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Berberine attenuates SPS-induced cognitive and affective deficits and corticolimbic injury in Wistar rats via restoration of BDNF and redox–inflammatory balance

Godson Emeka Anyanwu [a], Precious E. Ekwueme[b], Ayugha Nsan[b], Lilian Ezeugwu[b], Prisca Chiamaka Ogbe[b], Chinyere N. Anyanwu[c], Vivian O. Ojiakor[a] and Chima Paul Okechukwu Ugwu[d]

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ABSTRACT

Background: Single prolonged stress (SPS) is a validated PTSD-like paradigm that causes cognitive and affective dysfunction and corticolimbic injuries due to the dysregulation of the HPA-axis and neurotrophic, redox, and neuroimmune pathways. Berberine is a bioactive isoquinoline alkaloid that demonstrates neuroprotective effects, although its effect on corticolimbic-pathology caused by SPS is yet to be fully characterized.

Objectives: To ascertain whether berberine attenuates SPS-induced neurobehavioral impairments and corticolimbic neuronal damage in Wistar rats.

Methods: Adult male Wistar rats were exposed to SPS (restraint, forced swim, and anesthetic-induced loss of consciousness) and left undisturbed for 7 days to allow stress consolidation. Then, rats were given oral berberine (50 or 200 mg/kg) or fluoxetine (10 mg/kg) in a single daily dose. Behavioral measures were Y-maze spontaneous alternation, Morris water maze, novel object recognition and sucrose preference. ELISA was used to quantify serum corticosterone. BDNF, SOD, catalase, MDA, and IL-1b/IL-6/TNF-a were assayed in the hippocampus and mPFC. CFV and NeuN immunohistochemistry were used to assess neuronal integrity and astrocytic reactivity was assessed using GFAP-immunoreactivity.

Results: SPS induced severe deficits in working memory, spatial and recognition memories and reward sensitivity, and high basal corticosterone. It increased lipid peroxidation and pro-inflammatory cytokines while reducing antioxidant enzyme activities in brain tissue homogenates (hippocampus and medial prefrontal cortex). Berberine improved behavioral outcomes and attenuated endocrine, biochemical, and histopathological abnormalities in a dose-dependent manner.

Conclusion: Berberine attenuates SPS-induced-cognitive/affective dysfunction and corticolimbic-lesion and establishes normalization of basal corticosterone, BDNF recovery, antioxidant defense, lipid peroxidation, cytokine elevations, and astrocytic activation. These findings support berberine as a multi-target candidate for mitigating stress-related-neurotoxicity.

KEYWORDS

Berberine; single prolonged stress (SPS); PTSD model; cognitive dysfunction; HPA axis / corticosterone; oxidative stress (MDA; SOD; Catalase); neuroinflammation (IL-1β; IL-6; TNF-α)

1. Introduction

A significant cause of stress-related neuropsychiatric disease is severe psychological trauma, and chronic dysregulation of stress systems is closely associated with cognitive impairment and affective disturbances. One of the major mechanisms is maladaptive HPA axis signaling, whose downstream effects impact neuronal integrity and synaptic plasticity across corticolimbic circuits [1,2]. Specifically, regions that are of great interest because of their stress sensitivity and the ability to sub-serve learning, memory, executive control, and reward-related processing include the hippocampus and the medial prefrontal cortex (mPFC), making them important substrates of trauma-related phenotypes of cognition and mood [3–5].

As a paradigm to model PTSD-like traumatic stress biology in rodents, the single prolonged stress (SPS) paradigm is common [6]. SPS is usually a series of stressors administered within a day (restraint, forced

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swim, anesthetic-induced loss of consciousness, etc.), with an uninterrupted incubation period, during which neurobehavioral abnormalities develop later [7]. The SPS generates the features that are pertinent to PTSD, such as persistent disruptions in the process of fear learning and unlearning and extended dysfunction of the corticolimbic system [7,8].

Chronic or traumatic stress exposure at the neural systems level is linked to dendritic remodeling, impaired synaptic connection, and broken neuroplasticity in networks of the hippocampus and prefrontal systems, which can be translated into spatial, recognition, and working memory deficits [9–11]. Changes in neurotrophic signaling (particularly, the reduction in brain-derived neurotrophic factor (BDNF)) have been reported to affect impaired synaptic upkeep and cognitive functioning due to stress [12, 13]. Corticolimbic vulnerability is also facilitated by converging evidence in animal models that indicates long-lasting behavioral abnormalities and dysregulation of neuroendocrine functions following exposure to traumatic stress [7].

In addition to synaptic and neurotrophic dysfunction, neuroinflammation is becoming a well-known mediator of neuronal stress-related damage. Exposure to stress may lead to the release of peripheral and central cytokines (e.g. IL-1b, IL-6, TNF-a) and glial activation, which adds to synaptic dysfunction and increased neuronal vulnerability [14–17]. Oxidative imbalance also increases this pathology: inhibition of endogenous antioxidant mechanisms (such as SOD and catalase) and enhancement of lipid peroxidation may enhance inflammatory signaling and deteriorate neuronal survival [18–20]. Combined, dysregulated neurotrophic support, neuroimmune activation and oxidative injury generate a detrimental corticolimbic microenvironment that has the capacity to contribute to both cognitive impairment and affective dysregulation.

Berberine (BBR) is an isoquinoline alkaloid that has a long history of medicinal use and has an increasing evidence base that supports the neuroactive effects of this plant-derived compound [21]. Recent work highlights berberine's pleiotropic actions relevant to stress neuropathology, including antioxidant and anti-inflammatory effects and modulation of neurotrophic signaling [22,23]. Berberine was found to reduce depression-like symptoms and block inflammatory responses and promote neuroplasticity associated with BDNF in preclinical stress-related models [24, 25]. Despite accumulating evidence, berberine's integrated neuroprotective profile under SPS, spanning endocrine stress readouts (basal corticosterone), multi-domain neurobehavior (spatial/recognition/working memory and anhedonia), oxidative injury (SOD, catalase, MDA), inflammatory cytokines (IL-1b, IL-6, TNF-a), and corticolimbic histopathology (NeuN, Cresyl Violet, GFAP), remains insufficiently characterized in a single coherent SPS design.

While previous studies have reported the neuroprotective and antidepressant-like effects of berberine in stress-related models, these investigations have largely examined isolated behavioral outcomes or singular molecular pathways. A comprehensive, systems-level characterization integrating endocrine stress responses, multi-domain neurobehavioral outcomes, redox–inflammatory balance, neurotrophic signaling, and structural neuronal integrity within a single prolonged stress (SPS) framework remains limited. The present study addresses this gap by providing a unified evaluation of berberine's effects across corticolimbic circuits, enabling a more mechanistic and translational understanding of its multi-target neuroprotective profile in trauma-related neuropsychiatric dysfunction.

Based on this, this paper examined the role of berberine in mitigating SPS-induced cognitive and affective impairment and corticolimbic neuronal injury in adult Wistar rats, and the role of neurotrophic (BDNF), oxidative/antioxidant (SOD, catalase, MDA), and inflammatory cytokine (IL-1b, IL-6, TNF-a), and glial (GFAP) pathways in the hippocampus and mPFC. We hypothesized that berberine would attenuate SPS-linked dysfunction by normalizing HPA-axis output, restoring BDNF-associated neuroplastic support, reinforcing antioxidant defenses, reducing lipid peroxidation, and suppressing cytokine-driven neuroinflammation.

2. Materials and methods

2.1. Ethical approval and reporting

All animal experiments were carried out based on the ARRIVE 2.0 recommendations on the care, use, and management of laboratory animals [26], and were approved by the Institutional Research Ethics Committee

of the University of Nigeria, Enugu with ethical number COMHREC/2025/03/027. The experimental standards complied with the National Institutes of Health Guidelines regarding the Care and Use of Laboratory Animals (NIH publication no. 8023, revised 1978) [27]. All endeavors were made to lessen the pain of animals.

2.2. Animals, housing and husbandry

Adult male Wistar rats (8–10 weeks old; 200–250 g) were obtained from the institutional breeding facility. Animals were acclimatized seven days prior to experimentation, and kept in groups of four to five rats in each cage under usual laboratory conditions with a 12:12 h light/dark cycle (lights on at 07:00), ambient temperature of 23 ± 2°C, and relative humidity of about 60%. Standard chow and water were given ad libitum except when the procedure involved a particular stress.

