Mesenchymal stem cells' therapeutic potential for endotoxin-induced brain and spleen injuries in rats

Marwa A. Masoud[a], Wedad A. Hassan[a], Nahla A. Hassaan[b], Hanaa A. Mansour[a]

[a]Department of Pharmacology, National Organization for Drug Control and Research (NODCAR), Giza, [b]Department of Zoology, Faculty of Science, Al-Azhar University, Cairo, Egypt

Correspondence to Marwa A. Masoud, PhD, Department of Pharmacology, National Organization for Drug Control and Research (NODCAR), 6-Abu Hazem Street, Pyramids Ave., Postal Code: 29, Giza, Egypt.
Tel: +20 100 265 3204; fax: +20 235 855 582; e-mail: drmerro@yahoo.com

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Background and objective
Inhalation of bacterial endotoxin induces an acute inflammation in various organs, especially the brain and spleen. This study examined the therapeutic effects of bone marrow mesenchymal stem cells (BM-MSCs) in lipopolysaccharide (LPS)-induced brain-spleen injuries in rats as compared with dexamethasone.
Materials and methods
A total of 32 male Wistar albino rats, weighing 180–200 g, were used in the study and were divided into four groups. Group 1 (normal) rats received 20 µl of saline in each nostril for two consecutive days. Group 2 animals received LPS (20 µl of LPS of *Escherichia coli* in each nostril for 2 consecutive days) that induced brain-spleen injuries and served as a positive control group. Group 3 animals were injected with dexamethasone (2 mg/kg, once, intraperitoneal). Group 4 animals received (1×10**6**) BM-MSCs in 500 µl PBS/rat via intraperitoneal injection once before acute injury induction with LPS. At the end of the experiment, the authors studied the sickness behavior by assessing open field behavior and measured oxidative and inflammatory parameters.
Results and conclusion
LPS-induced open field behavior impairments (decreased locomotion and rearing and increased immobility), with elevation of a number of inflammatory cells, especially neutrophils. Moreover, LPS-induced elevation of lipid peroxidation along with reduction of both reduced glutathione and superoxide dismutase in brain and spleen tissues and increased interleukin-1β and myeloperoxidase contents in rats, compared with normal group. These harmful effects were hindered after treatment with MSCs. In conclusion, MSCs prevented both sickness and depressive-like behavior via neuroinflammatory pathway and could be a novel approach to therapy for LPS-induced serious injuries in rats but needs further clinical studies.

Keywords:
brain, inflammation, lipopolysaccharides, mesenchymal stem cells, spleen

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Introduction

Sepsis is a result of bacterial infection in the circulation and is associated with organ dysfunction, hypoperfusion, hypotension, and activation of the immune system [1]. Severe sepsis is a serious condition, and its mortality rate is high. Therefore, the need for new effective adjunctive therapy remains strong. Lipopolysaccharides (LPS) induce inflammation and reactive oxygen species, so LPS was frequently used as a model of sepsis in experimental animals [2]. The spleen is important in innate and adaptive immunity, and it plays a central role in mounting a response against a systemic inflammation, such as that induced by LPS [3]. The spleen plays a central role in B-cell differentiation and dendritic cell maturation occurring in the white pulp [4] and is also involved in the removal of damaged erythrocytes, blood filtration, iron recycling, and storage of plasma cells [5].

Peripheral administration of LPS exhibits both depressive and anxiety-like behavior in an animal model by causing a systemic inflammation through an increase in the production of proinflammatory mediators such as tumor necrosis factor-α, interferon-γ, interleukin-6 (IL-6), and IL-1ß. Furthermore, these proinflammatory cytokines produce sickness behavior syndrome, such as hyperthermia, anorexia, sleepiness, reduction of locomotor activity, exploration, loss of body weight, and anhedonia [6,7]. Moreover, LPS-induced inflammation leads to significant reduction in the neurotrophic factors like brain-derived neurotrophic

factor, nerve growth factor, and neurotrophin-3 levels in different regions of the brain [8].

Stem cells are undifferentiated progenitor cells with a broad developmental potential and exhibit the capacity to give rise to cells identical to them or to cells of multiple lineages. Depending on their residency, they can be classified as embryonic stem cells and adult stem cells, whereas according to their potency, they are divided in totipotent, multipotent, and unipotent. Adult stem cells are multipotent and include mesenchymal stem cells (MSCs), hematopoietic stem cells, endothelial progenitor cells, and organ-specific stem cells [9].

Recent studies showed that stem cell therapy may have application in various disorders, including sepsis [10], hepatic [11] and renal failure [12], diabetes [13], and myocardial infarction [14]. MSCs are a multipotent endogenous population of progenitors with capabilities of high proliferation and self-renewal [15]. MSCs have been isolated from many types of tissues and organs, including bone marrow (BM), heart, lung, and umbilical cord blood [16,17].

Dexamethasone is a well-known steroid agent that regulates inflammation by inhibiting inflammatory mediators [18]. Previous studies experimented dexamethasone in the treatment of brain inflammatory diseases [19].

The antidepressant and antianxiety activities of MSCs in LPS-induced depressive-like behavior model have not been studied so far. Thus, in the present study, we investigated the possible antidepressant effects of MSCs through their effect on behavior, oxidative stress, and inflammation compared with dexamethasone. Furthermore, we assessed the effects of MSCs pretreatment on behavior alteration, oxidative stress, and proinflammatory cytokines contents in the brain and spleen following an immune challenge with LPS in rats.

