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Najeeb-ur-Rehman  
_Aga Khan University_

Malik Hassan Mehmood  
_Aga Khan University_

Khalid M. Alkharfy

Anwarul-Hassan Gilani  
_Aga Khan University_

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Prokinetic and laxative activities of *Lepidium sativum* seed extract with species and tissue selective gut stimulatory actions

Najeeb-ur-Rehmana, Malik Hassan Mehmooda, Malik Hassan Mehmooda, Khalid M. Alkharfyc, Anwarul-Hassan Gilani a, b, c,∗

a Natural Product Research Division, Department of Biological and Biomedical Sciences, The Aga Khan University Medical College, Karachi 74800, Pakistan
b Department of Pharmacology, Faculty of Pharmacy, University of Karachi 75270, Pakistan
c Department of Clinical Pharmacy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

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**A B S T R A C T**

**Aim of the study:** To provide ethnopharmacological basis for the medicinal use of *Lepidium sativum* seeds in indigestion and constipation.

**Materials and methods:** The in vivo studies were conducted in mice, while isolated tissues of mouse, guinea-pig and rabbit were suspended in tissue bath to measure isotonic contractions.

**Results:** The aqueous-methanolic extract of *Lepidium sativum* seeds (Ls.Cr) at 30 and 100 mg/kg showed atropine-sensitive prokinetic and laxative activities in mice, which were partially sensitive to atropine. In isolated gut preparations of mouse and guinea-pig, Ls.Cr (0.1–1 mg/mL) caused a concentration-dependent stimulatory effects both in jejunum and ileum, which was blocked in the presence of atropine. In rabbit jejunum, the stimulant effect of Ls.Cr remained unchanged in the presence of atropine, pyrilamine or SB203186, while in rabbit ileum, the stimulatory effect was partially blocked by atropine. The Ls.Cr was more efficacious in gut preparations of rabbit than in guinea-pig or mouse. The phytochemical analysis of the plant extract detected alkaloids, saponins and anthraquinones as plant constituents.

**Conclusion:** This study showed the prokinetic and laxative effects of *Lepidium sativum* in mice, which were partially mediated through a cholinergic pathway. The in vitro spasmodic effect of the plant extract mediated through a similar mechanism with species and tissue-selectivity, provides a rationale for the medicinal use of the seeds of *Lepidium sativum* in indigestion and constipation, and suggests studying the plant extracts on more than one species to get the wider picture.

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1. Introduction

*Lepidium sativum* Linn. (family: Cruciferae) is commonly known as “Common cress”, “Garden cress” or “Halim”. It is a small smooth erect annual herb and is cultivated as a culinary vegetable all over Asia (Baquar, 1989). Its seeds are popularly used as gastrointestinal (GI) stimulant, laxative, gastroprotective and digestive aid (Nadkarni, 1986; Fleming, 1998). In addition, the plant has been reported to have other properties, such as antibacterial, antiasthmatic, diuretic, aphrodisiac, and abortifacient; its leaves are also used by Europeans in salads (Nadkarni, 1986; Duke et al., 2002).

The plant has been reported to contain alkaloids (imidazole, lepidine, semilepidinoside A and B) (Maier et al., 1998), β-carotenes, ascorbic, linoleic, oleic, palmitic and stearic acids (Duke, 1992), cucurbitacins and cardenolides (Fleming, 1998). Moreover, a few phenolic constituents, such as, sinapic acid and sinapin were isolated from its seed extract (Schultz and Gmelin, 1952).

*Lepidium sativum* has been studied for its medicinal use as antihypertensive (Maghrani et al., 2005), diuretic (Patel et al., 2009), antiasthmatic (Archana and Anita, 2006), antiinflammatory, hypothermic, analgesic and coagulant (Al-Yahya et al., 1994), hypoglycemic (Patole, 1998) and bone fracture healing agent (Ahsan et al., 1989). However, there is no report in the literature validating its medicinal use in constipation or other GI disorders. In this investigation, we studied the seed extract of *Lepidium sativum* using the in vivo and in vitro assays, to provide a scientific base for its medicinal use in gut disorders, such as indigestion and constipation.

