Antipyretic, Analgesic and Anti-inflammatory Activities of
Strobilanthes formosanus

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ABSTRACT

The methanolic extract of Strobilanthes formosanus was investigated for its antipyretic, analgesic and anti-inflammatory activities in animal models. In unanesthetized rats, the extract (100-1000 mg/kg, i.p.) caused a dose-related fall in colonic temperature at room temperature (24 ± 1°C). The fever induced by interleukin-1β (10 ng/10 μL, lateral cerebral ventricle injection) was attenuated by treatment with the extract (50-1000 mg/kg, i.p.). However, the extract (1000 mg/kg) and acetaminophen (50 mg/kg) did not significantly attenuate pyrexia induced by PGE₂ (200 ng/10 μL, i.c.v.). The extract significantly and dose-dependently inhibited the writhing response of mice and decreased the licking time in both the early and late phases of the formalin test. A significant (P ≤ 0.05) inhibition of neutrophil degranulation induced by fMLP was also produced by the extract. This study established the antipyretic, analgesic and anti-inflammatory activities of S. formosanus.

Key words: Strobilanthes formosanus; Antipyretic; Analgesic; Anti-inflammatory.

INTRODUCTION

Strobilanthes formosanus (Moore) Hsieh et Huang (Acanthaceae) is a perennial herb endemic to Taiwan. The plant is used in Chinese folk medicine for the treatment of coughing, fever, mumps, sore-throats, hepatitis, and trauma. There are no chemically and pharmacologically investigations that support these traditional claims. Otherwise, a number of chemical constituents had been reported from this genus, such as terpenes from S. auriculatus, alkaloids and triterpenes from S. cusia and flavonoids from S. dyeriana. From pharmacological study, diuretic activity has been reported from the aerial part of S. boerhaavioide, uterine stimulant effect and vasoconstrictor activity from S. crispus, antimicociceptive, anti-inflammatory and antipyretic effects from the leaves of S. cusia, and antispasmodic activity from the entire plant of S. dalhausiana. Since most species of Strobilanthes were reported to possess a broad spectrum of pharmacological activities, we examined the antipyretic, analgesic and anti-inflammatory activities of S. formosanus.
MATERIALS AND METHODS

Plant Material
The plant material used in this study was collected from Nantou Shien of central Taiwan in November, 2000. A voucher specimen (No. ICPS-98003) was deposited in the herbarium of the Institute of Chinese Pharmaceutical Sciences, China Medical College, Taichung, Taiwan.

Preparation of SF Extracts
The air-dried entire plant of *S. formosanus* (SF) (8.7 kg) was exhaustively extracted with methanol (60 L × 5) at room temperature. The extract was filtered and concentrated under reduced pressure using a rotary evaporator to obtain a dark syrup (830 g). The extract was suspended in 0.5% carboxymethyl cellulose (CMC) for administration to animals.

Animals
Male ICR mice (20-25 g) and male Sprague-Dawley rats (220-250 g) were obtained from the National Laboratory Animal Breeding and Research Center, National Science Council, Taiwan. They were housed in standard cages at a constant temperature of 22 ± 2°C, relative humidity of 55 ± 5% with 12 h dark-light cycle. The animals were provided with pelleted diet and tap water *ad libitum*.

Acute Toxicity
Mice (10 per group) were administered intraperitoneal and oral doses of the SF extract. Mortality in each group within 72 h was recorded.\(^\text{12}\)

Antipyretic Activity

A. Rat training and the method of measuring colonic temperature
Sprague-Dawley rats were trained to be kept quietly under minimal restraint in special rat stocks for periods of several hours at a time. The animals had freedom to move their limbs and neck and remained in an alert condition. Temperature was measured in a temperature-controlled room (ambient temperature 24 ± 1°C). The colonic temperature was measured every minute with a copper-constantan thermocouple which was connected to a thermometer (Portable Hybrid Recorder, Yokogawa Hokushin Electric, Japan). The copper-constantan thermocouple was enclosed in polyethylene 200 tubing, sealed at one end, and then inserted 50 mm into the colon.\(^\text{13}\)

B. SF extract effect on the normal rats colonic temperature change under room temperature
The trained animals were used in groups of six per dose of SF extract (100-1000 mg/kg), acetaminophen (50, 100 mg/kg) or vehicle. The colonic temperature of each animal was allowed to stabilize for at least 60 min before any injections, and then observed for 5 h.\(^\text{13}\)

