An Investigation of the Antiinflammatory Effects of an Extract from Cladonia rangiformis Hoffm.

Halıs Süleyman, a,b Derya Yıldırım, a Ali Aslan, a Fatma Göçer, a Akçahan Gepdiremen, a and Zuhal Güvenalp c

Atatürk University, Faculty of Medicine, Department of Pharmacology; a 25240 Erzurum, Turkey. Atatürk University, Faculty of Education, Department of Biology; b 25240 Erzurum, Turkey, and Atatürk University, Faculty of Pharmacy, Department of Pharmacognosy, c 25240 Erzurum, Turkey. Received July 2, 2001; accepted August 24, 2001

In this study, the antiinflammatory effects of 50, 100 and 200 mg/kg doses of extract obtained from Cladonia rangiformis, so-called C-1, were investigated. The effects of C-1 on the acute phase of inflammation were studied in formaldehyde-induced edema. A cotton-pellet granuloma test was used to investigate the effects of C-1 on chronic inflammation. The antiedema potency of C-1 was compared with indomethacin. C-1 at the doses mentioned above showed 33.8% (p<0.005), 36.1% (p<0.005), 43.1% (p<0.001) inhibition, respectively. The corresponding antiinflammatory effect for indomethacin was determined as 72% (p<0.001). 200 mg/kg C-1 and 10 mg/kg indomethacin decreased the formation of granuloma tissue induced by cotton-pellet method at a rate of 57.3% (p<0.005) and 52.1% (p<0.005), respectively. It was seen that C-1 was more effective on chronic inflammation than on acute inflammation.

Key words Cladonia rangiformis, antiinflammatory effect, rat

Lichens are symbiotic organisms composed of fungi and algae.1) Cladonia rangiformis is a type of lichen which also includes fungi and algae. Its appearance is bush-like, its color is gray if it is dry. Its color changes to green when it gets wet. Species of lichens are distributed in most of the environmental habitats of the world. Cladonia rangiformis is distributed in hot–dry regions at an altitude of 1100 m.2) Lichens have been used extensively in traditional medicine in the world to treat a variety of disorders such as fever, epilepsy, tuberculosis, gout, external wounds, jaundice, etc.3)

Burkholder showed the antimicrobial effects of extracts from some types of lichens.4) Recently, they have been found to exhibit antihistamine, antiviral, spasmolytic and antimicrobial effects.5–8) One of the most notable properties of the lichens is that the compounds causing such effects are synthesized only by lichens. This property makes lichens popular for scientific studies.9)

Common substances involved in lichens include fatty acids, lactons, vitamins (B12, Folic acid), anthraquinones, depsides, depsidones, tridepsidones and substrates that are specific for lichens. Some of these substances are prolicenterinic, filodic, licherinic, lobaric and usnic acids.5,9,10) It is suggested that lichen's antibiotic effects are based on the most effective of these substances: usnic acid, licherinic acid, usnic acid, depsides and depidones.11) It was shown that 4-O-methylcrotylchloroephlic acid is a type of lichen acid which acts as a powerful inhibitor of prostaglandin biosynthesis, making it potentially useful as an antiinflammatory drug.9)

The preparation extracted from some types of lichens such as Cetraria islandica are currently used to treat mucosal inflammation in Europe.10)

It is well known that to investigate the effects of drugs on the acute phase of inflammation, models induced by pro-inflammatory agents such as carrageenan, dextrane, formaldehyde, serotonin, histamine and bradykinin in rat paws are employed.12–16) Chronic inflammation models produced by implanting a foreign body under the skin are used to study the effects of a drug on the proliferation phase of inflammation.15,17)

In this study, we aimed to investigate the antiinflammatory effects of an extract from C. rangiformis on acute and chronic phases of inflammation, and also to compare its antiinflammatory effect potencies with indomethacin, which is a well-known antiinflammatory drug.

