Antiinflammatory and Analgesic Effects of Phlomis lanceolata Boiss. and Hohen. Extracts and Examination of their Components

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Abstract: The purpose of this investigation was to study the anti-inflammatory and analgesic properties of total extract and four fractions (ether, ethyl acetate, n-butanol and water) from Phlomis lanceolata (Lamiaceae) in mice. The plant material was extracted with methanol. In order to estimate the polarity of the active compounds, the total extract was dissolved in water and the water soluble portion was successively partitioned between ether, ethyl acetate and n-butanol. The total extract and four fractions were analyzed by Thin Layer Chromatography (TLC) by use of specific reagents. Dose of 100 mg kg\(^{-1}\) of each extracts were used in carrageenan-induced paw edema, formalin and writhing nociception tests in mice. All compounds reduced paw edema in comparison to the control group at 1, 3, 5 and 7 h post carrageenan injection. The total, ether and aqueous extracts were similar to indomethacin while the ethyl acetate extract was weaker than indomethacin in reduction of paw edema. All extracts induced antinociception in both phases of formalin test. The total and ether extracts were as potent as indomethacin in both phases of formalin test. The ethyl acetate extract was weaker than indomethacin in the second phase of formalin-test while the n-butanol and aqueous extracts showed more antinociception than indomethacin in the second phase of formalin test. All extracts as well as indomethacin induced antinociception in writhing test in comparison to control. The total and aqueous extracts induced the same antinociception as indomethacin while ether, ethyl acetate and n-butanol showed weaker antinociception than indomethacin. Positive results for iridoids and phenolic compounds were indicated by phytochemical analysis of total extract. Phenolic compounds were found in all fractions whereas only n-butanol and aqueous fractions showed positive results for iridoid glycosides. The higher antinociceptive effects of n-butanol and aqueous extracts in the inflammatory phase of formalin test among different extracts tested, might back to the presence of iridoid glycosides, phenolic glycosides or other glycosides. These data suggest that different extracts of P. lanceolata produce different antinociceptive activities that could be due to the effect of one or a combination of the bioactive components in each extract.

Key words: Phlomis lanceolata, lamiaceae, anti-inflammatory, analgesia, antinociception, mouse

INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Herbal therapy is used to treat a large variety of ailments and symptoms, e.g. inflammation, fever and pain; however, there is not adequate experimental evidences about their effectiveness\(^{1,4}\). The genus Phlomis (Lamiaceae) is represented by 17 species in flora Iranica with ten species of this genus are endemic including P. lanceolata found only in Iran\(^{9}\). Some species of Phlomis have been used in folk medicine as stimulant, tonic\(^{9,10}\), wound healing\(^{10}\) and pain relief\(^{9}\). There are evidences indicating various activities such as anti-inflammatory, immunosuppressive, antmutagenic\(^{9}\), free radical scavenging\(^{9}\), antimicrobial\(^{4}\), antinociception\(^{12}\) and anti-ulcerogenic\(^{13}\) for Phlomis. Plants belonging to the genus Phlomis have been shown to contain different classes of glycosides comprising iridoids, flavonoids, phenylpropanoids,

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phenylethanoids and diterpenoids. The group of flavonoid is famous for its anti-inflammatory, anti-allergic, antithrombotic, vasoprotective, inhibition of tumor promotion and protection of gastric mucosa properties. These properties have been attributed to influence of flavonoids on production of prostaglandins and their antioxidant effects.[14] Some phenylpropanoid glycosides are known to possess diverse biological properties including cytotoxic, cytostatic, anti-inflammatory, antinociceptive, immunosuppressant and antimicrobial effects.[15] Many of iridoid glycosides isolated from plants showed significant biological activities, e.g. choleretic, purgative, hepatoprotective, vasoconstrictor, analgesic, sedative, anti-inflammatory and antimicrobial activities.[16] Recently, free radical scavenging effects of phenylpropanoid glycosides have been reported from some species of Phlomis.[17] Till now, P. lanceolata has not been the subject of any pharmacological research. This study describes the first investigation in pharmacological activity on this plant.

