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A Novelty-Induced Integrated Environmental Challenge to Assess Sedative-Anxiolytic Potential of Lychee (*Litchi Chinensis*) Honey

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Abstract

The assessment of neuropharmacological potential has always been challenging due to the variance found with different subjects, in different time intervals. Therefore, the present study was aimed to introduce a new approach by integrating behavioral fields together which allowed to utilize the same rodent for different experiments at the same time to minimize the variances. Experiments through Open Field, Hole Cross and Elevated Plus Maze fields were designed in such a way that same subject can experience all fields subsequently at the consecutive time intervals. Lychee honey at doses 2g/kg, 4g/kg and 6g/kg body weight was orally administered to Swiss Albino mice and their sedative and anxiolytic potential was evaluated against diazepam (1mg/Kg). At all doses the honey found to be highly effective as significantly reduced the locomotor and other exploratory activities. The data implied that the honey may act through the GABAA receptors and can prove as a potential source for neuro drug research.

Keywords: Open Field, Hole Cross, Elevated Plus Maze, Rearing, Grooming, Lychee Honey

1. Introduction

Lychee (*Litchi chinensis*) flower appears to be small, greenish white to light yellow color, occurs once in a year during late winter to early spring in Bangladesh. During the span bees collect nectar from the flower and turn into honey. Studies demonstrated that polyphenol, flavonoid and condensed tannin exist in the flower [1-3]. These compounds contribute to antioxidant, anti-carcinogenic, anti-inflammatory and cardio-protective effects, and to prevent degenerative

diseases [2,4-6]. The flower also contains gentistic acid and epicatechine which contributes to its antioxidant activity [7]. Reports suggest that honey derived from Lychee flower is one of the abundant sources of vitamins and minerals which boost the immune system [8]. It also has inhibitory effect on lipase activity and obesity [9]. Moreover, literatures confirmed its efficacy over hepatotoxicity and cadmium- and lead-induced cytotoxicity, due to the presence of proanthocyanidine [10,11]. Lychee honey is considered as a useful source that provides anti-free radicals, anti-tyrosinase and anti-bacterial activity against pathogenic bacteria causing skin diseases [12]. The moisture content found in litchi honey is usually lower than that of the other honeys. It is light amber in color and has a pleasant aroma. Studies have demonstrated the nutritional as well as medicinal values in many pharmacological fields, but very few investigated its neuropharmacological potential. The present study therefore focused on the sedative and anxiolytic potential of the honey in comparison with standard drugs.

To assess the neuropharmacological actions, numerous methods have been prescribed and adopted by neuroscientists but most of which described limitations for not having appropriate experimental subjects. As the behavior of rodent is highly variable depending on time and individuality of the subject, the major drawbacks in these studies reported as the inability to use the same subject for all subsequent tests, or, on the other hand the vast variability in behavior found using the same subject in different times. To minimize the uneven extrapolation of data caused by these limitations, a novel approach was taken by integrating experimental fields together.

2. Materials and Methods

2.1 Drugs and Chemicals

Diazepam was obtained from Square Pharmaceuticals Ltd. Lychee honey (1 kg) was collected from a cultivated hive in the lychee garden of Dinajpur district (25.63 ° N 88.65 ° E) of Bangladesh in the month of April 2018.

2.2 Preparation of the Test Samples

Collected viscous Lychee honey (LCH) were kept in airtight glass jar at room temperature (25˚C) and passed through a sieve (0.5 mm mesh) to remove non soluble particles (bee particles, wax, pollen, egg) and other coarse material.

2.3 Physical Properties of Test Samples

2.3.1 Determination of Moisture Content and Total Soluble Solids

Moisture content can be deduced from refractive index of honey [13]. A portable honey refractometer (Biobase BK-PRN3, China) with 58 – 92% Brix range, thermoregulated at 20˚C and calibrate with distilled water, was used for measuring the refractive index of honey. Total Soluble Solids were deduced from its Brix value and temperature correction was applied according to ISO 2173:2003 [14].

2.3.2 Determination of pH

The pH of the sample was measured using a pH meter (Biobase pH-10S, China), calibrated at pH 4.01 and 7.00. Honey sample was prepared as 10% (w/v) solution in distilled water and reading was taken in triplicate [13].

2.3.3 Determination of Optical density (OD)

Optical density was determined from a 10% (w/v) honey solution in distilled water by using a UV-VIS Spectrophotometer (Biobase BK-UV1800, China). Absorbance was taken at 530 nm using distilled water as blank. The method was performed as described by Wakhle in 1997 [15]. Absorbance values obtain was compared with standard set by United State Department of Agriculture (1985) [16].