The present work aims to evaluate the efficacy of BM-derived stem cells compared with dexamethasone in the treatment of brain-spleen injuries induced by bacterial endotoxin in rats.

Materials and methods

Animals

The present experimental study was carried out on white albino rats (*Rattus norvegicus*). The standard guidelines of National Organization for Drug Control and Research (NODCAR) were used in handling animals. The animals were selected from a pure strain, so genetic influence was kept at a constant and uniform level. Animals had free access to food and water *ad libitum*. They were maintained at 21–24°C and 40–60% relative humidity with 12-h light–dark cycle. All animals' procedures were performed in accordance with the institutional Ethics Committee and in accordance with the recommendations for the proper care and use of laboratory animals. Unnecessary disturbance of animals was avoided. Animals were treated gently, and squeezing, pressure, and tough maneuver were avoided. All procedures were carried out according with the research ethics committee for experimental studies at the National Organization for Drug Control and Research NODCAR/I/33/19 on September 18, 2019.

Bacterial lipopolysaccharide

LPS was obtained from *Escherichia coli* O55:B5, L2637, and purified by gel-filtration chromatography (Sigma Aldrich, St. Louis, Missouri, USA). LPS from *E. coli* was administered intranasally. Overall, 10 μg of endotoxin prepared in 20 μl of pyrogen-free saline was instilled into each nasal passage (total doses of 20 μg prepared in 40 μl/day) for 2 consecutive days according to Wagner *et al.* [20] and Suri *et al.* [21].

Isolation and characterization of BM-MSCs

BM was harvested by flushing the tibiae and femurs of 6-week-old male white albino rats with Dulbecco's modified Eagle's medium (GIBCO/BRL; Life Technologies, Grand Island, New York, USA) supplemented with 10% fetal bovine medium (GIBCO/BRL). Nucleated cells were isolated with a density gradient (Ficoll/Paque Pharmacia Biotech, Uppsala, Sweden) and resuspended in complete culture medium supplemented with 1% penicillin-streptomycin (GIBCO/BRL). The cells were incubated at 37°C in 5% humidified atmosphere for 12–14 days as primary culture or upon formation of large colonies. When large colonies developed (80–90% confluence), cultures were washed twice with PBS, and cells were trypsinized with 0.25% trypsin in 1 mmol/l EDTA (GIBCO/BRL) for 5 min at 37°C. After centrifugation (at 2400 rpm for 20 min), cells were resuspended with serum-supplemented medium and incubated in a 50 cm^2 culture flask (Falcon). The resulting cultures were referred to as first-passage cultures [22]. On day 14, the adherent colonies of cells were trypsinized and counted. BM-derived MSCs were characterized by their adhesiveness and fusiform shape and identified by staining with surface markers CD29, CD90 for

MSCs, and CD34 for hematopoietic cells using flow cytometry [23].

Experimental design

A total of 32 albino Wistar rats weighting initially between 180 and 200 g were randomly assigned into four groups of eight rats in each group:

(1) Group 1 (normal): rats received 20 µl of saline in each nostril for 2 consecutive days.
(2) Group 2 (positive control): rats received 20 µl of LPS (*E. coli* endotoxin) in each nostril (intranasal) (total dose 20 µg/rat/day) for two consecutive days according to Wagner *et al.* [20] with modification.
(3) Group 3 (LPS+DEX): animals were injected once intraperitoneal by 300 µl/rat dexamethasone (2 mg/kg) according to Lin *et al.* [24] followed by 20 µl of LPS (*E. coli* endotoxin) in each nostril (intranasal) (total dose 20 µg prepared in 40 µl/rat/day) for 2 consecutive days.
(4) Group 4 (LPS+MSCs): animals received (1×10^6) BM-derived MSCs in 500 µl PBS/rat (intraperitoneal) according to Maron-Gutierrez *et al.* [25]. After 30 min, rats received 20 µl of LPS (*E. coli* endotoxin) in each nostril (intranasal) (total dose 20 µg prepared in 40 µl/rat/day) for 2 consecutive days.

Tissue sampling

At the end of the experiment, 24 h after last manipulation, behavioral test [open field test (OFT)] was conducted. Then animals were decapitated, and blood samples were collected. Then, spleens and brains (hippocampus and cerebral cortex) were quickly dissected. The dissected organs were harvested and rinsed with ice-cold isotonic saline. Brains and spleens were divided into two portions: one was kept in 10% formalin for histopathological examinations, whereas the other in −80°C for estimating other biochemical parameters. The cerebral cortex (which included the hippocampus) and spleens were dissected and weighed and then homogenized in ice-cold saline to prepare 10% homogenate, which was used for the assessment of brain and spleen contents of lipid peroxides (MDA), reduced glutathione (GSH), IL-1β, myeloperoxidase (MPO), and superoxide dismutase (SOD) activity. The other cerebral cortex (which included the hippocampus) was homogenized in ice-cold solution of acidified n-butanol to obtain 10% homogenate for determination of brain contents of serotonin (5-HT), dopamine (DA), and norepinephrine (NE). Finally, the used animals were frozen till being incinerated.

Behavioral assays

OFT: the open field test was used to measure the motor activity, excitability, emotionality, and exploratory behavior in rodents. The apparatus used was a square-shaped wooden box 80××40 cm in height, with red sides and white floor divided by black lines into 16 equal squares of 4×4 [26]. Ambulation frequency was evaluated by recording the number of crossed squares, and rearing frequency was recorded when the animal stands on its hind limbs with or without support of the wall over a period of 3 min [27,28]. The light was maintained at minimum to avoid anxiety behavior. The apparatus was cleaned with a solution of 10% ethanol between tests to hide animal clues.

