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Research article

The preventive effects of *Saccharomyces boulardii* against oxidative stress induced by lipopolysaccharide in rat brain

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ABSTRACT

The brain is sensitive to oxidative stress, which can trigger microglial activation and neuroinflammation. Antioxidant therapies may provide neuroprotection against oxidative stress. In recent years antioxidant effects of probiotics and their possible mechanisms in oxidative stress-related models have been determined. In the current study, for the first time, we assessed the effects of *Saccharomyces boulardii* on oxidative stress provoked by lipopolysaccharide (LPS) in the rat brain. Four groups of animals were used, including the control, LPS, *S. boulardii* + LPS, and *S. boulardii* groups. All animals received either saline or *S. boulardii* (10^{10} CFU) by gavage for four weeks. Between days 14 and 22, all animals received either LPS (250 μg/kg) or saline by intraperitoneal (i.p.) injection. *S. boulardii* was able to inhibit lipid peroxidation and prevent the reduction of antioxidant levels, including glutathione and catalase in the model of oxidative stress induced by LPS in the rat hippocampus and cortex. Also, it increased the lowered ratio of glutathione/oxidized glutathione in both tissues. Serum levels of anti-inflammatory interleukin 10 (IL-10) and proinflammatory cytokines IL-6 and IL-8 increased and decreased, respectively. *S. boulardii* has potential antioxidant activities in oxidative stress-related model, possibly modulating gut microbiota, immune defense, and antioxidant enzyme activities that can be considered in preventing oxidative stress-related central nervous system (CNS) diseases.

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1. Introduction

Oxidative stress is marked by an inequity between reactive oxygen species (ROS) and the antioxidant defense system, stemming from either elevated ROS levels or a diminished antioxidant response [1,2]. Biological systems generate ROS as aerobic metabolic by-products, including hydrogen peroxide (H_2O_2), superoxide (O_2^-), hydroxyl radicals (OH•), and singlet oxygen ($1O_2$) [3,4]. There are two sources for the generation of cellular ROS: mitochondrial oxidative phosphorylation or cellular response to xenobiotics, cytokines, and bacterial invasion [2]. In this way, the released ROS cause lipid peroxidation under stress conditions resulting in the formation of products (e.g., malondialdehyde [MDA] and downregulation of antioxidant levels (e.g., glutathione [GSH]) [1,5]. The presence of ROS can lead to oxidative damage in cellular components [4].

The link between oxidative stress and several neurological diseases, including Parkinson's disease (PD), Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), depression, and memory loss, has been well established [6,7].

In this regard, lipopolysaccharide (LPS)-induced oxidative stress and mitochondrial dysfunction cause cell and tissue damage that can be considered the significant manifestations of acute and chronic inflammatory diseases [8] leading to ROS, nitric oxide (NO), cytokines, and chemokines generation [8–10]. LPS modulates inflammatory response through the secretion of the proinflammatory cytokines in the circulation. Then systemic inflammation can damage the blood-brain barrier (BBB) by several mechanisms including decreasing its integrity, increasing the permeability, and reducing the tight junction protein expression. Neuroinflammation and oxidative stress may be the next steps which are the most important molecular events in some neurodegenerative diseases, memory dysfunction, and mood disorders [11,12]. A single dose or chronic administration of LPS triggers the release of proinflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukine-6 (IL-6), and IL-1β in the brain [13–15], and oxidative stress [16]. Therefore, the LPS animal model is used as a suitable model for investigating neuroinflammation and neurodegeneration. Our previous study also showed neuroinflammation, memory impairment, and amyloid-beta deposition in the hippocampus of a LPS rat model [17].

In recent years, the antioxidant properties of probiotics and their potential mechanisms in models related to oxidative stress have been a topic of discussion [18–21]. The microorganism probiotics groups that are used include bacteria LAB (lactic acid bacteria), *Lactobacillus, and Bifidobacterium* which produce lactic acid, and the yeast *Saccharomyces boulardii* [22].

Numerous investigations have provided evidence of the protective properties of *S. boulardii* in diverse gastrointestinal disorders characterized by inflammation, such as inflammatory bowel diseases (IBD), Crohn's disease (CD), ulcerative colitis (UC), mucositis, intestinal infections, intestinal, lung, and hepatic injuries, and diabetic model [23–30]. *S. boulardii* is used worldwide to prevent and treat infectious diarrhea of various etiologies. It appears that *S. boulardii* may suppress the generation of proinflammatory cytokines or enhance anti-inflammatory mediators [31]. The principal aim of this study was to investigate, for the first time, the influence of *S. boulardii* on oxidative stress induced by LPS in the rat brain. Furthermore, serum anti-inflammatory and proinflammatory cytokines levels were examined.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (with weights ranging from 200 to 230 g) were procured from the Neuroscience Research Center and were randomly accommodated under standard conditions:

Rats were housed in standard polypropylene cages measuring 580 mm × 38 mm × 200 mm. Bedding consisting of wood shavings was provided, and the rats were given access to standard pellets and water ad libitum. The room was maintained at a constant temperature of 21 ± 2 °C, with a 12-h light/dark cycle. Environmental enrichment was provided in the form of nesting polyvinyl chloride pipes. All experiments were approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (Approval No. IR.SBMU.PHNS.REC.1398.129) and adhered to the guidelines set forth by the Animal Research: Reporting of In Vivo Experiments (ARRIVE).

2.2. Drugs

S. boulardii was obtained from Zist Takhmir Company (Iran). The DAILYEAST® capsule contains 10^{10} colony-forming units [CFU]). Ketamine and sedaxyl® were sourced from Bremer Pharma GMBH (Warburg, Germany) and KELA (Hoogstraten, Belgium), respectively. *S. boulardii* and LPS (Sigma, USA) were dissolved separately in saline and phosphate-buffered saline.

