Scientific Research Publishing

An Herbal Formula (GPC) Suppresses the Releases of Pro-Inflammatory Effectors in Lipopolysaccharide/Peptidoglycan-Activated RAW264.7 Macrophages and Reduces the Extent of Chemical-Induced Acute/Chronic Inflammation in Rodents

Pou Kuan Leong[1], Hoi Yan Leung[1], Wing Man Chan[1], Jihang Chen[1], Huawei Zhu[2], Jialing Ning[2], Yiting Yang[2], Chung Wah Ma[2], Kam Ming Ko[1*]

[1]Division of Life Science, Hong Kong University of Science and Technology, Hong Kong, China
[2]Infinitus (China) Company Ltd., Guangzhou, China
Email: *bcrko@ust.hk

Abstract

Pharyngitis (an inflammation in the pharynx) is a commonly occurring symptom of upper respiratory tract infection in patients suffering from common cold. The high prevalence of upper respiratory tract infection necessitates a safe and effective anti-inflammatory agent for pharyngitis. Chinese herbal medicine, which has been clinically prescribed for thousands of years, may offer a basis for the treatment of common cold as well as the amelioration of pharyngitis. In the present study, we investigated the *in vitro* and *in vivo* anti-inflammatory activities of a Chinese herbal formula, namely GPC, which is comprised of Glycyrrhiza Radix, Platycodonis Radix, Citri Reticulatae Pericarpium, Phyllanthi Fructus and Taraxaci Herba. Incubation with GPC (30, 100 and 300 μg/mL) suppressed the releases of tumor necrosis factor α (TNF-α), interleukin 6 (IL-6) and nitric oxide in lipopolysaccharide/peptidoglycan-activated RAW264.7 macrophages. In addition to the cell-based study, long-term treatment with GPC (0.35, 1.05 and 2.10 mL/kg/day × 30 doses) was found to reduce the extent of inflammation in animal models of carrageenan-induced paw edema (acute inflammation) as well as cotton pellet-induced granuloma formation (chronic inflammation) in mice. The ability of GPC to enhance the tracheobronchial expectorant action suggested its immunomodulatory activity in the respiratory tract. This postulation was supported by the observation that GPC reduced the degree of pha-

ryngitis and reversed the changes in plasma TNF-α and IL-6 levels in capsaicin-induced pharyngitis in rats. The ensemble of results suggests that GPC may offer a promising prospect for alleviating the extent of pharyngitis by virtue of anti-inflammatory activities.

Keywords

Pharyngitis, Glycyrrhiza Radix, Platycodonis Radix, Citri Reticulatae Pericarpium, Phyllanthi Fructus, Taraxaci Herba

1. Introduction

Inflammatory response, which is an essential component of innate immunity, enables the removal of pathogens and facilitates antigen presentation via a series of complex interplays among immune cells [1]. Upper respiratory tract infection, which triggers inflammatory responses in buccal cavity, nose, pharynx and trachea, is commonly found in patients suffering from common cold [2]. In this regard, group A *Streptococcus*, which causes 700 million infections each year [3], has been found to be the most prevalent cause of acute bacterial pharyngitis (*i.e.* an inflammation in the pharynx) in children, adolescents, and young adults [4]. Therapeutic intervention using anti-inflammatory agents on group A streptococcal pharyngitis is therefore widely needed. Chinese herbal medicine, which has long been prescribed for the treatment of upper respiratory tract infection, may offer an important resource for the search of such anti-inflammatory agents from naturally-occurring herbs.

In the present study, we investigated the *in vitro* and *in vivo* anti-inflammatory activities of a Chinese herbal formula, namely GPC, which is comprised of, Glycyrrhiza Radix, Platycodonis Radix, Citri Reticulatae Pericarpium, Phyllanthi Fructus and Taraxaci Herba. The component herbs are traditionally used for the treatment of common cold as well as the amelioration of pharyngitis. We first examined the effect of GPC on the releases of tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6), as well as the production of nitric oxide (NO) in lipopolysaccharide (LPS)/peptidoglycan (PGN)-activated RAW264.7 macrophages. Subsequently, the effects of long-term treatment with GPC in carrageenan-induced paw edema in mice (an animal model of acute inflammation) as well as cotton pellet-induced granuloma formation in mice (an animal model of chronic inflammation) were investigated. Furthermore, we examined the effect of long-term treatment with GPC on tracheobronchial expectorant action in mice. The effect of long-term treatment with GPC in capsaicin-induced pharyngitis and the associated releases of cytokines (TNF-α and IL-6) were also investigated in rats. To explore whether long-term treatment with AS1505 can cause hepatotoxicity, plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, which are biochemical parameters of hepatic damage, were measured in animals receiving long-term treatment with GPC.

