

RESEARCH ARTICLE

Anti-inflammatory Activity of the Water Extract from Treehom Remedy

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Abstract

Treehom remedy is a Thai traditional medicine used for relieving of pyrexia. Its composition are *Terminalia* sp., *Terminalia bellirica* Roxb., *Phyllanthus emblica* L., *Coriandrum sativum* Linn., *Aristolochia* sp., *Angelica dahurica* Benth., *Glycyrrhiza glabra* L., Sodium borate, *Trigonella foenum-graecum* L., *Terminalia chebula* Retz., *Rheum officinale* Baill. The aim of this study is to investigate the anti-inflammatory activity of the water extract from this remedy on LPS-activated J774A.1 macrophages. Nitric oxide production, COX-2 and iNOS expression were determined and compared between the extract- treated and untreated group. Our results demonstrated that the water extract of Treehom remedy significantly inhibited NO production from LPS-stimulated macrophages in concentration-dependent manner. The IC₅₀ value was 60.05 µg/ml. This extract did not affect J774A.1 cell viability at all concentration used in this study. The activity of this extract on iNOS enzyme showed the significant decreased in iNOS mRNA expression. This finding corresponded to the decrease in NO generation. In addition the extract was significantly decreased the expression of COX-2 mRNA enzyme. This enzyme is upregulated during inflammatory process. All these results showed the anti-inflammatory potential of Treehom remedy extract.

Keywords: Treehom remedy, inflammation, NO, iNOS, COX-2.

ฤทธิ์ต้านการอักเสบของสิ่งสกัดด้วยน้ำจากตำรับยาตรีหอม

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บทคัดย่อ

ตำรับยาตรีหอมเป็นยาสามัญประจำบ้านแผนโบราณ ใช้แก้เด็กท้องผูก ระบายพิษไข้ ตำรับยานี้ประกอบด้วย เนื้อมะขามเทศ เนื้อมะขามป้อม ลูกผักชีลา รากไคร้เครือ โกงสุล ชะเอมเทศ น้ำประสานทอง สะตุ ลูกชดควัว เนื้อมะขามเทศ โกงสุลน้ำเต้าใหญ่หนึ่งลูก การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาฤทธิ์ต้านการอักเสบของสิ่งสกัดด้วยน้ำจากตำรับยาตรีหอมต่อเซลล์แมคโครฟาจ J774A.1 ที่ถูกกระตุ้นด้วยไลโปพอลิแซ็กคาไรด์ โดยวัดปริมาณการสร้าง nitric oxide การแสดงออกของยีน COX-2 และ iNOS ผลการศึกษาพบว่า สิ่งสกัดด้วยน้ำจากตำรับยาตรีหอมสามารถยับยั้งการสร้างไนตริกออกไซด์ได้ตามความเข้มข้นของสิ่งสกัดที่ใช้ในการศึกษา โดยมีค่า IC₅₀ เป็น 60.05 µg/ml และสิ่งสกัดไม่เป็นพิษต่อเซลล์ที่ใช้ศึกษา อีกทั้งยังสามารถลดการแสดงออกในระดับ mRNA ของเอนไซม์ inducible nitric oxide synthase (iNOS) โดยผลการยับยั้งการสร้างไนตริกออกไซด์สอดคล้องกับผลการยับยั้งการแสดงออกของเอนไซม์ iNOS นอกจากนี้สิ่งสกัดสามารถลดการแสดงออกของเอนไซม์ cyclooxygenase 2 (COX-2) ที่ทำหน้าที่สร้าง prostaglandins (PGs) ซึ่งเป็นสารสื่อที่มีบทบาทสำคัญในกระบวนการอักเสบ จากผลการศึกษาดังกล่าวแสดงให้เห็นว่าสิ่งสกัดด้วยน้ำจากตรีหอมน่าจะเป็นสารที่มีศักยภาพต้านการอักเสบได้

คำสำคัญ: ตำรับยาตรีหอม, NO, iNOS, COX-2.

