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# Minocycline attenuates lipopolysaccharide-induced locomotor deficit and anxiety-like behavior via upregulation of the BDNF/CREB protein expression in the rat medial prefrontal cortex (mPFC).

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**Abstract**

**Introduction**: Neuroinflammation following lipopolysaccharide (LPS) administration induces locomotor deficit and anxiety-like behavior. In this study, minocycline was compared to memantine, the NMDA receptor antagonist, for its effects on LPS-induced locomotor deficit and anxiety-like behavior in rats.

**Methodology**: Adult male Sprague Dawley rats were administered either two different doses of minocycline (25 or 50 mg/kg/day, i.p.) or 10 mg/kg/day of memantine (i.p.) for 14 days four days prior to LPS (5 mg/kg, i.p.) injection. The locomotor activity and anxiety-like behavior were assessed using the open field test (OFT). The phosphorylated tau protein level was measured using ELISA while the expression and density of brain-derived neurotrophic factor (BDNF) and cAMP response element-binding protein (CREB) protein in the medial prefrontal cortex (mPFC) were measured using immunohistochemistry and western blot, respectively.

**Results**: In the mPFC, minocycline treatment reduced the locomotor deficit and anxiety-like behavior, reduced phosphorylated tau protein level, and upregulated BDNF/CREB protein expression comparable to memantine, with the higher dose of minocycline having better benefits.

**Conclusion**: Minocycline treatment attenuated LPS-induced locomotor deficit and anxiety-like behavior in rats, possibly via a decrease in phosphorylated tau protein levels and an increase in the expression of the BDNF/CREB proteins.

**Keywords**: Lipopolysaccharide, minocycline, memantine, medial prefrontal cortex, locomotor deficit, anxiety-like behavior

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## Introduction

A previous clinical study involving healthy volunteers has shown that exposure to endotoxins induced locomotion deficit, anxiety-like behavior, and neuroinflammatory response (Shishkina et al., 2020). Similarly, previous animal studies have shown that either intraperitoneal or cerebroventricular injection of lipopolysaccharide (LPS) induced locomotion deficits and anxiety-like behavior (Tufvesson-Alm et al., 2020). As a result, LPS has been used widely used in animal models to investigate the underlying mechanisms of locomotion deficit and anxiety-like behavior-induced neuroinflammatory diseases (Li et al., 2020; Zhang et al., 2018; Ge et al., 2015).

LPS stimulates the inflammatory pathway, which activates the formation of phosphorylated tau protein (neurofibrillary tangles) and amyloid accumulation (Liu et al., 2016; Badshah et al., 2016). Inflammatory cytokines influence the phosphorylation of the BDNF receptor (TrkB), thereby further interfering with BDNF signaling (Gibney et al., 2013), and tau protein at least partially mediates Aβ-induced brain-derived neurotrophic factor (BDNF) downregulation (Rosa et al., 2016). Downregulation of BDNF and TrkB expression in the hippocampus and cortex might lead to behavioral defects of depression and anxiety (Jiang & Salton, 2013). The cAMP response element-binding protein (CREB) regulates the transcription and subsequent expression of BDNF (Wu et al., 2019) and is also involved in locomotion and anxiety-like behavior (Valverde et al., 2004). Therefore, targeting tau protein and BDNF/CREB pathway is a potential strategy for the prevention and treatment of neurobehavioural disorders-induced neuroinflammatory diseases.

Minocycline (microglial inhibitor) is an approved therapeutic drug for the treatment of bacterial infection for over 30 years (Yong et al., 2004). It is a semi-synthetic second-generation tetracycline antibiotic that can cross the blood-brain barrier (BBB) into the cerebrospinal fluid as a result of its small molecular size (495 KDa) and high solubility (Plane

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et al., 2010). Recently, the developing research is focusing on its neuroprotective properties in *in vivo* and *in vitro* animal models as well as in clinical studies. Recent studies showed that minocycline improved synaptic transmission and integrity as well as neurologic function via reduction of phosphorylated tau protein level and upregulation of BDNF/CREB signalling pathways in several animal models (Motaghinejad et al., 2021; Salehi et al., 2019).

However, the protection of minocycline against LPS-induced locomotor deficit and anxiety-like behaviour remains unclear. Thus, this study was designed to evaluate the effects of minocycline in comparison to memantine, N-methyl-D-aspartate (NMDA) antagonist, on locomotion and anxiety-like behavior, phosphorylated tau protein level as well as BDNF and CREB proteins expression in mPFC of LPS rats. This study postulated that minocycline attenuates LPS-induced locomotor deficit and anxiety-like behavior via a reduction in phosphorylated tau protein level and upregulation of BDNF/CREB protein expression in medial prefrontal cortex (mPFC) rats.

