

Antiinflammatory activity of *ricinus communis* derived polymer

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Abstract

Aim: The present study aimed to evaluate the antiinflammatory activity of the polymer derived from *Ricinus communis* and its mechanism of action. **Methods:** The antiinflammatory activity was investigated in chronic and acute animal models and the mechanism of action involved in the antiinflammatory activity was determined by the *in vitro* phospholipase A₂ (PLA₂) enzyme assay. **Results:** In mouse ear edema (10.0 mg/ear) and granulomatous tissue formation (500 mg/kg) models, the polymer inhibited the inflammatory response in 75.08 ± 1.80% and 61.70 ± 1.80% of the cases, respectively (p < 0.001). Oral administration of the *Ricinus communis* polymer (500 mg/kg) inhibited 72.00 ± 1.20% of formalin-induced inflammation. Topical administration of the polymer on oral lesions of mice showed that the oral mucosa was recovered in 60.00 ± 1.40% (p < 0.05) of the cases. In *in vitro* assay, the phospholipase A₂ enzyme was inhibited by the *Ricinus communis* polymer (5.0 mg/mL) in a dose-dependent manner (84.60 ± 1.41%). **Conclusion:** the polymer derived from *Ricinus communis* showed a significant antiinflammatory activity, confirming that the pharmacological mechanism involved in this antiinflammatory action was related to the inhibition of the PLA₂ enzyme.

Key Words: polymer, *Ricinus communis*, phospholipase A2, antiinflammatory activity.

Introduction

Reconstruction of local bone defects resulting from trauma or bone tumors is a major problem in orthopedics and dental surgery. Currently, large amounts of autogenous bone grafts are used to manage these clinical situations. Although autogenous bone grafts, which combine osteoconductive, osteogenic and osteoinductive properties, are the gold standard for treating bone defects, only a few donor sites in the skeletal system are appropriate for supplying autogenous bone¹.

Polymers are the most versatile class of biomaterials, being extensively applied in medicine and biotechnology². A natural polyurethane resin obtained by polymerization of the polyester polyol is derived from the castor bean plant (*Ricinus communis*), which native of tropical regions³.

According to Kojima et al.³, this polymer has been developed for bone repair because of its capacity to stimulate new fibroblast formation and its progressively replacement by bone around and inside the material's porosities material with no late inflammatory response and no signs of systemic toxic effects.

The methanolic extract obtained from *Ricinus communis* root showed a significant antiinflammatory and free radical scavenging activity⁴. The pharmacological activity may be due to the presence of phytochemicals in the plant extract, like flavonoids, alkaloids and tannins, which have various biological actions⁴.

In Endodontics, a polymer detergent derived from *Ricinus communis* has been developed for use as a root canal irrigant⁵⁻⁸. The polymer detergent has similar antimicrobial activity to that of 0.5% sodium hypochlorite when used for irrigation of necrotic root canals⁵⁻⁸. Although sodium hypochlorite is still the most widely used root canal irrigant, studies have searched for alternative solutions and therapeutic resources that might improve the success rate of endodontic treatment⁸.

Barros et al.¹ reported that polymers offer the advantage

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that has biocompatibility with dental tissue, is osteoconductive and osteogenic, and provide space for the formation of new bone. The *Ricinus communis* polymer showed fibroblastic neof ormation progressively replaced by bone around and inside the porosities of material with absence of late inflammation reaction¹.

Specific inhibitors of phospholipase A₂ (PLA₂) have been sought for a variety of purposes. Since this enzyme is believed to control a number of processes ranging from mobilization of eicosanoids to metabolism of phospholipids, these inhibitors could be useful for controlling inflammatory processes such as rheumatoid arthritis, asthma and psoriasis⁹. Enzyme inhibition assays are important tools in the search for new drugs, and so the PLA₂ assay could determine the mechanism of action of *Ricinus communis* polymer in the antiinflammatory study. The present study investigated the acute and chronic antiinflammatory activity of the polymer obtained from *Ricinus communis* in mouse ear edema, formation of granulomatous tissue in rats, formalin-induced paw edema in mice and in oral mucosa lesions in mice. The antiinflammatory mechanism of action of *Ricinus communis* was assessed using the PLA₂ enzyme assay. Morphological analyses were also performed by means of topical antiinflammatory activity tests.