During the study, environmental enrichment (nesting material and cardboard tunnels) was provided. The positions of the cages were changed every week to reduce environmental bias. The animals were observed daily for their general health and welfare.

2.3. Experimental design, randomization, blinding, and sample size

Animals were behaviorally assessed at baseline prior to SPS exposure and were then assigned to groups using a computer-generated randomization sequence after acclimatization. To minimize experimental bias, group allocation was coded by an independent researcher not involved in data collection or analysis. Behavioral assessments, biochemical assays, histological processing, and image quantification were performed by investigators blinded to treatment allocation. Blinding was maintained throughout data acquisition and analysis, and treatment codes were only revealed after completion of statistical analyses.

Sample sizes were determined with reference to prior berberine studies and related experimental models, in which group sizes of 6–10 animals per group are commonly used across pharmacological, toxicological, and disease-model investigations [28–33]. In the present study, behavioral endpoints were prioritized as primary outcomes and therefore used $n = 10$ animals per group to improve sensitivity for detecting stress- and treatment-related differences across repeated measures. Biochemical and histological analyses were performed on terminal subsets of $n = 5$ animals per group because these endpoints required tissue harvesting and were intended to provide mechanistic support for the primary behavioral findings. Although a formal a priori power calculation was not performed, the study was designed to detect moderate-to-large effects based on established literature and prior experience with stress-model phenotypes.

Inclusion/exclusion criteria: Animals were excluded only for pre-defined reasons: unrelated illness, gavage complications, failure of perfusion/fixation, or loss of trackable behavioral files. Any exclusions and final n values per endpoint are reported.

Inclusion/exclusion criteria: Animals were only excluded on predetermined grounds, such as unrelated illness, gavage-related complications, failure of perfusion or fixation or loss of trackable behavioral information. All the exclusions and end sample sizes are reported on a per endpoint basis. Statistical outliers were not eliminated unless they were technical failures by predefined criteria.

2.4. Experimental timeline (see Figure 1)

Day −7 to −1: Acclimatization and baseline behavioral assessment
Day 0: Single prolonged stress (SPS) induction
Days 1–13: Stress consolidation period
Day 14: Post-stress behavioral assessment
Days 15–27: Daily drug or vehicle treatment
Day 28: Post-treatment behavioral assessment
Day 29: Basal morning blood collection, perfusion, and tissue harvesting.

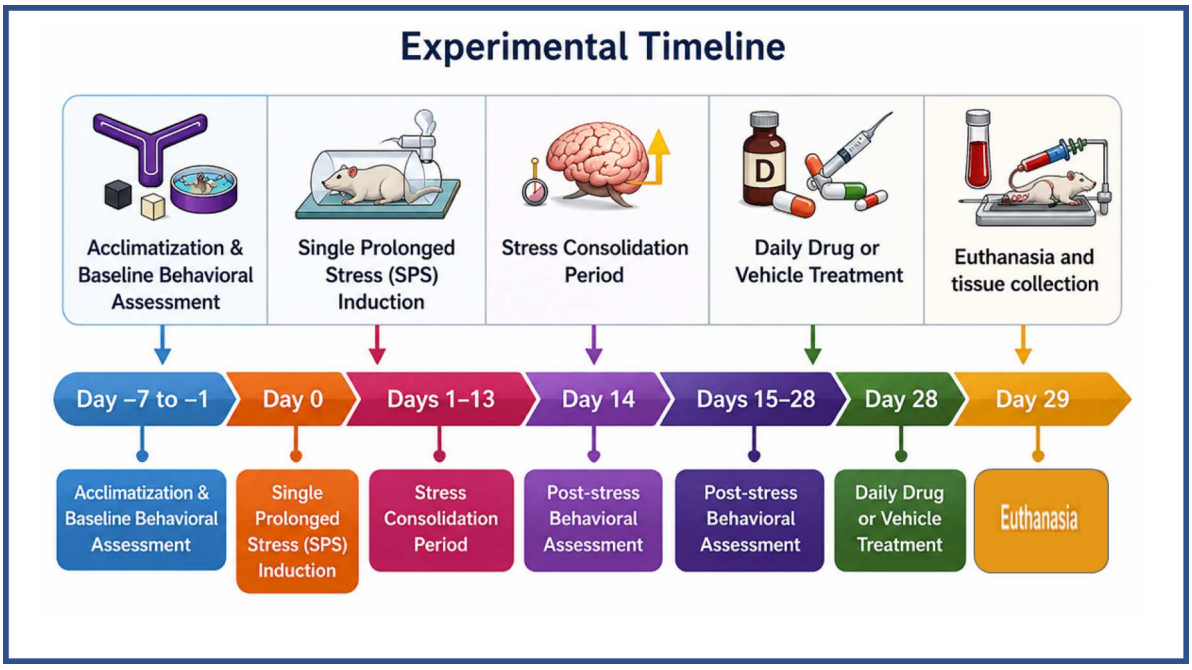


Figure 1. Schematic representation of the experimental timeline. Rats were acclimatized and underwent baseline behavioral assessment from Day −7 to Day −1. Single prolonged stress (SPS) was induced on Day 0, followed by a stress consolidation period (Days 1–13). Behavioral assessment was conducted on Day 14 to confirm stress-induced deficits. Animals then received daily drug or vehicle treatment from Days 15–27. Post-treatment behavioral assessment was performed on Day 28. On Day 29, animals were subjected to basal morning blood collection, transcardial perfusion, and tissue harvesting for biochemical, neurotransmitter, and histological analyses.

2.5. Single prolonged stress (SPS) model

Stress phenotype: A PTSD-like stress was induced with SPS. In the morning (09:00–13:00 h), on Day 1, the following SPS sequence was performed on animals:

1. Restraint stress: Rats were restrained for 2 h in well-ventilated Plexiglas restrainers.
2. Forced swim stress: Immediately following restraint, rats were subjected to forced swimming for 20 min in a cylindrical tank (height 50 cm; diameter 25 cm) filled with water (25 ± 1 °C; depth 30 cm). Animals were towel-dried and put in a warmed recovery cage of 15 min.
3. Loss of consciousness due to anesthetics: Rats were placed in an induction chamber with isoflurane (4–5% in oxygen) until they lost their righting reflex (usually 2–3 min) and then the anesthesia was stopped.

After SPS, animals were put back to their home cages and left alone, without any disturbance except normal husbandry to consolidate effects of stress. The control animals were manipulated but not subjected to SPS.

2.6. Drugs and treatment

2.6.1. Chemicals and reagents

Unless otherwise stated, all chemicals, antibodies, and assay kits were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). Berberine chloride (Cat. B3251) and fluoxetine hydrochloride (Cat. F132) were used for treatments. Treatments were given once daily between 08:00–09:00 h.

Histological reagents included Cresyl Violet acetate (Cat. C5042) and SIGMAFAST™ DAB (Cat. D4293). The primary antibodies were anti-NeuN (clone A60; Cat. MAB377, 1:500) and anti-GFAP (clone G-A-5; Cat. G3893, 1:1000). Biotinylated goat anti-mouse IgG (Cat. B7264) and VECTASTAIN(r) Elite ABC-HRP kit (Vector Laboratories; Cat. PK100) were used for immunodetection.

2.7. Experimental groups

1. Control + Vehicle
2. SPS + Vehicle
3. SPS + Fluoxetine (10 mg/kg)
4. SPS + Berberine (50 mg/kg)
5. SPS + Berberine (200 mg/kg)

Fluoxetine was used as a clinically relevant reference comparator on stress-related neurobehavioral change.

The doses of berberine (50 and 200 mg/kg, p.o.) were selected based on prior preclinical studies demonstrating neuroprotective and antidepressant-like effects within this range. Across rodent neurobiological models, effective doses of berberine typically range from 25 to 200 mg/kg, with consistent behavioral and neuroprotective benefits observed from approximately 50 mg/kg upward [34–36]. In ischemic stroke models, doses between 10 and 50 mg/kg have shown dose-dependent neuroprotection, with higher doses producing greater reductions in neurological deficits [34, 37]. Furthermore, meta-analytic evidence from Alzheimer's disease models indicates that although a broad dose range is effective (5–260 mg/kg), optimal behavioral and pathological outcomes are most consistently observed within the 50–100 mg/kg range [36, 38].