## Materials and methods

### Animals

Adult male Sprague Dawley rats were obtained from the Animal Research and Service Centre (ARASC), Universiti Sains Malaysia (USM). The rats were approximately 3 months old with bodyweight of 270 ± 20g. The rats were acclimatised to a new environment for one week prior to the start of the experiment. They were kept in polypropylene cages (32 x 24 x16 cm) and had free access to a standard pellet rodent diet (Altromin, Germany) and tap water. The rats were exposed to 12-hr light/dark cycles (lights off at 7 p.m., lights on at 7 a.m.) and held at 23$^{o}$C room temperature and 50 ± 5% relative humidity. The experimental protocol followed internationally accepted principles for laboratory animal use and care and was

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approved by the research and Ethics Committee of this university. The number of Animal Ethics Approval is: [USM/IACUC/2018/ (942) (114)].

**Experimental design**

The experimental timeline was shown in Figure 1. The rats were randomly divided into five groups (n=10) as follows: (i) control-treated with distilled water, (ii) LPS-treated with distilled water, (iii) LPS-treated with minocycline 25 mg/kg (Beheshti Nasr et al., 2013), (iv) LPS-treated with minocycline 50 mg/kg (Beheshti Nasr et al., 2013) and (v) LPS-treated with memantine 10 mg/kg (Hemmati et al., 2014).

Minocycline (USP, 12601Twinbrook Pkwy, Rockville, MD) and memantine (USP, 12601Twinbrook Pkwy, Rockville, MD) were administered intraperitoneally once daily for 2 weeks consequently, starting from day 1 to day14. LPS was obtained from *E.coli* 0111:B4 (Sigma-Aldrich, St. Louis, MO) and injected intraperitoneally once on day 5 at the dose of 5 mg/kg (Yaacob et al., 2018). After 2 weeks of treatment, all rats were subjected to OFT from day 15 to day 18.

**Open field test (OFT)**

The open field test (OFT) was used to assess locomotor activity and anxiety-like behavior in rats. The test was conducted between 8 a.m. and 12 p.m. The rats were brought into the behavioral room and acclimatised for 30 min prior to testing. After each trial, animals were returned to their cages. The apparatus consisted of a Perspex cage (height = 27 cm, length = 90 cm & width = 90 cm) and the bottom was divided into 25 small squares (16 × 16 cm). A video camera was placed 250 cm above the open field to record trials (Arc Soft Total media 3.5).

For testing, each rat was placed in the centre of the open field arena and locomotor activity was digitally recorded for 5 min. The open field apparatus was wiped with 70% ethanol between trials and dried before the next trial to avoid smelling bias. Locomotion was assessed

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by measuring the total distance travelled in the open field, speed and line crossings using Panlab Smart Video Tracking Harvard Apparatus (USA system version 3.0) and anxiety-like behavior were assessed by calculating rearing and grooming frequency, time spent in the centre of the open field and frequency of entries into the centre manually. The schematic representation of rat movement is shown in Figure 2. After behavioral assessment through OFT, rats were sacrificed by deeply anaesthetised with an overdose of sodium pentobarbital (60 mg/kg body weight). The brain was immediately collected and divided into right and left hemispheres. The brain tissues from the right hemisphere were preserved in 10% formalin for immunohistochemistry analysis.

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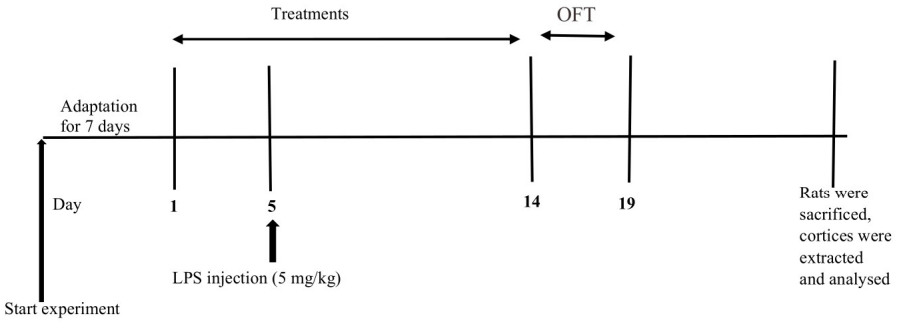