C. Surgery
Sprague-Dawley rats were anaesthetized with sodium pentobarbital (40 mg/kg, i.p.) and placed in a Kopf stereotaxic frame (Grass Instrument Co., W. Warwick, RI, USA) in the flat skull position. A 10 mm guide tube cannula (21 gauge stainless-steel tubing) was implanted in the lateral ventricle (LV) using the coordinates (anterior-posterior-0.8 mm to the left or right, lateral 1.5 mm in relation to the bregma, and 3.5-4.0 mm below the surface of the brain). After appropriately located craniotomy holes had been trephined, two self-tapping screws were attached to the calvaria of the parietal bones and the cannula guide tubes were inserted to the desired depth through the craniotomy holes. They were anchored with fast-drying dental cement to the cranial surface, which had been scraped clean of periosteum. Following surgery, the guide cannula
was plugged with a stylet and rats were returned to their cages for a minimum recovery period of one week.  

D. SF extract effect on induced fever colonic temperature change by interleukin-1β

The animals were pretreated with interleukin-1β (IL-1β, 10 ng/10 μL, i.c.v.) 30 min before the administration of SF extract (50, 100, 500, 1000 mg/kg body wt., i.p.), vehicle (0.5% CMC, i.p.) or acetaminophen (50, 100 mg/kg, i.p.). The colonic temperature of each animal was allowed to stabilize for at least 60 min before any injections, and then observed for 5 h.  

E. SF extract effect on induced fever colonic temperature change by prostaglandin E₂

Group 1 was pretreated with acetaminophen (50 mg/kg, i.p.) 30 min before administration of prostaglandin E₂ (PGE₂, 200 μg/10 μL, i.c.v.) and another group was given PGE₂ and SF extract (1000 mg/kg, i.p.) at the same time. The colonic temperature of each animal was allowed to stabilize for at least 60 min before any injections, and then observed for 5 h.  

Analgesic Activity

A. Acetic acid induced writhing method

Mice were used in groups of six per dose of plant extract or drug. The animals were pretreated with SF extract (10, 50, 100, 500, 1000 mg/kg, i.p.) or acetaminophen (50, 100 mg/kg, i.p.) for 5 min or 30 min, respectively, prior to i.p. injection of 1% acetic acid (0.1 mL/g). Five minutes after the i.p. injection of acetic acid, the number of writhings during the following 15 min were counted. Control mice received vehicle and the acetic acid experiment was repeated.  

B. Formalin test

Male ICR mice received 0.5% CMC as vehicle or various doses of SF extract (500, 1000, 1500 mg/kg) by intraperitoneal (i.p.) injection. Five min-
utes after 20 μL of 1% formalin was injected subcutaneously (s.c.) into the right hind paws of the mice using a microsyringe, the total hind paw licking time of each mouse was counted at 5 min intervals for 40 min. Acetaminophen (50 mg/kg, i.p.) was administered intraperitoneally 30 min prior to formalin injection. The first period (early phase) was recorded 0-5 min after the injection of formalin and the second period (late phase) was recorded 15-40 min after the injection of formalin.  

Anti-inflammatory Activities

A. Isolation of neutrophils

EDTA-mixed fresh blood was obtained from the abdominal aorta of pentobarbital (60 mg/kg, i.p.) anesthetized rats (Sprague-Dawley, 300-350 g). Neutrophils were separated from other blood cells by dextran sedimentation and centrifugation on a Ficoll-hypaque density gradient. Erythrocytes in the pellets were lysed by suspending the cells in 0.05% NaCl for 15 s followed by washing with 1.75% NaCl containing 0.25% bovine serum albumin. Cells were re-suspended in Hanks’ balanced salt solution (HBSS) containing 4 mM NaHCO₃ and 10 mM N-[2-hydroxyethyl] piperazine-N-[2-ethanesulfonic acid] (HEPES), pH 7.4 to a final concentration of 1 x 10⁷ cell/mL. The cell preparations consisted of 90 – 95% neutrophils (viability approximately 95% by trypan blue exclusion).  

B. Measurement of β-glucuronidase and lysozyme

A neutrophil suspension was pre-incubated at 37 °C with dimethylsulfoxide (DMSO) or crude extract for 3 min before the release reaction was triggered by the addition of 1 μM IMMP in the presence of 5 μg/mL cytochalasin B. Forty-five min later, the reaction was stopped by the addition of ice-cold Tyrode solution and centrifuged for 10 min at 1000 x g. β-Glucuronidase activity in the supernatant was determined by spectrophotometry at 550 nm af-
ter reaction with phenolphthalein-β-D-glucuro-
nidase as substrate. The release of β-glucuronidase
and lysozyme was expressed as percentage release
[release elicited by secretagogue spontaneous
release]/total content] × 100. The total content was
measured after treatment of the cell suspension
with Triton X-100. Spontaneous release was less
than 10%.20

STATISTICAL METHOD

The results are expressed as the mean ± S.E.
and the statistical significance of differences be-
tween groups was analyzed by one way analysis of
variance (ANOVA). P ≤ 0.05 was considered as sig-
ificant.

