

## Inhibitory effect of *Derris reticulata* ethanol extract on LPS-induced macrophage activation

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### Abstract

*Derris reticulata* Craib is a plant in Leguminosae family which has flavonoids as its major active compounds similar to other plants in genus *Derris*. It has been used as expectorant and thirst relief. In this study, we studied the effect of ethanol extract from stem of *D. reticulata* on LPS-induced macrophage activation. Murine macrophage, J774A.1 cells, were pretreated with 6.25-100 µg/ml the extract for 24 h and then stimulated with 100 ng/ml lipopolysaccharide (LPS) for 24 h. The extract inhibited nitric oxide production in LPS-activated J774A.1 cells in concentration-dependent manner with IC<sub>50</sub> at 62.5 µg/ml. The effect of this extract on phagocytosis activity of LPS-activated J774A.1 cells was also investigated. The extract at the concentrations of 50 and 100 µg/ml significantly inhibited zymosan phagocytosis of LPS-activated cells in a concentration dependent manner. These results demonstrated that flavonoids from *D. reticulata* may have potential to be an inflammatory agent.

**Keywords:** *Derris reticulata*, LPS-activated macrophage, phagocytosis, nitric oxide

### Introduction

Activated macrophages play important roles in innate and adaptive immune responses against a wide range of microorganisms as well as inflammatory response against infectious and non-infectious stimuli. These activated cells express and release a numerous molecules involving in those responses. They express several cytokine receptors, adhesion molecules, and various accessory molecules for immune responses on their cell surface. These cells increase ability to get rid of pathogens, debris cells and apoptotic cells by phagocytosis. They also generate several free radicals such as hydrogen peroxide, super oxide anion and nitric oxide (NO) for intracellular destroying pathogen. Activated macrophages express inducible nitric oxide synthase (iNOS) that catalyzes L-arginine to large amount of NO. They also produce and release more than 30 types of cytokines. These include pro-inflammatory cytokines (TNF- $\alpha$ , IL-1, IL-6, IL-8 and IL-12) that initiate to generate various inflammatory mediators such as prostaglandins (PGs) and leukotrienes (LTs) which cause the signs of inflammation; redness, swelling, fever and pain. Many clinically used anti-inflammatory agents target on PGs production or inhibit pro-inflammatory cytokine functions.

Many medicinal plants containing flavonoid compounds are used as traditional medicine for inflammation or fever treatment. Several plants in genus *Derris* are identified to have flavonoids as their major constituent (1). *Derris reticulata* Craib or “Cha-aem-nuea” is a plant in Leguminosae family that widely distributes throughout Thailand. Thai people use its stems and roots as a sweetening agent, an expectorant, an antitussive, a remedy for throat diseases and as a tonic agent (2). This study intended to investigate potential anti-inflammatory effect of the ethanol extract from stems of this plant.

## Materials and Methods

### Plant extract

Air-dried and grounded stems of *D. reticulata* was extracted with dichloromethane and then with absolute ethanol. The ethanol extract was dissolved in dimethylsulfoxide (DMSO). The constant final concentration of DMSO in this study was 0.2%.

### Cells

Murine macrophages J774A.1 were obtained from ATCC. The cells were subcultured 3 times weekly and maintained in DMEM containing 10% fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin and incubated at 37 °C in 5%CO<sub>2</sub>/ 95% air.

### Chemicals

The following reagents were obtained from Sigma, USA; nitroblue tetrazolium (NBT), LPS, zymosan A from *Saccharomyces cervisiae*, DMSO, 0.4% trypan blue dye. Chemicals and reagents for cell culture were from Gibco, USA. Griess reagent for nitric oxide was obtained from Promega, USA.

### Determination of NO production

1x10<sup>5</sup> cell/well J774A.1 cells in 96-well plates were treated with the ethanol extract at 6.25, 12.5, 25, 50 and 100 µg/ml for 24 h. The treated cells were stimulated with 100 ng/ml LPS for 24 h. The amount of NO release into the supernatant was determined in nitrite form by using Griess reagent. The reaction mixture was detected with a microplate reader at 540 nm. The amount of nitrite was calculated from sodium nitrite standard curve. The viability of the treated cells was determined by staining with resazurin for 2-4 h at 37 °C in 5%CO<sub>2</sub>/ 95% air. The reduction product resorufin in viable cells was detected by a microplate reader at 570 and 600 nm. The percentage of cell viability was calculated by comparing with LPS-activated condition.

