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Anxiolytic Effect of *Borago officinalis* (Boraginaceae) Extract in Male Rats

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Background: Medicinal plants with natural antioxidants have been shown to be beneficial in a variety of complications such as anxiety. The elevated plus-maze (EPM) is one of the most widely used models to assess anxiety in small rodents.
Objectives: This study was designed to characterize the anxiolytic-like activity of *Borago officinalis* (Linnaeus, family Boraginaceae) or Borage flowers extract, using an EPM test.
Materials and Methods: Male Wistar rats weighing 220-250 grams were used in the present study. Thirty minutes after an intraperitoneal (IP) injection of the Borage extract (50, 100, 200 mg/kg) or saline, each animal was placed in the EPM. Animal behaviors in the experimental sessions were recorded by a video camera located above the maze, interfaced with a monitor and a computer in an adjacent room. The time spent in the open arms, the percentage of entries into the open arms of the EPM and the numbers of entries into the closed arms were recorded for five minutes.
Results: Statistical analysis indicated that acute IP injection of Borage extract before an EPM trial significantly increased the time spent in open arms and percentage of open arms entries. Whereas, the extract had no effect on the number of closed arm entries.
Conclusions: Our results demonstrated that injection of Borage extract might have an anxiolytic profile in rats. However, the exact mechanism (s) related to the active compound (s) in Borage extract should be elucidated in future studies.

Keywords: Borago; Rat; Medicinal Plants; Antioxidants

1. Background

Medicinal plants have been used from ancient time for their medicinal values as well as to impart flavor to food (1). The use of plants in the management of illnesses has been continuously increased over time as they are readily and cheaply available healthcare alternatives (2). Nowadays, the crude extracts and dry powder samples from medicinal and aromatic plants and their species have been used for the development and preparation of alternative traditional medicine and food additives (3, 4). Various types of herbal medicines have been used as anxiolytics in different parts of the world (5). *Borago officinalis* (Linnaeus, family Boraginaceae) or Borage is an annual herb with nutritional value used in traditional medicine and culinary uses in some countries (6, 7). Borage is cultivated around the world but is native to Europe, North Africa and Asia (8, 9). This plant was investigated from some other points of view, mostly regarding their medicinal properties (10). Borage products, mainly seeds oil, flowers and leaves are used for medicinal and culinary purposes (11). The flowers are blue and rarely appear white or rose colored (12). In Iran, borage blooms during April until September (11). This plant is harvested in flowering period before the start of seed formation (13-15). It has been reported that borage has antipyretic, aphrodisiac, antispasmodic, demulcent, antihypertensive and diuretic effects. Furthermore, the products of Borage are used for the treatment of bronchitis, palpitations, cramps and diarrhea (16-18). This plant can be also considered useful for the treatment of diseases such as premenstrual pain, multiple sclerosis, rheumatoid arthritis and asthma (19-21). There is a variety of animal tests for the investigation of anxiolytic effects of substances (22). One of the most widely used models for detecting both anxiolytic- and anxiogenic-like effects of agents in small rodents is elevated plus-maze test (EPM) (23, 24). In this animal model, the increase percentage of entries into the open arms compared to the total entries reveals an anxiolytic effect of substances. An increase of the time and proportion of the entrances into the open arms without a changed locomotor activity is regarded as a powerful marker of anxiolytic substance effect (25-28).

2. Objectives

Medicinal plants with natural antioxidants have been shown to be beneficial in a variety of complications such as anxiety (29). Furthermore, increasing evidence suggests that impairment of antioxidant defense is important in the process of emotional disorders, such as depression and anxiety (30). On the other hand, it has been reported that borage extract constitutes a cheap source of antioxidant activity (11, 31-33). However, there are no

published reports in literature about the effect of *Borago officinalis* extract on anxiety. Therefore, this study was designed to characterize the anxiolytic-like activity of *Borago officinalis* flowers extract, using an EPM test.

3. Materials and Methods

3.1. Preparation of Plant Extract

Borage flowers were collected from Hamadan region (Iran) in the spring and identified at the Botanic Institute of Hamadan University of Medical Sciences. The plant flowers were separated, cleaned, washed, homogenized and then dried for storage. Then, dried flowers were ground and produced a crushed powder (19). The powdered material was soaked into 70% ethanol. The extract was then taken to the laboratory for the process of evaporation. The evaporation process involved total removal of ethanol and water with which the extraction took place from the extract. The extracts of flowers were dried at 40°C and finally freeze-dried (34). The doses of aqueous suspensions of Borage flowers extract are expressed as milligrams of dried extract per kilograms of rat body weight (mg/kg). The extracts were redissolved in their solvents before each individual experiment (35).