2. Material and methods

2.1. Preparation of the crude extract

The seeds of *Lepidium sativum* were purchased from a herbal store (Bin Menqash, Riyadh, Saudi Arabia) in March, 2010. The plant was authenticated by Dr. Mohammed Yusuf of King Saud University and the specimen has been preserved at the herbarium...
of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia, and also at the Natural Product Research Division, Department of Biological and Biomedical Sciences, Aga Khan University, Karachi with voucher # Ls-SE-04-10-98. By following a previously described method (Williamson et al., 1998), the seeds of Ls.Cr were soaked in 70% methanol for 3 d and filtered through muslin cloth and Whatman (Maidstone, UK) No. 1 filter paper simultaneously. This procedure was repeated three times, and all the filtrates were pooled and evaporated in rotary evaporator (model RE-111, Buchi, Flawil, Switzerland) under a reduced pressure to obtain finally, the crude extract of the seeds of Lepidium sativum (Ls.Cr). The yield of thick dark brown paste like mass was 15% (wt/wt).

2.2. Phytochemical screening

Phytochemical analysis of the crude extract of Lepidium sativum was performed qualitatively for the presence of alkaloids, saponins and anthraquinones as plant constituents according to a standard method (Evans, 2006). Briefly, alkaloids were detected by treating the plant material with Dragendorf’s reagent, resulting in the appearance of a precipitate at the bottom of the test tube. The presence of saponins was based in 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.

2.3. Drugs

Acetylcholine perchlorate (ACh), atropine sulphate, carbamylcholine (CCh), histamine hydrochloride, 5-hydroxytryptamine (5-HT), pyrilamine maleate and hexamethonium chloride were purchased from Sigma–Aldrich Chemicals Company (St Louis, MO, USA). SB203186 (1-piperidinylethyl-1H-indole-3-carboxylate) was purchased from Tocris (Ballwin, MO, USA). Chemicals used for making physiological salt solutions including potassium chloride, calcium chloride, glucose, magnesium chloride, magnesium sulfate, potassium dihydrogen phosphate, sodium bicarbonate, sodium dihydrogen phosphate and sodium chloride were obtained from Merck (Darmstadt, Germany). All chemicals used were of the analytical grade available and solubilized in distilled water.

2.4. Animals

A total of 90 BALB/c mice (weighing 20–25 g), 5 guinea-pigs (weighing 400–600 g) and 7 local breed rabbits (weighing 1–1.5 kg) of either sex were used, which 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 were fasted for 24 h before starting the experiment. In routine, they were given tap water ad libitum and a standard diet (Harkness and Wagner, 1995) consisting of (g/kg): flour 380, fiber 380, molasses 12, NaCl 5.8, nutritel 2.5, potassium metabisulfate 1.2, vegetable oil 38, fish meal 170 and powdered milk 150. The 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 Ethics Committee of The Aga Khan University.

2.5. In vivo experiments

2.5.1. Charcoal meal GI transit test

The method described by Al-Qarawi et al. (2003) was followed with slight modifications. Mice fasted for 12 h were divided into 7 different groups (6 animals in each). Two of the groups were treated per oral (p.o.) with increasing doses of Ls.Cr 30 and 100 mg/kg, acting as the test groups. One group was taken as negative control, treated with saline (10 mL/kg). The next group was administered CCh (1 mg/kg) as the positive control. After 15 min, the animals were given 0.3 mL of charcoal meal of distilled water suspension containing 10% gum acacia, 10% vegetable charcoal and 20% starch. The animals were sacrificed after 30 min and the abdomen was opened to excise the whole small intestine. The length of the small intestine and the distance between the pylorus region and the front of the charcoal meal was measured to obtain the charcoal transport ratio or percentage. In order to assess the involvement of acetylcholine (ACh)-like prokinetic effect of the extract and CCh, further groups of mice were pretreated with intraperitoneal (i.p.) injection of atropine (10 mg/kg) 15 min prior the administration of the extract or CCh.

