Antiinflammatory and Analgesic Activities of the Tissue Culture of *Saussurea involucrata*

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The tissue culture of *Saussurea involucrata* (TCSauI) *KAR. et KIR.* was studied to determine its antiinflammatory and analgesic activities in experimental animals. Similar to wild *S. involucrata*, TCSauI at doses of 75—300 mg/kg i.g. for 7 d markedly inhibited hindpaw edema induced by carrageenin in rats, ear edema induced by dimethylbenzene, and increased capillary permeability in the mouse abdominal cavity induced by acetic acid. Moreover, TCSauI had inhibitory activities against the writhing reaction induced by acetic acid and the hot plate reaction in mice. The present study provided evidence first that TCSauI has antiinflammatory and analgesic activities, suggesting the potential of the tissue culture technique to substitute for wild *S. involucrata* in the pharmaceutical industry.

Key words *Saussurea involucrata*; tissue culture; antiinflammation; analgesia

*Saussurea involucrata* *KAR. et KIR.* (Compositae), a rare Chinese medicinal herb on the verge of extinction, grows in the mountains at heights of 2800—3400 m in the Tianshan and A’er Tai areas in China. The flower and stem have long been used as an herbal medicine in most areas of China. In folk medicine, it is used for the treatment of rheumatoid arthritis, cough with cold, stomachache, dysmenorrhea, and altitude sickness, among others. Pharmacologic studies have demonstrated that *S. involucrata* and its constitutes have antiinflammatory, cardiotoxic, abortifacient, anticancer, and anti-fatigue actions.

The secondary metabolites in wild *S. involucrata* are mainly flavonoids, including apigenin, kaempferide, acacetin, luteolin, apigenin-5,6-dimethoxy-flavone, apigenin-6-methoxy-flavone, quercetin, rutin, etc. Other constitutes, such as alkaldoids, steroids, and lignans were also reported.

It is well known that *S. involucrata* grows very slowly. In recent years, the wild sources of *S. involucrata* have decreased dramatically due to the exhaustive collection for use in pharmaceutical preparations. To conserve the natural sources of *S. involucrata*, tissue culture is being developed, which might be used as a potential substitute for wild *S. involucrata* in the pharmaceutical industry. In the present study, therefore, the antiinflammatory and analgesic activities of the product of tissue culture of *S. involucrata* were investigated.

MATERIALS AND METHODS

Materials. Animals Wistar rats, weighing 120—220 g, and Swiss mice, weighing 18—22 g, were used in the studies. The Experimental Animal Center of Shenyang Pharmaceutical University supplied all animals. The animals were housed under standard conditions (22±2 °C, 50±10% relative humidity, 12-h light/dark cycles). Food and water were available *ad libitum*. During the experiments, half male and half female animals were used in each group.

Plant Material The wild *S. involucrata* (WSauI) used in this study was purchased from a local market in the Xinjiang region (China), and identified by Professor Yilin Chen (Institute of Botany, Chinese Academy of Sciences). The specimen of WSauI was deposited in the China National Herbarm (No. 020805, Beijing, China). The dried flowers (2.0 kg) of WSauI were ground and extracted three times with 95% ethanol. The extracts were concentrated under vacuum evaporation to afford brown extracts (yield 6.3%). The content of total flavonoids in the dried extract was 1.07%.

Tissue Culture Material Explants of stems and leaves of WSauI were cultured on MS medium with naphylacetic acid 2.0 mg/l, 6-benzylaminopurine 0.5 mg/l, 3% sucrose, and 0.65% agar for the inducement of callus and tissue culture. The culture conditions were: temperature, 24±1 °C; light intensity, 58.4 μmol·m−2·s−2; and light period, 12 h/d. The calli were dried at 60 °C and ground through a 40-mesh sieve. The tissue cultures of *S. involucrata* (TCSauI) (5.0 kg) were extracted three times with 95% ethanol. The extracts were concentrated under vacuum evaporation to afford brown extracts (450 g). The content of total flavonoids in the brown extracts was 2.6%. We inspected the quality stability of culture patches by determining the total flavonoids in TCSauI using spectrophotometric and HPLC methods. Our studies on the chemical constituents and quality control of TCSauI showed that different culture patches of TCSauI had good stability (data not shown).

All doses used in the experiments were the weight of the extract of the callus tissue. All samples were suspended in distilled water containing 0.5% carboxymethylcellulose sodium (CMC-Na) before use. Other reagents were purchased from commercial channels.

