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Morin decreases cortical pyramidal neuron degeneration via inhibition of neuroinflammation in mouse model of schizophrenia

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ABSTRACT

Neuroinflammation plays a prominent role in the pathophysiology and progression of schizophrenia. Thus, suppression of neuroinflammation may retard the progression of the disease. This study was designed to investigate whether morin, a bioactive compound with antipsychotic-like activity could reduce biomarkers of neuroinflammation and neurodegeneration in lipopolysaccharide (LPS)- and ketamine (KET)-induced schizophrenic-like behavior in mice. Animals were treated once daily intraperitoneally with morin (100 mg/kg), haloperidol (1 mg/kg), risperidone (0.5 mg/kg), or saline (10 mL/kg) in combination with LPS (0.1 mg/kg) for 14 consecutive days. However, from days 8–14, overt schizophrenia-like episode was produced with i.p. injection of KET (20 mg/kg) once daily. Schizophrenic-like behaviors: positive (open-field test), negative (social-interaction and social-memory tests) and cognitive (Y-maze test) symptoms were assessed on day 14. Thereafter, the levels and expressions of biomarkers of neuroinflammation were estimated in the striatum (ST), prefrontal cortex (PFC) and hippocampus (HC) using spectrophotometry, ELISA and immunohistochemistry. The effects of morin on cortical pyramidal neurons were estimated using Golgi-impregnation staining technique. LPS in combination with KET significantly ($p < 0.05$) induced schizophrenia-like behaviors, which was attenuated by morin. Morin significantly ($p < 0.05$) decreased tumor necrosis factor-α, interleukine-6 levels and myeloperoxidase activity in the ST, PFC and HC of mice treated with LPS + KET. Moreover, morin reduced regional brain expressions of cyclooxygenase-2, inducible nitric oxide synthase and nuclear factor kappa-B, and also rescued loss of pyramidal neurons in the PFC. Taken together, these findings suggest that morin reduces schizophrenic-like symptoms induced by LPS + KET via mechanisms related to inhibition of the release of pro-inflammatory mediators and suppression of degeneration of cortical pyramidal neurons in mice.

1. Introduction

Schizophrenia is a severe form of psychotic disorder that affects about 1% of the World population [1]. The manifestation of schizophrenic symptoms in the late adolescent or adulthood [2,3] has been ascribed to neuroimmune, neuroprogressive or epigenetic risk factors [4]. Although the etiology of schizophrenia remains elusive, there have been growing evidences supporting both neurodegenerative and neuroprogressive hypotheses [5]. The neurodegenerative hypothesis epitomized by hyperdopaminergic subcortical activity accounts for the positive symptoms (e.g., hyperactivity) [6]. The negative (e.g., social withdrawal) and cognitive (e.g. memory impairment) symptoms are linked to the neuroprogressive hypothesis [5,7]. The neuroprogressive hypothesis states that schizophrenia may be related to neuroinflammation as shown by increased serum and brain concentrations of inflammatory cytokines and proteins [8–10]. Moreover, intrauterine, perinatal or prenatal exposure to infections that activate inflammatory signaling pathways may contribute to the onset of schizophrenia [11].

Epidemiological evidences have shown that offspring of mothers who were infected with bacteria or parasites including *Toxoplasma*

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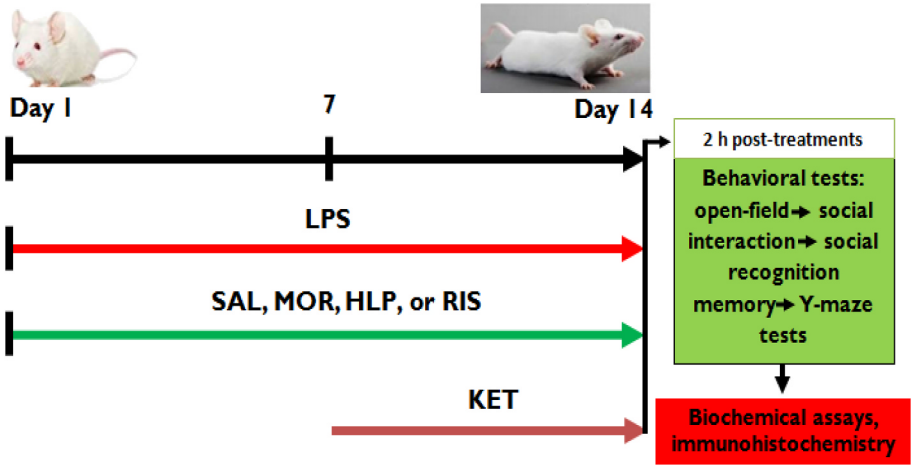


Fig. 1. Experimental protocol.

gondii during gestation showed increased vulnerability to schizophrenia during adulthood [12]. Similar findings have also been reported in individuals exposed to prenatal or postnatal infections with influenza and other viral pathogens [13]. Thus, systemic maternal or early childhood immune alterations may be involved in the genesis of the disease [14]. These evidences have led to the design of animal models involving administration of infectious agents to model certain features of schizophrenia [5,14,15]. However, lipopolysaccharide (LPS), a non-infectious component of a gram-negative bacteria is commonly used to induce behavioral and biochemical changes that characterized neuropsychiatric disorders including schizophrenia [11,15].

Preclinical studies have shown that systemic administration of LPS produced neuropsychiatric disorders via activation of innate immune system and release of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukine-6 (IL-6) [14,15]. Indeed, peripheral administration of TNF-α and IL-6 have been reported to cause neurobehavioral deficits such as social withdrawal [16] and amnesia [17]. Intraperitoneal injection of LPS has also been shown to alter cortical and subcortical dopaminergic and 5-hydroxytryptaminergic systems via increased release of TNF-α and IL-6 [18]. Besides, IL-6 has been implicated in the up-regulation of kynurenine, an endogenous antagonist of *N*-Methyl-D-aspartate (NMDA) receptor [19] suggesting altered glutaminergic system occurs in response to chronic infections or neuroinflammation [17,18]. Increased dopaminergic activity due to systemic administration of NMDA receptor antagonists has also been established [20,21]. This finding has led to the use of NMDA receptor antagonists such as ketamine (KET) for inducing schizophrenia-like symptoms in laboratory animals.

The use of KET as an animal paradigm of schizophrenia has gained increased recognition over the years [21–23]. Induction of schizophrenia-like behaviors by KET has been attributed to the blockade of NMDA receptors located on the inhibitory GABAergic (gamma amino butyric acid) neurons in the cortical and subcortical regions of the brain [21,22]. Previous studies have also shown that KET potentiates oxidative stress and neuroinflammatory responses in rodents pretreated with LPS during early life that orchestrate neurobehavioral changes relevant to schizophrenia [11,23]. These findings further implicate chronic inflammation in the neurobiology of schizophrenia. Neuroinflammation may also plays prominent roles in the cytoachitectural disorganization of neuronal populations including derangement of cortical pyramidal neurons that characterized the disease [17,24]. Thus, it has been proposed that compounds with anti-neuroinflammatory and neuroprotective activities may improve schizophrenic symptoms in clinical settings [10].

Morin is a naturally occurring bioactive flavonoid found in several fruits and vegetables [25]. Previous studies have revealed that morin exhibited immunomodulatory [26], anti-oxidant [27], and anti-cancer [28] properties. Morin was also shown to suppressed LPS-induced neuroinflammation [29] and attenuated diabetic-induced cognitive decline in rats [27]. It also protected against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced loss of dopaminergic neurons [29], and reduced amyloid beta-induced neurodegeneration [30]. We have reported in our previous studies that morin exhibited anti-psychotic-like activity [31] and prevented neurobehavioral deficits in sleep deprived mice [32]. Morin was also shown in our recent study to have prevented and reversed KET-induced schizophrenia-like behavior via enhancement of GABAergic neurotransmission and brain derived neurotrophic factor (BDNF), and suppression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-2 (Nox-2)-induced oxidative damage in mice [24]. This present study was designed to determine whether morin could influence schizophrenic-like behavior, biomarkers of neuroinflammation and neurodegeneration induced by combination of LPS and KET in mice.

2. Materials and methods

2.1. Laboratory animals

Male Swiss mice were obtained at 6 weeks old from the Central Animal House of the College of Medicine of University of Ibadan, Ibadan, Nigeria. They were kept in plastic cage (42 × 30 × 27 cm) at standard conditions with a 12-h light/dark cycle. They were allowed to have access to standard rodent pellet food and water ad libitum throughout the period of experiments. The experimental procedures were approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/App/12/2016/01).

2.2. Drug and treatments

Morin [MOR (100 mg/kg)], haloperidol [HLP (1 mg/kg)], risperidone [RIS (0.5 mg/kg)] (Sigma-Aldrich, St. Louis, USA) were dissolved in normal saline and administered intraperitoneally (i.p.). LPS [lipopolysaccharide, *Escherichia coli* serotype, 055:B5; (0.1 mg/kg, i.p.)] (Sigma-Aldrich, St. Louis, MO, USA) and ketamine hydrochloride [KET (20 mg/kg, i.p.)] (RotexMedica, Germany) were dissolved and diluted in normal saline respectively. Saline [SAL (10 mL/kg)] solution was also administered intraperitoneally, as normal control. The doses of MOR [24], HLP [24], RIS [22], LPS [11,23], and KET [7] used in this study were based on results obtained from previous studies and preliminary investigations.

