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Research paper

Antipsychotic effect of diosgenin in ketamine-induced murine model of schizophrenia: Involvement of oxidative stress and cholinergic transmission

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ABSTRACT

A decrease in the levels of antioxidant arsenals exacerbate generation of reactive oxygen/nitrogen species, leading to neurochemical dysfunction, with significant impact on the pathogenesis of psychotic disorders such as schizophrenia. This study examined the preventive and reversal effects of diosgenin, a phyto-steroidal saponin with antioxidant functions in mice treated with ketamine which closely replicates schizophrenia-like symptoms in human and laboratory animals. In the preventive phase, adult mice cohorts were clustered into 5 groups (n = 9). Groups 1 and 2 received saline (10 mL/kg, *i.p.),* groups 3 and 4 were pretreated with diosgenin (25 and 50 mg/kg), and group 5 received risperidone (0.5 mg/kg) orally for 14 days. Mice in groups 2–5 additionally received a daily dose of ketamine (20 mg/kg, *i.p.*) or saline (10 mL/kg/day, *i.p.*). In the reversal phase, mice received intraperitoneal injection of ketamine or saline for 14 consecutive days prior to diosgenin (25 and 50 mg/kg/*p.o.*/day) and risperidone (0.5 mg/kg/*p.o.*/day) treatment from days 8–14. Mice were assessed for behavioral changes. Oxidative, nitrergic markers, and cholinergic (acetylcholinesterase activity) transmission were examined in the striatum, prefrontal-cortex and hippocampus. Diosgenin prevented and reversed hyperlocomotion, cognitive and social deficits in mice treated with ketamine relative to ketamine groups. The increased acetylcholinesterase, malondialdehyde and nitrite levels produced by ketamine were reduced by diosgenin in the striatum, prefrontal-cortex and hippocampus, but did not reverse striatal nitrite level. Diosgenin increased glutathione, and catalase levels, except for hippocampal catalase activity when compared with ketamine controls. Conclusively, these biochemical changes might be related to the behavioral deficits in ketamine-treated mice, which were prevented and reversed by diosgenin.

1. Introduction

Schizophrenia is a chronic psychiatric and disabling brain disorder characterized by psychotic symptoms and cognitive and functional deficit (Ermakov et al., 2021). While the occurrence of this disorder remains over 1% of the global population, the pathophysiology remains unclear with diverse associated pathologies (Monte et al., 2013; Ben-Azu et al., 2018a). However, research on heterogeneity of schizophrenia consists of inflammatory, immune and genetic factors, and other causative biological linkages that have gained a significant interest over the past decade (Ben-Azu et al., 2023a). Interestingly, these factors and the biological pathways are united by the involvement of oxidative stress (Monte et al., 2013; Ben-Azu et al., 2018a). In this context, the vulnerability-stress-biological alteration models are supported with remarkable growing evidences (Ben-Azu et al., 2018a, 2019, 2022, 2023a, 2019, 2018a; Ermakov et al., 2021; Omeiza et al., 2023). There are enormous reports of redox imbalance in the etiology of schizophrenia in numerous literatures (Hellemans et al., 2010; Koga et al.,

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2016; Ben-Azu et al., 2023b) and meta-analyses (Fraguas et al., 2017; Hoen et al., 2013; Wang et al., 2016). Regardless of the heterogeneity of data involved, the pre-eminence of pro-oxidant processes and antioxidant system deficiency, indicating, the condition of generalized oxidative stress is a strong-point in schizophrenia and has popularly remained a drug target for the treatment of the disease (Monte et al., 2013; Ben-Azu et al., 2018a, 2018b, 2019, 2023b). Most importantly, alterations in non-enzymatic antioxidant system such as vitamins C, E, glutathione, and other biochemical substrates result in brain reduction of polyunsaturated fatty acids (PUFAs) contents. As such, these models imply that the predisposition of subjects affected by oxidative stress is dependent on the genetic makeup, which notably exacerbates inflammatory responses and alterations of other biological pathways later in life notably implicated in the disease progression (Smigielski et al., 2020).

Schizophrenia is reported to have several etiological roots that are yet to be fully understood, but not limited to the already reported neurotransmitter perturbations such as alterations in dopamine, glutamate, serotonin, gamma amino butyric acid (GABA), and acetylcholine (ACh) neurotransmissions (Chatterjee et al., 2012; Howes et al., 2015; Omeiza et al., 2023) as well as neuro-immune and neurotrophic protein derangements in critical brain regions (Zhang et al., 2016; Ben-Azu et al., 2021, 2019). In addition, clinical and preclinical findings have shown evidences of neuro-immunological imbalance, notably involving over-reactivity of microglia and astrocyte sensomes, including the pathological release of neuronal cytokines and neuro-inflammatory proteins release in schizophrenia (Ben-Azu et al., 2019; Comer et al., 2020; Ben-Azu et al., 2023).

Animal model of ketamine (KET) treatment in experimental mice have been shown to stimulate broad clinical symptoms and observable features similar to those found in schizophrenic patients (Krystal et al., 1994). KET is a psychotomimetic that acts on glutamate system by inhibiting *N*-methyl-D-aspartate (NMDA) receptors (Beck et al., 2020; Ben-Azu et al., 2019; Omeiza et al., 2023). Administration of KET in experimental animals induce both positive (perceptual changes and delusions) and negative symptoms (blunted affect and emotional/social withdrawal) as well as cognitive deficits consisting of learning and memory impairments (Monte et al., 2013; Ben-Azu et al., 2018b; McCutcheon et al., 2019). It is evidenced in several studies that KET-induced experimental schizophrenia was associated with oxido-nitrosative stress cascade, profoundly depleting endogenous antioxidant system, up-regulating inducible nitric oxide (NO) synthase (iNOS), release of NO, and integrative upstream release of inflammatory cytokines and proteins with concomitant disruption of glutamatergic system (Monte et al., 2013; Ben-Azu et al., 2018c; de Araújo et al., 2021). Specifically, Abram et al. moreover established that KET induces NMDAR hypofunction by refashioning the thalamic hyper-connectivity similar to that found in schizophrenic patients, across their illness course, including the clinical high-risk for psychosis period preceding the onset of psychosis. Thus, proposing that KET model significantly mimics the onset, progress and/or severity of psychotic symptoms in the patients (Usman et al., 2019; Abram et al., 2022; Ben-Azu et al., 2019; Okubo Eneni et al. (2020)). Interestingly, notable findings have shown that KET-induced experimental schizophrenia were abated by second generation antipsychotic drugs such as risperidone, aripriparzole and clozapine (Ben-Azu et al., 2018b; Okubo Eneni et al. (2020); Eneni et al., 2023).

Therapeutically, the use of antipsychotic drugs is currently considered as a treatment measure for attenuating schizophrenia-like symptoms and associated pathophysiologies in pre-clinical (Monte et al., 2013; Ben-Azu et al., 2023b; Eneni et al., 2023) and clinical (Patel et al., 2014; Dietrich-Muszalska et al., 2021; Hong and Bang, 2020) settings. However, adopting nutritional pharmacotherapy as a treatment option for schizophrenia condition shows that dietary measure involving nutraceuticals products remain a cost-effective and a naturally viable approach to prevent and extenuate schizophrenia and its associated pathologies (Ben-Azu et al., 2018, 2019; Oshodi et al., 2021; Ishola et al., 2021; Ugwu et al., 2022; Emudainohwo et al., 2023).

Notably, diosgenin (DG) is a natural steroidal saponin, biosynthesized from cholesterol through isoprenoid pathway in several medicinal plants (Avula et al., 2014). Previous investigations reported that it exhibits high biocompatibility with low toxicity profile when administered acutely or sub-chronically to rodents (Cayen et al., 1979; Qin et al., 2009). Further studies have shown that DG and its derivatives possess significant antioxidative, anti-inflammatory, neuroprotective and anti-apoptotic ability against different brain diseases (Li et al., 2018; Mahmoudi et al., 2021; Tohda et al., 2013, 2012). Pre-clinically, DG has demonstrated potent effects in the treatment and management of nervous system diseases such as Parkinson's and Alzheimer's diseases (He et al., 2018; Li et al., 2018; Yang and Tohda, 2018). Also, as a nootropic agent, DG improves memory performance and mitigates Alzheimer's disease pathology associated with cognitive deficit, notably including oxidative damage in rodents (Mahmoudi et al., 2021; Tohda et al., 2013, 2012; Yang and Tohda, 2018). The above-mentioned restorative properties exhibited by DG strongly suggest that it is a promising candidate for the treatment of schizophrenia disease. Hence, this study was designed to investigate the preventive and reversal effects of DG in experimental KET model relevant to schizophrenic disorder in mice.

