In vitro Anti-inflammatory Effects of Thai Herb Essential Oils

ฤทธิ์ต้านอักเสบของน้ำมันหอมระเหยจากสมุนไพรไทยในหลอดทดลอง

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ABSTRACT

Thai herb essential oils have been used in traditional medicine for a long time. Several studies have reported a variety of its actions such as antibacterial, antifungal, antioxidant, anticancer and cytotoxic activities. In addition, many reports showed some essential oils also have anti-inflammatory effects in murine cell lines, however, the reports in human cell have been limited. Therefore, the goal of this study was to evaluate the anti-inflammatory activities of 10 Thai herb essential oils on lipopolysaccharide (LPS) induced inflammation in phorbol 12-myristate 13-acetate (PMA) differentiated THP-1 macrophage. THP-1 was pretreated with Thai herb essential oils for 1 hour prior to LPS. Then, the cells were harvested and subject to assay for cyclooxygenase 2 (COX-2) activity and cuture medium were collected to estimate prostaglandin E_2 (PGE_2) level. Among ten species of essential oils, Lesser galanga ([*Boesenbergia pandurata* (Roxb.)Schltr] oil showed the excellent activity to inhibit COX-2 production and PGE_2 with 100% and 93.3%, respectively. The COX-2 IC_{50} of tested oil was 51.2 µg/ml. Thus, the results suggested that Lesser galanga essential oils are potential for inflammatory condition and may compose of several active compounds with anti-inflammatory properties. Further experiments are required to deeply analysis of their active compounds, mechanism of actions and application into medicinal use for anti-inflammatory purpose.

บทคัดย่อ

น้ำมันหอมระเหยจากสมุนไพรไทยมีการนำมาใช้ในยาแผนโบราณมาเป็นเวลานานและมีงานวิจัยหลายเรื่องที่ รายงานฤทธิ์ของน้ำมันหอมระเหยเหล่านี้ เช่น ฤทธิ์ต้านเชื้อแบคทีเรียและเชื้อรา ฤทธิ์ต้านอนุมูลอิสระ และฤทธิ์เป็นพิษต่อเซลล์ นอกจากนี้ยังมีรายงานจำนวนมากแสดงให้เห็นว่าน้ำมันหอมระเหยบางชนิดมีฤทธิ์ในการต้านการอักเสบอีกด้วย แต่ส่วนใหญ่มักเป็นการทดลองที่ทำในเซลล์ของหนู ส่วนการทดลองในเซลล์มนุษย์นั้นมีน้อยมาก ดังนั้นการทดลองนี้จึงมีเป้าหมายที่จะประเมินฤทธิ์ต้านการอักเสบของน้ำมันหอมระเหยจากสมุนไพรไทยทั้ง 10 ชนิด โดยใช้เซลล์ THP-1 ที่ถูกกระตุ้นให้เกิดการอักเสบด้วยสาร lipopolysaccharide (LPS) หลังจากนั้นจะเก็บเซลล์เพื่อทดสอบการทำงานของเอนไซม์ cyclooxygenase-2 (COX-2) และเก็บอาหารเลี้ยงเซลล์เพื่อวัดปริมาณ prostaglandin E_2 (PGE_2) จากผลการทดลองพบว่าน้ำมันหอมจากกระชาย [*Boesenbergia pandurata* (Roxb.) Schltr] ให้ผลยับยั้งเอนไซม์ COX-2 ได้ดีที่สุด โดยยับยั้งกิจกรรมของเอนไซม์ COX-2 ได้ 100% ยังยั้งสาร PGE_2 ได้ 93.3% และความเข้มข้นต่ำสุดที่สามารถยับยั้งเอนไซม์ (COX-2) ได้ 50% (inhibitory concentration 50, IC_{50}) คือ 51.2 µg/ml ผลการทดลองนี้ชี้ให้เห็นว่าน้ำมันหอมระเหยจากกระชาย มีฤทธิ์ที่น่าสนใจในการต้านอักเสบและมีสารสำคัญหลายชนิดที่มีคุณสมบัติต้านการอักเสบ โดยขณะนี้กำลังอยู่ระหว่างการศึกษาวิเคราะห์สารสำคัญ กลไกการออกฤทธิ์ เพื่อประยุกต์ใช้เป็นยาสำหรับต้านการอักเสบต่อไปในอนาคต

