Anti-nociceptive and anti-inflammatory activities of the extracts of *Stauntonia chinensis*

Chen Ying[1], Wu Ning[1], Liu Ying[1], Gao Hao[2], Dong Hua-Jin[1], Su Rui-Bin[1], Yao Xin-Sheng[2] and Li Jin[1]*

[1]Beijing Institute of Pharmacology and Toxicology, 27th Taiping Road, Beijing, PR China
[2]Institute of Traditional Chinese Medicine & Natural Products, College of Pharmacy, Jinan University, Guangzhou, PR China

Abstract: The aim of this investigation was to study the anti-nociceptive and anti-inflammatory activities of *Stauntonia chinensis* (*S. chinensis*) and the possible action mechanisms of effective fractions. The anti-nociceptive and anti-inflammatory activities of *S. chinensis* extracts, including the 60% EtOH extract (YMG), the *n*-BuOH extract (YMGB) and the aqueous residue (YMGW) of YMG, and the fractions from YMGB (YMGB1~YMGB7) were investigated by using the mouse acetic acid-induced writhing test and the rat formalin test. The effect of these extracts on the PGE_2 production was tested as well. In the mouse acetic acid-induced writhing test and the rat formalin test, YMGW and YMGB displayed anti-nociceptive and anti-inflammatory activities, suggesting that they were the active ingredients of YMG. Among the fractions isolated from YMGB, YMGB1, YMGB3, YMGB4 and YMGB6 were the main active ingredients producing anti-nociceptive activity and YMGB3, YMGB5, YMGB6 and YMGB7 were the main active ingredients producing anti-inflammatory activity. Additionally, YMGW, YMGB and its separations reduced the production of PGE_2, which might be the mechanism of them producing anti-inflammatory activity. These results demonstrated the active ingredients of *S. chinensis* producing anti-nociceptive and anti-inflammatory activities, which is valuable to validate the substance basis of *S. chinensis*'s pharmacological actions.

Keywords: *Stauntonia chinensis* DC; Antinociceptive activity; Anti-inflammatory activity; PGE_2 production

INTRODUCTION

Stauntonia chinensis DC (Lardizabalaceae) is a widespread medicinal plant traditionally used in China for a long time to treat several diseases (Jiangsu New Medical College, 1977). The crude extracts of *Stauntonia chinensis* (*S. chinensis*) are shown to have analgesic and sedative activities (Shanghai First Pharmaceutical Factory and Shanghai Zhong Hau Pharmaceutical Factory, 1976). Furthermore, it has been found that the crude extracts have many other pharmacological actions, such as anti-inflammation, nerve blocking, radio sensitization and so on. In addition, the preparations, such as tablets and injections of *S. chinensis* DC, have been widely applied for the treatments of rheumatism arthralgia, hyperostosis, trifacial and sciatic neuralgia clinically. Several phytochemical studies reveal that nortriterpenoid saponins (Wang *et al*., 1989a,b, 1990, 1991), lignan glycosides (Wang *et al*., 1989c, 1992a) and saponarin (Wang *et al*., 1992b) are the principal constituents of *S. chinensis* by ethanol extraction. In our previous studies on the chemical constituents of *S. chinensis*, a series of saponins were separated from YMGB6 and YMGB7, which were separated from *n*-BuOH extracts of *S. chinensis* and reported (Gao *et al*., 2007, 2008a,b, 2009). Furthermore, the findings on the chemistry and biology of *S. Chinensis* discovered hederagenin (13), the related aglycone, which was the derivative of the extract of YMGB7, but not saponin exhibited a significant anti-inflammatory activity (Gao *et al*., 2009). It is indicated that all the traditional uses of the herb are most likely related to anti-nociceptive and anti-inflammatory actions. However, up to date, there is rare report about the thorough study of the anti-nociceptive and anti-inflammatory activities of the extracts of *S. chinensis*. Thus it is worthwhile to confirm the anti-nociceptive and anti-inflammatory activities of the extracts of *S. chinensis* and to find a scientific substance basis for the traditional utilization of *S. chinensis*. In order to further reveal the pharmacological actions of *S. chinensis*, the present study evaluated the anti-nociceptive and anti-inflammatory activities of the extracts of *S. chinensis* in mouse acetic acid-induced writhing test and rat formalin test. Furthermore, the possible mechanism of several effective fractions producing anti-inflammation was investigated.

MATERIAL AND METHODS

Preparation of S. chinensis extracts

According to our previous report (Gao *et al.*, 2009), the extracts of *S. chinensis* were prepared. These extracts included the 60% EtOH extract (YMG), the *n*-BuOH extract (YMGB) and the aqueous residue (YMGW) of YMG, and the fractions from YMGB (YMGB1~ YMGB7).