2. Materials and method

2.1. Drugs and reagents

Diosgenin (DG), risperidone (RIS), antioxidant reagents were purchased from Sigma-Aldrich, St. Louis, MO, USA and Burgoyne Burbidges & Co., Mumbai, India. Ketamine hydrochloride was bought from Rolex Medica, Germany. Other chemicals bought for this study were of analytical grades with the highest purities.

2.2. Experimental animal

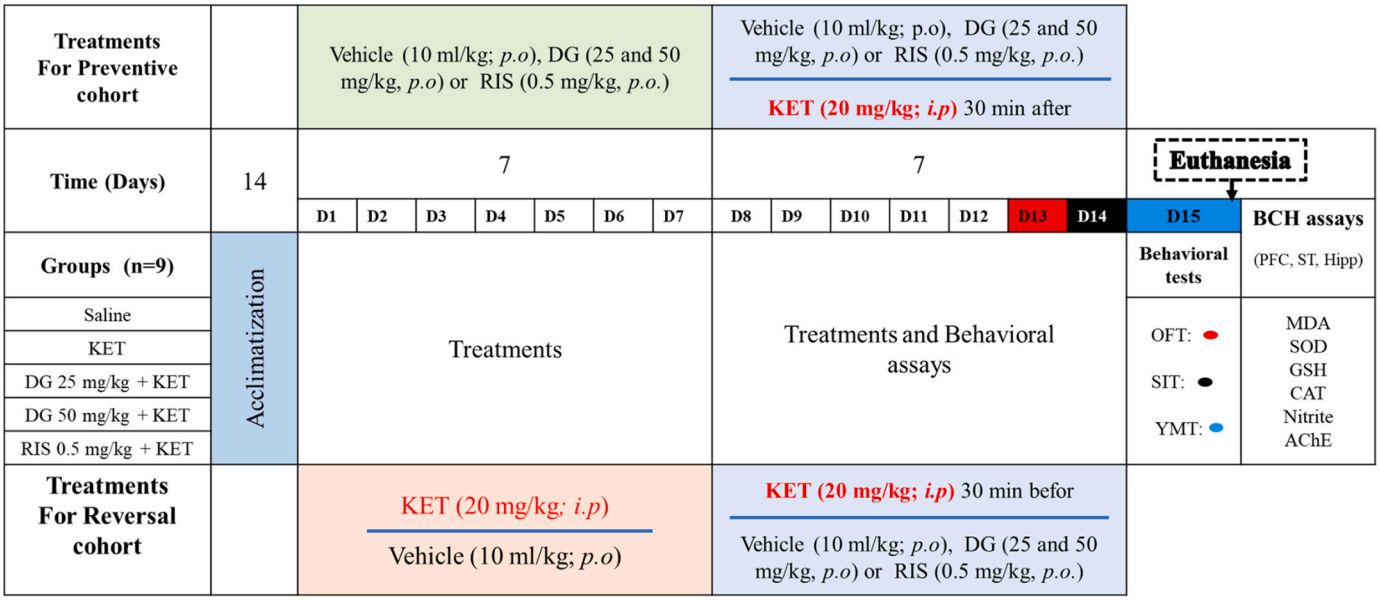
Male Swiss albino mice (20–25 g) were from the Laboratory Animal Centre of the College of Medicine, Delta State University, Abraka and kept in the University central animal house (23 ± 2 ∘C; 12-hr light/12-hr dark cycle, 40–70% relative humidity) with unlimited access to food and water. All treatment and experimental protocol were approved by Delta State University Animal Care and Use Research Ethics Committee of the Faculty of Basic Medical Sciences (REC/FBMS/DELSU/23/185) in compliance International Regulatory Agencies such as National institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985).

2.3. Dose selection

The study was assigned into two separate cohorts to evaluate the preventive and reversal effects of DG on KET-induced schizophrenia behavior and oxidative alterations as previously described (Ben-Azu et al., 2022; Monte et al., 2013). The doses of KET (20 mg/kg) (Ben-Azu et al., 2023, 2022), DG (25 and 50 mg/kg) (Leng et al., 2020; Sathya et al., 2020), and RIS (0.5 mg/kg) were selected according to findings from preliminary and previous reports. Saline (0.9%) was used to constitute DG and RIS, prior to oral (*p.o.*) gavage administration and were administered at a volume of 10 mL/kg of individual animal weights; whereas KET was diluted in 0.9% saline and given intraperitoneally (*i.p.*) as established from previous protocols (Ben-Azu et al., 2023; Ben et al., 2022; Chatterjee et al., 2012).

2.4. Treatment protocols

In the preventive cohort, the animals were organized into 5 treatment groups ($n = 9$). The animals assigned into groups 1 and 2 received saline (10 mL/kg, *p.o.*) pre-treatments, groups 3 and 4 were given DG



Scheme 1. Treatment protocol.

(25 and 50 mg/kg, *p.o*) while animals in group 5 was given RIS (0.5 mg/kg, *p.o.*) daily for 14 days. Thereafter, from day 8 to 14, the animals in groups 2–5 were daily injected with KET (20 mg/kg, *i.p.*) 30 min after oral administration of the vehicle and DG respectively. In the reversal cohort, the animals were also organized into 5 treatment groups ($n = 9$). Groups 1 received saline (10 mL/kg, *p.o.*) while groups 2–5 were daily injected with ketamine (20 mg/kg, *i.p.*) for 14 days. From days 8 to 14, the animals in group 2 additionally had saline (10 mL/kg, *p.o.*), groups 3 and 4 were administered DG (25 and 50 mg/kg, *p.o*) while group 5 received RIS (0.5 mg/kg, *p.o.*) once daily 30 min after KET injection. Behavioral phenotypes of schizophrenia such as explorative activity in open field test (OFT), social interaction test (SIT), and spatial working memory with Y-maze test was carried out. To avoid KET interference effect on gait and posture, OFT and SIT assessments were done on days 13 and 14 respectively, while Y-maze test (YMT) was done on day 15 twenty-four hour after treatments by animal behavioral experts who were blinded to the experimental groups (Scheme 1).

2.5. Behavioral tests and weight measurement

Explorative activity and locomotor behavior were evaluated in the animals using the OFT for 5 min according to earlier described protocol (Monte et al., 2013). Briefly, the apparatus which is a wooden box (28 ×28×25 cm) contains 16 equal squares (7 ×7 cm) lines with a front view glass wall. In the apparatus, we placed animals in the left-hand corner of the box and explorative activity consisting of number of lines crossing of each mouse was recorded for 5 min with a stop watch.

For the SIT assessment, 10 min investigation time was allotted for each animal as described earlier by Monte et al. (2013) and others (Ben-Azu et al., 2018a) using a plexiglass box separated into three chambers (60 ×40 cm) namely A (social chamber), B (middle chamber) and C (non-social chamber), connected by a small door (6 ×6 cm) on two sides of the middle chamber. In this test, chambers A and C contained iron nested restraining cages while chamber B (middle chamber) was without a restraining cage but was used to introduce mouse into the chambers. Briefly, experimental mouse was presented through chamber B for a 5 min exploration and familiarization period in all three-chambers. Thereafter, the mouse was removed, and a novel same-sex attractant mouse was placed inside the restraining cage in chamber A while the restrain cage chamber C was without. Immediately, the experimental mouse was returned through chamber B for exploration between chambers A-C for another 5 min. At the end, percentage (%) social preference was calculated for each animal following the period spent visiting chambers A and C using the formula: (% exploration time in the social chamber) - (% exploration time in the opposite chamber).

The YMT was used to investigate the spatial working memory in the experimental mice. This was judged based on the pattern and number of arms visitations for 5 min in a 3-armed (A, B and C) Y-maze apparatus (33 ×11×12 cm) separated proportionally at 120 °C. Correct alternation was defined as sequential entries into arms A to C (ABC, BCA, CAB etc) while cognitive impairment behavior was defined by wrong alternations such as BAB, CCA, BBA etc. At the end, cognitive function was scored as % correct alternation according to previous studies (Monte et al., 2013; Ben-Azu et al., 2022a). Body weights were also measured as previously described on the days 8 and 15 (Ben-Azu et al., 2023b).

