Anti-inflammatory Effects of Components in Shuxiong Tablet and Its Possible Formulary Rationality

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Abstract: **Objective** To determine whether the anti-inflammatory properties of Shuxiong Tablet (SXT) and the effective components group of SXT (ECGS) are equivalent and to assess the formulary rationality. **Methods** ECGS consisted of *Panax notoginsen* saponion (PNS), hydroxysafflor yellow A, and ferulic acid plus volatile oil of *Ligusticum chuanxiong*, which was based on the active ingredients and their ratios in SXT. We compared the anti-inflammatory actions of ECGS and SXT using the xylene-induced edema model and the carrageenan-induced edema model, as well as the analgesic activity of them using the acetic acid-induced writhing model. Moreover, cultured macrophages were incubated with media containing serum isolated from SXT-, ECGS-, or every component of ECGS-treated rats, to compare the depress effects on lipopolysaccharide (LPS)-stimulated NO production and inducible nitric oxide synthase (iNOS) expression. **Results** ECGS and SXT had equivalent anti-inflammatory actions and analgesic effects at an equipotent dosage in a dose-dependent manner. The drug-containing media could inhibit the LPS-stimulated NO production and iNOS expression in cultured macrophages. A 2 × 2 × 2 ANOVA revealed that three effective components could produce synergistic effect on the inhibition of NO production, and PNS was the capital component. **Conclusion** ECGS and SXT display an equivalent anti-inflammatory effect, and the formula follows traditional Chinese medicine compatibility principle, which shows obvious formulary rationality.

Key words: anti-inflammation; effective components group; formulary rationality; Shuxiong Tablet
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Introduction

Shuxiong Tablet (SXT), a combination of three traditional Chinese herbs including *Notoginseng Radix* et *Rhizome*, *Carthami Flos*, and *Chuanxiong Rhizoma*, is one of the herbal formulas that has been traditionally used in China to treat many inflammatory diseases, such as angina pectoris, myocarditis, myocardial infarction and tissue damage. Each tablet is composed of 100 mg *Notoginseng Radix* et *Rhizome* [rhizome of *Panax notoginseng* (Burk.) F. H. Chen, Araliaceae], the water extracts of 100 mg *Carthami Flos* (flower of *Carthamus tinctorius* L., Compositae), and 200 mg *Chuanxiong Rhizoma* (rhizome of *Ligusticum chuanxiong* Hort, Umbelliferae) (Pharmacopoeia Committee of P. R. China, 2005). According to the concepts of traditional Chinese medicine (TCM), *Notoginseng Radix* et *Rhizome*, *Carthami Flos*, and *Chuanxiong Rhizoma* are often used singly or combined with other herbs to prevent cardiovascular disease (CVD) and to treat tissue injury. It has been reported that the major active ingredients of *Notoginseng Radix* et *Rhizome* are *Panax notoginsen* saponions (PNS) (Dong *et al*, 2003), which would reduce oxidation and lipid actions (Chan and Tomlison, 2000; Jia, Liu, and Li, 2010), improve local circulation, and protect cardiovascular cells from ischemic damage (Kim *et al*, 1995; Wu *et al*, 2006). The volatile oil of *Chuanxiong Rhizoma* (VCO) (Liang, He, and Yang, 2005), which contains phthalide-type compounds and the ferulic acid (FA) (Wang *et al*, 2008) are the major active components in the water extracts of *Chuanxiong Rhizoma*. We didn't find chuanxiongzine in our water extracts of *Chuanxiong Rhizoma*. VCO plays a part in treating cardiovascular and cerebrovascular diseases

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(Natio, Kubota, and Shimoda, 1995) and FA inhibits endothelial and vascular smooth muscle cell proliferation (Hou *et al*, 2004). Hydroxysafflower yellow A (HSYA) is the main active component from the water extracts of the safflower (Meselhy *et al*, 1993). Previous studies have shown that HSYA could significantly reduce the myocardial infarct size, increase serum SOD activity, and scavenge the oxygen free radicals (Winter, Risley, and Nuss, 1962; Wang *et al*, 2008).

