Global Science Research Journals

ISSN: 2449-1942 Vol. 6 (1), pp. 071-075, March, 2018

http://www.globalscienceresearchjournals.org/

Global Journal of Neurology and Neurosurgery

Full Length Research Paper

Evaluation of anxiolytic and novelty induced behaviours following bee-honey consumption in rats

O. A. Oyekunle[1]*, M. A. Akanmu[2] and T. P. Ogundeji[1]

[1]Department of Physiology, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.
[2]Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

Abstract

Bee honey has been shown to have many trace elements. Antibacterial and antioxidant properties of bee honey have been reported and employed for therapeutic purposes. The present work evaluates the anxiolytic values and effect on novelty induced behaviours of bee honey consumption. Wistar rats of either sex were given different concentrations of bee honey (10, 20 and 40%) at the dose of 0.5 ml/100 g p.o. The control rats were allowed access to distilled water *ad lib*. Anxiolytic value was accessed in the Hole Board test while novelty induced behaviours were accessed in the Open-Field test. The result showed a significant dose dependent increase in exploratory activities of the experimental animals in the test groups when compared with the control (P<0.05) in the hole board test. In novelty induced behaviour tests, bee honey also produced a significant dose dependent increase in locomotor, rearing and grooming activities with the 40% concentration producing the highest level of significance when compared with the control (P<0.01). The results suggest that bee honey consumption mitigates anxiety and exerts excitatory effect on the central nervous system especially at the highest non-sedative dose.

Keywords: Anxiolytic, locomotor, hole board, bee honey, rearing, grooming neurobehaviour, open-field.

INTRODUCTION

Honey is produced by honey bee (genus *Apis*) from nectar of flowers. Honey gets its sweetness from the monosaccharide fructose and glucose and has approximately the relative sweetness as that of granulated sugar (http//www.honey.com, 2008; http//www.food.oregonstate.edu/sugar, 2008). It contains essential trace elements which have been implicated in brain functions - Copper (Prohaska, 1987); Zinc (Dreosti, 1983; Prohaska, 1987; Sandstead 1985; Wallwork, 1987); Iron (Beard et al., 1993; Yehuda, 1990; Beard, 2003) and Manganese (Dreosti and Richard, 1985). Bee honey has also been reported to be rich in vitamins-Riboflavin, Naicin, Pantothenic acid, Folic acid and Ascorbic have been reported to have neuromodulatory role. Bee honey has a pH range of 3.2 to 4.5 (Al-Waili, 2004) and this corroborates its antibacterial activity (Wahdah, 1998). It appears to be effective in killing drug resistant biofilms which are implicated in chronic rhinosinusitis (Science Daily, 2008). Antioxidants in honey have been employed in the treatment of colitis (Bilsel et al., 2002) such claims are consistent with its uses in traditions of folk medicine (Molan, 1992).

Role of free radical has been suggested in pathogenesis of diseases including neurodegenerative disorders (Halliwell, 1994; Wulf, 2002). These free radicals are produced during normal cellular metabolic functions and include hydroxyl radical (OH^-), superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2) and nitric oxide (NO) (Halliwell and Gutteridge, 1999; Frei, 1994). These reactive species have high chemical reactivity and the brain is highly susceptible to such oxidative damages, due to its high polyunsaturated fatty acid content and less balance is crucial in neurodegenerative/neuronal processes and antioxidant directly or indirectly protect cell from adverse effect of drugs, xenobiotics and toxic radical reactions (Halliwell, 1994; Wulf, 2002). In view of the above, this present study was designed to evaluate

*Corresponding author. E-mail: oalanre@gmail.com. Tel: 0803 074 2928.

the possible involvement of trace elements and antioxidant constituents of bee honey in relieving anxiety and neurobehavioural characteristics.

MATERIALS AND METHODS

Bee honey

The pure bee honey was procured from LAUTECH VENTURES and produced by the University's apiary under the management of the faculty of agricultural sciences, Ladoke Akintola University of Technology, Ogbomoso.