MATERIALS AND METHODS

Lichen Cladonia rangiformis was collected from Giresun, a city located in the Black Sea region of Turkey, in August of 1999. The material was identified by Dr. A. Aslan, a staff member of the Botany Department at Atatürk University. The plants are deposited in the herbarium of the Faculty of Pharmacy, Atatürk University (Number: 91).

Lichen Extract The air-dried and powdered lichen material (300 g) was extracted with methanol at 40 °C. The first extraction was completed for 4h. After the extract was filtrated, it was concentrated in a rotavapor at 40 °C. The methanol extract obtained at the end of this process was then solved in water. This watered extract was consumed using petrol ether. The remaining extract was lyophilized and used in this study. The extract was named C-1 and was kept at +4 °C.

Animals In this study, 48 adult male Wistar albino rats, weighing 180—200 g, obtained from Atatürk University, Faculty of Medicine, Department of Pharmacology Experimental Animal Laboratory, were used. The rats were fed standard laboratory chow and water before the experiment. The animal laboratory was windowless with automatic temperature (22±1 °C) and lighting controls (14 h light/10 h dark). Rats were divided into eight groups, each containing six individuals, then each of the groups were kept in different cages. In all experiments, the ethics guidelines for investigations using conscious animals were obeyed and the procedures were approved by the University ethics committee.

Chemicals The chemicals used in this study were formaldehyde (Sigma), thiopenthal sodium (Abbott) and indomethacin (Deva).

Antiinflammatory Studies Antiinflammatory effects of
C-1 were investigated in an aseptic arthritis model, which was induced by formaldehyde and the cotton pellet granuloma test. The ratio of the antiinflammatory effect of C-1 was calculated by the following equation: antiinflammatory activity (%) = \(1 - \frac{D}{C}\) · 100 where \(D\) represents the percentage difference in paw volume after C-1 was administered to the rats, and \(C\) represents the percentage difference in volume in the control group.17)

**Inflammation Model Induced by Formaldehyde in Rats**

In this series of experiments, the effects of C-1 and indomethacin on the acute phase of inflammation were investigated. Doses of 50, 100 and 200 mg/kg of C-1 were administered orally once a day for a period of 2 d. An hour after the last dose was administered, 0.2 ml of formaldehyde (1%, w/v) was injected into the rat hind paw. Before formaldehyde injection, the paw volume for each rat was measured separately by means of plethysmometer. Edema caused by formaldehyde was measured at 3, 6 and 24 h the first day, and measured once per day on the following days until inflammation disappeared.18) The antiinflammatory potency of C-1 was determined by comparing it with a group in which a control group is 0.86±0.10 ml, whereas the mean paw volume for the mentioned doses of C-1 and indomethacin was 0.57±0.05, 0.51±0.05, 0.42±0.05 and 0.50±0.16 ml, respectively. C-1, at doses of 50, 100, 200 mg/kg, decreased edema induced by formaldehyde 33.8% (\(p<0.005\)), 36.1% (\(p<0.005\)) and 43.1% (\(p<0.001\)) at the sixth hour, respectively. 10 mg/kg dose of indomethacin showed 72.1% (\(p<0.001\)) inhibition against formaldehyde-induced edema (Table 1). On the other hand, while the mean value for the paw volumes of the control group is 0.86±0.10 ml, that of 50, 100, 200 mg/kg doses of C-1 and 10 mg/kg dose of indomethacin are calculated as 0.57±0.05, 0.55±0.06, 0.49±0.06 and 0.24±0.08 ml, respectively (Fig. 1). At the end of the 24th hour, the mean paw volumes of all groups of rats nearly approached the control values, and the values calculated for antiedema effects were meaningless and therefore not mentioned here.

**The Effects of C-1 and Indomethacin on the Proliferation Phase of Inflammation**

It was seen that indomethacin and C-1 have reasonable antiinflammatory effects which could be calculated depending on the weight of cotton pellets.