2. Materials and Methods

2.1. Reagents

Fetal bovine serum (FBS) was obtained from Life Technologies (Grand Island, NY, USA). RPMI 1640 medium, dexamethasone, LPS, PGN, capsaicin, Evans Blue, indomethacin and phenol red (sodium salt) were purchased from Sigma-Aldrich Co (St. Louis, MO, USA). Carrageenan (λ-form) was bought from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). TNF-α and IL-6 ELISA kits were purchased from Shanghai ExCell Biology Inc. (Shanghai, China). The herbal formula GPC was provided by Infinitus (Guangzhou, Guangdong, China). GPC is an aqueous extract comprising Glycyrrhiza Radix, Platycodonis Radix, Citri Reticulatae Pericarpium, Phyllanthi Fructus, Taraxaci Herba in a ratio of 2:1:1:1:2 (w/w, dried herb). ALT and AST assay kits were purchased from Stanbio Laboratory (Boerne, TX, USA). All other chemicals were of analytical grade.

2.2. Cell Culture

A murine RAW264.7 macrophage cell line was purchased from American Type Culture Collection (Rockville, MD, USA). RAW264.7 cells were cultured in a monolayer using DMEM supplemented with 10% FBS, 100 units/mL penicillin, 0.1 mg/mL streptomycin and 1mM sodium pyruvate. RAW 264.7 cells were kept at 37°C in a humidified atmosphere of air and 5% CO_2. RAW264.7 cells used for the experiments were seeded at a density of 1.25×10^5 cell/mL on a 48-well culture plate and were allowed to grow to 60% - 80% confluence within 24 h prior to incubation with GPC.

2.3. Lipopolysaccharide/Peptidoglycan Induced Releases of Nitric Oxide and Cytokines in GPC-Incubated RAW264.7 Cells

RAW264.7 cells were incubated with GPC [30, 100 and 300 μg/mL, 1% (v/v) in PBS-A] for 24 h. The GPC-incubated cells were exposed to LPS (1 μg/mL) or PGN (30 μg/mL) for 24 h. Then, the concentration of nitrite (which is an indirect measure of nitric oxide production) in the culture medium was measured using the method of Griess [5]. The LPS/PGN-induced releases of TNF-α and IL-6 were measured using ELISA kits according to manufacturer's instruction.

2.4. Animal Care

Male Balb/c mice (8 - 10 weeks old, 25 - 30 g) and male Sprague Dawley rats (8 - 10 weeks old, 450 - 500 g) were maintained under a 12-h dark/light cycle at about 22°C, and allowed food and water ad libitum in the Animal and Plant Care Facilities at the Hong Kong University of Science and Technology (HKUST). All experimental protocols were approved by the University Committee on Research Practice at the HKUST.

2.5. Long-Term Treatment with GPC

Male Balb/c mice (8 - 10 weeks old, 25 - 30 g) were orally administered with GPC (0.35,

1.05 and 2.10 mL/kg/day × 30 doses). Male Sprague Dawley rats orally administered with GPC (0.18, 0.53 and 1.05 mL/kg/day × 30 doses). The doses of 1.05 mL/kg/day in mice and 0.53 mL/kg/day in rats are equivalent to the daily recommended dose for human. Twenty-four hours after the last dosing with GPC, mice/rats were anesthetized by intraperitoneal injection with a mixture of 100 mg/kg ketamine and 10 mg/kg xylzine in sterile saline. Heparinized blood samples were drawn from ketamine/xylzine-anesthetized mice/rats by cardiac puncture. Rodents were then sacrificed by cardiac excision under anesthesia. Plasma samples were obtained by centrifugation at 400 ×g at 4˚C for 10 min. The potential hepatotoxicity of long-term GPC treatment in mice/rats was assessed by measuring plasma activities of ALT and AST using assay kits.

