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Anti-diarrheal activity of (−)-Epicatechin from *Chiranthodendron pentadactylon* Larreat: Experimental and computational studies

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ABSTRACT

Ethnopharmacological relevance: *Chiranthodendron pentadactylon* Larreat is frequently used in Mexican traditional medicine as well as in Guatemalan for several medicinal purposes, including their use in the control of diarrhea.

Aim of the study: This work was undertaken to obtain additional information that support the traditional use of *Chiranthodendron pentadactylon* Larreat, on pharmacological basis using the major antisecretory isolated compound from computational, in vitro and in vivo experiments.

Materials and methods: (−)-Epicatechin was isolated from ethyl acetate fraction of the plant crude extract. In vivo toxin (*Vibrio cholera* or *Escherichia coli*)-induced intestinal secretion in rat jejunal loops models and sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis on *Vibrio cholera* toxin were used in experimental studies while the molecular docking technique was used to conduct computational study.

Results: The antisecretory activity of epicatechin was tested against *Vibrio cholera* and *Escherichia coli* toxins at oral dose 10 mg/kg in the rat model. It exhibited the most potent activity on *Vibrio cholera* toxin (56.9% of inhibition). In the case of *Escherichia coli* toxin its effect was moderate (24.1% of inhibition). SDS–PAGE analysis revealed that both (−)-epicatechin and *Chiranthodendron pentadactylon* extract interacted with the *Vibrio cholera* toxin at concentration from 80 μg/mL and 300 μg/mL, respectively. Computational molecular docking showed that epicatechin interacted with four amino acid residues (Asn 103, Phe 31, Phe 223 and The 78) in the catalytic site of *Vibrio cholera* toxin, revealing its potential binding mode at molecular level.

Conclusion: The results derived from computational, in vitro and in vivo experiments on *Vibrio cholera* and *Escherichia coli* toxins confirm the potential of epicatechin as a new antisecretory compound and give additional scientific support to anecdotal use of *Chiranthodendron pentadactylon* Larreat in Mexican traditional medicine to treat gastrointestinal disorders such as diarrhea.

1. Introduction

Chiranthodendron pentadactylon Larreat (Sterculiaceae) is commonly known as "flor de manita" and "mano de león" in Mexico, while in Guatemala, it is locally called as "manita" (Linares et al., 1988; Cáceres, 1996; Chanfón, 2007). *Chiranthodendron pentadactylon* is a small to medium sized evergreen tree which grows along the wet mixed oak–pine and deciduous mountain forests from Guatemala and southern Mexico. In Mexican traditional medicine it is used for the treatment of several diseases such as heart illness, diarrhea, dysentery, epilepsy, cancer, ulcers, headache, eye's pain and inflammation, as well as an analgesic (Linares et al., 1988; Argueta et al., 1994).

Previous pharmacological investigations reported the antiprotozoal, vasorelaxant antibacterial, antipropulsive, and antisecretory activities of this plant (Perusquía et al., 1995; Alanís et al., 2005; Velázquez et al., 2006; Calzada et al., 2006, 2010). Mostly the

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phenolic compounds such as flavonoids are responsible for the antisecretory effect of "flor de manita". Among them, epicatechin exhibited the most potent antisecretory activity (Velázquez et al., 2009). Thus, the present work was undertaken to obtain additional information that support the traditional use of *Chiranthodendron pentadactylon* Larreat, on pharmacological basis using epicatechin from computational, in vitro and in vivo experiments.

2. Materials and methods

2.1. Plant material

The flowers from *Chiranthodendron pentadactylon* (Sterculiaceae) were collected by Dr. Fernando Calzada in October 2001 in Ozumba, State of Mexico, Mexico. The plant material was authenticated by MS Abigail Aguilar-Contreras of the Herbarium IMSSM of Instituto Mexicano del Seguro Social (IMSS) where the voucher specimen is conserved under reference number 14404.

2.2. Extraction from Chiranthodendron pentadactylon

The air-dried and finely powdered flowers (10.0 kg) were extracted by maceration at room temperature with MeOH (2×20 L). After filtration the extracts were combined and evaporated *in vacuo* to yield 1.2 kg of red residue.