Hematological parameters

(1) Total leukocytic count
This took place according to the method of Ingram and Minter [29] and Nourbakhsh *et al.* [30]. The whole blood was diluted at 1 : 20 in glacial acetic acid solution (1%), that is, 20 µl was taken from whole blood+380 µl white blood cell diluting solution.
(2) Differential leukocytic count
Leishman stain was used for the differential leukocytic count. The stain allows determining different elements of white blood cells, which were scored by the method of Hayhoe and Flemans [31]. This included four types of cells in this study: neutrophils, lymphocytes, monocytes, and eosinophils. The percentage of each type was calculated relative to the total leukocytic count.

Biochemical parameters

(1) Determination of brain-spleen reduced GSH
Determination of brain-spleen reduced GSH contents was done using Biodiagnostic kit (Biodiagnostic, Cairo, Egypt), according to the method described by Beutler *et al.* [32] and expressed as mg/g wet tissue.
(2) Determination of brain-spleen lipid peroxides (MDA)
Lipid peroxide contents were assessed using Biodiagnostic kit according to the method described by Satoh *et al.*[33] and expressed as nmol/g wet tissue.
(3) Determination of brain-spleen SOD activity
Determination of brain SOD activity was done using a kinetic colorimetric Biodiagnostic kit according to the method described by Nishikimi *et al.* [34].
(4) Determination of brain-spleen IL-1β contents
IL-1β content was estimated by enzyme-linked immunosorbent assay using a rat IL-1β sample.

IL-1β determination was done using a test reagent kit (eBioscience Co., San Diego, California, USA) according to the manufacturer's instructions and expressed as pg/g wet tissue.

(5) Determination of brain-spleen MPO activity
Determination of brain MPO activity was done using a kinetic colorimetric method described by Bradley *et al.* [35].

(6) Determination of brain serotonin (5-HT), DA, and NE contents
For the determination of neurotransmitters, a 10% (w/v) homogenate was prepared in acidified n-butanol. Each homogenate was centrifuged at 5000 rpm (4C) for 10 min. The resultant supernatant was used for determination of monoamines, namely, serotonin (5-HT), DA, as well as NE according to Ciarlone [36], and they were expressed as μg/g wet tissue.

Histopathological examination

At the end of the experiments, brains and spleens were dissected from rats in the different experimental groups and then fixed in 10% formalin prepared in saline for 12 h. Specimens were washed under tap water followed by adding alcohol (methyl, ethyl, and absolute ethyl) for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56°C in hot air oven for 24 h. Paraffin wax tissue blocks were prepared for sectioning at 4 μm thickness by a sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, and stained with hematoxylin and eosin (H&E) stain for histopathological examination using a light microscope [37].

Statistical analysis

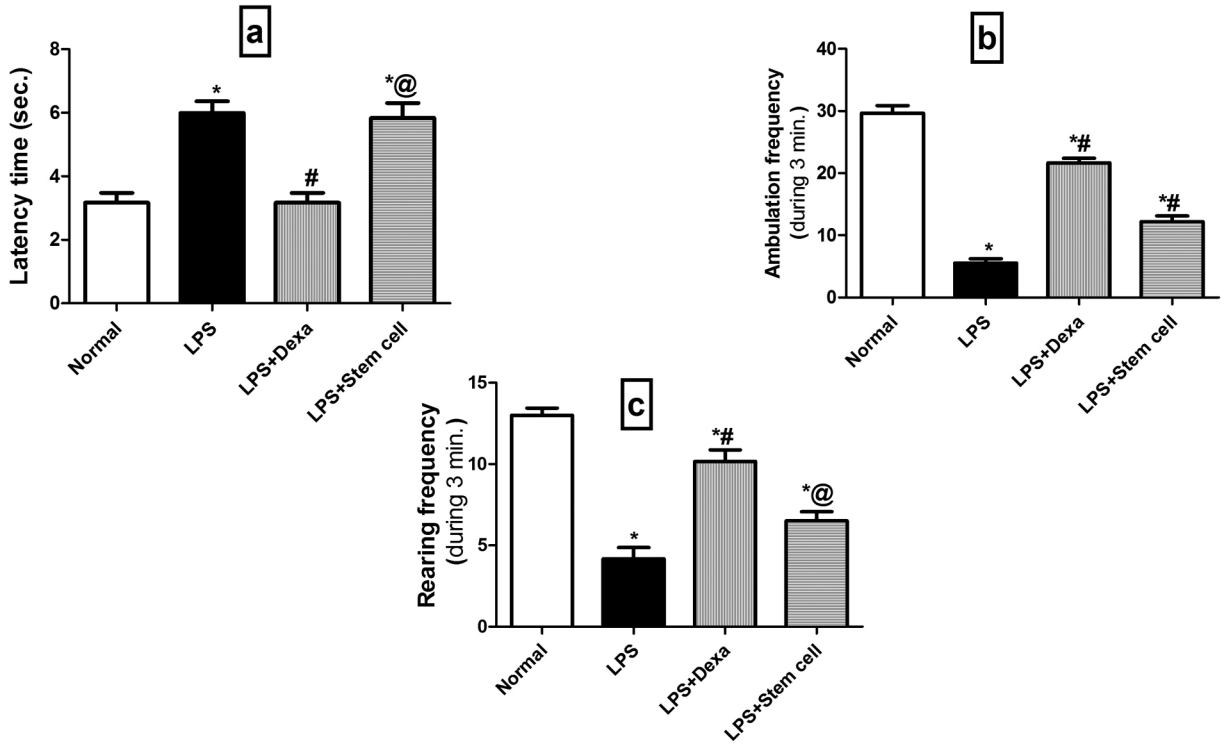
All in-vivo results were presented as mean±SEM. Data were expressed as the mean±SEM. Statistical analysis was carried out by one-way analysis of variance followed by Tukey–Kramer multiple comparisons test to calculate significance of the difference between treatments. Values of P less than 0.05 were considered significant. Statistical analysis was done using GRAPHPAD PRISM software (version 5; San Diego, California, USA).

