Toxicological, Antidiarrheal and Spasmolytic Activities of Solanum paniculatum

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Abstract

Solanum paniculatum is popularly known as “jurubeba-verdadeira”. In folk medicine, its roots, stems, and leaves are used as tonics, anti-inflammatory, carminatives, diuretics, and for gastrointestinal disorders. This species is listed in the Brazilian Pharmacopoeia and belongs to the “Relação Nacional de Plantas Medicinais de Interesse ao SUS”. Based on folk medicine data of the Solanum genus, we decided to investigate whether the crude ethanol extract from S. paniculatum aerial parts presents toxicological, antidiarrheal, and spasmolytic activities. The crude ethanol extract from S. paniculatum aerial parts did not produce in vitro or in vivo toxicity and showed dose-dependent antidiarrheal activity, inhibiting equi-potently both the defecation frequency (ED50 = 340.3 ± 35.1 mg/kg) and liquid stool formation (ED50 = 370.1 ± 19.4 mg/kg) in mice. Conversely, the crude ethanol extract from S. paniculatum aerial parts did not inhibit normal intestinal transit, even though it has shown a dose-dependent reduction of both the castor oil-induced intestinal transit (Emax = 36.9 ± 1.3%, ED50 = 242.0 ± 8.6 mg/kg) and intestinal fluid content (Emax = 74.8 ± 2.4%, ED50 = 328.9 ± 15.9 mg/kg). Additionally, the crude ethanol extract from S. paniculatum aerial parts was approximately 2-fold more potent in antagonizing the phasic contractions induced with histamine (IC50 = 63.7 ± 3.5 µg/mL) than carbobach 10−6 M (IC50 = 129.3 ± 14.1 µg/mL). Therefore, we concluded that the crude ethanol extract from S. paniculatum aerial parts presents antidiarrheal activity in mice related to the inhibition of small intestinal motility and secretion as well as nonselective spasmolytic activity on the guinea pig ileum.

Introduction

Diarrhea is one of the most common diseases and is characterized primarily by an increased number of evacuations (3 or more in 24 h), a decrease in the stool consistency, and the presence of blood and/or mucus that, in some cases, leads to electrolytic disorder [1]. Approximately 2 million children worldwide die due to diarrhea every year, making diarrhea the third most common cause of death in children younger than 5 years in poor countries [2]. An alternative strategy used against diarrhea is the use of natural products in special medicinal plants. Included in the Brazilian biodiversity, the Solanum genus is the most representative of the Solanaceae family with 260 species [3] and is characterized by the production of a variety of glycoalkaloids [4] and a high occurrence of flavonoids, alkalamides, and glycosides [5]. Some species of Solanum are used in folk medicine for diarrhea treatment, such as S. dasphyllum Schumach & Thonn [6], Solanum marginatum L.f. [7], Solanum tuberosum L. [8], and Solanum sisymbriifolium Lam. [9]. Solanum paludosum Moric. [10] and Solanum asterophorum Mart. [11] have shown antidiarrheal activity in mice. Additionally, there are evidences that some species exhibit spasmolytic activity on the guinea pig ileum, such as Solanum megalonyx Sendtnr, S. asterophorum [12, 13], Solanum jahrense Agra & Nee [14], and Solanum agrarium Sendtnr [15]. Solanum paniculatum L. is a shrubby species popularly known as “jurubeba-verdadeira” [16]. Its roots, stems, and leaves are used as tonics, anti-inflammatory, carminative, diuretics, and digestive in folk medicine [17–19]. Additionally, a decoction of the leaves is used to treat intestinal parasites and gastrointestinal disorders [20, 21]. Furthermore, this species is listed in the Brazilian Pharmacopoeia for digestive disorders [22] and...
Results and Discussion

In this study, we investigated, for the first time, the toxicological, antidiarrheal, and spasmyloytic activities of SP-EtOHAP. In vitro and in vivo protocols were employed. This study was subsidized because diarrhea is a serious public health problem and constitutes a major morbidity and mortality cause in children younger than 5 years, particularly in children under one year [25, 26].

*S. paniculatum* has a relative potential to advance in productive stages, allowing for the development of products of interest to SUS (Sistema Único de Saúde, Brazil’s public health-care system), since it is largely used in folk medicine to treat gastrointestinal disorders, and *Solanum* species have been used for the diarrhea treatment and show antidiarrheal activity in mice.

