ORIGINAL RESEARCH

Effect of Oral Honey Administration on Sleep-Deprived Male Mice

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ABSTRACT

Background: Sleep is a biologic process that is essential for life and optimal health. Sleep plays a critical role in brain function and systemic physiology. The deleterious health consequences of sleep deprivation are associated with risks for a wide variety of medical conditions. Honey's potential role in restorative sleep may have implications in improving long term health.
Objective: To determine the effect of honey on sleep deprivation in male mice.
Method: The study was carried out using four (4) groups of young male mice (N=5-6 per group) deprived of sleep for a period of 6 hours. Bee honey was administered orally at three dose levels; 10 %, 20 % and 40 %^{V}/v respectively to three groups while the control group was administered normal saline (vehicle). Novelty-Induced Behaviour (NIB), Elevated plus-maze (EPM), Hole board and Y-maze models were used to evaluate the effects of honey on the mice.
Results: In the NIB model, a significant ($p<0.05$) decrease in locomotion was observed dose-dependently. The same observation was recorded for rearing behaviour while a biphasic effect was observed in grooming behaviour. The results obtained in the other models showed that locomotor activity was significantly decreased suggesting that honey has central inhibitory effect. In the hole board test and EPM, there were significantly ($p<0.05$) decreased activity of the mice due to the administration of honey.
Conclusion: This study demonstrates that honey exerts dose-dependent central inhibitory effects in sleep-deprived male mice therefore suggesting possible amelioration of sleep deprivation effects.

Keywords: Oral Honey, Sleep Deprivation, Behavioural Test, Neurological Effect, Mice

INTRODUCTION

Sleep is a biologic process that is essential for life and optimal health. Sleep plays a critical role in brain function and systemic physiology; including metabolism, appetite regulation and the functioning of the immune, hormonal and cardiovascular systems[1]. Normal healthy sleep is characterized by sufficient duration, good quality, appropriate timing and regularity, and the absence of sleep disturbances and disorders[1].

The National Sleep Foundation's Sleep Duration Recommendations[2] for each night is as follows: new-borns (0 to 3 months): 14 to 17 hours; infants (4 to 11 months): 12 to 15 hours; toddlers (1 to 2 years): 11 to 14 hours; pre-schoolers (3 to 5 years): 10 to 13 hours; school-age children (6 to 13 years): 9 to 11 hours; teenagers (14 to 17 years): 8 to 10 hours; young adults (18 to 64 years): 7 to 9

hours; and older adults (over 65 years): 7 to 8 hours. Several sleep disorder classifications exist. However, they are typically manifested in one of the following three ways: failure to obtain the necessary amount or quality of sleep (sleep deprivation), an inability to maintain sleep continuity (disrupted sleep) and events that occur during sleep (e.g., sleep apnoea, restless legs syndrome)[1]. Total sleep is known to comprise both slow wave (non-rapid eye movement) sleep and paradoxical (or rapid eye movement) sleep[3].

Reports have shown that total sleep deprivation is becoming a feature of our lifestyle[4]. Thus, disruption of the sleep–wake cycle will adversely affect an individual's daily performance, safety and health. The public health implications of sleep deprivation (SD) are enormous. The deleterious health consequences of SD are associated with risks for a wide variety of medical conditions (e.g., hypertension, obesity, diabetes, depression and compromised immunological function)[5,6,7].

SD also accounts for 20 % of all serious car accidents, which is equivalent to those attributed to alcohol[8]. SD adversely affects cognitive performance interfering with attention and reaction time and this can impair judgment and decision making. The mechanisms associated with impaired cognitive function with SD are poorly understood[8].

Research has explored honey as an enigmatic gel that has gastroprotective, hepatoprotective, reproductive, antioxidant, hypoglycaemic, antihypertensive, antibacterial, antifungal, anti-inflammatory, immunomodulatory, wound healing, cardio-protective and antitumor effects[8,9,10,11,12,13,14]. Unfortunately, research on the nootropic and neuropharmacological effects of honey is scarce[8].

Honey has been shown to enhance longer and higher quality sessions of restorative sleep. In a double-blind, randomized, placebo-controlled study to assess the effect of honey on nocturnal cough and sleep quality, results showed a symptomatic relief of children's nocturnal cough and sleep difficulty due to Upper Respiratory Tract Infections (URTI) when compared with placebo treatment[15]. Sleep deprivation effects are extremely important to public health because poor sleep has been associated with cardiovascular and metabolic disorders including hypertension, obesity, diabetes mellitus and cognitive degeneration. Honey's potential role in restorative sleep may have implications in improving long term health[16].

Animal and human studies have been carried out to determine the effects of sleep deprivation. Exploratory behaviours seen in animal models often involve the excitatory neural systems such as the cholinergic and dopaminergic systems, whereas anxious behaviour often involves the inhibitory neural system, specifically γ-aminobutyric acid (GABA)[17]. Several lines of experimental evidence support the hypothesis that the neuropharmacological effects of honey are mediated via dopaminergic and nonopioid central mechanisms[10,17,18].