2.3. Experimental procedure

Animals were randomly allocated into four groups of eight animals. The control (Con) group was given saline (10 ml/kg) by gavage for four weeks and saline (1 ml/kg) by intraperitoneal (i.p.) injection for nine days (between days 14 and 22). The LPS group received saline (10 ml/kg) by gavage for four weeks and LPS (250 μg/kg/day) by i.p. injection for nine days. The *S. boulardii* (Sb) + LPS group was given Sb (10^{10} CFU/ml) by gavage for four weeks and LPS (250 μg/kg) by i.p. injection for nine days [17].

The remaining group received Sb (10^{10} CFU/ml) by gavage for four weeks and saline (1 ml/kg) by i.p. injection for nine days. On day 29, the animals underwent anesthesia through ketamine/xylazine administration (60/6 mg/kg, i.p.), and blood samples were collected to measure the levels of inflammatory cytokines. Subsequently, animals were euthanized through decapitation, and the

hippocampus and cortex were carefully dissected and preserved at −80 °C for subsequent analysis. Fig. 1 is the timeline of the experimental procedure.

2.4. Sample preparation

The hippocampus and cortex samples were prepared according to the manufacturing protocols for each parameter. At first, to measure MDA levels, hippocampus and cortex tissues (approximately 100 mg) were homogenized in a cold 1.15 % KCl solution containing butylated hydroxytoluene (BHT) to prevent additional peroxidation. After centrifugation, the resulting supernatants were collected for further analysis as mentioned in the Biochemical Assays section (2.5.).

In addition, hippocampus and cortex tissues weighing 50–100 mg were homogenized in lysing buffer (500–1000 μl) and then centrifuged. The resulting supernatants were used for the assessment of various parameters including superoxide dismutase activity (SOD), total antioxidant capacity (TAC), catalase (CAT), glutathione (GSH), oxidized glutathione (GSSG), and glutathione peroxidase (GPx). The next steps completely were described in the Biochemical Assays section (2.5). To examine inflammatory cytokines concentration in serum, the blood samples were centrifuged at 3500 rpm for 15 min and serum samples were collected and stored at −80 °C.

2.5. Biochemical Assays

2.5.1. Measurement of lipid peroxidation

MDA, serving as an indicator of lipid peroxidation, was measured using the MDA assay kit according to the manufacturer's instructions (Teb Pazhouhan Razi Company, Tehran, Iran). MDA reacts with thiobarbituric acid (TBA) to produce an MDA-TBA adduct, resulting in the formation of a red-colored complex [32]. Previously collected brain supernatants (100 μl), as outlined in section 2.4, were combined with detergent (100 μl) and a chromogenic solution (comprising thiobarbituric acid, alkali, and acetic acid, 200 μl). The resulting mixture was heated in a boiling water bath for 1 h. Subsequently, the tubes were transferred to an ice bath for 10 min to stop the reactions, followed by centrifugation to remove any residual substances. The absorbance was determined at 530 nm and the quantification of lipid peroxides was based on the MDA standard curve, with results expressed as μmol/mg tissue protein. This kit has been utilized in multiple earlier studies to analyze the levels of MDA in the brain [33,34].

2.5.2. Superoxide dismutase activity

The measurement of SOD activity was conducted indirectly using a Nasdox™ assay kit (Navand Salamat Company, Urmia, Iran) based on its ability to inhibit pyrogallol autoxidation [35]. Fifty μl of brain sample supernatants, as outlined in section 2.4, were combined with 250 μl of the supplied reaction mixture from the kit and incubated at room temperature for 5 min. The absorbance was measured at 405 nm, and the SOD activity was calculated using the formula (OD of sample/OD of control) and reported as U/mg protein. This kit has been employed in various prior studies to evaluate SOD levels in *in vivo* experiments [36,37].

2.5.3. Total antioxidant capacity assay

The antioxidant potential of the samples, in terms of their ability to convert ferric iron (Fe^{3+}) into ferrous iron (Fe^{2+}), was determined using the ferric reduction antioxidant power (FRAP) method [38]. The Naxifer™ assay kit from Navand Salamat Company in Urmia, Iran, was utilized for the analysis. The brain sample supernatants (5 μl), as detailed in section 2.4, were combined with 250 μl of the kit's working solution. The change in color resulting from the reaction was measured at 590 nm after 5 min using a microplate reader. TAC was calculated by employing the standard curve and then represented as mmol Fe^{2+}/mg tissue protein. This kit has been utilized in numerous prior studies to evaluate TAC levels in *in vivo* experiments [37,39].

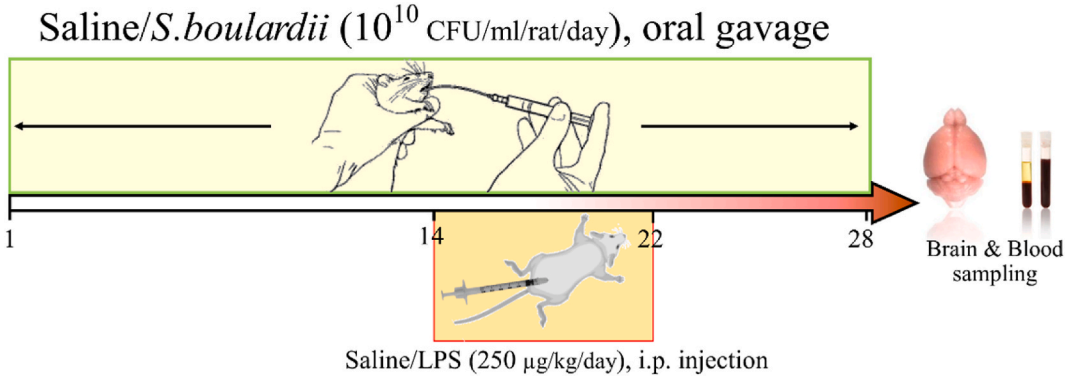

Fig. 1. Timeline of the experimental procedure.

