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ORIGINAL ARTICLE

Mahanimbine-Induced Neuroprotection via Cholinergic System and Attenuated Amyloidogenesis as well as Neuroinflammation in Lipopolysaccharides-Induced Mice

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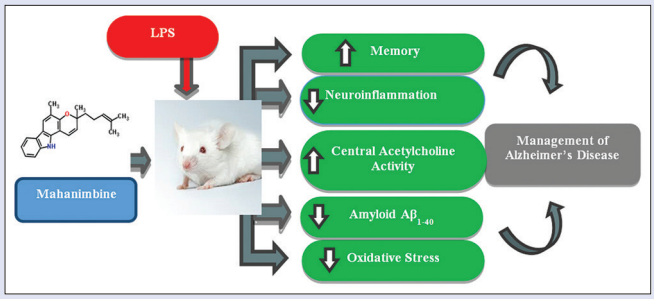
ABSTRACT

Background: *Murraya koenigii* leaves are traditionally used in India and other South Asian countries as a spice in regular food dishes to improve taste and aroma. These leaves are known for the rich content of mahanimbine, a key carbazole alkaloid. Although there are numerous reports that support the neuroprotective role of various alkaloids, the effect of mahanimbine against memory impairment remains to be elucidated. **Objective:** The present study aimed to explore the neuroprotective potential of mahanimbine against lipopolysaccharides (LPS)-induced memory deficit in Institute of Cancer Research (ICR) mice. **Materials and Methods:** Group of mice were being fed with mahanimbine (1, 2, and 5 mg/kg, *p. o.*) for 30 days. Subsequently, neuroinflammation was induced with LPS (250 μg/kg, *i. p.*) for 4 days. Morris water maze (MWM) assessment was conducted to assess spatial memory. The brain was then collected and subjected to amyloid-beta (Aβ) ($Aβ_{1-42}$ and $Aβ_{1-40}$) measurement, acetylcholine (ACh) and acetylcholinesterase (AChE) assays and neuroinflammatory analyses (interleukin [IL]-1 β, tumor necrosis factor alpha [TNF-α], IL-10 transforming growth factor beta [TGF-β], and cyclooxygenase [COX]). **Results:** The MWM test showed that treatment with mahanimbine significantly enhanced memory of LPS-challenged mice by decreasing both escape latency as well as escape distance. Pretreatment of the LPS-challenged mice with mahanimbine improved central cholinergic transmission by increasing ACh level through inhibition of AChE. It also significantly attenuated $Aβ_{1-40}$ level. While anti-inflammatory cytokines (TGF-β and IL-10) were upregulated, mahanimbine significantly inhibited pro-inflammatory cytokines (IL-1 β and TNF-α), the total activity of COX, and expression of COX-2 gene in LPS-induced group. **Conclusion:** The overall findings supported the neuroprotective potential of mahanimbine against LPS-induced neuroinflammation.

Key words: Acetylcholine, beta-amyloid, mahanimbine, *Murraya koenigii*, neuroinflammation

SUMMARY

- Mahanimbine from *Murraya koenigii* leaves elicited potential neuroprotection against lipopolysaccharides (LPS)-challenged ICR mice. The present findings indicated that mahanimbine could improve memory capacity and central cholinergic transmission as well as reduce neuroinflammation and amyloid-beta$_{1-40}$ level despite the influence of LPS.



Abbreviations used: Ach: Acetylcholine; AChE: Acetylcholinesterase; AD: Alzheimer's disease; APP: Amyloid precursor protein; Aβ: Amyloid-beta; ANOVA: Analysis of variance; BACE1: β-Secretase 1; COX: Cyclooxygenase; cDNA: Complementary DNA; ED: Escape distance; EL: Escape latency; LPS: Lipopolysaccharides; mRNA: Messenger RNA; MWM: Morris Water Maze; OECD: Organisation for Economic Co-operation and Development; TGF-β1: Transforming growth factor beta1; TNF-α: Tumor necrosis factor alpha.

