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Black seed honey—A powerful ingredient of prophetic medicine; its neuropharmacological potential

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ABSTRACT

Introduction: *Prophetic Medicine* is a rich branch of natural prophylactic medicine recommended by the *Prophet Muhammad (Peace Be Upon Him)* and is scientifically proved to be highly effective against almost all types of ailments. Honey and Black cumin are the most investigated ingredients of prophetic medicine for the scientific explanation of their benefits. The present study was designed to explore the neuropharmacological potential of Honey derived from Black Seed flower.

Materials and Methods: For evaluation of its neuropharmacological potential, modified open field, hole cross, and elevated plus maze (OF-HC-EPM) approach was applied on female Swiss Albino mice, which was basically an integration of OF-HC-EPM experimental cascade to trial with the rodents after single oral administration where parameters for sedative and anxiolytic behavior such as square crossing, hole crossing, grooming, rearing, entry, and duration in open or close arms were observed.

Results: Dose-dependent (2, 4, and 6 g/kg of body weight) analysis was performed against standard Diazepam (1 mg/kg). At low doses (2 g/kg), honey possesses anxiolytic action avoiding sedation, whereas at high doses (4 and 6 g/kg), it caused mild sedative response yet developed decision-making capability and increased confidence. On the contrary, Diazepam was associated with sedative followed by anxiolytic activity.

Conclusion: From the study, it was concluded that black seed honey exhibits non-sedative anxiolytic activity in dose-dependent manner in comparison to the established standard drug where sedation is often considered a limitation. Thus, as exhibited by black seed honey, it can be a good source for new drug development.

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Black seed honey; square crossing; rearing; grooming; hole crossing; elevated plus maze

Introduction

Honey and Black Cumin (often called Black Seed) are considered natural healers for almost all diseases. The benefit of these two agents is highly acknowledged by every society and culture from ancient till modern age. Black Cumin and Honey are among the most powerful ingredients from the branch of *Prophetic Medicine*. The limitless advantage of honey is stated in the *Holy Qur'an* in a Chapter named '*An-Nahl*' meaning '*The Bee*' as '*And your Lord inspired to the bee, "Take for yourself among the mountains, houses, and among the trees and [in] that which they construct. Then eat from all the fruits and follow the ways of your Lord laid down [for you]." There emerges from their bellies a drink, varying in colors, in which there is healing for people. Indeed in that is a sign for a people who give thought*' [1]. The *Prophet Muhammad* (*Peace Be Upon Him*) said "Honey is a medicine for the body and the Qur'an is a medicine for the soul. Benefit

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yourselves from the use of the Qur'an and honey." [2]. In another narration, Prophet emphasized that *"There is healing in Black Cumin for all diseases except death"* [3]. By nature, bees collect nectar from the black seed flowers and build hive to store as food. Hence, honey derived from the Black Cumin flower is religiously believed to contain dual benefits for human health though such claim is yet to be verified through scientific investigation.

Black seed honey is usually found during the cultivation period of the seed in the winter season. Black seed (*Nigella sativa*) is an annual herb that belongs to the family Ranunculaceae. Flower comes out as yellow, bluish-white, and also purplish. In Bangladesh, it is widely known as *Kalo Jira* that is cultivated during the period from November to March [4–7]. There have been many investigations on the black seed so far on their neuropharmacological potential [8–13]. However, very few studies on its honey for such potential have been reported. Thus, the present study was aimed to assess the sedative-anxiolytic potential of black seed honey using the modified integrated open field, hole cross, and elevated plus maze (OF-HC-EPM) experimental method.

Materials and Methods

Collection and preparation of the sample

Approximately 1.2 kg of Black Seed Honey (BSH) was collected from Al Affan (a commercial source), sourced from a harvested hive from the Shariatpur Sadar Upazila (23°12.5'N 90°21'E) of Shariatpur district of Bangladesh in February 2019. The coarse particles including bee materials, wax, pollen, and egg were removed by filtering the honey through a 0.5 mm mesh. Then it was kept in an impermeable and airtight glass jar at 25°C ± 2°C.

Physicochemical properties

Measurement of moisture content and total soluble solids

According to the method described by Bogdanov *et al*. [14], the moisture content and total soluble solids (TSS) were determined for the honey sample. After calibration, a portable honey refractometer (Biobase BK-PRN3, China) with Brix capacity of 58%–92% was used. A honey drop was spread over the prism of refractometer and the moisture and TSS level were observed. Temperature correction was applied to deduce the value [15].

