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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

comparative analysis compared to the control group revealed that the group treated only with CH and SN at a dose of 80 mg/kg showed a significant difference of 20 and 46% respectively, but not, the dose of 40 mg/kg. Nevertheless, there is a tendency to decrease the percentage.

Moreover, the administration of the mixture of CH-SN infusion caused a decrease in the percentage of activated carbon advancement, a response directly proportional to the dose used. The results reported were: 65% (Mix-10), 60% (Mix-20), 45% (Mix-40) and 25% (Mix-80). The comparison of these percentages with the control group shows a significant difference in the group treated with Mix-40 (36%) and Mix-80 (65%), suggesting that the 40 and 80 mg/kg doses of CH-SN exert an antimotility effect (Fig. 1).

Anti-diarrheal effect of the mixture of chamomile and star anise tea

In the second experiment, diarrhea was induced feeding castor oil to the mice, the results indicate that the time before the onset of diarrhea was longer in the group treated with loperamide (163 ± 16.2 min) than controls (36 ± 4.2 min) (Fig. 2A). This demonstrates the antidiarrheal effect of loperamide, because the latency to the onset of diarrhea is three times higher than that of the control group (Fig. 2A).

The onset of diarrhea in animals treated only with CH, at doses of 40 mg/kg (38.3 ± 6.2 min) and 80 mg/kg (63.5 ± 5.02 min) or SN at 40 mg/kg (66.5 ± 2.34 min) and 80 mg/kg

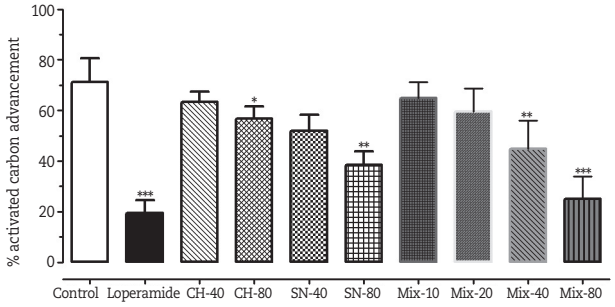

Figure 1 – Effect of chamomile and star anise mixture infusion on the intestinal motility of mice. Ten groups were formed (n = 10 per group): control, loperamide (5 mg/kg), CH (40 and 80 mg/kg), AS (40 and 80 mg/kg), Mix-10, Mix-20, Mix-40 and Mix-80. Activated carbon was administered and % of activated carbon advancement subsequently analyzed. The data plotted are the mean ± SEM. (One way ANOVA, Dunnett's post-test, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

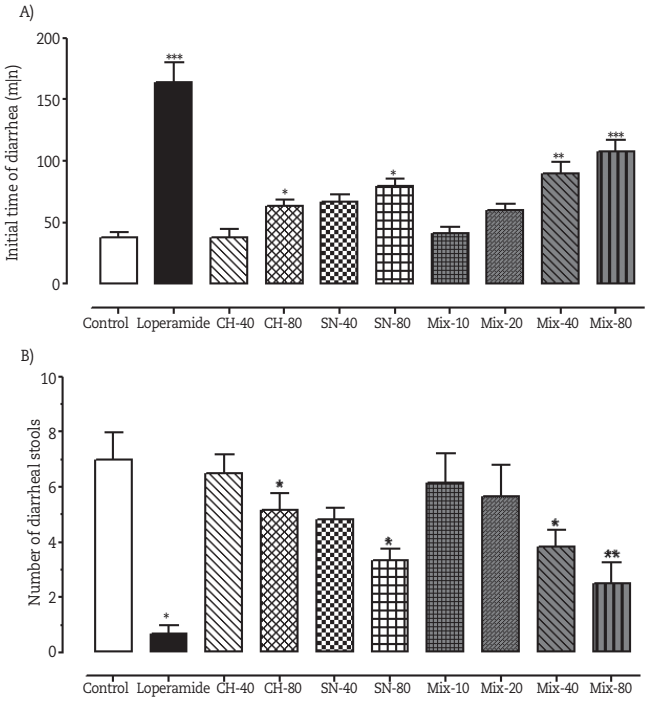

Figure 2 – Effect of the chamomile and star anise mixture infusion on diarrhea induced with castor oil in mice. The animals were divided into 10 groups (n = 10 per group): control, loperamide (5 mg/kg), CH (40 and 80 mg/kg), AS (40 and 80 mg/kg) Mix-10, Mix-20, Mix-40 and Mix-80. Once castor oil was administered, the time of the onset of diarrhea (A) and the number of diarrheal stools (B) were charted. The data plotted are the mean ± SEM. (One way ANOVA, Dunnett's post-test, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

(79.8 ± 5.8 min), shows the anti-diarrheal effect, separately. While the infusions of the CH-SN mixture exerted a protective effect against the diarrhea-inducing castor oil. The increased proportions of the infusion mixture showed a gradual increment in the lapse of time before the onset of diarrhea, with significant results for Mix-40 (90 ± 8.1 min) and Mix-80 (108 ± 9.6 min) when compared to the control group, and the statistical analysis indicated a difference of 136 and 184%, respectively (Fig. 2A).

In terms of diarrhea severity, it was observed that the number of stools for the control group and the group treated with loperamide was of 7 ± 1 and 0.6 ± 0.33, respectively. Thus, this result confirms the antidiarrheal action of loperamide (91% vs control group). Likewise, the CH- and SN- treated group were 6.50 ± 1.6 and 5.16 ± 0.6 at 40 mg/kg and 4.83 ± 0.40 and 3.33 ± 0.42 at 80 mg/kg, respectively, whereas the groups administered Mix-40 (3.8 ± 0.6) and Mix-80 (2.5 ± 0.7) of the CH-SN mixture had a significantly lower number of diarrheal stools compared with the control group (Fig. 2B). These results indicate that the combination of CH-SN had a protective effect against castor oil-induced diarrhea in mice.

