

Research Paper

Diosgenin reverses posttraumatic stress disorder in mice by augmenting neurochemical release and inhibiting HPA axis dysfunction, oxidative stress, and neuroinflammation

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ABSTRACT

Post-traumatic stress disorder (PTSD) is a mental disorder linked to neurochemical, hypothalamic-pituitary-adrenal (HPA)-axis dysregulations, inflammatory and pro-oxidant challenges in response to traumatic events. It is one of the leading causes of neurocognitive declines, hence prompting the need for a pharmacological intervention. However, the impact of diosgenin, a naturally occurring steroidal saponin with adaptogenic-like action, on PTSD-induced neuropsychiatric disturbances and its underlying mechanisms are unknown. In this study, we investigated the outcome of diosgenin treatment in a multimodal traumatic, single prolonged stress (SPS)-induced PTSD in mice. Following the SPS-induced 7 days of PTSD, mice ($n = 9$) were thereafter treated with diosgenin (25 and 50 mg/kg) or fluoxetine (10 mg/kg) orally from days 8–20 (14 days). Locomotor, cognitive-, depressive- and anxiety-like behaviors were investigated. We assayed for changes in adrenal weight, serum glucose and corticosterone concentrations. Neurochemical, inflammatory, oxido-nitrergic dysfunctions and monoamine oxidase-B and acetylcholinesterase activities, were measured in the striatum, prefrontal-cortex and hippocampus. The results revealed that the SPS challenge inhibited locomotor, spatial/non-spatial memory functions, increased anxiety and depressive-like features, which were reversed by diosgenin. Diosgenin reduced SPS-induced increased monoamine oxidase-B, acetylcholinesterase activities, TNF- α , IL-6, malondialdehyde and nitrite levels in the striatum, prefrontal-cortex and hippocampus. Antioxidants such as glutathione, superoxide-dismutase, and catalase levels in SPS-mice brains were increased by diosgenin. Moreover, diosgenin attenuated SPS-induced hyper-HPA-axis mediation of PTSD by decreasing serum corticosterone, glucose levels and adrenal gland hypertrophy. Herewith, we suggest that diosgenin convenes adaptogenic-like protection against mice exposed to PTSD by enhancing antioxidant machinery, neurochemical modulations, and inhibition of oxido-nitrergic, inflammatory, and HPA-axis dysfunctions.

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1. Introduction

Neuropsychiatric disorders are widely acknowledged as a leading cause of social and behavioral disability, significantly reducing the quality of life for those affected. This, in turn, contributes to the economic burden of disease in both developing and developed countries (Chin and Vora, 2014; Porceli et al., 2019; Li et al., 2023). Amongst these, posttraumatic stress disorder (PTSD), anxiety-depression, increased/decreased reflexia, and suicidal ideation are common neurological and neuropsychiatric disorders that often result from adverse life experiences (Holliday et al., 2020). Clinical and preclinical studies have reported that psychosocial, environmental, emotional, or biological stressors stand as major factors disrupting the internal milieu of the central nervous system (CNS), but they are notably contingent on the levels of severity and duration of exposures to stressors (Ben-Azu et al., 2022a). Well-studied among other risk factors of neurological disorders is PTSD, which has been reported both in human and animal subjects to cause a significant decline in neurocognitive abilities and significantly exacerbate the development of neuropsychiatric phenotypes (Souza et al., 2017; Zhang and Standifer, 2022).

PTSD is a psychological disorder which develops mostly from exposure to extremely violent situations such as gun robbery scenes, unexpected medical illness, accidents, natural disasters, childhood molestation, or sexual attack/rape (Astwoli et al., 2015; Souza et al., 2017). The hallmark symptom of PTSD is heightened anxiety occurring from re-experiencing of traumatic events. This could possibly suggest and indicate amplification of the biochemical pathways involved in the mechanisms of storage of the traumatic memories post-exposure (Ben-Azu et al., 2022b). Interestingly, a substantial amount of evidence supports and validates the use of the single but intense prolonged stress (SPS) paradigm as a rodent model of PTSD (Yamamoto et al., 2009). SPS is a preclinical model of PTSD consisting of multiple traumatic exercises such as psychological, physiological, and pharmacological stressors, which is notably reversible by popular antidepressant agents such as fluoxetine, a selective serotonin reuptake inhibitor (SSRI) (Yamamoto et al., 2009). SPS-induced PTSD in rodents is characterized by recurrent fear response to external, psychosocial, or psychological cues, which is associated with altered serum glucocorticoid concentrations indicative of hypothalamus-pituitary-adrenal (HPA) axis dysfunction (Wang et al., 2012). Moreover, Zhang et al. (2012) reported that SPS stimulates changes in mineralocorticoid receptor expression in the medial prefrontal cortex in rats after the development of PTSD. Studies have also shown that prolonged traumatic stress causes significant alteration of neuronal morphology and functions (Zhang et al., 2010; Nisar et al., 2020). Some brain regions, such as the amygdala, bilateral cingulate cortex, and left hippocampal volumes, were reportedly decreased after traumatic stress exposure (Shin et al., 2006; O'Doherty et al., 2015). In addition, altered neurotransmitter activities defined by heightened norepinephrine and dopamine production but with low synthesis of serotonin and GABA neurotransmitter and imbalances in the cellular status of pro-oxidants and inflammatory molecules are also reported as some of the frontliners that trigger PTSD in animals subjected to SPS (Malikowska-Racia et al., 2019; Xiang et al., 2019; Karanikas et al., 2021). Reports have shown that exposure to SPS exacerbated sleep abnormalities, enhanced anxiety, excess arousal and fear of learning new skills, as well as dampened spatial and non-spatial memory functions and impaired social interaction (Souza et al., 2017).

Anxiolytic, antidepressant, and antipsychotic drugs are the most commonly prescribed for PTSD treatment or management. Despite this treatment paradigm, about 40–50 % of patients respond less and thereafter suffer from the devastating health condition of this debilitating disease (Lee and Lee, 2018). Hence, this prompts the need to develop pharmacological agents that can attenuate or slow down the disease progression, suffering and destructive effects on the neurocellular structures experienced by patients with PTSD. Over the years, increased attention has been shifted toward the use of naturally

occurring compounds as alternative therapeutic agents for treating psychiatric symptoms and the biochemical alterations observed in PTSD (Deligiannidis and Freeman, 2010).

Diosgenin is a naturally occurring steroidal saponin found in *discorea* species (Mahmoudi et al., 2021). Based on existing accumulated evidence, diosgenin has been biologically and pharmacologically investigated and the reports show that it possesses strong therapeutic effects against several disorders such as cancer, cardio-metabolic syndrome (diabetes, hyperglycemia and hyperlipidemia) (Ahmed et al., 2014; Roghani-Dehkordi et al., 2015), reproductive disorders (Khosravi et al., 2019) and skin aging (Chen et al., 2015). Additionally, in pre-clinical neuroscience, diosgenin was previously investigated, and the report also showed that it elicits neuroprotective functions typified by the reversal effects of the functional and structural alterations and induction of neural regeneration in a mouse model of diabetic neuropathy (Kang et al., 2011). As further shown in the study done by Tohda et al. (2012, 2023), the treatment with diosgenin also reduces amyloid plaques and neurofibrillary tangles and abated axonal degeneration in mice brains, which was associated with memory improvement notably via activation of membrane-related rapid response steroid-binding receptors in Alzheimer's disease model (Tohda et al., 2012, 2013). Interestingly, diosgenin has been suggested as an antipsychotic by normalizing N-methyl-D-aspartate glutamatergic receptor activity (Ben-Azu et al., 2024a, 2024b) and a nootropic agent used to prevent cognitive shutdown and oxidative damage following D-galactose-induced senescence in mice (Chiu et al., 2011). However, there is a paucity of scientific evidence demonstrating the therapeutic potential of diosgenin after exposure to traumatized events such as SPS-induced PTSD and its potential underlying mechanisms. Therefore, this study was designed to investigate whether diosgenin treatment could reverse the biochemical changes associated with SPS-induced PTSD in mice.

