کارگاه‌های آموزشی مرکز اطلاعات علمی

مقاله نویسی علوم انسانی

اصول تنظیم قراردادها

آموزش مهارت های کاربردی در تدوین و چاپ مقاله
The Antinociceptive Effects of Hydroalcoholic Extract of Borago Officinalis Flower in Male Rats Using Formalin Test

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ABSTRACT

Introduction: Borago officinalis flower (borage) is a known sedative in herbal medicine; the aim of the present study was to evaluate the antinociceptive effect of borage hydroalcoholic extract in formalin test male rats.

Methods: Fifty-six adult male albino Wistar rats were randomly divided into seven groups: Control groups of A (intact), B (saline), and C (Positive control) plus test groups of D, E, F, and G (n=8). The groups D, E, and F received 6.25, 12.5, and 25 mg/kg, Borago officinalis flower hydroalcoholic extract before the test, respectively but group G received 25 mg/kg borage extract and aspirin before the test. A biphasic pain was induced by injection of formalin 1%. The obtained data were analyzed by SPSS software ver. 17 employing statistical tests of Kruskal-Wallis and Mann-Whitney. The results were expressed as mean±SD. Statistical differences were considered significant at P<0.05.

Results: The results revealed that the acute and chronic pain behavior score in test groups of D, E, and F significantly decreased compared to groups A and B, but this score did not show any difference compared to group C. Moreover, chronic pain behavior score in group G was significantly lower than all other groups.

Discussion: The results indicated that Borago officinalis hydroalcoholic extract affects the acute and chronic pain behavior response in formaline test male rats.
associated biomarkers (Lee et al., 2014). The literature review indicates that seed oil and flowers of borage contain many unsaturated fatty acids such as palmitic, linoleic, stearic, and γ-linolenic acids (Morteza, 2014; Ramandi et al., 2011). Since Borago officinalis L. flowers are used as a sedative in traditional medicine, this experiment was designed to evaluate the antinociceptive effects of hydroalcoholic extract of Borago officinalis L. flowers in mature male rats using formalin test.

2. Methods

2.1. Animals

Fifty-six adult male albino Wistar rats, weighing 250±30 g were selected. After weighing, the animals were divided into 7 (intact, placebo, positive control, and test) groups (n=8 in each group) and they were individually kept in cages (one rat in each cage). All animals had free access to water and food. The rats were placed in a room at 23±3°C with a fixed 12:12 h synthetic dark-light period (Timer Model: SUL180a, AC 220V, China, 6 am to 6 pm) with humidity of 45% to 60%. During the trial period, the air was suitably recycled. This experiment followed the principles and procedures of the Iranian Council on animals and approval from the Committee of Animal Research, Zahedan University of Medical Sciences.

2.2. Study design

After a week of habituation, the animals were randomly assigned to the following groups (n=8):

Control group A (intact): They did not receive any agent before the test.

Placebo group B (placebo): They received normal saline 30 minutes before the test.

Positive group C (Aspirin): They received 100 mg/kg aspirin solution 30 minutes before the test by gavage (Adeyemi et al., 2008).

Test group D: They received 6.25 mg/kg Borago officinalis flower hydroalcoholic extract by gavage 30 minutes before the test.

Test group E: They received 12.5 mg/kg Borago officinalis flower hydroalcoholic extract by gavage 30 minutes before the test.

Test group F: They received 25 mg/kg Borago officinalis flower hydroalcoholic extract by gavage 30 minutes before the test (Kweka et al., 2012).

And finally Test group G: They received 25 mg/kg Borago officinalis flower hydroalcoholic extract and 100 mg/Kg aspirin at the same time 30 minutes before the test by gavage.

2.3. Drugs

The compounds used in the present study were formicdehyde (37%) and crude aspirin (Germany Merck Corporation). Physiological saline was purchased from a city drug store and maintained in suitable temperature (refrigerator).

2.4. Preparation of the extracts

Borago Officinalis flowers were purchased from a local medicine market in Kerman, Iran and identified by the experts. The flowers were turned into powder by hand. Extraction was performed by mixing 200 g of dried powder in ethylic alcohol (70%) for 24 hours in Soxhlet extractor. The prepared extract was filtered through a gauze cloth followed by filtration throughout a normal filter paper Whatman No. 1 (Etebari et al., 2012). The product was a dark brown hydroalcoholic extract, which was dried later in incubator for 1 day.

2.5. Preparation of aspirin solution

Crude aspirin powder (Germany Merck Corporation) was purchased from Pharmacy School in Zabol, Iran. Before the test measurements, aspirin was weighed and daily mixed with saline.

2.6. Measure

2.6.1. Pain behavior response scoring

Acute pain was assessed using formalin test (1%). At first, rats were located in open Plexiglas observation chambers for 30 min to be adjusted to their new environment. Then, they were removed for formalin test administration. After formalin administration, the rats were placed in the previous Plexiglas observation box measuring 40 cm×20 cm×20 cm. These Plexiglas observation chambers had a mirror placed below the floor of the chamber at a 45° angle to allow an unobstructed view of the rat’s paws. Pain behavior responses were measured at the beginning by subcutaneous injection of formalin 1% (50 μL/ paw) with a 30-gauge needle into the dorsal
surface of the right hind paw. Formalin-induced flinching behavior was biphasic.