Although the clinical use of SXT to treat CVD has been reported (Zhao, 2002), the active constituents and the pharmacological properties of their potency are not yet clarified. It has been reported that inflammation plays an important role in the occurrence and progression of CVD (Franks, 2006). Since the three crude materials in SXT have anti-inflammatory effects (Friedl *et al*, 2001; He *et al*, 2008; Sudheer *et al*, 2008), we believe that the major cardiovascular protecting mechanisms of SXT are mediated through their anti-inflammatory action. In addition, nitric oxide (NO) is one of the inflammatory mediators, which is produced by various cell types (macrophages etc.) in response to infection. NO mediates a proinflammatory response (Cross and Wilson, 2003) which may lead to CVD (Frostegard *et al*, 1999), and excessive NO production is one of the mechanisms that lead to an inflammatory state. The role of NO in inflammatory heart diseases is a double-edged sword. The expression of NO directly exhibits antiviral effects by inhibiting viral proteinases (Badorff *et al*, 2000). On the other hand, NO leads to programmed cell death of cardiomyocytes, as a result of oxidative stress (Shimojo *et al*, 1999). It is likely that unrestricted NO synthesis by activated macrophages contributes to cardiac tissue damage; therefore, compounds that regulate NO synthesis in macrophages may provide a treatment to prevent damage or improve function following cardiac tissue damage.

In the present study, we aimed to make a components mixture of SXT, which composed of PNS, HSYA, and FA plus VCO and all according to their ratios in SXT, to obtain an effective components group of SXT (ECGS). We compared the anti-inflammatory actions of ECGS and SXT using the xylene-induced edema model, the carrageenan-induced edema model, and the acetic acid-induced writhing model to determine if ECGS and SXT have equivalent anti-inflammatory effects at an equipotent dosage. In order to assess the formulary rationality of this compound preparation, *in vitro* cultured macrophages are grown in media containing serum from SXT-, ECGS-, or every component of ECGS-treated rats, to determine if they are able to inhibit lipopolysaccharide (LPS)-stimulated NO production and inducible nitric oxide synthase (iNOS) expression.

Materials and methods

Materials and reagents

Notoginseng Radix et *Rhizome*, *Carthami Flos*, and *Chuanxiong Rhizoma* were obtained by Yonggang in Bozhou, Anhui Province, China, and all the herbs were identified by Dr. MA Shi-ping in China Pharmaceutical University. Aspirin was supplied by AstraZeneca Co., Ltd (Wuxi, China). PNS (97.9%) was purchased from Kunming Pharmaceutical Corporation (Kunming, China). FA (99.2%) was provided by Shanghai Chemical Reagent Company, Medical Corporation (Shanghai, China). HSYA (98.5%) was provided by Shandong Natural Medicinal Engineering Research and Development Center (Shandong, China). VCO (98.8%) was purchased from Jiangxi Jishui Tong Ren Natural Medicated Oil Company (Jishui, China). The xylene, CGN, and acetic acid were purchased from Sigma-Aldrich (St. Louis, MO). Phenol red-free RPMI 1640, ampicillin, streptomycin, and fetal bovine serum (FBS) were purchased from Gibco BRL (Grand Island, NY). The NO assay kit was purchased from Beyotime Biotechnology Co. (Jiangsu, China). The monoclonal antibody for β-actin, rabbit polyclonal antibody against iNOS, and horseradish peroxidase-conjugated secondary antibodies were supplied by Santa Cruz Biotechnology (Santa Cruz, CA). LPS and thioglycolate broth were purchased from Sigma (St. Louis, MO). Mammalian protein extraction reagent (RIPA) and bicinchoninic acid (BCA) protein assay kit were purchased from Beyotime (Haimen, China).

Preparation of the SXT and ECGS formula

CMC-Na suspension (0.5%) of SXT was prepared in 200 mL by putting fine powder of *Notoginseng Radix* et *Rhizome* (50 g) into the water extracts of the *Chuanxiong Rhizoma* (100 g) and *Carthami Flos* (50 g) (Pharmacopoeia Committee of P. R. China, 2005). Consequently, the concentrations of crude *Notoginseng Radix* et *Rhizome*, *Chuanxiong Rhizoma*, and *Carthami*

Flos in the suspensions were as follows: 0.25, 0.50, and 0.25 g/mL suspensions, respectively, and the concentration of SXT, converted to crude drugs, was 1.00 g/mL. The content for each of the contents in SXT, including PNS, HSYA, FA, and VCO, were determined by HPLC (Qi, *et al*, 2007): PNS, 0.805%; HSYA, 0.250%; FA, 0.100% and VCO, 0.125%. The content for PNS, HSTA, and VCO were in agreement with previously reported figures (Pharmacopoeia Committee of P. R. China, 2005; Chen *et al*, 2004), but the percentage of FA was only in agreement with the total FA found in crude *Chuanxiong Rhizoma* (Wang *et al*, 2008). We suspect that the higher percentage of FA in SXT comes from some coniferyl ferulate that is hydrolyzed into FA when heating (Wang *et al*, 2008).

