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The effect of *Ferula szowitsiana* extract on chemical pain in male Wistar rats

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Abstract: Investigations on the use of medicinal plants are appealing to researchers. In Iranian traditional medicine, *Ferula szowitsiana* is used as an analgesic. This study investigates the effect of the extract of aerial parts of *Ferula szowitsiana* on an experimental model of chemical pain induced by subplantar injection of formalin. The *Ferula szowitsiana* hydroalcoholic extract was prepared with ethanol, Tween 80 and saline at a ratio of 8:1:1 serving as a solvent. Wistar Rats in the weight range of 200-250 g were divided into 10 groups randomly (n=7) according to treatment applied as follows: control; solvent (intraperitoneal, i.p.); solvent (intrathecal, i.t.); extract (50, 100, 200, 400 mg/kg i.p.); extract (80 μg/10 μL i.t); naloxone (2 mg/kg i.p.); extract (400 mg/kg) and naloxone (i.p.). To study the chemical pain, subplantar administration of formalin was performed in all groups. The results showed that the extract could reduce chemical pain in the neurogenic and inflammatory phase dose dependently as compared to the control group. The magnitude of the response after intrathecal administration of a dose of 80 μg/10μL was comparable to that observed with 400 mg/kg of extract administered via the intraperitoneal route. The administration of naloxone as an opioid receptor antagonist reversed the analgesic effect of the extract completely. According to the results, it seems that at least a significant part of the analgesic effect of *Ferula szowitsiana* extract is exerted through its effect on the central nervous system, and the analgesic effect of the extract in acute and inflammatory chemical pain is due to its effect on the opioid system.

Key words: *Ferula szowitsiana*; chemical pain; formalin test; naloxone; opioid system

INTRODUCTION

Pain is one of the unpleasant feelings and emotional experiences accompanied by real or potential tissue damage [1]. This is one of the most critical sensations that results in awareness of danger, physical damage or illness. Pain can arise from chemical, physical or thermal stimuli [2]. Chemical pain in the test after subplantar injection of formalin can be observed in two consecutive stages. The acute stage (neurogenic pain) occurs at the very beginning, with predominantly Aδ and C fibers being involved, and it then quickly subsides. After a short pause (inter-phase), the tonic phase (inflammatory pain phase) begins. The pain signal related to this stage is mainly transmitted by C fibers and to a lesser extent by Aδ fibers. In this stage, the pain increases with a mild slope and then decreases gradually. This phase is mostly due to inflammatory agents released from damaged tissue [3]. The introduction of new analgesic drugs with fewer side effects is important for researchers [4]. *Ferula szowitsiana* is one of the natural analgesic plants used in Iranian traditional medicine [5]. About 86% of the plant's ingredients are monoterpenes such as α pinene, β pinene, limonene, caryophyllene and myrcene [4, 6]. These compounds, with effects on opioid, cannabinoid and adenosine receptors, can block the pain sensation [7-9]. The search for new pain-blockers with the fewest possible side effects is a big part of drug economy. Thus, we studied the analgesic effect of *Ferula szowitsiana* extract.

MATERIALS AND METHODS

Animals

All research and experimental operations on rats were approved by the Ethical Committee of Ferdowsi University of Mashhad (FUM) and according to provisions

of international scientific ethics and the protection of laboratory animals [10]. Male Wistar rats (200-250 g) were used. Animals were kept in the animal house in the Department of Biology at the FUM under a 12-h light/dark cycle at 22±2°C, in plexiglass cages with access to food and water *ad libitum*. The rats were randomly divided into ten groups (n=7) according to the treatment applied as follows: control (received no treatment); solvent (i.p.); solvent (i.t.); extract (50, 100, 200, 400 mg/kg i.p.); extract (80 µg/10 µL i.t); naloxone (2 mg/kg i.p.); extract (400 mg/kg) and naloxone (2 mg/kg i.p.). For evaluating pain, 30 min after i.p. and 5 min after i.t. administration of extract or solvent, formalin was injected into the subplantar and animal behavior was monitored for 60 min. In the group that received naloxone (an opioid receptor antagonist), 20 min after extract administration, naloxone was injected, followed 10 min later by a subplantar injection of formalin, and pain behavior was monitored for 60 min.

