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Physalis angulata extract exerts anti-inflammatory effects in rats by inhibiting different pathways

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ABSTRACT

Physalis angulata is a popular medicine used in Brazil due to its anti-inflammatory effects, but the pharmacological mechanisms underlying these actions remain to be better understood. In the present work, lyophilized aqueous extract from the roots of *Physalis angulata Linneu* (AEPa) was used to control the inflammatory response induced by the injection of 1% carrageenan into subcutaneous rat's air pouches. Adenosine deaminase (ADA) activity, nitrite level, and prostaglandin E_2 (PGE_2) level were used to evaluate the action of inflammatory mediators. Tumor growth factor-β (TGF-β) level was used as a bioindicator of immunomodulatory response. Rats were injected with vehicle, indomethacin, or AEPa (0.5 mg/kg, 1 mg/kg, and 5 mg/kg i.p.), 1 h before carrageenan administration. AEPa at 0.5 mg/kg had no effect. However, 1 mg/kg of AEPa showed significant anti-inflammatory effects, decreasing exudate volume, total number of inflammatory cells, ADA activity, nitrite level, and PGE_2 level in 50%, 41%, 20%, 60%, and 41%, respectively. The anti-inflammatory effects of 5 mg/kg AEPa appeared to be more effective than those of 1 mg/kg AEPa (84%, 80%, 43%, 70%, and 75%, respectively). In addition, TGF-β level was upregulated to 9700 pg/ml after 5 mg/kg AEPa, in comparison with 160 pg/ml in the vehicle-treated group, and 137 pg/ml in the indomethacin-treated group. The results indicate that AEPa exerts powerful anti-inflammatory and immunomodulatory activities, interfering with the cyclooxygenase pathway, lymphocyte proliferation, NO, and TGF-β production.

1. Introduction

Physalis angulata (Solanaceae) is a widespread indigenous herb found in tropical areas of Africa, Asia, and America, including the Amazon (Pio Corrêa, 1926; Lin et al., 1992). It grows up to 1 m with small stem, cream-colored flowers, and light yellowish-orange, edible fruits wrapped by a layer of leaves (Pio Corrêa, 1926). This herb has been used in traditional medicine as analgesic, anti-rheumatic, to treat sore throat and abdominal pain. It is considered as antipyretic, antinociceptive, antidiuretic, and anti-inflammatory for hepatitis and cervicitis (Lin et al., 1992; Bastos et al., 2006). In the Amazon, *Physalis angulata* is popularly known as "camapu" and its juice is used as sedative, depurative, anti-rheumatic, and for the relief of earache.

Physalis angulata extracts upregulate the activity of spleen natural killer cells (NK cells) in tumor-bearing mice (Won et al., 1988). The plant phytochemistry is known to contain glucocorticoids, physalin, and withangulatin. It has been found that the latter compound has an inhibitory action in macrophage culture (Lin et al., 1992; Soares et al., 2003) and in rat's brain tumor cells (Lee et al., 1991).

Inflammatory response is a complex process mediated by a variety of signaling molecules locally released by nerve endings, mast cells, platelets, and leucocytes. Some of these molecules and their precursors are used as inflammation markers, such as adenosine deaminase (ADA), prostaglandins, and nitric oxide (NO). ADA is an enzyme involved in the purine catabolism, catalyzing the deamination of adenosine to inosine (Kalkan et al., 1999). ADA is widely distributed in human tissues especially in lymphoid tissues (Sullivan et al., 1977). Its main physiological activity is related to T lymphocytic proliferation (Hovi et al., 1976) and maturation (Shore et al., 1981). Prostaglandins and NO are important mediators of inflammation and other pathophysiological processes. The

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production of PGE_2 at injury sites is due to its ability to cause vasodilatation and to increase edema induced by phlogists that increase vascular permeability (Appleton et al., 1996). PGE_2 is a monocyte/macrophage product, which regulates the immune response and activates several cell classes (Ninnemann, 1988). Nitric oxide modulates both acute and chronic inflammatory reaction and has a well-established role in the endothelial-dependent control of vascular tonus (Olesen et al., 1994). NO released from endothelium is activated by soluble guanylate cyclase, and is responsible for the cytotoxicity of macrophages and neutrophils (Ohshima and Bartsch, 1994).

TGF-β is a secreted protein that regulates proliferation, differentiation, and death of various cell classes. All immune cell lineages, including B and T lymphocytes, as well as macrophages, release TGF-β. This factor negatively regulates proliferation and differentiation of these cell classes. Thus, TGF-β is a potent immunosuppressor and changes in TGF-β signaling is linked to autoimmunity, inflammation, and cancer (Moustakas et al., 2002). TGF-β1 also has a major role in the regulation of immune cell functions. TGF-β1-knockout mice develops severe multiorgan inflammation, which begins in the neonatal period and results in early death (Monteleone et al., 2004).