Figure 1

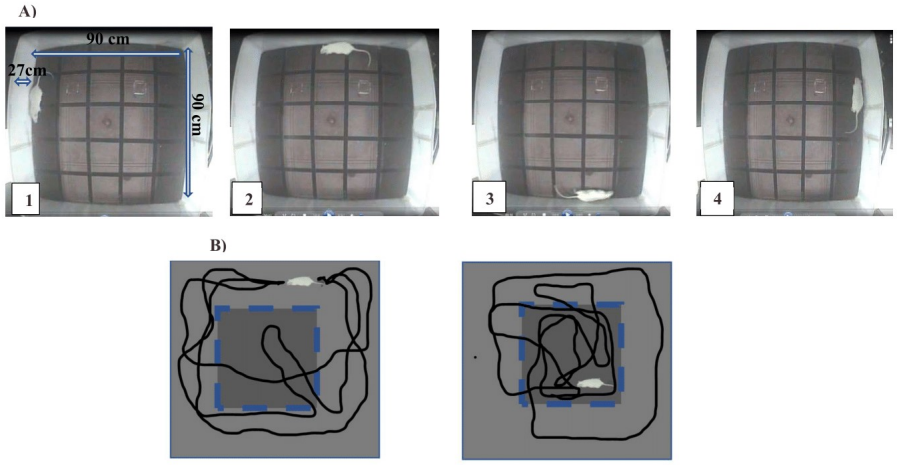


Figure 2

Figure 1: Experimental timeline

Figure 2: Rat's activity in the open field test (OFT) (A). OFT setup consists of a square arena divided into lines (1). The rat was allowed to explore the arena for 5 min (2-4). The total distance, speed, line crossings, rearing and grooming frequency and time spent, and frequency to enter the central square are recorded. Diagram showing movement of rats (black lines) in peripheral and central arena of OFT (B).

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**Immunohistochemistry for BDNF and CREB positive cells expression**

The paraffin sections were dewaxed by immersion in xylene I and II solutions for 2 min each. After that, the slides were hydrated in decreasing dilutions of ethanol for 2 min each. For Antigen (Ag) retrieval, the slides were placed in a pressure cooker containing Tris EDTA buffer at a temperature of 90ºC for 3 min and after that left to cool down inside $dH_2O$ for 2 min. The slides were placed in a sequenza immunostainer, a few drops of hydrogen peroxide ($H_2O_2$) blocking agent were added into the slides and the slides were incubated for 5 min. Then, the slides were washed with $dH_2O$ for 2 min and immersed in Tris buffer saline-Tween 20 (TBST) buffer twice for 5 min each. Primary antibodies (Santa Cruz; mouse BDNF and CREB; dilution = 1:200 & 1:100) were added and slides were incubated overnight at - 4ºC. The slides were rinsed in TBST twice for 5 min each, secondary antibodies (Santa Cruz; Anti-mouse BDNF and CREB; dilution = 1:500 & 1:200) were added to slides and were incubated for 1 hr at room temperature. The slides were rinsed in TBST twice for 5 min each and flooded in 3, 3'-Diaminobenzidine (DAB) for 5-10 min at room temperature. Then, the slides were washed with $dH_2O$ for 2 min and dipped in haematoxylin for 5 sec. Next, the slides were dehydrated in increasing dilutions of ethanol for 2 min each, immersed in xylene I and II for 2 min each and mounted by placing coverslips onto the slides using cytoseal. The sections known to express the BDNF and CREB positive cells in the mPFC regions were imaged under 40 and 100× magnification using an image analyser connected to a light microscope (Olympus Corporation, Japan). The BDNF and CREB positive cells in the mPFC regions were counted using Image-J software (http:// imagej.nih.gov/ij). The counting was done within a 100 × 100 mm grid placed in the mPFC region using three random sections for each rat. Only clearly visible brown DAB colour cells were considered as BDNF and CREB positive cells.

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**Western blotting for BDNF and CREB protein expression level**

The brain tissues from the left hemisphere were taken for WB and ELISA analysis. For WB analysis, the proteins were extracted from mPFC tissue using radioimmunoprecipitation assay (RIPA) buffer. The homogenates were centrifuged at 12 000 g at -4ºC for 15 min (Hettich Zentrifugen, Germany). The protein's quantifications in each supernatant were calculated using a Bradford protein assay kit (Bio-Rad, USA). Proteins (60µg) were denatured with sodium dodecyl sulfate (SDS) sample buffer and separated using 10% SDS-polyacrylamide gel electrophoresis (PAGE). The proteins were transferred to a polyvinylidene fluoride (PVDF) microporous membrane (Membrane Solutions, USA) and then blocked with 5% skim milk for 1 hr at room temperature. The membrane was incubated with primary antibodies (Santa Cruz, mouse BDNF and/or CREB; dilution 1:500 each) overnight at -4ºC. Secondary antibodies (Santa Cruz; anti-mouse BDNF and CREB; dilution = 1:5000) were added and incubated for 1 hr at room temperature and the protein bands on the membranes were detected with Clarity™ Western ECL substrate kits (Bio-Rad, USA). The relative density of the protein bands was evaluated by densitometry using Fusion FX Chemiluminescence Imaging apparatus (Viber Lourmat, Germany) and quantified by Image J software (NIH, USA).