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INTRODUCTION

Alzheimer's disease (AD) is reported as the most prevalent form of dementia. It is clinically characterized by progressive deficit in memory, deficiency in cognitive function, and inappropriate behavior. The major pathological markers of AD include formation of extraneuronal senile plaques and intraneuronal neurofibrillary tangles; these are primarily composed of amyloid-beta (Aβ) and aggregated microtubule-associated protein tau, respectively.[1] Deposition of Aβ and neuroinflammation play vital roles in AD pathogenesis. Neuroinflammation is attributed to synthesis of inflammatory cytokines (transforming growth

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factor [TGF]-β, tumor necrosis factor [TNF]-α, interleukin [IL]-6, and IL-β) that can further enhance amyloid precursor protein (APP) expression and Aβ formation.[2] Increased production of cytokines will also result in upregulation of β-secretase 1 (β-site amyloid precursor protein–cleaving enzyme 1 [BACE1]), an enzyme that generates Aβ by cleavage of APP.[3]

The neuroanatomical studies indicated that cholinergic neurons which project into the hippocampus and neocortex are primarily affected by AD. Therefore, augmenting brain cholinergic neurotransmission has been focused as symptomatic treatment against AD.[4,5] Acetylcholinesterase (AChE) is also known to control cortical cholinergic neurotransmission by catalyze the metabolic cleavage of acetylcholine (ACh) in the synaptic cleft after depolarization and thus stops synaptic transmission. At present, AChE inhibitors are approved clinically to enhance cholinergic neurotransmission in AD patients.[6]

Murraya koenigii Linn originates from the family of *Rutaceae*. It is commonly known as curry leaves in English as well as "*Pokok kari*" locally. The bark, leaves, and root of this plant are used in indigenous medicines as a carminative, stimulant, stomachic, and tonic. Various parts of *M. koenigii* are also used in ayurvedic and other traditional medicines for treatment of pain, curing piles, inflammation, rheumatism, itching, traumatic injury and snake bite.[7] *M. koenigii* has also been reported to possess cytotoxic, antioxidative, cholesterol-lowering, antibacterial, and antiulcer properties.[8,9] The bioactive carbazole alkaloid constituents of *M. koenigii* include O-methyl mahanine, isomahanine, O-methyl murrayanine, koenimbine, bismahanine, bispyrafoline, euchrestine, bismurrayafoline, murrayanol, mahanimbine, girinimbine, and mahanine.[10] Among the many compounds, mahanimbine is the key carbazole alkaloid that can be isolated from *M. koenigii* leaves. Mahanimbine is widely documented for its ability in reducing blood glucose and total cholesterol levels as well as increasing high-density lipoprotein.[11] The effect on memory impairment, however, is not well studied. Our previous reports demonstrated that supplementation of mice with *M. koenigii* leaves for 30 days exhibited antiamnesic effect.[12] In a later study, total alkaloidal extract of *M. koenigii* leaves reversed memory deficit/loss and elevated ACh activity in scopolamine- and age-induced amnesia models.[13] In addition, mahanimbine isolated from *M. koenigii* leaves inhibited AChE activity *in vitro* at a potent IC_{50} value (0.03 ± 0.09 mg/mL).[14] As an extension of our findings, the present study aimed to uncover the neuroprotective effect of mahanimbine against lipopolysaccharides (LPS)-induced neuroinflammation models.

MATERIALS AND METHODS

Extraction and isolation of mahanimbine

The *M. koenigii* fresh leaves were purchased from Puncak Alam area (Malaysia). The collected leaves were authenticated by a taxonomist from Biodiversity and Environment Division, Forest Research Institute, Malaysia, and a voucher specimen (PID 24101011) was submitted in the herbarium. The procedures of extraction and isolation were followed according to Tachibana *et al.*[10,15]

Animals

Male ICR mice were supplied by the Laboratory Animal Facility and Management, Universiti Teknologi MARA (UiTM), Puncak Alam, Malaysia. The animals, which weighed 25–35 g and of 8–12 weeks of age, were housed in polyacrylic cages and maintained at room temperature (21°C–25°C). All the animals were allowed to access the standard diet and water *ad libitum*. The rodents were housed in groups of about six per cage and acclimatized for at least 5 days before experiment. Experiments were carried out between 0800 and 1800 h. The experimental design was approved by the Research Committee on the Ethical Use in Research, UiTM, Malaysia (Reference No.: 37/2014).

Acute toxicity study

The acute toxicity study of mahanimbine was performed according to the 423 guideline of the Organisation for Economic Co-operation and Development. Initially, three mice were randomly selected for this study and administered with 5 mg/kg mahanimbine orally.[16]

Drug administration

Mice were distributed randomly into six groups ($n = 6$). The animals from the control and LPS-treated groups were subjected to administration of vehicle (0.5% w/v Carboxymethyl cellulose (CMC)) for 30 days (day 1–30). Mahanimbine groups (three groups: 1, 2 and 5 mg/kg), on the other hand, were being treated orally for 30 days (day 1–30). The Morris water maze (MWM) training trials were then conducted over 3 days (day 23–25). The actual behavioral assessments using MWM were conducted 4 h after an intraperitoneal injection of LPS (250 μg/kg) daily (except for the control group) over the last 4 days of treatment (day 26–30). After 24 h (day 31) of the last acquisition test, each of the mouse was exposed to a probe trial for 60s. Figure 1 illustrates the treatment timeline.

