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Anti-Inflammatory Effects of *Moringa Oleifera* Lam Leaf Extract in Lipopolysaccharide-Activated Microglia

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Abstract

Moringa oleifera Lam has been used as a medicinal plant in many Asian countries including Thailand. The plant has a variety of pharmacological effects such as antioxidant, anti-inflammation, hypolipidemic, anti-infection, anticancer, and antidiabetic. The leaves and young pods, which contain various nutritional compounds including proteins, fatty acids, and vitamins, are widely consumed. In this study, we investigated the effects of hexane, ethyl acetate, methanol and ethanol crude extracts of *M. oleifera* Lam leaves on the viability of microglial cells and on the lipopolysaccharide-induced microglial activation. which is widely used as a model for neuroinflammation. Our findings suggested that all of the crude extracts at concentrations ranging from 10^{-9} to 10^{-5} g/mL did not significantly affect the viability of microglial cells and exhibited similar effects. Subsequently, we focused on ethanol extract for investigation of the molecular mechanism involving neuroinflammation. We found that the ethanol extract of *M. oleifera* Lam leaves at the concentrations of 10^{-9} to 10^{-5} g/mL significantly reduced inducible nitric oxide synthase (iNOS) protein expression and nitric oxide (NO) production, accompanied by the reduction of phospho-NF-κB and phospho-IκBα expressions. The results suggested that the ethanol extract of *M. oleifera* Lam leaves reduced NO production through reduction of iNOS protein expression following the inhibition of NF-κB/IκBα signaling pathways. Our study underscored the therapeutic potential of *M. oleifera* Lam leaves extract in neurodegenerative diseases associated with neuroinflammation.

Keywords: *Moringa oleifera*, LPS, Microglia, Nitric oxide, NF-κB

Introduction

Aging is the major risk factor of neurodegenerative disorders. At the cellular level, aging is associated with the accumulation of oxidative stress, which is a major contributor to the pathogenesis of neurodegenerative diseases, especially Parkinson's disease and Alzheimer's disease [1]. With the increase in the world's aging population, the number of neurodegenerative patients is expected to surge, causing enormous healthcare challenges, particularly in developing countries. Microglia are the resident macrophage-like cells that play roles as the immune cells in the central nervous system to maintain homeostasis and defense against brain injuries [2]. Microglia can play both positive and negative roles. The positive one is to promote neuronal survival [3] through the release of neurotrophic and anti-inflammatory molecules and the elimination of invading pathogens. However, upon over-activation or chronic exposure to noxious insults, microglia release cytotoxic substances including nitric oxide (NO), superoxide anion, and pro-inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) [4-6]. These molecules are correlated with the development of neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease [7].

Inflammatory processes in the brain often trigger the induction of inducible nitric oxide synthase (iNOS), which is transcriptionally regulated by interferon regulatory factor-1 (IRF-1), and nuclear factor-κB (NF-κB) [8]. Bacterial lipopolysaccharide (LPS) can activate transcriptions of pro-inflammatory genes including NF-κB leading to the production of various inflammatory molecules such as NO, TNF-α and IL-1β and was widely used to induce microglia activation [9]. LPS induces neuroinflammation by activating of Toll-like receptors (TLR) pathway [10], and further activating the downstream pathways, such as NF-κB through the recruitment of myeloid differentiation primary response protein 88 (MyD88) [10,11]. The

mammalian NF-κB consists of homo-or heterodimer of the Rel protein family, with the heterodimer of p65 and p50 subunits being the most commonly activated form [12]. In resting cells, NF- κB is retained in the cytoplasm in a quiescent form by binding to inhibitors of kappa B (IκB) proteins, consisting of IκBα, IκBβ, and IκBε [13]. Upon inflammatory signal activation, IκBs are phosphorylated by IκB kinase (IKK) complex, which contains of catalytic subunits of IKKα and IKKβ and a regulatory subunit of NEMO (NF-κB essential modulator) or IKKγ. Phosphorylated IκBs subsequently undergo ubiquitination and subsequent proteasomal degradation, leading to the release of the NF-κB complex (p50 - p65) and its translocation to the nucleus, where it activates various target inflammatory genes, including iNOS, leading to nitric oxide (NO) production [14]. Excessive production of NO causes oxidative stress, disrupting mitochondrial oxidative phosphorylation, causing DNA damage, and resulting in neuronal death [15,16]. Moreover, excessive production of NO by activated microglia is associated with the progression of the neurodegeneration [17,18]; while inhibition of NO production demonstrated significant neuroprotection [19-21].

