ORIGINAL ARTICLE

Evaluation of the anti-inflammatory, antinociceptive and antipyretic activities of the extracts from *Smilax corbularia* Kunth rhizomes in mice and rats (*in vivo*)

Wantana Reanmongkol[1], Arunporn Itharat[2] and Pisit Bouking[3]

Abstract

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The effects of either ethanol or aqueous extract from rhizomes of *Smilax corbularia* Kunth (*S. corbularia*) on anti-inflammatory activity in carrageenin-induced edema in rats, antinociceptive activity using writhing, hot plate and formalin tests in mice and the antipyretic activity in yeast-induced fever were examined. Oral administration of the ethanol extract of *S. corbularia* rhizomes significantly suppressed the paw edema induced by carrageenin in rats while the aqueous extract had no effect. Neither the ethanol extract nor aqueous extract significantly affected the antinociceptive tests in mice and yeast-induced fever in rats. These results suggest that the ethanol extract of *S. corbularia* rhizomes possess anti-inflammatory activity, and that its actions on the inflammation may be different from those of aspirin.

Key words : *Smilax corbularia*, extract, anti-inflammatory, antinociceptive, antipyretic

[1]Ph.D.(Pharmacology), Assoc. Prof., [3]Dip.(Science), Technician, Department of Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla 90112 Thailand. [2]Ph.D. (Pharmacognosy), Assoc. Prof., Applied Thai Traditional Medicine Centre, Faculty of Medicine, Thammasart University, Klong Luang, Pathum Thani, 10120 Thailand.
Corresponding e-mail: wantana.r@psu.ac.th

บทคัดย่อ

วันทนา เหรียญมงคล[1] อรุณพร อิฐรัตน์[2] และ พิศิษฐ์ บัวกิ่ง[1]

ประเมินฤทธิ์แก้อักเสบ แก้ปวด และแก้ไข้ของสารสกัดจากเหง้าหัวข้าวเย็นเหนือ (*Smilax corbularia* Kunth) ในหนูทดลอง

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ทำการทดสอบฤทธิ์ทางเภสัชวิทยาของสารสกัดชั้นเอทานอลและสกัดชั้นน้ำจากเหง้าของหัวข้าวเย็นเหนือ ต่อฤทธิ์ลดการบวมที่อุ้งเท้าซึ่งเกิดจากการเหนี่ยวนำด้วยคาร์ราจีนินในหนูขาว ผลการระงับปวดซึ่งเกิดจากกรดอะเซติกในหนูถีบจักร และผลต่อการลดไข้ซึ่งเกิดจากการเหนี่ยวนำโดยยีสต์ในหนูขาว เมื่อป้อนสารสกัดชั้นเอทานอลและชั้นน้ำจากเหง้าของหัวข้าวเย็นเหนือ (400-1600 มก./กก.) เข้าทางปากในหนูทดลอง พบว่าสารสกัดชั้นเอทานอลขนาด 1600 มก./กก. สามารถลดการบวมอย่างมีนัยสำคัญที่อุ้งเท้าหนูขาวซึ่งเกิดจากการเหนี่ยวนำด้วยคาร์ราจีนิน สารสกัดชั้นเอทานอลและชั้นน้ำจากเหง้าของหัวข้าวเย็นเหนือ ไม่สามารถลดอาการปวดในหนูถีบจักร และไม่สามารถลดไข้ซึ่งเกิดจากการเหนี่ยวนำด้วยยีสต์ในหนูขาว จากผลการทดลองนี้เสนอว่า สารสกัดชั้นเอทานอลจากเหง้าของหัวข้าวเย็นเหนือมีฤทธิ์แก้อักเสบ โดยมีกลไกการออกฤทธิ์ต่อการอักเสบที่แตกต่างจากแอสไพริน

[1]ภาควิชาเภสัชกรรมคลินิก คณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์ อำเภอหาดใหญ่ จังหวัดสงขลา 90112 [2]ศูนย์การแพทย์แผนไทยประยุกต์ คณะแพทยศาสตร์ มหาวิทยาลัยธรรมศาสตร์ อำเภอคลองหลวง จังหวัดปทุมธานี 10120

Smilax corbularia Kunth, family Smilacaceae is known in Thai as Hua-Khao-Yen-Nea or Hua-Khao-Yen-Wog (Koyama, 1981). The rhizomes of this plant have been used for treatment of cancer by formulating with other four species of Hua-Khao-Yen (Pornsiriprasert *et al*., 1986). The extract of *S. corbularia* increased natural killer cell, monocyte and macrophage (Vongsakul and Ketsaard, 1995).