Behavioral assessments of memory by morris water maze

The MWM is made up of a black circular pool (height: 35 cm, diameter: 100 cm) divided into four quadrants of equal area: north east (NE), south east, north west, and south west (SW) and an escape platform (14.5 cm height and 4.5 cm diameter) submerged 1.0 cm below the opaque surface at the center of the SW quadrant. A video tracking system with SMART-LD Program (Panlab, Spain) was used to record the swimming of each mouse. To train the animals, each mouse was subjected to three training sessions per a day from three fixed points of each quadrant, allowing it to explore the platform for 3 days (Days 29–31). During the actual test (Days 32–35), each animal would swim from a fixed starting position (NE) and escape latency (EL), swimming speed and escape distance (ED) recorded. The animal would be gently guided by hand to the platform, if it crossed the cutoff time (60s). A probe test was examined without the platform on day 36, 24 h after the 3-day acquisition test, to assess memory consolidation. The time spend by each mouse in the target quadrant (SW) was recorded for 60s by allowing them to swim freely in the pool.[17]

Collection of brain samples

On the day 36, after MWM test all the mice were sacrificed by cervical decapitation under light anesthesia using a combination of ketamine (100 mg/kg, *i. p.*) and xylazine (20 mg/kg, *i. p.*). Immediately after

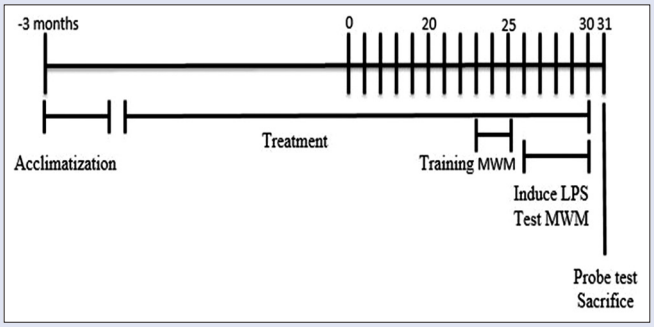

Figure 1: Treatment timeline

cervical decapitation, half of the brain was homogenized with cold phosphate-buffered saline for biochemical analysis while the other half was kept in RNA later for cyclooxygenase (COX)-2 gene expression.

Determination of acetylcholine level and acetylcholinesterase activity

The EnzyChrom™ ACh assay kit (BioAssay System, USA) was utilized to determine the level of ACh in brain homogenate. In addition, the level of the AChE was estimated using QuantiChrom™ AChE Assay kit (BioAssay System, USA).

Determination of amyloid-beta$_{1-40}$ and amyloid-beta$_{1-42}$ levels

The both of the amyloids levels in brain homogenate were evaluated using the standard ELISA bioassay kits from Cloud Clone Corp (Houston, USA).

Determination of cytokines level

Cytokine (i.e., IL-1 β, TNF-α, IL-10, and TGF-β1) levels in brain homogenate were evaluated using the Procarta® Immunoassay kit-Polystyrene beads from Affymetrix, eBioscience (Vienna, Austria).

Determination of total cyclooxygenase activity

The Cayman COX activity assay kit (Ann Arbor, USA) was followed to determine the activity of total COX in brain homogenate.

Quantification of cyclooxygenase-2 gene expression using real-time polymerase chain reaction

RNeasy Mini kit from Qiagen (Valencia, CA) was utilized to isolate total RNA from mouse brain. The isolated RNA (1 μg) was reverse-transcribed into cDNA using the Reverse Transcriptase Kit from Qiagen (Valencia, CA). For real-time polymerase chain reaction (RT-PCR), cDNA (2 μL) was amplified using a SYBR Green Q-PCR master mix (Qiagen, Valencia, CA) in a Corbett instrument (Qiagen, Germany). The RT-PCR conditions were: 95°C for 5 min followed by 40 cycles at 95°C for 10 min and 60°C for 30 min. The expression of all genes were determined and normalized against the internal control, β-actin. cDNA sequences of mouse COX-2 (forward 5'-GTGTGCGACATACTCAAGCAGGA-3': reverse 5'-TGAAGTGGTAACCGCTCAGGTG-3') and β-actin (forward 5'-TGACAGGATGCAGAAGGAGA-3': reverse 5'-GCTGGAAGGTG GACAGTGAG-3') were obtained from previous reports. Rotor-Gene 6000 software (Qiagen, Germany) was used to analyze the recorded fluorescence measurements. Furthermore, comparative C_t (threshold cycle) method was used to quantify gene expression of COX-2.

