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Comparison of Antinociceptive Effects of Total, Water, Ethyl Acetate, Ether and *n*-butanol Extracts of *Phlomis anisodonta* Boiss. and Indomethacin in Mice

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Abstract: The objective of the present study was to evaluate the anti-inflammatory and analgesic activities of the crude and fractioned extracts (ether, ethyl acetate, *n*-butanol and water) of *Phlomis anisodonta* (Lamiaceae) in the models of carrageenan-induced paw edema and acetic acid-induced writhing in mice. Dried powdered material from the aerial parts of *Phlomis anisodonta* was extracted with methanol (80%) twice. The methanol extract was separated into four fractions: after drying the methanol extract, the solid residue was dissolved in water and the water-soluble portion was successively partitioned to produce the above-mentioned fractions. The total extract, ether fraction and ethyl acetate fractions significantly reduced the number of writhings compared to the control group. Water and *n*-butanol fractions showed no analgesic activity. Neither the total extract nor four fractions were more effective than indomethacin in acetic acid-induced test. In carrageenan-induced inflammation test, only water and ethyl acetate fractions showed antiinflammatory activity compared to the control group. The total extract, *n*-butanol and ether fractions showed no antiinflammatory activity. Neither the total extract nor four fractions were more effective than indomethacin. Different types of glycosides found in the genus *Phlomis* could be responsible for observed effects. Variations in antiinflammatory and analgesic effects of different fractions of this plant might be attributed to the fact that different fractions of *P. anisodonta* contain active compounds in variable ratios and activities. A comprehensive analysis for determination and quantification of these compounds is required).

Key words: *Phlomis anisodonta* Boiss., Lamiaceae, inflammation, nociception, pharmacology

INTRODUCTION

Studies on some species of *Phlomis*, including *P. anisodonta* extracts, showed antinociceptive effects and other potential therapeutic effects (e.g., treatment of diseases of the respiratory tract and diabetes)^[1-4]. Some compounds isolated from this genus showed significant biological activities, e.g., antiinflammatory, antinociceptive^[1], cytotoxic^[5], antimicrobial^[6], free radical scavenging^[4]; however, there is not adequate experimental evidence about their effectiveness^[7-10].

The genus *Phlomis* (Lamiaceae) is represented by 17 species in flora Iranica in which ten species of this genus are endemic including *P. anisodonta*^[11].

Plants belonging to the genus *Phlomis* contain different classes of glycosides comprising iridoids, flavonoids, phenylpropanoids, phenylethanoids and diterpenoids^[2,5,12].

Some phenylpropanoid glycosides are known to possess diverse biological properties including cytotoxic, cytostatic, antiinflammatory, antinociceptive, immunosuppressant and antimicrobial effects^[3]. Many of iridoid glycosides isolated from plants indicated significant biological activities, e.g., choleric, purgative, hepatoprotective, vasoconstrictor, analgesic, antiinflammatory and antimicrobial activities^[2]. Recently, free radical scavenging effects of phenylpropanoid glycosides have been reported from some species of

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Phlomis^[3]. Regarding above-mentioned points, the present study was designed in order to evaluate analgesic and antiinflammatory activities of different fractions from *P. anisodonta* in mouse writhing nociception and rat carrageenan inflammation tests.

MATERIALS AND METHODS

Plant material: Aerial parts of *Phlomis anisodonta* were collected during the flowering stage from Mazandaran province (north of Iran) in June 2002. A Voucher specimen [No. 6531 THE] has been deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences. To prepare the extract from *P. anisodonta*, the plant material was air dried, powdered and extracted twice with methanol (80%) in percolator. The combined methanol extracts were evaporated and allowed to dry under reduced pressure to give a solid mass. The percentage yield on dried starting material was 30% (w/w) for dried hydroalcoholic extract of *P. anisodonta*. The crude extract was dissolved in water and the water soluble portion was successively partitioned between ether, ethyl acetate and *n*-butanol (yields 10.49, 2.86, 4.89 and 11.76%, respectively). Analysis of the crude extract and four fractions of *P. anisodonta* has been reported by our team previously^[1].