Several isolation and identification of the compounds from *Smilax* spp. rhizome have been reported. Glycosides and glucosides included flavonol glycosides, flavanonol glucosides, steroidal glycosides, dihydroflavonol glycosides, maltol glucosides, phenylpropanoid glycosides (Yuan *et al*., 2004; Cheng *et al*., 2003; Chen *et al*., 2000; Chen *et al*., 1996; Guo *et al*., 2004; Yi *et al*., 1998); phenolic compounds (Li *et al*., 2002) and steroidal saponins (Bernardo *et al*., 1996; Ju *et al*., 1994; Jia and Ju, 1992; Kubo *et al*., 1992; Sashida *et al*., 1992; Ju and Jia, 1992) were identified.

Some pharmacological activities of *Smilax* spp. rhizome have been studied. Oral administration of the extract from *S. sarsaparilla* at the dose of 500 mg/kg reduced the paw edema induced by carrageenan in rats (Ageel *et al*., 1989). The methanol extract of rhizomes of *S. glabra* (100 mg/kg, i.p.) reduced the blood glucose of normal mice and KK-Ay mice (Fukunaga *et al*., 1997). The aqueous extract (400, 800 mg/kg, p.o.) from rhizome of *S. glabra* inhibited the swelling of the adjuvant arthritis in rats (Jiang and Xu, 2003). The ethyl acetate, butanol and aqueous extracted fractions from *S. china* root showed high levels of DPPH free radical scavenging activity (Lee *et al*., 2001). The decoction of *S. china* (90 and 180 mg/kg, p.o.) could significantly inhibit inflammatory swelling on adjunctive arthritis mouse (Lu *et al*., 2003).

Although some pharmacological studies of *Smilax* spp. have been reported, no studies of *S. corbularia* rhizomes have previously been conducted on anti-inflammatory, analgesic and antipyretic actions.

In the present study, in order to evaluate the potential anti-inflammatory effect of the extracts of *S. corbularia* rhizomes, we investigated the anti-inflammatory activity in experimental animal model using carrageenin-induced paw edema in

rats. The analgesic and antipyretic activities were also examined using writhing, formalin and hot plate tests in mice and yeast-induced fever in rats.

Materials and methods

Plant material

The rhizomes of *S. corbularia* Kunth (Smilacaceae) (Hua-Khao-Yen-Nea) were collected from Amphor Mae Taeng, Chiang Mai Province. Authentication of plant material was carried out at the herbarium of the Department of Forestry, Bangkok, Thailand, where the herbarium voucher has been kept. A duplicate set has been deposited in the herbarium of Southern Center of Thai Medicinal Plants at Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla, Thailand. The voucher number is SKPA 179190315

Preparation of the extracts from the rhizomes of *S. corbularia*

Small pieces of the rhizome of *S. corbularia* were dried at 50°C, powdered and extracted by methods corresponding to those practised by Thai traditional doctors. For the ethanolic extract, dried ground rhizome of *S. corbularia* (100 g) was percolated with 95% ethanol, then concentrated to dryness under reduced pressure. The water extract was obtained by boiling dried ground rhizome of *S. corbularia* (100 g) for 30 min in distilled water (300 ml), all extracts being filtered and freeze dried. The percentage yields of the ethanol and aqueous extracts were 12.0% and 9.2%, respectively. The ethanol and aqueous extracts were used as the test extract. All doses were expressed in terms of crude extract (mg/kg body weight).

Animals

All animals used in this study were obtained from the Southern Animal Facility, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand. Male Swiss mice and Wistar rats with the weight ranging from 30-38 g and 150-220 g, respectively, were used. The rats were handled for 5-10 min daily for several days before experiments. The animals were housed for at least one week in the laboratory animal room prior to testing. Food and water were given *ad libitum* unless otherwise specified. All procedures described were reviewed and approved by the Institutional Committee for Ethical Use of Animals, Prince of Songkla University, Thailand (Ref.11/49).

