

## Evaluation of Antinociceptive, Anti-inflammatory and Antipyretic Effects of *Strobilanthes cusia* Leaf Extract in Male Mice and Rats

Yu-Ling Ho,<sup>\*,†</sup> Kuo-Ching Kao,<sup>\*</sup> Huei-Yann Tsai,<sup>\*,‡</sup> Fu-Yu Chueh<sup>\*</sup>  
and Yuan-Shiun Chang<sup>\*,‡</sup>

<sup>\*</sup>*Institute of Chinese Pharmaceutical Sciences, China Medical College  
Taichung 404, Taiwan, R.O.C.*

<sup>†</sup>*Nursing Department, Hungkuang Institute of Technology  
Taichung Shien 433, Taiwan, R.O.C.*

<sup>‡</sup>*Pharmacy Department, China Medical College Hospital  
Taichung 404, Taiwan, R.O.C.*

**Abstract:** The leaf of *Strobilanthes cusia* (Acanthaceae), popularly known as Da-Ching-Yeh, has been commonly used in traditional Chinese medicine. It is used for influenza, epidemic cerebrospinal meningitis, encephalitis B, viral pneumonia and mumps. It is also used to treat sore throat, aphthae and inflammatory diseases with redness of skin, etc. In this study, we evaluated the antinociceptive, anti-inflammatory and antipyretic effects of methanol extract of *Strobilanthes cusia* leaf. The results showed that the extract significantly inhibited the writhing responses of mice and decreased the licking time on both the early and late phases of the formalin test in a dose-dependent manner. It also reduced the paw edema induced by carrageenan in rats. In addition, it potently attenuated pyrexia induced by lipopolysaccharide.

**Keywords:** *Strobilanthes cusia*; Antinociceptive; Anti-inflammatory; Antipyretic.

### Introduction

*Strobilanthes cusia* (Nees) Kuntze of the Acanthaceae family is widely distributed in northern and central Taiwan (Editorial Committee of the Flora of Taiwan, 1998). The aerial part of the plant was used locally for indigo dyeing in ancient time. The leaf of *Strobilanthes cusia* is used as traditional Chinese medicine and is commonly called Da-Ching-Yeh (大青葉) in Taiwan. In China, people call it Nan-Ban-Lan-Yeh (南板藍葉) and its root and rhizome are called Nan-Ban-Lan-Gen (南板藍根). In Chinese medicine (Institute of Pharmaceutical

Correspondence to: Dr. Yuan-Shiun Chang, Institute of Chinese Pharmaceutical Sciences, China Medical College, 91, Hsueh-Shih Road, Taichung 404, Taiwan, Republic of China. Tel: (+886) 4-2203-0380, Fax: (+886) 4-2208-3362, E-mail: yschang@mail.cmc.edu.tw

Sciences, Academy of Medical Science, PRC, 1994), Nan-Ban-Lan-Yeh is used to treat influenza, epidemic cerebrospinal meningitis, encephalitis B, viral pneumonia, and mumps. It is also used for sore throat, aphthae, inflammatory diseases with redness of skin, etc. (Editorial Committee of Chung-Hwa-Pen-Tsao, 1999). The entire fresh plant of *Strobilanthes cusia* has antifungal activity and is used to treat athletes foot (Honda and Tabata, 1979).

In this study, we evaluated the antinociceptive, anti-inflammatory and antipyretic effects of *Strobilanthes cusia* leaf extract. The results showed that the extract significantly inhibited the writhing responses of mice and decreased the licking time on both the early and late phases of the formalin test. It also inhibited the carrageenan-induced paw edema in rats. In addition, it attenuated pyrexia induced by lipopolysaccharide.

## Materials and Methods

### *Plant Materials*

In this study, commercially available, dried leaves of *Strobilanthes cusia* (SC-L) were purchased from a local market. They were identified by comparative anatomical studies, and authenticated by Dr. Chung-Chuan Chen, professor of pharmacognosy, China Medical College. A voucher specimen (No. Chen 460) was deposited in the herbarium of the School of Pharmacy, China Medical College, Taichung, Taiwan, R.O.C.

