行政院國家科學委員會專題研究計畫　成果報告

以動物模式探討香茹草之抗發炎功能

計畫類別：個別型計畫
計畫編號：NSC91-2320-B-041-006-
執行期間：91 年 08 月 01 日至 92 年 07 月 31 日
執行單位：嘉南藥理科技大學食品衛生系

計畫主持人：吳明娟

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中　華　民　國 92 年 10 月 31 日

行政院國家科學委員會補助專題研究計畫成果報告

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共同主持人：夏彩蘭

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執行單位：嘉南藥理學院生物科技研究所

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一、中文摘要

在前一年計畫中(NSC90-2320-B-041-012)我們利用老鼠巨噬細胞株發現，香茹的乙醇抽出物具有很強的抑制 iNOS 表達的能力。因此本計劃我們繼續探討香茹乙醇抽出物在老鼠的腹腔巨噬細胞及脾臟細胞的抗發炎功能。我們發現香茹乙醇抽出物可抑制 LPS 誘發的腹腔巨噬細胞的 NO, PGE_2, IL-1β, IL-6 及 IL-12 的產生。對 PHA 誘發的脾臟細胞的 INF-γ亦有顯著的抑制效果。由此可知香茹的乙醇抽出物對初級細胞所產生的促發炎因子具顯著抑制功效。

關鍵詞：香茹、腹腔巨噬細胞、脾臟細胞、NO、 PGE_2

Abstract

In the previous study, we showed that the ethanol extract of Hsiang Ju (*Glossogyne tenuifolia* Cassini) (GT) inhibited LPS-mediated NO release in RAW 264.7 cell line. The primary mechanism responsible for this effect was through transcriptional repression of iNOS expression. In the present study, we further investigated the anti-inflammatory effects of GT on LPS-activated mouse peritoneal macrophages and PHA-stimulated spleenocytes. Our results demonstrated that GT inhibited NO, PGE2, IL-1b, IL-6 and IL-12 release in LPS-activated peritoneal macrophages in dose-dependent manners. Moreover, GT also exerted concentration-dependent inhibition of interferon-γ in PHA-stimulated spleenocytes. These results demonstrated GT possessed strong anti-inflammatory activities in primary cells.

Keywords: **Keywords**: *Glossogyne tenuifolia*, Nitric oxide (NO), Prostaglandin E2 (PGE_2), peritoneal macrophages, spleenocytes

Introduction

Inflammatory responses are typically present as a series of vascular and cellular reactions initiated by injury or infection. Activation of macrophages is a key event in the initiation and propagation of these defensive reactions. When stimulated by pathologic stimuli or injury, macrophages release nitric oxide, prostaglandin E2, interleukin (IL)-1, IL-6, IL-12, and other proinflammatory cytokines that augment the host's defense against invasion by microbes. The release of inflammatory mediators is essential for host survival from infection, and is also required for the repair of tissue injury [1, 4, 5]. These beneficial effects, however, are critically dependent on the magnitude of the immune response, because large amounts of macrophage-derived inflammatory mediators can also cause collateral damage to normal cells [1, 2, 3] and are potentially lethal when the release is sufficient to cause systemic exposure [6]. Thus, inhibiting the overproduction of inflammatory mediators is an important therapeutic goal for drug development.

Glossogyne tenuifolia Cassini (*Hsiang Ju*) is a plant native to Penghu, also known as the Pescadore Islands, Taiwan and has a long history of being used as an antipyretic, detoxication, and anti-inflammatory remedy in folk medicine among local residents. However, no mechanistic information regarding its action responsible for its activity is available.

In this study, we investigated the detailed anti-inflammatory activities of the

ethanol extract of *G. tenuifolia* (GT) toward pro-inflammatory cytokine release on the LPS-stimulated murine peritoneal macrophages and PHA-activated spleenocytes. The obtained results provide support for the traditional use of this plant in Taiwanese medicine against inflammatory-based disorders.

Results

1. *Effects of GT on pro-inflammatory cytokine release in LPS-activated mouse peritoneal macrophages*

Peritoneal macrophages were isolated from 8 ~12-week-old BALB/c mice which were treated with 4% thioglycollate for 4 days. The isolated peritoneal macrophages (1x 10^5/well) were cultured in 96-well plates in the medium containing LPS (1 μg/ml) and various concentrations of GT. After 5, 7 and 10 h of incubation, supernatants were collected for IL-6, IL-1β and TNF-α, as well as IL-12 analyses, respectively. After 24 h of incubation, supernatants were collected for NO and PGE_2 analyses.

