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Original article

Antinociceptive Effect of *Elaeagnus angustifolia* Fruits on Sciatic Nerve Ligated Mice

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Abstract

Objective(s)
The role of *Elaeagnus angustifolia* fruit as an analgesic agent in acute pain has been proved earlier. In this study, the effects of aqueous extracts of three parts of this fruit (pericarp, medulla and seed) on chronic pain were investigated in mice.
Materials and Methods
A partial nerve injury was made using a tight ligature around the sciatic nerve, then doses (0.5, 1, 1.5 g/kg, i.p.) of pericarp, medulla and seed extracts were injected in nerve ligated mice. The effect of different doses of three parts of this fruit on chronic pain was examined 14 days after sciatic nerve ligation using the hot-plate test. Controls received saline (5 ml/kg, i.p.) and imipramine (40 mg/kg).
Results
In the hot plate test, intraperitoneal injection of different doses of three parts of this fruit showed considerable analgesic effect on nerve ligated mice that was dose dependent with duration of action of 120 min.
Conclusion
Administration of the aqueous extracts of pericarp, medulla and seed of *E. angustifolia* fruit indicated significant analgesic effect on chronic pain in nerve ligated animals.

Keywords: Antinociception, *Elaeagnus angustifolia,* Neuropathic pain, Sciatic Nerve

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Introduction

Chronic neuropathic pain is a form of pain that caused by neural injury and persisted at least 3 to 6 months or longer. It is a severe clinical problem with limited treatment options. It is reported that different therapeutic agents such as tricyclic antidepressants, anticonvulsants, membrane stabilizers can alleviate neuropathic pain (1, 2).

Elaeagnus angustifolia L. subspecies Elaeagnaceae is cultivated from the northern areas of Asia to the Himalayas and Europe because of its capacity to grow in different environmental conditions (3). *E. angustifolia* is a shrub or tree up to 7 m high. The fruits are elliptical and reddish-brown (4). Multiple-stemmed trees are main parts in protection plantings, such as field windbreaks and living snowfences. *E. angustifolia* is a good example of these multiple-stemmed trees. It has an irregular globe shape (5). Species of this family have a lot of medical utilization. In folk medicine, *E. angustifolia* (Russian olive) fruit and flower are used as a tonic and antipyretic agent. Also, it is used for treatment of urinary diseases, gastric disorders, diarrhea, nausea, vomiting, jaundice, asthma and flatulence (6, 7). In Iranian traditional remedies, *E. angustifolia* fruit has been used as an analgesic agent for reducing of pain in rheumatoid arthritis (8). Recent pharmacological studies have shown muscle relaxant activity, antibacterial, anti-inflammatory and antinociceptive effects (9-11, 16). Flavonoids have been considered one of the most important constituents in *E. angustifolia*. Antinociceptive and anti-inflammatory activities of some flavonoids have been reported previously (10-13).

Many patients that received chemical therapeutic agents for long time suffered from interactions, possible side effects and toxicity of them. So, it is necessary to investigate potential of herbal remedies for the discovery of new bioactive compounds that lead to the development of novel neuropathic analgesics. Due to the reported use of this plant in folk and modern medicine in treatment of pain and because of its flavonoids constituents and the lack of any report on the effect of this plant on chronic pain (sciatic nerve injury model), the present study was carried out.

Materials and Methods

Animals

Male albino mice (25-32 g each) were obtained from a randomly bred colony maintained on special diet (Khorassan Javane Co, Mashhad, I.R. Iran) in the animal house of Mashhad University of Medical Sciences. Animals were housed in a colony room under a 12/12 hr light/dark cycle at 21±2 °C and had free access to water and food. All experiments were carried out in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines on the use of animals.

Preparation of the aqueous extract

E. angustifolia fruit was collected from around Kashmar (Khorassan province), Iran, in autumn. It was authenticated by Ferdowsi University and samples vouchers were preserved for reference in the Herbarium of Department of Pharmacognosy, School of Pharmacy, Mashhad (Taheri, 97-0501-1).

Three parts of this fruit (pericarp, medulla and seed) were isolated, cleaned and dried in shadow and powdered by mechanical grinder. Then, the powders of different parts (50 g) were added to 500 ml boiling distilled water and boiled for 15 min and the mixtures were subsequently filtered through cloth and concentrated in vacuo at 40 °C. The residues were suspended in normal saline.