Material and Methods

Animals

Fasted male Wistar rats (200-250 g) and male Swiss mice (20-25 g) obtained from the Central Animal House of Sacred Heart University (USC) were used. The animals had free access to tap water. Experimental protocols were approved by the institutional Ethics Committee and were conducted according to recommendations of the Canadian Council on Animal Care¹⁰. All experiments were performed in the morning, according to current guidelines for laboratory animal care and ethical guidelines for the investigation of experimental inflammation in conscious animals¹¹.

Chemical Agents and Reagents

A natural detergent obtained from *Ricinus communis* was used in this research. This substance was produced by the Group of Analytic Chemistry and Technology of Polymers from University of São Paulo, São Carlos, SP, Brazil. The technology created at the Institute of Chemistry of São Carlos produced a polymer that had characteristics of a bone substitute in Brazil¹².

Other substances were also used in the experiments: arachidonic acid, hydrocortisone, indomethacin, dexamethasone, formalin, sodium taurocholate, calcium chloride, phosphatidylcholine dipalmitate, phospholipase A₂ and sodium hydroxide (Sigma-Aldrich Corp., St Louis, MO, USA), acetone, propolis (Herbarium Laboratório Botânico Ltda, Colombo, PR, Brazil.), tween 80, ethyl alcohol and xylene (Merck KGaA, Darmstadt, Germany),

xylazine hydrochloride (Bayer S/A, São Paulo, SP, Brazil), ketamine (Laboratórios Pfizer Ltda, São Paulo, SP, Brazil).

Acute Toxicity in Animals

The acute toxicity of the *Ricinus communis* polymer was investigated by intraperitoneal and oral administration in male mice (n=50) weighing 20-25 g. In this assay, increasing doses of the test substance were orally and intraperitoneally administered to groups of 10 animals *per* dose (100, 300, 500, 700, 1,000 mg/kg). The animals were observed for 14 days and the mortality rate was recorded¹³.

Mouse Ear Edema Induced by Arachidonic Acid

The *in vivo* antiinflammatory activity of *Ricinus communis* polymer was assessed in the mouse ear edema model using arachidonic acid to induce inflammation¹⁴. Control mice received only the irritant agents, whereas experimental mice also received the *Ricinus communis* polymer (5 and 10.0 mg/ear) applied topically together with the irritant agent. Arachidonic acid was dissolved in acetone at concentrations of 10 mg/mL and each mouse received 0.5 µg/ear of arachidonic acid on the left ear. The drugs were applied topically to the inner surface of the ear with an automatic pipette in a volume of 5 µL of arachidonic acid. The right ear (control) received 20 µL of acetone (vehicle). The mice were killed by cervical dislocation 1h after treatment with arachidonic acid. Each ear was removed with a metal punch (6 mm diameter disc) and the edema was calculated by subtracting the weight of the right ear (control) from the left treated ear. Hydrocortisone (217 µg/ear) was used as a positive control.

Drug effects were expressed as percentage of inhibition, according to the following equation: $[\text{weight of left minus right control ears}] - [\text{weight of left minus right treated ears}] \times 100, \text{ weight of left control ear}$

Phospholipase Activity

The inhibition of PLA₂ activity (purified from *Apis mellifera* bee venom) by the polymer obtained from *Ricinus communis* was assayed by measuring the decrease in the pH of the incubation mixture using a pH electrode in a closed stirring chamber.

The test substance was incubated for 30 min with the PLA₂ enzyme and added to the assay medium containing 4 mM sodium taurocholate, 12 mM calcium chloride and 7 mM phosphatidylcholine dipalmitate (Sigma-Aldrich Corp.). This technique is reliable under pH 5.0¹⁵. In the present study, the mean initial pH of the phospholipids mixture was 8.0. Positive controls were set up using purified PLA₂ (0.33 µg/mL) from bee venom. The total incubation volume was 2.5 mL. The four different concentrations of the polymer used in these *in vitro* experiments were 2.5, 3.0, 3.5 and 5.0 mg/mL. The PLA₂ inhibition was calculated according to the following equation: $[\text{D pH (treated/min)}, \text{D pH (control/60 min)}] \times 100 = \% \text{ enzymatic reaction}$

Granulomatous Tissue Formation

Cotton rolls (Johnson and Johnson, New Brunswick, NJ, USA) were cut into 5-mm pieces and sterilized in groups of four pellets (160 mg). Rats were anesthetized with xylazine hydrochloride (50 mg/kg) and ketamine hydrochloride (180 mg/kg) and then the pellets were implanted subcutaneously into four symmetrically distributed positions in the abdomen¹⁶⁻¹⁷.