Carrageenin-induced paw edema

According to the method described by Winter *et al.* (1962), the initial right hindpaw volume of the rats was measured using a plethysmometer (Ugo Basile) and then 0.1 ml of 1% (w/v) carrageenin was subcutaneously injected into the subplantar region of the right hind paw. The volume of right hind paw was measured at 1, 2, 3, 4, and 5 hr after carrageenin injection, and the edema volume was determined. The data were expressed as paw volume (ml), compared with the initial hindpaw volume of each rat. Cosolvent, each extract of *S. corbularia* or aspirin was orally administered 30 min before carrageenin injection. Each group comprised 6 rats.

Antinociceptive activities

1. Writhing test

Writhing behaviour was tested, in which 0.6% acetic acid solution (10 ml/kg body weight) was injected intraperitoneally and the number of writhings and stretchings was counted over a 20-min period as previously reported (Koster *et al.*, 1959; Hendershot and Forsaith, 1959). The plant extract of each extract (400, 800 and 1600 mg/kg), a reference analgesic drug, aspirin (200 mg/kg), or cosolvent was orally administered 30 min before acetic acid. Each group used 10 mice.

2. Hot plate test

The hot plate test was carried out according to the method described by Woolfe and MacDonald (1944). Mice were placed on a hot plate maintained at 55°C ± 1°C. Latency of nociceptive response such as licking, flicking of a hind limb or jumping was measured. Starting thirty minutes after p.o. administration of the test agents except morphine (15 min after administration), the nociceptive response was measured every 15 min over a 60 min period. Morphine sulfate was

injected subcutaneously. The cut-off time was 45 sec. Only the mice that showed nociceptive responses within 15 sec were used for the experiments. Each group used 10 mice.

3. Formalin test

Thirty minutes after administration of the extracts of *S. corbularia* (400, 800 and 1600 mg/kg, p.o.), aspirin (200 mg/kg, p.o.) or cosolvent except morphine (15 min after administration), 20 µl of 2.5% formalin in saline was injected subcutaneously to a hindpaw of the mice. Morphine sulfate was injected subcutaneously. The time spent licking the injected paw was recorded and the data were expressed as total licking time in the early phase (0-5 min) and the late phase (15-30 min) after formalin injection (Hunskaar *et al*., 1985). Each group used 10 mice.

Antipyretic activity

Antipyretic actvity of drug was measured by slightly modifying the method described by Adams *et al*. (1968). Male Wistar rats were fasted overnight with water ad lib before the experiments. Pyrexia was induced by subcutaneously injecting 20% (w/v) brewer's yeast suspension (10 ml/kg) into the animals' dorsum region. Seventeen hours after the injection, the rectal temperature of each rat was measured using a digital thermometer (SK-1250MC, Sato Keiryoki Mfg. Co., Ltd., Japan). Only rats that showed an increase in temperature of at least 0.7°C were used for the experiments. Test agent or cosolvent was administered orally and the temperature was measured at 1, 2, 3, 4 and 5 hr after drug administration. Each group used 6 rats.

Chemicals

The following drugs were used: morphine sulfate, brewer's yeast, carrageenin lambda (AR grade, Sigma Chem. Co., St. Louis, U.S.A.), aspirin, Tween 80 (AR grade, Srichand United Dispensary Co., Ltd., Bangkok, Thailand), formalin, sodium chloride (AR grade, Carlo Erba, Germanny), acetic acid (AR grade, J.T, Baker Inc., Phillipsburg, U.S.A.), ethanol (AR grade, Merck, Germany), Propylene glycol (Vidhyasom Co., Ltd, Bangkok, Thailand). The extracts of *S. corbularia* and aspirin were dissolved in cosolvent solution (propylene glycol : tween 80 : water = 4:1:4) and administered orally in a constant volume (10 ml/kg for mice and 5 ml/kg for rats) 30 min before the experiments. Morphine sulfate was dissolved in 0.9% sodium chloride solution and administered subcutaneously. All drug solutions were prepared immediately before starting the experiments.

Statistical Analysis

Data are expressed as mean ± SEM and were analyzed statistically by one-way ANOVA procedures, followed by Dunnett's test. A difference was considered significant at $p<0.05$.

Results

Effect of the ethanol and aqueous extracts on carrageenin-induced paw edema in rats

Only the ethanol extract of *S. corbularia* rhizomes significantly decreased the paw edema induced by carrageenin in rats at the dose of 1600 mg/kg whereas the aqueous extract of *S. corbularia* rhizomes had no significant effects. Aspirin (200 mg/kg), a reference drug, significantly reduced the carrageenin-induced paw edema (Table 1).