**Corresponding author:* e-mail: jinli9802@163.com

Assessment of anti-nociceptive and anti-inflammatory activities of Stauntonia chinensis extracts

Animals and Drugs

Kunming mice and Sprague-Dawley (SD) rats obtained from Beijing Laboratory Animal Center (China) were used for experiments. Animals were kept in a room maintained under an ambient temperature of 22±1°C and relative humidity of 50-60%, with a 12h light-12 h dark cycle (lights on between 8:00 AM and 8:00 P.M.). They were supplied ad libitum with standard rodent food and water. All experiments were conducted in accordance with the US guidelines (NIH publication #85-23, revised in 1985) for laboratory animal use and care and the experimental protocol was approved by the Committee on Animal Care and Use of Beijing Insititute of Pharmacology and Toxicology Acetylsalicylic acid (AsA, white powder, purity N99%) was purchased from Alfa Aesar A Johnson Matthey Company.

Drug administration

The extracts of *Stauntonia chinensis* and AsA were dissolved in ethanol (480µl) and DMSO (360µl), then diluted in distilled water to 6ml. Drugs were administered in volume of 20ml/kg (po) in mice and rats. Distilled water contained 8% ethanol and 6% DMSO was administered as a vehicle control.

Mouse acetic acid-induced writhing test

This test consists in inducing nociception in mice by an i.p. injection of 0.6% acetic acid administration in a volume of 0.4ml in mice. The extracts of *Stauntonia chinensis* (500mg/kg), AsA (200mg/kg) and vehicle (20 ml/kg) were administered by oral route 40 min prior to acetic acid injection. The induced nociceptive behaviour is characterized by abdominal contractions known as writhing, described as an exaggerated extension of the abdomen combined with the outstretching of the hind limbs. The numbers of writhing were counted within 15 min after i.p. injection of acetic acid 5 min. The inhibition rate was calculated according to the following equation: Inhibition rate = (mean writhing number $_{vehicle}$− writhing number $_{tested\ compounds}$) /mean writhing number $_{vehicle}$×100% (Koster *et al*., 1959; Xu and Chen, 2003).

Next, the ED_{50} values of YMGB1, YMGB4, YMGB6 and YMGB7 were tested and calculated by the method of Bliss (probit regression analysis). This method maximizes the log-likelihood function to fit a parallel set of Gaussian sigmoid curves to the dose-response data, and ED_{50} values and 95% confidence intervals were provided.

Rat formalin test

Male Sprague-Dawley (SD) rats weighing 180-220g were placed in clear plastic chambers with a mirror placed at a 180° angle to allow an unobstructed view of the paws. The extracts of *Stauntonia chinensis* (400mg/kg), AsA (200mg/kg) and vehicle (20ml/kg) were administered by oral route 40min prior to the formalin injection. To induce nociception, rats were injected into the plantar surface of the right hind paw with 50µl of dilute formalin (5%) by using a 50-gauge needle. Immediately after, each rat was placed into a glass cylinder provided with mirrors to enable a total panorama of the nociceptive behaviour. All observations were carried out by a blinded investigator.

The first phase was obtained immediately after injection and lasted 10 min; this was known as early phase (neurogenic phase). A second period was observed 10–60min after formalin injection and denominated late phase (inflammatory phase). Nociceptive behavior was quantified as the number of flinches of the injected paw during 2 min periods every 10 min up to 60 min after injection. The area under the curve (AUC) for both phases was estimated, and a significant area reduction was interpreted as antinociception (Husnkaar and Hole, 1987). Percent of antinociception was calculated according to the following equation: Percent of antinociception = (mean AUC $_{vehicle}$− AUC $_{tested\ compounds}$) /mean AUC $_{vehicle}$×100%. Determination of the paw volume before and 60 min after administration was used for assessment of anti-inflammation (Ji *et al*., 2006; Amaury *et al*., 2006). The dose-response curves of YMGW, YMGB5 and YMGB6 were tested in the rat formalin test.

Assessment of the PGE_2 production

Materials

The human colon adenocarcinoma cell line HT-29 was purchased from Chinese Academy of Medical Science, Institute of Basic Medical Cell Center. Fetal bovine serum was purchased from Hyclone-pierce (Hyclone 1,South Logan, UT, USA). Cell culture reagents were purchased from Gibco Incorporation. TNF-α Arachidonic acid (AA) were purchased from sigma Chemical Co. (St. Louis, MO). PGE_2 ELISA kit was purchased from Cayman Chemical Co. All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO), unless otherwise stated.

Cell culture

HT-29 cells were cultured in DMEM/F12 medium supplemented with 10% fetal bovine serum, 100U/ml penicillin and 100U/ml streptomycin at 37°C with humidified atmosphere consisting of in 95% air and 5% CO_2.

Drug preparation

The stock solutions (2.5mg/ml) of *Stauntonia chinensis* extracts were prepared by dissolving it in 10µl dimethyl sulfoxide (Sigma Chemical Co., St. Louis, MO) and diluted with 9.99 ml DMEM/F12 and stored at 4°C.