Two hours after implanting the cotton pellets, the animal groups (n=10 each) were treated orally by gastric gavage with tween (10 mL/kg), dexamethasone (0.2 mg/kg), and the *Ricinus communis* polymer at doses of 250 and 500 mg/kg. Daily application of these substances continued for 6 days. On the 7th day, the animals were killed by cervical dislocation, the cotton pellets removed, dried (60°C) and weighed. The difference between the initial and final dry weight corresponded to the weight of the granulomatous tissue formed.

Formalin-Induced Paw Edema in Mice

The method applied was similar to that described by Henriques et al.¹⁸. Groups of male animals were treated orally by gastric gavage with tween (10 mL/kg), indomethacin (30 mg/kg) used as positive controls or with the *Ricinus communis* polymer (250 and 500 mg/kg, p.o. respectively). The polymer was administered 30 min before the injection of 2% formalin in PBS (30 µL/paw) into the sub plantar area of the left hind paw. Paw volume was measured 4 h after formalin injection. Edema was calculated as the difference (µL) between the injected and control paw. The area under the curve (AUC) versus Å paw volume was calculated for each animal and edema was expressed as the mean ± SEM of AUC.

Effect of *Ricinus communis* in Oral Mucosa Lesions of Mice

Male Swiss mice were used in this study. An ulcerated lesion was produced with topical application of NaOH (40%) in the oral mucosa of anesthetized animals¹⁹⁻²⁰. Lesions of the experimental group were treated with a daily topical administration of the polymer derived from *Ricinus communis* at dose of 250 and 500 mg/kg. Lesions of negative and positive control animal groups were treated with a solution of tween 80 and a propolis solution (propolis 11%), respectively. Fourteen days later, the animals were sacrificed. The ulcerated lesions were counted and classified according to level of severity presented²¹.

Histological Analysis

Tissues obtained from the ears and oral mucosa lesions of mice were fixed in Bouin for 24 h, then dehydrated through ascending concentrations of ethyl alcohol, cleared with xylene, embedded in Histosec (Merck; 11609), and prepared for microtomy. Eight-micrometer-thick sections were then deparaffinized and rehydrated through

descending concentrations of ethyl alcohol. These samples were stained with hematoxylin and eosin for morphological analysis, using hematoxylin for 15 min. The samples were washed in running water for 10 min and sections turned from red to blue. Sections were stained with eosin for 10 min and washed in water, dehydrated, placed in 95% alcohol, cleared and mounted in resin²². Photomicrographs were obtained with an Axiophot in Photomicroscope (D-7082; Carl Zeiss, Jena, Germany). The lesions were counted by histomorphometry analysis.

Statistical Analysis

Results were reported as means ± SEM and were analyzed statistically by analysis of variance followed by Tukey's test. P values of less than 0.05 were considered significant.

Results

Acute Toxicity in Animals

Ricinus communis polymer at doses of up to 1,000 mg/kg did not cause any mortality in male albino mice (n=30) during 14 days after intraperitoneal injection and oral administration.

Mouse Ear Edema Induced by Arachidonic Acid

The mouse ear edema reached a maximum at 1h after arachidonic acid application. The *Ricinus communis* polymer significantly inhibited swelling, considerably reducing the vascular permeability response to arachidonic acid application. The polymer (5.0 and 10.0 mg/ear) inhibited the inflammation induced by arachidonic acid in a concentration-dependent manner in $54.40 \pm 1.60\%$ and $75.08 \pm 1.80\%$ ($p < 0.001$, respectively) (Table 1; Figure 1). The positive control, hydrocortisone ($217 \mu\text{g}/\text{ear}$) inhibited $77.00 \pm 1.20\%$ of the topical inflammation ($p < 0.001$). Histologically, the topical application of arachidonic acid caused neutrophil accumulation (Figure 1A). In this experiment, a rapid, albeit transient edema, was induced accompanied by erythema.

Ricinus communis polymer application immediately after the irritant agent inhibited acute inflammation caused by arachidonic acid. In this treatment, neutrophils were absent (Figure 1B), confirming the antiinflammatory effect mentioned by *Ricinus communis* polymer.

