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Anti-inflammatory Activity of *Cissampelos sympodialis* Eichl. (Menispermaceae) Leaf Extract

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SUMMARY. The aqueous fraction of the ethanolic extract obtained from the leaves (AFL) of *Cissampelos sympodialis* Eichl. (Menispermaceae) was evaluated for anti-inflammatory activity, as it is used in folk medicine for this purpose. In mice the AFL (100 mg/kg, ip) inhibited both the 12-O-tetradecanoylphorbol 13-acetate and capsaicin- induced ear edema by 58% and 37%, respectively. The effective dose of AFL to inhibit the carrageenan-induced rat paw edema was 50 mg/kg (24%). Preliminary results of experiments on cell migration showed that the administration (subcutaneous route) of AFL at 100 and 200 mg/kg in rats inhibited the carrageenan-induced neutrophil migration measurement after the administration of the irritant by 53 and 50%, respectively. The results show that the AFL has anti-inflammatory activity.

RESUMEN. "Actividad antiinflamatoria del extracto etanólico de las hojas de *Cissampelos sympodialis* Eichl. (Menispermaceae)". La fracción acuosa del extracto etanólico obtenido de las hojas (AFL) de *Cissampelos sympodialis* ha sido evaluada con respecto a la actividad antiinflamatoria, ya que en medicina popular es usada para esta finalidad. En ratones el AFL (100mg/kg ip) ha inhibido edema de la oreja por el 12-O-tetradecanoylphorbol 13-acetate y capsaicin en un 58% y 37%, respectivamente. La dosis eficaz de AFL para inhibir el edema de la pata de rata por carragenina fue 50 mg/kg (el 24%). Los resultados preliminares de experimentos sobre la migración celular mostraron que AFL en dosis 100 y 200 mg/kg sc en ratas después de la administración de la carragenina inhibió la migración de neutrófilos en un 53% y 50%, respectivamente. Los resultados muestran que el AFL posee actividad antiinflamatoria.

INTRODUCTION

Cissampelos sympodialis Eichl. (Menispermaceae) is a plant popularly known in Brazil as "milona". The water infusion of its root is used in folk medicine for the treatment of asthma, arthritis, bronchitis and urinary infections [1], where inflammation is a common component of these diseases. For example, asthma is essentially a T helper type 2 (Th2) cell cytokine profile-driven chronic airway inflammation. Indeed, cytokines such as IL-4, IL-5 and/or IL-13 control various stages of the disease and interact to maintain and amplify the inflammatory response [2,3].

Recently, Piuvezam *et al.* [4] demonstrated that the aqueous fraction of the ethanolic extract obtained from the leaves of *C. sympodialis* (AFL) increases the production of the anti-inflammatory cytokine (IL-10) and inhibits the T cell proliferative response by concanavalin-A-treated BALB/c spleen cells.

Several other pharmacological studies have demonstrated that the AFL increases intracellular cyclic adenosine monophosphate (cAMP) levels in guinea pig alveolar leukocytes [5] and in human peripheral neutrophils [6]. cAMP is known to suppress inflammation by down-regulating neutrophil activity [7]. The AFL also inhibits both histamine-induced bronchospasm in normal guinea pigs and antigen-induced anaphylactic responses in ovalbumine-sensitized guinea pigs [8].

The above results indicate that the AFL may

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PALABRAS CLAVE: Actividad antiinflamatoria, migración celular, *Cissampelos sympodialis* Eichl., Menispermaceae.

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have anti-inflammatory activity, which was investigated in the present work.

MATERIAL AND METHODS

Plant material and preparation of the extract

Leaves from *Cissampelos sympodialis* Eichl. (Menispermaceae) were obtained from the Botanical Garden of the Laboratório de Tecnologia Farmacêutica/Universidade Federal da Paraíba (voucher specimen Agra 1456). The leaves were dried at 50 ºC in an oven and pulverized. The powder was extracted with 80% ethanol in water at 70 ºC for 5 days. The dried extract was dissolved in water, filtered and known volumes were dried to determine the final concentration of the water-soluble component. All doses are expressed in terms of the concentration of the water-soluble components (mg/kg of body weight). The yield was 22% on average, based on solid residues present.