Prostaglandin E_2 (PGE_2) production in HT-29 cells

PGE_2 is a major product produced by COX-2 from arachidonic acid and is often used to acsess COX-2

activity in cells (Vaccarino & Melzack, 1992). To inactivate COX-1, HT-29 cells (4×10^3 cells/well) were pre-treated with 100μmol/L AsA for 10h. Then the cells were washed with PBS for three times. After that, the cells were incubated with TNF-α 50μg/L for 5h. Then the cells were treated with fraction samples (100μg/ml) for 30 min after washed by PBS for three times. After treatment, the test solutions were removed and replaced with 100μmol/L arachidonic acid diluted in 0.1% DMEM/F12 for 10 min. The PGE_2 level in the cell supernatants was measured using a commercial ELISA kit (Cayman) according to the manufacturer's instructions.

DATA ANALYSIS

The data were expressed as mean ± standard error of the mean (SEM). All data were compared by one-way ANOVA followed by Dunnett's t-test. $P<0.05$ was considered statistically significant.

RESULT

Anti-nociceptive activity of S. chinensis extracts in the mouse acetic acid-induced writhing test

The first study was carried out to investigate the anti-nociceptive activities of all of the extracts from *Stauntonia chinensis* in the mouse acetic acid-induced writhing test. We found the vine-stem extract of *S. chinensis* (YMG) exhibited notable anti-nociceptive activity and both the n-BuOH extract of YMG (YMGB) and the aqueous residue (YMGW) produced significant anti-nociception, in which the anti-nociceptive percentage were 36.5% and 53.4%, respectively. This result suggested that both YMGW and YMGB were the active ingredients of YMG producing anti-nociceptive activity. Among the fractions isolated from YMGB, all the fractions except YMGB2 and YMGB5 administered orally at the dose of 500mg/kg produced significant anti-nociceptive activities (fig. 1).

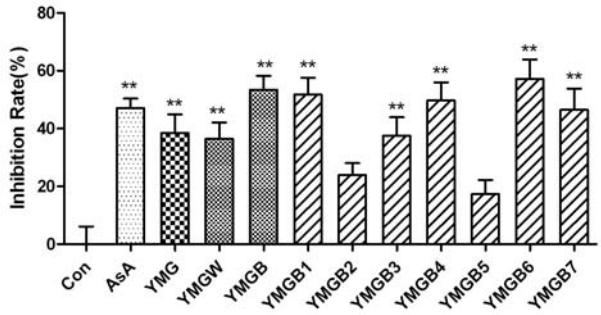

Fig. 1: Anti-nociceptive activity of *Stauntonia chinensis* extracts in the mouse acetic acid-induced writhing test. Mice were pretreated orally with ASA (200mg/kg), *Stauntonia chinensis* extracts (500mg/kg) or vehicle (20ml/kg) for 40 minutes prior to acetic acid application (0.6%, i.p.). The numbers of writhing were counted within 15 min after injection of acetic acid 5 min. Each data point represents the mean ±SEM. n=20. **P*<0.05, ***P*<0.01, compared with control group, one-way ANOVA followed by Dunnett's t-test.

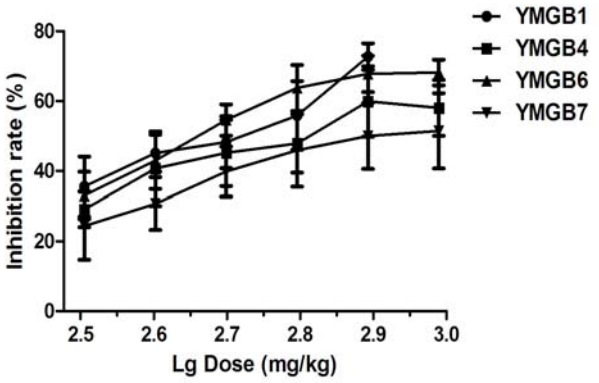

Fig. 2: Dose-response curves of the anti-nociceptive effect of YMGB1, YMGB4, YMGB6 and YMGB7 (320-976 mg/kg, p.o) in the acetic acid-induced writhing test. Mice were pretreated orally with YMGB1, YMGB4, YMGB6 and YMGB7 (320-976 mg/kg) for 40 minutes prior to acetic acid application (0.6%, i.p). The numbers of writhing were counted within 15 min after injection of acetic acid 5 min. Each data point represents the mean ±SEM. n=10.

Dose-dependent anti-nociceptive activity was observed at the tested dose levels. YMGB1, YMGB4, YMGB6 and YMGB7 (from 320 to 976 mg/kg, p.o) showed significant anti-nociceptive activities in dose-dependent manner (fig. 2). The anti-nociceptive efficiencies of YMGB1, YMGB4, YMGB6 and YMGB7 were 72.7%, 58.0%, 68.2% and 51.5%, respectively. The above four fractions showed the similar anti-nociceptive potency, of which the ED_{50} values were near to 350 mg/kg (table 1).