**Phosphorylated tau protein assay by Enzyme-linked immunosorbent assay (ELISA)**

For ELISA analysis, the mPFC tissues from the left hemisphere were extracted quickly and placed in ice-cold saline. The mPFC brain tissues were weighed and homogenised (10% w/v) in ice-cold phosphate buffer saline (PBS: 0.1 M, pH 7.4) to prevent enzyme degradation for 5 min. The homogenised tissues were centrifuged at 10 000 x g for 10 min at -4°C (Hettich Zentrifugen, Germany). The supernatants were allocated into Eppendorf tubes and preserved at -80 °C for ELISA analysis.

The phosphorylated tau protein level in mPFC tissue was quantified by Rat pτ ELISA kit according to the manufacturer's instructions (Elabscience®, China). Standard, blank and

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samples (100 µL each) were added to the 96-microwells plate. The microplate was sealed and incubated for 1 and 30 min at 37°C. The liquid was decanted from each well and 100 µL of biotinylated detection antibody working solution was added to each well. The microplate was sealed and incubated for 1 hr at 37°C. The solution was decanted from each well and 350 µL of wash buffer was added to each well. The solution was soaked for 1 min, aspirated from each well, and left to dry against clean absorbent paper (repeated 3 times). The horseradish peroxidase (HRP) conjugate working solution (100 µL) was added to each well and the microplate was sealed and incubated for 30 min at 37°C. The solution was decanted again from each well and repetition of the washing step 5 times was performed. A substrate reagent (100 µL) was added to each well and the microplate was sealed and incubated for 15 min at 37°C. Then the stop solution (50 µL) was added to each well. The optical density (OD value) of each well was measured at 450 nm wavelength on a microplate reader set (Thermo Fisher Scientific Inc. Waltham, MA, USA). Calculations were done by referring to the standard curve and data were expressed as phosphorylated tau protein (pg/mL).

**Statistical analysis**

The study data were analysed using Statistical Package for the Social Sciences (SPSS) software (SPSS Inc., USA) version 24 and presented as means ± standard errors of mean (SEM). Differences between groups were evaluated using a one-way Analysis of Variance (ANOVA) followed by the Bonferroni post hoc test. A probability value ($p$) of less than 0.05 was used to indicate a significant difference.

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## Results

### Effects of minocycline on food intake and body weight

Figure 3 displayed the changes in food intake and body weight throughout the experiment. Food intake and body weight were assessed on day 1 and day 19 of the experiment. Statistical analysis showed that there were no significant differences ($p$>0.05) between groups in mean food intake and body weight on day 1 of the experiment. However, the LPS rats exhibited significantly lower mean body weight ($p$<0.05) and mean food intake ($p$<0.05) when compared to the control group on day 19 of the experiment, indicating LPS induced weight loss and reduced food intake. Interestingly, the mean weight and food intake of LPS rats treated with minocycline 25 and 50 mg/kg and memantine were significantly higher ($p$<0.05) than untreated LPS rats, indicating that minocycline and memantine could prevent LPS induced weight loss and reduce food intake. There was no significant difference in food intake and body weight between two doses of minocycline and memantine treated LPS rats ($p$>0.05).

### Effects of minocycline on the locomotor deficit and anxiety-like behaviour

The effects of minocycline on the locomotor deficit and anxiety-like behavior have been shown in Figure 4. The total distance, speed and number of line crossings were used to assess the locomotion of experimental rats. The results exhibited that there was significantly lower total distance, speed and number of line crossings ($p$<0.05) in LPS injected rats in comparison to the control group. Unlike untreated LPS rats, the LPS rats treated with minocycline and memantine revealed significantly higher total distance, speed and number of line crossings ($p$<0.05) compared to the LPS group.

Anxiety-like behaviours were assessed by measuring rearing and grooming frequency, time spent in the centre of the open field and frequency of entries into the centre. There was a significant decrease in rearing frequency, time spent in the centre of the open field and frequency of entries into the centre ($p$<0.05) and an increase in grooming frequency ($p$<0.05)

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in LPS rats compared to control. Minocycline at both doses and memantine significantly increased rearing frequency, time spent in the centre of the open field and frequency of entries into the centre ($p$<0.05) and decreased grooming frequency ($p$<0.05) compared to LPS rats.

