

## Antinociceptive and anti-inflammatory effects of *Sambucus ebulus* rhizome extract in rats

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Received 10 September 1997; received in revised form 20 January 1998; accepted 20 March 1998

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### Abstract

In this study we used the chronic (formalin test) and acute (tail flick) pain models of rats for evaluation of probable analgesic and anti-inflammatory effect of *Sambucus ebulus* (Se) rhizome extract. Sodium salicylate (SS) was used as a positive control. A total of 300 mg/kg of SS (i.p.) had no effect on tail flick latency, while 100 and 200 mg/kg i.p. of extract increased this latency ( $P < 0.01$  and  $P < 0.001$ , respectively). In formalin test, SS (300 mg/kg i.p.) and extract (100 mg/kg i.p.) alleviated the animals' nociception in the second phases, while in the first phase, only the extract caused an anti nociceptive effect ( $P < 0.05$ ). A total of 200 mg/kg of the extract showed a significant effect on both phases ( $P < 0.001$ ), which was not reversed by naloxone (2 mg/kg i.p.). On the other hand in the acute anti-inflammatory test, the plant extract (200 mg/kg i.p.) showed a significant effect, (e.g. SS  $P < 0.01$ ) and was not reversed by naloxone (2 mg/kg i.p.). Therefore, it seems that the mechanism of the antinociceptive and anti-inflammatory actions of extract are not related to the opioid system, of course the comparison of chronic administration of SS and Se showed a rapid onset of action for Se rather than SS, and because of its effect on tail flick latency and both phases of formalin test, the site of its analgesic action is probably central. Our phytochemical studies indicate that methanol extract of plant rhizome contains flavonoids, steroids, glycosides and tannins. The LD<sub>50</sub> of the extract was 600 mg/kg. © 1998 Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** *Sambucus ebulus*; Formalin test; Tail flick; Antinociception; Anti-inflammation; Rat

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### 1. Introduction

It is believed that current analgesia inducing drugs such as opiates and NSAIDs are not useful in all cases, because of their side effects and potency. As a result, the search for other alterna-

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tives seems necessary and beneficial. In Iranian folk medicine, the leaves and rhizomes of the plant *Sambucus ebulus*, have been used topically for curing inflammatory joint diseases.

*S. ebulus* (Dwarf elder), from the family Caprifoliaceae, extensively grows in the northern regions of Iran. There are several reports concerning the anti-inflammatory, and antinociceptive effects of the plant *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).

Analgesic compounds available in the market still present a wide range of undesired effects, leaving an open door for new and better compounds (Elisabetsky et al., 1995). Thus, a study was made on the anti-inflammatory and antinociceptive effects of the plant *S. ebulus*.

In the present study, a methanol extract of *S. ebulus* rhizome was further evaluated in rats using the tail flick and formalin analgesiometric tests, as well as plethysmometric evaluation of paw edema volume for acute and subacute inflammation.

## 2. Materials and methods

### 2.1. 2.1. Plant collection and identification

Plant material was collected from the area of Gilan (Ziba-Kenar, Bandar-Anzali) in the northern province of Iran, in Spring 1995. Taxonomic identification was confirmed by Mr Bahram Zehezad, and the voucher number 90 was specified by Shaheed Beheshti University Herbarium.

### 2.2. Phytochemical procedure

#### 2.2.1. Methanol extract

Fresh rhizome (1 kg) was soaked in 1 l of methanol for 3 days, and the step was repeated twice. After distillation of solvent by flash rotae-

vaporator, moisture determination was performed as follow: 2 g of final extract placed in 60–65°C for 72h, then weighed and weight loss was used as a moisture indicator. Final extract was contained 22.5% wetwt.

#### 2.2.2. Preliminary chemical tests

Preliminary phytochemical properties of the extract were studied using the following reagents and chemicals (Trease and Evans, 1983):

Alkaloids with Mayer and Dragendorff's reagents, flavonoids with the use of Mg and HCl, tannin with 1% gelatin and 10% NaCl solutions, cardiac glycosides with FeCl<sub>2</sub> and H<sub>2</sub>SO<sub>4</sub>, cyanogenic glycosides with picrate paper, terpenoids with Liebermann–Burchard method and use of H<sub>2</sub>SO<sub>4</sub>, antrakinons with Borntrager's reaction and saponin with ability to produce suds.

