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Neuroprotective Potential of Mahanimbine against Lipopolysaccharides (LPS)-Induced Neuronal Deficits on SK-N-SH Cells and Antioxidant Potentials in ICR Mice Brain

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NSMA and VM carried out the laboratory tests, acquisition of the data and preparation of manuscript. Authors KR and SML carried out in vitro experiments and preparation of the manuscript. Authors AA and SS performed the in vivo experiments and analysis of the results. Author ABAM was involved in the preparation and revision of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Murraya koenigii* commonly known as curry leaves, is traditionally used in India and other South Asian countries as a spice for its characteristic flavor and aroma. Mahanimbine is a major carbazole alkaloid derived from *Murraya koenigii* leaves. There are numerous reports that support

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the neuroprotective role of various alkaloids. The present study investigated the neuroprotective potential of mahanimbine against lipopolysaccharides (LPS)-induced neuronal deficits of SK-N-SH cells and antioxidant potentials in ICR mouse brain.
Study Design: The targeted compound mahanimbine was subjected to both *in vitro* and *in vivo* studies.
Place and Duration of Study: The study was conducted in Faculty of Pharmacy, Universiti Teknologi MARA, Malaysia and College of Pharmacy, Qassim University, Kingdom of Saudi Arabia between June 2015 and August 2017.
Methodology: For the *in vitro* study, SK-N-SH cells were induced with the 100µg/ml of LPS. Then, neuroprotection and reactive oxygen species (ROS) assays were conducted to assess cell viability and the formation of ROS. On the other hand, ICR mice were being fed with mahanimbine (1, 2 and 5 mg/kg, p.o.) for 30 days for *in vivo* study. Neuroinflammation was thereafter induced by intraperitoneal injection of LPS (250 µg/kg) for 4 days. At the end of the treatment, the animals were sacrificed. The brain was collected for antioxidants assays, measuring oxidative biomarkers such as catalase, reduced glutathione, superoxide dismutase, glutathione reductase, and thiobarbituric acid (TBARs).
Results: SK-N-SH cells exposed to 100 µg/ml LPS showed a significant cell viability loss and increased level of ROS. However, pre-treatment of SK-N-SH cells with mahanimbine significantly prevented cell loss and consequently attenuated LPS-induced ROS formation. In addition, mahanimbine also inhibited β-secretase (BACE50 = 4µg/mL) that is important for production of β-amyloid (Aβ). For *in vivo* study, the biochemical analysis of the whole brain detected increased catalase (CAT) and glutathione reductase (GRD) levels, and significantly decreased malondialdehyde (MDA) level in mahanimbine treated groups as compared to LPS-induced but untreated group.
Conclusion: The overall findings supported the neuroprotective and antioxidant potential of mahanimbine against LPS-induced neurotoxicity.

Keywords: Mahanimbine; SK-N-SH cells; lipopolysaccharides; β-secretase; oxidative stress; antioxidant.

1. INTRODUCTION

Neuroinflammation is a process involving the activation of astrocytes and microglia cells by inflammatory mediators in various central nervous system (CNS) pathologies, including trauma, stroke, brain infection and neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) [1]. In the activated condition, microglia release several free radicals and cytokines involved in the neurodegenerative process. The lipopolysaccharides (LPS)-induced neurotoxicity through neuroinflammation is one of the essential tools for evaluating the neuroprotective effect of some compounds using *in vitro* as well as *in vivo* studies. In fact, LPS can directly activate microglia, which then causes the production of inflammatory mediators, for example, pro-inflammatory cytokines, nitric oxide (NO) and cyclooxygenase-2 (COX-2) [2].

β-secretase (BACE-1) is an important enzyme for the generation of β-amyloid (Aβ) peptide. The formation of Aβ peptide is from the sequential cleavage of amyloid precursor protein (APP) by BACE-1 and γ-secretase through the amyloidogenic pathway [3]. BACE-1 has been reported to be a key enzyme that initiates the pathway to the formation of Aβ proteins that results in the development of neurotic plaques in the brain of AD patients [4]. Based on previous studies, the expression and activity of BACE-1 were found elevated in the brain of AD patients [5]. Hence, the BACE-1 inhibition is one of the potential therapies to reduce the Aβ peptide accumulation in the brain region.

