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Effects of three purgative decoctions on inflammatory mediators

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Abstract

In traditional Chinese medicine (TCM), there are three *Cheng-Chi-Tang* decoctions (CCTDs) including: *Ta-Cheng-Chi-Tang* (TCCT), *Xiao-Chen-Chi-Tang* (XCCT) and *Tiao-Wei-Chen-Chi-Tang* (TWCCT), which are the frequently used purgative remedies to treat "internal heat"-induced symptoms like a bloated and painful abdomen, hard stools and fever, etc. Constituents in each formulation are *Rheum palmatum* L. (Polygonaceae), *Magnolia officinalis* Rehd. et Wils. (Magnoliaceae), *Citrus aurantium* L. (Rutaceae), *Mirabilitum* (mirabilite, crystals of sodium sulfate, Na_2SO_4) for TCCT; *Rheum palmatum*, *Magnolia officinalis*, *Citrus aurantium* for XCCT; and *Rheum palmatum*, *Mirabilitum*, *Glycyrrhiza uralensis* Fisch. (Leguminosae) for TWCCT. However, the underlying mechanisms for purging internal pathological heat are far from fully clarified, and few scientific investigations have been carried out to delineate the relationships between the anti-inflammatory effects and laxative potencies of these formulations. In this study, the anti-inflammatory effects of the three CCTDs on lipopolysaccharide (LPS)-induced nitric oxide (NO) and prostaglandin E (PGE_2) production in RAW 264.7 cells, carrageenan-induced paw edema in mice and the laxative effect in mice were explored. The results showed that TCCT inhibited LPS-induced NO and PGE_2 production and inducible nitric oxide synthase (iNOS) expression in RAW 264.7 cells more effectively than did XCCT or TWCCT. Moreover, paw edema of carrageenan-treated mice was significantly attenuated in mice pretreated with 1 g/kg TCCT. TCCT also showed the strongest purgative activity among the three formulations. These findings indicate that TCCT has anti-inflammatory effects in addition to its traditionally known purgative activities. It may have potential to treat inflammatory disease conditions.

Keywords: Traditional Chinese medicine; Anti-inflammatory; Inflammatory mediators; Purgative

1. Introduction

Inflammation is involved in the pathogenesis of many diseases. Proinflammatory stimuli activate cellular responses with increased production of many cytokines, including prostaglandins (PGs) and nitric oxide (NO) during the inflammatory process (Cirino, 1998). Many tissues can acutely or chronically generate excess NO and prostaglandin E (PGE_2) by overexpression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in response to various inflammatory stimulators, for example, increased NO and PGE_2 production has been noted in lipopolysaccharide (LPS)-induced macrophages, carrageenan-challenged animals and arthritic patients (Salvemini et al., 1996; Amin et al., 1999; Chen et al., 2001). NO and PGs have also been suggested to be involved in ileus, gallbladder inflammation and appendicitis (Nilsson et al., 1996; Nemeth et al., 2001; Bauer et al., 2002).

Patients suffering with acute intestinal obstruction, acute cholecystitis and acute appendicitis often display the signs and symptoms like abdominal distention, rigidity, tenderness, constipation and fever (Kowalak and Hughes, 2002). Similar symptoms and signs have been described in *Shan Han Lun*, a classical piece of traditional Chinese medicine (TCM) literature of the Han dynasty (about 200 A.D.), as heat pattern of abdominal pain. According to *Shan Han Lun*, when the contracted evil heat binds internally, symptoms and signs manifested included abdominal fullness and pain that refuses pressure, constipation, thirst, fever and delirium in severe cases (Windrige and Wu, 1994; Deng, 1999a). The traditional principle of treatment is to purge off the evil internal heat (Deng, 1999b). There are three