### 2.3. Pharmacological evaluation

#### 2.3.1. Antinociceptive activity

Male NMRI rats (220–270 g) were used for these studies. A total of 45 min prior to testing, the animals were placed individually in a transparent Plexiglas box (30 × 30 × 30 cm) which also served as an observation chamber. The extract and drug were administered intraperitoneally (i.p.), in a volume of 1 ml/kg, 25 min before tail flick and 30 min before formalin test. The extract was diluted with distilled water.

**2.3.1.1. Tail flick.** Acute nociception was assessed using a tail flick apparatus (type 812-HSE) following the method of D'Amour and Smith (1941). Briefly, each animal was placed in a restrainer, 2 min before treatment, and baseline reaction time was measured by focusing an intensity controlled beam of light on the distal one-third portion of the animals tail (light intensity = 7). The extract or drug was administered (i.p.) immediately after this step and 25 min later, the post drug reaction time was measured. A 10 s cut off time was used in order to prevent tissue damage. Reversibility of nociception by naloxone (2 mg/kg i.p.) was tested by administering it 10 min prior to measurement.

**2.3.1.2. Formalin test.** The procedure used was similar to that described previously (Dubuisson and Dennis, 1977). Briefly, 50  $\mu$ l of 2.5% formalin was injected under the plantar surface of the left hind paw. Rats were observed in the above described box and the severity of pain was recorded using the following scores: 0, rats walked or stood firmly on the injected paw; 1, partially elevated or favored the paw; 2, elevated the paw without contact with the floor; or 3, licked, bit or shook the paw. A mirror was placed with a 45° angle underneath the floor in order to allow an unobstructed view of the formalin injected paw by the observer. Two distinct nociceptive time periods are shown to be induced following formalin injection, the first represent phasic pain and the second period represent tonic pain. Reversibility by naloxone (2 mg/kg i.p.) was tested by administering it 15 min before formalin injection.

### 2.3.2. Anti-inflammatory activity

Male NMRI rats (220–270 g) were used for these studies. The method used was the same as the formalin induced paw edema (Kumar and Basu, 1994) except that 50  $\mu$ l formalin was injected sub-plantar to the left hind paw. Extract and drug were given i.p. 30 min before formalin induced paw edema. In acute anti-inflammatory tests, measurement of paw edema was performed 1 h after edema induction. The edema volume was measured with a plethysmometer according to Panthong et al. (1989). Reversibility by naloxone (2 mg/kg i.p.) was tested by administering it 15 min before formalin injection.

In the chronic anti-inflammatory test, daily measurement and then drug injection was performed up to the 7th day.

### 2.3.3. Acute toxicity

LD<sub>50</sub> was assumed using 50% death within 72 h following i.p. administration of the extract at different doses (500, 700, 1000 and 2000 mg/kg). Male mice weighing 20–25 g were used in this experiment.

## 2.4. Statistical analysis

The results are presented as mean  $\pm$  S.E.M. Statistical significance between groups was analyzed by means of one way ANOVA, followed by Student's-*t* test.  $P < 0.05$  were considered significant. In tail flick studies, paired *t*-test was used.

## 3. Results

### 3.1. Preliminary chemical test

Preliminary phytochemical tests showed that methanol extract of *Se* contains flavonoids, steroids, tannins, glycosides and cardiac glycosides.

### 3.2. Tail flick

Sodium salicylate (300 mg/kg i.p.) did not show any analgesic effect in this test, but the methanol extract significantly enhanced the tail flick reaction time in a dose-dependent manner (Table 1). This antinociceptive effect was not reversed by pretreatment with naloxone.

