Antinociceptive activity of aqueous extract of *Lepidium sativum* L. in mice

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Abstract. In the present study the aqueous extract of *Lepidium sativum* L. (family: Brassicaceae) was investigated for possible antinociceptive effect in Swiss - albino male mice. In this experiment three groups of male mice were used (n=6). Two models were used to study the effects of the extracts on nociception, acetic acid-induced writhing test and hot plate test in mice. *Lepidium sativum* L. extract was administered in the dose of 20 mg/kg orally 30 minutes prior to pain induction. The aqueous extract showed significant (p<0.05) analgesic activity evidenced by increase in the reaction time by hot plate method and significant (p<0.05) reduction in acetic acid - induced writhings in mice with a maximum effect of 27.00% reduction. These effects were compared with the control and standard drug, diclofenac sodium (50 mg/kg, p.o).

The results indicate that aqueous extract of *Lepidium sativum* L. possesses a significant antinociceptive activity in central and peripheral pain models in mice and therefore, it can be used as supplemental therapy in acute or chronic pain conditions.

Key words: Antinociception, aqueous extract, *Lepidium sativum* L., Hot plate, Writhing test

1. Introduction

Natural products of plant origin as alternative sources of drugs are still a major part of traditional medical systems in developing countries. In developed countries as well, the use of traditional plant extract in the treatment of various diseases has been attracting more attention because of its minor side effects (1). *Lepidium sativum* L. known as pepper grass or garden cress belongs to the family Brassicaceae (Cruciferae). Garden cress is an annual erect herbaceous plant, growing up to 30 cm that is native to Egypt and west Asia but is widely cultivated in temperate climates throughout the world (2). This plant is known to contain phenolic compounds, with chlorogenic acid as predominant component (3). Phytochemical screening of *Lepidium sativum* L. seeds revealed presence of triterpenes, alkaloids, flavanoids, tannins, coumarins and saponins (4). In Turkish folk medicine *Lepidium sativum* L. or ‘tere otu’ is used as digester, carminative and appetizer (5) and it is known to be useful in therapy of hemorrhoids (6). Previous studies have demonstrated the protective effect of *Lepidium sativum* L. against carcinogenic compounds (7) and growth inhibition of antibiotic- resistance strain of *Pseudomonas aeruginosa* (8). In another study conducted on asthmatic subjects it was demonstrated that four week long treatment with *Lepidium sativum* L. seed powder statistically significantly improved various parameters of pulmonary function (9). The aqueous extract of *Lepidium sativum* L. has been reported to exhibit a potent hypoglycemic activity in normal and streptozotocin induced diabetic rats (10). Hypoglycemic activity of this plant can be explained by a potent inhibition of renal glucose reabsorption (11). It is also shown that aqueous extract of *Lepidium sativum* L. exhibits antihypertensive and diuretic activities (12). Extensive ethnobotanical surveys conducted in Morocco revealed wide usage of garden cress seeds in the management of hypertension (13) as well as in diabetes and renal disease (14). *Lepidium sativum* L. is a component of Sudard, poly-herbal formulation containing extracts of 11 medicinal plants used in the ayurvedic system of...
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medicine for the treatment of inflammation and pain associated with rheumatoid arthritis which showed good anti-inflammatory, anti-arthritic and analgesic activities in the experimental animal models (15). The present study was to investigate the antinociceptive activity of aqueous extract of Lepidium sativum L. using hot plate and acetic acid-induced writhing tests.

2. Materials and methods

2.1. Animals

The study was carried out on Swiss - albino male mice (30-40 g), maintained under standard laboratory conditions of food and water. Animals were housed at room temperature of 24±1°C with 12h light / dark cycle. The animals were housed in groups for a minimum of 3 days prior to pharmacological experiment. The experimental protocols have been approved by the Local Ethical Committee on Animal Experimentation of the Yuzuncu Yil University, Van, Turkey. The minimum number of animals and duration of observation required to obtain consistent data were employed. “Principles of laboratory animal care” (NIH publication number 85–23, revised 1985) guidelines were followed. After experiment was completed animals were kept under observation for 7 days for acute or sub acute toxicity.

2.2. Plant material

Specimens of garden cress (Lepidium sativum L.) were collected from gardens in Van region (eastern Turkey) in June 2009. The taxonomical identity of plant was confirmed in Faculty of Biology, Yuzuncu Yil University, Van. Aerial parts of plants were dried in shed at room temperature, ground and kept in amber glass bottles.

