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Analgesic and anti-inflammatory potential of aerial parts of the *Daphne mucronata* Royle extract in mice: Opioid-independent action

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ABSTRACT

Objective: To investigate the analgesic and anti-inflammatory property and possible involvement of opioid receptors of ethyl acetate extract from aerial parts of *Daphne mucronata* (*D. mucronata*) in mice by formalin test.

Methods: Single doses of 2.5, 5.0 and 10.0 mg/kg of body weight of ethyl acetate extract of *D. mucronata* were intraperitoneally administered to the mice 30 min before analgesic test. The anti-nociceptive effect of preparations was evaluated based on the formalin in mice.

Results: The results indicated that the extract (2.5, 5.0 and 10.0 mg/kg) increased the pain threshold of mice and induced analgesia in both phases of formalin test. Like morphine sulfate (5.0 mg/kg, *i.p.*), the extract also showed more effective analgesic effect on the late phase of formalin test. Pre-treatment of animals with naloxone (5.0 mg/kg *i.p.*) did not inhibit the effects of the extract.

Conclusions: Our findings suggest that *D. mucronata* contains potential analgesic and anti-inflammatory compounds which support its traditional use. Moreover, it seems that the analgesic and anti-inflammatory effects of the extract is mediated by non-opioid mechanisms. Further pharmacological studies are required to determine whether the analgesic mechanisms are actually responsible for such properties.

1. Introduction

Daphne mucronata Royle (Thymelaeaceae) (*D. mucronata*), popularly known as Kheweshk, is an indigenous plant grown in Iran [1]. It is traditionally used as a herbal medicine for the treatment of various diseases such as cancer [2]. The *Daphne* genus consists of about 100 species [3,4]. Only a limited number of scientific studies have been conducted on *D. mucronata* so far. Primary phytochemical studies have demonstrated coumarins and diterpenes as the main active constituents of *D. mucronata* [5,6]. Derivatives of these substances have shown analgesic activity in different animal models of pain [7,8]. Scientific studies on other species of *Daphne* such as *Daphne pontica* L. and *Daphne oleoides* [9] have provided more documents on the analgesic and anti-inflammatory potential of this plant. However, the analgesic and anti-inflammatory properties and also the involvement of opioid receptors in mediating of these effects have not been reported.

The present study attempted to evaluate the analgesic and anti-inflammatory effects of ethyl acetate extract of the aerial parts of *D. mucronata* on mice using formalin test. The involvement of opioid receptors in analgesic effects of the extract was also determined.

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All experimental procedures involving animals were conducted in accordance to UK Animals (Scientific Procedures) Act, the European Communities Council Directive, the French Directives concerning the use of laboratory animals and the current laws of Iran and approved by the Animal Research Committee of Shahed University.

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2. Materials and methods

2.1. Plant material

Aerial parts of *D. mucronata* were collected from Kermanshah Region (Kermanshah Province, Iran). This plant species has been identified by herbarium of Shahed University, Tehran, Iran.

2.2. Preparation of the ethyl acetate extract

Aerial parts of *D. mucronata* were washed, chopped, air-dried under shade and powdered. The powder was stored in an airtight container. About 100 g of dried powder was soaked in 500 mL of ethyl acetate for 48 h with occasional shaking and then it was passed through muslin cloth and filtered through the filter paper. The extract was finally dried using a freeze dryer. First plant extracts were prepared by dissolving the plant extract in dimethyl sulfoxide. The final concentrations of dimethyl sulfoxide in the injection solution after dilution were less than 0.01% (v/v). In the present study three doses of the extract (2.5, 5.0 and 10.0 mg/kg) were selected based on our preliminary studies in order to evaluate their analgesic effects.

2.3. Animals

Male mice (20–25 g) were obtained from the animal house of Tehran University, Tehran, Iran. The animals were kept in a room with controlled temperature of (22 ± 2) °C and a 12 h light/dark cycle with free access to food and water *ad libitum*. The experimental protocol was approved by the Ethics Committee for Animal Research of the Shahed University of Tehran.

2.4. Formalin test

Formalin test was performed by assessing the formalin-induced paw licking response in mice. About 20 μL of 1% formalin, prepared in 0.9% saline, were subcutaneously injected into the dorsal hind paw of male mice (20–25 g) immediately placed in transparent box for observation. The extract was injected at doses of 2.5, 5.0 and 10.0 mg/kg *i.p.* 30 min before formalin injection to the mice. The positive control group received morphine (Daru Pakhsh, Iran) at a dose of 5.0 mg/kg.