2.6. Effect of GPC Treatment on Carrageenan-Induced Paw Edema in Mice

Male Balb/c mice were randomly divided into 6 groups of 4 - 8 animals: 1) vehicle control; 2) carrageenan control; 3) carrageenan + dexamethansone (positive control); 4) carrageenan + GPC (0.35 mL/kg); 5) carrageenan + GPC (1.05 mL/kg) and 6) carrageenan + GPC (2.10 mL/kg). Mice were orally administered with GPC for 30 doses, as previously described. For the positive control group, mice were intraperitoneally injected with dexamethasone (10 mg/kg) 30 minutes before the carrageenan challenge. Thirty minutes after the last dosing with GPC, mice were anesthetized by intraperitoneal injection with a mixture of 100 mg/kg ketamine and 10 mg/kg xylzine in sterile saline. The thickness of left hind limb in ketamine/xyline-anesthetized mice was measured (as initial thickness), followed by an injection with carrageenan (50 μL, 1% (w/v) in sterile saline). The changes in paw thickness were measured using a digital caliper (Mitutoyo, Japan) and monitored for 4 h. Mice were then sacrificed by cardiac excision under ketamine chloride-induced anesthesia and paw tissue was obtained. Paw tissue was homogenized with 2.5 mL homogenizing buffer [10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 100 μM ethylenediaminetetraacetic acid, 0.32 M sucrose] using a tissue homogenizer ULTRA-TURRAX T25 (IKA laboratory technology, Wilmington, NC, USA) at 95,000 rpm, and the cytosolic fraction was obtained by centrifugation at 10,000 ×g for 20 min at 4˚C [6].

2.7. Effect of GPC Treatment on Cotton Pellet-Induced Granuloma Formation in Mice

Male Balb/c mice (~8 weeks of age) were randomly divided into 5 groups of 6 - 8 animals in each group: 1) control, 2) indomethacin (positive control), 3) GPC (0.35 mL/kg), 4) GPC (1.05 mL/kg) and 5) GPC (2.10 mL/kg). Mice were orally administered with GPC for 30 doses, as described earlier. For the positive control group, the mice were orally administered with indomethacin (10 mg/kg/day × 7 doses). At the 24th dose of GPC (or the 1st dose of indomethacin), mice were anesthetized by a mixture of ketamine/xylzine in sterile saline. Dorsal hairs were shaved and the skin was wiped with 70% [(v/v) in H_2O] ethanol in preparation for cotton pellet implantation. The sterilized

and pre-weighed cotton pellet was aseptically implanted into a subcutaneous pouch. A cotton pellet was implanted on the left dorsal and right dorsal sides, respectively, in each mouse [7]. Twenty-four hours after the last dosing with GPC or indomethacin, the implanted cotton pellets were removed from ketamine/xylzine-anesthetized mice and they were weighed (showing wet weight) [7]. The wet cotton pellets were dried in an oven at 60°C for 24 h and then weighed (showing dry weight). The net increase in wet weight (as compared with the initial weight) of the cotton pellet is indicative of the degree of inflammatory response in transudative phase, whereas the extent of proliferative phase is indicated by the net increase in dry weight. The weights of two pellets from each individual animal were averaged. Data were expressed as net wet weight (*i.e.* wet weight − initial weight) and net dry weight (*i.e.* dry weight − initial weight). Percentage inhibition was computed by the equation:

[(net dry/wet weightcontrol − net dry/wet weightdrug)/(net dry/wet weightcontrol)] × 100%.

2.8. Effect of GPC Treatment on Tracheobronchial Expectorant Action in Mice

The tracheobronchial expectorant action was assessed by phenol red secretion test. In brief, male Balb/c mice were randomly divided into 5 groups of ≥6 animals: 1) control, 2) ammonium chloride (1 g/kg, positive control), 3) GPC (0.35 mL/kg), 4) GPC (1.05 mL/kg) and 5) GPC (2.10 mL/kg). Mice were orally administered with GPC for 30 doses, as previously described. For the positive control, mice were orally administered with a single bolus dose of ammonium chloride (1g/kg) at 30 min prior to the phenol red injection. After the last dosing with GPC/positive control, mice were intraperitoneally injected with phenol red (500 mg/kg in saline). At thirty-minutes post-injection, the mice were sacrificed and the tracheas (0.3 cm) were excised and incubated with an incubation buffer (0.9% saline with 0.2 M NaOH) at room temperature for 30 min under vortex mixing. An aliquot of 200 μL of the supernatant from each incubated sample was measured spectrophotometrically at 550 nm, using Victor3 Multi-Label Counter (Perkin-Elmer, USA). The amount of phenol red eliminated in the tracheobronchial secretion was estimated using a phenol red standard calibration curve [8].

2.9. Effect of GPC Treatment on Capsaicin-Induced Pharyngitis in Rats

Male Sprague Dawley rats were randomly divided into 6 groups of ≥6 animals: 1) control; 2) capsaicin control; 3) capsaicin + dexamethasone (1 mg/kg, positive control); 4) capsaicin + GPC (0.18 mL/kg); 5) capsaicin + GPC (0.53 mL/kg) and 6) capsaicin + GPC (1.05 mL/kg). Rats were orally administered with GPC for 30 doses, as previously described. For the positive control group, mice were orally administered with dexamethasone (1 mg/kg/day × 10 doses). Twenty-four hours after the last dosing with GPC/dexamethasone, the ketamine/xylzine-anaesthetized rats were intravenously injected (via lateral tail vein) with Evans Blue dye (60 mg/kg in saline). At 10 min post-injection with Evan Blue dye, capsaicin (0.3 mM, dissolved in a mixture of 10% ethanol − 10%