Bee honey concentrations

Bee honey concentrations were prepared as follows:

For 10%:

0.5 ml of bee honey dissolved in 4.5 ml distilled water.

For 20%:

1 ml of bee honey dissolved in 4 ml distilled water.

For 40%:

2 ml of bee honey dissolved in 3 ml of distilled water.

Dose basis

0.5 ml/100 g body weight.

Animal

40 Wistar rats of both sexes and non-pregnant weighing 100 -120 g were used in this investigation. The rats were kept in plastic cages in the Animal house of Pharmacology Department, Faculty of Pharmacy, Obafemi Awolowo University (O.A.U), Ile-Ife, Osun State, with an unrestricted access to both food and water. After one week of habituation, animals were subjected to the experiments. For each of the experiment, each animal was tested only once. All tests were carried out between 8.30 am and 4.30 pm. The guidelines on Animal experiments were strictly followed.

Experimental protocol

Each experimental procedure consists of 4 groups with 5 rats in each group. Group1 (control group) was given water *ad lib* while groups 2-4 (treatment groups) were given 10, 20 and 40% bee honey concentration respectively with the aid of oral canula.

Hole board test

In this procedure, as described by Boissier and Simon (1960), one hour after the administration of bee honey concentration the animal was placed on a board (40 x 40 cm) with 16 holes (symmetrically distributed in four rows) for 5 min. The number of times the animal dips its head into the hole during the 6 min period was registered. The results obtained were expressed as mean total number of head dips (Lister, 1987).

Open-field test

For each test session, the animals were allowed to explore the testing environment, a quiet well -ventilated room, for at least 30 min. Spontaneous motor activity is monitored for 30 min in the modified Open-Field test as described by Eckeli et al. (2000). Briefly, the structure consist of a rectangular arena composed of a hard board floor (diameter 60 x 60 cm) with a surrounding wall of dimension 64 x 64 x 64 cm^3, both made of white painted wood. The floor was divided by permanent red marker into squares of 4.2 cm^2 at the bottom (on the external surface). One hour after oral administration of bee honey, each rat was introduced into the arena. Locomotor activity was evaluated by counting the number of floor unit crossed on the floor of the square with all paw (during 30-min period) . Grooming activity was evaluated by counting the numbers of licking of the body and pubis with mouth and face washing actions. Rearing behaviour was evaluated by counting the number of times in which the animal stood on its hind limbs with its forearm against the wall of the observation cage or in the air during the 30 min period after oral administration of bee honey. Before introducing each animal into the arena, the arena was cleaned with methylated spirit to eliminate the possible bias due to the odour that could be left by the previous subject.

Statistical analysis of results

Results of the experiments and observations were expressed as mean ± standard error of mean (SEM). The significance of differences between groups was determined using one-way analysis of variance (ANOVA) followed by post-hoc Dunnett test. In all the observations, statistical significance was considered at $P< 0.05$.

RESULTS

Figure 1 showed the result obtained from the Hole Board test which is a model for anxiolytic investigation. It showed gradual increase in numbers of head dip obtained with increasing concentrations of bee honey. Only 20 and 40% concentrations of bee honey produced significant difference when compared with the control. Figures 2a-c showed the results obtained from the Open-Field test. There was concentration dependent increase in locomotor activities but only the 20 and 40% concentration produced significant difference ($P<0.05$) when compared with the control. In rearing behaviour only 40% bee honey concentration produced a significant difference ($P<0.05$) when compared with the control. In grooming activity, only 20 and 40% bee honey concentrations produced significant difference when compared with the control. In all the novelty induced behaviours, the most significant effects were observed at 40% bee honey concentration.

