

The Antinociceptive Effects of Hydroalcoholic Extract of *Bryonia dioica* in Male Rats

Mohammad Zarei^{1,2}; Saeed Mohammadi^{3,*}; Nasreen Abolhassani⁴; Mahtab Asgari Nematian⁵

¹Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, IR Iran

²Department of Physiology, Hamadan University of Medical Sciences, Hamadan, IR Iran

³Department of Biology, Faculty of Basic Sciences, Islamic Azad University, Hamadan, IR Iran

⁴Department of Biology, Faculty of Basic Sciences, Science and Research Branch, Islamic Azad University, Tehran, IR Iran

⁵Department of Biology, Hamadan Branch, Payam-noor University, Hamadan, IR Iran

*Corresponding author: Saeed Mohammadi, Professor Mussivand Blvd, Hamadan Branch, Islamic Azad University, Hamadan, IR Iran. Tel: +98-8134494000, Fax: +98-8134494026, E-mail: smiauhphd.sm@gmail.com

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Background: Side effects of synthetic analgesic drugs in the clinical practice have drawn researchers' attention on developing the herbal medicine as more appropriate analgesic agents.

Objectives: This study aimed to investigate the antinociceptive effect of hydroalcoholic leaf extract of *Bryonia dioica* (HEBD) on male rats.

Materials and Methods: In this experimental study, 42 adult male rats were divided into 7 groups: control, HEBD (80, 100, and 300 mg/kg, ip), morphine (1 mg/kg, ip), indomethacin (1 mg/kg, ip), and naloxone (1 mg/kg ip). In order to assess the analgesic effects of the extract, writhing, tail-flick, and formalin tests were used. Also, Tukey post hoc and 1-way analysis of variance (ANOVA) tests were used to analyze the data.

Results: Using writhing, tail-flick, and formalin tests, dramatic antinociceptive effects were observed for HEBD at the dose of 300 mg/kg with $P < 0.01$. The results of data analysis also revealed that there was no significant difference between indomethacin and dose of 300 mg/kg of extract in formalin test. LD50 of the plant was 4200 mg/kg.

Conclusions: The experimental data verified that HEBD leaves displayed remarkable antinociceptive activity. Furthermore, the nonselective opioid receptor antagonist, naloxone blocked the antinociceptive effect of the extract in all tests, suggesting that HEBD may act both at peripheral and central levels.

Keywords: Pain; Plants, Medicinal; Bryonia; Formaldehyde; Rats

1. Background

Feeling pain is one of the primary reactions of an organism as well as one of the important requirements in its protection against potential damaging stimuli to the tissues. Tissue damage is a result of production and release of several inflammatory mediators, many of which are able to sensitize the primary afferent nociceptors and consequently cause pain (1, 2).

Most of the times, the conventional drugs are not enough to manage the inflammation and pain. Moreover, for most patients the common treatments lack appropriate effects so that long-term consumption of these drugs leads to severe side effects (3, 4).

Herbal medicines are important resources of new chemicals with very strong therapeutic effects (5). In Iranian traditional medical practice, although prescribing herbal medicine to treat the pain and inflammation is customary, the origin and structure of such plants have often remained unknown. Therefore, learning about the pharmaceutical effects of these plants' extracts can be ap-

plied as a logical research approach in order to discover new drugs (6, 7).

Bryonia dioica commonly known as white-bryony belongs to *Cucurbitaceae* family. This plant is a climbing perennial herb with tuberous roots, which grows in temperate Europe, North Africa, and Western Asia (8).

Bryonia dioica is used for both internal and external uses. It is taken orally in small quantities for the treatment of various inflammatory conditions, bronchial complaints, asthma, intestinal ulcers, hypertension, and arthritis. Externally, it is applied as a rubefacient to muscular and joint pains and pleurisy. It has been reported that the plant is used in folk medicine as a drastic purgative, emetic, bitter tonic, and an anti-diabetic agent (9-11).

2. Objectives

The researchers have reviewed the related literature and concluded that no research study has been conducted to investigate the antinociceptive effect of hydroalcoholic ex-

tract of *Bryonia dioica* in male rats. Therefore, in the present study, the antinociceptive effects of *Bryonia dioica* leaf were investigated using formalin, writhing, and tail-flick tests.

3. Materials and Methods

3.1. Extract Preparation

One kilogram of fresh *Bryonia dioica* leaves were prepared (confirmed by the botanist of Avicenna University of Hamedan) in July 2012, a voucher specimen of the plant (voucher number: 128) was deposited in the herbarium of the Department of Biology, Faculty of Basic Sciences, Avicenna University, Iran. For preparation of hydroalcoholic extract, dried leaves of the plant (100 g) were macerated with 500 mL of EtOH:H₂O (7:3) for 48 h. The extract was then shaken, filtered, and evaporated in a rotating evaporator (Heidolph, Laborota 4000, Schwabach, Germany) under reduced pressure until its dryness.