### 3.3. Formalin test

The effect of methanol extract of the rhizome in early (0–10 min) and late (15–60 min) phases of formalin test are shown in Table 2. Sodium salicylate was active in the late phase ( $P < 0.001$ ). The extract was also effective in both early and late phases of formalin test in a dose-dependent manner. Results obtained from the effect of *Se* extract at the two phases of formalin test show a dose dependent activity. The antinociceptive effect of *Se* extract (200 mg/kg i.p.) was not reversed by naloxone pretreatment (2 mg/kg, Table 2).

### 3.4. Acute anti-inflammatory tests

Results presented in Table 3 show that i.p. administration of methanol extract of *S. ebulus* rhizome inhibited the formalin induced edema, especially at the dose of 200 mg/kg ( $P < 0.01$ ) and this activity was not reversed by naloxone pre-

Table 1  
Effect of *Sambucus ebulus* extract, sodium salicylate and extract plus naloxone on tail flick test

Group ( <i>n</i> = 7)	Dose (mg/kg)	Reaction time		
		Pre-drug	Post-drug	$\delta$
DW	—	3.42 ± 0.14	3.44 ± 0.14	0.014 ± 0.12
SS	300	3.62 ± 0.13	3.17 ± 0.21	0.45 ± 0.29
Se	50	4.72 ± 0.20	6.07 ± 0.61*	0.34 ± 0.55
Se	100	5.02 ± 0.24	7.52 ± 0.61**	2.5 ± 0.65
Se	200	4.60 ± 0.28	7.38 ± 0.75**	2.78 ± 0.62
N	2	4.46 ± 0.27	4.60 ± 0.43	0.18 ± 0.23
N+Se	2+200	4.46 ± 0.25	9.58 ± 0.29	4.94 ± 0.43

DW, distilled water; SS, sodium salicylate; Se, *Sambucus ebulus* extract; N, naloxone reaction time;  $\delta$ , mean ± S.E.M.

\*  $P < 0.01$ , \*\*  $P < 0.001$ , evaluated by paired *t*-test vs. DW group.

treatment (2 mg/kg i.p.). Differences between sodium salicylate 300 mg/kg i.p. and 50, 100 and 200 mg/kg i.p. doses of extract were not significant.

### 3.5. Subacute anti-inflammatory tests

Results presented in Fig. 1 show that i.p. administration of a 100 mg/kg dose of *S. ebulus* methanol extract chronically inhibited the development of formalin induced edema, and so recovery time (time for reduction edema volume to 1/2 of maximum edema volume in control group) was less than sodium salicylate 300 mg/kg i.p. (1 day).

Fig. 2 shows the mean of formalin induced edema volume in 7 days. Both extract and sodium salicylate produced significant inhibitory effects, but the extract was more potent than sodium salicylate ( $P < 0.01$ ,  $P < 0.05$ , respectively).

### 3.6. Acute toxicity

Methanol extract produced 80% lethality when administered i.p. at doses > than 700 mg/kg and 30% lethality at dose 500 mg/kg, therefore the LD<sub>50</sub> was estimated to be ~ 600 mg/kg.

## 4. Discussion

In this study it was shown that i.p. administration of methanol rhizome extract produce anti-

inflammatory activity in both acute and chronic inflammatory tests. In neurogenic inflammation, some peripheral end of capsaicin sensitive sensory neurons release substance P and other inflammatory peptide mediators. The peripheral end of these neurons also contain inhibitory opioid receptors. Opiates may be considered to be inhibitors of substance P containing neurons and inhibitors of developing inflammation (Barnes et al., 1990), but since the anti-inflammatory action of the extract was not reversed by naloxone—an opioid antagonist—it is concluded that the mechanisms underlying the anti-inflammatory action of the extract in acute inflammatory test might be unrelated to the opioid system.

Our results suggest that the extract (100 mg/kg) possesses a potent anti-inflammatory activity in chronic administration and its potency was higher than sodium salicylate (300 mg/kg i.p.) so that 50% recovery time was ~ 1.5 days for the extract and 2.5 days for sodium salicylate. Other mechanisms are underlying the anti-inflammatory action, like the exogenous effect of steroids or endogenous release of glucocorticoids, interaction with prostaglandin biosynthesis, interaction with tachykinin, or other inflammatory mediators (Barnes et al., 1990), which should be studied for this extract.