Animals

Male Wistar rats (250-300 g) and male Swiss mice (25-30 g) were used throughout the experiments. Experimental animals were maintained with free access to water. Control animals received the vehicle only. Both the intraperitoneal (ip) and subcutaneous (sc) routes were used as our previous studies have shown that oral administration was ineffective (data not shown). All experiments were carried out with strict adherence to ethical guidelines.

Models of inflammation

12-O-tetradecanoylphorbol 13-acetate (TPA) or capsaicin-induced mouse ear edema.

Edema was induced according to the method described by Merlos *et al.* [9] and Mantione & Rodríguez [10]. Irritant dermatitis was induced on the right ear by topical application of 0.01% 12-O-tetradecanoylphorbol 13-acetate (TPA) or capsaicin (12.5 mg/ml), both dissolved in acetone. The left ear (control) received the vehicle (acetone). AFL was injected at 50, 100 and 200 mg/kg, ip and dexamethasone (Dex.) was administrated ip at 2 mg/kg one hour before application of the irritant agents. Animals were killed by cervical dislocation after either six hours (TPA) or one hour (capsaicin) after the induction of inflammation. An 8-mm diameter punch biopsy was performed on each ear.

Carrageenan-induced rat paw edema

Carrageenan-induced edema assay was carried out according to Winter *et al.* [11]. Edema was induced on the right hind foot of a rat by subplantar injection of 0.1 ml of a solution of 1% carrageenan in 0.9% NaCl (w/v). Swelling of the carregeenan and contralateral saline-injected foot was measured before injection the carrageenan (time 0) and at 1.5, 3 and 5 hours after injection of carrageenan. The control group received indomethacin (10 mg/kg, oral administration), a reference anti-inflammatory substance, or 0,9% NaCl solution under the same experimental conditions. AFL was administrated in doses of 50, 100 and 200 mg/kg ip one hour before carrageenan administration. The degree of pedal edema was determined by measuring the left hind paw volume by water plethysmography (Ugo Basile). The percentage inhibition of edema was calculated for each animal group in comparison with its vehicle-treated control group. Each treated group was composed of 5 rats.

Carrageenan induced neutrophil migration in the rat

Groups of 6 Wistar rats were treated with AFL (50, 100, 200 mg/kg, sc) or vehicle. One hour after treatment, animals were injected with 1ml of carrageenan solution (500 µg/ml, ip.). Four hours after the inflammatory stimuli the animals were killed by cervical dislocation. The peritoneal cavities were washed with 10 ml of a 3% albumin solution diluted in PBS and supplemented with 5 UI/ml of heparin. A volume of 6 ml was collected from the peritoneal cavities. The total cell number was counted in a Neubauer chamber with Turk solution and expressed as numbers of cells/ml. Differential cell counts were performed after cytocentrifugation and staining with HEMA 3 stain. At least 200 cells were counted and results were expressed as number of cell population/ml [12].

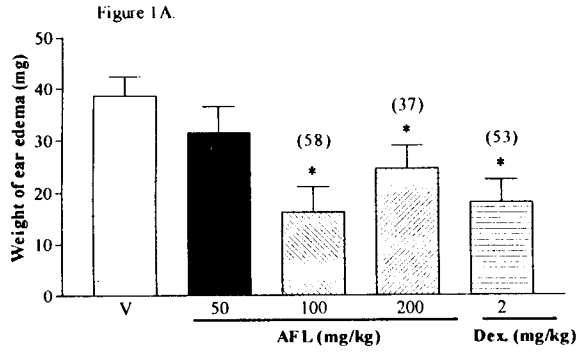
Statistical analysis

Data are expressed as mean ± S.E.M. Differences between control and treatment groups were tested for significance ($P<0.05$) using Student's t-test.

