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Antinociceptive Effects and Acute Toxicity of *Nectaroscordum koelzi* ex-tract in Mice

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ABSTRACT

The objective of this study was to assess the antinociceptive activity and acute toxicity of *Nectaroscordum koelzi* fruit extract in mice. Totally, 90 BALB/c mice were used to determine the antinociceptive effect of the extract using rotarod, tail-flick, and hot plate tests. The acute toxicity was determined at doses of 1, 2, 4, 6 g/kg for 2 days. The maximum non-fatal dose and the LD_{50} value of *N. koelzi* fruit extract were 1.97 g/kg and 2.91 g/kg, respectively. The findings showed that in tail-flick and hot plate tests, 250, 500, and 750 mg/kg of *N. koelzi* fruit extract had a dose-dependent analgesic effect, 30min after administration and a significant difference was observed between the groups receiving the extract and saline ($p<0.05$). Moreover, the results showed no significant ($p>0.05$) changes with injecting the above-mentioned doses of *N. koelzi* fruit extract in sensory-motor test. The findings revealed the potent antinociceptive activity of *N. koelzi* fruit extract in mice. However, the exact mechanisms, which are responsible for the pharmacological effects have not been investigated, yet.

Keywords: *Allium, Pain; analgesic, mice, herbal medicines.*

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INTRODUCTION

Nociception is the neural procedure of processing and encoding noxious motivations caused by nociceptors (pain receptors) that alerts the unexpected chemical, thermal or mechanical modifications [1]. Pain is defined as such tissue damage or considered in terms of tissue damage or both. There are different types of pain including nociceptive, inflammatory, neuropathic, and functional pains that are due to neurobiological mechanisms [2]. Reviews have proven that nearly 20% of the world population has experienced at least one type of pain; so it can have a negative influence on the individuals' health and routine activities including relationships, cognitive ability, and working capabilities [3]. At the moment, there are lots of synthetic agents are available to relieve pain; however, these drugs have unpleasant side effects including gastrointestinal bleeding and cardiovascular disorders [4, 5], which restrict their remedial aids and have constrained to discover new drugs with low side effects. Medicinal herbs have long been applied to prevent and treat numerous diseases around the world [6-8]. Today, we are witnessing an ever-increasing trend towards the use of medicinal

plants and their products to treat diseases in all countries [9, 10].

Nectaroscordum koelzi from the genus of *Allium* (family Alliaceae) grows in Iran, Iraq, Turkey, North Caucasus, and Transcaucasus called "Piaze tabestaneh" in Persian. In folk medicine, root, leaf, bark, and fruit of this plant have been extensively applied to prevent and treat diseases such as bladder and kidney stones, joint pains, and rheumatism [11, 12]. This study was conducted to determine the antinociceptive activity of *N. koelzi* extract on BALB/c mice using Hot plate and Tail-flick tests as well as its effects on motor coordination by rotarod test.

MATERIALS AND METHODS

Herbal materials

Nectaroscordum koelzi fruits were collected from the rural regions of Khorramabad Mountains (Lorestan, Iran) in May 2017. Identification of the collected plants was performed by a botanist at the Razi Herbal Medicine Research Center, Khorramabad, Iran. A voucher specimen of the herbal materials was deposited at the Herbarium of Agriculture and Natural Resource Research Center, Khorramabad, Iran.

Preparing the methanolic extract

200 g of air-dried plant material was applied to extraction by methanol (80%) for three consecutive days at room temperature using the percolation method. The obtained extract was filtrated through a filter paper, concentrated in vacuum at 50 °C by means of a rotary evaporator (Heidolph, Germany) and stored at -20 °C, until use [13, 14].

Acute toxicity test

Various doses of *N. koelzi* extract (0.5, 1, 2, and 3 g/kg) were intraperitoneally injected to 6 mice to evaluate the toxicity. The dead animals were counted 48 h after treatment. Determination of LD_{50} values was done by using the Probit test in SPSS software [15].

Animals

Male BALB/c mice weighing 25-30 g were kept in light-dark cycles (12:12-h), at room temperature (22±2 ºC) and water and food access, *ad libitum*. Mice were permitted to adapt to the laboratory situation 30 minutes before starting the test. The present investigation was ethically permitted by the Ethics Committee of Lorestan University of Medical Sciences, Khorramabad, Iran.