### *Preparation of the SC-L Extracts*

The plant materials of SC-L (2.0 kg) were macerated five times with 10 L methanol at room temperature for 3 days. The methanol extracts were filtered and concentrated to give dark syrup under reduced pressure. The yield was 215 g, 10.8% of the starting materials. The extract was dissolved in 0.5% carboxymethylcellulose (CMC) as vehicle to make up different concentrations prior to administration to experimental animals.

### *Animals*

Male ICR mice (20–25 g), male Sprague-Dawley rats (220–260 g) and male Wistar rats (150–180 g) obtained from the National Laboratory Animal Breeding and Research Center, National Science Council, Taiwan, were used throughout this study. Animals were housed in standard cages (five rats or mice per cage) at a constant temperature of  $22 \pm 2^\circ\text{C}$  and 12:12 h light-dark cycle with lights on at 07:00 h, and were provided with pelleted diet and tap water *ad libitum*.

### *Writhing Test*

The methods of Koster *et al.* (1959) and Collier *et al.* (1968) were used to induce writhing by acetic acid. Mice were used in groups of eight per dose of SC-L extract or indomethacin.

The animals were kept singly in a clear plastic observational chamber (14 × 14 × 15 cm), and were pretreated with SC-L extract (15.63, 62.5, 125, 250, 500 and 1000 mg/kg) or indomethacin (4 mg/kg) by intraperitoneal (i.p.) injection 30 minutes prior to i.p. injection of 1% acetic acid in a volume of 0.1 ml/10 g per animal. Five minutes after the i.p. injection of acetic acid, the number of writhes exhibited by mice was counted for 10 minutes. Control mice received vehicle and the acetic acid experiment was repeated.

#### *Antinociceptive Test*

Formalin-induced licking paw test was modified from the method described by Dubuisson and Dennis (1977). Male ICR mice received 0.5% CMC as vehicle or various doses of SC-L extract (62.5, 125, 250, 500 and 1000 mg/kg), or indomethacin (4 mg/kg) by intraperitoneal (i.p.) injection. Thirty minutes later, 25 µl of 1% formalin was injected subcutaneously (s.c.) into the right hind paw of mice with microsyringe. Each mouse was then placed in an individual clear plastic observational chamber (14 × 14 × 15 cm), and the total licking time of hind paw made by each mouse was counted every 5-minute intervals for 40 minutes. The first period (early phase) was recorded 0–5 minutes after the injection of formalin and the second period (late phase) was recorded 15–40 minutes after the injection.

#### *Carrageenan-induced Paw Edema in Rats*

This anti-inflammatory test was performed according to the method of Winter *et al.* (1962). Edema in the right hind paw of Wistar rats was induced by injecting subcutaneously 0.1 ml of 1% (w/v) carrageenan (Sigma Chemical Co., USA) in saline into the footpad. The paw volume of each rat was measured before carrageenan injection and at 30-minute intervals up to five times with a Plethysmometer 7150 (UGO, Basil, Italy). The drug test groups were treated with SC-L extract (125, 250, 500 and 1000 mg/kg, i.p.) 30 minutes before carrageenan injection. The animals in the control group received vehicle only, while a standard reference group received indomethacin (4 mg/kg, i.p.) in 0.5% CMC. The edema rate of each group was calculated as follows:

$$\text{Edema rate (E) \%} = \frac{V_t - V_o}{V_o} \times 100\%$$

where  $V_o$  is the volume before carrageenan injection (ml);  $V_t$  is the volume at  $t$  h after carrageenan injection (ml).

#### *Antipyretic Activity Test*

The method of Santos and Rao (1998) was modified and used for the assessment of antipyretic activity of the plant extracts. Sprague-Dawley rats were used in groups of six per dose of plant extract or drug. They were previously trained to be kept quietly under minimal restraint in a special rat stock for periods of several hours at a time. Each animal had freedom to