M. oleifera Lam (syn. *M. ptreygosperma* Gaertn.), commonly known as drumstick, horse radish tree, or "Maroom" in Thai, is a member of Moringaceae family. *M. oleifera* leaf contains high concentration of nutritional compounds including β-carotene, proteins, vitamin C, vitamin E, calcium, potassium, flavonoids, (e.g kaempferol, quercetin), and phenolic compounds (e.g. chlorogenic acid, gallic acid, ellagic acid) [22]. Various parts of *M. oleifera* Lam have been used in traditional medicine to treat a range of conditions such as inflammation, infections, cardiovascular diseases, gastrointestinal disorders, and hepatorenal diseases [23]. Several parts of this plant have also been consumed as food, with young pods and leaves served as table vegetables. Previous studies demonstrated that aqueous extract of *M. oleifera* leaves possess cholesterol-lowering ability in rats [24] and rabbits [25]. Additionally, bioactive components obtained from ethanolic extracts of *M. oleifera* leaves can reduce blood pressure in rats [26]. Moreover, hexane, chloroform, ethyl acetate, and methanol extracts of *M. oleifera* leaves all exhibited a remarkable inhibitory effect against coliform bacterial growth [27].

The use of indigenous plants that possess anti-oxidative as well as anti-inflammatory activities for the prevention of diseases could provide a great economic benefit. *M. oleifera* Lam is among the plants with beneficial properties, since several studies demonstrated the anti-oxidative effects of various parts of this plant. Nonetheless, relatively few studies demonstrated the effect of this plant on the inflammatory processes in the brain, there was a recent study reported the anti-inflammatory effect of *M. oleifera* leaf extract in BV2 microglial cells [28]. Therefore, in this study, we aimed to investigate the anti-inflammatory effects and elucidate the underlying molecular mechanisms of the crude extracts, particularly the ethanol extract of *M. oleifera* leaves on the LPS-stimulated microglia model, which recapitulates neuroinflammation. Our results may provide insights on the neuroprotective potential of ethanol extract of *M. oleifera* leaves and the underlying mechanisms in the neurodegenerative diseases caused by neuroinflammation.

Materials and methods

Chemicals and reagents

Dulbecco's Modified Eagle's Medium (DMEM), Trypsin-EDTA, and Tryphan Blue were purchased from Gibco BRL (Grand Island, NY, USA). Fetal bovine serum (FBS) was purchased from PAA Laboratories (Morningside, Queensland, Australia). 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT), mouse monoclonal anti-β-actin antibody, LPS (*Escherichia coli* serotype 026:B6), and protease inhibitor cocktail were purchased from Sigma (St. Louis, MO, USA). Rabbit polyclonal anti-iNOS antibody was purchased from Santa Cruz Biotechnology (CA, USA). Anti-phospho-NF-κB p65 (Ser536) antibody, mouse monoclonal anti-phospho-IκBα (Ser32/36) (5A5) antibody, and biotinylated protein ladder were purchased from Cell Signaling Technology (Beverly, MA, USA). HRP-conjugated goat anti-rabbit Ig and HRP-conjugated goat anti-mouse IgG antibodies were purchased from Invitrogen (Carlsbad, CA, USA). The nitrocellulose membrane was purchased from GE Healthcare (Little Chalfont, Buckinghamshire, UK). The enhanced chemiluminescense (ECL) detection agent was purchased from Pierce (Rockford, IL, USA).

