Anti-inflammatory activity and concentrations of polyphenols and flavonoids in the ethanolic extract of dodonaea viscosa (Sapindaceae)

Raquel M. M. Necchi*, Cauriane Ercolani**, Sérgio L. Dalmora***,
Melânia P. Manfron****

ABSTRACT: Both anti-inflamatory activity of ethanolic extract of D. viscosa in induction model of granulomatous tissue and the total polyphenols and flavonoids content were evaluated in this study. The animals treated with the sample showed 54.46 ±3.99% inhibition of the formation of granulomatous tissue while the control group treated with nimesulide, showed 58.55 ±1.82% confirmed significant antiinflammatory activity. The measurements of total polyphenols and flavonoids in the extract were significant when compared to the standards, which confirms the popular use of this medicinal plant.

Descriptors: Antiinflammatory, Dodonaea viscosa, Flavonoids, Granuloma, Polyphenols, Sapindaceae.

Atividade anti-inflamatória e concentrações de polifenóis e flavonóides no extrato etanólico de Dodonaea viscosa (Sapindaceae)

RESUMO: A atividade antiinflamatória do extrato etanólico de D. viscosa em modelo de indução de tecido granulomatoso, o teor de polifenóis totais e flavonóides foram avaliados. Os animais tratados com a amostra apresentaram 54,46 ± 3,99% de inibição da formação de tecido granulomatoso enquanto que o grupo controle (tratado com nimesulida) apresentou 58,55 ± 1,82% confirmando uma significativa atividade antiinflamatória. As dosagens de polifenóis totais e flavonóides no extrato foram significativas quando comparados aos padrões, o que comprova o uso popular desta planta medicinal.

Descritores: Antiinflamatória, Dodonaea viscosa, Flavonóides, Granuloma, Polifenóis, Sapindaceae.

*Doutoranda em Ciências Farmacêuticas na Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil.
**Mestre em Ciências Farmacêuticas pela Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil.
*** Doutor em Ciências Biológicas pela Universidade Federal de São Paulo (UNIFESP), São Paulo, SP, Brasil.
****Doutora em Ciências Biológicas pela Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP), São Paulo, SP, Brasil.

Introduction

Family Sapindaceae comprises about 1600 species grouped in 14 genera.[1] The morphological characteristics of this family are simple and alternate leaves, tetramerous and androgynous flowers, fruit of capsule-samaroide type which include 3-4 wards, petals with glandular or squamous appendix, asymmetrical extra-staminal disk, tricarpellate ovary, seed usually with aryl and fruit commonly winged. [2-4]

Dodonaea viscosa (L) Jacq. is a shrub or tree, with sparse canpy that reaches 4 to 8m of height, distributed in tropical and subtropical regions. In Brazil, it occurs on the coast, sandbanks, plains and slopes of hills and popularly known as "vassoura-vermelha, vassourão, vassoura-do-campo". [5;2]

In traditional medicine the "vassoura-vermelha" is used to treat pain, fever, cramps, gout, rheumatism, sexually transmitted diseases and even tumors.[6] In a broad sense, the extracts are widely used as analgesic, antiinflammatory, antiviral, spasmolytic, laxative agents, antimicrobial and hypotension.[7]In India, the infusion of the leaves is used to treat rheumatism, hemorrhoids, fractures and snake bites. [8;7]

Studies of chemical composition show that the aqueous and ethanolic extracts of D. viscosa leaves has heterosides cardiotonic, flavonoids, tannins, saponins, essential oils, coumarins, gum, mucilage, organic acids. [9-11] Compounds were isolated like Aliarin, Dodonic acid, viscosol stigmosterol[9], isorhamnetin[12] dodonosid A and B[13], penduletin, quercetin[14], doviscogenin and four kaempferol methyl ethers[15]. In biological assays the extract of leaves showed local anesthetic activity, smooth muscle relaxant[16], antibacterial against gram-negative bacteria[17;18], antifungal activity against Candida albicans [19;20], anti-inflammatory activity by the method of paw edema rats[11], antiviral and anti-ulcerogenic[21] front of coxsackievirus B3 and influenza A[22]. Sukkawala e Desai[23] found that the ethanol extract of leaves of D. viscosa presents properties like anti-ascariasis, anthelmintic, hypotensive, vasoconstrictor and relaxation of uterine muscle in different experimental models.

