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Comparative Analgesic and Anti-Inflammatory Effect of *Salviae miltiorrhizae* Pieces and its Products in Mice

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ABSTRACT

In this paper, three decoctions of different Danshen medication forms (crude SM pieces, SM formula granule, liquored SM pieces) were compared to explore their comparative analgesic and anti-inflammatory effect. In three mice models, the hot plate test and the writhing test were used to analyze their analgesic effect, and the ear edema test was carried out to assess their anti-inflammatory effect. Compared with the corresponding model control group, the results suggested that the three different SM decoctions all had definite effect in prolonging the latency to licking a hind paw or jumping, reducing the writhing rate within 30 min and narrowing the deviation between the weight of ears. Furthermore, liquored SM pieces and SM formula granule had better analgesic and anti-inflammatory effect than crude SM pieces and no obvious differences appeared between liquored SM pieces and SM formula granule.

Keywords- Analgesic effect, Anti-inflammatory effect, *Salviae miltiorrhizae*.

INTRODUCTION

The traditional Chinese medicine Danshen, derived from the dry root of *Salviae miltiorrhizae* Bge (SM) (Fig 1), has been widely applied to the treatment of irregular menstruation, algomenorrhea, angina pectoris, hepatauxe, etc[1-3]. In recent years, aqueous Danshen extract has been documented to possess analgesia and anti-inflammatory properties *in vitro*[4,5]. Many different medication forms for SM are used in clinic, including crude SM pieces, SM formula granule, liquored SM pieces, SM tablet and SM capsule, etc. Moreover, different forms are often used interchangeably. In recent years, SM formula granule is widely used in all medication forms. However, no document suggested the different pharmacological activities of these SM medication forms. In order to explore the comparative analgesic and anti-inflammatory effect of *Salviae*

miltiorrhizae pieces and its products, crude SM pieces, SM formula granule and liquored SM pieces were compared by carrying out hot plate test, writhing test and ear edema test.

MATERIALS AND METHODS

Reagent and animals

Crude SM pieces, SM formula granule, liquored SM pieces were purchased from Tianjiang pharmaceutical Co., Ltd. (China, Jiangyin). Chemical reagents used in the study are analytically pure. Aspirin was acquired from Dayang Pharmaceutical Technology Corporation (Beijing, China. Kunming mice were offered by Shandong University of TCM. Hot plate was from Beijing gene and life science Corporation (China).

Decoction preparation

Crude SM pieces and its liquored SM pieces were prepared into decoction in accordance with the Chinese Pharmacopoeia. *Viz*, SM pieces were powered and extracted via reflux with water for 3h. Then the filtrate of the extract was concentrated to 0.2 g·mL^{-1} crude drug. SM formula granule were heated and dissolved in water to a final concentration of 0.2 g·mL^{-1} crude drug.

Hot plate test

The hot plate test is assessed the effect of analgesic by measuring reaction time[6]. When mice were placed on a heated surface, they would perform a specified behavior (hind paw-licking or jumping) in response to painful thermal stimuli. In the test, fifty mice were randomly and averagely divided into positive control group (aspirin), negative control group (normal saline), SM pieces group, SM formula granule group and liquored SM pieces group. Before the experiment, Kunming mice were selected for the reaction time between 5 to 30 s. The hot plate was kept at a temperature of 55 ± 0.1 °C. The reaction time for mouse was recorded before and 60, 120 and 180 min after the intragastric administration of the medicine (0.015 mL g^{-1} body) or normal saline (0.015 mL g^{-1} body) respectively.

Writhing test

The writhing test is a chemical visceral pain model by measuring the effect of analgesic activity[7]. Each mouse was injected intraperitoneally with 0.2 ml acetic acid solution (0.7%, v/v) half an hour after the intrathecal administration of the drugs (0.015 mL g^{-1} body) or normal saline (0.015 ml g^{-1} body). Five minutes later, mice were individually placed into glass cylinders and their abdominal muscles contracted with a stretching of the hind limbs. The effect of the analgesic was expressed by the number of writhes observed by each mouse for 30 min.

Ear edema test

Anti-inflammatory activity was assessed by conducting ear edema test as described by Gad[8]. Groups of mice were given the intraperitoneal administration of the drugs (0.2 mL) or normal saline (0.2 mL). Half an hour later, 0.2 mL of xylene was spread on the surface of left ear of each mouse under anaesthesia, while the right ear was considered as control. Four hours after the intraperitoneal administration, all mice were sacrificed and both ears were cut off along their ears baseline. Ear swelling inhibition was determined by the formula:

$$\text{Ear swelling inhibition} = \frac{\text{A-B}}{\text{A}} \times 100$$

Where A and B denotes ear swelling of the negative group and the drug groups, respectively.

Statistic analysis

Each experiment was repeated thrice, and all data were expressed as mean ± standard deviation (SD) and analyzed by

SPSS11.0 statistical software. Statistical significance was determined by ANOVA followed by student's *t*-test. *P* value of less than 0.05 was considered statistically significant.

RESULTS

Effect of SM decoction on the hot plate response

The results indicated that the SM decoction prolonged the reaction time for nociception above the control value from the beginning to 90 min obviously. In a series of SM decoction, SM formula granule decoction is superior to the crude SM pieces decoction, but no significance occurred in the experiment (Fig.2).

Effect of SM decoction on the writhing response

Compared with the negative control, the SM formula granule decoction extended the latency and reduced the number of writhes apparently, and the aspirin was the best analgesic drug among the six groups. The experiment data also showed that the SM formula granule decoction possessed better analgesic effect than crude SM decoction (Fig. 3).

