

## Effects of the hexane extract from *Glycosmis parva* on LPS-induced macrophage activation

Suchintana Chumseng<sup>1\*</sup>, Chandhanee Itthipanichpong<sup>2</sup>, Nijisiri Ruangrunsi<sup>3</sup>, Chaisak Chansriniyom<sup>3</sup>, Wacharee Limpanasithikul<sup>2</sup>

<sup>1</sup> Interdepartmental Program of Pharmacology, Graduate School, Chulalongkorn University, Thailand.

<sup>2</sup> Department of Pharmacology, Faculty of Medicine, Chulalongkorn University, Thailand.

<sup>3</sup> Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

\* Presenting Author

### Abstract

The *in vitro* effect of the hexane extract from branches of *Glycosmis parva* on LPS-induced macrophage activation was investigated. Murine macrophages, J774A.1 cells, were used in this study. The extract suppressed nitric oxide production from LPS-activated J774A.1 cells in a concentration dependent manner with IC<sub>50</sub> 44.70 µg/ml. It decreased the mRNA expression of the inducible nitric oxide synthase (iNOS) which is responsible for NO production as well as the cyclo-oxygenase 2 (COX-2) which is responsible for prostaglandins (PGs) synthesis in these activated macrophages. The extract also inhibited the mRNA expression of pro-inflammatory cytokines, including TNF-α, IL-1β and IL-6. The results from this study indicated that the hexane extract from *G. parva* may have anti-inflammatory activities against activated macrophages which play a significant role during inflammatory process.

**Keywords:** *Glycosmis parva*, macrophage, inflammatory.

### Introduction

Macrophages play an essential role in the innate and adaptive immunities as well as the inflammatory process. These cells are activated by directly recognizing pathogens via pattern recognition receptors (PRRs) such as toll like receptors (TLRs) or by several cytokines from immune cells such as interferon-γ from Th1 cells. Activated macrophages express and secrete several enzymes and mediators that involve in immune response and inflammatory process. They express inducible nitric oxide synthase (iNOS) for nitric oxide (NO) synthesis, cyclooxygenase-2 (COX-2) for prostaglandin (PG) synthesis. The cells also express several cytokines and chemokines as well as pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and IL-8) that activate iNOS and COX-2 expression. They also play a key role in many inflammatory diseases such as rheumatoid arthritis and atherosclerosis (1). Some mediators of activated macrophages including PGs and pro-inflammatory cytokines are targets of clinically used anti-inflammatory agents including corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs), selective COX-2 inhibitors and cytokines inhibitors (anti-TNF-α drugs and IL1 receptor antagonist).

Many medicinal plants have been used as alternative medicines for treatment of various inflammatory diseases. *Glycosmis parva* Craib is a small shrub widely distributes in Thailand. Acridone alkaloids and sulfur-containing propanamides are major compounds found in plants of genus *Glycosmis*. Anti-infective and anticancer activities of these compounds have been reported. This study aimed to investigate anti-inflammatory activity of the hexane extract of *G. parva* on LPS-induced J774A.1 cells activation.

## Materials and methods

**Plants extract:** The precipitated pellet from methanol extract from branches of *G. parva* was partition in hexane. The hexane extract was dissolved in dimethylsulfoxide (DMSO) and further diluted to various final concentrations in DMSO 0.2%.

**Cells:** The murine macrophage cells J774A.1 were obtained from American Type Culture Collection (ATCC). The cells were 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.

**Other materials:** Reagents for cell culture, TRIzol and Taq polymerase were obtained from Gibco, USA. Reverse transcription system kit was purchased from Promega, USA. Other chemical reagents were from Sigma USA.

### Determination of nitric oxide production

J774A.1 cells (2x10<sup>5</sup> cells/ml) were treated with the hexane extract of *G. parva* at the concentrations 6.25-50 µg/ml for 24 h, and then activated with 100 ng/ml LPS for 24 h. The amount of NO as nitrite form in the supernatants of the treated cells was determined using Griess reagent.

### Identification of mRNA expressions of pro-inflammatory cytokines, iNOS and COX2

J774A.1 cells (2x10<sup>5</sup> cells/ml) were treated with the hexane extract of *G. parva* at the concentrations of 25 and 50 µg/ml for 24 h, and then activated with 100 ng/ml LPS for 6 h or 24 h to determine cytokines or iNOS and COX-2 expression, respectively. Total RNA was isolated from the treated cells and then reversed to cDNA. The cDNA was used as the template to amplified mRNA of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), iNOS and COX-2 with suitable primers. The PCR products were run on 1.5% agarose gel electrophoresis and semi-quantitatively determined by gel documentation.

