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RESEARCH ARTICLE

The anxiolytic effect of bee pollen hydroalcoholic extract in mice

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ABSTRACT

Background: Anxiety is a common human mental disorder. Medicinal therapy of this disease is associated with many side-effects. Thus, search for new medication with fewer side-effects seems inevitable. Bee pollen (often referred to as a life-giving dust) has various therapeutic properties. **Aims and Objectives:** In this study, potential anxiolytic effects of hydroalcoholic extract of bee pollen was examined in mice. **Materials and Methods:** This study was conducted on 20-25 g male mice in three groups of eight. Animals received intraperitoneal injections of hydroalcoholic extract of bee pollen at 200, 400, 800, and 1600 mg/kg doses, diazepam at 1 mg/kg dose, and saline at 10 ml/kg dose. 30 min after injection, animals were studied in elevated plus maze device for 5 min. **Results:** The results obtained showed significantly longer presence in open arms of elevated plus maze device of animals that received 800 and 1600 mg/kg doses of hydroalcoholic extract of bee pollen compared to animals that received diazepam. However, no significant difference was observed between 1600 and 800 mg/kg doses. **Conclusion:** The above results confirmed anxiolytic effect of hydroalcoholic extract of bee pollen on mice.

KEY WORDS: Bee Pollen; Anxiety; Elevated Plus Maze; Mice

INTRODUCTION

Anxiety is a natural feeling experienced in threatening situations. A mental state of apprehension and concern together with physiological changes (increased heart rate, blood pressure, and muscle tone) prepares the individual for fight or flight.

In other words, anxiety is an unpleasant and vague pervasive apprehension that is often accompanied by symptoms of autonomic nervous system stimulation.[1,2] An abnormal anxiety is disproportionate to the threatening factor and lasts longer. Clinical characteristics of anxiety disorders are the same as normal anxiety, with no difference in type, but in duration and severity.[3]

Anxiety disorders are among major human problems, such that 25% of American people suffer from the disorder. In general, chemical medications used in the treatment of anxiety disorders have many side-effects including dependence, withdrawal syndrome (if discontinued), drowsiness, and lethargy.[3] Thus, search continues for an effective medication with fewer side-effects. A potential alternative is medicinal plants used in the treatment of various diseases.

Active compounds in herbal medicines are in a state of biological equilibrium due to their association with other compounds; hence, they do not accumulate in the body and have fewer or no side-effects, and accordingly, they are significantly superior to chemical medicines.

Bee pollen is a mixture of flower pollen and nectar and bee salivary compounds and is used as feed in all hive development stages.[4] A collection of this natural produce is

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a recent advancement that is primarily dependent on basic techniques for separation of pollen from honey bees after entering the hive.[4] The main composition of bee pollen consists of carbohydrates, fibers, proteins, and fats. In fact, bee pollen is a complete food because it contains essential amino acids. However, its composition depends on the plant origin and other factors including climate, soil type, and beekeeping activities.[4] Bee pollen is considered a healthy food, with a wide range of therapeutic properties such as antimicrobial, antifungal, antioxidant, anti-inflammatory, and liver protection properties.[4]

Yildiz et al. showed that bee pollen can prevent depression in humans through inhibiting monoamine oxidase.[5] Pascoal et al. demonstrated antimicrobial, anti-inflammatory, and anti-mutation properties of bee pollen.[6] Yildiz et al. conducted a study on prevention of liver damage by bee pollen in rats with liver damage induced by carbon tetrachloride. Their results showed that use of bee pollen accelerated recovery from liver damage caused by carbon tetrachloride poisoning and it was therefore proposed as an alternative therapy for liver damage.[7] Kolesarova et al. studied the effects of bee pollen on ovarian activity and showed that bee pollen is a strong regulator of ovarian activity in rats.[8] Kacániová et al. investigated the effects of bee pollen on five species of bacteria in intestinal microbial flora in chickens and reported that bee pollen had the most antimicrobial effect on *Klebsiella oxytoca*.[9] Kafadar et al. showed that bee pollen reduced the rate of bone loss due to osteoporosis in rats that had undergone ovariectomy.[10] Attia et al. studied the effects of bee pollen on growth features in New Zealand white rabbits and concluded that bee pollen significantly improved these features in pollen receiving group compared to other groups.[11] Karampour et al. showed that bee pollen causes recovery of memory in mice.[12] Karampour et al. showed that bee pollen causes antiepileptic effect on mice.[13] Bee pollen can be found in Iran and it is used in traditional medicine for its anxiolytic properties. Therefore, we decided to investigate its anxiolytic properties in an animal model.

