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Topical anti-inflammatory activity of *Solanum corymbiflorum* leaves

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ABSTRACT

Solanum corymbiflorum is popularly known as “baga-de-veado” and its leaves are applied on inflamed legs, scabies, tick bite, boils, mastitis, low back pain and otitis. The aim of this study was evaluate anti-inflammatory *in vivo* activity and relate this activity with antioxidant compounds present in the extract of *S. corymbiflorum* leaves. The extract from *S. corymbiflorum* leaves topically applied was able to reduce the croton oil-induced ear edema and myeloperoxidase (MPO) activity with maximum inhibition of 87 ± 3% and 45 ± 7%, rescpectively in the dose of 1 mg/ear. Similar results were found for positive control dexamethasone, which presented inhibitions of ear edema and MPO activity of 89 ± 3% and 50 ± 3%, respectively in a dose of 0.1 mg/ear. These findings are due, at least in part, the presence of polyphenols (195.28 mg GAE/g) and flavonoids, as chlorogenic acid (59.27 mg/g), rutin (12.72 mg/g), rosmarinic acid, caffeic acid and gallic acid found by high performance liquid chromatography (HPLC) analysis. This species showed potencial antioxidant by 1,1-diphenyl-2-picrylhydrazyl (DPPH), and carbonyl groups in proteins methods which may be related with the presence of this compounds. This species possess anti-inflammatory activity confirming their popular use for the local treatment of skin inflammatory disorders.

1. Introduction

Solanum corymbiflorum (Sendtn.) Bohs (syn. *Cyphomandra corymbiflora*), popularly known as “baga-de-veado”, is native in the southern states of Brazil and in Argentina, where is known as “ka’a Kururu” (Herb of frog) (Soares and Mentz, 2006). In the folk medicine its leaves can be applied on inflamed legs, scabies, tick bite, boils, mastitis, low back pain and otitis (Keller and Prance, 2012). However, to our knowledge, there are no studies related to popular use of *S. corymbiflorum* to treat skin inflammatory diseases. Taking into consideration the different uses of this species related to skin anti-inflammatory effects, there is a hypothesis that this activity can be confirmed using an inflammation model induced by topical application of croton oil (da Cunha et al., 2001).

The mechanism inflammatory is attributed, in part, to release of reactive species from activated cells, such as neutrophils and macrophage. Thus, free radicals are mediators that provoke or sustain inflammatory processes and consequently, their neutralization by radical scavengers can attenuate the inflammatory process (Conforti et al., 2008). In this sense, vegetal extracts with antioxidant effects have been established as a therapeutic approach for treating inflammation (Nijveldt et al., 2001). Moreover, phenolic compounds exhibit a wide range of biological effects including antibacterial, anti-inflammatory, antiallergic, hepatoprotective and anticarcinogenic actions. Many of these biological functions have been attributed to their free radical scavenging capacity (Krishnaiah et al., 2011).

The aim of this study was evaluate the anti-inflammatory *in vivo* activity of the extract of *S. corymbiflorum* leaves and relate their anti-inflammatory activity with antioxidant compounds present in this extract.

2. Material and methods

2.1. Chemicals

All chemicals were of analytical grade. Solvent for the extractions, folin–ciocalteau reagent, iron sulfate, hematoxylin–eosin and paraffin were purchased from Merck (Darmstadt, Germany). Croton oil, hexadecyltrimethylammonium bromide (HTAB), tetramethylbenzidine (TMB), dexamethasone, gallic, ascorbic, rosmarinic, chlorogenic and caffeic acids, quercetin, rutin, DPPH,

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Tris–HCl, thiobarbituric acid and DCFH-DA were acquired from Sigma Chemical Co. (St. Louis, MO, USA). Isoflurane (Baxter, São Paulo, Brazil), sodium acetate, acetone, absolute ethanol, acetic acid, formaldehyde (all from Vetec, Rio de Janeiro, Brazil) were used.

2.2. Plant collection and extractions

S. corymbiflorum leaves (300 g of an individual) were collected in Gaurama (Rio Grande do Sul state, Brazil) in October (2012). A dried voucher specimen is preserved in the herbarium of the Department of Biology at Federal University of Santa Maria by register number SMBD 13159. The leaves were dried at room temperature and powdered in a knife mill. The powder of leaves was macerated at room temperature with 70% ethanol for a week with daily shake-up. After filtration, the extract was evaporated under reduced pressure to remove the ethanol, the aqueous extract was dried in a stove (temperature above 40 °C) to produce the extract.

2.3. Anti-inflammatory activity

2.3.1. Animals

Male Swiss mice (25–30 g) were kept in a temperature-controlled room (22 ± 2 °C) under a 12 h light-dark cycle. Animals were acclimatized to the laboratory for at least 1 h before the experiments and were used only once. All of the experiments were carried out between 8:00 a.m. and 5:00 p.m. The data reported in this study were carried out in accordance with current ethical guidelines for the investigation of experimental pain in conscious animals (Zimmermann, 1983) and were approved by the Ethics Committee of the Federal University of Santa Maria (process number 5786050215/2015). The number of animals and the amount of irritant agent were the minimum necessary to demonstrate the consistent effects of the drug treatments.