RESULTS

A. Acute Toxicity

The LD₅₀ of the SF extract when adminis-
tered intraperitoneally and orally were 3.9 g/kg and
more than 10 g/kg, respectively (Table 1).

B. Antipyretic Activity

Table 2 shows the time course of the effects of
SF extract on normal body temperature in rats. In

C. Analgesic Activity

In control mice, the number of writhes during
the 15 min test period was 46.0 ± 2.2 (n = 6). The

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Change in colonic temperature (Δ°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.03 ± 0.08</td>
</tr>
<tr>
<td>SF</td>
<td></td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>-0.40 ± 0.09**</td>
</tr>
<tr>
<td>500 mg/kg</td>
<td>-0.80 ± 0.06***</td>
</tr>
<tr>
<td>1000 mg/kg</td>
<td>-1.07 ± 0.10***</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>0.10 ± 0.00</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>0.07 ± 0.07</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>0.07 ± 0.07</td>
</tr>
</tbody>
</table>

SF extracts were injected at 0 min. Data are shown as mean ± S.E. (n = 6). *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 compared with the vehicle group.

Table 1. The Acute Toxicity of SF Extract in Mice

<table>
<thead>
<tr>
<th>S. formosanus</th>
<th>LD₅₀ Dose (g/kg)</th>
<th>95% confidence limits (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.p.</td>
<td>3.99</td>
<td>3.69-4.32</td>
</tr>
<tr>
<td>p.o.</td>
<td>&gt; 10</td>
<td>-</td>
</tr>
</tbody>
</table>

normal rats, SF extract (100, 500, 1000 mg/kg, i.p.)
produced a dose-related fall in colonic tempera-
ture at room temperature and reached its maximum at 30
min after injection of each dose of SF extract. In
contrast, acetaminophen had no effect on body
temperature. The fever induced by IL-1β (10 ng/10 μL,
i.e.v.) was also attenuated by treatment with SF ex-
tract. Table 3 shows that i.e.v. administration of
IL-1β (10 ng/10 μL) plus SF extract (50, 100, 500,
1000 mg/kg, i.p.) affected the basal level of colonic
temperature after administration of SF extract 120
min after administration of IL-1β. The pyrexia was
attenuated by SF extract (50, 100, 500, 1000 mg/kg,
i.p.) and acetaminophen (50, 100 mg/kg, i.p.). Ta-
ble 4 shows that the fever induced by PGE₂ (20
ng/10 μL, i.e.v.) was not markedly attenuated by
treatment of SF extract and acetaminophen.
Table 3. Time Course of the Effects of SF Extract on the Hyperthermia Induced by Intracerebroventricular Injection (i.c.v.) of IL-1β in Rats

<table>
<thead>
<tr>
<th>Treatment (i.p.)</th>
<th>ΔT (°C) after IL-1β administration for</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>120 min</td>
</tr>
<tr>
<td>IL-1β 10 ng/10 μL</td>
<td>1.90 ± 0.08</td>
</tr>
<tr>
<td>IL-1β + SF</td>
<td>2.23 ± 0.17</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>1.68 ± 0.08</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>1.75 ± 0.10</td>
</tr>
<tr>
<td>500 mg/kg</td>
<td>1.73 ± 0.19</td>
</tr>
<tr>
<td>1000 mg/kg</td>
<td>1.80 ± 0.10</td>
</tr>
<tr>
<td>IL-1β + Acetaminophen</td>
<td>2.18 ± 0.13</td>
</tr>
</tbody>
</table>

SF extracts were injected 120 min after IL-1β injected at 0 min. The colonic temperature of vehicle-injected rats was 37.72 ± 0.22 °C at time 0 min. ΔT denotes the difference between the control values before injection and after injection. The values are shown as mean ± S.E. (n = 6). *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 compared with the IL-1β group.

