Original article **P29**

Inhibitory effect of methanol and hexane extracts of *Moringa oleifera* Lam leaves on NO production in LPS-activated microglia

Busarat Karachot[1*], Anusorn Thampithak[1], Nattinee Jantaratnotai[1], Yamaratee Jaisin[1], Benjawan Meesarapee[1], Patoomratana Tuchinda[2], Yupin Sanvarinda[1,3]

[1]Department of Pharmacology, [3]Center for Neuroscience, Faculty of Science, Mahidol University, Thailand.
[2]Department of Chemistry, Faculty of Science, Mahidol University, Thailand.
* Presenting Author

Abstract

Moringa oleifera Lam(*M.oleifera*), is generally found in tropical areas, South Asia and Thailand. It has been used for the treatment of various diseases including inflammation. However, little is known about its anti-inflammatory activity. We, therefore studied the effect of crude extracts from leaves (Methanol and hexane fractions) of *M. oleifera* on the production of nitric oxide (NO), a pro-inflammatory substance in highly aggressively proliferating immortalized (HAPI) microglial cells activated by lipopolysaccharide(LPS) and found that the extracts from *M. oleifera* significantly suppressed nitric oxide production in a dose dependent manner. This preliminary result demonstrates that the anti-inflammatory activity of the crude extracts of *M. oleifera* leaves is due partly through the inhibition of NO production and suggests that *M. oleifera* may have neuroprotective potential in neurodegenerative diseases caused by neuroinflammation.

Keywords: *Moringa oleifera* Lam, Microglia , Nitric oxide .

Introduction

Central nervous system (CNS) composes of neurons and glia cells such as microglia and astrocytes. Microglia are known as residence brain immune system, responsible for homeostasis regulation and defense against injury. If microglia were chronically activated, they produced nitric oxide and other pro-inflammatory factors which caused damage to neurons and led to the development of neurodegenerative diseases.

Moringa oleifera Lam or in Thai "Maroom" a member of Moringaceae, has many pharmacological effects. Almost all parts of this plant have been used for various ailments in the indigenous medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, hematological and hepatorenal disorders.

The plant has been reported to be highly potent anti-inflammatory agent. Therefore, the objective of this study is to investigate the anti-inflammatory effects of crude extracts (methanol and hexane fractions) obtained from *Moringa oleifera* Lam leaves in the inflammation model, stimulating of HAPI microglial cell with lipopolysaccharide (LPS) ,on nitric oxide production.

Methods

Crude extracts (hexane and methanol fractions) of *Moringa oleifera* Lam leaves were obtained from Department of Chemistry, Faculty of Science, Mahidol University, Thailand.

Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% heat-inactivated fetal bovine serum (FBS) was used to maintain HAPI microglial cells at 37°C under humidified 5% CO_2 and 95% air atmosphere. In all experiments, cells were left to

acclimate for 24h before any treatments. Pretreatment of *M.oleifera* leaf extract was done by adding the extract 1 h before LPS treatment to HAPI cells.

The number of cell viability was determined by MTT assay. HAPI cells were cultured onto 96-well plate at a density of $2x10^4$ cells/well overnight. Then the cells were treated with various concentrations of crude extracts of *M.oleifera*. The medium was removed at 24 h and 10 mg/ml MTT in Hank's balanced salt solution was added to each well and further incubated for 4 h in a humidified atmosphere at 37 °C, 5% CO_2. After that MTT was removed and cells were lysed with 100 µl DMSO, microplate reader was used to determine the absorbance at the wavelength of 570 nm and at a reference wavelength of 665 nm.

Nitric oxide (NO) production from LPS-treated cells was determined by using Griess assay. HAPI cells ($5x10^5$ cells/well) were plated onto 6-well plate and treated with LPS (100 ng/ml) for 24 h. Then the cell culture supernatant from each sample was collected and the equal volume of Griess reagent was added. Optical density at 545 nm of the sample was determined by a microplate reader. Dilution of sodium nitrite with culture media at concentrations between 0 to 100 µM was used as standard curve.

Results

Effects of *M. oleifera* extracts on cell viability

The cells ($2x10^4$ cells/well) were treated with various concentrations of *M. oleifera* ranging from 10^{-12}g/ml to10^{-3}g/ml for 24 h then performed MTT assay. As shown in Fig. 1A, hexane extract at concentrations from 10^{-12}g/ml to 10^{-4}g/ml had no effect on cell survival. However, the viability of the cells was remarkably reduced to only 10% (of control) by high concentration (10^{-3}g/ml) of *M.oleifera* extract. Similar to hexane extract, (Fig.1B) methanol extract at concentrations from 10^{-12}g/ml to 10^{-5}g/ml had no effect on cell survival. However, the viability of the cells was remarkably reduced to 10% (of control) at 10^{-3}g/ml of *M. oleifera*.