Cytokines result in up-regulation of BACE1 and the over production of Aβ peptide is postulated to induce lipid peroxidation, protein oxidation and formation of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) [6]. Excessive free radical production over the capacity of the antioxidant defense mechanism leads to oxidative stress, a condition that is closely associated with AD pathogenesis. The changes induced by Aβ peptide, however, can be reversed by antioxidants and vitamin E [7]. The SK-N-SH is a neuronal cell line established in cell culture from human metastatic neuroblastoma tissue and maintained *in vitro* and

it also extensively used as a target cell line in cell-mediated cytotoxicity assay [8]. These cells exhibit a neuronal phenotype and have multiple neurochemical markers. Thus, this cell line is very useful for the assessment of neuroprotection [9].

Murraya koenigii Linn belongs to the family of Rutaceae. It is commonly known as curry leaves in English as well as 'Pokok kari' in Malay. The leaves, bark and root of *M. koenigii* are used in indigenous medicines as a tonic, stomachic, stimulant and carminative [10]. The bioactive carbazole alkaloid constituents of *M. koenigii* include O-methyl mahanine, isomahanine, O-methyl murrayanine, koenimbine, bismahanine, bispyrafoline, euchrestine, bismurrayafoline, murrayanol, mahanimbine, grinimbine and mahanine [11]. Isomahanine, O-methyl murrayanine, bismahanine, and bismurrayafoline, in particular, possess radical scavenging ability against 1,1- diphenyl-2-picryl hydrazyl (DPPH) radical [12]. Among the many compounds, mahanimbine is the major carbazole alkaloid derived from *M. koenigii* leaves. Mahanimbine has been widely documented for its ability in reducing blood glucose and total cholesterol levels as well as increasing high density lipoprotein (HDL) [13]. Moreover, a carbazole alkaloid mahanimbine from *M. koenigii* leaves was found to inhibit AChE activity *in vitro* [14]. As a continuation of our research work, the present study aimed to evaluate the neuroprotective effect of mahanimbine on *in vitro* using SK-N-SH neuroblastoma cells and *in vivo* in mouse models of LPS-induced neuroinflammation.

2. MATERIALS AND METHODS

2.1 Reagent

SK-N-SH cell was purchased from ATCC, USA. Eagle's minimum essential medium (MEM), fetal bovine serum (FBS), penicillin/streptomycin, trypsin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), lipopolysaccharide (LPS), dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Company (St. Louis, Mo., USA). 2'7'-Dichlorodihydrofluorescein diacetate (H2- DCF-DA) and BACE-1 assay kit were obtained from Invitrogen (USA). Hanks' balanced salt solution (HBSS), MEM without phenol-red was purchased from Gibco (Carlsbad, CA). Catalase assay kit, superoxide dismutase assay kit, glutathione assay kit, glutathione reductase assay kit and TBARs Assay kit from Cayman Chemical Company, USA.

2.2 Extraction and Isolation of Mahanimbine

M. koenigii leaves were air dried and powdered. The dried and powdered leaves *M. koenigii* was defatted using petroleum ether for three days at room temperature, filtered and the solvent was evaporated to dryness. The powdered leaves was then sprinkled with 10% (v/v) ammonia (NH_3) solution, left to soak overnight and re-extracted with chloroform ($CHCl_3$) for another three days. The $CHCl_3$ extract was concentrated under reduced pressure using a rotary evaporator before being subjected to extraction with 5% (v/v) hydrochloric acid (HCl). It was later basified with 25% (v/v) NH_3 solution and re-extracted with $CHCl_3$. The $CHCl_3$ extract was then washed with distilled water and sodium chloride solution and dried with sodium sulphate. The crude alkaloid was loaded into a column chromatography (packed with silica gel) and eluted with methanol and $CHCl_3$. Each collected fraction was tested for the presence of alkaloids by Mayer's test and Dragendorff's reagents. The most potent fractions were pooled together and subjected to preparative TLC for isolation of the compounds. The compound was identified as mahanimbine by comparing NMR, HPLC and Mass spectral data with values published previously [12,15].

2.3 *In vitro* BACE-1 Assay

Mahanimbine, at concentration ranging between 0.1-1000 µg/mL, were assayed for BACE1 inhibition using a fluorescence resonance energy transfer (FRET) assay that uses baculovirus-expressed BACE-1 and a specific substrate based on the Swedish mutation of amyloid precursor protein (APP) [16]. This peptide substrate becomes highly fluorescent upon enzymatic cleavage. A mixture of 10 µL of test compound (diluted in assay buffer), 10 µL of BACE1 substrate (Rh-EVNLDAEFK-quencher, in 50 nM ammonium bicarbonate) and 10 µL of BACE1 enzyme (1.0 U/mL) were incubated at room temperature for 60 min in dark condition. Subsequently, 10µL of BACE1 stop buffer (2.5 M sodium acetate) was added to the mixture. Fluorescence was read using a spectrofluorometer (TECAN) under excitation at 545 nm and emission at 585 nm. Percentage inhibition of the enzyme was then calculated.