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Cheng-Chi-Tang decoctions (CCTDs) from *Shan Han Lun*. The three CCTDs include *Ta-Cheng-Chi-Tang* (TCCT), *Xiao-Chen-Chi-Tang* (XCCT) and *Tiao-Wei-Chen-Chi-Tang* (TWCCT), and all belong to the purgative category in TCM, i.e. these formulas will induce a laxative effect in humans (Hsu and Hsu, 1980). The ingredients of the three formulas are similar, and so are their clinical indications except that XCCT and TWCCT are used when milder symptoms are present. Nowadays, TCCT is indicated in diseases like acute intestinal obstruction without complications, acute cholecystitis and acute appendicitis (Liu, 1988; Qi et al., 2004). Other researchers have shown that TCCT was also effective in treating posttraumatic respiratory distress syndrome (Liu et al., 1992); reducing acute-phase protein levels in patients with multiple organ failure syndromes (Zhao et al., 1998), and inflammatory mediators in patients after a tumor operation (Wang and Qi, 1999). The clinical indications and the researches have led us to speculate that purgative formulas may have anti-inflammatory activity. However, few studies are available on the mechanism of anti-inflammatory activities of purgative formulas.

In this survey, we studied the plausible anti-inflammatory activities of the three CCTDs, due to their applicability for treating diseases like acute appendicitis, acute cholecystitis and reducing inflammatory mediators. We used LPS-stimulated RAW 264.7 murine macrophages and carrageenan-induced paw edema in mice to examine the inhibitory effects of TCCT, XCCT and TWCCT on the inflammatory mediators. The purgative actions of TCCT, XCCT and TWCCT were also assessed in this study in order to assure their purgative effects. Our findings indicate that among the three CCTDs, TCCT has anti-inflammatory effects in addition to its traditionally known purgative activities. It may have potential to treat inflammatory disease conditions when purgation and anti-inflammation are simultaneously indicated.

2. Materials and methods

2.1. Cell culture

RAW 264.7 murine macrophages were obtained from the American Type Culture Collection (Rockville, MD, USA). Cells were cultured in Dulbecco's-modified Eagle's medium from Sigma (St. Louis, MO, USA) and 10% heat-inactivated fetal bovine serum (FBS) from Gibco BRL (Grand Island, NY, USA), and then incubated at 37 °C in a humidified incubator containing 5% CO_2.

2.2. Animals

ICR male mice weighing 20 ± 2 g were obtained from the National Science Council, Taipei, Taiwan, and maintained in plastic cages at 21 ± 2 °C with free access to pellet food and water. They were kept on a 12-h light:12-h dark cycle. All mice used in this experiment were cared according to the Ethical Regulations on Animal Research of our university.

2.3. Preparation of the three CCTDs

TCMs used in this study were purchased from a traditional Chinese medicinal store in Taipei, Taiwan. The medicinal plants and materials used in the experiment included the root and bark of *Rheum palmatum* L. (Polygonaceae), the bark of *Magnolia officinalis* Rehd. et Wils. (Magnoliaceae), the immature fruit of *Citrus aurantium* L. (Rutaceae), the root of *Glycyrrhiza uralensis* Fisch. (Leguminosae) and *Mirabilitum* (mirabilite, crystals of sodium sulfate, Na_2SO_4). The medicinal materials were authenticated by Associate Prof. H.C. Chang, National Laboratories of Food and Drugs, Department of Health, Executive Yuan, Taipei, Taiwan. Voucher specimens (nos. RP-0001, MO-0001, CA-0001, GU-0001 and M-0001) were deposited at the Herbarium of the College of Pharmacy, Taipei Medical University. Table 1 lists the constituents and their proportions used to prepare the three CCTDs according to the Unified Formula announced by the Committee on Chinese Medicine and Pharmacy of Department of Health in Taiwan. Specifically the herbs were immersed in distilled water and boiled until half of the original amount was left. The extract was then filtered and freeze-dried. The yield of the extraction was about 25% (w/w). Ten milligrams of the freeze-dried TCM powder was dissolved in 1 ml of 10% dimethyl sulfoxide (DMSO) from Sigma and stored at −20 °C until use.

2.4. Chromatographic analysis of the three CCTDs

The HPLC system consisted of a Shimadzu (Kyoto, Japan) LC-10ATvp liquid chromatograph equipped with a DGU-14A degasser, an FCV-10ALvp low-pressure gradient flow control valve, an SIL-10ADvp auto injector, an SPD-M10Avp diode array detector and an SCL-10Avp system controller. Peak areas were calculated with Shimadzu Class-VP software (version 6.12 sp5).