Table 1: The ED_{50} values of *Stauntonia chinensis* extracts in the acetic acid-induced writhing test (n=10).

Compounds	ED_{50} (mg/kg)	95% Confidence interval (mg/kg)
YMGB1	346	303.9~384.0
YMGB4	346	215.6~420.5
YMGB6	336	299.7~365.7
YMGB7	360	322.8~389.3

Anti-nociceptive and anti-inflammatory activities of S. chinensis extracts in the rat formalin test

Compared with the vehicle control, YMG, the primary extract of *S. chinensis*, displayed significant anti-nociceptive activity against formalin-induced paw withdrawal in both the early and late phases and anti-inflammatory activity against formalin-induced paw edema (fig. 3). Both YMGW and YMGB showed significant anti-nociceptive activities in either early or late

phases, but the anti-nociceptive activity of YMGW was stronger than that of YMGB at the same dosage of 400 mg/kg (p.o.) (figs. 3A1, 3B and 3C). In addition, YMGW significantly attenuated the formalin-induced paw edema and YMGB showed a tendency (fig. 3D), suggesting that YMGW was the main active ingredients of YMG reducing inflammatory reaction. Among the fractions isolated from YMGB, all the fractions except YMGB7 (400mg/kg, po.) produced significant anti-nociceptive activity (fig. 3A2-A3 and fig. 3B), suggesting that these six fractions were the active ingredients of YMGB producing analgesia. Furthermore, all the fractions except YMGB2 (400mg/kg, po.) caused a significant analgesia in the early phase, while YMGB1, YMGB3 and YMGB5 caused significant analgesia in the late phase (fig. 3C). In the mean time, YMGB3, YMGB5, YMGB6 and YMGB7 showed significant anti-inflammatory activity, compared with the vehicle control in the paw volume change value (fig. 3D).

We chose YMGW, YMGB5 and YMGB6, which have better anti-nociceptive activities or anti-inflammatory activities to further test their dose-response curves. The anti-nociceptive and anti-inflammatory activities of YMGW showed dose-dependent manners from 100 to 800mg/kg (fig. 4). Additionally, the anti-nociceptive and anti-inflammatory activities of YMGB5 and YMGB6 were dose-dependent from 200 to 800mg/kg (figs. 5 and 6).

Attenuation of S. chinensis extracts to the PGE_2 production

PEG_2, generated by inducible COX-2, is an important mediator to induce inflammatory processes, therefore, the effects of *S. chinensis* extracts on the PGE_2 production were observed. HT-29 cells were pretreated with AsA to inactivate COX-1, and then the cells were incubated with TNF-α to induce COX-2 over-expression. The results showed that YMGW (100μg/ml, the safe concentration on HT-29 cell in MTT assay, data was not shown) significantly reduced the PGE_2 content produced by COX-2.and YMGB showed a tendency (fig. 7). Among the fractions isolated from YMGB, YMGB3, YMGB4, YMGB5, YMGB6 and YMGB7 (100μg/ml) were also able to significantly reduce the production of PGE_2 (fig. 7). These results suggested that inhibiting PGE_2 production might be the mechanism of these extracts producing anti-inflammatory actions.

DISCUSSION

The outcome of the present research demonstrated that *S. Chinensis* produces a significant anti-nociceptive effects in different kinds of induced pain in rodents. Some studies on the chemical constituents of *S. Chinensis* has reported, the proportions of YMGB6 and YMGB7 that rich in saponins were hardly half of the contents of extracts from YMGB (YMGB1 ~YMGB7). Previous studies indicated that a series of saponins were separated from YMGB6 and YMGB7 reported (Gao *et al*., 2007, 2008a,b, 2009). The results herein showed that YMGB1, YMGB3, YMGB4, YMGB5, YMGB6 and YMGB7 produced anti-nociceptive and anti-inflammatory activities in both of the mouse acetic acid-induced writhing test and the rat formalin test. It indicated that constituations of Saponins and non-saponins are most likely contributed to the antinociceptive and anti-inflammatory actions.

It is important to mention that pain and inflammation are associated with many pathophysiological processes of various clinical conditions like arthritis, cancer and vascular diseases (Suffness and Pezzuto, 1991; Mukherjee, 2003). The findings of our present study indicate that the extracts of *S. chinensis* possess significant anti-nociceptive and anti-inflammatory activities in the mouse acetic acid-induced writhing test and the rat formalin test. An intraperitoneal injection of acetic acid can produce peritoneal inflammation (acute peritonitis), which has been associated with increased levels of prostaglandins in the peritoneal fluids (Deraedt *et al*, 1980). Formalin-induced paw pain produces a distinct biphasic nociception, both of an early and late phase is due to the release of peripheral mediators like serotonin, histamine, bradykinin, prostaglandins and at least to some degree, the sensitization of central nociceptive neurons. (Huang *et al*., 2011a,b). The results herein revealed that both the aqueous and n-BuOH extracts of YMG (YMGW and YMGB) were the active ingredients of *S. chinensis* DC. producing anti-nociceptive and anti-inflammatory activities in these two inflammatory pain models, the mouse acetic acid-induced writhing test and the rat formalin test. Furthermore, the fractions of YMGB1, YMGB3, YMGB4 and YMGB6 were the main active ingredients of YMGB producing anti-nociceptive activity, and the fractions of YMGB3, YMGB5, YMGB6 and YMGB7 were the main active ingredients of YMGB producing anti-inflammatory activity.