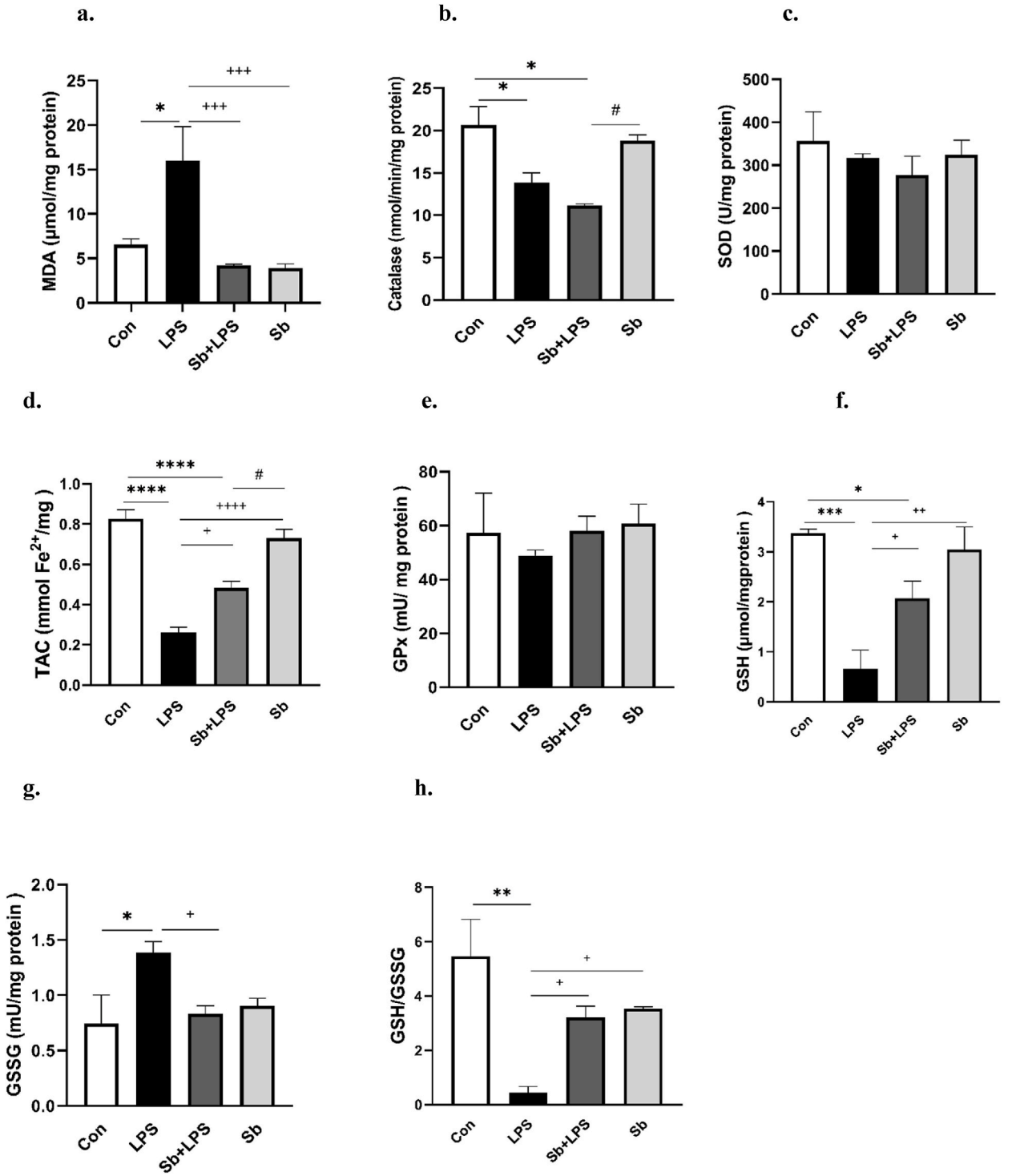

Fig. 2. Panels a–h show the effects of *S. boulardii* on the MDA, CAT, SOD, TAC, GPx, GSH, GSSG and GSH/GSSG ratio respectively in the hippocampus. Data are presented as mean ± SEM. *P < 0.05, **P < 0.01, and ***P < 0.001, ****P < 0.0001, compared to control group. $^{+}$P < 0.05, $^{++}$P < 0.01, $^{+++}$P < 0.001 and $^{++++}$P < 0.0001 compared to LPS group, #P < 0.05 compared to Sb group, N = 8. Control (Con), lipopolysaccharide (LPS), *S. boulardii* + lipopolysaccharide (Sb + LPS), *S. boulardii* (Sb).

2.5.4. Catalase activity

Catalase activity was determined by monitoring its interaction with hydrogen peroxide (H_2O_2) and methanol, resulting in the production of formaldehyde. The formaldehyde generated, in conjunction with a chromogen, was detected at 550 nm [40]. For the measurement of catalase activity, 20 μl of the supernatant, as described in section 2.4, was utilized following the instructions provided by the Nactaz™ assay kit from Navand Salamat Company in Urmia, Iran. Catalase activity was calculated using a standard curve and reported as nmol/min/mg tissue.

2.5.5. Glutathione, glutathione peroxidase, and oxidized glutathione assay

GPx initiates the oxidation of GSH to generate GSSG, subsequently employed in reducing cumene hydroperoxide within the glutathione peroxidase test method. Subsequently, GR transforms GSSG back to GSH through the reduction of NADPH [41]. Consequently, the GPx activity, measured at 340 nm, exhibits a decrease due to the utilization of NADPH [42]. The supernatants collected earlier (50 μl), as described in section 2.4, were utilized for the evaluation of GPx following the instructions provided by the Nagpix™ assay kit from Navand Salamat Company in Urmia, Iran. Absorbance was continuously recorded at 340 nm, and the obtained value was determined using the GPx standard curve. The result was expressed as mU/mg tissue protein. The assay had a sensitivity of detecting GPx activity as low as 0.5 mU/ml in the samples.