Effect of the ethanol and aqueous extracts on nociceptive responses

Writhing test

Neither the ethanol extract nor aqueous extract of *S. corbularia* rhizomes significantly affected the number of writhings and stretchings induced by intraperitoneal 0.6% acetic acid while the reference drug aspirin (200 mg/kg) produced significant protective effects towards the acetic acid-induced pain (Table 2).

Hot plate test

Neither of the extracts from the rhizomes of *S. corbularia* (400, 800 and 1600 mg/kg, p.o.) nor aspirin (200 mg/kg, p.o.) had any significant protective effects on heat-induced pain in mice. In contrast, a centrally acting analgesic drug, morphine sulfate (10 mg/kg, s.c.), markedly increased pain latency (Table 3).

Table 1. Effect of the ethanol extract and aqueous extract of *S. corbularia* and aspirin on carrageenin-induced paw edema in rats.

Drug	Dose (mg/kg, p.o.)	Paw volume (ml)					
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
Cosolvent	-	4.11±0.19	4.88±0.20	5.82±0.36	6.83±0.45	7.36±0.26	7.49±0.35
Aspirin	200	3.55±0.07	3.92±0.12*	3.99±0.12*	4.10±0.14*	4.32±0.10*	4.59±0.08*
S. corbularia	400	4.14±0.18	4.79±0.19	5.95±0.30	6.96±0.43	7.32±0.39	7.51±0.40
(ethanol)	800	3.99±0.07	4.58±0.16	5.85±0.43	6.89±0.51	7.25±0.38	7.59±0.44
	1600	3.86±0.08	4.21±0.07*	5.31±0.28	6.20±0.32*	6.61±0.30*	6.95±0.37
Cosolvent	-	4.50±0.03	5.36±0.13	6.50±0.15	7.52±0.18	7.93±0.14	8.06±0.08
Aspirin	200	4.50±0.06	4.88±0.05*	5.09±0.05*	5.46±0.14*	5.65±0.11*	5.69±0.12*
S. corbularia	400	4.47±0.10	5.31±0.14	6.56±0.24	7.33±0.30	7.56±0.41	7.50±0.42
(aqueous)	800	4.58±0.04	5.65±0.07	6.96±0.05	7.95±0.14	8.27±0.09	8.23±0.13
	1600	4.46±0.10	5.26±0.14	6.52±0.16	7.47±0.13	7.54±0.42	7.36±0.38

The initial hind paw volume of the rat was determined volumetrically. Thirty min after test agent administration (p.o.), 1% carrageenin in saline was subcutaneously injected in a volume of 0.1 ml into the right hind paw at time 0 and the paw volume was measured at 1 hr intervals for 5 hr. Each point represents the mean ± S.E.M of 6 rats. *p<0.05, compared with the control group (Dunnettís test).

Table 2. Effect of the ethanol extract and aqueous extract of *S. corbularia* and aspirin on acetic acid induced writhing in mice.

Drug	Dose (mg/kg p.o.)	No. of writhing (counts/20 min)
Cosolvent	-	31.0±2.5
Aspirin	200	15.3±3.7*
S. corbularia	400	30.9±3.1
(ethanol)	800	30.0±1.5
	1600	34.8±2.2
S. corbularia	400	30.4±4.5
(aqueous)	800	24.8±2.2
	1600	32.1±2.7

The ethanol extract and aqueous extract of *S. corbularia* were orally administered. After 30 min, 0.6% acetic acid solution (10 ml/kg) was intraperitoneally injected in mice. Immediately after injection, the number of writhings was counted over a 20-min period. Each datum represents the mean ± S.E.M. from 10 mice. *p<0.05, compared with the control group (Dunnett's test).

Formalin test

Neither the ethanol extract nor aqueous extract of *S. corbularia* rhizomes significantly reduced the licking activity in both phases while aspirin decreased the licking activity in the late phase as shown in Table 4. In contrast, the reference antinociceptive drug morphine sulfate (10 mg/kg, s.c.) significantly reduced the licking activity against both phases of formalin-induced nociception.