The compounds quantified of SP-EtOHAP were chlorogenic acid, sitosterol, and stigmasterol. Chlorogenic acid (0.92%) was quantified by high-performance liquid chromatographic-diode array detection (HPLC-DAD) and was the most abundant phenolic compound that can be considered a chemical marker for this species because it was also isolated from the roots. The quantification of sitosterol and stigmasterol steroids was possible using HPLC-evaporative light scattering detection (HPLC-ELSD). The concentration of stigmastanol (1.40%) was twice as high as sitosterol (0.71%). Interestingly, these two compounds are always isolated together from plant species; however, in this study, it was possible to analyze and quantify the two separate substances using a C-18 column coupled to HPLC-ELSD. The values of LOD were 28.5, 0.13, and 0.14 µg and the values for LOQ were 86.3, 0.39, and 0.41 µg for chlorogenic acid, sitosterol, and stigmastanol, respectively.

Because some *Solanum* species have cytotoxic effects, such as *Solanum asperum* Rich. and *S. asterophorum*, and present moderate hemolytic activity in rat erythrocytes [27,28], we initially decided to observe the cytotoxic potential and possible acute toxicity of SP-EtOHAP using in vitro and in vivo models, respectively.

In the cytotoxicity assay, SP-EtOHAP ([125, 250, 500, and 750 µg/mL, n = 3]) did not induce rat erythrocytes lysis (Fig. 1), producing no damage to rat erythrocyte membranes in the range of concentrations used. Erythrocytes provide a simple model to study the toxic or protective effect of a variety of substances [29] because they are a cell type highly susceptible to lysis [30]. Therefore, our results suggest that possibly other stronger cell types do not suffer toxic effects from the extract. Furthermore, in the in vivo assay, the extract did not induce physiological changes based on the parameters of hyperactivity, aggression, tremors, and convulsions, among others. Both male and female mice (n = 6) did not show these behavioral changes after SP-EtOHAP administration (2500 or 5000 mg/kg p.o. and 1000 or 2000 mg/kg i.p.) along the observation period of 4 h. These results indicate that SP-EtOHAP did not promote toxic signs or changes in the level of the central nervous system. Additionally, there were no animal deaths during the observation period of 14 days after SP-EtOHAP administration, making it impossible to determine the LD50.

Thus, in the absence of any sign of toxicity, we continued our antidiarrheal and spasmyloytic investigations.

Diarrhea is a result of changes in the intestinal electrolyte and water transport, resulting in a decrease of absorption combined with an increase in secretion. As a result of these changes, there is an increase in the amount, frequency, and volume of stools as well as a change in the consistency, increasing the water content [31]. To verify a possible antidiarrheal effect of the extract, we used castor oil as a diarrheal agent because it is widely used to screen drugs with this property [32].

SP-EtOHAP (125, 250, 500, and 750 mg/kg, p.o., n = 6) inhibited castor oil-induced diarrhea (Fig. 2) equipotently and in a dose-dependent manner in terms of the frequency of defecation (ED50 = 340.3 ± 35.1 mg/kg) and number of liquid stools (ED50 = 370.1 ± 19.4 mg/kg). In order to obtain the maximum antidiarrheal effect, we used the dose of 750 mg/kg since the dose of 500 mg/kg presented an effect of approximately 50%. In acute toxicity experiments, we found that 5000 mg/kg (p.o.) did not induce toxicity in mice, giving us a margin of safety for its use in the next experiment.

These results suggest that SP-EtOHAP has active substances in its composition with antidiarrheal activity that might inhibit the intestinal transit or increase water and electrolyte absorption in the gastrointestinal tract, both of which are altered in diarrhea [33]. Given these premises, the antidiarrheal effect of SP-EtOHAP was investigated, measuring the intestinal transit and fluid accumulation in mice.

Interestingly, SP-EtOHAP (125, 250, and 500 mg/kg, p.o., n = 6) did not inhibit the normal intestinal transit, unlike atropine, which decreased intestinal transit from 84.9 ± 0.9% (control) to 63.6 ± 0.3%. However, the dose of 500 mg/kg increased the distance traveled by the marker to 95.1 ± 1.7% (Fig. 3A), suggesting that SP-EtOHAP may have a laxative effect under normal physiological conditions, resulting in a possible side effect of the extract-based pharmaceutical preparations. In contrast, SP-EtOHAP (62.5, 125, and 250 mg/kg, p.o., n = 6) inhibited the castor oil-induced intestinal propulsion in a dose-dependent manner.
Atropine inhibited (38.0 ± 1.9%) the distance traveled by the marker compared with the negative control (Fig. 3B). Thus, we demonstrated that the antidiarrheal effect of the extract involves alterations in intestinal motility under pathological conditions.

