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AS MEN OF HONOUR WE JOIN HANDS

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Ameliorating Effects of Honey on Ethanol, Caffeine, Morphine and Scopolamine- Novelty Induced Behaviors and Memory Impairment in Male Albino Mice

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ABSTRACT

Background: Honey is a natural substance produced by honey bees and was found to be useful to humankind since ancient times. It has medicinal properties and found to possess inhibitory effects on the Central Nervous System (CNS).

Methods: Thus, we evaluated its ameliorating effects of honey on scopolamine, morphine, caffeine and ethanol induced behavioral models: Novelty-Induced Behaviors (NIB), learning and memory impairment in male mice.

Results: The results indicated that honey showed a significant effect on morphine and scopolamine-induced locomotor activity {[morphine: [F (3,19) = 11.736; p = 0.0003) and scopolamine: [F (3,19) = 29.673; p = 0.0001)]}. Honey significantly reduced ethanol, morphine, scopolamine and increased the caffeine effects on rearing behavior [ethanol: [F (3,19) = 13.724; p = 0.0001); morphine: [F (3,19) = 18.167; p = 0.0001); scopolamine: [F (3,19) = 5.523; p =0.008 and caffeine: [F (3,19) = 3.506; p = 0.039)] when compared with control groups. In grooming, honey significantly reduced effect of morphine and increased scopolamine-induced behavior [morphine: F (3,19) = 12.895; p = 0.0002) and scopolamine: [F (3,19) = 9.465; p = 0.0008)]. Honey produced a significant effect on ethanol and morphine with spatial working memory in mice [ethanol: [F (3,19) = 5.236; p = 0.010) and morphine: [F (3,19) = 10.080; p = 0.0006)]. In elevated plus maze test, honey significantly increased the transfer latency of ethanol: [F (3,19) = 0.08805; p = 9656); morphine: [F (3,19) = 1.610; p = 0.2265; scopolamine: [F (3,19) = 0.1695; p = 0.9154) and (Caffeine: [F (3,19) = 0.1736; p = 0.9127]) on spatial working memory impairment in mice.

Conclusion: In conclusion, honey has significant inhibitory effects on ethanol, morphine, scopolamine and caffeine pharmacological effects on the CNS.

INTRODUCTION

Honey is a natural substance produced by honey bees of genus Apis, order Hymenoptera, family Apidae and subfamily Apinae known to be useful to humans since ancient times[1-4]. Honey has been reportedly used in the treatment of various diseases such as; salve for sore eye[4], wounds and diseases of the intestine[2,5] as a preservative agent[6] and a vehicle for transporting medicinal properties of plants[4]. Honey has been found to possess antibacterial activities[7,8], antioxidant property[9,10] anti-inflammatory activity[4], wound healing property[4], antimutagenic and antitumor activity[7]. Honey is a mixture of sugars and other compounds. It consists of carbohydrate, protein, glucose, fructose, sucrose, dextrin, formic acid volatile acid and

pollen grains, wax, thiamine, riboflavin, niacin, all the minerals, enzymes, maltose, melezitose, pentosans, gums and colouring matter[11,12]. It also consists of organic compounds (acetic, butanoic, formic, choline, citric, succinic, lactic, malic, pyroglutamic, gluconic acids) which may serve as precursors of neurotransmitters that are essential for brain functions[4,13]. The activity of the nervous system is mediated by many kinds of inter neurons releasing one or another neurotransmitter such as: noradrenaline, gamma aminobutyric acid (GABA), dopamine, glutamate (Glu), acetylcholine (ACh), serotonin[14,15]. Several chemicals (ethanol, caffeine, morphine, scopolamine etc) when exposed to the central nervous system are capable of producing a myriad of effects on the activities and functions of these neurotransmitters via activating them. Honey is used as a sweetener in beverages containing caffeine and also in the production of alcoholic drinks. It has been suggested that pre-treatment with Tualang honey has protective effects against hypoxia-induced memory deficits in male rats[16]. Hence, this study was designed to investigate the ameliorating effects of honey on scopolamine, morphine, caffeine and ethanol-induced novelty induced behavior (NIB) and memory impairment in male mice.