Cell culture

Highly aggressive proliferation immortalized (HAPI) microglial cells were generously provided by Dr. James R. Connor (Hershey Medical Center, Hershey, PA, USA). The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 5 % heat-inactivated fetal bovine

serum (FBS) at 37 °C under 5 % CO_2 and 95 % air. Before each experiment the cells were seeded and allowed to grow for 24 h to reach 80 % confluence.

Plant materials

M. oleifera Lam leaves were collected in Bangkok, Thailand. The leaves were harvested in the morning in around November, after the rainy season. However, they have been reported that to obtain the maximum biological activities of the bioactive compounds, the leaves should be harvested in the early morning [29] and the bioactive contents are highest in February [30]. The voucher herbarium specimen has been deposited in the Forestry Herbarium, Royal Forest Department, Bangkok (BKK-PC-1). Crude extracts were kindly provided by Professor Dr. Patoomratana Tuchinda, Department of Chemistry Faculty of Science Mahidol University, Thailand. The dried, finely-ground leaves of *M. oleifera* Lam. (3.8 kg each) were successively percolated with hexane, ethyl acetate, methanol, and ethanol (5×11 L each) at room temperature. After the removal of each solvent under reduced pressure, crude extracts (about 20 % yield) were obtained. The crude extracts were dissolved in dimethyl sulfoxide (DMSO) at 100 mg/mL as stock solution and further diluted with DMSO for cell treatment. The final concentration of DMSO applied to the cells was 0.1 % in all experiments.

Assessment of cell viability

The cell viability was measured by the quantitative colorimetric with MTT assay. HAPI cells were cultured onto 96 well-culture plates at a density of 2×10^4 cells/well. The cells were incubated for 24 h with various concentrations of the hexane, ethyl acetate, methanol, and ethanol extracts of *M. oleifera* leaves ranging from 10^{-9} - 10^{-3} g/mL, or pretreated with the hexane, methanol, and ethanol extracts at the concentrations of 10^{-7} - 10^{-5} g/mL for 1 h before exposure to LPS (100 ng/mL) for 24 h. In each experiment, the medium was removed and 1 mg/mL of MTT in HBSS was added in each well. The cultures were further incubated for 4 h with a humidified atmosphere at 37 °C under 5 % CO_2. The supernatant was removed and the purple formazan crystals formed were dissolved in 100 µL of DMSO. The absorbance was measured at 570 nm on a microplate reader (Bio-Tek instruments, Winooski, VT, USA).

Assessment of NO production

NO production was measured by detecting the level of nitrite, the stable and non-volatile break down product, using Griess reagent. In brief, the cells were plated onto 6-well plates at a density of 5×10^5 cells/well and were treated with various concentrations of hexane, methanol, and ethanol extracts of *M. oleifera* Lam leaves ranging from 10^{-7} - 10^{-5} g/mL for 1 h before exposure to LPS for 24 h. At the end of the treatment, 100 µL of culture medium from each sample was transferred to the 96 well-culture plate and mixed with equal volume of Griess reagent, followed by incubation at room temperature for 15 min. The optical density was measured at 545 nm with microplate reader (Bio-Tek Instruments, Winooski, VT, USA) within 30 min. Sodium nitrite in the culture media at the concentration of 0 - 100 µM was used as standard.

Western blot analysis of iNOS, phospho-NF-κB p65, and phospho-IκBα expression

The cells were plated onto a 6-well plate at a density of 5×10^5 cells/well for 24 h before the experiment. The cells were pretreated with different concentrations of the ethanol extract of *M. oleifera*, ranging from 10^{-7} - 10^{-5} g/mL for 1 h, before stimulation with LPS (100 ng/mL). The samples were collected at 6 h after LPS exposure for the detection of iNOS and phospho-NF-κB p65, or at 1 h after LPS exposure for the detection of phospho-IκBα expression.The cells were washed 3 times with PBS before lysis with RIPA buffer. The cell lysates were centrifuged at 12,000 rpm for 15 min at 4 °C. The supernatant was collected and protein concentration was measured by Bradford assay. The protein samples (20 µg) were separated with SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked with 5 % skimmed milk in TBST, incubated with primary antibodies at 4 °C overnight, and then with HRP-conjugated secondary antibodies (1:5000) for 1 h at room temperature, according to the manufacturer's instruction. Protein bands were developed by ECL reagents and were exposed to autoradiography film. For iNOS, phospho-NF-κB p65 and phospho-IκBα antibodies were used at 1:1000 dilution. The expression levels were normalized with β-actin (1:5000 dilution).