The pain intensity was then scored and rated according to the following numerical scales:

0: Quadrupeds are placed on the floor and the weight is evenly distributed.
1: The injected paw lightly touches the floor or another part of the animal's body and little or no weight is placed upon it.
2: The injected paw is not in contact with any surface. The un-injected paw is placed firmly on the floor.
3: The injected paw is licked, bitten or shaken.

The mice were observed for 60 min after the injection of formalin and the time (s) spent in each scale (0, 1, 2 and 3) was recorded. Rating was averaged over 3 min blocks. Numerical ratings are calculated through the following formula:

$$\text{Pain rating} = (T1 + 2T2 + 3T3)/180$$

where T1, T2 and T3 are the time (s) spent in categories 1, 2 and 3, respectively during each 3 min block. The nociceptive response includes two phases; the first phase is the neurogenic pain response which was recorded during the first 5 min after formalin injection. The second phase is the inflammatory response which was recorded 5–60 min after formalin injection.

2.5. Participation of opioid system

To investigate the possible participation of the opioid system on antinociceptive effect of *D. mucronata* extract, the mice were pre-treated (30 min) before receiving the extract (10.0 mg/kg, *i.p.*) or morphine (5.0 mg/kg, *i.p.*) with naloxone (5.0 mg/kg, *i.p.*). The nociceptive response was recorded immediately after the formalin injection.

2.6. Statistical analysis

GraphPad Prism for Windows version 5 was used for all data and statistical analysis. All experimental groups consisted of six animals. The results were expressed as mean ± SEM. The time-course curves were subjected to Two-way (treatment x time) repeated measures analysis of variance (ANOVA) followed by Bonferroni *post-hoc* test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Formalin test results

Figure 1 shows the effect of different doses of extract (2.5, 5.0 and 10.0 mg/kg) on the time course of formalin induced nociception in mice ($n = 6$), compared to morphine (5.0 mg/kg). Injection of formalin evoked a retinue biphasic behavior in nociceptors of the mice (Figure 1). The extract injection significantly reduced the pain behavior of mice in both phases of formalin test and also induced effective analgesia in mice ($P < 0.01$) (Figure 2). The extract showed effective anti-nociceptive changes at 2.5–10.0 mg/kg in both phases of formalin test, compared to morphine (5.0 mg/kg).

3.2. Opioid independence analgesic action

Naloxone (5.0 mg/kg, *i.p.*) did not significantly change the antinociceptive effect of the extract in both phases of the test (Figure 3A, B). Whereas, morphine-induced analgesia (5.0 mg/kg) was completely reversed at the presence of naloxone.

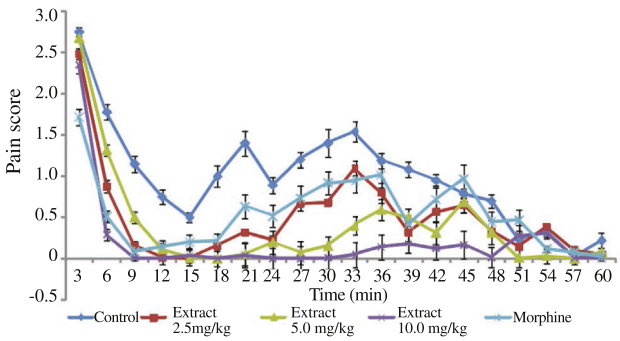

Figure 1. Time course of the formalin test response after administration of *D. mucronata* ethyl acetate extract in mice.
D. mucronata royal extract (2.5, 5.0, 10.0 mg/kg, *i.p.*) or morphine (5.0 mg/kg, *i.p.*) administrated 30 min before formalin 2.5% injection to the mouse hind paw. The analgesic response in all groups (including morphine treated group), only compared with control that received saline, intraperitoneally.