Tween 20% - 80% distilled water) was applied to the pharyngeal mucosa using cotton swab, while control animals received the vehicle. In brief, capsaicin solution was gently applied with the aid of a cotton swab for 5 seconds at each time point for three times consecutively. At 60 min following capsaicin application, all rats were sacrificed by exsanguination. Fifty microliters of 5% Na2EDTA in saline was injected into the rat via the inferior vena cava and then perfused with pre-warmed Krebs-Henseleit buffer (120 mM NaCl, 25.4 mM NaHCO$_3$, 4.8 mM KCl, 1.2 mM KH$_2$PO$_4$, 0.86 mM MgSO$_4$·7H$_2$O, 1.25 mM CaCl$_2$·2H$_2$O and 11 mM glucose, pH 7.4) for 20 min at a rate of approximately 28 mL/min to expel the intravascular Evan Blue dye. The bilateral musculus masseter of the rat was incised and the lower jaw was removed to enable the extirpation of the pharynx. The portion of pharynx spanning from the caudal end of the soft palate to the epiglottis was isolated and weighed (approximately 40 - 50 mg). The Evan Blue dye trapped in the tissue was extracted in formamide at 55˚C for 24 h and the amount of Evan blue dye was measured spectrophotometrically at 600 nm. The content of dye in tissue was expressed as microgram of dye per gram of tissue wet weight. The extravasation of Evan Blue dye into the pharyngeal tissue is a quantitative evaluation of the capsaicin-induced plasma exudation in rat pharyngeal mucosa [9].

In parallel with the above experiment, another set of experiments was conducted using the same protocol except for the administration of Evan Blue dye. The plasma samples of GPC/dexamethasone-treated, capsaicin-challenged rats were collected and subjected to the measurement of TNF-α and IL-6 using ELISA kits.

2.10. Statistical Analysis

Data were analyzed by one-way Analysis of Variance (ANOVA). Post-hoc multiple comparisons were performed using TUKEY test. P values <0.05 were regarded as statistically significant. All statistical analyses were performed using GraphPad Prism 6.0 and SPSS 17.0.

3. Results

3.1. Effect of GPC Incubation on Lipopolysaccharide (LPS) or Peptidoglycan (PGN)-Activated Releases of Pro-Inflammatory Effectors in RAW264.7 Macrophages

Incubation with GPC tended to decrease the production of TNF-α (by 31% at 300 μg/mL) in LPS-activated RAW264.7 macrophages (Figure 1(a)). GPC incubation (at 10, 30 and 300 μg/mL) also suppressed the production of IL-6 (43, 58 and 76%, respectively) and NO (13, 29 and 73%) in LPS-activated RAW264.7 cells. Dexamethasone incubation (5 μM) drastically suppressed the releases of TNF-α (74%), IL-6 (73%) and NO (87%) in LPS-activated RAW264.7 cells.