DISCUSSION

Nutritional factors such as lack of trace elements and vitamins in the diet can influence the neurobehavioural profile of the organism and precipitate anxiety-like syndrome. The neuronal changes mediating these

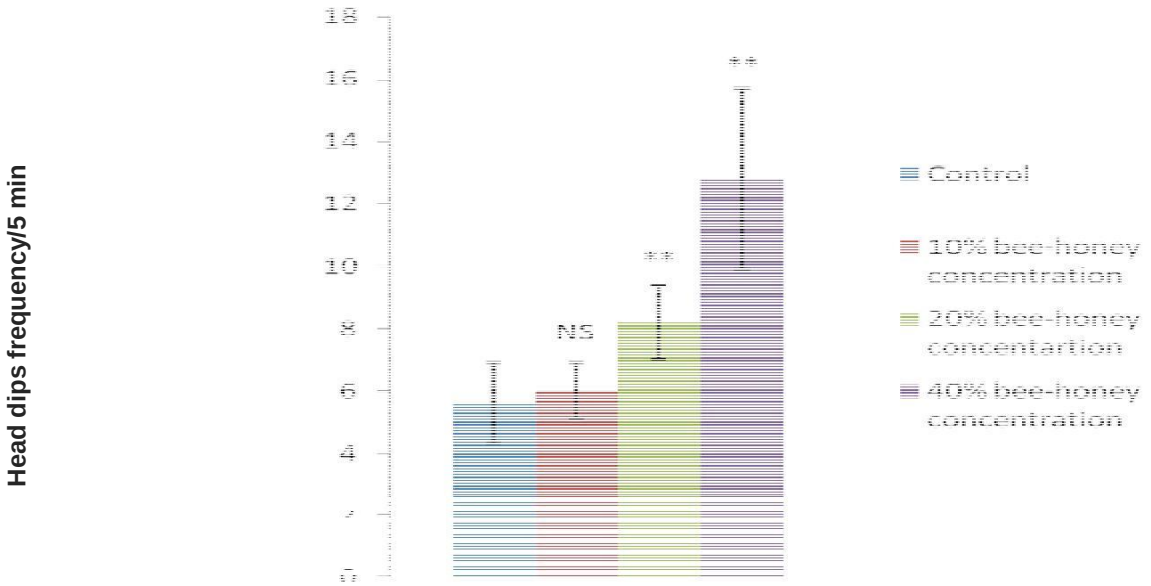

Figure 1. Effects of bee honey on Hole Board exploratory behaviour in rats. Each column and bar represents Mean ± SEM of number of head dips 1 hour after oral administration of water (control) and bee honey concentrations. ** P< 0.05 (N = 5).