Table 1 - Effect of topical application of *Ricinus communis* on arachidonic acid (0.5 mg/ear)-induced mouse ear edema

Treatment	Dose	Inhibition (%)
Control (acetone)	10.0 mL/ear	-
Hydrocortisone	217.0 µg/ear	77.00 ± 1.20(**)
<i>Ricinus communis</i> polymer	5.0 mg/ear	54.40 ± 1.60 (**)
<i>Ricinus communis</i> polymer	10.0 mg/ear	75.08 ± 1.80 (**)

Each value is the mean ± SEM for 7 animals. Each value differed significantly from the respective control value. ANOVA $F_{(3, 24)} = 35.0$. ** $p < 0.001$, Tukey's test

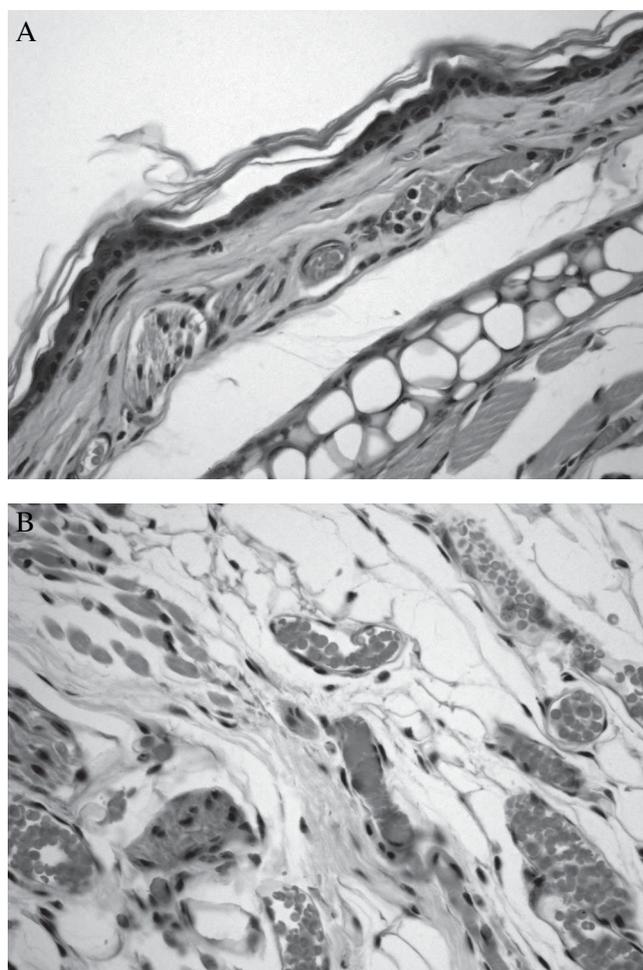


Fig. 1 - Arachidonic acid (AA)-induced mouse ear edema. A: animals treated with AA and acetone show a typical acute inflammation with neutrophils (arrow) inside blood vessels. Bar = 100 μ m, original magnification: 40x. B: acute inflammation was inhibited (no neutrophils inside blood vessels) in animals treated with AA and 10 mg/ear of Ricinus communis. Bar = 500 μ m, original magnification 40x.

Phospholipase Activity

At concentrations up to 5.0 mg/mL, the polymer obtained from Ricinus communis had a significant inhibitory activity against PLA₂ enzyme (0.33 μ g/mL bee venom) (Table 2). The polymer inhibited the PLA₂ activity around 84.60 \pm

Table 2 - Inhibitory action of Ricinus communis on phospholipase A₂ activity.

Drug	Concentration (mg/mL)	Inhibition (%)
Ricinus communis polymer	2.5	35.70 \pm 1.20
Ricinus communis polymer	3.0	67.70 \pm 0.98 (*)
Ricinus communis polymer	3.5	80.00 \pm 1.34 (*)
Ricinus communis polymer	5.0	84.60 \pm 1.41 (*)

Data are reported as the mean % inhibition \pm SEM for 5 experiments (N=5). The concentrations (2.5; 3.0; 3.5; 5.0 mg/mL) of the Ricinus communis polymer are significantly different (p<0.05) (3.0, 3.5 and 5.0 *** 2.5 mg/mL). * p<0.05, Tukey's test

1.41% (p<0.05). These results showed that the possible mechanism of action of Ricinus communis involved in its antiinflammatory activity would occur by PLA₂ inactivation, that releases arachidonic acid during the beginning of the inflammatory cascade.