Accordingly, 50 mg/kg was selected as a moderate, well-established neuroactive dose, while 200 mg/kg was included to evaluate dose-dependent effects and ensure sufficient central nervous system engagement in the SPS model.

2.8. Behavioral assessments

A blinded experimenter did behavioral testing during the light phase. Animals were habituated to the testing room for ≥30 min prior to testing. Between animals, apparatus surfaces were wiped with 70 percent ethanol.

2.9. Y-maze spontaneous alternation test

The Y-maze was used to test working memory; the three arms of the Y-maze were 40 cm long, 10 cm wide, and 15 cm high and were placed at angles of 120°. Rats were put on the end of one arm and given 8 min to roam freely in the maze. An arm entry was documented once all four paws had entered an arm.

Spontaneous alternation (%) was calculated as:

$$\text{Spontaneous Alternation (\%)} = (\text{Number of Alternations} / (\text{Total Arm Entries} - 2)) \times 100$$

The maze was cleaned between trials to eliminate olfactory cues.

2.10. Morris water maze (MWM)

The spatial learning and memory was tested in a circular pool (180 cm diameter) filled with water (24 ± 1 degC) that was made opaque with non-toxic white paint. The target quadrant had a submerged hidden platform (10 cm diameter) that was placed approximately 1 cm below the surface.

Each day, rats were trained four times and this was repeated on four days. The maximum trial time was 60 s; the animals were allowed 15 s to be on the platform between trials. A probe trial 60 s (platform removed) was performed 24 h after the last training session. The time taken to escape the latency (training), time taken in the target quadrant, platform crossings, and the speed of swimming were captured on EthoVision XT (Noldus).

2.11. Novel object recognition (NOR)

NOR testing was done in an arena of 60 × 60 cm. The sample phase entailed exposure to two identical objects over a period of 5 min. One of the objects was substituted with a new object of a different shape

and texture after a 1 h retention period. The identity of objects and position were balanced between animals and sessions. Exploration was characterized as a nose orientation of within 2 cm of the object.

Discrimination index (%) was calculated as:

$$\text{Discrimination Index (\%)} = 100 \times (\text{T_novel} - \text{T_familiar})/(\text{T_novel} + \text{T_familiar})$$

Where:

- T_{novel} = time spent exploring the novel object
- $T_{familiar}$ = time spent exploring the familiar object

2.12. Sucrose preference test

Anhedonia was measured through a two bottle choice test. Rats were habituated to water and 1% sucrose for 48 h. Rats were exposed to the two bottles after 20 h of food and water deprivation. Percentage preference of sucrose was calculated as intake of sucrose/ total fluid intake x 100. Total fluid intake was also measured to correct the nonspecific consumption effects.

2.13. Tissue collection

Rats were anesthetized with ketamine/xylazine (100/10 mg/kg, i.p.) twenty-four hours following the last behavioral test. Blood was collected between 08:00–10:00 h via cardiac puncture within ≤3 min of cage disturbance to minimize handling-induced corticosterone elevation. Serum was separated and stored at −80 °C.

Animals were perfused transcardially with ice-cold 0.9% saline. Brains were removed and bisected midsagittally; one hemisphere was snap-frozen (−80 °C) for biochemical analyses and the other fixed in 4% paraformaldehyde for histology.

2.14. Sampling and homogenization of brain regions

Dorsal hippocampus (bregma −3.3 to −4.0 mm) and medial prefrontal cortex (bregma +2.7 to +3.2 mm) were dissected on dry ice. Tissue homogenization was done in ice-cold lysis buffer with protease inhibitors and centrifuged at 12,000 g for 15 min at a temperature of 4 °C. Concentrations of proteins were obtained through a BCA assay (Sigma-Aldrich Cat. BCA1).

2.15. Biochemical assays

Cytokines (IL-1b RAB0277, IL-6 RAB0311, TNF-a RAB0480), BDNF (RAB1138), SOD (CS0009), catalase (MAK531), corticosterone (RAB0030), and MDA (MAK085) were measured as per the instructions of the manufacturers. Each of the samples was measured twice and adjusted to total protein.

2.16. Histology and immunohistochemistry

Systematic random sampling was used to collect coronal sections (30 um) (1-in-6). Neuronal density and pyknotic neurons (cells with shrunken soma and hyperchromatic condensed nuclei) were determined by staining with Cresyl Violet. NeuN and GFAP immunohistochemistry was done by use of standardized protocols. All the groups were stained in the same staining runs in all sections.

2.17. Quantitative analysis and image acquisition

Quantitative analysis of histological and immunohistochemical staining was performed using ImageJ software (NIH, U.S.A.). For each animal, three non-overlapping sections per brain region (hippocampus and medial prefrontal cortex) were analyzed, and three representative fields per section were captured at standardized magnification under identical imaging conditions.

For Cresyl Violet staining, neuronal integrity was assessed by counting viable neurons characterized by clear cell bodies, distinct nuclei, and preserved morphology, while pyknotic or shrunken cells were identified and counted separately. NeuN-positive cells were quantified as a measure of neuronal density using threshold-based segmentation to identify positively stained nuclei, and results were expressed as the number of positive cells per field.

GFAP immunoreactivity was quantified as percentage area fraction using thresholding techniques, with consistent threshold settings applied across all images to ensure comparability.

The percentage of pyknotic neurons was calculated using the formula:

$$\%\text{Pyknotic neurons} = (\text{Number of pyknotic neurons} / \text{Total number of neurons counted}) \times 100$$

where the total number of neurons represents the sum of viable and pyknotic cells within the defined field.

All image acquisition and analyses were performed by investigators blinded to group allocation.

2.18. Statistical analysis

Data are expressed as mean ± SEM. Normality was assessed using Shapiro–Wilk tests. Repeated-measures ANOVA was used for MWM training and longitudinal behavioral measures. Biochemical and histological endpoints were analyzed by one- way ANOVA followed by the post hoc test by Tukey. The statistical significance was determined as $p < 0.05$. GraphPad Prism 9.5 was used to conduct analyses.

3. Results

3.1. Statistical overflow

Unless otherwise indicated, group differences were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test for single-timepoint biochemical/histological outcomes (Figure 2 and Figures 4–8). The results of longitudinal behavioral measures (Figure 3) were done using two-way repeated-measures ANOVA (factors: group x time) and then Tukey post hoc. The statistical significance was determined at $p < 0.05$ and the corresponding n values are presented in the legends of the respective figures.

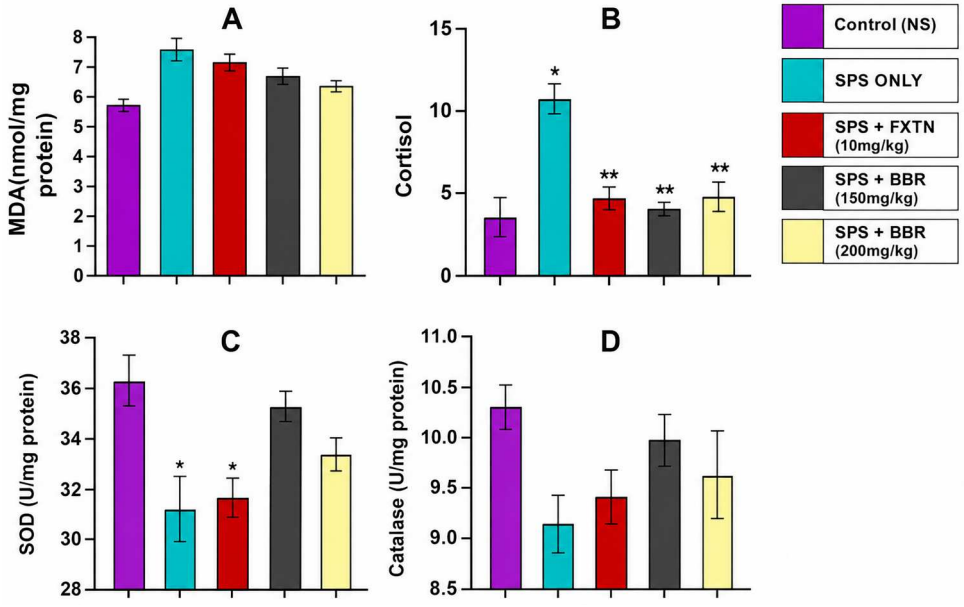


Figure 2. Effects of berberine on stress-induced oxidative imbalance following single prolonged stress (SPS). (A) Malondialdehyde (MDA) levels, an index of lipid peroxidation. (B) Serum corticosterone levels measured at basal morning time points. (C) Superoxide dismutase (SOD) activity. (D) Catalase activity.