Glutathione levels were determined using the Nargul™ reduced glutathione (GSH) assay kit (Navand Salamat Company, Urmia, Iran) based on 2,2′-dinitro-5,5′-dithiodibenzoic acid (DTNB) measurement [43]. The brain supernatants collected earlier (20 μl), as described in section 2.4, were combined with GSH buffer (20 μl), and subsequently, an equal amount of DTNB (Ellman's reagent) and glutathione reductase (120 μl) were added. The mixture was incubated for 30 s. Afterward, a 60 μl cofactor solution was introduced, and the resultant absorbance was recorded at 412 nm. The measurement of GSH levels was conducted by utilizing a standard curve, and the outcomes were reported in μmol/mg tissue protein.

To assess GSSG, 25 μl of brain supernatants, as outlined in section 2.4, were combined with GSH buffer, enzyme, cofactor, and DTNB according to the instructions provided by the Naglu™ assay kit from Navand Salamat Company in Urmia, Iran. Subsequently, the absorbance at 412 nm was measured, and the obtained value was determined using the GSSG standard curve. The result was then reported as μm/mg tissue protein.

2.6. Serum cytokine levels

Following the collection of blood samples, the serum concentrations of IL-10 (DY522, Bio-Techne, USA), IL-6 (CSB-E04640r, Cusabio, USA), and IL-8 (abx576575, Abbexa, USA) were determined using ELISA (Enzyme-Linked Immunosorbent Assay) kits following the manufacturer's instructions. The serum cytokine concentrations were ascertained via a standard curve and presented in pg/ml.

2.7. Statistical analysis

The analysis was conducted using GraphPad Prism version 9.5.0. The normality of data variances was assessed using the Kolmogorov–Smirnov test. Variances among the experimental groups were analyzed through a one-way analysis of variance (ANOVA) followed by post hoc Tukey's and Bartlett's tests for multiple comparisons. Statistical significance was determined when $P < 0.05$. The data is presented as the mean ± SEM (standard error of the mean).

3. Results

3.1. Effects of S. boulardii on oxidative stress parameters in the hippocampus

As presented in Fig. 2a, the MDA levels exhibited a significant increase in the hippocampal tissues of the LPS group compared to the Con group ($P < 0.05$). Conversely, the administration of *S. boulardii* with LPS effectively inhibited the LPS-induced elevation in MDA levels ($P < 0.001$, Fig. 2a). Both Sb + LPS and Sb-treated groups had no significant difference in MDA levels with control animals.

Catalase activity significantly decreased in the hippocampal tissues of the LPS-treated group in comparison with the Con group ($P < 0.05$). Similarly, as shown in Fig. 2b, the Sb + LPS-treated group significantly reduced catalase activity versus the Con group ($P < 0.05$). Consequently, catalase activity was not significant between the Con group and Sb-treated animals and also between Sb + LPS and LPS-treated (Fig. 2b). In other words, probiotic *S. boulardii* was unable to increase the catalase activity in the Sb + LPS group.

The groups did not exhibit any significant differences in SOD activity, as depicted in Fig. 2c. As shown in Fig. 2d, the TAC levels displayed a significant decrease in the hippocampal tissues of the LPS and Sb + LPS-treated groups versus the Con group ($P < 0.0001$). There was no difference in the TAC levels between the control animals and the Sb-treated group. However, the difference in TAC levels between Sb + LPS and LPS groups was significant ($P < 0.05$). As indicated in Fig. 2e, no significant variances were detected in the GPx activity within the hippocampus among the groups. As indicated by the findings presented in Fig. 2f, the GSH levels in the hippocampal tissues were notably reduced in both the LPS and Sb + LPS groups when compared to the Con group ($P < 0.001$ and $P < 0.05$, respectively). The Sb-treated animals had no difference in GSH levels from the control group. Remarkably, the Sb + LPS-treated group exhibited significantly higher GSH levels in comparison to the LPS-treated group ($P < 0.05$). As shown in Fig. 2g, in the LPS group, GSSG levels significantly increased in hippocampal tissues versus the Con group ($P < 0.05$). Both Sb + LPS and Sb-treated animals had no substantial difference in GSSG levels with the control group. However, pretreatment of *S. boulardii* with LPS significantly reduced

