January 2012

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Studies on Antidiarrheal and Antispasmodic Activities of Lepidium sativum Crude Extract in Rats

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This study was aimed to provide the pharmacological basis for the medicinal use of Lepidium sativum in diarrhea using in vivo and in vitro assays. The seed extract of Lepidium sativum (Ls.Cr) at 100 and 300 mg/kg inhibited castor oil-induced diarrhea in rats. In isolated rat ileum, Ls.Cr (0.01–5 mg/mL) reversed carbachol (CCh, 1 μM) and K+ (80 mM)-induced contractions with higher potency against CCh, similar to dicyclomine. Preincubation of rat ileum with a lower concentration of Ls.Cr (0.03 mg/mL) caused a rightward parallel shift in the concentration–response curves (CRCs) of CCh without suppression of the maximum response, while at the next higher concentration (0.1 mg/mL), it produced a non-parallel rightward shift with suppression of the maximum response, similar to that of dicyclomine. Ls.Cr shifted the CRCs of Ca++ to the right with suppression of the maximum response, similar to verapamil. These data suggest that Lepidium sativum seed extract possesses antidiarrheal and spasmyloytic activities mediated possibly through dual blockade of muscarinic receptors and Ca++ channels, though additional mechanism(s) cannot be ruled out and this study explains its medicinal use in diarrhea and abdominal cramps. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: Lepidium sativum; antidiarrheal; anticholinergic; Ca++ antagonist

INTRODUCTION

Lepidium sativum Linn. (Cruciferae) locally known as ‘garden cress’ or ‘hab arachad’ has been used widely in different parts of the world for its wide therapeutic application. In different regions of Saudi Arabia, Lepidium sativum seeds are used commonly to treat abdominal discomfort, such as dysentery and diarrhea, in addition to its other beneficial effects, such as febrifuge, diuretic, antirheumatic, antiallergic and antiulcer (Ageel et al., 1987; Duke et al., 2002). In various countries of Africa, Lepidium sativum seeds are thought to be a better medicinal remedy to cure respiratory disorders such as bronchitis and asthma (Kloos, 1976). The herb is also used to compensate vitamin C deficiency and to strengthen the immune system (Fleming, 1998).

Lepidium sativum is documented to possess alkaloids, riboflavin, α-tocopherol, β-carotenes, β-sitosterol, ascorbic, linoleic, oleic, palmitic and stearic acids. It is considered a good source of mono-unsaturated fatty acids and L-arabinose (Duke, 1992). Moreover, cucurbitacins and cardenolides have also been identified as plant constituents (Fleming, 1998).

Lepidium sativum has been studied pharmacologically for its laxative (Rehman et al., 2011b), antibacterial (Darwish and Aburjai, 2010), bronchodilatory (Rehman et al., 2011a), contraceptive effects (Sharief and Gani, 2004) and in inflammatory bowel disease (Rahimi et al., 2010). There is no study available showing its efficacy in hyperactive gut disorders, such as diarrhea. This study attempted to provide a rationale for its medicinal use in diarrhea.

MATERIALS AND METHODS

Plant material and preparation of the crude extract. The seeds of Lepidium sativum were bought from the local herbal market of Riyadh, Saudi Arabia in March, 2010. The sample was identified with the help of a taxonomist (Dr Mohammed Yusuf, Department of Pharmacognosy, King Saud University) and the specimen was preserved at the herbarium of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia, and also at the herbarium of the Natural Product Research Division, Department of Biological and Biomedical Sciences, the Aga Khan University, Karachi with voucher # Ls-SE-03-10-98. By following a previously described method (Williamson et al., 1998), the seeds of Ls.Cr were soaked in 70% methanol for 72 h followed by filtration through muslin cloth and Whatman (Maidstone, UK) no.1 filter paper simultaneously. This procedure was repeated three times, and all the filtrates were combined and evaporated in a rotary evaporator (model RE-111, Buchi, Flawil,
Switzerland) under reduced pressure to obtain finally the crude extract of the seeds of Lepidium sativum (Ls.Cr). The yield of the thick dark brown pasty-like mass was 15% wt/wt.

**Phytochemical screening.** Phytochemical analysis of the crude extract of Lepidium sativum was performed qualitatively for the presence of alkaloids, tannins saponins and anthraquinones as plant constituents according to a standard method (Evans, 2006). Briefly, alkaloids were detected by treating the plant material with Dragendorff’s reagent, resulting in the appearance of a precipitate at the bottom of the test tube. The plant material was deemed positive for tannins when a green mass was 15% wt/wt.