The mean weights of the moist pellets implanted under the skin in the rats from the control group were evaluated as 210±37.4 mg, from the rats administered 200 mg/kg C-1 as 87±25 mg (\(p<0.005\)), and from the indomethacin-administered rats as 76±24 mg (\(p<0.001\)) (Table 2). According to

---

**Table 1. Effects of C-1 and Indomethacin on Formaldehyde-Induced Paw Edema in Rats**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Animals</th>
<th>Dose (mg/kg)</th>
<th>Mean values for paw volumes</th>
<th>Differences in volume of paw</th>
<th>Antiinflammatory effects (%)</th>
<th>(p)-Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before inf.</td>
<td>After 6 h inf.</td>
<td>(ml)</td>
<td>(%)</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>—</td>
<td>0.81</td>
<td>1.67</td>
<td>0.86±0.10</td>
<td>106</td>
</tr>
<tr>
<td>C-1</td>
<td>6</td>
<td>50</td>
<td>0.87</td>
<td>1.44</td>
<td>0.57±0.05</td>
<td>66</td>
</tr>
<tr>
<td>C-1</td>
<td>6</td>
<td>100</td>
<td>0.81</td>
<td>1.36</td>
<td>0.55±0.06</td>
<td>68</td>
</tr>
<tr>
<td>C-1</td>
<td>6</td>
<td>200</td>
<td>0.89</td>
<td>1.38</td>
<td>0.49±0.06</td>
<td>55</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>6</td>
<td>10</td>
<td>0.91</td>
<td>1.15</td>
<td>0.24±0.08</td>
<td>26</td>
</tr>
</tbody>
</table>

**RESULTS**

The Effects of C-1 and Indomethacin on the Inflammation Induced by Formaldehyde At the end of 3 h, C-1 at the doses of 50, 100 and 200 mg/kg decreased edema induced by formaldehyde at the rate of 16% (\(p<0.05\)), 24.8% (\(p<0.05\)) and 43.8% (\(p<0.001\)), respectively. At the same time, the effect of 10 mg/kg indomethacin was 34.6% (\(p<0.005\)). Mean paw volume of control group rats was 0.68±0.09 ml, whereas the mean paw volume for the mentioned doses of C-1 and indomethacin was 0.57±0.05, 0.51±0.05, 0.42±0.05 and 0.50±0.16 ml, respectively. C-1, at doses of 50, 100, 200 mg/kg, decreased edema induced by formaldehyde 33.8% (\(p<0.005\)), 36.1% (\(p<0.005\)) and 43.1% (\(p<0.001\)) at the sixth hour, respectively. 10 mg/kg dose of indomethacin showed 72.1% (\(p<0.001\)) inhibition against formaldehyde-induced edema (Table 1). On the other hand, while the mean value for the paw volumes of the control group is 0.86±0.10 ml, that of 50, 100, 200 mg/kg doses of C-1 and 10 mg/kg dose of indomethacin are calculated as 0.57±0.05, 0.55±0.06, 0.49±0.06 and 0.24±0.08 ml, respectively (Fig. 1). At the end of the 24th hour, the mean paw volumes of all groups of rats nearly approached the control values, and the values calculated for antiedema effects were meaningless and therefore not mentioned here.

The Effects of C-1 and Indomethacin on the Proliferation Phase of Inflammation It was seen that indomethacin and C-1 have reasonable antiinflammatory effects which could be calculated depending on the weight of cotton pellets.

The mean weights of the moist pellets implanted under the skin in the rats from the control group were evaluated as 210±37.4 mg, from the rats administered 200 mg/kg C-1 as 87±25 mg (\(p<0.005\)), and from the indomethacin-administered rats as 76±24 mg (\(p<0.001\)) (Table 2). According to
these results, the antiproliferative effects of C-1 and indomethacin were calculated as 58.6% and 63.9%, respectively. Weights of cotton-pellets for the group given C-1 and the group given indomethacin were 20.5±5.5 mg (p<0.005) and 23.0±4.8 mg (p<0.005), respectively, after they were dried. When the antiproliferative effects were calculated on the basis of dry weight pellets, the inhibition of inflammation by C-1 and indomethacin were established as 57.3 and 52.1%, respectively.