GPC incubation suppressed the production of IL-6 (35, 48 and 74%) and NO (53, 64 and 83%) in PGN-activated RAW264.7 cells (Figure 1(b)). However, GPC incubation did not inhibit the releases of TNF-α in PGN-activated RAW264.7 cells. Dexamethasone incubation suppressed the production of TNF-α (61%), IL-6 (81%) and NO (82%)
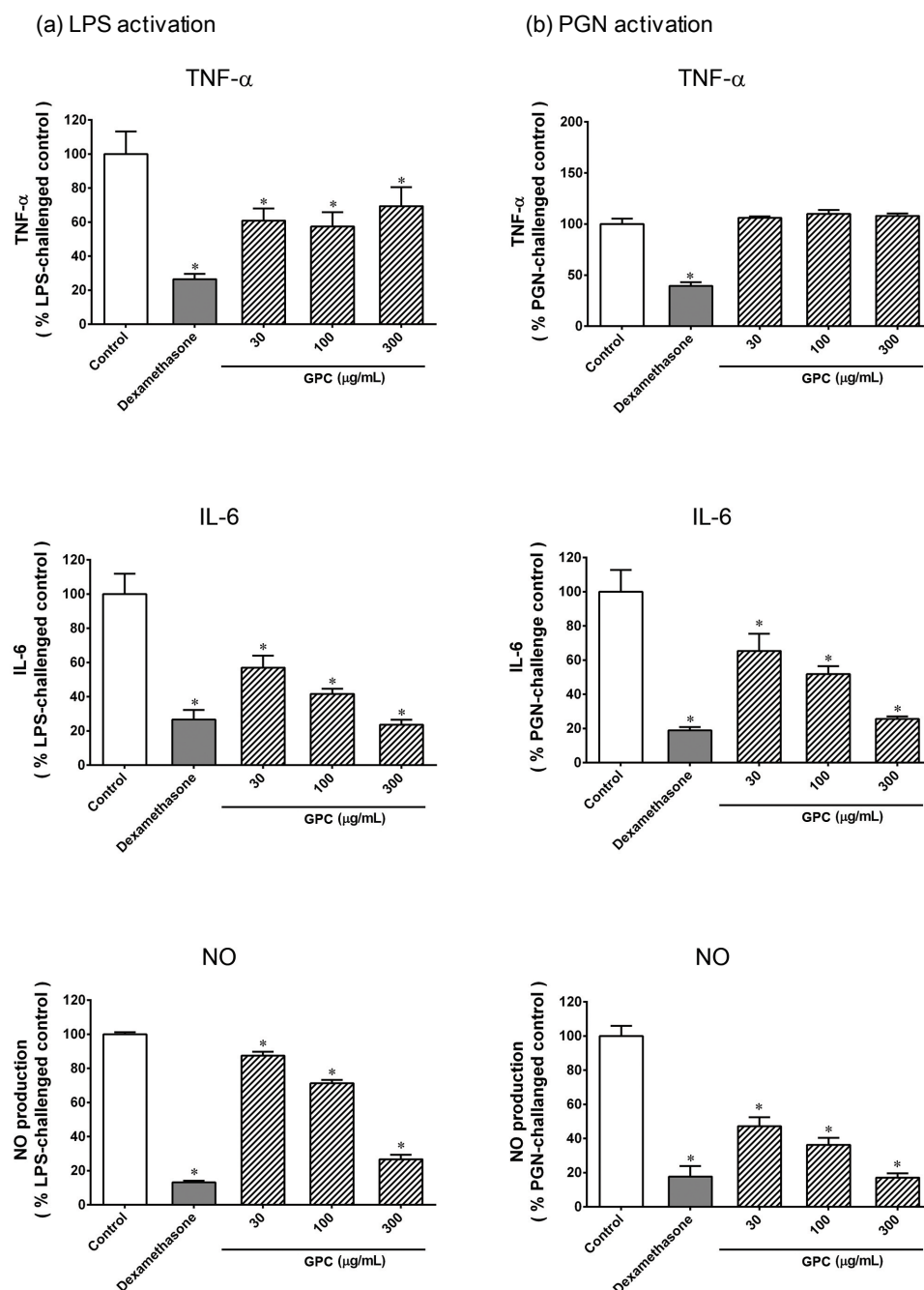
(a) LPS activation   (b) PGN activation

Figure 1. Effect of GPC incubation on lipopolysaccharide (LPS) or peptidoglycan (PGN)-activated releases of pro-inflammatory effectors in RAW264.7 macrophages. Following the incubation with GPC (μg/mL) or dexamethasone (μM) for 24 h, RAW264.7 macrophages were exposed to LPS (1 g/mL, panel A) or PGN (30 g/mL, panel B) for 24 h. After LPS (or PGN) incubation, the levels of TNF-α, IL-6 and NO in the culture medium were measured. Data are expressed as % LPS-challenged control by normalizing relative to the value of LPS stimulated cells (panel (a)) or expressed as % PGN-challenged control by normalizing relative to the value of PGN stimulated cells (panel (b)). Values given are means ± SEM, with n ≥ 3. *Significantly different from the LPS-challenged control in panel (a) (or the PGN-challenged control in panel (b)).

in PGN-activated RAW264.7 cells.

3.2. Effects of Long-Term Treatment with GPC on Carrageenan-Induced Paw Edema in Mice

Carrageenan injection caused a significant increase (104%) in paw thickness in mice. Long-term treatment with GPC suppressed the carrageenan-induced increase in paw thickness (Figure 2), with the extent of inhibition being 36% (at 2.1 mL/kg), when compared with the un-treated control. Dexamethasone treatment significantly inhibited the carrageenan-induced increase in paw thickness (by 45%).

3.3. Effect of Long-Term GPC Treatment on Cotton Pellet-Induced Granuloma Formation in Mice

Long-term treatment with GPC (at 2.1 mL/kg) suppressed the increases in both net wet weight (12.7%) and net dry weight (18.1%) of cotton pellets in mice induced with granuloma formation (Table 1). Indomethacin treatment also inhibited the increases in wet weight (14.1%) and dry weight (24.7%) of cotton pellets.