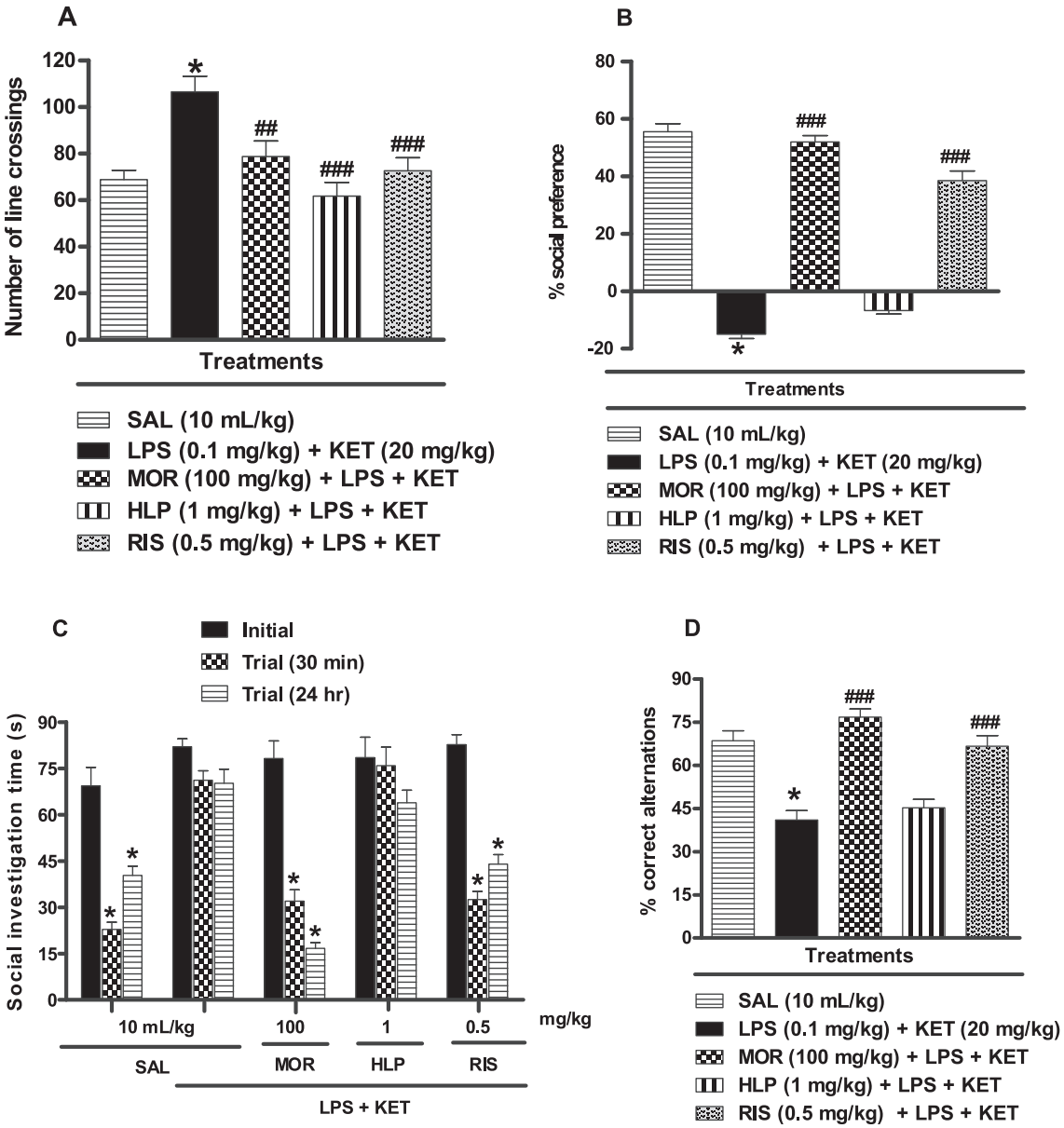


Fig. 2. Morin attenuates lipopolysaccharide and ketamine-induced behavioral alterations in mice. (A) Hyperlocomotion in open field test; (B) Social withdrawal in social interaction test; (C) Social recognition memory deficit in social memory test [C Bars represent the mean ± SEM (n = 8 animals/group). *p < 0.05 compared to initial exposure (two-way ANOVA followed by Bonferroni *post-hoc* test)]; (D) Spatial working memory impairment in Y-maze test. A, B and D Bars represent the mean ± SEM (n = 8 animals/group). *p < 0.05 compared to SAL group and $^{\#\#}p$ < 0.01, $^{\#\#\#}p$ < 0.001 compared to LPS + KET group (one-way ANOVA followed by Bonferroni *post-hoc* test). SAL = Saline, LPS = Lipopolysaccharide, KET = Ketamine, MOR = Morin, HLP = Haloperidol, RIS = Risperidone.

2.3. Experimental design

The effect of MOR on LPS and KET-induced schizophrenia-like behavior was evaluated in mice using modified protocols of preventive and reversal models of schizophrenia [22]. In the preventive protocol, repeated neuroimmune activation was induced with LPS [purified lyophilized *Escherichia coli* endotoxin (serotype, 055:B5; 0.1 mg/kg, i.p.)] [11,23], and overt schizophrenia-like episode was produced using sub-chronic dose of KET (20 mg/kg, i.p.) in the reversal protocol [7,22]. However, individual groups of LPS or KET [7,11] alone were not included in this study because of ethical purposes and for their well established effects in literature [7,11]. Briefly, following randomization into 5 treatment groups (n = 8 mice/group); the animals in group 1, which served as normal control received SAL (10 mL/kg, i.p.) while group 2, which served as negative control was given LPS (0.1 mg/kg, i.p.) daily for 14 days. However, mice in group 3 received MOR (100 mg/kg, i.p.), group 4 had HLP (1 mg/kg, i.p) while group 5 received RIS (0.5 mg/kg, i.p.) along with LPS for 14 consecutive days. However, 30 min later, the animals in groups 2–5 were given a daily dose of KET (20 mg/kg, i.p.) from days 8–14 (Fig. 1).

2.4. Behavioral tests

Two hours after the last treatment on day 14, schizophrenia-like behavioral phenotypes were assessed using open-field, social interaction, social recognition memory and Y-maze tests. The behavioral tests were carried out by trained observers who were blind to the test groups between 8:30 a.m. and 1:00 p.m., with 30 min in between each test to reduce stress and were carried out in the following order: open-field, social interaction, social recognition memory and Y-maze tests.

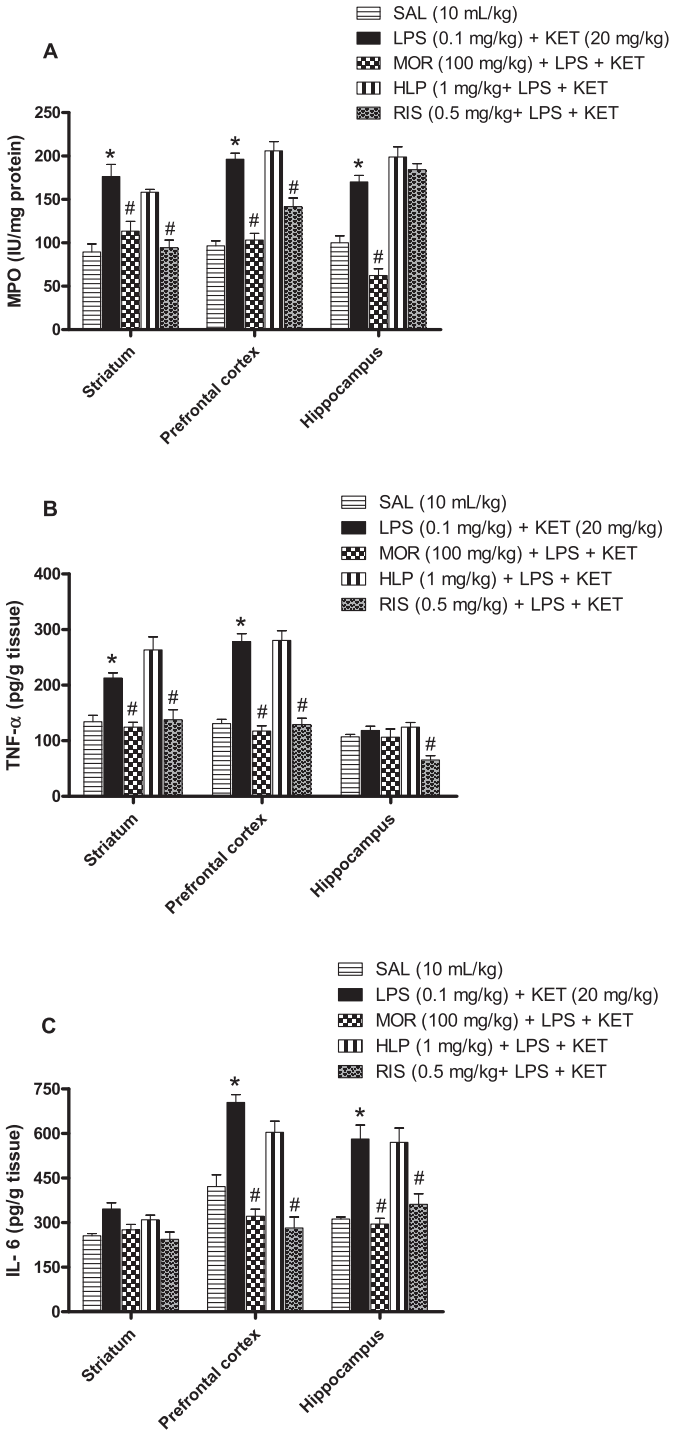


Fig. 3. Morin inhibits the release of inflammatory enzyme and proinflammatory cytokines in lipopolysaccharide and ketamine treated mice.(A) Myeloperoxidase (MPO) enzyme activity; (B) Tumor necrosis factor-alpha (TNF-α); (C) Interleukin-6 (IL-6).A, B and C Bars represent the mean ± SEM ($n = 8$ animals/group). $^{*}p < 0.05$ compared to SAL group and $^{\#}p < 0.05$ compared to LPS + KET group (two-way ANOVA followed by Bonferroni *post-hoc* test). SAL = Saline, LPS = Lipopolysaccharide, KET = Ketamine, MOR = Morin, HLP = Haloperidol, RIS = Risperidone.

2.4.1. Open-field test (OFT)

The effect of MOR on locomotion activity in mice was assessed in an open-field chamber. The OFT apparatus consists of a wooden box measuring 35 × 30 × 23 cm with visible lines drawn to divide the floor into 36 (20 cm × 20 cm) squares with a frontal glass wall, and placed in a sound free room. Before each test, animals were kept in the test room at least 1 h before the open-field test to avoid environmental stress on outcome. Mice were placed individually in the rear left square and allowed to explore the chamber freely. The number of squares crossed with all paws (crossing) were observed and counted for 5 min [22]. The observation cage was cleaned with 70% ethanol after each assessment to remove olfactory cue from previous animal.

2.4.2. Social interaction test (SIT)

The effect of MOR on social withdrawal was assessed using the SIT model. The testing chamber consisted of a Plexiglas box (60 × 40 cm) divided into three chambers (A, B and C). Mice moved between the chambers through a small opening (6 × 6 cm) in the dividers. An iron restraining cage was placed in each of the two side chambers (A and C), with chamber A containing the probe mice. The test (experimental) mouse was placed in the center chamber (chamber B) and allowed 5 min of exploration time in all the chambers. At the end of the 5 min, an unfamiliar mouse was placed in one of two restraining cages in chamber A, while chamber C was without mouse. Afterwards, the test mouse was again placed in chamber B and allowed to explore between chamber A and chamber C in the social test box for a period of 5 min. The time spent in the two chambers, A and C was estimated as the % time spent in the social chamber − % time spent in the opposite chamber. After each test session, the observation apparatus was cleaned with 70% ethanol to remove sensational residual odor [22].

2.4.3. Social recognition memory assessment

The effect of MOR on social recognition memory was assessed as described by Gao et al., [33]. The test is based on the increased tendency of an experimental mouse to explore a non-experimental (unfamiliar) mouse at first exposure and the decrease in the duration of exploration upon second exposure (fixed inter-trial intervals). The decrement in exploration time between the first and second exposures is used as an indication of social recognition memory. Social recognition memory test (SMT) was conducted in an observation chamber measuring 29 × 18 × 12 cm. The SMT consists of two exposure sessions: the pretest (training; first exposure) session and test (second exposure) session. Prior to the first exposure, test mice (experimental mice) and unfamiliar mice (non-experimental social interacting mice) were brought to the observation room and were left to acclimatize to the new environment for 1 h. The training session was carried out by placing each experimental mouse into an observation chamber (29 × 18 × 12 cm) and allowed to habituate to the test environment for 15 min. Thereafter, the non-experimental (unfamiliar) mouse was placed into the same observation chamber with the experimental mouse. The duration of social investigatory behaviors, which include direct contact or sniffing, nosing, grooming, pawing or inspection of body parts of the unfamiliar (non-experimental) mouse were scored by a trained observer for a period of 5 min. After the first exposure (training session), the unfamiliar mice were returned to their home cages for an interval of 30 min (for short term memory) and 24 h (for long term memory; consolidation of social recognition memory). The re-exposure (test sessions) were carried out 30 min and 24 h after the pretest session between the same experimental and non-experimental counterparts. The time spent(s) by the experimental mice in exploration of unfamiliar mice at inter-trial intervals were measured. The social recognition memory was defined as a decrement in the exploration time between the first and second (fixed inter-trial intervals) exposures for the short- and long-term social recognition memories [33]. The test box was also cleaned with 70% ethanol to avoid instinctive odorant cues from previous animal to the next.