2.3.4 Determination of Honey Density

1 ml honey was drawn with a syringe, both empty and filled weight was measured using an Automatic Electronic Analytical Balance (Biobase BA2004N, China). Mass of the honey was determined from the difference of these two weights. Finally, Density was calculated as described by Kinoo et al. in 2012 [17] using the below formula.

2.4 Acute Toxicity Test

Before execution of the in-vivo experimental methods, acute toxicity was observed. Lychee Honey was orally administered to 20 experimental animals at the dose of 5g, 7.5g, 10g & 15g per kg of

body weight. Rodents were then observed for next 72 hours for any number of deaths or any unusual symptoms or behavior.

2.5 Experimental Animal

Female Albino mice, 45 days of age having 27-32 g body weight, were selected for the evaluation. Rodents were accustomed with a 12 h light/dark cycle, ambient temperature, air ventilation and ad libitum food and water at animal house of Institute for Pharmaceutical Skill Development and Research. Total six groups were formed which consisted five mice in each and orally challenged with respective agents.

Group 1: Blank (No gavage), Group 2: Control (Distilled Water), Group 3: Diazepam (1mg/kg), Group 4: LCH-2 (2g/kg body weight, equivalent to 25% w/v in 0.15ml distilled water), Group 5: LCH-4 (4g/kg body weight, equivalent to 50% w/v in 0.15ml distilled water), Group 6: LCH-6 (6g/kg body weight, equivalent to 75% w/v in 0.15ml distilled water).

2.6 Experimental Design

A novelty induced environmental challenge was designed by combining three apparatus in a continuous series [18]. After oral gavage, mice were placed in Open Field, Hole Cross and Elevated Plus Maze (EPM) sequentially in a row and allowed to explore each for three minutes. For first interval, mice experienced 0-3rd min at open field, 4-6th min at hole cross (however for simplifying, the time denoted as 0 min for hole cross) and 7-9th min at EPM (the time denoted as 0 min for EPM). For each rodent, the cascade was repeated in 30, 60, 90 and 120 minute intervals accordingly.

2.6.1 Open Field Test

Mice were placed in an open cubic box measuring a dimension of 60x60x60 cm having a tiled (5x5 cm) floor alternatively colored black and white. This method was performed as described by Billah et al. (2016) [19] to asses sedative-anxiolytic activities by observing parameters such as Number of Square crossed, Grooming and Rearing.

2.6.2 Hole Cross Test

In this experiment mice were freely allowed to cross a 3 cm hole made on a partition at 7 cm floor height which divided a 30x20x14 cm box into two equal compartments. Number of holes crossed was counted during the observation as parameter of exploratory behavior. This method was performed as described by Nawrin et al. (2015) [20].

2.6.3 Elevated Plus Maze Test

In Elevated Plus Maze (EPM) apparatus, mice were allowed to move in any direction of a Plus shaped mirror-designed two open arms intersecting with two closed arms. Each arm had a length of 14 cm, width of 5 cm and the close arm had wall height of 14 cm. Open and close arm entry and duration were observed as parameter indicating anxiolytic potentials as described by Hawiset et al., (2011) [21].

2.7 Statistical Analysis

Statistical analysis of data was done by utilizing the method of one-way analysis of variance (ANOVA) followed by Dunnett's t tests using SPSS 24 for windows. The results obtained were compared with the control group. P values < 0.05, 0.01 and 0.001 were considered statistically significant.

3. Results

3.1 Physicochemical properties of Honey Sample

Table 1 showed the physicochemical properties of lychee honey. The moisture content found 16.1 g/100g which was superior to that of minimum international standard i.e. 21 g/100g as mentioned in USDA. An optical density of 0.40 indicated an extra light amber color and the honey found to be neutral in its pH.

Table 1: Physicochemical Properties of Lychee Honey

Parameters	Observations *
Moisture Content (g/100g honey)	16.1 ± 0.04
Total Soluble Solids (% Brix)	82.5 ± 0.07
Density (w/v)	1.6937 ± 0.01
Optical Density (at 530nm)	0.40 ± 0.01
pH (1-14)	7.1

*All methods performed in triplicate.

3.2 Acute Toxicity Test

With the administered doses, no unusual symptoms or behavior or death was observed within 72 hours which suggestively allowed to design safe doses for further in-vivo tests.