The mobile phase was composed of 0.05% trifluoroacetic acid–acetonitrile (v/v) with gradient elution (0 min, 82:18; 13 min, 82:18; 40 min, 60:40; 47 min, 40:60; 50 min, 30:70;

Table 1
Constituents and their proportions in TCCT, XCCT and TWCCT

Plant name	Parts used	TCCT (g)	XCCT (g)	TWCCT (g)
Rheum palmatum L.	Root and bark	8	14	12
Magnolia officinalis Rehd. et Wils.	Bark	3	7	–
Citrus aurantium L.	Immature fruit	16	7	–
Mirabilitum	Crystals of Na_2SO_4	6	–	12
Glycyrrhiza uralensis Fisch.	Root	–	–	6

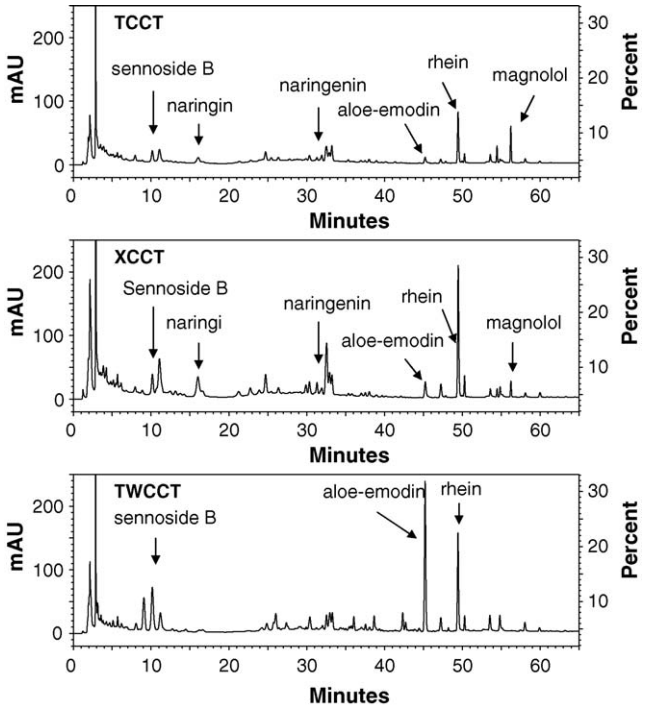

Fig. 1. HPLC fingerprints of the three CCTDS.

60 min, 25:75 and 65 min, 0:100). Solvents were filtered through a 0.45-μm FP Vericel (PVDF) membrane filter from Pall Corporation (Ann Arbor, MI, USA). A Purospher STAR RP-18e reversed-phase column (250 mm × 4 mm i.d.) and a Purospher STAR RP-18e guard column (4 mm × 4 mm i.d.) (Merck, Darmstadt, Germany) were used. The flow-rate was 1.0 ml/min with UV absorbance detection at 254 nm. The analysis involved 20 μl of sample solution. The operation was carried out at room temperature (25 °C).

HPLC of the three formulas was first performed as previously reported (Wang et al., 2002b), to verify the major compounds in each formula. The following compounds were identified for each decoction: TCCT—sennoside B (with a retention time (R_t of 10.2 min), naringin (R_t of 16.1 min), naringenin (R_t of 31.3 min), aloe-emodin (R_t of 45.3 min), rhein (R_t of 49.5 min) and magnolol (R_t of 56.3 min); XCCT—sennoside B (R_t of 10.2 min), naringin (R_t of 16.1 min) and naringenin (R_t of 56.3 min); and TWCCT—sennoside B (R_t of 10.2 min), aloe-emodin (R_t of 45.3 min) and rhein (R_t of 49.5 min) (Fig. 1).

2.5. Cell viability

Cell viability was determined by the mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan as previously described (Wang et al., 2002a). Briefly, RAW 264.7 cells (1×10^5 cells/well) were cultured in 96-well plates for 24 h after treatment with each CCTD extract at a concentration from 50 to 200 μg/ml. After the indicated time of treatment, cells were incubated with MTT for 4 h, then solubilized in isopropanol containing 0.04N HCl. Finally, the products were evaluated by measuring the optical density for each well at 600 nm, using an MRX microplate reader (Dynex Technologyies, Guernsey, Channel Islands, UK).