Diarrhea is a consequence of the disorders of intestinal function in which there are excessive intestinal secretion, impaired intestinal absorption, and/or fast traffic [34]. Therefore, we decided to investigate whether the extract inhibits castor oil-induced intestinal fluid accumulation. SP-EtOHAP (125, 250, and 500 mg/kg, p.o., n = 6) inhibited, in a dose-dependent manner, the liquid content (ED50 = 328.9 ± 15.9 mg/kg), demonstrating that the extract presents an antidiarrheal effect also due to changes in intestinal secretion, however, less potently than the intestinal motility under pathological conditions as we verified (Fig. 4). This effect is desirable because it decreases intestinal secretion, thereby increasing water absorption and combating dehydration.

An intestinal smooth muscle model is important for investigating the action mechanisms of drugs that may be used in pathophysiological processes, such as intestinal cramps and diarrhea. Because several plants of the Solanaceae family have antispasmodic activity, we decided to investigate whether SP-EtOHAP has any effect on intestinal smooth muscle to, at least partially, confirm the observed antidiarrheal activity.

To evaluate the spasmolytic effect, phasic contractions were induced with carbachol (CCh) or histamine 10^{-6} M on the guinea pig ileum. SP-EtOHAP (9–729 µg/mL, n = 5) antagonized the phasic contractions induced by these contractile agents in a concentration-dependent manner (IC50 = 129.3 ± 14.1 and 63.7 ± 3.5 µg/mL, respectively), and was approximately 2-fold more potent in antagonizing the histamine-induced phasic contractions (E_{max} = 36.9 ± 1.3%, ED50 = 242.0 ± 8.6 mg/kg).
80.7 ± 3.1 and 94.0 ± 0.8%, respectively; Fig. 5). Thus, this effect appears to be due to a mechanism at the level of the histaminergic receptor, however, we do not discard that other downstream mechanisms may be involved.

These data suggest that SP-EtOHAP does not present a cytotoxic effect and systemic toxicity in mice. Additionally, the extract presents antidiarrheal activity in mice due to the inhibition of small intestinal motility and secretion as well as nonselective spasmolytic activity on the guinea pig ileum. Thus, this study provides scientific support to the medicinal use of *S. paniculatum* in gastrointestinal disorders, such as diarrhea, and proves the efficacy of the plant in such conditions. Therefore, this species may be used further as an herbal medicine for the relief of these disorders.

Material and Methods

Plant material

The roots and aerial parts of *S. paniculatum* were collected in Brazil, Pernambuco, municipality of Recife, in 2011 and identified by Maria de Fátima Agra (PhD) from PPGPNSB/CCS/UFPB. Voucher specimens (51691) are deposited at the Herbarium Vasconcelos Sobrinho, UFPE. The powdered roots (640.0 g) and aerial parts (2.3 kg) of *S. paniculatum* were extracted with EtOH. The EtOH extract was concentrated under vacuum in a rotaevaporator. This work used chlorogenic acid, stigmasterol, and sitosterol compounds as phytochemical markers for analysis and quality control because these substances have been isolated from *S. paniculatum* roots. Part of the EtOH extract (40.0 g) from *S. paniculatum* roots was dissolved in MeOH:H₂O (1:1) and extracted with hexane and EtOAc. These solvents were evaporated, providing the hexane (3.9 g), EtOAc (5.8 g), and MeOH:H₂O (22.6 g) fractions. Sitosterol and stigmasterol were isolated in a mixture from the hexane fraction (20 mg), and chlorogenic acid was isolated from the EtOAc and MeOH:H₂O (90 mg) fractions. For quantification, the compounds sitosterol and stigmasterol were purchased from Sigma-Aldrich.

