

Original article:**EVALUATION OF THE IN VIVO ANTI-INFLAMMATORY ACTIVITY OF A FLAVONE GLYCOSIDE FROM *CANCRINIA DISCOIDEA* (LEDEB.) POLJAK**

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ABSTRACT

The anti-inflammatory effect of selagin-7-O-(6''-O-Acetyl)- β -D-glucoside, isolated from the medicinal herb *Cancrinia discoidea* (Ledeb.) Poljak, was evaluated for its anti-inflammatory activity in the carrageenin- and serotonin-induced rat paw oedema models of acute inflammation and the cotton pellet-induced granuloma rat model of chronic inflammation. Flavone glycoside at doses of 5, 10, or 20 mg/kg, the clinical anti-inflammatory indomethacin at 10 mg/kg, or vehicle were administered orally before injection of the pro-inflammatory compound. The test compound showed significant anti-inflammatory activity against paw edema induced by carrageenin or serotonin, most notably at the highest test dose of 20 mg/kg. In the cotton pellet-induced granuloma model, the compound showed dose-dependent anti-inflammatory activity, with the highest effect at 20 mg/kg. In all three assays, the flavone glucoside compound was more active at 20 mg/kg than indomethacin at 10 mg/kg.

Keywords: *Cancrinia discoidea* (Ledeb.) Poljak, selagin-7-O-(6''-O-Acetyl)- β -D-glucoside, anti-inflammatory activity

INTRODUCTION

Inflammation is a physiological reaction to injury or to infectious, allergic, or chemical irritation. Inflammatory processes are complex biochemical phenomena characterized by tissue edema, pain, and leukocyte infiltration (Vickerstaff and Bielory, 1990). The most common clinical treatments for inflammatory diseases are nonsteroidal or steroidal anti-inflammatory compounds (Rainsford, 2007). The use of steroidal drugs as anti-inflammatory agents is now controversial, however, due to their multiple side effects (Schäcke et al., 2002). Therefore, it is necessary to develop new agents with more powerful anti-inflammatory activities but with fewer side effects. Indigenous medicinal plants are possible

sources of novel anti-inflammatory with more tolerable side effects.

The genus *Cancrinia*, classified in subtribe *Matricariinae* of the *Anthemideae*, consists of about 30 species of subshrubs and herbaceous perennials native to Central Asia. *Cancrinia discoidea*, a perennial herb of genus *Cancrinia*, is mainly distributed in the mountains of western China and Central Asia (Yu and Li, 1997). This plant has been harvested both as an important edible invigorant and medicinal herb to cure several ailments such as inflammation, dermal ulcer, bleeding, and stomach-ache in traditional folk medicine. In addition to use in traditional medicine, *C. discoidea* has been described as an important invigorant. In a previous study, we isolated a number of fla-

vonoids from *C. Discoidea*, including a new flavone glycoside, selagin-7-O-(6''-O-Acetyl)- β -D-glucoside, and demonstrated that this flavone glycoside exhibited effective inhibitory activity against human neutrophil O_2^- generation (Zhu and Tian, 2010).

The aim of this study was to investigate the anti-inflammatory potential of this new flavone glycoside (Figure 1). In the present work, the flavone glycoside was evaluated for its anti-inflammatory activity in two acute and one chronic model of inflammation in rats, and the anti-inflammatory response was compared to the clinical anti-inflammatory indomethacin.

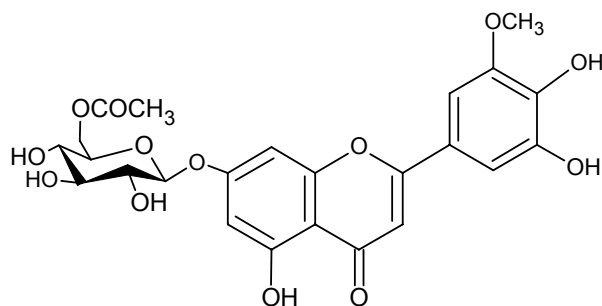


Figure 1: Structure of selagin-7-O-(6'' -O-Acetyl)- β -D-glucoside

MATERIALS AND METHODS

Test materials

The isolation and structure elucidation of selagin-7-O-(6'' -O-Acetyl)- β -D-glucoside was reported before (Zhu and Tian, 2010). Carrageenin, serotonin and indomethacin were purchased from Sigma-Aldrich.

