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EVALUATION OF ANALGESIC, ANTI-INFLAMMATORY AND ANTIPYRETIC ACTIVITIES OF THE ETHANOL EXTRACT FROM *SPERANSKIA TUBERCULATA*

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Abstract

Background: *Speranskia tuberculata* (Bunge) Baill, has been used to prevent and treat many diseases in Chinese folk medicine, nevertheless, few investigations had been reported.

Materials and Methods: Animals were orally administered STE at the doses of 125, 250, 500 mg/kg. The analgesic effect was estimated in mice by hot-plate test and the acetic acid-induced writhing test. The anti-inflammatory effect was assessed using rat paw edema model elicited by fresh egg white and the mouse ear edema model caused by dimethylbenzene. The antipyretic effect was determined using the lipopolysaccharide (LPS)-induced rat fever model. In addition, the acute oral toxicity of STE was studied.

Results: STE significantly and dose-dependently reduced the number of writhing responses in mice, prolonged reaction time of mice against heat stimulation, depressed egg white-induced paw edema in rats and the dimethylbenzene-caused ear edema in mice, but did not alleviate LPS-induced pyrexia in rats. No death of mice was observed when orally administered STE up to 52.8 g/kg (approximately 2080 times of clinical dose used).

Conclusion: STE possesses evident analgesic and anti-inflammatory activities, but has no antipyretic effect. Furthermore STE has a favorable safety. These findings support the applications of *Speranskia tuberculata* as an analgesic and anti-inflammatory drug in folk medicine.

Key Words: Herb, pain, inflammation, Pyrexia, Safety

Introduction

Speranskia tuberculata (Bunge) Baill. (Euphorbiaceae) is a medicinal herb widely distributed in Liaoning, Jilin, Henan, Hebei, Shanxi and other provinces in China. In Chinese folk medicine, the whole plant has been used to prevent and treat arthralgia due to wind-dampness, beriberi caused by cold-wetness evil and pyogenic infections because of its good efficacy for expelling wind and dampness, relaxing muscles and joints, promoting blood circulation, and relieving pain. The chemical composition of this plant includes β-sitosterol, 18-hydroxy(-)-manool (Li et al., 2000) and alkaloids (Shi et al., 2000). Its aqueous extract is an effective anti-mitotic microtubule-binding agent, which significantly inhibits the growth of human breast carcinoma MDA-MB-231 cells (Mazzio et al., 2014). To our knowledge, the analgesic, anti-inflammatory and antipyretic effects of this plant have not been reported so far. In the present study, the above mentioned effects of the ethanol extract of *Speranskia tuberculata* (STE) were evaluated in animals to substantiate and expand its clinical applications. In addition, the acute oral toxicity of STE was determined.

Materials and Methods

Plant material and extraction

The whole plant was collected from Shenyang city in Liaoning Province in September 2013. The identification was performed by Professor

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Han-Ming Zhang, a botanist from the Department of Pharmaceutical Botany, School of Pharmacy, Second Military Medical University. A voucher specimen (YZ130901) was deposited in the Herbarium of the Department of Pharmaceutical Botany, School of Pharmacy, Second Military Medical University. The dried plant (500 g) was pulverized and the refluxing extraction was carried out with 8 L 70% (v) ethanol at 75°C two times for 1 hr each time. After filtration, the extract was concentrated under reduced pressure and dried at 50°C for *in vivo* evaluation. The yield was 10.13%.

Animals and treatment

ICR mice (20–25 g) and male Wistar rats (200–220 g) were purchased from the Experimental Animal Center of the Second Military Medical University (Shanghai, China) and housed in a regulated environment (20 ± 2°C), with a 12-hr light/dark cycle (08:00–20:00, light). The animals were allowed free access to feed and water. Each experimental group consisted of 10 animals.

The ethanol extract of *Speranskia tuberculata* (STE), aspirin, indomethacin (IMC), dexamethasone (DEX), paracetamol (PAE) and tramadol (TRA) were respectively dissolved in distilled water prior to administration. Three groups of animals orally received 125, 250, and 500 mg/kg STE, respectively. The positive control group was given the corresponding positive drug, that is, aspirin (100 mg/kg) IMC (10 mg/kg), DEX (5 mg/kg), PAE (100 mg/kg) and TRA (30 mg/kg). Another group (negative control) was given the same volume of distilled water. The evaluation was performed after 1 hr of oral administration.

