

Anti-inflammatory Effects of an Aqueous Extract of *Capraria biflora* L.

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SUMMARY. The antiinflammatory effects of leaves of *Capraria biflora* L. were investigated. The aqueous extract 10% was administered at different doses in two models of inflammation: the carrageenin-induced paw edema in rats and the peritonitis induced by carrageenin in mouse. In two tests, the dose of 200 mg kg⁻¹ of the extract showed anti-inflammatory activity like indometacin and the effect was dose dependent. It is possible that the antiinflammatory effect of this plant may obey to more than one mechanism and that the flavonoids could be involved in it.

RESUMEN. "Efectos antiinflamatorios de un extracto acuoso de *Capraria biflora* L.". Se investigaron los efectos antiinflamatorios de las hojas de *Capraria biflora* L. El extracto acuoso fue preparado al 10% y se administró a ratas en el modelo del edema plantar inducido por carragenina y a ratones en el modelo de peritonitis inducida por el mismo agente. En ambos ensayos, la dosis de 200 mg kg⁻¹ del extracto mostró un efecto similar a la indometacina y el efecto fue dosis-dependiente. Los efectos antiinflamatorios de esta planta podrían obedecer a diversos mecanismos y los flavonoides estar involucrados en ellos.

INTRODUCTION

Capraria biflora L. (Scrophulariaceae) is a perennial shrub, distributed in North and South America¹⁻³ and it is extensively used as a medicinal plant for a wide variety of ailments. It has been used to treat fever and for their diuretic, stimulant and digestive properties and also is regarded as a tonic and beneficial to digestion⁴. Several investigations demonstrated that in the roots there is a compound known as bifluorin with antibiotic activity⁵.

In Cuba, *Capraria biflora* L. is used in traditional medicine for the treatment of menstrual pain, pelvic inflammation and rheumatic disorder⁶. Their properties remain to be pharmacologically evaluated and nothing is known about the active constituents responsible for these effects.

In the present study, the anti-inflammatory effects of aqueous extract of this plant are evaluated.

MATERIALS AND METHODS

Plant material and extraction

The plant material was collected in the Botanical Garden of the Central University of Las Villas and identified by Dr. Cristóbal Ríos Albuerne. A voucher specimen of *Capraria biflora* L. (Nº 08132) was deposited in the herbarium of the mentioned Garden.

Leaves were dried at room temperature. The powdered leaves were extracted with hot water (100 °C) in a Soxhlet apparatus for three hours. The aqueous extract (10% w/v) was concentrated by lyophilization to give a brown mass. This extract was resuspended in distilled water for the pharmacological tests.

Animals

Female Wistar rats (170-240 g) and male and female Swiss mice (25-30 g) (CENPALAB, National Center for Production of Laboratory Animals, Havana, Cuba) were used. The animals

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were maintained at room temperature with water and food *ad libitum*.

Limit toxicity^{7, 8}

Groups of 10 male and female mice were treated orally with 2 g kg⁻¹ of aqueous extract or saline as recommended by the Growth and Economic Help Organization (OECD). Mice were observed for 14 days. The signs observed were motor activity, rare reactions, phonation, pain sensibility, sound sensibility, tact sensibility, social behavior, abnormal tail, aggressive behavior, ataxia, convulsions, muscle tone, paralysis, somatic response, posture reflex, prostration, shivers, exophthalmia, eye irritation, cornea reflex, nystagmus, pupil reflex to light, defecation, salivation, miction and death. After mice sacrifice, organs like heart, kidneys, intestines, liver and lungs were macroscopically observed.

Carrageenin-induced paw edema in rats⁹

Groups of 6 rats were used. Edema was induced by subcutaneous injection (0.1 ml) of 1% freshly prepared suspension of carrageenin in normal saline, into the plantar side of the right hind paw of each rat. Aqueous extract of *Capraria biflora* L. (50, 100, 200 mg kg⁻¹ in saline, i.p.) and Indometacin (10 mg kg⁻¹) as a standard drug were given 30 min before the carrageenin injection. The volume of the paw was measured by means of a caliper dial (Mitutoyo) before and 5 h after carrageenin injection. Three replications were done for each measurement and values were accepted when the variable-ness coefficient was equal or less than 4%. The percentage of inflammation increase was calculated in relation to paw volume before treatment with carrageenin. Control animals received the vehicle only.

Peritonitis induced by carrageenin¹⁰

Groups of 10 mice were treated with aqueous extract of *Capraria biflora* L. (50, 100, 200 mg kg⁻¹ in saline, i.p.). One hour afterwards all animals received an intraperitoneal injection of 0.25 ml carrageenin (0.75% in saline). The animals were killed by cervical dislocation 5 hours later. The peritoneal cavity was washed with 2 ml of buffer phosphate solution and the peritoneal exudate was collected. The number of migratory leukocytes was determined in a Neubauer camera. Three replications were done for each measurement. Indometacin (7 mg kg⁻¹) was the reference drug.