2.4 Cell Culture

SK-N-SH, a human neuroblastoma cells was used as a neuronal model in this study. The SK-N-SH cells were obtained from the American Type Culture Collection (ATCC, No.HTB-11). These cells were cultivated in minimum essential medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin in a humidified incubator maintained at 37ºC under 5% CO2/ 95% air. The medium was changed every 2 days and sub-culture was performed when the cells reached 60% confluent. For the moment, the media was discarded and the cells were washed using phosphate buffer saline (PBS). Next, trypsin was added to detach the cells followed by centrifugation to collect the pellet. Then cells were plated at appropriate densities according to the experimental design.

2.5 Cell Viability Assay

Cell viability was determined using the conventional [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) reduction assay as described previously [17]. SK-N-SH cells were seeded at 2 x 10^4 cells per well onto a 96-flat bottom well plate and incubated overnight to allow cell attachment. The cells were pre-treated with mahanimbine at final concentrations of 0.005, 0.05, 0.5, 5 and 50 µg/mL for 24 h. Treated cells were further incubated with 100 µg/mL LPS for yet another 24 h. α-Tocopherol (10 µg/mL) was used as a standard reference for comparing the drug efficacy. Briefly, 50µL MTT solution (5mg/mL) was added onto each well and incubated at 37ºC for 4 h. The purple formazan crystals formed by living cells were solubilized in dimethyl sulfoxide (DMSO) and absorbance was determined colorimetrically at 570nm using a microplate reader (Tecan).

2.6 Measurement of Intracellular ROS Generation

The production of reactive oxygen species (ROS) was evaluated using 2', 7'-dicholorofluorescein diacetate (DCFH-DA), a fluorescent probe. DCFH-DA is hydrolyzed by intracellular esterase to form non fluorescent 2'7'-dichlorofluorescein (DCFH) which is then converted to highly fluorescent 2'7'-dichlorofluorescein (DCF) in the presence of ROS [18,19]. Briefly, SK-N-SH cells were seeded at 2 x 10^4 cells per well onto a 96-flat bottom well plate and incubated at 37ºC overnight. The cells were then pre-treated with either mahanimbine at concentrations 0.005, 0.05, 0.5, 5 and 50 µg/mL or α-tocopherol at 10 µg/mL for 24 h before being induced with 100 µg/mL LPS. DCFH-DA was added to each well and incubated at 37ºC for 30 min. ROS production was measured immediately using a microplate reader at 485 nm excitation wavelength and 530 nm emission wavelength. The intensity of DCF fluorescence corresponds to the amount of ROS.

2.7 Animals

The experiments were carried out using male ICR mice obtained from the Laboratory Animal Facility and Management (LAFAM), Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam, Malaysia. The animals, which weighed 25-35 g and of 8-12 weeks old, were housed in polyacrylic cages and maintained at room temperature (21-25ºC) and relative humidity of 45-65% with a controlled light-dark cycle. All mice had access to standard laboratory food and water *ad libitum*. The rodents were housed in groups and each of them consists with six number. The mice were acclimatized for at least five days prior to experiment. The experiments were carried out between 0800 h to 1800 h. The experimental procedure was approved by the Research Committee on the Ethical Use in Research (UiTM Care) Universiti Teknologi MARA, Malaysia (37/2014) and the care of laboratory animals was carried out as per the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

2.8 Vehicle

Mahanimbine (1, 2 and 5 mg/kg) and Piracetam (400 mg/kg) were suspended in 0.5% (w/v) carboxymethyl cellulose sodium (CMC) and administered to animals orally (p.o.). The LPS (serotype 055: B5, Sigma, St. Louis, MO, USA) was dissolved in 0.9% (w/v) normal saline and administered to the animals through intra-peritoneal injection (i.p.).