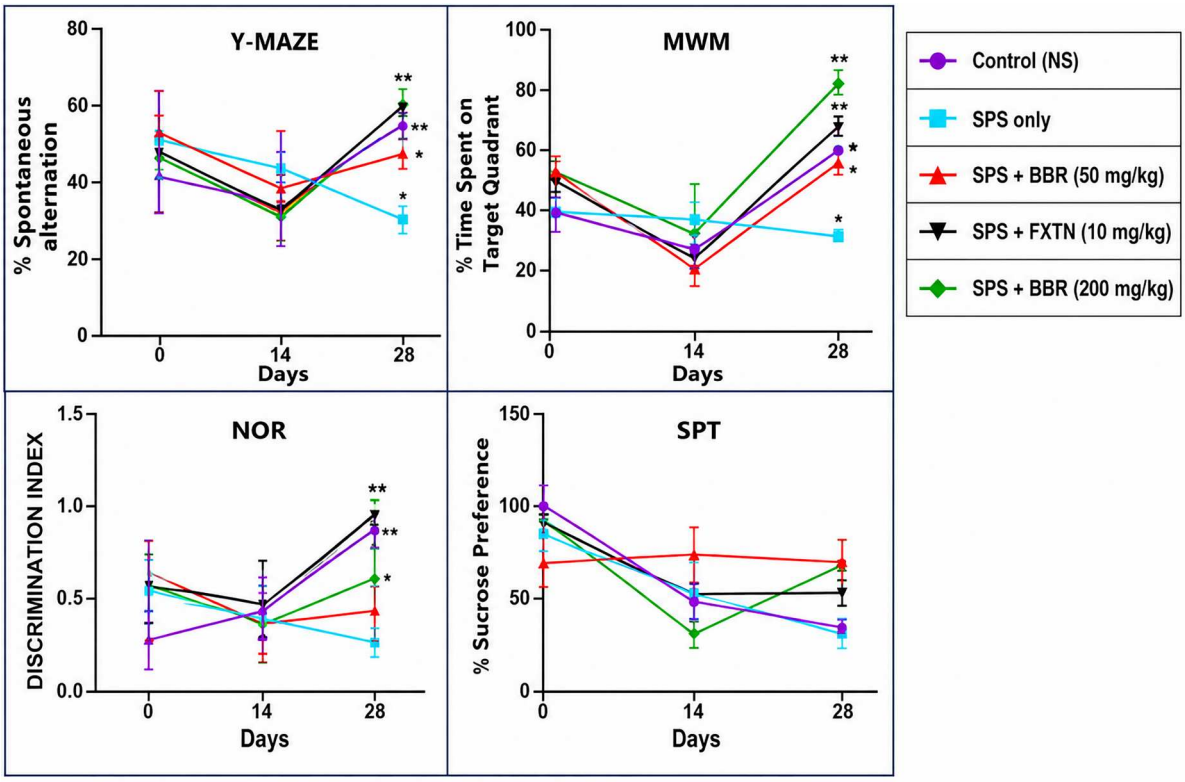


Figure 3. Effects of berberine on SPS-induced neurobehavioral deficits. (A) Y-maze spontaneous alternation (%SA) assessed longitudinally at baseline, post-stress, and post-treatment. SPS reduced spontaneous alternation, indicating impaired working memory, while berberine (50 and 200 mg/kg) improved alternation rates, with greater efficacy at 200 mg/kg. (B) Morris water maze (MWM) performance expressed as percentage time spent in the target quadrant (memory retention) across time points. SPS decreased target-quadrant preference, whereas berberine restored retention toward control levels. (C) Novel object recognition (NOR) discrimination index measured at baseline, post-stress, and post-treatment. SPS blunted novel-object preference, while berberine improved recognition memory, with the higher dose showing superior recovery. (D) Sucrose preference test (SPT) (% sucrose preference) assessed longitudinally as an index of anhedonia. SPS produced a sustained reduction in sucrose preference, which was attenuated by berberine in a dose-dependent manner. Fluoxetine (FXTN, 10 mg/kg) served as the reference comparator. Data are presented as mean ± SEM ($n = 5$ rats per group). Group labels: Control (NS), SPS only, SPS + FXTN (10 mg/kg), SPS + BBR (50 mg/kg), SPS + BBR (200 mg/kg). Statistical analysis: two-way repeated-measures ANOVA (factors: group × time) followed by Tukey's post hoc test. *$P < 0.05$ vs Control (NS); *$P < 0.05$ vs SPS only; #$P < 0.05$ vs SPS + FXTN; α$P < 0.05$ vs SPS + BBR (50 mg/kg); β$P < 0.05$ vs SPS + BBR (200 mg/kg).

3.2. Stress induction and dysregulation of the HPA-axis

There was a significant group difference in basal morning serum corticosterone (one-way ANOVA, $p < 0.05$; Figure 2(B)). Relative to the control (NS) group, SPS exposure created a significant increase in corticosterone, which validates chronic dysregulation of the HPA-axis. Post hoc analysis revealed that the treatment of berberine significantly lowered the SPS-induced corticosterone increase, with the highest dose (200 mg/kg) generating the greatest normalization in comparison with SPS-only animals; fluoxetine also reduced corticosterone in comparison with SPS-only (Figure 2(B)).

3.3. Berberine attenuates SPS-induced oxidative imbalance in whole-brain homogenate

There were significant differences in the measurements of oxidative stress indices in whole-brain homogenate (one-way ANOVA, $p < 0.05$, MDA, SOD, catalase and Figure 2(A, C, D)). The lipid peroxidation levels were greatly enhanced by SPS as indicated by a high level of MDA compared to controls (Figure 2(A)). The effect of berberine on the reduction of MDA was significant relative to the SPS-only animals with a dose-responsive effect (greater reduction at 200 mg/kg) and fluoxetine had a partial effect (Figure 2(A)).

SPS also interfered with endogenous antioxidant defenses, and SOD and catalase activities were lower than controls (Figure 2(C–D)). Tukey post hoc comparisons showed that berberine significantly restored

SOD activity compared to SPS-only with higher restoration at 200 mg/kg (Figure 2(C)). Catalase had a recovery pattern with treatment (Figure 2(D)), and the difference between the groups was indicated to be significant by the overall ANOVA model and the post hoc trends as shown by the figure.

3.4. Berberine improves SPS-induced cognitive and affective dysfunction

There was a difference in behavioral performance with time and treatment conditions (two-way repeated-measures ANOVA, group x time interaction $p < 0.05$ with post hoc tests used to point out the between-group differences at the appropriate timepoints).

3.4.1. Working memory (Y-maze spontaneous alternation)

At the post-stress time point, SPS decreased spontaneous alternation performance compared to baseline (Figure 3(A)). Post hoc analysis after treatment showed that berberine caused significant spontaneous alternation improvement over SPS-only animals at the post-treatment evaluation, with BBR200 having the greatest recovery; fluoxetine too improved alternation relative to SPS-only but to a smaller degree than high-dose berberine (Figure 3(A)).

3.4.2. Spatial memory retention

The outcome of MWM varied between groups over time (two-way repeated-measures ANOVA, $p < 0.05$; Figure 3(B)). SPS decreased memory retention (reduced target- quadrant preference through the post-stress/ post-treatment trajectory). Berberine enhanced the retention compared to SPS-only, where BBR200 generated the most significant improvement at the post-treatment time; fluoxetine partially enhanced the improvement compared to SPS-only (Figure 3(B)).