Given the determined percentages of the contents in SXT, we added 1.61 g of PNS, 0.5 g of HSYA, 0.2 g of FA, and 0.25 g of VCO into a 0.5% CMC-Na solution to obtain 200 mL of an ECGS suspension. Consequently, the concen- tration of ECGS in the suspension was 12.8 mg/mL, and at an equipotent concentration to SXT, converted to crude drugs, was 1.00 g/mL.

Animals

Both male SD rats (200－250) g and mice (18－22) g were purchased from the Laboratory Animal Center of Henan Province, China. Animals were housed in a room with an ambient temperature of 21－25 ℃ and a relative humidity of 50%–60%. They had free access to food and water. All experimental procedures carried out in this study were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of China Pharmaceutical University.

Xylene-induced ear edema

Ear edema was induced in male mice according to the modified method published (Pongprayoon *et al*, 1991) with some additional modifications. The mice were divided into six groups and ig administered one of six treatments: vehicle (0.5% CMC-Na), Aspirin (200 mg/kg), the three dosages of ECGS (50, 25, and 12.5 mg/kg, respectively) or SXT (1.95 g/kg, which was equipotent in crude drug dosage with the 25 mg/kg ECGS). One hour after administration of the test drugs, xylene was topically applied to the right ears of mice (30 μL per ear). The left ear was used as the negative control. One hour after xylene treatment, the mice were sacrificed and both ears were removed. Circular sections were removed from both ears using a 7 mm diameter cork borer and weighed on a FA1004 Electronic Balance (Shanghai, China). The increase in weight caused by the irritant was calculated by subtracting the weight of the untreated left ear section from that of the right ear. Results obtained with the test drugs were compared with those obtained from the vehicle only group.

CGN-induced hind paw edema

Paw edema was produced in mice by CGN following the modified methods (Winter, Risley, and Nuss, 1962). Male mice were divided into six treatment groups as described above. A volume of 0.03 mL of 1% CGN in normal saline solution was intradermally injected into the plantar side of the right hind paw of the mouse. The left paw was used as the negative control. Test drugs and vehicle were given 1 h prior to CGN. Four hours after injection, the mice were sacrificed and both hind paws were removed at the joints of paw. The increase in weight caused by the irritant was calculated by subtracting the weight of the untreated left paw section from that of the right paw. Results obtained with the test drugs were compared with those obtained from the vehicle only group.

Acetic acid-induced writhing test

A slight modification of the acetic acid-induced writhing method was used (Koster, Anderson, and Debeer, 1959). Male mice were again divided into six treatment groups as described above. One hour after administration of the vehicle or test drugs, a 0.7% aqueous solution of acetic acid was ip administered to each mouse at a dose of 10 mL/kg. The number of writhes produced in these animals was counted for 15 min. Writhing was defined as a contraction of the abdominal muscles together with a stretching of the hind limbs.

LPS-induced production of NO and expression of iNOS in macrophages *in vitro*

Serum preparation The rats were randomly grouped into ten groups: blank (vehicle only) group, Aspirin group, SXT group, PNS group, HSYA group, FA-VCO group, PNS-HSYA group, PNS-FA-VCO group, HSYA-FA-VCO group, and ECGS group. Each of the constituents was ig administered at the following doses corresponding to their proportion in SXT: SXT (6.48 g/kg, crude drugs); PNS (52.2 mg/kg); HSYA (16.2 mg/kg); FA-VCO (FA 6.48 mg/kg + VCO 8.10 mg/kg), PNS-HSYA (PNS + HSYA = 52.2 + 16.2 mg/kg),

PNS-FA-VCO (PNS + FA + VCO = 52.2 + 6.48 + 8.10 mg/kg), HSYA-FA-VCO (HSYA + FA + VCO = 16.2 + 6.48 + 8.10 mg/kg), and ECGS (83.0 mg/kg). In addition, Aspirin dose was 60 mg/kg. The animals were dosed with an equipotent dosage so that each ingredient and each combination corresponded in dosage with the crude drug concentrations in SXT. All the drugs and constituents were dissolved in 0.5% CMC-Na.