Statistical analysis

Statistical significance was assessed by one-way ANOVA followed by a Tukey comparison (Graph Pad Prism program version V (Graph Pad software, San Diego, CA, USA), p-value < 0.05 was considered statistically significant.

Results and discussion

Effect of *M. oleifera* extracts on cell viability

The potential cytotoxic effects of *M. oleifera* extracts were determined using MTT assay. The extracts of *M. oleifera* at the concentrations of 10^{-9} to 10^{-5} g/mL did not alter the cell viability. However, the hexane extract at the concentrations of 10^{-4} and 10^{-3} g/mL significantly caused the cell viability to decrease to 89.13 ± 4.92 % and 0.58 ± 0.08 % of the control, respectively (**Figure 1(A)**). After treatment with the ethyl acetate extract at the concentrations of 10^{-4} and 10^{-3} g/mL, the cell viability significantly decreased to 49.60 ± 1.58 % and 0.27 ± 0.03 % of the control, respectively (**Figure 1(B)**). The methanol extract at the concentrations of 10^{-4} and 10^{-3} g/mL, significantly decreased the cell viability to 79.35 ± 1.51 % and 0.72 ± 0.08 % of the control, respectively (**Figure 1(C)**), whereas the ethanol extract at 10^{-4} and 10^{-3} g/mL decreased the cell survival to 84.89 ± 1.17 % and 0.37 ± 0.03 % of the control, respectively (**Figure 1(D)**).

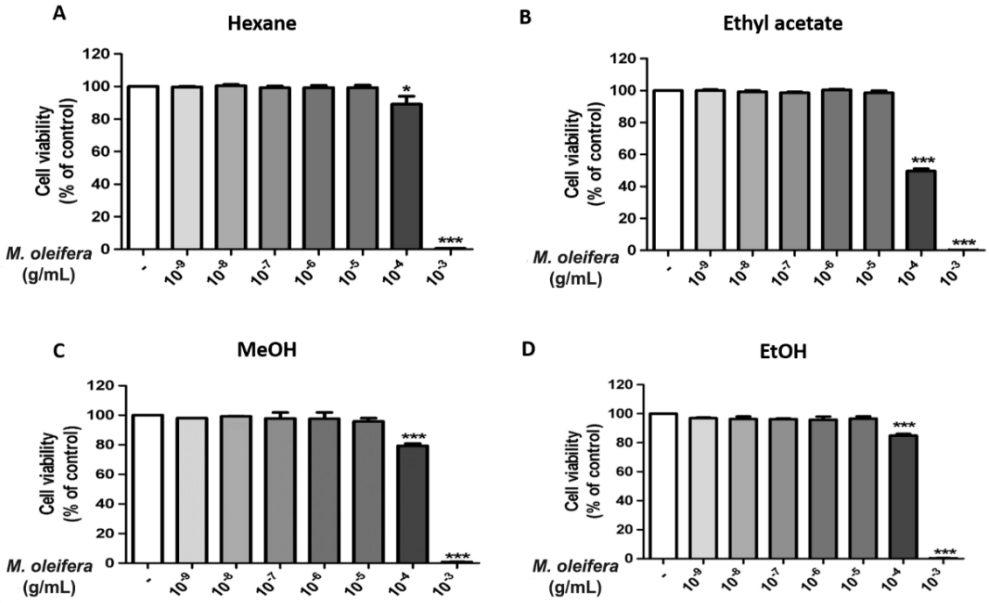

Figure 1 Effects of *M. oleifera* leaves extracts on the viability of HAPI microglia. (A) effects of hexane extract, (B) effects of ethyl acetate extract, (C) effects of methanol extract, (D) effects of ethanol extract. Values are expressed as percentage of the control group. Data are the mean ± SEM from 3 independent experiments, $^{*}p < 0.05$, $^{***}p < 0.001$ compared with the control group.