2. Experimental procedures

2.1. Drugs and reagents

Drugs (diosgenin, fluoxetine) and reagents were bought from Sigma-Aldrich, St. Louis, USA, and Burgoyne Burbidges & Co., Mumbai, India. Corticosterone, tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), dopamine, and serotonin ELISA kits were acquired from Oxford Biomedical Research and BioLegend, USA. Other chemicals bought for this study were of analytical grade and the highest purity.

2.2. Animal

We obtained the Animal Care and Use Research Ethical number (REC/FBMS/DELSU/22/149) from the Ethical Committee of Basic Medical Sciences of Delta State University based on International regulatory practice. Swiss mice (20–25 g) obtained from the Delta State University Medical Science Animal Facility were kept under laboratory conditions (23 $\pm$ 2 °C; 12-hr light/12-hr dark cycle, relative humidity 40–70 %), with free access to food and water.

2.3. Single prolonged stress protocol

The SPS protocol is a multiparadigm traumatic event composed of single continuous exposures to three different stressful exercises, including psychological (restraint), physiological (forced group swimming), and pharmacological (chemical exposure to ether). This procedure was done to initiate and exacerbate symptoms and phenotypes of PTSD using experimental animals. The procedure was carried out between 8 a.m. and 1 p.m. as initially described (Serova et al., 2013; Laukova et al., 2014) and approved by the Animal Use Protocol ID: REC/FBMS/DELSU/22/149 by the Faculty of Basic Medical Science's Ethical Regulatory Committee on the Use and Care of laboratory animals. Briefly, animals were immobilized for 2 h in a 50 mL falcon tube

container (restraint stress device) with the pointed edge opened and covered with wire gauze to allow sufficient breathing and restrict head and body movement. Thereafter, mice were placed in a transparent cylinder (50 cm high, 24 cm diameter) filled to 2/3rd with 24 °C water for 20 min. The animals were then aired and allowed to recover for another 15 min prior to diethyl ether exposure until a comatose state was reached. Following the exposures to these three stressful events (SPS induction), all animals were singly placed in home cages to regain consciousness in a new environment and then allowed to stay in an unperturbed condition for 7 days to develop the psychiatry states of PTSD prior to treatment.

2.4. Study design and drug preparation

Diosgenin and fluoxetine were dissolved in 5 % dimethyl sulfoxide (DMSO). Mice were orally administered with diosgenin (25 and 50 mg/kg), fluoxetine (10 mg/kg) or 5 % DMSO, 10 mL/kg, which also served as a vehicle (VEH) for 14 consecutive days starting from day 8 to 21, after the 7 days of isolation. Doses of diosgenin (25 and 50 mg/kg) used in this study were chosen based on the findings from preliminary investigations (Leng et al., 2020), while the dose of fluoxetine used was adopted from previous studies (Dulawa et al., 2004). However, the effects of diosgenin and fluoxetine alone were not investigated based on earlier findings (Cui et al., 2023).

The mice were divided into five groups ($n = 6$ per group):

- Group 1 (non-stressed control): mice received vehicle (10 mL/kg, $p.o$) once daily for 14 days, starting from day 8 to day 21.
- Group 2 (stressed control): mice received vehicle (10 mL/kg, $p.o$) once daily post-SPS induction for 14 days, starting from day 8 to day 21.
- Groups 3 and 4 (stress + diosgenin treatment): mice received oral treatment with 25 and 50 mg/kg of diosgenin daily post-SPS induction for 14 days, starting from day 8 to day 21.
- Group 5 (stress + fluoxetine treatment): mice received oral treatment with 10 mg/kg of fluoxetine once daily post-SPS induction for 14 days, starting from day 8 to day 21.

2.4.1. Behavioral examination

All the behavioral investigations were done and analysed by trained experts blinded to the study from the 19th to 22nd day following SPS induction and treatment, between 7:00 a.m. and 2:00 p.m., with 40 - 45 min between successive tests to minimize the stress effect on results. Tests were performed on different days in the order presented below.

2.4.1.1. Spontaneous motor activity. Locomotor assessment: a paradigm developed for examining motor and explorative despair in laboratory rodents was done with the open field test maze on day 19 in an open arena described by (Izídio et al. (2005)). In this test, the number of lines crossed by the mice was recorded for 5 min.

2.4.1.2. Tests for memory function. On day 19, the Y-maze test and novel object recognition test (NORT), which are indicators of cognitive and immediate short-term spatial and non-spatial memory declines, were also carried out. The Y-maze test relied on the total number of alternations calculated as percentage alternation (Monte et al., 2013) and object recognition, as previously described, was evaluated in the experimental mice as previously described (Ben-Azu et al., 2020). Notable, the Y-maze test was based on the number of correct alternations in a three-arm identical apparatus (labelled as A, B, and C). The correction was described as entries into all three arms within 5 min, and complete alternation was defined as previously described by Monte et al. (2013). However, NORT was based on the ability of the mice to discriminately identify a novel object from the familial object following

training and test phases, each of which each lasted for 5 min (Ben-Azu et al., 2020).

2.4.1.3. Test for anxiety-like and social behavior. We measured the anxiety-like response of the experimental mice on day 20th using the light and dark box (LDB), elevated plus maze and hole board maze, which test and indicate aversive-like behavior (Pellow et al., 1985; Adebayo et al., 2022). The social withdrawal, isolation and interaction response in mice was investigated on day 21 using social interaction and recognition tests (2019; Emudainohwo et al., 2022) to affirm further the level of neuropsychiatric state of PTSD in the experimental mice as previously described (Gao et al., 2009; Kaidanovich-Beilin et al., 2011).

2.4.1.4. Tests for depressive-like features. Finally, tail suspension and forced swimming tests, estimated by increase or decrease in immobility (passive swimming) time that indicate whether the animals are depressed or not, were also conducted on day 21, and each test was carried out for a period of 5 min (Borsini et al., 1989; Dereli et al., 2018; Ben-Azu et al., 2018a). All the behavioural experiments were recorded with a recording camera for 5 min each, and the video file was further analyzed with the ANYmaze neurobehavioral analysis software (ANY-maze, Stoelting Co., USA).

2.4.2. Blood sugar estimation

Blood was collected from the experimental mice's tail vein on day 15 after 8 h overnight fast for blood sugar estimation using a commercialized glucometer machine (AccuChek).

2.4.3. Tissue preparation for adrenal weight measurement and biochemical analyses

The experiment ended post-fasting blood sugar estimation on day 15, and thereafter, the mice were sacrificed under ketamine hydrochloride anesthesia. Each adrenal gland was weighed with a sensitive weighing balance to assess for adrenal hypertrophy. The cardiac puncture was done for the collection of blood, and then the brain excision for the hippocampus, striatum and pre-frontal cortex dissection and isolation was done, followed immediately by homogenization in cold phosphate buffer (10% w/v, 0.1 M, pH 7.4). Collected blood samples and brain (hippocampus, striatum and prefrontal cortex), homogenates were centrifuged for 15 min/3000 rpm and 10 min/10,000 rpm at 4 °C to get serum and supernatants. Finally, the serum and supernatants were stored (−20 °C) until biochemical assays were performed.

2.4.4. Estimation of protein content

Protein levels of the serum and cortico-striatal and hippocampal samples were determined using the protocol previously described by Gornall et al. (1994).