3.2. Animals

Male Wistar rats weighing 220-250 grams were transported from animal house to a room adjacent to the test laboratory 72 hours before the test. They were housed under a controlled condition; 12:12 dark/ light cycle (lights on at 08:00 AM) at 22 ± 2°C. Animals had free access to food and water. Rats were randomly assigned to different treatment groups (n = 10). Animals were divided into four groups: control group and Borage extract groups [50, 100, 200 mg/kg intraperitoneal (IP)]. Animals were tested under the same experimental conditions. All animals received humane care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication 86-23 revised 1985) and the study was also approved by the local ethics committee of Hamadan University of Medical Sciences.

3.3. Elevated Plus-Maze Test

EPM design was similar to that originally described by Lister (36). In brief, the apparatus was composed of two open (50 cm × 10 cm × 1 cm) and two enclosed (50 cm × 10 cm × 50 cm) arms with a central platform (10 cm × 10 cm) to form a plus sign. The maze was elevated 50 cm above the floor. Thirty minutes after an IP injection of the Borage extract (50, 100, 200 mg/kg) or saline, animals behavior in the percentage of entries into the arms and time spent in each arms were recorded during five minutes (37). Animal behaviors in the experimental sessions were recorded by a video camera located above the maze, interfaced with a monitor and a computer in an adjacent room. The open-arm entries and open-arm time were used as indices of anxiety and the number of entries into the closed arms reduction of spontaneous locomotion in rat. After the test, the maze was carefully cleaned with a wet tissue paper (10% ethanol solution).

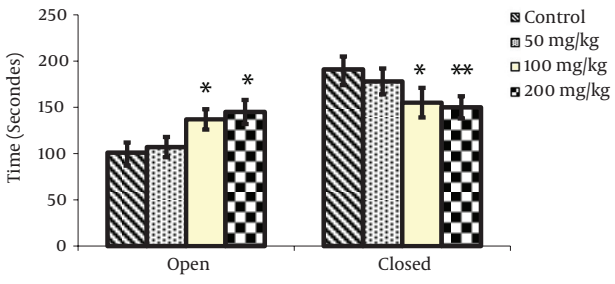
3.4. Statistical Analysis

Calculation of the total time spent in each of the arms, number of entries into the closed arms of EPM and percentage of entries into the each arms compared to total entries were performed using computerized analysis. The difference between the groups was determined by one-way Analysis of Variance (ANOVA) followed by Tukey post hoc test. In all cases differences were considered significant if $P < 0.05$. Results were expressed as Mean ± SEM.

4. Results

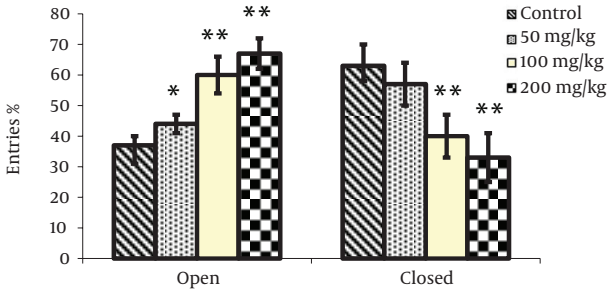
One-way ANOVA indicated that compared to the control group, extract of *Borago officinalis* groups spent more time in the open arms. Tukey post hoc test analysis showed that extract-treated group spent more time in the open arms in the doses of 100 and 200 mg/kg ($P < 0.05$), whereas effect of 50 mg/kg was not significant compared with the control group. The effects of different doses of *Borago officinalis* extract on the duration of time spent in the open arms are shown in Figure 1. One-way ANOVA indicated that compared to the control group, acute doses of *Borago officinalis* exerted significant effects on the percentage of entries into the open arms (Figure 2). A Tukey's post hoc analysis revealed significant effects of *Borago officinalis* treatment on the percentage of entries into the open arms (50 mg/kg; $P < 0.05$ and 100 and 200 mg/kg; $P < 0.01$). The number of entries into the closed arms was not significantly different between the *Borago officinalis* treated and control groups (Figure 3).

Figure 1. The Effects of Borage Extract (50, 100, 200 mg/kg) on the Time Spent in the Open and Closed Arms of the EPM Within the Five-Minute Test Period. Rats Were 10 in the Treated Groups



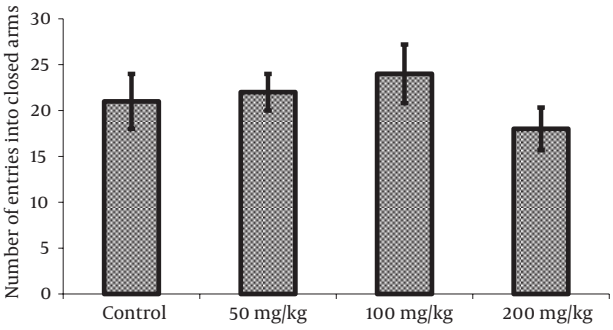
Comparisons were made using a one-way anova followed by post hoc tukey test. *$P < 0.05$ and ** $P < 0.01$ vs. control.

