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Original Article

Solanum paniculatum root extract reduces diarrhea in rats

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ABSTRACT

Solanum paniculatum L., Solanaceae, locally known as "jurubeba", is widely used in Brazil for culinary purposes, and in folk medicine to treat of diverse disorder including gastric dysfunctions. In this study we investigated the antidiarrheal activity of *S. paniculatum* roots extract in rats at different concentrations (125, 250 and 500 mg/kg, *p.o*) using different experimental models such as castor oil-induced diarrhea, enteropooling and gastrointestinal motility, determined by *in vivo* experimental models. The major compound of root extract was characterized as chlorogenic acid based in the IR, 1D and 2D NMR analysis. All the extract doses achieved antidiarrheal potency, as indicated by reduced weight of feces in castor oil-induced diarrhea, decreased intestinal motility and significantly inhibited castor oil-induced enteropooling compared to the vehicle group. The highest dose (500 mg/kg) produced greater antimotility effect and better reduction of enteropooling, similar to the reference drug Loperamide (5 mg/kg). Extract from *S. paniculatum* L. roots had antidiarrheal activity, as shown by the lower weight of the feces as well as decrease in the accumulation of intestinal fluid and slower transit, justifying the traditional use of plant for diarrhea.

Introduction

The family Solanaceae A.L. Jussieu comprises more than 3000 species distributed in 106 genera, and their species have a cosmopolitan distribution. The most economically important genus of the family is *Solanum*, with approximately 1500 species distributed all over the world (Nurit et al., 2007). Among the species of *Solanum* genus, *Solanum paniculatum* L. is to be more that highlights for its many medicinal uses, wide distribution and especially for being recognized as herbal medicine by the Brazilian Pharmacopeia, whose roots and stems are indicated in the treatment of anemia and liver and digestive disorders. The species *S. paniculatum* L., known popularly either as jurubeba or jurupeba, is used commonly in Brazilian folk medicine for the treatment of diverse disorders, such as liver and gastric dysfunctions and hangover as well as for culinary purposes (Mesia-Vela et al., 2002; Botion et al., 2005; Agra et al., 2007; Sabir and Rocha, 2008; Vieira et al., 2013). The plant is a component of a variety of pharmaceutical formulations including: syrups, infusions and decoctions, ethanol extracts, and elixirs (Júnior et al., 2015). Ethnopharmacological studies have revealed that leaves, roots, stems and fruits of *S. paniculatum* are used to treat disorders of the liver and kidney, anemia, tuberculosis, malaria and hypertension, and in anti-inflammatory, antipyretic and stimulant formulations (Joachimovits, 1954; Ribeiro et al., 1986; Agra et al., 2007). Chemical studies revealed a variety of compound groups in *S. paniculatum* tissues, such as steroids, terpenes, alkaloids (including jurubebina, jubebine and solanine), and saponins (including sojuripidine, isojurubidine, isopaniculidine and jurubidine) (Vieira et al., 2010; Ramos and Ramos, 2012).

Diarrhea continues to be a major health problem throughout the world, especially causing of malnutrition in children under five years old. It is also a major cause of the high morbidity and mortality, particularly among children. In developing countries, the principal causes of diarrhea is associated with the enterotoxins that are produced and secreted from bacterial organisms like *Vibrio cholerae*, *Salmonella*, *Shigella* and *Escherichia coli* (Walker et al., 2013).

Considering the pharmacological potential of *S. paniculatum* and the use of plants for the treatment of diarrhea, this study investigated the activity of *S. paniculatum* extract against diarrhea induced by various methods in rats.

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Materials and methods

Plant materials, extraction and isolation

Solanum paniculatum L., Solanaceae, specimens were collected in the city of Camaragibe, Pernambuco state, northeastern Brazil, in January 2012. Botanical identification was done by Instituto Agronômico de Pernambuco (IPA) and a voucher specimen was deposited at the Dárdano de Andrade Lima Herbarium of IPA (88503). The roots were dried at 40 °C by 72 h and the dried material was milled to a fine powder in a MACSALAB mill.

Dried roots (46 g) were firstly submitted Soxhlet extraction with 200 ml of dichloromethane for 2 h and followed by extraction using 300 ml of ethanol for a period of 4 h. The solvent was filtered and concentrated *in vacuo* to yield 4.4 g (9.6%) of fatty-free crude extracts with presence of precipitate. Part of extract was washed twice with chloroform, filtrated and dried to yield a white solid identified as chlorogenic acid.