2.4.5. Enzyme-linked immunosorbent assay

Serum corticosterone and cortico-striatal and hippocampal TNF- α , IL-6, dopamine and serotonin were all estimated according to the manufacturer's protocols. All readings were thereafter collected with a Spectramax M-5 (Molecular Devices, Sunnyvale, CA) plate reader, and then the level of serum corticosterone and cortico-striatal and hippocampal TNF- α , IL-6, dopamine and serotonin were calculated from the standard curve of the ELISA kits and further expressed as pg/mg protein (Ben-Azu et al., 2022a).

2.4.6. Estimation of monoamine oxidase acetylcholinesterase and glutamic acid decarboxylase enzyme levels

Experiments on monoamine oxidase-B (MAO-B) assay were done following incubation of benzylamine alongside brain supernatant and other reagents, such as 10 % zinc sulphate and NaOH (1 M), for 2 min. Absorbent spectrophotometric readings were acquired at 450 nm (Schurr and Livne, 1976). Acetylcholinesterase enzyme activity was

quantified based on acetylthiocholine-dependent reaction (Ben-Azu et al., 2022b). Glutamic acid decarboxylase assay was investigated using a previously established L-Glutamic acid sodium-dependent assay method (Yu et al., 2011).

2.4.7. Estimation of cortico-striatal and hippocampal oxidative stress indices

Pro-oxidant and anti-oxidant molecules were carried out on the cortico-striatal and hippocampal homogenates supernatants. Contents of pro-oxidant molecules in the cortico-striatal and hippocampal brain regions were determined by direct measurement of the lipid peroxidation marker, malondialdehyde, using the existing method (Buege and Aust, 1978) and nitrite concentration as nitrergic stress indicator was assayed (Green et al., 1982). Enzymic antioxidants such as catalase were obtained through hydrogen peroxide as a substrate previously reported by Claiborne (1995), and superoxide dismutase was estimated according to superoxide inhibition of adrenaline described by Ben-Azu et al. (2022b), while the non-enzymic antioxidant such as glutathione was estimated as earlier described (Umukoro et al., 2020) was based on Ellman's method.

2.5. Statistical analysis

The data collected were presented as means $\pm$ S.E. with the mean values between groups compared by the analysis of variance (ANOVA) followed by Bonferroni post-hoc test. In all our analyses, we used GraphPad Prism software developed by Inc., La Jolla, USA, version 5.0., in which we considered $p < 0.05$ as statistically significant (Fig. 1).

3. Results

3.1. Diosgenin treatment improves exploratory activity and mitigates neuropsychiatric phenotypes in PTSD-challenged mice

As presented in Fig. 2a-f and Table 1, SPS exposure alters motor activity with increased symptoms of neuropsychiatric phenotypes in the animals. In comparison with the vehicle control, the animals exposed to SPS exhibited exploration ($[F4, 40] = 8.589, p < 0.0001$), social behavior (% social preference: ($[F4, 40] = 9.972, p < 0.0001$)) and stress resilience (social interaction ratio: ($[F4, 40] = 4.744, p = 0.0032$)) (Fig. 2a-c). However, diosgenin (25 and 50 mg/kg) reversed these changes compared to the untreated SPS-induced group. The data recorded also showed that diosgenin treatment decreased the immobility time in the SPS-induced mice following the tail suspension test ($[F4, 40] = 15.90, p < 0.0001$; 25 and 50 mg/kg) and forced swimming

test ($[F4, 40] = 12.78, p < 0.0001$; 50 mg/kg) assessments relative to the untreated SPS-induced mice respectively (Fig. 2d-e). Meanwhile, measuring the anxiety-like behavior in the SPS-induced animal as depicted in Fig. 2f-g and Table 1 to corroborate the depressive-like features observed, the data showed that SPS-induced mice spent significantly greater time in the dark compartment and less time in the light compartment relative to the vehicle control. Further, the percentage of open-arm entry and duration decreased significantly, instigating an increase in the index of open-arm avoidance in the SPS-induced mice, which validates the observed anxiogenic behavior when compared with the vehicle control (Table 1). However, all this effect was averted by the diosgenin treatment (25 and 50) mg/kg in a similar manner to the fluoxetine treatment (10 mg/kg) compared to the untreated SPS-induced mice (Fig. 2f-g and Table 1).

3.2. Diosgenin treatment improves short-term spatial and non-spatial memory functions in mice with ptsd challenges

Memory functions were investigated in the experimental animals, as presented in Fig. 3a-b. The data collected showed a decrease in memory index as evidenced by % alternation in the Y-maze test ($[F4, 40] = 5.866, p = 0.0008$) and discrimination index in the NORT ($[F4, 40] = 5.075, p = 0.0021$). However, diosgenin treatment increased the spatial (25 and 50 mg/kg) and non-spatial (50 mg/kg) memory functions in a similar fashion as the fluoxetine treatment (10 mg/kg) in the animal when compared with the untreated SPS-induced (Fig. 3a-b).

3.3. Diosgenin treatment positively regulates the hpa axis in mice with ptsd challenges

Glucose homeostasis in association with the HPA-axis and adrenal gland weight is presented in Fig. 4a-c. SPS increased significantly ($[F4, 20] = 11.02, p < 0.0001$) in mice's adrenal weight relative to the vehicle control, suggesting adrenal hypertrophy. However, the diosgenin (25 and 50) mg/kg and the fluoxetine treatment (10 mg/kg) reduced the increase in the adrenal gland comparative to the untreated SPS-induced mice (Fig. 4a). The results also showed a significant rise in blood sugar ($[F4, 20] = 7.368, p = 0.0008$) and serum corticosterone levels ($[F4, 20] = 6.144, p = 0.0022$) in the SPS-induced animals relative to the vehicle control (Fig. 4b-c). Moreover, treatment with diosgenin variedly reduces the blood sugar (25 mg/kg) and serum corticosterone (50 mg/kg) level ($P < 0.001$) in a similar fashion as the fluoxetine treatment (10 mg/kg) in comparison with the untreated SPS-induced.

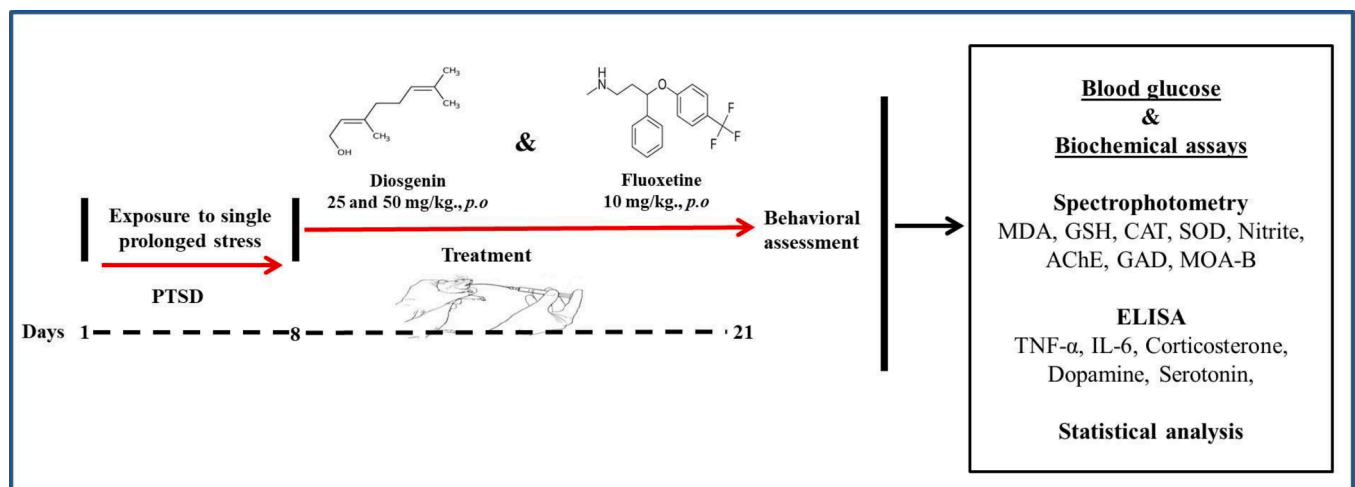


Fig. 1. Schematic representation of experimental design.

