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## Separation of Active and Toxic Portions in *Sambucus ebulus*

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**Abstract:** Methanol extract of fruits, leaves and roots of *Sambucus ebulus* were investigated for anti-inflammatory activity in rats (successively, after hexane and ethyl acetate extractions). Nearly all extracts produced statistically significant inhibition of edema induced by carrageenan at all doses when compared to the control groups. Anti-inflammatory effect was generally dose-dependent. The highest activity showed in fruits and leaves that at 600 mg kg<sup>-1</sup> i.p. inhibited 86 and 71% inflammation respectively (76% for diclofenac at 100 mg kg<sup>-1</sup> i.p.). No extracts exhibit any toxicity up to 2 g kg<sup>-1</sup> body weights intraperitoneally in mice. Ethyl acetate extract were withdrawn because of severe nociceptive response in rats. This extract showed severe toxicity (in particular, severe liver abscess) in all mice at all tested doses (125-1500 mg kg<sup>-1</sup> i.p.)

**Key words:** *Sambucus ebulus*, anti-inflammatory activity, toxicity, carrageenan

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### INTRODUCTION

Pain and inflammation are still one of the main health problems of the world's populations (Ahmadiani *et al.*, 1998). For chronic diseases such as osteoarthritis and rheumatoid arthritis a long time usage of antiinflammatory drugs is necessary. Current analgesia and antiinflammatory drugs such as opiates, glucocorticoids and NSAIDs are not useful in all cases, because of their side effects and potency. As a result, the search for other alternatives seems necessary and beneficial. This leaving an open door for new and better compounds (Elisabetsky *et al.*, 1995). In Iranian traditional medicine, the leaves and rhizomes of the plant *Sambucus ebulus* (Caprifoliaceae) have been used topically for curing painful joint diseases. Four species of the genus *Sambucus* are growing in Iran. Of these species, *S. ebulus* extensively grows in the northern regions of Iran (Ghannadi and Ghassemi-Dehkordi, 1997). There are several reports concerning the antinociceptive effects of *S. ebulus* in Iranian traditional medicine. A

literature survey of medicinal plants used as analgesics and anti-rheumatics was carried out amongst Iranian people living on the coast of the Caspian Sea. The survey indicated that traditionally, these people use leaves, rhizomes and roots of *S. ebulus* for treating bee and nettle bites, arthritis and sore-throat (Ognyanov *et al.*, 1979; Samsamshariat *et al.*, 1981; Zargari, 1981; Petkov, 1986; Mirhaydar, 1994). In addition it has been reported to be an insect repellent, antihemorrhoid, anti *Helicobacter pylori* activity, useful in the treatment of burns and infectious wounds, edema, eczema, urticaria, cold, inflammation and rheumatism (Tuzlaci and Tolon, 2000; Yesilada *et al.*, 1999; Guarrera, 1999). Recently we report an interesting anti-inflammatory activity of hexane extract of fruits of *Sambucus ebulus* (Ebrahimzadeh *et al.*, 2006). When we injected the next extract, ethyl acetate fraction, severe nociceptive effect was observed in mice. Here we report the toxic effect of ethyl acetate fraction. In addition, methanolic fraction (the third extract), showed again strong anti-inflammatory activity without any toxicity up to 2 g kg<sup>-1</sup> in rats.

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**MATERIALS AND METHODS**

*Sambucus ebulus* fruits were collected from 5th km of Sari-Qaemshahr road in Mazandaran (a northern state in Iran) in September 2005 and identified by confirmed by Department of Pharmacognosy. A voucher specimen (No. 87) has been deposited in Sari School of Pharmacy Herbarium. Fruits were dried at room temperature and powdered before extraction. One hundred grams of the powdered sample was fractionated by successive solvent extraction at room temperature by percolation with hexane (400 mL×3) then ethyl acetate (400 mL×3) and finally methanol (400 mL×3) successively. The resulting ethyl acetate and methanol extracts were concentrated over a rotary vacuum evaporator until a solid extract sample was obtained. The resulting crude extracts were freeze-dried. The extract was prepared in phosphate buffer (pH = 7.4) and tween 80 (4: 1) for pharmacological studies. Male Wistar rats weighing 180-200 g and male Swiss albino mice weighing 25-30 g were used for all experiments. They were housed in groups of six under standard light (7.00 to 19.00) and temperature (22±1°C) with food and water *ad libitum*. The animals were transferred to the laboratory at least 1 h before the start of the experiment. The experiments were performed during day (08:00-16:00 h). Each animal was used once only.

Antiinflammatory activity by Carrageenan-induced paw edema in rats (Eddy and Leimback, 1953; Shafiee *et al.*, 1998). Carrageenan (50 µL of 1% suspension) was injected into the subplanar tissue of the right hind paw of each rat. The methanol extract (100, 200 or 400 mg kg<sup>-1</sup>) or diclofenac sodium (100 mg kg<sup>-1</sup>) were

administered intraperitoneally to rats 30 min. before carrageenan. Control group received an equal volume of vehicle. The volume of edema was measured with a plethysmometer prior and 3 h after carrageenan injection. The degree of swelling induced was the ratio of the volume of hind paw before (b), to the volume of hind paw after carrageenan treatment (a). A ratio<1.5 after drug administration was considered to be a significant inhibitory effect

Acute toxicity assays were conducted as suggested by Kennedy *et al.* (1986). Methanol and ethyl acetate extracts were dissolved in tween 20- normal saline (1: 10) and given intraperitoneally. Dose- interval were progressively increased so that each dose was 50% higher than the preceding one (0, 125, 250, 500, 1000 and 1500 mg kg<sup>-1</sup> body wt.). The control mice were given solvent. Mice were closely observed for toxic symptoms and behavioral changes for the first 4 h of administration and behavior modifications, deaths or any other sign of toxicity and their respective latencies were recorded up to 14 days. Mice that died during the observation period and all surviving ones (killed on post-treatment day 14 by cervical dislocation) were subject to necropsy.