This paper aims to conduct anti-inflammatory activity of ethanol extract of leaves of D. viscosa by the method of induction of granulomatous tissue in rats and determine the amount of total polyphenols and flavonoids.

Materials and methods

Collection of botanical material and obtaining the plant extract

The leaves of Dodonaea viscosa were collected in Santa Maria, Rio Grande do Sul, Brazil. A sample of plant material was identified and deposited in the Herbarium of Biology Department of UFSM as voucher specimen SMDB 12.274. The ethanol extract of leaves of D.viscosa was obtained by cold maceration in 70% ethanol solvent and dry in hothouse.

Animals

We used male Wistar rats, weighing between 180 and 200 g, divided into 3 groups of 6 animals. The rats were kept in a vivarium of the Department of Industrial Pharmacy of UFSM with light and dark cycle of 12 hours and 22 ± 2 ºC, free access to food (standard rodent diet)

and water. All procedures were performed according to recommendations of the International Committee for the care of animals and in accordance with established national regulations for animal experimentation. The study was approved by the ethics committee and Animal Research, Federal University of Santa Maria, on the number 23081.018819/2005-99.

The induction of granulomatous tissue

The animals under aseptic conditions were anesthetized with ether and a sterile cotton cylinder was implanted in the subcutaneous tissue of rats according to Meier et al.,[24] and Niemegeers et al.,[25]. The three groups consisted of six animals, divided into positive control, negative control and test, were treated respectively with Nimesulide 5 mg/kg/day, propylene glycol 20% (v/v) and D. viscosa.

Treatment was delivered orally by gavage for seven days, given 1.5 ml of hydroetanolic extract of D. viscosa at a dose of 200 mg/kg/day, divided into three daily doses of 0.5 ml every 4 hours. After treatment, animals were sacrificed in a CO_2 chamber and the cotton removed by dissection and dried at 60°C for 24 hours in an hothouse to obtain the final weight on an analytical balance.

The weights of the granulomas were expressed in grams, calculated the percentage of inhibition of granuloma tissue by the difference between initial and final weight of cotton.

Dosage of total polyphenols

The dosage of polyphenols was performed through the Folin-Ciocalteau, with some modifications. [26,27] Based on the solution of 0.5 mg/ml of the ethanol extract of D. viscosa, solutions were prepared in volumetric flasks at concentrations of 50, 40, 30, 20, 10, 6 and 3 g/ml. In each one was added 1 ml of Folin-Ciocalteau, await 5 minutes, and 2 ml of sodium carbonate (Na_2CO_3) 20% with wait for 10 minutes. The solutions were homogenized, capped and protected from light and kept at room temperature. The absorbance was measured at 730nm using water as a blank.

The test was performed in triplicate. The standard curve was obtained using standard solutions of gallic acid at the same concentration of the sample. The regression curve obtained was $Y = 0.0027+0.0465x$ ($r^2 = 0.9998$).

Dosage of flavonoids

The dosage of flavonoids was carried out according to Rio[28] modified, where it was used rutin as standard in solution of aluminum chloride. Two grams of dried leaves were pulverized and extracted with 150 ml of 70% methanol in soxlet for 3 hours; The extract was filtered and the volume completed to 250 ml. After that, 15 ml of the extract was placed in a volumetric flask plus 1 ml of solution of $AlCl_3$ (5 g AlCl 3 in 100 ml of methanol), and the volume completed to 50 ml. After resting for 30 min., it was read at 425 nm on a spectrophotometer UV/visible to obtain the concentration of flavonoids.

The analysis was performed in triplicate. Absorbance data of the sample was compared with a standard curve constructed of rutin prepared with 70% methanol (10, 20, 50, 100, 150,

200, 300, 400, 800 µg/1,1 ml) plus 75 ml of $AlCl_3$ 5% methanol and 3.9 ml of 70%. The regression curve obtained was Y = 0.0027+0.0465x (r^2 = 0.9998).

