

## Inhibitory effect of *Harrisonia perforata* root extract on macrophage activation

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### Abstract

Root of *Harrisonia perforata* Merr. (*Simaroubaceae*) is one of compositions in Thai herbal remedy named Bencha-Loga-Wichien which has been used as an alternative medicine for the treatment of pyresis. The direct effect of *H.perforata* on LPS-activated macrophage, J774A.1 cells, were investigated in this study. The ethanol extract of *H.perforata* root inhibited LPS-activated nitric oxide (NO) production in a concentration-dependent manner. This inhibition was corresponded to the decrease in mRNA expression of inducible nitric oxide synthase (iNOS) in the extract treated LPS-activated J774A.1 cells. The extract also decrease the mRNA expression of the cyclooxygenase-2 (COX-2) which is the enzyme required for large amount of prostaglandin synthesis in activated macrophage during inflammatory process. These results indicated that the ethanol extract of *H.perforata* root has direct inhibitory effect on macrophage activation. It may have not only antipyretic effect but also anti-inflammatory activity.

**Keywords:** *Harrisonia Perforata*, inflammation, NO, iNOS, COX-2.

### Introduction

Inflammation is a protective response of host against pathogen, chemical or physical stimuli. Several immune cells involve in the inflammatory process as well as macrophages. Macrophages in the inflammation area are activated by the components of pathogenic microorganism invading the host such as LPS which is an endotoxin derived from the cell wall of Gram negative bacteria. Activated macrophages express various components on their cell surface and synthesize several intracellular and extracellular mediators involve inflammation such as pro-inflammatory cytokines (TNF- $\alpha$ , IL-1, IL-6 and IL-8), NO, superoxide free radicals and PGs. These mediators lead to cardinal signs of inflammation; pain, edema, red and fever. Many anti-inflammatory agents inhibit synthesis or functions of these pro-inflammatory and inflammatory mediators[1]. Several alternative medicine as well as herbal medicines are also used to treat inflammation. *Harrisonia perforata* (khon-thaa) root is one in five roots in Bencha-Loga-Wichien remedy which is used in Thai traditional medicine as antipyretics. Several compounds from this plant roots were identified including 2-hydroxymethyl-3-methylalloptaeroxylin, heteropeucenin-7-methylather, perforatic acid, lupeol, 5-hydroxy-6-7-dimethoxycoumin,  $\beta$ -sitosterol, campesterol, stigmasterol,  $\beta$ -sitosteryl-3-O-glucopyranoside, stigmasteryl-3-O-glucopyranoside, chloresteryl-3-O-glucopyranoside[2]. The ethanol extract of *H.perforata* has been reported to have anti-asthmatic, anti-infective and antipyretic activities[3][4]. In this study we investigate the direct inhibitory effect of the ethanol extract from roots of *H.perforata* on LPS-activated macrophages, J774A.1.

## Methods

**Plant extract:** The 95% ethanol extract from dried root powder of *H.perforata* was dissolved in dimethylsulfoxide (DMSO) as the stock solution. The final concentration of this extract were prepared in DMSO 0.2%.

**Cells:** The murine macrophage, J774A.1, were obtained from ATCC. The cells were maintained in the completed 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.

### Effect of the extract on NO production:

J774A.1 cells (2x10<sup>5</sup> cells/ml) were treated with the ethanol extract of *H.perforata* roots at 3.125-50 µg/ml (5 concentrations) for 24 h. Ten µM dexamethasone was used as the positive control. The treated cells were activated with 100 µg/ml LPS for 24 h. The supernatants were used to measure the amount of released NO in nitrite form by Griess reagent. Nitrite concentration was determined by a standard curve prepared with sodium nitrite. The percentage of NO inhibition of the ethanol extract was compared to the LPS – activated cells without the extract. The treated cells were determined for their viability by staining with rezasurin(blue dye) which is reduced in viable cells to its substrate, rezorufin(red product). The amount of rezorufin production was determined at 570 and 600 nm. The percentage of cells viable of the extract-treated cells was compared to the LPS-activated cells without the extract

### Effect of the extract on the mRNA expression of iNOS and COX-2 :

J774A.1 cells (2x10<sup>5</sup> cells/ml) were treated with the ethanol extract at the concentrations of 12.5,25 and 50 µg/ml for 24 h. Ten µM dexamethasone was used as the positive control. The treated cells were then activated with 100 ng/ml LPS for 24 h. Total RNA was isolated from the treated cells using Trizol reagents and then reversed to cDNA using reverse transcription system kit. The cDNA was used as the template to amplified mRNA of iNOS and COX-2 with specific primers for iNOS and COX-2 genes. The PCR products were run on 1.5% agarose gel electrophoresis and measured their densities by gel documentation.

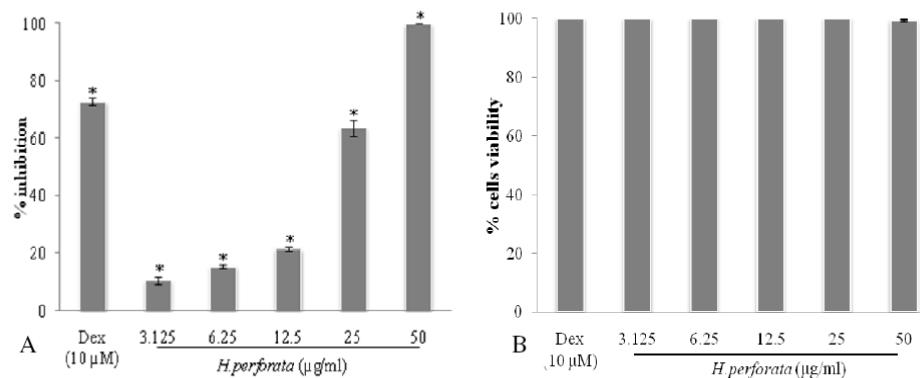
### Statistical analysis:

Data were presented as means.±.S.E.M. One-way ANOVA with Tukey's Honestly Significant Difference (HSD) post hoc test was used to determine the statistical significance analysis. The p-value<0.05 was considered as statistically significance.

