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Original Article

Anti-inflammatory, and antinociceptive effects of *Campomanesia adamantium* microencapsulated pulp

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ABSTRACT

Guavira fruits have antimicrobial, antioxidant, antinociceptive, and anti-inflammatory activities. Spray drying has been widely used in the food industry presenting good retention in bioactive compounds used to transform the pulp/fruit juice into powder form. Therefore, the present study has evaluated the anti-inflammatory and antinociceptive activities of the microencapsulated pulp of *Campomanesia adamantium* (Cambess.) O.Berg, Myrtaceae, by spray drying. Different groups of mice were treated with the doses of 100 and 300 mg/kg of microencapsulated "guavira" pulp and inflammatory parameters were assessed in a carrageenan paw edema-model and leukocyte migration with pleurisy model, while the antinociceptive activity was assessed using the formalin method and CFA-induced hyperalgesia model. A significant reduction in leukocyte migration and in paw edema was observed in rodents in all time after carrageenan injection for both doses of microencapsulated pulp of *C. adamantium* when compared with control group. Microencapsulated pulp of *C. adamantium* also reduced licking time at the first (nociceptive) and second (inflammatory) phases in the formalin model. In CFA-induced cold and mechanical hyperalgesia, depressive behavior, and knee edema, all parameters analyzed were significantly inhibited by microencapsulated pulp of *C. adamantium*. Microencapsulation by spray drying proved to be a technique that promotes bioavailability and the preservation of bioactive components in guavira pulp.

Introduction

Among the Brazilian Cerrado, there is a native plant species known as *Campomanesia adamantium* (Cambess.) O.Berg, Myrtaceae, (guavira) (Lorenzi, 2000) that is widely found in isolated fields in Midwestern and southeastern Brazil. The fruits grow on small bushes and have specific characteristics, such as bright colors from green to yellow, with a strong and citric aroma (Fernandes et al., 2014). Its fruit has the potential to be used *in natura* in the food industry and as flavoring in the drink industry due to its juiciness, mineral content, fiber, and interesting bioactive substances from the nutritional and functional points of view as phenolic compounds. In addition to their pleasant taste, the fruits are considered a source of vitamin C (Breda et al., 2012), which is an important micronutrient involved in several biological functions in the human body (Pascoal et al., 2014).

In folk medicine "guavira" fruits are used as antirheumatic, antidiarrheal, hypocholesterolemic, anti-inflammatory (Ramos et al., 2007), and to the treatment of cystitis and urethritis (Pascoal et al., 2014). Previous studies with the fruits have observed antimicrobial (Pavan et al., 2009; Cardoso et al., 2010), antioxidant (Coutinho et al., 2010), antinociceptive, and anti-inflammatory activities (Ferreira et al., 2013), as well as apoptotic and antiproliferative activities in PC-3 human prostate carcinoma cells (Fernandes et al., 2014). Phytochemical investigations have found the presence of flavanones and chalcones in the ethyl acetate extract from its fruits (Pavan et al., 2009) and phenolic contents in the ethyl acetate, ethanol, and hexane extracts from the leaves, particularly flavonoids (Coutinho et al., 2010). Ferreira et al. (2013) has demonstrated the presence of myricitrin, quercetin, and myricetin in ethyl acetate in the extract from the leaves of *C. adamantium*. The hydro-alcoholic extract of *C. adamantium* fruit

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peels has anti-inflammatory, antihyperalgesic, and antidepressant activities in rodents (Souza et al., 2014).

The fruits of *C. adamantium* are restricted to the period of harvesting (Coutinho et al., 2008). Conservation alternatives to improve the availability of pulp, such as spray drying, has been widely used in the food industry (Tonon et al., 2009, 2013) presenting good retention in bioactive compounds used to transform the pulp/fruit juice into powder form (Chen et al., 2014), allowing prolonged storage and greater stability of the product, giving it a longer shelf life (Souza et al., 2014; Mahdavi et al., 2014). Together with this idea, recent technological advances, such as the microencapsulation technique, have helped to solve these issues and renewed interest in natural products in drug discovery (Lescano et al., 2014).

Phenolic compounds, particularly flavonoids, have free-radical scavenging properties and also inhibit lipid peroxidation. The presence of flavonoids and chalcones in the human diet can reduce the risk of cancers and tumors and also exhibit several activities, such as antibacterial, antifungal, anti-inflammatory, antileishmanial, antimalarial, and anti-HIV protease (Hodek et al., 2012; Tewtrakul et al., 2003; Djeridane et al., 2006; Cabrera et al., 2007; Nowakowska, 2007).

Therefore, the study of microcapsuled fruits of *C. adamantium* enables scientific knowledge of the pharmacological properties of the plant, considering that there may be loss of bioactive compounds and the microencapsulation technique promotes its preservation. Thus, this study aims to evaluate the anti-inflammatory parameters and antinociceptive effects of the microencapsulated pulp of *C. adamantium* (MPCA) in rodents.