Effect of SM decoction on ear edema response

In the ear edema experiment, the edema extent of negative control is serious, but the data of the SM decoction groups is relative lighter. Compared with the crude SM decoction, the SM formula granule decoction had a better effect in the aspect of anti-inflammatory (Fig. 4).

DISCUSSION

Salviae miltiorrhizae Bge, a traditional Chinese medicine, has the function of extending blood vessel, suppressing platelet aggregation, improving cerebral circulation and relieving pain[9-11]. In this experiment, the results testified that three SM decoctions possessed obviously analgesic and anti-inflammatory activities. However, their pharmacological mechanisms still remain unclear. It documented that salvianolic acid A and tanshinone may be the effective chemical constituents for analgesic and anti-inflammatory[12,13]. Our results also showed that SM formula granule and liquored SM pieces had better analgesic and anti-inflammatory effect than crude SM pieces, and the highest activity was SM formula granule, but no statistic significance occurred amid the three medicinal agents described above. Our experiments partly explained the clinical significance for the preparation of SM. For the liquored SM, liquored process can increase the amount of dissolution of the active ingredients for tanshinone, and thereby enhance its effect[14-17]. While SM formula granule decoction showed the highest pharmacological activities, because SM formula granules were carried out the serial processing, such as extraction, concentration, separation, drying and granule process, resulting in the high concentration of the active ingredients and improved medicinal property[18].

CONCLUSION

Although SM possess analgesia and anti-inflammatory properties, our study preliminary confirmed the fact that SM formula granule is superior to the crude SM pieces in clinic for analgesic and anti-inflammatory.

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Figure 1. The original plant of *Salvia miltiorrhiza* Bge

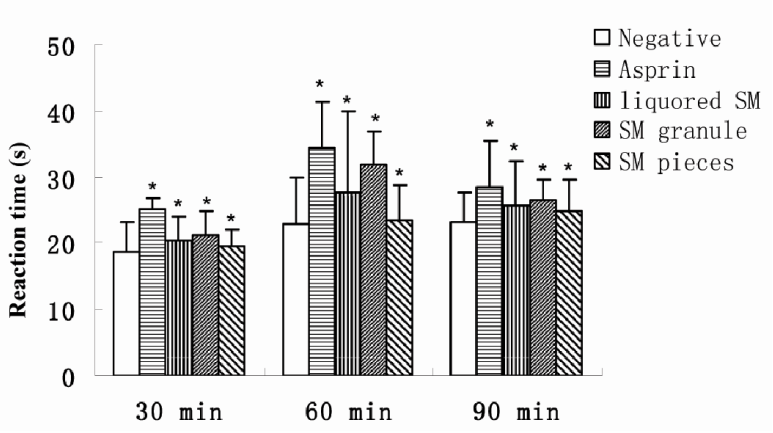

Figure 2. Effect of SM decoction on the hot plate response. Ordinate and abscissa represent reaction and measuring time. Results were expressed as means ± SD for the groups of 10 mice. Each experiment was performed thrice independently. *P<0.05 compared to the negative control as determined by the Student's t-test

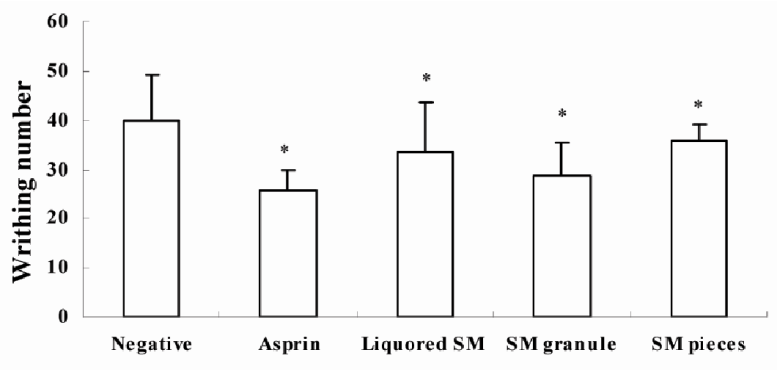

Figure 3. Effect of SM decoction on the writhing response. Ordinate represents number of writhing and abscissa represents medication. Results were expressed as means ± SD for the groups of 10 mice. Each experiment was performed thrice independently. *P<0.05 compared to the negative control as determined by the Student's t-test

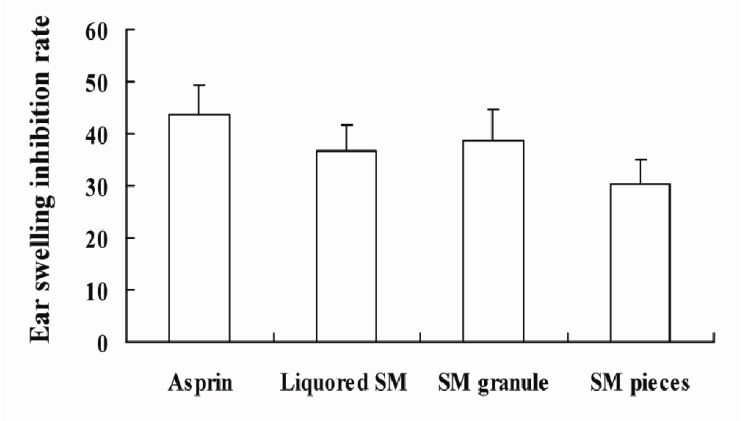

Figure 4. Effect of SM decoction on ear edema response. Ordinate and abscissa represent ear swelling inhibition rates and medication, respectively. Results were expressed as means ± SD for the groups of 10 mice. Each experiment was performed thrice independently. *P<0.05 compared to the negative control as determined by the Student's t-test