Figure 2. The Effects of Borage Extract (50, 100, 200 mg/kg) on the Percentage of Entries Into the Open and Closed Arms of the EPM Within the Five-Minute Test Period



Rats were 10 in the treated groups. Comparisons were made using a one-way ANOVA followed by post hoc Tukey test. * $P < 0.05$ and ** $P < 0.01$ vs. control.

Figure 3. The Effects of Borage Extract (50, 100, 200 mg/kg) on the Number of Entries Into the Closed Arms of the EPM During the Five-Minute Test Session



Data represent Means ± SEM. Animals were 10 in the treated groups.

5. Discussion

Borage is a medicinal plant with different usages in pharmaceutical, industrial and forage fields used in production of drinks and salads (7). Many natural substances and plants exert well accepted medicinal effects on animal behaviors and are able to act on chronic conditions such as anxiety that do not respond well to current therapeutic treatments (38). Substances and drugs derived from traditional plants may have possible therapeutic correlation in the remedy of disorders such as anxiety (5). Based on the above topics, the purpose of this study was to distinguish anxiolytic-like activity of the extract prepared from *Borago officinalis*. The results of present study demonstrated that the extract of *Borago officinalis* increased both the percentage of the time spent in the open arms of the maze and percentage of entries into the open arms of the maze. In the other word, the extract was able to produce anxiolytic effect in rats. An increase in the percentage of entrances into the open arms and time spent in open arms, lacking a changed locomotors activity are confirmed as a potent sign for an anxiolytic compounds effect (23). Borage is of great interest among medical and nutritional research groups due to its high content of some useful compounds (10, 11, 39). Linolenic acid and palmitic acid are collected from flowers and high levels of α-Linolenic acid is in mature leaves (40). Borage oil has high content of α-linolenic acid (33, 39). Borage oil has been reported to lower serum cholesterol, phospholipids and triglyceride levels (41). Borage oil is considered to improve task performance through attenuation of blood pressure, heart rate and temperature elevation in response to stress (42). Due to its folkloric use as an antispasmodic remedy and based on the traditional use of *Borago officinalis* in asthma, *Borago officinalis* extract was tested for its possible spasmolytic and bronchodilator effects (17). *Borago officinalis* seed oil is used in pharmaceutical products, especially for its high content in α-linolenic acid, an essential and unusual fatty acid (10, 43). It has been shown that palmitic, linoleic and stearic acids were dominant in the seed oil of borage (44). Wettasinghe and Shahidi investigated the antioxidant and reactive oxygen species (ROS) scavenging properties of borage meal extract (45, 46). Extensive research has been conducted to reveal multiple neural substrates and mechanisms that contribute to the etiology of depression and anxiety, among which the imbalance between oxidation and antioxidant defense system has gained attention (30). Some studies demonstrated the role of oxidative stress in anxiety of rodents (47, 48). According to this, it has been shown that induction of oxidative stress in mice occurs in same time with anxiety (49). Diets including high levels of sugar were reported to increase the oxidation of proteins in brain and to cause anxiety in animals. Increased anxiety has been positively correlated with increased ROS levels. Interestingly, the induction of oxidative stress by a non-pharmacological method leads to anxiety like behaviors in rats (47). Among natural antioxidants, phenolic antioxidants are extensively available in plants (50-52). Effectiveness of antioxidant activity of plant extracts in vitro is probably caused by their ability to act as free radical scavengers (53, 54). It has been demonstrated that administration of Borage was protective against Aα-induced memory and antioxidant deficit (6). Moreover, different works remarked the antioxidant activity of Borage extracts, especially related to their content in phenolic compounds (11, 46, 55). The use of Borage has become more popular, because its oil is one of the sources of α-linolenic acid (15, 56-58). Moreover, several studies evaluated the associations between antioxidant activity of Borage extract and its α-linolenic acid content (31, 32). Borage increased the antioxidant power of homogenate samples of hippocampus (46, 59). The protective effect of Borage on memory can be related to its function of scavenging free radicals and high content of α-linolenic acid (39). In conclusion, our results demonstrated that injection of Borage extract might have an anxiolytic profile

in rat. The presence of polyphenols, flavonoids and substances such as α-linolenic acid in Borage flowers extract reinforces the anxiolytic effects of this plant found in this study. However, the exact mechanism(s) related to the active compound (s) in Borage extract should be elucidated in future studies.

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Authors' Contributions

Study concept and design: Alireza Komaki, Bahman Rasouli and Siamak Shahidi. Acquisition of data: Bahman Rasouli and Alireza Komaki. Analysis and interpretation of data: Alireza Komaki and Bahman Rasouli. Drafting of the manuscript: Alireza Komaki and Siamak Shahidi. Critical revision of the manuscript for important intellectual content: Alireza Komaki, Bahman Rasouli. Statistical analysis: Alireza Komaki and Siamak Shahidi. Administrative, technical and material supports: Alireza Komaki and Bahman Rasouli. Study supervision: Alireza Komaki and Siamak Shahidi.

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