Analgesic test

In the writhing test (Marzouk et al., 2011; Mahmoudi et al., 2013; Zhu et al., 2011), male mice were intra-peritoneally injected with 1% acetic acid (10 ml/kg) after 1 hr of oral administration of STE or IMC. The number of abdominal constrictions was counted for 15 min.

In the hot plate test (Kesim et al., 2012; Ma et al., 2011; Saha et al., 2013), the temperature of the hot plate was kept at 55±0.5ºC and the latency time was recorded through observation of either licking hind paws or jumping movements to avoid heat injuries. A day before the experiment, the pretreatment latencies were determined and the mice with only initial nociceptive response between 5-30 s were selected for experiment. After 30, 60, 90 and 120 min of oral administration of STE or TRA, the latency time of mice was recorded. The cut-off time was set to 60 s against thermal injuries.

Anti-inflammatory test

In the ear edema test (Zhu et al., 2011), mice received STE or DEX before 1 hr of application of dimethylbenene (40 µl/ear) in the right ears. The ear swelling was evaluated through recording the weight difference between the left ear and the right ear after 2 hr of drug treatment.

In the rat paw edema test (Yu et al., 2012). Acute inflammation was induced through subplantar injection of 0.1 ml of freshly prepared 10% (v/v) egg white into the right hind paws of the rats. Paw volume was measured plethysmometrically after 0, 0.5, 1, 2, 3, 4, and 6 hr of injection using a paw edema detector. Rats were orally pre-administered STE or IMC before 0.5 hr of injection.

Antipyretic test

The assessment of the antipyretic activity of STE was performed in accordance with the method reported (Zhu et al., 2011; Santos et al., 1998). The temperature in external auditory meatus was measured using an electronic ear thermometer (IRT 3020, Kunshan Reying Photoelectric Co. Ltd., Jiangsu). Rats were randomly divided into five groups according to their basal temperature before experiment. After 0.5 hr of intra-peritoneal injection of LPS (1 mg/kg), rats were orally administered STE or PAE. The temperature was measured every 30 min interval within 6 hr.

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Acute toxicity

The acute toxicity test[5] was performed to evaluate any possible toxicity of STE. According to different responses to the extract, mice were orally administered different doses of STE. The maximum dose was 52.8 g/kg, while the control group received only distilled water. All animals were observed for any gross effect or mortality within 7 days.

Statistical analysis

Data analysis was performed by SPSS 13.0 statistical package. Multiple comparisons were carried out by one-way ANOVA followed by LSD t-test. A value of $P < 0.05$ was considered statistically significant and all results are presented as mean ± SD.

Results

In the writhing test, intra-peritoneal injection of 1% acetic acid evidently elicited writhing responses in mice. However STE decreased the number of writhing responses in a dose-dependent manner within 15 min (Figure 1). In the hot plate test, STE did not reveal a demulcent effect with 30 min of administration. However, there was a significantly antinociceptive activity exhibited after 60, 90 and 120 min of administration (Figure 2).As shown in Figure 3, dimethylbenzene markedly elicited mice ear edema, which was depressed by STE significantly and dose-dependently when compared with the control group. Table 1 exhibits the effects of STE on rat paw edema, which was elicited by subplantar injection of fresh egg white and persisted for 6 hr. However, STE effectively inhibited the percentage of rats paw edema lasting for 6 hr, when compared with the model group of rats given distilled water.

After injection of LPS, the temperature remarkably rose in rats. However, STE did not decrease the raised temperature significantly when compared with the control group of rats given distilled water (data not shown). Although the mice were given 52.8 g/kg of STE, no mortality was observed during the assessment period (7 days). So the minimum lethal dose of STE is more than 52.8 g/kg, which is equivalent to approximately 2080 times of clinical dose.