Introduction

Inflammation is an important mechanism in response of tissues and cells to protect against foreign substances. It is involved many inflammatory mediators secreted from macrophages. Macrophages play role in inflammatory process. Activated macrophages produce and secrete many inflammatory mediators and pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), interleukin-1 (IL-1) and the enzymes involved in inflammation as well. These enzymes are inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX) which are responsible for nitric oxide and prostaglandins generation during inflammatory process. COXs are at least divided into two isoforms; a constitutive form (COX-1) and an inducible isoforms (COX-2) (Abu-Ghefreh et al 2009). During inflammation, COX-2 is selectively induced by pro-inflammatory cytokines at the site of inflammation. Inflammatory reactions also induce the production of reactive oxygen species (ROS) by activated macrophage. The inflammatory reaction is beneficial when its effects are limited to the pathogens while prolonged inflammation is able to be the causes of various kinds of chronic diseases, such as Alzheimer's disease, cancer, rheumatoid arthritis, psoriasis, etc. Nonsteroidal anti-inflammatory drugs (NSAIDs) are drugs commonly used for management of inflammation. They inhibit cyclooxygenase (COX) enzyme and it is resulting in inhibition of prostaglandins production at the site of inflammation. Inhibition of gastrointestinal prostaglandins is associated with mechanism-based toxicities that limit their usefulness. Recently, natural medicines from plants

have been widely researched as antiinflammatory agents since they are considered to be efficacious and safe (Kassuya et al 2009, Sharma et al 2007, Shukla et al 2010). Treehom remedy is a Thai traditional medicine which are composed of several ingredients including *Terminalia* sp., *Terminalia bellirica* Roxb., *Phyllanthus emblica* L., *Coriandrum sativum* Linn., *Aristolochia* sp., *Angelica dahurica* Benth., *Glycyrrhiza glabra* L., Sodium borate, *Trigonella foenum-graecum* L., *Terminalia chebula* Retz., *Rheum officinale* Baill. It is approved as the folk remedy for relieving of pyrexia. As far as we known, the anti-inflammatory effect of the water extract from Treehom remedy have not been studied. Therefore, the aim of this study is to investigate anti-inflammatory activity of the water extract from Treehom remedy on LPS-activated J774A.1 macrophages.

Materials and Methods

Preparation of treehom remedy extract

Treehom remedy was extracted using 95% ethanol until exhausted. The marc was extracted with warm water. This extract was evaporated by lyophilization until dryness and dissolved in complete media as the stock solution.

Cells

The murine macrophage cell lines J774A.1 were purchased from the American Type Culture Collection (ATCC). The cells were maintained in the completed Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum, 100 U/ml penicillin and 100 μ g/ml streptomycin at 37 $^{\circ}$ C, 97% humidity, and 5% CO₂.

Effects of the extract on nitric oxide production in LPS-stimulated J774A.1 cells

The J774A.1 cells were pretreated with the water extract of Treehom remedy at 6.25-100 µg/ml and incubated for 24 h. Dexamethasone 10 µM were used as the reference compound. The treated cells were stimulated with 100 ng/ml LPS and incubated at 37 °C, 5% CO₂ for 24 h. The supernatant was collected for determination of NO production by Griess reaction assay. Nitrite concentration was calculated by using nitrite standard curve and determined the percentage of NO inhibition. The treated cells were also determined for viability by resazurin reduction assay. Then the amount of resorufin was determined by microplate reader at 570, 600 nm.

Effect of the extract on mRNA expression of cytokines, iNOS and COX-2 in LPS-stimulated J774A.1 cells

The J774A.1 cells were pretreated with the water extract of Treehom remedy at the 25-100 µg/ml. Dexamethasone 10 µM was used as the reference compound. Total RNA was isolated from the treated cell by TRIzol reagent and then reversed to cDNA by reverse transcription system kit. Then cDNA was used as the template to amplified mRNA of iNOS and COX-2 with the specific primers. The *PCR products* were determined on 1.5% agarose gel electrophoresis and stained with ethidium bromide (EtBr₂). Band intensity was measured by gel documentation.

Statistical analysis

Data were presented as mean ± S.E.M. One-way ANOVA with Turkey'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.