Table 4. Time Course of the Effects of SF Extract on the Hyperthermia Induced by Intracerebroventricular Injection (i.c.v.) of PGE2 in Rats

<table>
<thead>
<tr>
<th>Treatment (i.p.)</th>
<th>Change in colonic temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>PGE2 20 ng/10 μL</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>SF + PGE2</td>
<td>-0.03 ± 0.03</td>
</tr>
<tr>
<td>1000 mg/kg</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

SF extracts were injected at 0 min. Data are shown as mean ± S.E. (n = 6). *P ≤ 0.05, **P ≤ 0.01 compared with the PGE2 group.

treatment of animals with SF extract (10-1000 mg/kg) produced a significant and dose dependent inhibition of the control writhes (Table 5). The inhibition by 100 mg/kg extract was similar to that produced by 50 mg/kg acetaminophen (31.89, 33.33, respectively).

The extract demonstrated a dose-dependent relationship in both phases of formalin induced

Table 5. Effect of the SF Extract on Acetic Acid-induced Writhing in Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>No. of writhings</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>-</td>
<td>46.00 ± 2.18</td>
<td>-</td>
</tr>
<tr>
<td>S. formosanus</td>
<td>10</td>
<td>43.50 ± 1.67*</td>
<td>5.43</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>36.50 ± 2.43*</td>
<td>20.65</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>31.33 ± 1.71**</td>
<td>31.89</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>21.67 ± 1.73***</td>
<td>52.89</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>2.17 ± 0.66***</td>
<td>95.28</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>50</td>
<td>30.67 ± 2.11**</td>
<td>33.33</td>
</tr>
</tbody>
</table>

Data are shown as mean ± S.E. of six observations.
*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 compared with vehicle group.
Table 6. Effect of the SF Extract on Formalin-inducedResponses in Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>0-5 min</th>
<th>% Inhibition</th>
<th>15-30 min</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>-</td>
<td>69.99 ± 2.47</td>
<td>-</td>
<td>159.26 ± 5.20</td>
<td>-</td>
</tr>
<tr>
<td><em>S. formosanus</em></td>
<td>500</td>
<td>57.69 ± 3.37</td>
<td>17.59</td>
<td>117.19 ± 10.35**</td>
<td>26.42</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>25.84 ± 3.27***</td>
<td>63.08</td>
<td>69.18 ± 5.64***</td>
<td>56.56</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>6.58 ± 1.30***</td>
<td>90.60</td>
<td>20.73 ± 4.47***</td>
<td>86.98</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>50</td>
<td>47.59 ± 3.02***</td>
<td>32.00</td>
<td>48.11 ± 4.48***</td>
<td>69.79</td>
</tr>
</tbody>
</table>

Data are shown as mean ± S.E. of six observations. **P ≤ 0.01, ***P ≤ 0.001 compared with vehicle group.

Table 7. The Inhibitory Effect of the SF Extract on the Release of β-Glucuronidase and Lysozyme during Neutrophil Degranulation Induced by FMLP

<table>
<thead>
<tr>
<th>Drugs</th>
<th>(µg/mL)</th>
<th>β-Glucuronidase</th>
<th>% Inhibition</th>
<th>Lysozyme</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>28.9 ± 1.1</td>
<td>***</td>
<td>26.7 ± 2.2</td>
<td>***</td>
</tr>
<tr>
<td>SF</td>
<td>(1)</td>
<td>24.5 ± 0.4</td>
<td>17.5 ± 2.7</td>
<td>18.7 ± 2.1*</td>
<td>29.5 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>(3)</td>
<td>21.7 ± 0.5**</td>
<td>28.4 ± 3.0</td>
<td>14.0 ± 2.5**</td>
<td>48.0 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>13.9 ± 0.8**</td>
<td>52.6 ± 4.0</td>
<td>11.1 ± 1.6*</td>
<td>58.1 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>(30)</td>
<td>8.1 ± 0.6**</td>
<td>70.0 ± 3.3</td>
<td>8.8 ± 0.4**</td>
<td>65.3 ± 5.8</td>
</tr>
<tr>
<td>IC50</td>
<td></td>
<td>9.2 ± 0.2</td>
<td>5.4 ± 1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TFP</td>
<td>(3 µM)</td>
<td>27.8 ± 1.3</td>
<td>4.2 ± 3.9</td>
<td>22.9 ± 2.4</td>
<td>13.6 ± 4.6</td>
</tr>
<tr>
<td></td>
<td>(10 µM)</td>
<td>9.5 ± 1.3**</td>
<td>67.2 ± 2.9</td>
<td>11.0 ± 0.6**</td>
<td>57.6 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>(30 µM)</td>
<td>5.0 ± 0.4**</td>
<td>81.5 ± 1.9</td>
<td>1.7 ± 1.1**</td>
<td>94.4 ± 3.4</td>
</tr>
<tr>
<td>IC50</td>
<td></td>
<td>9.3 ± 0.2</td>
<td>8.1 ± 0.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n = 3, *P ≤ 0.05, **P ≤ 0.01. Trifluoperazine (TFP): positive control.