Granulomatous Tissue Formation

Treatment for 6 days with dexamethasone and the polymer of R. communis inhibited the formation of granulomatous tissue induced by implantation of subcutaneous cotton pellets into the abdominal region (Table 3). The polymer at the dose of 250 and 500 mg/kg inhibited the inflammatory process by 55.70 \pm 1.20 and 61.70 \pm 1.80%, respectively (p<0.001). Dexamethasone was effective in inhibiting inflammation by 72.30 \pm 1.60% (p<0.001).

Formalin-Induced Paw Edema in Mice

Intradermal injection of formalin (1%, 20 μ L), into one of the hind paw of normal rats (control group-treated orally with tween) caused a local inflammatory response, which reached a maximum intensity of edema at hour 4 after application of the phlogistic agent.

Ricinus communis polymer (250 and 500 mg/kg, p.o.) significantly decreased paw swelling by 61.80 \pm 1.30% and 72.00 \pm 1.20%, respectively (Table 4, p<0.001). Treatment of the animals with indomethacin (30 mg/kg), the reference antiinflammatory, significantly reduced the intensity of edema induced by formalin around 80.00 \pm 2.00% (p<0.001).

Table 3 - Effect of oral administration of Ricinus communis on rat granuloma tissue formation

Treatment	Dose	Dry weight of granuloma (mg)		Inhibition (%)
		Initial	Final	
Tween	10mL/kg	258.0 \pm 2.8	491.3 \pm 1.8	-
Dexamethasone	0.2 mg/kg	245.02 \pm 2.1	172.72 \pm 1.2	72.30 \pm 1.60 (**)
Ricinus communis polymer	250 mg/kg	243.7 \pm 1.6	188.0 \pm 1.4	55.70 \pm 1.20 (**)
Ricinus communis polymer	500 mg/kg	238.0 \pm 1.2	176.3 \pm 1.3	61.70 \pm 1.80 (**)

Each value is the mean \pm SEM for 10 animals. The Ricinus communis polymer at the dose of 250, 500 mg/kg and the positive control (dexamethasone) were significantly different compared to the respective control value. ANOVA F_(3,36) = 35.0. **p<0.001, Tukey's test

Table 4 - Effect of *Ricinus communis* on formalin-induced mouse paw edema

Treatment	Dose	Inhibition (%)
Tween	10 mL/kg	-
Indomethacin	30 mg/kg	80.00 ± 2.00 (**)
<i>Ricinus communis</i> polymer	250 mg/kg	61.80 ± 1.30 (**)
<i>Ricinus communis</i> polymer	500 mg/kg	72.00 ± 1.20 (**)

Each value is the mean ± SEM for 7 animals. Each value differed significantly from the respective control value, ANOVA $F_{(3, 24)} = 34.6$ ($p < 0.001$). ** $p < 0.001$, Tukey's test

Effect of *Ricinus communis* in Oral Mucosa Lesions of Mice

In experiments of oral mucosa lesions induced by sodium hydroxyl 40%, the polymer obtained of *Ricinus communis* (250 and 500 mg/kg) healed respectively 35.00 ± 1.20% and 60.00 ± 1.40% of the oral mucosa lesion ($p < 0.05$) (Table 5; Figure 2). The positive control (propolix) healed 78.00 ± 1.60 ($p < 0.05$). When only NaOH was applied topically, it was observed that the surface epithelium was destroyed (Figure 2A). In Figure 2B, the oral lesion was healed and the surface epithelium was recovered in mice treated with the *Ricinus communis* polymer.

Table 5 - Topical effect of *Ricinus communis* on oral mucosa lesions of mice

Treatment	Dose	Inhibition (%)
Tween	10 mL/kg	-
Propolix	10 µl	78.00 ± 1.60 (*)
<i>Ricinus communis</i> polymer	250 mg/kg	35.00 ± 1.20(*)
<i>Ricinus communis</i> polymer	500 mg/kg	60.00 ± 1.40 (*)

Data are reported as the mean % inhibition ± SEM for 9 animals.