Statistical analysis

The results were expressed as mean ± standard error of mean (SEM). The statistical analysis was performed using Student t test and the results were considered significant when $p<0,05$.

Results

The treatment of animals during seven days with extract of D. viscosa leaves led to a significant reduction in weight of granuloma. In the experiments, the animals treated with nimesulide had 58.55 ± 1.82% inhibition of the inflammatory process and the group treated with test extract of D. viscosa showed inhibition of 54.46 ± 3.99% compared to negative control.

The measurements of total polyphenols and flavonoids were 16.89±1,02% e 15.20±1,35% compared to the standards of gallic acid and rutin, respectively.

Discussion

The results of inhibition of granuloma extract of D. viscosa and the nimesulide standard were similar showing a significant antiinflammatory activity.

Granuloma, at the end of the sixth day, is characterized by the formation of vascularized fibrous capsule, containing fibroblasts and infiltrating mononuclear cells. Thus, the lower the fibrous capsule developed, the greater the effect of anti-inflammatory drug tested. [29,30]This model is used to evaluate the activity of a particular drug on a chronic inflammatory process. Bastos et al.,[31] conducted a study using this methodology to determine the antiinflammatory activity of minocycline and doxycycline, both second-generation tetracycline, when it was possible to observe a significant inhibition of formation of granuloma tissue for both drugs compared to dexamethasone control.

According to Almeida et al.,[11] the aqueous and ethanolic extracts of D. viscosa has anti-inflammatory activity by testing the paw edema of rats when they received 600 and 800 mg/kg, respectively, inhibiting edema in 83.56% after 30 minutes and 55.98% after one hour. This method induce edema in back paw of rats and compare it with the contralateral paw, which is considered the control of the test. The swelling is then expressed by the difference in volume between the paws measured by plethysmometer after a certain period of time.[32] Although evaluate the acute inflammation, the results are concur to those made in this study, which significantly inhibited the induction of granulomatous cells characterized in chronic inflammation.

The antioxidant activity and the values found in the dosage of polyphenols and flavonoids in the extract of D. viscosa are significant, which justifies the popular use of this plant. The phenolic compounds are potent antioxidants and have a wide variety of good biological

activities like anti-inflammatory action. Flavonoids are characterized chemically by presenting the fenilbenzopirane core ($C_6C_3C_6$). Their anti-inflammatory activity is due to inhibition of arachidonic acid metabolism, involved in the formation of inflammatory mediators (prostaglandins and leukotrienes), by the ability to remove free radicals and to chelate divalent cations. [33,34]

Data from in vitro models of various structurally different flavonoids demonstrate antioxidant efficacy in relation for many conditions of oxidative stress. According Teffo et al.,[15] the extract of D. viscosa leaves exhibits antioxidant activity by the method of capture of the radical 2, 2 diphenyl picryl hydrazine (DPPH), and were attributed to flavonoids such as kaempferol isolated from the sample, which confirms the reasons for use in the treatment of infections in traditional medicine.

The tannin rich plants are also used in tradicional medicine to treat inflammatory processes. According to Scholz et al.,[35] the protocianidines of the peels of Anacadium occidentale showed anti-inflammatory by blocking mast cell degranulation in rats. The protoantocianidinas are natural antioxidants that act as scavengers of free radical and inhibiting enzymes such as phospholipase, cyclooxygenase and lipooxogenase and reduces lipid peroxidation. [36;37] Inhibition of these mediators of inflammation also may occur with the extract of D. viscosa, that presented significant antioxidant activity[15].

Conclusion

The ethanol extract of leaves of D. viscosa administered orally, inhibited the formation of granuloma tissue in rats, confirming anti-inflammatory activity. Also present significant levels of total polyphenols and flavonoids proving some of their uses in folk medicine.

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Raquel Medina Martins Necchi

Endereço para correspondência — Programa de PósGraduação em Ciências Farmacêuticas, Universidade Federal de Santa Maria, Prédio 26, Santa Maria, RS, Brasil. CEP: 97105900

Email: ranecchi@hotmail.com

Currículo lattes: http://lattes.cnpq.br/4029402462196629

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