MATERIALS AND METHODS

Chemical Reagents

Normal Saline, Absolute ethanol (20%v/v) [1.0 g/kg], freshly prepared caffeine (E Merck, Darmstadt, Germany)-40 mg/kg, Morphine (10 mg/kg), scopolamine hydrobromide (1 mg/kg) (Sigma, St. Louis, MO, USA) (weighed caffeine dissolved in measured volume of normal saline based on a particular dose).

Animals

The animals used were young male Swiss albino mice purchased from and housed in the Animal house of the Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. The animals weighed between 21.5 ± 0.2 g. Animals were housed in groups of ten in plastic cages, maintained under standard dark-light cycle (lights on between 7.00am and 6.00 pm) and at room temperature (25°C). Food and water were available *ad libitum* and the houses cleaned regularly. All rules applying to animal safety and care were observed. All behavioral tasks were conducted between 10:00 h and 16:00 h. Animal treatment and maintenance were carried out in accordance with the Principles of Laboratory Animal Care[17] and with the Animal Care and Use Guidelines of Obafemi Awolowo University, Ile-Ife, Nigeria.

Drug Administration and Experimental Procedures

Honey sample obtained from Idanre was prepared using normal saline as the vehicle to obtain thea dose of 10 % v/v. The freshly prepared honey was always administered to different groups of mice orally at a maximum volume of 10 µl/g body weight and normal saline was administered to the control group, ethanol (20 %v/v), caffeine, morphine and scopolamine were dissolved in 0.9% saline and were administered at maximum volume of 10 µl/g body weight at the doses used. Honey (10 % v/v, p.o) was given 45 min before each test and memory impairment was induced in mice with scopolamine (1 mg/kg, i.p.) or morphine (10 mg/kg, i.p) caffeine (40 mg/kg, i.p.) and ethanol (1.0 g/kg, i.p.) 45 min after each treatment with honey. Groups of animals were tested 1 hour after administration (10 µl/g body weight) of vehicle (Saline), honey sample (10 % v/v, p.o), scopolamine (1.0 mg/kg, i.p.), ethanol (1.0 g/kg, i.p.); caffeine (40 mg/kg, i.p.) or morphine (10 mg/kg, i.p.) in the open field (Novelty-induced behaviours-20 min), Y-maze (for spontaneous alternation- 6 min) and elevated plus maze (latency to closed arm entry) in a sequential order behaviorally. These animals were retested again for latency to closed arm entry in elevated plus maze 24 h later (as stated under elevated plus maze model for nootropic effect) as described by Akanmu *et al.*[17]

EXPERIMENTAL MODELS

Novelty-induced behavior (Locomotion, Rearing and Grooming)

Fifty male albino mice were divided into ten groups (n=5per group) based on honey sample, vehicle (normal saline), ethanol (20 %v/v), caffeine, morphine and scopolamine, ethanol (20 %v/v) and honey, caffeine and honey, morphine and honey and scopolamine and honey. The spontaneous open field effect was measured in arena of a rectangular structure that is composed of a hard board floor measuring (36x36x26 cm) and made of white painted wood. The floor is divided by permanent red markings into 16 equal squares at the bottom. Generally spontaneous motor activity was monitored for 20 minutes. After treatment, each mouse was introduced into the field and the total locomotion (number of floor units entered with all paws), rearing frequency (number of times the animal stood on its hind legs or with its fore arms against the walls of the observation cage or free in the air) and frequency of

grooming (number of body cleaning with paws, picking of the body and pubis with mouth and face washing actions) within each 10 minutes interval were recorded as described by Gomes *et al*.[18]. The arena was cleaned with 5% alcohol to eliminate olfactory bias and the arena allowed to dry before introducing a fresh animal.

NOOTROPIC EFFECTS

For the evaluation of nootropic effect, the groups of male albino mice were subjected to Y-maze and elevated plus-maze models 1 hour after treatment with honey (alone) or with prior administration of drugs used to induce memory impairments.