3.4. Effects of Long-Term Treatment with GPC on Phenol Red Expectorant in Mice

Long-term treatment with GPC increased the amount of expectorant in mice, with the degree of stimulation being 30% (at 2.1 mL/kg) (Figure 3). NH_4Cl treatment caused a
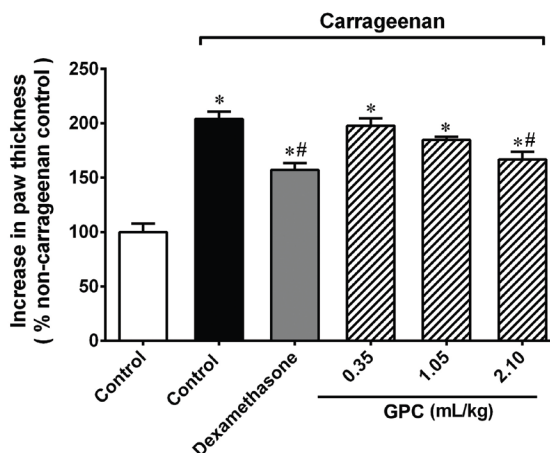

Figure 2. Effects of long-term treatment with GPC on carrageenan-induced paw edema in mice. Male Balb/c mice were administered with GPC (0.35, 1.05 and 2.10 mL/kg/day × 30 doses, p.o.) or dexamethasone (10 mg/kg × 1 dose i.v., positive control), as described in Materials and Methods. The left hind limb of the GPC-treated mice was injected with carrageenan (50 μL, 1%, w/v in sterile saline). Time-dependent changes in the thickness of the paw were monitored. The area under the curve (AUC) was calculated by plotting % initial thickness of paw against time. Data are expressed as % control by normalizing with the value of vehicle control group. Value given are means ± SEM, with n ≥ 4. *Significantly different from the non-carrageenan vehicle control group. #Significantly different from carrageenan vehicle control group.

Table 1. Effect of long-term GPC treatment on cotton pellet-induced granuloma formation in mice.

Group	Net wet weight (mg)	Net dry weight (mg)
Control	252.85 ± 4.29	17.30 ± 0.46
Indomethacin (10 mg/kg)	217.11 ± 5.59* (14.1)	13.03 ± 0.44* (24.7)
GPC (0.35 mL/kg)	258.47 ± 8.88 (-2.2)	16.93 ± 0.42 (2.13)
GPC (1.05 mL/kg)	231.02 ± 3.92 (8.6)	17.29 ± 1.14 (0.06)
GPC (2.10 mL/kg)	220.72 ± 3.06* (12.7)	14.17 ± 0.29* (18.1)

Values in parentheses indicate % inhibition. Data are expressed in mean ± SEM (n ≥ 6). * Significantly different from the control.
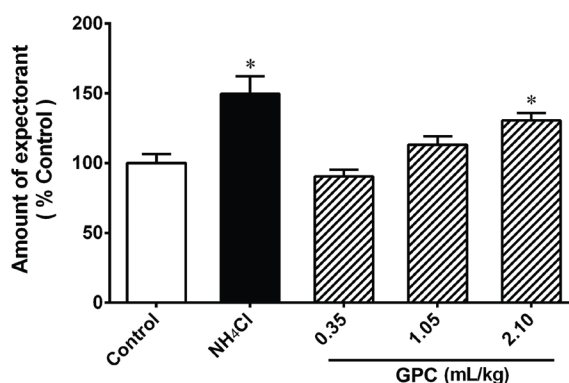

Figure 3. Effects of long-term treatment with GPC on phenol red expectorant in mice. Male Balb/c mice were administered GPC (0.35, 1.05 and 2.10 mL/kg/day × 30 doses, p.o.) or ammonium chloride (1 g/kg × 1 dose p.o., positive control), as described in Materials and Methods. After the last dosing with GPC/ ammonium chloride, mice were intraperitoneally injected with phenol red (500 mg/kg in saline). At 30 minutes post-injection, the mice were sacrificed and the tracheas (0.3 cm) of mice were excised and incubated with an incubation buffer at room temperature for 30 min under vortex mixing. The amount of phenol red in each sample was measured spectrophotometrically at 550 nm and estimated using a phenol red standard calibration curve. Data are expressed as % control by normalizing with the value of vehicle control group. Value given are means ± SEM, with n ≥ 11. * Significantly different from the vehicle control group.

significant increase (50%) in the amount of expectorant in mice.

3.5. Effects of Long-Term Treatment with GPC on Evans Blue Extravasation of Pharyngeal Tissue and Plasma Cytokine Levels in Capsaicin-Challenged Rats

Capsaicin challenge caused a drastic increase (208%) in the extent of Evan Blue extravasation in pharyngeal tissue of rats (**Figure 4(a)**). Long-term treatment with GPC suppressed (37 and 55% at 0.53 and 1.05 mL/kg, respectively) the Evan Blue extravasation. Dexamethasone treatment inhibited the extent of Evan Blue extravasation to a larger extent (by 87%) than those of GPC.