MATERIALS AND METHODS

Animals

In this study, 48 NMRI mice (weighing 20-25 g) were used. Animals were procured from Animal Rearing and Proliferation Center of Jondishapour University of Medical Sciences and were kept in the Animal House of Ahvaz School of Pharmacy at 23±2°C and 50% humidity in 12 hourly light-dark cycles. Mice used pellets and tap water.

Assessment of Anxiety

Anxiety was measured using elevated plus maze, which is an unconditional model for inducement, assessment, and determination of anxiolytic or anxiogenic effects of medications. This apparatus contains two open and two closed opposing arms and a 5 cm^2 × 5 cm^2 in the center. This apparatus should be held 45 cm above the floor. Each mouse was initially placed in the middle of the apparatus facing the open arm, and test was conducted for 5 min. Entering open arms and seeing the height scared the mice and made them run quickly toward the closed arms. The best criterion for measuring anxiety is duration of stay in open arms, where longer stay indicates reduced anxiety. The time when hind legs crossed the doors was the criterion for entering and exiting different parts of apparatus and frequent entry and duration of stay in open arms was considered as reduced anxiety index in mice.

Preparation of Medications

Diazepam ampules were prepared from Caspian Pharmaceutical Co. (Rasht, Iran). Bee pollen extract.

Extract preparation

In this study, hydroalcoholic extract of bee pollen was used. Bee pollen was procured from beekeeping farms in Ardebil Province. In this method, 500 g of powder was weighed and poured into a beaker, and ethanol 70% was added to a height of 2 cm above pollen level. The mixture was kept for 72 h, and stirred several times every day. Afterward, the mixture was filtered and then kept in a dish. The residue was rinsed in ethanol 70%, and then filtered and added to the previous extract.

The extract obtained was condensed in a rotary vacuum distiller, and dried in an oven at 35°C. The dry extract thus obtained was weighed and then kept in a suitable container in a cool and dry place.

Injection method

Peritoneal injections of 200, 400, 800, and 1600 mg/kg doses of extract, 1 mg/kg dose of diazepam (positive control), and 10 m/kg dose of saline (negative control) were performed, and 30 min later, animals were tested for 5 min.

Statistical analysis was performed using one-way ANOVA with *post-hoc* least significant difference test. $P < 0.05$ was considered significant.

RESULTS

Different doses of hydroalcoholic extract of bee pollen (except 200 mg/kg dose) resulted in significantly longer stay in open arms compared to diazepam and saline ($P < 0.05$). However, the stay in open arms was significantly shorter with 200 mg/kg dose and significantly longer with other doses (except 400 mg/kg dose) of bee pollen extract compared to diazepam (Figure 1).

Different doses of hydroalcoholic extract of bee pollen (except 200 mg/kg dose) resulted in a significant increase in percentage frequency of presence in open arms compared to diazepam and saline. However, percentage frequency of presence in open arms was significantly lower with 200 mg/kg dose and significantly higher in other doses compared to diazepam. Moreover, compared to 200 mg/kg dose, 400, 800, and 1600 mg/kg doses of bee pollen extract significantly increased frequency of presence in open arms (Figure 2).

Different doses of hydroalcoholic extract of bee pollen (except 200 mg/kg dose) resulted in a significant decrease in motor activity compared to diazepam and saline. On the other hand, 800 mg/kg and 1600 mg/kg doses of bee pollen extract significantly reduced motor activity compared to diazepam. Compared to 200 mg/kg dose, 400, 800, and 1600 mg/kg doses of bee pollen extract significantly decreased motor activity, while no significant difference was observed between 200 and 400 mg/kg doses (Figure 3).

A significant difference was observed between the group receiving 800 mg/kg dose of bee pollen and diazepam group in duration of stay in open arms (Figure 4).

A significant difference was observed between the group receiving 800 mg/kg dose of bee pollen and diazepam group in frequency of presence in open arms (Figure 5).

A significant difference was observed between the group receiving 800 mg/kg dose of bee pollen and diazepam group in motor activity (Figure 6).