2.9 Drug Administration and Collection of Brain Samples

Mice were divided randomly into six groups (n=6). The animals from the control and LPS-treated groups were subjected to the vehicle (0.5% w/v CMC) for 30 days. Meanwhile, the mahanimbine (1, 2 and 5 mg/kg) was administered orally for 30 days to three different groups. At the end of the treatment, the animals 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 cervical decapitation, the brain was carefully isolated and stored in cold PBS for biochemical analysis. The stored brain was homogenised using a glass WiseStir Homogeniser. The homogenized brain was then centrifuged at 4000 rpm and 4ºC for 10 min to collect the supernatant. The supernatant was kept at -80ºC for biochemical analysis.

2.10 Measurement of Antioxidants and Malondialdehyde (MDA) Levels in Brain Homogenate

The enzymatic and non-enzymatic antioxidants were measured using standard assay kits. Antioxidant activities of catalase (CAT), glutathione (GSH), superoxide dismutase (SOD) and glutathione reductase (GRD) in the brain homogenate were evaluated using Cayman assays kits (Ann Arbor, MI, USA). Whilst, the lipid peroxidation was evaluated based on the MDA level in the brain homogenate by thiobarbituric acid reactive substances (TBARs) assay kit. The absorbance of the assays was measure using TECAN Infinite M200 microplate reader (TECAN, Durham, USA).

2.11 Statistical Analysis

In vitro inhibitory activity and data using the SK-N-SH cells were presented as mean values ± standard deviation (SD) of three parallel measurements. Other *in vivo* results were expressed as mean ± standard error means (SEM). Data were analyzed using the One- way ANOVA procedure in Graph Pad version 6, when there was a difference, Tukey–Kramer post hoc test was used to identify pairs that differed significantly. A probability value of 0.05 was considered as significant.

3. RESULTS

3.1 Potential of Mahanimbine in Inhibition of β-Secretase (BACE-1) Enzyme

Mahanimbine exhibited BACE1 inhibitory activity with an IC_{50} value of 4 µg/mL. This compound inhibited BACE 1 activity between 15.27% to 99.86% at concentrations ranging from 1-1000 µg/mL. Fig. 1 represents the percentage of BACE1 inhibition against various concentrations of mahanimbine.

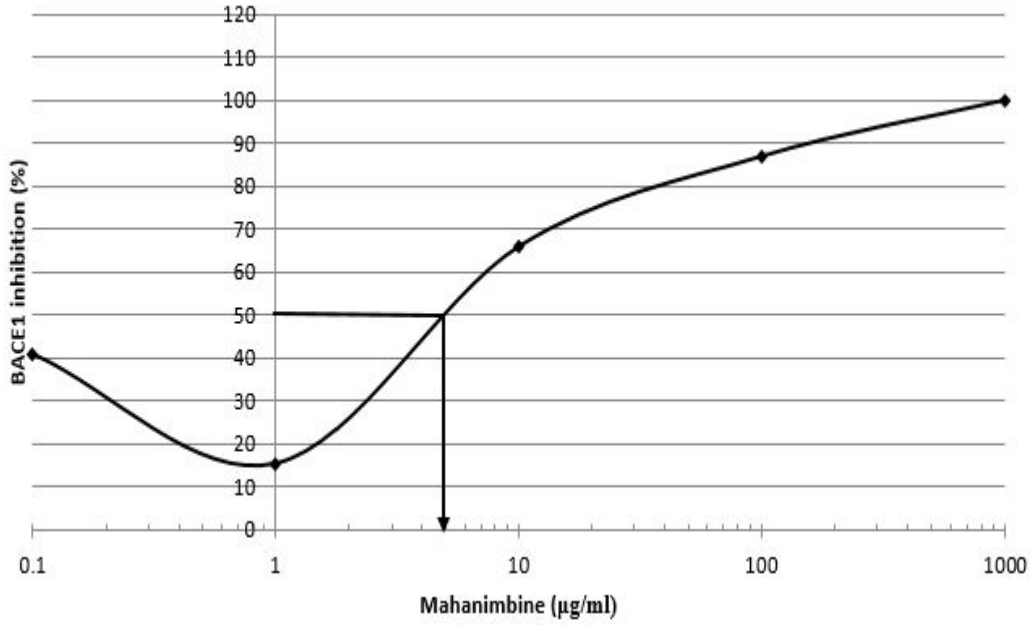

Fig. 1. Inhibitory activity mahanimbine on BACE1. Results are expressed as mean ± SD of triplicate wells from three independent experiments. Mahanimbine exhibited BACE1 inhibitory activity with an IC_{50} value of 4µg/mL

