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Nitric oxide inhibitory activity of Thai medicinal plants called Kod-Tung-Ha

Sunita Makchuchit[1*], Arunporn Itharat[2]**

[1]Faculty of Medicine, Thammasat University, Thailand.
[2]Applied Thai Traditional Medicine Centre, Faculty of Medicine, Thammasat University, Thailand.
* Presenting Author, ** Corresponding author

Abstract

Thai herbal medicine called *Kod-Tung-Ha* composes of five plants: *Angelica dahurica*, *Angelica sinensis*, *Artemisia annua*, *Atractylodes lancea* and *Ligusticum sinense*. These plants were examined for their inhibitory activities against lipopolysaccharide (LPS) induced nitric oxide (NO) production in RAW 264.7 cell lines. These plant extracts were also tested for the inhibitory effect on LPS-induced TNF-α release in RAW 264.7 cell lines. The extraction methods imitated by Thai folk doctors using such as maceration in ethanol or ethanolic extract and boiling by water or water extract. The results were found that the ethanolic extract of *Atractylodes lancea* exhibited the most potent inhibitory activity nitric oxide production (IC_{50} = 9.70 μg/ml) an also possessed potent activity against TNF-α release with an IC_{50} value of 24.35 μg/ml., followed by an ethanolic extract of *Angelica sinensis* (IC_{50} = 12.52 μg/ml). The water extract of all plants were apparently inactive. These results can support the use of these plants in combination as *Kod-Tung-Ha* for treatment of inflammatory-related diseases through the inhibition of NO and TNF-α release.

Keywords: Nitric oxide, Lipopolysaccharide, TNF-α release, RAW 264.7, Kot-Tung-Ha.

Introduction

Nitric oxide (NO) is one of the inflammatory mediators causing inflammation in many organs. This inorganic free radical has been implicated in physiologic and pathologic processes, such as vasodilation, non-specific host defense, ischemic stroke and acute or chronic inflammation[1)]. NO is produced from L-arginine by a chemical reaction catalyzed by the enzyme inducible nitric oxide synthase (iNOS) in living systems. After stimulation with bacterial lipopolysaccharide (LPS), many cells including macrophages express the iNOS which is responsible for the production of large amount of NO[2)]. Kot-Tung-Ha is composed of five plants: *Angelica dahurica* root, *Angelica sinensis* root, *Artemisia annua* aerial, *Atractylodes lancea* rhizome and *Ligusticum sinense* rhizome. These plants have been used for antipyretic[3,4)], analgesic[3)], anti-inflammatory[3,5,6,7)], cardiovascular diseases[8,9)], digestive disorders[6)] and cold treatment[10)]. In spite of the fact that all of these plants were reported to be tested for anti-inflammatory activity, but NO-inhibitory activity of some plants were not reported.

The aim of this study was to test for inhibitory activity of Kot-Tung-Ha against LPS-induced NO production in RAW 264.7 cell lines.

Materials and Methods

Plant material preparation and extraction

The plants were purchased from China. The voucher specimens are deposited at the herbarium of Southern Center of Thai Medicinal Plants at Faculty of Pharmaceutical Science, Prince of Songkla University, Songkhla, Thailand. Plants material were washed and then dried at 50°C, powdered and extracted by methods similar to those used by Thai traditional

doctors. In brief, for ethanolic extracts, dried plant material (300 g) was macerated by 95% ethanol for 3 days, 2 times, filtered and dried by using an evaporator. For water extracts, dried plant material (100 g) were boiled in distilled water for 30 minutes, filtered and dried by using a lyophilizer.

Anti-inflammation by nitric oxide inhibitory assay

In these experiments, RAW 264.7 murine macrophage leukemia cell lines used in this study were received from Assoc. Prof. Dr. Supinya Tewtrakul, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University. RPMI-1640 medium, Fetal bovine serum (FBS) and Penicillin-streptomycin (P/S) were purchased from Gibco. Lipopolysaccharide (LPS) and 3-(4,5-dimethyl-2- thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) were purchased from Sigma. 96-well microplates were purchased from Costar Corning. ELISA test kit was purchased from R&D Systems Inc.