* $p < 0.05$, Tukey's test

Discussion

The development of polyurethane resins derived from *Ricinus communis* widened the scopes in different fields of medical and dental research, as they were shown to have biocompatibility and potential applicability in several areas⁸. In Endodontics, a detergent derived from *Ricinus communis* has been proven to have similar antimicrobial activity as that of 0.5% sodium hypochlorite when used for irrigation of necrotic root canals⁵⁻⁶.

In the present study, we examined the antiinflammatory activity of the polymer obtained from seeds of *Ricinus communis*. This substance is a detergent derived from castor bean oil, has similar antimicrobial activity to that of 0.5% sodium hypochlorite when used for irrigation of necrotic root canals, is biocompatible with the periapical tissues, increases dentinal permeability and has similar ability to remove smear layer from the root canals as that of 17% EDTA⁸.

Studying the extract of leaves and root of *Ricinus communis*, Ilavarasan et al.⁴ observed a significant

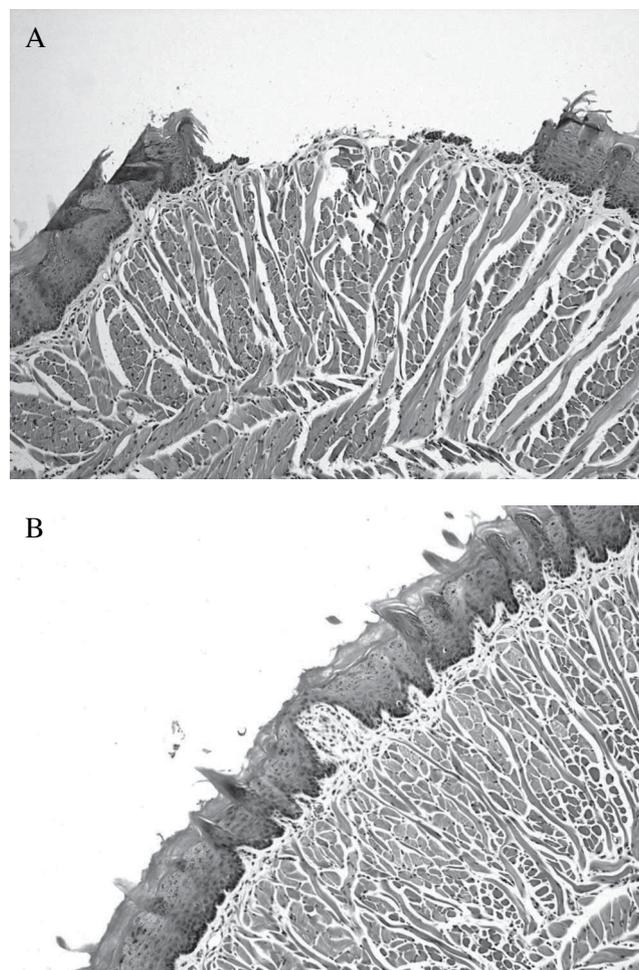


Fig. 2. Effect of *Ricinus communis* on oral mucosa lesions of mice. **A:** oral lesion caused by sodium hydroxide (40%) applied topically and treated with tween. Damage to epithelial tissue is noted and indicated by arrow. Bar = 100 µm, original magnification = 10x. **B:** the photomicrography show oral mucosa epithelium intact (regenerate in 14 days) by the *Ricinus communis* polymer (500 mg/kg) after induction of lesion by sodium hydroxyl 40%. Bar = 500µm, original magnification = 10x.

antiinflammatory activity in rats, possibly due to the presence of flavonoids, alkaloids and tannins present in the plant extract. It was also reported that flavonoids obtained from *Ricinus communis* root extract would explain its free radical scavenging activity. The excessive generation of reactive oxygen species (ROS) leads to a variety of pathological processes, such as inflammation, diabetes, hepatic damage and cancer⁴.

Inflammation is generally defined as the response of living tissue to an injurious stimulus²³. The usual features of inflammation include the activation of epithelial cells and resident macrophages, and the recruitment and activation of neutrophils, eosinophils, monocytes and lymphocytes²⁴. Leukotriene and prostaglandin synthesis is involved in arachidonic acid-induced ear edema²⁵. Chemically induced edema represents an acute local inflammation eliciting a complex series of physiological events involving many

processes in which components of a plant extract may interact, inhibiting kinins and prostaglandins on vascular permeability that appear to be involved with inflammatory process²⁶⁻²⁷.