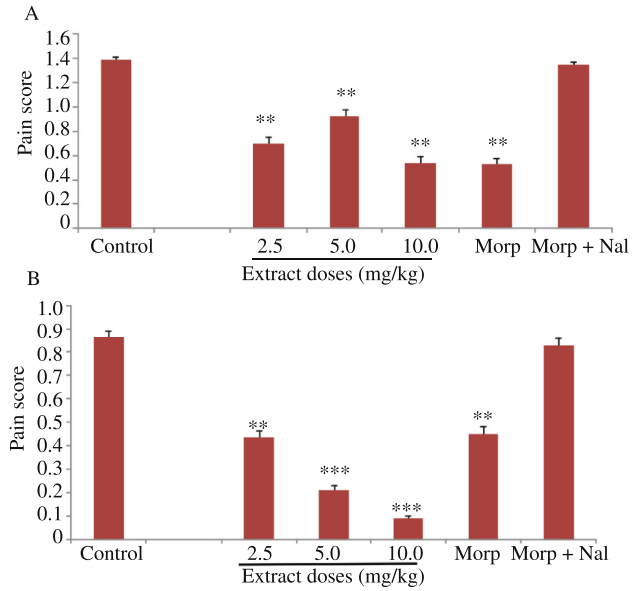

Figure 2. Pain score of mice in the early (A) and late (B) phases of formalin test after administration of different doses of the extract (2.5, 5.0, and 10.0 mg/kg, *i.p.*) and morphine (5.0 mg/kg, *i.p.*).
Each point represents the mean ± SEM, $n = 6$. The asterisks denote the significance levels compared with the control group (ANOVA). *: $P < 0.01$; **: $P < 0.001$; ***: $P < 0.000\,1$. Morp: Morphine; Nal: Naloxone.

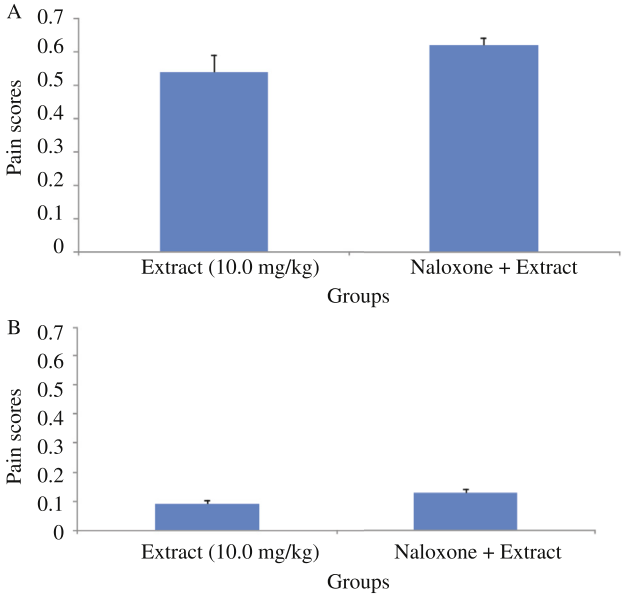

Figure 3. Pretreatment with naloxone showing no significant effect on the most effective analgesic dose (10.0 mg/kg) of the *D. mucronata* extract in the early (A) and late (B) phases of formalin test.

4. Discussion

The present study revealed the analgesic effect of *D. mucronata* extract on both phases of formalin test. This finding indicates that the analgesic effect of the extract mediated both centrally and peripherally. Naloxone pretreatment revealed that the pain modulatory effect of extract was opioid-independent in both phases of formalin test. In support of our findings, it has been demonstrated that many species of this plant (Thymelaeaceae) were being used in the treatment of various kinds of pain for hundreds of years in Asia [10]. The analgesic and anti-inflammatory property was also found in other *Daphne* genus [11].

Based on previous studies on pharmacological activity of some species of *Daphne* genus, it has been shown that they significantly delayed the adjuvant-induced nociceptive response and eased the paw swelling. In addition, they evidently inhibited the production of prostaglandin E2 and interleukin-1β and enhanced the activities of superoxide dismutase and catalase in the tissue of paws injected with the adjuvant. Furthermore, they remarkably reduced the content of nitric oxide and inactivated the activity of nitric oxide synthase in brain tissue of experimental animals [9]. The results of an *in vitro* study indicated the inhibitory effect of *D. mucronata* extract on tumor necrosis factor alpha receptor [12]. These pain modulatory mechanisms can be considered as non-opioid analgesic mechanisms [13]. More effective analgesic action of the extract on the late phase of formalin test indicated that there are other peripheral anti-inflammatory effects. Thus, the anti-inflammatory effect of such coumarins as daphnetin, found in the extract, was demonstrated [14].

It seems that the extract might act via activation of several opioid-independent pain controlling systems. Further studies are needed to identify exact mechanisms responsible for the anti-nociceptive and anti-inflammatory properties of *D. mucronata* extract.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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