2.4.4. Y-maze test (YMT)

The effect of MOR on spatial working memory was assessed using YMT based on changes in spontaneous alternation behavior. The

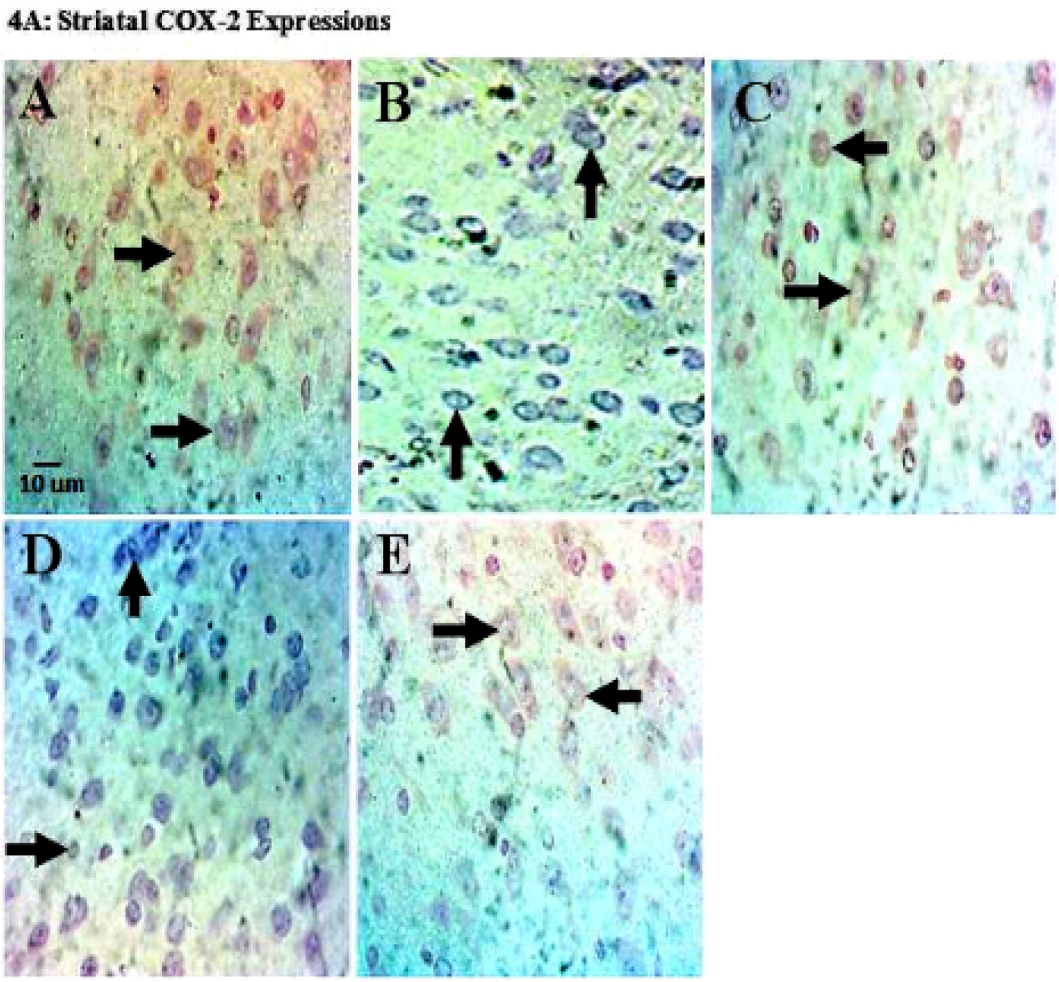


Fig. 4. Representative photomicrographs of the effect of morin on lipopolysaccharide and ketamine-induced immunohistochemical changes and expressions of COX-2 immunopositive cells in the *striatum* (A), *prefrontal cortex* (B) and *hippocampus* (C) of mice brains. A = SAL 10 mL/kg, B = LPS 0.1 mg/kg + KET 20 mg/kg, C = MOR 100 mg/kg + LPS + KET, D = HLP 1 mg/kg + LPS + KET, and E = RIS 0.5 mg/kg + LPS + KET. Vertical arrow indicates: High immunopositive cell expression and Horizontal arrow indicates: Low immunopositive cell expression. SAL = Saline, LPS = Lipopolysaccharide, KET = Ketamine, MOR = Morin, HLP = Haloperidol, RIS = Risperidone.

animals were placed individually in the Y-maze apparatus, which consisted of three identical arms (33 × 11 × 12 cm each) symmetrically separated at 120°. Each mouse was placed at the end of arm A, and allowed to explore all the three arms (labeled A, B, C) freely and the number of arm visits and sequence (alternation) of arm visits were observed for 5 min. Alternation behavior was defined as consecutive entries into all the three arms (i.e., ABC, CAB or BCA but not ABA, BAB or CAC). The percentage of alternation was calculated as total alternation number/(total number of entries − 2) × 100 [22]. After each test session, the observation chamber was cleaned with 70% ethanol to remove residual odor.

2.5. Preparation of brain tissues for enzyme-linked immunosorbent assays (ELISA)

After the behavioral assessments, mice in respective groups were sacrificed under deep ether anesthesia as previously described [24], and the brains were immediately harvested and weighed. Thereafter, specific brain regions: striatum (ST), prefrontal cortex (PFC) and hippocampus (HC) were isolated out on flat ice tray, weighed, homogenized and centrifuged at 5400*g* for 10 min at 4 °C. The supernatants were immediately frozen and stored at −80 °C until they are needed for assay.

2.5.1. Estimation of brain tumor necrosis factor-alpha (TNF-α)

Specific brain regions (ST, PFC and HC) of TNF-α concentration were estimated using ELISA MAX™ Deluxe kit (BioLegend, USA; CAT NO 430904) according to the manufacturer's instructions. All reagents, standard solutions and samples were brought to room temperature before use. Briefly, TNF-α enzyme immunoassay was carried out by adding 100 µL of brain samples, standards and controls to each wells of an overnight (18 h, 4 °C) mouse TNF-α capture antibody incubated 96 well plate. After which, the plate was sealed with adhesive foil and incubated for 2 h room temperature (25 °C) on a shaker (approx. 500 rpm). Then, 100 µL of biotinylated goat polyclonal anti-mouse TNF-α detection antibody and avidin-horseradish peroxidase (avidin-HRP) solutions were added to each wells; the plate was sealed and incubated for 1:3 min at room temperature (25 °C) on a shaker (approx. 500 rpm). Thereafter, 100 µL of the chromogenic substrate [3, 3′, 5, 5′- tetramethylbenzidine (TMB)] was added to each well and incubated in the dark for 15 min at room temperature (25 °C) before the addition of stop solution (100 µL) and the absorbance was read at 450 nm within 15 min using Spectramax M-5 (Molecular Devices, Sunnyvale, CA) multifunctional microplate reader equipped with Softmax Pro v 5.4 (SMP 5.4). Thereafter, a log-log logistic 4-parameter curve-fitting was used to determine regional brain sample concentrations in pg/mL.

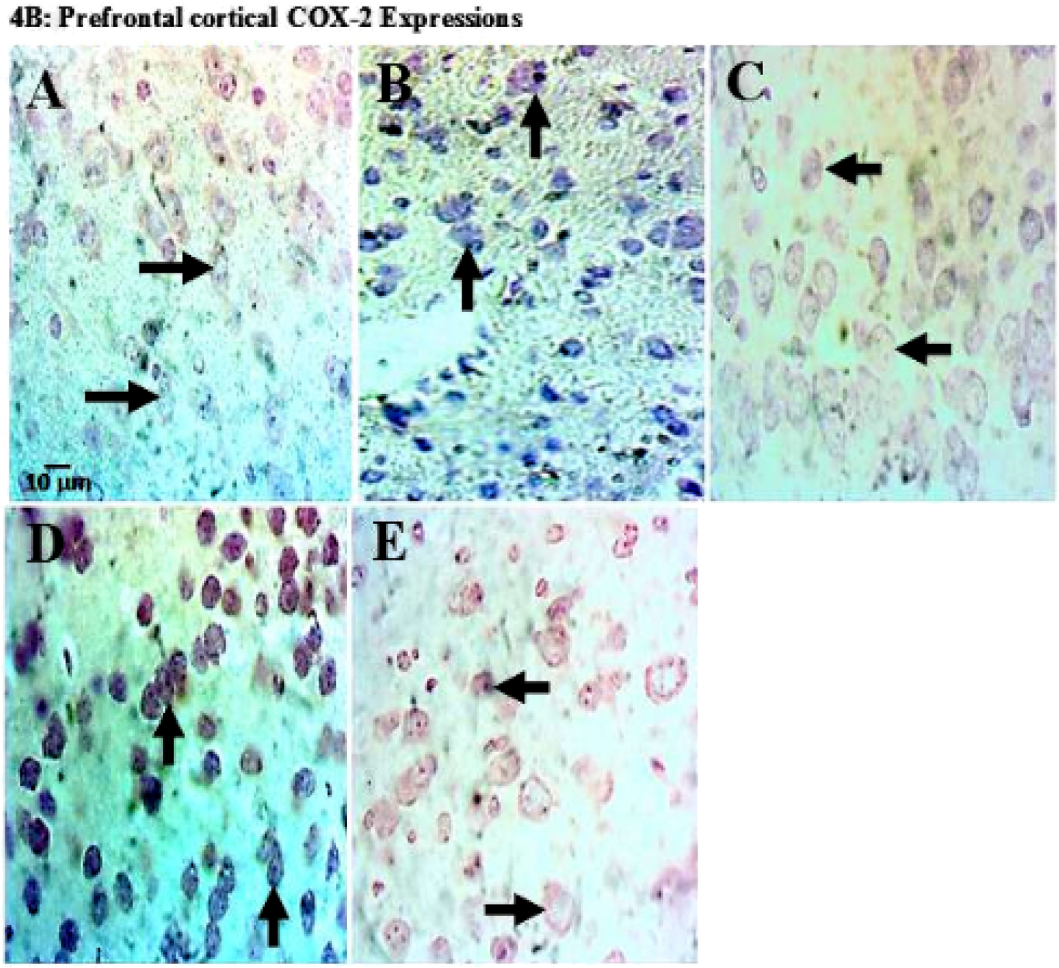


Fig. 4. (*continued*)

2.5.2. Determination of brain interleukin-6 (IL-6)

Regional brain IL-6 concentrations were also estimated using ELISA MAX™ Deluxe kit (BioLegend, USA; CAT NO 431304) according to the manufacturer's instructions. Briefly, all reagents, standard solutions and samples were brought to room temperature before use. IL-6 enzyme immunoassay was carried out by adding 100 µL of standards, control and regional brain samples to each wells of an overnight (18 h, 4 °C) mouse IL-6 capture antibody incubated 96 well plate. After which, microplate was sealed with adhesive foil and incubated for 2 h room temperature (25 °C) on a shaker (approx. 500 rpm). Then, biotinylated rat monoclonal anti-mouse IL-6 detection antibody (100 µL) and avidin-HRP (100 µL) solutions were added to each wells, and plates were sealed and incubated for 1:3 min at room temperature (25 °C) on a shaker (approx. 500 rpm). Thereafter, 100 µL of the chromogenic substrate (TMB) was added to each wells and incubated in the dark for 20 min at room temperature (25 °C). The absorbance was read at 450 nm within 15 min using Spectramax M-5 (Molecular Devices, Sunnyvale, CA) multifunctional microplate reader equipped with Softmax Pro v 5.4 (SMP 5.4), after the addition of 100 µL of stop solution on a shaker to achieve homogenous solutions. A log-log logistic 4-parameter curve-fitting was used to determine regional concentration of IL-6 for the ST, PFC and HC in pg/mL.

2.5.3. Determination of brain myeloperoxidase (MPO) activity

The myeloperoxidase activity was determined in the ST, PFC and HC according to the method described [34]. In brief, each brain supernatant was suspended in extraction buffer (0.5% hexadecyltrimethylammonium bromide in 50 mM potassium phosphate buffer; pH 6.0) and frozen at 20 °C. The process of freeze-thawed and sonication for 10 s cycle was repeated three times. The suspension was finally centrifuged at 15,000 rpm at 4 °C for 15 min. MPO activity was assayed by adding 0.2 mL of supernatant to 2.8 mL of mixed solution (containing 0.167 mg/mL O-dianisidine in 50 mM potassium phosphate buffer and 0.15 mM H_2O_2). The change in absorbance at 450 nm was monitored over 3 min using UV/VIS Spectrophotometer (INESA). One unit of MPO was defined as that giving a change in absorbance of 0.001 per min and the specific activity expressed as unit of MPO per milligram of protein.

2.5.4. Estimation of brain protein levels

Protein levels of the ST, PFC and HC was measured according to the method previously described [35] using the Biuret method. Accordingly, 1 mL of the diluted brain supernatant was collected and added to 3 mL of Biuret reagent in triplicate. The mixture was incubated at room temperature for 30 min and the absorbance was read at 540 nm using distilled water as blank. Bovine serum albumin (1 mg/mL) was used as standard and was measured in the range of 0.01–0.1 mg/mL.