RESULTS

Effect of the AFL treatment on TPA or capsaicin-induced mouse ear edema

The results obtained in TPA-induced mouse ear edema are shown in Figure 1A. The treatment with AFL at doses of 100 and 200 mg/kg inhibited the edema significantly by 58 and 37%, respectively. At these doses the weight of ear



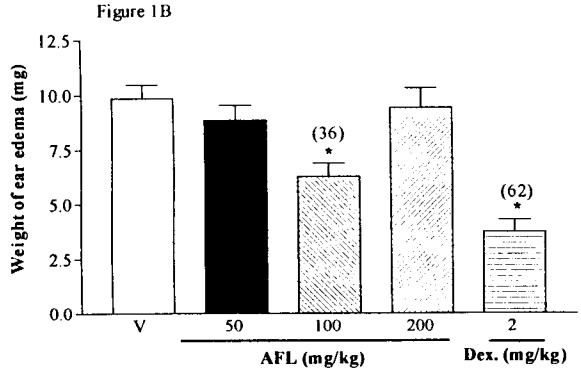


Figure 1. Effect of AFL and dexamethasone on TPA (A) or capsaicin (B)-induced mouse ear edema. Agents were administered ip one hour before the injection of the irritant agent, AFL (plant extract), Dex. (dexamethasone), V (vehicle). The percentage inhibition of edema was calculated as follows: the weight of the untreated ear was subtracted from the treated ear for each group. The mean irritant edema was obtained by subtracting the average vehicle control ear weight from the average TPA-treated or capsaicin ear weight. The percentage inhibition of edema was calculated by the subtraction of average vehicle edema value from the average AFL or Dex. Edema value and the mean were divided by the average vehicle edema value. The resultant value was multiplied by 100. Values are mean ± S.E.M. (n=4). The numbers in parenthesis indicate the percent inhibition of ear edema. *P<0.05 compared with control group (Student's t-test).

edema was significantly ($p<0.05$) lower than in the control group (vehicle). At a dose of 100 mg/kg of AFL the edema inhibition (58%) was similar to that observed with 2 mg/kg of dexamethasone (53%). AFL did not significantly reduce the weight of the ear edema at dose 50 mg/kg (Figure 1A). In the capsaicin-induced mouse ear edema test, AFL exerted a significant inhibitory effect (36%, $p<0.05$) only at a dose of 100 mg/kg (Figure 1B). No inhibitory effect was observed at a dose of 200 mg/kg. This phenomenon may be due to the presence of compounds in the extract that antagonize the activity of other compounds.

Effect of the AFL treatment on the carrageenan-induced hind paw edema

In control animals, the subplantar injection of carrageenan produced a local edema that increased progressively to reach a maximal intensity 5 hours after the injection of the phlogistic agent. AFL produced a small but significant inhibitory effect on the carrageenan -induced hind paw edema only at 5 h at doses of 50 and 100 mg/kg by 20 to 24% respectively (data not shown).

Effect of the AFL treatment on carrageenan-induced cell migration to the rat peritoneal cavity

The sc administration of AFL to carrageenan-injected rats was effective in promoting inhibition of neutrophil-migration to peritoneal cavity. This effect was more effective at doses of 100 (53%) and 200 mg/kg (50%) of AFL (Table 1). However the extract, at these doses, did not significantly inhibit the total leukocyte numbers.

DISCUSSION

The results presented here suggest that the AFL shows a significative anti-inflammatory activity. Thus the AFL (100 mg/kg ip.) inhibited

Groups	Total Leukocytes	Neutrophils	% of Inhibition	
Treatment (mg/kg)	x 10^6 cells/ml	x 10^6 cells/ml	Leukocytes	Neutrophils
Vehicle	8.29 ± 0,9	7.14 ± 1,0	-	-
AFL - 50	6.40 ± 0,7	5.54 ± 0,5	23	24
AFL - 100	5.52 ± 0,5	3.33 ± 0,9 *	33	53
AFL - 200	5.06 ± 1,0	3.60 ± 0,9 *	39	50