3.4.3. Recognition memory (novel object recognition)

NOR discrimination index was significantly influenced by group and time (two-way repeated-measures ANOVA, $p < 0.05$; Figure 3(C)). At the post-stress test, SPS reduced novelty preference when compared to controls. Post hoc analysis revealed that berberine had a significant improvement in the discrimination index compared to SPS-only at post-treatment, and more so with an increased level of improvement at 200 mg/kg, whereas fluoxetine had a lesser recovery pattern (Figure 3(C)).

3.4.4. Anhedonia (sucrose preference test)

Sucrose preference displayed a significant group × time effect (two-way repeated-measures ANOVA, $p < 0.05$; Figure 3(D)). SPS induced a sustained decrease in sucrose preference across time relative to controls. Post hoc analysis revealed that berberine was not only significantly reducing anhedonia induced by SPS, but BBR200 gave the best restoration to the levels of control at the later timepoint; fluoxetine also enhanced the preference of sucrose in comparison to SPS-alone (Figure 3(D)). The lack of significant group variation in total fluid intake (as described) is a factor that promotes an anhedonia-specific response and not a non-specific drinks response.

3.5. Berberine suppresses SPS-induced pro-inflammatory cytokine elevation

Brain pro-inflammatory cytokines were significantly different between groups (one-way ANOVA, $p < 0.05$ for IL-1 beta, TNF-alpha and IL-6; Figure 4(A–C)). SPS was found to significantly enhance IL-1 beta and IL-6 compared to controls (Figure 4(A, C)), with a similar elevation pattern for TNF-alpha plotted (Figure 4(B)) and associated with SPS. Post hoc comparisons showed that berberine suppressed SPS-associated increases in cytokines, with the direction and significance of individual pairwise comparisons (vs control and vs SPS-only) indicated by the statistical markers that are shown in Figure 4. Fluoxetine resulted in partial cytokine normalization consistent with the group means plotted (Figure 4(A–C)).

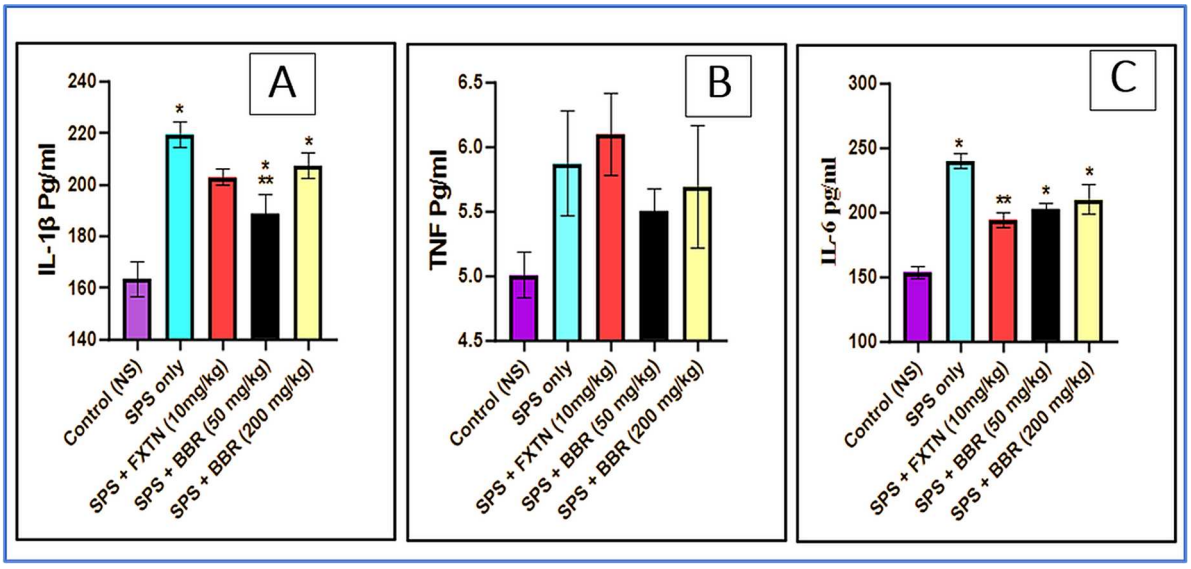


Figure 4. Effects of berberine on SPS-induced pro-inflammatory cytokine levels. Brain concentrations of IL-1β, TNF-α, and IL-6 in control and SPS-exposed rats treated with fluoxetine or berberine. Data represent mean ± SEM ($n = 5$). *$P < 0.05$ vs control; **$P < 0.01$ one-way ANOVA with Tukey post hoc test.

3.6. Berberine normalizes corticolimbic BDNF immunoreactivity

BDNF-positive cell counts were also found to be significantly different in each group in both regions (one-way ANOVA, $p < 0.05$; Figure 5(A-f) and Figure 5(B-f)). SPS decreased immunoreactivity of BDNF in the hippocampus (CA3) and prefrontal cortex in terms of decreasing the number of BDNF-positive neurons

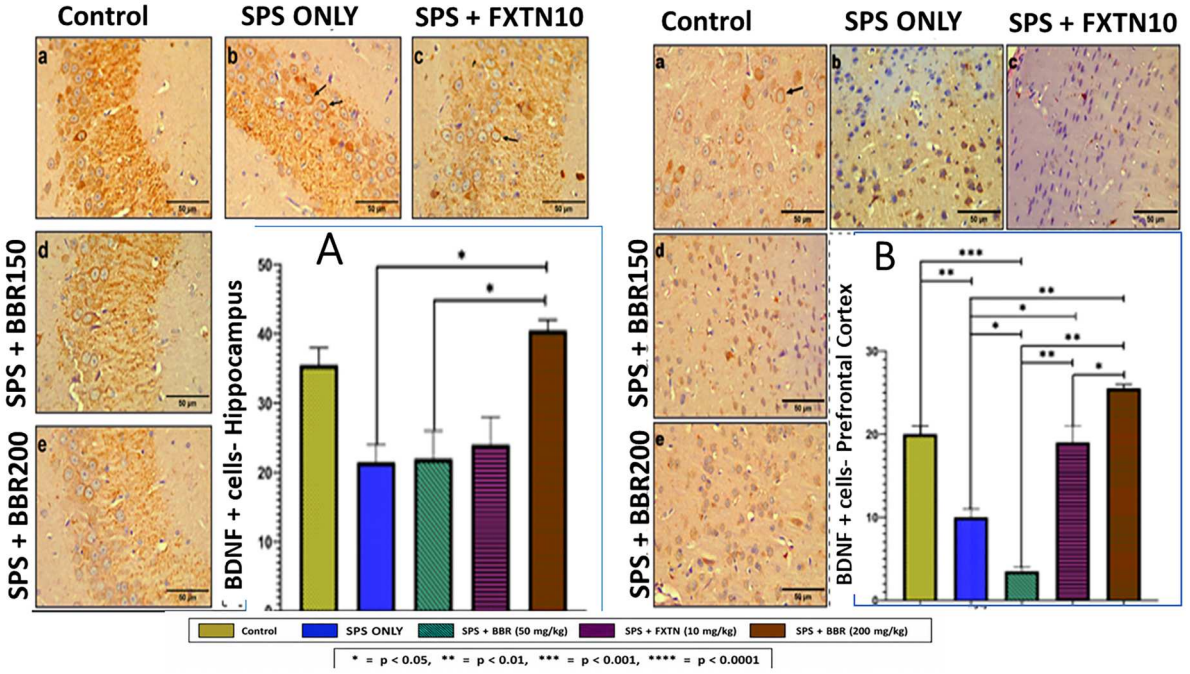


Figure 5. Berberine restores BDNF immunoreactivity in corticolimbic regions following single-prolonged stress (SPS). (A) Hippocampus (CA3). Representative photomicrographs of BDNF immunohistochemistry in (a) Control, (b) SPS only, (c) SPS + fluoxetine (FXTN10; 10 mg/kg), (d) SPS + berberine (BBR50; 50 mg/kg), and (e) SPS + berberine (BBR200; 200 mg/kg). SPS reduced BDNF immunoreactivity, evidenced by fewer BDNF-positive neurons and weaker staining intensity (arrows). Berberine increased BDNF expression in a dose-dependent manner, with BBR200 showing the greatest restoration toward control. (f) Quantification of BDNF-positive cells in the hippocampus.

and intensity of staining in the sections from SPS-only (Figure 5(A-b); Figure 5(B-b)). Post hoc testing revealed that berberine significantly enhanced the density of BDNF-positive cells as compared to SPS only in a dose-dependent manner with BBR200 resulting in the greatest restoration toward control levels; fluoxetine also increased the density of BDNF as compared to SPS only (Figure 5(A-f); Figure 5(B-f)).