3.2 Effect of Mahanimbine on Cell Viability in LPS-Induced SK-N-SH Cells

Fig. 2a shows the effect of mahanimbine on cell viability in LPS-induced SK-N-SH cells. Exposure of SK-N-SH cells to 100 µg/mL LPS for 24 h resulted in 70% cell death. Nevertheless, SK-N-SH cells pre-treated with 0.05 µg/mL, 0.5 µg/mL and 5 µg/mL mahanimbine significantly enhanced cell survival (by 40-55%) in the presence of LPS (P<0.05; P<0.001; P<0.05, respectively). Pre-incubation of SK-N-SH cells with the lowest (0.005 µg/mL) and highest (50 µg/mL) concentrations of mahanimbine, however, did not yield significant effect. It is noteworthy that pre-treatment with mahanimbine at 0.5 µg/mL (middle dose) exhibited neuroprotective effect that is comparable to that of standard drug α-tocopherol (10 µg/mL).

3.3 Mahanimbine Reduced the Level of Intracellular ROS against LPS-Induced Oxidative Stress in SK-N-SH Cells

Exposure of SK-N-SH to 100 µg/mL LPS alone significantly increased (P<0.001) the level of intracellular ROS by 90%. Mahanimbine was found to be non-toxic to cells at concentrations 0.005 µg/mL, 0.05 µg/mL, 0.5 µg/mL and 5 µg/mL (cell viability > 70%; Fig. 2b). Pre-treatment of SK-N-SH cells with the mahanimbine (0.005 µg/mL, 0.05 µg/mL, 0.5 µg/mL and 5 µg/mL) significantly attenuated (P<0.05; P<0.05; P<0.01; P<0.001; respectively) the level of intracellular ROS induced by LPS (by 30-50%). The standard drug α-tocopherol (10 µg/mL) also significantly reduced (P<0.001) ROS stress by 60% when compared to LPS alone (LPS control). The results suggested that pre-treatment with mahanimbine protected SK-N-SH cells against LPS-induced oxidative stress.

3.4 Mahanimbine Attenuated Oxidative Stress and Enriched the Antioxidants in the Brain of LPS-Induced Mice

Fig. 3a shows the potential of mahanimbine against lipid peroxidation production. A significant increase (P<0.001) of brain malondialdehyde (MDA) level was observed in LPS-induced group (2.81±0.36 µM) as compared to the control group (1.58 ±0.04 µM), indicating elevation of oxidative stress by LPS. Animals administered with mahanimbine at 1 mg/kg, 2 mg/kg and 5mg/kg, however, showed significant (P<0.001, P<0.001, P<0.01; respectively) attenuation of oxidative stress levels as compared to LPS-treated group. The MDA levels were 1.51 ±0.08 µM, 1.37 ±0.13 µM and 1.76 ±0.2 µM, respectively.

