JABET
Journal of Advanced Biotechnology and Experimental Therapeutics

ORIGINAL ARTICLE

J Adv Biotechnol Exp Ther. 2020; 3(2): 128-134
eISSN: 2616-4760, https://doi.org/10.5455/jabet.2020.d117
Published by www.bsmiab.org

Possible neuropharmacological effects of *Apis cerana indica* beehive in the Swiss Albino mice

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Academic Editor: Dr. Md. Abdul Hannan, Dongguk University, South Korea.
Received:30 January 2020; Accepted: 17 March 2020; Published: 05 May 2020.

ABSTRACT: The water-soluble extract is a gummy semi-solid content of *Apis cerana indica* beehive (WSE-BH). The present study reports the neuropharmacological effects of beehive derived from *Apis cerana indica*. The neuropharmacological results evaluated by modified open field, hole cross, elevated plus maze and hole board (OF-HC-EPM-HBT) test by Swiss Albino mice of both sexes after single oral administration where parameters for sedative and anxiolytic activity was square movements, hole crossing, time spent in open arm and head dipping which is the unpunished or unlearned response. A time-dependent manner activity observed by WSE-BH (200 and 400 mg/kg) and diazepam (1 mg/kg) against negative control normal saline. At low dose (200 mg/kg), the OF and HC possess significant reducing effects in time dependence manner while EPM and HBT exhibited significant anxiolytic activity avoiding sedation, whereas at 400 mg/kg exhibited an irregular effect. The current results were suggesting that WSE-BH might a good source of anxiolytic and sedative effects at low dose concentration.

KEYWORDS: Beehive, *Apis cerana indica,* anxiolytic, sedative, neuropharmacological effects

INTRODUCTION

Four hundred fifty million people suffered from mental disorders, with 121 million in depression [1]. Anxiety is a common health disorder in the world that causes a problem in the health care system [2] and moderates quality of life [3]. Anxiety is a profoundly predominant mental and physiological state described by psychomotor pressure, perceptive hyperactivity, and cautiousness disorders and causing one-eighth of the absolute populace of the world and turned into a significant territory of research enthusiasm for psychopharmacology [4, 5]. Synthetic anxiolytic drugs such as benzodiazepines are the most common medications for anxiety disorders. Unfortunately, they have a few adverse effects, for example, tolerance and physical dependency, amnesia, loss of sexual drive, weakness, gastrointestinal (GI) effects and body weight changes, sedation, and relaxation of muscle, which lead patients to look for alternative treatments [6].

Apitherapy (Apisis a Latin word that implies honey bee) is the act of utilizing honey bee items, for example, honey, propolis, jelly, pollen and venom of bee for disease or treatment proposes. It mentioned as "the science and art of the utilization of bee items, to maintain health care" [7, 8]. Bee items are parts of traditional medicine. Bee items have been found to show antioxidants, anti-inflammatory, and antimicrobial activities. It has been additionally demonstrated that characteristics of bee items hinder tumor cell development and metastasis and induce apoptosis of malignancy cells. Henceforth, these bioactive natural items may demonstrate to be helpful in malignant growth treatment [9]. In recent years, interest has increased in bee products for medical purposes.

In our study, the beehive of *Apis cerana indica* belongs to *Apidae* Family and genus *Apis* has been used. According to *Qur'an*, honey is a medicine [10]. Pharmacologically, it has been used as antimicrobial

[11], GIT diseases [12, 13], diabetes [14], anti-inflammatory and immunomodulatory [15, 16], antioxidant activities [17] and cardiovascular diseases [18]. The *A. cerana* reported containing fructose (37.27 - 40.51 %) along with glucose (35.12 - 38.04 %) while reducing sugar 73 % [19]. The genus *Apis* reported to have copper, zinc, iron which are essential for brain functions [20].

Our present study design to evaluate the neuropharmacological activity (anxiolytic and sedative) of a water-soluble extract of the beehive by using a modified open filed and hole crossed for sedative activity and elevated plus maze and hole board test for anxiolytic activity whereas the assessment is the unpunished or unlearned response while acute toxicity also studied.