Figure 1 demonstrated that GT (0.025~0.075 mg/ml) exhibited significant inhibition of NO release in a dose-dependent manner. Based on the MTT test, cell viability after 24 h of 0.075 mg/ml of GT treatment was greater than 95% when compared with the control groups (data not shown). This indicated that the level of GT was not toxic to macrophages. The results implied that the inhibition of nitrite production by GT was not due to cell death.

Figure 2 demonstrated that GT at 0.025 mg/ml exerted more than 67% of inhibition of PGE_2 production in LPS-activated peritoneal macrophages. Higher concentration of GT, however, did not enhance the effect.

Figure 3 showed that GT (0.01~0.075 mg/ml) inhibited concentration-dependent inhibition on IL-1β release. Figure 4 also demonstrated that GT (0.01~0.075 mg/ml) exerted dose-dependent repression on IL-6 production. Significant inhibition of IL-12 release was observed when GT concentration was greater than 0.05 mg/ml (Fig.5)

No significant inhibition was observed for LPS-activated TNF-α production by GT in tested range, although slight but insignificant effect was observed at highest tested concentration, 0.075 mg/ml (Fig. 6).

2. Effects of GT on Th1-specific cytokine, interferon-γ release in PHA-stimulated spleenocytes

Because IFN-γ produced Th1 lymphocytes and NK cells is another important mediator in inflammatory /immune processes, we examined in spleen cells whether GT modulates the release of this cytokine. Spleen cells (4 x10^6/well) obtained from BALB/c mice were treated with GT for 30 min followed by the administration of PHA (25 μg/ml) in 24-well plates. IFN-γ levels were determined from the supernatants after 3 days of treatment. Fig. 7 shows that IFN-γ production was suppressed by GT in a dose-dependent manner in PHA-induced cells. GT failed to alter cellular viability in any of the experiments, as determined with the MTT assay (data not shown).

Discussion and conclusions

In sepsis, challenge of cells with LPS stimulates cascades leading to activation of multiple mitogen-activated protein kinase family members, NF-κB, as well as phosphorinositide-3 kinase and nonreceptor tyrosine kinases of the Src family. The major finding of this study is that the ethanol extract of *Glossogyne tenuifolia* (GT) can inhibit inflammatory mediator, NO, PGE_2, IL-1β, IL-6, IL-12 and interferon-γ release in activated primary cells, murine peritoneal macrophages and spleenocytes.

We have previously shown that GT blocked LPS-stimulated inflammatory mediator production through NF-κB dependent cell line RAW 264.7. Moreover, luteolin-7-glucoside and oleanolic acid are the active components for the anti-inflammtory activity of GT [7]. However, unlike in RAW 264.7 cells, neither luteolin-7-glucoside nor oleanolic

acid exerted significant inhibitory effect in activated primary cells (data not shown). Inhibitory potency of GT toward TNF-α was much weaker than toward other pro-inflammatory cytokines, IL-1β, IL-6 and IL-12, in peritoneal macrophages. Contrary results were observed in RAW 264.7 cells. These results clearly demonstrated that various anti-inflammatory mechanisms exist in different cells.

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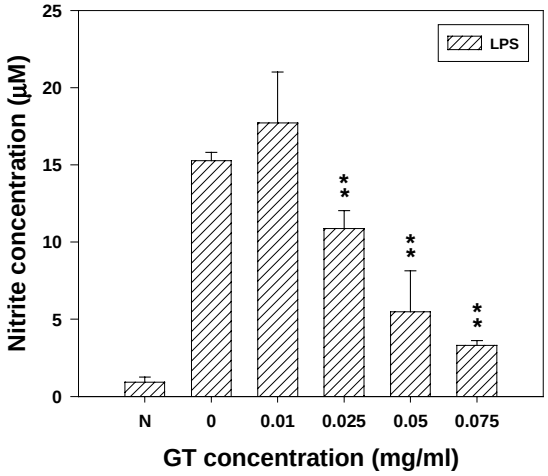

Fig. 1. Effect of GT on LPS-stimulated nitrite formation in peritoneal macrophages.