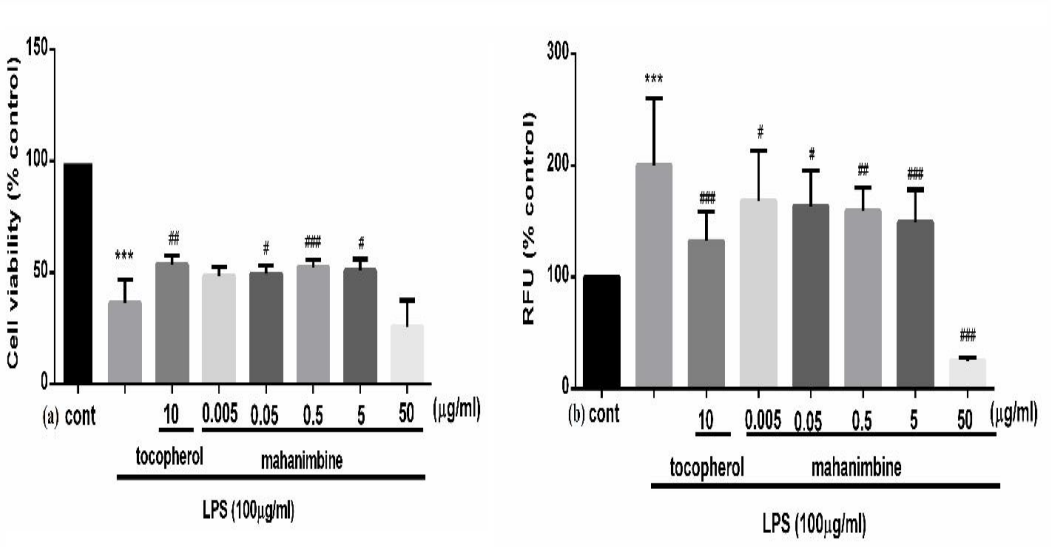

Fig. 2. Effect of mahanimbine on cell viability and ROS against LPS-induced in SK-N-SH cells. (a) The viability of cells was assessed by MTT assay. Percentage of cell viability was relative to the untreated control cells. (b) ROS level was measured by the oxidation of DCFH-DA. Each bar represents the mean ± SD of three independent experiments. *P < 0.05, **P<0.01 and ***P<0.001 vs control; # P< 0.05, ##P<0.01 and ###P < 0.001 vs LPS

The antioxidant capacity of mahanimbine was indicated by the activities of catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) and glutathione reductase (GRD). For catalase (Fig. 3b), the LPS-induced group showed significant decline (1.49 ± 0.06 nmol/min/mL; P<0.001) in its activity when compared to control group (18.51 ± 1.84 nmol/min/mL). Mice that were administered with 1 mg/kg, 2 mg/kg and 5 mg/kg mahanimbine significantly increased (P<0.001) catalase levels to 26.08 ±3.04 nmol/min/ml, 31.19 ±3.75 nmol/min/ml and 32.50 ±3.22 nmol/min/ml as opposed to the LPS-treated control group.

For GSH (Fig. 3c), mahanimbine did not result in significant changes to its declined activity in the presence of LPS (P>0.05). For glutathione reductase (GRD) (Fig. 3d), LPS alone suppressed GRD [60.33±1.57 nmol/min/mL (P<0.001)] activity when compared to the control group (99.12 ± 8.93 nmol/min/mL). Administration of 1 mg/kg mahanimbine showed a significant increase [85.57 ± 2.63 nmol/min/mL (P<0.05)] of GRD as compared to LPS-induced control mice. The higher doses of mahanimbine, however, did not significantly affect GRD. The values were 78.05 ±6.26 nmol/min/mL and 78.76 ±5.76 nmol/min/mL for 2 mg/kg and 5mg/kg, respectively.

For superoxide dismutase (SOD) (Fig. 3e), LPS-induced mice showed significant reduction (2.18 ± 0.17 U/mL; P<0.01) in its activity as compared with control group (2.93 ±0.07 U/mL). Mice that were being administered with 1mg/kg, 2mg/kg and 5mg/kg mahanimbine did not show any significant difference. The levels, however, were slightly higher (2.30 ± 0.14 U/mL, 2.52±0.09 U/mL and 2.60 ± 0.19 U/mL, respectively) when compared to LPS-induced control group.

4. DISCUSSION

Neuro-inflammation in AD contributes to the cytotoxicity of AD lesions thus consequently contributing to neurodegeneration in AD [20]. Based on the previous studies, there was increasing evidences that neuro-inflammatory mechanisms are implicated in the pathogenesis of neurodegenerative disorders such as AD. In fact, the occurrence of neuro-inflammation