3.3 Open Field Test

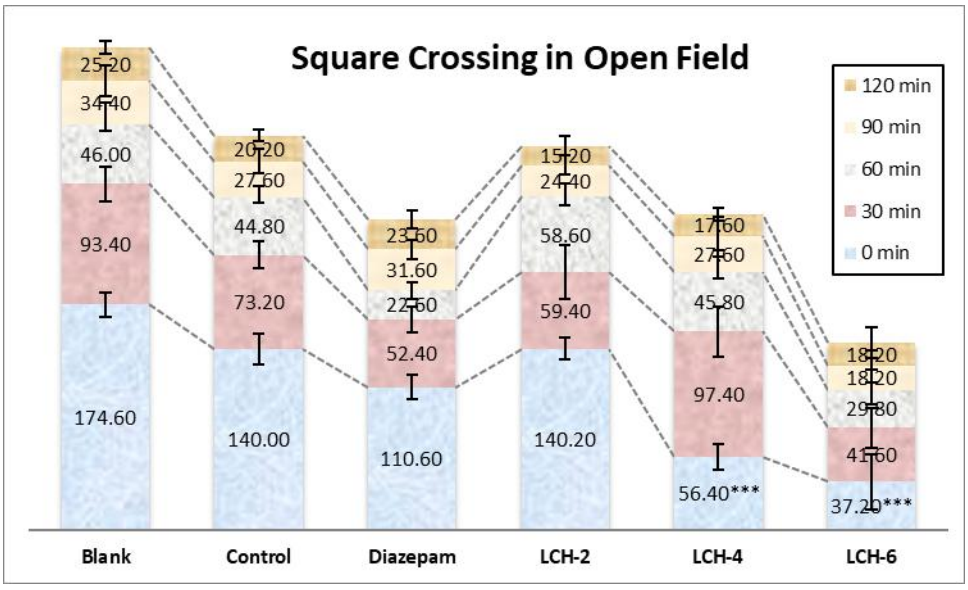

Figure 1: Number of Square crossed by the mice in different time intervals

Values are mean ± S.E.M., (n = 5); * P < 0.05, ** P < 0.01, *** P < 0.001; Dunnett t-test (2 sided) treated one group as control (water) and compared all other groups against it.

Figure 1 showed that Diazepam decreased the number of square crossed by the mice as immediate response (0 min) and in higher doses (4g/kg and 6g/kg),lychee honey significantly reduced the number compared to control. The trend was consistent even in late phases (90 and 120 min) where LCH in all doses found to be more effective than the standard.

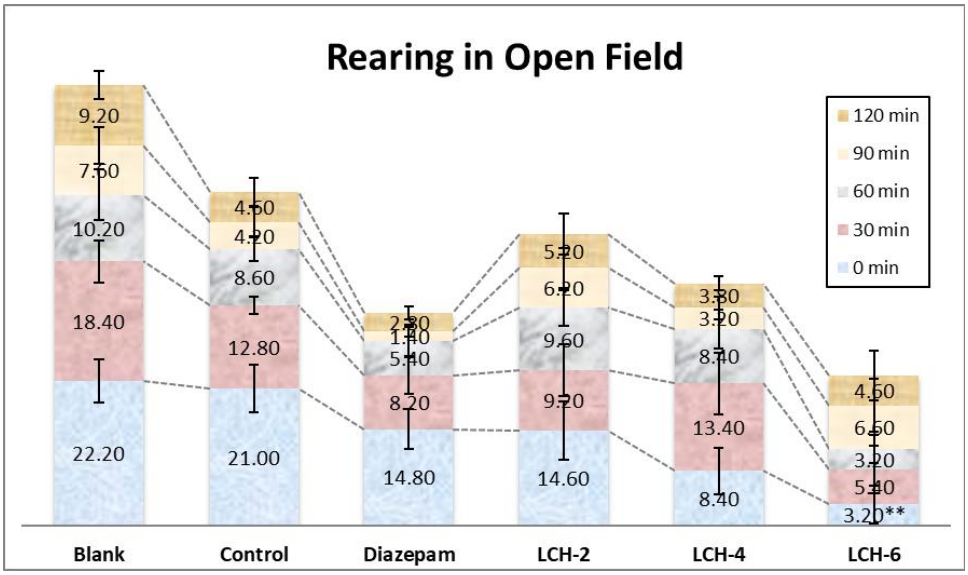

Figure 2: Number of vertical movement (rearing) done by the mice in different time intervals

Values are mean ± S.E.M., (n = 5); * P < 0.05, ** P < 0.01, Dunnett t-test (2 sided) treated one group as control (water) and compared all other groups against it.

