RESEARCH ARTICLE

Effect of Wisumpayayai Ethanol Extract on Lipopolysaccharide-Activated Macrophages

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Abstract

Wisumpayayai is an herbal remedy which has been used as anti-flatulence and anti-dyspepsia medication. This study aimed to investigate the effect of the ethanol extract of Wisumpayayai remedy on phagocytosis, nitric oxide (NO) production and inducible nitric oxide synthase (iNOS) expression in lipopolysaccharide-activated J774A.1 macrophage. The extract 12.5-100 µg/ml significantly decreased phagocytic activity of the activated macrophage in a concentration-dependent manner. The extract also suppressed nitric oxide production in these cells with the IC50 value of 37.39 µg/ml. The expression of inducible nitric oxide synthase which is responsible for NO production in activated macrophage during inflammation was decreased by the extract 50-100 µg/ml. The results obtained from this study indicated that Wisumpayayai ethanol extract are able to inhibit macrophage functions. This remedy may have other potential pharmacological properties beyond anti-flatulence and anti-dyspepsia effects.

Key Words: Wisumpayayai, macrophage, phagocytosis, nitric oxide

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ฤทธิ์ของสิ่งสกัดเอทานอลจากตำรับยาวิสัมพยาใหญ่ต่อเซลล์แมคโครฟาจที่ถูกกระตุ้นด้วยไลโปโพลีแซคคาไรด์

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บทคัดย่อ

วิสัมพยาใหญ่เป็นตำรับยาแผนโบราณใช้แก้อาการท้องขึ้น อืดเฟ้อ จุกเสียด การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของสิ่งสกัดเอทานอลจากตำรับยาวิสัมพยาใหญ่ต่อ กระบวนการจับกินสิ่งแปลกปลอมของเซลล์แมคโครฟาจ การสร้าง nitric oxide และการแสดงออกของยีน iNOSของเซลล์แมคโครฟาจ J774A.1 ที่ถูกกระตุ้นด้วยไลโปโพลีแซคคาไรด์ ผลการทดสอบพบว่าเมื่อให้สิ่งสกัดเอทานอลของตำรับยาวิสัมพยาใหญ่ที่ความเข้มข้น 12.5–100 μg/ml ลดกระบวนการจับกินสิ่งแปลกปลอมของเซลล์แมคโครฟาจได้ตามความเข้มข้นของสารสกัด อีกทั้งมีฤทธิ์ยับยั้งการสร้างไนตริกออกไซด์ โดยมีค่า IC_{50} 37.39 μg/ml และสิ่งสกัดที่ความเข้มข้น 50–100 μg/ml สามารถลดการแสดงออกในระดับ mRNA ของเอนไซม์ inducible nitric oxide synthase (iNOS) ซึ่งเป็นเอนไซม์ที่ใช้สร้างไนตริกออกไซด์ ที่ถูกกระตุ้นเมื่อเกิดการอักเสบ ผลจากการศึกษานี้แสดงให้เห็นว่าสิ่งสกัดเอทานอลของตำรับยาวิสัมพยาใหญ่มีฤทธิ์ยับยั้งการทำงานของเซลล์แมคโครฟาจ ดังนั้นสิ่งสกัดเอทานอลของตำรับยาวิสัมพยาใหญ่น่าจะมีศักยภาพต้านการอักเสบได้

คำสำคัญ: ตำรับยาวิสัมพยาใหญ่, แมคโครฟาจ, ไนตริกออกไซด์, ฟาร์โกไซโตซีส

Introduction

It is known that macrophages play key role in inflammatory process, especially in chronic inflammation. Macrophages are major tissue phagocytes in innate immunity (Gordon and Taylor 2005). Activated macrophages generate various pro-inflammatory cytokines (TNF-α, IL-1, IL-6 and chemokines) and inflammatory mediators (prostaglandins, leukotrienes and reactive oxygen/nitrogen species) (Ma et al 2003). Anti-inflammatory agents, such as corticosteroids and non-steroidal anti-inflammatory drugs (NSIADs) can inhibit these cytokine and mediator production in activated macrophages (Dinarello 2010). Thai people commonly use not only modern anti-inflammatory medications but also traditional medicines to relieve inflammation. Wisumpayayai is a household traditional remedy approved by the Ministry of Public Health of Thailand as anti-flatulence and anti-dyspepsia medication. It is composed of 20 herbal plants and 14 of these plants have been reported to have anti-inflammatory activities (Table 1). This study intended to investigate in vitro anti-inflammatory activity of this remedy on activated macrophages by using mouse macrophage J774A.1 cells activated by lipopolysaccharide (LPS).

