

栂(*Aesculus turbinata*)の実の抗炎症作用

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Antiinflammatory Effect of Japanese Horse Chestnut (*Aesculus turbinata*) Seeds

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ABSTRACT. The antiinflammatory effects of Japanese horse chestnut (*Aesculus turbinata*) seeds were examined *in vivo* and *in vitro*. The extract of this seed (HCSE) inhibited croton oil-induced swelling of the mouse concha. HCSE inhibited cyclooxygenase (COX) -1 and -2 activities, but had no effect on 15-lipoxygenase and phospholipase A₂ activities. Inhibition of COX-2 occurred at a lower concentration of HCSE than for COX-1. Japanese horse chestnut seeds contain coumarins and saponins, but these chemicals did not inhibit COX activities. These results suggest that the antiinflammatory effect of Japanese horse chestnut seeds is caused, at least partly, by the inhibition of COX. The inhibitor of COX in this seed may be a chemical(s) other than coumarins and saponins.

KEY WORDS: antiinflammation, cyclooxygenase, Japanese horse chestnut.

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Horse chestnut (*Aesculus hippocastanum*), which is native to Europe, is a deciduous tree up to 20 m high with 7 digitate big leaves. In autumn, this tree bears many fruits containing 1 or 2 large shiny brown seeds. The extract of this seed has been used traditionally for medical treatment of chronic venous insufficiency, traumatic edema, hemorrhoids, etc. in Europe [14]. It is also utilized as a constituent of commercial drugs in Japan.

On the other hand, Japanese horse chestnut (*Aesculus turbinata*) seeds have been used as food in Japan. It is also utilized as a folk medicine to cure bruises and sprains in some prefectures. However, there are few pharmacological studies on this seed. In the present study, therefore, the antiinflammatory effects of Japanese horse chestnut seeds *in vivo* and their effects on some inflammable enzymes *in vitro* were examined to search for a novel biochemical function of this unutilized bioresource.

Sample preparation: Mature Japanese horse chestnut seeds were gathered from trees along the streets of Morioka City. They were washed with tap water and dried in the sun for several days. The shelled seeds were milled and soaked in 4 volumes of 50% ethanol for two weeks at room temperature. The extract (HCSE) was filtered using filter paper (No. 5C, Advantec Toyo, Tokyo, Japan) for use in *in vivo* experiments. The dry matter weight of this extract solution was 125 mg/ml. A portion of the filtrate was freeze-dried and then dissolved in solvents appropriate for the *in vitro* experiments.

***In vivo* experiment:** Croton oil (Sigma Chemical, St. Louis, U.S.A.) was diluted with acetone at a concentration of 20 or 40 mg/ml. Male 7-week-old ICR mice were purchased from Clea Japan, Inc. They were kept in an animal room with the temperature conditioned to 22–24°C, and allowed free access to a commercial diet (MF, Oriental Yeast, Tokyo, Japan) and tap water throughout the acclimation period and experiment. After a one week acclimation period, 10 μ l of croton oil was smeared on the inside of the left concha and dried with a blower. Immediately after

treatment with croton oil, 10 μ l of 50% ethanol (control) or HCSE was smeared onto the same part of the concha. The thickness of concha was measured with a dial thickness gauge (Ozaki Mfg. Co., Ltd., Tokyo, Japan) just before and 3, 24, and 48 hr after exposure to croton oil. The swelling rate of the concha was calculated as follows:

$$\text{Swelling rate (\%)} = (T - T_0) / T_0 \times 100,$$

where T_0 = thickness before exposure to croton oil, and T = thickness after exposure to croton oil.

In this experiment, 10 animals were assigned to each treatment group and the swelling rate was compared using the t-test.

***In vitro* experiment:** The effects of HCSE on cyclooxygenase (COX), 15-lipoxygenase (15-LOX), and phospholipase A₂ (PLA₂) activities were examined *in vitro* by means of their respective assay kits (Cayman Chemical, Michigan, U.S.A.). The COX assay kit contained the COX-1 and -2 enzymes, so the effects on these two isozymes could be determined separately and specifically. The procedure used was detailed in the manual of these kits. The effects of coumarin, esculetin, saponin, and escin on the activities of COX-1 and -2 were also examined because the seeds may contain these chemicals. These *in vitro* experiments were carried out in duplicate or triplicate, and statistical analysis was conducted using the method of Dunnett.