Statistical analysis

Each data was stated as mean ± standard error means in results. Graph Pad version 6 (GraphPad Software Inc., United States) was employed for one-way ANOVA to compare between multiple groups and Tukey-Kramer *post hoc* test to compare between two groups. The level 0.05 was followed as significant between the groups.

RESULTS

Acute toxicity study

No mortality was observed at 50 mg/kg (*p. o.*) mahanimbine. Three doses (1, 2, and 5 mg/kg) were therefore selected for subsequent assessment of memory and neuroprotective potential.

Mahanimbine-enhanced memory in lipopolysaccharides-induced mice

Figure 2a shows that LPS-induced control animals (250 μg/kg, *i. p.*) required longer EL for day 1–3 (20.15 ± 1.90 s, $P < 0.01$; 19.78 ± 1.70 s, $P < 0.01$; 17.38 ± 1.14s, $P < 0.001$; respectively) when compared to the control mice (12.32 ± 0.75 s, 11.64 ± 1.77 s and 8.96 ± 1.29 s, respectively). These results confirmed the LPS-induced memory deficit in mouse. Treatment with different doses of mahanimbine, however, significantly reversed memory impairment induced by LPS. Mice treated with 1 mg/kg mahanimbine exhibited greater extend of improvement against memory impairment when compared to the other two doses (2 and 5 mg/kg). The EL values were 12.74 ± 1.83 s ($P < 0.05$), 7.03 ± 0.74 s ($P < 0.001$), and 6.40 ± 0.29 s ($P < 0.001$) for days 1–3, respectively. The EL values for mice treated with 2 mg/kg mahanimbine were 12.24 ± 1.48 s ($P < 0.01$), 8.44 ± 1.71 s ($P < 0.001$) and 6.91 ± 0.67 s ($P < 0.001$) on days 1–3,

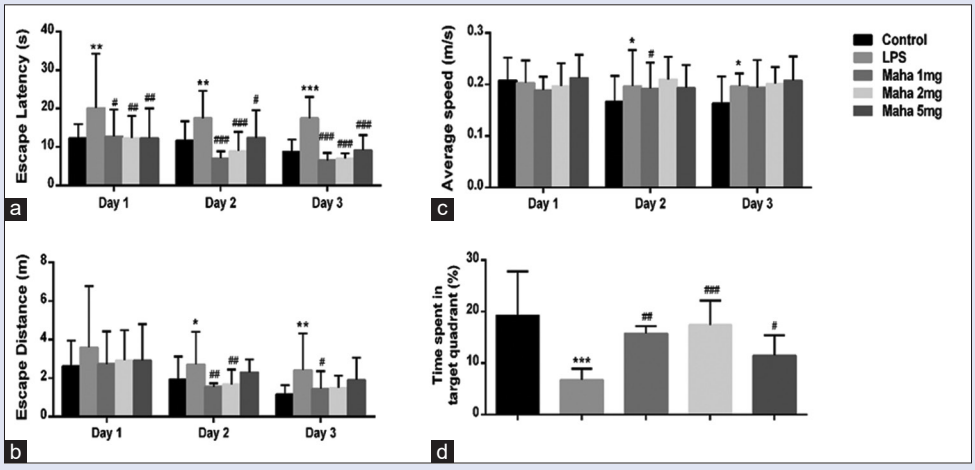

Figure 2: Pretreatment lipopolysaccharides-induced mice with mahanimbine enhanced memory. (a) Hidden platform acquisition. Escape latency represents time taken to escape to the platform from the water. (b) Distance travelled. Escape distance represents the distance toured to escape to the platform from the water. (c) Motor function of animals as reflected by their swimming speeds. (d) Probe test. Percentage of time in target quadrant is calculated as ratio of time spent in target quadrant area relative to the time spent in the rest of the pool. Each bar represents mean ± standard error means ($n = 6$). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ versus control group; #$P < 0.05$, ##$P < 0.01$, and ###$P < 0.001$ versus lipopolysaccharides-induced group

respectively. The EL values for mice treated with 5 mg/kg mahanimbine were 12.26 ± 1.14 s ($P < 0.01$), 12.38 ± 0.66 s ($P < 0.05$) and 8.77 ± 0.42 s ($P < 0.001$), respectively.