Table 1. Effect of AFL on carrageenan-induced cell migration in rat peritoneal cavity. Carrageenan (500 µg/ml) was injected one hour after treatment with AFL at 50, 100, 200 mg/kg sc or saline (vehicle). The cells were collected 4 hours after the injection of the phlogistic agent. Data are mean ± S.E.M. (n=6). *P<0.05 compared with control group (Student's t-test).

the TPA-induced ear edema by 58% and the capsaicin-induced ear edema by 36%. The AFL caused slight inhibition (20-24%) of the rat paw oedema induced by carrageenan 5 hours after injection of the phlogistic agent. The carrageenan-induced cell migration in the rat cavity showed that the AFL inhibited neutrophil migration by 53%. Preliminary studies showed that the AFL has no analgesic activity in the experimental models of acetic acid induced writhing and tail flick tests [13].

The AFL inhibited the TPA ear edema at a dose of 100 mg/kg ip. Inflammation induced by TPA can activate the protein kinase C in a manner similar to that of endogenous diacylglycerol [14]. Activation of the protein kinase C, a Calcium-dependent enzyme, produces neutrophil [15], platelet and mast cell degranulation [16] and induces smooth muscle contraction [17].

Earlier pharmacological studies demonstrated that the AFL inhibits the spontaneous tonus of the trachea by stimulating β-adrenoceptors [8]. It also antagonizes contractions of the trachea caused by a variety of endogenous mediators related to capsaicin and arachidonic acid in normal tissues and by ovalbumin in sensitized trachea [18]. These effects appear to be mediated by AMPc and cAMP-dependent protein kinase A activity [6]. However the AFL also inhibits phosphodiesterases (PDE) type IV and V from guinea pig lungs [18].

In addition, a bisbenzylisoquinoline alkaloid (Warifteine) isolated from the *Cissampelos sympodialis* root also produced spasmolytic action and inhibited the KCl-induced Ca^{+2} influx through voltage operated Ca^{+2} channels in the ileum [19,20]. Therefore, it is possible that the AFL inhibitory activity on TPA-induced ear edema was due to compounds present in the extract which increase the cAMP levels, inhibit the release of Ca^{+2} from intracellular stores and may be related to a decrease of inflammatory mediators.

The AFL inhibited neutrophil migration induced by carrageenan in a dose-dependent manner. In this model, neutrophil migration is mediated by chemotactic factors such as IL-8, leukotriene B4 and other molecules produced by macrophages [21]. The inhibition of neutrophil migration in this experimental model may be related to a decrease in IL-8 production. In support of this hypothesis, we demonstrated that AFL stimulated the production of IL-10 by concanavalin-A activated mouse spleen cells [4]. It is reported that IL-10 is an anti-inflammatory cytokine, which decreases the production of TNF-α, IL-1, IL-6 and IL-8 by macrophages [22]. The migration and activation of neutrophils at the inflammatory site are also controlled by Ca^{+2}-dependent mechanisms. Since AFL has been shown to inhibit the release of Ca^{+2} from intracellular stores, we can postulate that AFL may also inhibit the neutrophil migration through this pathway.

The AFL inhibited capsaicin-induced mouse ear edema. In this experimental model, substance P and other tachykinins [23] mediate the inflammatory response. The inhibitory effect of AFL on capsaicin-induced ear edema suggests that the extract may have compounds, which interfere with these mediators. We did not demonstrate the AFL mechanisms of action directly but it is possible to suggest that substances in the extract may affect the release or the action of mediators cited above.

The AFL slightly inhibited carrageenan-induced paw edema in rats. There is acute inflammation in which the early phase of edema (until 1 hour) is histamine-, 5-hydroxitryptamine (5-HT)- and bradykinin-dependent. The second phase of inflammation which occurred 3-5 h after the administration of the irritant is induced by the release of bradykinin, proteases, prostaglandins, lysossomal products and by neutrophil infiltration [24]. Therefore, the AFL may not directly interfere with mediators in this inflammatory model.

The data presented here demonstrate that the AFL has anti-inflammatory activity. This activity may be related to compounds which regulate mechanisms of the TPA-induced edema, and of the carrageenan-induced neutrophil migration. Further studies need to be carried out to identify molecules associated with these effects.

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