Key Words : Essential oils, COX-2, PGE_2

คำสำคัญ: น้ำมันหอมระเหย เอนไซม์ไซโคลออกซิจิเนสทู โพรสตาแกลนดินอีทู

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Introduction

Inflammation is a common mechanism of the immune system that protect against injuries caused by physical wounds and non-self-invaders. The inflammation response is vascular changes and white blood cell recruitment at the site which has been invaded or can be stimulated throughout the body. Although, the purpose of the inflammatory process is to eradicate the invaders and start repairing process to maintain general physiological functions, but long-term over-inflammation might cause tissue damages and associated with many diseases such as rheumatoid arthritis, inflammatory bowel disorders, Alzheimer's disease and also cause of various physical dysfunctions. During inflammation, white blood cells secrete many inflammatory cytokines (interleukin-1β [IL-1β], interleukin-6 [IL-6] and tumor necrosis factor-α [TNF-α]) and involved inflammatory mediators such as nitric oxide (NO) and prostaglandin E_2 (PGE_2), which are generated by inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) respectively (Kim et al.,2013). Nowadays, numerous evidences have pointed out that cyclooxygenase (COX) is main enzyme that plays the important role in chronic inflammatory diseases. It exists in two isoforms, COX-1 and COX-2. Normally, COX-1 is responsible for protecting the gastric mucosa and maintaining homeostasis while COX-2 is playing a crucial part in the inflammatory process (Abdel-Azeem et al.,2009 and El-Sayed AAA et al.,2012). The role of COX-2 in driving inflammation is converts arachidonic acid (AA) that release from membrane phospholipid by phospholipase A_2 (PLA_2) into prostaglandin H_2 (PGH_2) and finally prostaglandin E synthase (PGES) metabolite into PGE_2 which is mediator that related to the classic signs of inflammation: redness, warmth, swelling and pain (Ricciotti E et al.,2011). PGE_2 act as vasodilators to promote vascular permeability and increased blood flow into the inflamed tissue leading to redness, swelling and edema. Furthermore, warmth and pain result from the action of PGE_2 stimulates peripheral sensory neurons and the preoptic area of the hypothalamus to promote pyrogenic effects. Therefore, this study is focusing on COX-2 and PGE_2 which would be beneficial in the treatment of pain and inflammation without interfering physiological processes.

There are several reports about anti-inflammatory effect of Thai herbs, such as allylpyrocatechol (APC) isolated from *Piper betle* Linn. (Betel) in LPS-stimulate Raw 264.7. The APC was inhibited NO and PGE_2 production in a dose dependent manner as also reduced iNOS, COX-2, IL-12p40 and TNF-α mRNA expression (Sarkar et al.,2008). *Boesenbergia pandurata* (Roxb.) Schltr (Lesser galanga) extracts inhibited LPS induction of PGE_2 in RAW 264.7 cell lines. Panduratin A and hydroxypanduratin A showed strong activities against PGE_2 release, with IC_{50} values of 10.5 and 12.3 µM, respectively, and moderate effects on TNF-α (IC_{50} = 60.3 and 57.3 µM, respectively (Tewtrakul et al.,2009) . The different constituents of *Zingiber cassumunar* Roxb. (Plai) markedly decreased COX-2 and PGE_2 production in LPS-stimulated human dental pulp cells (Aupaphong et al.,2013). Moreover, phenylbutenoid dimers 1 and 2 that isolated from the rhizomes of *Zingiber cassumunar* Roxb. demonstrated potent COX-2 inhibitiory activity with the IC_{50} values of 2.71 and 3.64 µM, respectively in LPS induced RAW 264.7 (Han et al.,2005).

There are several studies have reported various anti-inflammatory effect of essential oil, however, the reports on actions of Thai herb essential oil on the inflammatory activity in human cells have been limited. In this study, 10 Thai herb essential oils were evaluated for their anti-inflammatory functions on human

monocytic cell line (THP-1). The cyclooxygenase-2 activities was examined using the enzymatic assay kit and the prostaglandin E_2 was determined by enzyme immunoassay.