2.6. Measurement of nitrite formation

NO was measured as nitrite production in the medium after 24 h of incubation with or without the extracts, or *N*-nitro-L-arginine methyl ester (L-NAME, 1 mM, as positive control, Sigma) and/or LPS (500 ng/ml). Briefly, nitrate in the medium was converted to nitrite and measured spectrophotometrically after the Griess reaction (Wang et al., 2002b). Anti-inflammatory activity was presented in terms of NO production inhibition percentage.

2.7. Measurement of prostaglandin E_2 (PGE_2)

The amount of PGE_2 produced by cells in the media was assessed with a commercially available enzyme immunoassay system (Amersham Pharmacia Biotech, Buckinghamshire, UK), as previously described (Wang et al., 2002b). Briefly, 100 μg of supernatant of the cultured medium was collected for determination of the PGE_2 level according to the manufacturer's instructions. Indomethacin (10 μM, supplied by TTY Biopharm, Taiwan) was used as the reference drug in the in vitro study.

2.8. Western blot assay of iNOS and COX-2

The expressions of iNOS and COX-2 by LPS-stimulated RAW 264.7 cells were investigated by Western blot analysis. RAW 264.7 cells exposed to extracts for 24 h were collected into tubes and then washed with PBS. Protein samples were prepared according to our previous paper (Wang et al., 2002b). Total protein (25 μg) was used for Western blot analysis. Proteins were transferred to a nitrocellulose membrane. Membranes were probed using antibodies specific to COX-2, iNOS and actin and visualized using a BCIP/NBT kit from Gibco BRL according to the manufacturer's instructions.

2.9. Carrageenan-induced paw edema in mice

Edema in the left hind paw of mice was induced by injection of 50 μl of 1% (w/v) carrageenan from Sigma in saline into the subplantar region. The perimeter of the paw was measured 1 h before the injection and after 1–6 h using calipers. The rectal temperature was also measured each time the paw perimeter was measured. TCCT at different doses (0.5 and 1 g/kg) and indomethacin (10 mg/kg, as a reference substance) were given orally, 1 h before the injection. The control group were given vehicle (0.1 ml/10 g). Another group of mice received no treatment at all, and was designated as the blank group. Each group consisted of five animals. The edema inhibition rate was calculated as follows:

$$\text{Inhibition rate}\,(\%) = \left(1 - \frac{E_T - E_B}{E_C - E_B}\right) \times 100$$

where E_T is the foot perimeter value of the treated group, E_B the perimeter of the blank group and E_C is the perimeter of the control group.

2.10. Purgative test

The purgative effects of the three CCTDs were investigated with procedures described previously with some modification (Yagi and Yamauchi, 1997). ICR mice were divided into four groups of five animals and fed orally with pellets or 100 mg/kg of TCCT, XCCT, or TWCCT extract powder as a suspension mixed with carbon as the colored marker, and were observed until the control group defecated. The time to the excretion of the first carbon-colored feces, the number of fecal stains on the blotting paper per animal and the form of the stool for each group were recorded. The time to excretion of the colored stool was considered as a measure of the large-intestine propulsion, while the number and form of the stools were considered as a measure of the purgative activities of the extract (Yagi and Yamauchi, 1997).

2.11. Statistical analysis

All analyses were performed with the use of STATA version 8. The data were statistically assessed first by one-way analysis of variance (ANOVA). Difference between drug-treated groups and control group was then evaluated by Bonferroni's *t*-test. $P < 0.05$ was considered significant. All data are expressed as mean ± S.D.

3. Results

3.1. HPLC fingerprints of the three CCTDs

The major compounds in the three CCTDs were analyzed with HPLC. Major peaks identified for each decoction are listed here: TCCT—sennoside B, naringin, naringenin, aloe-emodin, rhein and magnolol; XCCT—sennoside B, naringin, naringenin, aloe-emodin, rhein and magnolol; and TWCCT—sennoside B, aloe-emodin and rhein. The major compounds identified were similar between TCCT and XCCT. However, naringin, naringenin and magnolol were not identified in TWCCT, which could be explained by the absence of the fruit of *Aurantis immaturus* and the cortex of *Magnoliae officinalis* from this formulation. Magnolol is a major compound isolated from the cortex of *Magnolia officinalis*. Naringin and naringenin are flavonoids found frequently in citrus fruits and grapes, such as *Aurantis immaturus* (Tsai et al., 1999; Li et al., 2002). Sennoside B, rhein and aloe-emodin are the constituents found in *Rheum palmatum* (Wang et al., 2002b).

