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The analgesic effects of hydroalcoholic extract of Varthemia Persica in male rats in both acute and chronic pains

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Abstract

Objectives: Varthemia Persica DC is confined to Iran plateau and is the only existing species of that genus in Iran. Study of flavonoids in Varthemia Persica shows that there are five flavonoids in this herb. Due to lack of scientific studies on its pharmaceutical effects and biological properties as well as including various flavonoids and due to the fact that some of them have antinociceptive effects, we examined in present study its antinociceptive effect using formalin test on male rats.

Materials and Methods: In this experimental study, the experiments were performed on 40 male wistar rats weighting 190 to 200 grams, which were divided into 5 groups, each consisting of 8 rats. To evaluate the analgesic effects, the formalin induced pain-test was used. All animals were pre-treated with an oral dose of extracts (100, 200, 300 mg/kg). The control group received no drug and the witness group at only received distilled water. The first step is quick and acute pain which hits peak during 5 minutes. Then pain intensity is reduced for 5 to 10 minutes and twenty minutes after formalin injection, the second step of pain which is also called chronic pain restarts and lasts 60 minutes after formalin test Statistical analysis of the data was performed using ANOVA and t-test where appropriate.

Results: Results of this study showed that hydroalcoholic extract of Varthemia Persica can significantly reduce the pain depending on the dose in acute phase ($p<0.05$). However, in chronic phase, the high dose of the extract could reduce the pain.

Conclusion: It seems that flavonoids can probably reduce intracellular calcium through inhibiting of NMDA receptors. It also reduces activities of nitric oxide synthase enzyme and calcium dependent phospholipids A2, which results in antinociceptive effects.

Key word: Varthemia Persica DC, analgesia, flavonoids, formalin test

Introduction

Pain is caused by various kinds of stimulants, such as mechanical twisting of tissue, high temperature, low pH, chemicals (such as neuroactive substances that are released when being injured), and hyperosmotic solutions, that have no common forms [1]. At present, control of pain is done by using nonsteroidal anti-inflammatory drugs (NSAIDs) and opioid analgesics. There are many evident that show neurochemical systems, such as opioid system, involvement in pain controlling [2]. Opioid analgesics, especially morphine, have a high efficiency in relieving both acute and chronic pain. Repeated use of morphine can cause progressive decrease in its effects such that one would require more dose of morphine to achieve the same effect, which, in turn, is followed by addiction and many side effects too [3]. In this phenomenon, proteins such as protein kinase C, calcium calmodulin, protein kinase A, and protein kinases related to CGMP, which play part in phosphorylation of N-Methyl-D-aspartate (NMDA), are increased quantitatively. Therefore, nitric oxide content increases and NO/CGMP/PKG course is begun [4]. A method to attain new analgesics, with more efficiency and less restricting effects, is focusing on and attention to herbs and natural substances. Today, studying herbal species which are traditionally used as analgesics is considered a useful research strategy to prepare new analgesics [5]. Varthemia Persica DC is an aromatic herb from Chicorium intybus strain. It is confined to Iran plateau and is the only existing species of that genus in Iran. Study of flavonoids in Varthemia Persica shows that there are five flavonoids in this herb including quercetin, kaempferol, myricetin, luteolin, and apigenin [6]. Due to lack of scientific studies on its pharmaceutical effects and biological properties as well as including various flavonoids and due to the fact that some of them have antinociceptive effects, we examined in present study its antinociceptive effect using formalin test on male rats.

Substances and methods

Extract preparation

To prepare extract, aerial parts of the herb were collected and dried and powdered under appropriate conditions and away from sunlight. 500 grams of the resulted powdered were mixed with ethylic alcohol and distilled water at equal proportions by maceration method (soaking method) and stored for 24 hours. Within this period, the content of container was shaken alternatively to solve the extract in alcohol thoroughly. Then purified extract was centrifuged at 4500 rpm for eight minutes. The resulted liquid was poured onto an aluminum foil and placed into an incubator at 50°C to be dried. At the next step, desired amount of extract was solved into distilled water to gain different concentrations [7].

Grouping of animals

To perform this research, 40 adult male Wistar rats with approximate weight of 190-200 g were selected and were kept under equal conditions regarding weather, light, and nutrition for a week before the test.