High-performance liquid chromatography analysis

Analytical separation was performed in an HPLC-DAD system consisting of two Shimadzu LC-20AT pumps, a degasser DGU-20A5, an auto-injector SIL-20AC, an oven CTO-20 A, a photodiode array detector SPD-M20 A with a CBM-20 A interface, and a Phenomenex Luna® column C-18 (150 × 4.6 mm; 5 µm, 100 Å) protected by a holder. HPLC data acquisition was performed by LC solution software. The optimized analytical separations of chlorogenic acid were carried out using a mobile phase that consisted of 1% acetic acid in water (solvent A) and methanol (solvent B) with the following gradient: 0–30 min: 5 to 30% of B; 30–40 min: 30 to 100% of B; and 45 min: 100% of B. A flow rate of 1.0 mL/min at 40°C and an injection volume of 20 µL were employed. The UV spectra were recorded at 320 nm. The samples were filtered through a 0.45-µm nylon membrane (Whatman). Five solutions of different concentrations of chlorogenic acid (25 µg to 800 µg/mL) were injected in triplicate, and both the regression equation and the linearity factor were determined. For sterol (stigmasterol and sitosterol) analysis, the HPLC system consisted of 1% acetic acid in water (solvent A) and methanol (solvent B) with the following gradient: 0–30 min: 5 to 30% of B; 30–40 min: 30 to 100% of B; and 45 min: 100% of B. A flow rate of 1.0 mL/min, a 40°C column chamber, a drift tube temperature of 70°C, and nitrogen at 2 mL/min. The calibration was made at concentrations ranging from 15.6 to 125 µg/mL in triplicate. The LOD was calculated based on a signal-to-noise ratio (S/N) of three, while the LOQ was determined at an S/N of ten. The noise level was measured during the analysis of one of the samples and involved a portion of the chromatogram that was separated from the region containing the chlorogenic acid, stigmasterol, and sitosterol peaks.

Animals

For the experimental protocols, we used Wistar rats (*Rattus norvegicus*) weighing 250–300 g, Swiss mice (*Mus musculus*) weighing 25–35 g, and guinea pigs (*Cavia porcellus*) weighing 350–500 g of both sexes. The mice were from Central Bioterium of the Universidade Federal de Alagoas (UFAL) and the rats and guinea pigs were from the “Professor Thomas George” Bioterium of CBiotec/UFPB. Previously, the animals were maintained in a 12-h light-dark cycle under a controlled temperature (21 ± 1°C) with free access to food and water. All of the experimental procedures were approved by the Ethics Committee in Research (CEP) of UFAL, certificate no. 010489/2009–15, and the Ethics Committee on Animal Use (CEUA) of CBiotec/UFPB, certificate no. 0905/13.

Fig. 5 Effect of SP-EtOHAP on phasic contractions induced by carbachol 10⁻⁶ M (A) and histamine 10⁻⁶ M (B) on the guinea pig ileum. Columns and vertical bars represent the mean and S.E.M., respectively (n = 5). One-way ANOVA followed by Bonferroni’s post-test, **p < 0.001 (control vs. SP-EtOHAP).
Chemicals
Magnesium sulfate heptahydrate (MgSO₄), potassium chloride (KCl), chloride hydrate (CaCl₂), glucose, sodium bicarbonate (NaHCO₃), sodium chloride (NaCl), and sodium dihydrogen phosphate (NaH₂PO₄) were purchased from Vetec Química Fina Ltda. Atropine (99%), carbachol chloride (CCh), Cremophor®, and histamine were obtained from Sigma-Aldrich. Carboxymethylcellulose was obtained from Formula. Castor oil was obtained from Farmax. Loperamide (99%) was obtained from Janssen Cilag Farmacêutica Ltda. and activated charcoal was obtained from Proquímios. All substances were diluted in distilled water, and the extract was solubilized in Cremophor® (3%), whose concentration never exceeded 0.01% (v/v). At this concentration, this chemical is devoid of contractile or relaxant effects in the studied organ, according to previously obtained data (data not shown). Chlorogenic acid (99%) was isolated from the roots of S. paniculatum. Stigmasterol (99%) and sitosterol (95%) were obtained from Sigma-Aldrich.

Evaluation of the hemolytic effect of the crude ethanol extract from Solanum paniculatum aerial parts in rat erythrocytes
After 12 h of fasting, a blood sample of the rats was collected, mixed with 0.9% NaCl, and 10 mM CaCl₂, and centrifuged at 5000 rpm for 3 min to obtain the erythrocytes. Triton X-100 1% (100 µL, positive control) or SP-EtOHAP (different concentrations) was added to the erythrocyte suspension. The negative control was an erythrocyte suspension plus 0.9% NaCl and 10 mM CaCl₂. Hemolysis was quantitated by spectrophotometry at 540 nm and expressed as a percentage [35].