The presence of saponins was based on the appearance of froth upon vigorous shaking of diluted samples. Lastly, anthraquinones were screened, by treatment of the plant material with NH₄OH reagent added after dissolving it in 1% HCl and purifying with benzene. The appearance of a pink, violet or red color demonstrated their presence.

**Chemicals.** Dicyclomine, carbamylcholine (CCh), potassium chloride (KCl), verapamil, acetylcholine perchlorate (ACh) and atropine sulphate were purchased from Sigma-Aldrich Chemicals Company, St Louis, MO, USA. Castor oil was purchased from Karachi Chemical Industries (Pvt.) Ltd. F/25 S. I. T. E., Karachi (Pakistan). All the chemicals used were of analytical grade and dissolved in distilled water/saline. Stock solutions of all the chemicals were made fresh on the day of experiment.

**Animals.** Sprague-Dawley (SD) rats (180–220 g) of either sex were housed at the animal house of the Aga Khan University under a controlled environment (23–25 °C). The animals were kept in plastic cages (47 × 34 × 18) with sawdust (changed at every 48 h) and fasted for 24 h following the start of the experiment but routinely were given tap water and standard diet. Experiments were performed with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (National Research Council, 1996) and approved by the Ethical Committee of the Aga Khan University.

**Castor oil-induced diarrhea.** To assess the antidiarrheal activity, a previously described method was followed (Shah et al., 2010a). Sprague-Dawley rats (n = 25) of either sex were fasted for 24 h before starting the experiment. The animals were housed in individual cages and divided into five groups (n = 5 each). The first group received saline in vehicle (10 mL/kg, p.o.) and served as the negative control. The next two groups received different doses of the plant extract (Ls.Cr) 100 and 300 mg/kg. Subsequent groups were pretreated with the positive control dicyclomine (50 and 100 mg/kg). After 1 h of the treatment, each animal received castor oil (10 mL/kg, p.o.) through a feeding needle. At 6 h after dosing the castor oil, the individual rat cages were inspected (by an observer not knowing about the treatment) for the presence or absence of unformed fecal pellets; the absence of unformed stool was recorded as a positive result, indicating protection from diarrhea.

**Rat ileum.** Sprague-Dawley rats of local breed and either sex, starved for 24 h, were humanely killed by cervical dislocation. The ileum was dissected out, immersed in Tyrode’s solution and cleaned of the mesentery. Respective segments 2–3 cm long were mounted in a 10 mL tissue organ bath filled with Tyrode’s solution, aerated continuously with a mixture of 95% O₂ and 5% CO₂ (carbogen) and maintained at 37 °C. The composition of the Tyrode’s solution in mM was: 2.68 KCl, 136.9 NaCl, 1.05 MgCl₂, 11.90 NaHCO₃, 0.42 NaH₂PO₄, 1.8 CaCl₂ and 5.55 glucose, (pH 7.4). A preload of 1 g was applied to each tissue, while intestinal contractions were measured using an isotonic transducer 50–6360 (Harvard Apparatus, Holliston, MA, USA) coupled with a student oscillograph (Harvard Apparatus)/PowerLab model ML-845, data acquisition system (ADInstruments; Sydney, Australia). Each tissue was allowed to equilibrate for at least 30 min before the addition of any drug. Afterwards, each tissue was stabilized with a submaximal concentration of acetylcholine (ACh, 0.3 μM). In order to observe the spasmolytic effect of the test substance on induced contractions, high K⁺ (80 mM) and carbachol (CCh, 1 μM) were used as spasmodic agents, which were used to determine the different inhibitory mechanisms, such as Ca²⁺ antagonist and anticholinergic. The test material was added in a cumulative fashion to obtain the concentration-dependent inhibitory responses. The relaxation of the tissue preparation was expressed as the percentage of the control contraction mediated by the added spasmonog (Shah et al., 2010b).