Previous studies show that the crude extracts of *S. chinensis* DC. have analgesia, the mechanisms of which might be related to anti-nociceptive stimulus, anti-inflammation and nerve blocking (Shanghai First Pharmaceutical Factory and Shanghai Zhong Hau Pharmaceutical Factory, 1976). In the formalin test, YMG, YMGW, YMGB and all the fractions from YMGB except YMGB2 caused a significant analgesia in the early phase, which might be due to the nerve blocking actions. In the late phase, YMG, YMGW and YMGB, and the fractions of YMGB1, YMGB3 and YMGB5 produced significant anti-nociceptive activities, which might be due to the inhibition to the inflammatory response. This is consistent with our results that YMG, YMGW and

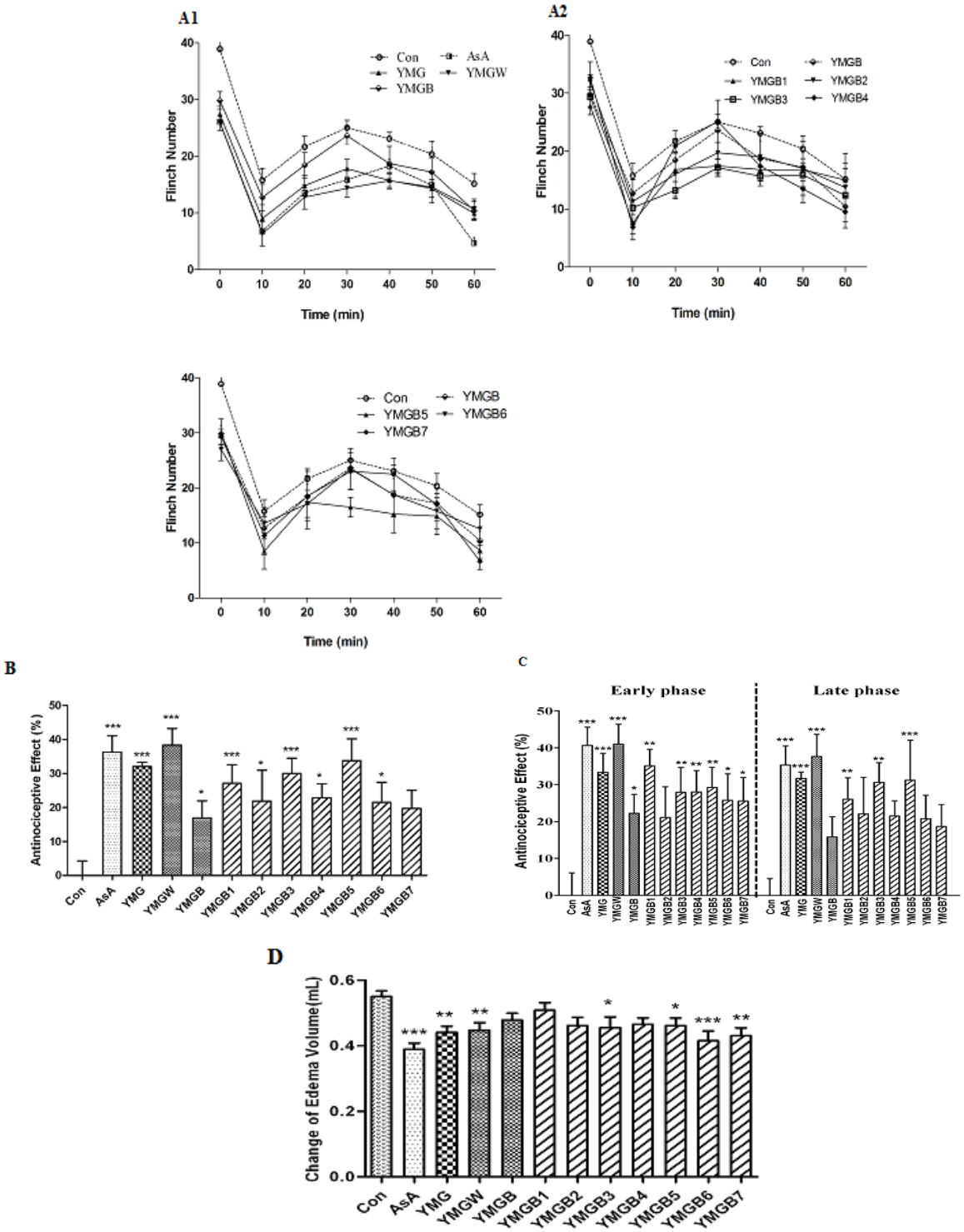

Fig. 3: Anti-nociceptive and anti-inflammatory activities of *Stauntonia chinensis* extracts in the rat formalin test. Rats were pretreated orally with AsA (200 mg/kg), *Stauntonia chinensis* extracts (400mg/kg) or vehicle (20ml/kg) for 40 minutes prior to formalin (5%, 50 µl/rat) application. And then, the nociceptive