move his limbs and neck in an alert condition (Hsieh *et al.*, 1998). Fever was induced with 1 mg/kg of lipopolysaccharide (Sigma Chemical Co., USA) injected intraperitoneally (Nava and Carta, 2000). The animals were pretreated for 90 minutes with lipopolysaccharide before administration of SC-L extract (15.63, 31.25, 125, 250 and 500 mg/kg, i.p.), vehicle (0.5% CMC, i.p.) or indomethacin (4 and 10 mg/kg, i.p.). Temperature was measured in a temperature-controlled room (ambient temperature  $24 \pm 1^\circ\text{C}$ ). The colonic temperature was measured every minute with a copper-constantan thermocouple connected to a thermometer (YOKOGAWA, HR1300). The copper-constantan thermocouple was enclosed in polyethylene 200 tubing, sealed at one end, and inserted 60 mm into the colon. The colonic temperature of each animal was allowed to stabilize for at least 60 minutes before any injections. Basal values were taken immediately before the injection (time 0) of SC-L extract, indomethacin or vehicle alone. In addition, colonic temperature of animals treated with SC-L extract alone (15.63, 31.25, 125, 250 and 500 mg/kg, i.p.) was also measured.

### Statistical Analysis

The results were expressed as mean  $\pm$  SE which was subjected to analysis of variance (ANOVA). In the case of significant variation, the mean values were compared by one-way ANOVA test and were considered statistically significant when  $p < 0.05$ .

## Results

### Writhing Test

One percent acetic acid produced a substantial number of writhes in control animals pretreated with vehicle. At doses of 62.5–1000 mg/kg i.p., the SC-L extract significantly inhibited the writhing responses of mice caused by the intraperitoneal administration of acetic acid in a dose-dependent manner. At a dose of 15.63 mg/kg, the number of writhing responses did not change significantly when compared with the control (Fig. 1).

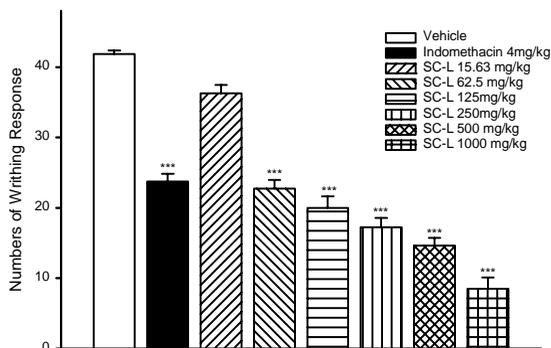


Figure 1. The effect of *Strobilanthes cusia* leaf (SC-L) methanolic extract on writhing responses test in mice. Data are shown as mean  $\pm$  SE (n = 8). \*\*\*p < 0.001 compared with control.

*Antinociceptive Test*

Various doses of SC-L extract (62.5, 125, 250, 500 and 1000 mg/kg, i.p.) show dose-dependent antinociceptive effects on both the early and late phases of the formalin test (Fig. 2). SC-L extract (62.5–1000 mg/kg) significantly reduced the licking time on the early phase and inhibited the licking time on the late phase.

*Carrageenan-induced Paw Edema in Rats*

Percentages of edema, calculated for each group for SC-L extract and indomethacin are presented in Fig. 3. The rat's footpad became edematous soon after injection of carrageenan. Edema rate of the right footpad reached its peak at 3 hours (69.40%). Administration of SC-L extract at 1000 mg/kg i.p. significantly inhibited the development of pad swelling from 1.5 to 5 hours after carrageenan injection, and at 500 mg/kg, it reduced the paw edema from 1.5 to 3 hours. At 250 mg/kg, it affected the edema rate significantly when compared with the control at 3 hours. At 125 mg/kg, the change in percentage of edema was insignificant when compared with the control at any time point.