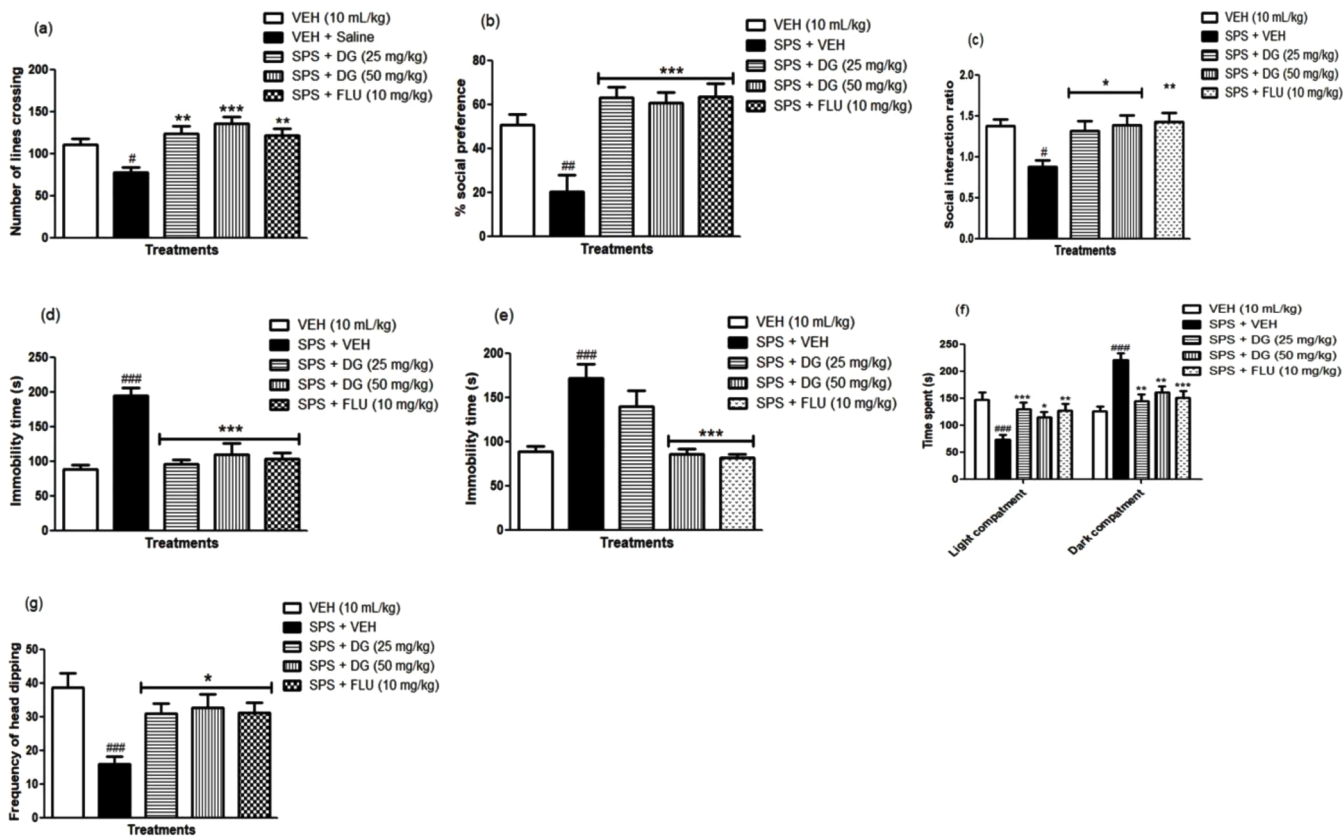


Fig. 2. Diosgenin treatment improves exploratory activity and mitigates neuropsychiatric phenotypes in PTSD-challenged mice: open-field test, OFT (a), social interaction test, SIT (b), social interaction ration, SIR (stress resilience test) (c), tail suspension test, TST (d), forced swim test, FST (e), elevated-plus maze, EPM (f), and hole board test, HBT (g) Bars represent the mean $\pm$ S.E.M of 9 animals/group. # p < 0.05, ### p < 0.001 Vs VEH group; * p < 0.05, ** p < 0.01, *** p < 0.001 Vs SPS group. VEH = Vehicle, SPS = Single prolonged stress, DG = Diosgenin, FLU = Fluoxetine.

Table 1

Effect of diosgenin on single prolonged stress-induced anxiety-like behaviour in mice using elevated-plus maze test.

GROUPS	DOSE (mg/kg)	OPEN ARM ENTRY	OPEN ARM DURATION	%OPEN ARM ENTRY	%OPEN ARM DURATION	INDEX OF OPEN ARM AVOIDANCE
VEH	10 mL/kg	2.05 $\pm$ 0.09	26.80 $\pm$ 1.98	36.48 $\pm$ 1.77	8.99 $\pm$ 0.90	77.27 $\pm$ 4.84
SPS		0.96 $\pm$ 0.03 [#]	12.48 $\pm$ 0.38 [#]	8.11 $\pm$ 0.28 [#]	5.39 $\pm$ 0.57	90.56 $\pm$ 8.49 [#]
DG	25 mg/kg	4.03 $\pm$ 0.32*	96.38 $\pm$ 7.94*	59.22 $\pm$ 4.93*	46.39 $\pm$ 6.28*	47.20 $\pm$ 4.85*
	50 mg/kg	4.00 $\pm$ 0.49*	81.75 $\pm$ 5.74*	54.94 $\pm$ 2.74*	42.56 $\pm$ 5.93*	51.25 $\pm$ 3.62*
FLU	10 mg/kg	5.83 $\pm$ 30.82*	109.95 $\pm$ 6.93*	53.84 $\pm$ 4.03*	51.37 $\pm$ 3.44*	52.11 $\pm$ 4.88*

Values represent the mean $\pm$ S.E.M of 9 animals/group.

[#] p < 0.001 compared to VEH group.

* p < 0.05 compared to SPS group (one-way ANOVA followed by Bonferroni *post-hoc* test). VEH = Vehicle, SPS = Single prolonged stress, DG = Diosgenin, FLU = Fluoxetine.

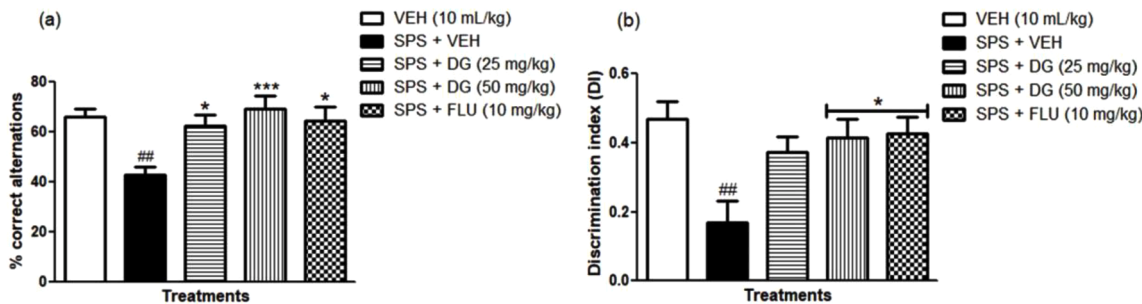


Fig. 3. Diosgenin treatment improves short-term spatial and non-spatial memory functions in mice with PTSD challenges: spatial working in the (a) and non-spatial in the NORT working (b) memories. Bars represent the mean $\pm$ S.E.M of 9 animals/group. ## p < 0.01 Vs VEH group; * p < 0.05, *** p < 0.001 Vs SPS group. VEH = Vehicle, SPS = Single prolonged stress, DG = Diosgenin, FLU = Fluoxetine.