MATERIALS AND METHODS

Plant material: The fresh aerial parts of Phlomis lanceolata Boiss. and Hohen were collected during the flowering stage from Ardebil province (northwest of Iran) in June 2002. Voucher specimen [No. 6535 THE] has been deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. The plant material was air dried, powdered and extracted twice with methanol (80%) in percolator. The combined methanol extracts were evaporated to dryness under reduced pressure to give solid residues. The percentage yield on dried starting material was 15% (w/w) for dried hydroalcoholic extract of P. lanceolata. In order to estimate the polarity of the active compounds, the total extract was dissolved in water and the water soluble portion was successively partitioned between ether, ethyl acetate and n-butanol (yields 0.83, 1.19, 3.14 and 8.55% w/w, respectively). The total extract and four fractions were analyzed by Thin Layer Chromatography (TLC) using specific reagents.[17,18]

Animals: Male albino N-MRI mice from animal house of Faculty of Pharmacy, TUMS weighting 20-30 g were kept under standardized conditions (temperature 22±2°C, normal lighting) and fed with the normal laboratory diet. The protocol of study was approved by TUMS ethical committee.

Administration of reference and test compounds: Each drug was dissolved in appropriate solvents as follows: indomethacin (5 mg kg⁻¹, i.p[19] as a reference drug and extracts were dissolved in 2.5% v/v Tween 80 in water-DMSO (10:0.5), sonicated for 10 min at room temperature and administered intraperitoneally (i.p.). Carrageenan (Sigma) 3.5% w/v[20] was dissolved in normal saline and formalin (Merek) 0.5%[21] dissolved in water. Control animals received 2.5% v/v Tween 80 in water-DMSO (10:0.5) only as vehicle. Acetic acid 0.7% was prepared, in water to induce abdominal constriction in mice[22,23]. The dose 100 mg kg⁻¹ of each extract was prepared and administered[24]. All treatment compounds were diluted in a way to obtain an injection volume of 10 mL kg⁻¹ (animal weight) and administered i.p.

Anti-inflammatory activity:
Carrageenan-induced paw edema: Paw edema in mice was induced by injection of 0.05 mL of carrageenan (3/5% w/v)[25]. Indomethacin (5 mg kg⁻¹, i.p.) and the total, ether, ethyl acetate, n-butanol and aqueous extracts (100 mg kg⁻¹, i.p.) were administered 30 min before subplantar injection of the edematosus agent. The control group received only the carrageenan. The paw diameter was measured at intervals of 1, 3, 5 and 7 h using a Colis (Helios, Germany) after carrageenan injection[26]. The difference between the left and the right paw diameters (indicating the degree of inflammation) was determined in comparison to control animals.

Analgesic activity:
Formalin test: In this test, the time (seconds), which each mouse spent licking its posterior left paw after subplantar injection of formalin (0.5%, 25 µL) was considered as response to pain[27]. Immediately after formalin injection, animals were placed individually in a glass cylinder (20 cm wide, 25 cm long) on a flat glass floor and a mirror was arranged at the angle of 45°C under the cylinder to allow clear observation of the paws of the animals. The reaction time to pain was measured with a chronometer during the first phase (0-5 min, neurogenic pain) and during the second phase (10-30 min, inflammatory pain). Indomethacin (5 mg kg⁻¹, i.p.) and dose 100 mg kg⁻¹ of each extract (total, ether, ethyl acetate, n-butanol and aqueous) were administered 30 min before formalin injection. Control animals received only the vehicle used to dilute the substances.

Acetic acid-induced abdominal writhing: In this test, each mouse was received 0.7% aqueous solution of acetic acid (10 mg kg⁻¹, i.p.) and then individually housed in a glass cylinder on a flat glass floor and a mirror glass was arranged at an angle of 45°C under the cylinder. Nociception was recorded by counting the number of
writhes after injection of acetic acid during 30 min. A writhes is indicated by abdominal constriction and stretching of at least one hind limb\(^{[22-24]}\). Indomethacin (5 mg kg\(^{-1}\), i.p.) and dose 100 mg kg\(^{-1}\) of each extract (total, ether, ethyl acetate, \(n\)-butanol and aqueous) were administered 30 min before acetic acid injection. Control animals received only the vehicle used to dilute the substances.

### Statistical analysis

Comparison between groups was made by one-way Analysis of Variance (ANOVA) followed by Tukey's multiple comparison test using GraphPad software. Differences with \(p<0.05\) between experimental groups were considered statistically significant.

