EVALUATION OF ANTI-INFLAMMATORY EFFECTS OF ETHANOLIC EXTRACT OF SALVIA HYPOLEUCA

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Summary

In this study the anti-inflammatory activity of ethanolic extract of Salvia hypoleuca was evaluated. Salvia hypoleuca has a large history of herbal use because of pharmaceutical characteristics and the medicinal values of the Saliva hapoleuca have been mentioned in ancient literature as useful in disorders. The effects of ethanolic extracts of Salvia hypoleuca were studied on carrageenan induced paw edema. Results of this study indicated that the ethanolic extract decreased the edema induced in hind paw. It has been concluded that ethanolic extract of Salvia hypoleuca (200 mg/kg b.w.) has a good anti-inflammatory activity against carrageenan induced paw edema.

Key words; Salvia hypoleuca, Anti-inflammatory, carrageenan, paw edema.

Introduction

The use of herbal medicine has become increasingly popular worldwide and medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The genus Salvia, one of the most important genus of Lamiaceae family, is widely used in flavouring and folk medicine all around the world (1). Fifty-eight species of this genus are documented in the Flora of Iran; 17 of them are endemic (2). The plants of the genus Salvia, which consist about 900 species (3) are generally known for their multiple pharmacological effects such as analgesic and, antioxidant (4), hepatoprotective (5), hypoglycemic activities (6), and

antiischemia (7,8). In our continuing efforts at identifying medicinal plants with anti-inflammatory activity and establishing scientific evidence for activity, the acclaimed potency of the root bark of this plant in inflammatory conditions stimulated our interest to screen the extract for effect on inflammation.

Materials and Methods

Plant material

Salvia hypoleuca was collected from Guilan province, and then was identified by a botanist. Its leaves and fruits were dried under shade and powdered. The extract was prepared by the maceration method (80% ethanol in 300 gr/lit for 48 hours), filtered with filter paper. After filtration ethanol was removed by rotary evaporator. The extract was dissolved in normal saline and administrated orally into rats.

Animals

Adult Wistar rats of both sexes weighing between 200-250 g were used for experiment and were obtained from from Razi Institute, (Karaj, Iran) and maintained according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, Razi Institute, Karaj, Iran. They were housed in standard environmental condition like, ambient temperature (250 ± 10C), relative humidity (55±5%) and 12/12h light dark cycle. Animals had free access to standard pellet diet and water ad libitum.

Anti-inflammatory activity by Carrageenan induced rat paw edema method

The rat paw edema method of Winter et al (1962) was used (9). Albino rats of either sex weighing 200 – 250 g were divided in 4 groups (N=6). Group-I received 0.5% CMC suspension (control), Group-II, III and IV received ethanolic extract (100, 150, 200 mg/kg, P.O) of Salvia hypoleuca respectively. Group-V received Diclofenac (reference standard 1mg/kg, P.O) (10). Animals were

treated with drugs by oral route and subsequently 1 h after treatment; 0.1ml of 1% suspension of carrageenan in normal saline was injected into the subplanter region of left hind paw to induce edema. The paw volume was measured initially at 0, 1, 2, 3 and 4hr after carrageenan injection using digital paw edema meter (520-R, IITC Life Science - USA). The difference between the initial and subsequent values gave the actual edema volume which was compared with control.

The inhibition of inflammation was calculated using the formula, % inhibition =100 (1-Vt/Vc), Where 'Vc' represents edema volume in control and 'Vt' edema volume in group treated with test extracts.

Statistical analysis

Data analysis was carried out using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. $P < 0.05$ was considered statistically significant.

Results

The effect of Salvia hypoleuca extract (100, 150, 200 mg/kg) in carrageenan induced paw edema in rats is shown in Table 1 and 2. The met extract of S. hypoleuca (200 mg/kg) prevented the formation of edema induced by carrageenan and thus showed significant anti-inflammatory activity ($p<0.05$). This does (200 mg/kg) reduced the edema induced by carrageenan by 23.45% after 3h injection of noxious agent as compared to the control vehicle treated group. Diclofenac sodium at 10mg/kg inhibited the edema volume by 12.60 %. On carrageenan induced acute inflammation model the extract (200 mg/kg) produced better inhibition of paw edema.

Treatment groups (n=6)	**Dose (mg/kg)**	**Edema Diameter (cm)**				
		0hr	**1hr**	**2hr**	**3hr**	**4hr**
Group I	**10 ml/kg**	**0.93** ± 0.003	**0.96** ± 0.004	**0.97** ± 0.003	**1.00** ± 0.021	**1.02** ± 0.03
Group II	**100**	**0.88** ± 0.02a	**0.86** ± 0.006 a	**0.82** ± 0.005a	**0.81** ± 0.03a	**0.78** ± 0.04a
Group III	**150**	**0.86**± 0.006a	**0.84** ± 0.01a	**0.83** ± 0.006a	**0.80** ± 0.004a	**0.78** ± 0.001a
Group IV	**200**	**0.79** ± 0.008a	**0.77**± 0.006a	**0.76** ± 0.009a	**0.74** ± 0.01a	**0.73** ± 0.004a
Group V	**10**	**0.91**± 0.004b	**0.90**± 0.008a	**0.88** ± 0.005a	**0.85** ± 0.007a	**0.82** ± 0.004a

Table: 1 Effect of Salvia hypoleuca extract on carrageenan induced paw edema in rats.

Each value is mean ± SEM N=6 rats
a P < 0.01
b P < 0.05
One way ANOVA followed by Dunnet Multiple comparison test
Statistically significant when compared to control

Treatment	Percentage inhibition (%) at various times intervals			
	1hr	2hr	3hr	4hr
Ethanolic Extract 100 mg/kg	10.74	**12.45**	**18.98**	**26.86**
Ethanolic Extract 150 mg/kg	**12. 32**	**13.51**	**18.97**	**26.15**
Ethanolic Extract 200 mg/kg	**25.86**	**28.98**	**31.00**	**32.95**

Table 2: Percentage of inhibition of paw edema exhibited by ethanolic extract of Salvia hypoleuca.

Discussion

Carrageenan induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1 – 2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages (11,12). Folkloric treatment of inflammation of various etiologies, using medicinal plants, is well known to masters of the art of traditional medicine practice.

The significant inhibitory activity shown by the extract of Salvia hypoleuca (100, 150, and 200 mg/kg) over a period of 4 h in carragenan-induced inflammation was quite similar to that exhibited by the group treated with diclofenac sodium. The highest percentage inhibition activity was found in the dose of 200 mg/kg. These results indicate that the extract acts in later phases in dose dependent manner, probably involving arachidonic acid metabolites, which produce an edema dependent on neutrophils mobilization (13). Also, this extract may have inhibited the release of pro-inflammatory mediators of acute inflammation such as histamine and prostaglandin. Given the data it can be concluded that it is concluded that the ethanolic extract of Salvia hypoleuca (200 mg/kg) having good anti-inflammatory activities and it shown dose dependent activities. The results support the traditional use of this plant in inflammatory conditions and suggest the presence of biologically active components which may be worth further investigation and elucidation.

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