Figure 2b presents the effect of mahanimbine against ED. Mice injected with LPS alone travelled the longest distance before finding the hidden platform on day 2–3 trials (2.72 ± 0.22 m [$P < 0.05$] and 2.43 ± 0.27 m [$P < 0.01$], respectively) when compared to the control group (1.93 ± 0.16 m and 1.17 ± 0.19 m, respectively). There were no significant deviations observed in-between the groups on day 1. Mice fed with 1 mg/kg mahanimbine, however, travelled significantly shorter distance (1.56 ± 0.07 m [$P < 0.01$] and 1.46 ± 0.18 m [$P < 0.05$], respectively) for day 2–3 when compared to LPS-induced animal. A significant decline (1.68 ± 0.11 m [$P < 0.01$]) in ED was also recorded in mice treated with 2 mg/kg mahanimbine on day 2. On the other hand, neither animals treated with LPS alone nor mahanimbine in the presence of LPS showed significant changes in average swimming speed when compared to the control group [Figure 2c].

As for the probe test [Figure 2d], LPS-induced mice spent significantly lesser time (6.70% ±0.69% [$P < 0.001$]) in the target quadrant (SW) when compared to the control group (19.22% ±2.61%). Mice administered with mahanimbine at 1, 2, and 5 mg/kg, however, spent significantly longer time (15.77% ±0.58% [$P < 0.01$], 17.42% ±1.92% [$P < 0.001$] and 13.10% ±1.07% [$P < 0.05$], respectively) in the target quadrant when compared with LPS-induced mice.

Mahanimbine improved the cholinergic activity in lipopolysaccharides-induced mouse brain

In reference from Figure 3a, the level of the ACh was significantly declined ($P < 0.01$) by LPS-injection (11.55 ± 1.52 μM) as compared to the control group animals (27.98 ± 1.60 μM). Administration of LPS-challenged mice with mahanimbine (1, 2, and 5 mg/kg), however, had significantly increased the level of ACh in brain homogenate (27.48 ± 2.44 μM [$P < 0.01$], 24.40 ± 2.87 μM [$P < 0.05$], 23.19 ± 2.62 μM [$P < 0.05$], respectively) when compared to LPS-induced mice. Overall, administration of low dose mahanimbine (1 mg/kg) produced better results in increasing brain ACh level.

From Figure 3b, the activity of AChE in LPS-treated group (409 ± 8.89 U/L) was significantly elevated ($P < 0.05$) as compared with the control group (346 ± 18.91 U/L). Nevertheless, 1 mg/kg (314.39 ± 15.17 U/L; $P < 0.001$) and 2 mg/kg (337.82 ± 4.55 U/L; $P < 0.01$) mahanimbine significantly inhibited the activity of AChE when compared to the LPS-induced control group. Moreover, treatment with 5 mg/kg mahanimbine (376.96 ± 16.03 U/L) did not show any significant differences.

Mahanimbine reduced neuroinflammation markers in brain homogenate of lipopolysaccharides-induced mice

The ability of mahanimbine in suppressing neuroinflammation was indicated by reduced levels of IL-1 β and TNF-α (pro-inflammatory cytokines) and increased levels of IL-10 and TGF-β1 (anti-inflammatory cytokines) in the brain. LPS had significantly elevated TNF-α ($P < 0.05$) and IL-1 β ($P < 0.001$) levels and significantly reduced ($P < 0.05$) IL-10 and TGF-β1 levels when compared to the control animals. The production of IL-1 β was significantly reduced under treatment with 1 mg/kg (0.92 ± 0.05 pg/mL; $P < 0.05$) and 2 mg/kg (0.96 ± 0.04 pg/mL; $P < 0.05$) mahanimbine when compared to LPS-induced group (1.29 ± 0.10 pg/mL) [Figure 4a]. There were no significant changes observed at the highest dose (5 mg/kg) of mahanimbine. Although LPS-induced TNF-α [Figure 4b] level was reduced under treatment with 1, 2, and 5 mg/kg mahanimbine (1.83 ± 0.17 pg/mL, 2.17 ± 0.17 pg/mL, and 2.17 ± 0.17 pg/mL, respectively), the reductions were not significant when compared to LPS-injected group (2.5 ± 0.224 pg/mL).