Interestingly, minocycline 50 mg/kg exerts significantly higher total distance, speed and number of line crossings, rearing frequency, time spent in the centre of the open field and frequency of entries into the centre ($p$<0.05) and lower grooming frequency ($p$<0.05) than minocycline 25 mg/kg and memantine 10 mg/kg. No significant differences between minocycline 25 mg/kg and memantine ($p$>0.05) have been observed.

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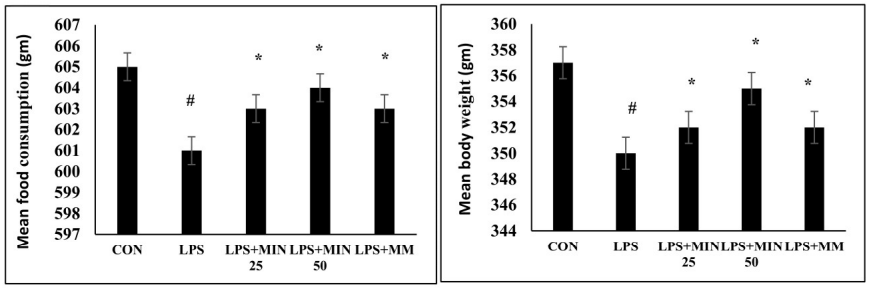


Figure 3.

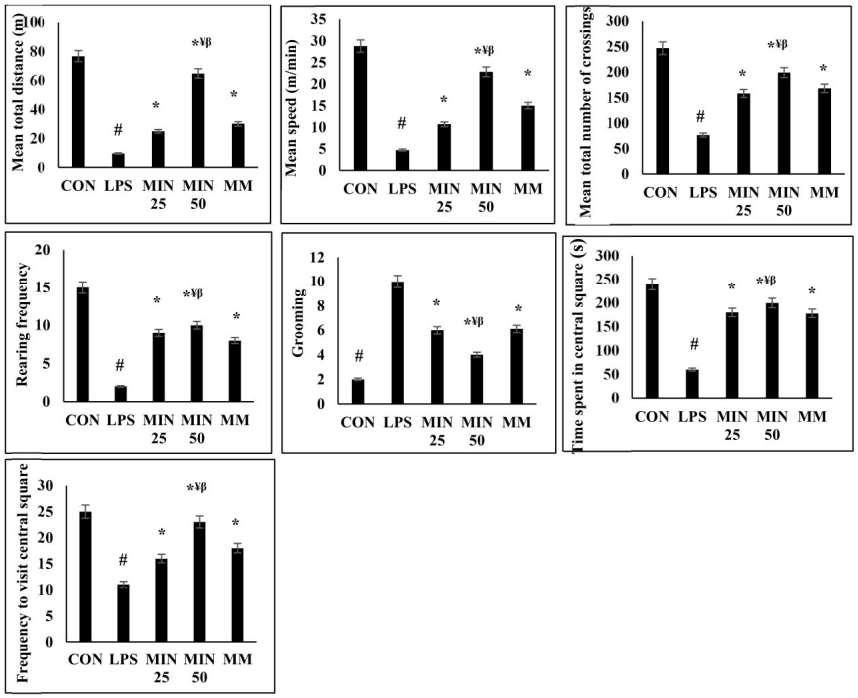


Figure 4

Figure 3: Mean food intake and body weight for all groups over a three-week experimental period.

Figure 4: Mean of the total distance (1); speed (2); the number of line crossings (3); rearing (4); grooming (5); time spent in the central square (6) and frequency to visit the central square (7) of all experimental groups during the open field test.

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One-way ANOVA test followed by Bonferroni post hoc test. Values are expressed as mean ± SEM. # $p<0.001$ versus control group; * $p<0.05$ versus LPS group, ¥, $p<0.05$ versus MIN 25, β, $p<0.05$ versus MM.

**Effects of minocycline on the expression of BDNF and CREB positive cells**

Figures 5 and 6 displayed the expression and quantification of BDNF and CREB positive cells. The LPS rats showed a more significant decrease in the number of the BDNF and CREB positive cells in mPFC tissues ($p<0.05$) compared with the control group. The minocycline and memantine treated LPS rats exhibited a more significant increase in BDNF and CREB positive cells ($p<0.05$) compared to the LPS group. There were no significant differences between minocycline (50 mg/kg), minocycline (25 mg/kg) and memantine (10 mg/kg) in the number of BDNF and CREB positive cells ($p>0.05$).