3.2. Animals

Forty-two male Wistar rats (220 - 250 g) were purchased from Pasteur Institute of Iran. The animals were kept at a controlled temperature of $23 \pm 1^\circ\text{C}$, with a light/dark cycle of 12:12 hours, with the light period beginning at 06:00 hour, and they were given food and water ad libitum. All experiments were conducted between 10:00 and 16:00 hour. The study was conducted in concordance with the IASP guidelines on the use of laboratory animals (12).

3.3. Drugs and Extracts Administration

The animals were divided into 7 groups each with 6 members: control, HEBD (80, 100, and 300 mg/kg, ip), morphine (1mg/kg, ip), indomethacin (1mg/kg, ip), and naloxone (1 mg/kg ip). Sulfate morphine, naloxone and indomethacin were purchased from Darou Pakhsh (Iran) and acetic acid with formalin from Merck Inc (Germany).

3.4. Writhing Test

On the experiment day, 30 minutes before running the experiments, the animals were sent into a standard experiment glass box to get used to the conditions. The hydroalcoholic extract of *Bryonia dioica* leaves was solved in sterile physiologic serum and injected intraperitoneally in doses of 80, 100, and 300 mg/kg. After 15 minutes, acetic acid on the scale of 1 mg/kg of the body weight with the density of 6% was injected and immediately after the intraperitoneal injection of the acetic acid, the number of abdominal contractions was counted for 30 minutes (both legs stretched). It is also necessary to mention that each animal was used only once (13). In the control group, after intraperitoneal injection of saline, the writhing test was ran.

3.5. Tail-flick Test

Acute nociception was assessed using a tail flick analgesimeter (by Borj Sanat Iran Company). following the method of D'Amour and Smith (14). Briefly each mouse

was placed in a restrainer, 30 minutes after treatment and baseline reaction time was measured by focusing a beam of light on the distal one-third portion of the tail. At 15 minutes intervals the reaction time was recorded until 2 hours. A 15 seconds cut off time was used in order to prevent tissue damage. Percent of maximum possible antinociceptive effect was calculated for each time and compared.

3.5. Formalin Test

In this experiment, suggested model of Dubuisson and Dennis, was used in order to evaluate the chronic pain. One hour before the test, the animals were sent into the special box of formalin test in order to get used to the experiment condition. The box was made of Plexiglas in the dimensions of $30 \times 30 \times 30$ cm. Positioned in 45° , a mirror was inserted below the box and in front of the observer to observe the animal's behaviors more clearly. Thirty minutes after the intraperitoneal injection of the drugs, there was a subcutaneous injection of 50 μL of 2.5% formalin solution into the subplantar surface of the left hind paw; then the animal was sent to the test special box again. The animal's behavior was observed and labeled for 60 minutes as following: once every 15 seconds, the motor response to pain was rated and recorded on a scale of 0, 1, 2, and 3. The numbers indicate the following reactions: number 0, the animal moves with complete balance and its weight distributed equally on both feet; number 1, the animal could not tolerate its body weight on the being-injected foot or take care of that foot; number 2, the animal raised the painful claw and has no contact with the box floor; and number 3, the animal licks the painful claw, chewed or moved severely. The average of first 5-minute grades was considered as phase 1 (acute phase) and the average of minutes 15 to 60 was considered as the phase 2 (chronic phase) (15).

3.6. Lethal Dose (LD₅₀)

The acute toxicity was determined by the previous laboratory model (16). Various doses of the extract were injected separately and intraperitoneally to the male rats. The number of deaths of the animals was counted within the next 72 hours and the LD₅₀ of the plant extract was determined.

3.7. Statistical Analysis

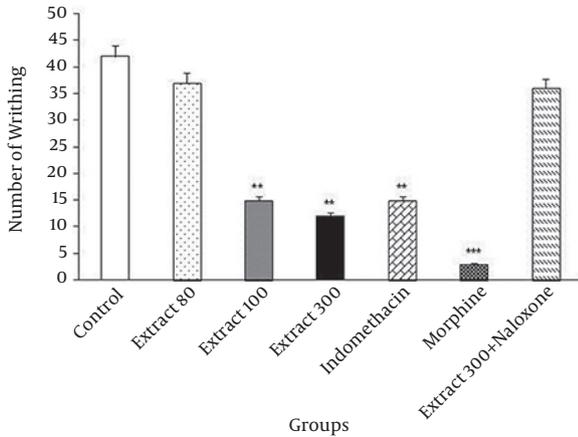
The data were presented in the form of mean \pm S.E.M. Analysis of variance (ANOVA) and then Tukey test was performed. After the data were obtained from different experimental groups, they were analyzed using SPSS 18 software and $P < 0.05$ was considered as the significant indicator.