### RESULTS AND DISCUSSION

From Table 1, it appears that all compounds reduced paw edema in comparison to the control group at 1, 3, 5 and 7 h post carrageenan injection significantly. Data indicate that total, ether and aqueous extracts are similar in reduction of edema when compared to indomethacin. The ethyl-acetate extract was weaker than indomethacin in reduction of paw edema.

All extracts as well as indomethacin induced antinociception in both phases of formalin test in comparison to control. Data indicate that total and ether extracts are as potent as indomethacin in reduction of formalin-induced nociception in first and second phases. The ethyl acetate extract was weaker than indomethacin in the second phase of formalin-test. The \(n\)-butanol and aqueous extracts showed more antinociception than indomethacin in the second phase of formalin test (Table 2).

All extracts as well as indomethacin induced antinociception in writhing test in comparison to control. The total and aqueous extracts induced the same antinociceptive effect as indomethacin while ether, ethyl acetate and \(n\)-butanol showed weaker antinociception than indomethacin (Table 3).

Positive results for iridoids and phenolic compounds such as flavonoids and phenylpropanoids was indicated by phytochemical analysis using TLC and specific reagents for total extract. Phenolic compounds found in four fractions whereas only butanol and aqueous fractions showed positive results for iridoid glycosides.

Results of the present study confirmed analgesic and anti-inflammatory potency of *P. lanceolata* extracts on various models tested in mice with some minor variations comparable to indomethacin. The ethyl acetate extract showed less anti-inflammatory effects than indomethacin in carrageenan test and second phase (inflammatory) of formalin test and also less visceral antinociception in abdominal writhing test. The \(n\)-butanol and aqueous extracts showed more antinociception than indomethacin in the second phase of formalin test. Several mediators such as histamine, serotonin, kinin, acetycholine, substance P, prostaglandins and leukotrienes play role in induction of pain and inflammation\(^{[22,25,26]}\). Based on bibliography, no study has been conducted on interactive effects of *P. lanceolata* with these substances and its mechanism of action remains to be elucidated by further studies. Supporting the present results, our previous study indicated antinociceptive effects of *Phlomis olivieri* Berth., *Phlomis anisodontia* Boiss. and *Phlomis persica* Boiss. total extracts on mouse writhing test\(^{[12]}\).

The present phytochemical analysis using TLC and specific reagents indicated positive results for iridoids.
and phenolic compounds such as flavonoids and phenylpropanoids for total extract. Phenolic compounds were found in four fractions whereas only n-butanol and aqueous fractions showed positive results for iridoid glycosides. Some phenylpropanoid and iridoid glycosides isolated from species of Phlomis and other plants showed antinociceptive and anti-inflammatory effects. Some of them inhibited the action of cyclooxygenase-2 (COX-2) enzyme and decreased the production of prostaglandins. Evaluation of anti-inflammatory activity of phenylpropanoid glycosides e.g. acacetin and forsythoside-B showed that these compounds have higher inhibitory potencies on COX-2 than on COX-1. These results are interesting since COX-2 is mainly associated with inflammation and the COX-1 inhibition cause side effects which are often observed with non-steroidal anti-inflammatory drugs (NSAID). On the other hand iridoid glycosides such as logamin, aucubin and verberanil showed anti-inflammatory activity in carrageenin-induced mouse paw edema and some iridoids e.g. agnuside showed analgesic effect in writhing test and anti-inflammatory activity that could be related to inhibition of COX-2 enzyme. In addition, some flavonoids have shown inhibition of COX-2 enzyme expression. Higher antinociceptive effects of n-butanol and aqueous extracts among different extracts tested, in the inflammatory phase of formalin test might back to presence of iridoid glycosides. However, this study does not establish the mechanism(s) by which different extracts of P. lanceolata exhibit antinociception in tested models but confirms the analgesic and anti-inflammatory properties of different extracts of P. lanceolata comparable to indomethacin as a reference drug and suggests a good future for them in therapy of pain in human. Differences in antinociceptive effects observed by different extracts could be due to the effect of one or a combination of the bioactive components in each extract. Further studies shall aim at isolating, characterizing and purifying compounds in each fraction with potential bioactive properties. In addition, studies on the mechanism of actions of isolated compounds will be necessary.

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REFERENCES