3.2. Anti-inflammatory effects of the three CCTDs on LPS-induced RAW 264.7 cells

We examined the effect of the three CCTDs on LPS-induced PGE_2 and NO production in RAW 264.7 cells. RAW 264.7 cells were treated with LPS (500 ng/ml) in the presence or absence of various concentrations of TCCT, XCCT, or TWCCT, L-NAME (1 mM) or indomethacin (10 μM) and the supernatants were harvested after 24 h. The harvested medium was assayed for NO levels by the Griess reaction and for PGE_2 levels by the EIA method. At 200 μg/ml, both TCCT and XCCT showed significant inhibitory effects (71.8 ± 1.92 and 63.14 ± 1.35, respectively) while L-NAME produced 71.1% inhibition on LPS-induced NO produced by RAW 264.7 cells (Fig. 2a). Indomethacin (10 μM) had no inhibitory effect on NO (data not shown). TCCT also showed a dose-dependent inhibitory effect on NO produced by LPS-induced RAW 264.7 cells (Fig. 2b). At 200 μg/ml, both TCCT and TWCCT showed significant inhibitory effects on LPS-induced PGE_2 produced by RAW 264.7 (Fig. 3). The inhibition percentage of indomethacin (10 μM) was 99.6%, while L-NAME had no inhibitory effect on PGE_2. Among the three CCTDs, only TCCT showed significant inhibitory effects on both NO and PGE_2. Cell viabilities were not affected in the presence of 200 μg/ml TCCT, XCCT, or TWCCT as determined by the MTT assay (data not shown).

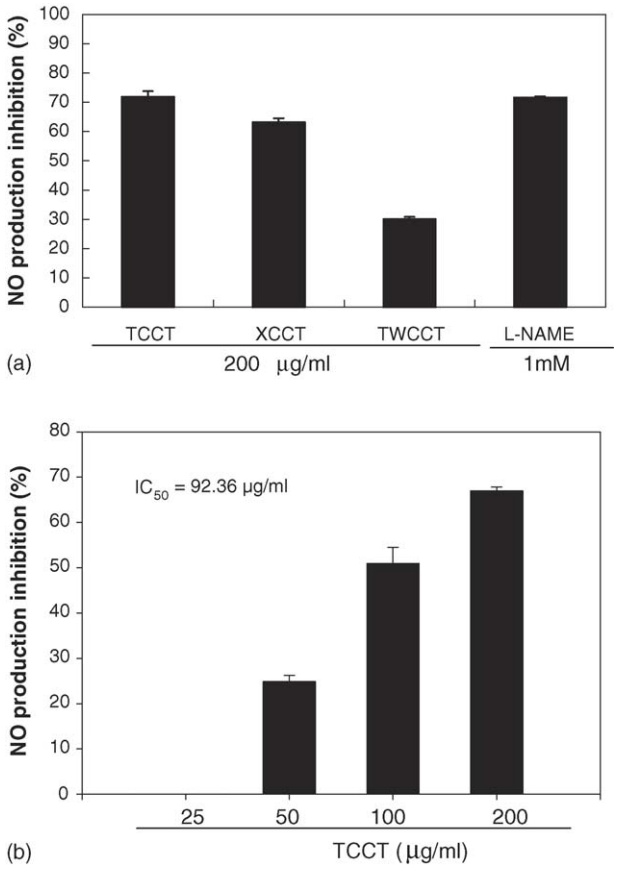

Fig. 2. Inhibitory effects of the three CCTDs (a) and of TCCT (b) at different concentrations on nitrite production by LPS-stimulated RAW 264.7 cells. Values are given in percentage of inhibition of nitrite concentration compared to the control. Bars represent the mean (±S.D.) of at least three independent experiments, each performed in triplicate.