Rearing was found to be decreased both by the test samples and the standard from the initial stages after administration and this pattern was evident till the late phases. LCH-6 reduced the activity significantly in all intervals compared to Diazepam (Figure 2).

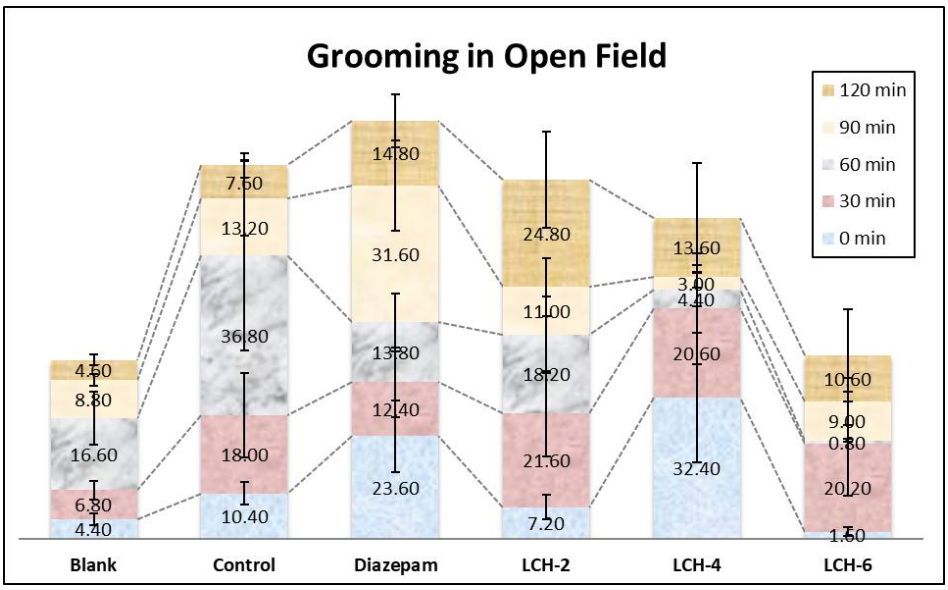

Figure 3: Grooming by the mice in different time intervals

Values are mean ± S.E.M., (n = 5); Dunnett t-test (2 sided) treated one group as control (water) and compared all other groups against it.

LCH-4 significantly increased (32.40) the grooming response in mice compared to Diazepam (23.60) immediately after oral gavage (Figure 3). Diazepam found to increase the activity at 90 min and 120 min whereas LCH in all doses found to reduce the activity in 60 min and 90 min however increased it at 120 min.

3.4 Hole Cross Test

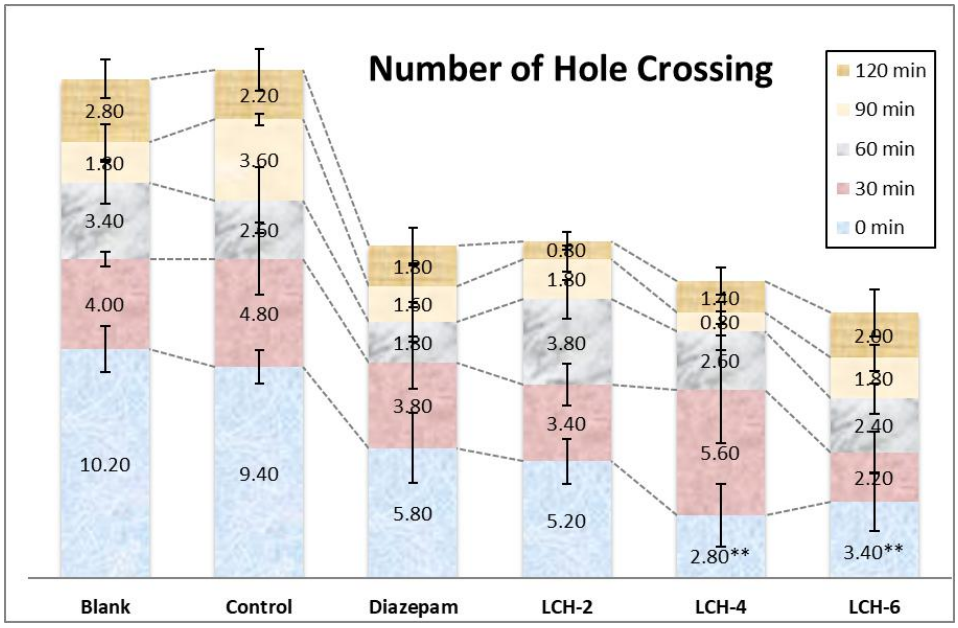

Figure 4: Number of hole crossed by the mice in different time intervals

Values are mean ± S.E.M., (n = 5); * P < 0.05, ** P < 0.01, Dunnett t-test (2 sided) treated one group as control (water) and compared all other groups against it.