MATERIALS AND METHODS

Collection and preparation of the extract

Approximately 1kg of beehive collected from hilly area of Bandarban of Chittagong division, Bangladesh in August 2019. The beehive was identified by co-author Mohammad Sohel and supervisor by A.S.M. Ali Reza (Assistant Professor) Department of Pharmacy, International Islamic University Chittagong, Kumira-4318, Chittagong, Bangladesh.

The collected beehive dried and ground by mechanical drier (NOWAKE, Japan). A total of 620 g powder was found. From that, only (80 g) was soaked in 150 ml water for ten days at room temperature with irregular shaking. Filtration followed by Whatman filter paper No. 1. The evaporation was done by a water bath at 65 °C to get a gummy semi-solid water-soluble extract of the beehive (12.15 g of WSE-BH). The percentages of the yield of a water-soluble extract of beehive 15.19 %. The extract preserved in an amber glass vial at refrigerator (4 °C) until further used.

Chemicals

Diazepam (10 mg/2mL) purchased from Opsonin Pharma Limited, Dhaka, Bangladesh and NaCl, (Merck, Mumbai, India.) were used in this study.

Experimental animals

Swiss albino mice weighing about 27-32 g of both sexes used in this experiment were procured from the International Islamic University Chittagong, Kumira-4318, Chittagong, Bangladesh. All the mice were habituated in the animal house under room temperature (25 ± 2 °C) with proper food and water supply maintenance.

Experimental design (OF-HC-EPM- HBT)

Four separately groups (n=5) were formed. The Negative control group received normal saline (0.9 % NaCl) at 10 mL/kg, whereas the positive control diazepam received 1 mg/kg intraperitoneally. The test group received 200 and 400 mg/kg dose in accordance with their body weight by oral gavage.

The sedative and anxiolytic activity of beehive evaluated by the previously described method with few modifications [21, 22]. Open field (OF), hole-cross (HC), and elevated plus maze (EPM), hole-board test (HBT) was sequentially presented to evaluate sedative and anxiolytic activity by single dosing. After administration, mice placed in OF for 0-3 minutes, at HC for 4-6 minutes, at EPM for 7-9 minutes, and at HBT for 10-12 minutes. The animals allowed calming for 18 minutes before the next series starting. The series sequentially followed for 30, 60, 90, 120 minutes, respectively. The study approved by the Institutional Animal Ethical Committee, Department of Pharmacy, International Islamic University Chittagong, Bangladesh according to governmental guidelines under the reference Pharm/P&D/147/13-19.

Open field test

The sedative-anxiolytic activity of WSE-BH evaluated as behavioral parameters such as the number of square movements by the previously described method [23]. The open field devices a square box (60 × 60 × 60 cm) with 25 square of equal dimension (5 × 5 cm) marked as black and white.

Hole-cross test

The test performed in accordance with the previously described method with modification [24]. Hole cross test devices were having a size of 30 × 20 × 14 cm with a fixed partition in the middle, whereas a 3cm in diameter hole was made at a 7 cm height.

Elevated plus maze test

The elevated plus maze (EPM) consisted of two opened arms (35 × 5 cm) crossed with two closed arms (35 × 20 cm). The arms were connected together with a central square (5 × 5 cm). The apparatus was elevated to a height of 25 cm in a dimly illuminated room. The

percentage of Time spent in open arms recorded for anxiolytic properties [25].

$$\% \text{ time spent in open arm} = \frac{\text{time spent in open arm}}{\text{time spent in open arm} + \text{time spent in closed arm}} \times 100$$

Hole-board test

The study was conducted using a wooden board measuring 20 cm × 40 cm with 16 equally spaced holes. The HBT based on head dipping, which measures anxiety behavior with exploratory activity [26].

Statistical analysis

The result characterized in mean ± SEM (n=5). The statistical analysis followed by unpaired t-test of GraphPad Prism (ver 7) in comparison with negative control (normal saline 0.9 % NaCl) where $P < 0.05$ considered as statistically significant.

RESULTS

WSE-BH extract decreases the number of movements in a dose-dependent manner

The sedative activity of WSE-BH summarized in Figure 1. The number of movements high at 200 mg/kg with significant ($P < 0.05$) time-dependency while 400 mg/kg showed an irregular decreased with the time. The positive control diazepam (standard) showed a sharp decreased from 0 to 120 minutes. The number of movements at 90 minutes for 200 mg/kg (18.00 ± 7.21, $P < 0.05$), and diazepam (22.00 ± 1.73, $P < 0.05$) in comparison to negative control. The last observation (120 minutes) exhibited 23.00 ± 3.06 ($P < 0.05$), 12.33 ± 3.38 ($P < 0.01$) movements by 200 mg/kg and diazepam (1mg/kg), respectively.