The primary flinching behavior was an acute phase (0–10 min) followed by a moderately short and quiet period, and then a lengthened constant response (15–60 min). Pain behavioral responses were measured every minute, and averaged at 5-minute intervals for 1 hour (Long et al., 2013; Wheeler-Aceto et al., 1990; and Dubuisson, Dennis, 1977). The scores were as following:

0 = the injected paw does not show any reaction, indicating no pain

1 = the injected paw has a little reaction, by picking up the leg, indicating mild pain

2 = the injected paw is completely elevated and the heel is not in contact with surface, indicating moderate pain

3 = the injected paw is licked, bitten, or shaken, indicating severe pain.

2.6.2. Statistical analysis

The obtained data were analyzed by performing Kruskal-Wallis and Mann-Whitney statistical tests using SPSS software ver. 17. The results were expressed as mean±SD. Statistical differences were considered significant at P<0.05.

3. Results

The results obtained from the present study showed that the injection of formalin (1%) in rats paw induced a characteristic pattern of pain behavior, which was biphasic. The primary phase initiates immediately after formalin injection, falling slowly after 10 min. The second phase started at about 15 min later and lasted for 60 min. Our results indicated that acute pain behavior score in group C (2.62±0.13), D (2.51±0.21), E (2.31±0.13), F (2.31±0.21), and G (2.11±0.17), significantly decreased compared to control groups A (3.13±0.19) and B (3.21±0.17, P=0.01) (Figure 1, P<0.002). On the other hand, this value in groups D, E, and F did not show any differences compared to group C. This value in group G significantly decreased compared to other groups (Figure 2, P=0.001, P=0.01).

In addition, chronic pain behavior score in group C and test groups D, E, and F significantly decreased compared to control groups A and B. On the other hand, this value

Figure 1. Acute pain behavior response score in group A, B, C and test groups D, E, F and G. *=P<0.05 compared to A and B**, P<0.05 compared to all groups, n=8.

Figure 2. Chronic pain behavior response score in group, A, B, C and test groups D, E, F and G. *=P<0.05, compared to A & B groups, **=P<0.05 compared to all groups, n=8.

4. Discussion

Our findings indicated that acute pain behavior score in groups C, D, E, F, and G significantly decreased compared to control groups A and B but this value in group G was significantly lower than those values in other groups. Moreover, chronic pain behavior score in groups C, D, E, F, and G significantly decreased compared to control groups A and B. This value did not show any significant differences among groups C, D, E, and F, but
this value in group G significantly decreased compared to other groups.

Moreover, our results showed that injection of formalin 1% into the paw of the rats induced a biphasic pain. The primary stage (acute pain) occurred at once, falling slowly after 10 minutes. The chronic pain started at about 15 min and lasted for 60 min (Jahanbani et al., 2014). The formalin test is a suitable model for biphasic experimental pain, which initiated at distinct periods. The first period (phase 1) is acute or neurogenic pain happening around 3 min after formalin injection. The second period (phase 2) is chronic or inflammatory pain, which happens around 30 minutes after injection. Phase 1 develops due to the direct stimulation of pain receptors, while phase 2 is the result of sensitization happening throughout those inflammatory phenomena (Velázquez-González et al., 2014). Sui et al. (2014) reported that inflammatory pain may be due to altering the mitochondrial functions such as dysfunction of their energy generating system, permeability, reactive oxygen species generation, or intracellular calcium mobilization apoptotic pathways (Sui BD et al., 2013).

Reviewing of the literature showed that the antinociceptive effects of Borago officinalis flower extracts have not been studied before; however, Wauquier et al. in 2012 reported that diets containing Borago officinalis L extract could improve bone inflammation in patients suffering from osteoporosis. The crude extract of borage (Borago officinalis L.) has antioxidant activity and causes the free radical concentration to decrease in tissues (Bandoniene et al., 2002). In 2008, Conforti et al. reported that traditional medicine such as Borago officinalis L. (Boraginaceae) contained the highest number of sterols and have shown anti-inflammatory activity in vitro, as well as antioxidant and anti-radical properties in vivo (Conforti Fet al., 2008). Ramandi et al. (2011) investigated that Borago officinalis flower oil contains fatty acids and terpenes.

In addition, Bandoniene et al. (2002) showed that the crude Borage officinalis leaves extract had a main antioxidative composite, which was recognized as rosmarinic acid (Etebari et al., 2012). Al-Khamees et al. (2011) reported that borage oil obtained from the seeds of the borage plant (Borago officinalis) is an important and abundant source of γ-linolenic acid, and borage oil is beneficial for diabetic neuropathy symptoms and other organ dysfunctions (Etebari et al., 2012). Our findings revealed that Borago officinalis hydroalcoholic flower extract had antinociceptive effects in rats and these findings were supported by previous studies. On the other hand, our results are in line with Gilani et al. (2007), who reported that Borago officinalis extract had anti-spasmodic, bronchodilator, and cardiovascular inhibitory properties probably mediating through Ca²⁺ channel obstruction which might justify the predictable utilization of the plant in restless gastrointestinal, respiratory, and cardiovascular disorders. Our findings also revealed that administration of Borago officinalis hydroalcoholic flower extract decreases acute and chronic pain behavior in test groups D, E, F and G and this might be due to its antioxidant effects that possibly improved the injured cells induced by formalin injection or inhibited the trigger of the depolarization in pain sensory neurons.

Our findings revealed that hydroalcoholic extract of Borago Officinalis flowers has antinociceptive effects in formalin test male rats; however, this mechanism needs further investigation.

Acknowledgements

This study was supported by the Deputy Research Center at Zahedan University of Medical Sciences. We are also grateful to the supervisor of department of physiology, Faculty of Medicine Zahedan University of Medical Sciences.

Conflict of Interest

The authors declared no conflict of interest.

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