Acute and chronic inflammatory processes can be induced by several means, and antiinflammatory agents exert their effects through different manners²⁸⁻²⁹. For screening of new antiinflammatory compounds, the croton oil-induced mouse ear edema assay is widely used in conjunction with the *in vitro* phospholipase A₂ assay³⁰⁻³¹. Enzyme inhibition assays are important tools in the search for new drugs. It has been established that the inflammation induced by arachidonic acid involves an increase in PLA₂ activity, which, in turn, leads to the release of arachidonic acid and subsequent biosynthesis of leukotrienes and prostaglandins, thus also involving the lipoxygenase pathway³².

Bresnick et al.³³ stated that PLA₂ catalyzes the sn-2 hydrolysis of phospholipids releasing free fatty acids, predominantly arachidonic acid and lysophospholipids. These products can have biological actions or be further metabolized to form a variety of proinflammatory lipid mediators including prostaglandins, leukotrienes platelet-activating factor and thus the inhibition of PLA₂ by pharmacological agents should have led to an antiinflammatory effect.

Glycosides obtained from *Ipomoea imperati* also showed activity in both tests mentioned above, reducing mouse ear edema induced and inhibiting bee-venom phospholipase A₂ (PLA₂) activity³².

The cotton pellet-induced granuloma was used as a chronic model to evaluate the antiinflammatory effects of natural products and have good predictive value for screening antiinflammatory agents³⁴. This method is a suitable test for assessing the antiinflammatory activity drugs and widely used to evaluate the transudative and proliferative components of chronic inflammation³⁵. In chronic inflammatory states, the efficacy of antiinflammatory agents can be indicated by inhibiting the increase in fibroblasts and the infiltration of neutrophils and exudation³⁶⁻³⁷.

When macrophages, epithelioid cells and multinucleate giant cells predominate at the site of inflammation, the lesion is named nodular chronic inflammation or granuloma. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma. Dry weight correlates well with the amount of granulomatous tissue formed³⁸.

Catanzaro Guimarães et al.³⁸ reported that the inflammatory process is represented by edema, which is formed in the early stages of granuloma development, or as a result of immune reactions participating in the pathogenesis of these lesions. These reactions originate immune complexes that activate the complement system to generate vasodilator mediators. The latter mechanism is represented by cells, collagen fiber and newly formed blood vessels³⁸.

The formalin's test is a well-known model of chemically induced inflammation and nociception³⁹. The intra-plantar injection of diluted formaldehyde causes inflammation that release mediators such as bradykinin, histamine,

sympathomimetic amines, tumor necrosis factor- α and interleukins⁴⁰⁻⁴³. In addition to these mediators, local prostaglandin levels are responsible for the progress of nociception and are targeted by most non-steroidal antiinflammatory drugs⁴³.

In the formalin assay, edema that is maintained during the plateau phase occurs due to kinin-like substances. Later, the swelling phase prevails due to the release of prostaglandin-like substance⁴⁴. Thereby, the formalin assay is well suited for the comparative bioassay of antiinflammatory agents since the relative potency estimates obtained for most drugs tend to reflect clinical experience⁴⁵. In experiments of oral mucosa lesions induced by sodium hydroxyl 40%, the *Ricinus communis* polymer was tested. Ulcers are defined as local defects on the surface of tissues or organs. These defects are produced by loss of surface epithelium with exposure of connective tissue. Contact of the mucous membrane with physical or chemical agents represents the main source of mouth ulcers⁴⁶. Oral ulcers are one of the most common complaints in the dental practice and are usually caused by mechanical trauma induced by ill-fitted dentures, orthodontic appliances and fractured crowns and restorations⁴⁷.

With major loss of cells involved in oral lesion formation, the inflammatory and repair processes occur by secondary union and involve a series of vascular, cellular, neurological and humoral events. Ulcer healing is a dynamic process of filling mucosal defects with proliferating and migrating of epithelial cells as well as connective tissue, resulting in the reconstruction of the mucosal architecture⁴⁸.

In conclusion, the findings of the present study showed a potent antiinflammatory activity of *Ricinus communis* polymer, this antiinflammatory effect being related to the phospholipase A₂ enzyme inhibition. This study establishes the therapeutic rationale of using *Ricinus communis* polymer in various inflammatory events, which has antimicrobial activity and has been used for root canal irrigation.

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