2.6. Preparation of brain tissues for immunohistochemistry

Mice brains in the respective groups for immunohistochemistry were anaesthetized with ether and perfused transcardially with sterile phosphate buffered saline (PBS) and later with 10% buffered formaldehyde. Thereafter, brains were rapidly harvested, and fixed with 10% phosphate buffered formaldehyde at 25 °C for 48 h. The ST, PFC and HC (cornusammonis 1, CA1) of the brains were then subjected to

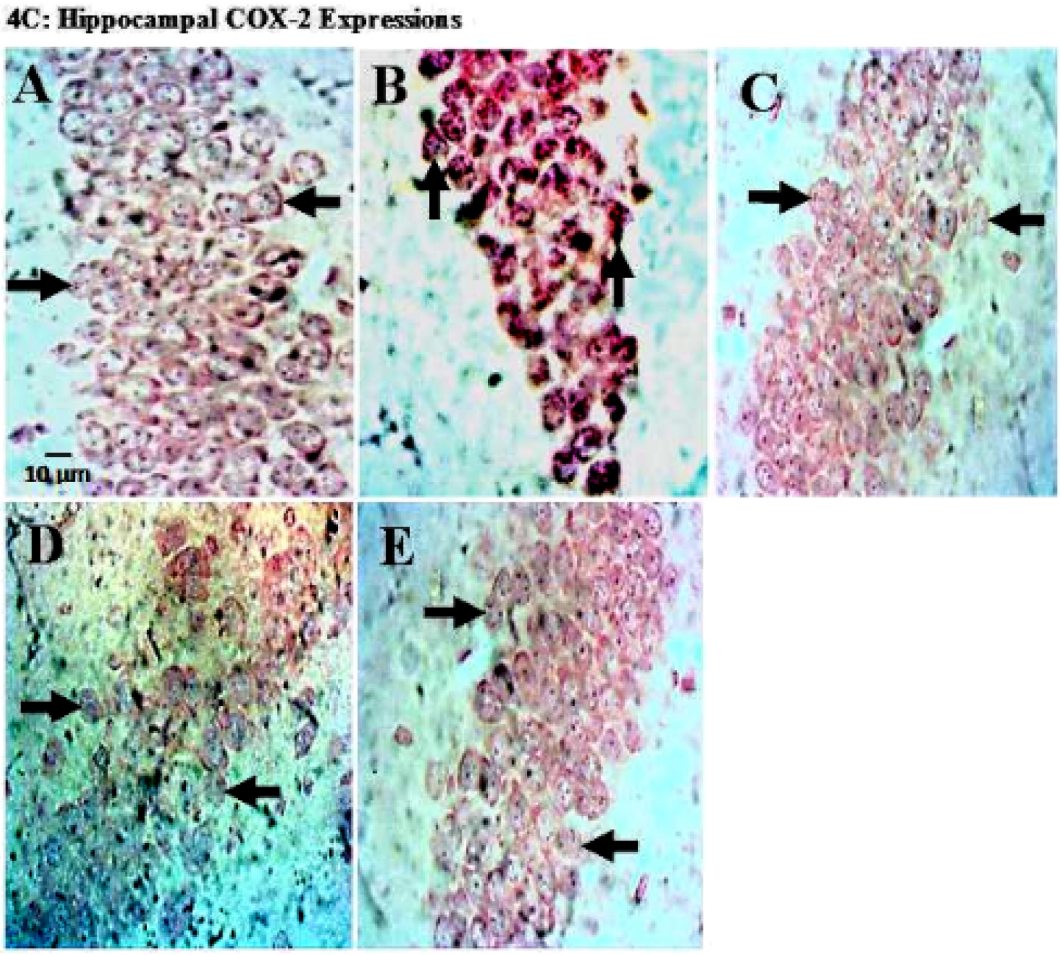


Fig. 4. (*continued*)

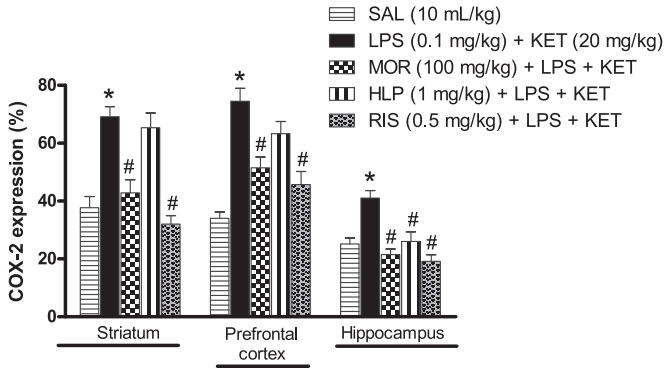


Fig. 5. Morin reduces the expressions of COX-2 immunopositive cells in the *striatum*, *prefrontal cortex* and *hippocampus* in lipopolysaccharide and ketamine treated mice. Bars represent the mean ± SEM ($n = 3$ animals/group). $^{*}p < 0.05$ compared to SAL group and $^{\#}p < 0.05$ compared to LPS + KET group (two-way ANOVA followed by Bonferroni *post-hoc* test). SAL = Saline, LPS = Lipopolysaccharide, KET = Ketamine, MOR = Morin, HLP = Haloperidol, RIS = Risperidone.

the routine method for paraffin wax embedment to get paraffin wax embedded tissue blocks. The expressions of cyclooxygenase-2 (COX-2), inducible nitric-oxide synthase (iNOS) and nuclear transcription factor-kappa B (NFκB) immunopositive cells of the ST, PFC and HC of mice treated with LPS and KET were determined using immunohistochemistry kits (Santa cruz, Germany) according to the manufacturer's protocol and modified method of Edelstein et al. [36]. Briefly, for each antibody, brain tissue sections [ST (caudate and putamen), PFC and HC (CA1) regions] were subjected to the process of deparaffinization and hydration using xylene and graded alcohols (100, 90 and 80%) for 5 min, respectively. Thereafter, slides were washed twice with normal saline and incubated with peroxidase block for 5–10 min at room temperature (25 °C). Tissue sections were rinsed with normal saline, placed in citrate buffer tank and heated in a water bath for 3–5 min for antigen retrieval. Slides were washed three times with PBS containing 0.02% Tween 20, before adding protein blocking solution for 5–10 min at room temperature (25 °C). Tissue sections were incubated with primary antibody (1:300) for 20–30 min at room temperature (25 °C). Afterwards, slides were incubated with one-step horseradish peroxidase polymer for 20–30 min at room temperature (25 °C), after rinsing with PBS 4–6 times and. Also, tissue sections were rinsed 4–6 times with PBS containing 0.02% Tween 20 and 2–3 times with normal saline. Few drops of ready to use 3, 3′-diaminobenzidine (DAB) reagent were added on each tissue samples and kept to incubate for 6–10 min at room temperature (25 °C) before washing with PBS 5–7 times and then with normal saline. Thereafter, slides were incubated with hematoxylin for 30–60 s, rinsed with normal saline and allowed to dry before mounting with suitable mountant. Images revealed using Leica ICC50 E Digital Camera (Germany) which was connected to a computer interface (MagnaFire) and an Olympus BX-51 Binocular research microscope, and expression of immunopositive cells were analyzed using Image J software (NIH, Bethesda, MD, USA) [36].

2.7. Golgi staining for pyramidal neurons of the prefrontal cortex

In order to get detailed information on the morphology of

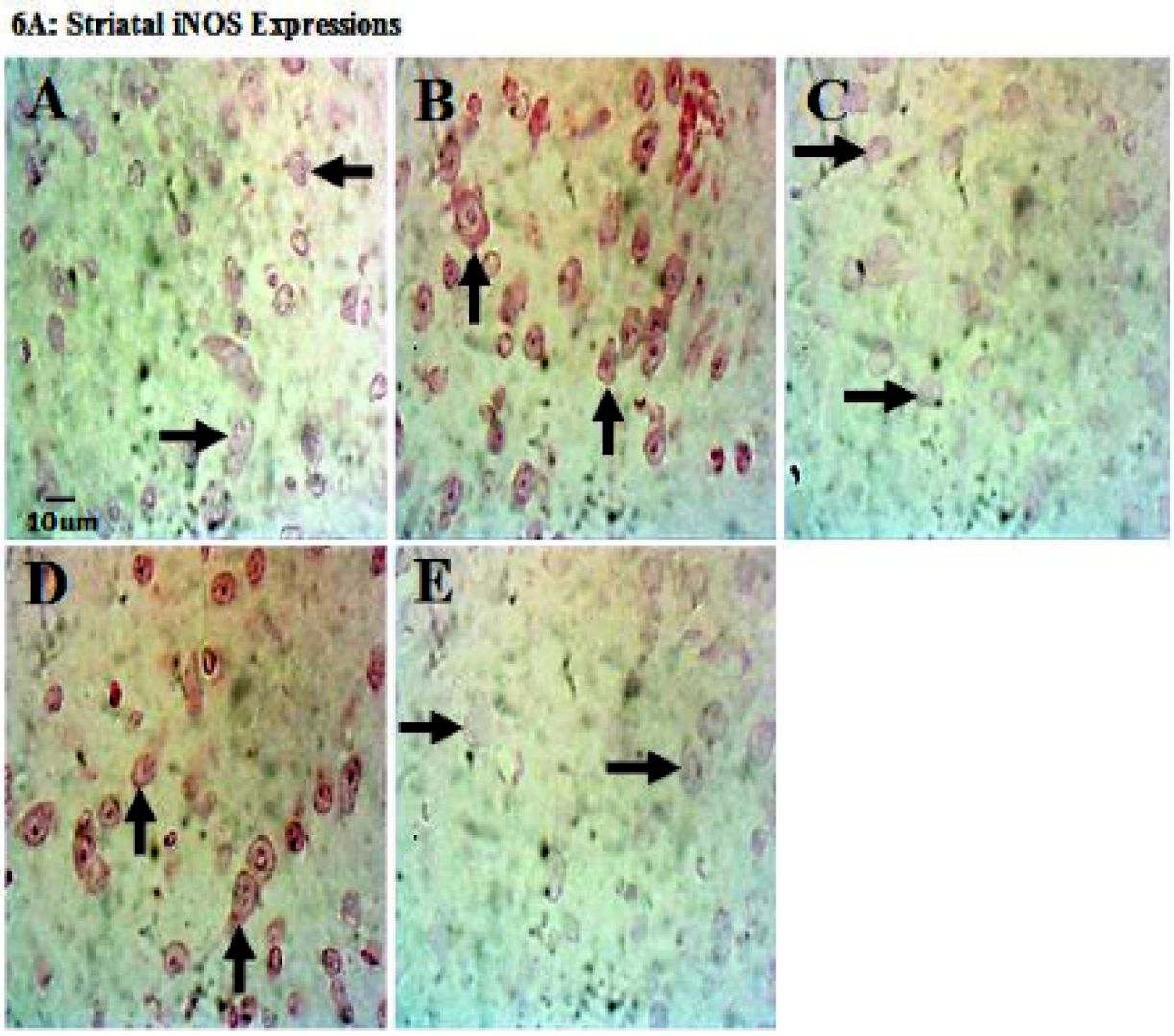


Fig. 6. Representative photomicrographs of the effect of morin on lipopolysaccharide and ketamine-induced immunohistochemical changes and expressions of iNOS immunopositive cells in the *striatum* (A), *prefrontal cortex* (B) and *hippocampus* (C) of mice brains. A = SAL 10 mL/kg, B = LPS 0.1 mg/kg + KET 20 mg/kg, C = MOR 100 mg/kg + LPS + KET, D = HLP 1 mg/kg + LPS + KET, and E = RIS 0.5 mg/kg + LPS + KET. Vertical arrow indicates: High immunopositive cell expression and Horizontal arrow indicates: Low immunopositive cell expression. SAL = Saline, LPS = Lipopolysaccharide, KET = Ketamine, MOR = Morin, HLP = Haloperidol, RIS = Risperidone.

pyramidal neuron of the PFC, Golgi silver impregnation staining technique was adapted according to the method previously described [37]. After 24 h of perfusion, mice brains were immersed in potassium dichromate solution for 5 days (5 changes every 24 h) and then silver nitrate for 3 days (3 changes every 24 h). Thereafter, the brain tissues were infiltrated for 30 min in molten wax, embedded in paraffin wax and cooled overnight at 4 °C. The paraffin blocks were trimmed and sectioned at 60 μm, transferred into graded series of alcohol (80%, 90%, and two changes of 100%) for 2 min and cleared in xylene for 10 min. Stained brain tissues were thereafter mounted on glass slides using DPX as mountant. The slides were then viewed using Leica DM 500 digital light microscope (Germany) and images were later captured using Leica ICC50 E digital camera (Germany). The transverse diameters of the soma body and dendritic aborization of the pyramidal neurons of the PFC were measured and data analyzed using computerized image analyzer (Image J/Micro-Manager 1.4). Photomicrograph calibrations were also done using Image J/Micro-Manager 1.4 [36].