Materials and Methods

Plant extract

The ethanol extract of Wisumpayayai remedy was prepared by soaking this remedy power in 95% ethanol in Soxhlet extractor. After extraction the solvent was removed by rotary evaporator until dry. The dry extract was dissolved in dimethylsulfoxide (DMSO) and diluted to various final concentrations in the constant 0.2% DMSO solution.

Control

In all experiments, 10 μM dexamethasone and 0.2% DMSO solution were used as reference and negative controls, respectively.

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 μg/ml of penicillin and 100 μg/ml of streptomycin and then incubated at 37 ˚C in 5% CO2/95% air. These cells were used in the density of 2×105 cells/ml in all experiments.

Table 1. The composition of herbal plants in Wisumpayayai remedy

✓*1.Coriandrum sativum* L.	✓*6.Conioselinum univitatum*	✓*11.Terminalia chebula*	*16. Syzygium aromaticum* L.
✓*2.Diospyros decandra*	✓*7.Angelica sinensis*	✓*12.Acorus calamus* L.	*17. Cinnamomum verum*
✓*3.Myristica fragrans*	✓*8.Artemisia pallens*	✓*13.Zingiber officinale*	*18. Aristolochia* sp.
✓*4.Angelica dahurica*	✓*9.Cinnamomum bejolghota*	✓*14.Piper retrofractum*	*19. Tinospora crispa* L.
✓*5.Atractylodes lancea*	✓*10.Terminalia arjuna*	*15. Amomum krervanh.*	*20. Melastoma saugnineum*

✓ Have evidence of Anti-inflammatory activities

Effect of The ethanol extract of Wisumpayayai remedy on NO production in activated macrophage.

J774A.1 cells were treated with 6.25-100 µg/ml the ethanol extract and 100 ng/ml LPS at 37 ˚C for 24 h. The supernatant was collected for determination of nitric oxide content by Griess test. The inhibitory effect of the extract and the reference drug (dexamethasone 10 µM) on nitric oxide production in LPS-activated macrophage were compared to the solvent control.

Determination of the effect of the ethanol extract of Wisumpayayai remedy on cell viability.

After removing the supernatant for determining amount of NO, the remaining treated cells were assessed their viability by resazurin reduction assay. (Anoopkumar-Dukie et al 2005)

Effect of The ethanol extract of Wisumpayayai remedy on phagocytic activity of LPS-activated J774A.1 cells with Nitroblue Tetrazolium Dye Reduction Test (NBT).

J774A.1 cells were treated with 12.5-100 µg/ml of the extract and 100 ng/ml LPS for 24 h. The treated cells were washed with DMEM media. Then 800 µg/ml zymosan and 600 µg/ml NBT were added for 1 h. The cells were washed with methanol and 2M KOH solution and DMSO were added. The amount of NBT reduction in cell was determined at 570 nm. The percentage of phagocytic inhibition of the ethanol extract was compared to the LPS-activated condition without the extract. (Park ea al 1968)

Effect of The ethanol extract of Wisumpayayai remedy on the expression of iNOS

J774A.1 cells were treated with 25-100 µg/ml of the extract and 100 ng/ml LPS for 24 h. Total RNA was isolated from the treated cells using Trizol reagent and reversed to complementary DNA (cDNA) using ImProm-IITM Reverse Transcription System kit. The cDNA was used as the template to amplified mRNA of iNOS with specific primer for iNOS gene. The PCR product was run on 1.5% agarose gel electrophoresis, stained with ethidium bromide and density determination by gel documentation compared to the solvent control. (Mullis and Faloona 1987)

Statistical analysis

Data were expressed 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 of differences between the values for the various experimental and control group. The p-value < 0.01 was considered as statistically significance.