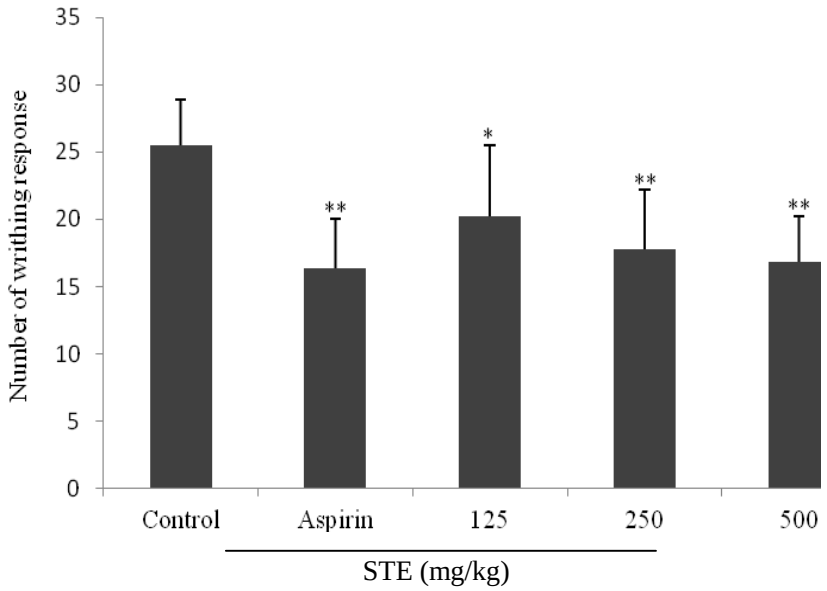

Figure 1: Effects of STE on writhing reflex in mice. After intraperitoneal injection of acetic acid (1%, 10 ml/kg), the writhing number of mice was counted immediately for 15 min. Data are presented as mean ± SD, n = 10. *P < 0.05, **P < 0.01, significance versus control.

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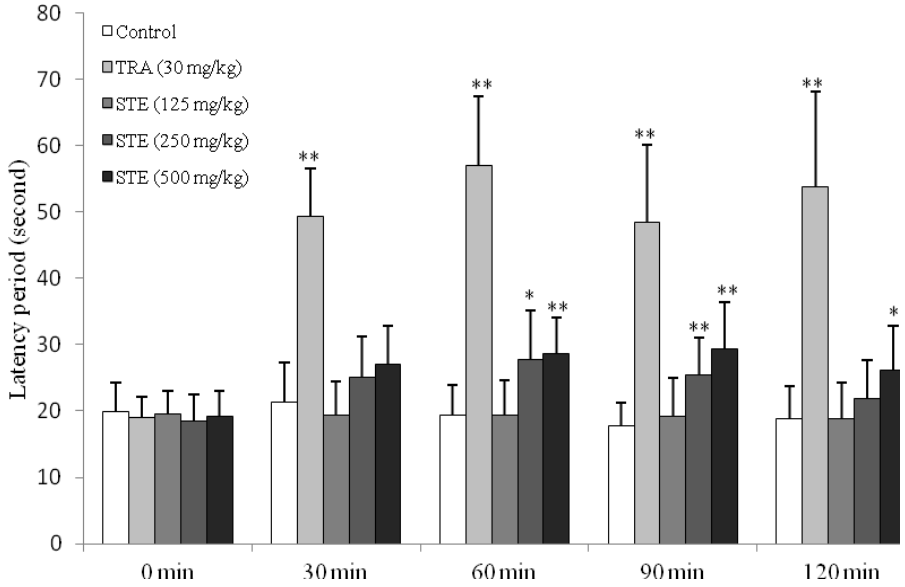

Figure 2: Effects of STE on heat-stimulated mice. After mice were put on the hot plate maintained at 55 ± 0.5℃, the latency time was recorded immediately. Data are presented as mean ± SD, n = 10. *P < 0.05, **P < 0.01, significance versus control.

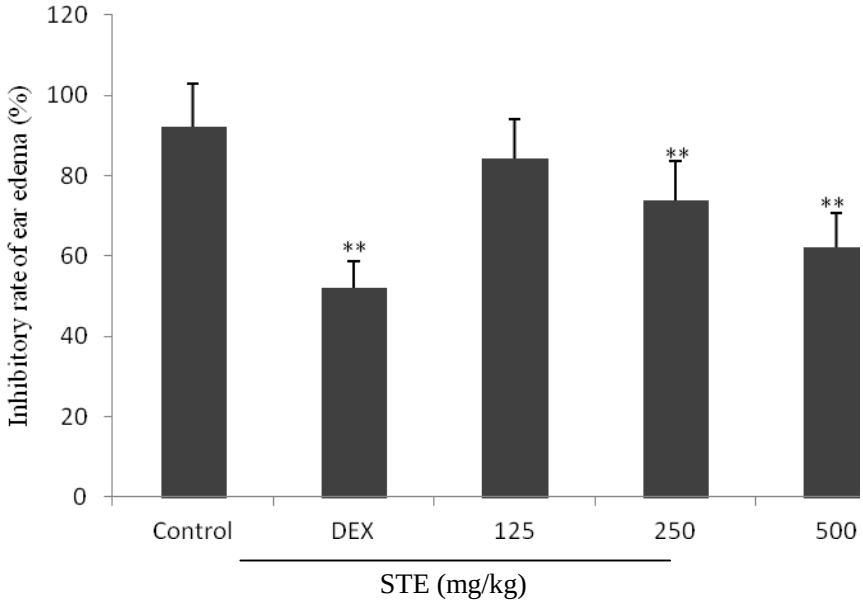

Figure 3: STE inhibits the ear edema of mice. Before 1 h of application of dimethylbenzene (40 µl/ear) to the right ears, mice received STE or DEX. Data are presented as mean ± SD, n = 10. *P < 0.05, **P < 0.01, significance versus control.