Next, effects of the three CCTDs on iNOS and COX-2 expressions in LPS-induced RAW 264.7 cells were observed by Western blot assay. Similarly, RAW 264.7 cells were treated with LPS (500 ng/ml) in the presence or absence of various concentrations of TCCT, XCCT, or TWCCT, and cells were harvested after 24 h. TCCT showed dose-dependent inhibition of iNOS expression by LPS-induced RAW 264.7 cells. COX-2 expression was not greatly affected by the three decoctions (Fig. 4).

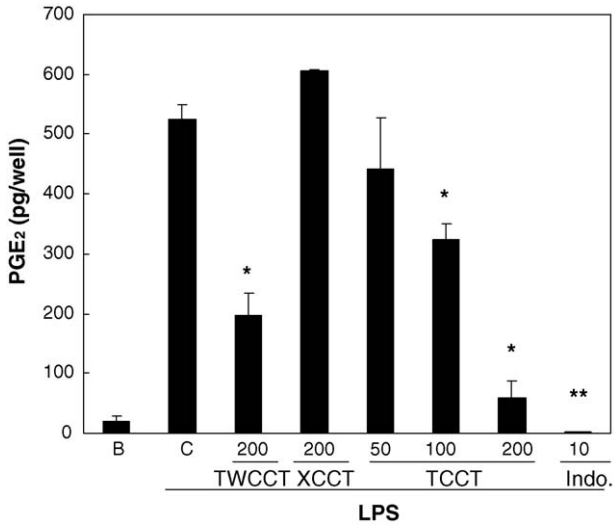

Fig. 3. Inhibitory effect of the three CCTDs on PGE_2 production by LPS-stimulated RAW 264.7 cells. B represents unstimulated cells and C represents solvent control. The concentration of three CCTDs were in μg/ml and indomethacin (Indo.) was in μM. Bars represent the mean (±S.D.) of at least three independent experiments, each performed in triplicate.

3.3. Inhibitory effect of TCCT on carrageenan-induced paw edema in mice

Since TCCT more readily inhibited NO and PGE_2 production and iNOS expression in the above in vitro study than did XCCT or TWCCT, we continued to examine the in vivo anti-inflammatory effect of TCCT using carrageenan-induced paw edema in ICR mice. Intraplantar injection of carrageenin in the mice induced an acute, time-dependent biphasic increase in paw edema with maximal swelling reached at 6 h, which is in agreement with response reported previously (Seibert et al., 1994; Salvemini et al., 1996; Speroni et al., 2005). Although the mean paw perimeter measurement at 5 h was higher than the mean perimeter measured at 3 h, the difference was not statistically significant (Fig. 5). Although a small dose of indomethacin was used (10 mg/kg), the inhibitory percentage of indomethacin on paw swelling increased from 25% at 2 h to 67% at 5 h (Table 2). Administration of 0.5 or 1 g/kg TCCT exerted inhibitory effect on the development of paw swelling also (Fig. 5), but only TCCT

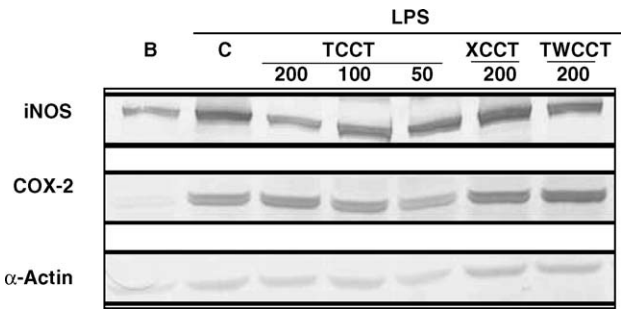

Fig. 4. Effects of the three CCTDs on iNOS and COX-2 expression by LPS-stimulated RAW 264.7 cells. B represents unstimulated cells and C represents solvent control. The concentration of three CCTDs were in μg/ml. Results are representative of three separate experiments.

