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Original article

Effect of Oral Administration of *Astragalus ecbatanus* Chloroform Extract on Acute and Chronic Pain in Balb/C Mice

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Abstract

Background and objectives: *Astragalus* spp., have been used as a pain reliever in traditional medicine; therefore, in this study, we decided to evaluate the antinociceptive effects of *Astragalus ecbatanus* chloroform extract (AECE) in acute and chronic pain in male mice. **Methods:** The extract was obtained from aerial parts of *A. ecbatanus* with maceration method. The antinociceptive effect of AECE was determined by tail-flick, hot-plate, formalin, and rotarod tests followed by the oral intake of mice with AECE at the doses of 200, 400, and 800 mg/kg for 14 days in male Balb/C mice. **Results:** The results showed AECE at the concentrations of 400 and 800 mg/kg revealed a mean latency time of 6.4 and 7.2 s, respectively; representing a remarkable ($p<0.05$) antinociceptive activity compared with the control group. AECE, especially at the doses of 400 and 800 mg/kg, significantly increased the time until the occurrence of painful behaviors (licking or jumping) compared to the control group ($p<0.001$). The results showed AECE, especially in concentrations of 400 and 800 mg/kg, markedly ($p<0.05$) reduced the pain behaviors in the first phase (acute) and the second (chronic) phase of the formalin test compared to the control group. **Conclusion:** According to the reducing pain effect of this plant in both pain tests and in both stages of the formalin test, it can be concluded that *Astragalus ecbatanus* reduces both acute pain and chronic pain and can relieve pain both peripherally and centrally.

Keywords: *Astragalus ecbatanus*; extract; herb; mice; pain

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Introduction

Pain is an unpleasant sensory experience caused by acute or potential tissue damage and is considered as an indicator for identifying diseases. So far, many effective efforts have been made in the field of understanding the mechanisms of pain and treating its types by researchers and doctors [1]. Currently, pain control is done using opioid painkillers and non-steroidal anti-inflammatory drugs. Opioid analgesics, especially morphine, are highly effective in relieving acute and chronic pain [1,2]. These drugs exert their effects by affecting the three opioid receptors located in the central nervous system, especially the spinal cord and brain stem, but by inducing tolerance and physical dependence, as well as increasing sensitivity to pain or hyper allergy, they cause side effects [3,4]. Also, milder painkillers such as non-steroidal anti-inflammatory drugs (NSAIDs) are

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known to exert their effect by preventing the synthesis of eicosanoids (such as prostaglandins) by inhibiting the enzyme cyclooxygenase (COX-oxygenase-Cyclo) and inhibiting pain in its initial stages in the peripheral part [3].
Their long-term use does not lead to tolerance or dependence, but by inhibiting COX, they cause digestive complications such as bleeding in the digestive system [4]. Considering the adverse side effects of chemical drugs and paying exorbitant costs for their preparation, researchers are looking for new drugs in the field of pain reduction to have fewer side effects than existing drugs. Medicinal plants and medicines derived from them have been known as important sources of treatment by human since ancient times [5]. Even today, due to the ease of access to these drugs, there is a lot of focus on their use and research on their properties. Herbs of the genus *Astragalus* include nearly 3000 species of herbs and shrubs, mostly perennials, with more than 250 taxonomic divisions in the world [6]. These herbs are widely distributed throughout the temperate region of the world and so far about 800 species have been identified in the pastures and mountainous areas of Iran [7]. Many species of *Astragalus* have long been used in traditional medicine to treat diabetes, nephritis, stomach ulcers, high blood pressure, and chronic bronchitis [8,9]. In addition, various pharmacological properties of this genus such as antioxidants, boosting immunity system immune system, antihypertensive, antimicrobial and anti-inflammatory effects have been proven [10,11]. *Astragalus ecbatanus* Bunge is one of the native plants in Iran, especially in its western regions, which has been traditionally used for treating painful, anti-stress, nervous disorders, and inflammatory illnesses [10]. In recent years, the antinociceptive and analgesic effects of some *Astragalus* species such as *A. hamosus* (at doses of 700 and 1000 mg/kg of the hydroalcoholic extract), aqueous extract of *A. arbusculinus* gum extract (at doses of 300 and 1000 mg/kg), and ethanolic extract *A. fasciculifolius* (at doses of 400-1200 mg/kg) have been proven in animal models [12-14]. In this study, we decided to evaluate the antinociceptive effects of *A. ecbatanus* chloroform extract (AECE) in two acute and chronic phases in male mice.