### Statistical analysis

Data were expressed as mean ± standard deviation. One-way ANOVA with Tukey's Honestly Significant Difference (HSD) post hoc test was used to determine the statistical significance of differences between the values for the various experimental and control groups. The p-value < 0.05 was considered as statistically significance.

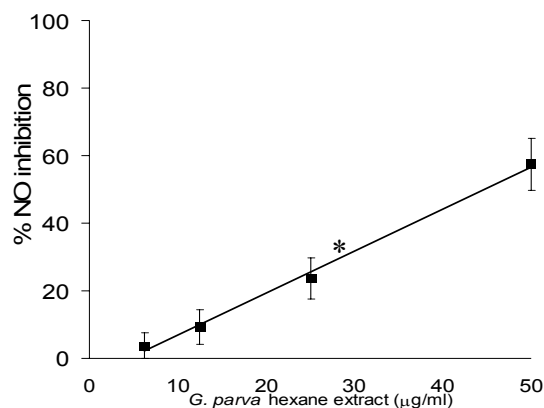
## Results

### Effect of *Glycosmis parva* hexane extracts on LPS stimulated-macrophages

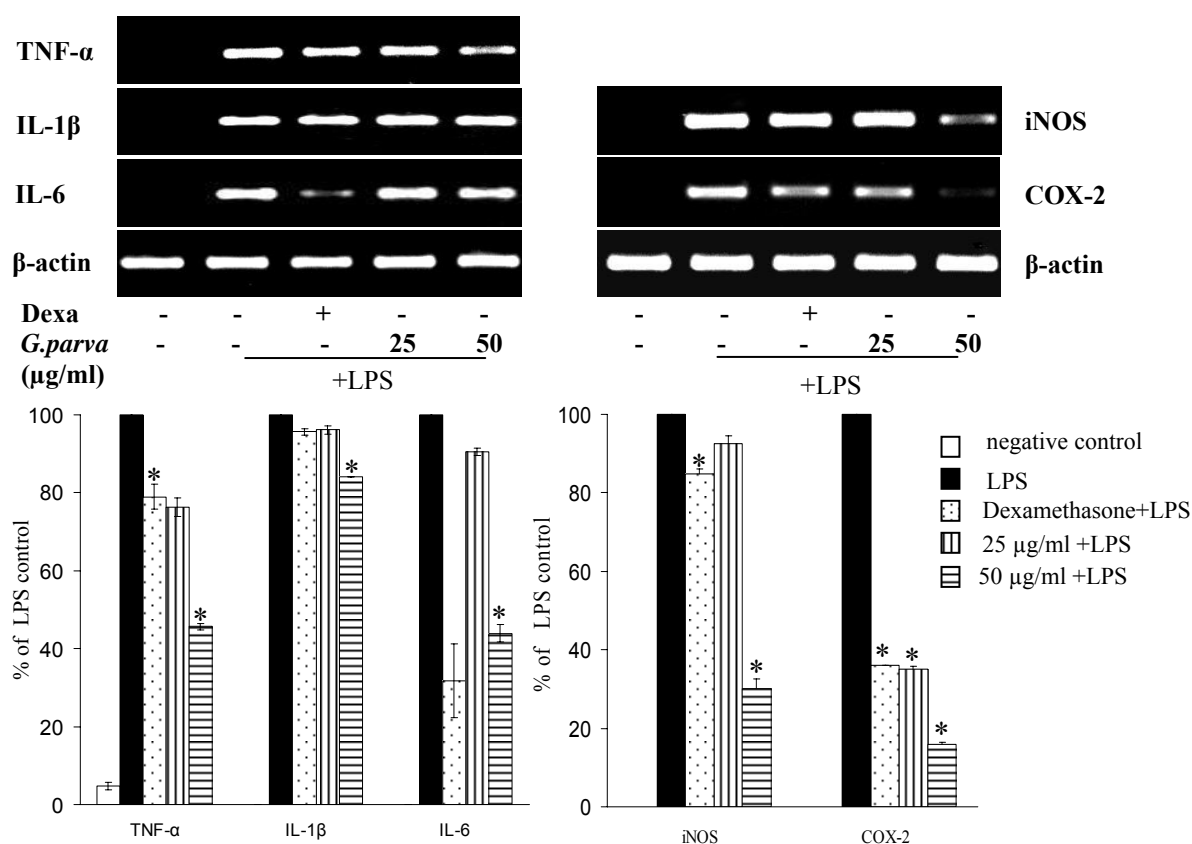
The extract inhibited NO production in LPS-stimulated J774A.1 cells in a concentration-dependent manner with IC<sub>50</sub> values at 44.70 µg/ml (Fig.1)