2.8. Statistical analysis

Data were expressed as Mean ± S.E.M. (standard error of mean). Following a normality test, all behavioral data were analyzed using one-way analysis of variance (ANOVA) (except for social recognition memory data, which was analyzed using two-way ANOVA) followed by Bonferroni *post-hoc* test for multiple comparisons where appropriate. Biochemical data was analyzed using two-way ANOVA followed by Bonferroni *post-hoc* test. Data analyses were performed using Graph Pad Prism software version 5 (GraphPad Software, Inc. La Jolla, CA 92037 USA). A level of $p \leq 0.05$ was considered as statistically significant for all tests.

3. Results

3.1. Morin attenuates hyperlocomotion induced by lipopolysaccharide and ketamine in mice

The effect of MOR on LPS and KET-induced hyperlocomotion is

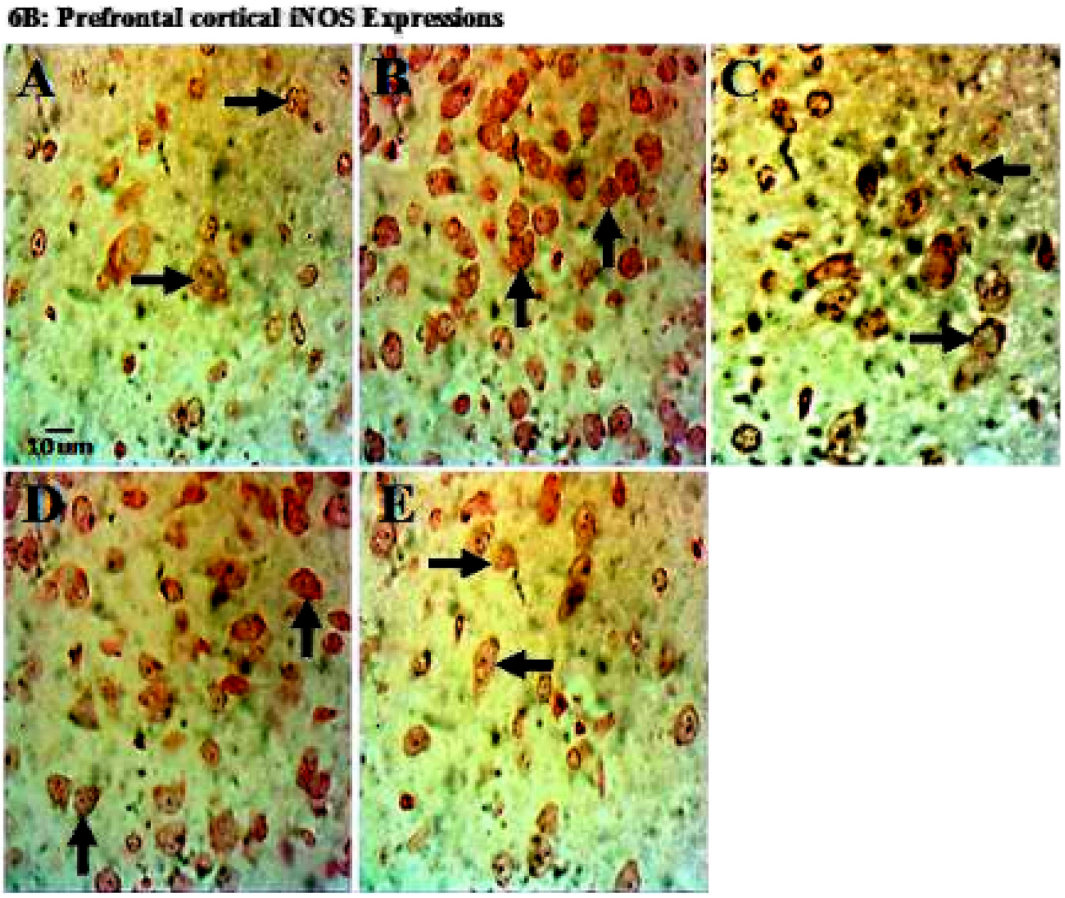


Fig. 6. (*continued*)

shown on Fig. 2A. One-way ANOVA showed that there were significant differences between treatment groups: numbers of line crossing [F (4, 35) = 8.608, $p < 0.0001$] (Fig. 2A). Post-hoc test revealed that chronic injection of LPS along with KET significantly ($p < 0.001$) increased spontaneous locomotor activity relative to SAL-treated mice. However, MOR ($p < 0.01$), HLP ($p < 0.001$) or RIS ($p < 0.001$) prevented the hyperlocomotion in a significant manner (Fig. 2A).

3.2. Morin decreases social isolation induced by lipopolysaccharide and ketamine in mice

In the social interaction test, there was a significant difference between treatment groups: % social preference [F (4, 35) = 204.2, $p < 0.0001$]. Chronic treatment with LPS (0.1 mg/kg, i.p.) for 14 days followed by KET (20 mg/kg, i.p.) treatment from days 8 to 14 caused a significant ($p < 0.001$) decrease in % social preference relative to SAL group (Fig. 2B). As shown in Fig. 2B, MOR or RIS significantly ($p < 0.001$) attenuated deficit in social behavior induced by LPS in combination with KET. However, treatment with HLP ($p > 0.001$) did not prevent the deficit in social interaction (Fig. 2B).

3.3. Morin attenuates social recognition memory impairment induced by lipopolysaccharide and ketamine in mice

In the social recognition memory test, one-way ANOVA showed that there were significant differences between treatment groups: short-term memory [F (4, 35) = 40.52, $p < 0.0001$] and long-term memory [F (4, 35) = 37.46, $p < 0.0001$] (Fig. 2C). Post hoc-test revealed that LPS in combination with KET significantly ($p < 0.001$) decreased social recognition memory between conspecifics at inter-trial interval of 30 min and 24 h. However, MOR or RIS significantly increased the social recognition memories based on decrease in time of exploration upon re-exposure of conspecific companion mice at 30 min ($p < 0.001$) and 24 h ($p < 0.001$) suggesting enhancement of short- and long-term social recognition memories. However, HLP did not significantly ($p > 0.05$) modify short- and long-term social recognition memory in mice treated with LPS and KET (Fig. 2C).

3.4. Morin reduces lipopolysaccharide and ketamine-induced spatial working memory impairment in mice

There was a significant difference between treatment groups: % alternation behavior [F (4, 35) = 23.32, $p < 0.0001$] (Fig. 2D). Moreover, repeated exposure of LPS and KET significantly ($p < 0.001$) decreased % alternation behavior in comparison with SAL group (one-way ANOVA). However, MOR or RIS ameliorated memory deficit induced by LPS in combination with KET. In contrast, HLP ($p > 0.05$) could not prevent the deficit in cognitive performance (Fig. 2D).

3.5. Morin inhibited brain myeloperoxidase activity in lipopolysaccharide and ketamine-treated mice

Two-way ANOVA revealed that there were significant differences between treatment groups in the activity of MPO: ST [F (4, 35) = 17.05, $p < 0.0001$], PFC [F (4, 35) = 55.53, $p < 0.0001$] and HC [F (4, 35) = 43.74, $p < 0.0001$]. Post-hoc tests showed that LPS together with KET significantly ($p < 0.001$) increased MPO activity in the ST, PFC and HC relative to SAL-treated groups. However, the increase in MPO activity in the ST and PFC were reduced by MOR or RIS in a significant ($p < 0.05$) manner (Fig. 3A). Although RIS ($p > 0.05$) did not reduce MPO activity in the HC, MOR significantly ($p < 0.001$) reduced the activity of this enzyme in the HC. On the other hand, HLP

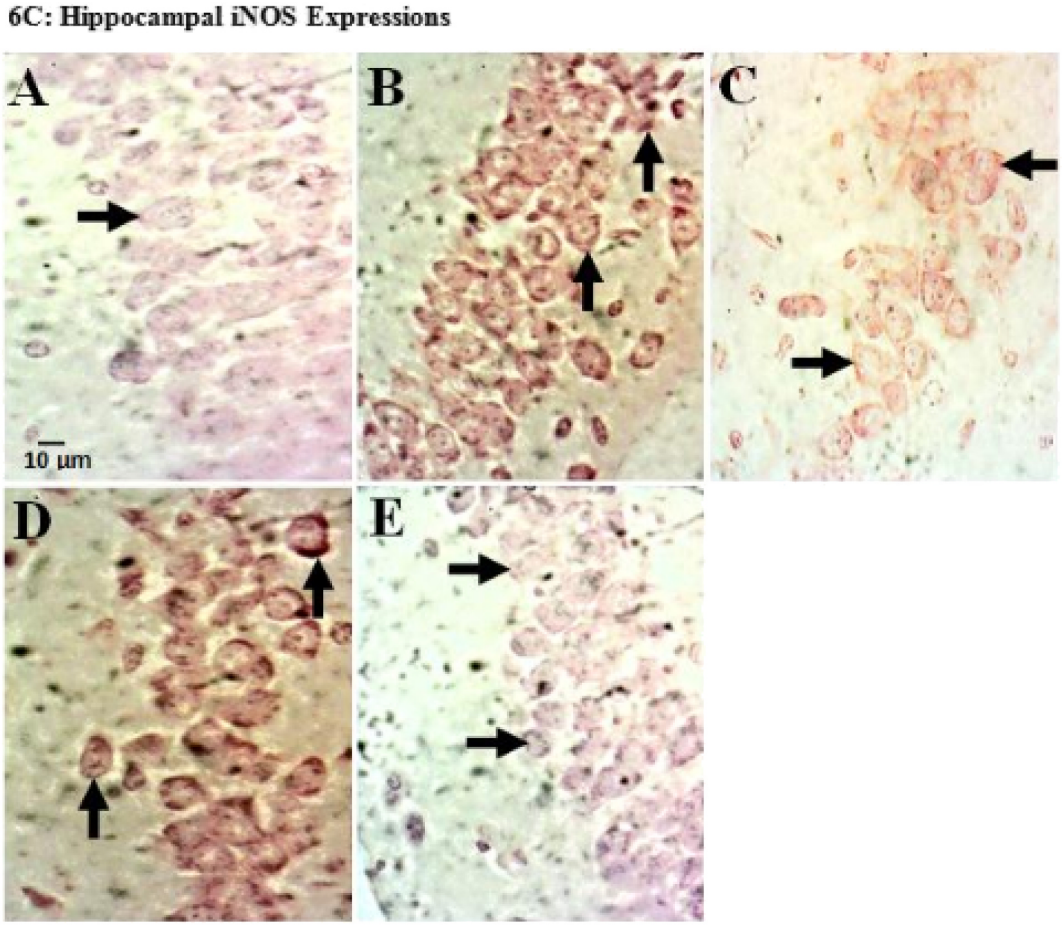


Fig. 6. (*continued*)

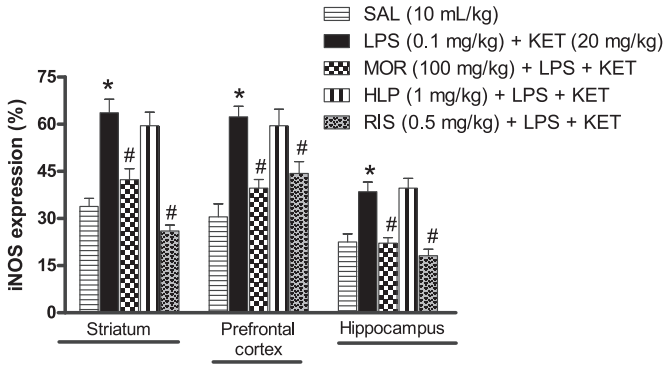


Fig. 7. Morin reduces the expressions of iNOS immunopositive cells in the *striatum*, *prefrontal cortex* and *hippocampus* in lipopolysaccharide and ketamine treated mice. Bars represent the mean ± SEM ($n = 3$ animals/group). $^{*}p < 0.05$ compared to SAL group and $^{\#}p < 0.05$ compared to LPS + KET group (two-way ANOVA followed by Bonferroni *post-hoc* test). SAL = Saline, LPS = Lipopolysaccharide, KET = Ketamine, MOR = Morin, HLP = Haloperidol, RIS = Risperidone.

