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Research paper

Antidiarrheal and intestinal antiinflammatory activities of a methanolic extract of *Qualea parviflora* Mart. in experimental models

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ABSTRACT

Ethnopharmacological relevance: An ethnopharmacological survey indicated that the bark from *Qualea parviflora* Mart. (Vochysiaceae) could be used to treat gastrointestinal disorders, such as diarrhea and intestinal inflammation. The objective of this study was to evaluate the effects of a methanolic extract from the bark of *Qualea parviflora* (QP) in an experimental model of diarrhea and intestinal inflammation induced in rodents.

Material and methods: The antidiarrheal and antispasmodic effects of QP were investigated by measuring intestinal motility, diarrhea, and intestinal fluid accumulation in rodents after challenging with a cathartic agent. In addition, the effects of QP on the contractility of the isolated mice-ileum preparation were determined. Acute intestinal inflammation was induced in male Wistar rats by the rectal administration of trinitrobenzenesulfonic acid (TNBS) in 50% ethanol (0.25 mL). QP was administered orally (for 5 days) prior to the induction of inflammation. The colonic injury and extent of inflammation were assessed by macroscopic damage scores and lesion length. The enhanced colonic mucosal injury, inflammatory response, and oxidative stress were evaluated by myeloperoxidase (MPO) activity; the tumor necrosis factor alpha (TNF-α), interleukin 1β (IL1-β), and malondialdehyde (MDA) levels; and the glutathione (GSH) content.

Results: Oral treatment with QP (500 mg/kg) delayed the onset of diarrhea, reduced the amount of liquid stool, and decreased the severity of the diarrhea and the evacuation index in rodents challenged with castor oil ($p < 0.01$). Additionally, QP (150–500 μg/mL) demonstrated effective antispasmodic activity against carbachol-induced contractions of mouse ileum *in vitro*. Oral treatment (25 and 50 mg/kg/day) with QP significantly reduced the intestinal inflammation induced by TNBS in rats (52% and 45%, respectively). Improvement of colonic mucosal injury by treatment with QP was demonstrated by a decrease in MDA levels and an increase in GSH content in colonic tissue. QP also prevented intestinal inflammation as evidenced by reduced cytokine levels (TNF-α and IL1-β) and low MPO activity.

Conclusions: The ethnopharmacological usefulness of the bark from *Qualea parviflora* against diarrhea containing blood and mucus was supported by the observed antidiarrheal, antispasmodic, and intestinal antiinflammatory properties of this medicinal plant.

1. Introduction

Qualea parviflora Mart. (Vochysiaceae) is a common tree found in different habitats in the Brazilian savanna. An infusion of the bark from this medicinal plant is used in traditional medicine to treat diarrhea containing blood and mucus (Silva et al., 2000). This species is also used as an antiulcerogenic, antidiarrheal, antiinflammatory, antiseptic, and astringent treatment by local communities in regions where the plant naturally thrives (Rodrigues and Carvalho, 2001). The ethnopharmacological properties of *Qualea parviflora* have been evaluated in pre-clinical studies, which have demonstrated the beneficial effects of this plant in treating some gastrointestinal diseases. An integrative study demonstrated that the methanolic extract of the bark of *Qualea*

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parviflora is an effective antiulcerogenic and antimicrobial treatment that has no detectable acute toxic effects (Mazzolin et al., 2010). The gastroprotective effect of *Qualea parviflora* is directly related to the maintenance of sulfhydryl compound (SH) and glutathione (GSH) levels in the gastric mucosa rather than the prevention of the damaging effects induced by either ethanol or indomethacin. The antioxidant effect of *Qualea parviflora via* the action of the natural antioxidants SH and GSH was also demonstrated by an *in vitro* lipid peroxidation assay, which showed that the IC_{50} values for the *Qualea parviflora* extract were as low as those for quercetin (Mazzolin et al., 2010). Based on these results, complementary studies are needed to determine whether the beneficial effects of this antioxidant plant species are applicable to the treatment of other gastrointestinal disorders, such as diarrhea and intestinal inflammation, in which oxidative stress is an important etiological factor.

Antioxidants have the ability to counteract the harmful effects of oxidative agents and can therefore either treat or prevent oxidative stress-related diseases. Several studies have shown the benefits of using natural antioxidant compounds to treat gastric ulcers and diarrhea (Chatterjee et al., 2011; Luis-Ferreira et al., 2012; Rodrigues et al., 2012; Santos et al., 2012) and to prevent and/or treat inflammatory bowel disease (IBD) (Faria et al., 2012; Hartmann et al., 2012; Récio et al., 2012; Witaicenis et al., 2012; Algieri et al., 2013).

Diarrhea is defined as a decrease in stool consistency and an increase in the volume or frequency of defecation for a period of days or weeks. This ailment may be divided into several categories; however, not all types of diarrhea are easily categorized because some categories overlap. Although relatively common in the population, patients presenting with diarrhea can become a diagnostic challenge (Juckett and Trivedi, 2011). Diarrhea is a prevalent symptom of IBD and affects most patients diagnosed with IBD. Diarrhea may represent the first perceived manifestation of the intestinal inflammation that causes these patients to seek medical attention, and this symptom persists throughout the course of the disease (Wenzl, 2012). These episodes may result in bloody inflammatory diarrhea, abdominal pain, nausea, loss of appetite, rectal bleeding, perianal fistulae, weight loss, fever, and anemia (Juckett and Trivedi, 2011).