Material and Methods

Ethical consideration

This study was approved by the ethics committee of Lorestan University of Medical Sciences, Khorramabad, Iran, with the ethics number of IR.LUMS.REC.1401.190.

Chemicals

n-Hexane, chloroform and methanol were purchase from Merk, Germany. Folin-Ciocalteu, aluminum chloride, morphine and formaldehyde were prepared from Sigma-Aldrich, USA.

Plant material

Aerial parts of *A. ecbatanus* Bunge were collected from the rural regions of Nurabad district, Lorestan province, Western, Iran in June 2021. After identifying the herbal materials by a botanist, a voucher specimen was archived at the Herbarium of Razi Herbal Medicines Research Center, Khorramabad, Iran (No. LUMS-26354).

Preparing the chloroform extract

Air dried plant material (200 g) was powdered and defatted with -n hexane. Maceration method with 70% methanol was used for extraction (72 h). The obtained extract was concentrated by a rotary evaporator at 50 °C and 100 rpm, under vacuum. The obtained extract was kept at −20°C until testing [15].

Phytochemical analysis

The primary phytochemical analysis of the *A. ecbatanus* chloroform extract was performed to confirm the presence of tannins, saponins, alkaloids, flavonoids, and glycosides, etc based on the previous investigation [16].

Total phenolics content

In this test, 3 mL of Folin-Ciocalteu solution with 0.3 mL AECE was mixed with 7% sodium carbonate and the mixture was incubated one hour at room temperature. Finally, by a spectrophotometer, the optical density of the tested tube was read at 760 nm. The total phenolics content of AECE was displayed as mg gallic acid equivalents (GAE) /g dry weight [17].

Total flavonoids content

In brief, 300 µL of AECE was mixed with 2% aluminum chloride (300 µL) and 150 µL of 3% aqueous acetic acid. The suspension was then mixed with 90% ethanol to reach a volume of 5 mL and was incubated at room temperature for 30 min. Finally, by a spectrophotometer, the optical density of the tested tube was read at 760 nm and

total flavonoids content was displayed as mg quercetin equivalent per gram dray weight of plant (mg QE/ g DW) [18].

Animals

In this study, 152 male Balb/C mice weighing between 25-30 g were used, which were randomly placed in groups of 10 mice each. The mice were kept under suitable laboratory conditions with 12 hours of light and 12 hours of darkness at room temperature (22 ± 1°C). They were placed in laboratory conditions one hour before testing. In addition, efforts were made to maintain and work with animals based on the recommendations of the laws for the protection of laboratory animals as well as existing protocols.

Tail flick test

For this test, 40 Balb/C mice in five groups were orally administrated with various concentrations of AECE (200, 400, and 800 mg/kg), morphine (1 mg/kg) and normal saline; their thermal pain threshold was evaluated. In tail flick test, thermal light with an intensity of 5 is shone on the end of the animal's tail by the tail flick device, and the tail flick latency is measured in seconds from the time the heat radiation starts until the tail is removed. In order to avoid tissue damage, the maximum time of irradiating the tail with light is 10 seconds. For each animal, the tail withdrawal delay was measured three times, and the average of the three measurements was reported as the delay time (TFL). A time interval of 5 minutes was considered between each measurement [19].

Formalin test

At first, 40 Balb/C mice in five groups were orally administrated with various concentrations of AECE (200, 400, and 800 mg/kg), morphine (1 mg/kg) and normal saline. The device related to this test is a 30 cm × 30 cm × 30 cm glass chamber with mirror walls. The chamber was cleaned and disinfected with alcohol after each test. Each animal was placed in the chamber for 10 min before injection to get familiar with the environment. Fifty microliters of 1.5% formalin solution (with 0.55% formaldehyde) was injected subcutaneously in the upper lip (near the nose) and then the time of rubbing the injection area was recorded by a video camera. (Sometimes the animal uses both front limbs to rub). Between 0 and third minutes is the primary phase of formalin injection (acute) and between the 12^{th} and 39^{th} minutes is considered the secondary (chronic) phase (the total duration of the experiment will be min 45) [20].