From our data, the ethyl acetate extract at high doses seemed to be more toxic to the cells than the others; therefore, only the hexane, methanol, and ethanol extracts of *M. oleifera* at the concentrations of 10^{-7}, 10^{-6} and 10^{-5} g/mL were used for the subsequent experiments. As shown in **Figure 2**, treatment the HAPI cells with hexane (A), methanol (B) and ethanol (C) extracts of *M. oleifera* for 1 h prior to stimulation with LPS (100 ng/mL) for 24 h, as well as LPS alone at the concentration of 100 ng/mL, did not decrease the cell viability.

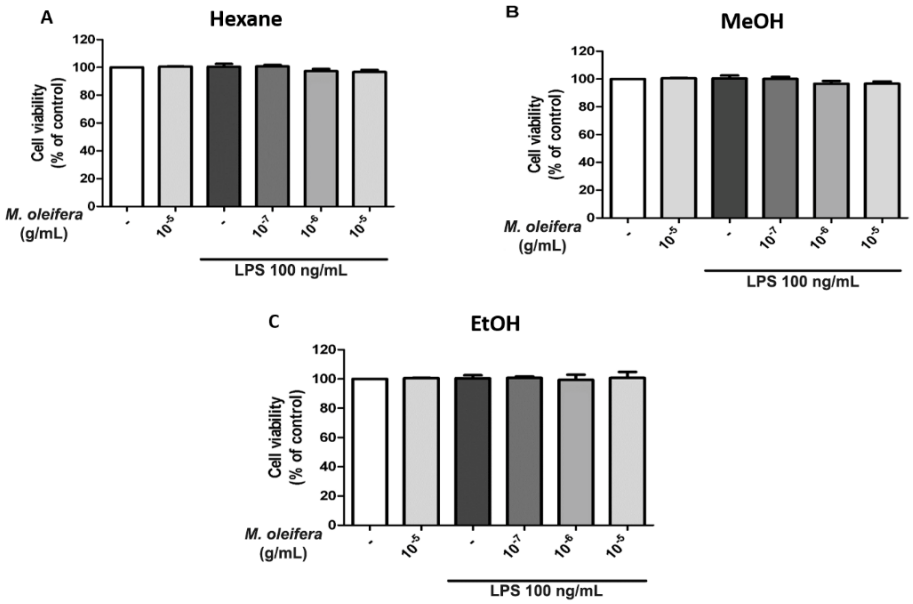

Figure 2 Effects of *M. oleifera* leaves extracts on the viability of HAPI microglia treated with LPS 100 ng/mL for 24 h. (A) Effects of the hexane extract, (B) effects of the methanol extract, (C) effects of the ethanol extract. LPS at 100 ng/mL did not have an effect on the viability of the cells.

Effects of *M. oleifera* extracts on LPS-induced NO production

The cells were treated with various concentrations of *M. oleifera* extracts, ranging from 10^{-7} to 10^{-5} g/mL for 1 h prior to stimulation with LPS (100 ng/mL) for 24 h. The extracts at the concentrations of 10^{-7}, 10^{-6} and 10^{-5} g/mL significantly reduced the levels of NO production to 88.05 ± 2.14, 67.54 ± 2.05, 35.12 ± 1.86 % (hexane extract), to 86.67 ± 2.04, 66.67 ± 2.53, 30.05 ± 1.01 % (methanol extract), and to 86.96 ± 1.27, 63.88 ± 2.04, 34.64 ± 2.28 % (ethanol extract) of LPS-treated cells, respectively (**Figures 3(A) - 3(C)**).