Table 1: Effects of STE on egg white-induced paw edema in rats

Group	Animal (N)	Dose (mg/kg)	Percentage of paw edema (%)	
			0.5 h	1 h
Control	10	Distilled water	66.7±7.7	69.7±7.4
IMC	10	10	50.8±6.3**	48.5±5.9**
STE	10	125	64.9±7.9	61.8±5.6*
STE	10	250	60.0±7.6	58.8±7.3**
STE	10	500	57.7±7.0*	55.7±6.4**

Percentage of paw edema (%)

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2 h	3 h	4 h	6h
68.8±8.9	51.5±6.7	38.2±5.6	26.7±4.5
50.2±5.3**	44.8±4.9**	22.6±4.4**	13.0±3.6**
60.2±7.1*	54.9±6.8	36.9±5.2	25.7±3.9
54.8±5.4**	47.9±5.6	30.7±4.0**	21.7±2.9*
52.6±5.8**	43.0±5.6*	33.3±4.2*	20.5±4.0*

Before 0.5 hr of subplantar injection of 0.1 ml of 10% (v/v) fresh egg white into the right hind paws, rats were orally administered STE or DEX. Paw volume was measured at different times after injection using a paw edema detector. Data are presented as mean ± SD. *P < 0.05, **P < 0.01, significance versus control.

Discussion

Although *Speranskia tuberculata* has been widely applied to prevent and treat various diseases in Chinese folk medicine, few investigations are reported on its chemical composition and pharmacologic effects, which obstructs the further clinical uses. In the present study, several classic animal models were used to evaluate the analgesic, inflammatory and antipyretic activities of STE. The experimental results exhibited that STE evidently reduced mice writhing number induced by acetic acid, prolonged mice reaction time against heat stimulation, depressed rat paw edema caused by fresh egg white and mice ear edema elicited by xylene. At the same time, STE exhibited good security in the acute oral toxicity test, which did not cause any death of mice even up to 52.8 g/kg.

It is known that the acetic acid-induced writhing mouse is a nonselective antinociceptive screening model. After intra-peritoneal injection of acetic acid into mice, acute inflammation arising in the peritoneal area elicits a painful reaction due to the release of endogenous substances, such as prostaglandins E_2 and $F_{2\alpha}$, which stimulate nervous endings (Daud et al., 2006; Gyires et al., 1984). In the current study, STE evidently antagonized the writhing responses of mice to acetic acid. The hot plate test was carried out further to confirm whether this antinociceptive activity is related to central analgesia substances. Our results showed that STE significantly prolonged latency time of mice in the hot-plate test, suggesting that this analgesic effect is performed through the participation of the central nervous system.

Fresh egg white-elicited paw edema of rats is well suitable for assessment of anti-inflammatory agents (Basu et al., 1991). Many inflammatory mediators such as prostaglandins and pro-inflammatory cytokines play an important role in pain caused by chemical stimulation (Arunachalam et al., 2009). As shown in Table 1, STE noticeably reduced egg white-elicited paw edema of rats, suggesting inhibitory release of some inflammatory mediators, especially prostaglandins. The dimethylbenzene-induced ear edema test was employed further to evaluate the anti-inflammatory activity of the extract. As a result, STE also inhibited ear edema significantly and dose-dependently, displaying the significant anti-inflammatory activity.

These results seem to show that the anti-inflammatory and analgesic activities of STE are associated with prostaglandins, which can elicit inflammation, pain and pyrexia. Therefore the antipyretic test was carried out further to substantiate this speculation. It is unexpected that STE did not antagonized rat pyrexia elicited by intra-peritoneal injection of LPS (data not shown).

Safety is vital in clinical uses of one drug. So we further evaluated the acute oral toxicity of STE, which did not cause any death of mice at the dose of 52.8 g/kg (equal to approximately 2080 times of clinical dose used), showing a favorable safety.