2.5.2. Laxative activity test

In accordance with the previous method (Haruna, 1997), mice fasted for 6 h before the experiment were placed individually in cages lined with clean filter paper. The animals were divided into 7 groups (6 animals in each); the first group acting as the negative control and administered saline (10 mL/kg, p.o.), while the next group received CCh (1 mg/kg, i.p.), which served as the positive control. The third and fourth groups received orally, 30 and 100 mg/kg of Ls.Cr, respectively. To determine the mechanism underlying its laxative effect, separate sets of mice (group # 5–7) were pre-treated with atropine (10 mg/kg, i.p.) 1 h before administration of the extract or CCh. After 18 h, the feces production (total number of feces and total number of wet feces per group) in all animals was counted, and the percentage increase in wet feces relative to that of total fecal output was recorded, which was considered as the laxative effect (Mehmood and Gilani, 2010).

2.6. In vitro experiments

By following the previously described methods (Ghayur and Gilani, 2005; Ghayur et al.; Gilani et al., 2005), different gut preparators (jejenum and ileum) from mouse, guinea-pig and rabbit were obtained subsequent to cervical dislocation of the animals; the abdomen was cut opened, required tissues were isolated out. Tissue preparations (ileum or jejunum) of 2–3 cm long were mounted in 10 mL tissue baths containing Tyrode’s solution maintained at 37 °C and aerated with a mixture of 5% carbon dioxide and 95% oxygen (carbogen). The composition of Tyrode’s solution (mM) was KCl 2.68, NaCl 136.9, MgCl₂ 1.05, NaHCO₃ 11.90, NaH₂PO₄ 0.42, CaCl₂ 1.8, and glucose 5.55 (pH 7.4). A preload of 1 g was applied to each tissue, and the contractile responses were recorded using isometric transducer 50–6360 (Harvard Apparatus, Holliston, MA, USA) coupled with either a student oscillograph (Harvard Apparatus) or PowerLab (ML-845) data acquisition system (AD Instruments; Sydney, Australia) and a computer using chart software (version 5.3). The tissues were allowed to equilibrate for a period of 30 min, and then stabilized with sub-maximal concentration of acetylcholine (ACh, 0.3 μM). The tissues were presumed stable only after the reproducibility of the said responses. The Lepidium sativum seed extract was examined later for any spasmodic activity on the ileum and jejunum preparations of mouse, guinea-pig and rabbit at concentrations ranged from 0.003 to 3.0 mg/mL.

2.7. Statistical analysis

All the data expressed are mean ± standard error of mean (S.E.M., n = number of experiments) and the median effective concentrations (EC₅₀ values) with 95% confidence intervals (CI). One-way analysis of variance (ANOVA) followed by Dunnett’s test
or unpaired t-test was used to assess the laxative activity, while one-way ANOVA followed by Tukey’s test was employed for the effect of plant extract in charcoal meal transit. The concentration-response curves (CRCs) were analyzed by non-linear regression and two-way ANOVA followed by Bonferroni’s post-test correction or unpaired t-test was used for multiple comparisons of CRCs with the respective control. All the graphing, calculations and statistical analysis were performed using GraphPad Prism 4 for windows (GraphPad Software, San Diego, California, USA).

3. Results

3.1. Phytochemical analysis

Preliminary phytochemical analysis of the seed extract of \textit{Lepidium sativum} revealed the presence of alkaloids, saponins and anthraquinones.