On the other hand, IL-10 level was significantly increased under treatment with 1 mg/kg (3.67 ± 0.22 pg/mL; $P < 0.01$) and 2 mg/kg (3.5 ± 0.13 pg/mL; $P < 0.01$) mahanimbine when compared to LPS-induced animals (2.67 ± 0.21 pg/mL) [Figure 4c]. Based on Figure 4d, 2 and 5 mg/kg mahanimbine significantly increased the level of TGF-β1 (2116.5 ± 158.19 pg/mL and 2143.69 ± 183.44 pg/mL ($P < 0.01$), respectively) when compared to LPS-induced mice (1344 ± 98.92 pg/mL). Low-dose mahanimbine (1 mg/kg) appears to produce better results in modulating the production of IL-1 β, TNF-α l, and IL-10 levels against LPS-induced control. On the contrary, 2 and 5 mg/kg mahanimbine suppressed pro-inflammatory cytokine TGF-β1 at a greater extend when compared to its lower dose (1 mg/kg).

Figure 4e illustrates significant elevation (10.32 ± 0.04 U/mL; $P < 0.001$) of total COX activity in the brain of LPS-induced mice when compared to control group (6.28 ± 0.18 U/mL). The activity of COX was significantly attenuated ($P < 0.001$) by 1, 2 and 5 mg/kg mahanimbine (3.77 ± 0.09 U/mL, 3.96 ± 0.09 U/mL and 4.62 ± 0.08 U/mL, respectively) when compared to LPS group.

Figure 4f shows that the expression of COX-2 mRNA level was significantly elevated in LPS group (6.95 ± 0.93; $P < 0.001$). The mRNA expression of COX-2 in LPS-induced mice was, however, significantly attenuated ($P < 0.001$) under treatment with mahanimbine at all doses.

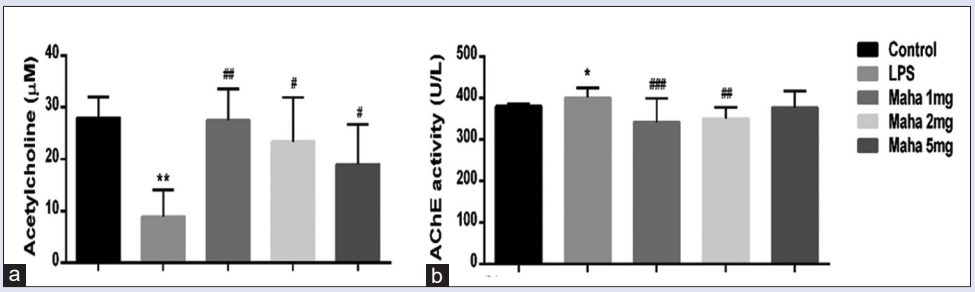

Figure 3: Pretreatment with mahanimbine of lipopolysaccharides-induced mice improved cholinergic activity in brain. (a) Acetylcholine level in the brains of lipopolysaccharides-induced mice. (b) Acetylcholinesterase activity in the brains of lipopolysaccharides-induced mice. Each bar represents mean ± standard error means ($n = 6$). *$P < 0.05$, **$P < 0.01$, versus control group; #$P < 0.05$, ##$P < 0.01$, and ###$P < 0.001$ versus lipopolysaccharides-induced group