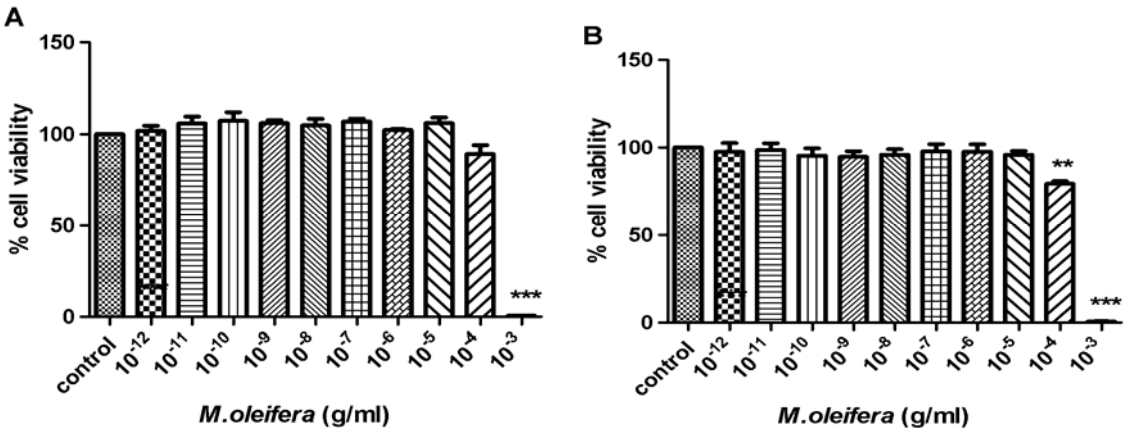

Fig 1. Effects of hexane (A) and methanol (B) extracts of *M.oleifera* leaves on HAPI cell viability. After 24-h incubation with various concentrations of the extract, cell viability was measured with MTT assay(%).

Inhibition of NO production by *M.oleifera* extracts

To determine the effect of *M.oleifera* extracts on NO production, The hexane and methanol extracts of *M.oleifera* at concentrations from 10^{-8}g/ml to 10^{-6}g/ml were added to HAPI cells for 1 h before adding LPS(100ng/ml). After stimulation with LPS for 24 hrs, the levels of NO in the culture media were determined with Griess assay. Both crude extracts of *M.oleifera* significantly reduced the levels of NO production from LPS-stimulated cells in a dose- dependent manner. In Fig. 2A, at concentration of 10^{-6}g/ml *M.oleifera* (hexane extract) was able to inhibit NO production more than 50 %, compared with LPS alone. As shown in

Fig. 2B, at concentration of 10^{-6}g/ml, *M.oleifera* (methanol) was able to inhibit NO production more than 70%, compared with cells activated only with LPS.

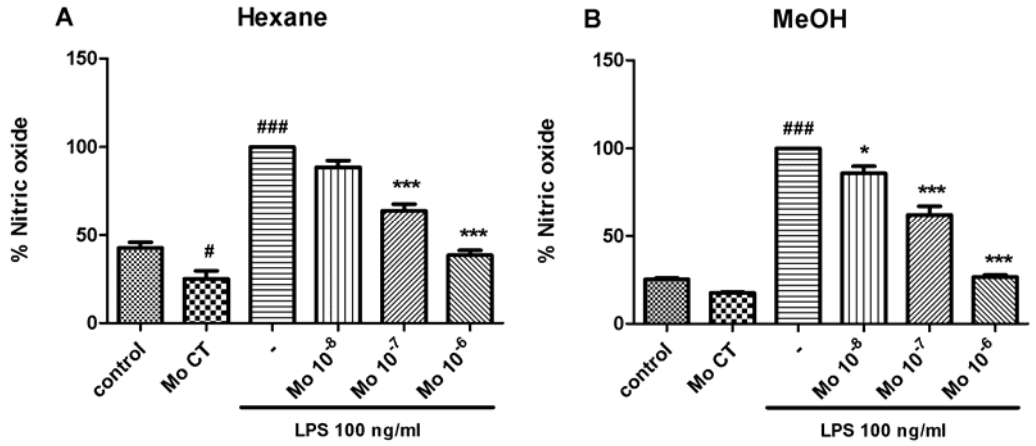

Fig. 2. Effects of hexane (A) and methanol (B) extracts of *M.oleifera leaves* on LPS-stimulated NO production in HAPI microglias.The cells ($5x10^5$ cells/well) were cultured with various concentrations of *M.oleifera* extract for 1 h before administration of 100 ng/ml LPS**.** After incubated with LPS for 24 h, the culture supernatants were assayed for NO production (%).

Discussion

The result from the present study demonstrates that both crude extracts (methanol and hexane fractions) of *M.Oleifera* Lam leaves have anti-inflammatory effect because both fractions significantly reduced NO production in HAPI microglial cells activated by LPS. It is well known that LPS stimulates microglia and macrophage to produce nitric oxide and other pro-inflammation cytokines such as interleukine-6(IL-6), and tumor necrosis factor-alpha etc. The reduction of NO production of *M. oleifera* leaf crude extracts on microglia may be due to the direct effect on inhibition of the inducible nitric oxide synthase (iNOS). Since upon activation the iNOS was expressed in microglia.

Conclusion

The results from the present study suggests that both crude extracts (methanol and hexane) obtained from leaves of *Moringa oleifera* Lam. have anti-inflammatory effect. It has been demonstrated that the active components from ethanol crude extract from *M.Oleifera* leaves contains isothiocyanate and thiocarbamate. The reduction of nitric oxide production in our experiments may come from the action of these 2 active components. However, further study is necessary.

Acknowledgement

This project was partly supported by Department of Chemistry, Faculty of Science, Mahidol University

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