Capsaicin challenge also caused an increase in plasma level of IL-6 (31%) in rats (**Figure 4(b)**), but plasma level of TNF-α was decreased (by 59%) (**Figure 4(c)**). The

(a) Evans blue extravasation
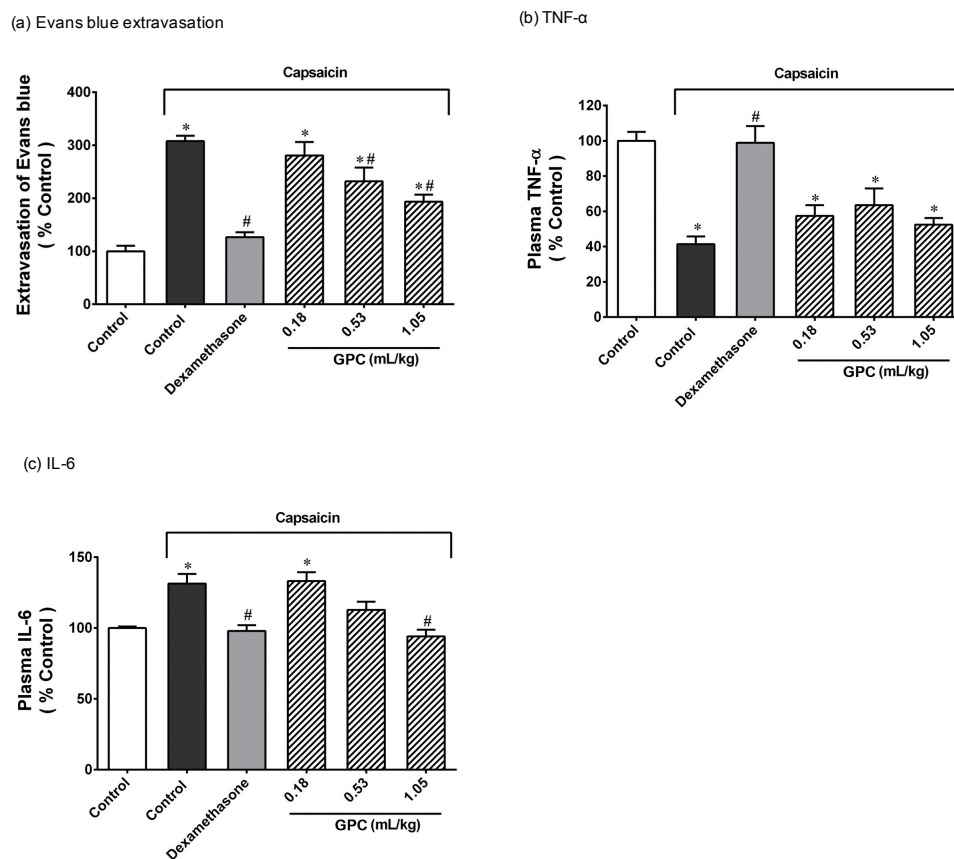
(b) TNF-α

(c) IL-6

Figure 4. Effects of long-term treatment with GPC on Evans Blue extravasation of pharyngeal tissue and plasma cytokine levels in capsaicin-challenged rats. Male Sprague Dawley rats were orally administered with GPC (0.18, 0.53 and 1.05 mL/kg× 30 doses) or dexamethasone (1 mg/kg/day × 10 doses). Twenty-four hours after the last dosing with GPC/dexamethasone, the ketamine/xylzine-anaesthetized rats were intravenously injected with Evans Blue dye (60 mg/kg in saline) via the lateral tail vein. At 10 min post-injection with Evan Blue dye, capsaicin (0.3 mM) was applied to the pharyngeal mucosa using cotton swab, while control animals received the vehicle, as described in Materials and methods. The content of Evan blue dye in tissue is expressed as microgram of dye per gram of issue wet weight (panel A). Plasma samples of capsaicin-challenged GPC/dexamethasone-treated rats were collected and subjected to the measurement of TNF-α (panel B) and IL-6 (panel C) using ELISA kits. Data are expressed as % control by normalizing with the value of vehicle control group. Value given are means ± SEM, with n ≥ 3. *Significantly different from the non-capsaicin vehicle control group. #Significantly different from capsaicin vehicle control group.

long-term GPC treatment decreased plasma level of IL-6 (59% - 100%) in capsaicin-challenged rats, but it slightly (but insignificantly) increased plasma TNF-α level in capsaicin-challenged rats (**Figure 4(a)** and **Figure 4(b)**). Dexamethasone treatment suppressed plasma IL-6 (100%) level in capsaicin-challenged rats, but it drastically increased the plasma TNF-α level (by 139%), when compared with un-treated capsaicin control.