Y-Maze Model (Spatial Working Memory)

Fifty male albino mice were divided into ten groups (n=5per group) based on honey sample, vehicle (normal saline), ethanol (20 %v/v), caffeine, morphine and scopolamine, ethanol (20 %v/v) and honey, caffeine and honey, morphine and honey and scopolamine and honey. Spontaneous alternation is a measure of spatial working memory and Y-maze has been used as a measure of spontaneous alternation, short-term memory, general locomotor activity and stereotypic behavior[19,20]. Y-maze model was used to assess the ameliorating effects of honey with ethanol, caffeine, morphine and scopolamine on spatial working memory. The Y-maze consists of three equally spaced arms (120° apart; 41 cm long × 15 cm high × 5 cm wide). In this test, each mouse was placed in one arm of the compartments 1 hour after administration of honey and 45 minutes after the drugs administration and allowed to move freely for 6 min. The following parameters defined were used to determine the percentage alternation as described by Krebs-Kraft *et al*.[21] and Nasri *et al*.[22]

(i) An arm entry was defined as the body of the mouse (except for its tail) completely entering into an arm compartment.
(ii) Alternation was defined as entering three different arms consecutively
(iii) The maximum spontaneous alternation is the total number of arms entered minus two (2)

The spontaneous alternation percentage (SA %) is defined as a ratio of the arm choices that differed from previous two choices (successful choices) to total choices during the run (total entry minus two) [18,19,22].

Elevated plus maze (EPM) test

Fifty male albino mice were divided into ten groups (n=5per group) based on honey sample, vehicle (normal saline), ethanol (20 %v/v), caffeine, morphine and scopolamine, ethanol (20 %v/v) and honey, caffeine and honey, morphine and honey and scopolamine and honey. The elevated plus maze is a behavioral model used in evaluating learning and memory[20]. The apparatus consists of two open arms and two covered arms (16 cm × 12 cm × 5 cm) extended from a central platform (5 cm × 5 cm) elevated to height of 25 cm from the floor. On the first day of the experiment after honey and drug administration, each mouse was placed at the end of an open arm, facing away from the central platform as described by Joshi and Megeri[24]. The transfer latency (TL), defined as the time taken by the mouse to move from her original position with its four legs to one of the covered arms, was recorded on the second day. When the animal did not enter into one of the covered arms within 90 s, it was gently pushed into one of the two covered arms and the TL was recorded as 90 s [25]. After each session, each mouse was allowed to explore the maze for 10 s and returned to its cage. Memory retention was examined after 24 h[20,24].

Statistics Analysis

Data obtained were expressed as mean ± standard error of mean (SEM), subjected to statistical analyses using computer software GraphPad® InStat version 5.01[27] and $p<0.05$ was taken as accepted level of significant different from control. To compare the differences between the first and the second transfer latencies in groups in the Elevated plus maze test, Wilcoxon t-test was used. All other behavioral data was analyzed by One way analysis of variance (ANOVA) and post hoc tests (Student's Newman-Keuls) were carried out to determine the source of a significant main effect or interaction.

RESULTS

Open Field Test (Novelty-induced behavior)

Locomotion

Effects of ethanol (EtOH:1.0 g/kg, i.p.), morphine (MPH:10 mg/kg, i.p.), scopolamine (SCM:1 mg/kg, i.p.) or caffeine (CAF:40 mg/kg, i.p.) administered 45 min after each treatment with honey (HNY:10 % v/v, p.o.) or saline (SAL) on locomotor activity in the open field test in mice are presented in figures 1a-d respectively. Honey had no significant effects on ethanol and caffeine-induced locomotor activity {[ethanol: [$F(3,19) = 2.214$; $p = 0.1261$) and caffeine: [$F(3,19) = 2.545$; $p = 0.0926$)]}. However, honey alone decreased the locomotor activity in mice and significantly reduced effects of morphine and scopolamine-

induced locomotor activity {[morphine: [F (3,19) = 11.736; p = 0.0003)] and scopolamine: [F (3,19) = 29.673; p = 0.0001)]} when compared with the control.