Determination of pH

After a two-point calibration at pH 4.01 and 7.00 with buffer standards, pH of a 10% (w/v) honey solution was identified with Biobase pH-10S (China) pH meter. The method was repeated for three times with freshly prepared solution [16].

Determination of optical density (OD)

Optical Density of a 10% (w/v) honey solution was determined at 530 nm using the Biobase BK-UV1800 UV-VIS Spectrophotometer (China) where distilled water served as blank. The United State Department of Agriculture (USDA) (1985) guideline for color determination of honey was followed [17,18].

Determination of honey density

The method was performed as described by Kinoo *et al.* [19] with slight modification. 1 ml honey was weighted in a 1 ml syringe through Biobase BA2004N (China) automatic electronic analytical balance and density was measured using the below formula

$$\text{Density of Honey} = \frac{(\text{Weight of the honey} + \text{syringe}) - \text{Empty weight of the syringe}}{\text{Volume of the honey}}$$

Experimental animal

Female Swiss Albino mice having 27–32 g of body weight at age of 45 days were selected for all the tests performed in this study. They were habituated in the animal house of Institute for Pharmaceutical Skill Development and Research with comfortable room temperature (25°C ± 2°C) at 12 h light/dark cycle. They were allowed to consume *ad libitum* food and water.

Acute toxicity test

A 72 hours observation was made on 20 mice after oral gavage of high doses of honey (5, 7.5, 10 and 15 g per kg of body weight). Any number of deaths or any unusual symptoms or behavior observed was recorded. This assisted to draw a safe dose line prior execution of the *in vivo* experiments.

Grouping of animals

Six individual groups were formed and treated with the following agents. Group 1: Blank (No gavage), Group 2: Control (Distilled Water), Group 3: Diazepam (1mg/kg), Group 4: BSH-2 (2 g/kg body weight, equivalent to 25% w/v in 0.15 ml distilled water), Group 5: BSH-4 (4 g/kg body weight, equivalent to 50% w/v in 0.15 ml distilled water), and

Group 6: BSH-6 (6 g/kg body weight, equivalent to 75% w/v in 0.15 ml distilled water).

The (OF-HC-EPM) experimental design

A combined methodological approach was executed by integrating three widely accepted conventional experimental designs as described by Billah *et al.* [20]. Open Field, Hole Cross, and Elevated Plus Maze apparatus were arranged sequentially in a cascade and mice at a single-dose oral administration were placed in all three fields in a row. As for first exposure, mice were placed at the open field for 0–3rd minute, at hole cross for 4–6th minute (however, for simplifying, the time denoted as 0 minute for hole cross) and at EPM for 7–9th minute (the time denoted as 0 minute for EPM). The series was repeated in 30-, 60-, 90-, and 120-minute intervals accordingly.

Open field test

In this test, mice were placed at one corner of Open Field, an open cubic box (60×60×60 cm) with a tiled (5×5 cm) floor alternatively colored black and white [21]. Behavioral parameters such as Number of Square crossing, Grooming, and Rearing were observed for investigation of sedative-anxiolytic potential [22].

Hole cross test

The test was performed as described by Takagi *et al.* [23] with slight modification. A rectangular box (30×20×14 cm) was equally divided by a partition on which at 7 cm floor height, a 3 cm hole was constructed. Mice were freely allowed to cross the hole and the count was recorded as sign for spontaneous and exploratory behavior [24].

Elevated plus maze test

According to the prescribed method of Pellow *et al.* [25], a plus-shaped apparatus was designed by two oppositely intersecting open (14×5 cm) and closed arms (14× 5×14 cm) [25]. Entry and Duration in open and close arms were observed as parameters indicating anxiolytic potentials [26].

Statistical analysis

Statistical analysis of data was done by utilizing the method of one-way analysis of variance followed by Dunnett's t-tests using SPSS 25 for Windows 10. The results obtained were compared with the control group. p values < 0.05, 0.01, and 0.001 were considered as statistically significant.

Results

Physicochemical properties

Table 1 showed the physicochemical properties of Black seed honey. The honey is physically dark brown in color and acidic in nature (pH 4.2). Optical density at 530 nm was found as 0.326 which reflected white to extra light amber color according to USDA guideline. The moisture content found was within the internationally accepted limit (NMT 21 g/100 g).