Diazepam and LCH at all doses gradually reduced the number hole crossed by the mice (Figure 4). Both LCH-4 and LCH-6 in all stages found to significantly decrease the number compared to Diazepam.

3.5 Elevated Plus Maze Test

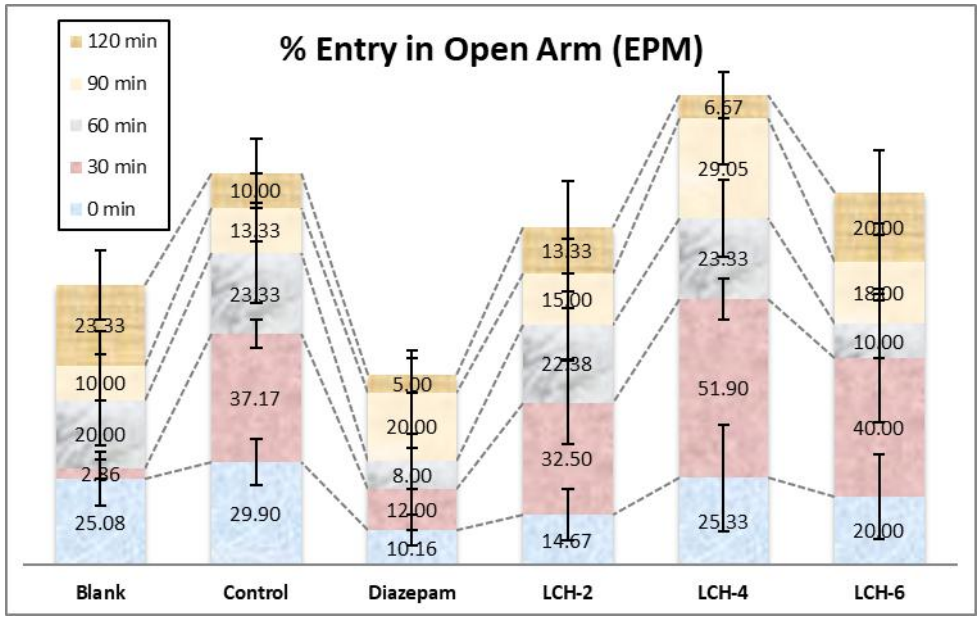

Figure 5: Percentage of open arm entry by the mice in different time intervals

Values are mean ± S.E.M., (n = 5); Dunnett t-test (2 sided) treated one group as control (water) and compared all other groups against it.

Figure 5 showed that the percentage entry in open arm against the entry in total arm was most increased by the LCH-4 (51.90%) and LCH-6 (40%) in second interval (30 min) compared to control (37.17%). Similar trend was also observed in 90 min and 120 min.

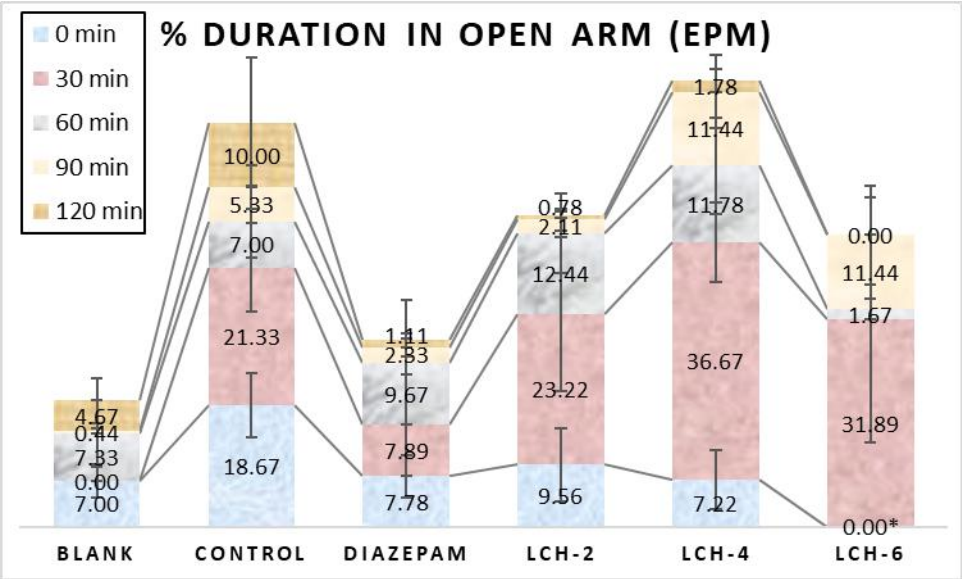

Figure 6: Percentage of duration spent in open arm by the mice in different time intervals

Values are mean ± S.E.M., (n = 5); * P < 0.05, Dunnett t-test (2 sided) treated one group as control (water) and compared all other groups against it.