Tail flick test

The thermal pain threshold of 30 BALB/c mice in five test groups (6 mice per each group; given 250, 500, and 750 mg/kg of *N. koelzi* extract, as well as normal saline and morphine) was evaluated using the tail-flick test according to the method explained elsewhere [16]. Briefly, when the animal is exposed to the concentrated burning light on the middle one-third of the tail, withdraws its tail after a while, i.e. latency time. The intensity of light on the tail-flick apparatus (Sparco, Iran) was set to make latency time of 2 to 4 s in the intact animal. 10 s was considered as a cutoff time to prevent any tissue damage. For each test set, the latency time was recorded 3 times with an interval of 15 minutes; the mean was considered as a thermal pain threshold (tail-flick latency).

Hot plate test

The pain sensitivity of 30 BALB/c mice in five test groups (6 mice per each group; given 250, 500, and 750 mg/kg of *N. koelzi* extract as well as normal saline and morphine) was evaluated using an apparatus (LE710 model, Lsi LETICA, Spain) containing a Plexiglas wall with a height of 30 cm and a plate with a diameter of 19 cm. The plate temperature was adjusted to 55±0.2 °C. The time between the test beginning and jumping or licking front paw was considered as the time of response to thermal pain (with a maximum cutoff equal to 1 min) [17].

Rotarod test

Motor coordination was assessed using the rotarod test. 4 groups, each containing 5 animals were already trained to stay on a rolling rod (3 cm in diameter) rotating at 8 rpm for 3 minutes. The extract was given to the groups (75, 150 and 300 mg/kg, i.p.) except the control group that received purified water. 30 minutes after extract administration, the animals were put on the rolling rod and the number of mice falling during the procedure (3 min) was recorded [18].

Statistical analysis

The obtained results were expressed as mean ± SEM. Data analysis was carried out by using SPSS statistical package version 17.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA with Tukey's post-hoc test was used to assess differences between the experimental groups (20). In

addition, P<0.05 was considered statistically significant.

RESULTS

Acute toxicity

The maximum non-fatal dose and the LD50 value of *N. koelzi* fruit extract were 1.97 g/kg and 2.91 g/kg, respectively.

Tail flick test

Figure 1 shows the antinociceptive activity of *N. koelzi* extract in the tail-flick test. The results demonstrated that the administration of 250, 500, and 750 mg/kg of *N. koelzi* extract with the mean latency times of 5.3, 6.3, and 8.1 seconds, respectively showed a significant anti-nociceptive effect in comparison to the control (saline) group (p<0.05). In addition, the results showed that the latency time in the group receiving 750 mg/kg of the extract was significantly more than that of morphine (p=0.01).

Hot plate test

As shown in Figure 2, 250, 500, and 750 mg/kg of *N. koelzi* fruit extract demonstrated a dose-dependent analgesic effect, half an hour after administration and so, the difference between the groups receiving the extract and saline was significant (p<0.05). These results also showed that the reaction time of 750 mg/kg of extract had a more antinociceptive effect than that of morphine, however, no significant difference was observed (p>0.05).

Rotarod test

In this investigation, the effects of *N. koelzi* extract on BALB/c mice were evaluated by the rotarod test. The obtained findings revealed that there is no significant (p>0.05) modification after injecting 250, 350, and 750 mg/kg of *N. koelzi* extract in the sensory-motor test.

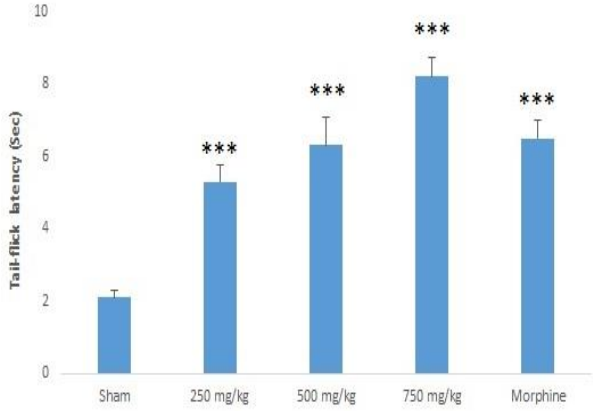

Figure 1. Effect of the *N. koelzi* fruit extract and morphine on the pain threshold of the mice in the tail-flick test.