Rats in each group were ig administered the relevant drugs twice daily for three consecutive days. One hour after administration on the third day, blood was aseptically collected from the main ventral artery and placed in tubes, which were allowed to stand at 25 ℃ for about 5 h, then the sera were isolated from whole blood by centrifugation at 2500 r/min for 20 min. The isolated sera were filtered two times through a 0.22 μm cellulose acetate membrane and then bottled. Finally, the sera were heated to 56 ℃ in a water bath for 30 min and stored at －20 ℃ (Bochu, Liancai, and Qi, 2005).

Cell culture Primary macrophages were collected from the peritoneal cavities of mice (8-week-old male C57BL/6) after ip injection of 1% thioglycolate broth (3 mL) 3 d before harvesting. The collected cells were centrifuged and washed. Cells were cultured in 96-well culture plates (2×10^6 cells/well), in RPMI 1640 medium containing 10% heat-inactivated FBS, 100 μU/mL ampicillin and 100 μg/mL streptomycin at 37 ℃ in 5% CO_2, and 95% air. After 2 h, non-adherent cells were removed by washing with phosphate buffered saline (PBS). Then, the experiments were carried out as follows.

The cells were divided into 11 groups. The negative control group was incubated with 1640 containing 10% blank serum. The other ten groups were stimulated with LPS (10 μg/mL) for 24 h, then the LPS-containing media was replaced with 1640 that contained 10% serum isolated from the rats treated with either vehicle or drugs and then the macrophages were incubated for another 24 h. Each group contained six parallel samples. After cells were incubated according to the grouping, NO production and iNOS expression were detected.

Nitrites release After the cells were incubated according to the aforementioned grouping, 50 μL culture solutions were collected from each well and put into the counterpart well on another plate. Then, NO production in the cells was measured using the Griess method (Hsu *et al*, 2005) and according to the instructions supplied with the NO assay kit. The absorbance of the test samples was read at 570 nm and compared with standard solutions of sodium nitrite prepared in the same culture media. The results were expressed in μmol/L.

Western blotting analysis of iNOS Whole cell lysates were centrifuged at 10 000 × *g* for 10 min to remove insoluble components. An aliquot was taken for protein determination using the BCA protein assay kit. Equal amounts of protein were subjected to 10% SDS-PAGE and electrotransferred to a nitrocellulose membrane. Membranes were incubated first with primary antibodies against iNOS and then with the horseradish peroxidase-conjugated secondary antibodies. The bands were visualized by autoradiography using a chemiluminescence system (Pierce). Data were normalized using β-actin as a control for loading.

Statistical analysis

The results were reported as the mean and standard deviation. Data in all tests were analyzed by one-way (ANOVA) followed by LSD post hoc test for multiple comparisons. In the experiment testing the LPS-induced NO production in macrophages, a 2 × 2 × 2 (PNS × HSYA × FA-VCO) mixed-factor ANOVA was performed for assessing the interaction among PNS, HSYA and FA-VCO on NO production. Statistical significance was accepted for $P < 0.05$.

Results

Xylene-induced ear edema and CGN-induced hind paw edema in mice

To determine if ECGS could significantly inhibit inflammation *in vivo*, mice were ig administered ECGS and then subjected to two models of acute inflammation (Fig. 1). Following administration of 50, 25 or 12.5 mg/kg ECGS, we found that ECGS showed dose-dependent anti-inflammatory activity in both xylene-induced ear edema (83%, 51%, and 17% inhibition, respectively) and CGN-induced paw edema (58%, 39%, and 16% inhibition, respectively). A reference compound, Aspirin, showed 80% and 64% inhibition at 200 mg/kg against ear edema and paw edema, respectively. Interestingly, the SXT showed 45% and 34% inhibition at 1.95 g/kg in the two models, respectively, which were similar to the effects of ECGS at 25 mg/kg, suggesting