Animals

Male and female Sprague Dawley rats, 42 to 45 days old, were obtained from the Laboratory Animal Center, Sun Yat-sen University. The animals were quarantined and acclimatized for a week before the treatment. Rats of either sex, weighing 180–210 g, were randomly selected and assigned to treatment and control groups using a computer randomization process. The animals were housed in autoclaved polyethylene cages (4 rats in each group per cage) and maintained on a 12:12 h light:dark cycle. The temperature and relative humidity

of the animal room were maintained at 23 ± 2 °C and 50-70 %.

Carrageenin-induced paw edema

Animals of either sex were divided into five groups of eight animals each. The first group (negative control) received the vehicle only (distilled water). Animals of the second, third, and fourth groups were treated orally with the flavone glycoside at doses of 5, 10, or 20 mg/kg. Group 5 (positive control) received indomethacin at 10 mg/kg. Thirty min after drug administration, edema was induced by the injection of 0.1 mL 1 % carrageenin in normal saline into the plantar aponeurosis of the right hind paw. Hind paw volume (an index of swelling) was measured using a plethysmograph before injection and at 1, 2, 3, 4, and 5 h after carrageenin injection (Winter et al., 1962). The difference between the initial and subsequent paw volume reading gave the actual oedema volume. The percent inhibition of inflammation was calculated using the formula:

$$\% \text{ inhibition} = 100(1 - V_t/V_c),$$

where V_c represents oedema volume in control and V_t the oedema volume in the group treated with the tested flavone or indomethacin.

Serotonin induced paw edema

Animals of either sex were divided into five treatment groups of eight animals each. The negative control group received distilled water vehicle only. Animals of the second, third, and fourth groups were treated orally with the flavone glycoside at 5, 10, or 20 mg/kg. The positive control group received indomethacin at 10 mg/kg. Thirty min after drug administration, edema was induced by the injection of 0.05 mL serotonin, freshly prepared in normal saline, into the plantar aponeurosis of the right hind paw. Hind paw volumes were measured using a plethysmograph before injection and at 1, 2, 3, 4, and 5 h after serotonin injection (Maity et al., 1998). The change in paw volume was taken as actual oedema

volume. The percent inhibition of inflammation was calculated using the formula:

$$\% \text{ inhibition} = 100(1 - V_t/V_c),$$

where V_c represents oedema volume in control and V_t the oedema volume in the group treated with the tested flavone or indomethacin.

Cotton pellet-induced granuloma

Rats of either sex were divided into five groups of eight each. Inflammation was induced by the method of Mosquera et al. (2011) with slight modifications. The animals were anesthetized with ketamine (50 mg/kg) (i.m.). The back skin was shaved and disinfected with 70 % ethanol and an incision made in the lumbar region. Subcutaneous tunnels were formed by blunted forceps and a sterilized, pre-weighed cotton pellet (15 ± 1 mg) was placed on both sides in the scapular region. The animals were treated orally with distilled water as negative control, indomethacin as the positive control drug (10 mg/kg), or 5, 10, or 20 mg/kg of flavone glycoside daily for 7 days. On day 8, the animals were sacrificed and the pellets were dissected out. They were dried in an oven at 60 °C until the weight stabilized. The net dry weights (initial minus final) were determined. Wet content and dry content were determined using the formulae:

$$\text{Wet content} = \text{weight of the cotton pellet (wet)} - \text{weight of the cotton pellet (dry)}$$

$$\text{Dry content} = \text{weight of the cotton pellet (dry)} - \text{weight of the cotton pellet.}$$

Statistical analysis

Data are presented as means \pm standard deviation (SD). For statistical analysis, data were analyzed by the one-way ANOVA followed by a Duncan post-hoc for pairwise comparisons between groups. A $p < 0.05$ indicated a significant difference between the groups.