Acute toxicity test

No fatality, morbidity, or abnormality was observed for tested animals after a 72-hour monitoring period. Thus, the given high doses were considered safe for acute use.

Open field

From Figure 1, it was evident that the number of square crossing was high with low dose (2 g/kg) of honey but with higher doses (4 and 6 g/kg) the number was reduced. Diazepam showed a sharp decrease from 30 to 120 minutes.

Diazepam and BSH-6 reduced the number of rearing (Fig. 2) but increased the duration of grooming (Fig. 3). On the contrary, BSH-2 and BSH-4 found with increased rearing response; however, decreased grooming compared to control.

Hole cross

Figure 4 depicts that only Diazepam and BSH-2 were found to reduce the number of hole cross compared to the control group. However, BSH-4 and BSH-6 failed to create any significant difference from the negative control.

Elevated plus maze

Dose-dependent elevation in the entry was observed in open arm which was found to decrease gradually with time (Fig. 5). Highest percentage of duration in

Table 1. Physicochemical properties of black seed honey.

Parameters	Observations*
Moisture Content (g/100 g honey)	19.4 ± 0.32
Total Soluble Solids (% Brix)	77.5 ± 0.80
Density (w/v)	1.6054 ± 0.01
Optical Density (at 530 nm)	0.326 ± 0.01
pH (1–14)	4.2

*All the methods performed in triplicate.

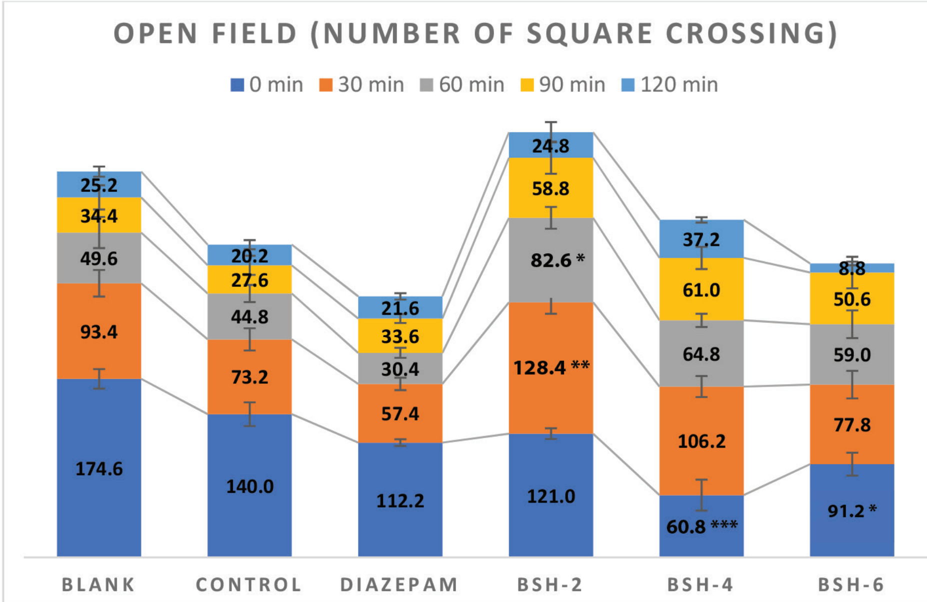

Figure 1. Number of square crossed by the mice in different time intervals. Values are mean ± SEM, (n = 5); $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; Dunnett t-test (two sided) treated one group as control (water) and compared all other groups against it.