3.2. In vivo findings

3.2.1. Effect of Ls.Cr on jejunum preparation of different animals

Ls.Cr treatment produced 53.8 ± 9.2% and 63.2 ± 5.3% \((n = 6)\) wet feces in mice at 30 and 100 mg/kg, respectively. The positive control group (CCh (1 mg/kg) produced 73.6 ± 6.8% wet feces, while the saline treated group did not form any wet feces. When Ls.Cr (30 and 100 mg/kg) was studied for its positive influence on wet feces in mice pretreated with atropine, the significant effect declined to 4 ± 4% and 7.8 ± 5%, respectively; further details are shown in Table 1.

3.2.2. Laxative activity

Ls.Cr treatment produced 53.8 ± 9.2% and 63.2 ± 5.3% \((n = 6)\) wet feces in mice at 30 and 100 mg/kg, respectively. The positive control group (CCh (1 mg/kg) produced 73.6 ± 6.8% wet feces, while the saline treated group did not form any wet feces. When Ls.Cr (30 and 100 mg/kg) was studied for its positive influence on wet feces in mice pretreated with atropine, the effect declined to 4 ± 4% and 7.8 ± 5%, respectively; further details are shown in Table 1.

3.3. In vitro findings

3.3.1. Effect of Ls.Cr on jejunum preparation of different animals

In mouse jejunum, the seed extract of \textit{Lepidium sativum} caused a concentration-dependent stimulant effect at 0.03–0.3 mg/mL, being more effective than in mouse ileum. Pretreatment of the tissue with atropine \((0.1 \mu M)\) blocked \((p < 0.001)\) the spasmodic effect of Ls.Cr, while the presence of hexamethonium, pyrilamine or SB203186 did not alter \((p > 0.05)\) its effect (Fig. 2A). The values shown in Fig. 2A represent 4–6 isolated tissue preparations obtained from 3 animals.

In guinea-pig jejunum, Ls.Cr \((0.01–0.1 \text{mg/mL})\) exhibited a mild spasmodic effect reaching its highest 17.4 ± 1.2% \((\text{mean ± S.E.M.; } n = 5)\) of ACh maximum. The efficacy of achieved contractile effect of the extract in jejunum preparation was found lower than observed in guinea-pig ileum. When the spasmodic effect of Ls.Cr was re-determined in the presence of different antagonists, it was fully blocked in the presence of atropine, while remained unchanged \((p > 0.05)\) in the presence of hexamethonium, pyrilamine or SB203186 (Fig. 2B). The values presented in Fig. 2B represent 4–5 isolated tissue preparations obtained from 2 animals.

Table 1

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Mean defecation/group</th>
<th>Mean number of wet feces/group</th>
<th>Mean % of wet feces</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saline (p.o., mL/kg)</td>
<td>10</td>
<td>1.4 ± 0.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Carbachol (i.p.)</td>
<td>1</td>
<td>11.4 ± 0.6**</td>
<td>8.4 ± 0.9**</td>
<td>73.6 ± 6.8</td>
</tr>
<tr>
<td>3</td>
<td>Ls.Cr (p.o.)</td>
<td>30</td>
<td>6.8 ± 1.1**</td>
<td>3 ± 0.4**</td>
<td>53.8 ± 9.2</td>
</tr>
<tr>
<td>4</td>
<td>Carbachol (i.p.) + Atropine (i.p.)</td>
<td>100</td>
<td>9.4 ± 1.8**</td>
<td>4.8 ± 0.4**</td>
<td>63.2 ± 5.3</td>
</tr>
<tr>
<td>5</td>
<td>Carbachol (i.p.) + Atropine (i.p.)</td>
<td>100</td>
<td>1.9 ± 0.8**</td>
<td>0.1 ± 0.09**</td>
<td>4.0 ± 1.7</td>
</tr>
<tr>
<td>6</td>
<td>Ls.Cr (p.o.) + Atropine (i.p.)</td>
<td>30 + 10</td>
<td>3.6 ± 0.5*</td>
<td>0.2 ± 0.2**</td>
<td>4 ± 4</td>
</tr>
<tr>
<td>7</td>
<td>Ls.Cr (p.o.) + Atropine (i.p.)</td>
<td>100 + 10</td>
<td>4.8 ± 0.7**</td>
<td>0.4 ± 0.2**</td>
<td>7.8 ± 5</td>
</tr>
</tbody>
</table>