Formalin test

We used formalin test to study antinociceptive effects of the extract. And for this purpose, we used eight adult male rats in each group. An hour after injecting 100, 200, and 300 mg/Kg of hydroalcoholic extract of Varthemia Persica, 50 µL formalin 2.5% was injected into rat's right sole. Then the rat was immediately kept within test case [8]. The control group received no treatment while witness group received 1 ml distilled water (solvent of extract), that is, as equivalent as consumed dose by experimental groups. Each rat's behavioral response to algogenic stimulant was rated for an hour at 15 seconds intervals based on the method proposed by Dubbison and Dennis [9,10].

In this observation, rates zero to three were

given every 15 seconds based the rat's behavior in appearing pain in the foot into which formalin has been injected. (Zero, where the rat uses his treated foot like the other foot; 1, where the rat puts his treated foot on the ground but doesn't lean on it; 2 where the rat doesn't put his treated foot on the ground; and 3 where the rat moves, licks, and bites his treated foot). Then, we calculated pain intensity of each rat at 5 minute intervals and analyzed mean pain intensity statistically. In formalin test, formalin injection into rat's sole would cause two-step incidence of pain. The first step is quick and acute pain which hits peak during 5 minutes. Then pain intensity is reduced for 5 to 10 minutes and twenty minutes after formalin injection, the second step of pain which is also called chronic pain restarts and lasts 60 minutes after formalin test.

Data analysis

Statistical analysis of results between groups was done by variance analysis, ANOVA, T. Test. A significant difference was considered between groups ($p<0.05$). The results are shown as standard deviation ± average.

Results

As shown in diagram 1, hydroalcoholic extract of Varthemia Persica (100, 200, 300 mg/Kg) has caused significant decrease in pain rate in acute pain step of formalin test compared to control and witness groups ($p<0.05$) (diagram 1).

Statistical analysis of results of analgesic effects

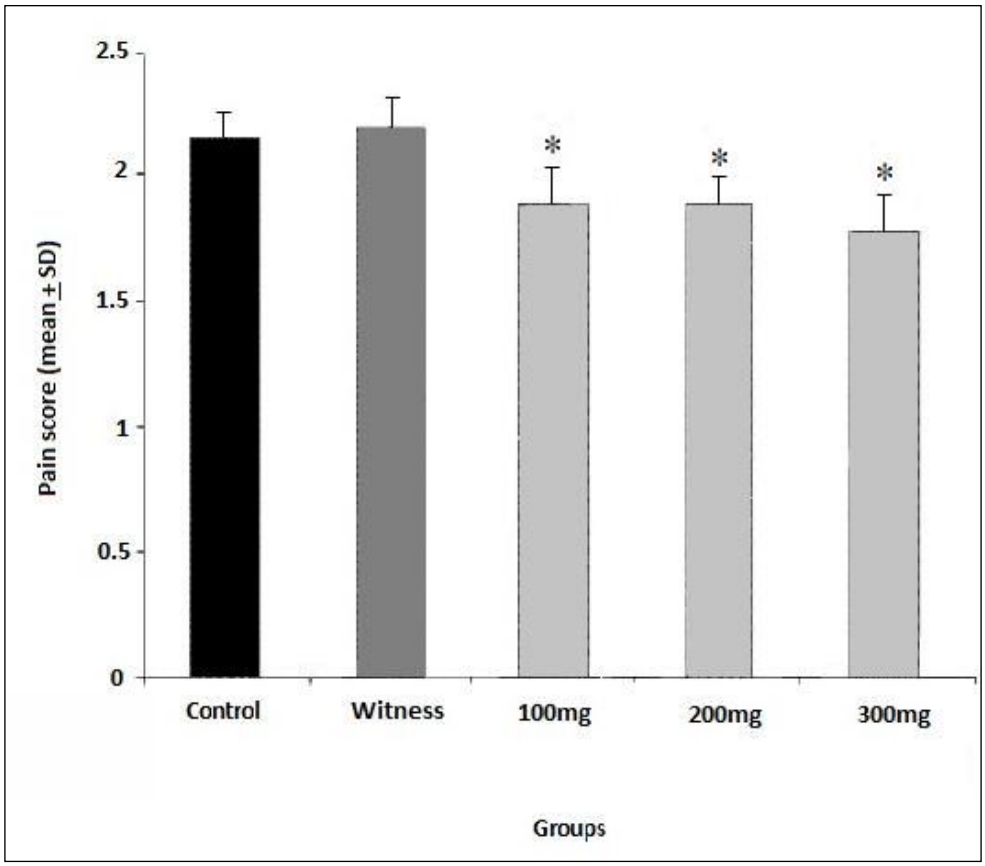

Diagram 1: comparison mean pain intensity in acute pain step between rats receiving different doses of extract of Varthemia Persica and control and witness groups *(p≤'3d0.05).

of hydroalcoholic extract of Varthemia Persica in chronic pain step shows that the extract can relieving pain in chronic step only by maximum quantity($p<0.05$) (diagram 2).