## Result

### Effects of the ethanol extract on NO production in LPS-stimulated J774A.1 cells

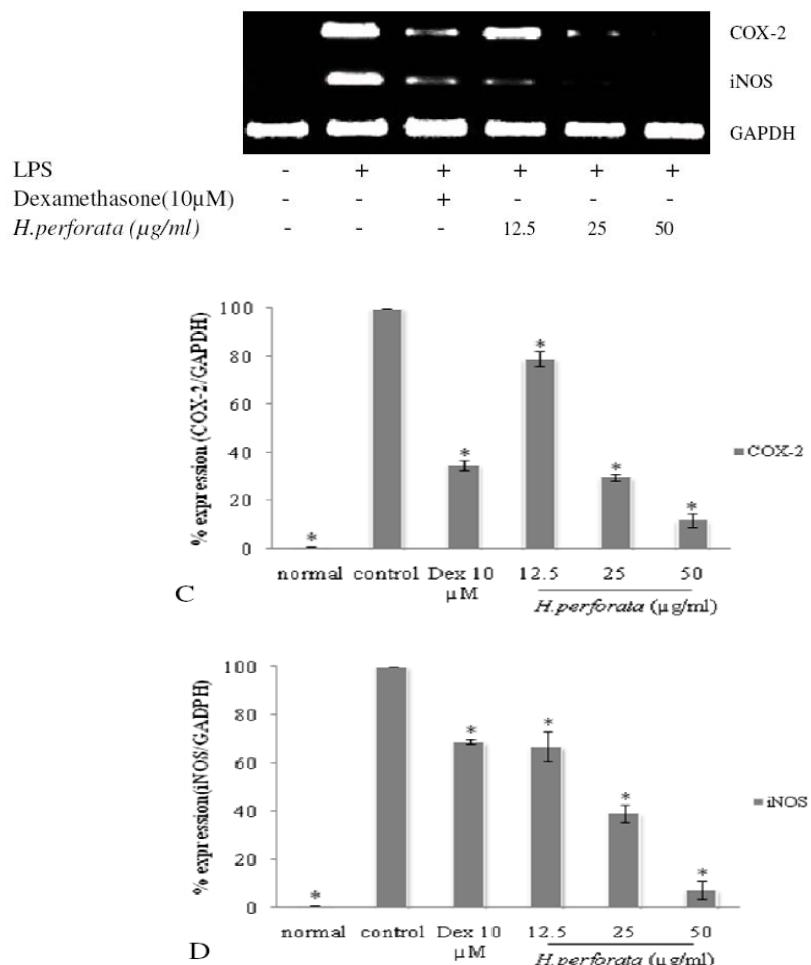
When compared to the untreated LPS-stimulated J774A.1, the extract suppressed NO production in LPS-activated cells in a concentration-dependent manner with its IC<sub>50</sub> 23.14 µg/ml (Fig. 1A). It didn't have any effect on J774A.1 cell viability at all concentrations used in the study (Fig.1B).



**Figure 1.** Effects of the ethanol extract from *H. perforata* roots at the concentrations 3.125-50 µg/ml on NO production(A) and on cell viability (B) in LPS-stimulated J774A.1 macrophage cells. The data are expressed as mean ± S.E.M from 4 independent experiments (n=4). \*P < 0.05 compared to untreated cells.

### Effects of the extract on the mRNA expression of iNOS and COX-2.

The extract decreased the mRNA expression of iNOS and COX-2 in a concentration dependent manner (Fig.2.). The inhibitory effect of the ethanol extract on iNOS mRNA expression is correlated to its effect on NO production.



**Figure 2.** Effects of the ethanol extract from *H. perforata* roots on mRNA expression of COX-2 (C) and iNOS (D) in LPS-stimulated J774A.1 macrophage cells. The data are expressed as mean ± S.E.M from 3 independent experiments (n=3). \*P < 0.05 compared to untreated cells.

## Discussion

*Harrisonia perforata* root is used one of the components in Thai traditional medicine, Bencha-Loga-Wichien, which has been used as antipyretics. Fever is one of the cardinal signs of inflammation and infection. Activated macrophages are the source of many pro-inflammatory cytokines and several mediators involve inflammatory process. In this study, we examined the effect of the ethanol extract of *Harrisonia perforata* root on macrophage activation. We demonstrated that the ethanol extract inhibited LPS-activated murine macrophage, J774A.1 cells. It suppressed NO production as well as iNOS mRNA expression in a concentration dependent manner in LPS-activated J774A.1 cells. It also significantly decreased the mRNA expression of COX-2 in the activated cells. It is known that iNOS and COX-2 are responsible to the large amounts of NO and prostaglandin E2 production, respectively. Both NO and PGE2 are the important inflammatory mediators in fever and inflammatory diseases. These results suggest that *H.perforata* extract may have therapeutic potential in reducing fever and inflammation.

## Conclusion

Our results demonstrated that the ethanol extract of *H.perforata* roots decreased the expression of iNOS and COX-2 which are responsible for NO and PGE2 production. *H.perforata* roots might the source of an effective therapeutic agent for not only fever but also various inflammatory diseases.

## Acknowledgements

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