Methodology

Chemicals

Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco, Karlsruhe, Germany), Fetal Bovine Serum (FBS; PAA, Cölbe, Germany), $NaHCO_3$, Penicillin/Streptomycin solution were obtained from GIBCO (Grand Island, NY). Phosphate-buffered saline (PBS), trypan blue, Phorbol 12-myristate 13-acetate (PMA; Sigma, St. Louis, MO, USA), lipopolysaccharide (LPS) from *Escherichia coli* 055:B5, γ-irradiated (Sigma, St. Louis, MO, USA), DMSO, Celicoxib (Sigma, St. Louis, MO, USA), COX Activity Assay Kit and PGE_2 Enzyme Immunoassay kit were purchased from Cayman Chemical (Ann Arbor, MI, USA).

Test compounds

Ten essential oils which were obtained from Holy basil leaves and flowering shrub (*Ocimum tenuiflorum* L.), Hairy basil leaves (*Ocimum americanum* L.), Sweet basil leaves and flowering shrubs (*Ocimum basilicum* L.), Betel leaves (*Piper betle* L.), Guava leaf (*Psidium guajava* L.), Kaffir lime leaves (*Citrus hystrix* DC.), Lemongrass leaves (*Cymbopogon citratus* (DC.) Stapf.), Galanga rhizomes (*Alpinia galangal* (L.) Wild.), Lesser galanga rhizomes [*Boesenbergia pandurata* (Roxb.) Schltr.] and Plai rhizomes (*Zingiber cassumunar* Roxb.) were purchased from Thai-China Flavours and Fragrances Industry Co., Ltd.

THP-1 culture, treatment and stimulation

THP-1 human monocytes was cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and penicillin/streptomycin at 37 °C and 5% CO_2 in a humidified incubator. Cells will be pelleted *via* centrifugation and assessed for viability using the Trypan-blue exclusion method. Viable cells were plated at a density of 1×10^7 cells/mL in 100 mm culture dish. To differentiate monocytes into macrophages, THP-1 cells were treated with 40 µg/mL of PMA for 48 hours. Followed by adding each essential oil at desired concentration and further incubated for 1 hour. The inflammation will be induced by adding 1 µg/mL of LPS and incubate for 24 hours. After incubation, the cells were harvested in Tris-HCl pH 7.8 containing 1 mM EDTA by cell scraper then centrifugation for further analysis of COX-2 activity assay. All supernatants will be kept at -80 °C for PGE_2 determination.

Assay of COX-2 Activity by COX Activity Assay kit

To analyze the mediation of COX-2, THP-1 cells were lysed in cold lysate buffer to release endogenous COX enzyme. Then, COX-2 level were measured with COX Activity Assay kit from Cayman Chemical (Ann Arbor, MI, USA). The measurement was carried out according to manufacturer's manual. The COX activity assay kit will be detected the colorimetric of oxidized N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD) at 590 nm that produced by peroxidase activity of COX. The COX-1 inhibitor (SC-560) will be added to subtract absorbance from total COX activity. After that, calculate the IC_{50} using Graph Pad prism software (Graph Pad Inc, San Diego, USA) with Equation: log concentration (inhibitor) vs. normalized response -- Variable slope.

Assay of Prostaglandin E_2 by Enzyme Immunoassay kit

PGE_2 level in cultured medium was evaluated by enzyme Immunoassay kit from Cayman Chemical

(Ann Arbor, MI, USA). The measurement according to the manufacturer's instructions. PGE_2 in the supernatants (PGE_2 Tracer) in constant concentration were competitive binding to PGE_2 monoclonal antibody that coated the bottom of the well. The plate will be washed to remove all unbound reagents, and added Ellman's reagent (which contains the substrate to AChE) each well for color development. Then, determine the intensity between 405 and 420 nm. The effects of 10 Thai herb essential oils on PGE_2 productions were calculated by comparison with a standard curve and represent as PGE_2 concentration. Finally, PGE_2 level be calculated and reported as percent inhibition.