Results

The behavioral test

In the open field test, the latency time (Fig. 1a), the ambulation (Fig. 1b), and rearing (Fig. 1c) frequencies of normal group were 3.17±0.29 s, 29.67±1.22, and

Figure 1



The neuroprotective effects of DEX and MSCs on latency time (a) ambulation (b) and rearing (c) frequencies against LPS-induced brain injury in rats. Each bar represents means (n=6)±SE. Statistical analysis was carried out by one-way analysis of variance followed by Tukey–Kramer multiple comparison test. *Significantly different from normal group at $P<0.05$. #Significantly different from LPS-infected group (positive control) at $P<0.05$. @Significantly different from DEX-treated group at $P<0.05$. DEX, dexamethasone; LPS, lipopolysaccharide; MSC, mesenchymal stem cell.

13.00±0.45, respectively. The latency time significantly increased in positive control group by 87% accompanied with a significant decrease in the ambulation and rearing frequencies by 82 and 68%, respectively, as compared with normal rats. As compared with the positive control group (LPS-treated group), treatment with DEX significantly decreased latency time by 47%, with a significant increase in ambulation and rearing frequencies by 3 and 1.5 folds, respectively; in addition, treatment with MSCs improved only ambulation frequency by 122%.

Hematological parameters

As illustrated in Table 1, infection of rats with LPS causes significant increment in leukocytes and neutrophils by 78 and 105%, respectively, accompanied by a significant decline in monocytes and lymphocytes by 43 and 46%, respectively, as compared with normal group. Both DEX and MSCs significantly decreased leukocytes and neutrophils by 42 and 46% and by 35 and 36%, respectively. In addition, DEX and MSCs significantly increased only lymphocytes by 129 and 112% respectively. However, DEX alone increased monocytes by 67% as compared with LPS-treated group.

Oxidative stress markers

Figure 2 depicts the cerebral and spleen contents of MDA (a,d) and GSH (b,e) for normal rats, which were 9.15±0.14 and 2.86±0.17 (nmol/g wet tissue), and 25.48±0.34 and 6.05±0.07 (mg/g wet tissue), respectively. The cerebral and spleen activities of SOD (c,f) of normal rats were 77.67±0.63 and 119.60±4.82 (U/g wet tissue, respectively. We found that LPS-induced oxidative stress in rats as evidenced by significant decrease in cerebral and spleen contents of GSH by 27 and 59%, respectively, and activity of SOD by 12 and 48%, respectively, whereas LPS caused a significant increment in cerebral and spleen MDA content by 79 and 93%, respectively, as compared with the normal group. In addition, treatment with DEX increased cerebral and spleen contents of GSH and SOD activities significantly by 37, 136, 11, and 78% respectively, as compared with LPS-treated group. On the other hands, treatment with MSCs increased cerebral and spleen contents of GSH and SOD activities significantly by 35, 252, 8.5, and 81% respectively, as compared with LPS-treated group. Moreover, as compared with DEX-treated group, administration of MSCs caused significant augmentation in spleen content of GSH by 49%.

- treatment with DEX

	Brain	Spleen
GSH content	↑37%	↑136%
SOD activity	↑11%	↑78%

- treatment with MSCs

	Brain	Spleen
GSH content	↑35%	↑252%
SOD activity	↑8.5%	↑82%

Inflammatory markers

As represented in Table 2, LPS-induced inflammation as evidenced by significant increase in cerebral and splee contents of IL-1β and MPO activities by 6 and 5 folds and 67 and 40%, respectively, as compared with normal group. Treatment with DEX and MSCs significantly decreased both cerebral and spleen contents of IL-1β and MPO activities by 60, 60, 67, and 70% and 51, 34, 58, and 49%, respectively, as compared with LPS-treated group. Moreover, treatment with MSCs significantly decreased cerebral and spleen contents of IL-1β by 19 and 26%, respectively, as compared with DEX-treated group.