This study was done to document the effect of oral honey administration on sleep deprivation effects in male mice.

METHODS

Materials

The following materials were used for the study: i) Normal saline ii) Honey (sourced from Idanre, Ondo State, Nigeria).

Animals

Young male albino mice weighing 23.8 ± 5 g were purchased from the animal house of the Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria. Animals were housed in plastic cages with stainless steel wire coverings. The cages were kept clean at all times, and food and water were provided *ad libitum*. The mice were housed in the animal house of the Pharmacology Department, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife. Generally, animals were handled for 5 minutes every day, 2 weeks before the experiment to habituate them to handling, presence of investigator and experimental environment. The animals were

allowed to acclimatize to the testing area for 15–30 minutes before administration of samples or normal saline (vehicle). The entire experimental period was between 8:00 am and 4:00 pm daily. The experimental protocols followed the guidelines established by the National Institute of Health or the Care and Use of Laboratory Animals and Ethical & Practical Principles of the Use of laboratory Animals[19].

Sample Preparation

Normal saline was used as the vehicle. 10 %, 20 % and 40 % solutions of honey were prepared by diluting 1 ml, 2 ml and 4 ml respectively of honey, with normal saline made up to 10 ml for each concentration. A dose of 0.5 ml/kg of each solution of pure honey was orally administered to animals in the respective test groups. The control group received normal saline at a similar dose of 0.5 ml/kg.

Test Administration

The animals were divided into four groups (N=5-6 per group). For each group, the animals were deprived of sleep for a period of 6 hours. Total sleep deprivation was achieved by a gentle tapping of the cage whenever drowsiness or attempts to engage in a sleeping posture was observed[3,20]. This is because mice are nocturnal animals and tend to sleep during daytime. After the period of sleep deprivation had elapsed, the control group of animals was given normal saline while the other three groups were given different doses (10 %, 20 % and 40 % respectively; 0.5 ml/kg) of the honey preparation. One hour after administration, the animals were subjected to the classical behavioural tests to examine the neurological effects of the test preparation.

Novelty-Induced Behaviour (NIB) Test

Each of these behavioural states: locomotion, rearing and grooming, were observed and scored at ten-minute time intervals for 30 minutes. This was to characterize the behavioural changes induced by the honey sample in the mice when placed in an open field. The apparatus used consist of a rectangular arena composed of a hardboard floor (60 × 60 cm^2) with a surrounding wall 64 cm in height, both made with white painted wood. The floor was divided by permanent red marker into squares of 9 cm at the bottom. One hour after administration, each mouse was introduced into the arena and total locomotion (expressed as the number of floor units entered), frequency of grooming (the number of body cleaning with paws, picking of the body and pubis with mouth and face washing actions) and rearing frequency (number of times the animal stood on its hind legs or with its forearm against the wall) for a total duration of 30 minutes, were recorded. Before introducing each animal, the arena was cleaned with 70 % alcohol to eliminate possible bias due to the odour that could have been left behind by the previously-tested animal[21].

Hole Board Test

The hole-board apparatus consists of a wooden board (40 × 40 $cm^{2)}$ raised 40 cm above ground. The board was divided into 16 squares symmetrically distributed in four rows and holes were cut into the centre of each square. Each animal was placed on the board, one hour after oral administration of either the vehicle or different solutions of honey (10 %, 20 % and 40 %^{V}/v). The frequency of head dips into the holes during a period of 6 minutes was registered[22]. Total locomotion was also recorded.

Elevated Plus Maze (EPM) Test

The apparatus is made of wood and has two narrow enclosed arms which are bordered by high walls and has two open arms which are essentially unprotected boards. The elevated plus maze, a modification of the apparatus validated for mice by Lister[23,24], consists of two open arms (30 cm× 5 cm × 0.25 cm) and two closed arms (30 cm × 5 cm × 15 cm) emanating from a common central platform (5 cm × 5 cm). The two pairs of identical arms are opposite each other. The entire apparatus is elevated to a height of 50 cm above floor level. At the start of the session, the mouse was placed at the centre of the maze, with its

head facing an open arm and allowed to explore the maze for 6 min. During this 6-min test period, the following measurements were recorded: the number of entries and the time spent in open and closed arms, and the exploratory behaviour (total number of arm entries). An entry with all four feet into one arm is defined as an arm entry. The plus maze was carefully wiped with 70 % alcohol after each animal. The results were expressed as percentage of time spent in open arms relative to total time spent in both open and closed arms; and percentage of number of entries into open arms relative to total entries into both open and closed arm entries. Anxiolytic effects are defined as an increase in the proportion of open arm entries divided by the total number of arm entries, and the time spent on open arms relative to the total time spent on both arms. Any decrease in these parameters indicates an anxiogenic effect.