3.7. Berberine preserves neuronal survival and integrity (NeuN immunohistochemistry)

NeuN-positive neuronal density was significantly different between groups in both the hippocampus and PFC (one-way ANOVA, $p < 0.05$; Figure 6(A-f); Figure 6(B-f)). SPS significantly decreased the number of NeuN-positive neurons compared to controls, which is consistent with stress-associated neuronal loss or decreased neuronal marker expression (Figure 6(A-b); Figure 6(B)-SPS). Post hoc analyses revealed that berberine significantly improved NeuN-positive cell counts compared to SPS-only, with BBR200 showing the most preservation, and fluoxetine showing intermediate recovery (Figure 6(A-f); Figure 6(B-f)).

3.8. Berberine maintains corticolimbic cytoarchitecture on Cresyl Fast Violet staining

Cresyl Fast Violet evaluation showed significant SPS-associated cytoarchitectural disruption of the hippocampus CA3 and PFC, including low neuronal packing and high pyknotic neurons (Figure 7(A,B)). Quantitative indices of the hippocampus were significantly different between groups (one-way ANOVA, $p < 0.05$; Figure 7(F-G)). SPS decreased neuronal/cell density and increased the pyknotic

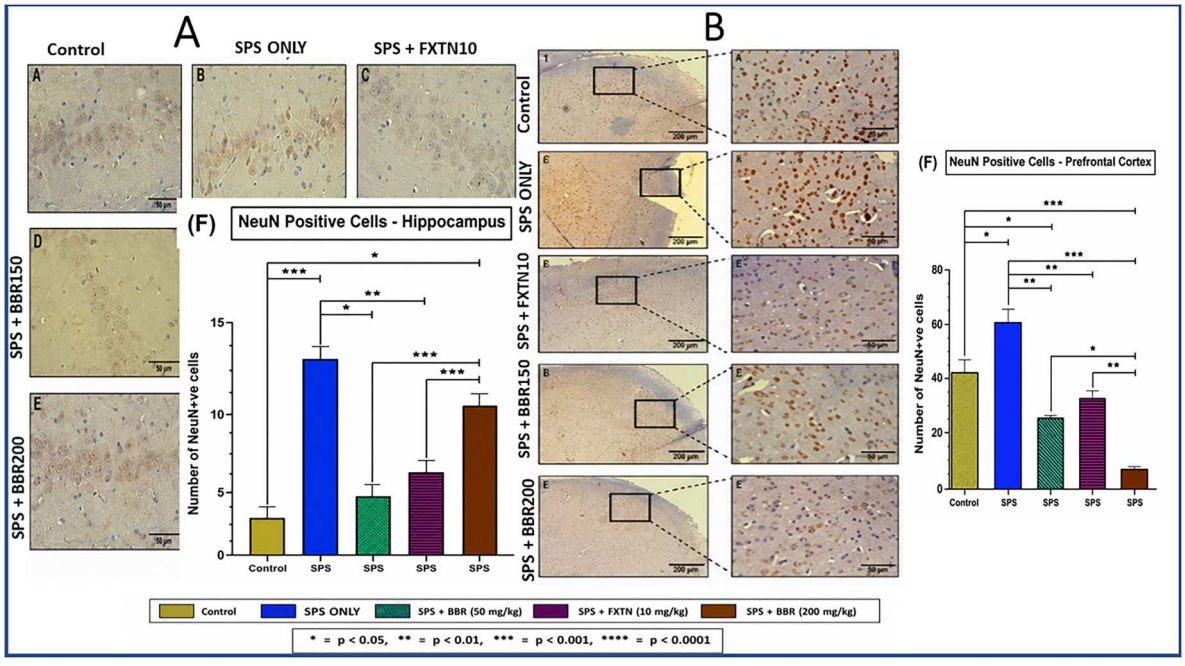


Figure 6. Berberine preserves neuronal survival in the hippocampus and prefrontal cortex following single-prolonged stress (SPS). (A) Hippocampus. Representative photomicrographs showing NeuN immunohistochemical staining in the hippocampus of (a) Control, (b) SPS-only, (c) SPS + fluoxetine (FXTN10; 10 mg/kg), (d) SPS + berberine (BBR50; 50 mg/kg), and (e) SPS + berberine (BBR200; 200 mg/kg)–treated rats. SPS markedly reduced the number of NeuN-positive neurons, indicating stress-induced neuronal loss. Berberine treatment dose-dependently increased NeuN immunoreactivity, with the high dose (200 mg/kg) providing substantial preservation of neuronal integrity. (f) Quantitative analysis of NeuN-positive cells in the hippocampus. (B) Prefrontal cortex (PFC). Representative low- and high-magnification photomicrographs of NeuN immunostaining in the PFC across the same experimental groups. SPS caused a pronounced reduction in NeuN-positive neuronal density, whereas berberine significantly restored neuronal survival in a dose-dependent manner, with greater efficacy observed at 200 mg/kg. (f) Quantification of NeuN-positive cells in the PFC. Data are expressed as mean ± SEM (n = 5 animals per group). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test; significance levels are indicated on the graphs (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; comparisons as indicated). Scale bar = 50 µm.