In all experiments, animals were randomly divided into six groups. TCSauI was administered at doses of 75, 150, 300 mg/kg i.g. WSauI was administered at the doses of 150 mg/kg i.g. Indomethacin (Chifeng Pharmacertical Factory, China) 10 mg/kg i.g. was administered only once during the entire experiment. Animals in the control group were administered 0.5% CMC-Na i.g. TCSauI and WSauI were administered once a day for 7 d since pilot studies showed that acute treatment with TCSauI or WSauI has no obvious pharmacologic activity.

Antinflammatory Effects Dimethylbenzene-Induced Ear Edema in Mice: The method described by Chen was...
used with slight modification. Dimethylbenzene (25 μl per mouse) was applied on both sides of the right ear 1 h after the last drug administration. The mice were killed by cervical dislocation 2 h after the application of dimethylbenzene. The right ear and left ear were amputated at the same place with a hole-opening utensil (ID = 7 mm) and weighed. The rate of ear edema was calculated according to the following equation:

\[ \text{edema rate} (\%) = \frac{(B - A) 	imes 100}{A} \]

Where B is the weight of the right ear and A the weight of the left ear.

Carrageenan-Induced Hindpaw Edema in Rats: The experiment was carried out following the method described by Winter et al.14) Carrageenan (1% in saline) was injected into the plantar surface of the right hind paw of the rats 1 h after the last drug administration. Then the hindpaw volume was measured once every hour for 4 h and the rate of paw edema was calculated according to the following equation:

\[ \text{edema rate} (\%) = \frac{(B - A) 	imes 100}{A} \]

Where B is the volume of the paw after carrageenan injection and A that before the injection.

Acetic Acid-Induced Increase in Capillary Permeability in Mouse Abdominal Cavity The experiment was carried out according to the method described by Chen.13) Evans blue (0.5% in saline, 0.1 ml per mouse) was injected intravenously 1 h after the last administration. Acetic acid (0.6% in saline, 0.2 ml per mouse) was injected intraperitoneally. Mice were killed by cervical dislocation 20 min after the injection of acetic acid. Then the abdominal cavity was washed several times with saline in a total of 4 ml per mouse. The washing solutions were collected and centrifuged for 5 min (1000 rpm). The content of Evans blue in the supernatant was determined with a spectrophotometer (Model 721, Shanghai, China) at 590 nm.

Analgesic Effects Acetic Acid-Induced Writhing in Mice: The method described by Siegmund et al.15) was used. Animals were injected intraperitoneally with acetic acid (0.8% in saline, 0.3 ml per mouse) 1 h after the last administration. The number of writhings occurring between 5 to 20 min after the injection of acetic acid was recorded.

Hot Plate Test in Mice: The hot plate test was carried out according to the method described by Eddy and Leimbach.16) Mice were placed on a hot plate maintained at 55 ± 0.5 °C and the time in seconds between the placement of mice on the platform and licking of the hindpaw or jumping was recorded as the response latency. Latency measures were recorded (before drug) and 30 min after the last drug administration. Mice exhibiting latency time greater than 30 s or less than 5 s were excluded. If the mice did not respond to the stimulus, the latency was recorded as 60 s.

Statistical Analysis The results are expressed as mean ± S.E.M. One-way analysis of variance (ANOVA) followed by Dunnett’s test was used to compare the drug effects with the blank control group.

RESULTS

Antiinflammatory Effects The results showed that at the dose of 300 mg/kg i.g. for 7 d, TCSaul had significant inhibitory effects on dimethylbenzene-induced ear edema in mice (Table 1).

Administration of carrageenan induced progressive edema reaching a maximum after 3 h. TCSaul at doses of 75, 150, and 300 mg/kg i.g. for 7 d showed significant suppressive effects on carrageenan-induced edema at 3 to 4 h (Table 2).

Moreover, TCSaul at doses of 150 and 300 mg/kg i.g. for 7 d showed significant inhibitory effects on the acetic acid induced increase in capillary permeability in the abdominal cavity of mice. WSAul 150 mg/kg showed even more significant effects (Table 3).

Analgesic Effects At doses of 75, 150, and 300 mg/kg i.g. for 7 d, TCSaul significantly inhibited the increase in the writhing reaction in mice induced by acetic acid in comparison with the control group (Table 4). It also significantly increased the pain threshold in the hot plate experiment in mice (Table 5).