Effect of the ethanol and aqueous extracts from *S. corbularia* rhizomes on yeast-induced fever in rats

There were no significant effects of ethanol and aqueous extracts of *S. corbularia* rhizomes

Table 3. Effect of the ethanol extract and aqueous extract of *S. corbularia*, aspirin and morphine on nociceptive response induced by heat in mice.

Drug	Dose (mg/kg, p.o.)	Latency of nociceptive response (sec)			
		15	30	45	60 min
Cosolvent	-	11.3±1.4	9.7±1.0	7.4±0.8	8.4±1.1
Aspirin	200	10.1±1.1	9.3±1.2	10.4±1.2	9.8±1.1
Morphine	10	32.0±2.6*	33.3±2.2*	33.1±2.8*	31.6±2.1*
S. corbularia	400	11.0±1.4	9.2±0.6	9.7±1.5	10.0±1.2
(ethanol)	800	10.3±1.1	7.7±1.0	8.7±1.5	8.3±0.9
	1600	10.3±1.0	10.2±1.2	9.5±0.7	9.0±1.1
Cosolvent	-	6.7±0.4	7.1±1.0	7.2±1.0	8.0±0.7
Aspirin	200	10.1±1.4	7.6±0.9	6.3±0.7	7.9±0.9
Morphine	10	28.5±1.7*	29.0±2.8*	29.8±1.4*	28.5±1.5*
S. corbularia	400	7.0±0.6	7.8±1.2	8.2±0.7	7.6±0.5
(aqueous)	800	7.1±0.5	8.3±1.1	8.8±1.0	7.8±1.0
	1600	7.6±0.6	7.9±0.6	8.7±0.7	7.1±0.8

Beginning 30 min after oral administration of test agents (or 15 min after morphine injection, s.c.), the nociceptive response was measured every 15 min over a 60-min period. Each datum represents the latency of nociceptive responses (sec) ± S.E.M. (n=10) * p< 0.01 compared with the control group (Dunnett's test)

Table 4. Effect of the ethanol extract and aqueous extract of *S. corbularia*, aspirin and morphine on hindpaw licking in the formalin test in mice.

Drug	Dose (mg/kg, p.o.)	Early Phase (sec)	Late Phase (sec)
Cosolvent	-	80.2±9.0	81.0±15.8
Aspirin	200	67.8±6.7	9.4±3.3*
Morphine	10	21.4±4.6*	0.5±0.3*
S. corbularia	400	86.1±4.3	53.8±9.2
(ethanol)	800	94.9±5.6	57.5±12.5
	1600	71.2±5.9	67.3±17.0
Cosolvent	-	88.3±6.7	66.1±7.5
Aspirin	200	76.2±8.7	6.1±3.1*
Morphine	10	22.4±5.2*	1.2±1.1*
S. corbularia	400	76.5±8.2	54.7±13.4
(aqueous)	800	70.5±8.2	77.3±9.9
	1600	72.8±6.5	48.0±9.7

Thirty min after test drug administration (p.o.), 2.5% formalin was subcutaneously injected to a hindpaw in a volume of 20 µl. Each datum represents the mean licking time ± S.E.M. from 10 mice in the early phase (0-5 min) and the late phase (15-30 min) after formalin injection. *p< 0.01 compared with the control group (Dunnett's test)

on yeast-induced fever whereas a reference drug aspirin reversed yeast-induced fever in rats (Table 5).

Discussion

The results demonstrate that the ethanol extract (1600 mg/kg) obtained from *S. corbularia* rhizomes exhibited anti-inflammatory activity by significant suppression of the paw edema induced by carrageenin at 1, 3 and 4 hr after carrageenin injection, though less potent than that of the aspirin (200 mg/kg), which significantly reduced paw edema at 1, 2, 3, 4 and 5 hr after carrageenin injection in rats. As the carrageenin-induced paw edema model is used for evaluation of anti-inflammatory activity of the compounds involving several chemical mediators such as prostaglandins, serotonin, histamine and bradykinin (Vinegar *et al*., 1987), it is possible that the active constituents including in the ethanol extract of *S. corbularia* rhizome may be involved in the inhibition of some inflammatory mediators in this activity.

The writhing test is generally used for screening of antinociceptive effects (Koster *et al*., 1959; Hendershot and Forsaith, 1959). Unfortunately, the ethanol and aqueous extracts of *S. corbularia* rhizome did not show any significant inhibition on acetic acid-induced writhing response, while the reference drug aspirin (200 mg/kg) produced significant protective effects against the acetic acid induced pain in mice.