**DISCUSSION**

In the present study, the antiinflammatory effects of extract from *Cladonia rangiformis*, named C-1, were investigated. It was seen that there are no ill effects of the extract on the behavior or health of the animals.

The effects of C-1 on acute inflammation were studied on formaldehyde-induced paw edema. It was seen that all the doses of C-1 reduced inflammation induced by formaldehyde. The effect of 50 mg/kg C-1 on formaldehyde-induced edema was insignificant at 3 h. While the antiedema effect of 100 mg/kg C-1 was smaller than indomethacin, the effect of 200 mg/kg C-1 was higher than indomethacin. C-1 reduced formaldehyde-induced inflammatory edema at all doses, at 6 h. It was also observed that there were no significant differences in the antiedema effects of the doses of C-1 in the range of 50—100 mg/kg. On the other hand, it was seen that the antiedema effect was increased at the doses of 200 mg/kg of C-1. Ten mg/kg dose of indomethacin was more potent than that of C-1.

The effects of C-1 and indomethacin at all doses, on formaldehyde-induced edema, were insignificant at 24 h.

Histamine, serotonin, bradykinin, prostaglandin and substance P have roles in formaldehyde-induced edema in rat hind paw. Histamine increases vascular permeability and has a role in the development of edema. Nitric oxide and substance P are involved in increased vascular permeability, which is produced by histamine.

The injection of formaldehyde into rat paw increases the release of bradykinin, which is a known inflammation mediator. Studies on animals have shown that bradykinin causes paw edema.

Prostaglandins cause hyperalgesia at lower doses and pain at higher doses. They produce edema by increasing the effects of histamine and bradykinin.

It is known that formaldehyde-induced inflammation usually involves two distinct phases. It has been proposed that the early or first phase reflects the direct stimulation of nociceptors, while the later or second phase may be associated with inflammation mediators. Some studies have shown that substance P receptor antagonists inhibit the later phase of formaldehyde-induced edema, and substance P has a role in this response.

C-1 and indomethacin are more effective at 6 h of inflammation, which means that the inhibition of inflammation mediators was produced by formaldehyde.

Chronic inflammation is a reaction arising when the acute response is insufficient to eliminate proinflammatory agents. Chronic inflammation includes a proliferation of fibroblasts and the infiltration of neutrophils and exudation. Chronic inflammation occurs by means of the development of proliferative cells. These cells can be either spread or granuloma form.

Non steroidal antiinflammatory drugs (NSAIDs) cause a decrease in granuloma tissue arising as a result of cellular reaction, which is released by inhibiting granulocyte infiltration to foreign body implanted. The effects of steroids on chronic inflammation are more significant than on acute inflammation. These effects depend on the inhibitory functions of macrophages and fibrosis.

It was seen that C-1 is more effective on chronic inflammation than on acute inflammation, and that both C-1 and indomethacin prevented weight increases of granuloma tissue induced by cotton-pellet at almost the same level. Formaldehyde-induced paw edema becomes visible after a short time following formaldehyde injection, and acute inflammation symptoms (tumor, rubour, and colour) reach the peak at 3—6 h. The cotton-pellet test is developed over seven days and acute inflammation symptoms are not evident. C-1 and indomethacin were used in the cotton-pellet test for seven days.

The presence of rangiformic acid, furmapotocetrarik acid, norrangiformic acid, atranorin acid, atranorin and usnic acid in lichen *C. rangiformis* has been reported. In addition, the antiinflammatory effect of usnic acid has been shown in carrageenan-induced paw edema and the cotton-pellet test. In our study, the antiinflammatory effect of C-1 is probably due to usnic acid, which is present in the constitution of *C. rangiformis*. Further studies are required in order to determine its mechanism.

**REFERENCES**