4. Results

4.1. Writhing Test

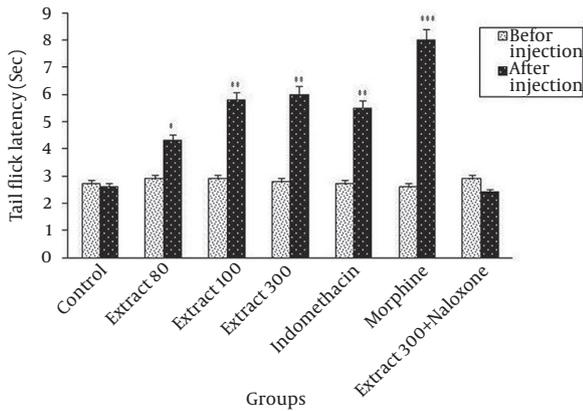
The results of this study revealed that there is a significance difference in the injection of 100 and 300 mg/kg

Figure 1. Effect of different doses of hydroalcoholic extract of *Bryonia dioica* on acetic acid induced writhing in rats.



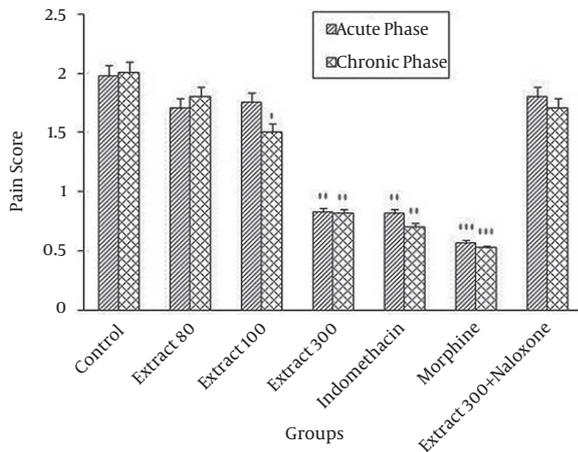
As compared with control: **P < 0.01 and ***P < 0.001 (n = 6, mean ± S.E.M).

Figure 2. Effect of different doses of hydroalcoholic extract of *Bryonia dioica* on the reaction time of rats



As compared with control: * P < 0.05, ** P < 0.01 and ***P < 0.001. (n = 6, mean ± S.E.M).

Figure 3. Effect of the hydroalcoholic extract of *Bryonia dioica* on formalin-induced nociception in rats



As compared with control: * P < 0.05, ** P < 0.01 and ***P < 0.001. (n = 6, mean ± S.E.M).

of the hydroalcoholic extract of *Bryonia dioica* leaf compared to the control group ($P < 0.01$). As shown in Figure 1, there is a significant difference between the morphine and indomethacin group and control group ($P < 0.001$ and $P < 0.01$, respectively).

4.2. Tail-flick Test

In this test, injection of 80 mg, 100 mg, and 300 mg/kg doses of the hydroalcoholic extract of *Bryonia dioica* in comparison with the control group showed a significant decrease ($P < 0.05$, $P < 0.01$, and $P < 0.01$, respectively). On the other hand, injection of morphine and indomethacin compared to the control group showed a significant decrease ($P < 0.001$ and $P < 0.01$, respectively) (as shown in Figure 2).

4.3. Formalin Test

As shown in Figure 3, in formalin test, injection of 300 mg/kg of the indomethacin extract and morphine led to decrease the pain score in acute phase ($P < 0.01$, $P < 0.01$ and $P < 0.001$, respectively). The injection of doses 100 mg and 300 mg of the extract all led to the significant decrease of the pain in chronic phase ($P < 0.05$ and $P < 0.01$). Also injection of indomethacin and morphine in comparison with the control group showed a significant decrease in both acute and chronic phase of formalin test ($P < 0.01$ and $P < 0.001$, respectively).

4.4. Lethal Dose (LD50)

LD₅₀ of the plant intraperitoneally was 4200 mg/kg.