2.6. Tissue preparation for biochemical analysis

The experiment was terminated on day 15 after the completion of behavioral test. The mice were anaesthetized with KET hydrochloride followed by cervical dislocation. The brain was excised and the hippocampus, striatum and pre-frontal cortex were dissected on cold ice tray. The dissected brain regions were immediately homogenized in a cold phosphate buffer (10% w/v, 0.1 M, pH 7.4) and then centrifuged for 10 min/10,000 rpm at 4 °C to obtain the supernatants. Finally, the supernatants were stored (−20 °C) until biochemical assessments.

2.6.1. Estimation of protein content

The level of protein in the striatum, prefrontal cortex, and hippocampus samples were determined with the previously described protocol by GORNALL et al. (1949). Distil water (0.9 mL), striatum, prefrontal cortex, and hippocampus (0.1 mL) and biuret reagent (3 mL) were thoroughly mixed together and left to incubate at 25 °C for 30 min. Finally, the absorbance was read at 540 nm using an UV/Vis Spectrophotometer (INESA 750 N, China). The standard (1 mg/mL bovine serum albumin) was then calculated within 0.01–0.1 mg/mL range (GORNALL et al. (1949)).

2.6.2. Estimation of lipid peroxidation

The lipid peroxidation activity in the striatum, prefrontal cortex, and hippocampus were measured by estimating the content of

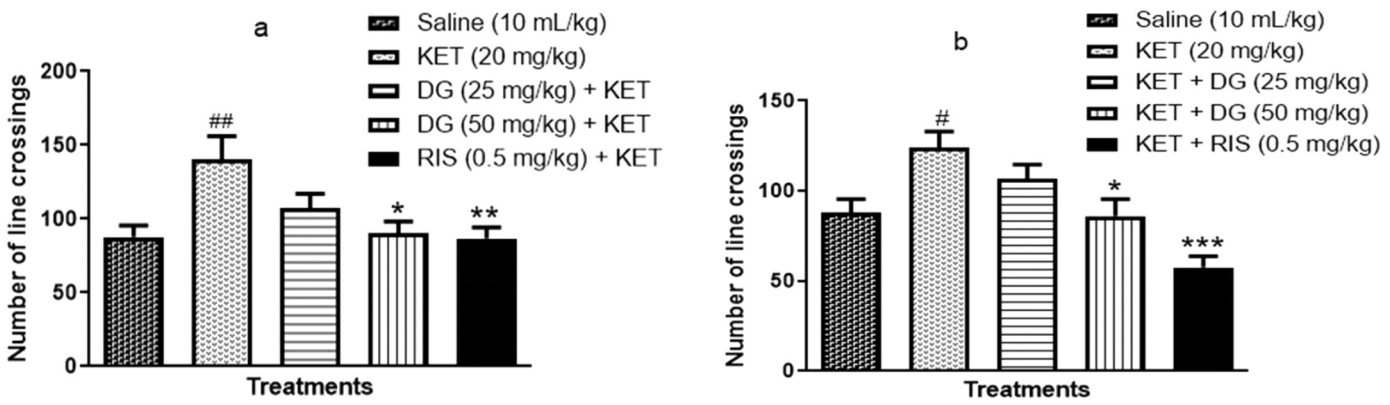


Fig. 1. Diosgenin (DG) abates ketamine-induced hyperlocomotion in the preventive (a) and reversal (b) treatment protocols. Bars represent the mean ± S.E.M of 9 animals / group. #$p < 0.05$, ##$p < 0.01$ vs saline group and *p < 0.05, * *p < 0.01, ** *p < 0.001 vs KET group **KET** = Ketamine, **RIS** = Risperidone.

malonylaldehyde (MDA) as described by Emudainohwo et al. (2023) and Nagababu et al. (1994). 100 µL aliquots of the different brain regions were mixed with 900 µL Tris-KCl buffer prior to addition of 500 µL of 30% TCA. 500 µL of 0.75% thiobarbituric acid (TBA) was then added to the mixture and heated in water bath for 45 min at 80 °C. Thereafter, the heated mixture was immediately cooled and centrifuged for 5 min at 3000 rpm. the absorbance level set at 532 nm (UV/Vis Spectrophotometer (INESA 750 N, China)). The formed MDA was calculated by molar extinction coefficient of 1.56 × 105/M/cm and expressed as nmol MDA mg-1 protein (Emudainohwo et al., 2023; Nagababu and Lakshmaiah, 1994).

2.6.3. Estimation of nitrite level

The striatal, prefrontal cortical, and hippocampal nitrite concentrations were estimated using the Griess reagent, which shows the amount of nitric oxide generation. 100 µL of greiss reagent (1:1 solution of 1% sulfanilamide in 5% phosphoric acid and 0.1% of N-1- naphthyl ethylenediamine dihydrochloride) was thoroughly mixed and added to 100 µL of the supernatant and then the absorbance was read at 540 nm wavelength with the UV/Vis-spectrophotometer (752 N INESIA, China). The striatum, prefrontal cortical, and hippocampal nitrite levels were calculated from the standard curve generated from sodium nitrite (0–100 µM) (Green et al., 1982).

2.6.4. Estimation of reduced glutathione level

The reduced glutathione (GSH) contents in the striatum, prefrontal cortex, and hippocampus were analyzed as according to Jollow et al. (1974) 100 µL aliquot of the striatum, prefrontal cortex, and hippocampus were added to 400 µL of 20% TCA and then centrifuged for 10 min at 4 °C set at 10,000 rpm. 2 mL of 0.6 M DTNB was added to the reacting mixture and then underwent 10 min incubation at room temperature. Finally, the absorbance was read at a wavelength of 412 nm

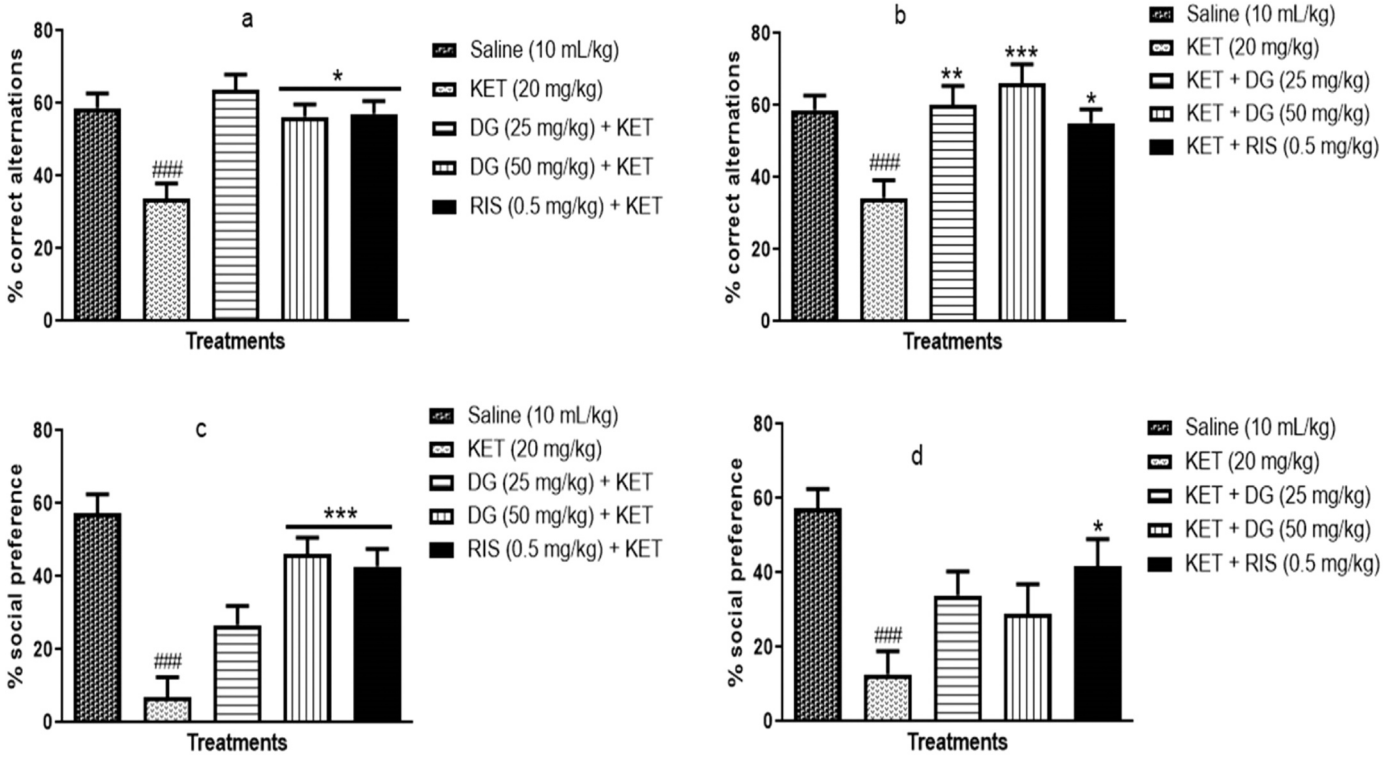


Fig. 2. Effect of diosgenin (DG) on ketamine (KET)-induced spatial working memory impairment and social interaction deficit in the preventive (a,c) and reversal (b, d) treatment protocols. Bars represent the mean ± S.E.M of 9 animals / group. #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ vs saline group and *p < 0.05, * *p < 0.01, * **p < 0.001 vs KET group. RIS = Risperidone.

