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Original Article

Hyptis pectinata gel prevents alveolar bone resorption in experimental periodontitis in rats

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ABSTRACT

Hyptis pectinata (L.) Poit., Lamiaceae, is an aromatic, abundant and broadly used plant species in Sergipe to treat oral and gastrointestinal pain and inflammation. The aim of the present study was to analyze the relation between periodontitis and changes in the corporal mass and alveolar bone structure after induction of experimental periodontal disease in rat treated or not treated with *H. pectinata* gel at 5% (GS5%) and 10% (GS10%), comparing their effects with doxycycline gel at 10% (D10%, positive control), vehicle gel (negative control) and a group with experimental periodontal disease, but non-treated. The gels were locally applied in the gingival region immediately after the experimental periodontal disease induction by ligature (3×/day, 11 days). Bone destruction was determined through clinical exam, histopathological analysis and cone beam computed tomography of the experimental animals ($n = 36$). After 11 days of periodontitis induction, all groups that received ligature presented a decrease in the corporal mass, except to the naïve group (without experimental periodontal disease) ($p < 0.05$). Computed tomography results have shown healthy bone structure in the group I and bone resorption for the test groups. Histopathological analysis confirmed the healthy bone structure for naïve group animals, while the test groups exhibited bone loss in several degrees. In particular, the non-treated group animals had an intense inflammatory process. When the periodontium of the animals treated with GS10% was histopathologically analyzed, insertion periodontium was preserved. The results for these groups were significantly different of the vehicle group ($p < 0.05$). According to the results, the gel based in the aqueous extract of *H. pectinata* at 10% can prevent bone loss in experimental periodontal disease similarly to doxycycline 10%.

Introduction

Pathological cases of gingivitis and periodontitis, the oral illnesses more prevalent in Brazil are target of special attention in needy communities, especially in the Brazilian Northeast, where the individuals with these pathologies normally have evolved cases, which are accompanied with the deterioration of the dental unit as well as their bone support (Botelho et al., 2007). They are frequently linked to oral bacteria, which are associated to many systemic diseases such as pneumonia and cardiovascular diseases. Therefore, it has been emphasized the need to oral care in the systemic control of health (Takarada et al., 2004).

Periodontitis, an important cause of teeth loss in adults, is a chronic inflammatory disease characterized by located bone resorption (Chambrone and Chambrone, 2006; Botelho et al., 2010). Recent researches have indicated the local utilization of doxycycline gels as an isolated treatment, which prevent tooth scaling and root planning and are effective in combating the periodontal disease (Tinoco and Tinoco, 2000). In addition, it has been suggested that the association of natural products with preventive care can reduce the high incidence of diseases that affect the dental element as the periodontal disease (Juiz et al., 2010; Siqueira et al., 2010; Guimarães et al., 2013). Recently, our group has developing study about the pharmacological use of natural products, as

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medicinal plants, to treat orofacial disroders (Quintans-Júnior et al., 2010; Bonjardim et al., 2011; Siqueira-Lima et al., 2014; Quintans et al., 2014).

Hyptis species are aromatic plants that present pharmacologically active substances with antimicrobial, antifungal, anti-HIV, analgesic and antiinflammatory activities, and cytotoxicity and insecticide properties (Bispo et al., 2001; Santos et al., 2008; Oliveira et al., 2011; Franco et al., 2011; Paixão et al., 2013). *H. pectinata* (L.) Poit., Lamiaceae, an aromatic perennial bush species with low foliar density and erect branches, is widely spread in the states of Sergipe and Alagoas. The plant can be easily found around cities to be used for treating oral, gastric disorders and fever (Martinez, 1989; Paixão et al., 2013). Its aqueous extract has previously shown antinociceptive and antiedematogenic effects by reducing symptoms up to 54% at 400 mg/kg (Bispo et al., 2001). Since the plant leaf crude aqueous extract showed orofacial antinociception (Paixão et al., 2013), it was found necessary to further investigate its action against other orofacial diseases such as periodontitis. Therefore, the aim of the present study was to analyze the relation between periodontitis and changes in the corporal mass and alveolar bone structure after induction of experimental periodontal disease (EPD) in rat treated or not treated with *H. pectinata* gels compared with the standard drug doxycycline.

Materials and methods

Plant material

Hyptis pectinata (L.) Poit., Lamiaceae, leaves were collected during the florescence period in the metropolitan area of Aracaju, Sergipe, Brazil (255 m, 10°55′56.1″ South, 37°06′34.7″ West). The plant material was identified and a voucher specimen was deposited in the herbarium of the Department of Biology in the Federal University of Sergipe under the number ASE-19005.