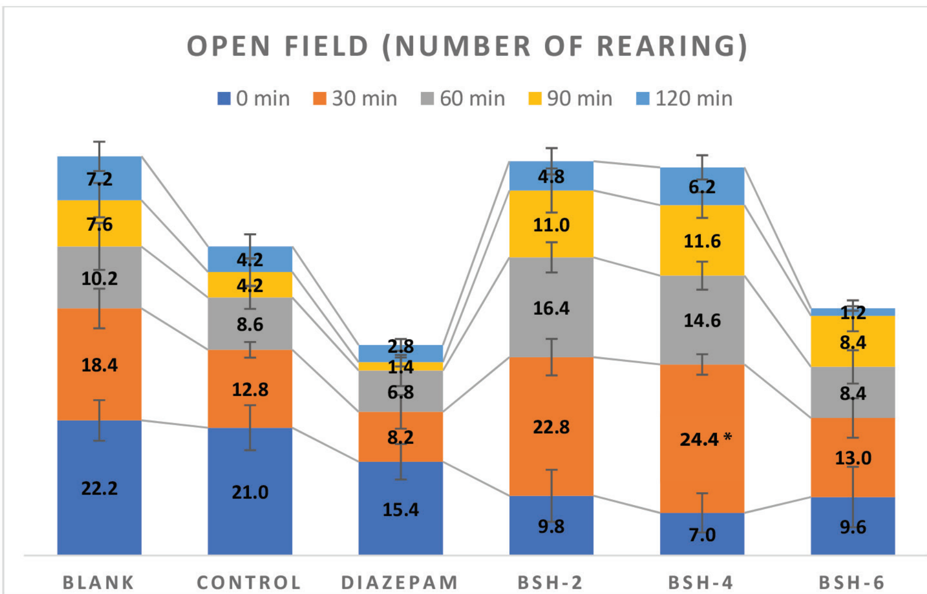

Figure 2. Number of vertical movement (rearing) performed by the mice at different time intervals. Values are mean ± SEM, (n = 5); $^*p < 0.05$, Dunnett t-test (two sided) treated one group as control (water) and compared all other groups against it.

open arm was observed by BSH-4 (Fig. 6). Diazepam established the lowest response both in entry and duration when compared to control.

Discussion

Open Field (OF), Hole Cross (HC), and Elevated Plus Maze (EPM) are the three widely adopted tools for investigating neurological conditions. Integration of these fields allowed to study comparative psychology, altered exploration, anxiety, and emotionality from Open Field, measurement of spontaneous movement from Hole Cross, and expression of anxiety-related behavior from EPM [23,27,28]. Though age, sex; prior test experience, presence of stimuli/inducer in test apparatus, forced feeding, and handling by experimenter greatly affect rodent's normal

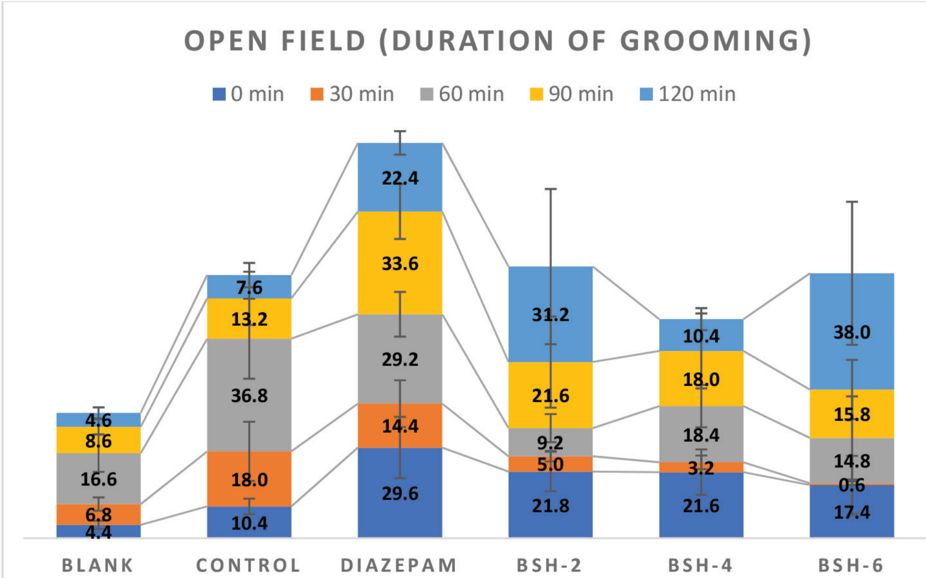

Figure 3. Grooming behavior exhibited by the mice at different time intervals. Values are mean ± SEM, (n = 5); Dunnett t-test (two sided) treated one group as control (water) and compared all other groups against it.