Plant extraction

Ferula szowitsiana were collected from the Sarakhs area in Khorasan Razavi province, and submitted to the herbarium of the Botanical Institute of FUM, with the voucher number 37837. Stems and leaves were dried. Fifty g of the dried plant were crushed and macerated in 70% ethanol and keep for 24 h in the dark. The obtained solution was filtered after 24 h. Ethanol, Tween 80 and saline at a ratio of 8:1:1, respectively, served as an extract solvent [11].

Formalin test

In order to assess the analgesic effects of tonic pain during which tissue damage occurs, the formalin test was used. In this test, 0.05 mL diluted formalin (2.5%) was injected into the paw subcutaneously (s.c.). After formalin injection, the animal was placed in an observation box and behavior were recorded for 60 min. Animal behavior was followed at 15 s, then every 5 min, and averaged in the report as follows: when the animal does not behave = 0; toe on the floor or limp =1; the foot is absolutely on top = 2; licking or biting its leg = 3. This test consists of two pain response phases. In the first phase (neurogenic pain), the animal's pain lasts about 5 min; in the second phase (inflammatory pain), pain is much more severe and lasts about 40 min. Between the two phases there is a 5 min interval when the pain is relieved [12, 13].

Intrathecal (i.t.) administration

The animals were anesthetized by an i.p. injection of ketamine (100 mg/kg) and xylazine (20 mg/kg). The animals were fixed in a stereotaxic apparatus for performing the surgery. A PE10 tube (total size 11 cm) was placed in the subarachnoid space below the atlas vertebra to the lumbar spinal cord area (8 cm of PE10 tube). The remaining 3 cm of the tube was used for drug administration. One week after recovery, when no motor deficits were observed in animals, the drug was injected i.t. at the desired volume [14]. In this study, for i.t. administration, 80 µg of the extract dissolved in 10 µL of the solvent was considered as an equal concentration to 400 mg/kg dose of the extract for 0.2 g tissue of the lumbar segment of the rat spinal cord.

Statistical analysis

The data were averaged and presented as means±SEM, then analyzed by one-way ANOVA, followed by the Newman-Keuls test to compare the averages ($p<0.05$), by Graph Pad Prism 5.

RESULTS

Comparing the results of the formalin test between the control and intraperitoneal (i.p.) and intrathecal (i.t.) administrations of different solvent groups did not show significant differences (Fig. 1). Therefore,

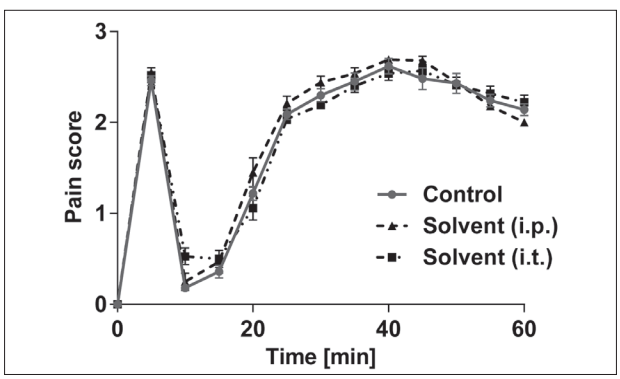

Fig. 1. Comparison of chemical pain caused by plantar injection of formalin, between control, i.p. and i.t. administration of solvent (ethanol, tween 80 and saline at a ratio of 8:1:1, respectively). Data are presented as means±SEM and n=7.