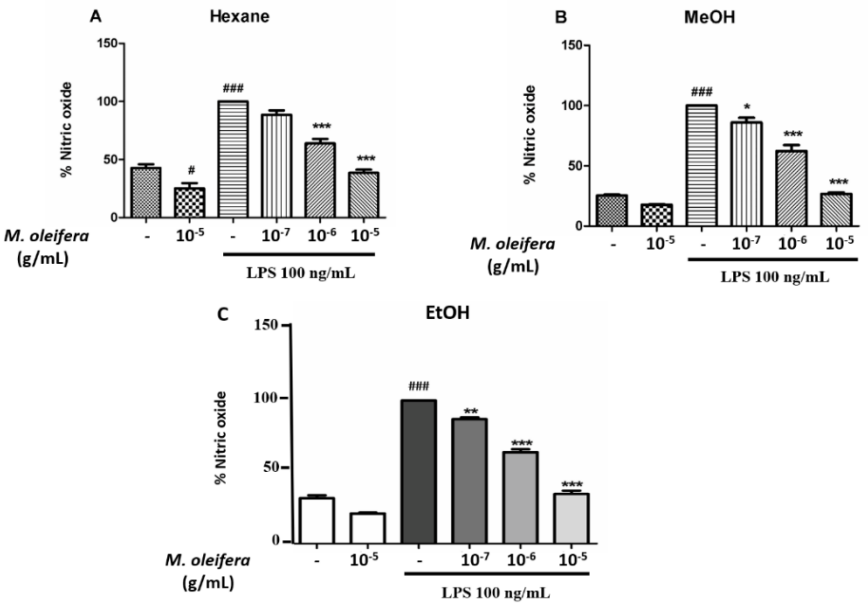

Figure 3 Effects of the extracts of *M. oleifera* Lam leaves on LPS-induced NO production. The extracts of *M. oleifera* Lam leaves dose-dependently reduced the NO production in LPS treated HAPI cells. (A) hexane extract, (B) methanol extract, (C) ethanol extract). The values are represented the mean ± SEM of 3 independent experiments. #$p < 0.05$, ###$p < 0.001$ compared to the untreated control, $^{*}p < 0.05$, $^{***}p < 0.001$ compared to the cells pretreated with LPS 100 ng for 24 h.

The ethanol extract was chosen for further investigation of the underlying molecular mechanism since the decrease in NO production after treatment with hexane, methanol, and ethanol extracts of *M.oleifera* leaves prior to LPS stimulation exhibited a similar pattern. Moreover, previous studies showed that the ethanol extract of *M.oleifera* leaves possesses high antioxidant activity and low cytotoxicity [22,31-33], and 1 report showed a close correlation between antioxidant and anti-inflammatory activities [34].

Effects of ethanol extract of *M. oleifera* on iNOS protein expression

To examine whether the inhibitory effect of ethanol extract of *M. oleifera* on NO production was associated with the decrease in iNOS expression, HAPI cells were treated with the ethanol extract of *M. oleifera* (10^{-7} to 10^{-5} g/mL) for 1 h prior to stimulation with LPS (100 ng/mL) for 6 h. The expression of iNOS protein was barely detectable in the unstimulated cells and in the *M. oleifera*-treated cells, while significantly increased upon LPS treatment (**Figure 4(A)**). The level of iNOS expression was markedly reduced in a concentration-dependent manner after HAPI microglia were pretreated with ethanol extract of *M. oleifera*. The expression of β-actin protein was not affected by LPS and by ethanol extract of *M. oleifera* treatments and was used as a control. Our result demonstrated that ethanol extract of *M. oleifera* at the selected concentrations inhibited the expression of iNOS protein in LPS-activated microglia.

Effects of ethanol extract of *M. oleifera* on phospho-NF-κB p65 protein expression

HAPI cells were treated with 10^{-7} to 10^{-5} g/mL of the ethanol extract of *M. oleifera* for 1 h prior to stimulation with LPS (100 ng/mL) for 6 h before Western blot analysis of phosphorylated-NF-κB p65 expression. Our results showed that LPS significantly induced NF-κB p65 phosphorylation, which was markedly suppressed by the ethanol extract of *M. oleifera* in a concentration-dependent manner (**Figure 4(B)**). This result demonstrated that ethanol extract of *M. oleifera* exerted its effect by inhibiting the phosphorylation of NF-κB p65.