**Effects of minocycline on the expression level of BDNF and CREB proteins**

Figure 7 demonstrated the protein density and mean IDV value for BDNF and CREB proteins. The mean IDV values of BDNF and CREB proteins in mPFC tissues of the LPS group were significantly decreased ($p<0.05$) compared to the control group. The mean IDV values of BDNF and CREB proteins in the LPS group treated with minocycline and memantine were significantly increased ($p<0.05$) compared to the LPS group. The mean IDV values of BDNF and CREB proteins in the LPS group treated with minocycline (50 mg/kg) were significantly higher ($p<0.05$) than minocycline (25 mg/kg) and memantine (10 mg/kg) treated LPS rats. There was no significant difference in mean IDV values of BDNF and CREB proteins between minocycline (25 mg/kg) and memantine groups ($p>0.05$).

**Effects of minocycline on phosphorylated tau protein expression level**

This study also showed significant differences in the mean expression level of phosphorylated tau protein among all experimental groups which was shown in Figure 8. The mean expression level of phosphorylated tau protein was significantly higher ($p<0.05$) in LPS

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injected group compared to the control group. The mean expression level of phosphorylated tau protein was significantly lower ($p$<0.05) in the minocycline and memantine-treated LPS groups in comparison to the LPS group. However, minocycline 50 mg/kg significantly reduced phosphorylated tau protein expression level ($p$<0.05) more than minocycline 25 mg/kg and memantine 10 mg/kg.

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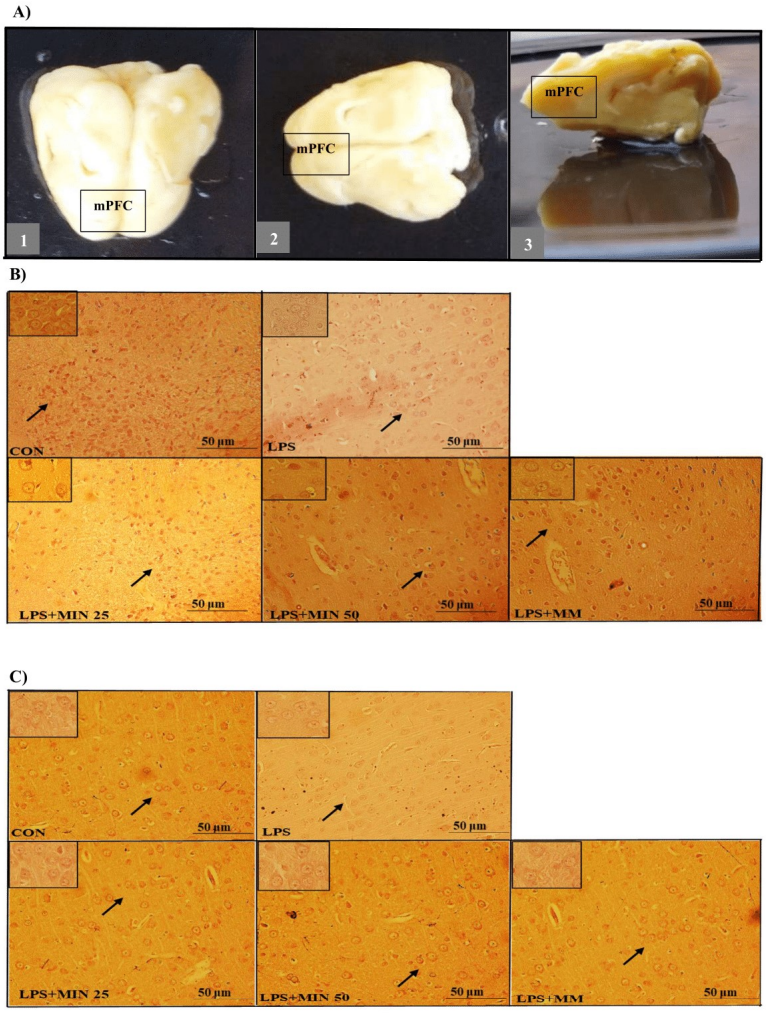


Figure 5

Figure 5: The localization of medial prefrontal cortex at bregma 3.20 mm and intraural 12.20 mm (A). Distribution of BDNF (B) and CREB positive cells (C) in the mPFC at 40× and 100× magnification. The black arrows indicate BDNF and CREB positive cells. CON=Control; LPS=Lipopolysaccharide; MIN 25=Minocycline 25 mg/kg; MIN 50=Minocycline 50 mg/kg; MM=Memantine 10 mg/kg.