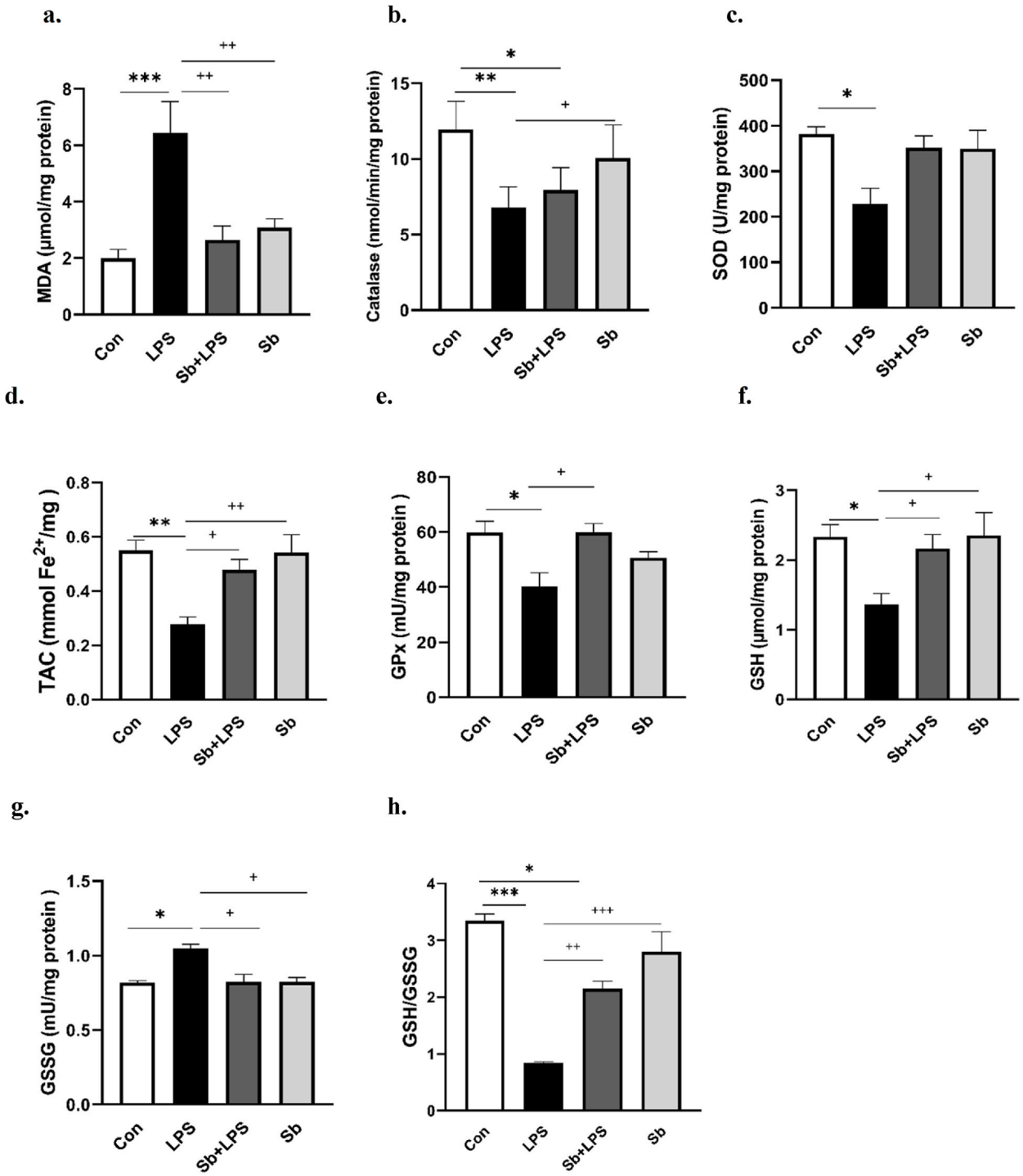

Fig. 3. Panels a–h show the effects of *S. boulardii* on the MDA, CAT, SOD, TAC, GPx, GSH, GSSG and GSH/GSSG ratio respectively in the cortex. Data are presented as mean ± SEM. *P < 0.05, **P < 0.01, and ***P < 0.001, compared to control group. $^{+}$P < 0.05, $^{++}$P < 0.01 and $^{+++}$P < 0.001, compared to LPS group, N = 8. Control (Con), lipopolysaccharide (LPS), *S. boulardii* + lipopolysaccharide (Sb + LPS), *S. boulardii* (Sb).

the elevation of GSSG levels in comparison with the LPS-treated alone ($P < 0.05$). As presented in Fig. 2h, the LPS-treated group demonstrated a significantly lower GSH/GSSG ratio versus the Con group ($P < 0.01$). This ratio was not significant between the Con and Sb + LPS groups and also between control and Sb-treated animals. In contrast, the Sb + LPS-treated group exhibited a significantly higher GSH/GSSG ratio compared to the LPS alone ($P < 0.05$).

3.2. Effects of S. boulardii on oxidative stress parameters in the cortex

As presented in Fig. 3a, our findings revealed a significant increase in MDA levels in the cortical tissues of the LPS group versus the Con group ($P < 0.001$). MDA levels in the Sb + LPS and Sb groups were not different from those in the Con group. However, pretreatment of *S. boulardii* with LPS could significantly prevent the rise in MDA levels in comparison with the LPS alone ($P < 0.01$).

As shown in Fig. 3b–a significant decrease was found in the catalase activity of cortical tissues of the LPS-treated group versus the Con ($P < 0.01$). Furthermore, pretreatment of *S. boulardii* with LPS significantly decreased catalase activity versus the Con group ($P < 0.05$). However, the difference in catalase activity between the Sb + LPS and LPS groups and between control and Sb-treated animals was not significant (Fig. 3b).

As shown in Fig. 3c, the SOD activity in the cortical tissues of the LPS group exhibited a significant decrease versus the Con group ($P < 0.05$). *S. boulardii* was effective in preventing the decline in SOD activity in the Sb + LPS group, which led to the absence of a significant difference between the Sb + LPS group and the Con group. There was no difference in the SOD activity between the control animals and the Sb-treated group. As shown in Fig. 3d, the TAC levels in the cortical tissues of the LPS group significantly decreased versus the Con group ($P < 0.01$). Both the Sb + LPS and Sb-treated animals had no substantial difference in TAC levels with the control group. Pretreatment of *S. boulardii* with LPS could significantly increase the TAC levels versus the LPS alone ($P < 0.05$). Notably, the TAC levels of the Sb + LPS-treated group were not different from the Con group.

As shown in Fig. 3e and f, the LPS-treated group exhibited significant decreases in GPx activity and GSH levels versus the Con group ($P < 0.05$). However, pretreatment of *S. boulardii* in the Sb + LPS animals could significantly increase GPx activity and GSH levels of cortical tissues versus LPS-treated alone ($P < 0.05$). Furthermore, the difference in GPx activity and GSH levels between the Sb + LPS and Con groups and also between control and Sb-treated animals were not significant.