In order to differentiate the competitive and non-competitive antagonistic effect of the test substance, first control CRCs of CCh, were constructed up to the maximal effect and then these CRCs were reconstructed in the presence of increasing concentrations of the test material. The rightward parallel shift in the CRCs of agonist, indicating the competitive antagonism (Arunlakshana and Schild, 1959), while non-parallel displacement with suppression of the maximum response, confirms the non-specific antagonisms (Mehmood et al., 2011). To confirm the CCB activity of the test material, the tissue was allowed to stabilize in normal Tyrode’s solution, which was then replaced with Ca²⁺-free Tyrode’s solution containing EDTA (0.1 mM) for 30 min,

**Table 1. Antidiarrheal activity of Lepidium sativum seed extract (Ls.Cr) in rats, on castor oil (10 mL/kg)-induced diarrhea**

<table>
<thead>
<tr>
<th>Treatment (p.o.), dose (mg/kg)</th>
<th>No. of rats out of five with diarrhea</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (10 mL/kg) + castor oil</td>
<td>5/5</td>
<td>0</td>
</tr>
<tr>
<td>Ls.Cr + castor oil</td>
<td>100 + 10</td>
<td>3/5</td>
</tr>
<tr>
<td></td>
<td>300 + 10</td>
<td>1*/5</td>
</tr>
<tr>
<td>Dicyclomine + castor oil</td>
<td>50 + 10</td>
<td>3/5</td>
</tr>
<tr>
<td></td>
<td>100 + 10</td>
<td>0/5</td>
</tr>
</tbody>
</table>

* p < 0.05 and b p < 0.01 vs saline + castor oil treated group (χ²-test).
Figure 1. Inhibitory effect of (A) the seed extract of *Lepidium sativum* (Ls.Cr), (B) dicyclomine, (C) verapamil and (D) atropine on K⁺ (80 mM) and carbachol (CCh)-induced contractions in isolated rat ileum preparations. Symbols represent mean ± SEM; n = 4–6.

Figure 2. Inhibitory effect of (A) the seed extract of *Lepidium sativum* (Ls.Cr), (B) dicyclomine, (C) atropine and (D) verapamil on carbachol (CCh) concentration–response curves in isolated rat ileum preparations. Symbols represent mean ± SEM; n = 4–5.
in order to chelate the Ca\(^{++}\) from the environment and the tissues. This solution was further replaced with K\(^{-}\)-rich and Ca\(^{++}\)-free Tyrode's solution of the following constitution (mM): KCl 50, NaCl 91.04, MgCl\(_2\) 1.05, NaHCO\(_3\) 11.90, NaH\(_2\)PO\(_4\) 0.42, glucose 5.55 and EDTA 0.1. After an incubation period of 30 min in normal Tyrode's solution, the concentration–response curves (CRCs) of Ca\(^{++}\) were constructed. When the control CRCs of Ca\(^{++}\) were found superimposable (usually after two cycles), the tissue was preincubated with different concentrations of the test substance for 60 min, to note the presence of possible CCB-like activity.

Statistical analysis. The data expressed as mean ± SEM values (\(n=\) number of experiments) and the median effective concentrations (EC\(_{50}\)) values with 95% confidence intervals. The chi-square (\(\chi^2\)) test was applied to differentiate the results in antidiarrheal activity assay. A value of \(p<0.05\) was considered significantly different. The concentration–response curves (CRCs) were analysed by non-linear regression. All the graphing, calculations and statistical analysis were performed using the GraphPAD program (GraphPAD, San Diego, California, USA).

RESULTS AND DISCUSSION

In an attempt to validate the medicinal use of *Lepidium sativum* seeds in gastrointestinal disorders, such as diarrhea (Duke *et al*., 2002), this study was planned to assess the antidiarrheal effect of the plant extract in rats, while its possible underlying mechanism(s) were evaluated using the isolated tissue preparations. The plant extract at a dose of 100 and 300 mg/kg showed a marked antidiarrheal effect in rats by producing 40% and 80% protection against castor oil-induced diarrhea, similar to dicyclomine, which showed 40% and 100% protection at the tested doses of 50 and 100 mg/kg as shown in Table 1. Dicyclomine is a standard antispasmodic drug acting through dual inhibition of muscarinic receptors and Ca\(^{++}\) channels (Downie *et al*., 1977), while verapamil, a standard Ca\(^{++}\) antagonist (Fleckenstein, 1977), was more potent against high K\(^{-}\) (Fig. 1C), and atropine, a known anticholinergic drug (Gilani *et al*., 1997), inhibited CCh contractions only (Fig. 1D). These data suggest that the relaxant effect of the plant extract is mediated through the dual inhibition of muscarinic receptors and Ca\(^{++}\) channels. However, the Ca\(^{++}\) antagonist effect completely reversed CCh and high K\(^{-}\)-induced contractions with EC\(_{50}\) values of 0.08 mg/mL (95% confidence interval, 0.06–0.09; \(n=5\)) and 1.58 mg/mL (1.4–1.8; \(n=5\)) respectively, being more potent against CCh (Fig. 1A), similar to dicyclomine (Fig. 1B), a dual inhibitor of muscarinic receptors and Ca\(^{++}\) channels (Downie *et al*., 1977), while verapamil, a standard Ca\(^{++}\) antagonist (Fleckenstein, 1977), was more potent against high K\(^{-}\) (Fig. 1C), and atropine, a known anticholinergic drug (Gilani *et al*., 1997), inhibited CCh contractions only (Fig. 1D). These data suggest that the relaxant effect of the plant extract is mediated through the dual inhibition of muscarinic receptors and Ca\(^{++}\) channels. However, the Ca\(^{++}\) antagonist effect