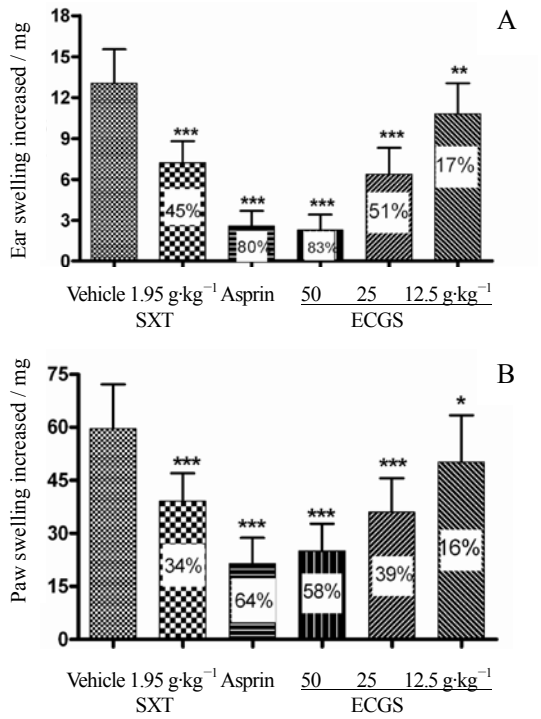

Fig.1 Inhibition of ECGS, SXT, and Aspirin against the mouse xylene-induced ear edema model (A) and the mouse CGN-induced paw edema model (B)

Values in each column represent percent inhibition of the edematic response. The data are expressed as the $\bar{x} \pm s$ (n = 10)

*P < 0.05 **P < 0.01 ***P < 0.001 *vs* the vehicle group

that the anti-inflammatory activity of ECGS is identical to SXT at the equipotent dosage.

Acetic acid-induced writhing test

Similarly, ECGS possessed strong analgesic activity in the acetic acid-induced writhing model at doses of 12.5, 25, and 50 mg/kg with the inhibition of writhing calculated at 19%, 55%, and 87%, respectively. As observed with the anti-inflammatory activity, ECGS displayed dose-dependent analgesic activity and a similar effectiveness in inhibiting writhing compared to SXT (50% inhibition at 1.95 g/kg) at the equipotent dosage. Aspirin also demonstrated significant analgesic activity, 86% inhibition at 200 mg/kg (Fig. 2).

Production of NO

To determine if ECGS, SXT or their components could modulate macrophage production of NO, cultured macrophages were subjected to LPS treatment and then incubated with control or drug-containing media and assayed for NO production. As shown in Fig. 3A, LSD post hoc tests showed that the LPS-treated NO production in the macrophages was increased in the vehicle only group (P < 0.001 *vs* control). However, NO production in the Aspirin-, SXT-,

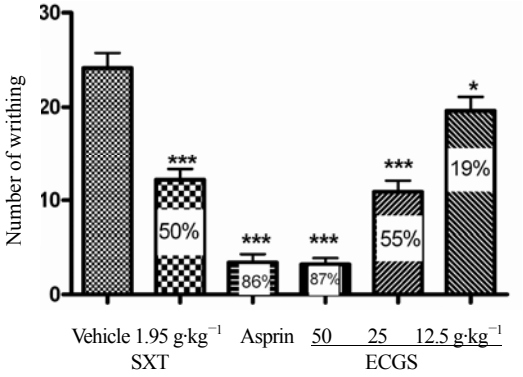

Fig. 2 Analgesic activity of ECGS, SXT, and Aspirin in mice

Values in each column represent the percent inhibition of writhings. The standard method for acetic acid-induced writhing in mice was employed. The vehicle group showed 24.1 ± 5.1 writhings during 15 min and the data are represented as the $\bar{x} \pm s$ (n = 10)

*P < 0.05 ***P < 0.001 *vs* the vehicle group

and ECGS- treated macrophage groups were decreased (all P < 0.001 *vs* model). Given equipotent dosages, SXT and ECGS elicited identical action on the inhibition of LPS-treated NO production. Compared to the vehicle only group, PNS-FA-VCO, PNS-HSYA, and HSYA-FA-VCO groups also displayed significant inhibition on NO production (P < 0.01, 0.01, 0.05, respectively), while no significant effects existed in PNS, HSYA, and FA-VCO groups (all P > 0.05). Interestingly, the ECGS group exhibited a more significant inhibition of NO production than other component groups (all P < 0.01).