RESULTS AND DISCUSSION

Inflammation is a ubiquitous response of living tissues to injury. It involves a complex cascade of enzyme activation, cytokine release, extravasation of fluid, cell migration, tissue breakdown, and repair (Vane and Bolting, 1995). Flavonoids are important constituents of plants that exhibit a variety of beneficial effects on human health. The anti-inflammatory properties of various flavonoid glycosides have been studied in order to establish and characterize their potential utility as therapeutic agents for the treatment of inflammatory diseases (Gil et al., 1994; Rotelli et al., 2003; Guardia et al., 2001). In the present study, three different animal models were employed to evaluate of anti-inflammatory potential of selagin-7-O-(6''-O-Acetyl)- β -D-glucoside.

The anti-inflammatory effect of the flavone glycoside in the carrageenin-induced rat paw oedema model is shown in Table 1 and the percent inhibition of inflammation is shown in Table 2. In all treated groups, subplantar injection of carrageenin (0.1 mL, 1 % w/v) produced strong oedema in the rat paws, reaching a maximum after 3 h and generally starting to decrease thereafter. Our results revealed that selagin-7-O-(6''-O-Acetyl)- β -D-glucoside possessed potent anti-inflammatory activity against the acute phase of inflammation. The experimental group with the highest test dose (20 mg/kg) exhibited a significant anti-inflammatory effect, starting after 3 h ($p < 0.05$); the activity was higher than observed for the standard drug indomethacin (10 mg/kg). The experimental groups with lower doses exhibited less inflammation than the negative control but more than the positive control. Percentage of inflammation and inhibition at different times for both genders of control and treated rats were not significantly different. Carrageenin-induced paw edema is a prominent experimental model for acute inflammation. Development of edema induced by carrageenan has been described as biphasic (Vinegar et al., 1969).

Table 1: Acute anti-inflammatory activity of the test compound in the carrageenin-induced rat paw oedema model

	Percentage of inflammation at time (h)						
	Initial	1 h	2 h	3 h	4 h	5 h	6 h
Negative control	8.3±0.4	31.0±4.5	40.2±5.8	49.5±8.1	43.7±7.8	40.5±5.4	25.6±4.8
5 mg/kg	8.2±0.5	29.2±4.2	36.6±5.6	34.6±6.6	30.2±7.5*	26.3±6.4*	14.2±3.6*
10 mg/kg	8.3±0.2	28.3±4.6	34.1±5.7	32.9±5.8*	23.6±4.2*	20.6±5.4*	5.4±3.2*
20 mg/kg	8.4±0.4	28.6±4.8	32.8±5.9	25.5±6.5*	16.3±6.8*	12.8±3.5*	4.0±2.0*
Indomethacin (10 mg/kg)	8.4±0.4	28.1±2.7	32.4±3.8	32.5±5.2*	26.4±5.9*	17.1±4.5*	4.9±2.5*

Values are expressed as mean ± SD (n = 8).

*p<0.05 compared to the negative control

Table 2: Acute anti-inflammatory activity against carrageenin-induced rat paw oedema expressed as: percent of inhibition of oedema formation at time (h)

	Percentage of inhibition at time (h)					
	1 h	2 h	3 h	4 h	5 h	6 h
5 mg/kg	5.8	9.0	30.1	30.9	35.1	44.5
10 mg/kg	8.7	15.2	33.5	46.0	49.1	78.9
20 mg/kg	7.7	18.4	48.5	62.7	68.4	84.4
Indomethacin (10 mg/kg)	9.4	19.4	34.3	39.6	57.8	80.9

Following carrageenan injection, there was a sudden elevation of paw volume compared to histamine and serotonin injection (Lo et al., 1982), after which increased vascular permeability was maintained by the release of kinins up to 2.30 h. From 2.30 to 6 h after carrageenan injection, the inflammatory mediators appeared to be prostaglandins, the release of which is closely associated with migration of leucocytes into the inflamed site (Brito and Antonio, 1998). It had been demonstrated that flavone glycoside is able to inhibit the enzymes responsible for prostaglandin synthesis, as well as other mediators of the inflammatory process.