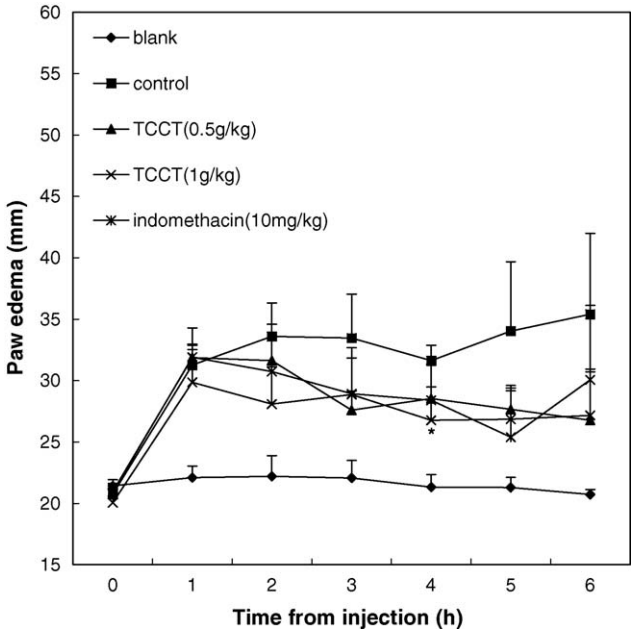

Fig. 5. Effect of TCCT on the paw perimeter of carrageenan-induced paw edema. Values represent the mean of five animals for each group. $^{*}P < 0.05$ is statistically significantly different from the control.

(1 g/kg) statistically and significantly reduced paw edema in the 4th hour after the carrageenan injection as compared to the control group. TCCT (1 g/kg) also significantly reduced the PGE_2 level in the serum of these mice when compared to the control group (data not shown). The results support the in vivo anti-inflammatory effect of TCCT.

3.4. Purgative activities of the three CCTDs

Shapeless stools, an increased number of fecal pieces or fecal stains, and a shortened time to the first excretion of the carbon-colored stool indicated the presence of diarrhea and increased propulsive activity of the large-intestine. Mice fed TCCT passed a greater number of shapeless stools, and an increased mean number of feces per animal. The time to the excretion of the carbon-colored stool was also fastest in mice fed TCCT. The above results suggest that TCCT had the strongest purgative activity among the three CCTDs (Table 3).

Table 2
Swelling inhibition percentage of different doses of TCCT and indomethacin on carrageenan-induced paw edema

Group	Time after injection of carrageenan (h)					
	1	2	3	4	5	6
TCCT (0.5 g/kg)	0	17.3	51.1	30.1	50.0	58.7
TCCT (1 g/kg)	15.3	48.2	40.4	47.2	56.3	56.1
Indomethacin (10 mg/kg)	0	25.1	39.8	31.4	67.8	36.4

All values are in percentage and the percentage of inhibition was calculated as described in Section 2.

Table 3
Purgative activities of TCCT, XCCT and TWCCT

Group	Onset of excretion (min)	Mean number of pieces of feces or stains	Form of feces
Control	125	1.0	B and C
TCCT	85	7.4	A and B
XCCT	110	5.8	B and C
TWCCT	103	3.0	A and B

A, shapeless stool with carbon color; B, formed stool with carbon color; C, formed stool with normal color.

4. Discussion

The three CCTDs are representative purgative formulas in TCMs, used when internal pathological heat is the etiology for producing symptoms like fever, abdominal pain and distention and constipation. However, literature discussing the mechanisms of expulsion of "internal heat" by TCM purgatives are limited. Nevertheless, at the turn of the 21st century, it is necessary to support these empirical theories with scientific investigations on the medicinal aspect of TCM in order to promote the safety and efficacy of TCM in maintaining the wellness of human beings in this modern world. In this survey, we studied the plausible anti-inflammatory activities of the three CCTDs, due to their applicability for treating diseases like acute appendicitis, acute cholecystitis and reducing inflammatory mediators. Specifically, the laxative effect and anti-inflammatory effect of three CCTDs on NO and PGE_2 production during in vivo and in vitro inflammatory conditions were investigated. The data suggested that the three CCTDs have different activity levels in terms of anti-inflammation and purgation, with TCCT simultaneously exhibiting the most anti-inflammatory and laxative effects. The findings indicate that among the three CCTDs, TCCT has anti-inflammatory effects in addition to its traditionally known purgative activities. From the in vitro results, the mechanism of TCCT in reducing paw edema might be due to its ability to inhibit NO and PGE_2 production. However, because many mediators are involved in the evolution of edema after carrageenan injection (Di Rosa et al., 1971; Salvemini et al., 1996), and purgative drugs might also reduce liquid in the edematous tissue without interfering any mediator or enzyme. The exact mechanism of action of TCCT in paw edema resolution is still not fully delineated at this point. In modern medicine, frequently used laxatives, such as sennosides and dulcolax do not have documented anti-inflammatory effects. Non-steroidal anti-inflammatory drugs (NSAIDs), the most frequently used anti-inflammatory agents, have no inhibitory effect on NO and have been reported to attenuate the laxative potency of sennosides (Yagi et al., 1988). Thus, the pharmacological effects of TCCT would be more similar to anti-inflammatory agents like diacerhein and its active metabolite rhein which do have laxative side effects, and inhibitory effect on NO production (Dougados et al., 2001; Wang et al., 2002b).