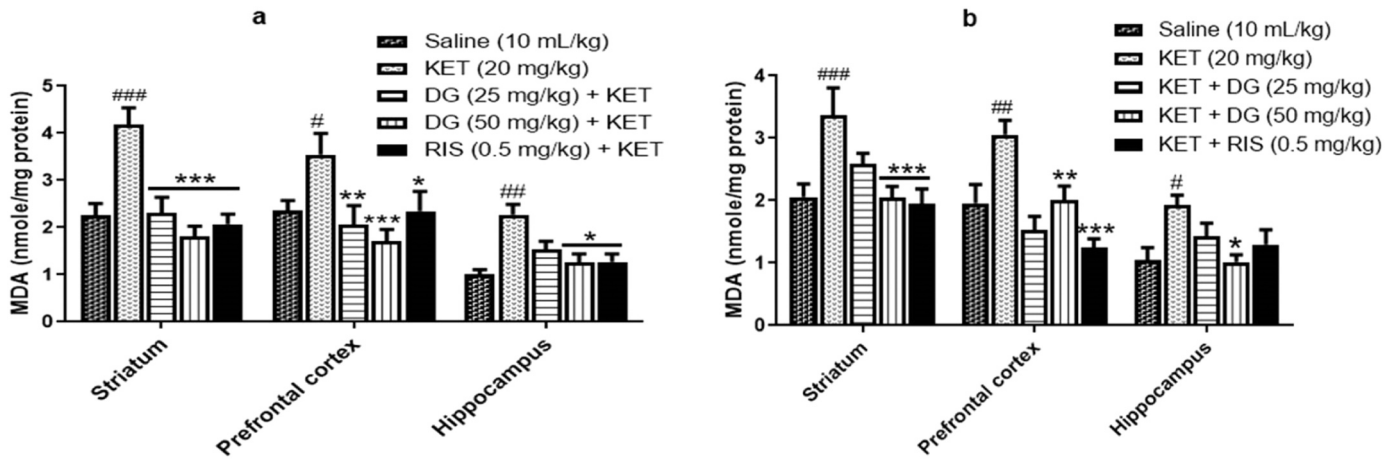


Fig. 3. Diosgenin (DG) reduces malondialdehyde (MDA) concentrations in the striatum, prefrontal cortex and hippocampus of mice in the preventive and reversal treatments with ketamine (KET). Bars represent the mean ± S.E.M of 7 animals / group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to saline group and $^{*}p < 0.05$, $^{* *}p < 0.01$, $^{* **}p < 0.001$ compared to KET group RIS = Risperidone.

(UV/Vis Spectrophotometer (INESA 750 N, China)) and expressed as μmol mg− 1 protein (Jollow et al., 1974).

2.6.5. Estimation of catalase Level

The activity and content of catalase was measured in the striatum, prefrontal cortical, and hippocampal brain regions by adopting the colorimetric assay method based on formation of yellow complex with ammonium molybdate and H_2O_2 as the enzyme substrate according to Goth et al. (1991). The absorbance level was set at 405 nm and read with a UV/Vis Spectrophotometer (INESA 750 N, China). The unit of the enzyme activity was expressed as kU/ mg protein (Góth, 1991).

2.6.6. Estimation of acetylcholinesterase Level

The amount of acetyl-cholinesterase was estimated as follows: 0.4 mL of aliquots of the striatum, prefrontal cortex and hippocampal supernatants was added to 2.6 mL of phosphate buffer (0.1 M, pH 7.4) and then mixed with 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB) (0.1 mL). Thereafter, acetylthiocholine iodide (0.1 mL) was added together to the reacting mixture and then read under UV/Vis Spectrophotometer (INESA 750 N, China) at wavelength of 412 nm at 10 min with the absorbance changes taken every 2 min interval and recorded. Acetylcholinesterase activity was read when the change in colour produced increases from the thiocholine after reaction with DTNB. Absorbance change per minute was determined and expressed as μmol/min/mg protein (Ben-Azu et al., 2022b).

2.7. Statistical analysis

The data (behavioral and biochemical data) were analyzed using one–way analysis variance (ANOVA) and expressed as Mean ± S.E.M followed by post–hoc test (Bonferroni) for multiple comparisons where appropriate, using Graph Pad Prism software, Inc., Lajolla, USA, version 5.0. A level of $p < 0.05$ was considered as statistically significant for all tests.

3. Results

3.1. Diosgenin treatment prevents and reverses ketamine-induced hyperlocomotion in mice

The preventive and reversal effects of DG on KET-induced hyperlocomotion are presented in Fig. 1a-b. The results showed that KET (20 mg/kg) injection significantly ($p < 0.05$) heightened the number of line crossings in the preventive and reversal cohorts relative to the saline-treated group, which indicates marked hyperlocomotor effect. However, DG (50 mg/kg, $p < 0.05$) in the preventive cohort significantly prevented KET-induced hyperlocomotion (Fig. 1a) while in the reversal cohort, DG (50 mg/kg, $p < 0.05$) significantly reversed the KET-induced hyperlocomotion behavior when compared with KET control groups (Fig. 1a). In similar effect, RIS (0.5 mg/kg) treatment in the preventive ($p < 0.01$) and the reversal ($p < 0.001$) cohort significantly reduced the hyperlocomotion behavior instituted by the KET (20 mg/kg,) induction relative to the KET control groups (Fig. 1a-b).

3.2. Diosgenin treatment prevents and reverses ketamine-induced spatial memory and social interaction deficit in mice

The effects of DG on KET-induced spatial working memory deficit and social preference are based on alternation behavior in the YMT and % social preference in the SIT in the preventive and reversal treatments (Fig. 2a-d). From the results obtained, KET (20 mg/kg) in both treatment cohorts significantly ($p < 0.001$) decreased percentage correct alternation behavior in the YMT compared to the saline-treated group. Meanwhile, exposure to DG (50 mg/kg, $p < 0.05$) treatment in the preventive cohort significantly abated the spatial working memory impairment caused by the KET induction when compared with KET control group (Fig. 2a). The treatment with both DG (25 mg/kg, $p < 0.01$) and DG (50 mg/kg, $p < 0.001$) significantly reversed the spatial working memory impairment caused by the KET induction when compared with KET control group (Fig. 2b). In the same pattern, RIS (0.5 mg/kg, $p < 0.05$) treatment in the preventive and the reversal cohorts significantly prevented and reversed the spatial working memory impairment caused by the KET (20 mg/kg) induction when compared to the KET control groups (Fig. 2a-b). Furthermore, treatment with DG (50 mg/kg) and RIS (0.5 mg/kg) in the preventive cohorts significantly ($p < 0.001$) increased the percentage social preference score when compared to the KET control group (Fig. 2c). But in the reversal cohorts, only RIS (0.5 mg/kg) treatment increased significantly ($p < 0.05$) the percentage of social preference score when compared to the KET control group (Fig. 2d).