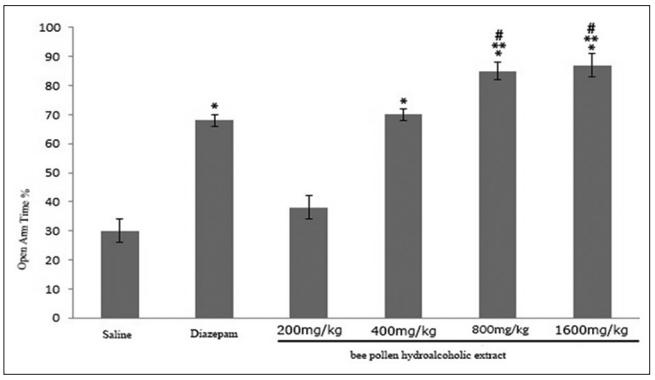

Figure 1: The effect of different doses of bee pollen hydroalcoholic extract compared to saline and diazepam on duration of stay of mice in open arms. *Significant difference between saline group and bee pollen group (200 mg/kg) ($P < 0.05$). **Significant difference between diazepam and bee pollen group (200 mg/kg) groups ($P < 0.05$). #Significantly different from bee pollen group (400 mg/kg) ($P < 0.05$)

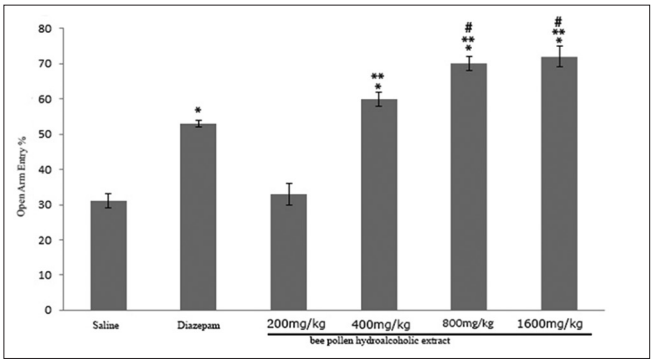

Figure 2: The effect of different doses of bee pollen hydroalcoholic extract compared to saline and diazepam on frequency of presence of mice in open arms. *Significant difference between saline group and bee pollen group (200 mg/kg) ($P < 0.05$). **Significantly different from diazepam group ($P < 0.05$). #Significantly different from bee pollen group (400 mg/kg) ($P < 0.05$)

DISCUSSION

Anxiety disorders are the most common psychological disorders and the leading cause of patients' visit to psychiatric clinics. Although many medicinal and nonmedicinal anxiety control methods have been devised, and many have been successful, there are still many people that suffer from anxiety for different reasons, and full

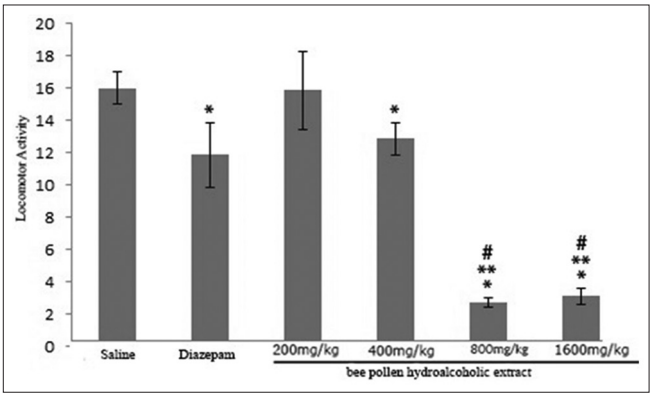

Figure 3: The effect of different doses of bee pollen hydroalcoholic extract compared to saline and diazepam on motor activity in mice. *Significantly different from saline group ($P < 0.05$). **Significantly different from diazepam group ($P < 0.05$). #Significantly different from bee pollen groups (200 and 400 mg/kg doses) ($P < 0.05$)

