

Study on the Anti-Inflammatory Effects of Ethanolic Extract of *Cynanchum acutum*

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Abstract: In this study the anti-inflammatory activity of ethanolic extract of *Cynanchum acutum* was evaluated. *Cynanchum acutum* has a large history of herbal use because of pharmaceutical characteristics and the medicinal values of the *Cynanchum acutum* have been mentioned in ancient literature as useful in disorders. The effects of ethanolic extracts of *Cynanchum acutum* 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 *Cynanchum acutum* (200 mg/kg b.w.) has a good anti-inflammatory activity against carrageenan induced paw edema.

Keywords: Anti-inflammatory, carrageenan, *Cynanchum acutum*, paw edema

INTRODUCTION

Cynanchum acutum (*Cynanchum* comes from the Greek words *kyon*, meaning dog and *anchein*, meaning strangle or poison. *acutum* means ending to sharp point, pertaining to its leaves) is an invasive plant species belonging to the family Apocynaceae. Taxonomically speaking, Apocynaceae (including Asclepiadaceae) is a monophyletic family (Judd *et al.*, 2002) containing 355 genera and 3700 species, the majority of them are poisonous and many have medicinal uses. The plant is quiet poisonous with few medical applications. It has been used as a purgative in the French pharmaceutical Codex (Garnier *et al.*, 1961) and its milky latex is used for skin and eye problems in Tunisian folk medicine (Sayed *et al.*, 2003; Boukef, 1986) and its seeds are edible in some parts of Iran. The photochemical investigations on *Cynanchum acutum* L. have revealed the presence of several natural compounds including β -sitosterol, lupeol, lupylin acetate and α -amyryl (Halim *et al.*, 1990), sarcostine, quercetin and quercetin 3-O- β -D-galactoside (El-Sayed *et al.*, 1994), four flavonoid glycosides: quercetin di-O-hexoside, quercetin 3-O-rhamnosyl (1 \rightarrow 2) glycoside, quercetin 3-O-galactoside and quercetin 3-O-xyloside (Heneidak *et al.*, 2006) and 2 simple coumarins: scopoletin and scoparone (El-Demerdash *et al.*, 2009) as well as of seven other flavonoids (Ghada *et al.*, 2008). Studies on other species of this genus which have close affiliations with *C. acutum* also have been done and the following products have been distinguished: steroidal glycosides (Liu *et al.*, 2007), carbohydrates (Yi-Bin *et al.*, 2004), alkaloids (Tian-Ying *et al.*, 2001), phenolic compounds

(Lou *et al.*, 1993) and triterpenes (Konda *et al.*, 1990). This close affiliation enables us to predict the presence of these products in *C. acutum*, since close genotypes ends to production of similar compounds.

Antidiabetic and antioxidant activity of methanolic extract of aerial parts of *Cynanchum acutum* has been reported and antiulcerogenic effects of ethanolic extract of the plant also have been shown in rats (Atta *et al.*, 2005). In our continuing efforts at identifying medicinal plants with anti-inflammatory activity and establishing scientific evidence for activity, we decided to identify the effects of ethanolic extract of *Cynanchum acutum* on inflammation.

MATERIALS AND METHODS

Plant material: *Cynanchum acutum* was collected from Sistan and Balouchestan province in May 2011 and then was identified by a botanist. The whole plant at flowering time was dried under shade and powdered. The extract was prepared by the maceration method (80% ethanol in 300 g/L for 48 h), 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 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

Table 1: Effect of *Cynancum acutum* extract on carrageenan induced paw edema in rats

Treatment groups (n = 6)	Dose (mg/kg)	Edema diameter (cm)				
		0 h	1 h	2 h	3 h	4 h
Group I	10 mL/kg	0.93±0.003	0.96±0.004	0.97±0.003	1.00±0.021	1.02±0.030
Group II	100	0.88±0.020a	0.86±0.006a	0.82±0.005a	0.81±0.030a	0.78±0.040a
Group III	150	0.86±0.006a	0.84±0.010a	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±0.010a	0.70±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

Each value is mean±SEM n = 6 rats; a: p<0.01; b: p<0.05

Table 2: Anti-inflammatory effects of *Cynancum acutum* (inhibition of paw edema) showed in a dose dependent manner in rat

Treatment	Percentage inhibition (%) at various times intervals			
	1 h	2 h	3 h	4 h
Ethanollic extract 100 mg/kg	9.32	11.34	16.65	25.97
Ethanollic extract 150 mg/kg	10.75	12.76	18.23	26.31
Ethanollic extract 200 mg/kg	24.87	27.63	31.85	33.96

like, ambient temperature (25±10°C), relative humidity (55±5%) and 12/12 h 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 was used. 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 *Cynancum acutum*, respectively. Group-V received Diclofenac (reference standard 1 mg/kg, P.O) (Brooks *et al.*, 1991). Animals were treated with drugs by oral route and subsequently 1 h after treatment; 0.1 mL of 1% suspension of carrageenan in normal saline was injected into the sub planter region of left hind paw to induce edema. The paw volume was measured initially at 0, 1, 2, 3 and 4 h after carrageenan an 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 (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 *Cynancum acutum* extract (100, 150, 200 mg/kg) in carrageenan an induced paw edema in rats is shown in Table 1 and 2. The met extract of

Cynancum acutum (200 mg/kg) prevented the formation of edema induced by carrageenan an and thus showed significant anti-inflammatory activity (p<0.05). This does (200 mg/kg) reduced the edema induced by carrageenan and by 31.85% after 3 h injection of noxious agent as compared to the control vehicle treated group. Diclofenac sodium at 10 mg/kg inhibited the edema volume by 16.46%. On carrageenan an induced acute inflammation model the extract (200 mg/kg) produced better inhibition of paw edema.

DISCUSSION

Carrageenan an 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 and 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, polymorph nuclear cells and prostaglandins produced by tissue macrophages. 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 *Cynancum acutum* (100, 150 and 200 mg/kg) over a period of 4 h in carrageenan an-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 (Just *et al.*, 1998). 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 *Cynanchum acutum* (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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