Rearing

Effects of ethanol (EtOH:1.0 g/kg, i.p.), morphine (MPH:10 mg/kg, i.p.), scopolamine (SCM:1 mg/kg, i.p.) or caffeine (CAF:40 mg/kg, i.p.) administered 45 min after each treatment with honey (HNY:0.1ml/g p.o.) or saline (SAL) on rearing activity in the open field test in mice are presented in figures 2a-d, respectively. Honey significantly reduced the effect of ethanol, slightly reduced the effect of scopolamine but increased the effect of caffeine on rearing induced behavior in mice when compared with the control. However, honey did not show any effect on morphine induced rearing behavior {ethanol: [F (3,19) = 13.724; p = 0.0001); morphine: [F (3,19) = 18.167; p = 0.0001); scopolamine: [F (3,19) = 5.523; p =0.008 and caffeine: [F (3,19) = 3.506; p = 0.039)]}.

Grooming

Effects of ethanol (EtOH:1.0 g/kg, i.p.), morphine (MPH:10 mg/kg, i.p.), scopolamine (SCM:1 mg/kg, i.p.) or caffeine (CAF:40 mg/kg, i.p.) administered 45 min after each treatment with honey [HNY:10 % v/v, p.o.] or saline (SAL) on grooming behavior in mice are presented in figures 3a-d, respectively. Honey had no significant effects on ethanol and caffeine-induced grooming behavior in mice [ethanol: [F (3,19) = 0.890; p = 0.4637) and caffeine: [F (3,19) = 2.091; p = 0.1417)]; however, it significantly increased the effect on scopolamine-induced grooming behavior but significantly reduced the effect on morphine induced grooming behavior in male mice when compared with control group [scopolamine: [F (3,19) = 9.465; p = 0.0008) and morphine: [F (3,19) = 12.895; p = 0.0002)].

LEARNING AND MEMORY

Spontaneous alternation testing (Y-maze)

Effects of ethanol (EtOH:1.0 g/kg, i.p.), morphine (MPH:10 mg/kg, i.p.) scopolamine (SCM:1 mg/kg, i.p.) or caffeine (CAF:40 mg/kg, i.p.) administered 45 min after each treatment with honey (HNY:10 % v/v, p.o.) or saline (SAL) on spontaneous working memory (Y-maze) in mice are presented in figures 4a-d, respectively. Honey has significant effects with ethanol and morphine on spatial working memory in mice [ethanol: [F (3,19) = 5.236; p = 0.010) and morphine: [F (3,19) = 10.080; p = 0.0006)] when compared with the control. However, it has no effects with scopolamine and caffeine on spatial working memory in mice [scopolamine: [F (3,19) = 2.780; p = 0.074) and caffeine: [F (3,19) = 0.5060; p = 0.6836)].

Elevated plus maze test

Effects of ethanol (EtOH:1.0 g/kg, i.p.), morphine (MPH:10 mg/kg, i.p.), scopolamine (SCM:1 0mg/kg, i.p.) or caffeine (CAF:40 mg/kg, i.p.) administered 45 min after each dose of honey (HNY:10 % v/v, p.o.) or saline (SAL) in the elevated plus maze are presented in figures 5a-d, respectively. In this test, honey produced no significant effects on TL1 {TL1[ethanol: [F (3,19) = 1.711; p = 0.2048); morphine: [F (3,19) = 0.8532; p = 0.4852; scopolamine: [F (3,19) = 1.447; p = 0.2664) and caffeine: [F (3,19) = 0.8275; p = 0.4979]} but significantly increased the TL2 of ethanol, morphine and scopolamine (TL2: ethanol: [F (3,19) = 0.08805; p = 9656); morphine: [F (3,19) = 1.610; p = 0.2265; scopolamine: [F (3,19) = 0.1695; p = 0.9154) and increased the TL2 of caffeine (Caffeine: [F (3,19) = 0.1736; p = 0.9127]) in male mice.