2.3. Preparation of the aqueous extract

1 g of powdered plant was extracted with 100 ml distilled hot water (72°C) for 30 min. The aqueous extract was then filtered, concentrated under vacuum and finally freeze-dried at -40°C. The yield of this process was 40.8 %. This extract was dissolved in distilled water just before use and given orally to mice at a dose of 20 mg/kg body weight. This dose was chosen as a therapeutical dose for Lepidium sativum L. aqueous extract based on literature (11, 12, 16).

2.4. Experimental design

Swiss albino mice were randomly divided into three groups of 6 animals each. Group I served as control (normal saline 0, 2 ml per animal, orally), group II was given diclofenac sodium (50 mg/kg, p.o) as standard drug (17) and group III was treated with test drug (20 mg/kg, p.o). All drugs were administrated orally, half an hour before the onset of pain stimulus in different models of nociception in albino mice.

2.5. Hot plate test

Hot Plate analgesia meter (Commat Ltd., Turkey) was used to determine the central component of nociception. Mice were placed individually on a hot plate set to 52.5 ± 0.5 °C and the time between placement of the mouse on the platform and shaking or licking of the paws or jumping was recorded as the reaction time or latency of the pain response. In order to avoid the damage to the paws of the animals, the time standing on the plate was limited to 30 sec (cut-off time). Hot plate test was performed on all animals individually in 30th, 45th and 60th minutes after treatment.

2.6. Writhing test

Acetic acid – induced writhing test was used to evaluate the antinociceptive activity against chemical noxious stimulus and peripheral analgesic activity of herbal extract. Abdominal contractions were induced by 0.6 % acetic acid solution (15 ml/kg, i.p.) in mice pretreated with normal saline, diclofenac sodium or aqueous extract of Lepidium sativum L. Five minutes after the injection of acetic acid, the number of abdominal contractions and stretches during the following 10 min was counted. Writhing movement was accepted as contraction of the abdominal muscles together with stretching of the hind limbs. Antinociceptive effect was expressed as the reduction of the number of writhing between control and pretreated mice (18). The percentage of the inhibition of writhes was calculated as:

\[
\% \text{Inhibition of writhes} = \frac{(\text{Control mean} - \text{Test mean})}{\text{Control mean}} \times 100.
\]

2.7. Statistical analysis

Experimental data from hot plate and acetic acid-induced writhing tests were expressed as mean ± SEM. Differences between given sets of data were considered to be statistically significant when p value was less than 0.05. Results were statistically evaluated using Kruskal-Wallis and Mann-Whitney U test.

3. Results

Aqueous extract of Lepidium sativum L. in dose of 20 mg/ kg body weight applied per oral, showed significant antinociceptive activity in both models used in this study. The results of hot plate test and acetic acid induced writhing test are shown in Table 1 and 2, and in Figure 1.
Table 1. Effects of aqueous extract of *Lepidium sativum* L. on hot plate response in mice

<table>
<thead>
<tr>
<th>Time after treatment</th>
<th>CG Mean ± SEM</th>
<th>DS Mean ± SEM</th>
<th>LS Mean ± SEM</th>
</tr>
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<tbody>
<tr>
<td>30 min</td>
<td>8.32 ± 1.77\textsuperscript{b}</td>
<td>12.07 ± 2.42\textsuperscript{a}</td>
<td>7.93 ± 1.75\textsuperscript{b}</td>
</tr>
<tr>
<td>45 min</td>
<td>7.68 ± 2.02\textsuperscript{a1}</td>
<td>12.28 ± 2.19\textsuperscript{a1}</td>
<td>10.15 ± 3.15\textsuperscript{b1}</td>
</tr>
<tr>
<td>60 min</td>
<td>7.28 ± 2.12\textsuperscript{a2}</td>
<td>11.92 ± 1.91\textsuperscript{a2}</td>
<td>10.95 ± 2.64\textsuperscript{b2}</td>
</tr>
</tbody>
</table>

Different lower cases represent different group means, CG =Control group, DS =Treated with diclofenac sodium, LS=Aqueous with *Lepidium sativum* L., \textsuperscript{a}Statistically different from CG and LS groups, p<0.05, \textsuperscript{a1}Statistically different from CG and LS groups, p<0.01, \textsuperscript{a2}Statistically different from CG and LS groups, p<0.01, \textsuperscript{b}Statistically different from CG and DS groups, p<0.05.

Fig. 1. Effects of aqueous extract of *Lepidium sativum* L. on hot plate response in mice.