Values shown are mean ± S.E.M. of 6 animals per group. *p < 0.05, **p < 0.01 and ***p < 0.001 show a comparison of group #2, 3 and 4 vs. group #1 (One-way ANOVA followed by Dunnett’s t-test), group #5 vs. group #2, group #6 vs. group #3 and group #7 vs. group #4 (unpaired t-test). The term p.o. represents per oral, while i.p. is for intraperitoneal injection.
Fig. 2. The stimulatory effects of the seed extract of *Lepidium sativum* (Ls.Cr) without and with atropine (0.1 μM), pyrilamine (1 μM), hexamethonium (0.3 mM) and SB203186 (1 μM) in isolated jejunum and ileum preparations of (A and D) mouse, (B and E) guinea-pig and (C and F) rabbit. The values shown are mean ± S.E.M. of 4–7 individual experiments conducted on the isolated tissue preparations from 5 to 7 different animals [mouse (6), guinea-pig (5) and rabbits (7)]. *p < 0.05, **p < 0.01 and ***p < 0.001 (two-way ANOVA, followed by Bonferroni's post-test correction or unpaired t-test).

in Fig. 2C represent 4–7 isolated tissue preparations obtained from 3 animals.

3.3.2. Effect of Ls.Cr on ileum preparation of different animals

In mouse ileum, the seed extract of *Lepidium sativum* caused a stimulatory effect at 0.03–1 mg/mL in isolated mouse ileum with efficacy of 22.3 ± 1.1% (n = 5) of the ACh maximum obtained at 3 μM (Fig. 2D). Pretreatment of the tissue with atropine (0.1 μM) blocked the spasmogenic effect of Ls.Cr, while hexamethonium (0.3 mM), pyrilamine (1 μM) or SB203186 (1 μM) pretreatment showed no influence (Fig. 2D). The values presented in Fig. 2D are mean ± S.E.M. of 4–6 preparations obtained from 3 animals.
In guinea-pig ileum, the plant extract exhibited concentration-dependent (0.01–1 mg/mL) contractions with efficacy of 65.8 ± 2.7% (n = 6) of ACh maximum. Pretreatment of the tissue with atropine (0.1 μM), but not with hexamethonium, pyrilamine or SB203186, abolished the spasmodic effect of Ls.Cr (Fig. 2E). The values shown in Fig. 2E are mean ± S.E.M. of 4–6 preparations obtained from 3 animals.

In rabbit ileum, Ls.Cr also showed a concentration-dependent (0.1–3 mg/mL) stimulatory effect with efficacy (98.8 ± 0.9%) similar to that of ACh (p > 0.05). Pretreatment of the tissue with atropine (0.1 μM) partially blocked the spasmodic effect at higher concentration (p < 0.001), while pretreatment of tissues with hexamethonium, pyrilamine or SB203186 had no effect (p > 0.05) as shown in Fig. 2F. The values presented in Fig. 2F are mean ± S.E.M. of 5–7 preparations obtained from 4 animals.

4. Discussion

Keeping in view the medicinal use of Lepidium sativum in gut disorders, such as indigestion and constipation, its seed extract was tested in mice, where it propelled charcoal meal through the small intestine and increased the production of wet feces, hence showing prokinetic and laxative activities, similar to the effect of carbachol, a standard cholinergic agonist and accelerator of intestinal contraction (Brown and Taylor, 2006). These gut stimulatory actions of the extract were found partially sensitive to atropine, a muscarinic receptor blocker (Gilani et al., 1997), indicating the presence of some ACh-like component(s) in addition to other gut stimulant constituent(s). ACh is a neurotransmitter of the parasympathetic nervous system and is known to cause gastrointestinal stimulation through the activation of muscarinic receptors (Brown and Taylor, 2006), hence, the presence of ACh-like constituents explains its medicinal use in constipation and as digestive aid.