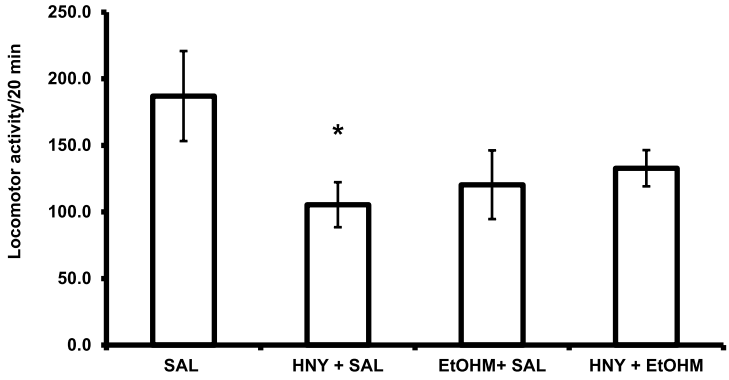

Figure 1a: Effects of ethanol [(EtOH:1.0 g/kg, i.p.] administered 45 min after treatment with honey [HNY:10 % v/v, p.o] or saline (SAL) on locomotor activity in the open field test in mice. n=5; values are presented as mean ± SEM; *p<0.05 when compared with SAL (control) group

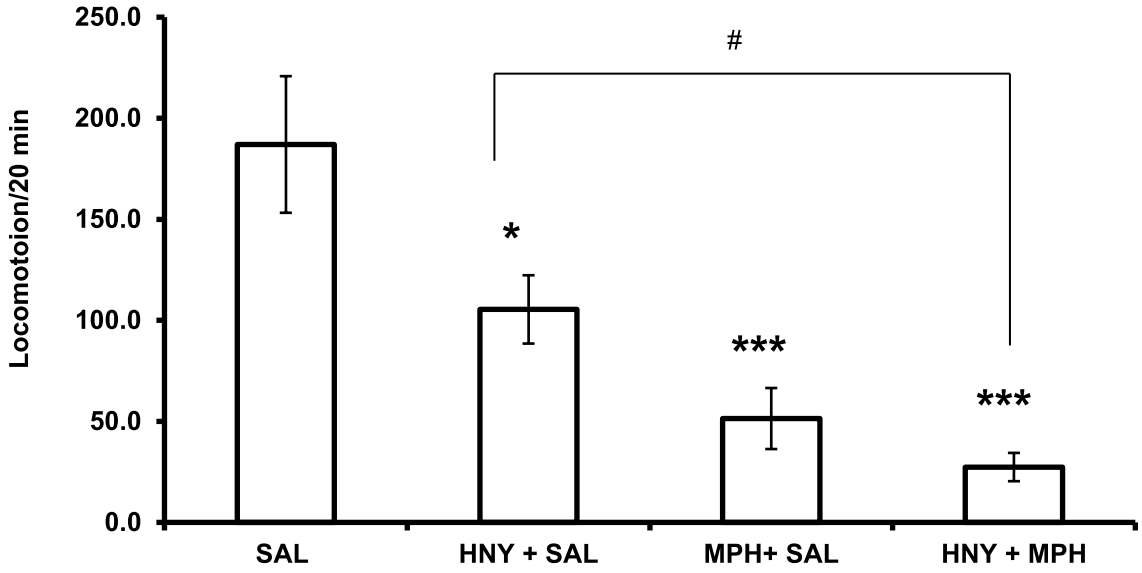

Figure 1b: Effects of morphine [MPH:10 mg/kg, i.p.] administered 45 min after treatment with honey [HNY:10 % v/v, p.o] or saline (SAL) on locomotor activity in the open field test in mice. n=5; values are presented as mean ± SEM; *p<0.05, ***p<0.001 when compared with SAL (control) group; #p<0.05 when compared with HNY + SAL treated group.