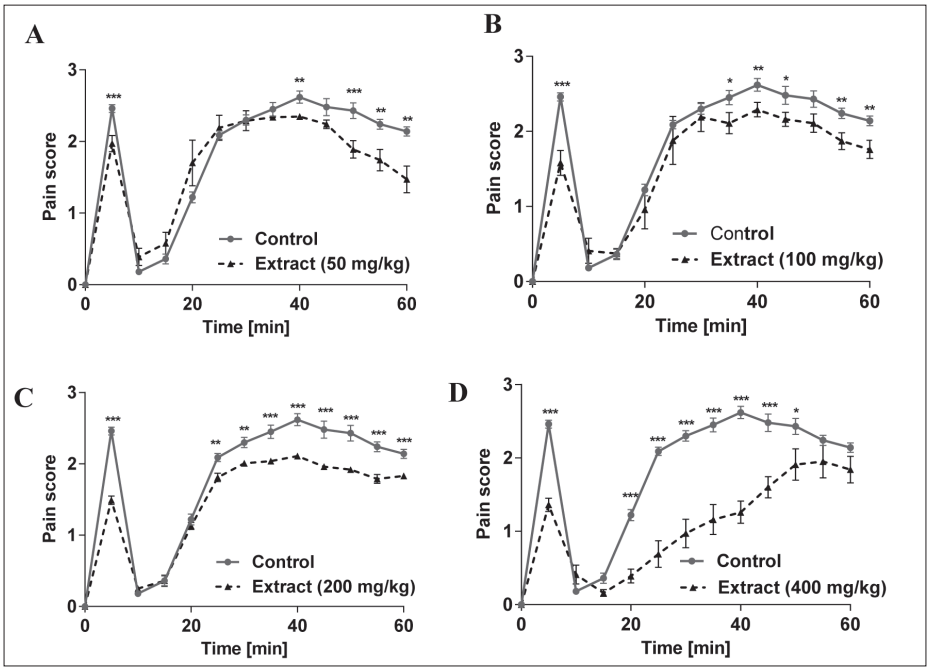

Fig. 2. Comparison of the result of the formalin test between control and groups that were administered the extract (50, 100, 200, 400 mg/kg i.p.): **A** – control vs. 50 mg/kg. **B** – control vs. 100 mg/kg. **C** – control vs. 200 mg/kg. **D** – control vs. 400 mg/kg. (P<0.01** and P<0.001*** compared to the control group). Data are presented as the means±SEM (n=7).

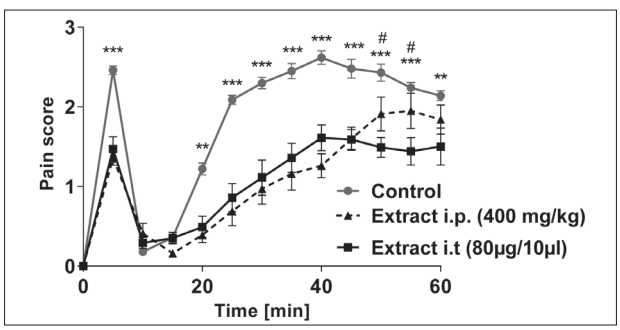

Fig. 3. Comparison of the results of the formalin test between the control and two routes of extract administration (400 mg/kg i.p., 80 µg/10µL i.t,) group. P<0.01**, P<0.001*** compared to the control group; P<0.05# compared to the i.p. administrated group. Data are presented as means±SEM (n=7).

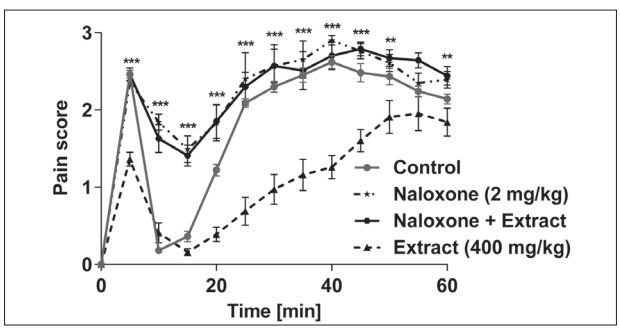

Fig. 4. Comparison of the results of the formalin test between control, extract (400 mg/kg i.p.), naloxone (2 mg/kg i.p.) and extract+naloxone groups; P<0.01** and P<0.001***. Data are presented as means±SEM and n=7.

