

ANTI-INFLAMMATORY ACTIVITY OF THE SOFT EXTRACT
AND OINTMENTS OF *Petiveria alliacea* L. IN RATS

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Summary

A preclinic pharmacological evaluation of the anti-inflammatory action of the soft extract (active principle) and ointments elaborated from the leaves of the specie *Petiveria alliacea* L. (anamú) was carried out. The model of biological valoration: "Subplant Edema by Carragenin", was used in male rats of the line *Sprague dawley*. The soft extract was administered by intraperitoneal injection using doses of 2000, 4000 and 6000 mg/kg of body weight and the ointments by topical application at concentrations of the 10, 30 and 50 % of the active principle. The positive controls utilized were: Indomethacin suspension 10 mg/kg of body weight and Niflumic Acid ointment 3 %, respectively. Three groups of rats were selected aleatorially according to the compounds evaluated and at the same time each of these groups was divided into three subgroups consisting of 6 animals each. The results demonstrated that the soft extract at the three doses tested and the ointments at the concentrations of 30 and 50 % possess effective antinflammatory activity, the ointment of 10 % gave negative results. The best antinflammatory efficacy is achieved with the highest doses and concentrations tested without showing external signs of toxicity, this is similar with the positive controls.

Key words: antinflammatory, preclinic pharmacology, anamú, *Petiveria alliacea* L.

Introduction

The plant specie *Petiveria alliacea* L. (anamú), one of the medicinal plants approved by the Ministry of Public Health of Cuba (MINSAP) (1) for integral studies, has been used from generation to generation with different therapeutic objectives (2) which include its anti-inflammatory action (1,3). Since there are not scientific studies to corroborate this action, the Pharmacy Department of the Oriente University, through one of their lines of investigation has carried out research about this and at the same time complying with the Critical Route of Investigation in Medicinal Plants established by MINSAP which demands among other aspects, the necessity of a preclinic pharmacological evaluation. In this investigation, an herbal drug from this plant specie is proposed with the intended use of treating Psoriasis after completing all the studies established by the Critical Route of Investigation in Medicinal Plants (4).

Methods

Recollection and processing of plant material: The leaves were identified taxonomically in the Tomás Romay museum in the city of Santiago of Cuba. Later the residual humidity and other pharmacognostic parameters were determined including phytochemical screening, to determine the quality of the drug (4, 5).

Preparation of soft extract: The soft extract was obtained according to the process described in the Ramal Norm of Public Health of Cuba 311 (NRSP) (6).

Preparation of the ointments: The ointments were obtained from the soft extract at 3 concentrations (10, 30 and 50 %) by the method of incorporation, they were packed in wide brimmed dark coloured glass bottles with a capacity of 30 g. according to the Cuban Norm (NC) 26-120:1985 (7).

Preclinic Evaluation in vivo of the anti-inflammatory activity of the soft extract and ointments: The Universal Model: Carragenin Induced Edema in Hind paws (8) was utilized in a sample of 108 *Sprague dawley* male rats with varying body mass of between 150 and 250 g., bred in the Laboratory of Antibodies and Experimental Biomodels (LABEX) of Santiago of Cuba and selected by simple aleatory sampling. The animals were maintained under normal environmental conditions of LABEX, according to the normalized laboratory procedures, that is, controlled temperature of 24 ± 2 °C, relative humidity of 60-65 % and a regime of 12 hours light- 12 hours darkness. Their diet of ratonina (CENPALAB) and acidified water according to the veterinary norm (9). The behaviour of the rodents was observed with respect to the food and water intake during the 15 days before the investigation to determine whether they experienced a good state of health.

All the experimental animals were deprived of food and water 12 hours before starting the experiment.

The compounds evaluated were:

- **Soft extract:** At doses of 2000, 4000 and 6000 mg/kg of body weight, respectively; Indometacin suspension 10 mg/Kg of weight as the positive control (8) and a hydroalcoholic mixture at 30 % as the negative control. They were administered by intraperitoneal injection.

These doses were established taking into account the following toxicological bibliographical reports: “Determination of the average lethal dose orally, demonstrated that mortality was not obtained, not even with the use of a higher dose of 8 g/kg of body weight (10); “Sole intraperitoneal administration of 10g/kg of weight of the decoction of leaves to rats did not provoke any external signs of toxicity during the period of observation of 7 consecutive days” (11); among others.

- **Ointments:** At concentrations of 10, 30 and 50 % of the active principle, ointment of Niflumic Acid at 3 % was used a positive control and a mixture of vasolanoline as the negative control. All these compounds were administered topically.

For both experiments, 54 male rats were used, these were selected aleatorially in 3 groups according to the compounds evaluated and at the same time each of these groups was divided into 3 subgroups of 6 animals each.