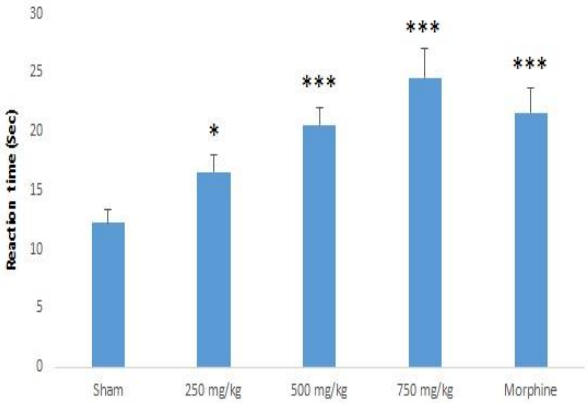

Figure 2. Effect of the *N. koelzi* fruit extract and morphine on the pain threshold of mice in the hotplate test.

DISCUSSION

In recent years, chronic inflammatory diseases are one of the major health risks in all countries around the world; while the non-steroid anti-inflammatory drugs are widely used for the treatment of these diseases [19]. Many studies have shown the anti-inflammatory and antinociceptive effects of a large number of medicinal herbs such as *Rosa damascene, Urtica pilulifera, Elaeagnus angustifolia, Zhumeria majdae, Crocus sativus ,Zataria multiflora*, *etc.* [18, 20-25]. Here, we aimed to assess the antinociceptive activity and acute toxicity of *N. koelzi* fruit extract in mice.

The obtained findings revealed that *N. koelzi* fruit extract had a remarkable antinociceptive activity in hot plate and tail-flick tests as a dose-dependent manner. Nowadays, the tail-flick test is described as a valuable experiment in assessing the analgesic effects of tested drugs [26]. Our findings demonstrated that the *N. kolezi* extract as a dose-dependent response reduced pain induced by thermal motivation. Subsequently, the tail-flick test was done to study spinal responses and detect central analgesic route [27] and suggested that this extract probably makes its analgesic activities through the CNS. Moreover, in the hot-plate test, *N. koelzi* extract demonstrated an analgesic dose-dependent effect and a significant difference between the groups receiving the extract and saline (p<0.05). These results also showed that the reaction time in the antinociceptive effect of 750 mg/kg of the

extract was more than that of morphine; however, no significant difference was observed.

Concerning the antinociceptive activities of plants in the *Allium* genus, Maghsoodi et al. [28] showed that the aerial parts of *Allium paradoxum* have considerable analgesic effects on BALB/c mice in the hot-plate test. Mahmoodi et al. [29] demonstrated that *A. hirtifolium* extract especially at the dose of 200 mg/kg, alone and along with aspirin, showed significant antinociceptive activity in formalin, tail-flick, and writhing tests. In the other study, Roghani et al. [30] reported that oral administration of *A. schoenoprasum* leaf for 56 days could reduce the nociceptive score in the formalin test in mice with diabetes mellitus. Furthermore, Jayanthi et al. [31] reported that *A. sativum* bulbs powder had favorable analgesic and anti-nociceptive activities, probably through both central and peripheral mechanisms.

Reviews have reported some phytochemical compounds including tannins, flavonoids, and saponins in various species of *Allium* genus [32]. Other studies also showed the analgesic effect of these constituents such as saponins and flavonoids [33, 34]. There have been reports of inhibition of nitric oxide enzyme induction and synthesis of cyclooxygenase-2 by triterpenoids, which facilitates pain relief [34, 35]. Flavonoids have many biologic effects on protein synthesis, cell differentiation, and angiogenesis in human [36]. Besides, various types of flavonoids, both glycoside and non-glycoside types have analgesic and anti-inflammatory effects [36-38]. Hence, phytoconstituents in this plant might be responsible for their antinociceptive effects; whereas its exact action mechanism is not well understood.

Regarding the fact that sedation may affect the response to damage and painful stimuli, the effects of the extract on motor coordination were assessed by sensory-motor test on mice by using a rotarod apparatus. The obtained results showed no significant changes in the sensory-motor test when injecting 250, 500, and 750 mg/kg of *N. koelzi* extract.

In terms of the toxicity, the maximum non-fatal dose and the LD_{50} of the intraperitoneal injection of the *N. koelzi* fruit extract were 1.97 g/kg and 2.91 g/kg, respectively. So, *N. koelzi* fruit extract did not have any significant toxicity on male BALB/c mice [39].

CONCLUSION

The results of our study revealed the promising antinociceptive activity of *N. koelzi* fruit extract in mice. However, the precise mechanism of pharmacological activities remains to be investigated.

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