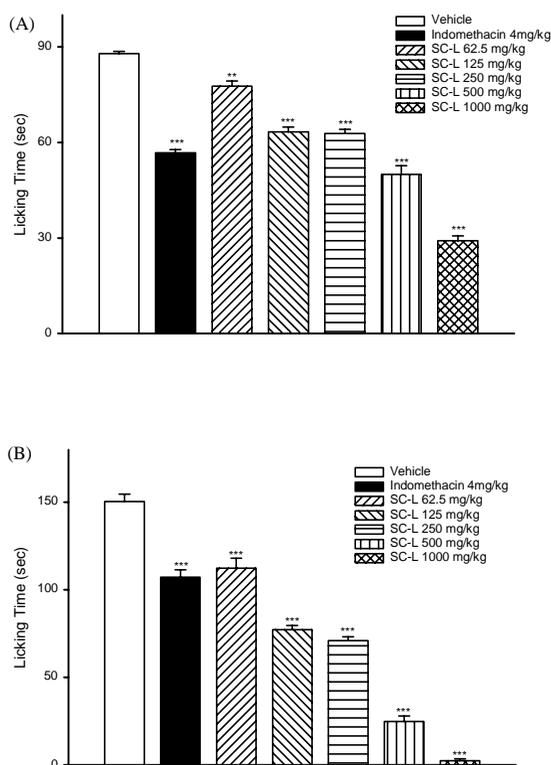


Figure 2. Effects of *Strobilanthes cusia* leaf (SC-L) methanolic extract on the early phase (A) and late phase (B) of formalin test in mice. Data are shown as mean  $\pm$  SE (n = 8). \*\*p < 0.01, \*\*\*p < 0.001 compared with control.

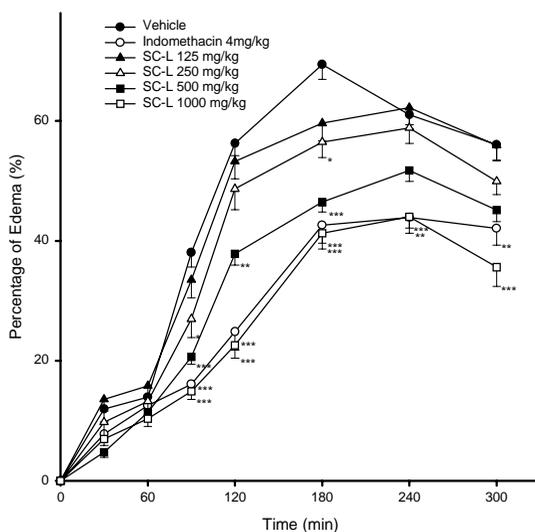


Figure 3. Influence of *Strobilanthes cusia* leaf (SC-L) methanolic extract on carrageenan-induced paw edema in Wistar rats. Data are shown as mean  $\pm$  SE (n = 8). \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001 compared with control.

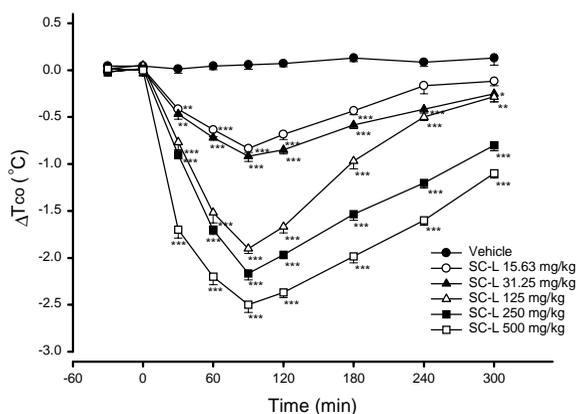


Figure 4. Time course of the effects of *Strobilanthes cusia* leaf (SC-L) methanolic extract on the change of colonic temperature in Sprague-Dawley rats. SC-L extract was injected at 0 minutes. Data are shown as mean  $\pm$  SE (n = 6). \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001 compared with vehicle group.

### Antipyretic Activity Test

Figure 4 shows the time course of the effects of SC-L extract on normal body temperature in rats without lipopolysaccharide pretreatment. In normal rats, SC-L extract (15.63, 31.25, 125, 250 and 500 mg/kg, i.p.) produced a dose-related fall in colonic temperature at room temperature ( $24 \pm 1^\circ\text{C}$ ). The colonic temperature dropped to a minimum at 1.5 hours with each dose. The fever induced by lipopolysaccharide (1 mg/kg, i.p.) was attenuated by treatment with SC-L extract. Intraperitoneal administration of lipopolysaccharide (1 mg/kg) plus SC-L extract (15.63, 31.25, 125, 250 and 500 mg/kg) affected basal level of colonic temperature after administration of SC-L extract for 90 minutes. Its hypothermia effect was potentiated by SC-L extract (Table 1).