2.3.2. Treatments

The extract of the *S. corymbiflorum* leaves, the irritant croton oil and dexamethasone were applied topically to the right ear of each mouse. Dexamethasone was used as a positive control.

2.3.3. Ear edema measurements

Skin dermatitis was induced by topical administration of croton oil and the inflammatory response was evaluated through of edema formation. The edema was quantified by the increase in ear thickness of mice upon inflammatory challenge. Ear thickness was measured before and after induction of the inflammatory response, using a digital micrometer (Digimess) in animals anesthetized with isoflurane (Silva et al., 2011). The micrometer was applied near the tip of the ear just distal to the cartilaginous ridges. The thickness was expressed in μm. To minimize variation, a single investigator performed the measurements throughout each experiment. The acetone (20 uL/ear) was used as vehicle group. The irritant agent (1 mg/ear), dexamethasone (0.1 mg/ear) and *S. corymbiflorum* extract (0.00001–1 mg/ear) were dissolved e applied topically in a constant volume of 20 μL of acetone to the right ear of each animal.

2.3.4. Croton oil-induced ear edema

Acute inflammation model was induced by a single topical application of croton oil at a concentration of 1 mg/ear in the right ear of the mice according to the method describe previously, with some modifications (da Cunha et al., 2001). The *S. corymbiflorum* extract (0.00001–1 mg/ear) or dexamethasone (0.1 mg/ear), used as a positive control, was applied topically immediately before of the croton oil treatment. Ear thickness was measured prior to and 6 h after the induction of inflammation. Six hours after the application of croton oil, the animals were sacrificed and ear samples (circles of tissue 6 mm in diameter) were collected for further analysis.

2.3.5. Myeloperoxidase activity (MPO) assay

MPO is an enzyme found in cells of myeloid origin and has been used as a biochemical marker of polymorphonuclear cells (mainly neutrophil) infiltration to the tissue. MPO activity was determined using an assay described previously (Oliveira et al., 2014), with some modifications. After 6 h of application croton oil, was assessed the MPO enzyme activity in the ear samples. Tissue samples were homogenized with a motor-driven homogenizer in 300 μl of acetate buffer (8 mM, pH 5.4) containing HTAB. The results were expressed as optical density (OD)/mL of the sample.

2.3.6. Histology

Separate groups of mice were used to verify the histological changes in mouse ear 6 h after croton oil administration or croton oil plus treatments. Mice were euthanized and the right ear was removed and fixed in an alfac solution (16:2:1 mixture of ethanol 80%, formaldehyde 40% and acetic acid). Each sample was embedded in paraffin wax, sectioned at 5 μm and stained with hematoxylin–eosin. A representative area was selected for qualitative light microscopic analysis of the inflammatory cellular response with a 20x and 40x objectives (Oliveira et al., 2014). To minimize a source of bias, the investigator did not know the group that they were analyzing.

2.4. Phytochemical analysis

2.4.1. Total polyphenols content

The amount of polyphenol was evaluated by method described by Chandra and Mejia (2004), using the Folin–Ciocalteau reagent. The extract samples were prepared at a concentration of 0.15 mg/mL. Absorbance was measured at 730 nm, in triplicate. Gallic acid was used in the calibration curve. The results were displayed in mg of gallic acid equivalents per g of extract (mg GAE/g).

2.4.2. Total flavonoids content

The flavonoids content was determined by the reaction with aluminum chloride using the method described by Woisky and Salatino (1998). Briefly, $AlCl_3$ solution was added to a aliquot of the sample and after 15 min the absorbance was verified at 420 nm. The data were calculated based on the calibration curve of rutin and expressed in mg of rutin equivalents per g of extract (mg RE/g).

2.4.3. Determination of total alkaloids

Total alkaloids were quantified by reaction of precipitation with Dragendorff's reagent by Sreevidya and Mehrotra (2003). The absorbance was measured at 435 nm and carried out in triplicate. For the results was used a calibration curve of bismuth nitrate which were expressed in mg of total alkaloids per g of extract (mg/g).

2.4.4. HPLC analysis polyphenols

HPLC analysis was performed on a Shimadzu HPLC system (Kyoto, Japan), Prominence Auto-Sampler (SIL-20A), equipped with Shimadzu LC-20 AT reciprocating pumps connected to a DGU 20A5 degasser, CBM 20A integrator, UV–VIS detector DAD SPD-M20A and LC Solution 1.22 SP1 software. Reversed phase chromatographic analyses were carried out under gradient conditions using a C-18 column (250 mm × 4.6 mm) packed with 5 μm diameter particles. The mobile phase comprising of solvent 1 (water containing 2% acetic acid) and Solvent 2 (methanol), according to method of Piana et al. (2013) with modifications.

The flow rate used was 0.6 mL/min and 40 μL of injection