WSE-BH extract reduces the number of hole cross in a time-dependent manner

The hole-cross test for sedative activity of WSE-BH depicted in Figure 2. The dose of 200 and 400 mg/kg and positive control diazepam (1 mg/kg) reduced the number of hole cross in a time dependence manner significantly ($P < 0.05$) in 0, 30, and 90 minutes. The number of hole-cross at first observation (0 minutes) for 200 mg/kg (5.33 ± 2.53, $P < 0.05$), 400 mg/kg (5.00 ± 1.15, $P < 0.001$) and diazepam (15.33 ± 0.88, $P < 0.01$) in comparison to negative control. The second observation (30 minutes) exhibited 4.33 ± 1.20 ($P < 0.05$), 2.67 ± 0.33 ($P < 0.001$) hole cross by 200 mg/kg and 400 mg/kg, respectively.

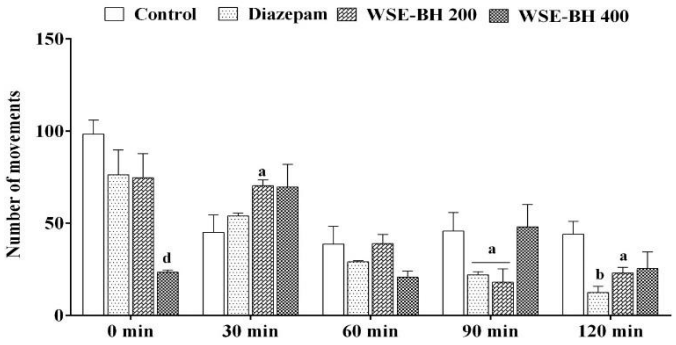

Figure 1. Effects of water soluble extract of beehive on open filed test by mice in different time intervals. The result characterized in mean ± SEM (n=5). [a] $P < 0.05$, [b] $P < 0.01$ and [d] $P < 0.0001$. The statistical analysis followed by unpaired t-test of GraphPad Prism (ver 7) in comparison with negative control (normal saline 0.9 % NaCl). WSE-BH - water soluble extract of beehive.

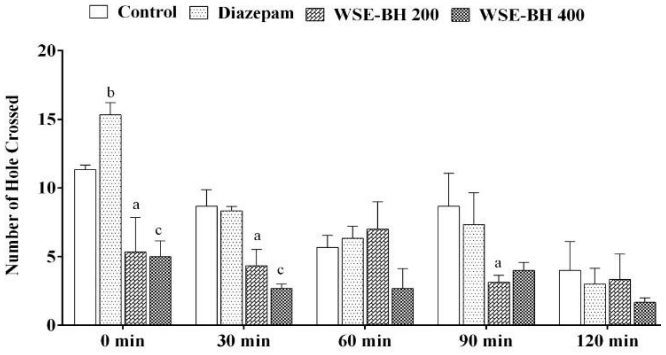

Figure 2. Effects of water-soluble extract of beehive on hole cross test by mice in different time intervals. The result characterized in mean ± SEM (n=5). a $P < 0.05$, and c $P < 0.001$. The statistical analysis followed by unpaired t-test of GraphPad Prism (ver 7) in comparison with negative control (normal saline 0.9 % NaCl). WSE-BH - water soluble extract of beehive.

WSE-BH extract reduces the time spent in open arm

In an elevated plus-maze (EPM) test, a significant ($P < 0.0001$) percentage of time spent observed by diazepam (74.97 ± 7.96 %) compared to the negative control group at second observation. A dose-dependent manner anxiolytic activity observed at 0 to 60 minutes while at 60 minutes 200 mg/kg exhibited (23.53 ± 3.89 %) and 400 mg/kg (12.71 ± 2.42 %, $P < 0.05$) in open arm time spent compared to negative control (Figure 3).