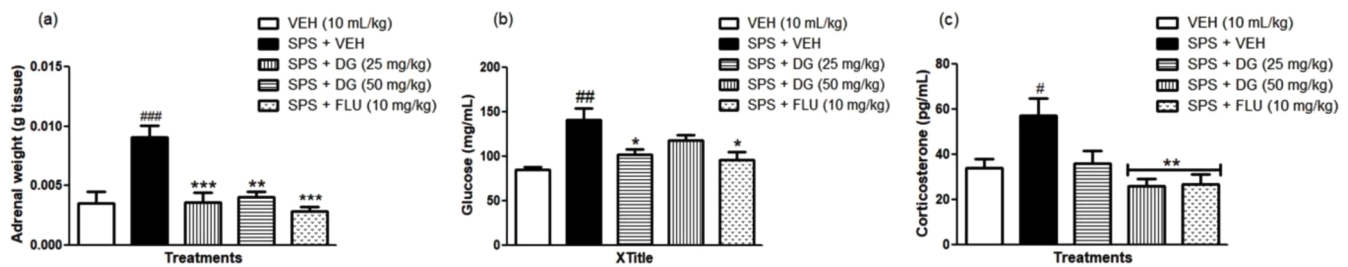


Fig. 4. Diosgenin treatment positively regulates the HPA axis in mice with PTSD challenges: adrenal weight (a), glucose (b), and corticosterone (c). Bars represent the mean $\pm$ S.E.M of 9 animals / group. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ Vs VEH group; * $p < 0.05$, *** $p < 0.001$ Vs SPS group. VEH = Vehicle, SPS = Single prolong stress, DG = Diosgenin, FLU = Fluoxetine.

3.4. Diosgenin treatment modulates acetylcholinesterase, monoamine oxidase-B and glutamic acid decarboxylase enzyme activities in the brain of PTSD-challenged mice

As represented, the acetylcholinesterase (Fig. 5a) (Treatment: [F8, 60] = 1.78; $p = 0.0981$, Interaction: [F4, 60] = 18.54; $p < 0.0001$ and Treatment $\times$ Interaction: [F2, 60] = 17.57; $p < 0.0001$) and MAO-B (Fig. 5b) (Treatment: [F8, 60] = 1.86; $p = 0.0839$, Interaction: [F4, 60] = 15.54; $p < 0.0001$ and Treatment $\times$ Interaction: [F2, 60] = 23.03; $p < 0.0001$) enzyme activities were profoundly increased but accompanied by decreased glutamic acid decarboxylase (Fig. 5c) in the corticostriatal and hippocampal regions of the SPS-induced mice compared to vehicle control. However, the treatment with diosgenin (25 and 50 mg/kg) and fluoxetine (10 mg/kg) inhibited acetylcholinesterase (Fig. 5a) and MAO-B (Fig. 5b) enzyme release and increased glutamic acid decarboxylase (Fig. 5c) in the different brain regions when compared to the untreated SPS groups.

3.5. Diosgenin treatment increases dopamine and serotonin release in the brain of PTSD-challenged mice

As shown in Fig. 6a-b, SPS induction caused a marked decrease in dopamine (Treatment: [F6, 48] = 3.02; $p = 0.0139$, Interaction: [F3, 48] = 17.15; $p < 0.0001$ and Treatment $\times$ Interaction: [F2, 48] = 13.82; $p < 0.0001$) and serotonin (Treatment: [F6, 48] = 1.56; $p = 0.1786$, Interaction: [F3, 48] = 24.37; $p < 0.0001$ and Treatment $\times$ Interaction: [F2, 48] = 15.59; $p < 0.0001$) levels in the cortico-striatal and hippocampal region of mice brain comparative to the vehicle controls (Fig. 6a-b). Diosgenin 50 mg/kg and fluoxetine 10 mg/kg treatments increased dopamine and serotonin concentrations in the different brain regions of the mice when compared to SPS control. Both the diosgenin 50 mg/kg and fluoxetine 10 mg/kg showed no marked effects on the dopamine

concentration in the hippocampus of the stressed-induced mice after treatment.

3.6. Diosgenin treatment improves endogenous antioxidant defense system and suppresses pro-oxidant activity in PTSD-challenged mice

From the data presented in Fig. 7a-c, the endogenous antioxidant activities (glutathione, superoxide dismutase, catalase) in the experimental mice in different brain regions decreased after SPS induction compared to the control. Diosgenin (25 and 50 mg/kg) and fluoxetine (10 mg/kg) treatments increased the endogenous antioxidants in the cortex, striatum and hippocampus of the mice brain relative to the untreated SPS-induced mice. In contrast, the superoxide dismutase and catalase activities in the hippocampal brain region after treatment with diosgenin (25 and 50 mg/kg) did not show significant changes ($p > 0.05$) when compared to the untreated SPS mice (Fig. 7a-c).

Furthermore, as shown in Fig. 7d-e, SPS exposure provoked redox disturbances in the cortex, striatum and hippocampus of the mice brain compared to the control mice. Moreover, estimating the mice brain pro-oxidant status after diosgenin (25 and 50) mg/kg treatment, our data showed a positive modulatory effect evidenced by decreased malondialdehyde concentration (Treatment: [F8, 60] = 3.40; $p = 0.0028$, Interaction: [F4, 60] = 27.94; $p < 0.0001$ and Treatment $\times$ Interaction: [F2, 60] = 13.00; $p < 0.0001$) (Fig. 7d). In a different manner, the nitroergic activity in the hippocampal brain region after treatment with diosgenin (25 and 50) mg/kg failed to produce any significant effect ($p > 0.05$) when compared to untreated SPS control (Fig. 7e).

3.7. Diosgenin treatment inhibits cortico-hippocampal pro-inflammatory cytokine release in the brains of mice with ptsd challenges

As presented in Fig. 8a-b, the release of pro-inflammatory cytokines

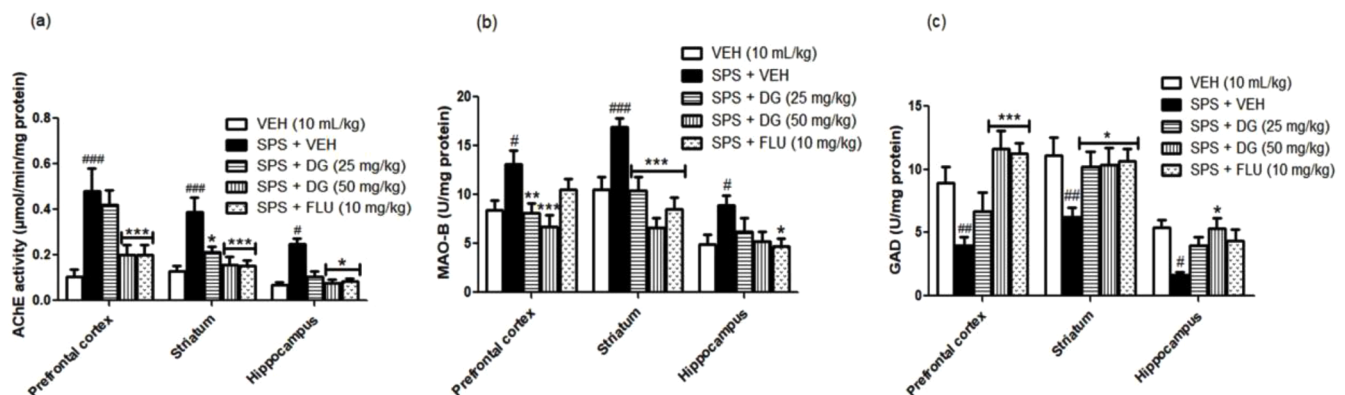


Fig. 5. Diosgenin treatment modulates acetylcholinesterase (a), monoamine oxidase-B (b) and glutamic acid decarboxylase enzyme (c) activities in the brain of PTSD-challenged mice. Bars represent the mean $\pm$ S.E.M of 9 animals/group. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ Vs VEH group; * $p < 0.05$, *** $p < 0.001$ Vs SPS group. VEH = Vehicle, SPS = Single prolong stress, DG = Diosgenin, FLU = Fluoxetine.