**Table 1. Effects of SC-L Extract on the Maximum Change of Colonic Temperature in the Hyperthermia Rats Induced by Lipopolysaccharide (LPS)**

Treatment	Change in Colonic Temperature ( $\Delta^{\circ}\text{C}$ )	
	Control	LPS
Vehicle (i.p.)	0.06 $\pm$ 0.05	1.15 $\pm$ 0.08
SC-L (i.p.)		
15.63 mg/kg	-0.83 $\pm$ 0.04*	0.77 $\pm$ 0.06
31.25 mg/kg	-0.92 $\pm$ 0.05*	0.63 $\pm$ 0.05 <sup>†</sup>
125 mg/kg	-1.90 $\pm$ 0.05*	0.28 $\pm$ 0.07 <sup>‡</sup>
250 mg/kg	-2.17 $\pm$ 0.07*	0.03 $\pm$ 0.07 <sup>‡</sup>
500 mg/kg	-2.50 $\pm$ 0.08*	-0.20 $\pm$ 0.11 <sup>‡</sup>
Indomethacin (i.p.)		
4 mg/kg	-0.05 $\pm$ 0.08	0.63 $\pm$ 0.05 <sup>†</sup>
10 mg/kg	-0.33 $\pm$ 0.10	0.10 $\pm$ 0.10 <sup>‡</sup>

Data are shown as mean  $\pm$  SE (n = 6).

\*p < 0.001 compared with the vehicle control group.

<sup>†</sup>p < 0.01, <sup>‡</sup>p < 0.001 compared with the LPS group.

## Discussion

The present study shows that SC-L extract significantly antagonised acetic acid-induced writhing and significantly attenuated the nociception produced by formalin injection. The acetic acid writhing test was normally used to study the peripheral analgesic effect of drugs. Although this test is a non-specific model (e.g. anticholinergic and antihistaminic and other agents also showed activity in this test), it was widely used for analgesic screening. It involved local peritoneal (cholinergic and histaminic receptors) and the acetylcholine and histamine mediators. SC-L extract dose-dependently inhibited the writhing responses of mice. This result illustrated the antinociceptive effects of SC-L extract might be peripherally mediated.

The licking time in the formalin test showed biphasic responses. The early phase (0–5 minutes) was mediated centrally by stimulating the nociceptor directly and by releasing substance P or bradykinin; and the late phase (15–40 minutes) was mediated peripherally via releasing some chemical transmitters (e.g. histamine, serotonin, prostaglandins, kinins, etc.) (Shibata *et al.*, 1989). SC-L extract decreased the licking time in the formalin test in mice on both the early and late phases. The result indicated that the antinociceptive effects of SC-L extract might be mediated by its central and peripheral effects.

Carrageenan rat paw edema is a suitable test for evaluating anti-inflammatory drugs which had been frequently used to assess the anti-edematous effect of natural products (Basu and Nag Chaudhuri, 1991). Various mediators were released by carrageenan in the rat paw. Thus, while the initial phase might be due to the release of histamine and serotonin, kinins may play a role in the middle phase (Di Rosa and Sorrentino, 1968), and prostaglandins could be the most important mediators in the final 3–5 hours post-carrageenan response (Vinegar *et al.*, 1969). Figure 3 showed that SC-L extract inhibited the middle and final phases of edema, suggesting that the extract had a non-selective inhibitory effect on the release or actions of these mediators.

Lipopolysaccharide, an integral component of the outer membrane of gram-negative bacteria, stimulates myeloid cells to synthesize cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), which induced marked changes in behavior, metabolic and the neuroendocrine systems. These cytokine-mediated events are part of a general homeostatic reaction and therefore served as the organism's first line of defense against infection (Hart, 1988). The studies of Kluger (1991) and Derijk *et al.* (1993) showed that lipopolysaccharide caused fever in rats. In the present study, we administered lipopolysaccharide to rats to investigate the antipyretic effect of SC-L extract. SC-L extract attenuates pyrexia by lipopolysaccharide, therefore suggesting that SC-L extract might affect TNF- $\alpha$ , IL-1 or IL-6 production to attenuate lipopolysaccharide-induced pyrexia.

In conclusion, the data obtained in this study demonstrate that SC-L has antinociceptive, anti-inflammatory and antipyretic activities and affirmed the claim by traditional medicine practitioners of their use in seasonal febrile diseases and inflammatory diseases with redness of skin and sore throat (Editorial Committee of Chung-Hwa-Pen-Tsao, 1999). However, further studies are necessary to fully elucidate the mechanism of action of *Strobilanthes cusia*.

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