Inflammation studies using air pouch models with carrageenan injection revealed increased vascular permeability, and exudate formation bearing huge numbers of polymorphonuclear (PMN) leucocytes (Hambleton and Miller, 1989). In this work, we used this experimental model to investigate AEPa anti-inflammatory effects. Our results suggest that AEPa has potent anti-inflammatory and immunomodulatory effects that interfere with the production of PGE_2 and NO, lymphocyte proliferation, and TGF-β level *in vivo,* validating the ethnomedicine use of this herb in the Amazon.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 200–220 g were obtained from the Animal Facilities of the Evandro Chagas Institute (Ananindeua, Pará, Brazil). They were randomly assigned to groups of 10 animals and maintained in plastic boxes with food and water *ad libitum* under a 12 h light/12 h dark cycle. Room temperature was maintained at 22 ± 1 °C. The animals were kept in the laboratory for at least 2 h before the experiments which were performed between 8 a.m. and 1 p.m. in order to avoid circadian influence. All the procedures involving animal care and experimentation were performed in accordance with the guidelines of the Ethical Committee for Research with Experimental Animals (CEPA) of the Federal University of Para. All efforts were made to minimize the number of animals used and their suffering.

2.2. Preparation of the aqueous extract (AE) of Physalis angulata

Plants were collected in the Pará State. A voucher specimen (Ref. 653) was deposited in the João Murca Pires Herbarium of the Emilio Goeldi Museum (Pará, Brazil) and then classified by Dr. Ricardo Secco of the Department of Botany of this institute. Roots of *Physalis angulata* were cut from the plant for extract production. The material weighting about 150 g was cleaned, extracted with 700 ml of Milli-Q water at boiling point, and concentrated to a final volume of 100 ml. Decoct was frozen and stored at −20 °C for subsequent lyophilization, producing 2.712 g of the extract.

2.3. Preparation of air pouches and induction of inflammatory process

Induction of air pouches was performed as described by Tao et al. (1999). The air was carefully removed inside a flow chamber to avoid contamination. The pouches were inflated with 20 ml of air injected into the intrascapular area and then re-inflated with 10 ml of air every 3 days. Nine days after the preparation of the air pouches, 1% Carrageenan–kappa in 0.9% NaCl was injected into the pouches to induce a local inflammatory process.

2.4. Sample collection

The samples were obtained 16 h after phlogist administration. A small incision was made in the pouch wall and the air pouch content was carefully removed using a sterile Pasteur pipette. To increase the total exudate volume and thus improving measurement precision, 3 ml of PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM, and 1.4 mM $Na_2HPO_4{\cdot}7H_2O$) were previously injected into the air pouch. In the next step, the exudate volume, total number of cells, nitrite content, adenosine deaminase activity, prostaglandin level and TGF-β level were determined.

2.5. Chemicals

The drugs used were: Carrageenan–kappa, DMEM, poly-L-lysine, phenic acid, sodium nitroprussiate, naphtylethylen, sulfanilamide and indomethacin (Sigma Chemical Co., St. Louis, MO, USA). Fetal serum bovine, penicillin–streptomycin (GIBCO, Auckland, New Zealand). All the other reagents were of analytical grade. The indomethacin was dissolved in 5% $NaHCO_3$ and Tween 80 plus 0.9% NaCl.

2.6. Treatment

Fifty rats were randomly divided in control and AEPa groups. The control group was intraperitoneally injected with 0.9% NaCl or indomethacin 10 mg/kg. The AEPa group was i.p. injected with 0.5, 1, or 5 mg/kg AEPa in 0.9% NaCl. The treatment was completed 1 h before carrageenan administration.

2.7. Cell culture

Three samples from each animal group, containing 150 μl of exudate were incubated in DMEM supplemented with 10% fetal bovine serum and penicillin–streptomycin 10,000 U/ml, in plates previously treated with poly-L-lysine (PLL 100 μg/ml). Cultures plates were kept in 5% CO_2/95% O_2 at 37 °C. After 24 h, five fields of the exudate culture were selected randomly and analyzed to get the total number of inflammatory cells.

2.8. Assay of nitrite level

Samples were initially diluted 1:2 in PBS and then 500 μl of each diluted sample were mixed with the same volume of Griess reagent (0.1% naphtylethylen + 1% sulfanilamide in 5% phosphoric acid) (Green et al., 1982). The absorbance values at $OD_{570\,nm}$ were measured and referred to a standard curve (sodium nitrite solution) to determine the NO_2^- concentration.

2.9. Assay of adenosine deaminase

Exudate ADA activity was determined according to the Rodrigues et al. (1994) colorimetric method. Exudate samples were incubated for 1 h at 37 °C in 0.5 ml of adenosine solution