Figure 3. Concentration–response curves of Ca\(^{++}\) constructed in the absence and presence of increasing concentrations of (A) the seed extract of *Lepidium sativum* (Ls.Cr), (B) dicyclomine and (C) verapamil in isolated rat ileum preparations. The symbols represent mean ± SEM; \(n=4–7\).
in the plant extract was observed at a slightly higher concentration, similar to dicyclomine.

The dual inhibitory mode involving anticholinergic and Ca++ channel blockade of the plant extract was also evaluated separately by constructing the CRCs of CCh in rat ileum. Preincubation of the plant extract at a lower concentration (0.03 mg/mL) caused a rightward parallel shift in the CCh curves without suppressing the maximum response (Fig. 2A), a characteristic typical of specific antagonist (Arunlakshana and Schild, 1959), as seen with dicyclomine at a lower concentration (0.01 μM) and with atropine at both tested concentrations (Fig. 2B, C). At the higher concentration (0.1 mg/mL), Ls.Cr caused a non-parallel shift with suppression of the maximum response (Fig. 2A), similar to the effect of dicyclomine at 0.03 μM (Fig. 2B), while verapamil showed non-parallel shifts with suppression of the maximum response at both tested concentrations (Fig. 2D).

K+ at a concentration higher than 30 mM (high K+) is known to cause smooth muscle contractions through opening of voltage-dependent L-type Ca++ channels, thus allowing inward movement of extracellular Ca++. Bolton (1979) resulting in sustained contraction. Thus, a substance that inhibits high K+-provoked contractions is considered as a blocker of Ca++ influx (Fleckenstein, 1977). The CCB activity was confirmed when pretreatment of tissue with the plant extract caused a rightward shift in the CRCs of Ca++ with suppression of the maximum response of Ca++ (taken as 100%) at 0.3 and 1 mg/mL, respectively (Fig. 3A), similar to the effect of dicyclomine, papaverine and verapamil (Fig. 3B and C), while atropine was devoid of such an effect (data not shown).

The assessed antidiarrheal effect of Lepidium sativum seed extract in rats, mediated possibly through the dual blockade of muscarinic receptors and Ca++ channels, provides evidence for its effectiveness in diarrhea, though additional mechanism(s) cannot be ruled out. This is also in line with the general concept in the Greco-Arab system that natural remedies contain effect enhancing and/or side effect neutralizing combinations of activities (Gilani and Rahman, 2005).

The phytochemical screening of Lepidium sativum seed extract revealed the presence of different chemical classes, such as alkaloids, saponins and anthraquinones. The literature has also shown the presence of β-sitosterol as a plant constituent. Moreover, alkaloids and β-sitosterol are known for their antispasmodic effect (Palombo, 2006), hence, the presence of these phytochemicals in Lepidium sativum may be responsible for its usefulness in diarrhea, though further studies are required to know the precise nature of chemical constituents mediating alleged biological activities.

CONCLUSION

This study shows that Lepidium sativum possesses antidiarrheal and antispasmodic activities mediated possibly through dual inhibition of muscarinic receptors and Ca++ channels, though additional mechanism(s) cannot be ruled out and this study provides a sound mechanistic support to the medicinal use of Lepidium sativum seeds in diarrhea.

Acknowledgement

This study was initiated during the visit of Dr Anwarul-Hassan Gilani to King Saud University as a part of Visiting Professor Programme, and partially supported by the Higher Education Commission, Government of Pakistan, as an indigenous PhD scholarship to Mr. Najeeb-ur-Rehman.

Conflict of Interest

The authors state no competing financial interests exist.

REFERENCES


USEFULNESS OF \textit{LEPIDIUM SATIVUM} IN DIARRHEA