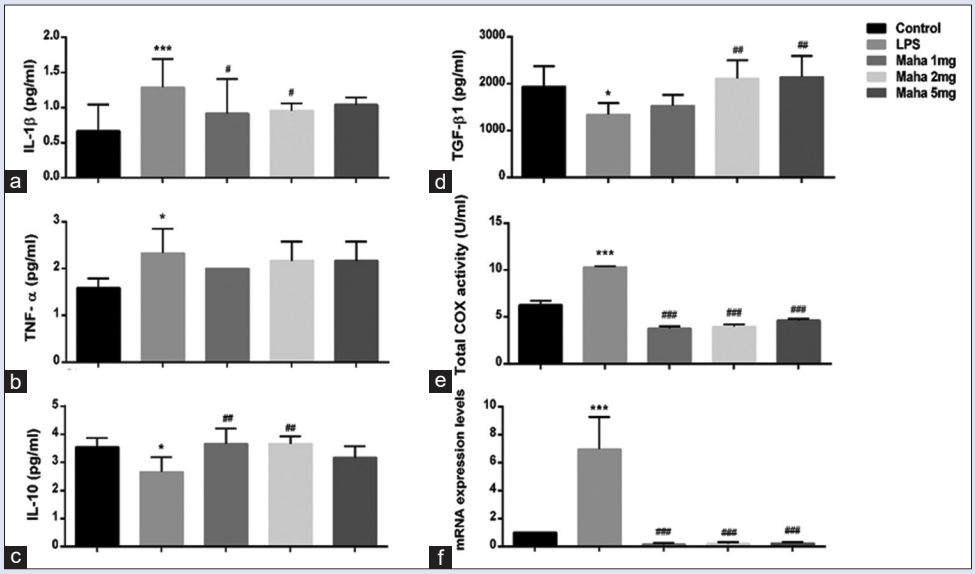

Figure 4: Inhibitory effect of mahanimbine against neuroinflammatory markers in lipopolysaccharides-induced mice. (a-d) Cytokine levels were determined using the multiplex assay kit. (e) Cyclooxygenase activity in the brain was measured using ELISA kit. (f) Cyclooxygenase-2 gene expression was validated using real-time-polymerase chain reaction. Each bar represents mean ± standard error means (n = 6). *P < 0.05 and ***P < 0.001 versus control group; #P < 0.05, ##P < 0.01, and ###P < 0.001 versus lipopolysaccharides-induced group

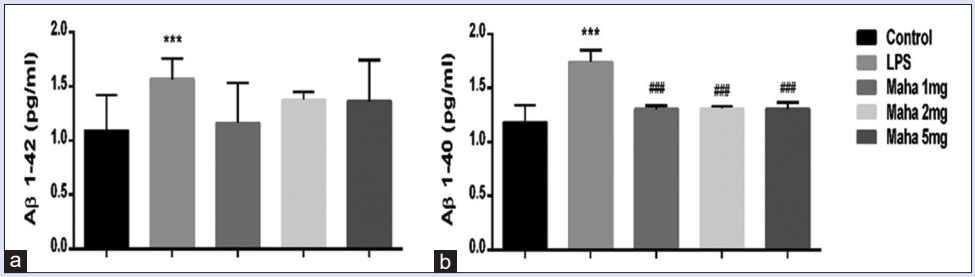

Figure 5: Mahanimbine suppressed formation of β-amyloid in lipopolysaccharides-induced mice. The level of amyloid-beta$_{1-42}$ and amyloid-beta$_{1-40}$ were measured using amyloid-beta ELISA kit. (a) Amyloid-beta$_{1-42}$ level in the brains of lipopolysaccharides-induced mice. (b) Amyloid-beta$_{1-40}$ level in the brains of lipopolysaccharides-induced mice. Each bar represents mean ± standard error means (n = 6). ***P < 0.001 versus control group; ###P < 0.001 versus lipopolysaccharides-induced group

Mahanimbine inhibited amyloid-beta$_{1-40}$ but not amyloid-beta$_{1-42}$ in the lipopolysaccharides-induced mouse

The level of Aβ_{1-42} in mouse brain was induced (P < 0.001) by LPS (250 μg/kg) intraperitoneal injections for 4 days [Figure 5a]. Treatment with 1 mg/kg (1.33 ± 0.07 pg/mL), 2 mg/kg (1.0 ± 0.03 pg/mL) and 5 mg/kg (1.51 ± 0.10 pg/mL) of mahanimbine slightly suppressed the level of Aβ_{1-42}, but without any significant difference as compared to LPS-induced group (1.58 ± 0.07 pg/mL). As for Aβ_{1-40} level, LPS injection significantly elevated the level of Aβ_{1-40} (P < 0.001) as compared to control [Figure 5b]. Treatment with all doses of mahanimbine, however, significantly attenuated (P < 0.001) the level of Aβ_{1-40} when compared to the LPS-induced group. These results are indicated that mahanimbine has a potential in inhibiting the formation of Aβ_{1-40} but not Aβ_{1-42}.

DISCUSSION

Neuroinflammatory mechanisms are increasingly implicated in the pathogenesis of neurodegenerative disorders such as AD.[18] In this regard, neuroprotective approach against neuroinflammation is deemed effective for management of AD. Mahanimbine is a key carbazole alkaloid that can be found in *M. koenigii* leaves. Capitalizing on the association of carbazole alkaloids with anti-inflammatory, antioxidant, anti-viral and anti-tumor activities,[11] thus, the present study was demonstrated the neuroprotective effect of mahanimbine using mouse model against neuroinflammation induced by the LPS.

Based on the MWM findings, the present study found that mahanimbine could improve memory loss due to the LPS-induced neuroinflammation. MWM is an extensive maze model for determine the spatial learning and memory in various rodent models.[19] The continuous 30 days of pretreatment with mahanimbine (1, 2 and 5 mg/kg) had significantly enhanced memory against LPS-induced memory impairment as manifested through a decrease in EL and ED. Since no changes were observed in swimming speed cross the groups, it was clear that LPS affected only the navigation parameters which were dependent upon learning and memory process but not the motor system. The probe test was performed after removal of hidden platform so as to evaluate memory retention and consolidation.[20] It was found that the groups of mice treated with mahanimbine spent higher time in the target quadrant, but the effects were not dose dependent. Treatment of low

doses (1 mg/kg and 2 mg/kg) of mahanimbine yielded greater effect on memory parameters when compared to high dose (5 mg/kg).