Instrumentation and chromatography materials

IR spectra were measured in KBr pellets with a Varian infrared spectrometer. 1D and 2D NMR spectra were recorded using Bruker spectrometer NMR (300 MHz and 500 MHz). HPLC analyses of extracts were performed in a Shimadzu LC10 instrument using a Phenomenex RP-18 column (250 mm × 4.7 mm, 5 μm), eluted in gradient mode starting with 5% aqueous formic acid/methanol (8:2) for 15 min, raising to 30% methanol in 20 min, with detection at 325 nm and flow rate of 1 ml/min. Four mg of the crude extract was suspended in 2 ml of methanol and applied to C18 cartridges. The cartridges were eluted with 4 ml of MeOH and the eluents were filtered (0.45 μm filter) prior to analysis. The standard of chlorogenic acid was acquired of the Sigma–Aldrich (Batch: SLBB6914V).

Experimental animals

Male albino rats weighing between 200 and 250 g were used for the experiments. The animals were obtained from the animal house of the Antibiotics Department of Federal University of Pernambuco, which is registered with the Brazilian College of Animal Experimentation under no. 18. All experiments were authorized by the Ethical Committee for Animal Care (protocol number: 23076.46150/2012-23 CCB-UFPE). The animals were kept in polypropylene boxes at a temperature of 22 ± 3 °C with a light-dark cycle of 12 h and received balanced feed and water *ad libitum*. The animals were deprived of food before each experiment.

Castor oil induced diarrhea

The method described by Awouters et al. (1978) was followed. Healthy albino rats of either sex (200–250 g) were randomly selected and divided into five groups of five animals each. They were deprived of food for 12 h prior to the test, with free access to water. Group 1 received the vehicle alone (1% Tween 80), while those in group 2 received Loperamide (5 mg/kg) as positive control. Animals in groups 3, 4 and 5 received *S. paniculatum* root extracts (125, 250, 500 mg/kg) respectively. Extract administration was by the oral route. The animals were housed singly in cages lined with pre-weighed filter paper. One hour after pretreatment with the extract, the animals were then given 1 ml of castor oil orally and the time between oil administration and appearance of first diarrheal drop was noted. Thereafter, they were observed for 4 h for the presence of diarrhea and total weight of feces excreted was obtained.

Gastrointestinal motility test

Male albino rats (200–250 g) were randomly divided into five groups of five rats each. They were deprived of food for 12 h before the test, but were allowed free access to water. Group 1 rats (control) were treated with the vehicle (1% Tween 80). Group 2 rats received Loperamide (5 mg/kg), while groups 3, 4 and 5 received different doses of the *S. paniculatum* root extracts (125, 250 and 500 mg/kg), respectively. Thirty minutes after drug administration, 1 ml of charcoal meal (5%) was administered orally to all animals and 30 min later, all the rats were sacrificed and the abdomen was opened. The small intestine was dissected out from the pylorus to the cecum and the total distance traveled by the charcoal plug along the small intestine was estimated for both the control and the treated groups. The percentage distance traveled by the charcoal meal from the pylorus to the cecum was noted.

Castor oil-induced enteropooling

In this method, as described by Robert et al. (1976), rats were deprived of food for 12 h before the experiment. The rats were divided into six groups of five each. The vehicle (1% Tween 80) was given to the first group. The second group received Loperamide (5 mg/kg), while groups 3, 4 and 5 received graded doses of SPRE (125, 250 and 500 mg/kg). Thirty minutes later, all the rats were treated with 1 ml of castor oil. A sham group did not receive any treatment. After 30 min, each rat was sacrificed and the whole length of the intestine from the pylorus to the cecum was dissected and the contents weighed.

Statistical analysis

Results were expressed as mean ± SEM. The significance of difference between means was determined using one-way analysis of variance and the statistical significance was set at $p < 0.05$. All data were analyzed using GraphPad Prism version 5.0 (GraphPad Software Inc., San Diego, CA, USA).

Results

Chemical profile

The analysis by HPLC root extract of *S. paniculatum* showed a major peak with retention time at 8 min (Fig. 1). The ethanolic crude extract of the *S. paniculatum* roots was submitted to purification steps, resulting in the isolation of major compound as a yellow amorphous powder determined as chlorogenic acid. Structural elucidation of chlorogenic acid was based on interpretation of spectral data, mainly IR, UV ^{1}H and ^{13}C NMR and 2D-NMR, including comparison with values described in the literature (Agbo et al., 2014).

Castor oil-induced diarrhea

The highest total weight of feces was obtained in the control group (11.17 ± 0.70 g), whereas the lowest weight of feces was recorded for animals that received Loperamide (3.81 ± 0.13 g) (Table 1). All the extract doses achieved antidiarrheal potency by means of reduced weight of feces compared to the control group.

Gastrointestinal motility test

In the control group, charcoal meal traversed most of the small intestine (73.88 ± 1.34%). In the extract test groups (125, 250 and 500 mg/kg), significant dose-dependent decreases in the transit of charcoal meal through the small intestine were observed (64.62 ± 1.87, 61.92 ± 1.80 and 55.86 ± 3.40), respectively (Table 2).