In the formalin test, interestingly, nociception occurs in two phases (Dubuisson and Dennis, 1977; Tjølsen et al., 1992). The first phase starts immediately after formalin injection and contin-

Table 2

Effect of *Sambucus ebulus* rhizome extract, sodium salicylate, and extract plus naloxone on early and late phases of formalin induced pain

Group (n = 7)	Dose (mg/kg)	Early phase		Late phase	
		Score of pain	% Inhibition	Score of pain	% Inhibition
DW	—	1.74 ± 0.05	—	2.07 ± 0.03	—
SS	300	1.64 ± 0.06	5.75	1.55 ± 0.08***	25.12
Se	50	1.47 ± 0.07**	18.36	1.81 ± 0.05***	12.56
Se	100	0.99 ± 0.07***	42.75	1.32 ± 0.05***	36.23
Se	200	0.20 ± 0.06***	88.33	0.97 ± 0.10***	53.14
N	2	1.80 ± 0.05	—	1.89 ± 0.05	9
N+Se	2+200	0.31 ± 0.05***	82.18	0.94 ± 0.09***	54.58

DW, distilled water; SS, sodium salicylate; Se, *Sambucus ebulus* extract; N, naloxone; score of pain, mean ± S.E.M. Student's *t*-test, \* *P* < 0.05, \*\* *P* < 0.01, \*\*\* *P* < 0.001 vs. DW group.

ues for 5 min, after which, nociception appears to diminish. The second phase is marked by a return to high levels of nociception beginning 15–20 min after formalin injection and continuing for ~ 60 min. The first phase is probably a direct result of stimulation of nociceptors in the paw, while the second phase may reflect the inflammation process, and at least to some degree, the sensitization of central nociceptive neurons (Coderre et al., 1990; Coderre and Melzack, 1992). This method is also very useful for elucidating the mechanism of pain and analgesia (Tjølsen et al., 1992).

Table 3

Acute effect of *Sambucus ebulus* methanol extract, sodium salicylate and naloxone plus extract, on formalin induced rat hind paw edema 1 h after formalin injection

Group (n = 7)	Dose (mg/kg)	Formalin induced paw edema	
		Volume of edema (mm <sup>3</sup> )	% Inhibition
DW	—	236.3 ± 10.5	—
SS	300	165.9 ± 12.7*	29.7
Se	50	238.9 ± 36.1	—
Se	100	186.8 ± 48.7	—
Se	200	151.3 ± 20.4*	35.9
N	2	248.5 ± 14.6	—
N+Se	2+200	159.7 ± 20.4*	23.4

DW, distilled water; SS, sodium salicylate; Se, *Sambucus ebulus* extract. Volume of edema, mean ± S.E.M. \* *P* < 0.01, evaluated by *t*-test vs. DW group.

Drugs such as narcotics which act mainly centrally, inhibit both phases of formalin induced pain, while drugs, such as aspirin, hydrocortisone, and dexamethasone which are primarily peripherally acting, only inhibit the late phase (Chen et al., 1995; Elisabetsky et al., 1995; Santos et al., 1995). In this study it was shown that the administration of methanol rhizome extract produced clear dose dependent antinociceptive effects on tail flick, and

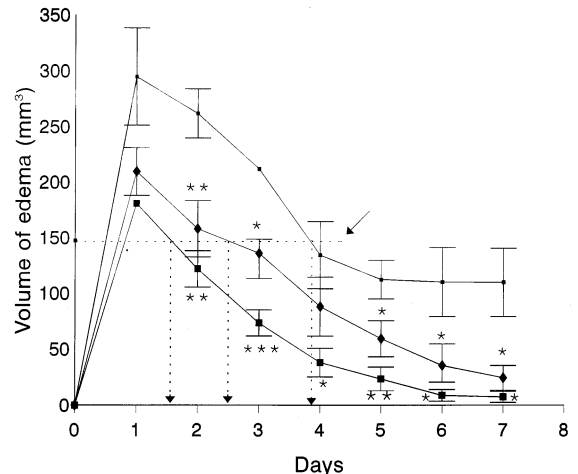


Fig. 1. Effects of chronic administration of *Sambucus ebulus* rhizome extract, 100 mg/kg per day (■), and sodium salicylate, 300 mg/kg per day (◆) on formalin induced paw edema. An arrow shows the time for 50% recovery of maximum edema in the control group. (■) distilled water. ANOVA, *P* < 0.05; Student's *t*-test, \* *P* < 0.05; \*\* *P* < 0.01 vs. distilled water group.