Hot plate test

For this experiment, 40 Balb/C mice in five groups were orally administrated with various concentrations of AECE (200, 400, and 800 mg/kg), morphine (1 mg/kg) and normal saline. The apparatus used for this test includeds a plate with the diameter of 20 cm and a Plexiglas wall with height of 30 cm (LE710 model, Lsi LETICA, Spain). The temperature of the plate was established to 55 ± 0.2 °C to assess the pain sensitivity of tested mice. The interval among the start of the test and the licking front paw or jumping was considered as response time to thermal pain (maximum cutoff was considered 60 s) [21].

Rotarod test

Rotarod experiment was applied to determine the motor coordination in tested mice. Balb/c mice were trained to retain for 180 s on a rolling rod (3 cm diameter) rotating at 8 rpm. Forty Balb/C mice in five groups were orally administrated with various concentrations of AECE (200, 400, and 800 mg/kg), morphine (1 mg/kg) and normal saline, mice were positioned on the rolling rod and the number of falls experienced by the mice during the technique (3 min) was recorded [20].

Statistical analysis

SPSS version 17.0 software was used for data analysis. One-way ANOVA test was used to compare quantitative variables between groups, and Tukey test was used if significant. In addition, the significance level was $p<0.05$ and confidence interval (CI) was considered 95%.

Results and Discussion

Based on the results of phytochemical analysis, the attendance of flavonoids, saponins, terpenoids, and polysaccharides was displayed in AECE. The findings of the contents of secondary metabolites revealed that the total phenolics and flavonoids content were 0.74 (mg GEA/ g DW) and 2.64 (mg QE/g DW), respectively.

Figure 1 shows the comparison of the time latency to the painful stimulus after the oral administration of various concentration of AECE. The results showed that AECE at the concentrations of 400 and 800 mg/kg revealed a mean latency time of 6.4

and 7.2 s, respectively; representing a remarkable ($p<0.05$) antinociceptive activity compared with the control group.

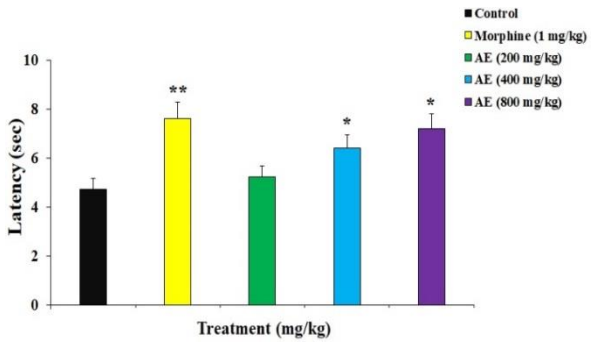

Figure 1. The effect of oral administration of different doses (200, 400 and 800 mg/kg) of *Astragalus ecbatanus* extract (AE) on the tail jump time in the tail flick test; *p<0.01 and **p<0.001: significant difference compared to the control group.

Figure 2 shows the effect of oral administration of AECE at the doses of 200, 400 and 800 mg/kg on pain behaviors caused by the hot plate test. The results of the repeated ANOVA test showed that AECE, especially at the doses of 400 and 800 mg/kg, significantly increased the time until the occurrence of painful behaviors (licking or jumping) compared to the control group ($p<0.001$). The results also showed that although the extract of AECE at 800 mg/kg caused a greater increase in the duration of pain behaviors compared to 1 mg/kg morphine; however, this difference was not significant ($p<0.05$).

Figure 3 displays the effect of oral administration of various concentrations of AECE different on formalin-induced pain behaviors in tested mice. The results showed AECE, especially at the concentrations of 400 and 800 mg/kg, markedly ($p<0.05$) reduced the pain behaviors in the first phase (acute) of formalin test compared with the control group. The results also showed that AECE, especially at the concentrations of 800 mg/kg, significantly ($p<0.001$) caused a decrease in pain behaviors in the second (chronic) phase of the formalin test compared to the control group.

According to the findings of the motor coordination experiment, there was no significant ($p>0.05$) difference in the sensory-motor test followed by the oral administration of different doses (200, 400 and 800 mg /kg) AECE.

Astragalus spp., have shown various pharmacological and therapeutic properties in traditional and modern medicines [8,9].