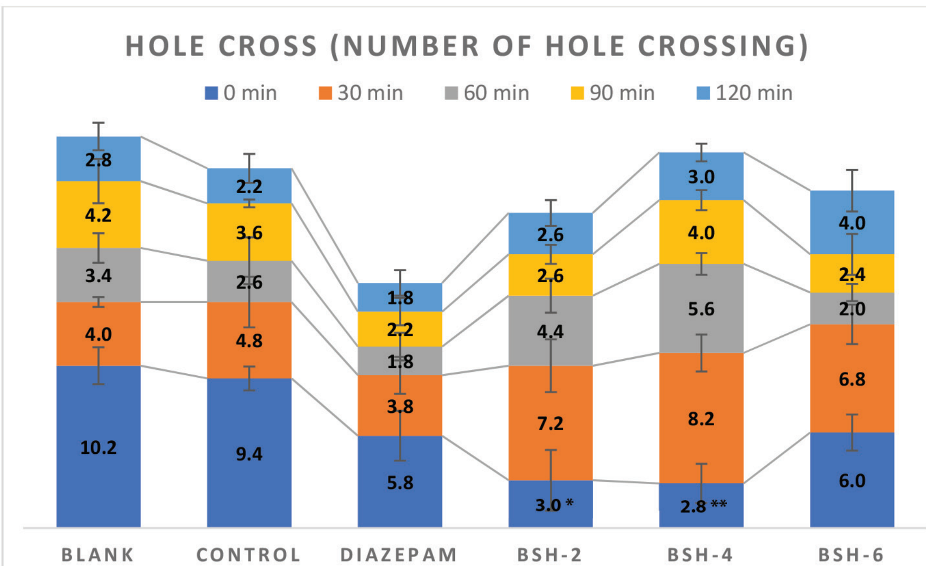

Figure 4. Number of hole crossed by the mice at different time intervals. Values are mean ± SEM, (n = 5); $^*p < 0.05$, $^{**}p < 0.01$, Dunnett t-test (two sided) treated one group as control (water) and compared all other groups against it.

behavior, major challenges found reported are first administration against repeated administration, utilizing same rodent for another experiment but in different time or using different rodent for different experiments [29,30]. To avoid these individual- and time-dependent gaps, mice were subjected to single oral administration to trial against different fields. Incorporation of a Blank group reduced the chance for false interpreted results. The behavioral parameters observed in OF-HC-EPM cascade represented a different neurological expression. In open field, square crossing symbolized locomotor or ambulatory activities, rearing denoted vertical movements due to non-specific excitability whereas grooming signified the decision-making behavior which was negatively related to the index of high activity state [22,23,31]. Activity of hole crossing represented spontaneous movements of rodents

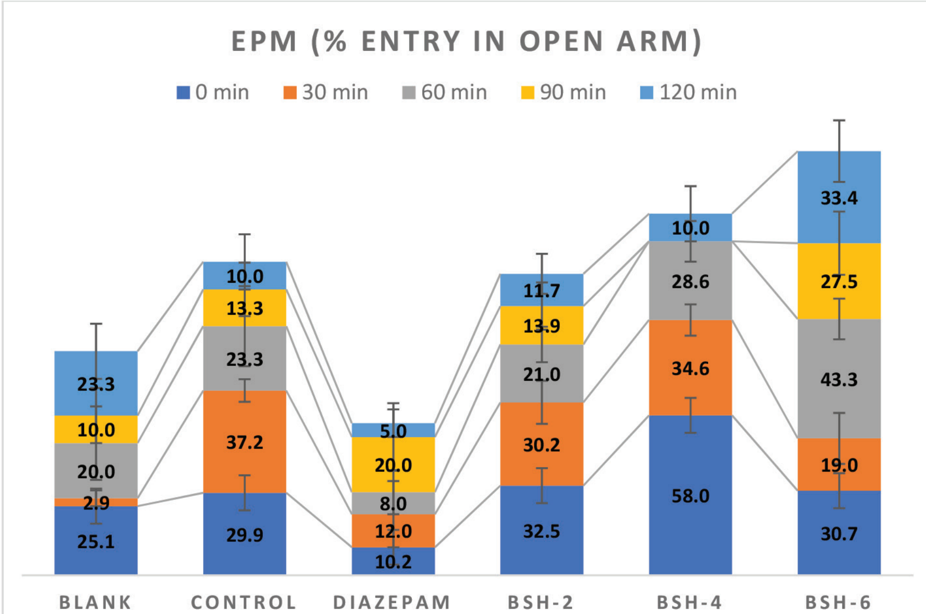

Figure 5. Percentage of open arm entry by the mice at different time intervals. Values are mean ± SEM, (n = 5); Dunnett t-test (two sided) treated one group as control (water) and compared all other groups against it.