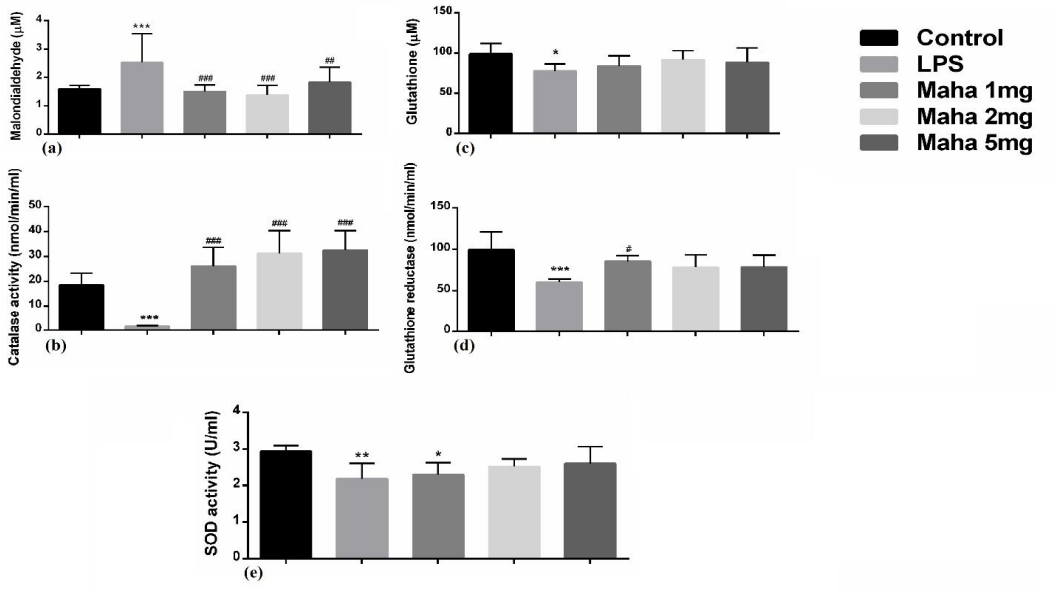

Fig. 3. Effect of mahanimbine on oxidative stress and antioxidants in brains of LPS-induced mice. The oxidative stress and antioxidants were measured by an ELISA kit. a) The level of MDA reflected to the oxidative stress in brain homogenate. b) Catalase activity in brain homogenate. c) Concentration of GSH in brain homogenate. d) GRD activity in brain homogenate. e) SOD activity in brain homogenate. The values are mean ± SEM (n=6). *P<0.05,P<0.01 and ***P<0.001 vs control group; #P<0.05, ##P<0.01 and ###P<0.001 vs LPS-induced group**

within the central nervous system (CNS) causes increase in the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) which then contributes to the process of neurodegeneration [21]. In that regard, a therapeutic approach against the neuro-inflammation would be an effective goal for management of neurodegenerative diseases especially AD. Mahanimbine is a major carbazole alkaloid from *M. koenigii* leaves. Carbazole alkaloids have been reported for their various pharmacological activities such as anti-inflammatory, antioxidant, anti-viral and anti-tumour activities [13]. Thus, the present study was carried out to demonstrate the neuroprotective effect of mahanimbine using SK-N-SH neuroblastoma cells and mouse model against neuro-inflammation induced by the LPS. *In vitro* BACE1 enzyme inhibitory activity by mahanimbine also established.

The neuroprotective effect using SK-N-SH neuroblastoma cells seems to be mediated by attenuating the cytotoxic effects of LPS when pre-treatment with mahanimbine and reduced ROS level. Regarding cell viability assay, the cytotoxicity potential of mahanimbine was confirmed on SK-N-SH neuroblastoma cells. The concentration of mahanimbine at 0.005 µg/ml, 0.05 µg/ml, 0.5 µg/ml and 5 µg/ml did not show any toxic effects towards the cells since almost 80% - 85% of viable cells was exhibited after treatment with the respective concentration of mahanimbine. Similar pattern was also observed for cells treated by α-tocopherol (10 µg/mL) used as a standard drug. However, the highest concentration of mahanimbine at 50 µg/ml caused approximately 85% of cell death and showed its toxic towards the cells. The IC_{50} (half maximal inhibitory concentration) value of mahanimbine was at 15 µg/ml against the cell line that was indicated that the concentration above 15µg/ml can be considered as toxic using SK-N-SH neuroblastoma cells. The further neuroprotective evaluation results represented that the same selected doses of mahanimbine (0.005 µg/ml, 0.05 µg/ml, 0.5 µg/ml and 5µg/ml) significantly increased the cell viability of SK-N-SH cells that induced by LPS. It is considered that mahanimbine acts as a neuroprotective agent against LPS-induced neuroinflammation at lower concentration even compared to established neuroprotective natural compound α-tocopherol.

The accumulation of ROS production may lead to the oxidative stress and also activate apoptosis signaling [22]. Moreover, oxidative stress is widely involved in the neural cell death related to the variety of chronic neurodegenerative disorders such as AD [23,24]. Thus the formation of ROS is an early response to the oxidative stress. Exposure of LPS initiated a rapid burst in ROS formation as validated by the DCFH-DA assay that has been utilized extensively as a marker for overall intracellular oxidative stress and it reflects the overall oxidative status of the cell [25]. Oxidative stress can be treated using antioxidants, thus based on the biological effect of carbazole alkaloid, it is one of the important class that exhibit antioxidant effect [26]. Previous study has found that carbazole alkaloid from *Murraya koenigii* had significant antioxidant and radical-scavenging activities [27]. Consistent with these findings, we observed that the level of ROS was elevated in cells exposed to LPS. However, the elevation of ROS could be prevented by pre-incubating cells with mahanimbine at 0.005 µg/ml, 0.05 µg/ml, 0.5 µg/ml and 5 µg/ml prior to the induction of LPS. These data have shown that, the neuroprotective effect of mahanimbine against LPS-induced neuroinflammation might be at least in a part due to its antioxidant activity.