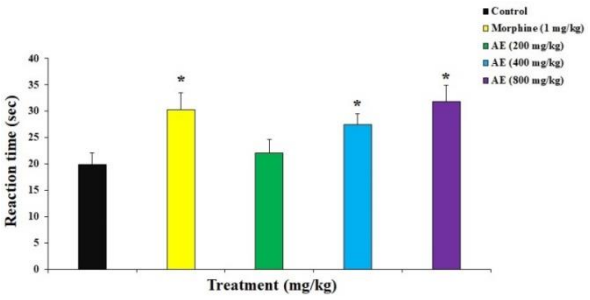

Figure 2. The effect of oral administration of different doses (200, 400 and 800 mg/kg) of *Astragalus ecbatanus* extract (AE) on the reaction time of the mice in the hot-plate test; *p<0.001: significant difference compared to the control group.

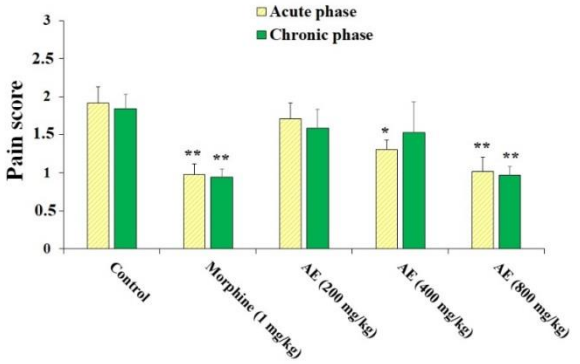

Figure 3. The effect of oral administration of different doses (200, 400 and 800 mg/kg) of *Astragalus ecbatanus* extract (AE) on pain behaviors caused by formalin injection in the acute (0-3 minutes) and chronic phase (12-39 minutes); *p<0.05 and **p<0.001 significant difference compared to the control group.

The antinociceptive and analgesic effects of some *Astragalus* species such as *A. hamosus*, *A. arbusculinus*, and *A. fasciculifolius* have been proven in animal models [12-14]. In this study, we initially tested the analgesic effects of different extracts and fractions of *A. ecbatanus* as a pilot, but the results of the initial studies showed that among the tested extracts and fractions, chloroform extract showed the most analgesic effectiveness. For this reason, in this study, we decided to evaluate the antinociceptive effects of *A. ecbatanus* in acute and chronic phases in mice. In tail flick test, we found that AECE at the concentrations of 400 and 800 mg/kg revealed a mean latency time of 6.4 and 7.2 s, respectively; representing a remarkable ($p<0.05$) antinociceptive activity compared with the control

group. Tail flick test is used to investigate the central analgesic effects of drugs and chemical compounds. In fact, this test is sensitive to drugs that act on the central nervous system [22]. The hot plate test is a model of severe supraspinal pain and has been used in studies of the properties of analgesic drugs for more than 50 years. The main characteristics of this test are the ability to provide a direct and accurate examination of the animal's response to treatments. It is also a simple and widely accepted application [23]. The acute pain induced by the hot plate test is not related to pathology; therefore, it is not similar to inflammatory and neuropathic pain in which constant symptoms of the disease develop [24]. Our results revealed that AECE, especially at the doses of 400 and 800 mg/kg, significantly increased the time until the occurrence of painful behaviors (licking or jumping) compared to the control group.

The formalin test is widely used to investigate the pain-causing mechanism and to study the analgesic effect of compounds [25]. The first phase (early phase) starts immediately after formalin injection and lasts for 3-5 minutes. The second phase or late phase (late phase) starts approximately 15-20 minutes after formalin injection and lasts up to one hour. The first is due to the direct stimulation of type C sensory fibers. While the second stage is actually an inflammatory response [26]. Non-steroidal anti-inflammatory drugs (aspirin, indomethacin) and steroidal (hydrocortisone, hexamethasone) relieve pain in the second stage of the formalin test, but in the first stage, they either have no effect or have little effect. Therefore, it seems that inflammatory processes and substances such as histamine, serotonin, prostaglandins and bradykinin play a role in the delayed phase [27]. In addition, it seems that the delayed phase is caused by changes in the central nervous system (posterior horn of the spinal cord), which is also influenced by the neural activity produced during the first stage of this test [28]. Our results showed that AECE, especially at the concentrations of 400 and 800 mg/kg, markedly ($p<0.05$) reduced the pain behaviors in the first phase (acute) of formalin test compared with the control group. The results also showed that AECE, especially at concentrations of 800 mg/kg, significantly ($p<0.001$) caused a decrease in pain behaviors in the second (chronic) phase of the formalin test compared to the control group.