Results

Effect of the ethanol extract of Wisumpayayai remedy on NO production in LPS-stimulated J774A.1

In this study, 100 ng/ml LPS potently stimulated NO production in J774A.1 cells (32.27 ± 1.06 µM nitrite). The extract significantly inhibited NO production in LPS-activated J774A.1 cells in a concentration-dependent manner, with IC_{50} of 37.39 µg/ml and without cytotoxicity on these cells.

Effect of the ethanol extract of Wisumpayayai remedy on phagocytic activity in LPS-activated J774A.1 cells

LPS increased phagocytic ability of J774A.1 when compared to the untreated cells. The extract 12.5-100 µg/ml inhibited phagocytic activity in LPS-activated J774A.1 cells in a concentration-dependent manner (Figure 2).

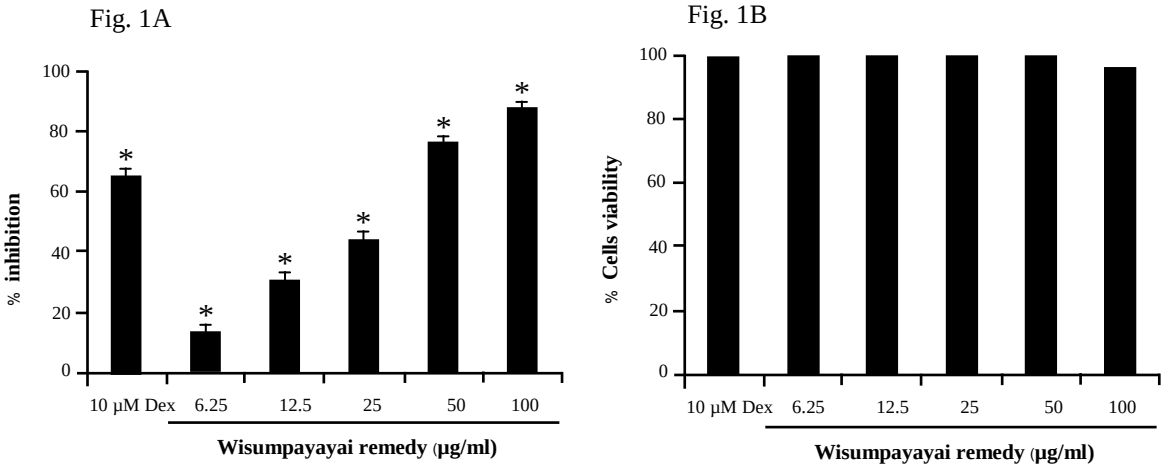

Figure1. Effect of the ethanol extract of Wisumpayayai remedy on NO production (A) and cell viability (B) in LPS-activated J774A.1 cells. Data represent as means ± S.E.M. of three independent experiment (n=3) performed in triplicate. *P < 0.01 compared with the solvent control.

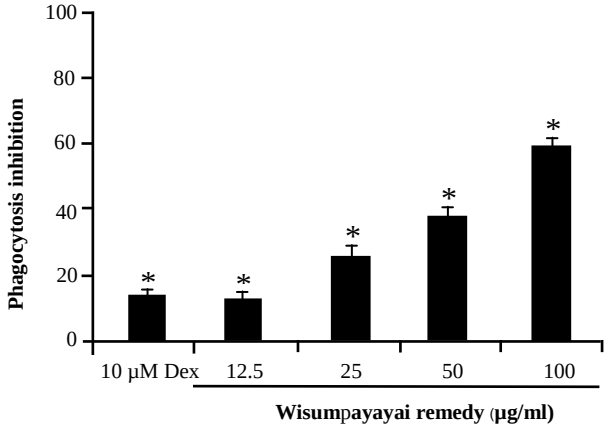

Figure2. Effect of the ethanol extract of Wisumpayayai remedy on phagocytic activity in LPS-activated J774A.1 cells. Data represent means ± S.E.M. of three independent experiment (n=3) performed in triplicate.
*P < 0.01 compared with solvent control.