3.3. Diosgenin treatment extenuates ketamine-induced lipid peroxidation activity in mice brain

As shown in Fig. 3a-b, KET treatment significantly ($p < 0.05$) increased lipid peroxidation as indicated by increase in MDA levels in the striatum, prefrontal cortex and hippocampus region in both preventive and reversal cohorts when compared to the saline-treated group

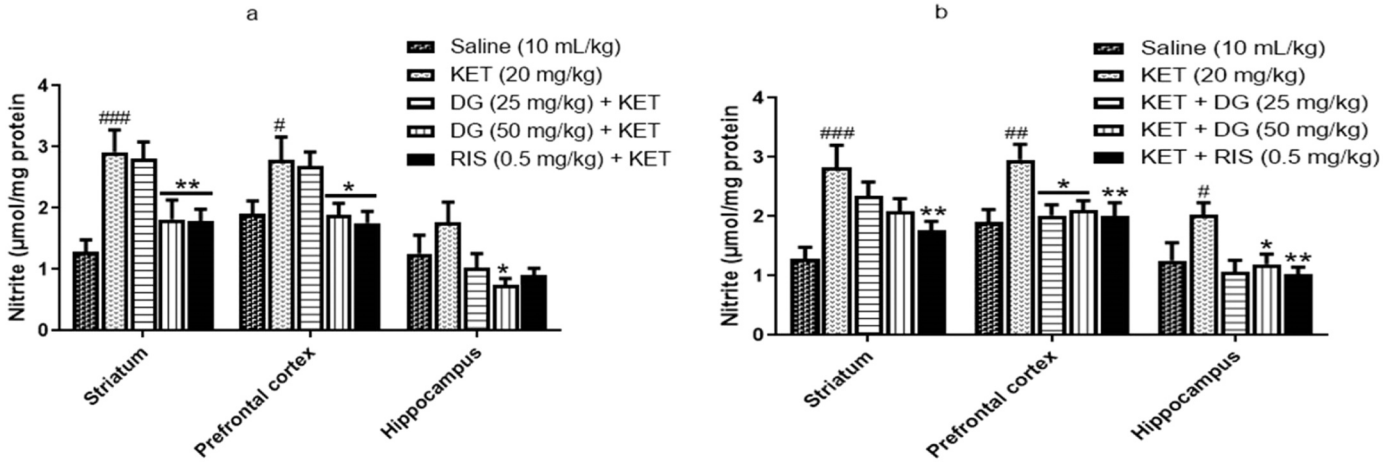


Fig. 4. Diosgenin (DG) reduces nitrite concentrations in the striatum, prefrontal cortex and hippocampus of mice brains in the preventive and reversal treatments with ketamine (KET). Bars represent the mean ± S.E.M of 7 animals / group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to saline group and $*p < 0.05$, $* *p < 0.01$ compared to KET group RIS = Risperidone.

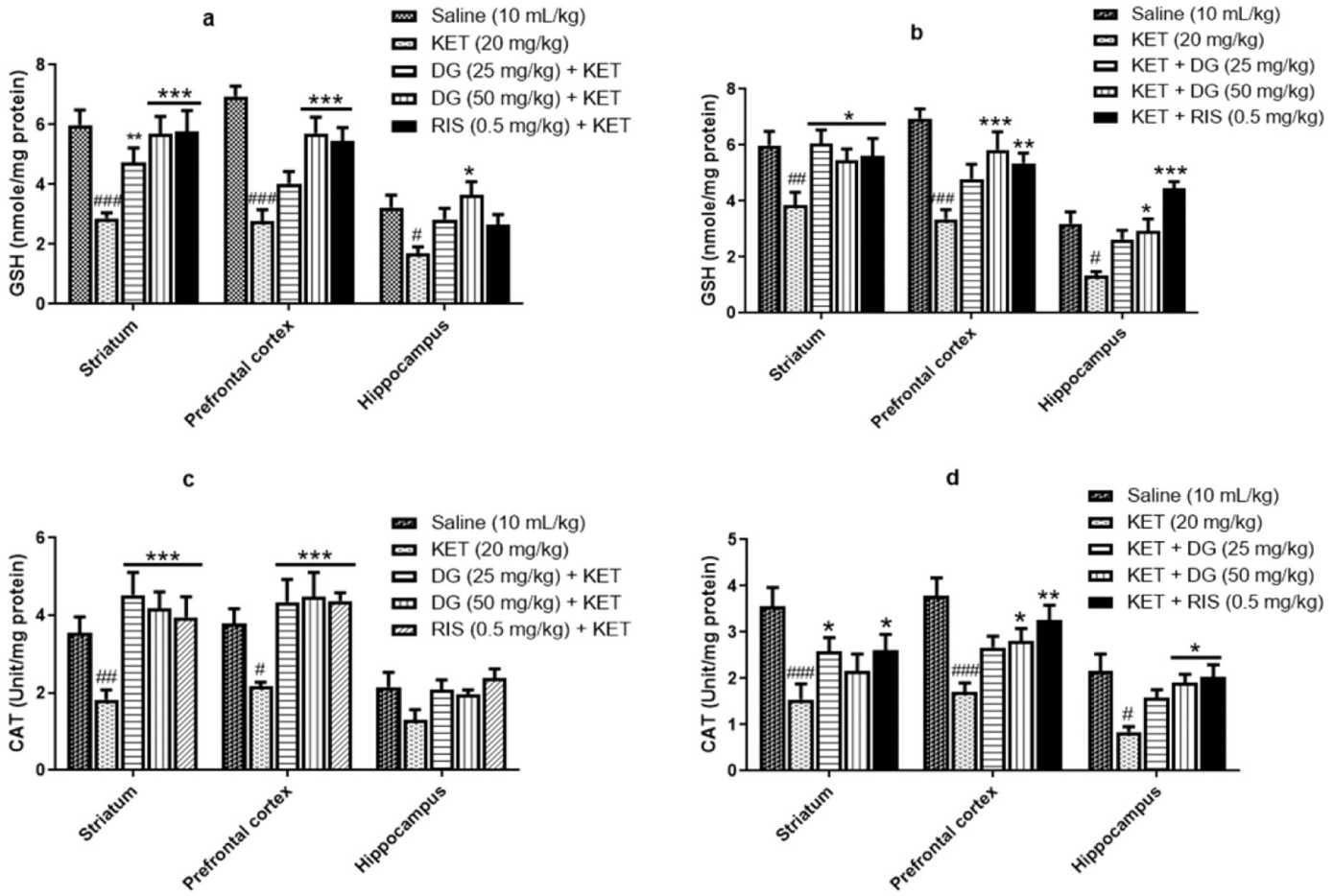


Fig. 5. Diosgenin (DG) enhanced glutathione (GSH) concentration and catalase (CAT) activity in the striatum, prefrontal cortex and hippocampus of mice brains in the preventive (a,c) and reversal (b,d) treatments in mice brains treated with ketamine (KET). Bars represent the mean ± S.E.M of 7 animals / group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to saline group and $*p < 0.05$, $* *p < 0.01$, $* **p < 0.001$ compared to KET group (two-way ANOVA followed by Bonferroni post hoc test). RIS = Risperidone.

(Fig. 3a-b). However, treatment DG (50 mg/kg) decreased the levels MDA in the striatum ($p < 0.001$), prefrontal cortex ($p < 0.01$) and hippocampus ($p < 0.05$) significantly in the preventive cohort relative to the KET control group (Fig. 3a). RIS (0.5 mg/kg) treatment significantly ($p < 0.001$) prevented the increase in MDA content in the striatum and the prefrontal cortex but not in the hippocampus relative to the KET control group (Fig. 3a). Moreover, treatment with DG (25 and 50 mg/kg) decreased the levels MDA in the striatum ($p < 0.001$) and the prefrontal cortex ($p < 0.01$, $p < 0.001$), while treatment with DG (25 mg/kg) decreased the levels of MDA in the hippocampus ($p < 0.05$)

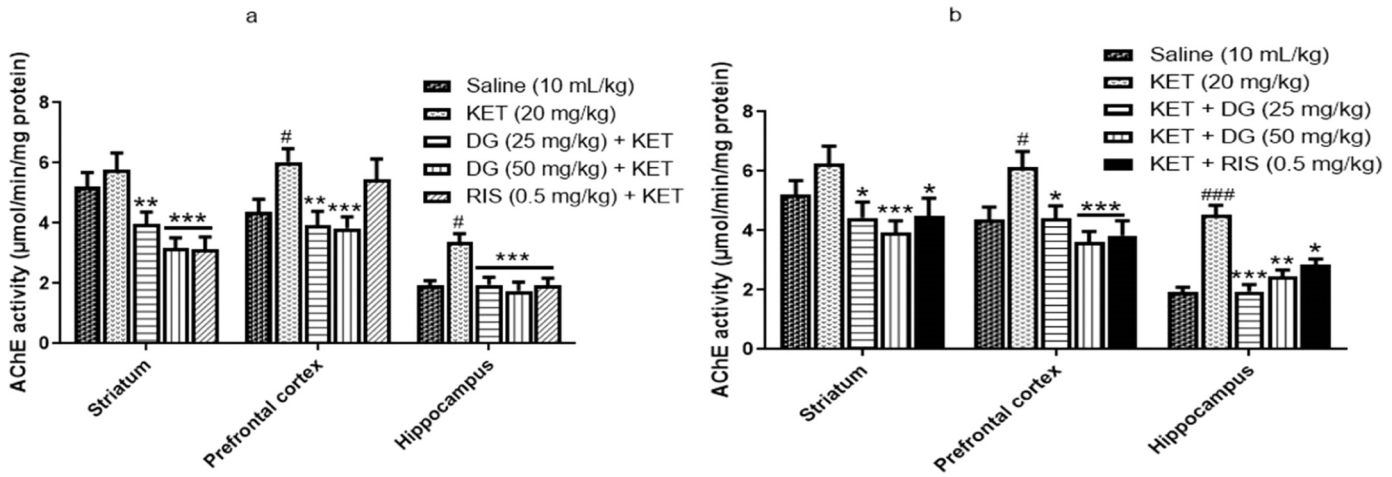


Fig. 6. Diosgenin (DG) reduces acetylcholinesterase activity in the striatum, prefrontal cortex and hippocampus of mice brains in the preventive (a) and reversal (b) treatments with ketamine (KET). Bars represent the mean ± S.E.M of 7 animals / group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to saline group and *p < 0.05, * *p < 0.01, * **p < 0.001 compared to KET group (two-way ANOVA followed by Bonferroni post hoc test). RIS = Risperidone.

