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Antisecretory activity of plants used to treat gastrointestinal disorders in Mexico☆

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Abstract

Aqueous and methanolic extracts from 26 medicinal plants used in Mexico to treat gastrointestinal disorders were screened to evaluate their antisecretory activity on cholera toxin-induced intestinal secretion in rat jejunal loops model. Extracts were tested at a dose of 300 mg/kg. From 56 samples tested, both extracts from *Chiranthodendron pentadactylon*, *Hippocratea excelsa* and *Ocimum basilicum* were the most potent with inhibition values ranging from 68.0 to 87.6%. On the other hand, the methanolic extract of *Geranium mexicanum* (aerial parts) and the aqueous extract of *Bocconia frutescens* showed the highest activity with inhibition values of 93.4 and 86.0%, respectively. The results obtained in this study give some scientific support to the use of the Mexican medicinal plants employed for the treatment of gastrointestinal disorders such as diarrhea.

Keywords: Mexican medicinal plants; Aqueous extracts; Methanolic extracts; Antisecretory activity; Cholera toxin

1. Introduction

Diarrheal disease is one of the most common causes of morbidity and mortality in many developing countries (Guerrant, 1985; Amstrong and Cohen, 1999). It is often caused by enterotoxins which are produced by bacteria such as enterotoxigenic *Escherichia coli*, *Salmonella typhi*, *Salmonella typhimurium*, *Clostridium difficile*, *Clostridium freundii*, *Aeromonas hydrophila*, *Yersinia enterocolitica*, *Campylobacter jejuni* and *Vibrio cholerae*. Enterotoxins have their effect on the enterocyte functions by stimulating the secretion of transepithelial electrolytes, thus increasing the osmotic flux of water and ions to the intestinal lumen. Specifically, heat-labile (LT) and heat-stable (ST) enterotoxins from *Escherichia coli*, *Vibrio cholerae* and *Campylobacter jejuni* increase net fluid secretion by affecting the enzymes adenylyl cyclase or guanylyl cyclase in the intestinal mucose (Guerrant, 1985; Torres et al., 1993; Mutschler et al., 1995; Hör et al., 1995; Torregosa et al., 1996; Raufman, 1998; Amstrong and Cohen, 1999).

To control diarrhea, the treatment of choice is oral rehydration solution (ORS). It has reduced the levels of mortality in children and elderly by dehydration, but not morbidity for diarrhea (Amstrong and Cohen, 1999; Turvill et al., 2000). To treat the secretory diarrhea there are some drugs, such as racecadotril and loperamide, that decrease intestinal hyper-

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secretion. Both drugs have side effects; racecadotril cause brochospasm, fever and vomiting, and loperamide should not be administrated to children younger than 6 years of age and patients with constipation, and intestinal obstruction, and in acute dysentery caused by bacterial infections because it can mask the symptoms and cause intestinal perforation (Brown, 1979; Rogé et al., 1993; Salazar et al., 2000).

On the other hand, there are some compounds that showed inhibitory properties on the intestinal secretion, such berberine, chlorpromazine, clonidine, nicotinic acid, indometacin, somatostatin and ethycrynic acid, but they were not developed as antidiarrheal drugs (Rabbani et al., 1982; Sack and Froehlich, 1982; Fedorak and Field, 1987).

In Mexico, the use of medicinal plants to treat gastrointestinal disorders such as diarrhea and dysentery is widespread (Campos, 1991; Aguilar et al., 1994). However, most of these plant species have not been investigated from a pharmacological point of view to demonstrate their antisecretory properties, which could lead to support their use as antidiarrheal and antidysenteric drugs in traditional medicine.

In this work, we screened 56 aqueous and methanolic extracts from 26 Mexican medicinal plants to assess their antisecretory activity using the cholera toxin-induced intestinal secretion in rat jejunal loops model. It is important to notice that none of these species or their isolated metabolites have been previously evaluated as antisecretory agents.

2. Materials and methods

2.1. Plant materials

The assessed plants were collected by the authors (Calzada and Velázquez) from different regions in Mexico: Mexico City, States of Hidalgo, Mexico, Sinaloa, Guanajuato and Yucatan. They were selected according to their use in Mexican traditional medicine to treat gastrointestinal disorders. Voucher herbarium specimens (Table 1) have been authenticated by MS Abigail Aguilar, Herbarium IMSSM of Instituto Mexicano del Seguro Social. Plant species, part used, voucher specimens and the percentage of inhibition of the intestinal secretion are shown in Table 1.

2.2. Preparation of crude extracts

The air-dried plant material (20 g) was extracted by maceration with 300 mL of MeOH for 1 week. Then the macerate was filtered and concentrated under reduced pressure at 40 °C. For aqueous extracts, 20 g of air-dried plant material was extracted by decoction with 100 mL of distilled water for 30 min, the solution was filtered and lyophilized. The yields are shown in Table 1.

2.3. Cholera toxin

Lyophilized powder (1 mg) of cholera toxin (Sigma) containing approximately 220,000 units/mg of protein was suspended in 1 mL of sterile water. For the study, aliquots of the toxin solution were dissolved in a 1× PBS (NaCl 8 g, KCl 0.2 g, $Na_2HPO_4{\cdot}7H_2O$ 0.115 g, KH_2PO_4 0.2 g/L) solution with 1% bovine serum albumin (Sigma) to obtain a concentration of 3 μg/mL.

2.4. Antisecretory assay

The antisecretory activity of the extracts was tested using a method previously described (Torres et al., 1993). 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 Hospital de Pediatria del Centro Medico Nacional Siglo XXI, IMSS, in accordance with the guidelines for care and use of laboratory animals. The effect of the extracts was studied on intestinal secretion indirectly by measuring the fluid accumulation in the intestine following cholera toxin administration to rats. Two jejunal loops were prepared in the rats and inoculated with 3 μg/mL of cholera toxin dissolved in 1× PBS with 1% bovine albumin. Rats ($n = 4$ per group by duplicated) were treated orally with each extract (300 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 antidiarrheal drug, After 4 h, the animals were sacrificed using ethyl ether. The antisecretory activity of the extracts was measured as the fluid secretion in the loops and expressed in percentage of inhibition.

2.5. Statistical analysis

Values are expressed as mean ± S.E.M. Statistical significance was determined using Mann–Whitney *U*-test. Values with $p < 0.05$ were considered significant.

3. Results and discussion

We tested 56 aqueous and methanolic crude extracts obtained from medicinal plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders. These were tested at oral doses of 300 mg/kg because at this dose most of the extracts showed antisecretory activity. In traditional medicine since infusions or decoctions are usually taken three times per day when diarrhea occurs, our results can be related with their traditional use because the used dose is approximately one cup of plant tea which is recommended by Mexican people to treat gastrointestinal disorders (Aguilar et al., 1994).

The antisecretory activity was tested using the cholera toxin-induced intestinal secretion in rat jejunal loops model. The antisecretory activity of the extracts tested is shown in Table 1. We found that both extracts from *Chiranthodendron pentadactylon*, *Hippocratea excelsa* and *Ocimum basilicum* were the most active with inhibition values ranging from 68.0 to 87.6% at 300 mg/kg. Methanolic extract of *Geranium mex-*