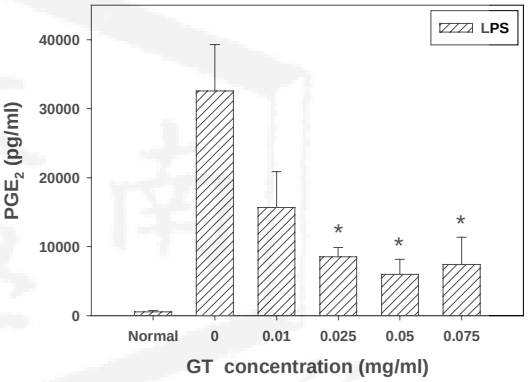

Fig. 2. Effect of GT on LPS-stimulated PGE_2 production in peritoneal macrophages

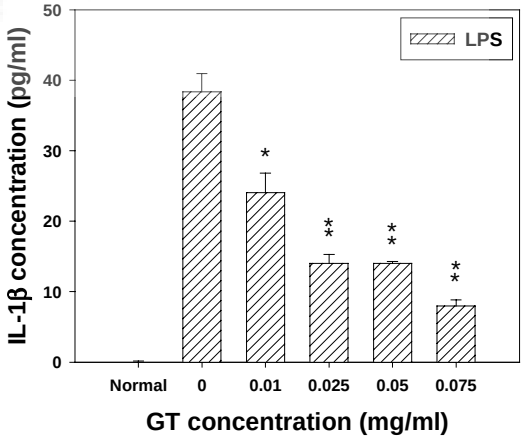

Fig. 3. Effect of GT on LPS-stimulated IL-1β release in peritoneal macrophages

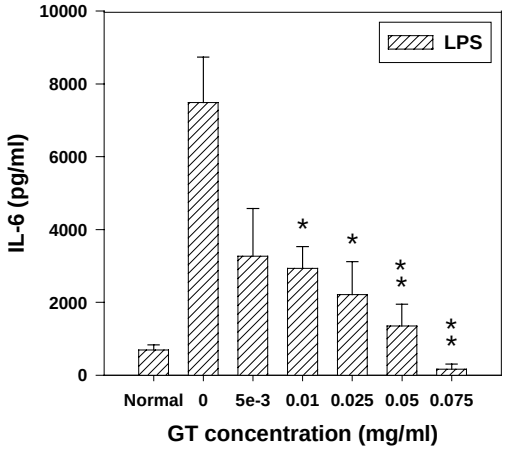

Fig. 4. Effect of GT on LPS-stimulated IL-6 release in peritoneal macrophages

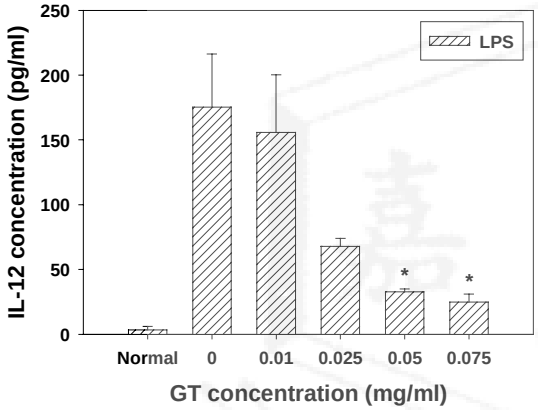

Fig. 5. Effect of GT on LPS-stimulated IL-12 release in peritoneal macrophages

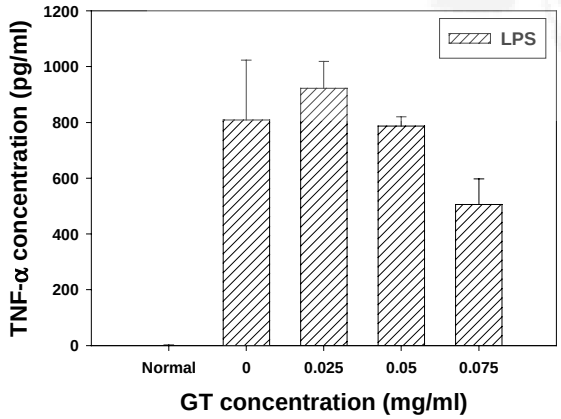

Fig. 6. Effect of GT on LPS-stimulated TNF-α release in peritoneal macrophages

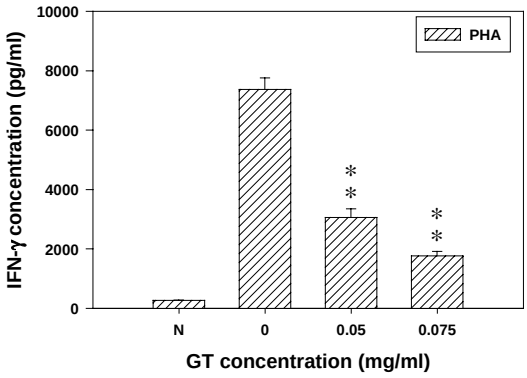

Fig. 7. Effect of GT on PHA-activated interferon-γ production in spleenocytes