Y-Maze Test

The Y-maze is composed of three identical arms mounted in the shape of a "Y"; each arm 50 cm long, 40 cm high and inclined at an angle of 120^{o}. The floor of each arm consists of wood (10 cm wide). This test was carried out to obtain results for locomotor activity and learning and memory. Each animal was placed in one of the arm compartments and was allowed to move freely for a period of 6 minutes without reinforcers. The parameters that were scored: (1) frequency of entry into each arm, (2) total arm entries and (3) spontaneous alternation. Spontaneous alternation percentage (SA%) was defined as a ratio of the arm choices that differed from the previous two choices ("successful choices") to total choices during the run ("total entry minus two"). The sequence of the arm entries, which are alternations, was manually recorded. An alternation is defined as an entry into all three arms in consecutive choices. For instance, each alternation is followed by a comma in the following sequence of arm entries (each arm is labelled A, B, or C): BCACBCABCABCABC. In this example, the mouse entered 15 arms, 11 of which are alternations. The number of maximum spontaneous alternation is then the total number of arms entered minus 2 and the percentage is calculated as:

$$\frac{\text{actual alternations}}{\text{maximum alternations}} \times 100$$

Therefore, the spontaneous alternation percentage in this case is 73.3 %. The apparatus was cleaned with 70 % alcohol in between sessions to eliminate the odour of the previous animal[25].

Data Analysis

The behavioural data were analysed by One-way Analysis of Variance (ANOVA) (Primer statistical software by Standton A. Glantz, version 3.01, McGraw-Hill Inc.) and Dunnett's test to compare the results between the control group (vehicle-treated) and each of all the tested groups. The results were expressed as Mean and Standard Error of Mean (SEM) and values of $p < 0.05$ were considered statistically-significant.

RESULTS

1) Novelty-Induced Behaviour (NIB) Test for Locomotion

The results obtained during the 30-minute period in the NIB test model showed that locomotion was significantly decreased in honey-treated sleep-deprived mice as shown in Fig. 1.

2) Novelty-Induced Behaviour (NIB) Test for Rearing

In rearing behaviour analysis, the results obtained showed that one-way ANOVA revealed a significant effect of honey administration in sleep-deprived mice and Dunnett's post hoc analysis revealed that doses of 20 %^{V}/v and 40 %^{V}/v of honey caused a significant decrease when compared with vehicle-treated sleep-deprived mice (Fig. 2).

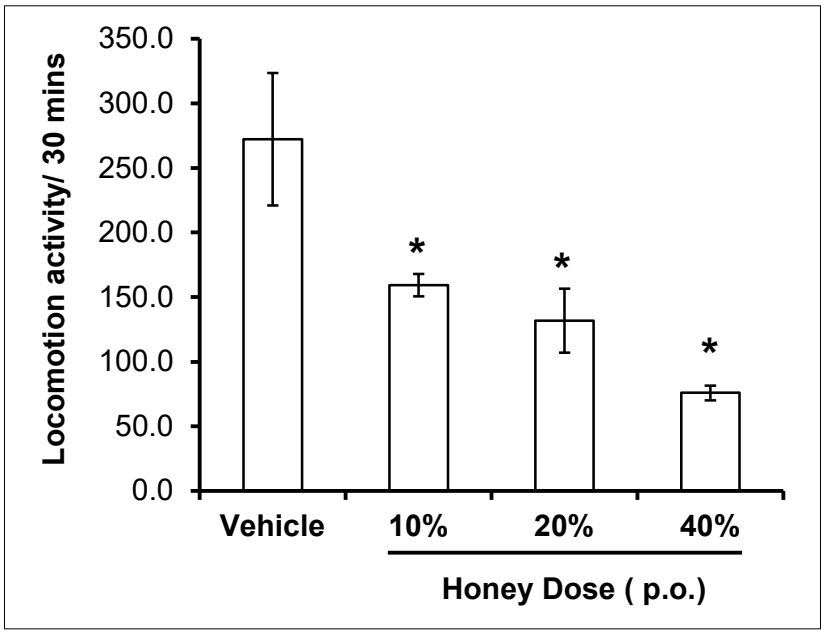

Fig. 1. The Effects of honey administration on Locomotor activity in Sleep-deprived mice. Each value represents the Means ± S.E.M. [Saline-treated (n = 6); 10 % ^{V}/v (n = 6); 20 % ^{V}/v (n = 6) 40 %^{V}/v (n = 5). *p < 0.05 vs vehicle-treated (control) group)]

