochemical) were expressed as mean ± S.E.M and analyzed using one-way analysis of variance and *post hoc* tests (Student's Newman-Keuls) for the multiple comparisons where appropriate using GraphPad InStat® Biostatistics software (GraphPad Software Inc. USA). A level of $P < 0.05$ was considered statistically significant for all tests.

3. Results

3.1. KET-induced hyperactivity

Locomotor activity, assessed by measuring movement counts over 5 min (in seconds) and the number of rearing events, showed that the KET group exhibited a significant ($P < 0.05$) increase in movement counts and rearing events in the OFT compared to the VEH and KET + RISP groups. Specifically, the KET group showed an average movement of 549.0 ± 19.9 and 16.40 ± 0.93 rearing events. The control group (VEH) showed an average movement of 377.4 ± 14.6 and 9 ± 0.71 rearing events, while the RISP-treated group (KET + RISP) showed an average movement of 446.0 ± 10.2 and 10.40 ± 0.93 rearing events, respectively (Table 1). The significant increase in both movement counts and rearing events in the KET group suggests that KET induces hyperactivity. The control group showed the lowest activity levels, while the KET + RISP group exhibited intermediate activity (Table 1), indicating that RISP may mitigate some of the hyperactive behavior induced by KET (20 mg/kg, i.p.) administration.

3.2. Exploratory performance in the EPM

The KET group showed poor exploratory performance in the EPM test, with significant increase ($P < 0.05$) in latency to enter the open arms. The mean latency value for the KET group was 34.30 ± 1.77 s, in contrast to 18.79 ± 0.47 and 24.36 ± 1.75 s for the VEH and KET + RISP groups, respectively (Table 2). The increased latency in the KET group suggests that KET impaired spatial memory, as evidenced by the poor performance after prior exposure and the increased time spent to enter the open arms. The control group showed the shortest latency, while the KET + RISP group showed an intermediate latency (Table 2), suggesting that RISP may have cognitive-enhancing effect, reducing the altered decision-making and exploratory performance deficits induced by KET administration.

3.3. KET increases SOD activity

Animals in the KET group, which received KET alone for the duration of the experiment, showed a significant

Table 1. Locomotor activity assessment

S/N	Treatments	Movement counts/5 min in activity cage (secs)	Numbers of rearing
1.	VEH	377.4±14.6**	9±0.71**
2.	KET	549.0±19.9	16.40±0.93
3.	KET+RISP	446.0±10.2**	10.40±0.93**

Notes: Ketamine-induced hyperactivity (spontaneous movement and rearing events) in mice.
Values represent mean±S.E.M of 5 animals per group. In one-way analysis of variance test (ANOVA), ($F^{[2}.000, 9.552] = 31.43$, $P<0.0001$; $F^{[2}.000, 11.^{40} = 20.88$, $P<0.0001$), VEH and KET+RISP groups showed significant difference when compared with KET group. **$P<0.05$ as compared with KET group.
Abbreviations: VEH: Vehicle; KET: Ketamine; RISP: Risperidone.

Table 2. Evaluation of anxiety-induced poor exploratory performance

S/N	Treatments	Latency period in open arms entries in EPM/5 min) (sec)
1	VEH	18.79±0.47**
2	KET	34.30±1.77
3	KET+RISP	24.36±1.75**

Notes: Ketamine-induced altered spatial working memory (cognitive dysfunction) in mice.
Values represent mean±S.E.M of 5 animals per group. In one-way analysis of variance test (ANOVA), ($F^{[2}.000, 8.551] = 28.95$, $P<0.0001$), VEH and KET+RISP groups showed significant difference when compared with KET group. **$P<0.05$ as compared with KET group.
Abbreviations: VEH: Vehicle; KET: Ketamine; RISP: Risperidone; EPM: Elevated plus maze.

($P < 0.05$) increase in SOD activity, with a mean value of 49.98 ± 0.51 unit/mg protein, compared to the VEH and KET + RISP groups, which showed mean values of 34.84 ± 1.06 and 36.52 ± 1.30 unit/mg protein, respectively (Figure 1).

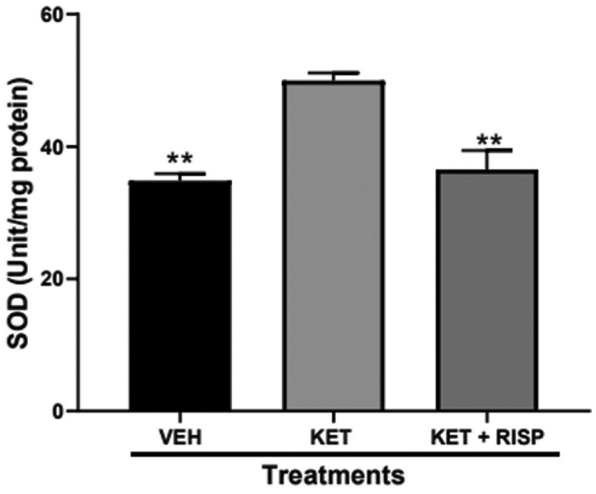


Figure 1. Superoxide dismutase activity in brain samples. Bars represent mean ± S.E.M of 5 animals per group. In one-way analysis of variance test (ANOVA), ($F^{[2}.000, 9.055] = 67.55$, $P < 0.0001$), VEH and KET + RISP groups showed significant differences when compared with KET group. **$P < 0.05$ as compared with KET group.
Abbreviations: VEH: Vehicle; KET: Ketamine; RISP: Risperidone; SOD: Superoxide dismutase.