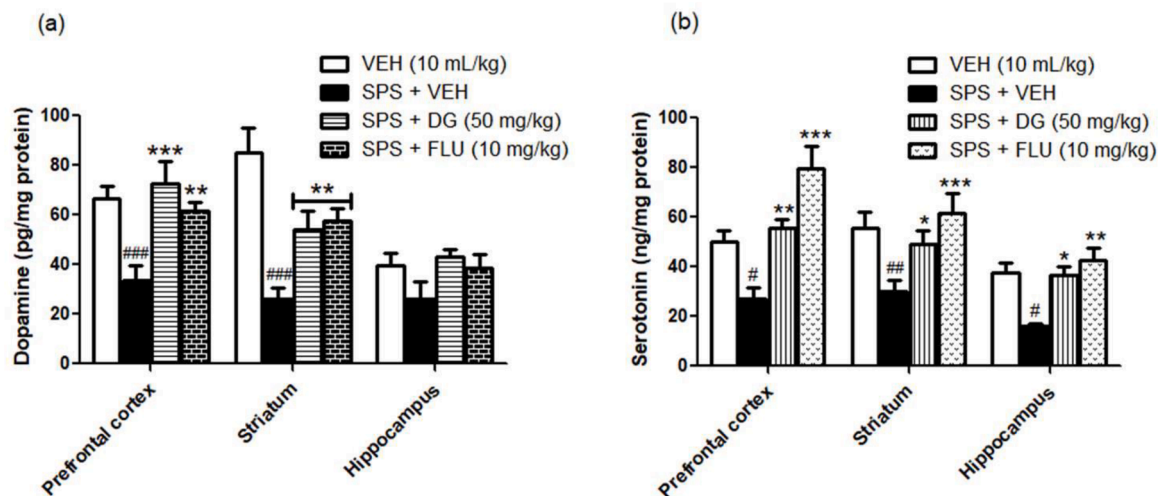


Fig. 6. Diosgenin treatment increases dopamine (a) and serotonin (b) release in the brain of PTSD-challenged mice. Bars represent the mean $\pm$ S.E.M of 9 animals/group. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ Vs VEH group; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ Vs SPS group. VEH = Vehicle, SPS = Single prolong stress, DG = Diosgenin, FLU = Fluoxetine.

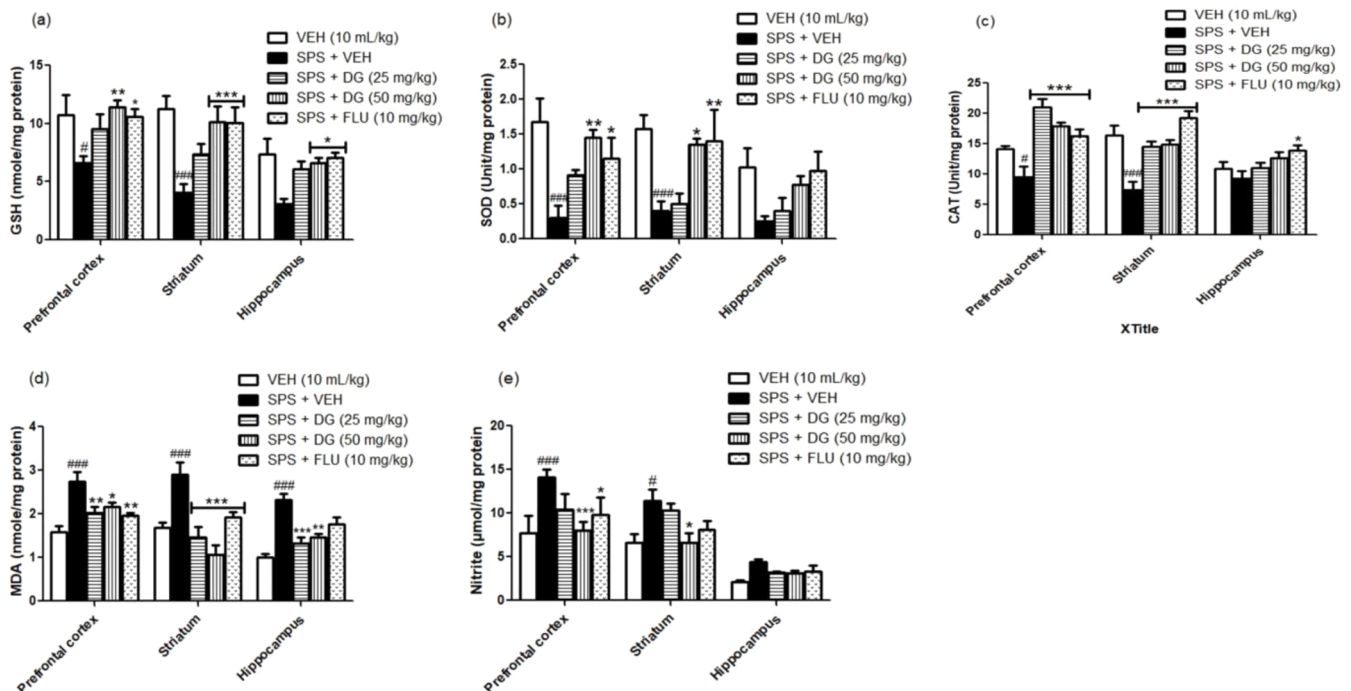


Fig. 7. Diosgenin treatment improves the endogenous antioxidant defense system (a, b, c) and suppresses pro-oxidant activity (d, e) in PTSD-challenged mice. Bars represent the mean $\pm$ S.E.M of 9 animals/group. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ Vs VEH group; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ Vs SPS group. VEH = Vehicle, SPS = Single prolong stress, DG = Diosgenin, FLU = Fluoxetine.

in the cortico-hippocampal regions was heightened after SPS exposure when compared to the control, which was halted by diosgenin (50 mg/kg) and fluoxetine (10 mg/kg) treatments as indicated by two-way ANOVA: TNF- α (Treatment: [F3, 32] = 0.77; $p = 0.5207$, Interaction: [F3, 32] = 21.88; $p < 0.0001$ and Treatment $\times$ Interaction: [F1, 32] = 4.20; $p = 0.0486$) (Fig. 8a) and IL-6 (Treatment: [F3, 32] = 1.67; $p = 0.1933$, Interaction: [F3, 32] = 16.64; $p < 0.0001$ and Treatment $\times$ Interaction: [F1, 32] = 11.50; $p = 0.0019$) (Fig. 8b) relative to the untreated SPS controls. However, 50 mg/kg of diosgenin did not have a significant effect ($p > 0.05$) in the hippocampus compared to the untreated SPS-induced mice (Fig. 8b).