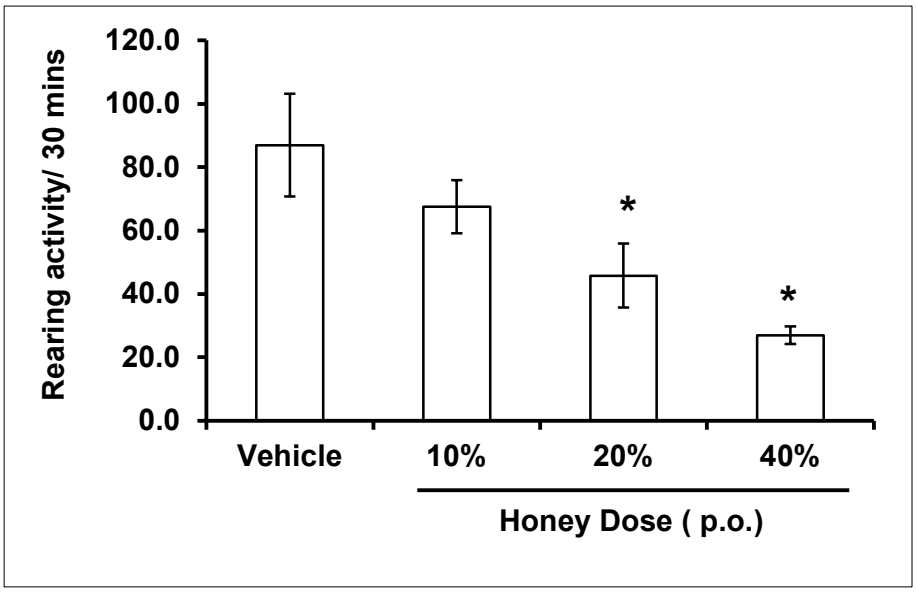

Fig. 2: The Effects of honey administration on rearing activity in Sleep-deprived mice. Each value represents the Means ± S.E.M. [Saline-treated (n = 6); 10 %^{V}/v (n = 6); 20 %^{V}/v (n = 6) 40 %^{V}/v (n = 5). *p < 0.05 vs. vehicle-treated (control) group].

3) Novelty-Induced Behaviour (NIB) Test for Grooming

In grooming behaviour during the 30-minute observation, the results showed that there was no significant effect of honey in sleep-deprived mice even though biphasic results were obtained with the various doses administered. One-way ANOVA and Dunnett's post hoc test showed there was no significant effect due to administration of honey (Fig. 3).

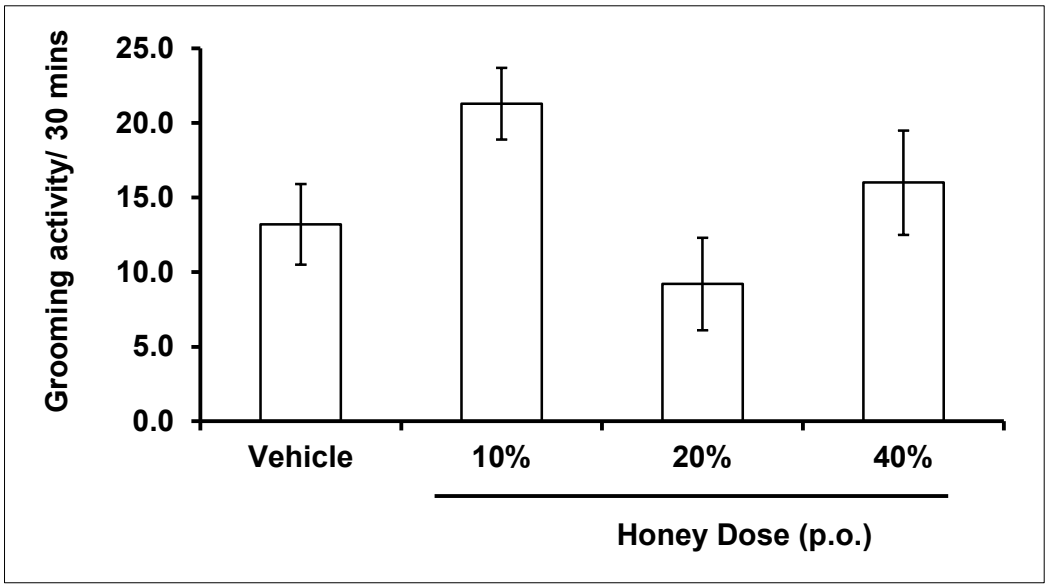

Fig. 3. The Effects of honey administration on grooming behaviour in Sleep-deprived mice. Each value represents the Means ± S.E.M. Saline-treated (n = 6); 10 %^{V}/v (n = 6); 20 %^{V}/v (n = 6) 40 %^{V}/v (n = 5)