### Determination of phagocytosis activity

1x10<sup>5</sup> cell/well J774A.1 cells in 96-well plates were treated with 25, 50 and 100 µg/ml ethanol extract for 24 h and then activated with 100 ng/ml LPS for 24 h. The treated cells were carefully washed twice with DMEM and then incubated with 800 µg/ml of zymosan and 600 µg/ml of NBT for 1h. The cells were washed 3 times with methanol, air-dried, and lysed in 120 µl of 2M KOH and 140 µl of DMSO. The oxidized NBT product in blue color was detected at 570 nm. The percentage of phagocytosis inhibition was determined by comparing to LPS-activated condition.

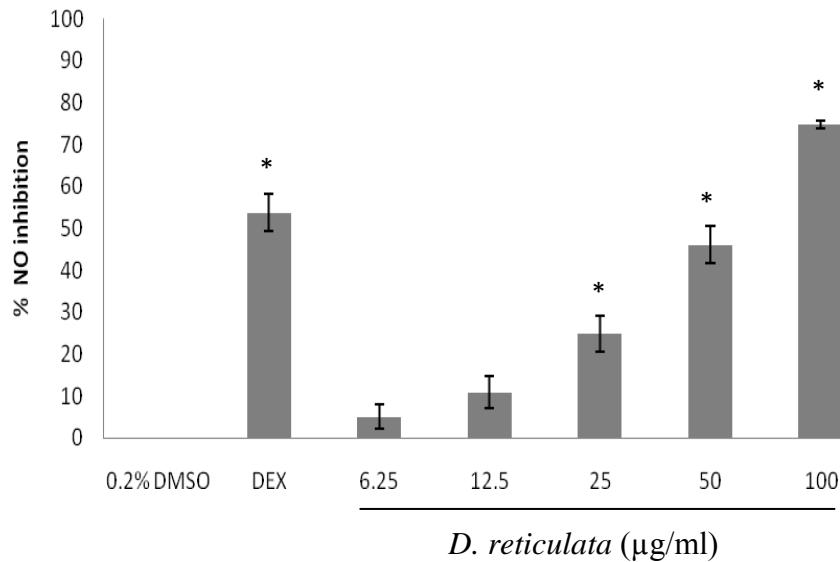
### Statistical analysis

All data were presented as mean ± S.E.M. Data analysis was performed on SPSS 17.0. Statistical comparisons were made by one-way ANOVA followed by Turkey's post hoc test. The p-value<0.05 was considered statistically significant.

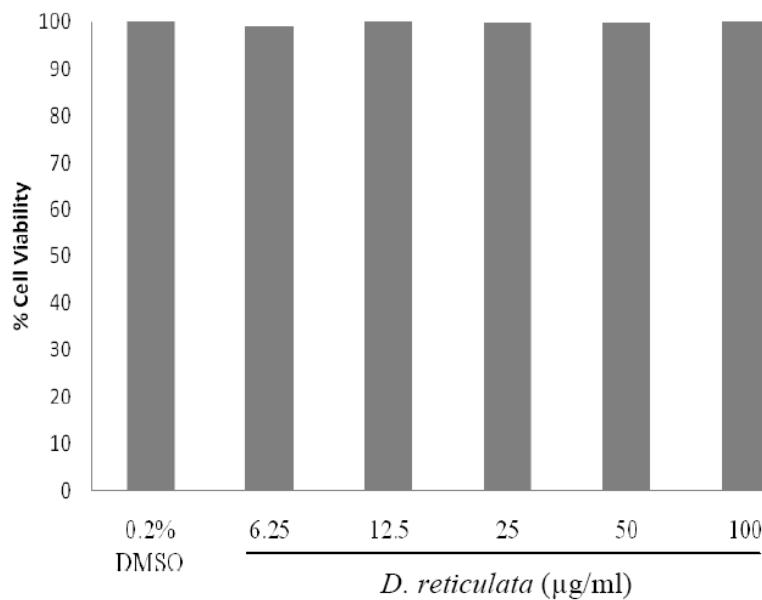
## Results

### Effect of the ethanol extract on NO production

*D. reticulata* ethanol extract decreased NO production in LPS-stimulated J774A.1 cells in a concentration-dependent manner with IC<sub>50</sub> 62.5 µg/ml (Fig.1). The extract at the concentration of 6.25, 12.5, 25, 50 and 100 µg/ml inhibited NO production by 5.1%, 10.9%, 24.9%, 46.1% and 74.8%, respectively. It didn't affect cell viability of the treated macrophages (Fig.2).