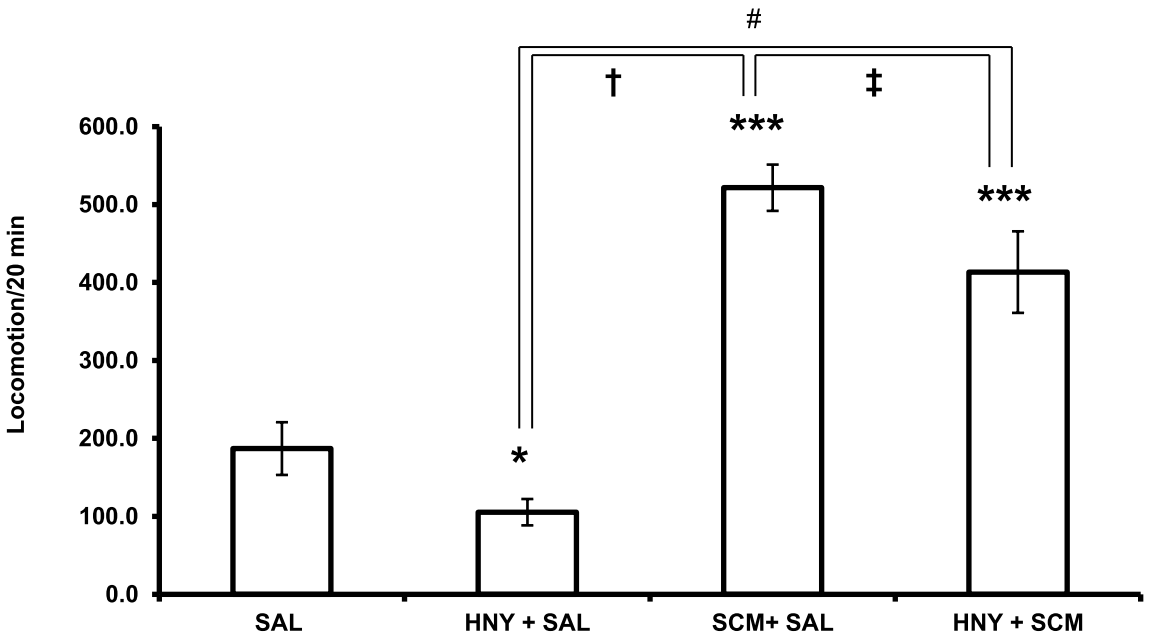

Figure1c: Effects of scopolamine [SCM:1 mg/kg, i.p] administered 45 min after treatment with honey [HNY:10 % v/v, p.o] or saline (SAL) on locomotor activity in the open field test in mice. n=5; values are mean ± SEM; *p<0.05, **p<0.01, ***p<0.001 when compared with SAL (control) group; #p<0.05 when compared with HNY + SAL treated group; †p<0.05 when compared with SCM + SAL treated group; ‡p<0.05 when compared with SCM +HNY treated group.