5. Discussion

The results of the present study, confirm the antinociceptive effect of hydroalcoholic extract of *Bryonia dioica* leaf. Standard tests of Writhing, tail-flick and formalin were used in order to investigate the antinociceptive effect of hydroalcoholic extract of *Bryonia dioica* leaf. One of the most important tests, which is usually used to screen possible antinociceptive mixtures is writhing test in which acetic acid as well as a chemical stimulation, is extensively used to evaluate peripheral antinociceptive activity (17). The hydroalcoholic extract of *Bryonia dioica* prevented abdominal constriction caused by acetic acid; therefore, it is estimated that its alleviative effects are supported by the environmental mechanisms. Intraperitoneal injection of acetic acid can cause the acute inflammation of the peritoneum (18). In this model, it seems that peripheral antinociceptive effects of *Bryonia dioica* are caused indirectly by internal mediators such as bradykinin, serotonin, histamine, substance-p, and prostaglandin. It is justified that all of these mediators are associated with the stimulation of peripheral nociceptive neurons (17). Also the concentration of glutamate and aspartate in the cerebrospinal fluid increased after the injection of acetic acid (18, 19). The present results demonstrate that systemic treatment of rat with HEBD

causes significant and dose-dependent antinociception. Glutamate is known to be the main excitatory amino acid in pain transmission. Therefore, substances capable of blocking either iGluRs (ionotropic glutamate receptors) or mGluRs (metabotropic glutamate receptors) may exhibit antinociceptive effects in several mammalian species (20).

Tail-flick test (in which the thermal stimuli are used) is among the most significant parameters of evaluating antinociceptive activities (14). The results of the present study indicate that injection of moderate or high doses of the extract decreases the pain resulted from thermal stimulation in tail-flick test. Since tail-flick test is used to investigate the spinal reflexes, and to identify central antinociceptive (21, 22), *Bryonia dioica* extract can be suggested as having central antinociceptive effects.

The advantage of using evaluative model of formalin pain is its ability to recognize the compounds that act through central pain route from peripheral pain (23). Subcutaneous injection of formalin creates two different nociceptive phases. Phase 1 is acute neurogenic phase, which is created around active nociceptive neurons under direct effect of formalin. The second phase, called chronic inflammatory phase is created due to activation of ventral horn neurons in spinal cord level (24). The results showed that *Bryonia dioica* extract leaves have an inhibitory effect over the pain. It is necessary to mention that decreasing effect is more prominent in the chronic phase than the acute phase. Restraining the chronic phase of the formalin test by the extract can be a result of inflammation, which releases compounds like prostaglandins F_2 and E_2 in some amounts sensitized by central nociceptive neurons (25). To evaluate opioid system interference caused by antinociceptive effect of this extract with naloxone, one of the antagonist drugs in opioid system was used, which prevents the activation of opioid receptors (26, 27). The results of the present study indicate that naloxone decreases the antinociceptive effect of the extract. Therefore, it seems that the effect of extract in pain relief is due to the opioid receptors.

Biologic or therapeutic activity of herbs has a close relationship with their chemical combinations (28). The phytochemical screening of roots of *Bryonia dioica* showed the presence of polyphenols, sterols, triterpenes, alkaloids, heterosides-c, carbohydrates and saponins. However, catechic tannins, gallic tannins, anthocyanes, coumarins, anthraquinones and heterosides-o were not detected. The biological or therapeutic activities of medicinal plants are closely related to their chemical compounds (29, 30). On the other hand, antinociceptive effects of saponins and alkaloids have been mentioned in various scientific references (31-33).

One of the important compounds of triterpenoids available in this plant is cucurbitacin. In a study, it has been stated that *Wilbrandia ebracteata* can leave an antinociceptive effect because of having cucurbitacin as well as inhibiting prostaglandin E_2 . Therefore, this compound

can be named as noticeable candidates to create antinociceptive effects of *Bryonia dioica* (34). It is also indicated that compounds such as luteolin and apigenin contain antinociceptive effects (35).

Various flavonoids such as kaempferol have been already proved as possessing anti-inflammatory and antinociceptive effects (36-39). The results showed that by inhibiting the activity of N-methyl-D-aspartate receptor, flavonoids cut intracellular calcium down. Consequently, the synthesizer enzyme of calcium-related nitric oxide and phospholipase A2 decreases too and with the reduction of nitric oxide and prostaglandins, especially the prostaglandin E_2 and F_2 reveals its antinociceptive effects (40).

Another compound available in the extract of this plant is tannin. There are reports stating that tannins have roles in creating antinociceptive and anti-inflammatory effects (41). Therefore, another part of antinociceptive effects of the extract is due to tannins inside the plant.

In conclusion, our data indicated that *Bryonia dioica* has a peripheral and central antinociceptive effects. The antinociceptive effect of the extract may be due to their content of flavonoids and/or tannins. However, the chemical constituents responsible for the pharmacological activities remain to be investigated.

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