Statistics

Data were statistically analyzed by Student's t-test or Mann Whitney's U test. Values are mean ± S.D., p<0.05 vs. control.

RESULTS AND DISCUSSION

The aqueous extract of *Capraria biflora* L. was not toxic at a dose of 2 g kg⁻¹. There were no changes in signs or organs observed.

The anti-inflammatory effects of an aqueous extract of *Capraria biflora* L. and indometacin are shown in Figure 1. In control rats, the edema induced by carrageenin was progressive reaching to 4 h. Previous treatment with 50, 100 and 200 mg kg⁻¹ of aqueous extract reduced the paw edema in rats by 38.75, 35.96 and 27.94%, respectively, after 4 h carrageenin injection, with dose-dependent response. Dose of 200 mg kg⁻¹ exhibited a stronger anti-inflammatory activity

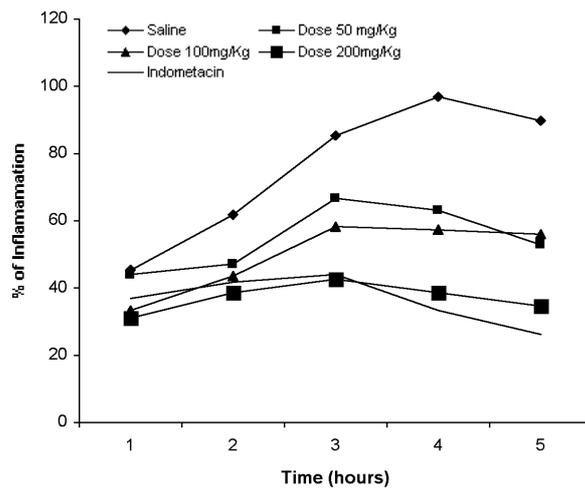


Figure 1. Effect of i.p. administration of different doses of aqueous extract of *Capraria biflora* L. on carrageenin-induced paw edema, n=6, p< 0.05, Mann Whitney's U test.

Treatment	Leukocyte count	P
Saline	3.88 ± 1.55	-
Indometacin	1.40 ± 0.92 *	65.2
Dose 50 mg/Kg	2.75 ± 1.38	29.12
Dose 100 mg/Kg	1.95 ± 1.41*	49.74
Dose 200 mg/Kg	1.60 ± 1.08*	58.76

Table 1. Effects of i.p. administration of different doses of aqueous extract of *Capraria biflora* L. on leukocyte count. n=10. P= Percentage of inhibition of leukocyte migration. *p<0.05 vs. control, Student's t-test.

comparable with that induced by indometacin ($p > 0.05$).

The carrageenan paw edema test produce an acute, non-specific inflammation that results from the sequential action of several mediators. Between 2 and 3 h after carrageenan induction of inflammation mainly prostaglandins and bradikinin take part in edema ¹¹. At this moment, the Indometacin produced the maximal inhibition of edema, showing a typical behavior of non-steroidal anti-inflammatory drugs. Other authors refer that the maximal effect occurs at 4 h ¹². Boughton *et al.* ¹³ have provided evidence that oxygen radical play an important role in the maintenance of carrageenan paw edema, too. Substances that inhibit the prostaglandin synthesis or scavenge the oxygen radical can decrease the inflammation.

In peritonitis test, the leukocyte count in control rats was $3.88 \pm 1.92 \times 10^3 \text{ mm}^{-3}$. Treatment with 50 mg/Kg of aqueous extract did not significantly reduce the leukocyte migration (29.4%) while 100 and 200 mg/Kg decreased it by 49.74 and 58.76%, respectively. This test con-

firms the anti-inflammatory effect of *Capraria biflora* L.

The carrageenan, the irritant agent of this test, provides a rapid infiltration of polymorphonuclear leucocytes to tissues ¹⁴. During sub-acute phase of inflammatory response leukocytes and monocytes are adhered to endothelial cell and cross the wall of blood vessels as a consequence of the attracting power of chemokines ¹⁵.

C. biflora aqueous extracts reduced the inflammation in two test in a way dose-dependent. Many metabolites could be involved in these results. It has been recently found that aqueous extract of this plant contains several metabolites such as alkaloids, flavonoids and tannins ¹⁶. Flavonoids are specially related with anti-inflammatory properties of some plants, as they decrease the formation of pro-inflammatory mediators (prostaglandins, leukotrienes, reactive oxygen species, nitric oxide) ¹⁷. By this reason we considered that the flavonoids present in *Capraria biflora* leaves could be responsible for the observed anti-inflammatory effect.

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