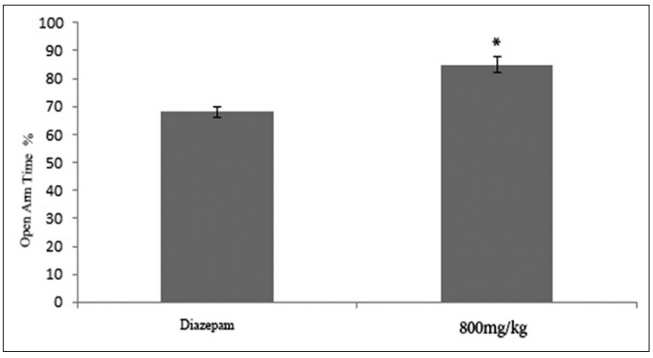

Figure 4: The effect of 800 mg/kg dose of bee pollen compared to 1 mg/kg dose of diazepam on duration of stay in open arms in mice. *Significant difference between bee pollen and diazepam groups

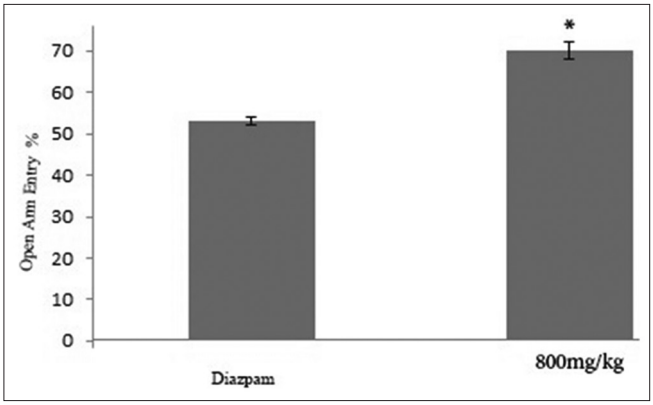

Figure 5: The effect of 800 mg/kg dose of bee pollen compared to 1 mg/kg dose of diazepam on frequency of presence in open arms in mice. *Significant difference between bee pollen and diazepam groups

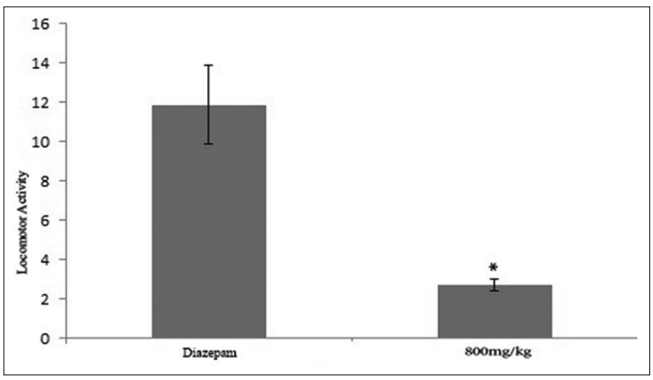

Figure 6: The effect of 800 mg/kg dose of bee pollen compared to 1 mg/kg dose of diazepam on motor activity in mice. *Significant difference between bee pollen and diazepam groups

control of this personal and social problem seems a long way off.[33]

Many chemical medicines including benzodiazepines (such as alprazolam and diazepam) are used in treatment or control of anxiety. Despite certain benefits, these drugs have several side-effects including dependence, which emerges after discontinuation. Thus, they should not be used for long periods.[12]

The place of traditional medicine and herbal remedies is obvious to everyone. Herbal medicines mediated by other compounds provide a state of physiological equilibrium and do not accumulate in the body and have fewer side-effects.

Because of the prevalence and importance of anxiety and fewer effects of herbal medicines and potential anxiolytic effect of bee pollen, this study investigated anxiolytic effect of hydroalcoholic extract of bee pollen in mice using elevated plus maze apparatus.

The results obtained showed that 400, 800 and 1600 mg/kg doses of bee pollen extract were able to prolong duration of stay in open arms of the apparatus, which indicated reduced anxiety level in mice. There was also a significant increase in frequency of presence in open arms at 800 and 1600 mg/kg doses. Therefore, hydroalcoholic extract of bee pollen reduces indicators and signs of anxiety in mice and it can be concluded that hydroalcoholic extract of bee pollen has anxiolytic properties through bonding to benzodiazepines in gamma-aminobutyric acid receptor, and thus moderates anxious reactions. Although no study has yet been conducted on anxiolytic properties of bee pollen, previous modern medicine studies on bee pollen confirm its many therapeutic properties including antimicrobial, antifungal, antioxidant, anti-inflammatory, and liver protection properties.[6]

CONCLUSION

The above results confirmed anxiolytic effect of hydroalcoholic extract of bee pollen on mice.

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