Original Article

Anti-nociceptive Effects of the Aerial Parts of Salvia nemorosa L. Extracts in Mice

H. Hosseinzadeh PhD, S. Amel PharmD

Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

- Abstract

**Background**-Several genera of Labiatae have anti-nociceptive properties with an efficacy similar to classical analgesic drugs.

**Objective**-In this study, the anti-nociceptive activity of Salvia nemorosa extract was evaluated.

**Methods**-Anti-nociceptive properties of the aerial parts of S. nemorosa were studied using hot plate and writhing tests on the aqueous decoction and ethanolic maceration extracts of the aerial parts of this plant.

**Results**-In contrast to the ethanolic extract, intraperitoneal injection of aqueous extract showed anti-nociceptive activity in the hot-plate test which was inhibited by naloxone. In the writhing test, however, considerable anti-nociceptive activity was observed in case of both aqueous as well as ethanolic extracts. Here again, naloxone had an inhibitory effect on the anti-nociceptive properties of only the aqueous extract.

**Conclusion**-This study shows that S. nemorosa had both central and peripheral anti-nociceptive activities that may be mediated by opioid receptors.

**Keywords**- Salvia nemorosa • anti-nociceptive activity • opioid activity • medicinal plants

**Introduction**

The genus Salvia from the Labiatae family, has 58 different species such as S. aegyptica, S. aethiopis, S. officinalis and S. nemorosa. 1 Labiatae, are generally known for their multiple pharmacological effects including their analgesic, anti-inflammatory2, antioxidant3, hepatoprotective4, hypoglycemic 5-7, antimicrobial 8, and CNS-depressant activities. 9 Through chemical investigations nemorone, a triterpene compound, 10 and glycosides such as salvionosides A-C11 have been isolated from this plant.

The following study was undertaken to study the anti-nociceptive activities of S. nemorosa (called Maryam-e Goli Kuhi in Persian) in mice.2, 9, 12

**Materials and Methods**

**Animals:**

Male Albino mice weighing 25-30 gr were selected from a random-bred colony and maintained on a special diet (Khorasan Javane Co. Mashhad, I.R. Iran) in the animal house of Mashhad University of Medical Sciences. The animals were housed in colony rooms 12/12 hours light/dark cycle at a temperature of 21±2° C with free access to food and water.

**Plant material:**
Salvia nemorosa was collected from Shandiz (an area with 30 Km distance from Mashhad, northern Iran). The plants were identified in the herbarium of Ferdowsi University. Voucher samples were preserved for reference in the herbarium of the Department of Pharmacognosy, School of Pharmacy, Mashhad (153-1914-4). After being dried in the shadow, the plants were subsequently grounded.

Preparation of extracts:

The extract of the plant was obtained using 2 methods, aqueous decoction, and maceration with ethanol. In the decoction method, one liter of hot water was added to 100 grams of the grinded plant, boiled for 15 minutes and finally filtered through cloth. This extract was then concentrated to the desired volume under reduced pressure.

In the maceration method, 200 grams of the grounded plant was added to 500-ml ethanol (85%, v/v) and left as such for three days. This macerated mixture was subsequently filtered and concentrated under reduced pressure at 50° C. The ethanolic extract was solubilized by Tween-80.

Anti-nociceptive study:

Hot-plate test:

Hot-plate test was assessed on mice, in which the temperature of the metal surface was maintained at 55±0.2° C for 40 seconds (cut-off time). Latency to a discomfort reaction (licking paws or jumping) was determined before and after drug administration.

Writhing test:

One hour after administration of extracts, the mice were given an intraperitoneal injection of 0.6% v/v acetic acid solution (volume of injection 0.1ml/10gr). Morphine was injected intraperitoneally 45 minutes before the injection of acetic acid. Naloxone was administered subcutaneously 15 minutes before the extracts and morphine injection. The number of writhes produced in these animals was counted for 30 minutes.

Maximum tolerated dose:

Different doses of the extracts were injected to separated groups of six mice. After 48 hours, the highest dose that failed to induce mortality was considered as the maximum tolerated dose.

Materials:

The following agents were used: morphine sulfate (Daru Pakhsh, I.R. Iran), naloxone hydrochloride (ToliDaru, I.R. Iran).

Statistical analysis:

The data were expressed as mean values ± SEM and tested with ANOVA followed by the multiple comparison test of Tukey-Kramer.

Results

The maximum tolerated doses of the aqueous and ethanolic extracts were 4 and 8 g/kg, respectively. Also it was seen that doses of 10 and 8 mg/kg of the respectively aqueous and ethanolic extracts killed all animals.
Morphine (2.5-10 mg/kg, IP) showed signiﬁcant anti-nociceptive activity in the hot-plate test. In this test, the intraperitoneal injection of the aqueous extract exhibits anti-nociceptive activity. Naloxone (1 mg/kg, SC) completely inhibited this activity (Fig. 1). The ethanolic extract did not show any significant activity in the hot-plate test.

Both extract forms of Salvia nemorosa showed marked anti-nociceptive activities in the writhing test. There were no signiﬁcant difference between the effect of the aqueous (0.5 mg/kg) and the ethanolic extract (1 and 2 mg/kg) compared with morphine (10 mg/kg) in reducing writhes numbers. Naloxone (1 mg/kg, SC) pretreatment only reduced the anti-nociceptive activities of the aqueous extract and morphine (Fig. 2).

Discussion

Present results indicate that the aqueous and ethanolic extracts of the aerial parts of S. nemorosa have anti-nociceptive activities with different proﬁles.

The maximum tolerated dose of the aqueous extract was found to be much higher than the ethanolic extract. This indicates that the aqueous extract is probably less toxic and better tolerated than the other extract.

Opioid agents exert their analgesic effects via supraspinal (μ1, μ3, δ1, δ2) and spinal (μ2, δ1, δ2) receptors.13 Hot-plate test is a speciﬁc central anti-nociceptive test.14 The aqueous extract showed anti nociceptive activity in the hot-plate test, and this effect was inhibited by naloxone. Therefore, it is possible that the extract had exerted its effect through central opioid receptors and promoted the release of endogenous opiopptides.

Both extracts showed signiﬁcant anti-nociceptive activities in the writhing test. They reduced the number of writhes more than 90%. In this test, the extracts showed considerable anti-nociceptive activity similar to morphine. Other substances such as opioid agonists, opioid partial agonists and non-steroidal anti-inﬂammatory agents show their anti-nociceptive activity in the writhing test.15 Since the anti-nociceptive activity of the ethanolic extract was not inhibited by naloxone, it means that they had no opioid effects and it is likely that the effect of the extracts is similar to non-steroidal anti-inﬂammatory drugs.

It is concluded that the aqueous and ethanolic extracts of the aerial parts of S. nemorosa have signiﬁcant anti-nociceptive effects. The aqueous extract has central and peripheral anti-nociceptive activity, which is partially mediated by opioid receptors. The ethanolic extract, however, possibly has only peripheral anti-nociceptive effects.

Acknowledgment

The authors are thankful to Dr. M. Ramezani, Assistant Professor, Department of Pharmaco-gnosy, School of Pharmacy, Mashhad, for his guidance.

References


AIM Home|Table of Contents