**RESULTS**

This extract did not exhibit any toxicity up to 2 mg kg<sup>-1</sup> when injected intraperitoneally in mice (Table 1). In acute toxicity assays of ethyl acetate fruits extract (Table 2), in the 125 and 250 dose groups, only a sluggish movement of animals a mild sedative effect or slight lethargy was found. There was no mortality in the

Table 1: Anti-inflammatory activity of methanol extract from *S. ebulus* different parts on carrageenan induced paw edema in rats<sup>a</sup>

Treatments	Dose (mg kg <sup>-1</sup> )	Initial paw thickness (cm) (b)	Paw thickness after 3 h (cm) (a)	a/b ratio <sup>b</sup>
Control	Vehicle	0.22 ± 0.07	0.43±0.05	2.16±0.64
Methanol/fruits	200	0.31±0.05	0.44±0.05**	1.42±0.1
	400	0.33±0.06	0.42±0.07***	1.26±0.06
	600	0.17±0.04	0.20±0.02***	1.21±0.17
Methanol/roots	200	0.32±0.06	0.50±0.04*	1.58±0.16
	400	0.33±0.04	0.43±0.05**	1.33±0.07
	600	0.14±0.03	0.18±0.02***	1.29±0.33
Methanol/leaves	200	0.32±0.05	0.41±0.03***	1.26±0.1
	400	0.33±0.04	0.41±0.04***	1.25±0.05
	600	0.36±0.06	0.42±0.06***	1.17±0.07
Diclofenac	100	0.18±0.04	0.23±0.06***	1.28±0.17

<sup>a</sup> An hour after treatment, 50 µL of 1% suspension of carrageenan were injected into the plantar side of hindpaw of rats; <sup>b</sup> A ratio less than 1.5 was considered to be a significant inhibitory effect. Values are mean±SD. (n = 6), \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, with respect to control (ANOVA followed by Newman-Keuls multiple comparisons test). Ethyl acetate extractions were withdrawn because of severe nociceptive response in rats

Table 2: Toxicity of a single dose of an ethyl acetate extract from *S. ebulus* fruits by intraperitoneal injection to mice

Organ weights (g)	<i>Sambucus ebulus</i> ethyl acetate extracts (mg kg <sup>-1</sup> . ip) <sup>a</sup>					
	0	125	250	500	1000	1500
Heart	0.18±0.04	0.24±0.05	0.16±0.08	0.20±0.00	0.24±0.05	0.22±0.08
Liver	2.62±0.10	2.64±0.19	2.30±0.44	2.82±0.55	2.82±0.64	2.52±0.22
Kidneys	0.56±0.08	0.76±0.15	0.56±0.11	0.66±0.15	0.54±0.11	0.64±0.18
Lungs	0.32±0.04	0.36±0.15	0.42±0.13	0.50±0.18	0.30±0.12	0.30±0.07

<sup>a</sup> Values are mean±SD. (n = 6), p>0.05, with respect to control (ANOVA followed by Newman-Keuls multiple comparisons test)

doses studied even after 14 days. In higher doses (500-1500), mice showed severe symptoms of lethargy and anorexia in the beginning days after treatment. Five mortalities were preceded by anorexia, lethargy, severe body-weight loss and lusterless skin. Mortalities occurred on 7th day for one mouse in 500 mg kg<sup>-1</sup> dose, on 6th day for another mouse in 1000 mg kg<sup>-1</sup> dose and on 4th day for three mice in 1500 mg kg<sup>-1</sup> dose. Ethyl acetate extract of *Sambucus ebulus* produced severe liver abscess in all mice at all tested doses..

### DISCUSSION

Hexane extract of fruits of *Sambucus ebulus* was safe and showed a high anti-inflammatory effect (Ebrahimzadeh *et al.*, 2006). The next portion, ethyl acetate extract, was responsible for toxic effect and cause mortality in mice and the last portion, the methanolic extract, again showed a high anti inflammatory effect and was safe up to 2 mg kg<sup>-1</sup>, ip. The results of present study support the folkloric utilization of leaves. All extracts (first and last, hexane and methanolic extracts) produced statistically significant inhibition of edema induced by carrageenan at all doses when compared to the control groups (Table 1). Anti-inflammatory effect was generally dose-dependent. The highest activity showed in methanolic extract of fruits and leaves that at 600 mg kg<sup>-1</sup> i.p. inhibited 86 and 71% inflammation, respectively. These activities were comparable to that of diclofenac at 100 mg kg<sup>-1</sup> i.p. which inhibited inflammation by 76% when injected intraperitoneally in mice. No extracts exhibit any toxicity up to 2 mg kg<sup>-1</sup> body weight when injected intraperitoneally in mice.

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