CG=Control group, DS=Positive control group, treated with diclofenac sodium, LS=Aqueous extract of *Lepidium sativum* L., \textsuperscript{a}Statistically different from CG and LS groups, p<0.05, \textsuperscript{a1}Statistically different from CG and LS groups, p<0.01, \textsuperscript{a2}Statistically different from CG and LS groups, p<0.01, \textsuperscript{b}Statistically different from CG and DS groups, p<0.05.

In the hot-plate test the extract considerably increased the animal’s reaction time to the heat stimulus. Values were found to be significant (p<0.05) at 60 min after treatment with 20 mg/kg of *Lepidium sativum* L’s aqueous extract. Values measured at 30 and 45 minutes after extract was given were found statistically insignificant.

Acetic Acid-induced Writhing: The extract decreased the number of acetic acid induced abdominal constrictions in mice and the values were found to be significant (p<0.05) at dose tested. Percent decrease, compared to control was 27% (Table 2).

As expected, diclofenac sodium in dose 50 mg/kg showed both peripheral and central antinociceptive action. In hot plate test diclofenac sodium showed significant elevation in pain threshold in comparison to control and indicated significant antinociceptive activity 15, 30 and 60 minutes after application (Table 1 and Figure 1). It also inhibited the acetic acid-induced writhing significantly (p<0.005) (decrease compare to control: 76, 32 %), as shown in Table 2. In three out of six animals in DS group, diclofenac sodium totally inhibited writhing behavior.

No toxicity or mortality was observed during observation period of seven days after the completion of experiment.

Table 2. Effects of aqueous extract of *Lepidium sativum* L. on acetic acid-induced writhing test in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>No of writhing (mean ± SEM)</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>19.00 ± 1.89\textsuperscript{a}</td>
<td>/</td>
</tr>
<tr>
<td>DS</td>
<td>4.50 ± 1.65\textsuperscript{c}</td>
<td>76,32</td>
</tr>
<tr>
<td>LS</td>
<td>13.87 ± 4.26\textsuperscript{b}</td>
<td>27,00</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Statistically different from control group, p<0.01  
\textsuperscript{b}Statistically different from diclofenac sodium and control groups, p<0.05
4. Discussion

Pain is known as one of most common healthcare problems. In a survey conducted in 15 European countries it was shown that chronic pain of moderate to severe intensity occurs in 19% of adult Europeans, seriously affecting the quality of their social and working lives (19). Although pharmacological pain management provides significant relief in several pain-related diseases, many patients turn to alternative medicine in order to avoid serious and commonly seen side effects of conventional drugs. Herbal based drugs used in pain therapy can contribute to restore the quality of life to a patient and may effect and enhance conventional pain management (20).

This study was designed to investigate potential antinociceptive effects of aqueous extract of Lepidium sativum L. aerial parts using hot plate test and acetic acid-induced writhing test. These two tests were selected in order to investigate both centrally and peripherally mediated effects of nociception. The above study showed that the Lepidium sativum L. aqueous extract at the dose tested (20 mg/kg) produced analgesia, both centrally and peripherally.

The hot plate test is a specific central antinociceptive test (21,22). In the present study, it was demonstrated that the animals treated with the extract did show increase in their response latency period in comparison with the control group at 60th minute after administration. It suggested that the extract might effect through central opioid receptors or promoted release of endogenous opiopeptides.

The acetic acid writhing assay is useful for evaluation of mild analgesic non-steroidal anti-inflammatory compounds (23). Writhing model is a sensitive test widely used for the evaluation of peripheral antinociceptive activity (24, 25). Raval and Ravishankar (26) reported that abdominal constrictions produced during the administration of acetic acid are related to sensitization of the analgesic receptors to prostaglandins. It is therefore possible that the extract of Lepidium sativum is effective due to its analgesic effect, probably by inhibiting the synthesis or action of prostaglandins.

Although analgesic effect demonstrated by Lepidium sativum L. is not comparable with effect of diclofenac sodium (p<0.05), the results support the traditional use of this plant in some painful conditions as supplemental therapy. The exact mechanism of action is not known at this stage; however, antinociceptive activity of this extract can be related with the chemicals such as triterpenes, alkaloids, flavonoids and phenolic compounds reported in the phytochemical screening of Lepidium sativum L. (2,3,4). In order to reveal exact mechanism of action and optimal dose range for Lepidium sativum L. aqueous extract further investigation will be performed.

References