Aqueous extract preparation

Leaves were dried in an oven at 37 °C with air renewal and circulation for 48 h until complete dehydration. Afterwards, they were triturated to give a fine powder (2 kg), which was extracted with distilled water (3:10, w/v) under constant agitation for 4 h at 35 °C. After filtration, the supernatant was lyophilized to give the dry crude aqueous extract (CAE, 32 g). CAE was distributed in seven pots and kept in a desiccator in the Laboratory of Natural Product Pre-clinical Pharmacology of the Federal University of Sergipe to be used in the subsequent experiments, when they were redissolved in water to give specific concentrations.

Gel preparation

CAE was maintained under dark to avoid the possible light degradation of some of the compounds present in it. For the gel base production, 0.2% Nipagim® (Batch GBG0020625, Pharma Special), 0.05% EDTA disodium (Batch 090707#2, DEG) and purified water were measured and heated together in a water-bath up to 70 °C. When the temperature was reached, the mixture was removed from the water-bath and slowly added to hydroxyethyl cellulose, which was stirred until complete homogenization and solubilization of the polymer. When this previous mixture reached 40 °C, Germal® previously solubilized in water was added to complete homogenization until the gel formation. Afterwards, triethanolamine was added for pH adjustment to the range 5.0–6.0 at environment temperature. *H. pectinata* extract was mixed with propilenoglicol (Batche 10030930C, Pharma Nostra) and incorporated in the gel at 5% or 10% considering gel mass.

The gels were stored in white polyethylene containers kept hermetically sealed at 8 °C until use. Stability studies to evaluate gel consistency on a 2-month period were performed with the formulations being kept in different conditions (4 °C, 37 °C and environment temperature) and the gel formulation viscosity was measured in regular intervals. The study indicated that gel viscosity, color and consistency did not change significantly during the period analyzed in the specified circumstances.

Animals

A previous training with the research team on anesthesia procedures, adaptation and handling of the animals and gels, and convenient placement of the bondages was done before the beginning of the experiment. The animals were positioned on the surgery table to allow the rat oral opening maintenance, facilitating the access to the maxilla posterior region of their teeth. Previous to the experiment, 36 male Wistar rats (100–200 g) were housed in temperature controlled rooms and received water and food ad libitum. All experiments were conducted in accordance with local guidelines on the welfare of experimental animals and with the approval of the Committee on Ethics in Animal Research of the Federal University of Sergipe (#47/09).

The experimental groups were divided in naïve (animals non-treated and not subjected to EPD), non-treated group (NT, animals subjected to EPD, but without treatment), vehicle-treated group (V, animals subjected to EPD and treated with vehicle gel), GS5% group (animals subjected to EPD and treated with local *H. pectinata* gel at 5%, w/w), GS10% group (animals subjected to EPD and treated with local *H. pectinata* gel at 10%, w/w) and D10% group (animals subjected to EPD and treated with local doxycycline gel at 10%, w/w). Doxycycline was utilized as a reference drug. The local treatment with the gel was first performed immediately after the surgical procedure and then three times/day until the animal sacrifices on the 11th day.

The animals in the test groups with periodontal diseases ($n = 6$, each group) received a ligature, while the animals in the control group (naïve, $n = 6$) were not induced for periodontitis, although they were submitted to capture, hold, daily weighing and sacrifice similarly to the test groups.

Induction of experimental periodontal disease (EPD)

The EPD protocol used in present study was performed similarly to Botelho et al. (2007, 2010). A sterilized nylon (3.0) thread ligature was placed around the cervix of the second upper-left molar of rats anesthetized with 10% ketamina (0.08 ml/100 g, *i.p.*) and xilazyne chlorhydrate (0.04 ml/100 g). The ligature was knotted on the buccal side of the tooth, resulting in subgingival position palatinally and in supragingival position orally. The contralateral right side was used as the unligated control. Animals were weighted daily.

Histopathological analysis

After sacrifice under anesthesia, animals had their maxillae excised. The specimens were fixed in 10% neutral buffered formalin and demineralized in 7% nitric acid. These specimens were then dehydrated, embedded in paraffin and sectioned along the molars in a mesio-distal plane for hematoxylin and eosin staining. Sections of 6 μm thickness, which included the roots of the first and second molars, were used. The areas between the first and second molars, where the ligature was placed, were analyzed under light microscopy using a 0–3 grade score, considering the inflammatory cell influx, and alveolar bone and cementum integrity, as described previously (Botelho et al., 2007): score 0, absence or only a discrete cellular infiltration (inflammatory cell infiltration is sparse