the surgical procedures for i.t. injection did not have any negative side effects. Doses of 50, 100, 200 and 400 mg/kg (i.p.) of the plant extract were compared with the control group in order to investigate the effects of extract in the formalin test. The results showed that the degree of chemical pain in both neurogenic and inflammatory pain compared to the control group was reduced significantly (p<0.01 ** and p<0.001***, respectively; Fig. 2). Also, i.t. administration of the extract (80 µg/10 µL) reduced the chemical pain in both neurogenic and inflammatory pain (p<0.01**, p<0.001***, respectively; Fig. 3) when compared to the control group. Comparison between the two routes of extract administration (400 mg/kg i.p. and 80 µg/10 µL i.t.) in Fig. 3 does not show significant differences between them in the neurogenic and at all moments of inflammatory pain phases, except at 50 and 55 min (Fig. 3). As shown in Fig. 4, the administration of nal-

oxone (2 mg/kg, i.p.) diminished the interphase interval between neurogenic and inflammatory pain phases in comparison with the control group ($p<0.001^{***}$). To investigate the mechanism of extract function and its relationship with the opioid pathway, the most effective dose of the extract (400 mg/kg i.p.) was used in combination with naloxone and the results were compared with extract administration alone. An i.p. administration of naloxone reversed the analgesic effect of the extract (Fig. 4).

DISCUSSION

Many factors at the peripheral and central levels can be involved in pain transmission and are in turn suitable targets for pain therapy. According to the results of formalin tests, the *Ferula szowitsiana* extract dose-dependently reduced pain behavior during chemical pain. Phytochemical studies of *Ferula szowitsiana* specified that this plant contains compounds such as α pinene, β pinene, caryophyllene, limonene and myrcene [6]. On the other hand, several studies have determined that these compounds can decrease pain through various mechanisms, including opioid, cannabinoid and adenosine systems [15, 16]. Therefore, this extract could reduce pain due to its ingredients. In order to investigate whether the pain reduction effect of the extract is mediated through peripheral or central nervous systems, the extract was administered at a concentration of 80 μg/10μL (the concentration with the most effective dose) via the i.t. route. The absence of significant differences between the two routes of administration (400 mg/kg i.p. and 80 μg/10 μL i.t.) in pain reduction suggests that the effect of *Ferula szowitsiana* extract on pain relief is because of its impact on the CNS. To identify the mechanism of extract action, naloxone was used as an opioid receptor antagonist. First, i.p. administration of naloxone resulted in the omission of the interphase interval observed in the control group, which is a reflection of the role of the opioid system in the interval phase and probably due to recall of endogenous opioids. Second, the coadministration of naloxone and the plant extract eliminated the analgesic effect of the extract in both acute and almost all inflammatory phases. This suggests that the analgesic effect of the extract on acute and inflammatory chemical pain was probably due to the effect on the opioid system (mostly μ opioid receptors) that are widely distributed in the dorsal horn of the spinal cord. When opioid agonists are administered systemically, a part of their effects is caused by the release of endogenous opioids, so that the systemic administration of exogenous opioids primarily acts on the μ receptors, which then leads to the release of endogenous opioids that at a later stage impact κ and δ receptors that also produce an analgesic effect [17]. According to previous findings, α and β-pinene have an analgesic effect through μ-opioid receptor activation [15]. On the other hand, myrcene could recall endogenous opioids and produce an analgesic effect [18]. Other studies have suggested that cannabinoid receptors type 2 (CB2) cause the release of the endogenous opioid β-endorphin that in turn can affect μ-opioid receptors and lead to anti-inflammatory and analgesic effects [19]. Therefore, caryophyllene as a CB2 receptor agonist [20] can enhance the effects of plant extracts through activation of the cannabinoid receptor. Another terpene, limonene, as an adenosine A2a receptors agonist, can reduce pain [21]. A2a receptor activation can suppress NMDA receptors, resulting in postsynaptic inhibition in the CNS and blockage of pain transmission messages [22].

In summary, these results show that the mechanism of extract action was probably through the CNS and that it impacted the opioid system. However, the exact mechanisms of action of *Ferula szowitsiana* extract in reducing chemical pain need further investigation.

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Conflict of interest disclosure: The authors declare that they have no conflict of interest.

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