Neurotransmitters

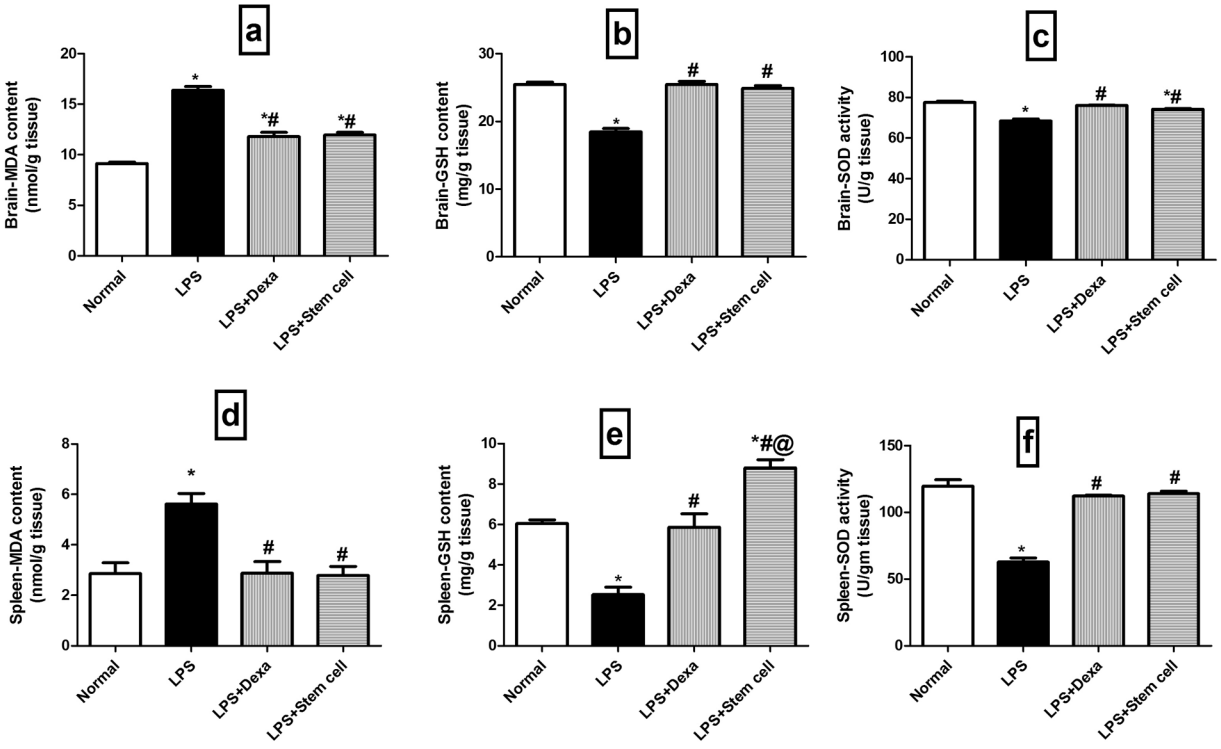
As illustrated in Table 3, we found that LPS-induced significant decline in cerebral contents of 5-HT, DA, and NE by 25, 27, and 25%, respectively, as compared

Table 1 The effects of dexamethasone and MSCs on total and differential leukocytic count in LPS-induced injury in rats

Groups	Parameters			
	Total leukocytic count ($\times 10^3$/cmm)	Peripheral blood neutrophils (%)	Peripheral blood monocytes (%)	Peripheral blood lymphocytes (%)
Normal	7.92±0.18	23.83±0.83	5.33±0.33	66.67±0.71
LPS	14.35±0.46*	49.33±0.88*	3.00±0.26*	23.83±1.25*
LPS+DEX	8.28±0.29#	31.67±1.41*#	5.00±0.36#	54.83±3.54*#
LPS+MSCs	7.68±0.53#	31.33±1.78*#	4.17±0.31	50.67±2.22*#

Each value represents means (n=6)±SE. Statistical analysis was carried out by one-way analysis of variance followed by Tukey–Kramer multiple comparison test. DEX, dexamethasone; LPS, lipopolysaccharide; MSC, mesenchymal stem cell. *Significantly different from normal group at $P<0.05$. #Significantly different from LPS-infected group (positive control) at $P<0.05$.

Figure 2



The neuroprotective effects of DEX and MSCs on cerebrum and spleen malondialdehyde (MDA; a,d), reduced glutathione (GSH; b,e) contents, and superoxide dismutase (SOD; c,f) activities against LPS-induced brain-spleen injury in rats. Each bar represents means (n=6)±SE. Statistical analysis was carried out by one-way analysis of variance followed by Tukey–Kramer multiple comparison test. *Significantly different from normal group at $P<0.05$. #Significantly different from LPS-infected group (positive control) at $P<0.05$. @Significantly different from DEX-treated group at $P<0.05$. DEX, dexamethasone; LPS, lipopolysaccharide; MSC, mesenchymal stem cell.

Table 2 The effects of dexamethasone and MSCs on IL-1β content and myeloperoxidase activity against LPS-induced brain-spleen injury in rats

Groups	Parameters			
	Brain IL-1β (pg/g tissue)	Spleen IL-1β (pg/g tissue)	Brain MPO (U/g tissue)	Spleen MPO (U/g tissue)
Normal	2.19±0.07	5.67±0.29	18.68±0.93	36.58±1.87
LPS	15.11±0.55*	31.71±0.81*	31.27±1.12*	51.45±1.63*
LPS+DEX	6.26±1.23*#	12.77±0.39*#	15.34±1.36#	33.70±2.73#
LPS+MSCs	4.96±0.03*#@	9.47±0.19*#@	13.08±1.01*#	26.04±1.65*#