Figure 1 shows the effect of HCSE on croton oil-induced swelling of the mouse concha. Three hours after exposure to croton oil, the concha of the control animals had swelled to more than twofold in thickness in both the 0.2 and 0.4 mg/ear groups. The swelling rate of the 0.2 mg/ear mice declined faster than the 0.4 mg/ear mice. The treatment with HCSE significantly alleviated the swelling in both groups.

Figure 2 shows the effect of HCSE on the activities of COX, 15-LOX, and PLA₂. HCSE inhibited COX-1 and -2 activities in a dose-dependent manner, but had no effect on the 15-LOX and PLA₂ activities. The inhibition of COX-2 occurred at a lower concentration of HCSE (≥ 1 mg/ml) than for COX-1 (4 mg/ml). Coumarin, esculetin, and saponin did

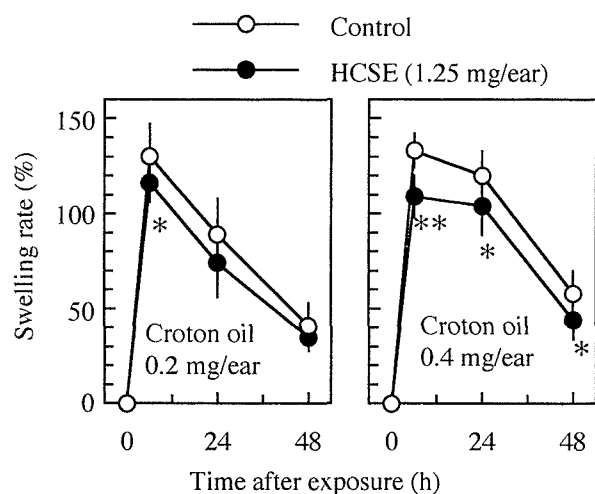


Fig. 1. Effect of HCSE on croton oil-induced swelling of the mouse concha. Mean $\pm$ SD, $n=10$; *: $p<0.05$; **: $p<0.01$.

not affect the activities of COX-1 and -2, while escin elevated the activity of COX-2 (Fig. 3).

The antiinflammatory effects of horse chestnut seeds and escin, a major active component of the seeds, have been reported previously, *e.g.* suppression of dextran-induced edema of rat's feet, reduction of exudative fluid in irritant-provoked peritonitis and pleurisy, and inhibition of the increase of vascular permeability induced by histamine or serotonin [2, 8, 11, 15]. However, this is the first report to our knowledge that demonstrates the antiinflammatory effect of Japanese horse chestnut seeds.

Many chemical mediators are involved in the onset and progress of inflammation. The metabolites of arachidonic acid play an especially important role in the pathogenesis of inflammation. The activation of arachidonic acid cascade may be implicated in croton oil-induced inflammation because phorbol esters, inflammable constituents of croton oil, increase the release of arachidonic acid and the expression of COX-2 [6, 7]. Since PLA₂, COX, and LOX partici-

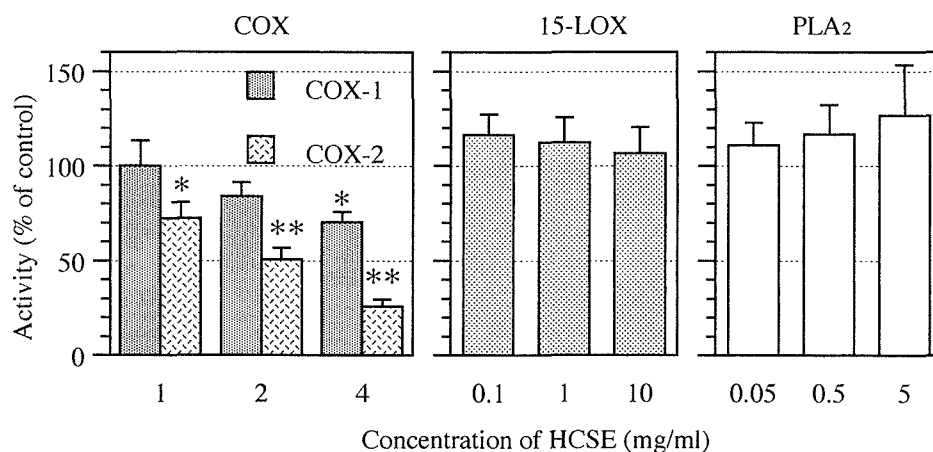


Fig. 2. Effect of HCSE on COX, LOX, and PLA₂ activities *in vitro*. Activities were expressed as a percentage of the control. Mean $\pm$ SD, $n=2-3$; *: $p<0.05$; **: $p<0.01$.