As shown in Fig. 3g, the LPS-treated group exhibited a significant rise in the GSSG levels of cortical tissues compared to the Con group ($P < 0.05$). However, when *S. boulardii* was administered with LPS, it prevented the increase in GSSG levels versus the LPS alone ($P < 0.05$).

The distinction between control animals and the Sb + LPS and Sb groups did not reach statistical significance in Fig. 3g. As presented in Fig. 3h, the GSH/GSSG ratio in the cortex exhibited a notable reduction in the LPS-treated group compared to the Con group ($P < 0.001$). Within the Sb + LPS-treated group, *S. boulardii* significantly elevated the diminished GSH/GSSG ratio in comparison to the LPS-treated group ($P < 0.01$). The difference in GSH/GSSG ratio was significant between the Sb + LPS and Con groups ($P < 0.05$) but was not significant between the control and Sb-treated animals.

3.3. Effects of S. boulardii on the serum cytokine levels

As presented in Fig. 4a, the LPS-treated group significantly decreased serum levels of IL-10 versus the Con group ($P < 0.01$).

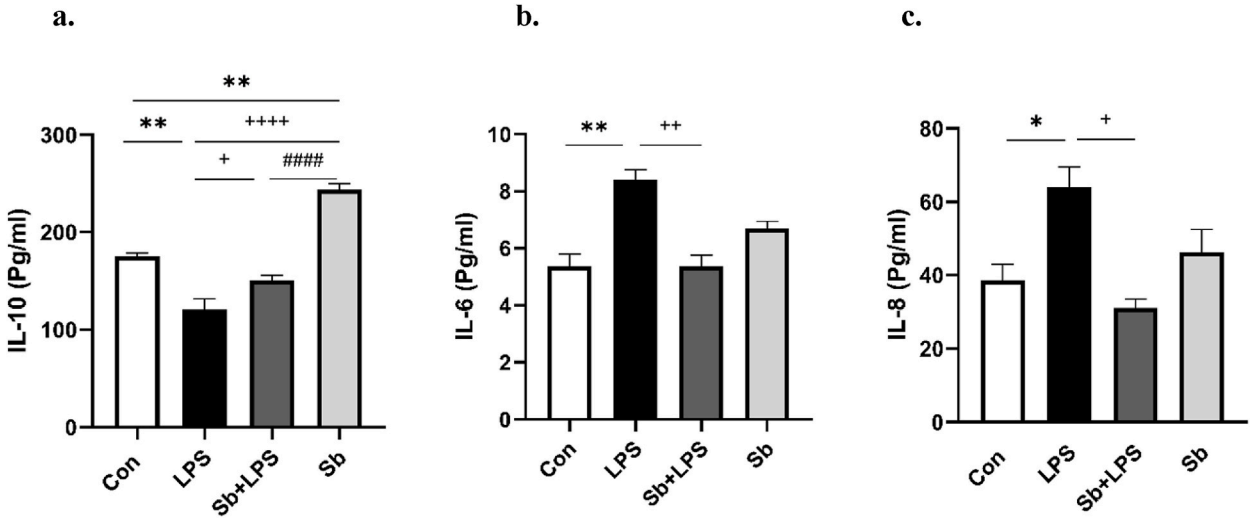

Fig. 4. Panels a–c show the effects of *S. boulardii* on the serum of IL-10, IL-6, and IL-8 respectively. Data are presented as mean ± SEM. *$P < 0.05$, and **$P < 0.01$, compared to control group. $^{+}P < 0.05$, $^{++}P < 0.01$, and $^{++++}P < 0.0001$ compared to LPS group, $^{\#\#\#\#}P < 0.0001$, compared to Sb group. N = 8. Control (Con), lipopolysaccharide (LPS), *S. boulardii* + lipopolysaccharide (Sb + LPS), *S. boulardii* (Sb).

Pretreatment of *S. boulardii* with LPS significantly elevated IL-10 levels compared to LPS treatment alone ($P < 0.05$). The IL-10 levels were not different between both groups of the Sb + LPS and Con (Fig. 4a). Besides, the Sb-treated animals showed a significant increase in IL-10 levels compared to controls.

As shown in Fig. 4b and c, the LPS-treated group showed significant increases in the IL-6 and IL-8 serum levels versus the Con group ($P < 0.01$ and $P < 0.05$, respectively). On the contrary, the Sb + LPS-treated group exhibited a notable reduction in the serum concentrations of IL-6 and IL-8 in comparison to the LPS-treated group ($P < 0.01$ and $P < 0.05$, respectively). Both the Sb + LPS and Sb-treated animals had no substantial difference in IL-6 and IL-8 levels with the control group.

4. Discussion

Our findings present novel evidence indicating that the utilization of *S. boulardii* has the potential to mitigate the rise in lipid peroxidation in the hippocampus and cortex of rats within an oxidative stress model induced by LPS. In this regard, it prevented the decrement of the levels of antioxidants, including GSH and catalase especially in the cortex, and the increment of GSSG levels against oxidative stress induced by LPS in both the hippocampus and cortex. Therefore, the ratio of GSH/GSSG was preserved after the administration of *S. boulardii* with LPS in both tissues. Also, *S. boulardii* could prevent a decrease in TAC induced by LPS. We observed that SOD and GPx activities did not change in the LPS model in the hippocampus but were reduced in the rat cortex. The concurrent administration of *S. boulardii* with LPS effectively mitigated the decline in SOD activity observed in the cortex. Additionally, an interesting point is that the lack of a difference between Sb and LPS + Sb groups in all parameters except for catalase activity in the hippocampus tissue indicates that probiotic *S. boulardii* could successfully modulate oxidant and antioxidant parameters in the context of oxidative stress and inflammation that induced by LPS.