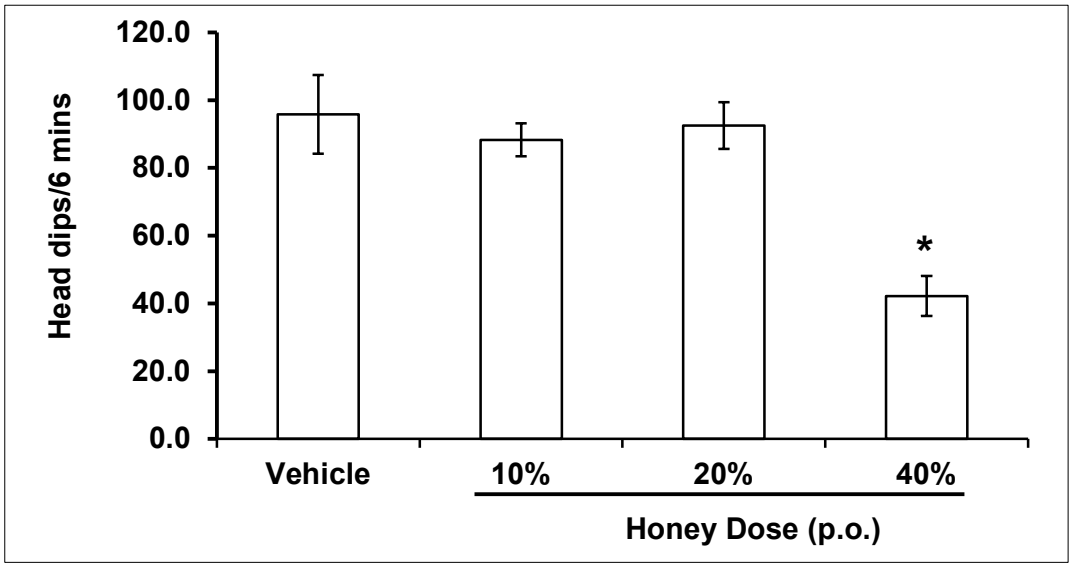

Fig. 4. The Effects of honey administration on number of head dips in Sleep-deprived mice. Each value represents the Means ± S.E.M. [Saline-treated (n = 6); 10 %^{V}/v (n = 6); 20 %^{V}/v; (n = 6); 40 %^{V}/v (n = 5). *p < 0.05 vs. vehicle-treated (control) group].

4. Hole Board Test

a. Head Dips

Results for the number of head dips are shown in Fig. 4 above. Honey at 10 %^{V}/v and 20 %^{V}/v did not elicit any significant effect on the frequency of head dips compared to the control group. However, honey at 40 %^{V}/v caused a significant reduction in the frequency of head dips relative to the control.

b. Locomotion:

The results on line crossing showed that oral honey administration decreased locomotor activity that only reached a significant level at the dose of 40 %^{V}/v (Fig. 5).

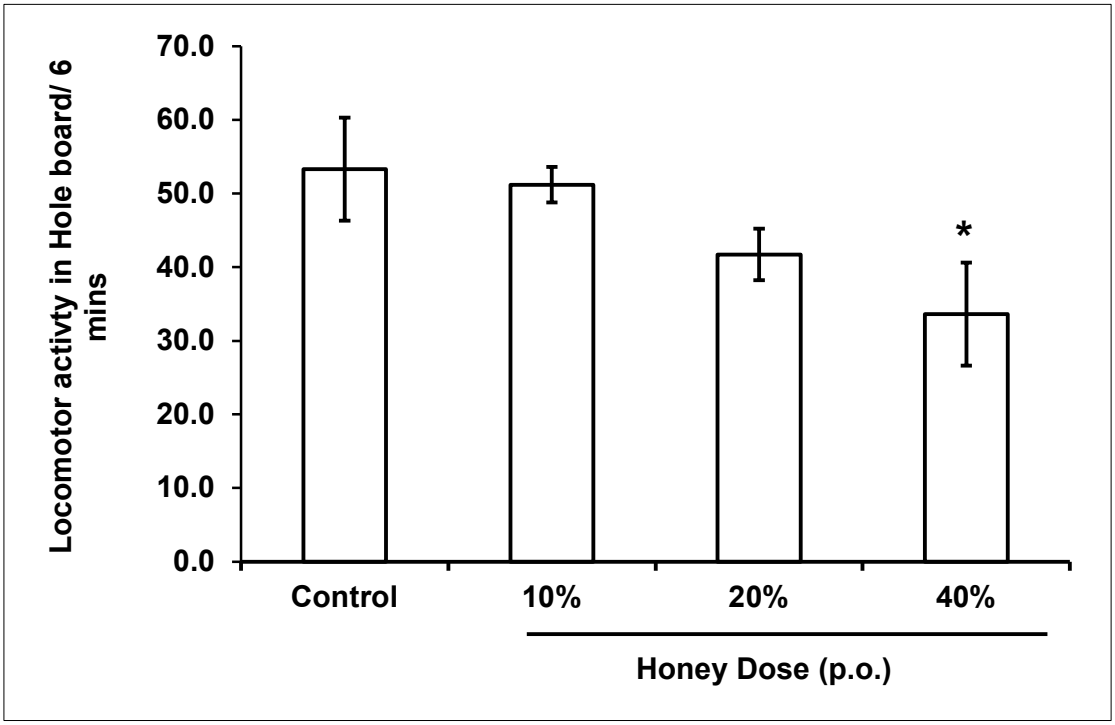

Fig. 5. The effects of honey administration on locomotor activity in sleep-deprived mice using hole board model. Each value represents the means ± S.E.M. [Saline-treated (n = 6); 10 %^{V}/v (n = 6); 20 %^{V}/v (n = 6); 40 %^{V}/v (n = 5) *p < 0.05 vs. vehicle-treated (control) group].

Table 1: Mean (±SE) number of entries and duration in both closed and open arms in the elevated plus-maze presented in total sleep-deprived mice treated with vehicle or honey (10 %^{V}/v, 20 %^{V}/v or 40 %^{V}/v).