4. Discussion

Investigations have shown that dysfunctions of the central nervous system caused by PTSD can lead to neurocognitive impairments and neuropsychiatric complications (Tolosa et al., 2020; Karanikas et al., 2021). Treating these behavioral symptoms with pharmacological agents has proven difficult. We investigated the therapeutic and neuroprotective potential of diosgenin on SPS-induced PTSD-like conditions. The experiment shows that SPS induces PTSD through the production of oxidative and inflammatory molecules and suppression of neuronal transmission. However, diosgenin treatment reversed SPS-like PTSD symptoms, improving behavior, memory functions, and stress

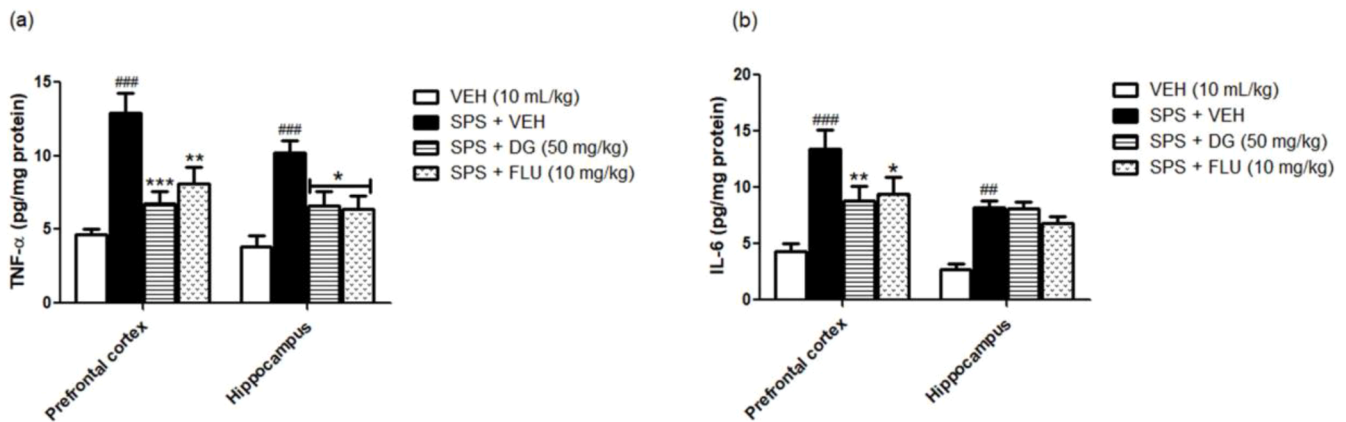


Fig. 8. Diosgenin treatment inhibits cortico-hippocampal pro-inflammatory cytokine release in the brains of mice with PTSD challenges. Bars represent the mean $\pm$ S.E.M of 9 animals / group. ## $p < 0.01$, ### $p < 0.001$ Vs VEH group; * $p < 0.05$, *** $p < 0.001$ Vs SPS group. VEH = Vehicle, SPS = Single prolong stress, DG = Diosgenin, FLU = Fluoxetine.

resilience. These effects were mediated by modulation of neurotransmissions and normalization of the HPA axis.

In this study, exposure to stressful cues led to a significant increase in blood glucose concentration. This is attributed to the activation of the HPA axis and the production of glucocorticoids, which promote gluconeogenesis and inhibit glucose uptake (Kuo et al., 2015). High levels of glucocorticoids due to stress have been linked to impairment in hippocampal-dependent learning and memory processes (Gerges et al., 2001; Ben-Azu et al., 2019). Conflicting reports in a study showed different effects on blood glucose levels in stressed mice (Iranmanesh et al., 1990). However, previous evidence supports increased glucose levels due to stress (Ugwu et al., 2022). This finding complements extreme circulating corticosterone levels in the SPS animals, which impaired glucose utilization and were reversed by diosgenin. Zhe et al. (2008) and Eagle et al. (2013) observed and reported significant adrenal gland deregulation with increased ACTH/corticosterone/mineralocorticoid release associated with glucocorticoid receptor over-expression in mice following introduction to SPS. However, the decrease in corticosterone levels in PTSD-challenged mice may be due to the neuroprotective and therapeutic potential of diosgenin in restoring adrenal gland activity and hypertrophy. This is supported by previous studies that observed significant adrenal gland deregulation in mice following exposure to stress (Mishima et al., 2015; Ugwu et al., 2022). However, the finding that diosgenin normalized the impaired HPA axis of SPS-induced PTSD mice suggests that it possesses adaptogenic-like activity capable of enhancing stress resilience, similar to previous findings (Ben-Azu et al., 2024c). Complementarily, a recent study moreover found that diosgenin mitigates the HPA axis dysregulation and impaired activity of hippocampal brain-derived neurotrophic factor induced by chronic restrain stress, which is one of the front liners and subunits of SPS that propagated the PTSD observed in this study (Cui et al., 2023).

Further examination of the behavioral response in learning and memory, social and activity and stress resilience in the mentally disturbed mice revealed interesting results, in which diosgenin treatment still exerted its positive effect. The measurement of locomotor activity was carried out to confirm the influence of SPS on PTSD-related reduction in explorative skills and dysfunction in motor function. The outcome confirmed that physical and psychological trauma-induced anxiogenic state, such as SPS-induced anxiety, is accompanied by impaired locomotor activity and reduced exploration (Ellmers et al., 2020; Faithi et al., 2022). Inherently, the outcome of the open-field test experiment is indicative of the fact that physical exploration and anxiogenic predicaments are believed to be associated with traumatic events notably related to the degeneration of neurons involved in the regulation of motor and anxious states (Faithi et al., 2022). However,

this impaired locomotion associated with heightened anxiety, which was evident by the lack of movement to the center of the open-field test, was ameliorated by diosgenin. Interestingly, the psychoimmunological study of *Dioscorea* (wild yam) in which diosgenin is the primary steroidal saponin, was shown to demonstrate potent anxiolytic and antidepressant effects in ovariectomized rats, a menopausal condition characterized by intense anxiety, motor, and memory impairments (Ho et al., 2007).

Interestingly, anhedonia, which is a core symptom of depression usually observed in humans with reduced ability to experience pleasure following exposure to environmental cues such as SPS, has been largely reported in patients with PTSD (Iniguez et al., 2014; Lazarov et al., 2020). From our analyses, the tail suspension, forced swim and social interaction tests indicate that chronic exposure to stressors exacerbates the expression of common and core endo-phenotypes of major depressive-like and social behavior disorder, including social avoidance and helplessness (Lee et al., 2020). Also, the present findings are further consistent with previous reports showing that SPS induction increases immobility time in the tail suspension and forced swim tests in mice (Lee et al., 2020). Apparently, the treatment with diosgenin decreased the duration of immobility time and increased the attempt to climb in both measurements in both the tail suspension and forced swim tests. Additionally, many investigations have shown that depression-associated symptoms and aberrant social behavior are typically accompanied by anxiety-related symptoms (Ben-Azu et al., 2018a; Chen et al., 2021, 2022a). In the present study, we complementarily found that mice predisposed to SPS-induced PTSD comorbidly expressed anxiety-like features alongside depressive-like behaviors (Lee et al., 2020; Sur et al., 2022). However, diosgenin administration after SPS exposure abated this anxiogenic state based on increased time spent in the light zone in the LDB and the frequent head dip in the hole board experiments. Thus, our results remarkably speculate that diosgenin treatment may ameliorate traumatic stress-induced contextual fear-mediated memory conditioning in the amygdaloid brain, which is evidently illuminated by the LDB and hole board tests. Remarkably, mental stress, depression, anxiety and impaired social behavior have previously been implicated in obstructing cortical regulation of online working memory function in humans (Christopher and MacDonald, 2005). Therefore, expounding that the burden of these symptoms may contribute to the observed reduction in short-term spatial and non-spatial memory functions as well as social recognition memory in the SPS-induced PTSD experimental mice. This effect was further abated by diosgenin administration. Indeed, this effect could be attributed to the neurotransmitters (e.g., dopamine and serotonin) and neurochemical enzymes (Lee et al., 2020; Sur et al., 2022).