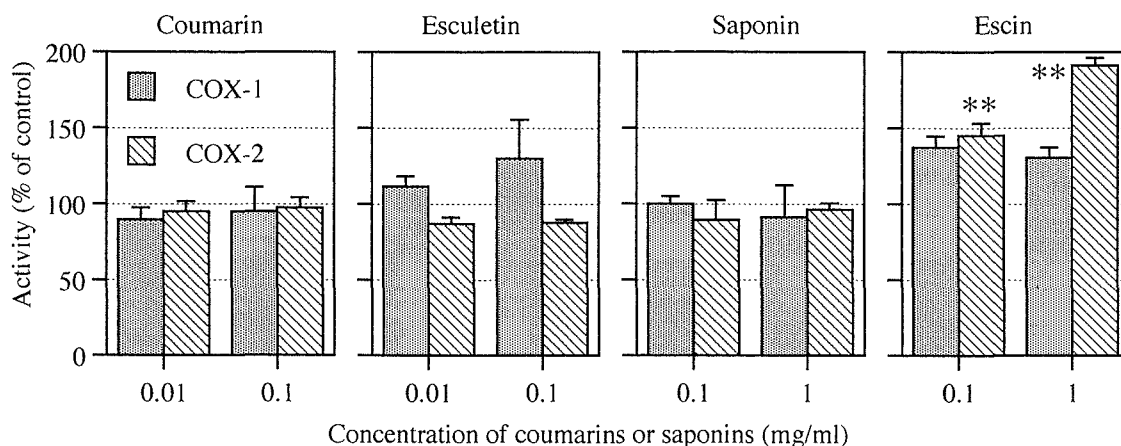


Fig. 3. Effect of coumarins or saponins on COX activities *in vitro*. Activities were expressed as a percentage of the control. Mean $\pm$ SD, $n=2-3$; **: $p<0.01$.

pate in the arachidonic acid cascade, inhibition of these enzymes would alleviate inflammation by decreasing the production of chemical mediators. In practice, many non-steroidal antiinflammatory drugs are COX inhibitors. It has also been reported that several COX inhibitors decrease phorbol ester-induced dermal inflammation in mice [10]. This suggests, therefore, that the antiinflammatory effects of Japanese horse chestnut seeds result, at least partly, from the inhibition of COX activities.

Some polyphenolic compounds, *e.g.* epigallocatechin gallate [6], resveratrol [7], and curcumin [1], inhibit phorbol ester-induced COX-2 expression. Avicins, a family of triterpenoid saponins, also inhibit expression of COX-2 [3]. Japanese horse chestnut seeds contain polyphenols and saponins, although their detailed compositions have not been clarified [4, 9, 12]. Therefore, not only inhibition of COX activity but also inhibition of COX-2 expression may contribute to the antiinflammatory effects of this seed.

There are two isoforms of COX. COX-1 is constitutively expressed in many tissues, while COX-2 is induced in inflamed tissue. COX-1 promotes basal production of cytoprotective prostaglandins in the gastric mucosa, so inhibition of COX-1 may contribute to gastric ulceration. Therefore, selectivity toward COX-2 is an important property of antiinflammatory drugs. HCSE inhibited the activity of COX-2 more strongly than that of COX-1, and significant inhibition of COX-2 appeared at a low concentration. This is a desirable nature for antiinflammatory agents.

Japanese horse chestnut seeds contain saponins and coumarins [4, 9]. A triterpenoid saponin, escin, is a major constituent of the seeds that has antiinflammatory effects, such as inhibition of edema and vascular permeability [8, 11]. Therefore, escin was expected to inhibit COX activities. However, neither escin nor saponin inhibited COX activities. On the other hand, Kimura *et al.* reported that several coumarin derivatives, *e.g.* esculetin, fraxetin, and fraxidin, decreased the formation of HHT (hydroxyheptadecatrienoic acid) [5]. Their findings suggest that these coumarin compounds inhibit COX activities. However, we could not reproduce this phenomenon (Fig. 3). Therefore, the COX

inhibitor in Japanese horse chestnut seeds may be a chemical(s) other than coumarins and saponins.

Some polyphenolic compounds have an inhibitory effect on COX activities [13]. Since Japanese horse chestnut seeds contain considerable amounts of polyphenols [12], COX inhibition may be caused by polyphenolic compounds in these seeds. We are now separating the active chemicals from the HCSE to identify the COX inhibitor in these seeds. The results will be reported in the near future.

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