Effects of ethanol extract of *M. oleifera* on phospho-IκBα protein expression

To evaluate the impact of the ethanol extract of *M. oleifera* on the phosphorylation of IκBα in LPS-stimulated microglia, HAPI cells were treated with various concentrations of the ethanol extract of *M. oleifera* (from 10^{-7} to 10^{-5} g/mL) for 1 h prior to LPS exposure (100 ng/mL) for 1 h. As shown in **Figure 4(C)**, LPS treatment significantly increased the phosphorylation of IκB. The ethanol extract of *M. oleifera* Lam leaves markedly suppressed LPS-induced IκBα phosphorylation in a concentration-dependent manner. The results clearly demonstrated that the ethanol extract of *M. oleifera* exerted an inhibitory effect on LPS-induced microglial cells through inhibition of IκBα - NF-κB axis.

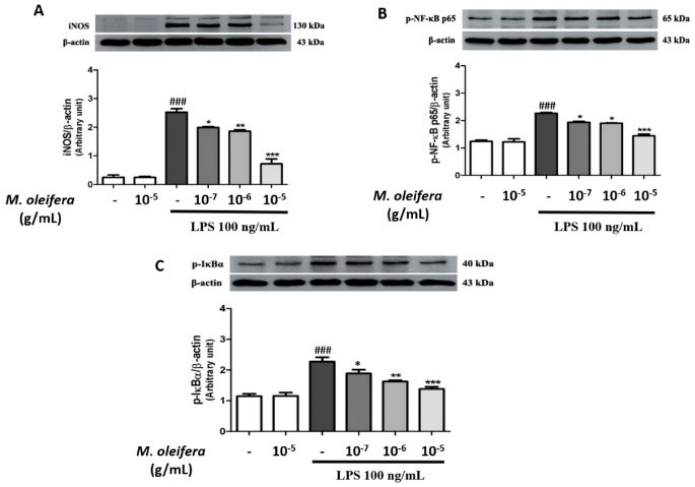

Figure 4 Effects of ethanol extract of *M. oleifera* Lam leaves on the expression of iNOS, p-NF-κB, p-IκB proteins. The cells were treated with various concentrations of the ethanol extract of *M. oleifera* from 10^{-7} to 10^{-5} g/mL for 1 h prior to stimulation with LPS (100 ng/mL) for 6 h. (A) The expression of iNOS protein was measured by Western blot analysis. The intensity of the bands was quantified by densitometry, normalized to that of β-actin protein. (B) Effects of the ethanol extract of *M. oleifera* Lam leaves on phospho-NF-κB p65, and (C) phospho-IκBα proteins expression. The values are expressed as mean ± SEM from 3 independent experiments. ###$p < 0.001$ compared to the untreated control group, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to the cells treated only with LPS 100 ng/mL.

In this study, we used LPS-activated microglia as a neuroinflammation model and investigated the anti-inflammatory activity of the ethanol extract of *M. oleifera* Lam leaves. LPS treatment significantly induced NO production, iNOS, as well as phospho-NF-κB p65 and phospho-IκBα expressions. Pretreatment of the microglial cells with the ethanol extract of *M. oleifera* resulted in suppression of LPS-induced iNOS, phospho-NF-κB p65, and phospho-IκBα expressions, suggesting its anti-inflammatory role. Since NF-κB regulates the expression of pro-inflammatory mediators such as iNOS, COX-2, and IL-6, further studies are needed to prove these effects, especially in animal models or clinical trials.

Accumulating evidence suggests that oxidative stress and brain inflammation play roles in the pathogenesis of various neurodegenerative disorders, including Parkinson's disease, Alzheimer's disease, multiple sclerosis, amyotrophic lateral sclerosis, and AIDS dementia [7]. In the present study, LPS was used to immune-stimulate the microglia. LPS at 100 ng/mL substantially increased the NO production without affecting the cell viability, which was consistent with the previous studies [35,36]. We used various concentrations of the *M. oleifera* Lam leaves extracts to determine the effects on the cell viability. At very high concentration (10^{-3} g/mL), the leaf extracts of *M. oleifera* substantially reduced the cell viability, with ethyl acetate extract exhibiting the highest reduction percentage. Previous studies showed that ethyl acetate fraction gave the highest yield of total phenolic compounds [37] and potent antioxidant activity [38]. However, Prabakaran *et al.* [37] suggested that the ethanol extract possesses a high antioxidant capacity and thereby could be used for therapeutic purposes. At lower concentrations, the plant extracts exerted no effect on cell survival, suggesting a good safety profile and that its effects on microglia were not due to the change in cell viability.