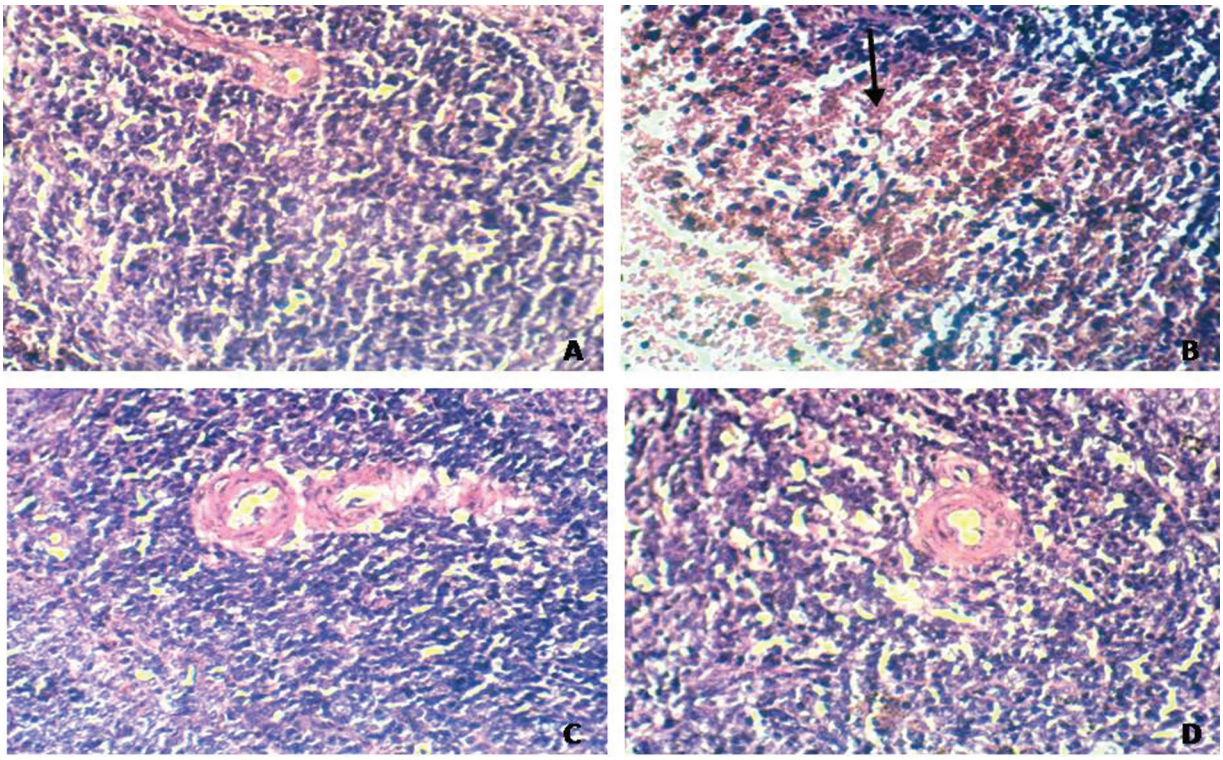
Each value represents means (n=6)±SE. Statistical analysis was carried out by one-way analysis of variance followed by Tukey–Kramer multiple comparison test. DEX, dexamethasone; IL, interleukin; LPS, lipopolysaccharide; MPO, myeloperoxidase; MSC, mesenchymal stem cell. *Significantly different from normal group at $P<0.05$. #Significantly different from LPS-infected group (positive control) at $P<0.05$. @Significantly different from DEX-treated group at $P<0.05$.

Table 3 The effects of dexamethasone and MSCs on neurotransmitters contents in LPS-induced brain injury in rats

Groups	Parameters		
	5-HT content (μg/g tissue)	DA content (μg/g tissue)	NE content (μg/g tissue)
Normal	0.85±0.03	33.04±0.55	2.84±0.10
LPS	0.64±0.04*	23.83±1.11*	2.09±0.03*
LPS+DEX	0.71±0.03*	26.70±0.77*	2.35±0.05*
LPS+MSCs	0.71±0.02*	27.84±0.80*#	2.38±0.09*

Each value represents means (n=6)±SE. Statistical analysis was carried out by one-way analysis of variance followed by Tukey–Kramer multiple comparison test. 5-HT, serotonin; DA, dopamine; DEX, dexamethasone; LPS, lipopolysaccharide; MSC, mesenchymal stem cell; NE, norepinephrine. *Significantly different from normal group at $P<0.05$. #Significantly different from LPS-infected group (positive control) at $P<0.05$.

Figure 3



The microscopic appearance of rat spleen tissue: (a) normal group showing normal lymphoid follicle. (b) LPS treatment shows focal splenic hemorrhage, necrosis, and lymphocytic depletion. (c and d) DEX or MSCs treatments correspondingly showed reduced inflammatory cells infiltration (hematoxylin and eosin, ×400). DEX, dexamethasone; LPS, lipopolysaccharide; MSC, mesenchymal stem cell.

with normal group. However, treatment with MSCs only improved DA content by 17% as compared with LPS-treated group.

Histopathological examinations results

Figure 3 illustrates the microscopic appearance of rat spleen tissue: (a) normal group showing normal lymphoid follicle, (b) LPS treatment shows focal splenic hemorrhage, necrosis, and lymphocytic depletion; and (c and d) treatments with DEX or MSCs correspondingly showed reduced inflammatory cell infiltration (H&E, ×400).