In conclusion, our findings showed that the ethanol extract of *Speranskia tuberculata* (STE) possesses the favorable analgesic, anti-inflammatory and antipyretic activities, and supports the clinical applications of this medicinal herb to arthralgia, arthritis, beriberi, and pyogenic infections. Nevertheless, it is necessary to further investigate the chemical composition and mechanism of action of this plant.

Conflicts of interest

Authors declare no conflict of interest.

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References

1. Arunachalam, G., Subramanian, N., Pazhani, G.P., Karunanithi, M., Ravichandran, V. (2009). Evaluation of anti-inflammatory activity of methanolic extract of *Solanum nigrum* (Solanaceae). Iran J. Pharm. Sci. 5: 151–156.
2. Basu, A., Chaudhuri, A.K. (1991). Preliminary studies on the anti-inflammatory and analgesic activities of *Calotropis procera* root extract. J. Ethnopharmacol. 31: 319–324.
3. Daud, A., Habib, N., Sanche, Z., Riera, A. (2006). Anti-inflammatory, anti-nociceptive and antipyretic effects of extracts of *Phrygilanthus acutifolius* flowers. J. Ethanopharmacol. 108: 198–203.
4. Gyires, K., Torma, Z. (1984). The use of the writhing test in mice for screening different types of analgesics. Arch. Int. Pharmacodyn. Ther. 267:131–140.
5. Kesim, M., Kadioglu, M., Okuyan, M., Muci, E., Erkoseoglu, I., Kalyoncu, N.I., Yaris, E. (2012). The evaluation of analgesic effects of simvastatin, pravastatin and atorvastatin in hot plate test. Eur. Rev. Med. Pharmacol. Sci. 16:789-796.
6. Li, C., Zhang, C.Z., Hu, F.D., Shi, J.G. (2000). Chemical constituents from *Speranskia tuberculata* (Bge.) Baill. Zhongguo Zhong Yao Za Zhi 25:291-292.
7. Ma, K.J., Zhu, Z.Z., Yu, C.H., Zhang, H., Liu, J., Qin, L.P. (2011). Analgesic, anti-inflammatory and antipyretic activities of the ethanol extract from *Desmodium caudatum*. Pharm. Biol. 49:403-407.
8. Mahmoudi, M., Eerahimzadeh, M.A., Pourmorad, F., Rezaie, N., Mahmouda, M.A. (2013). Anti-inflammatory and analgesic effects of egg yolk: a comparison between organic and machine made. Eur. Rev. Med. Pharmacol. Sci. 17:472-476.
9. Marzouk, B., Marzouk, Z., Fenina, N., Bouraoui, A., Aouni, M. (2011). Anti-inflammator and analgesic activities of Tunisian *Citrullus colocynthis* Schrad. immature fruit and seed organic extracts. Eur. Rev. Med. Pharmacol. Sci. 15:665-672.
10. Mazzio, E., Badisa, R., Mack, N., Deiab, S., Soliman, K.F. (2014). High Throughput Screening of Natural Products for Anti-mitotic Effects in MDA-MB-231 Human Breast Carcinoma Cells. Phytother. Res. 28: 856-867.
11. Saha, S., Hossain, F., Anisuzzman, M., Islam, M.K. (2013). Pharmacological evaluation of Musa seminifera Lour. Fruit. J. Integr. Med. 11:253-261.
12. Santos, F.A., Rao, V.S. (1998). A study of the anti-pyretic effect of quinine, an alkaloid effective against cerebral malaria, on fever induced by bacterial endotoxin and yeast in rat. J. Pharm. Pharmacol. 50: 225–229.
13. Shi, J.G., Wang, H.Q., Wang, M., Yang, Y.C., Hu, W.Y., Zhou, G.X. (2000). Polyoxygenated bipyridine, pyrrolylpyridine, and bipyrrole alkaloids from *Speranskia tuberculata*. J. Nat. Prod. 63:782-786.
14. Yu, C.H., Tang, W.Z., Peng, C., Sun, T., Liu, B., Li, M., Xie, X.F., Zhang, H. (2012). Diuretic, anti-inflammatory, and analgesic activities of the ethanol extract from *Cynoglossum lanceolatum*. J. Ethnopharmacol. 139:149-154.
15. Zhu, Z.Z., Ma, K.J., Ran, X., Zhang, H., Zheng, C.J., Han, T., Zhang, Q.Y., Qin, L.P. (2011). Analgesic, anti-inflammatory and antipyretic activities of the petroleum ether fraction from the ethanol extract of *Desmodium podocarpum*. J. Ethnopharmacol. 133: 1126-1131.