	Closed Arm		Open Arm	
Treatment	No. of entries	Duration (s)	No. of entries	Duration (s)
Vehicle (n=6)	10.8 ± 0.7	298.0 ± 14.2	2.5 ± 0.7	61.8 ± 14.2
Honey 10 % (n=6)	8.2 ± 1.9	332.6 ± 12.5	1.7 ± 0.8	27.3 ± 12.5*
Honey 20 % (n=6)	4.8 ± 0.9*	346.7 ± 7.6*	0.3 ± 0.3*	8.5 ± 8.5 *
Honey 40 % (n=6)	1.8 ± 0.6*	360.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*
One-way F (3,22)	10.584	6.101	4.289	6.332
ANOVA P-value	0.0003	0.004	0.02	0.003

*P<0.05 compared with vehicle-treated group (one-way ANOVA followed by Dunnette's t-test)

5. Elevated Plus-Maze (EPM) Test

Results obtained from the elevated plus maze test are presented as number of entries and time spent in both open and closed arms (Table 1). The administration of honey produced a significant decrease in the number of entries and duration of time spent in the open arms of the elevated plus maze at doses of 20 %^{V}/v and 40 %^{V}/v in sleep-deprived mice when compared with the vehicle-treated sleep-deprived mice. The results on

percentage of entries and time spent in both open and closed arms are presented in Table 2. The administration of honey produced a significant decrease in the percentage of number of entries and duration in the open arms at the 20 and 40 % v/v doses when compared with the vehicle-treated sleep-deprived mice. The total arm entries result is presented in Fig. 6 where it was observed that locomotor activity was decreased dose-dependently which was found to be significant ($p<0.05$) at doses of 20 %^{V}/v and 40 %^{V}/v in sleep-deprived mice when compared with vehicle-treated sleep-deprived mice.

Table 2: Mean (±SE) percent of entries and duration in both closed and open arms in the elevated plus-maze presented in total sleep-deprived mice treated with vehicle or honey (10 %^{V}/v, 20 %^{V}/v or 40 %^{V}/v).

	Closed Arm		Open Arm	
Treatment	Percent of entries	Percent of Duration	Percent of entries	Percent of Duration
Vehicle (n=6)	82.9 ± 4.2	82.8 ± 3.9	17.1 ± 4.1	17.2 ± 3.9
Honey 10 % (n=6)	86.8 ± 6.1	92.4 ± 3.5	13.3 ± 6.1	7.6 ± 3.5*
Honey 20 % (n=6)	95.8 ± 4.2	97.6 ± 2.4*	4.2 ± 4.2	2.4 ± 2.4 *
Honey 40 % (n=6)	100.0 ± 0.0*	100.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*
One-way F (3,22)	3.072	6.341	3.072	6.341
ANOVA P-value	0.05	0.003	0.05	0.003

*P<0.05 compared with vehicle-treated group (one-way ANOVA followed by Dunnette's t-test)

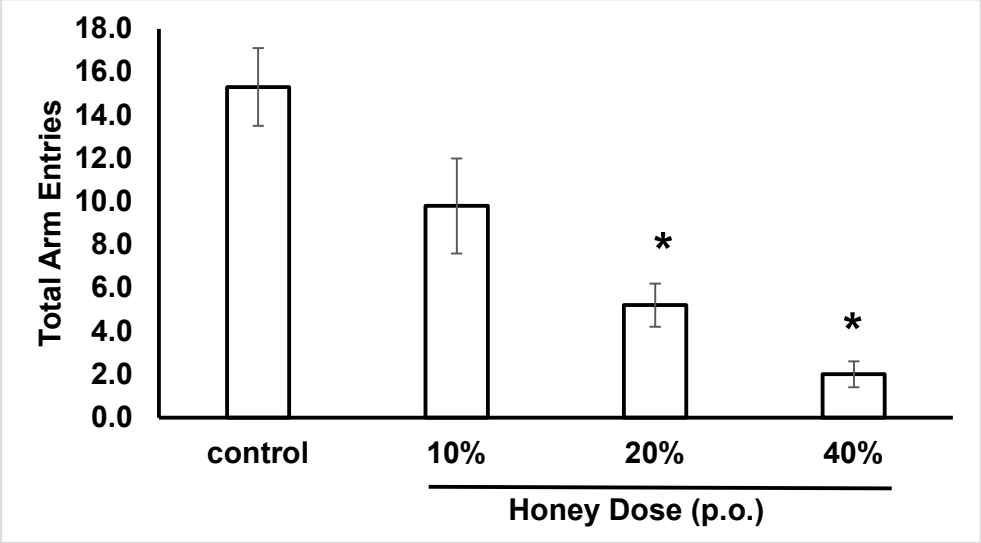

Fig. 6. The effects of honey administration on total arm entries in sleep-deprived mice using the elevated plus-maze model. Each value represents the Means ± S.E.M. [Saline-treated (n = 6); 10 %^{V}/v (n = 6); 20 %^{V}/v (n = 6); 40 %^{V}/v (n = 5). *p < 0.05 vs. vehicle-treated (control) group].