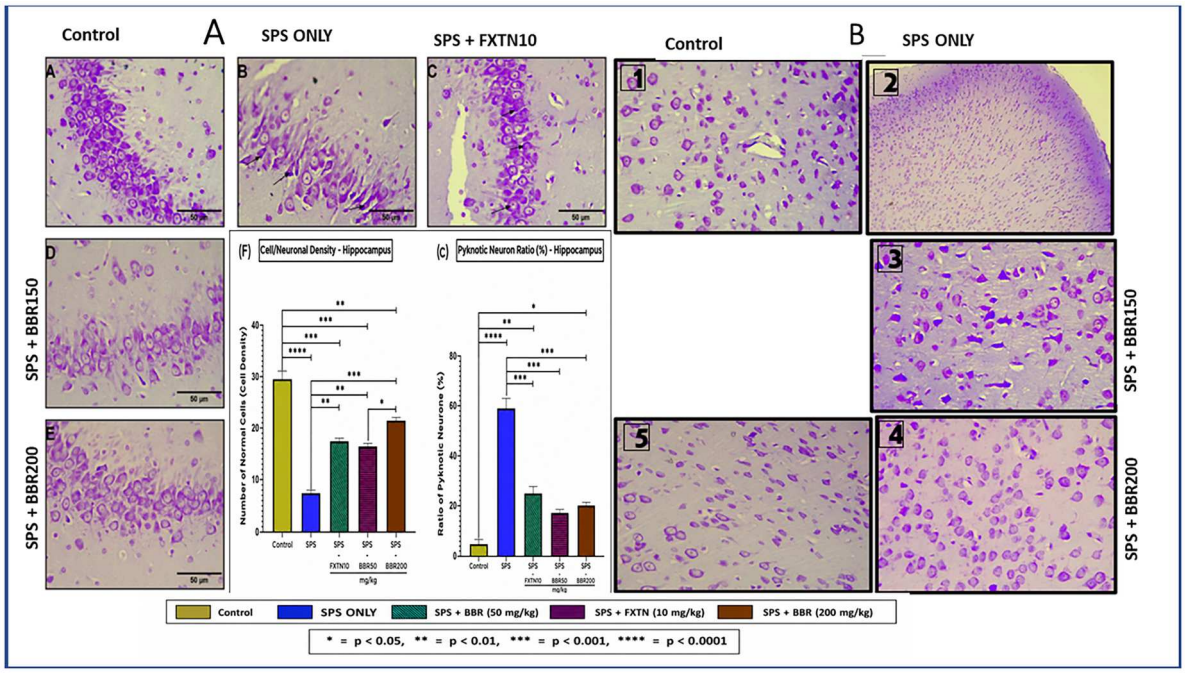


Figure 7. Berberine preserves corticolimbic cytoarchitecture following single prolonged stress (SPS) as assessed by Cresyl Fast Violet (CFV) staining. (A) Hippocampus (CA3). Representative CFV-stained hippocampal sections from Control, SPS only, SPS + fluoxetine (FXTN10; 10 mg/kg), SPS + berberine (BBR50; 50 mg/kg), and SPS + berberine (BBR200; 200 mg/kg). SPS produced marked disruption of hippocampal cytoarchitecture, characterized by reduced neuronal packing density and increased numbers of darkly stained, shrunken pyknotic neurons (arrows). Fluoxetine and berberine mitigated these degenerative features, with high-dose berberine showing the strongest preservation of neuronal morphology. (F–G) Quantification of hippocampal neuronal/cell density (F) and pyknotic neuron ratio (%) (G). Data are mean ± SEM ($n = 5$). One-way ANOVA followed by Tukey's post hoc test. Significance is indicated on the graph (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$). Scale bar = 50 µm. (B) Prefrontal cortex (PFC). Representative CFV-stained prefrontal cortical sections from the same experimental groups (Control, SPS only, SPS + FXTN10, SPS + BBR50, SPS + BBR200). Control sections show preserved cortical architecture with neurons displaying normal Nissl substance and organized cellular distribution. SPS exposure induced conspicuous neuronal injury, including disorganization of cortical cytoarchitecture, reduced neuronal packing, and frequent pyknotic neurons. Fluoxetine partially attenuated these alterations, whereas berberine produced a dose-dependent preservation of neuronal integrity, with BBR200 yielding near-normal cytoarchitecture. Scale bar = 50 µm.

neuron ratio compared to controls. Post hoc testing suggested that berberine significantly improved the density of neurons/cells and decreased the ratio of pyknotic neurons compared to SPS-only, with the highest preservation at 200 mg/kg, and fluoxetine partially reversed the changes associated with SPS (Figure 7(F–G)). In the PFC, representative sections also exhibit a decreased amount of cytoarchitectural disruption and decreased pyknotic profiles with berberine treatment, which is most apparent at the higher dose (Figure 7(B)).

3.9. Berberine suppresses SPS activation of astrocytes (GFAP)

GFAP immunoreactivity was significantly different between groups in the hippocampus and PFC (one-way analysis of variance, $p < 0.05$; Figure 8(A-f); Figure 8(B-f)). SPS caused increased GFAP-positive astrocytes and astrocytic hypertrophy compared with controls consistent with reactive astrogliosis (Figure 8(A-b); Figure 8(B-b)). Post hoc testing revealed that berberine significantly decreased GFAP immunoreactivity compared to that of SPS-only, with greater attenuation of GFAP immunoreactivity with berberine at 200 mg/kg; fluoxetine also decreased GFAP immunoreactivity compared with that of SPS-only, although fluoxetine did not normalize GFAP immunoreactivity as consistently as high-dose berberine (Figure 8(A-f); Figure 8(B-f)).

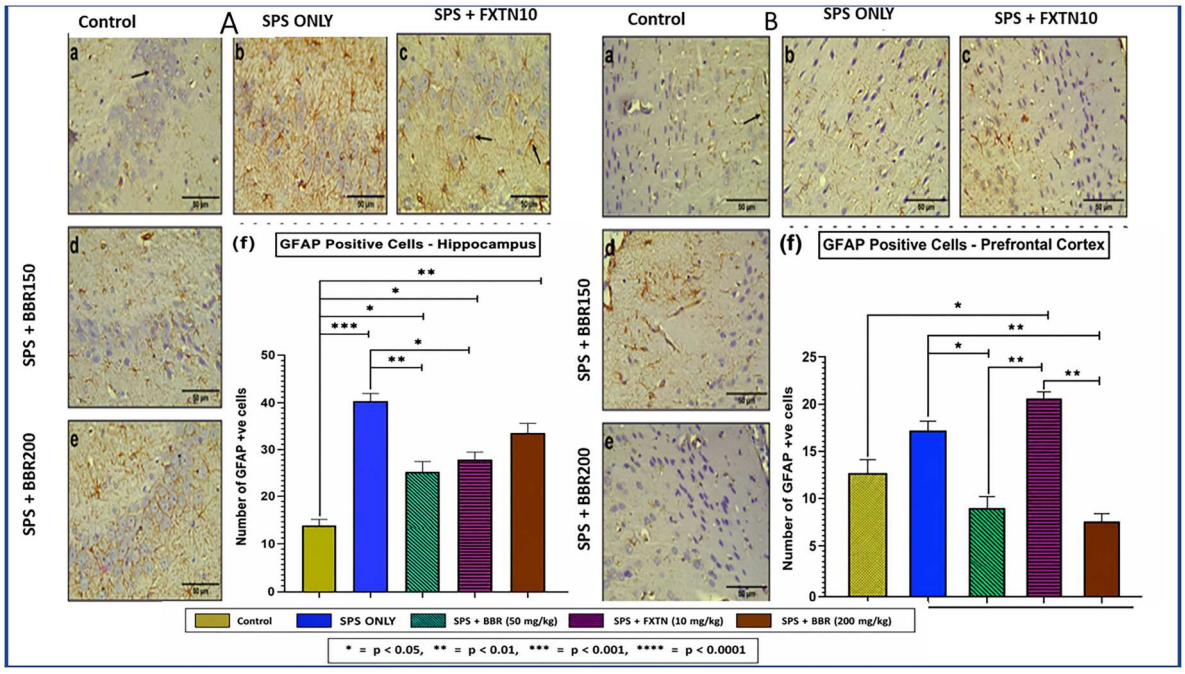


Figure 8. Effects of berberine on SPS-induced astrocytic activation in the hippocampus and prefrontal cortex. (A) Hippocampus. Representative photomicrographs showing GFAP immunohistochemical staining in the hippocampus of (a) Control, (b) SPS-only, (c) SPS + fluoxetine (FXTN10; 10 mg/kg), (d) SPS + berberine (BBR50; 50 mg/kg), and (e) SPS + berberine (BBR200; 200 mg/kg)–treated rats. SPS exposure markedly increased GFAP immunoreactivity, characterized by a higher number of GFAP-positive astrocytes and hypertrophic astrocytic processes (arrows), indicating reactive astrogliosis. Berberine treatment attenuated SPS-induced astrocytic activation in a dose-dependent manner, with the high dose (200 mg/kg) showing greater suppression of GFAP expression. (f) Quantification of GFAP-positive cells in the hippocampus. (B) Prefrontal cortex (PFC). Representative photomicrographs of GFAP immunostaining in the PFC across the same experimental groups. SPS induced a pronounced increase in GFAP-positive astrocytes and astrocytic hypertrophy, whereas berberine significantly reduced GFAP immunoreactivity, with more robust effects observed at 200 mg/kg. (f) Quantitative analysis of GFAP-positive cells in the PFC. Data are expressed as mean ± SEM ($n = 5$ animals per group). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test; significance levels are indicated on the graphs (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; comparisons as indicated). Scale bar = 50 µm.

4. Discussion

This study demonstrates that oral berberine reverses neurobehavioral and corticolimbic neuropathology induced by SPS on a convergent profile including (i) partial recovery of basal HPA-axis output (serum corticosterone), (ii) reverse of affective–cognitive impairment, (iii) recovery of antioxidant defenses with less lipid peroxidation, (iv) inhibition of pro-inflammatory cytokine signaling, (v) recovery of BDNF-dependent neurotrophic support, and (vi) attenuation of neuronal injury and reactive astrogliosis within hippocampal–prefrontal circuitry.