To further study the possible mode of the observed prokinetic and laxative properties of the extract, we used isolated jejunal and ileum preparations from different animals, such as mouse, guinea-pig and rabbit. In the gut preparations from mouse and guinea-pig, though, efficacy for the spasmodic effect of the plant extract varied, but the stimulant effect was completely blocked by atropine in both preparations, thus, showing a common mechanism, through cholinergic action. However, when tested in the gut preparations of rabbit, the stimulant effect in jejunum was not blocked by any of the antagonists studied including pyrilamine, a histaminic type-1 (H₁) receptor blocker (Sharif et al., 1994) or hexamethonium, a ganglion blocker (Wien et al., 1952) or SB203186, a serotoninergic receptor antagonist (Sander-Bush and Mayer, 2006), while atropine caused a partial blockade in ileum, but no effect on jejunum. This indicates that the rabbit tissue behaves differently from those of mouse and guinea-pig in the nature of gut stimulant effect, and clearly suggesting some additional mechanism(s), independent of histamine, nicotine or 5-hydroxytryptamine (5-HT, serotonin) receptors activation.

Taken together, these results demonstrate the presence of atropine-sensitive stimulatory constituent(s) in Lepidium sativum observed in all tissues except rabbit jejunum, and atropine-insensitive observed in both tissues of rabbit. The spasmodic effect of Ls.Cr on rabbit tissues seems involving more than one stimulant components, hence, showed the highest efficacy. Other mechanisms known for their gut stimulant property, which have not been ruled out in this study includes certain prostaglandins (Beubler and Kollar, 1988), platelet activating factor (Izzo et al., 1998), nitric-oxide-donating or releasing compounds (Mascolo et al., 1994), dopaminergic antagonists (Briere et al., 1995), cholecystokinin (Briere et al., 1999) and tachykinins (Severini et al., 2002).

Collectively, the data in jejunum and ileum preparations of mouse, guinea-pig and rabbit indicate a species and tissue-selective gut stimulatory effect of Lepidium sativum extract, which was evident in the following ways: (1) in rabbit jejunum preparations, the stimulatory effect of the crude extract was insensitive to atropine and was greater (p < 0.01) than the achieved effect of Ls.Cr on all other tissues, showing partially or fully atropine sensitive effect and (2) in ileum preparations, the stimulatory effect of the extract was partially sensitive to atropine and was more efficacious (p < 0.001) in rabbit as compared to its observed atropine-sensitive effect in guinea-pig and mouse. In different used animals, the efficacy of Ls.Cr for its stimulant effect was as rabbit > guinea-pig > mouse.

In imparting its spasmodic effect, Ls.Cr was found more efficacious in rabbit and guinea-pig ileum, while in mouse, it was more effective in jejunum. Such types of tissue or species-selective effects have also been noticed in various earlier studies with other plant extracts (McLeod et al., 1994; Ghayur and Gilani, 2005; Ghayur et al., 2005; Mehmoond and Gilani, 2010; Mehmoond et al., 2010). Based on these observations, a suggestion can be made that when studying the gut stimulatory effect of the plant materials using gut tissues from more than one species may be advisable to know the broader picture.

The presence of saponins and anthraquinones amongst the chemical constituents, which are known for their spasmodic effect (Akah et al., 1997; Pasricha, 2006), may explain the gut stimulant actions of Lepidium sativum.

5. Conclusion

This study shows that the prokinetic and laxative activities of Lepidium sativum in mice are partially mediated through muscarinic receptors, and the in vitro findings demonstrated the species and tissue-selective spasmodic effect mediated through a similar mechanism. Thus, this study provides sound mechanistic basis for the medicinal use of Lepidium sativum in gut disorders, such as indigestion and constipation, and the observed species and tissue-selective effect in this study suggest using more than one species for a better picture of the pharmacological profile when studying plant extracts.

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