Fig. 1A

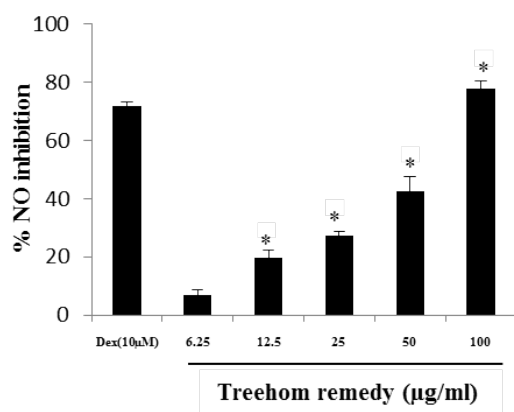


Fig. 1B

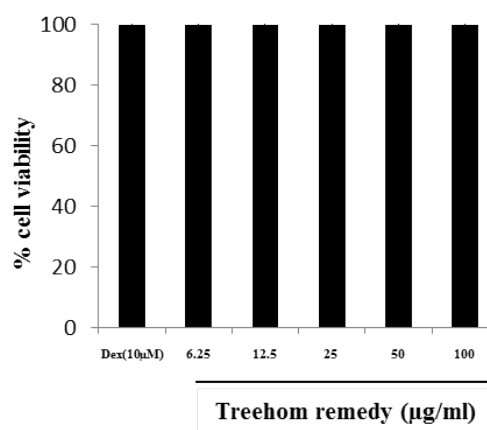


Figure1. Effects of the water extract from Treehom remedy at the concentrations 6.25-100 µg/ml on nitric oxide production (A) and cells viability in LPS-stimulated J774A.1 cells(B). The data are expressed as mean ± S.E.M from 3 independent experiments (n=3).

*P<0.05 compared to untreated cells.

Results

Effects of the extract on nitric oxide production in LPS-stimulated J774A.1 cells

Pretreatment with the water extract of Treehom remedy 6.25-100 $\mu\text{g/ml}$ significantly inhibited LPS-stimulated NO production in concentration-dependent manner (Fig. 1A). The IC_{50} value was 60.05 $\mu\text{g/ml}$. This extract did not affect J774A.1 cell viability at all concentration used in this study (Fig. 1B).

Effects of the extracts on mRNA expression of iNOS and COX-2 in LPS stimulated J774A.1 cells.

Figure2. demonstrated that the water extract of Treehom remedy 25-100 $\mu\text{g/ml}$ significantly - decreased mRNA expression of iNOS (73.97%, 60.83%, 39.64%)(A) and COX-2 (86.81%, 72.66%, 48.95%)(B) in dose dependent manner while dexamethasone 10 μM exhibited 49.68% and 49.84% mRNA expression of iNOS and COX-2 respectively.