The progression of neurodegenerative diseases and conditions involves crucial steps, including oxidative stress, lipid peroxidation, and subsequent modifications to proteins and lipids through oxidation and nitration. Microglial activation and neuroinflammation are triggered by oxidative stress. Consequently, antioxidant therapies hold promise for providing neuroprotection in the presence of oxidative/nitrative stress [45]. *S. boulardii* significantly prevented lipid peroxidation due to LPS-induced oxidative stress in rat hippocampus and cortex that could inhibit the progression of neuroinflammation. In parallel with our study, *S. boulardii* pretreatment alleviated ischemia-reperfusion-induced lung injury by significantly suppressing increases in proinflammatory cytokine of serum and lung tissue MDA and TNF-α levels in rats [30].

GSH is an endogenous non-protein thiol-containing tripeptide with antioxidant properties [5,45]. GSH is synthesized in the cytoplasm and exists in higher concentrations in the mitochondrial matrix which can oxidize to GSSG. Total GSH levels and GSH/GSSG ratio are two important indices of the protective ability of cells under oxidative stress [1,5]. Consequently, a reduction in both of these indicators elevates vulnerability to oxidative stress, leading to potential harm that could be significant in the advancement of various conditions including cancer, cardiovascular ailments, respiratory disorders, inflammatory diseases, diabetes mellitus, and neurodegenerative conditions like PD and AD [46]. In this study, the prophylactic administration of *S. boulardii* effectively maintained the GSH/GSSG ratio in both tissues, counteracting the decremental effect induced by LPS. These findings offer promising prospects for protecting against neuroinflammation caused by oxidative stress. This finding agrees with a previous study that has prevented chloride secretion due to rotavirus-non-structural protein 4 (NSP4) by inhibiting the imbalance between ROS and GSH/GSSH ratio [47]. Other endogenous antioxidative defense system includes SOD, CAT, and GPx, which act as scavengers, protect cells from active oxygen, and produce O_2 and H_2O [3,20].

Our results indicated that *S. boulardii* could preserve the SOD, CAT, and GPx activities in the cortex against oxidative stress induced by LPS, which can protect the antioxidative defense system. Similar to our finding, *S. boulardii* exhibited neuroprotective effects and improved memory impairment by preventing antibiotic-induced gut dysbiosis in mice and reducing inflammation and oxidative stress in the intestines and brain [11]. Also, *S. boulardii* mitigated oxidative stress by increasing colonic SOD and CAT activities and GSH levels and decreasing colonic MDA levels in UC induced by dextran sulfate sodium (DSS) in mice. Furthermore, it inhibited colonic inflammation by reducing proinflammatory cytokine levels such as IL-1, IL-6, and TNF-α possibly by inhibiting NF-κB and promoting Nrf2 pathways, essential targets in the treatment of UC [48]. In this study, SOD activity in the hippocampus did not significantly affect by LPS which may be related to the different responses of SOD to the oxidative stress model [49].

The study findings demonstrated that administering *S. boulardii* significantly elevated IL-10, an anti-inflammatory cytokine, in serum levels, and simultaneously reduced the proinflammatory cytokines IL-6 and IL-8 in the LPS model of oxidative stress. Likewise, the application of *S. boulardii* culture supernatant showed a reduction in the inflammatory response triggered by LPS in human dendritic cells. This resulted in a reduction in IL-6 and TNF-α levels, coupled with an elevation in IL-10 production [50]. *S. boulardii* exhibited anti-inflammatory ability against *Candida albicans* by diminishing the production of proinflammatory cytokines, through the regulation of interferon-gamma (IFN-γ) and IL-1β. Simultaneously, it promoted the secretion of anti-inflammatory cytokines like IL-4 and IL-10 [51]. Additionally, it reduced toll-like receptor 2 (TLR2) stimulation [52].

Moreover, *S. boulardii* has demonstrated its anti-inflammatory effects against *Clostridium difficile* by inhibiting the activation of MAP kinases ERK1/2 and JNK/SAPK, resulting in a reduction in IL-8 production [53]. Similarly, *S. boulardii* exhibited this effect against Salmonella by inhibiting MAP kinases ERK1/2, p38, and JNK, as well as nuclear factor kappa B (NF-κB) activation, resulting in decreased levels of IL-8 [54], IL-6, and TNF-α [29]. Furthermore, it inhibited the mRNA expression associated with TNF-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), CXC motif chemokine ligand 8 (CXCL8), and chemokine ligand 2 (CCL2) genes [31]. Moreover, *S. boulardii* exhibited the ability to up-regulate peroxisome proliferator-activated receptor gamma and down-regulate IL-8 expression in human HT-29 colonocytes and rat model colitis, respectively [55]. Also, *S. boulardii* inhibited the expression of proinflammatory cytokines and inducible nitric oxide synthase (iNOS) genes in the colonic mucosa of infected rats with

Blastocystis subtype-3 cysts [56].

The antioxidant effects of various probiotics against oxidative stress have been documented in several studies [57,58]. A meta-analysis of clinical trials on probiotics showed that their consumption could decrease high-sensitive C-reactive protein (hs-CRP) and MDA and increase GSH and TAC. As a result, they are effective against inflammation and oxidative stress biomarkers. Furthermore, their overall effects on serum TAC levels may be more evident on probiotic dose> 5 billion CFU/day [59]. On the other hand, several possible mechanisms for neuroinflammation induced by LPS have been discussed in a prior study: 1) cellular damage caused by ROS generation and cytokines activation, 2) astrocytes and microglia activation in the CNS that can potentiate the expression of IL-6 that is considered as a biomarker of neuroinflammation, 3) neurodegeneration and memory impairment [60].

We found that *S. boulardii* could preserve the antioxidant enzymes' activities against LPS-induced oxidative stress. The levels of serum proinflammatory cytokines IL6 and Il-8 and the anti-inflammatory IL-10 decreased and increased, respectively. Furthermore, we have shown that *S. boulardii* ameliorates neuroinflammation induced by LPS, amyloid-β deposition, and subsequent memory impairment or anxiety in animals, possibly by modulating the gut microbiota [17,61]. All of these observations confirm that *S. boulardii* exhibits protective effects against LPS-induced neuroinflammation.