Extracts from parts of *M. oleifera* demonstrated a wide range of activities, including hypolipidemic and anti-atherosclerotic effects [24,25]. A recent study showed the antioxidant effect of the methanol extract of *M. oleifera* leaf powder in an H_2O_2-induced oxidative stress model in human neuroblastoma cells (39). In this study, ethanol extract of *M. oleifera* Lam leaves significantly suppressed NO production in LPS-activated microglia (**Figure 3(C)**) which could be explained through the reduction of iNOS expression (**Figure 4(A)**). Our results are in line with a previous study showing that the phenolic glycosides from the fruit of *M. oleifera* inhibited LPS-induced NO production and iNOS protein expression in murine macrophage RAW 264.7 cell line [40]. It is possible that the phenolic glycosides mediated the effects observed in our study as well since they are abundant in various parts of *M. oleifera* [41]. In *M. oleifera* leaves, the major bioactive compounds identified are quercetin-3-*O*-glucoside, kaempferol, gallic acid, 3-caffeoylquinic acid, and 5-caffeoylquinic acid [41,42]. The antioxidant and anti-inflammatory activities of these compounds have been reported in several recent studies [43]. These results support the use of *M. oleifera* leaves extracts as nutraceutical products for health promotion and for the prevention of neurodegenerative diseases. Thus, they are also rich in vitamins and nutrients that surpass the quality achieved by a single synthetic compound.

In our study, LPS strongly induced IκBα and NF-κB p65 phosphorylation in microglial cells and pretreatment with the ethanol extract of *M. oleifera* Lam leaves attenuated the level in a concentration-dependent manner. Several inflammatory stimuli, including bacterial LPS, activate the transcription factor NF-κB in microglia [44], leading to the production of iNOS and other inflammatory cytokines [45,46]. In unstimulated cells, NF-κB is retained in the cytosol in an inactive form as a homodimer or a heterodimer by binding to the inhibitory protein IκBs. Phosphorylation of IκBs by IKKα/β causes proteasome degradation of IκBs, resulting in NF-κB nuclear translocation and subsequent binding to the DNA and transcriptional activities [47]. NF-κB p65 is phosphorylated at serine 536 upon IκB degradation by IKKα/β and the phosphorylation reflected the IKKα/β-IκBs-NF-κB signaling cascade [48]. We demonstrated that the ethanol extract of *M. oleifera* Lam leaves significantly inhibited NF-κB p65 phosphorylation (**Figure 4(B)**), together with IκBα phosphorylation (**Figure 4(C)**). Our findings suggested that the ethanol extract of *M. oleifera* Lam leaves exerted its inhibitory effect on microglial activation through NF-κB-dependent pathway. However further studies are needed to investigate the contribution of other pathways involving neuroinflammation such as mitogen-activated protein kinases (MAPK) and Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathways. Since we used crude extracts that can have batch variations of the bioactive components, depending on the farming area, harvesting time, and seasons, the anti-inflammatory activities of the pure compounds including quercetin-3-O-glucoside, kaempferol, gallic acid, 3-caffeoylquinic acid and 5-caffeoylquinic acid, which are found in *M. oleifera* ethanol leaves extract should also be further investigated in the present neuroinflammatory models.

Conclusions

The findings of this study indicated that the ethanol extract of *M. oleifera* Lam leaves contained bioactive compounds that possessed anti-inflammatory activity by suppressing NO production through NF-κB pathway inhibition, suggesting its use as a nutraceutical product for neuroprotection in various neuroinflammatory conditions. Further studies are needed to identify the bioactive components of ethanol extract of *M. oleifera* leaves, as well as, the precise molecular targets of these compounds along the NF-κB signaling pathway and other inflammatory pathways.

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