6. Y-Maze Test

In the Y-maze test, the results of spontaneous alternation revealed that honey administration had no significant effect on spatial memory in mice at doses administered (Fig. 7). The locomotor activity in the Y-maze was

significantly decreased with administration of honey that was significant at the dose of 40 %v/v when compared to saline-treated sleep-deprived mice (control) (Fig. 8)

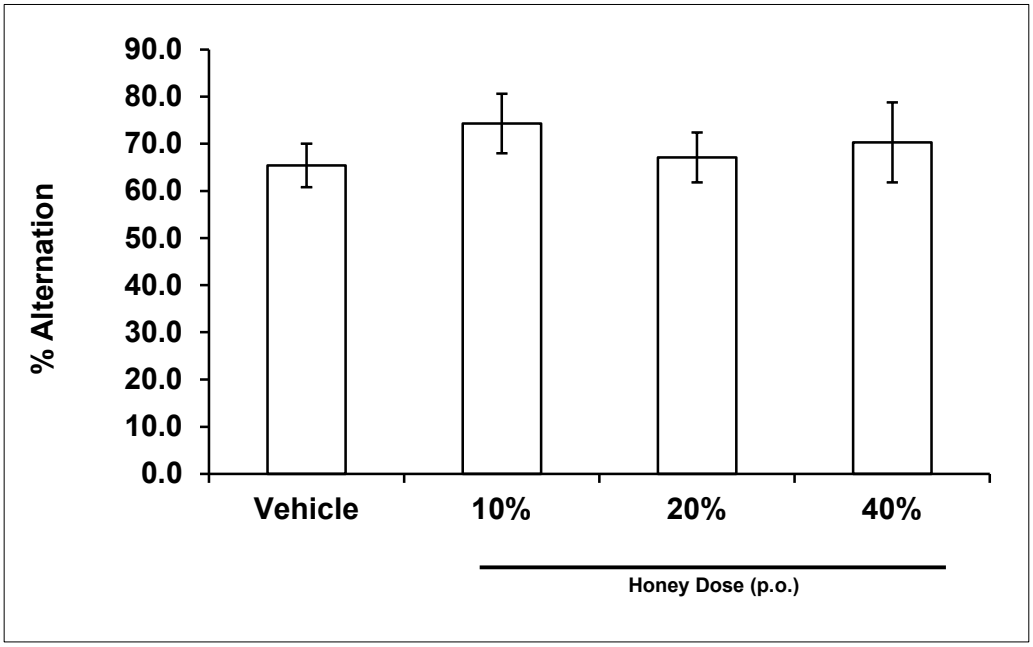

Fig. 7. The effects of honey administration on learning and memory in sleep-deprived mice using Y-maze model. Each value represents the means ± S.E.M. Saline-treated (n = 6); 10 %^{V}/v (n = 6); 20% ^{V}/v (n = 6) 40 %^{V}/v (n = 5).

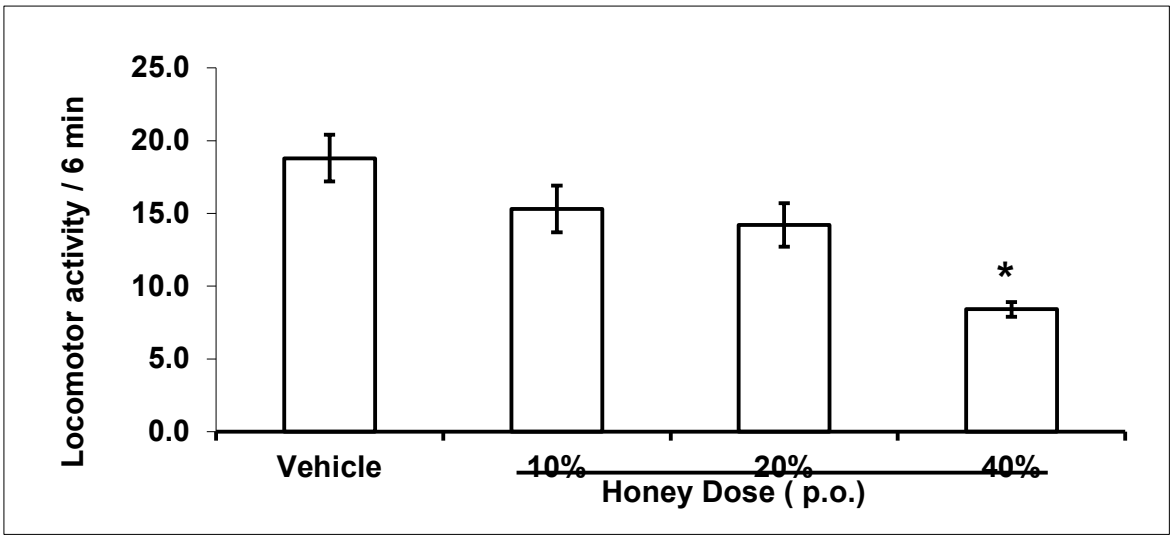

Fig. 8. The effects of honey administration on locomotor activity in sleep-deprived mice using Y-maze model. Each value represents the means ± S.E.M. [Saline-treated (n = 6); 10%^{V}/v (n = 6); 20%^{V}/v (n = 6) 40%^{V}/v (n = 5) *p < 0.05 vs. vehicle-treated (control) group].