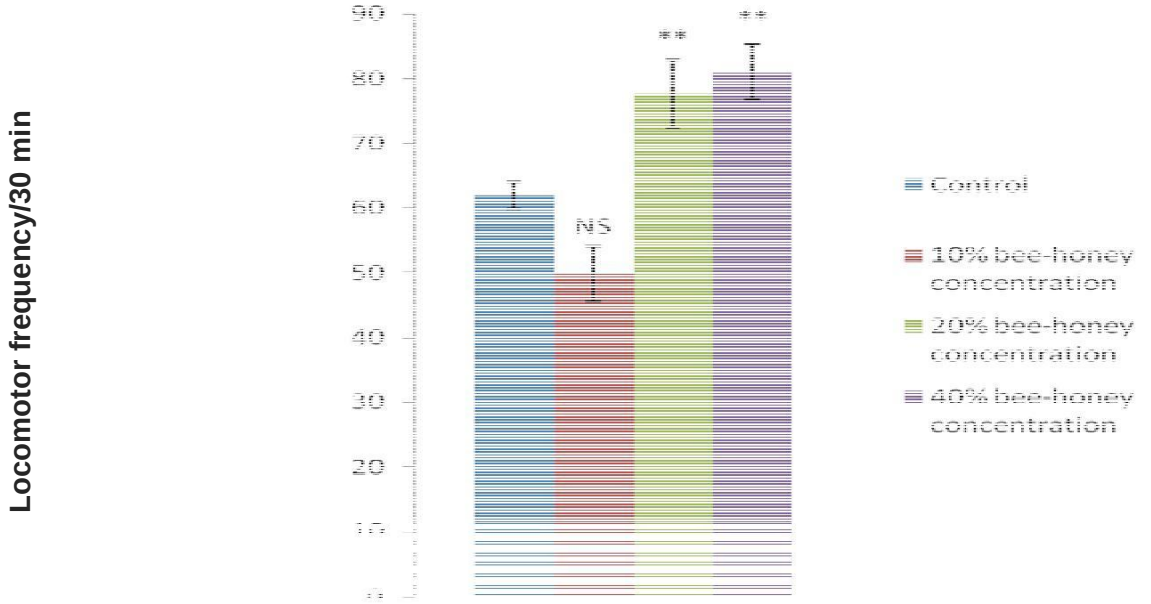

Figure 2a. Effects of bee honey on locomotor activities in rats after 30 min session. Each column and bar represents Mean ± SEM of frequency of locomotor activities 1 h after oral administration of water (control) and bee honey concentrations. **P<0.05 (N = 5).

behavioral deficits appear to be free radicals and the oxidative stress they generate (Cantuti-Castelvetri et al., 2000). Both Hole Board and Open Field tests have been effectively employed to access the neurobehavioural profile of animals under the influence of anxiogenic/anxiolytic agents. Drugs that reduced anxiety are known as anxiolytics and there is increased exploratory behaviour in novel situations (Bharttachanya and Statyan, 1997). The increased number of head dips and locomotor activities observed in Hole Board and Open field tests especially at 40% bee honey concentration are reliable indices of anxiolytic effects.

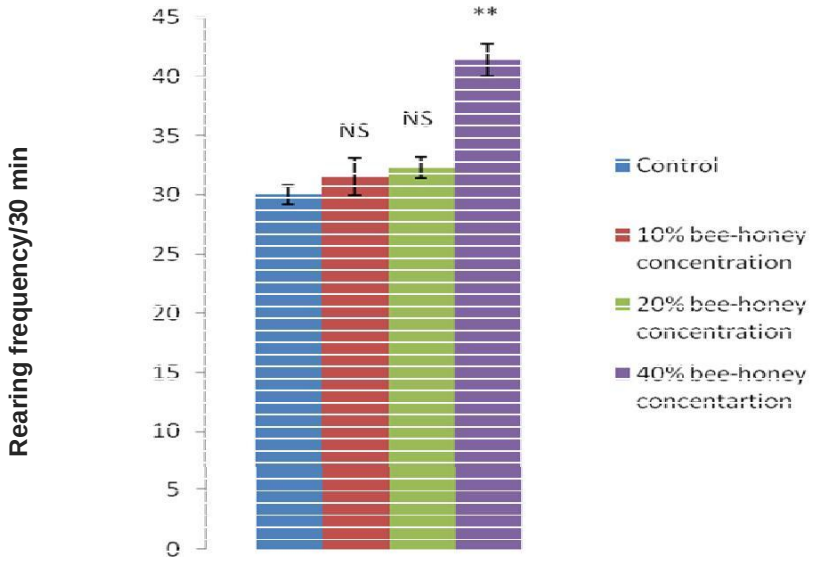

Figure 2b. Effects of bee honey concentrations on rearing behaviours in rats after 30 min session. Each column and bar represents Mean ± SEM of frequency of rearing behaviour 1 h after oral administration of water (control) and bee honey concentrations.**P<0.05 (N = 5).