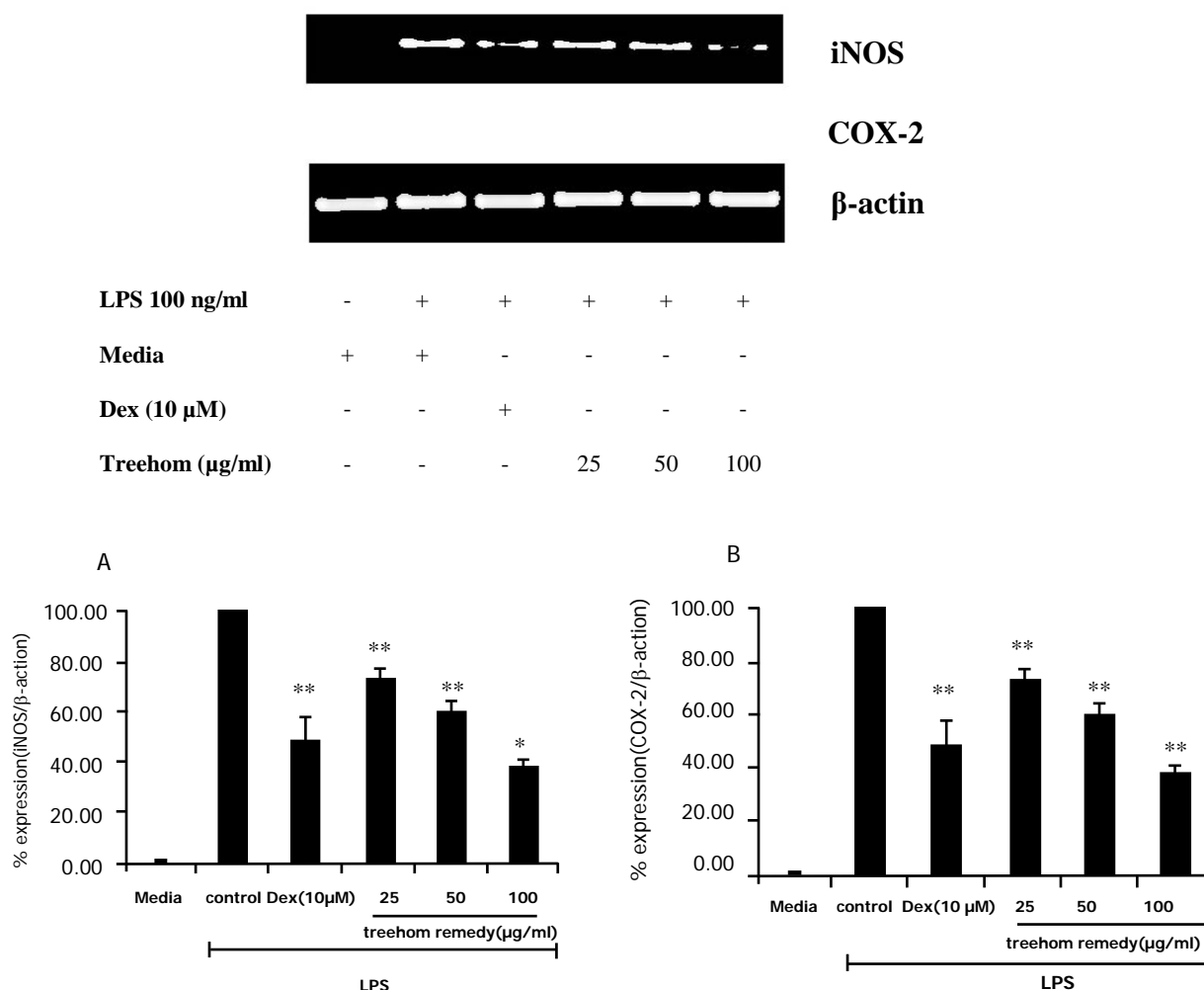


Figure2. Effects of the water extract from treehom remedy at the concentrations 25-100 $\mu\text{g/ml}$ on mRNA expression of COX-2 (A) and iNOS (B). The data are express mean \pm S.E.M from 5 independent experiments (n=5).

* $P < 0.05$ compared to LPS activated condition(control)

Discussion

Treehom remedy is a Thai traditional medicine used as antipyretic remedy. As pain, fever, redness and swelling are signs occurred during inflammation. Macrophages are stimulated during inflammatory process and their functions are including antigen presentation, phagocytosis, and immunomodulation through production of various cytokines and growth factors. In this study, we investigated anti-inflammatory activity of the water extract from Treehom remedy on LPS-activated macrophages. The extract suppressed nitric oxide production in dose dependent manner with the IC₅₀ value of 60µg/ml and corresponded to the decrease of iNOS enzyme mRNA expression determined by RT-PCR. Nitric oxide (NO) is known as a mediator of inflammatory response .(Sharma et al 2007).It possesses cytotoxic properties against pathogenic microbe and it also possesses damaging effects on host tissues in inflammatory response. Decrease in NO production is capable of reduction of inflammation. The extract also decreased COX-2 enzyme

mRNA expression at 50 and 100 µg/ml. COX-2 is induced in response to various pathological conditions such as inflammation, tumor, shock ect. Decrease in COX-2 expression is subsequent leading to inhibition of prostaglandins production and inflammatory response is being improved. These results demonstrate that the water extract of Treehom remedy may exhibit anti-inflammatory potential in LPS-activated J774A.1 macrophages.

Conclusion

We reported that the water extract of Treehom remedy decreased NO production, iNOS and COX-2 mRNA enzyme expression on LPS-activated J774A.1 macrophages. It may exhibit anti-inflammatory potential and further investigations are needed to clarify this action.

Acknowledgement

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