The herbs used to prepare the three CCTDs included root and bark of *Rheum palmatum*, the bark of *Magnolia officinalis*, the immature fruit of *Citrus aurantium*, *Mirabilitum* and root of *Glycyrrhiza uralensis*. *Rheum palmatum* was present in all three decoctions, and it is known as the principal constituent within these formulas. *Rheum palmatum* is a well-known laxative herb. Even though determination of the active components of medicines with mixtures of herbs is very complicated, using chemical constituents as reference standards helps to assure the quality of the herbal product and the validity of comparisons between studies using the same product. Moreover, major compounds identified in herbs or formulas may be helpful in delineating the underlying mechanisms. In this study, we identified the major compounds within the three CCTDs with HPLC. Anthraquinone derivatives including aloe-emodin, rhein and sennosides are the major constituents in *Rheum palmatum* (Wang et al., 2002b). Sennosides A and B are metabolized to rhein anthrone and rhein by the intestinal bacterial flora. Together, anthranoids affect large-intestine motility and absorption, resulting in a laxative effect (Van Gorkom et al., 1999), while *Mirabilitum* produces an osmotic effect in the intestines, resulting in a laxative effect. *Mirabilitum* was present in TCCT and TWCCT, but not in XCCT. In TCM, *Glycyrrhiza uralensis* is a herb that tonifies the spleen and qi (Mills and Bone, 2000). In many TCM formulations, *Glycyrrhiza uralensis* coordinates the action of a multiple-herb formula, including attenuating the stronger pharmacological effect of the principal herb. The milder laxative effect of TWCCT may be explained by the presence of *Glycyrrhiza uralensis*, as its presence may have attenuated the laxative effects of aloe-emodin, sennoside B and rhein, while the absence of *Mirabilitum* in XCCT could explain the milder laxative effect of XCCT than TCCT. Further investigations need to be conducted to delineate the complex interaction between the individual herbs constituents of these purgative formulas.

Rhein, a compound with a documented anti-inflammatory effect was identified in this study (Borderie et al., 2001; Wang et al., 2002b). Rhein is also the active metabolite of diacerhein, which is now being considered as a disease-modifying drug for osteoarthritis, as the drug has been shown to modulate many factors related to the pathogenesis of osteoarthritis (Tamura and Ohmori, 2001; Tamura et al., 2002). Magnolol has been found to exert an anti-inflammatory effect through inhibition of prostaglandin and leucotriene formation (Hsu et al., 2004), and other proinflammatory cytokines (Park et al., 2004). Naringenin has been found to suppress iNOS formation and NF-κB activation in RAW 264.7 cells activated by LPS (Tsai et al., 1999). Significant anti-inflammatory effects exhibited by TCCT may have been contributed by the combined effect of rhein, magnolol and naringenin.

Many diseases, including inflammatory diseases are not yet effectively controlled or treated by standard medical practice, and this has contributed to the increasing use of natural products in industrialized countries. Patients suffering from chronic inflammatory conditions, e.g. patients with rheumatoid arthritis or osteoarthritis have turned to the use of complementary and alternative medicines including herbal products to restore their health (Kaboli et al., 2001). This study will be helpful to health providers in making decisions about using purgatives for treating patients. Future clinical evaluations of the effectiveness of TCCT in alleviating inflammatory conditions in patients with concomitant constipation are also warranted.

Acknowledgments

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