($p > 0.05$) did not affect MPO activity in the brain regions (Fig. 3A).

3.6. Morin reduces release of tumor necrosis factor-alpha in mice brains treated with lipopolysaccharide and ketamine

Fig. 3B showed that LPS given in combination with KET produced a significant ($p < 0.001$) increase in TNF-α concentrations in the ST ($p < 0.01$) [F (4, 35) = 17.77, $p < 0.0001$] and PFC ($p < 0.001$) [F (4, 35) = 56.16, $p < 0.0001$] but not in the HC [F (4, 35) = 10.90, $p < 0.0001$] relative to SAL groups. However, MOR ($p < 0.001$) or RIS ($p < 0.01$) significantly decreased the concentrations of TNF-α in the ST. As shown in Fig. 3B, MOR or RIS also reduced TNF-α level in the PFC ($p < 0.001$). There was no significant change in the hippocampal TNF-α level, although RIS ($p < 0.001$) decreased hippocampal TNF-α concentration. There were no significant changes in the concentrations of TNF-α in the group treated with HLP in the brain regions (Fig. 3B).

3.7. Morin decreases release of interleukin-6 in mice brains treated with lipopolysaccharide and ketamine

Two-way ANOVA revealed that there were significant differences between treatment groups in the concentrations of IL-6: PFC [F (4, 35) = 45.10, $p < 0.0001$] and HC [F (4, 35) = 22.70, $p < 0.0001$] (Fig. 3C). Post-hoc test showed that LPS along with KET significantly increased IL-6 concentrations in the PFC ($p < 0.001$) and HC ($p < 0.001$) when compared with SAL groups. However, there was no significant change in IL-6 level in the ST ($p > 0.05$) (Fig. 3C). As presented in Fig. 3C, the increase in the concentrations of IL-6 in the PFC and HC induced by LPS and KET were significantly ($p < 0.001$) reduced by MOR or RIS.

3.8. Morin reduces expression of cyclooxygenase-2 in mice brains treated with lipopolysaccharide and ketamine

The photomicrographs of the effect of MOR on LPS and KET-induced immunohistochemical changes and expressions of COX-2

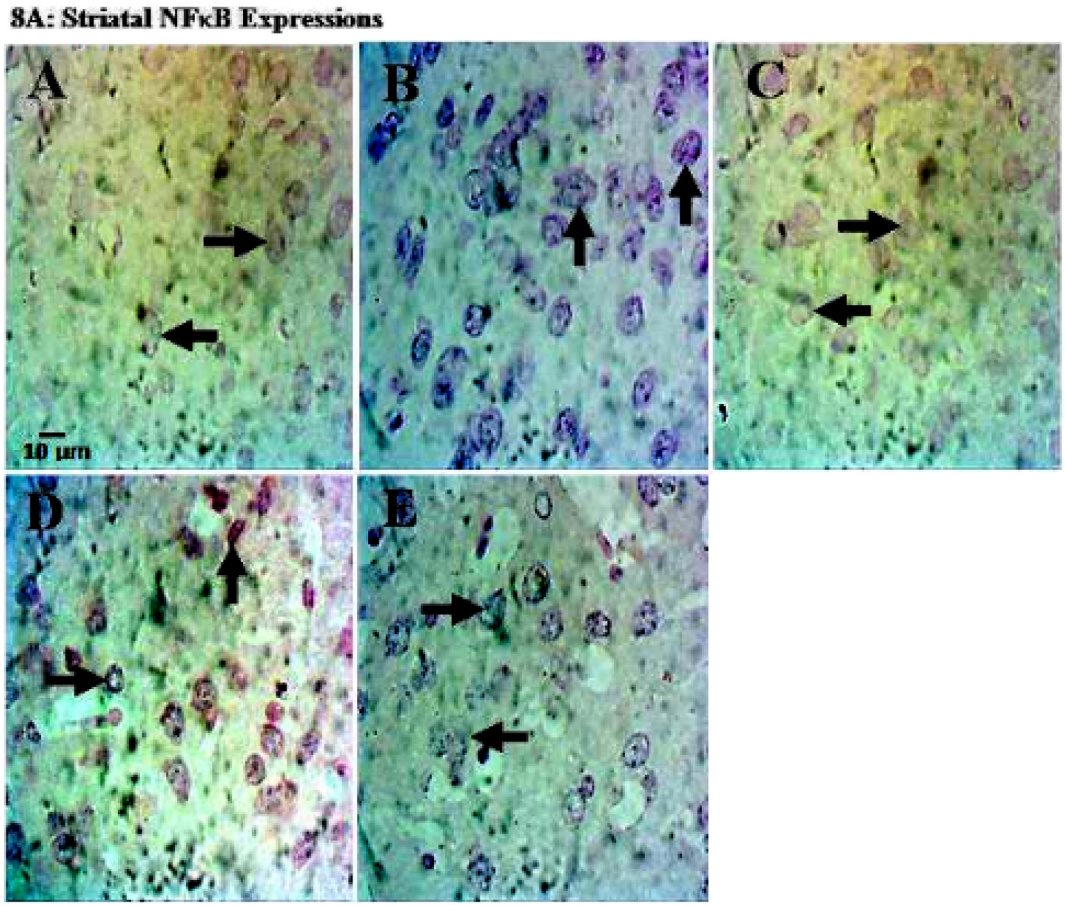


Fig. 8. Representative photomicrographs of the effect of morin on lipopolysaccharide and ketamine-induced immunohistochemical changes and expressions of NF-κB immunopositive cells in the *striatum* (A), *prefrontal cortex* (B) and *hippocampus* (C) of mice brains. A = SAL 10 mL/kg, B = LPS 0.1 mg/kg + KET 20 mg/kg, C = MOR 100 mg/kg + LPS + KET, D = HLP 1 mg/kg + LPS + KET, and E = RIS 0.5 mg/kg + LPS + KET. Vertical arrow indicates: High immunopositive cell expression and Horizontal arrow indicates: Low immunopositive cell expression. SAL = Saline, LPS = Lipopolysaccharide, KET = Ketamine, MOR = Morin, HLP = Haloperidol, RIS = Risperidone.

immunopositive cells in the ST, PFC and HC of mice are shown in Figs. 4A–C and 5. LPS given in combination with KET produced a significant increase in the expressions of COX-2 immunopositive cells in the ST ($p < 0.001$) [F (4, 10) = 14.61, $p = 0.0004$], PFC ($p < 0.001$) [F (4, 10) = 25.89, $p < 0.0001$] and HC ($p < 0.001$) [F (4, 10) = 20.68, $p < 0.0001$] in comparison to SAL groups (two-way ANOVA). However, post-hoc test revealed that MOR ($p < 0.01$) or RIS ($p < 0.001$) but not HLP ($p > 0.001$) reduced the increased expressions of COX-2 immunopositive cells in the ST and PFC (Fig. 5). Meanwhile, MOR ($p < 0.001$), RIS ($p < 0.001$) or HLP ($p < 0.01$) significantly decreased the expressions of COX-2 immunopositive cells in the HC (Fig. 5).

3.9. Morin inhibits expression of inducible nitric oxide synthase in mice brains treated with lipopolysaccharide and ketamine

Administration of LPS along with KET significantly increased expressions of iNOS immunopositive cells in the ST ($p < 0.001$) [F (4, 10) = 19.35, $p < 0.0001$], PFC ($p < 0.001$) [F (4, 10) = 15.44, $p = 0.0003$] and HC ($p < 0.01$) [F (4, 10) = 14.72, $p = 0.0003$] relative to SAL groups (Fig. 6A–C). However, the increased expressions of iNOS immunopositive cells in the ST and PFC were reduced by MOR or RIS ($p < 0.001$). MOR ($p < 0.01$) or RIS ($p < 0.01$) also attenuated hippocampal expressions of iNOS immunopositive cells in mice injected with LPS and KET (Fig. 7).

3.10. Morin inhibits lipopolysaccharide and ketamine-induced expression of nuclear factor kappa-B in mice brains

The photomicrographs of the effect of MOR on LPS and KET-induced immunohistochemical changes and expressions of NF-κB immunopositive cells in the ST, PFC and HC of mice are shown in Figs. 8A–C and 9. Two-way ANOVA showed that there were significant differences between treatment groups in expression of NF-κB: ST ($p < 0.05$) [F (4, 10) = 8.010, $p = 0.0037$], PFC ($p < 0.001$) [F (4, 10) = 30.88, $p < 0.0001$] and HC ($p < 0.01$) [F (4, 10) = 14.53, $p = 0.0004$]. Post-hoc analysis by bonferroni test revealed that LPS plus KET produced a significant increase in the expressions of NF-κB immunopositive cells in the brain regions when compared with SAL control. However, post-hoc test showed that MOR ($p < 0.01$) or RIS ($p < 0.001$) but not HLP ($p > 0.001$) significantly suppressed the increased expressions of NF-κB in the ST, PFC and HC (Fig. 9).

3.11. Morin decreases degeneration of soma size and dendritic spine of pyramidal neurons of the prefrontal cortex of mice brains treated with lipopolysaccharide and ketamine

The photomicrographs of the effect of MOR on LPS and KET-induced alterations on somata size and dendritic spine of pyramidal neurons of the PFC of mice brains are shown in Figs. 10 and 11A–B. As shown in Fig. 10, LPS given along with KET significantly decreased somata size ($p < 0.05$) [F (4, 10) = 100.7, $p < 0.0001$] (Fig. 11A) and

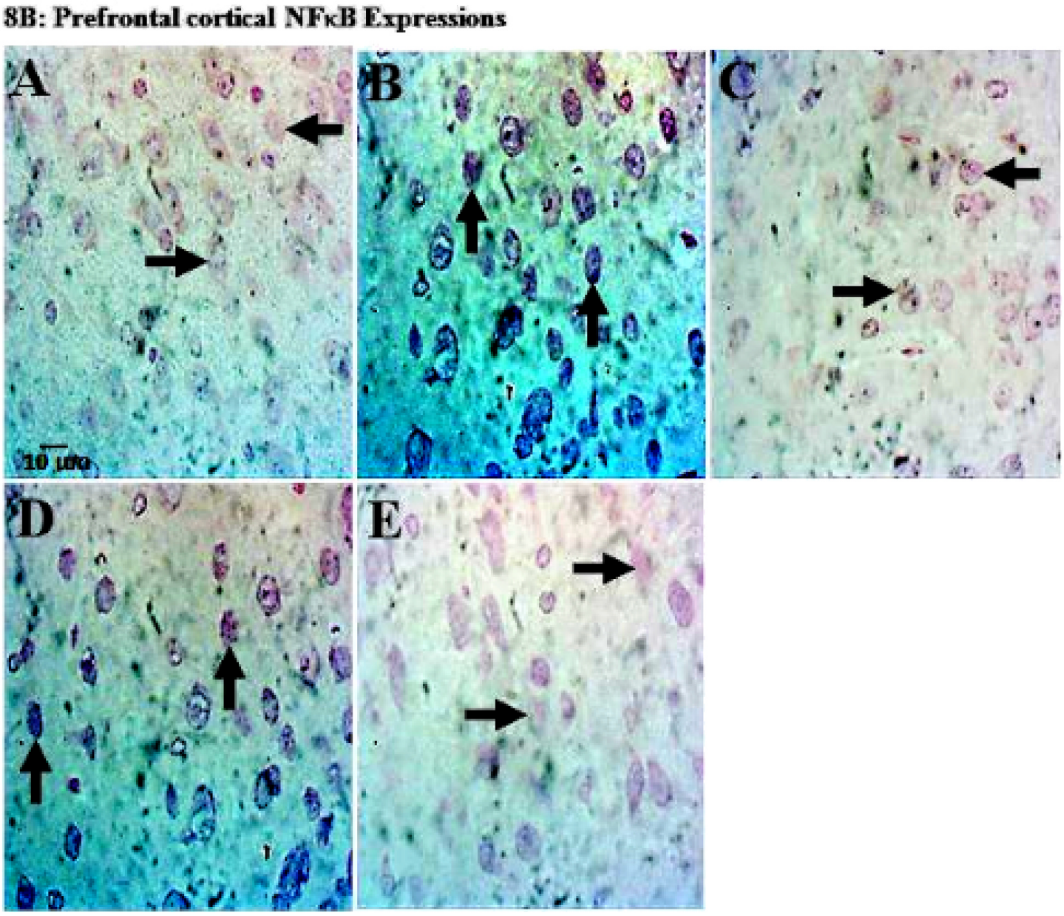


Fig. 8. (*continued*)