Nerve injury pain model

The mice were anesthetized with intraperitoneal (i.p.) ketamine 100/xylazine 10 (mg/kg). A partial nerve ligation of sciatic nerve was made by tying a tight ligature with 7-0 silk suture around approximately 1/3 to 1/2 the diameter of the sciatic nerve located in the right-paw side. In sham operated mice, the nerve was exposed, but not ligated (14).

Antinociceptive study

Hot-plate test

The hot-plate test was assessed on group of 10 male mice. The temperature of the metal surface

was maintained at 55±0.2 °C. Latency to a discomfort reaction (licking paws or jumping) was determined 14 days after the surgery. The cut-off time was 40 sec. Doses of (0.5, 1, 1.5 g/kg, i.p.) pricarp, medulla and seed extracts were administered. Controls received saline (5 ml/kg, i.p.) and imipramine (40 mg/kg) (Approved by Sobhan company) (15).

Statistical analysis

The data were expressed as mean values ±SEM and tested with analysis of variance followed by the multiple comparison test of Tukey–Kramer. Discrepancies with $P< 0.05$ were considered significant.

Results

Our result indicated that there was no significant difference between the control and sham group ($P> 0.05$). Latency to a discomfort reaction of ligated animals was significantly decreased at 14 days after the partial ligation of the sciatic nerve. The hyperalgesic response after the nerve injury is shown in Figure 1. The results of i.p. injection of pericarp aqueous extract showed that the dose of 0.5 g/kg had no significant effect. Doses of 1 and 1.5 g/kg had analgesic effect on the chronic pain in ligated animals that was dose dependent. The maximum effect of antinociceptive was at 60 min after the injection with duration of about 90 min (Figure 2). The analgesic effect of medulla aqueous extract on the chronic pain in ligated animals was dose dependent. The onset of effect was at 30 min with duration of about 90 min. The maximum effect of antinociceptive was at 60 min after the injection (Figure 3). Administration of seed aqueous extract indicated the antinociception on chronic pain in ligated animals that was dose dependent. The onset of effect was at 30 min with duration of about 120 min. The maximum effect of analgesic effect was at 60 min after the injection (Figure 4).

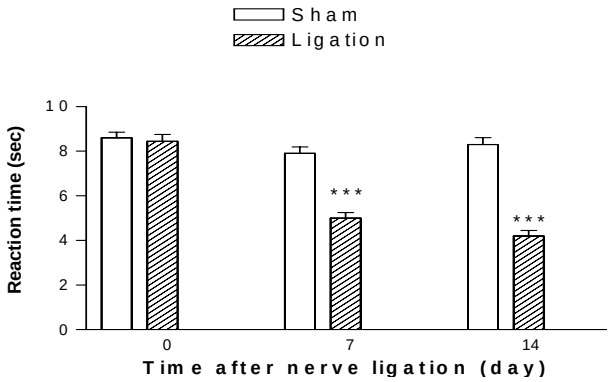

Figure 1. Changes in paw withdrawal thresholds to thermal stimulation in sham-operated or nerve ligated mice. Just before the ligation, and 7 and 14 days after the surgery, withdrawal latencies of the operated paw from the heat thermal stimulus were measured. Each column represents the mean±SEM for 10 mice. *** $P< 0.001$ vs. sham.

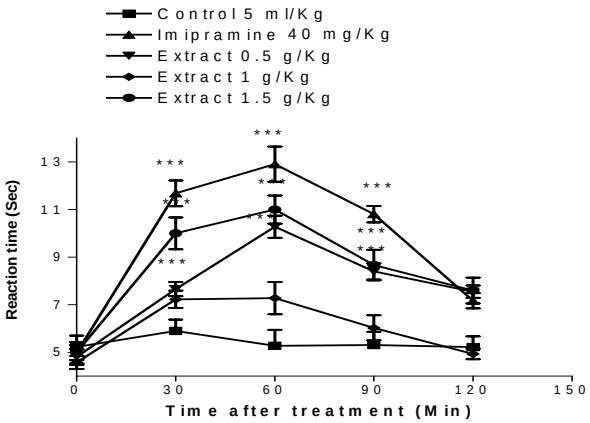

Figure 2. Effect of the aqueous extract of *E. angustifolia* pericarp (i.p.) on the pain threshold of mice in the hot-plate test after the sciatic nerve ligation. Each point represents the mean±SEM of reaction time for n= 10 experiments on mice. (**$P< 0.001$ and **$P< 0.01$, Tukey-Kramer test).