A 2 × 2 × 2 (PNS × HSYA × FA-VCO) mixed-factor ANOVA revealed a significant interaction among PNS, HSYA and FA-VCO [$F_{(1,20)}$ = 7.09, P = 0.011] on inhibiting NO production, with a significant effect by either PNS [$F_{(1,20)}$ = 38.11, P < 0.001], HSYA [$F_{(1,20)}$ = 15.86, P < 0.001] or FA-VCO [$F_{(1,20)}$ = 38.20, P < 0.001] alone. Moreover, this analysis revealed a significant interaction between PNS and FA-VCO [$F_{(1,20)}$ = 9.37, P = 0.004] and between PNS and HSYA [$F_{(1,20)}$ = 6.71, P = 0.013]; However, only a marginally significant interaction was observed between HSYA and FA-VCO [$F_{(1,20)}$ = 3.99, P = 0.053].

iNOS expression

It is well established that in macrophages NO is produced by iNOS. Consistent with the findings shown in Fig. 3, Western blotting analysis revealed that ECGS, SXT as well as Aspirin, directly inhibited the expression of iNOS in the LPS-treated macrophages. In fact, the highest inhibitions of iNOS expression in the LPS-treated macrophages were observed in the ECGS, SXT

and Aspirin groups (Fig. 3B). The PNS-FA-VCO group displayed a stronger effect on the down- regulation of iNOS expression than either the PNS-HSYA or HSYA-FA-VCO group. However, no marked changes in iNOS expression were observed in the PNS, HSYA, and FA-VCO groups and no iNOS expression was detected in the control group.

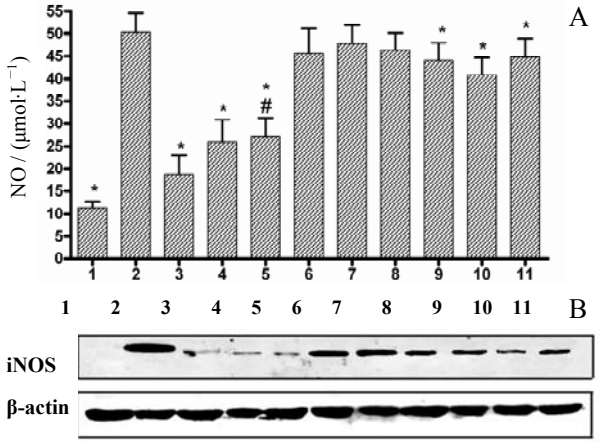

Fig. 3 Effect of ECGS on LPS-treated NO production and LPS-induced iNOS expression in macrophages

A: Effect of ECGS on LPS-treated NO production in the macrophages

B: Effect of ECGS on LPS-treated iNOS expression in the macrophages

The test groups were: control (1), vehicle only (2), Aspirin (3), SXT (4), ECGS (5), FA + VCO (6), HSYA (7), PNS (8), PNS + HSYA (9), PNS + FA + VCO (10), HSYA + FA + VCO (11). Macrophages were pretreated with 10 μg·mL^{-1} LPS for 24 h. Then, the cells were respectively treated with either blank serum (the vehicle group) or serum collected from rats treated with the aforementioned drugs for another 24 h. The data are shown as the $\bar{X} \pm s$ (n = 10). The * denotes significance in comparison with the vehicle group and the # denotes significance in the ECGS compared with all the component groups (FA + VCO, HSYA, PNS, PNS + HSYA, PNS + FA + VCO and HSYA + FA + VCO). A significant interaction among PNS, HSYA and FA-VCO was revealed ($F_{(1,20)}$ = 7.09, P = 0.011). The exact P values for each comparison are given in the text

Discussion

The present investigation clearly demonstrates that ECGS, the effective components of SXT, inhibits NO production by macrophages *in vitro*, at least partly, through down-regulation of iNOS expression. Not only did ECGS possess anti-inflammatory activity *in vivo* against several animal models of inflammation, but also the potency of the ECGS anti-inflammatory activity was higher than that of Aspirin. In addition, ECGS significantly inhibits acute inflammation in a dose-dependent manner and it has an identical anti-inflammatory activity as SXT at the equipotent dosage.

NO is an important mediator in the inflammatory process and is produced at inflamed sites in response to iNOS expression. High levels of NO have been reported in a variety of pathological processes including various forms of inflammation, circulatory shock, and carcinogenesis (Ohshima and Bartsch, 1994; Szabó, 1995). Therefore, an inhibitor of iNOS might be effective as a therapeutic agent for inflammatory diseases (Ianaro *et al*, 1994). SXT has been used as an herbal formula to treat myocardial diseases and tissue damage, which are characterized by chronic inflammation and mediated by macrophages (Szalay *et al*, 2006). In this study, inhibition of NO production in LPS-treated murine macrophages was used to assess the anti-inflammatory activity of SXT or its effective constituents *in vitro*. The rationale for this test lies in the fact that iNOS is induced in macrophage when it is stimulated by LPS (MacMicking, Xie, and Nathan, 1997; Chi *et al*, 2003). Based on these findings, it is suggested that one of anti-inflammatory processes of the ECGS or SXT is the suppression of excessive iNOS expression, resulting in the depression of NO production. By normalizing of NO levels in inflammatory heart or other diseased areas, the injured tissue could be restored by treatment with an iNOS suppressive drug.