Time spent in percentage at open arm compared to the total arm was observed in 30, 60 and 90 min by the honey in all doses (Figure 6). maximum rise was observed at 30 min by LCH-4 (36.67%). However, diazepam failed to increase the duration at any intervals.

4. Discussion

Open Field, Hole Cross and EPM are widely acceptable experimental methods to evaluate sedative-anxiolytic potential. The theories behind introducing these fields were to challenge the rodents to a novel environment. However the behavioral changes caused by these environments often got influenced by rodent's identical neurologic conditions. The major challenges reported were variations due to first administration against repeated administration, utilizing same rodent for another experiment but in different time or using different rodent for different experiments. Keeping the drawbacks in consideration, the present study undertook a newly modified approach to integrate the experimental fields so that to utilize the same rodents with single oral administration for exposing to different fields which had allowed to nullify the risk of individual and time dependent variance. The current study design too potentiated a mild risk for inducing over stress to the rodents due to multiple handling and diversity of environmental exposure, however, all subjects experienced the harmony. Moreover, incorporation of a Blank group (no treatment) served as the control for such associated risks.

Benzodiazepines act as positive allosteric modulators of the GABAA (γ -aminobutyric acid type A) receptor complex by binding to alpha-gamma subunit interface which increases neuronal chloride-ion influx, resulting in hyperpolarization of postsynaptic membranes [22]. These potentiations of GABAA receptor at α2/α3 subunit isoforms in limbic system, thalamus, hypothalamus and cerebral cortex produce calming effects which is responsible for facilitating the anxiolysis process [23]. Diazepam showed similar effects by reducing locomotion and other exploratory behaviors. From the data it can be suggested that diazepam worked in two phases. In sedation phase, immediately after administration it started to reduce and drastically decreased the locmotor activities upto 60 minutes. In second phase, it started to facilitate with limited activities

(square crossing and grooming) indicating the process of anxiolysis. Lychee honey at dose 4g/kg body weight significantly reduced the behavioral response immediately after oral administration followed by a sharp increase in 30 minutes which found to be decreased gradually in next all intervals. Honey at 2g/kg and 6g/kg dose followed the same pattern with exception to that of sharp increase at second interval.

The decrease in ambulatory activities such as in case of square or line crossed in open field indicates the sedative potential, the degree of which attributes to the counts at different intervals. Lychee honey at 6g/kg dose found to be more effective compared to diazepam which suggests the calming effect potentially by GABAergic interaction. Grooming is negatively related to index of high activity states [24] and is an important element for interpretation of decision-making behavior [25]. It also indicates the consummatory and washing activities when subjected to feeding as showed initially by LCH-4 [26]. Apart from reducing the locomotor activities, Diazepam and LCH was able to produce the decision-making behavior which implies the process of anxiolysis at late phases by a possible interaction with α2/α3 subunit of GABAA receptors. Combined with ambulation rearing is described as nonspecific excitability level of rodents upon different exposure [27]. This has been reported to be correlated with hippocampal slow wave activity [28]. Diazepam and LCH-6 might have modulated their sedative function by interfering with α1 subunit of GABAA receptors adjacent to the γ subunit [29].

Data from Hole cross test also suggested that the α1GABAA receptor subtype might be critically involved in the sedative potential of Diazepam [29] and LCH in higher doses as both minimizing the exploratory activity represented in terms of number of hole crossed.

Anxiety is characterized by stress and fear [30]. Presence of the rodent in open arm is considered the sign for mitigation of fear [31]. GABAA receptors containing an α2 subunit in limbic system, thalamus, hypothalamus and cerebral cortex position thought to be involved in alleviation of this fear to produce anxiolytic effect [23].

5. Conclusion

The present study design proved to be an effective approach for investigation of sedative-anxiolytic potential of drugs or agents. However, further research is needed for a constructive validation and to explain the interpretation of behavior. From the study it can be concluded that, at higher doses Lychee Honey possess great sedative-anxiolytic potential which need to be substantiated with in depth analysis for the involvement of neurotransmitters and their associated receptors.