DISCUSSION

Tissue culture has the advantages of resource conservation and industrial potential. However, the efficacy of the products

Table 1. Effects of TCSaul and WSAul on Dimethylbenzene-Induced Ear Edema in Mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg/d, i.g.)</th>
<th>No. of animals</th>
<th>Rate of ear edema (%) (Mean ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>10</td>
<td>110.50 ± 8.39</td>
</tr>
<tr>
<td>TCSaul</td>
<td>75</td>
<td>10</td>
<td>97.48 ± 9.67</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>10</td>
<td>95.56 ± 8.85</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>10</td>
<td>77.13 ± 13.47*</td>
</tr>
<tr>
<td>WSAul</td>
<td>150</td>
<td>10</td>
<td>107.70 ± 11.77</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>10</td>
<td>51.05 ± 5.49***</td>
</tr>
</tbody>
</table>

*p < 0.05, ***p < 0.001 vs. control.

Table 2. Effects of TCSaul and WSAul on the Hind Paw Edema Induced by Carrageenan in Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg/d, i.g.)</th>
<th>No. of animals</th>
<th>Edema rate at different time (% (Mean ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>10</td>
<td>23.03 ± 5.93</td>
</tr>
<tr>
<td>TCSaul</td>
<td>75</td>
<td>10</td>
<td>16.67 ± 3.48</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>10</td>
<td>22.43 ± 4.23</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>10</td>
<td>20.36 ± 4.19</td>
</tr>
<tr>
<td>WSAul</td>
<td>150</td>
<td>10</td>
<td>27.18 ± 3.78</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>100</td>
<td>10</td>
<td>9.83 ± 3.54</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01, ***p < 0.001 vs. control.
of tissue cultures is crucial if they are used as substitutes for medicinal plants. The present study, using different animal models first demonstrated that TCSauI has significant antiinflammatory and analgesic actions, which imply the potential usefulness of the tissue culture of S. involucrata.

Previous studies have shown that the ethanolic extract and flavones of wild S. involucrata inhibit egg albumen-induced edema in the rat hind paw, through the activation of the cortical function of the adrenal gland. In the present study, using dimethylbenzene-induced ear edema in mice and carrageenan-induced hindpaw edema in rats, we demonstrated that the tissue culture of this plant had significant antiinflammatory effects. Moreover, it was observed that TCSauI showed the most pronounced effect 3 h after carrageenan induction of hindpaw edema in rats, suggesting that the action is related to the inhibition of prostaglandin release.

The present study demonstrated that both TCSauI and the extract of wild S. involucrata inhibited acetic acid-induced increase in capillary permeability in the abdominal cavity of mice, which would further decrease the inflammatory reactions by inhibition of exudation. The inhibitory effects of TCSauI and the extract of wild S. involucrata on acetic acid-induced writhing and hot plate-induced increase in reaction time suggested that both samples have analgesic activity which was not only mediated peripherally but also centrally. It has been reported that flavonoids in WSAul showed inhibitory activities on the central nervous system in mice.

In the present study, the pharmacologic potencies of TCSauI and WSAul were not the same. It appeared that WSAul was more potent in the inhibition of pain reactions than TCSauI. The difference in efficacy and/or potency between TCSauI and WSAul may be due to the different parts used in extraction since the extracts of WSAul were from the flowers, and those of TCSauI were from the stems and leaves, which might have different levels of flavonoids. Moreover, the different contents of other constituents may be another reason. It is suggested that alkaloids from wild S. involucrata might have antiinflammatory activities, and poly saccharides of S. involucrata can scavenge superoxide anions, which is one crucial factor in inflammatory reactions. Further studies are needed to evaluate the effects of individual constituents that contribute to the antiinflammatory or analgesic effects of S. involucrata.

However, the effects of TCSauI appeared not to depend on the dose. Previous data also showed the inhibition of inflammation by WSAul may not be strictly dose dependent. Since both TCSauI and WSAul contain different constituents, as mentioned above, their mechanisms of action may be complex, and further efforts are required to elucidate the mechanisms by which TCSauI and WSAul exert their broad biological effects.

In conclusion, the present study demonstrated that TCSauI has the antiinflammatory and analgesic actions, which are also exhibited by the wild plant. These results illustrate the potential use of tissue culture techniques to substitute for the exhaustive harvesting of wild S. involucrata in the pharmaceutical industry.

REFERENCES