Thermic painful stimuli are known to be selective to centrally, but not peripherally, acting analgesic drugs (Chau, 1989). In the present study, morphine, a centrally acting analgesic drug, produced an inhibitory effect on the nociceptive response in this test, while the extracts of *S. corbularia* rhizomes and aspirin, a peripherally acting analgesic drug, failed to affect the response.

The formalin test is another pain model, which assesses the way an animal responds to moderate, continuous pain generated by injured tissue (Tjolsen *et al*., 1992). Centrally acting drugs such as morphine inhibited both of the early and late phases equally while peripherally acting drugs such as aspirin only inhibited the second phase. (Dubuisson and Dennis, 1977; Hunskaar and Hole, 1987). In the present study, the extracts of *S. corbularia* rhizomes had no significant suppression of the licking activity in either phase of the

Table 5. Effect of the ethanol extract and aqueous extract of *S. corbularia* and aspirin on brewer's yeast-induced fever in rats.

Drug	Dose (mg/kg, p.o.)	Average rectal temperature (ºC)					
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
Cosolvent	-	37.8±0.1	37.3±0.1	37.1±0.1	37.0±0.1	37.0±0.1	37.1±0.1
Aspirin	200	37.6±0.1	36.8±0.1*	36.2±0.1*	36.3±0.1*	36.3±0.1*	36.4±0.1*
S. corbularia	400	37.7±0.1	37.2±0.1	37.0±0.1	36.9±0.1	36.9±0.1	37.0±0.2
(ethanol)	800	37.6±0.1	37.2±0.1	37.0±0.1	36.9±0.1	36.8±0.1	36.8±0.1
	1600	37.5±0.1	37.1±0.1	36.8±0.1	36.8±0.1	36.9±0.1	36.8±0.1
Cosolvent	-	38.1±0.2	37.7±0.2	37.5±0.1	37.3±0.2	37.3±0.2	37.3±0.1
Aspirin	200	38.0±0.1	37.2±0.2*	37.0±0.3*	36.8±0.2*	36.6±0.2*	36.6±0.2*
S. corbularia	400	38.1±0.1	37.5±0.1	37.2±0.1	37.1±0.1	37.0±0.1	37.0±0.1
(aqueous)	800	37.8±0.1	37.5±0.2	37.2±0.2	37.1±0.2	37.1±0.2	37.1±0.1
	1600	38.0±0.1	37.7±0.1	37.3±0.1	37.2±0.1	37.2±0.1	37.3±0.1

Twenty percent of yeast suspension was subcutaneously injected into the dorsum region of rats. Seventeen hours after injection, rectal temperature was measured (time 0) and then drugs were orally administered. The temperature was again measured at 1, 2, 3, 4 and 5 hr after drug administration. Each datum represents the mean rectal temperature (ºC) ± S.E.M. (n = 6) *p<0.05, compared with the control group (Dunnett's test).

formalin-induced pain in mice. Morphine significantly reduced the licking activity in both phases while aspirin decreased the licking activity only in the late phase.

Neither the ethanol extract nor aqueous extract of *S. corbularia* rhizomes showed any significantly effect on yeast-induced fever in rats while the reference drug aspirin suppressed fever induced by yeast in rats by inhibiting the synthesis of prostaglandin E2 (Dascombe, 1985; Vane, 1987).

The ethanol extract (1600 mg/kg) of *S. corbularia* rhizome suppressed the paw edema induced by carrageenin in rats but had no significant effect on nociceptive response induced by chemicals or heat in mice or pyrexia induced by yeast in rats, compared with aspirin (200 mg/kg), a nonsteroidal anti-inflammatory drug, which possessed analgesic, antipyretic and anti-inflammatory activities by inhibition of prostaglandin synthesis via cyclooxygenase activity (Vane,1987). Thus, the anti-inflammatory action of the ethanol extract from *S. corbularia* rhizomes may act at some site(s) of action that is (are) different from that of aspirin. Nevertheless, further experiments are needed to elucidate its anti-inflammatory action.

In conclusion, the ethanol extract of *S. corbularia* rhizome shows an anti-inflammatory activity, but its action on inflammation may be different from that of aspirin.

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