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Conflict of Interests

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

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References

1 Aljadi, A.M. Kamaruddin, M.Y. Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys, ***Food Chemistry***, 2004 ,85, 513–518. DOI:10.1016/S0308-8146(02)00596-4

2 Liu SC, Lin JT, Wang CK, Chen HY, Yang DJ. Antioxidant properties of various solvent extracts from lychee (*Litchi chinensis* Sonn.) flowers, ***Food Chemistry***, 2009, 114, 577–581. DOI:10.1016/j.foodchem.2008.09.088

3 Yang DJ, Chang YY, Hsu CL, Liu CW, Wang Y, Chen YC. Protective effect of a litchi (*Litchi chinensis* Sonn.) flower-water-extract

on cardiovascular health in a high-fat/cholesterol-dietary hamster, ***Food Chemistry***, 2010, 119(4), 1457-1464. DOI:10.1016/j.foodchem.2009.09.027

4 Chang, Y. Y. Yang, D. J. Chiu, C. H. Lin, Y. L. Chen, J.W. & Chen, Y C. Antioxidative and anti-inflammatory effects of polyphenol-rich litchi (*Litchi chinensis* Sonn.)-flower–water-extract on livers of high-fat -diet fed hamsters, ***Journal of Functional Foods***, 2013, 5, 44–52. DOI:10.1016/j.jff.2012.08.002

5 Biglari, F. AlKarkhi, A.F.M. & Easa, A.M. Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran, ***Food Chemistry***, 2008, 107, 1636–1641. DOI:10.1016/j.foodchem.2007.10.033

6 Shahidi F. Natural antioxidants: Chemistry, health effects, and applications, Urbana, ***IL: AOCS Press*** 1997. DOI:10.1002/food.19970410536

7 Chen YC, Lin JT, Liu SC, Lu PS, Yang DJ. Composition of flavonoids and phenolic acids in lychee (*Litchi Chinensis* Sonn.) Flower extracts and their antioxidant capacities estimated with human LDL, erythrocyte, and blood models. ***J Food Sci*** 2011; 76(5): C724-728 DOI: 10.1111/j.1750-3841.2011.02164.x

8 Ibrahim SR, Mohamed GA. *Litchi chinensis*: medicinal uses, phytochemistry, and pharmacology. ***J Ethnopharmacol*** 2015; 174: 492-513 DOI: 10.1016/j.jep.2015.08.054

9 Wu,Y.H.S. Chiu, C.H. Yang, D. J. Lin, Y. L. Tseng, J. K. & Chen, Y. C. Inhibitory effects of litchi (*Litchi chinensis* Sonn.) flower-water extracts on lipase activity and diet-induced obesity, ***J. Funct Foods***, 2013, 5, 923–929. DOI: 10.1016/j.jff.2013.02.002

10 Bhoopat L, Srichairatanakool S, Kanjanapothi D, Taesotikul T, Thananchai H, Bhoopat T. Hepatoprotective effects of lychee (*Litchi chinensis* Sonn.): a combination of antioxidant and anti-apoptotic activities. ***J Ethnopharmacol*** 2011; 136(1): 55-66 DOI: 10.1016/j.jep.2011.03.061

11 Hwang, J.Y. Lin, J.T. Liu, S.C.; Hu, C.C.; Shyu, Y.S. Yan, D.J. Protective role of litchi (*Litchi chinensis* Sonn.) flower extract against cadmium- and lead-induced cytotoxicity and transforming growth factor β1-stimulated expression of smooth muscle α-actin estimated with rat liver cell lines, ***J. Funct. Foods***, 2013, 5 (2), 698-705. DOI:10.1016/j.jff.2013.01.013

12 Jantakee K, Tragoolpua Y. Activities of different types of Thai honey on pathogenic bacteria causing skin diseases, tyrosinase enzyme and generating free radicals. ***Biol Res*** 2015; 48: 4 DOI: 10.1186/0717-6287-48-4

13 Bogdanov, S. Physical properties of honey. In: Book of Honey, Chapter 4. ***Bee Product Science*** 2009. www.bee-hexagon.net.

14 International Standard Organisation, Fruit and Vegetable Products—Determination of Soluble Solids—Refracto- metric Method, 2nd Edition, ***International Organisation for Standardisation***, Geneva, 1998.

15 Wakhle, D.M. Beekeeping technology – production, characteristics and uses of honey and other products, In: Mishra, R.C. (Ed.), Perspectives in Indian Apiculture, ***Agro-Botanica, Bikaner*** 1997, pp. 134–139.