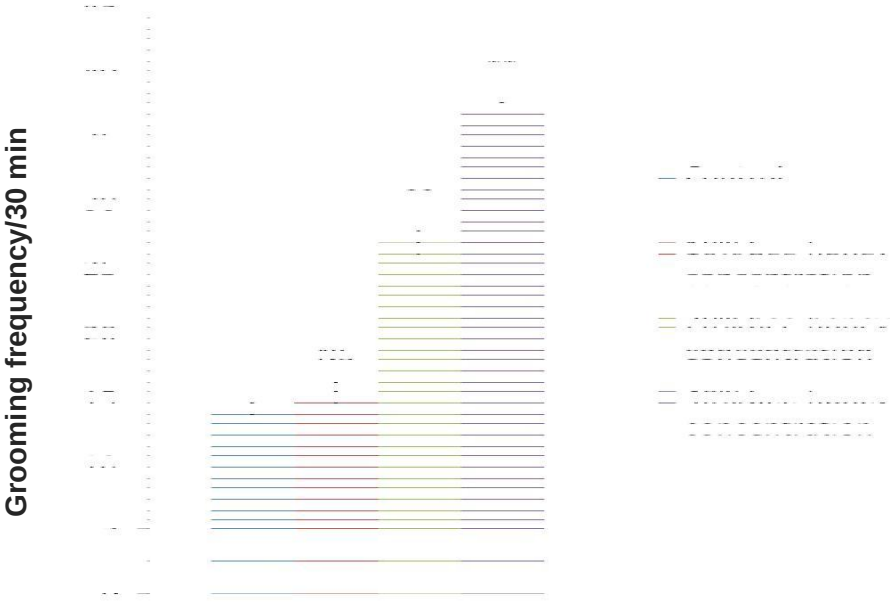

Figure 2c. Effects of bee honey on grooming behaviours in rats after 30 min session. Each column and bar represents Mean ± SEM of frequency of grooming activities 1 h after oral administration of water (control) and bee honey concentrations. **P<0.05 (N = 5).

Neuronal lesion are oftentimes associated with various degrees of behavioural impairments, and among the prime candidates responsible for producing the neuronal changes mediating these behavioral deficits appear to be free radicals and the oxidative stress they generate.

Therefore, there have been a number of studies which have examined the putative positive benefits of antioxidants in altering these neuronal/behavioral decrements, with varying degrees of success (Morris et al., 2002; Sofic et al., 2002).

From the result, the presence of antioxidants (majorly vitamins) in bee honey tends to mop up free radical generated in the CNS thereby alleviating anxiety. Biochemically, serotonin and its receptors have long been viewed as neurotransmitter involved in behavioural changes (Iversen, 1984; Briley et al., 1990). Of the 14 or more mammalian serotonin receptors subtypes that have been described in literatures at least four have been implicated in anxiety in various animal models (Lucki, 1996). The role of iron in neurotransmitter metabolism has been investigated and reported, it is now known that iron is essential for a number of enzymes involved in neurotransmitter synthesis (Beard et al., 1993; Ashkenazi et al., 1982) including tryptophan hydroxylase (serotonin), tyrosine hydroxylase (norepinephrine (NE) and Dopamine). In addition, iron is a cofactor for ribonucleotide reductase and is essential for the functioning of a number of electron transfer reactions related to both lipid metabolism and brain-energy metabolism (Wigglesworth and Baum, 1988). Therefore, the behavioural modulatory activity of bee honey could be as a result of the presence of this essential micronutrient (iron) in its composition. It has been suggested that there is an involvement of mesolimbic dopamine system in the novelty induced grooming. Selective dopamine D1 and D5 receptors agonists elicit an intense grooming behaviour while dopamine D2 receptor agonists reduce this behaviour (Griebel et al., 1994). Bee honey consumption appears to influence novelty induced behaviours in the animals as observed in the results especially at 40% concentration. The increased excitatory activity in the central nervous system could be due to certain component of bee honey acting probably via D1 and D5 receptors. This observation could be linked with the fact confirming co-localization of iron with dopaminergic neurons throughout the brain (Yehuda, 1990; Wigglesworth and Baum, 1988). In conclusion, it could be said that pure bee honey possess behavioural modulating potentials and may be used as an anxiolytic agent and may also be used to stimulate the central nervous system (antidepressant). Pure bee honey can be used as supplement in special diet formulation for subjects with mood disorders.

ACKNOWLEDGEMENTS

Our sincere gratitude goes to Mr. E.O. Akinbolusere, Chief Technologist, Department of Physiology, Ladoke Akintola University of Technology, Ogbomoso. Technical staff of Department of Pharmacology, Obafemi Awolowo University, Ile-Ife.

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