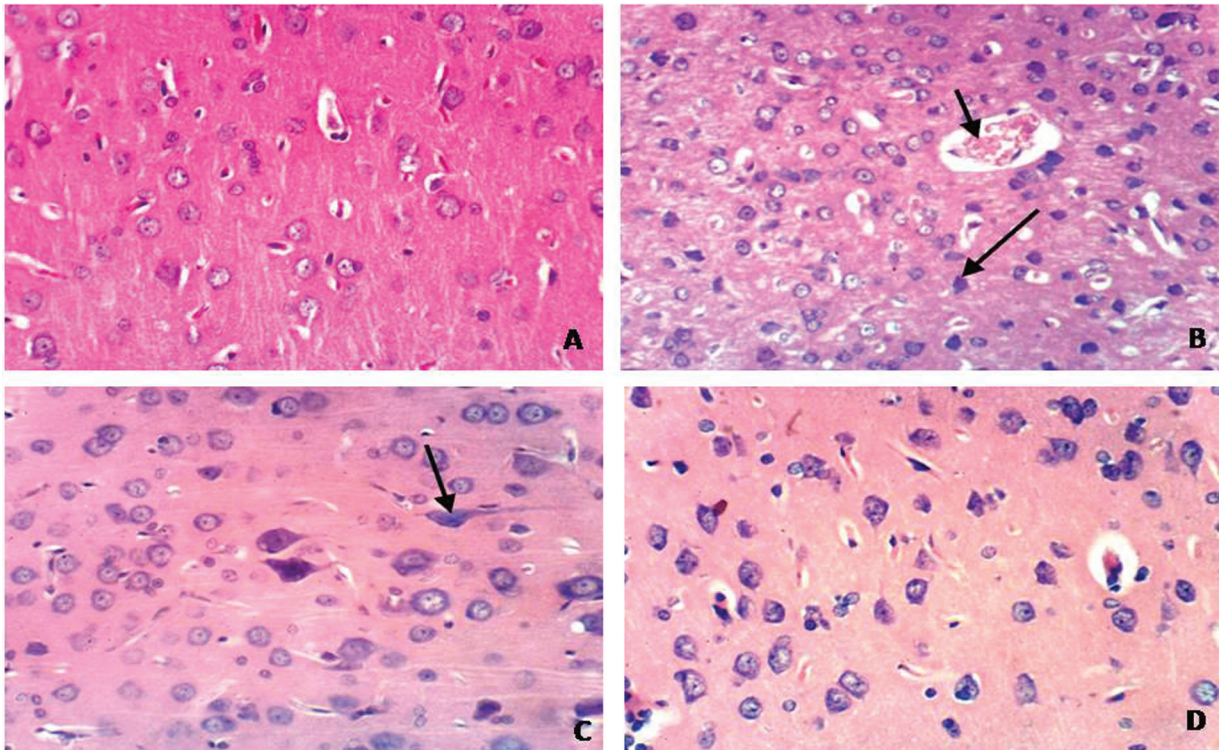
Figure 4 demonstrates the photomicrographs of rat cerebral cortex sections: (a) normal control group showed no histopathological alterations. Sections of rat treated with LPS (b) showed congestion of cerebral blood vessel, necrosis, and pyknosis of neurons. Section of rat treated with DEX (c) showed necrosis and pyknosis of some neurons. MSCs-treated group showed normal histopathological appearance (d) (H&E, ×400).

Figure 5 shows photomicrographs of rat hippocampus sections: (a) normal control group showed no histopathological changes, (b) sections of rat treated with LPS showed necrosis of neurons, and (c and d) hippocampal section of rat treated with DEX or MSCs correspondingly showed normal histopathological appearance (H&E, ×400).

Discussion

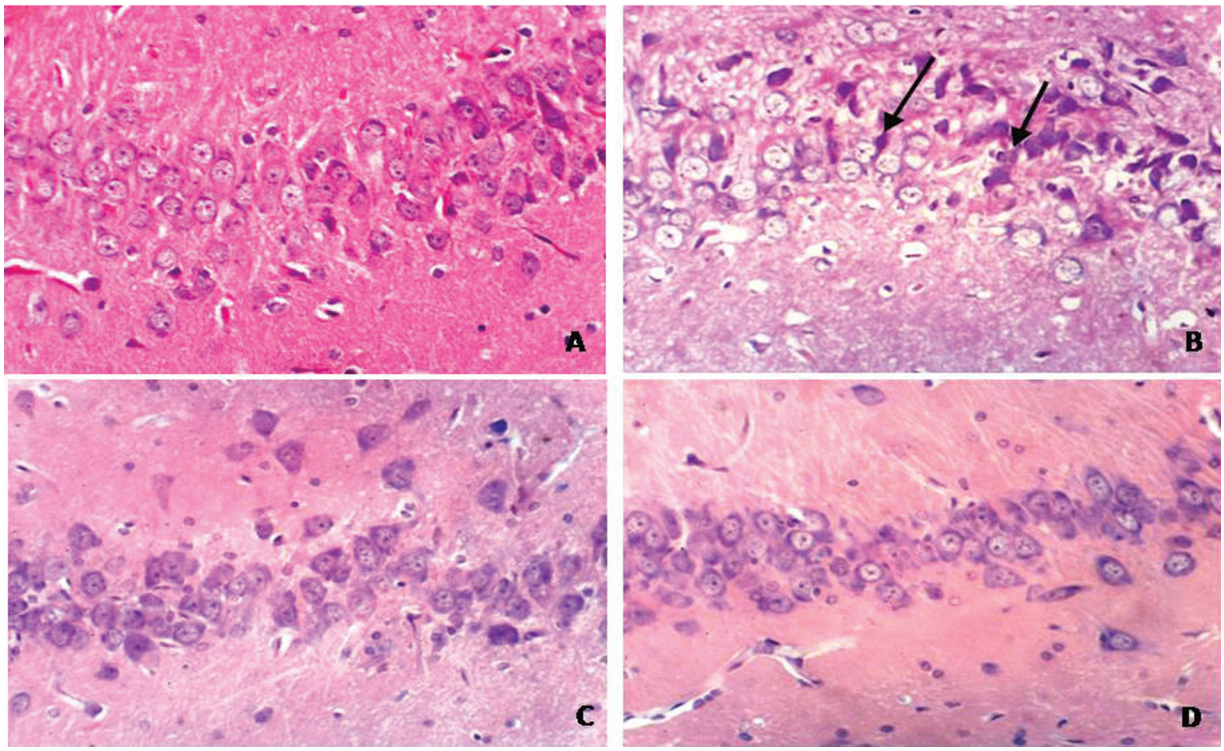
Endotoxin, or more accurately termed bacterial LPS, is recognized as the most potent microbial mediator implicated in the pathogenesis of sepsis and septic shock. The sudden release of vast quantities of LPS into the bloodstream is evidently deleterious to the host, initiating the release of a dysregulated and potentially lethal array of inflammatory mediators in the systemic circulation [38]. In the present study, intranasally injected LPS increased secretion of inflammatory cytokine IL-1β and increased activity of MPO in the brain and spleen tissues. Moreover, subsequent oxidative stress insult was noticed, resulting in an increased content of MDA and decreased GSH and SOD. Similarly, the results of Pallarès *et al.* [39] revealed that LPS stimulates the inflammatory response, resulting in the secretion of cytokines such as IL-6, IL-1β, IL-10, and tumor necrosis factor-α by the immune cells. Both the expressions of proinflammatory cytokines and oxidative stress have been implicated in brain damage and consequently poor motor performance [40].