16 United State Department of Agriculture, United States Standards for Grades of Extracted Honey, ***United State Department of Agriculture***, Washington DC, 1985.

17 Kinoo, M.S. Mahomoodally, M.F. Puchooa, D. Anti-Microbial and Physico-Chemical Properties of Processed and Raw Honeys in Mauritius, ***Advances in Infectious Diseases***, 2012, 2, 25-36. DOI:10.4236/aid.2012.22005

18 Billah MM, Rayhan MA, Yousuf SA, Nawrin K, Rayhan J, Khengari EM. A Novel Integrated (OF-HC-EPM) Approach to Study Anxiety Related Depressive Behavior in Mice Model: A Comparison of Neuro Standards, ***Advances in Pharmacology and Pharmacy,*** 2019, 7(3): 39-48. DOI:10.13189/app.2019.070301.

19 Billah, M.M. Nawrin, K. Ahmed, K.T. Jabed, M.S.U. Islam, M.N. Uddin, M.M. GABA mediated response of aqueous, ethanol and ethyl acetate extracts of *Dicranopteris linearis* leaf in Swiss Albino mice, J. ***HerbMed. Pharmacol.***, 2016, 5(1), 1-6.

20 Nawrin, K. Billah, M.M. Jabed, M.S.U. Roy, A. Ahmad, A.K.M.R.; Islam, M.N. Antipyretic, Antidiabetic, Thrombolytic and CNS Depressant Potential of Ethanol Extract

of Crotalaria Verrucosa L. Leaves, ***Am. J. Biomed. Sci.***, 2015, 7(4), 198-204; DOI: 10.5099/aj150400198

21 Hawiset T, Muchimapura S, Wattanathorn J, Sripanidkulchai B. Screening neuropharmacological activities of *Kaempferia parviflora* (Krachai dam) in healthy adult male rats, ***Am. J. Appl. Sci***., 2011, 8(7), 695-702. DOI: 10.3844/ajassp.2011.695.702

22 Calcaterra NE, Barrow JC. Classics in chemical neuroscience: diazepam (valium). ***ACS Chem Neurosci*** 2014; 5(4): 253-260 DOI: 10.1021/cn5000056

23 Zakusov VV, Ostrovskaya RU, Kozhechkin SN, Markovich VV, Molodavkin GM, Voronina TA. Further evidence for GABA-ergic mechanisms in the action of benzodiazepines. ***Arch Int Pharmacodyn Ther*** 1977; 229(2): 313-326 [PMID: 23084]

24 Prescott, R. Some behavioral -effects of variables which influence general level of activity of rats, ***Anim. Behav.***, 1970, 18, 791-796.

25 Sestakova N, Puzserova A, Kluknavsky M, Bernatova I. Determination of motor activity and anxiety-related behaviour in rodents: methodological aspects and role of nitric oxide. ***Interdiscip Toxicol*** 2013; 6(3): 126-135 DOI: 10.2478/intox-2013-0020

26 Walsh RN, Cummins RA. The Open-Field Test: a critical review. ***Psychol Bull*** 1976; 83(3): 482-504 [PMID: 17582919]

27 Ivinskis, A. The reliability of behavioural measures obtained in the open-field, ***Aust. J. Psychol.***, 1968, 20(3), 173-177, DOI: 10.1080/00049536808255754

28 Luscher BP, Baur R, Goeldner M, Sigel E. Influence of GABA(A) receptor alpha subunit isoforms on the benzodiazepine binding site. ***PLoS One*** 2012; 7(7): e42101 DOI: 10.1371/journal.pone.0042101

29 Rowlett JK, Platt DM, Lelas S, Atack JR, Dawson GR. Different GABAA receptor subtypes mediate the anxiolytic, abuse-related, and motor effects of benzodiazepine-like drugs in primates. ***Proc Natl Acad Sci USA*** 2005; 102(3): 915-920 DOI: 10.1073/pnas.0405621102

30 Shin LM, Liberzon I. The neurocircuitry of fear, stress, and anxiety disorders. ***Neuropsychopharmacology*** 2010; 35(1): 169-191 DOI: 10.1038/npp.2009.83

31 Billah, M.M. Hasan, M.R. Nawrin, K. Mohiuddin Habib, M.R. Evaluation of Analgesic and Sedative-anxiolytic Potential of *Paderia foetida* Leaf Extract, ***Am. J. Biomed. Sci.***, 2015, 7(2), 98-104. DOI: 10.5099/aj150200098