behavior was observed for 60 min. A1, A2 and A3: time- response curves of antinociception, both of the vehicle control and aspirin groups were the same in A1, A2 and A3. B: anti-nociceptive effects (%). C: anti-nociceptive effect (%) (early phase and late phase divided). D: anti-inflammatory effects. Each data point represents the mean±SEM, n=6-8. **P*<0.05, ***P*<0.01, ****P*<0.01, compared with control group, one-way ANOVA followed by Dunnett's t-test.

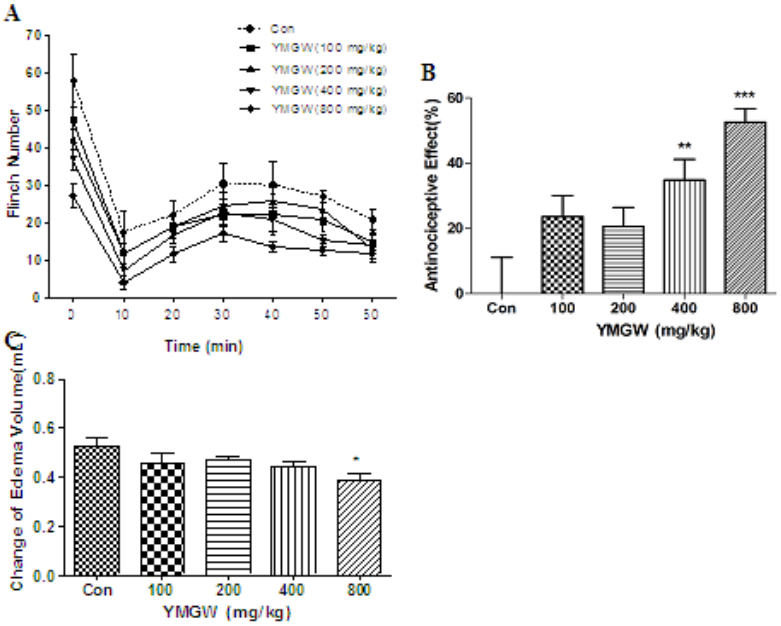

Fig. 4: Dose-dependent manner of YMGW on the anti-nociceptive and anti-inflammatory activities in the rat formalin test. Rats were pretreated orally with YMGW at the dose of 100, 200, 400 and 800 mg/kg or vehicle (20 ml/kg) for 40 minutes prior to formalin (5%, 50 µl/rat) application. And then, the nociceptive behavior was observed for 60 min. A: time- response curve of anti-nociception. B: anti-nociceptive effect (%). C: anti-inflammatory effect. Each data point represents the mean ±SEM, n=6. **P*<0.05, compared with control group, one-way ANOVA followed by Dunnett's t-test.