Evaluation of acute toxicity in mice
After 12 h of fasting, male and female mice (n = 12) were treated with 0.9% NaCl plus Cremophor® (10 mL/kg, negative control) or SP-EtOHAP at 2500 and 5000 mg/kg (p.o.) or 1000 and 2000 mg/kg (i.p.). The general signs and symptoms of toxicity, such as analgésia, contortions, sedation, and others, were recorded within 4 h. The animals were also evaluated over 14 days to monitor lethality. Based on this test, doses for the pharmacological studies were determined [36].

Effect of the crude ethanol extract from Solanum paniculatum aerial parts on castor oil-induced diarrhea in mice
Male and female mice were divided into groups (n = 6, each) and were treated orally with NaCl 0.9% plus Cremophor® (10 mL/kg, negative control), loperamide (10 mg/kg, positive control), or SP-EtOHAP (different doses). After 30 min of treatment, castor oil was administered orally (0.01 mL/g) to each animal to induce diarrhea. The animals were separated and placed in individual boxes lined with white paper. The animals were then inspected for 4 h to count the number of stools and their consistency, classifying the stools into solid or liquid, after which the total number of stools and the number of liquid episodes were determined [37]. The inhibitory effect of SP-EtOHAP was evaluated based on the ED₅₀ value, the dose of a drug that produces 50% of its maximal effect.

Effect of the crude ethanol extract from Solanum paniculatum aerial parts on intestinal transit in mice
Male and female mice were divided into three groups (n = 6, each) and after 12 h of fasting they were treated orally with 0.9% NaCl plus Cremophor® (10 mL/kg, negative control), atropine (2 mg/kg, positive control), or SP-EtOHAP (different doses). After 30 min, 5% activated charcoal solubilized in carboxymethylcellulose (0.5%) (0.01 mL/g) was administered. The animals were euthanized by cervical dislocation 30 min after the administration of activated charcoal (marker), the abdominal cavity was opened, and the small intestine was removed. The total length of the small intestine and the distance traveled by the activated charcoal were measured [38]. The same procedures described in the previous methods were performed, except that castor oil (0.01 mL/g) was administered orally 30 min before the activated charcoal [39,40]. The results were expressed as a percentage of the distance traveled by the marker in relation to the total length of the small intestine. The inhibitory effect that was exerted by SP-EtOHAP was evaluated based on an ED₅₀ analysis.

Effect of the crude ethanol extract from Solanum paniculatum aerial parts on castor oil-induced intestinal fluid accumulation in mice
Male and female mice were divided into groups (n = 6, each) and after 24 h of fasting, they were treated orally with 0.9% NaCl plus Cremophor® (10 mL/kg, negative control), atropine (10 mg/kg, positive control), or SP-EtOHAP (different doses). The castor oil (2 mL per animal) was then administered orally. After 30 min of castor oil administration, the mice were euthanized, the small intestine was dissected from the pylorus to the cecum, and the contents were expelled into a graduated cylinder to measure the volume of the fluid [41].

Effect of the crude ethanol extract from Solanum paniculatum aerial parts on carbachol- and histamine-induced phasic contractions on the guinea pig ileum
Guinea pigs were fasted for 18 h and euthanized by cervical dislocation followed by the sectioning of the cervical vessels and the removal of the ileum. Segments of approximately 2 to 3 cm in length were suspended in a 5-mL organ bath and stabilized for 30 min in modified Krebs solution (mM): NaCl (117.0), KCl (4.7), MgSO₄ (1.3), NaH₂PO₄ (1.2), CaCl₂ (2.5), NaHCO₃ (25.0), and glucose (11.0) [42] at 37°C and bubbled with a carbogen mixture (95% O₂ and 5% CO₂) in a resting tension of 1 g. After stabilization, two similar phasic contractions were induced by carbachol (CCh) or histamine 10⁻⁶ M (control). SP-EtOHAP was then added to the organ baths at different concentrations in distinct experiments and a third contraction was obtained and compared with the control. The inhibitory effect of SP-EtOHAP was evaluated based on the IC₅₀ (concentration value of a drug that produces 50% of its maximum inhibitory response) and maximum effect (E₅₀) values, as assessed through the concentration-response curves in both the absence (control) and presence of the extract [43].

Statistical analysis
The results are expressed as a percentage of the mean ± S.E.M., and were statistically analyzed by one-way ANOVA followed by Bonferroni’s post-test for multiple comparisons. The null hypothesis was discarded when p < 0.05. The ED₅₀, IC₅₀, and EC₅₀ values were calculated by nonlinear regression. The data were analyzed using GraphPad Prism® software version 5.01 (GraphPad Software, Inc.).
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Conflict of Interest

The authors state that they have no conflict of interest.

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