The central control of body temperature, but it can induce leukocytes and phagocytic macrophages to release endogenous pyrogens, such as IL-1, tumor necrosis factor, interferon and platelet-activating factor. These endogenous pyrogens then act on the central control of body temperature and lead to fever by prostaglandins, somatostatin, thyrotropin releasing factor, monoamines and other unknown protein factors. IL-1β is important in the fever mechanism of endogenous pyrogens. IL-1β injection by the i.c.v. route can induce a dose-dependent, apparent and continuously reaction in rats (above 6 hours). In this study, SF extract (100-1000 mg/kg) caused a dose-related fall in colonic temperature at room temperature (24 ± 1 °C). However, acetaminophen (50, 100 mg/kg) did not produce hypothermic effect in normal rats. The fever induced pain. (Table 6). The positive control acetaminophen (50 mg/kg) also produced significant (P ≤ 0.05) inhibition in the early and late phases.

**D. Anti-inflammatory Activity**

The SF extract inhibited the release of β-glucuronidase and lysozyme upon neutrophil degranulation induced by FMLP with IC50 values of 9.2 ± 0.2 and 5.4 ± 1.0 µg/mL, respectively (Table 7).

**DISCUSSION**

Fever, a disease symptom, is often described in clinical. Pyrogen, an induced fever substance, includes exogenous pyrogen and endogenous pyrogen. Exogenous pyrogen (endotoxin) is a large and complex molecule which can not directly act on

The SF extract inhibited the release of β-glucuronidase and lysozyme upon neutrophil degranulation induced by FMLP with IC50 values of 9.2 ± 0.2 and 5.4 ± 1.0 µg/mL, respectively (Table 7).
by IL-1β was also attenuated by treatment with SF extract (50-1000 mg/kg) and acetaminophen (50, 100 mg/kg) in rats. It showed that SF extract possessed both hypothermia and antipyretic effects and acetaminophen only had latter effect. Neither extract nor acetaminophen attenuated pyrexia induced by PGE2. The results showed that S. formosanus had antipyretic effects and might possess the same thermoregulation mechanism of PGE2 as acetaminophen.

The present study showed that SF extract significantly antagonized acetic acid-induced writhing and significantly attenuated the nociception produced by formalin injection. The acetic writhing test is normally used to study the peripheral analgesic effects of drugs. Although this test is a nonspecific (e.g., anti-cholinergic, antihistaminic and other agents also show activity in this test), it is widely used for analgesic screening and involves local cholinergic and histaminic receptors, and the mediators acetylcholine and histamine.18,24 SF extract significantly and dose-dependently inhibited the writhing responses of mice. This result indicates that the analgesic effects of SF extract might be mediated by its peripheral effect. The licking time in the formalin test shows biphasic responses; the early phase (0-5 min) is mediated by the central effect via direct stimulation of the nociceptor and release of substance P or bradykinin whereas the late phase (15-40 min) is mediated by the peripheral effect via release of chemical transmitters (e.g. histamine, serotonin, prostaglandins, kinins, etc.).24 The SF extract decreased the licking time in the formalin test in mice in both the early and late phases. This result indicates that the analgesic effects of the SF extract might be mediated by both central and peripheral effects.

In inflammatory reactions, activated neutrophils release mediators such as β-glucuronidase, lysozyme and platelet-activating factor.25 These substances lead to bronchial smooth muscle contraction, vasodilatation, increased vascular permeability and inflammatory disease.26,27 Neutrophils also release active substances such as hydrogen peroxide, superoxide anions and hydroxyl radicals which play important roles in producing cellular damage and are associated with aging and rheumatism.28,29 If the releasing reaction of neutrophils can be controlled, it will help prevent and cure inflammatory diseases. Furthermore, the SF extract showed significant inhibitory effects against β-glucuronidase and lysozyme release. The IC50 values of the extract were 9.2 and 5.9 μg/mL, respectively. The data indicate that SF extract could inhibit inflammation.

In conclusion, the data obtained in this study demonstrated that S. formosanus might have antipyretic, analgesic and anti-inflammatory activities. However, further studies are necessary to fully elucidate the mechanism of action of the plant.

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