The present study also evaluated the antioxidant activity using *in vivo* model which was LPS-induced mice model and pre-treatment with mahanimbine for 30 days. Lipopolysaccharides (LPS) is an endotoxin from outer membrane of Gram negative bacteria and acts as a neurotoxin to induce chronic inflammation and oxidative stress in the brain [28]. Multiple administrations of LPS increase the expression of inflammatory related markers and lead the immune activation and ROS production in brain. These events are reported to accumulate beta amyloids (Aβs) in the cerebral cortex and hippocampus [29]. We found that, LPS-induced mice expressed high levels of malondialdehyde (MDA) that indicated the enhancement of lipid peroxidation and produced oxidative stress in the brain. However, the LPS-induced MDA level was significantly inhibited by pre-treatment with mahanimbine. The present findings revealed that mahanimbine displayed a significant protection against LPS-induced oxidative stress that might be due to its antioxidants properties. Therefore, the study was continued with the evaluation of antioxidant activities like catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) and glutathione peroxidase (GRD). The lower activities of CAT, SOD, GSH and GRD in LPS-induced group indicated the impairment in

antioxidant defensive ability and free radicals scavenging capacity. Among the above listed antioxidants, the levels of CAT and GRD were significantly elevated by treatment with mahanimbine against LPS-induced that decline the antioxidant activities. SOD is an antioxidant enzyme that has a major role in the elimination of vulnerable superoxide anion by conversion to hydrogen peroxide and oxygen. The treatment of mahanimbine can able to increase the SOD levels in minor, but not significantly. The similarity of the results was found also in the levels of GHS. It is known that the level of the GHS is verified with reduction of glutathione disulfide (GSSG) to glutathione (GSH) by glutathione reductase. The present results are highlighted that the selected doses of mahanimbine showed failure in alteration of above enzymes levels in this study. From the findings, we concluded that, attenuation of ROS level in SK-N-SH and decreased lipid peroxidation in LPS-induced mice were due to the antioxidant properties of mahanimbine, especially on improving the CAT and GRD activities, and consequently had ability to prevent the memory impairment.

The β-site APP-cleaving enzyme 1 (BACE-1) or β-secretase is an important enzyme that is involved in metabolizing the amyloid precursor protein (APP) and generating the toxic peptide which is β-amyloid (Aβ) in the brain [30]. As mentioned in the previous study, Aβ plays a critical role in AD pathogenesis and the aggregation of Aβ triggers a complex pathological cascade which leads to neurodegeneration [31]. Further study demonstrated that reduction of Aβ levels with the deletion of BACE-1 gene enable to prevent memory impairment and hippocampal cholinergic dysfunction in BACE1(-/-).Tg2576(+) biogenic mice model [32]. These results suggest that, inhibition of BACE-1 is a valid therapeutic strategy for AD. Interestingly, previous study had been determined that the total alkaloidal extract of *Murraya koenigii* leaves significantly inhibited the activity of BACE-1 [33]. Concomitantly, the present study found that mahanimbine from *Murraya koenigii* leaves inhibited the activity of BACE-1 enzyme with an IC50 value of 4 µg/ml.

5. CONCLUSION

Taken together, LPS induced the neuro-inflammation in SK-N-SH cells through several pathways such as causing cell death and increasing the level of intracellular ROS. However, mahanimbine protected the SK-N-SH cells against LPS-induced neuro-inflammation via increasing the viability of cells and inhibiting ROS overproduction. Besides, mahanimbine inhibited the activity of BACE1 enzyme. Furthermore, the compound showed its antioxidant potential by elevating the levels of the antioxidant enzymes catalase and superoxide dismutase as well as reducing the level of malondialdehyde. Based on above *in vitro* and *in vivo* findings, we suggest that mahanimbine may be a useful agent for the prevention of neuroinflammation associated development or progression of AD.

CONCENT

It is not applicable.

ETHICAL APPROVAL

Regarding ethical issues, the present experimental procedure was approved by the Research Committee on the Ethical Use in Research (UiTM Care) Universiti Teknologi MARA, Malaysia (37/2014).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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