The results of the current study show that the AECE reduces the pain caused by thermal and chemical stimuli in a dose-dependent manner. Such an effect on these two types of stimuli is characteristic of central analgesics such as morphine, which inhibit both pain caused by inflammatory processes and pain caused by non-inflammatory processes [29]. Therefore, this plant extract has morphine-like effects which indicates its central analgesic effect.

Previous studies showed that phenolic and flavonoids compounds are considered as the main compounds of *Astragalus* spp. and it has been proven that phenolic and flavonoid compounds have a tendency to bind to GABA A receptors [30], where in relation to chronic pain pathology, it has been shown that significant analgesia occurs with GABA A receptor stimulation [31]. Therefore, the phenols and flavonoids in the extract of this plant may cause pain relief by stimulating GABA A receptors.

Conclusion

According to the reducing pain effect of *Astragalus ecbatanus* in both pain tests and in both stages of the formalin test, it can be concluded that this plant reduces both acute pain and chronic pain and both peripherally and centrally.

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Author contributions

Javad Ghasemian Yadegari and Ezatollah Fazeli Moghadam designed and concepted the study; Hazhir Golmohammadi and Setareh Dastyarhaghighi performed the experiments and data analysis; Mehrdad Ghoulami performed the critical review' Hossein Mahmoudvand was the supervisor and wrote the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

References

[1] Lamont LA, Tranquilli WJ, Grimm KA. Physiology of pain. *Vet Clin North Am Small Anim Pract*. 2000; 30(4): 703–728.

[2] Crofford LJ. Chronic pain: where the body

meets the brain. *Trans Ame Clin Climatol Assoc*. 2015; 126: 167–183.
[3] Gureje O, Von Korff M, Simon GE, Gater R. Persistent pain and well-being: a World Health Organization study in primary care. *Jama*. 1998; 280(2): 147–151.
[4] Els C, Jackson TD, Kunyk D, Lappi VG, Sonnenberg B, Hagtvedt R, Sharma S, Kolahdooz F, Straube S. Adverse events associated with medium-and long-term use of opioids for chronic non-cancer pain: an overview of Cochrane reviews. *Cochrane Database Syst Rev*. 2017: 10: 1–43.
[5] Gagnier JJ, van Tulder MW, Berman B, Bombardier C. Herbal medicine for low back pain: a Cochrane review. *Spine*. 2007; 32(1): 82–92.
[6] Rios JL, Waterman PG. A review of the pharmacology and toxicology of *Astragalus*. *Phytother Res*. 1997; 11(6): 411–418.
[7] Li X, Qu L, Dong Y, Han L, Liu E, Fang S, Zhang Y, Wang T. A review of recent research progress on the *Astragalus* genus. *Molecules*. 2014; 19(11): 18850–18880.
[8] Shahrajabian MH, Sun W, Cheng Q. A review of *Astragalus* species as foodstuffs, dietary supplements, a traditional Chinese medicine and a part of modern pharmaceutical science. *Appl Ecol Environ Res.* 2019; 17(6): 13371–13382.
[9] Miraj S, Kiani S. *Astragalus membranaceus*: a review study of its anti-carcinoma activities. *Der Pharm Lett*. 2016; 8(6): 59–65.
[10] Ghasemian Yadegari J, Nazemiyeh H, Asnaashari S, Fathiazad F. Chemical characterization of the essential oil from aerial parts of *Astragalus maximus* from Northwest of Iran. *Adv Biosci Clin Med*. 2015; 3(3): 32–39.
[11] Turgut-Kara N, Arı S. Micropropagation of *Astragalus maximus* Willd. *Biotechnol Biotechnol Equip*. 2006; 20(1): 20–22.
[12] Shojaii A, Motaghinejad M, Norouzi S, Motevalian M. Evaluation of anti-inflammatory and analgesic activity of the extract and fractions of *Astragalus hamosus* in animal models. *Iran J Pharm Res*. 2015; 14(1): 263–269.
[13] Shojaii A, Motevalian M, Rahnama N. Evaluation of anti-inflammatory and analgesic activities and the phytochemical study of *Astragalus arbusculinus* gum in animal models. *J Basic Clin Physiol Pharmacol*. 2015; 26(4): 369–374.
[14] Shahrani M, Asgharzadeh N, Torki A, Asgharian S, Lorigooini Z. *Astragalus fasciculifolius* manna; antinociceptive, anti-inflammatory and antioxidant properties in mice. *Immunopathol Persa*. 2021; 7(1): 1–7.
[15] Ghasemian Yadegari J, Khudair Khalaf A, Darabi R, Mahmoudvand H. Antiparasitic effects and cellular mechanism of *Astragalus maximus* chloroform extract against clinical isolates of *Giardia lamblia*. *Res J Pharmacol*. 2022; 9(3): 5–13.
[16] Evans WC. Trease and Evans pharmacognosy. 14th ed. London: WB Saunders Company Limited, 1972.
[17] Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Meth Enzymol.* 1999; 299: 152–178.
[18] Phuyal N, Jha PK, Raturi PP, Rajbhandary S. Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of *Zanthoxylum armatum* DC. *Sci World J*. 2020; Article ID 8780704.
[19] Mahmoudvand H, Ziaali N, Ghazvini H, Shojaee S, Keshavarz H, Esmaeilpour K, Sheibani V. *Toxoplasma gondii* infection promotes neuroinflammation through cytokine networks and induced hyperalgesia in BALB/c mice. *Inflamm*. 2016; 39(1): 405–412.
[20] Raboisson P, Dallel R. The orofacial formalin test. *Neurosci Biobehav Rev*. 2004; 28(2): 219–226.
[21] Mahmoudvand H, Khaksarian M, Ebrahimi K, Shiravand S, Jahanbakhsh S, Niazi M, Nadri S. Antinociceptive effects of green synthesized copper nanoparticles alone or in combination with morphine. *Ann Med Surg*. 2020; 51: 31–36.
[22] Carlisson KH, Jurna I. Depression by flupirtine; a novel analgesic agent of motor and sensory response of nociceptive system in the rat spinal cord. *Eur J Pharmacol*. 1987; 143(1): 87–99.
[23] Dubisson D, Dennis SG. The formalin test a quantivie study of the analgesic effects of morphin, meperidne and brainstem stimulation in rat and cats. *Pain*. 1997; 4(2):