Cholinergic neurons play vital roles in neurodegenerative diseases and other aging related memory deficits.[21] In line with the cholinergic hypothesis, higher deficiencies in ACh activities were commonly identified among senile dementia patients.[22] This is attributed to AChE found in the synaptic cleft which rapidly metabolizes ACh to choline and acetate and inhibits its neuronal signaling.[23] In the present study, exposure to LPS resulted in lower level of ACh and higher level of AChE activity. It was reported that LPS could also lower ACh synthesis through declined choline acetyltransferase function and weakened cholinergic transmission between the neurons.[24] Nevertheless, 30 days of pretreatment with mahanimbine improved the central cholinergic transmission by increasing ACh level and inhibiting AChE activity in the brain.

The current study also demonstrated the influence of mahanimbine against the elevated $A\beta_{1-42}$ and $A\beta_{1-40}$ in LPS-challenged mice. LPS-induced animals showed elevation of $A\beta_{1-42}$ and $A\beta_{1-40}$ level and impairment of memory parameters when compared to the control group. Increased $A\beta_{1-42}$ level could be associated with elevated expression of the amyloidogenic protein which could be responsible for neuronal damages that could lead to memory dysfunction.[2,3] Mahanimbine exhibited potential inhibition of $A\beta_{1-40}$ level but not $A\beta_{1-42}$ level.

Inflammation usually occurs to eliminate primary causes and tissue injury resulting from original insult. Neuroinflammatory responses are also correlated with the accumulation of $A\beta_{1-42}$ in the brain. Inflammatory reaction in the brain involves the microglia, astrocytes and neurons. On activation, the cells would induce prostaglandins, pro-inflammatory cytokines, ROS, macrophage inflammatory proteins, chemokines, nitric oxide, and inflammatory mediators.[25] The present study demonstrated the influence of mahanimbine against cytokine in LPS-challenged mice. Cytokines are small and nonstructural proteins that function through binding to specific cell surface receptors. The activity can be upregulated or downregulated by transcription factors via intracellular signaling mechanisms that could lead to either anti-inflammatory or pro-inflammatory reactions.[26] The current study showed elevated levels of IL-1 β as well as TNF-α and suppressed levels TGF-β1 as well as IL-10 by LPS. This confirmed the occurrence of neuroinflammation in the brain. Plata-Salamán *et al.* reported similar observations whereby imbalance between cytokines may lead to cytokine actions that could synergistically induce a cytotoxicity and amplified cycle of cellular activation.[27] Nevertheless, pretreatment with mahanimbine inhibited pro-inflammatory (IL-1beta and TNF-alpha) and increased anti-inflammatory cytokines (TGF-β1 and IL-10). The current results indicated that mahanimbine may act as a potential anti-inflammatory agent against LPS-induced neuroinflammation. Besides, the present study assessed the influence of mahanimbine against 2 inflammatory markers, which included total COX activity and COX-2 expression. COX exists in two isoenzymes, COX-1 and COX-2, both of which are involved in the formation of prostaglandins and other lipid mediators from arachidonic acid.[28] The two distinct isoforms of COX differ in terms of their tissue distribution, regulatory mechanisms and preferential coupling to upstream and downstream enzymes in CNS.[29] COX-1 is related to homeostatic production of prostaglandins synthesis, whereas COX-2 activity is induced by inflammatory stimuli.[30] The current results showed that mahanimbine has the potential in inhibiting total COX activity and COX-2 expression and subsequently reducing neuroinflammation.

CONCLUSION

Taken together, the results showed that mahanimbine could act as a potential neuroprotective agent against LPS-induced animal models. Mahanimbine was found to attenuate LPS-induced memory impairment. It improved the cholinergic activity in the brain by increasing ACh level and limiting the AChE activity. Furthermore, it showed anti-inflammatory activities by reducing release of pro-inflammatory TNF-alpha and IL-1 β, increasing release of anti-inflammatory IL-10 and TGF-β1, and inhibiting total COX activity and COX-2 gene expression. The present that mahanimbine would be a promising compound in prevention of neuroinflammation that is associated with development or progression of AD.

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Conflict of interest

There are no conflicts of interest.

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