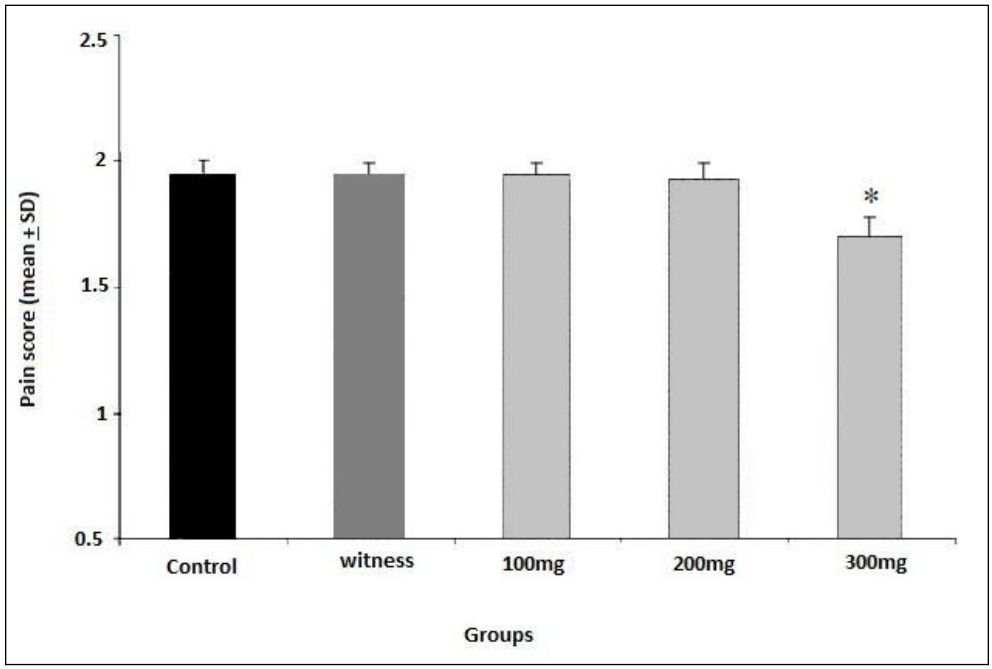

Diagram 2: comparison mean pain intensity in chronic pain step between rats receiving different doses of extract of Varthemia Persica and control and witness groups *($p<0.05$).

Discussion

According the findings from present study, hydroalcoholic extract of Varthemia Persica relieves acute pain of formalin test more than chronic pain. It seems that acute pain step is induced by formalin injection, direct stimulation of pain receptors, and activities of nervous fibers type C while in chronic pain step, they are a set of inflammatory reactions in injured tissue and functional changes in posterior horn of spinal cord that induce pain [9,10]. These functional changes are induced by stimulations of type C nervous fibers. Substances such as substance P, bradikinine, histamine, and prostaglandines are involved in this stimulation [12]. Studies by other researchers show that extract of Varthemia Persica contains flavonoids [6]. Therefore, regarding to presence of flavonoid compounds in this herb, some parts of analgesic effects of Varthemia Persica is probably related to these compounds. Flavonoids control phospholipase, lipoxygenase, and cyclooxygenase which effect directly on prostaglandines and cause analgesic effects. Flavonoids are considered one of the controllers of nitric oxide synthesizing enzyme and prevent nitric oxide production, which increases following formalin injection [13]. prostaglandines stimulates pain receptors both directly and by promoting their sensitivity to other agents like bradikinine. Therefore, flavonoids improve inflammation by controlling cycloxygenase in inflamed tissue and prevent formation of prostaglandines [14,15].

Also, studies have shown that flavonoids decrease intracellular calcium by controlling activity of N-Methyl D-aspartate followed by decrease in

activity of nitric oxide synthesizing enzyme and phospholipase dependent to calcium. By decrease in nitric oxide and prostaglandines, analgesic effects would appear [13]. The presence of flavonoid compounds in Varthemia Persica accounts for its analgesic effects by above mentioned mechanisms.

According to the results from present study and studies by other researchers, analgesic effect of hydroalcoholic extract of Varthemia Persica can be attributed to flavonoids existing in Varthemia Persica although more studies are needed to offer more exact answers on determining its effective substance.

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