Animals: Male albino *n*-MRI mice (weighing 20-30 g) from animal house of School of Pharmacy, TUMS 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: Indomethacin (5 mg kg⁻¹) and the 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^[13]. Carrageenan and acetic acid were administered according to methods^[14,15]. The dose 100 mg kg⁻¹ of each extract was prepared and administered^[1]. All treatment compounds were diluted in a way to obtain an injection volume of 10 mL kg⁻¹ (animal weight) and administered intraperitoneally (i.p.).

Antinociceptive activity

Acetic acid-induced abdominal writhing: Writhing behavior was tested, in which 0.7% acetic acid solution (10 mg kg⁻¹ body weight) was injected 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° under the cylinder. The number of squirms and stretching was

counted for 30 min as previously reported^[16,17]. 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.

Anti-inflammatory activity

Carrageenan-induced paw edema: Paw edema in mice was induced by injection of 0.05 mL of carrageenan 1% w/v. Indomethacin, the total extract and four fractioned parts (ether, ethyl acetate, *n*-butanol and aqueous fractions) were administered 30 min before subplantar injection of the edematous agent to the right paw of the mouse. The control group received only the carrageenan. The paw diameter was measured at intervals of 0, 1, 2, 3 and 4 h using a Colis (Helios, Germany) after carrageenan injection. The difference between this value at the time zero and another four time points was calculated (indicating the degree of inflammation) and was compared to the amount for control group^[18].

Statistics: The SPSS statistical software package version 10 was used for statistical analysis. Data were analyzed using Student's t-test. Results are expressed as mean±SEM. p<0.05 is considered statistically significant.

RESULTS

Effect of *P. anisodonta* on squirms in acetic acid-induced writhing test:

The number of squirms after acetic acid injection and administration of the total extract and four fractions (for 30 min) is shown in Fig. 1.

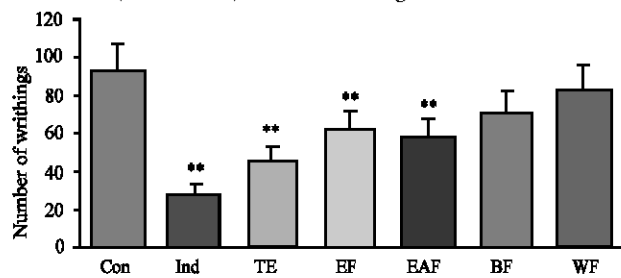


Fig. 1: Effects of *P. anisodonta* Boiss. different extracts and indomethacin on acetic acid-induced writhing test in mice. Total Extract (TE), Ether Fraction (EF), Ethyl Acetate Fraction (EAF), *n*-Butanol Fraction (BF), Water Fraction (WF) and Indomethacin (Ind). Each column represents the mean±SE of six animals. ** represent that the differences between control and treated groups is significant at p<0.01. The difference between Ind and all treated groups are significant (p<0.05) meaning that TE, EF, EAF, BF and WF are less effective than Ind

The total extract, ether fraction and ethyl acetate fractions significantly reduced the number of writhings compared to the control group ($p < 0.01$). Water and *n*-butanol fractions showed no analgesic activity. Neither the total extract nor four fractions were more effective than indomethacin

Effect of *P. anisodonta* on paw diameter in carrageenan-induced inflammation test: In the Table 1, data for anti-inflammatory activity of the total extract and four fractions in carrageenan-induced paw edema are shown. The right paw diameter after different time intervals was used as criteria for evaluation of inflammation. Generally, data indicate that only water and ethyl acetate fractions possessed antiinflammatory activity compared to the control group. The total extract, *n*-butanol and ether fractions showed no antiinflammatory activity. In this regard, neither the total extract nor four fractions were more effective than indomethacin.