2.3. Isolation of epicatechin from the MeOH extract of Chiranthodendron pentadactylon

Epicatechin was isolated from MeOH extract of *Chiranthodendron pentadactylon* according to the method of Velázquez et al. (2009). Briefly, the MeOH extract (200 g) was suspended in 10% MeOH–water (350 mL) and successively partitioned with CH_2Cl_2 and EtOAc (350 mL × 3, 6.0 g). The EtOAc fraction (6 g), was subjected to column chromatography (CC) on a Sephadex LH-20 (25 g, Farmacia) using $CHCl_3$ in EtOH (9.5:0.5; 8:2; 7:3; 6:4; 5:5; 3:7; 1:9; v/v), MeOH (10) and water (10) to give eight fractions (F-1 to F-8). Fraction F-5 (300 mg) was resolved by high pressure liquid chromatography (HPLC) with a Spherirorb S5ODS2 column (5% formic acid–acetonitrile, 80:20, v/v flow rate of 3.2 mL min^{-1}, λ 280 nm) allowing the isolation of (−)-epicatechin (40 mg). Epicatechin was identified by comparison of the spectroscopic data (^1H and ^{13}C NMR, UV, IR, and [α]), MP, TLC and HPLC with authentic sample available in our laboratory (Velázquez et al., 2009).

2.4. Animals

Male Sprague–Dawley rats (200–250 g) were obtained from the animal house of the IMSS. The experimental protocols were approved by the Animal Care and Use Committee of Pediatry Hospital from Centro Medico Nacional Siglo XXI, IMSS. Investigations using experimental animals were conducted in accordance with the official Mexican norm NOM 0062-ZOO-1999 titled Technical specifications for the production, care and use of laboratory animals (SAGARPA, 2001).

2.5. Antisecretory assay

The antisecretory activity of methanol extract and (−)-epicatechin was tested using a method previously described by Velázquez et al. (2006, 2009). The antisecretory effect was studied on intestinal secretion indirectly by measuring the fluid accumulation in the intestine following *Vibrio cholerae* (Sigma) or *Escherichia coli* (heat-stable enterotoxins STa, Sigma) toxin administration to rats. Two jejunal loops were prepared in the rats and inoculated with 3 μg/mL of toxin dissolved in 1× PBS with 1% bovine albumin. Rats ($n=11$ per group by duplicate) were treated orally with epicatechin (10 mg/kg in 1 mL of a 2% DMSO solution in water), or vehicle (2% DMSO solution in water). Loperamide (10 mg/kg) was used as an antisecretory drug. After 4 h, the animals were sacrificed and the intestinal loops were removed, weighted and measures. The antisecretory activity of the samples was measured as the fluid secretion in the loops and expressed in percent of inhibition.

2.6. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE)

Vibrio cholerae or *Escherichia coli* toxin (10 μg) dissolved in water was treated with epicatechin (0, 80 μg, 160 μg, 320 μg, and 640 μg) or methanol extract (300 μg). After this, 3.4 μL of 2-mercaptoethanol was added and the mixture was kept for 10 min at 40 °C. Then denaturated proteins were analyzed by SDS–PAGE according to the Laemmli method (Laemmli, 1970) and stained with Coomassie-blue.

2.7. Computational study

To examine the interaction between epicatechin and cholera toxin, docking simulations were done on the 3-D structure of toxin. Molecular docking simulations were performed using version 3.0.5 of the program AutoDock along with AutoDockTools using the hybrid Lamarckian Genetic Algorithm (LGA). This program was chosen because its algorithm allows full flexibility of small ligands. It has been shown that it successfully reproduces many crystal structure complexes and includes an empirical evaluation of the binding free energy. The preparation of toxin and epicatechin input structure and the definition of the binding sites were carried out under a GRID-based procedure. First, a rectangular grid box was constructed over toxin ($126 \times 126 \times 126$ Å^3). The setting up of the grids was performed with 60 points in each dimension, with a spacing of 0.375 Å between the grid points. In this study the X-ray structure of cholera toxin was taken from the protein Data Bank. Previously, the toxin structure was cleaned of its water molecules. All docking simulations were carried out with LGA and were realized with an initial population of 100 randomly placed individuals and a maximum number of energy evaluations (1×10^7). Resulting docking orientations within 1.0 Å in the root-mean square deviation tolerance of each other were clustered together and represented by the result with the most favorable free energy of binding.

2.8. Statistical analysis

The results are expressed as the mean ± S.E.M, Mann–Whitney *U*-test. Values with $p < 0.05$ were considered significant.

3. Results and discussion

Diarrhea is a common symptom of gastrointestinal infections caused by a wide range of pathogens, including bacteria, viruses and protozoa. Among these Rotavirus, *Escherichia coli*, *Shigella*, *Campylobacter*, *Salmonella*, *Vibrio cholerae*, and *Cryptosporidium* are responsible for most acute cases of diarrhea. In the case of *Escherichia* and *Vibrio* the acute watery diarrhea occurs when the balance between absorption and secretion in the small intestine is