4.1. SPS generates a chronic stress response and berberine suppresses the basal HPA-axis pathology

Single prolonged stress is a well-tested paradigm of traumatic stress, which replicates the behavioral and neurobiological adaptations of the key characteristics of PTSD, such as sustained changes in stress physiology and corticolimbic functioning [7]. In the current experiment, the SPS amplified basal morning serum corticosterone, which is in line with the continuation of neuroendocrine stress load and not a fleeting effect. Berberine dose dependently lowered corticosterone, which is comparable to the comparator effect of fluoxetine. This effect of glucocorticoids on the endocrine system is biologically significant, as chronic glucocorticoid signals can facilitate hippocampal/PFC synaptic dysfunction and intellectual impairment [39, 40]. This trend confirms the fact that the action of berberine is not limited to downstream anti-oxidant/anti-inflammatory effects, but can also involve upstream stress-axis regulation.

4.2. Berberine improves reward-related behavior and cognitive performance after SPS

SPS produced a sustained reduction in sucrose preference, supporting an anhedonia-like phenotype that is widely used as a translational proxy of reward-system dysfunction in stress-linked disorders [28]. This decrease was dose-dependently reversed by berberine, and reinforced the conclusion of enhanced reward sensitivity and not the general effect of fluid intake (observing that fluid intake was regulated in the Results narrative). These results are consistent with previous evidence of antidepressant-like effects of berberine in rodent models including evidence of monoaminergic and NO-cGMP signaling [22, 24, 41, 42].

At the cognitive level, SPS disrupted working memory (Y-maze spontaneous alternation), spatial memory retention (MWM target quadrant time), and recognition memory (NOR discrimination index). This trend is in line with the established susceptibility of hippocampal-prefrontal networks to traumatic/chronic exposure to stress [12, 43, 44]. Berberine enhanced activity in these areas, which is evidence of an extensive functional rescue as opposed to a task artifact. Previous literature also gives support to the benefits of berberine on cognitive enhancement in the context of oxidative/inflammatory brain injury [23, 45, 46], which aligns with the multi-domain recovery of behaviors in this case.

4.3. Restoration of redox homeostasis: berberine strengthens antioxidant defenses and reduces lipid peroxidation

Oxidative imbalance is a key mediator of the stress-induced neuronal susceptibility, which connects mitochondrial stress, lipid peroxidation, and subsequent neuroimmune response [18, 19, 20]. In this case, SPS reduced antioxidant enzyme activities (SOD, catalase) and raised MDA, which is an indication of weakened endogenous protective mechanisms and accelerated membrane oxidative damage. These alterations (defenses, damage) were reversed by Berberine and indicate a consistent antioxidant profile. This is compatible with evidence that under conditions of hippocampal injury, berberine can alleviate local oxidative stress and astrogliosis [47] and help to interpret the results as indicating that redox restoration would be a possible mechanistic contributor to the observed neuroprotective effect.

4.4. Berberine suppresses cytokine signaling and reduces glial activation, consistent with anti-neuroinflammatory actions

SPS elevated IL-1β, IL-6, and TNF-α, which are in line with stress-induced neuroimmune activation and cytokine-related plasticity and cognitive impairment [14, 48, 49]. Berberine inhibited these increases in cytokines in both corticolimbic regions, or showed central anti-inflammatory effects. This is in line with past research that suggests that berberine reduces neuroinflammation and improves behavioral responses in animals exposed to stress [24] and is in line with the existence of inflammasome-related regulation mechanisms in stressed models [25].

Simultaneously, SPS enhanced the GFAP immunoreactivity of the hippocampus and PFC, which not only validates reactive astrogliosis as an injury phenotype, but also promotes it. Berberine decreased the activation of GFAP and indicated that the decrease in cytokine levels was associated with a decrease in glial reactivity. As astrocytic activation may exert an exacerbating effect on the imbalance of oxidative stress and synaptic homeostasis, a reduction in astrogliosis offers the consistent cellular counterpart to functional recovery [19, 20, 50].

4.5. Berberine restores BDNF-dependent neurotrophic support and links molecular recovery to behavioral rescue

BDNF is a major modulator of synaptic plasticity, learning, and corticolimbic resilience that is often impaired by chronic exposure to stress with cognitional and affective downstream effects [12]. The SPS decreased the BDNF immunoreactivity in the hippocampus and PFC and berberine reinstated the BDNF to normal control levels. This recovery correlates well with the recoveries in MWM/NOR/Y-maze performance and sucrose preference. Mechanistically, berberine has been linked to BDNF restoration and

neuroinflammatory suppression in stress paradigms [24, 25], reinforcing the plausibility that trophic recovery is not merely epiphenomenal but part of an integrated therapeutic response.

4.6. Histological evidence supports true neuroprotection: preserved cytoarchitecture and neuronal markers

A key advantage of this data set is that of convergence between functional recovery and structural preservation. SPS induced neuronal degeneration on Cresyl Fast Violet staining (decreased neuronal/cell density and increased ratio of pyknotic neurons) in the hippocampus and PFC, associated with decreased neuronal density of NeuN-positive neurons. Berberine maintained cytoarchitecture, decreased pyknotic profiles and restored NeuN-positive cell density in both areas. This trend promotes actual neuroprotection and not merely symptomatic behavioral change. Blending of (i) decrease of oxidative damage, (ii) decrease of cytokine load, (iii) normalization of the trophic support and (iv) maintenance of neuronal integrity enhances the biological plausibility of the involvement of a multi-node protecting process. This combination of reduced oxidative damage, lower cytokine burden, normalized trophic support, and preserved neuronal integrity is highly consistent with a multi-target neuroprotective mechanism [51, 52].

4.7. Integrated interpretation and translational relevance

Taken together, the results are compatible with an interactive model where SPS causes a sustained stress response (↑ corticosterone) that facilitates redox failure (↓ SOD/catalase; ↑ MDA) and cytokine release (↑ IL-1b/IL-6/TNF-a) that results in reactive astrogliosis (↑ GFAP), loss of trophic support (↓BDNF), and structural neuronal loss in hippocampal-prefrontal circuitry (↓ NeuN; ↑ pyknotic neurons). Berberine breaks this cascade on several nodes - endocrine, oxidative, inflammatory, trophic and structural ones, resulting in quantifiable improvement in cognition and reward response. The multi-target profile is in line with general pharmacological evidence of berberine on mood- and stress-related neurobiology [22, 34, 25]. While these findings collectively suggest involvement of HPA-axis regulation, redox balance, inflammatory signaling, and neurotrophic support, the present study demonstrates associative relationships, and direct causal mechanisms cannot be conclusively established within this experimental design. Given the limitations of existing therapies for subsets of stress-related and treatment-resistant presentations [53, 54], these data provide support for the further development of berberine as an adjunct or lead compound for stress-linked cognitive/affective dysfunction.

4.8. Limitations and future directions

1. Mechanistic necessity vs association: The study shows convergent pathway normalization but does not show whether the restoration of the brain-derived neurotrophic factor (BDN) or the reduction of cytokines is critical for behavioral recovery. Pathway-interference designs (e.g. TrkB antagonism; inflammasome/cytotokine pathway blockage) can be added to the future work to test causality [12].
2. Characterization of the HPA-axis: Only the basal morning corticosterone was measured; stress-reactivity problems and diurnal profiling might improve the assessment of HPA dynamics in SPS [40].
3. Neuroimmune depth: our study focused on GFAP which supports astrocyte activation, but microglial markers (e.g. Iba1) and synaptic proteins (e.g. PSD95, synaptophysin) would better resolve the neuroimmune–synaptic interface.
4. Sex as a biological variable: This study was based on male rats only; sex-dependent stress biology is well-known, and follow-up cohorts must be supplemented with this variable to make the literature translationally complete.

5. Conclusion

Berberine improved cognitive and affective dysfunction induced by SPS and preserved neuronal integrity in the corticolimbic areas. These effects were balanced with partial normalization of basal corticosterone,

reinstatement of antioxidant defenses with less lipid peroxidation, suppression of cytokine upsurge, restoration of BDNF and suppression of astrogliosis. These findings indicate that berberine attenuates SPS-induced behavioral and neurobiological alterations and is associated with modulation of HPA-axis activity, oxidative stress, inflammatory signaling, and BDNF expression. These pathways represent potential mechanisms underlying its neuroprotective effects rather than definitive causal pathways. The results of the study can be used together to justify a multi-target neuroprotective agent, berberine, in stress-related neurotoxicity and cognitive dysfunction.

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Data availability statement

Data are available upon reasonable request. All data generated or analyzed during this study are included in this article.

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