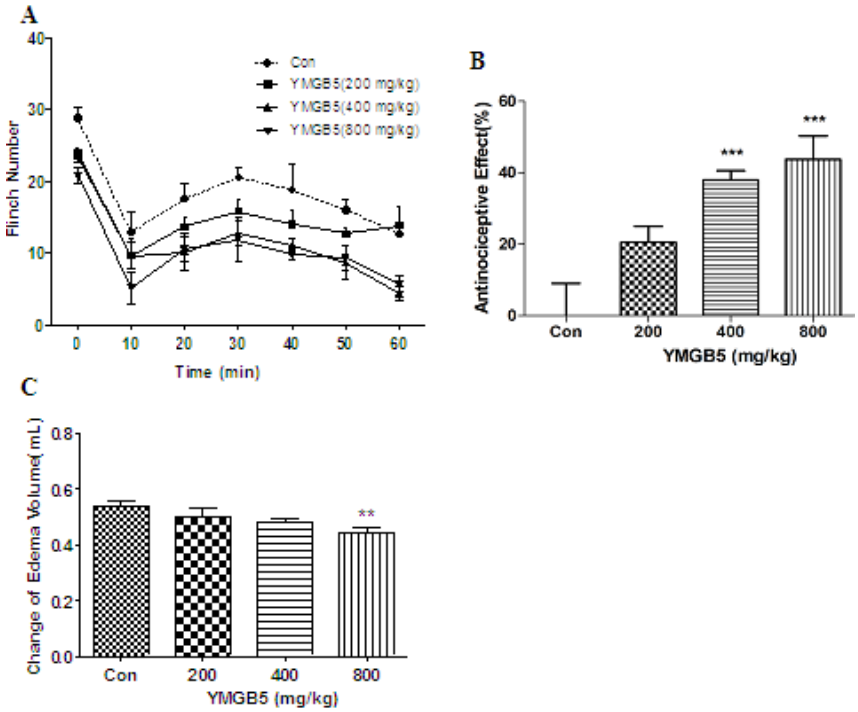

Fig. 5: Dose-dependent manner of YMGB5 on the anti-nociceptive and anti-inflammatory activities in the rat formalin test. Rats were pretreated orally with YMGB5 at the dose of 200, 400 and 800 mg/kg or vehicle (20ml/kg) for 40 minutes prior to formalin (5%, 50µl/rat) application. And then, the nociceptive behavior was observed for 60 min. A: time-response curve of anti-nociception. B: anti-nociceptive effect (%). C: anti-inflammatory effect. Each data point represents the mean ±SEM, n=6. **P*<0.05, ***P*<0.01, compared with control group, one-way ANOVA followed by Dunnett's t-test.

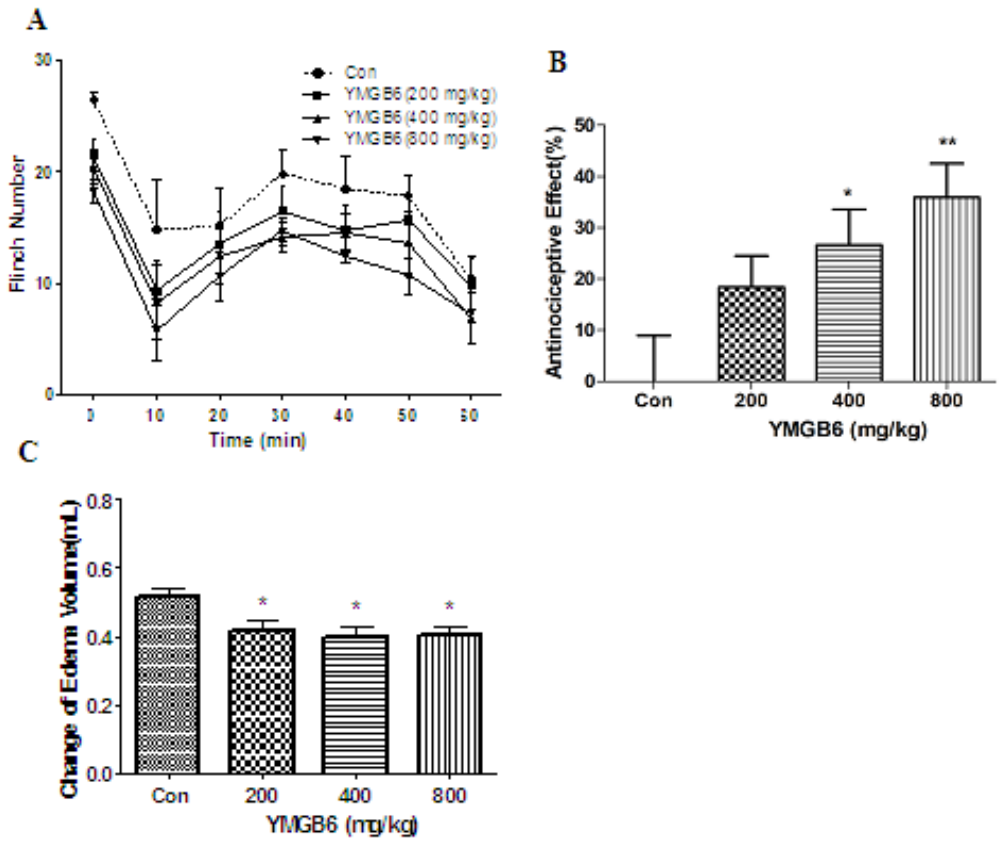

Fig. 6: Dose-dependent manner of YMGB6 on the anti-nociceptive and anti-inflammatory activities in the rat formalin test. Rats were pretreated orally with YMGB6 at the dose of 200, 400 and 800 mg/kg or vehicle (20 ml/kg) for 40 minutes prior to formalin (5%, 50 µl/rat) application. And then, the nociceptive behavior was observed for 60 min. A: time-response curve of anti-nociception. B: anti-nociceptive effect (%). C: anti-inflammatory effect. Each data point represents the mean ±SEM, n=6. **P*<0.05, ***P*<0.01, compared with control group, one-way ANOVA followed by Dunnett's t-test.

YMGB, and the fractions of YMGB3, YMGB5, YMGB6 and YMGB7 had significant anti-inflammatory activity of the attenuation to the formalin-induced paw edema.