DISCUSSION

Sleep deprivation is stressful and has been associated with adverse consequences for health and cognitive performance[26]. The Total Sleep Deprivation (TSD) model used in this study led to deprivation of both slow wave sleep and paradoxical sleep and this is known to cause significant increase in anxiety-like behaviour in rodents[27]. In this present study,

various behavioural models such as the NIB, Hole board, EPM and Y-maze models were used to determine the effects of honey in sleep-deprived mice. The results obtained after oral administration of honey at different doses of 10 %^{V}/v, 20 %^{V}/v and 40 %^{V}/v in mice using the Open field test showed that honey significantly decreased locomotor activity dose-dependently. Similar results on locomotor activity using other models such as hole-board, Y-maze and EPM showed similar central inhibitory effects. These results for locomotion are similar to the results obtained in a study that was carried out to assess the neuropharmacological effects of Nigerian honey in male mice[16]. Furthermore, rearing behaviour was significantly decreased dose-dependently in the open field test also suggesting a central inhibitory activity. In rodents, both locomotion and rearing are considered excitatory behaviours[28]. A major excitatory neurotransmitter involved in movement is dopamine and it is abundant in the nigrostriatal system[29]. Increase in locomotion has been attributed to increase in dopamine levels in the mesolimbic dopaminergic system[30]. *In vivo* imaging studies suggest that sleep deprivation increases dopamine levels in human brain and increased activity of the dopaminergic pathway causes an increase in motor activity[31]. Zant and colleagues reported that there was an increase in dopaminergic turnover (increase in the extracellular levels of the dopamine metabolites; 3, 4-dihydroxyphenylacetic acid-DOPAC and homovanillic acid-HVA) in the basal forebrain of rats due to total sleep deprivation for 6 hours[32]. Several lines of experimental evidence support the hypothesis that the neuropharmacological effects of honey are mediated via dopaminergic and non-opioidergic central mechanisms[10,17,18]. Previous study by Saito and colleagues revealed that TSD potentiated the hyperlocomotion effect of acute amphetamine in mice [3]. Honey has also been shown to have an inhibitory effect on the central nervous system[16]. In this study, honey produced an inhibitory action on the effects of sleep deprivation as shown by the significant reduction in locomotion in all the models used in this study. It can therefore be inferred from this study that the mechanism of action of honey in sleep-deprived mice may be mediated via the dopaminergic system. Total sleep deprivation has been known to increase grooming behaviour[3] and in this study, it was observed that the effect of honey on grooming behaviour in sleep deprived mice was biphasic; the low dose of honey (10 %^{V}/v) potentiated the grooming behaviour while the median dose of honey (20 %^{V}/v) reversed the effect on grooming. Generally, it was observed that honey had no significant effect on grooming behaviour of sleep-deprived mice. The hole board test is a behavioural model that can be used to determine explorative and motor activities in mice [33]. The results obtained in the hole board test showed that honey at the dose of 40 %^{V}/v significantly decreased the explorative behaviour in sleep deprived mice suggesting possible anxiogenic/sedative effects[34]. In the plus maze, the desire to explore a novel environment acts as a positive motivation to enter the open arms, whereas the aversive aspects of the arm act as a negative motivation[35]. The preference for the closed arms appears to reflect an aversion to the open arms generated by fear and anxiety induced by height and open arms[36]. The elevated plus maze is therefore widely used to study anxiety and anxiety-related disorders. In this present study, honey caused a significantly dose-dependent decrease in the number of entries and the time spent in open arms of the elevated plus-maze in sleep deprived mice. Furthermore, the results obtained showed that the doses of honey used in this study might have affected the motor activity of the mice due to its central inhibitory effect. The total arms entries were significantly decreased dose-dependently. Previous study has shown that the results obtained from the exploratory activity is an indication of either anxiogenic or anxiolytic behaviour of mice in a novel environment. Increased exploration is an intrinsic manifestation of reduced anxiety in animals. Thus, decrease in total arms entries

showed that there was a decrease in exploratory behaviour suggesting an anxiogenic effect. Sleep has been reported to facilitate learning, memory consolidation and retrieval in humans and animals while sleep deprivation impairs learning and memory in humans, mice and rats. It is also known that sleep deprivation impairs object recognition in mice[37]. In this study, the results from spontaneous alternation test using the Y-maze model showed that there was no significant effect of honey on the spatial working memory in the sleep-deprived mice suggesting that honey may not be effective in reversing the effect of sleep deprivation on learning and memory at doses used.

CONCLUSION

This study shows that honey has a significant action on the effects of sleep deprivation and may be useful in alleviating problems associated with sleep deprivation.

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CONFLICTS OF INTEREST

There are no conflicts of interest to disclose. The study was also self-funded.

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