significantly in the reversal cohort relative to the KET control group (Fig. 3b). Similarly, RIS (0.5 mg/kg) treatment significantly ($p < 0.001$) prevented the increase in MDA content in the striatum as well in the prefrontal cortex ($p < 0.05$) and hippocampus ($p < 0.05$) relative to the KET control group (Fig. 3b).

3.4. Diosgenin treatment reduces ketamine-induced nitrosative activity in mice brain

As indicated in Fig. 4a-b, KET treatment significantly ($p < 0.05$) increase nitrosative activity as shown by an increase in nitrite levels in the striatum, prefrontal cortex, and hippocampus regions in both preventive and reversal cohorts relative to the saline-treated group (Fig. 4a-b). However, treatment DG (50 mg/kg) reduced the levels nitrite in the striatum ($p < 0.05$), prefrontal cortex ($p < 0.05$) and hippocampus ($p < 0.05$) significantly in the preventive cohort relative to the KET control group (Fig. 4a). The RIS (0.5 mg/kg) treatment significantly ($p < 0.5$) prevented the increase in nitrosative activities in the striatum and the prefrontal cortex but not in the hippocampus in comparison to the KET control group (Fig. 4a). In addition, the treatment with DG (25 and 50 mg/kg) decreased the levels nitrite in the prefrontal cortex ($p < 0.05$), while the treatment with DG (50 mg/kg) alone decreased the levels of nitrite in the hippocampus ($p < 0.05$) significantly in the reversal cohort relative to the KET control group (Fig. 4b). Similarly, RIS (0.5 mg/kg) treatment significantly ($p < 0.01$) prevented the increase in nitrite content in all the three brain regions relative to the KET control group (Fig. 4b).

3.5. Diosgenin treatment improves the endogenous antioxidant enzyme activity in the ketamine-induced mice brain

As presented in Fig. 5a-d, injection with KET (20 mg/kg) significantly ($p < 0.001$) decreased the activity of endogenous enzymes as indicated by decreased levels of GSH and CAT in the striatum, prefrontal cortex, and hippocampus regions of the mice brains when compared to the saline-treated groups in both cohorts. KET induced changes in GSH concentration in the striatum ($p < 0.001$), prefrontal cortex ($p < 0.001$) and hippocampus ($p < 0.05$) of mice brains in the preventive cohort (Fig. 5a). Treatment with DG (25 and 50 mg/kg) profoundly increased the levels GSH in the striatum ($p < 0.01$, $p < 0.001$), while treatment with DG (50 mg/kg) alone increased the levels GSH in the prefrontal cortex ($p < 0.001$) and hippocampus ($p < 0.05$) significantly relative to the KET control group (Fig. 5a). Further, in the reversal cohort, KET induced changes in GSH concentration in the striatum ($p < 0.01$), prefrontal cortex ($p < 0.001$) and hippocampus ($p < 0.05$) of mice brains (Fig. 5b), but treatment with DG (25 and 50 mg/kg) elevated the levels GSH in the striatum ($p < 0.05$) significantly relative to the KET control group (Fig. 5b). Also, treatment with DG (50 mg/kg) increased the levels GSH in the prefrontal cortex and hippocampus significantly relative to the KET control groups (Fig. 5b). In the preventive cohort, RIS (0.5 mg/kg) treatment increased GSH content in the striatum and the prefrontal cortex while no significant effect was observed in the hippocampus relative to the KET control group (Fig. 5a). In similar effect, the treatment with RIS (0.5 mg/kg) in the reversal cohort consistently elevated the concentrations of GSH in the striatum prefrontal cortex and the hippocampus respectively when compared to the KET control group (Fig. 5b).

More so, KET injection incited significant changes in the activity of CAT in the striatum ($p < 0.01$) and prefrontal cortex ($p < 0.05$) alone of the mice brains relative to the saline-treated groups in the preventive cohort (Fig. 5c). Interestingly, treatment with DG (25 and 50 mg/kg, $p < 0.001$) and RIS (0.5 mg/kg, $p < 0.001$) profoundly increased the levels CAT in the striatum and the prefrontal cortex when compared to the KET control group (Fig. 5c). However, in the reversal cohort, KET injection completely caused significant reduction in the content of CAT in all the brain sections: striatum ($p < 0.001$), prefrontal cortex ($p < 0.001$) and hippocampus ($p < 0.05$) relative to the saline-treated (Fig. 5d). But the treatment with DG (25 mg/kg, $p < 0.05$) in the striatum and treatment with DG (50 mg/kg, $p < 0.05$) in both prefrontal cortex and the hippocampus elevated the content of CAT when compared to the KET control group (Fig. 5d). In addition, RIS (0.5 mg/kg) treatment significantly increased the content of CAT in the striatum ($p < 0.05$), prefrontal cortex ($p < 0.01$), and the hippocampus ($p < 0.05$) when compared to the KET control group (Fig. 5d).

3.6. Diosgenin treatment reduces acetylcholinesterase activity in ketamine-induced mice brain

As presented in Fig. 5a-b, KET treatment significantly ($p < 0.05$) increases acetylcholinesterase (AChE) activity, suggesting reduced ACh concentration in the striatum, prefrontal cortex, and hippocampus regions in both preventive and reversal cohorts relative to the saline-treated group (Fig. 6a-b). The treatment with DG (25 and 50 mg/kg) reduced AChE activity in the striatum ($p < 0.01$, $p < 0.001$), prefrontal cortex ($p < 0.01$, $p < 0.001$) and hippocampus ($p < 0.001$) significantly in the preventive cohort relative to the KET control group (Fig. 6a). The RIS (0.5 mg/kg) treatment significantly ($p < 0.5$) prevented the increase in AChE in the striatum and the hippocampus but not in the prefrontal

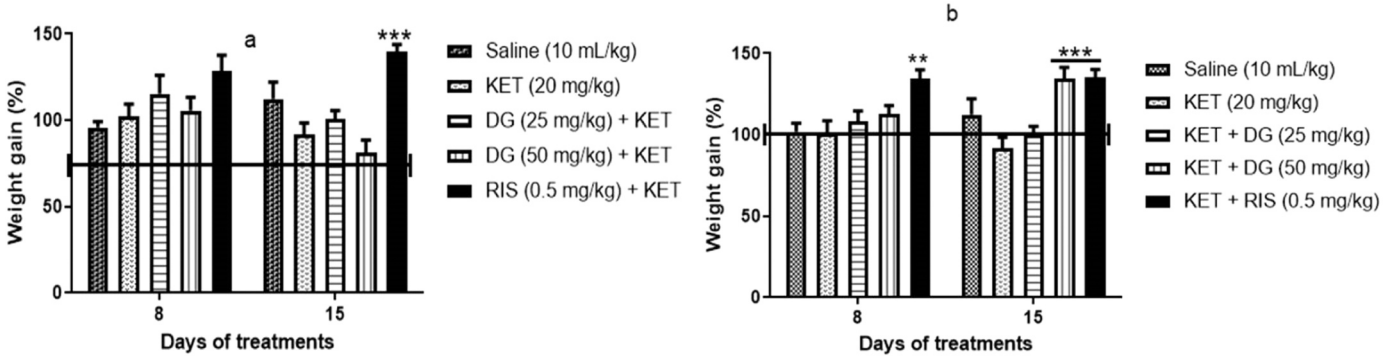


Fig. 7. Effects of diosgenin treatment on body weight gain in the preventive (a) and reversal (b) treatments with ketamine (KET). Bars represent the mean ± S.E.M of 7 animals / group. * *p < 0.01, * **p < 0.001 compared to KET group (two-way ANOVA followed by Bonferroni post hoc test). RIS = Risperidone.

cortex when compared to the KET control groups (Fig. 6a). Also, the treatment with DG (25 and 50 mg/kg) as well as RIS (0.5 mg/kg) significantly ($p < 0.05$, $p < 0.01$, $p < 0.001$) decreased the levels of AChE enzyme activity in all the three brain regions in the reversal cohort relative to the KET control group (Fig. 6b).