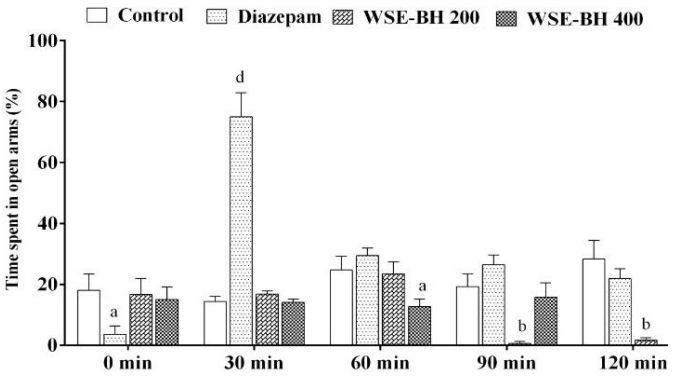

Figure 3. Effects of water soluble extract of beehive on percentage of time spent in open arm on elevated plus maze test by mice in different time intervals. The result characterized in mean ± SEM (n=5). [a] $P < 0.05$, [b] $P < 0.01$, and [d] $P < 0.0001$. The statistical analysis followed by unpaired t-test of GraphPad Prism (ver 7) in comparison with negative control (normal saline 0.9 % NaCl). WSE-BH - water soluble extract of beehive.

WSE-BH extract maximizes the number of head dipping in a time-dependent manner

The result of hole-board test depicted in Figure 4. A dose dependent manner significant number of head dipping observed by 200 mg/kg at 0 to 120 minutes observation while dose dependency of 400 mg/kg exhibited from 30 to 120 minutes. In second observation (30 minutes)**,** the number of head dipping was maximum whereas the diazepam exhibited (35.33 ± 2.91, $P < 0.01$) and the 400 mg/kg (31.00 ± 0.58, $P < 0.01$).

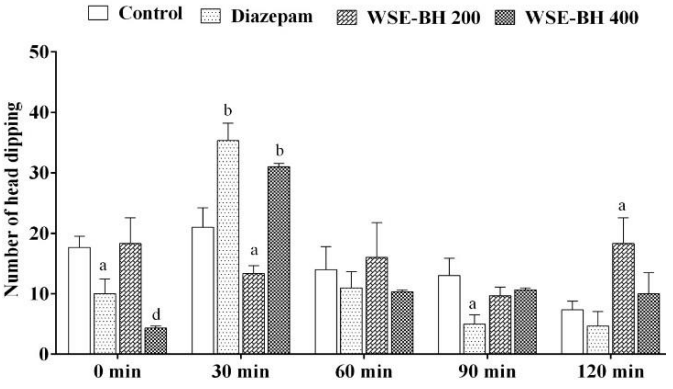

Figure 4. Effects of water soluble extract of beehive on number of head dipping on hole board test by mice in different time intervals. The result characterized in mean ± SEM (n=5). [a] $P < 0.05$, [b] $P < 0.01$, and [d] $P < 0.0001$. The statistical analysis followed by unpaired t-test of GraphPad Prism (ver 7) in comparison with negative control (normal saline 0.9 % NaCl). WSE-BH - water soluble extract of beehive.

DISCUSSION

Anxiety and stress-related conditions are extreme mental conditions that affect daily works performance. The perception of Charles Darwin that animals and humans individually share the same mechanism in expressing the emotion (mainly rodents). The advancement of animal models of anxiety and stress has recognized the pharmacological action and potential clinical impacts of a few medications. The open filed, hole cross, elevated plus maze and hole board test assessed for unlearned/unpunished responses whereas the model used for neurological conditions. Accumulation of these tools allowed us to study the sedative activity by open filed, hole board test and anxiolytic activity by elevated plus maze and hole-board test [27-29].

The administration of doses for animal model evaluated by acute toxicity study, whereas the WSE-BH exhibited a safe dose up to 4000 mg/kg. Hence a depression mode observed at the first 6 hours observation. The depression of mice in the first 6 hours might be because of the sugar level in the honey of *A. cerana*, which is fructose (37.27 - 40.51 %) along with glucose (35.12 - 38.04 %) [19] as it might also be available at the beehive. Sugar (specifically fructose) consumption might increase inflammation in humans [30] and also the risk of depression [31]. So, in our study, we choose a lower dose of 200 and 400 mg/kg for evaluation of the neuropharmacological activity.