Materials and methods

Plant material

Fruits of *C. adamantium* (Cambess.) O.Berg, Myrtaceae, were collected at "Cerrado," Brazil, on November 2014. A voucher specimen was deposited in the herbarium of the Faculty of Biological Sciences of UFGD (DDMS 4602). Fruits were sanitized and the pulp, peel, and seeds were separated. The pulp was packed in rigid polypropylene containers and stored at −18 °C until use.

High-performance liquid chromatography (HPLC)

The solvents employed were methanol (HPLC grade, Tedia Company, Fairfield, OH, USA) and acetonitrile (HPLC grade, Tedia Company, Fairfield, OH, USA). Samples of the pulp of *C. adamantium* were prepared with 1 g of pulp extract dissolved in 5 ml of methanol in an ultrasound for 20 min. Samples of MPCA were prepared with 5 g of microcapsules that were extracted with 25 ml of methanol in an ultrasound for 20 min and the dried material into a chapel was reconstructed in 5 ml of methanol.

The samples and standards were analyzed using an analytical HPLC (Varian 210) system, with a ternary solvent delivery system and an autosampler. A photodiode array detector was monitored at $\lambda = 200–800$ nm. The HPLC column was C-18 (25 cm × 4.6 mm; particle size, 5 μm; Luna, Phenomenex, Torrance, CA, USA), with a small pre-column (2.5 cm × 3 mm) containing the same packing to protect the analytical column. The flow rate and injected volume were 1.0 ml min^{-1} and 20 μl, respectively. All chromatographic analyses were performed at 22 °C.

The elution was conducted using in 0 min, acetonitrile 12%, water 50%, and methanol 38%; in 40 min, acetonitrile 10%, water 10%, and methanol 80%; and in 45 min, returning to the initial condition.

The substances used in HPLC analysis were isolated from the leaves of *C. adamantium*. Compounds were purified by HPLC, resulting in purity between 86% and 96%. The substances were dissolved separately in methanol to a concentration of 10 μg/ml degree chromatographic preparation of stock solutions used in the analysis by HPLC. The standards were easily identified by their UV absorption spectra and retention times. The substances found in the extracts were unambiguously identified by performing coinjection experiments in which aliquots of samples and standards were mixed and diluted to a known volume and analyzed by HPLC.

Microcapsulation of Campomanesia adamantium *fruits*

After preliminary tests and through other studies described in the literature, microcapsules were produced using maltodextrin 8% (DE 20 Maltogill, Cargill, Uberlândia, Brazil), gum Arabic 8% (Synth, Brazil), and chitosan 8% (Purifarma, São Paulo, Brazil) purchased from JKLAB (Química Diagnóstica e Segurança Ltda) (Oliveira et al., 2014). Samples were prepared using 24% encapsulating agent, 16% distilled water, and 60% pulp. The mixture of each formulation was homogenized in an Ultra-Turrax at a speed of 18,000 rpm until complete dissolution of the carrier agent, obtaining samples comprising 30% solids (encapsulating agent and pulp). The atomization process was conducted in a concurrent flow pattern using a mini spray dryer – LM (model MSD 1.0 LABMAQ). Samples were fed into the atomizer at a flow rate of 0.5 l h^{-1} with a 1.2 mm nozzle diameter, air flow of 35 l min^{-1}, and a drying air temperature of 180 °C. The determination of moisture content and ascorbic acid (AOAC, 2000) were performed on fresh and microencapsulated pulp of guavira.

Experimental animals

Male and female Swiss mice (20–25 g) were obtained from Universidade Federal da Grande Dourados (UFGD) biotherium. The animals were kept in collective cages under controlled temperature (23 ± 1 °C) and light conditions (12-h light/dark cycle), and had access to food and water *ad libitum*. The 23/2014 protocol was approved by the Ethics Committee on Animal Use (CEUA/UFGD).

Pleurisy

Different groups of female Swiss mice ($n = 5$ animals/group) were orally treated with MPCA at doses of 100 and 300 mg/kg or vehicle (0.9% saline solution, also called the control group). The positive control group received dexamethasone subcutaneously at a dose of 1 mg/kg. Pleurisy was induced in experimental groups by intrapleural injection of 100 μl of 1% carrageenan diluted in saline, after 1 h of treatment, as previously described (Velo et al., 1973). The naive group received 100 μl of sterile saline by intrapleural injection. After 4 h, animals were euthanized and the pleural cavity was washed with 1 ml phosphate-buffered saline. An aliquot of 20 μl of lavage (exudate) was collected from the pleural cavity, and diluted with Turck solution (1:20) and used for total leukocyte count in a Neubauer chamber (Kassuya et al., 2009).

Formalin-induced nociception

Sixty min before formalin injection, male Swiss mice ($n = 6$ animals/group) were divided into groups: dexamethasone (1 mg/kg, subcutaneous route), MPCA (100 and 300 mg/kg, oral route), and vehicle (saline solution, 0.9%, oral route). After respective treatment, 20 μl of saline containing 2.5% of formalin was injected in the right hind paw. Nociceptive response (paw licking) in seconds was evaluated from 0 to 5 min (phase 1 – neurogenic pain) and from 15 to 30 min (phase 2 – inflammatory response) after injection of

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