The anti-inflammatory effect of the flavone glycoside in the serotonin-induced rat paw oedema model is shown in Table 3, and the percent inhibition of inflammation

is shown in Table 4. The experimental group receiving the highest test dose (20 mg/kg) exhibited a significant anti-inflammatory effect, starting 3 h after serotonin injection ($p < 0.05$). Again, the inflammatory response was lower than observed for indomethacin at 10 mg/kg, while the experimental groups receiving lower doses showed less inflammation than the negative control but more than the positive control. Percentage of inflammation and inhibition at different times for both genders of control and treated rats were not significantly different. The flavone glycoside mediates these anti-inflammatory actions by either inhibiting the synthesis, release, or action of inflammatory mediators involved in inflammation.

Table 3: Acute anti-inflammatory activity of the test compound in the serotonin-induced rat paw oedema model

	Percentage of inflammation at time (h)						
	Initial	1 h	2 h	3 h	4 h	5 h	6 h
Negative control	12.4±0.6	28.5±5.6	51.3±6.8	63.4±6.9	42.2±7.6	24.8±6.8	12.6±5.2
5 mg/kg	12.5±0.5	28.2±5.3	48.2±6.7	53.3±5.8	29.4±6.2*	16.1±6.3*	7.1±3.5*
10mg/kg	12.3±0.6	27.8±6.1	45.2±5.9	45.7±5.9	24.5±5.6*	13.4±5.4*	5.4±2.7*
20 mg/kg	12.4±0.6	26.7±5.9	43.2±5.6	37.5±6.2*	18.3±7.2*	10.7±4.6*	2.6±1.5*
Indomethacin (10 mg/kg)	12.3±0.5	26.5±5.6	43.2±5.4	38.4±7.1*	25.8±7.6*	12.4±5.4*	4.8±3.5*

Values are expressed as mean ± SD (n = 8); * p<0.05 compared to the negative control

Table 4: Acute anti-inflammatory activity against serotonin-induced rat paw oedema expressed as the percent of inhibition of oedema formation at time (h)

	Percentage of inhibition at time (h)					
	1 h	2 h	3 h	4 h	5 h	6 h
5 mg/kg	1.1	6.0	15.9	30.3	35.1	43.7
10 mg/kg	2.5	11.9	27.9	41.9	46.0	57.14
20 mg/kg	6.3	15.8	40.9	56.6	56.9	79.4
Indomethacin (10 mg/kg)	7.0	15.8	39.4	38.9	50	61.9

In order to assess the test compound's efficacy against the later proliferative phase of inflammation caused by tissue degeneration and fibrosis, we used the cotton pellet granuloma test. Table 5 shows the effect of the flavone glycoside on cotton pellet-induced granuloma formation in rats. Maximal inhibition (45.1 %) of granuloma formation was observed at a dose of 20 mg/kg (p < 0.05), and this effect was greater than that of indomethacin at 10 mg/kg (41.7 % inhibition). Treatment groups with lower doses of the flavone glycoside showed a smaller anti-inflammatory effect than the positive control. Percentage of inhibition for both genders of control and treated rats were not significantly different.

In the cotton pellet granuloma model, inflammation and granuloma develop over a period of several days. This model tests bioactivity against the proliferative phase of inflammation. This later phase of inflammation involves the proliferation of macrophages, neutrophils, and fibroblast, and multiplication of small blood vessels, which are the basic sources of the highly vascularized reddish mass termed granulation tissue (Bhattacharya and Nag Chaudhuri, 1992). Hence, a decrease in the granuloma weight indicates a suppression of the proliferative phase mediated by the flavone glycoside.

Table 5: Chronic anti-inflammatory activity of the test compound on cotton pellet induced granuloma model in rats

Group	Wet content of granuloma (mg)	Inhibition (%)	Dry content of granuloma (mg)	Inhibition (%)
Negative control	48.6±10.2	–	324.6±46.2	–
5 mg/kg	43.2±10.5	11.1	243.5±30.6	25.0
10 mg/kg	38.1±13.9	21.6	206.8±25.7*	36.3
20 mg/kg	35.4±11.7	27.2	178.3±35.4*	45.1
Indomethacin (10 mg/kg)	36.4±12.6	25.1	189.2±30.7*	41.7

Values are expressed as mean ± SD (n = 8); * p<0.05 compared to control

CONCLUSION

Taken together, the results strongly suggest that selagin-7-O-(6"-O-Acetyl)- β -D-glucoside isolated from *C. discoidea* possesses anti-inflammatory effects, supporting the use of *C. discoidea* in traditional medicine.

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