Long-term treatment with GPC at the highest tested dose (2.10 mL for mice, 1.05 mL for rats) for 30 doses did not produce any undetectable changes in plasma ALT and

AST activities in mice/rats (data not shown).

4. Discussion

Pathogen-associated molecular patterns are highly conserved molecular moieties that can be recognized by the innate immune system. As such, LPS (a commonly found molecule on gram-negative bacterial surface) as well as PGN (a commonly found molecule on gram-positive bacterial surface) can be recognized by toll-like receptor 4 and 6, respectively, in monocytes, with a subsequent activation into pro-inflammatory macrophages [10]. In this regard, a cell model of LPS/PGN-activated macrophages is commonly used for screening anti-inflammatory agents. In the present study, the finding that GPC incubation reduced the extent of inflammatory response in LPS/PGN-activated RAW264.7 macrophages, as evidenced by decreases in the releases of TNF-α, IL-6 and NO, is corroborated by the anti-inflammatory effects produced by long-term GPC treatment in rodent models of acute inflammation (carrageenan-induced paw edema) as well as chronic inflammation (cotton pellet-induced granuloma formation) in mice. In addition, the ability of GPC to enhance the tracheobronchial expectorant action may suggest its immunomodulatory activity in the respiratory tract. This postulation was supported by the observation that GPC reduced the degree of capsaicin-induced pharyngitis in rats. While the biochemical mechanism underlying the differential action of capsaicin challenge in modulating plasma levels of TNF-α and IL-6 in rats is yet to be investigated, the suppression afforded by GPC treatment on capsaicin-induced pharyngitis was associated with the reversal changes in plasma TNF-α and IL-6 levels in capsaicin-irritated rats. The result indicates the ability of GPC to protect against capsaicin-induced pharyngitis. Given the high prevalence of upper respiratory tract infection, the ability of GPC to ameliorate capsaicin-induced pharyngitis may be used for alleviating the respiratory tract inflammation caused by infections.

The anti-inflammatory response induced by GPC is likely attributed to the pharmacological activity of each component herb. Recently, Fu et al. have made a survey on component herbs used in classical Chinese herbal prescriptions for treating respiratory diseases. It was found that Glycyrrhizae Radix was the most frequently used herb among the selected prescriptions [11]. This observation suggests that Glycyrrhizae Radix may play a pivotal role in the anti-inflammatory action of GPC. In support of this, various compounds isolated from Glycyrrhizae Radix were found to elicit anti-inflammatory response *in vitro* and *in vivo*. As such, diammonium glycyrrhizinate was found to attenuate the extent of hepatitis in concanavalin A-injected mice, presumably via the suppression on the release of pro-inflammatory cytokines [12]. Glycyrol was shown to suppress the levels of pro-inflammatory cytokines as well as inflammatory effector molecules in LPS-activated RAW264.7 macrophages [13]. Licochalcone A w, **which serves as a screening study,** as found to attenuate the allergic airway inflammation in ovalbumin-induced asthma mice [14]. The aqueous extract of Platycodonis Radix, which is also a component herb in GPC, was found to inhibit the nuclear factor-κB-mediated expression of pro-inflammatory effectors in LPS-activated human cul-

tured airway epithelial cells [15] as well as suppress the airway inflammation in ovalbumin-induced asthma mice [16]. Despite the fact that the anti-inflammatory effect of Citri Reticulatae Pericarpium and Phyllanthi Fructus on respiratory tract has not been reported, they were found to produce anti-inflammatory effect in LPS-activated vascular epithelial cells [17] and LPS-activated BV2 microglia cells [18], respectively. Interestingly, Taraxaci Herba was found to potentiate the attenuating action of colchicine on Behcet's disease-like symptoms in herpes simplex virus-infected mice, possibly through modulating the profile of cytokines [19]. The finding suggests that Taraxaci Herba may serve as a complementary herb in potentiating the anti-inflammatory action of other component herbs in GPC. Further investigation is required to investigate whether the component herbs of GPC can act additively or synergistically in eliciting an anti-inflammatory response.

5. Conclusion

In conclusion, the ensemble of results suggests that GPC may offer a promising prospect for producing an anti-inflammatory action, particularly for alleviating the extent of pharyngitis. Further studies are required to investigate the role of individual herb component in GPC in producing anti-inflammatory response as well as the biochemical mechanism underlying the anti-inflammatory action of GPC.

Acknowledgements

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