Figure 4



Photomicrographs of rat cerebral cortex sections: (a) normal control group showed no histopathological alterations. Sections of rat treated with LPS (b) showed congestion of cerebral blood vessel, necrosis, and pyknosis of neurons. Section of rat treated with DEX (c) showed necrosis and pyknosis of some neurons. MSCs-treated group showed normal histopathological appearance (d) (hematoxylin and eosin, ×400). DEX, dexamethasone; LPS, lipopolysaccharide; MSC, mesenchymal stem cell.

Figure 5



Photomicrographs of rat hippocampus sections: (a) normal control group showed no histopathological changes, (b) sections of rat treated with LPS showed necrosis of neurons. (c and d) Hippocampal section of rat treated with DEX or MSCs correspondingly showed normal histopathological appearance (hematoxylin and eosin, ×400). DEX, dexamethasone; LPS, lipopolysaccharide; MSC, mesenchymal stem cell.

Furthermore, LPS-administered rats showed significantly reduced brain content of neurotransmitters and were consequently associated with behavior changes, including prolonged open field latency time and reduced ambulation and rearing frequencies. Such behavioral deficits were reflected as impaired motor activity, excitability, and exploratory behavior in rodents [41]. LPS significantly reduced NE, DA, and 5-HT concentrations in brain cortex [42,43]. Yegla and Foster [44] had referred this effect in LPS-treated rats, exhibiting as a sickness behavior response, manifesting as longer response latencies, cognitive slowing, and impairment of attention. They suggested that acute activation of the peripheral immune system, via LPS injection, induces cognitive dysfunction mediated by neuronal inflammation.Upon tissue injury, MSCs migrate to the damaged organ and this suggested that MSCs could provide an ideal cell source for repair of injured organs including the CNS [45,46].

The present study pointed to the MSCs' remarkable immunomodulatory effect on the brain and spleen tissue contents of IL-1β and MPO activity. Similar studies revealed that treatment with MSCs showed reduced MPO^+ cell counts [47–49]. The immunomodulatory status induced by MSCs therapy was associated with a potent in-vivo antioxidant effect in LPS sick rats, as significant elevation of both brain and spleen tissues contents of GSH and SOD was concomitant with significant decrease of MDA. Our results are consistent with Lanza *et al.* [50] MSCs secrete antioxidant factors, including SOD, which promotes neuronal survival [51].

Based on the results of this study, it seems possible that MSCs normalize the neurotransmitters serotonin, DA, and NE contents in brain tissue of the LPS rat model. Similar findings were attained that MSCs protect catecholaminergic and serotonergic neuronal perikarya and transporter function from oxidative stress [52]. MSCs provide localized neuroprotection in an inflammation-driven rat model assessing the ability to protect against LPS-induced substantia nigra pronounced neuroinflammation, which resulted in partial loss of nigrostriatal dopaminergic neurons [53]. MSCs infusion may produce neurotrophic factors allowing partial recovery of dopaminergic pathway [54]. MSCs exhibit neuroprotective effects against hypoxic brain injury through exerting antioxidative effects and providing energy to the brain, which was revealed as significantly increased 5-HT level in the rat cortex and midbrain of MSCs-treated groups [55]. Moreover, MSCs are likely to be beneficial based on their ability to improve behavioral outcomes [56,57].

Conclusion

Our results confirmed the immune-modulatory effects of MSCs in the treatment of LPS-induced inflammatory brain and spleen damage as compared with dexamethasone. Stem cell therapy can clearly modulate the immune response, not only in the peripheral lymphoid organs but also within the CNS. The use of MSCs, which can differentiate into multiple cell types, including neurons, is an attractive idea of regenerative medicine, in particular, for neurodegenerative disorders through antioxidative stress influence and inflammatory cytokine alterations, affecting neurotransmitters balance and improving histological inflammatory criteria in the brain and the spleen.

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The manuscript has been read and approved by all the authors, that the requirements for authorship as stated earlier in this document have been met, and that each author believes that the manuscript represents honest work.

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Conflicts of interest

There are no conflicts of interest.

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