However, the active ingredients of *S. chinensis* DC. producing analgesia in the mouse acetic acid-induced writhing test is not fully consistent with those obtained in the rat formalin test. For example, YMGB5 shows better anti-nociceptive activity in the rat formalin test, whereas it was ineffective in the mouse acetic acid-induced writhing test. This might be due to the different mechanisms generated from the pain of these two models. The mouse acetic acid-induced writhing response is considered as visceral inflammatory pain model, while the rat formalin test is mainly of neurogenic phase and inflammatory phase, which occurs through the activation of the ventral horn neurons at the spinal cord level. Additionally, the anti-nociceptive and anti-inflammatory activities of one fraction is not entirely consistent. In the rat formalin test, YMGB1 showed a better anti-nociceptive effect, but it rarely had anti-inflammatory activity; and YMGB7 had a stronger anti-inflammatory activity, but its anti-nociceptive activity was weaker. The above results indicated that inhibiting the inflammatory response may be one of the anti-nociceptive mechanisms of *S. chinensis* extracts.

Based on the evidence that the extracts of *S. chinensis* had inhibitory actions on the acetic acid- and formalin-induced inflammation and pain, we investigated the preventive efficacy of extracts of *S. chinensis* to the production of PGE_2 using the HT-29 human colon cancer cell model, in which COX-2 is highly expressed (Shao *et al*., 2000). During inflammatory processes, large amounts of the inflammatory mediators, such as PGE_2, is generated by inducible COX-2. (Salvemini *et al*., 2003). COX-2 is induced by pro-inflammatory stimuli, including TNF-α, in cells *in vitro* and in inflamed sites *in vivo* (Fang J.Q., 2002). In recent years, non-steroidal anti-inflammatory drugs targeting at inducible COX-2 can inhibit colorectal tumor cell proliferation and reverse its precancerous lesions, and COX-2 enzyme is over expression in colorectal tumors and leads to prostate hormone levels increased (Chen *et al*., 2001). In this study, there is a

significant decrease in PGE_2 production with the treatment of the extracts of *S. chinensis* in HT-29 cells (fig. 7). There was certain correlation between the decrease in PGE_2 production and the anti-inflammation of the *S. chinensis* fractions. The fractions of YMGB3, YMGB5, YMGB6 and YMGB7 displayed significant anti-inflammatory activities in the formalin-induced paw edema, and these four fractions reduced PGE_2 production as well. Additionally, YMGB1 had no effect on the the formalin-induced paw edema, and it also failed to decrease PGE_2 production. These indicated that the anti-inflammatory activity of *S. chinensis* extracts was related to the reduction of PGE_2 production.

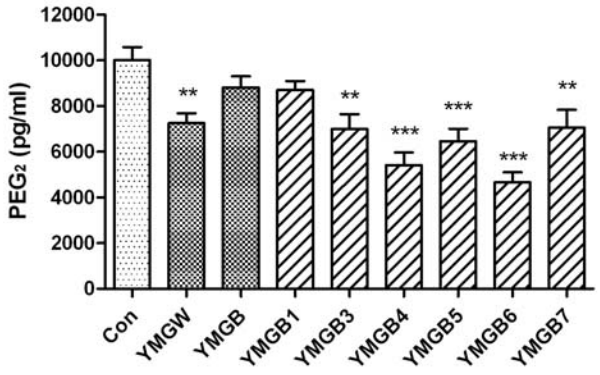

Fig. 7: Reduction of *Stauntonia chinensis* extracts to the PGE_2 production. HT-29 cells were stimulated with TNF-α (50μg/L) for 5h and pretreated with *Stauntonia chinensis* extracts for 30 min before exogenous arachidonic acid was added. After 10 min, the amount of PGE_2 production in the supernatant was assayed. Each bar represents the mean ± SEM of five experiments. $^{**}P<0.01$, $^{***}P<0.001$ compared with control group, one-way ANOVA followed by Dunnett's t-test.

CONCLUSIONS

In conclusion, it is to our knowledge the first study demonstrating that both the aqueous and n-BuOH extracts of YMG (YMGW and YMGB) were the active ingredients of *S. chinensis* producing anti-nociceptive and anti-inflammatory activities. Furthermore, the fractions of YMGB, such as YMGB1, YMGB3, YMGB4 and YMGB6 were the main active ingredients of YMGB producing anti-nociceptive activity, and the fractions of YMGB3, YMGB5, YMGB6 and YMGB7 were the main active ingredients of YMGB producing anti-inflammatory activity. The reduction of PGE_2 production contributed to the anti-inflammatory activity of *S. chinensis* extracts. These results demonstrated the active ingredients of *S. chinensis* producing anti-nociceptive and anti-inflammatory activities, which is valuable to validate the substance basis of *S. chinensis*'s pharmacological actions.

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