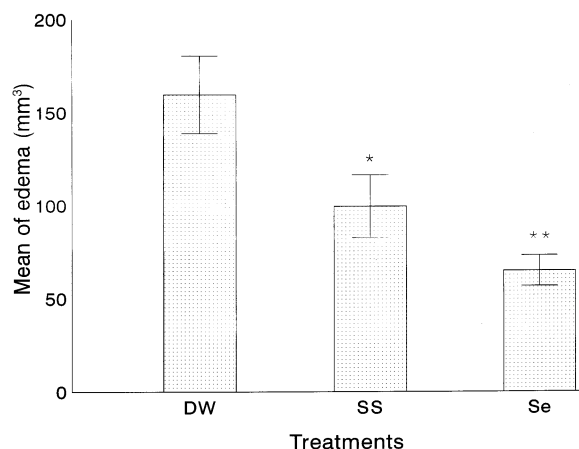


Fig. 2. Effects of chronic administration of *Sambucus ebulus* methanol extract 100 mg/kg per day (Se), and sodium salicylate 300 mg/kg per day (SS) on mean of paw edema at 7 days after formalin injection. \*  $P < 0.05$  and \*\*  $P < 0.01$  vs. distilled water (DW) group evaluated by Student's *t*-test.

also on both phases of formalin test. These findings suggest that central mechanisms are involved in the antinociceptive activity of the extract. The second phase of formalin test is related to a peripheral inflammatory process. This extract was able to inhibit this inflammation, so it can be deduced that peripheral mechanisms might also be involved in antinociceptive effects of Se.

The tail flick test is very sensitive to centrally acting drugs (Carlsson and Jurna, 1987). Thus, analgesic effects of the extract in the tail flick test model provide another document for central antinociceptive action of the extract.

In contrast to the report for morphine (Santos et al., 1995), antinociceptive actions of extract were not reversed by the opioid antagonist, naloxone. So the mechanism(s) underlying the antinociceptive action of the extract in both tail flick and formalin tests seems to be unrelated to the opioid system.

Considering the significant antinociceptive effects of the Se extract (100 and 200 mg/kg i.p.) on tail flick, and lack of ability of sodium salicylate (300 mg/kg i.p.) in changing the animals' nociception in this test, it can be proposed that the extract does not exert its antinociceptive effects in this pain model only by inhibiting the prostaglandin synthesis pathway. Other mecha-

nisms underlying antinociceptive actions of Se extract, such as the endogenous release of glucocorticoids or exogenous effect of steroids, interaction with  $\alpha$ -2 adrenoceptor or serotonergic system, L-arginine derived from nitric oxide or nitric oxide-related pathway, and interaction with tachykinin pathway (Santos et al., 1994), remain to be investigated.

These results give experimental support for the topical use of *S. ebulus* as an anti-rheumatic drug in folk medicine by people of northern Iran.

Our chemical studies carried out with *S. ebulus* indicate that methanol rhizome extracts of these plants contain many classes of compounds, including flavonoids, steroids, glycosides and tannins. On the other hand, there are some reports concerning the anti-inflammatory effects of flavonoids and steroids (Panthong et al., 1989, Recio et al., 1995), so the possibility of flavonoids and steroids, as responsible compounds for antinociceptive and anti-inflammatory effects of *S. ebulus* increases, and leave an open door for pharmacological investigations on the potential anti-inflammatory activity of this compounds in the plant extract.

### Acknowledgements

The authors are grateful to Dr B. Zehzad from Department of Botany, Shaheed Beheshti University, for approving the authentic of the plant used in this research and also Dr M.H. Pourgholami for critically reviewing the manuscript.

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