Inhibitory effects on NO production by murine macrophage-like RAW 264.7 cells were evaluated by the following method[1,11]. The RAW 264.7 cells were cultured in RPMI-1640 medium supplemented with 10% FBS and 1% P/S in 96-well plates with 1×10^5 cells/well for 1 h. The cells were stimulated with 5 μg/ml LPS together with test samples at various concentration for 48 h. NO production was determined by measuring the accumulation of nitrite in the culture supernatant using the Griess reagent. Cytotoxicity was determined using the MTT colorimetric method. The absorbance at 570 nm was measured.

Inhibitory effects on the release of TNF-α from RAW 264.7 cells were evaluated using Quantikine mouse TNF-α ELISA test kit.

The inhibition of NO production and TNF-α production were calculated using the following equation and IC_{50} values was calculated from the Prism program.

$$\text{Inhibition (\%)} = \frac{A - B}{A - C} \times 100$$

A–C: NO_2^- concentration (μM) for NO production and TNF-α concentration (pg/ml) for TNF-α production [A: LPS (+), sample (-); B:LPS (+), sample (+); C: LPS (-), sample (-)]

Results and Discussion

Ethanolic and water extracts form five plants of Kot-Tung-Ha were investigated for their inhibitory activities against LPS induced NO production in RAW 264.7 cell lines. Among these plants, an ethanolic extract of *A. lancea* exhibited the highest inhibitory activity against NO inhibitory effect with IC_{50} value of 9.70 μg/ml (Table 1), whereas other plants possessed high and moderate activity. The inhibitory activity of these plants were weaker than that of positive control, indomethacin (non-steroidal anti-inflammatory drug, NSAID, IC_{50} = 25.0 μM or 8.95 μg/ml). These plants were also tested for the inhibitory effect on LPS-induced TNF-α release in RAW 264.7 cells. The result revealed that ethanolic extract of *A. lancea* also possessed the most potent activity against TNF-α release with an IC_{50} value of 24.35 μg/ml, while other plants exhibited inactive. From this study, *A. lancea* showed strong inhibition on both NO and TNF-α releases. Regarding the biological activities, it has been reported that *A. lancea* exhibited potent inhibitory activities in 5-lipoxygenase, cyclooxygenase-1 and anti-tumor activity[6,12].

Table 1. Inhibitory effect of Kot-Tung-Ha on LPS-induced NO production and TNF-α release from RAW 264.7 cells.

Plant	Solvent	Inhibition of NO production		Inhibition of TNF-α release	
		% Inhibition at conc. 100 μg/ml	IC_{50}±SEM (μg/ml)	% Inhibition at conc. 100 μg/ml	IC_{50}±SEM (μg/ml)
Angelica dahurica	Ethanol	88.33±1.76*	44.23±2.71	-	-
	Water	13.50±0.35*	>100	-	-
Angelica sinensis	Ethanol	95.60±0.71*	12.52±2.31	86.59±0.17*	-
	Water	42.13±4.56	>100	-	-
Artemisia annua	Ethanol	96.47±2.23*	17.06±2.69	87.68±0.92*	-
	Water	35.17±3.08*	>100	-	-
Atractylodes lancea	Ethanol	94.03±3.22*	9.70±0.54	81.62±0.69*	24.35±1.19
	Water	39.27±4.41*	>100	-	-
Ligusticum sinense	Ethanol	92.00±4.70*	16.48±2.03	3.66±1.15	>100
	Water	20.43±5.06	>100	-	-
Indomethacin		80.30±1.50 (μM)	25.0 (μM) (8.95 μg/ml)	-	-

* Cytotoxic effect was observed.

Conclusion

In summary, the result obtained in this work indicated that the ingredients or plants in Kot-Tung-Ha, which were used to treat fever and cold, possessed strong active against LPS induced NO production in RAW 264.7 cell lines such as *Angelica sinensis*, *Artemisia annua*, *Atractylodes lancea* and *Ligusticum sinense*. The ethanolic extracts of *A. lancea* showed strong inhibition on both NO and TNF-α releases. These result supports using these plants for treatment of fever, cold and inflammatory-related diseases by Thai folk medicine.

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