3.7. Effects of diosgenin treatment on body weight gain in the ketamine-induced mice

Fig. 7a-b showed that KET treatment did not affect the change in body weight ($p > 0.05$) in both preventive and reversal cohorts when compared to the saline-treated group (Fig. 7a-b). However, both doses of DG treatment were unable to cause any change in body weight in the preventive cohort relative to the KET control group (Fig. 7a). The RIS (0.5 mg/kg) significantly ($p < 0.5$) enhanced body weight gain after 15 days of treatment when compared to the KET control group (Fig. 7a). Further, in the reversal cohort relative to the KET control group, the treatment with DG (25 and 50 mg/kg) did not cause any change in body weight but RIS (0.5 mg/kg) significantly improved body weight gain after 8 ($p < 0.01$) and 15 ($p < 0.001$) days of treatment (Fig. 7b).

4. Discussion

The findings of this study confirm that DG treatment in KET-induced experimental psychosis reduces hyperlocomotion, social interaction withdrawal and spatial working memory impairment via regulation of antioxidant system in the experimental mice brains in addition to normalization nitrosative and cholinergic neurotransmission. Schizophrenia-associated behavioral and cognitive shutdown have been previously established and reported, as demonstrated by hyperlocomotion and neuropsychiatric phenotypes with poor performance especially in KET-induced psychotic animals in the OFT, SIT and Y-maze (Monte et al., 2013; Ben-Azu et al., 2019; de Araújo et al., 2021), which are also consistent with the behavioral data obtained in this study.

DG is largely and currently gaining significant attention due to its huge pharmacological potentials, in addition to its intriguing simple means of action, validating and broadly increasing the knowledge acquired through its conventional use. Different mechanistic and preclinical investigations have been done on DG to better understand its benefits and significance against manifold illnesses (Chen et al., 2015; Huang et al., 2017). Interestingly, the overall findings from multiple studies proposed that DG could be adopted as a novel multi-target-based pharmacotherapeutic or chemopreventive drug for the treatment of variety of chronic diseases such as metabolic and neurological disorders (Mahmoudi et al., 2021). Of note, DG was reported to show high level of biocompatibility with low toxicity. For instance, acute oral treatment with DG showed no signs of acute toxicity (Qin et al., 2009). Also, experimental sub-chronic toxicity administrated further showed no significant changes in biochemical parameters (Cayen et al., 1979), which therefore, confirms the safety of oral administration of DG.

Green et al. reported that cognitive shutdown and behavioral changes in schizophrenic condition involve a broad array of social cognitive domains (Green et al., 2019; Smith et al., 2000). He demonstrated that it is the core feature of schizophrenia, and one with veritable implications for possible treatment and prognosis. From the findings obtained in this study, KET injection significantly elicited shift in normal behavioral functions as evidenced with hyperlocomotion linked to the positive symptoms of psychosis and reduced social behavior and impairment to spatial memory as evident by reduction in sociability and disorient spontaneous alternation behavior which is also linked to the negative symptoms of psychosis. NMDAR hypofunction have been investigated as one of the leading culprits in the pathogenesis of schizophrenia. Recently, Abram et al. reported that KET, an NMDAR antagonist triggers transient schizophrenia-like behaviors via alteration of the thalamic connectivity when given at sub-anesthetic doses to healthy volunteers (Abram et al., 2022). Thus, affirming that NMDAR hypofunction induces the thalamic hyper-connectivity, notably occurring via the blockage of NMDA receptors, particularly co-localized in the GABAergic inhibitory system remarkably involved in the regulation of dopamine and glutamate release in the striatal brain areas (Abram et al., 2022). This pathological mechanism has been adjudged as one of the major contributory processes that induces and exacerbates severity of some positive symptoms such as hallucination and hyperlocomotion seen in psychotic patient (Abram et al., 2022). Investigations have also shown that blockade of the D_2 receptors present on the striatal pathways as well as 5-$HT_{2A/C}$-release of serotonin induce large proportion of the positive and negative symptoms, thus necessitating the search for drugs with D_2-5-$HT_{2A/C}$ antagonism capacities in the treatment of the disease (Coyle, 2006; Chatterjee et al., 2012; Ben-Azu et al., 2021). Other investigations have also shown that KET injection in experimental psychosis markedly alter the suppresses neurotrophic factor such as brain derived neurotrophic factor (BDNF) and causes neuropathologic release of cytokine, notably leading loss synaptic plasticity in the limbic and cortical brains regions such as striatum, prefrontal cortex and the hippocampus implicated in the pathogenesis., These consequently affect the cognitive function and neurobehavior of the animals (Ben-Azu et al., 2023, 2019, 2018; Ben et al., 2022). Further, Sobota et al. reported that KET effects increases amygdala activity, strongly leading to decrease social interaction and increase anxiety-like behavior in mice (Sobota et al., 2015). Although large number of reports have shown that no specific region of the brain can singly answer the extent of symptoms and damage noticed in schizophrenia, coordination abnormalities across regions of the brain likely produce diverse cognitive and behavioral impairments (Barch, 2014; Li et al., 2019; Van Den Heuvel and Fornito, 2014). Consistent to previous studies, our observations revealed that schizophrenia-like behaviors were produced in animals after continuous KET injection (Oshodi et al., 2021).

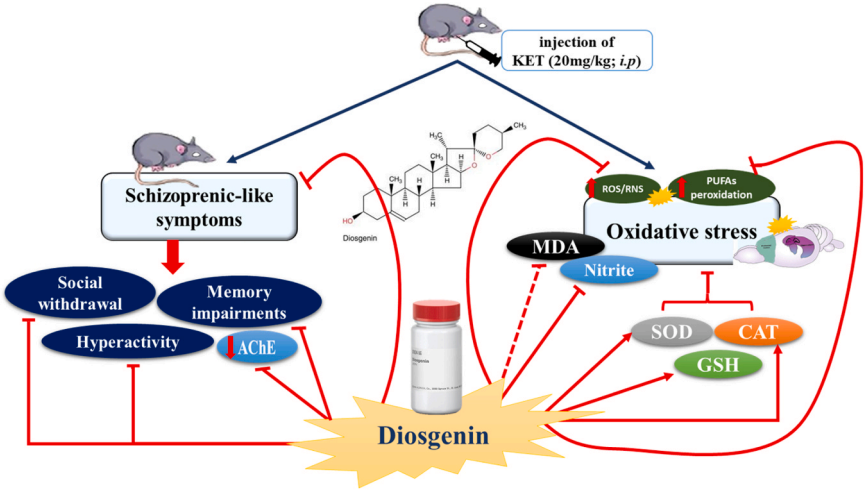
In this study, we observed that preventive and reversal treatment of

DG attenuated psychotic and behavioral deficits phenotypes mediated by the KET injection, including decrease hyperlocomotor activity, increase social interaction preference as well as improving the memory functions, thereby suggesting the antipsychotic potential of DG in experimental mice. Interestingly, our findings are similar to the effects of DG in previously related studies including anxiolytic effect and enhancement of spatial working memory functions in in rodents (Mahmoudi et al., 2021). Meanwhile, as reported in this study, it is important to note that repeated DG treatment in both cohorts elicited reduction in hyperactivity in the OFT as seen with limited number of line crossings either following the 7- or 14-days treatment in the reversal and preventive studies. In similar manner, the spatial working memory functions were also improved following the treatment with DG in the animals after 7 or 14 days of the reversal and preventive treatments. But in close proximity with previous investigations, our finding corroborated and affirms the therapeutic potential of DG to prevent behavioral despair in experimental models mimicking neurological disorders (Cai et al., 2020; Cui et al., 2023; Som et al., 2022). Examination of KET's effect in the preventive or reversal cohorts, we found that DG profoundly prevented sociability following 14 days treatment by improving the interaction time in the animals but failed to reverse KET-induced social withdrawal when compared with KET groups. Taken together, these findings could possibly suggest the ability of DG to both prevent the negative symptoms of schizophrenia for cases of families with genetic background but not for already ongoing schizophrenia episodes.