DISCUSSION

Generally, from the experiments performed in this study, analgesic and antiinflammatory activities from *P. anisodonta* are expectable. This is probably the first experimental evidence for antiinflammatory effect, although antinociceptive effects have been reported for some species of *Phlomis* (e.g., *P. olivieri* Benth. and *P. persica* Boiss.). This study confirmed the previously reported antinociceptive effect for *P. anisodonta* Boiss.^[1] Furthermore, neither the total extract nor four fractions were more effective than indomethacin as a standard drug in both tests performed. In this study, no phytochemical analysis was done to identify (or quantify) active ingredients in the extract and four fractions separately. However, glycosides like diterpenoids, iridoids, phenylpropanoids, phenylethanoids and flavonoids have been identified from the genus *Phlomis*^[19]. It seems that among the substances identified, mainly phenylpropanoid

and iridoid glycosides possess antinociceptive and anti-inflammatory activities^[3,4] and their presence was proved using a preliminary analysis in total extracts of some *Phlomis* like *P. anisodonta*^[1]. Some other biological effects have been described for these two types of glycosides^[3]. Evaluation of antiinflammatory activity of phenylpropanoid glycosides e.g., acetoside and forsythoside-B has shown their higher inhibitory potencies on cyclooxygenase-2 (COX-2) than on cyclooxygenase-1 (COX-1). This could have promising consequences since COX-2 is mainly associated with inflammation and COX-1 inhibition causes side-effects which are often observed with Non-steroidal Antiinflammatory Drugs (NSAIDs)^[20,21]. On the other hand, iridoid glycosides such as loganin, aucubin and verbenalin have shown antiinflammatory activity in carrageenan-induced mouse paw edema^[22] and analgesic effect has been proved for some iridoids like anguside, in writhing test that could be related to the inhibition of COX-2 enzyme^[23,24]. In addition, some flavonoids possess inhibitory effect on COX-2 expression^[25]. On the basis of obtained results it is suggested that ether and ethyl acetate fractions contain active compounds which mainly possess analgesic effect. In parallel, aqueous and ethyl acetate fractions contain active substances with antiinflammatory activity. In the case of total extract, ratio of antiinflammatory to analgesic compounds is not high enough to show the activity; and/or some compounds may counteract the antiinflammatory activity. These findings suggested that different extracts of *P. anisodonta* produce different activities that could be due to the effect of one or a combination of the bioactive components in each extract and also support the validity of the use of *P. anisodonta* in inflammation and nociception in medicine. Finally, the present results strongly support our previous findings about good analgesic and antiinflammatory effects of the extract from other species of this plant named *Phlomis lanceolata* Boiss. and Hohen.^[26] and *Phlomis olivieri* Benth.,

Table 1: Effect of the total extract and fractions of ether, ethyl acetate, *n*-butanol and water of *P. anisodonta* in comparison to indomethacin and control on carrageenan-induced paw edema in mice

Treatment	Difference of right paw diameter at different time intervals after carrageenan injection (mm)			
	1 h	2 h	3 h	4 h
Control	0.87±0.13	1.16±0.09	1.20±0.10	1.16±0.09
Indomethacin	0.41±0.04**	0.64±0.16*	0.57±0.10**	0.37±0.02**
Total	0.72±0.13	1.05±0.14	1.00±0.11	1.01±0.05
Ether	0.81±0.14	1.17±0.15	1.00±0.03	0.99±0.01
Ethyl acetate	0.42±0.11*	0.51±0.17*	0.58±0.18*	0.50±0.20*
<i>n</i> -butanol	0.71±0.07	0.96±0.10	0.97±0.07	0.92±0.05
Aqueous	0.58±0.11*	0.71±0.06**	0.83±0.11*	0.70±0.06*

Each point is the mean±SE of six animals. * and ** represent that the differences between control and treated groups are significant at $p < 0.05$ and $p < 0.01$, respectively

Phlomis anisodonta Boiss. and *Phlomis persica* Boiss.^[1]. A complete phytochemical analysis is needed to identify and quantify the active ingredients (mainly glycosides) in the total extract and different fractions to attribute activities observed to those substances.

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