4. Discussion

This study investigated the behavioral phenotypes and alterations in SOD enzymatic activity in a mouse model of schizophrenia induced by repeated sub-anesthetic doses of KET. Rodent models are widely utilized to study the biochemical and behavioral aspects of schizophrenia, providing insights into its underlying pathophysiology.[7,16] Behavioral paradigms such as the OFT and the EPM are particularly effective in evaluating hyperactivity and anxiety, respectively.[7] The EPM is also employed to assess altered exploratory behavior, which serves as a measure of cognitive impairments[13,27] integral to schizophrenia pathophysiology.[16] In these models, EPM assesses anxiety-induced decision-making processes during exploratory performance as spatial working memory by evaluating rodents' ability to adapt their exploratory behavior in response to environmental cues as evaluated by latency period to enter the open arms.[13,27] Frequent entries and preference for the closed arms, resulting in prolonged latency time to enter the open arms, suggest deficits in decision-making processes during exploration of the habituated environment. This behavior serves as a measure of anxiety-associated poor exploratory performance.[27] The OFT is particularly effective in evaluating hyperactivity.[12] Notably, alterations in the endogenous antioxidant system, marked by changes in antioxidant enzyme activity, have linked oxidative stress to behavioral deficits in schizophrenia.[2] Activities of endogenous antioxidant enzymes underscore the critical role of oxidative stress in schizophrenia pathophysiology. Alterations in brain biochemical activities, including induced oxidative stress and compensatory enzymatic activities of SOD, have been reported following the administration of psychotomimetic drugs like KET.[29,30] often coupled with schizophrenia-like behavioral manifestations.[6] Single and repeated administrations of KET are commonly employed to alter glutamate neurotransmission, serving as a model for psychosis-like effects. By acting as an NMDA receptor antagonist, KET induces a dissociative state characterized by disorganization and sensory perception.[14] In this study, findings from the OFT demonstrated that repeated sub-anesthetic doses of KET induced significant hyperactivity in a mouse model of schizophrenia. Increased horizontal and vertical movement, as well as rearing events, in the

KET-treated mice suggests heightened motor activity, which is the characteristic of KET's psychostimulant properties.[31,32] The enhanced locomotor activity observed in experimental animals following the administration of KET is often considered a model for the positive symptoms of schizophrenia, including arousal and disorganized behavior. This behavioral response may be attributed to heightened dopaminergic activity, particularly in the nucleus accumbens,[33] and disrupted cortical connectivity induced by the hypofunction of glutamatergic activity.[8] As such, deficit in behavior that followed repeated sub-anesthetic doses of KET administration may be triggered by the combined effects of direct or indirect dopamine agonists and NMDA receptor antagonists.[34] Cognitive function, as inferred from the EPM performance, was also impaired in the mice that received KET alone. The KET group demonstrated a prolonged latency period to enter the open arms ($P < 0.05$), which may reflect high level of anxiety-induced poor task performance due to deficits in decision-making processes.[13,27,30] Altered exploratory performance and frequent entries into the closed arms as indicatives of phenotypic anxiety were observed in KET group during the EPM test. This finding is consistent with previous findings linking anxiety overload to impaired normal cognitive processes in schizophrenia models.[27,35] This behavioral change might be due to the disruption of normal glutamatergic neurotransmission caused by KET, leading to impaired cognitive and memory functions.