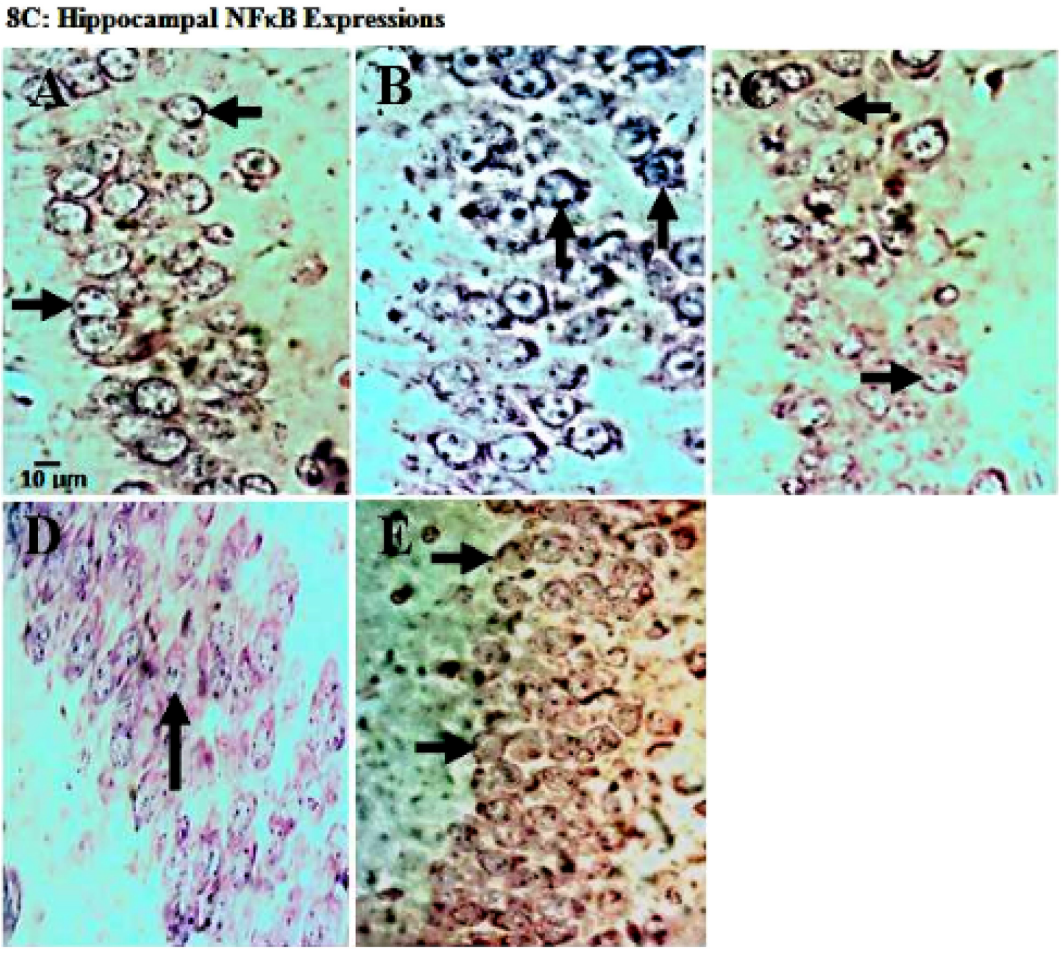
number of dendritic spines [F (4, 10) = 47.50, $p < 0.0001$] (Fig. 11B) of the pyramidal neurons relative to SAL controls, suggesting reduced neural networks. However, MOR produced significant protection against the deleterious effects of LPS and KET on soma size ($p < 0.05$) (Fig. 11A) and spine density ($p < 0.001$) (Fig. 11B) of the pyramidal neurons. Although RIS did not increase ($p > 0.05$) the soma size of the pyramidal neuron, it significantly ($p < 0.001$) increased dendritic spine density. In contrast, HLP caused a profound ($p < 0.001$) loss of prefrontal cortical pyramidal neurons, as evidenced by absence of soma body (Fig. 11A) and dendritic spines (aborization) (Fig. 11B) of the pyramidal neurons.

4. Discussion

The results of this study showed that repeated intraperitoneal injection of LPS prior to KET caused a significant neuroinflammation, as evidenced by increased levels of inflammatory enzyme (MPO), cytokines (TNF-α and IL-6) and proteins (COX-2, iNOS and NF-κB) in the ST, PFC and HC. Also, LPS and KET decreased the pyramidal neurons and its arbor in the PFC and induced schizophrenia-like behaviors typified by hyperlocomotion, social withdrawal and cognitive impairments. However, MOR significantly reduced schizophrenia-like behaviors and suppressed the brain levels of biomarkers of neuroinflammation as well as loss of cortical pyramidal neurons induced by LPS and KET in mice.

Current studies have shown that neuorinflammation contribute to the exacerbation of schizophrenic symptoms [9,38]. Indeed, LPS has been reported to produce a range of behavioral symptoms including social withdrawal and impaired learning and memory in mice via activation of the innate immune system [15,16]. On the other hand, chronic intraperitoneal injection of KET induces schizophrenia-like behaviors such as hyperlocomotion, stereotypy, deficits in pre-pulse inhibition, social interaction and memory [7,21–24,33]. However, early exposure to LPS prior to KET treatments has been shown to exacerbates KET-induced schizophrenia-like behaviors in rats [11,23]. In this study, we have shown that repeated injection of LPS prior to chronic injection of KET in mice exhibited behavioral perturbation consisting of hyperlocomotion, social withdrawal, social memory deficit and spatial cognitive impairment reminiscent of schizophrenia. The blockade of NMDA receptors located on subcortical and cortical GABAergic inhibitory brain areas that leads to increased dopamine and glutamate outflow has been linked to schizophrenia-like behavior induced by KET [21]. LPS has also been reported to alter the activities of subcortical and cortical GABAergic and glutaminergic neurotransmissions via release of proinflammatory cytokines [17,39]. Moreover, activation of the immune system in response to infectious agents has been shown to alter dopaminergic and glutaminergic activities in rats as well as increase the sensitivity to NMDA receptor antagonists [7,17,20,23]. Agreeably, Réus et al., [11] showed that the activity of KET on NMDA receptors is also linked to activation of inflammatory transcription factors such as NF-κB and release of proinflammatory cytokines [40]. The findings that MOR ameliorated the schizophrenia-like behaviors induced by LPS and KET further confirmed its potential benefit in the relieve of the positive, negative and cognitive symptoms associated with the disease [24,31].

Intraperitoneal injections of LPS and KET have been reported to exacerbate schizophrenia-like behaviors via microglia activation, which in turn produces loss of neurochemical ensembles particularly of cortical pyramidal neurons that characterized schizophrenic disorder [15,41]. Activation of microglia cells is known to cause the release of proinflammatory enzymes and cytokines that mediates neuroinflammation [26]. Myeloperoxidase (MPO) for example, is a potent



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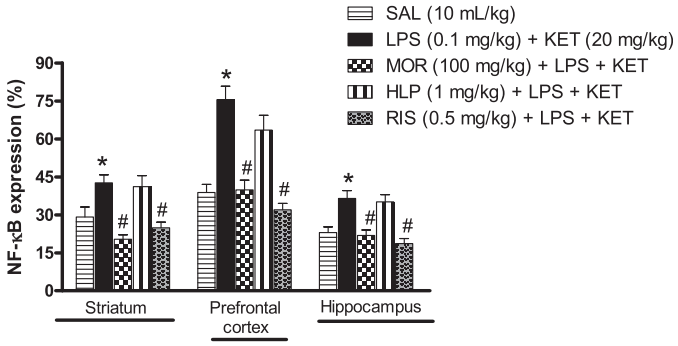

Fig. 9. Morin inhibited the expressions of NF-κB immunopositive cells in the *striatum*, *prefrontal cortex* and *hippocampus* in lipopolysaccharide and ketamine treated mice. Bars represent the mean ± SEM ($n = 3$ animals/group). $^{*}p < 0.05$ compared to SAL group and $^{\#}p < 0.05$ compared to LPS + KET group (two-way ANOVA followed by Bonferroni *post-hoc* test). SAL = Saline, LPS = Lipopolysaccharide, KET = Ketamine, MOR = Morin, HLP = Haloperidol, RIS = Risperidone.

inflammatory enzyme stored in the azurophilic granules of polymorphonuclear neutrophils and macrophages [42]. However, increased MPO activity in the serum of patients with schizophrenic disorders has been reported [42]. MPO has been implicated in microglia cell activation, up-regulation of pro-oxidants and proinflammatory cytokines, and distortion of blood brain barrier permeability [43]. In this study, LPS and KET increased MPO activity in the ST, PFC and HC accompanied by elevation in the concentrations of TNF-α and IL-6 in these brain regions. These findings further confirmed previous investigations, which showed that subtle inflammation contributes to the pathophysiology of schizophrenia [8,17,42]. Relevant to this background, studies have shown that increased brain levels of IL-6 is positively correlated with the clinical and molecular features of schizophrenia including: severity of symptoms [8], treatment resistance [44] and up-regulation of production of kynurenine, which is a pathologic endogenous NMDA receptor antagonist that increases midbrain dopamine firing and loss of pyramidal neurons [17,19,45]. Indeed, peripheral administration of IL-6 to animals has been reported to cause increased dopaminergic and 5-hydroxytryptaminergic turnover in the PFC and HC [39]. However, it has been suggested that the relative capacity of antipsychotic drugs to attenuate the release of proinflammatory enzymes and cytokines may be an important contributing factor underpinning the clinical efficacy of antipsychotic drugs [8,9,42,46]. Thus, the ability of MOR to suppress the release of MPO, TNF-α and IL-6 in a similar manner to RIS in the ST, PFC and HC of LPS and KET-treated mice might be playing a significant role in its antipsychotic effect.