The animals received a single dose of the trial compounds and half an hour later, were injected with 0.1 mL (10 mg) of carragenin at 1 % (distilled water) in the subplanta region of the right hind paw. After 4 hours, the volume of the inflamed paw was measured with a pletismograph. The anti-inflammatory activity was expressed in percentage of inhibition of the edema. The anti-inflammatory activity is considered effective when it is above 30 %. The results were statistically analyzed using the program, STAGRAPHICS PLUS, version 3.1, 1997.

Results

Processing of plant material: The residual humidity determined was 7.22 %.

Preclinic Evaluation in vivo of the anti-inflammatory activity of the soft extract and ointments: In the tables # 1 and 2, the averages of the values of volume of the rats paws treated with the evaluated compounds and the percentage values of inhibition of the edema induced by Carragenin as well as the Standard deviation (S) and the statistical significance for $p < 0.05$ are observed.

Table # 1: Average values of volume of the rats paws and percentage of inhibition of edema in the soft extract.

Substance used. Soft Extract (Dose mg/Kg)	$\bar{X} \pm S$ (mm de Hg)	% of inhibition
2000	14.75 \pm 2.2 ^a	28.61
4000	10.5 \pm 1.9 ^b	49.18
6000	9.75 \pm 2.0 ^b	52.81
Indometacin Suspension 10 mg/kg	12.66 \pm 2.0 ^{a,b}	38.73
Hidroalcoholic mixture 30 %	20.66 \pm 1.8 ^c	0

Diferents letters represent significant differences between groups ($p < 0.05$)

Table # 2: Average values of volume of the rats paws and percentage of inhibition of edema in the ointments.

Substance used.	$\bar{X} \pm S$ (mm de Hg)	% de inhibition
Ointment 10 %	19.50 \pm 1.6 ^b	8.58
Ointment 30 %	12.75 \pm 1.5 ^a	40.23
Ointment 50 %	12.50 \pm 1.7 ^a	41.40
Ointment of Niflumic Acid	11.00 \pm 1.4 ^a	48.43
Vasolanoline mixture	21.33 \pm 1.0 ^b	0

Diferents letters represent significant differences between groups ($p < 0.05$)

Discussion

Processing of plant material

The taxonomy identification verified that the specie *Petiveria alliacea* L. was used. The residual humidity lies within the established range found in the literature (4) for non- official drugs (less than 14 %), therefore it does not favour the growth of microorganisms. The results of the pharmacognostic analysis for the dry crude drug coincide with anterior experimental studies (12, 13) for the specie, showing the physical chemical quality of the same.

Preclinic Evaluation in vivo of the anti-inflammatory activity of the soft extract and ointments

In the table # 1, it is observed that as the dose of the soft extract increases, there is notable decrease in volume of the paws of the rats treated with Carragenin and an increase of the percentage value of inhibition of edema, therefore the antiinflammatory activity differs in each one of the doses evaluated, offering the best protection against inflammation at the dose of 6000 mg/kg of body weight and with no observation of external signs of toxicity. If the value medium is compared with that of the hydroalcoholic mixture of 30 % for each one of the three doses, and at the same time with the one of Indometacin, significant differences are observed from a statistical point of view with the negative control but not with the positive control, this permits to affirm that the extracted substances of the plant in that solvent have an antiinflammatory effect and this is relatively close to the one observed with Indometacin, but not for the negative control.

The doses of 4000 and 6000 mg/kg of weight are the most efficient and they don't show significant statistical differences between each other thereby making it possible to use them indiscriminately and achieve a similar antiinflammatory effect.

In table # 2, as the concentration of the active principle contained in the ointments increases, the antiinflammatory activity attained is higher (therefore using the form of administration mentioned earlier on permits a higher level of liberation from the base, diffusion and penetration) (14), thus offering better protection against inflammation at the concentration of 50 % and not 10 %.

Comparing the average values of the volume difference of the compound evaluated at different concentrations with the mixture of vasolanoline (21.33 mm Hg), it is observed that there no existing significant differences from the statistical point of view between the ointment at 10 % (19.5 mm Hg) and the negative control although, these are observed between the latter and the ointments at 30 and 50 % (12.75 and 12.5 mm Hg, respectively). Taking the earlier explanation into account, it is logical to expect significant differences between the ointment at 10 % and Niflumic Acid (11 mm Hg) used as the positive control, which reaffirms the inactivity of the former and not being like this for the other two ointments where there are no significant statistical differences with respect to the positive control, therefore the antiinflammatory activity of the same is compared to that of Niflumic Acid (a renowned non-steroid antiinflammatory).

The percentages of edema inhibition by the ointments at 30 and 50 % (40.23 and 41.4 %, respectively) are similar and higher than 30 %, therefore they can be utilized indiscriminately during therapy because of their effective antiinflammatory activity, with non-existing significant statistical differences between their average values of the volume.

The anti-inflammatory action of these compounds could be due to the presence of flavonoids in the same, to which the anti-inflammatory properties are attributed, since they inhibit phospholipase A₂, lipooxygenase o the sintetase prostaglandin endoperoxide; as well as for its anti-oxidant properties (15, 16, 17).

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