In this study, it was shown that no one single component (PNS, HSYA or FA+VCO) contained in ECGS has a significant effect on the inhibition of NO production in LPS-treated macrophages. However, a significant effect on inhibiting NO production and down-regulating iNOS expression in the LPS-treated macrophages, and an interaction among these components were observed in the ECGS group，suggesting that a significant synergistic action existed in the ECGS, a combination of PNS, HSYA, and FA + VCO. In addition, the components PNS + FA-VCO, PNS + HSYA or HSYA + FA-VCO, exhibit much weaker effect than ECGS on the inhibition of NO production. These data also suggest that the PNS might be the most important component in the ECGS formula, since the aforementioned components when containing PNS, PNS + FA-VCO or PNS + HSYA, display a marked interaction, but the components of HSYA + FA-VCO don't display that. The utility of TCM is based on the natural compounds, which are characterized by their chemical structure complexity and their multi-target nature (Kawashima *et al*, 2004). Since compound of Chinese

materia medica (CMM) is a mixture of medicinal herbs, there is a possibility that the chemicals interact to modify the individual pharmacological activities. These results indicate that therapeutic approaches using a single ingredient are not adequate for elucidating the pharmaceutical properties of SXT, and there must be a synergistic effect among the active components in ECGS: One is a key component (PNS) and the others are assistant components (FA, VCO, and HSYA), which could enhance its anti-inflammatory action. These results also indicate that the most effective anti-inflammatory component is ECGS, a combination of PNS, HSYA and FA + VCO, and deleting one component in the ECGS would markedly reduce it's anti-inflammatory action. In addition, ECGS has an identical anti-inflammatory activity compared to SXT at the equipotent dosage, strongly indicating that these constituents are the only effective components contained within SXT. Perhaps ECGS will clinically replace SXT in the future.

The research shows that inflammation is a complex process involving multiple inflammation-related cytokines (Hofmann *et al*, 2007), such as tumor necrosis factor (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), intercellular adhesion molecule-1 (ICAM-1), and so on. However, activated nuclear factor (NF-κB) can increase the expression of aforementioned inflammatory cytokines. TNF-α or IL-1β, as an inducing factor, can activate cyclooxygenase-2 (COX-2) and nitric oxide iNOS, and increase their expression, then increase the synthesis of prostacyclin (PGE_2), NO etc., the substances which induce inflammation. Three components in the ECGS can just impact the pathways of inflammatory process. For example, PNS is able to reduce the expressions of inflammatory mediators, such as ICAM-1, TNF-α etc., by inhibiting the NF-κB activity in Peritoneal macrophages or endothelial cells (Wang, Huang, and Peng, 2001; He *et al*, 2009); VCO may inhibit the expression of COX-2 protein and mRNA in hypothalamus of rats to reduce the content of PGE_2 (Yang *et al*, 2009), and FA is also an effective inhibitor of IL-8 production (Hirabayashi *et al*, 1995); HSYA can inhibit platelet aggregation induced by platelet activating factor (PAF), to inhibit the formation of blood clots and improve blood micro- circulation (Meselhy *et al*, 1993); In addition, VCO, FA, and HSYA are also able to inhibit the activity of NF-κB (Ling *et al*, 2008; Yan *et al*, 2001; Chen *et al*, 2008). Therefore, we suppose that these active ingredients in the SXT can simultaneously act through multi-target and multi-pathway to promote anti-inflammation effect, as this study shows that three components in the ECGS could produce synergistic effect on inhibited production of NO.

Conclusion

The present study suggests that the effect of ECGS, which contains PNS, HSYA, FA, and VCO, has an anti-inflammatory activity that is equivalent to SXT at an equipotent dosage. Furthermore, it was found that the four ingredients contained in the ECGS synergize with each other to achieve the anti-inflammatory properties. This is the first report defining the effective components group from SXT that elicit the anti-inflammatory effects and is a certification for formulary rationality of SXT.

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