It is believed that the centre for neurobehavioral shift in psychosis is based on the changed in neurotransmitters activities or release such as dopamine, serotonin, glutamate, ACh, adrenaline, aspartate, glycine, and GABA imbalance (Chatterjee et al., 2012; Ben-Azu et al., 2018c; Oshodi et al., 2021; Ishola et al., 2021). The severity of schizophrenia depends largely on the level of neurochemical imbalances affecting the neural circuitry observed in several forms of schizophrenia (Chatterjee et al., 2012; McCutcheon et al., 2019). Irregularities in the cholinergic system notably based on ACh concentration in the cortex has been reported to be responsible for cognitive symptoms of the disease (Omeiza et al., 2023). Though, nicotinic agonist and muscarinic antagonist are currently the putative antipsychotic and cognitive enhancing treatment in schizophrenia (Abram et al., 2022; Dietrich-Muszalska et al., 2021). More so, the M_1 receptor is one of the most abundant cholinergic muscarinic receptors abundantly found in the forebrain and the hippocampus and is a major contributor to the cognitive function in non-schizophrenia brain. However, M_1 cholinergic receptor activation leads to the potentiation of excitatory current via the NMDA receptors and contributes the cognitive functions and neural circuits in schizophrenic patient (Howes et al., 2015). In this study, we observed that KET treatment increased AChE enzyme flux in the prefrontal cortex and hippocampus of mice relative normal control. As a result, we suggest that KET may be reducing ACh concentration and subsequently attenuating cholinergic transmission and suppressing the nicotinic and M_1 receptor activities in the brain regions of the animals as indicated in their behaviors. However, treatment with DG reverses this effect by reducing AChE enzyme activity, evidently suggesting increased cholinergic system in the prefrontal cortex and hippocampus of the animals. Thus, the reduced AChE enzyme activity observed in this study might be associated with cognitive function, locomotion and sociability of the mice particularly in the preventive study. Also, important to mention that in this study DG was found to significantly reduce AChE enzyme activity both in the prefrontal cortex and hippocampus, unlike risperidone that failed to decrease AChE level in the prefrontal cortex relative to KET group. Besides, reduced AChE level was particularly linked improve neuroprotective functions of cholinergic-antiinflammatory role of ACh, which is in turn regulated by AChE enzyme activity (Pollak et al., 2005; Pavlov et al., 2009). By advantage, this experimental assertion gives a proof of concept that cholinergic system enhancing mechanisms either via AChE enzyme reduction as seen by DG or cholinergic receptor agonism from other studies could be responsible for the central neuroprotective and neurorestorative functions of ACh (Halder and Lal, 2021).

To elucidate more on the overwhelming evidences of redox imbalance in schizophrenia, the involvement of oxidative stress in deregulating neuronal lipid and altering mitochondrial metabolites instituting a shift in neurochemical homeostasis and changed receptor activity cannot be ignored as it is regarded as one of the front-liners of the disease (Murray et al., 2021; Upthegrove and Khandaker, 2020). Perhaps, the reported dysfunction in NMDA receptor was indeed associated with changes in oxidative pathway as a result of multiple mechanisms, notably involving Ca^{2+}-mediated redox reactions (Mouri et al., 2007). Undeniably, an imbalance in Ca^{2+} haemostasis is believed to provoke and exaggerate neuronal oxidative stress inducing endoplasmic reticulum stress and neuronal membrane dysregulation (Mouri et al., 2007). Also, intracellular Ca^{2+} store is known to regulate the release of neurochemicals in the synaptic terminals, thus regulating neurotransmitter-dependent behavior and plasticity (Ermakov et al., 2021). Nevertheless, in schizophrenia, synaptic transmission and plasticity disruptions appear mostly because of alteration of NMDAR which plays an import role in redox which is also controlled by Ca^{2+} homeostasis (Oguro-Ando et al., 2021).

Given these linkages, it is believed that conditions or stressors that led to depletion of GSH content and alterations of endogenous enzymatic antioxidants such as SOD and CAT cause neuropsychiatric



Scheme 2. Experimental hypothesis.

disorders linked with oxidative injury (Ben et al., 2022). In agreement with previous investigations (Monte et al., 2013; Ben-Azu et al., 2016, 2018a, 2018b, 2018d), KET injection significantly inhibited the production of GSH together with CAT enzyme, and increased MDA and nitrite concentrations in the cortical and limbic structures such as striatum, prefrontal cortex, and hippocampus respectively, suggesting a state of neuronal oxidative stress. In addition, report from post-mortem investigations in the cerebrospinal fluid, cortices, and caudate putamen of schizophrenia patients showed that low GSH content are mostly present (Do et al., 2000; Gawryluk et al., 2011), which further contributes to the severity of the disease (Nakao et al., 2021; Vallée, 2022). Interestingly, enhanced GSH and CAT functionality with decreased activity of lipid peroxidation and nitrite were observed in the preventive and reversal treatments with DG, suggesting its modulatory effect on the antioxidant system. Although we also observed that KET injection did elicit any marked changes in the hippocampal CAT level in the preventive cohort, DG treatment did not produce any significant effect following KET induction in the animals. It is believed that a very high level of reactive oxygen species (ROS) or reactive nitrogen species (RNS) in physiological concentration involving cell-signalling activities accompanied by electron transfer reactions favours oxidative stress (Do et al., 2000; Gawryluk et al., 2011). The imbalance between production of free radicals ROS/RNS and antioxidant defence system may result in cellular or molecular damage as observed in this study. Perhaps, the ROS/RNS released may cause membranal lipids because of damaged tissue evidenced by an increased MDA concentration in the brain. Basically, some of the damaged brain tissues might further escalate the activation of inflammation and immune processes (Comer et al., 2020). Thus, oxidative stress reported in this study may further be an inducer as well as product of inflammation.

It is important to also mention that the alterations GSH and nitrite levels in the hippocampus due to KET treatment were abated by DG in the prevention study, further reinforcing the neuroprotective potential of DG unlike risperidone. It is pertinent to suggest that since the treatment effects of DG in both cohorts behaves in similar manner as the RIS treatment in some of the biochemical indices in this study, DG administration could present potential anti-psychotic-like effect in psychotic disorders. Generally, DG treatment was found to demonstrate therapeutically beneficial biochemical effects in both protocols via enhancing the antioxidant defence system and reducing the brain concentration of MDA by buffering antioxidant arsenals (Scheme 2) in the specific brain regions. Although the neuro-protective and -reversal effects of DG elicited in this study were of different duration of treatments ranging between 7 and 14 days respectively, DG significantly demonstrated effects in both treatment protocols. In the context of comparison with risperidone, DG elicits significant biochemical effects, notably explaining and substantiating its ability to prevent and reversal schizophrenia-like features in experimental mice exposed to KET. Importantly, the treatment of both 7 and 14 protocols were shown to devoid of obesogenic potential evidenced by increased body weights unlike risperidone which is the standard drug used in this study.

Conclusively, DG administration prevents and reverses schizophrenia-related behavior in mice submitted to KET, notably by inhibiting lipid peroxidation and nitrosative activity and enhancing antioxidant and cholinergic systems in the mice brains.

CRediT authorship contribution statement

Benneth Ben-Azu, Olusegun G. Adebayo, Benjamin Oritsemuelebi, God'swill E. Uyere, Micheal T. Emuakpeje, Lenatababari Kumanwee: Conceptualization. **Benneth Ben-Azu, Benjamin Oritsemuelebi, Emmanuel O. Chidebe, God'swill E. Uyere, Micheal T. Emuakpeje, Lenatababari Kumanwee**: Data curation. **Olusegun G. Adebayo**: Writing – original draft preparation. **Benneth Ben-Azu, Olusegun G. Adebayo, Chukwuebuka B. Nwogueze, Aliance Romain Fokoua**: Writing – review & editing. **Benneth Ben-Azu, Benjamin Oritsemuelebi, Emmanuel O. Chidebe, Olusegun G. Adebayo, Chukwuebuka B. Nwogueze**: Supervision. **Benneth Ben-Azu, Olusegun G. Adebayo, God'swill E. Uyere, Micheal T. Emuakpeje, Lenatababari Kumanwee**: Funding acquisition. All authors have read and agreed to the publishing of the manuscript.

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Declaration of Competing Interest

Authors declare that they have no conflict of interest.

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Compliance with Ethical Standard

All experiments were approved and performed under the guidelines of Faculty of Basic Medical Sciences, Delta State University Animals Ethic Committee (REC/FBMS/DELSU/23/185) and the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication number: 85–23, revised 1985).

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