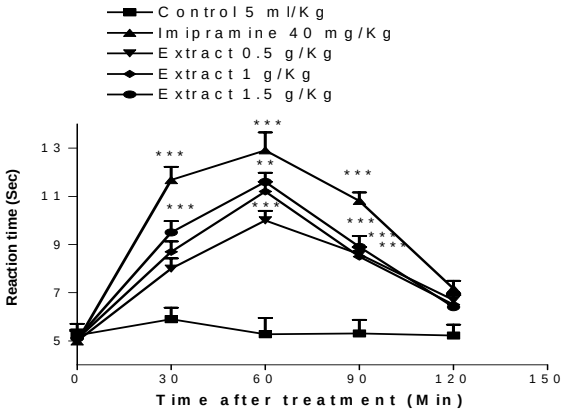

Figure 3. Effect of the aqueous extract of *E. angustifolia* medulla (i.p.) on the pain threshold of mice in the hot-plate test after the sciatic nerve ligation. Each point represents the mean±SEM of reaction time for n= 10 experiments on mice. (***$P< 0.001$ and ** $P< 0.01$, Tukey-Kramer test).

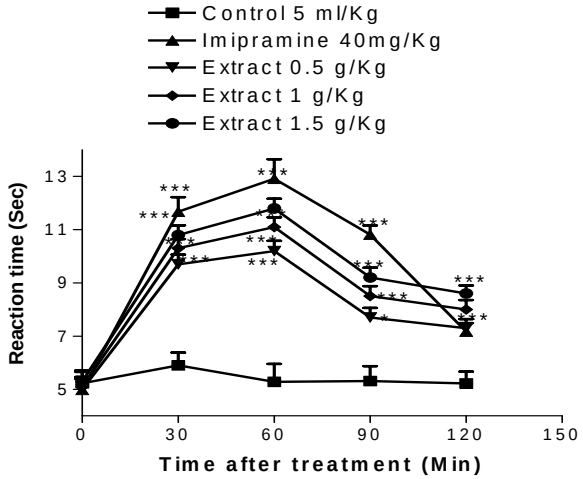

Figure 4. Effect of the aqueous extract of *E. angustifolia* seed (i.p.) on the pain threshold of mice in the hot-plate test after the sciatic nerve ligation. Each point represents the mean±SEM of reaction time for n= 10 experiments on mice. (***P< 0.001 and *P< 0.05, Tukey-Kramer test).

Discussion

Antinociceptive and anti-inflammatory effects of *E. angustifolia* fruit in mice were investigated in the previous studies (10, 11, 16). The ligation of sciatic nerve model is a specific method for the evaluation of antinociceptive activity on the chronic neuropathic pain. In the present study, mice with sciatic nerve injury displayed a marked hyperalgesia, and this persistent painful state lasted for 14 days. In the hot plate test, intraperitoneal administration of the aqueous extracts of three parts of *E. angustifolia* fruit showed significant antinociceptive activity on the chronic pain in nerve ligated animals that was dose-dependent. So, *E. angustifolia* fruit may have chronic antinociceptive effect.

It was demonstrated that some chemicals which were released into the nerve terminals, caused chronic pain, such as bradykinin and substance P (17). Other studies have shown that various flavonoids as well as biflavonoids produce significant antinociceptive activity (12, 13, 18, 19) due to the inhibitory effect on release of arachidonic acid and bradykinin (20). As this plant is a source of flavonoids, so it may be one of the mechanisms of antinociceptive effect against the chronic pain. Also, in the previous study, the muscle relaxant activity of *E. angustifolia* fruit was shown and antinociception in sciatic nerve ligated mice may be due to this effect (9).

Antinociceptive activity of intrathecal administration of serotonin and norepinephrine that potentiated by tricyclic antidepressant drugs, has been indicated previously (21, 22). Harman and harmaline alkaloids induced serotonin increase through the inhibitory effect on monoamine oxidase (23). It was shown that these alkaloids occur in this plant (24). Therefore, another mechanism of antinociceptive effect against the chronic pain of this plant may be due to the increase of serotonin in the central nervous system. Further investigations are necessary to evaluate the main antinociceptive mechanism of *E. angustifolia.*

Conclusion

Intraperitoneal administration of the aqueous extracts of pericarp, medulla and seed of *E. angustifolia* fruit showed significant analgesic effect on the chronic pain in nerve ligated animals. This plant may be effectively used as a protective agent against the neuropathic pain for clinical applications in human.

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