Diazepam, among the most well-known Benzodiazepines, acts by allosteric modulators of the γ-aminobutyric acid type A (GABA A) receptor complex. It links to the alpha-gamma subunit interface and proliferation of neuronal chloride-ion influx, which hyperpolarizes postsynaptic membranes [32]. The potentiation's of GABA A receptor at α2/α3 subunit in the limbic system, thalamus, hypothalamus, and ganglion produce reducing or calming effects which facilities anxiolytic activity [22]. The calming or reducing effects observed at WESE-BH by experimental models at a low dose of diazepam (1 mg/kg) followed by open field and hole-cross test at 200 mg/kg where a significant ($P < 0.05$) result observed in time dependence manner while at high dose 400 mg/kg exhibited an irregular movement and crossing with time. Similar to open field and hole cross test, the EPM for anxiolytic activity exhibited significant activity at a lower dose while the hole board test exhibited the maximum number of significant ($P < 0.01$) head dipping at second observation (30 minutes) by 400 mg/kg. In contrast, the 200 mg/kg observed similar activity at first (0 minutes) and fifth observation ($P < 0.05$). An earlier study by bee honey demonstrated the anxiolytic and locomotion or sedative activity, whereas a similar

activity was observed a low dose concentration by reducing the anxiety [33-50].

CONFLICTS OF INTEREST

Authors declared that they have no conflict of interest.

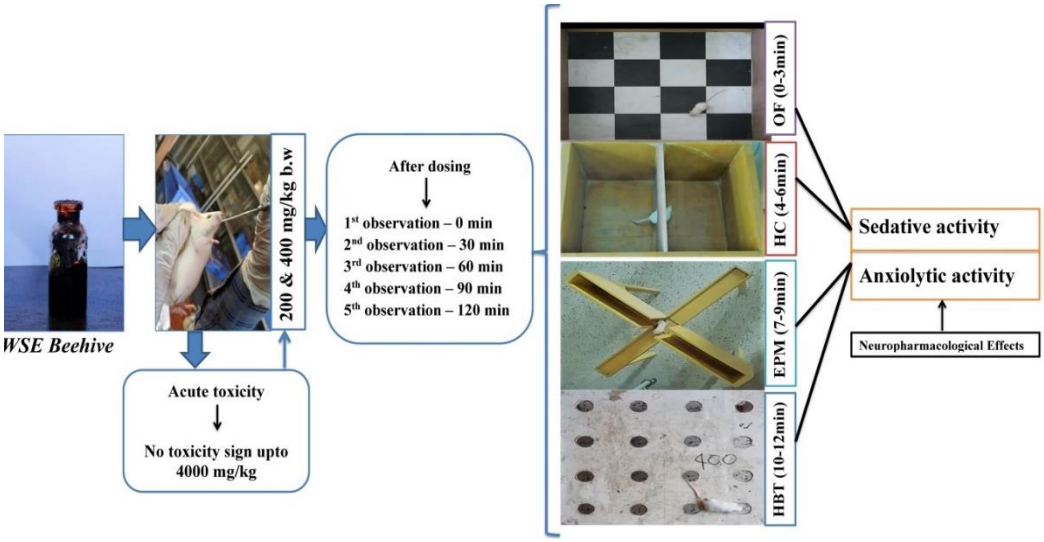

Figure 5. Graphical representation of neuropharmacological effects of water-soluble extract (WSE) of beehive.

CONCLUSIONS

Results are suggesting that Beehive of *A. cerana* could be a potential source of anxiolytic and sedative agents at a lower dose (Figure 5). Further study required to predict the possible mechanism of water-soluble extract of the beehive.

ACKNOWLEDGEMENT

Authors are very much thankful to the Department of Pharmacy, International Islamic University Chittagong, Bangladesh for research facilities and other logistic supports. This work is conducted with the individual funding of all authors.

AUTHOR CONTRIBUTIONS

AMT planned and designed the research. ASMAR and MSN arranged the whole facilities for the research and supervised the whole research. MS collected the beehive and prepared the extract with ASMAR. MU, MHM, MH and FBK conducted the entire laboratory works with AMT. AMT, MSN, ASMAR and TBE imparted in study design and interpreted the results putting efforts on statistical analysis and also participated in the manuscript draft and has thoroughly checked and revised the manuscript for necessary changes in format, grammar and English standard. All authors read and agreed on the final version of the manuscript.

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