### Effect of the extract on the expressions of cytokines, iNOS and COX-2 in LPS activated J774A.1

The hexane extract of *G. parva* at 50 µg/ml significantly inhibit the mRNA expression of pro-inflammatory cytokines; TNF-α, IL-1β, IL-6, as well as iNOS and COX-2 (Fig.2).



**Figure 1.** Inhibitory effects of the hexane extract of *G. parva* on NO production of LPS-activated J774A.1 cells. The percentage of inhibition is expressed as mean  $\pm$  SD of 5 independent experiments. \* significantly difference between the untreated and the extract-treated cells at  $p < 0.001$ .



**Figure 2.** Inhibitory effect of the hexane extract on the mRNA expressions of pro-inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$  and IL-6), iNOS and COX-2 in LPS-activated J774A.1 cells. The percentage of inhibition is expressed as mean  $\pm$  SD of two independent experiments.

\* significantly difference between the untreated and the extract treated cells at  $p < 0.001$

## Discussion

The hexane extract from branches of *G. parva* on activated macrophages was investigated in this study. The results demonstrated that the extract inhibited several mediators in LPS-activated J774A.1 cells. It suppressed the mRNA expressions of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, iNOS and COX-2 as well as NO production. These mediators not only play roles in innate immune response but also initiate and induce neighbor tissue injury and lead to inflammatory diseases such as rheumatoid arthritis (1). Inhibition of these mediators is

current or trend therapeutic strategy for treatment of inflammation. TNF- $\alpha$  is the major pro-inflammatory cytokine that induces the expression of other pro-inflammatory cytokines, iNOS and COX-2. Inducible NOS is responsible to excess NO production (2). NO in activated macrophages can interact with superoxide anion to generate powerful free radical, peroxynitrite. This free radical can cause tissue injury during inflammation (3). COX-2 is the inducible COX that induces large amount of PG synthesis in activated macrophages. PGs are the important mediators of inflammation response (4). Our study suggests that *G. parva* contains compound(s) which suppress the production of inflammatory mediators in activated macrophages. It has been reported that acridone alkaloids and sulfur-containing propanamides are major compounds found in *G. parva* (5). This plant also contains  $\beta$ -sitosterol and stigmasterol which have been demonstrated to suppress the production of inflammatory mediators in LPS-activated macrophage (6). It needs further investigation to identify the compound that has anti-inflammatory activity in this medicinal plant.

### Conclusion

This study demonstrated that the hexane extract from the branches of *G. parva* inhibits the production of several inflammatory mediators in LPS-activated macrophages. *G. parva* might be a source for development of anti-inflammatory agent(s).

### Acknowledgments

This study was supported by a grant from the Graduate School, Chulalongkorn University.

### References

1. Copsted lee-Ellen C and Banasik Jacquelyn L. Inflammation and immunity. Pathophysiology. Saunders Elsevier 2005; 4: 189-221.
2. Dinarello CA. Proinflammatory cytokines. Chest 2000; 118:503-8.
3. Bogdan C. Nitric oxide and the immune response. Nature Immunology 2001; 2: 907 –16.
4. Marco E, Raymond N. Cyclooxygenase-2: A therapeutic target. Annual Review 2002; 53:35-57.
5. Chansriniyom C, Ruangrunsi N, Lipipun V, Kumamoto T, Ishikawa T. Isolation of acridone alkaloids and *N*-[(4-Monoterpenyloxy)phenylethyl]-substituted sulfur-containing propanamide derivatives from *Glycosmis parva* and their anti-herpes simplex virus activity. Chemical & Pharmaceutical 2009; 57: 1246-50.
6. Kongsubsopa J. Chemical constituents of *Glycosmis parva* branches. Thesis Master of Science in Pharmacy, Chulalongkorn University, 2000: 4-88.