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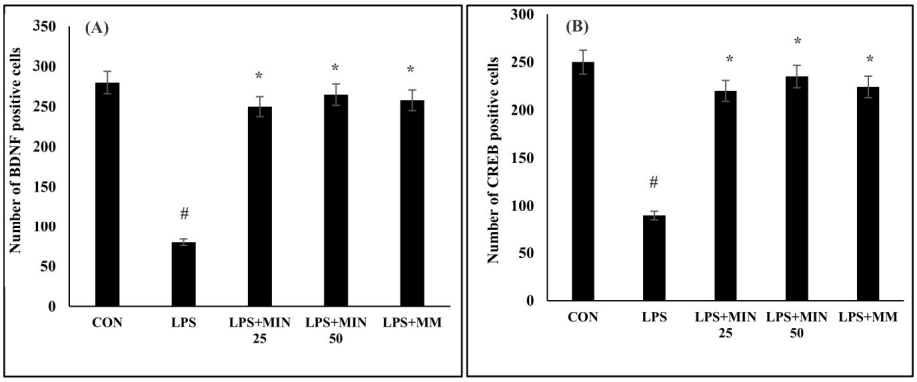


Figure 6

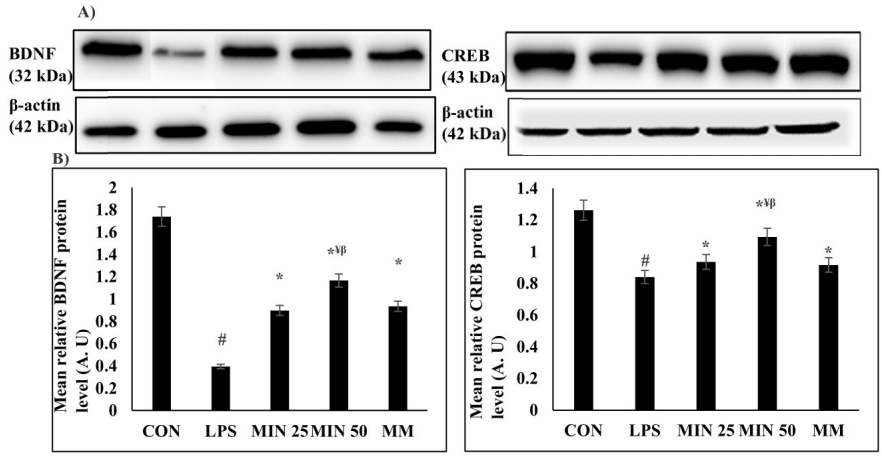


Figure 7

Figure 6: Total number of BDNF (A) CREB positive cells (B) in the mPFC. One-way ANOVA test followed by Bonferroni post hoc test. Values are expressed as mean ± SEM. # $p<0.001$ versus control group; *$p<0.05$ versus LPS group.

Figure 7: Effects of minocycline on the mean relative of BDNF and CREB protein levels in the mPFC. An example of western blot results for all groups (A). The lower panel demonstrates the loading control. (B) Quantification analysis of IDV between the groups. The data were

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normalized by the control group. One-way ANOVA test followed by Bonferroni post hoc test. Values are expressed as mean ± SEM, n=10 animals in each group. $^{\#}$ $p<0.05$ versus control group; *$p<0.05$ versus LPS group, $^{¥}$, $p<0.05$ versus MIN 25, $^{\beta}$, $p<0.05$ versus MM.

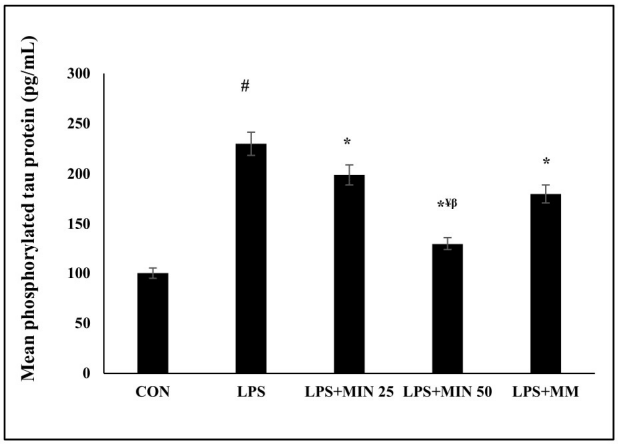


Figure 8

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Figure 8: Effects of minocycline on the mean phosphorylated tau protein level in the mPFC. One-way ANOVA test followed by Bonferroni post hoc test. Values are expressed as mean ± SEM. # $p<0.001$ versus control group; *$p<0.05$ versus LPS group, ¥, $p<0.05$ versus MIN 25, β, $p<0.05$ versus MM.

**Discussion**

This study demonstrated two main findings: 1) LPS induced locomotor deficit and anxiety-like behavior that was accompanied by decreased food intake and body weight, elevated phosphorylated tau protein level and decreased expression of BDNF and CREB proteins in the mPFC and 2) Both doses of minocycline significantly improved locomotor deficit and anxiety-like behavior. This study further verified the anxiolytic effects of minocycline, and minocycline reduced phosphorylated tau protein level as well as enhanced BDNF and CREB protein expressions in the mPFC of LPS rats comparable to memantine effects.

