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Original article

A mixture of chamomile and star anise has anti-motility and antidiarrheal activities in mice

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ABSTRACT

Diarrhea is a serious public health problem in Mexico and other countries. A widely used alternative in the treatment of diarrhea is the use of herbal medicines. Infusions of chamomile and star anise possess anti-inflammatory and antimotility properties that could help alleviate gastrointestinal disorders. The aim of this study was to determine the effect of the mixture of chamomile and star anise infusions on gastrointestinal activity in mice. A gastrointestinal assessment of the mixture of chamomile and star anise was carried out in mice, and the percentage of advance of administered activated carbon through the intestinal tract of the animals was measured. Furthermore, the diarrhea model was induced with castor oil. The infusions were prepared using a mix with a 50:50 ratio of the herbs, and were administered at Mix-10, 20, 40 and 80 (mg/kg) orally. The results indicate that Mix-40 and Mix-80 decreased the completion percentage of the activated carbon, delayed the appearance of diarrhea and decreased the number of evacuations in comparison with the control group. This suggests that the combination of chamomile and star anise can be used as an alternative antidiarrheal treatment.

Introduction

Diarrheal disease has long been recognized as a leading cause of morbidity and mortality (Snyder and Merson, 1982). Mainly affecting children, acute diarrhea causes an estimated 5 to 8 million deaths per year. The majority of cases of acute diarrhea occur in underdeveloped countries (Khan et al., 2004). Infectious intestinal diseases (which include diarrhea) were among the top 20 causes of death in Mexico (Programa Nacional de Salud 2007-2012), 2005 included.

Herbal medicines are an alternative widely used for the treatment of diarrhea. They constitute an indispensable component of the traditional medicine practiced worldwide due to accessibility, ancestral experience and economic viability. Despite the availability of a vast spectrum of pharmacological approaches for diarrheal management, the

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vast majority of people in developing countries rely on herbal drugs for its management (Afroz et al., 2006). The World Health Organization (WHO) has encouraged the study of the treatment and prevention of diarrheal diseases based on traditional medical practices (Atta and Mouneir, 2004).

One of these medicinal plants is chamomile (CH) (*Matricaria chamomilla* L.), which belongs to the Asteraceae family (Koehn and Carter, 2005). This plant is used in traditional medicine to treat wounds, ulcers, eczema and other ailments (Rombi, 1993; Awang-Dennis, 2006). CH has long been valued as a digestive relaxant and has been used to treat various gastrointestinal disturbances including indigestion, diarrhea and vomiting (Sakai and Misawa, 2005; Crotteau et al., 2006). Furthermore, CH has been used to treat colic, croup, and fevers in children (Peña et al., 2006). Several studies suggest that these protective effects are due to its anti-inflammatory, antioxidant and astringent properties (Weiss, 1998).

Star anise (SN) (*Illicium verum* Hook. f.) is another well-known herbal medicine used in many cultures primarily to treat infantile colic (Ize-Ludlow et al., 2004; Rojas et al., 2005), because its active ingredients include anethole and terpene hydrocarbons (phellandrene, limonene, dipentene) (Ramos-Montes de Oca et al., 2008), which are responsible for its antispasmodic action (Hall et al., 2002). Recently, it has been reported that the combination of plant infusions with antispasmodic properties could elicit more effective results in the treatment of gastrointestinal diseases (Srijana et al., 2010). However, there are no reports on the therapeutic use of the mixed infusions of CH and SN for the treatment of gastrointestinal disorders.

Therefore, the present study aims to evaluate the gastrointestinal activity of the mixture of CH and SN using an *in vivo* mice model.

Material and methods

Plant material and aqueous preparation

The chamomile and star anise were grown on campus grounds of the Benemérita Universidad Autónoma de Puebla (BUAP), Mexico. Intact flowers were dried at 40°C and samples were identified by Dr. Roberto Calva Rodriguez, and a voucher sample was deposited in the botanical garden at BUAP (file16-13). To prepare the aqueous extracts the leaves were grounded, and infused in phosphate-buffered water. Preparation was done just before use and the extracts were administered by gavage.

Animals

Adult albino male mice CD1 (20-25 g) were obtained from Bioterio Claude Bernard of BUAP. All the procedures described in this study are in accordance with The Mexican Council, in accordance with the Guide for the Care and Use of Laboratory Animals and by the ethics committee of the BUAP (VIEP-3447-2013). Animals were housed individually in a temperature and humidity-controlled environment and were kept under controlled-light conditions (12h:12h light-dark cycle) with food and water *ad libitum*. Prior to the experiments, all animals were kept fasting for 24h with free access to water.

Testing gastrointestinal motility

Ten groups were formed (n = 10 per group): negative control (isotonic saline (SSI)), positive control (loperamide, 5 mg/kg), CH (40 and 80 mg/kg), AS (40 and 80 mg/kg) and four mixtures of CH - AS groups (Mix-10, Mix-20, Mix-40 and Mix-80). The mixtures groups were treated with 50:50 mixture aqueous preparations of CH and AS at 10, 20, 40 and 80 mg/kg. Oral administration was used in all treatments. After 30 min, all animals were administered a suspension of gum arabic (5%) and activated carbon (10%). Thirty minutes later, the animals were sacrificed by cervical dislocation and the intestines were carefully removed from the abdominal cavity. The length of the intestine from pylorus to cecum and the distance traveled by the activated carbon were measured. The rate of advance of the label in the intestine of the mice of each group was calculated (Williamson et al, 1996; Romero et al., 2009).

Induction of diarrhea model

Ten groups were formed (n = 10 per group): negative control (SS), positive control (loperamide, 5 mg/kg), CH (40 and 80 mg/kg), AS (40 and 80 mg/kg) and four mixtures of CH-AS groups (Mix-10, Mix-20, Mix-40 and Mix-80). The mixtures groups were treated with a 50:50 mixture aqueous preparation of CH and AS at doses of 10, 20, 40 and 80 mg/kg. Oral administration was used in all treatments. Thirty minutes later, castor oil was administered to each animal. Mice were placed in individual boxes and the latency period to onset of diarrhea was observed for 6 h, and the number of stools per animal group was counted. (Williamson et al, 1996, Romero et al., 2009).

Statistical analysis

All data were expressed as mean ± standard error (SE) and were analysed using multivariate analysis of variance (MANOVA) followed by Dunnett's test. A probability ≤ 5% was considered significant.

Results

Antimotility effect of the chamomile and star anise tea mixture

To observe the possible antimotility effect of the CH-SN mixture infusion, we determined the rate of advance of activated carbon in the small intestine of mice as a parameter to evaluate the effects on intestinal motility. The results indicate that the activated carbon of the control group advanced 71% along the small intestine, while the loperamide-treated group was 19%. The comparative analysis between groups indicates that the group administered loperamide registered a significant decrease (73%) in the percentage of activated carbon advancement compared to the control group, a finding that shows the pharmacological effect of loperamide on intestinal motility.

On the other hand, the groups treated only with CH or SN at doses of 40 and 80 mg/kg, showed a percentage of advancement of the activated carbon of 63, 57, 51 and 38%, respectively. The