Serotonin and dopamine release or exogenous treatment with their

agonists and MAO inhibitors have been keenly reported to facilitate reward processes, social interaction and anhedonic behaviors (Lee et al., 2020). For example, the chronic treatment with SSRIs as reported by Harrison et al. (2001) and El Yacoubi et al. (2011) was shown to reverse the psychostimulant withdrawal-induced anhedonia effect in rats. Indeed, measurements of serotonin and dopamine in the cortico-striatal and MAO assay in the cortico-striatal and hippocampal regions from this study further complement in a similar manner the observed effects of diosgenin and fluoxetine. Essentially, diosgenin-induced elevated release of dopamine and serotonin in the PTSD-treated mice is strongly attributed to the reduced anhedonia-like phenotype and social withdrawal experienced by the PTSD-related mice. Specifically, we found that diosgenin inhibited the enzyme MAO-A, responsible for the metabolism of monoamines, including serotonin and dopamine, thus possibly increasing the synaptic pools of serotonin and dopamine in the different brain regions examined in this study. The role of dopamine in combination with serotonin release cannot be overemphasized as it is believed and reported that both neurotransmitters positively modulate behavioral functions and reduce the development of neuropsychiatric phenotype when produced in a relevant concentration in the presence or absence of a stimulant (Ben-Azu et al., 2018a). Obviously, our findings showed that diosgenin significantly increased dopamine concentrations in the cortico-striatal subfields, although there was a non-significant increase in dopamine concentration in the hippocampal brain region after treatment with the highest dose of diosgenin and fluoxetine. Taken together, these outcomes indicated that diosgenin treatment inhibited depression and improved social interaction behavior by elevating the serotonin and dopamine levels via inhibition of MOA enzyme activity in the relevant brain regions (Lee et al., 2020; Sur et al., 2022; Ben-Azu et al., 2024c).

Complex networks of neuronal connections, such as the cholinergic, glutamatergic and GABAergic neuronal systems, converge and project to several brain regions, which modulate memory formation and motor activities (Trang and Khandhar, 2022). Notably, inhibiting or decreasing these neuronal systems primarily interferes with learning and memory processes and increases anxiety (Huang et al., 2022). Interestingly, in the present study, PTSD downregulated the cortico-striatal and hippocampal cholinergic activity by increasing acetylcholinesterase enzyme with a concomitant decrease in glutamic acid decarboxylase in the traumatized mice. This observation further established the memory derangement and neuropsychiatric phenotypes revealed by the reduced exploratory motor activity, decreased spatial, non-spatial working, social recognition memory functions and anxio-depressive-like behavior reported in this study. Multiple specific targeted studies have investigated the role of cholinergic impairment paralleling cognitive deficits consisting of breakdown in spatial/non-spatial memories, executive control inhibition, and blockade of irrelevant cues into consciousness resulting in dysfunction in hyper-attentional behavior as well as fear-related phenotype (hyperarousal cluster symptom) (Yanpallewar et al., 2022). Also, the altered glutamic acid decarboxylase-GABAergic enzyme as the synthetic pathway to the major inhibitory neurotransmitter, GABA, in the pathogenesis of PTSD-related anxiety has received overwhelming attention (Bremner et al., 2000; Fang et al., 2018). Of note, Stupin et al. (2021) consequently postulated that chronic PTSD is associated with reduced neuronal volume, precisely the hippocampus, which may be because of atrophy or decreased neuronal activity, specifically mediated by co-transmission of acetylcholine and GABA (Takács et al., 2018). Given that the basal forebrain cholinergic system is widely believed to regulate cortical function via the formation of GABAergic synapses, we may also speculate that this effect might be implicating the alteration previously reported in the cholinergic/GABAergic systems in the PTSD-challenged mice. Thus, we proposed that the relative capacity of diosgenin to normalize acetylcholinesterase and glutamic acid decarboxylase enzyme activities in PTSD mice would suggest a neuroprotective adaptogenic-like property. Interestingly, diosgenin treatment abated the SPS-induced PTSD-associated increase

in acetylcholinesterase activity and glutamic acid decarboxylase depletion, which suggests improved cholinergic and GABAergic neurotransmissions.

Neuronal dysfunctions are linked to high oxidative stress levels, which can lead to neurotransmission deregulation and increased risk of neurological diseases (Ben-Azu et al., 2018b,d). Mental stressors can provoke redox imbalances and inflammation in the brain, affecting antioxidant profiles and predisposing to neuropsychiatric diseases (Ben-Azu et al., 2019, 2022b, 2024d; Chen et al., 2022). Reductions in endogenous antioxidant profiles such as superoxide dismutase, glutathione, and catalase have been reported in individuals with significant neuropsychiatric diseases (Chen et al., 2022; Ugwu et al., 2022). This study also evaluated oxidative status in the cortico-striatal and hippocampal brain regions after exposure to the SPS-induced traumatic event. Our data indicated that exposure to SPS stressor provoked an increase in pro-oxidant molecules, marked by exaggerated concentrations of malondialdehyde and nitrite levels accompanied by a concomitant decrease in endogenous antioxidants such as superoxide dismutase, glutathione, and catalase in the cortico-hippocampal brain region of the experimental mice. To substantiate the presence of oxidative stress, inflammatory cytokines were also evaluated in the striatum, prefrontal cortex and hippocampus and were found to be elevated in the same manner as the pro-oxidant molecules in the SPS-exposed mice. In the brain, oxidative stress initiates resident neuroimmune cells like microglia and astrocytes through complex events that cause the release of inflammatory mediators such as TNF- α and IL-6 in the cells. Such a cellular environment warrants TNF- α and IL-6 synthesis-mediated expression of other transcription factors and genes that regulate inflammatory response (Chen et al., 2022). Separately, PTSD has been associated with elevated expression of pro-inflammatory cytokines and oxidative stress biomarkers (Lee et al., 2022). These findings suggest that PTSD up-regulated neuroinflammation, a pathway associated with the pathological biochemical release of TNF- α and IL-6 and associated oxidative/nitrergic stress markers in the cortico-hippocampus in the mice. However, these outcomes from this study showed that an increase in the cortico-hippocampal malondialdehyde, nitrite, TNF- α and IL-6 activities were ameliorated by the diosgenin administration post-exposure with a concomitant increase in superoxide dismutase, glutathione, and catalase levels, suggesting antioxidant and anti-neuroinflammatory functions.

Together, our data shows that diosgenin treatment improved PTSD symptoms in mice by boosting neurochemical release, normalizing enzyme activities, and inhibiting inflammatory pathways in the brain region. It also improved glucose levels and HPA axis dysregulation.

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Availability of data and material

Not applicable

Code availability (software application or custom code)

Not applicable

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/22/149) and the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication number: 85–23, revised 1985).

Data availability

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

CRedit authorship contribution statement

Benneth Ben-Azu: Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Olusegun G. Adebayo:** Writing – review & editing, Writing – original draft, Resources, Methodology, Data curation, Conceptualization. **Adaeze Adebisin:** Writing – review & editing, Resources, Funding acquisition, Formal analysis. **Kenneth C. Oparaji:** Writing – review & editing, Validation, Software, Funding acquisition. **Vivian O. Ojiakor:** Writing – review & editing, Visualization, Software, Funding acquisition, Formal analysis. **Gift C. Pender:** Writing – review & editing, Resources, Funding acquisition. **Bensandy O. Odeghe:** Data curation, Funding acquisition, Validation, Writing – review & editing. **Noah A. Omeiza:** Writing – review & editing, Software, Resources, Data curation. **Halimat A. Abdulrahim:** Writing – review & editing, Resources, Funding acquisition. **Vivian Ezieshi:** Writing – review & editing, Resources, Funding acquisition, Data curation, Conceptualization. **Glory Ighosotu:** Writing – review & editing, Resources, Funding acquisition. **Emmanuel Omo-Odudu:** Writing – review & editing, Visualization, Resources, Funding acquisition. **Ekene I. Monye:** Writing – review & editing, Resources, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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