Schizophrenia pathophysiology is intricately linked to oxidative stress, marked by a disruption between the production of reactive oxygen species (ROS) and antioxidant defense mechanisms. Elevated ROS activity, primarily resulting from NMDA receptor dysfunction, disrupts glutamate homeostasis, contributing to excitotoxicity and synaptic damage. These biochemical alterations are closely associated with the development of schizophrenia's positive, negative, and cognitive symptoms. Among the key components of the antioxidant defense system, SOD plays a pivotal role by converting superoxide radicals into hydrogen peroxide (H_2O_2) and oxygen, thereby preventing the formation of peroxynitrite through the interaction of nitric oxide (NO) with superoxide radicals.[29] The altered SOD enzymatic activity observed in schizophrenia is of particular interest, as it provides insights into the oxidative mechanisms underlying the disorder and the potential neuroprotective or neurotoxic implications of SOD enzymatic activity. While heightened SOD enzymatic activity may reflect a compensatory mechanism against excessive ROS production,[19,20] it also suggests an overwhelmed antioxidant defense system, which can lead to persistent oxidative damage.[17] In this study, the impact of repeated sub-anesthetic doses of KET administration on SOD enzymatic activity in a mouse model of schizophrenia was investigated. The findings revealed that repeated KET administration significantly elevated SOD enzymatic activity, which may indicate an enhanced endogenous antioxidant system resulting from an increased oxidative stress response. These results align with existing literature emphasizing oxidative stress as a central component of schizophrenia pathology and the potential of KET to exacerbate this condition under certain dosing regimens.[22] Also, the observed increase in SOD enzymatic activity may represent a compensatory response to counteract elevated ROS levels induced by KET's pharmacological actions.[19,35] Elevated SOD enzymatic activity may reflect attempts to preserve overall redox balance, although it can simultaneously exacerbate the neurobiological and behavioral symptoms of schizophrenia.[36] Moreover, KET's effect on NMDA receptors may lead to increase NO generation via the NO/Ras/extracellular-regulated kinase 1/2 pathway,[37] and it may also interfere with mitochondrial function, particularly complex I activity, which further contributes to oxidative stress.[29,36] This disruption impairs oxidative phosphorylation, increases mitochondrial NO synthase activity, and generates superoxide radicals and NO.[37-39] These alterations likely trigger heightened SOD enzymatic activity as a compensatory response. Interestingly, preconditioning studies involving volatile anesthetics have demonstrated that transient inhibition of complex I, accompanied by a moderate increase in NO or superoxide radicals, activates SOD, resulting in a subsequent increase in its activity.[38,39] However, in schizophrenia models, KET's persistent effects may overwhelm this protective mechanism.[38,39] Furthermore, heightened SOD enzymatic activity may signify increased neuroinflammation, as oxidative stress activates pro-inflammatory pathways, leading to cytokine release and neuronal damage.[3,36] Neuroinflammation exacerbates schizophrenia symptoms, creating a cycle of oxidative damage and inflammation.[36]

The relationship between oxidative stress and schizophrenia symptoms was further underscored by behavioral correlations observed in the study. Elevated SOD enzymatic activity may contribute to phenotypic hyperactivity and cognitive impairments, which could be associated with the reinforcing role of oxidative stress and antioxidant dysregulation in schizophrenia. These findings highlight the potential of antioxidant therapies to alleviate schizophrenia-related symptoms.

Treatment with the atypical antipsychotic drug RISP (0.5 mg/kg, intraperitoneally) provided significant therapeutic benefits in the study. RISP mitigated KET-induced hyperactivity in the OFT and improved cognitive performance in the EPM test. Importantly, RISP

administration significantly ($P < 0.05$) reduced elevated SOD activity in KET-treated mice. This reduction was accompanied by behavioral symptom amelioration, consistent with clinical findings in schizophrenic patients receiving RISP.[40] The ability of antipsychotic medications to modulate the oxidative stress defense system may contribute to their therapeutic efficacy.

5. Conclusion

This study confirms that KET-induced NMDA receptor blockade leads to hyperactivity, cognitive impairment, and elevated SOD enzymatic activity, mirroring key schizophrenia-like phenotypes. Elevated SOD enzymatic activity was observed as a response to repeated sub-anesthetic doses of KET administration. The heightened SOD enzymatic activity likely represents a compensatory mechanism aimed at mitigating the effects of oxidative stress induced by KET administration. However, this response may also contribute to the persistence or exacerbation of neurobiological and behavioral symptoms associated with schizophrenia.

The findings suggest that oxidative stress plays a central role in the pathophysiology of KET-induced schizophrenia models. Elevated SOD enzymatic activity highlights the dysregulation of antioxidant defenses in response to excessive ROS production. Targeting this oxidative imbalance with antioxidant therapies holds potential for alleviating neurobiological and behavioral symptoms. Further investigations could focus on assessing oxidative and antioxidant markers, inflammatory cytokines, and neurotransmitter parameters to explore their modulation by therapeutic interventions.

These results emphasize the interplay between SOD enzymatic activity and NMDA receptor dysfunction in schizophrenia and underscore the importance of antioxidant approaches that restore redox balance. Such approaches may also improve behavioral and cognitive outcomes in schizophrenia models.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: All authors
Formal analysis: Yusuf Usman, Adegbuyi Oladele Aderibigbe
Investigation: Yusuf Usman
Methodology: All authors
Writing-original draft: Yusuf Usman
Writing-review & editing: Adegbuyi Oladele Aderibigbe, Fatai Adewale Fehintola

Ethics approval and consent to participate

All experimental procedures were approved by the Animal Care and Use Research Committee (ACURC), Faculty of Basic Medical Sciences, Federal University of Health Sciences Ila-Orangun (FUHSI/BMS/AREC/2401). All the procedures were in-line with the guidelines set forth by the NIH for the care and use of laboratory animals.

Consent for publication

Not applicable.

Availability of data

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Further disclosure

The paper has been uploaded to or deposited in a preprint (bioRxiv repository, with MS ID#: BIORXIV/2024/610179. doi: https://doi.org/10.1101/2024.08.28.610179 https://biorxiv.org/cgi/content/short/2024.08.28.610179v1).

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