Discussion

With their healing properties, plants are still the most well-known and accessible resource for a large proportion of the Mexican population, which explains the acknowledgement given by WHO to their great value as therapeutics in public health schemes. There is very strong scientific evidence related to the efficiency of herbal medicine. In Mexico approximately 4000 plant species have been attributed with medicinal properties (15% of the total flora). This number coincides with reports from various regions of the world by specialists in the field, which estimate that 1 in 7 plant types possess some healing property (Mantilla-Holguín et al., 2005). Since ancient times, plants have been used to develop drugs, flavorings and aromatic oils, amongst other things. The skills used in these processes have been passed from one generation to another, and are currently recognized and the subject of significant investigation in diverse disciplines as chemistry, biology, botany and pharmacology (Srivastava et al., 2010). Notably, the findings obtained over time have revealed a wide variety of therapeutic properties, including the antimotility and anti-diarrheal effects of some plants.

The pathophysiological mechanisms of diarrhea include increased intestinal motility, increased intestinal secretion disorders and inflammatory gastrointestinal processes. The main processes involved in acute diarrhea are the excess secretion by the mucosa in response to toxins produced by microbes within the intestinal lumen; and the alteration of the gastrointestinal mucosa produced by an underlying inflammatory process that alters the permeability and causes the exudate absorption capacity to decrease (Riverón Corteguera, 1999). The drugs and natural products that are used to attenuate acute diarrhea exert their effects on the processes above-mentioned.

CH and SN are two natural products that exert an anti-motility effect and modulate diarrhea. CH has been used for centuries as a medicinal plant for its anti-inflammatory and analgesic properties (McKay and Blumberg, 2006; Srivastava and Gupta, 2009). It is consumed in the form of tea and has been approved in Germany for the management of various inflammatory diseases of the gastrointestinal tract, as well as for topical application in the treatment of various skin and inflammatory disorders of mucosal surfaces, such as the oral cavity and anogenital areas (Ross, 2008). Several constituents of chamomile, including apigenin 7-*O*-glucoside, luteolin, terpene compounds, chamazulene, and (-)-α-bisabolol, patuletin, quercetin, myricetin, and rutin, have been studied regarding their anti-inflammatory activities. Of these, chamazulene, α-bisabolol, and apigenin have been shown to possess the highest anti-inflammatory activity against pro-inflammatory agents (McKay and Blumberg, 2006).

SN has carminative, antispasmodic, antiseptic, antimicrobial, anti-diarrheal activities, and is used to treat colics and as a tranquilizer. Researchers attribute these effects to the presence of two coumarin derivatives: 7-hydroxycoumarin and 7-methoxy-coumarin (Hall et al., 2002). However, SN also contains a dicycloether, and anethole, both of which have anti-motility activities. However, the consumption of high amounts of star anise tea (LD_{50} 4 g/kg, orally in mouse) may cause neurotoxicity and hepatotoxicity. The dose of SN used in this study ranged between 10 and 80 mg/kg, which is well below the LD_{50}. Bibliographical evidence and the findings of this study suggest that the combination of CH and SN may be more effective in reducing motility and diarrheal activity than the treatments used in conventional medicine.

Loperamide is a piperidine-derivative butyramide with μ-receptor activity and is an orally active anti-diarrheal drug (Brunton et al., 2005). This study confirmed the anti-motility effects of loperamide as evidenced by the much lower progress through the intestinal tract of the activated carbon than that found in controls. Sairam et al. 2003 showed similar results when comparing the anti-diarrheal effect of *Mangifera indica* using loperamide as a positive control in mice.

The anti-motility action of different mixtures of chamomile and star anise was demonstrated using activated carbon motility in the gut, as initially proposed by Ahmed et al. (2010) and Medha et al. (2010) to evaluate the intestinal contraction caused by plants and *Desmostachya tinctorum bipinnata*. The results of this study show that the use of Mix-40 and Mix-80 efficiently reduces the motility caused by activated carbon in comparison to the control group as well as to the groups treated with chamomile or star anise only (Fig. 1). This suggests that the combination of both herbal infusions reduces the motility effect, possibly through an additive action by active ingredients present, however, further studies are required to test this hypothesis on humans.

Castor oil in contact with the acid released intraluminal rhinoleic lipases, irritating the mucosa. The result is an alteration of the cell membrane due to the water and electrolyte secretion, significantly increasing the presence of prostaglandins in gastrointestinal cells (Ahmed et al., 2010). In this study of the anti-diarrheal effect of the chamomile

and star anise mixture, the results showed that the mixture of infusions decreased the diarrhea induced by castor oil in mice. In this regard, the administration of Mix-40 and Mix-80 led to a delay of the diarrhea onset caused by castor oil, and consequently, the number of diarrheal stools was lower in treated mice than the control group, similar to the report by Romero et al. (2009).

This protective effect is probably due to the constituents of the two infusions, which contain flavonoids and glycosides, among other inflammatory anetholes, and together reduce the harmful effect of ricinoleic acid on the mucosa, and hence diarrhea does not occur to such an acute extent (Meenakshi et al., 2008).

In conclusion, these results show for the first time that the chamomile and star anise mixture (Mix-40 and Mix-80) exerts an anti-motility effect and decreases an induced diarrhea in mice. The results suggest that the use of this combination of herbal infusions to treat and alleviate gastrointestinal problems is efficient.

Authors' contributions

AD and IVP contributed to the care of laboratory animals and the development of experimental models in the laboratory. LAC, VB, ST and RCM contributed to statistical analysis and interpretation of results. CRR, DA and IRCM contributed to the drafting and critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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