IBD is comprised of two main types of chronic disorders, ulcerative colitis and Crohn's disease, and is categorized as a gastrointestinal disease with unclear etiology. IBD is thought to involve several pathological factors, including immunological abnormalities, oxidative stress, gut microflora, an abnormal epithelial barrier, and inflammatory factors (Baumgart and Carding, 2007). However, some authors claim that oxidative stress may be one of the most important components of the pathophysiology of IBD. Reactive oxygen species (ROS) and nitric oxide (NO), both of which are generated as a consequence of the stimulation of immune cells, play an important role in IBD by regulating intestinal inflammation and increasing the susceptibility to injury in the absence of normal oxidative defense mechanisms (Dryden et al., 2005). It has been proposed that the antiinflammatory effects of corticoids and aminosalicylates are partially due to their ability to ameliorate oxidative stress (Cronstein et al., 1992; Miyachi et al., 1987). Salicylazosulfapyridine (SASP), 5-aminosalicylic acid (5-ASA), glucocorticoid, antiTNF-monoclonal antibodies, and immunosuppressants have been used to treat IBD for many years. However, these drugs produce several side effects, including allergic reactions, liver damage, and kidney damage (Baumgart and Sandborn, 2007). Most conventional treatments for the management of IBD have serious adverse effects that reduce patient compliance, which has led researchers to study complementary and alternative medicines that can promote the remission of disease activity with improved safety and tolerability. A variety of plants that have been historically used in traditional medicine for the management of IBD have been investigated, and different mechanisms have been proposed to explain the effectiveness of medicinal plants in the treatment of colitis, including antiinflammatory, antimicrobial, antioxidant, antiulcer, wound healing, and antidiarrheal properties (Rahimi et al., 2009; Récio et al., 2012). Although *Qualea parviflora* has been used to treat diarrhea with blood and mucus (one of the most apparent symptoms of IBD), no reports have described the therapetic effects of this plant against this disease. In the present study, we investigated the use of a methanolic extract from the bark of *Qualea parviflora* as an antidiarrheal and antispasmodic treatment and its acute intestinal antiinflammatory effect in rodents.

2. Materials and methods

2.1. Preparation of the methanolic extract

Qualea parviflora bark was collected by Dr. Hiruma-Lima from the Ypê Garden (savanna region) in Porto Nacional (Tocantins State – TO), Brazil. A voucher specimen was identified by Dr. S.F. Lolis from UNITINS in Porto Nacional and deposited under No. 9226 at the UNITINS Herbarium. The air-dried powdered bark (500 g) was successively extracted three times with methanol (48 h, 4 L) at room temperature. The solvent was evaporated at 60 °C under reduced pressure to yield 10.7 g (2.14% yield) of the *Qualea parviflora* methanolic extract (QP). Phytochemical profiles indicate that this extract contains several ellagic acid derivatives, triterpenes, and saponins (Nasser et al., 2006, 2008).

2.2. Animals

Male Swiss mice (40–50 g) and Wistar rats (150–250 g) were used for the antidiarrheal and antiinflammatory experiments, respectively. The animals were obtained from the Central Animal House (UNESP) in Botucatu, S.P. and housed in the Physiology Department under controlled temperature (23 ± 2 °C) and a 12 h light/dark cycle. They were provided a certified Labina (Purina, Brazil) diet and tap water *ad libitum*. Before each experiment, the animals were deprived of food for either 6 or 16 h as described in each experimental model. Standard drugs and the QP extract were administered orally using a saline solution (SAL) (10 mL/kg) as the vehicle. The protocols used were approved by the UNESP Institutional Animal Care and Use Committee and followed the recommendations of the Canadian Council on Animal Care (Olfert et al., 1993) (Protocol 42/04 – CEEA).

2.3. Antidiarrheal activity

2.3.1. Castor oil-induced diarrhea

Groups of male mice ($n=7$–8) were fasted for 16 h prior to receiving an oral dose of vehicle (SAL, 10 mL/kg), QP (12.5, 25, 50, 125, 250, and 500 mg/kg), or loperamide (10 mg/kg) 30 min before the oral administration of castor oil (0.2 mL/animal) (Awounters et al., 1978). Immediately after castor oil administration, each animal was placed in an individual cage lined with blotting paper and observed for 4 h. The following parameters were observed: onset of diarrhea, number of solid, semi-solid, and liquid feces, and total frequency of fecal outputs. A numerical score based on stool consistency was assigned: 1 (solid stool), 2 (semi-solid stool), and 3 (liquid stool). Each group received an evacuation index (EI) expressed by the following formula: $EI = 1 \times (\text{no. stool } 1) + 2 \times (\text{no. stool } 2) + 3 \times (\text{no. stool } 3)$ (Mukherjee et al., 1998).

2.3.2. Castor oil-induced intestinal fluid accumulation

The enteropooling assay described by Robert et al. (1976) was used to measure fluid accumulation, with some modifications.