In addition, increased expressions of key inflammatory proteins such as COX-2, iNOS and NF-κB have also been reported in patients with schizophrenia [46]. These inflammatory proteins have been implicated in the progression of the disease through the liberation of proinflammatory cytokines [46]. In fact, the negative and cognitive symptoms induced by LPS have been linked to up-regulation of these inflammatory proteins [46,47]. COX-2 for example, has been shown to be involved in neurotransmitter modulation and cortical activity-dependent synaptic remodeling via increased production of prostaglandin-E_2 (PGE_2) [62]. PGE_2 is a molecule of the proinflammatory

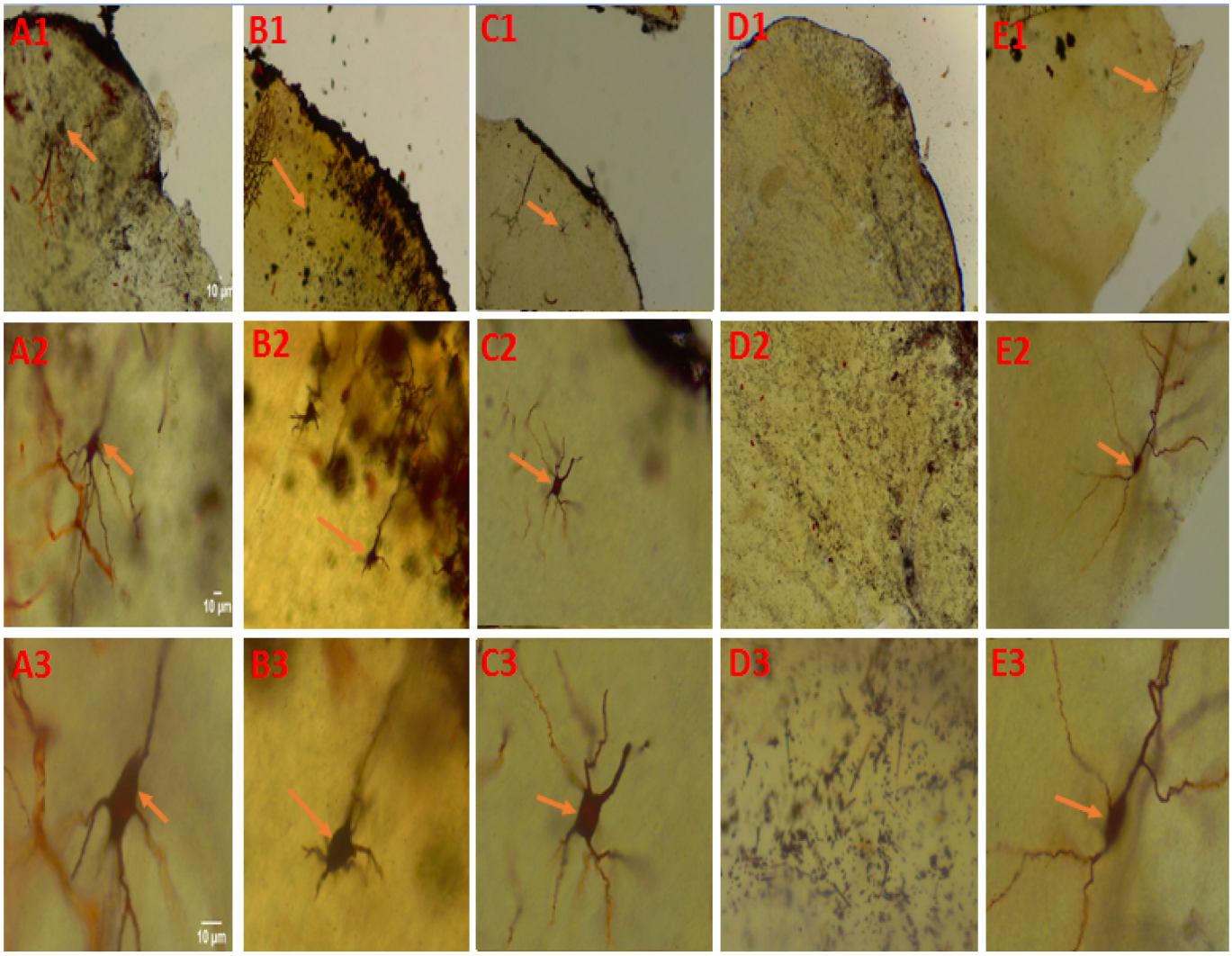

Fig. 10. Representative photomicrographs (Golgi stained sections) of the effect of morin on lipopolysaccharide and ketamine-induced alterations of soma size and dendritic spine arborization of *pyramidal neurons* in the *prefrontal cortex* of mice brains. A (A1–A3) = SAL 10 mL/kg revealed normal soma size and dendritic spine (arborization) B (B1–B3) = LPS 0.1 mg/kg + KET 20 mg/kg decreased soma size and dendritic spine projections, C (C1–C3) = MOR 100 mg/kg + LPS + KET revealed increased soma size and dendritic spines, D (D1–D3) = HLP 1 mg/kg + LPS + KET showed the absence of both soma body and dendritic spines of pyramidal neurons, and E (E1–E3) = RIS 0.5 mg/kg + LPS + KET revealed non-significant increased soma, and marked increase in spine lengths. Upper panel, A1–E1 represents groups at ×100 magnification; middle panel, A2–E2 is at ×400 magnifications while the bottom panel, A3–E3 is at ×1000 magnification. Scale bar for all figures = 10 μm. SAL = Saline, LPS = Lipopolysaccharide, KET = Ketamine, MOR = Morin, HLP = Haloperidol, RIS = Risperidone.

cascade, which stimulates the production of proinflammatory cytokines [46]. Also, neuroimmune activation has been linked with increased expression of iNOS in different brain regions and is responsible for the augmented production of nitric oxide (NO) [5,46]. Both preclinical and clinical studies have also shown elevated level of NO in the brains of schizophrenic mice [22,48] and patients [49]. Previous studies have revealed that increased iNOS expression cause nitrergic stress-induced alteration in glutaminergic neurotransmission and increased generation of free radicals [5,21,46]. Thus, it might be speculated that the antipsychotic-like effect exhibited by MOR in the LPS and KET-treated mice, may be mediated partly through inhibition of the up-regulation of COX-2 and iNOS in the brain.

Previous clinical studies using schizophrenic patient genes have identified NF-κB as a hub, where different, diverse signal transcription factors and cytokines, critical for schizophrenic genetic vulnerability factors, converge [50]. Indeed, LPS has been shown to cause microglia activation and increased expression of NF-κB via altered transcriptional machinery including increased immunoreactivity of Nox-2 pathway [26,46]. This action has been shown to cause progressive neuroinflammation, neurocellular degeneration, dysfunctional neurochemicals and altered forebrain GABAergic-mediated synaptic plasticity [15,46,51]. Notwithstanding, chronic KET injection also induced degeneration of neural networks including the loss of GABAergic interneurons, cortical parvalbumin interneurons and dendritic arborization via increased Nox-2 [40,41,52]. The combined actions of LPS and KET was proposed to further intensify the progressive neuroinflammation and increase the severity of schizophrenic symptoms [23,53], as observed in this study. Herein, LPS plus KET-induced schizophrenia was found to be accompanied by increased expressions of NF-κB in the ST, PFC and HC, and degeneration of cortical pyramidal neurons as evidenced by reduced pyramidal soma size and decreased dendritic spines. Thus, the result of this study further confirmed the findings of previous studies, which showed that LPS [46], KET [7] alone, or LPS plus KET-induced schizophrenia [11,23] is associated with neuroinflammation and loss of pyramidal neurons [40,41]. Thus, the ability of MOR in a similar manner to RIS to suppress the expression of NF-κB and prevent degeneration of pyramidal neurons, also suggest anti-neuroinflammatory and neuroprotective properties.

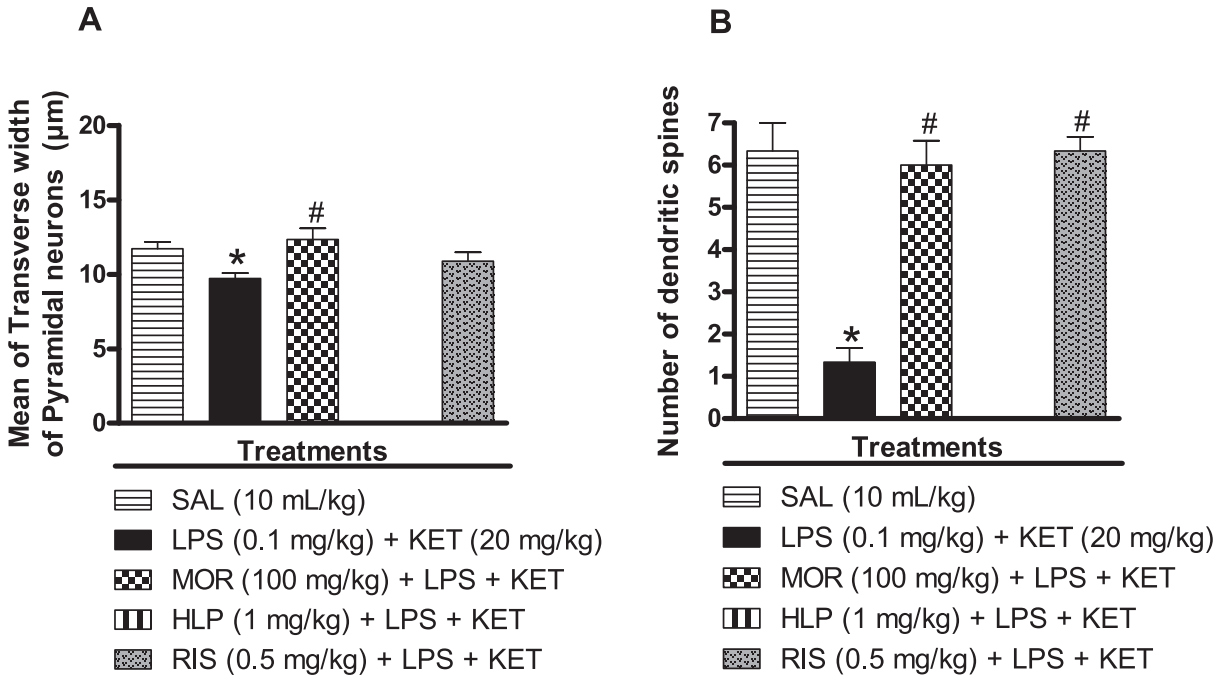

Fig. 11. Morin recovered the decreases in soma size (A) and dendritic spine density (B) of pyramidal neurons of the *prefrontal cortex* of mice brains treated with lipopolysaccharide and ketamine. Bars represent the mean ± SEM ($n = 3$ animals/group). $^{*}p < 0.05$ compared to SAL group and $^{\#}p < 0.05$ compared to LPS + KET group (one-way ANOVA followed by Bonferroni *post-hoc* test). SAL = Saline, LPS = Lipopolysaccharide, KET = Ketamine, MOR = Morin, HLP = Haloperidol, RIS = Risperidone.

On the other hand, HLP could not rescue the degeneration of pyramidal neuron; but rather worsen it. This finding is congruent with previous investigations, which showed that HLP may cause loss of neurons and decrease in nuclear size, suggesting neurotoxic effect [54,55]. Different studies have shown that chronic treatment with haloperidol induced neurotoxicity via: increased oxidative toxicity [56,57], release of proinflammatory cytokines, increased expression of NF-κB and apoptosis in neuronal cell cultures and mice brains [58,59]. Haloperidol-induced oxidative and neuroinflammatory processes have been linked to changes in synaptic signals, decreased expressions of GABAergic-related enzymes, degeneration of GABAergic-related neurons, reduced synaptosomal transport of GABA into pre-synaptic vesicles and fluctuation of dopamine levels [60,61]. Thus, these cellular mechanisms may perhaps contribute to haloperidol-induced tardive dyskinesia [59,61]; as well as the worsening effects on negative and cognitive symptoms, and degeneration of cortical neurons observed in this study [57]. Nonetheless, the protective effects of MOR or RIS are in agreement with previous studies, which showed that MOR or RIS inhibited LPS-activated iNOS, release of cytokine in BV2 microglia cells, and suppression NF-κB expression via inhibition of microglia activation in the brains of LPS-treated mice [26,46,57]. Earlier on, MOR was also shown to increase antioxidant enzymes [48,57] via increased nuclear factor erythroid 2-related factor 2 and CREB activation, and inhibition of expression of Nox-2 in the PFC in LPS- [26] and KET-treated mice [24] respectively. It is worthy of note that MOR is a safe promising natural product with potent immunomodulatory and anti-oxidant properties [25,62], which currently underpins its neuroproctive potentials [24,26]. Taken together, the anti-schizophrenic-like activity exhibited by morin in this study may be mediated via mechanisms related to inhibition of the release of proinflammatory mediators and suppression of degeneration of cortical pyramidal neurons in mice.

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Conflicts of interest

Authors declare that they have no conflict of interest.

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