161–174.
[24] Chen CL, Broom DC, Liu Y, Denooji JC, Li Z, Cen C, Abdel Samad O, Jessell TM, Woolf CJ, Ma Q. Run x1 determines nociceptive sensory neuron phenotype and is required for thermal and neuropathic pain. *Neuron.* 2006; 49(3): 365–377.
[25] Tgolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. *Pain*. 1991; 51(1): 17–25.
[26] Ngassapa DN. Comparison of functional characteristics of intradental A- and C-nerve fibres in dental pain. *East Afr Med J*. 1996; 73(3): 207–209.
[27] Manabu S, Tsuyako O, Hiroshi T, Reizo I. Modified formalin test: characteristic biphasic pain response. *Pain*. 1989; 38(3): 347–352.
[28] Rosland JH, Tjolsen A, Mahle B, Hole K. The formalin test in mice-effect of formalin concentration. *Pain*. 1990; 42(2): 235–242.
[29] Atta AH, Alkofahi A. Anti-nociceptive and anti-inflammatory effects of some Jordnian medicinal plant extrats. *J Ethnopharmacol*. 1998; 60(2): 117–124.
[30] Salah SM, Jager AK. Two flavonoids from *Artemisia herba-alba* Asso with in vitro GABA-A benzodiazepine receptor activity. *J Ethnopharmacol*. 2005; 99(1): 145–146.
[31] Knabl J, Witschi R, Hosl K, Reinold H, Zeilhofer UB, Ahmadi S, Brockhaus J, Sergejeva M, Hess A, Brune K, Fritschy JM, Rudolph U, Mohler H, Zeilhofer HU. Reversal of pathological pain through specific spinal GABA A receptor subtypes. *Nature*. 2008; 451(7176): 330–334.

Abbreviations

AECE*: Astragalus ecbatanus* chloroform extract; NSAIDs: non-steroidal anti-inflammatory drugs; GAE: gallic acid equivalents; QE: quercetin equivalent