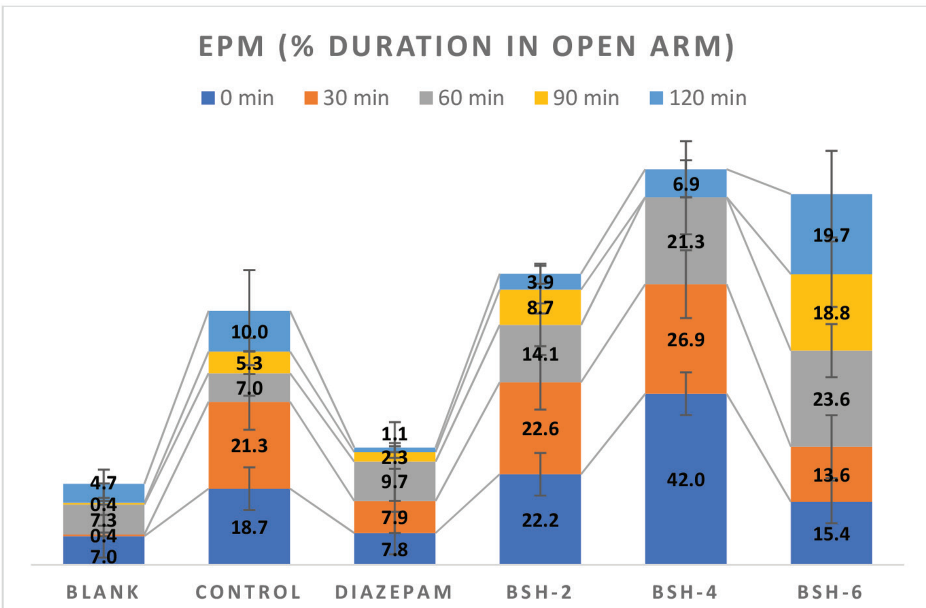

Figure 6. Percentage of duration spent in the open arm by the mice at different time intervals. Values are mean ± SEM, (n = 5); Dunnett t-test (two sided) treated one group as control (water) and compared all other groups against it.

[26]. Entry and time spent in the open arm of EPM indicated mitigation of fear [30].

Diazepam, among the most popular Benzodiazepines, act as positive allosteric modulators of the GABAA (γ-aminobutyric acid type A) receptor complex. It binds to alpha-gamma subunit interface and increases neuronal chloride-ion influx which hyperpolarizes postsynaptic membranes [32]. The anxiolytic effect is attributed to these potentiations of GABAA receptor at α2/α3 subunit isoforms in the limbic system, thalamus, hypothalamus, and cerebral cortex which produce relaxing effects and facilitate the anxiolytic process [33]. Such effects were observed through reduced ambulatory and exploratory behaviors. Honey derived from black cumin showed diversified response with different experimental fields. At low doses, unlike diazepam, it increased square crossing, rearing, and hole crossing which was indicative of its non-sedative anxiolytic effects, whereas at high doses, increased grooming response in open field along with entry and duration in the open arm of elevated plus-maze apparatus supported its hypothesis for sedative-anxiolytic potential.

Honey is not just a sugar rather a complex combination of enzymes, organic acid, trace materials, and yet unidentified compounds. It is an abundant source of protein, calcium, phosphorus, iron, niacin, vitamin C, and other minerals [34]. Honey is attributed to both internal and external healing. Alongside, Black Seed contains high protein, carbohydrate, essential fatty acid, vitamin A, B1, B2, C, niacin, calcium, potassium, and iron [35]. Flavonoids present in black seeds are being widely studied for its emerging evidence to induce improvements in memory, learning, and cognition [11,36–38]. Earlier study with an open field and EPM demonstrated an increase in explorative activity and antianxiety behavior. Chronic daily treatment with black seed was reported to increase the levels of serotonin/5-hydroxytryptamine (5-HT) and decrease the levels of hydroxy indole acetic acid (5HIAA) in the brain, both inducing the coordination of behavior including reducing anxiety via the production of serotonin [10].

Conclusion

Black Seed honey is a very popular natural product for its nutritional and medicinal values. From a religious point of view, it is considered a panacea. On neuropharmacological investigation, the honey exhibited non-sedative anxiolytic properties on rodents, whereas standard anxiolytic drugs like diazepam are often associated with sedative effects. Thus, the components of this honey can be a great source of further investigation which may lead to an invention of alternate new drug development without side effect.

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Abbreviations

BSH	Black Seed Honey,
OF	Open Field,
HC	Hole Cross,
EPM	Elevated Plus Maze
GABAA	γ-aminobutyric acid type A
USDA	United State Department of Agriculture

Conflict of interest

All authors agreed on the article before submission and had no conflict of interests.

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