S. boulardii possibly exerts part of its vital activity by modulating gut microbiota. The production of microbial metabolite short-chain fatty acids (SCFAs), including isobutyrate and valerate, has been reported to increase with *S. boulardii* supplementation in various studies which may be necessary for preventing and treating diseases [62–64]. *S. boulardii* showed anti-inflammatory and protective effects in the DSS model of UC in mice by decreasing the inflammatory cytokine levels (TNF-α and IL-8), protecting intestinal histological structures and tight junctions, which upregulation the percentage of S24-7 in the intestinal flora may be considered a fundamental mechanism of its activities [65].

In the current study, we examined the effect of probiotic *Saccharomyces boulardii* on brain oxidative stress in a LPS rat model which is known and used as a model for investigating neuroinflammation, neuronal loss, and neurodegeneration. According to previous studies, we assumed that the gut epithelium barrier and blood-brain barrier were disrupted following LPS-induced inflammation and then restored after probiotic treatment. Peng et al. (2021) discussed completely the mechanisms of BBB permeability and disruption by LPS [66]. Another study that showed BBB dysfunction and permeability in the LPS animal model was performed by Li et al. (2020). They found that LPS markedly disrupted the BBB and reduced Occldin and ZO-1 in the microvessels samples isolated from the brains of LPS-induced mice [67]. In this regard, recent studies revealed that probiotics can modulate BBB integrity. The BBB permeability and the expression of tight junction proteins were normalized in germ-free mice colonized with a commensal microbiota [68]. The probiotic *Lactobacillus reuteri* improved BBB dysfunction and cognitive deficit in offspring of LPS-induced dams [69].

There was no study addressing the effect of *S. boulardii* on BBB integrity and function. But, according to our results, the anti-inflammatory effects of *S. boulardii* in the circulation especially through its metabolites like SCFAs may affect some properties in BBB including its integrity and permeability to reverse the harmful effects of LPS which can result in decreasing oxidative stress in the brain samples.

Moreover, the intrinsic antioxidant activity of *S. boulardii* is evident. Previous research has shown that cysteine ethyl ester, a GSH inducer, effectively enhances intracellular GSH levels in various Saccharomyces strains, including *S. cerevisiae*, *S. bayanus*, and *S. boulardii*. This discovery suggests the possibility of merging the antioxidant properties of GSH to counteract oxidative stress with the probiotic characteristics of *S. boulardii* in the treatment of gastrointestinal disorders [70]. Furthermore, the synthesis of GSH by *S. boulardii* (CNCM I-745) is consistent with the results obtained in a prior study [45]. *S. boulardii* has intracellular ROS scavenging activity, and this antioxidant activity may be related to the presence of vanillin/vanillic acid as an active constituent in its fermentation broth [71].

S. boulardii has antimicrobial, metabolic, and enzymatic activities, increasing immune defense in the gut and decreasing the synthesis of inflammatory cytokines, antitoxin, and modulatory effects on intestinal flora [72]. Our results determined that *S. boulardii* has the potential to be neuroprotective against oxidative stress induced by LPS. On the other hand, *S. boulardii* indicates some advantages including growing at a temperature of 37 °C despite different strains of *S. cerevisiae*. Additionally, it displays resistance to low pH levels and demonstrates tolerance to bile acids [72–74]. It is naturally resistant to antibiotics [44]. It does not colonize the host gastrointestinal tract [75]. It does not engage in the DNA exchange of resistance genes with bacteria during antibiotic treatment [76,77].

5. Conclusions

The findings demonstrated for the first time that *S. boulardii* has potential antioxidant activities in oxidative stress-related model, possibly modulating gut microbiota, immune defense, and antioxidant enzyme activity that can be considered in preventing oxidative stress-related CNS diseases.

In this regard, the future directions for research in the field of the gut-brain axis should explore a new approach that considers it as a potential factor influencing brain health. Furthermore, the use of probiotics such as *S. boulardii*, with their neuroprotective effects, may be considered as a therapeutic intervention in CNS disorders related to neuroinflammation. However, the mechanisms behind the associations between the actions of *S. boulardii* and neurological functions need to be further investigated in future studies. This study also emphasizes the importance of fully understanding specific aspects of *S. bouvardia*'s mechanisms such as analyzing the gut composition when manipulated with this probiotic and evaluating the immunological, endocrine, and nervous pathways that *S. boulardii* can impact the BBB and the brain.

6. Limitations

The current study investigated the antioxidant impact of probiotic *S. boulardii* in the LPS-induced model for the first time. However, we had some limitations such as a confounding factor related to the complete dissimilarity of gut microbiota composition between animals and humans in this research context, which could complicate translating the results to human-based conditions. However, due to the limitations in human brain tissue collection, extensive study of animal samples is common. Moreover, in animal ethics, we had to limit the gavage time in our study to avoid damaging the animals for a long time. The limitation in the brain sample size was another challenge in evaluating all the oxidative stress markers in our research.

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Data availability

Data has been included in the article.

Ethics statement

All experiments were approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.PHNS.REC.1398.129) and performed according to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

CRediT authorship contribution statement

Fatemeh Babaei: Writing – original draft, Investigation, Formal analysis, Data curation. **Ava Navidi-Moghaddam:** Writing – original draft, Investigation, Formal analysis, Data curation. **Ariyan Naderi:** Investigation. **Shiva Ghafghazi:** Investigation. **Mohammadreza Mirzababaei:** Visualization, Investigation. **Leila Dargahi:** Writing – review & editing, Methodology. **Ghazaleh Mohammadi:** Writing – review & editing, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Marjan Nassiri-Asl:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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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