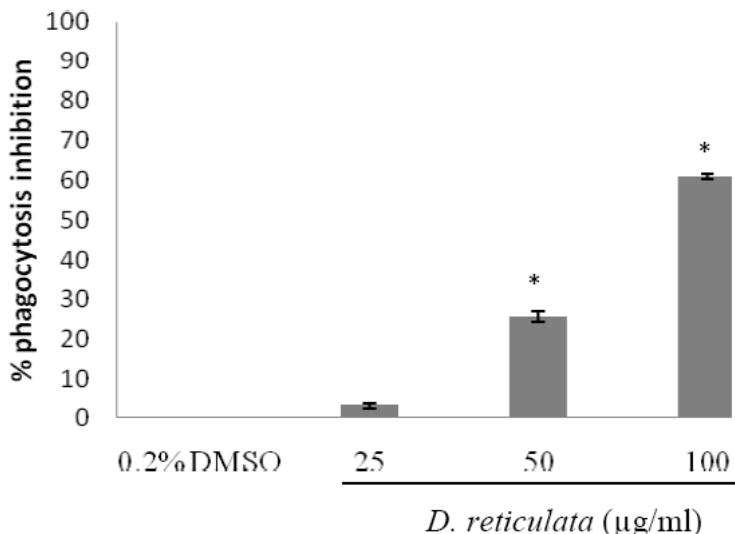
**Figure 1:** Inhibitory effect of *D. reticulata* ethanol extract on NO production in LPS stimulated-J774A.1 cells. The cells were treated with 6.25-100  $\mu\text{g/ml}$  extract and then stimulated with 100 ng/ml. Five  $\mu\text{g/ml}$  dexamethasone (DEX) was used as the positive control. The NO production was determined by Griess reagent. The percentage of NO inhibition compared to LPS-activated condition is presented as mean  $\pm$  S.E.M. of four independent experiments ( $n=4$ ); \*  $p<0.05$  compared to untreated control (LPS-activated cells).



**Figure 2:** Effect of the ethanol on cell viability. Viability of the treated cells from figure 1 was determined by resazurin assay. The data are presented as mean  $\pm$  S.E.M.

#### Effect of the ethanol extract on phagocytosis

When compared to the LPS-activated condition, the extract at 25, 50, and 100  $\mu\text{g/ml}$  inhibited phagocytosis in a concentration-dependent manner.



**Figure 3:** Effect of *D. reticulata* ethanol extract on phagocytosis in LPS stimulated-J774A.1 cells. The cells were treated at dose 25-100  $\mu$ g/ml extract. Phagocytosis of the treated cells was determined by zymosan-NBT assay. Data are presented as mean  $\pm$  S.E.M. of three independent experiments (n=3). \*  $p<0.05$  compared to LPS-activated condition (0.2% DMSO).

### Discussion and conclusion

This study intended to investigate the pharmacological effects of the ethanol extract from stems of *Derris reticulata* on macrophage activation. Activated macrophages are important immune cells that generate and release numerous mediators involving in inflammatory response against infectious and non-infectious stimuli (3). These mediators include pro-inflammatory cytokines (TNF- $\alpha$ , IL-1, IL-6, IL-8), oxygen free radicals, NO and prostaglandins. They play important roles in inflammatory process and the signs of inflammation. Many clinically used anti-inflammatory agents target on both synthesis and functions of these mediators such as NSAIDs, corticosteroids, and TNF- $\alpha$  and IL-1 inhibitors. We demonstrated that the ethanol extract from stems of *D. reticulata* suppressed the production of NO in LPS-activated J774A.1 cells in a concentration-dependent manner. This extract also decreased phagocytosis activity of activated macrophages which can lead to generation of inflammatory mediators in these cells. These results demonstrate the anti-inflammatory potential of compounds presenting in *D. reticulata* ethanol extract.

### Acknowledgements

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