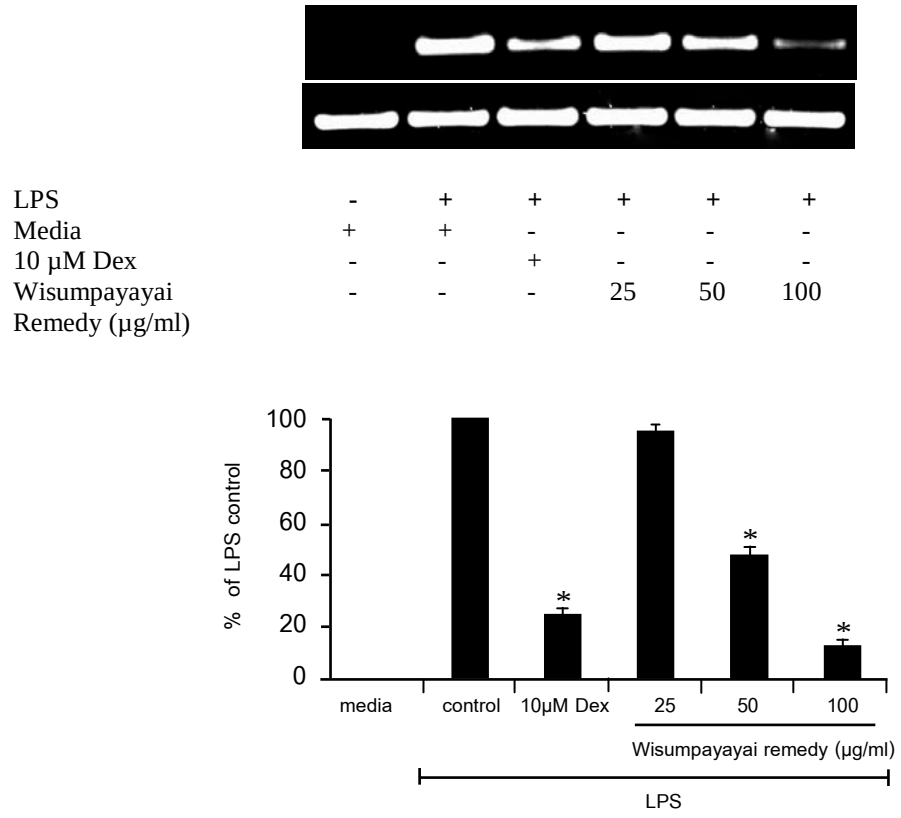

Figure 3. Effect of the ethanol extract of Wisumpayayai remedy on the mRNA expression of iNOS in LPS-activated J774A.1 macrophage cell determined by RT-PCR. Data represent means ± S.E.M. of three independent experiment (n=3).
*$P < 0.01$ compared with untreated cell.

Effect of the ethanol extract of Wisumpayayai remedy on the mRNA expression of iNOS

LPS increased the mRNA expression of iNOS which is the enzyme responsible for a large amount of nitric oxide production in activated macrophage. The extract at the concentration of 50 and 100 µg/ml significantly inhibited the mRNA expression of iNOS in the LPS-activated J774A.1 cells (Figure 3). This inhibitory effect was correlated to inhibitory effect of NO production of the extract.

Discussion

It is well established that anti-inflammatory drugs act on activated macrophages. They can inhibit several macrophage functions, especially the production of pro-inflammatory cytokines and inflammatory mediators which play important roles in inflammatory process. LPS, a lipoglycan of the outer membrane of Gram negative bacteria, is commonly used for activation of macrophage function in evaluation of anti-inflammatory substances. It potently stimulates production of pro-inflammatory cytokines, nitric oxide, prostaglandins and several other

inflammatory mediators in macrophages (Erridge et al 2002). The results obtained from this study demonstrated inhibitory effect of the ethanol extract of Wisumpayayai remedy on LPS-activated macrophage. The extract decreased the expression of iNOS gene, which is an inducible gene expressed in macrophage at activated condition. Enzyme iNOS is responsible for a large amount of NO production from the activated macrophage in inflammatory response. NO also plays role as a free radical in eliminating of the pathogen during phagocytosis (Guzik et al 2005). From this experiment, the extract were able to inhibit NO production in LPS-activated J774A.1 cells in a concentration-dependent manner. It also inhibited phagocytic activity of the activated cells in a concentration - dependent manner.

Conclusion

In summary, this study primarily evaluated the effect of ethanol extract of Wisumpayayai remedy on LPS-activated macrophage function, the results indicated that it possessed inhibitory effect on activated macrophages by decrease NO production,phagocytic activity and iNOS expression. More investigations, both *in vitro* and *in vivo*, are needed to clarify these actions.

Acknowledgement

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