Our study also revealed that the LPS rats gradually reduced food intake and gained less body weight throughout the experimental period compared to the control group consistent with the previous study (Fan et al., 2005). The aetiology of weight loss and reduced food intake in the LPS rat model appears to be multifactorial and several hypotheses have been postulated to explain it. LPS was known to induce sickness behaviours and growth failure, which were manifested by a reduction in activity, exploration, social interaction and consumption of food and drink, fever, protein loss, hypersomnia and activation of the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic system (Castanon et al., 2001). The minocycline and memantine treated LPS groups showed restoration of body weight gain that was comparable to the control group, suggesting that minocycline and memantine treatment ameliorated LPS-induced weight loss. Previous reports suggested that restoration of body weight and food intake in minocycline treated LPS rats was attributed to its anti-glial cells mediated neuroinflammation (Fan et al., 2005). The inhibitory effect of minocycline on glial cell-

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mediated neuroinflammation in our study (unpublished data) was in line with the previous study and could be the underlying pathway of minocycline protection against LPS-induced decreased food intake and body weight.

This study confirmed that intraperitoneal LPS (5 mg/kg) significantly induced locomotor deficit as exhibited by reduced mean total distance, speed and line crossings and anxiety-like behavior as showed by reduced rearing, time spent in the central square and frequency to visit the central square and increased grooming frequency. Similar findings have been observed in other studies where LPS induced locomotor deficit and anxiety-like behavior in several animal models (Kinoshita et al., 2009; Murray et al., 2020). The LPS-induced locomotor deficit and anxiety-like behavior were reversed by minocycline (25 and 50 mg/kg) and memantine (10 mg/kg) administration for 2 weeks which was in line with other studies that showed minocycline improved locomotor activity and exerted anxiolytic effects in several animal models (Afshary et al., 2020; Rooney et al., 2020).

Interference with neurotrophin expression and function in the frontal, parietal, temporal and occipital cortex has been proposed as one of the underlying mechanisms of LPS-induced neurobehavioural impairment. A previous study demonstrated that LPS stimulates pro-inflammatory cytokine production which inhibits brain BDNF expression via the activation of the hypothalamic-pituitary-adrenal (HPA) axis (Adzic et al., 2015). Additionally, several reports proposed that proinflammatory cytokines stimulate amyloid deposition and neurofibrillary tangles formation, which mainly consist of hyperphosphorylated tau protein, that trigger inflammatory and oxidative stress pathways. The inflammatory cytokines interfered with BDNF signalling by reducing the phosphorylation of the BDNF receptor (TrkB) (Cappoli et al., 2020). The downregulation of the BDNF/CREB signalling pathway leads to disturbance of synaptic neurotransmission, alteration of LTP and subsequent neurobehavioural dysfunction (Ge et al., 2015; Schnydrig et al., 2007; Yang et al., 2014). These findings were in

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line with our findings that LPS increased phosphorylated tau protein level as well as decreased BDNF and CREB protein expressions in mPFC, while minocycline and memantine treatment were able to reverse them by decreasing phosphorylated tau protein level and increasing BDNF and CREB protein expressions.

Thus, to the best of our knowledge, this study showed for the first time that minocycline attenuated locomotor deficit and anxiety-like behavior, reduced phosphorylated tau protein and upregulated BDNF and CREB proteins expressions in mPFC of LPS rats that were comparable to memantine effects. This study postulated that the anti-tau protein property of minocycline upregulated BDNF/CREB signalling pathway in mPFC and protected against LPS-induced locomotor deficit and anxiety-like behavior. Furthermore, the neuroprotective effects of minocycline observed in this study were dose-dependent which is the higher the dose, seen the better the neuroprotective effects even when compared to the memantine.

## Conclusion

Minocycline, in a dose-dependent manner, improved locomotor deficit and anxiety-like behavior, decreased phosphorylated tau protein and upregulated BDNF/CREB protein expression in mPFC of LPS rats. Higher dose of minocycline showed better results compared to memantine. Thus, minocycline can be used as a preventive-therapeutic drug for neuroinflammatory diseases.

## Declaration of conflicting interests

The author(s) declared that there were no possible conflicts of interest in the research, writing, or publishing of this paper.

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## Ethical Approval

The number of Animal Ethics Approval is: [USM/IACUC/2018/ (942) (114)].

## Informed consent

No informed consent was distributed.

## Author contribution

IL planned the experiment and write the manuscript, EYAQ performed the experiment and write the manuscript, IL, ZA, RZ review the manuscript.

## Data availability

The datasets generated during and/ or analysed during the current study are available from the corresponding author on reasonable request.

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