Results

In this study, we first measured the effect of 10 Thai herb essential oils on COX-2 activity. THP-1 were supplemented with culture medium with or without 100 µg/ml of 10 Thai herb essential oils for 1 hour and activated with 1 µg/mL of LPS to stimulated COX-2 for 24 hour. The result showed that Lesser galanga essential oil exhibited the best COX-2 inhibitory effect with 100% inhibition whereas guava leaf and lemongrass gave 54.6% and 50.5%, respectively (Table 1).

After that, culture medium were collected for evaluate PGE_2 level whether perform with PGE_2 Enzyme Immunoassay kit. As shown in Table 1, percentage inhibition of PGE_2 in the conditioned media treated with Betel vine essential oil showed the most potent inhibitor of PGE_2 production with 99.9% while Holy basil, Plai and Lesser galanga displayed 99%, 94.8% and 93.3%, respectively. From the result of COX-2 and PGE_2 inhibition, Lesser galanga essential oils demonstrated the most interesting percentage inhibition. Therefore, The 50% inhibition concentration (IC_{50}) of this essential oil was investigated. The IC_{50} of Lesser galanga essential oil was 51.2 µg/ml (Figure 1).

Table 1 Effect of 10 Thai herb essential oils and their inhibitory effect of COX-2 and PGE_2 level on LPS-activated THP-1 cells.

Essential oil	% COX-2 inhibition	% PGE_2 inhibition
DMSO	0	0
Celecoxib	45.5	90.7
Kaffir lime	0	69.2
Plai	18.2	94.8
Galanga	0	90.1
Holy basil	40	99
Hairy basil	18.2	87.8
Guava	54.5	60.7
Lesser galanga	100	93.3
Betel	26.3	99.9
Sweet basil	21.9	0
Lemongrass	50.4	93.3

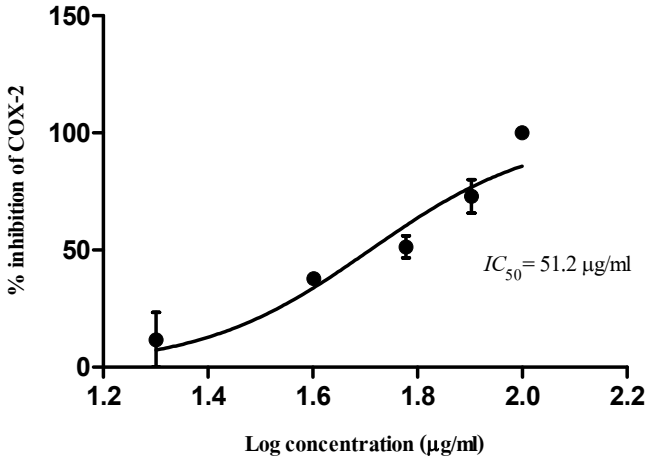

Figure 1 Effect of Lesser galanga essential oils on COX-2 enzyme activity on LPS-activated THP-1 cells. To derive IC50, THP-1 macrophages were incubated with different concentration of Lesser galanga essential oil for 1 hour. Then stimulated COX-2 using 1 µg/mL of LPS for 24 hour. Cell were collected and measured COX-2 activity by COX Activity Assay kit from Cayman Chemical

From the result of COX-2 and PGE_2 inhibition, Lesser galanga essential oils demonstrated the most interesting percentage inhibition. Therefore, The 50% inhibition concentration (IC_{50}) of this essential oil was investigated. The IC_{50} of Lesser galanga essential oil was 51.2 µg/ml (Figure 1). Therefore, this results indicates the capacity of Lesser galanga essential oil to inhibit COX-2 activity of THP-1 cell.

Discussion and Conclusion

Among of 10 Thai herb essential oils, Lesser galanga essential oil displayed the most promising effects with 100% COX-2 inhibition and 93.3% PGE_2 inhibition. Moreover, Lesser galangal oil was inhibit COX-2 activity in dose-dependent manner with IC_{50} value of 51.2 µg/ml on LPS stimulated THP-1. Therefore, we expected there were anti-inflammatory ingredient in essential oil. Lesser galanga oils that analysed by GC and GC-MS showed the major constituents such as camphor, geraniol and (*E*)-β-ocimene (Jantan *et al*., 2001). The further studies will be performed on the pure compound, to find out the active compound, mechanism of actions, finally apply into medicinal use of anti-inflammatory purpose.

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