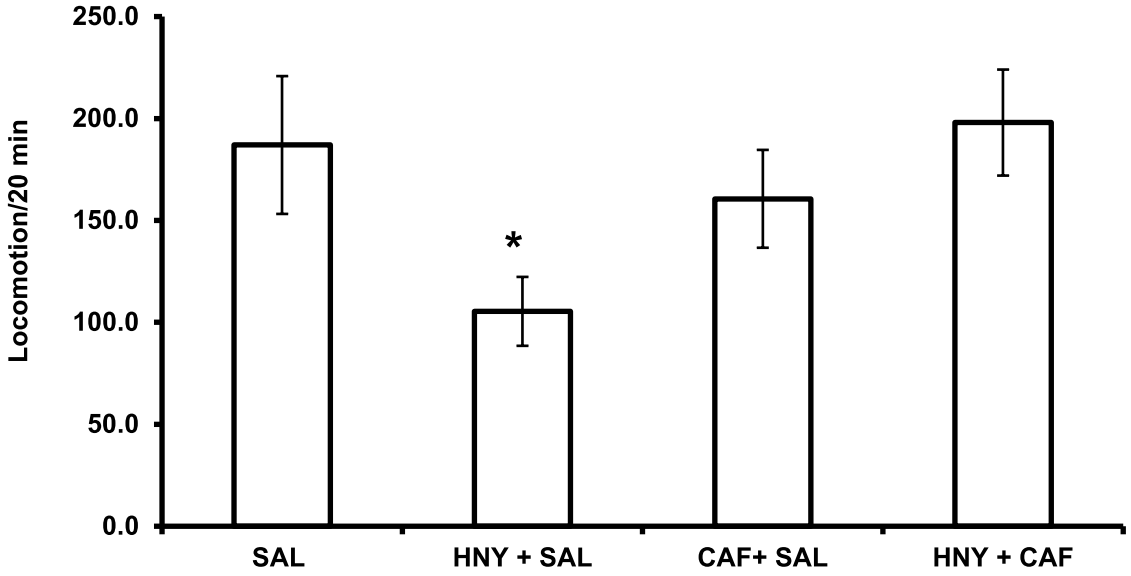

Figure1d: Effects of caffeine [CAF:40 mg/kg, i.p] administered 45 min after treatment with honey [HNY:10 % v/v, p.o] or saline (SAL) on locomotor activity in the open field test in mice, n=5; values are mean ± SEM; *p<0.05 when compared with SAL (control) group.

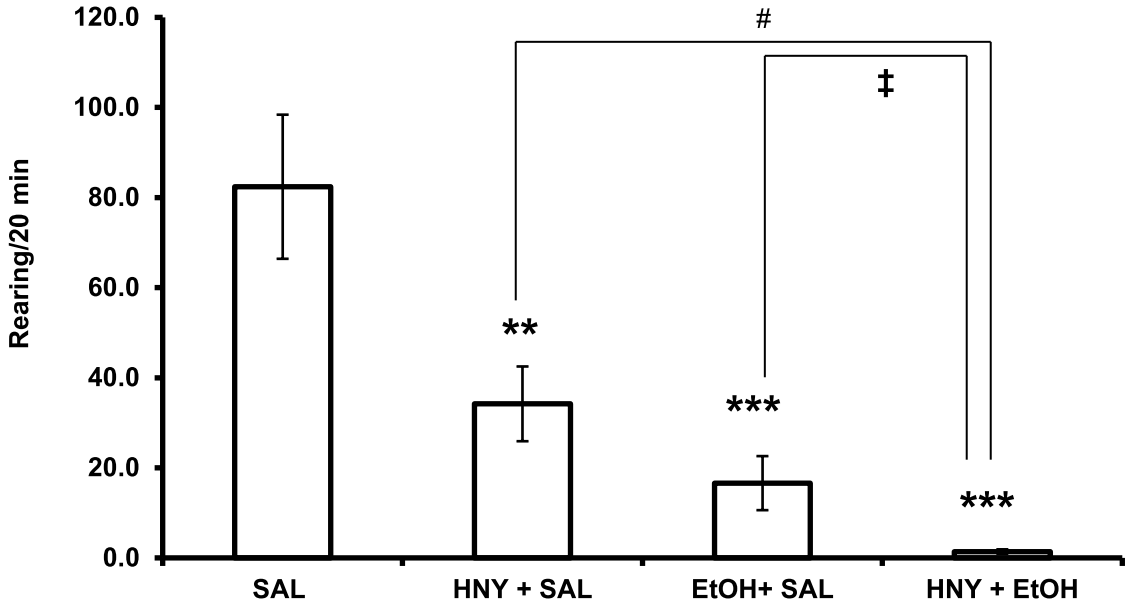

Figure2a: Effects of ethanol [(EtOH:1.0 g/kg, i.p.] administered 45 min after treatment with honey [HNY:10 % v/v, p.o] or saline (SAL) on rearing activity in the open field test in mice.n=5; values are mean ± SEM; **p<0.01, ***p<0.001 when compared with SAL (control) group; #p<0.05 when compared with HNY + SAL treated group; ‡p<0.05 when compared with EtOH +HNY treated group.

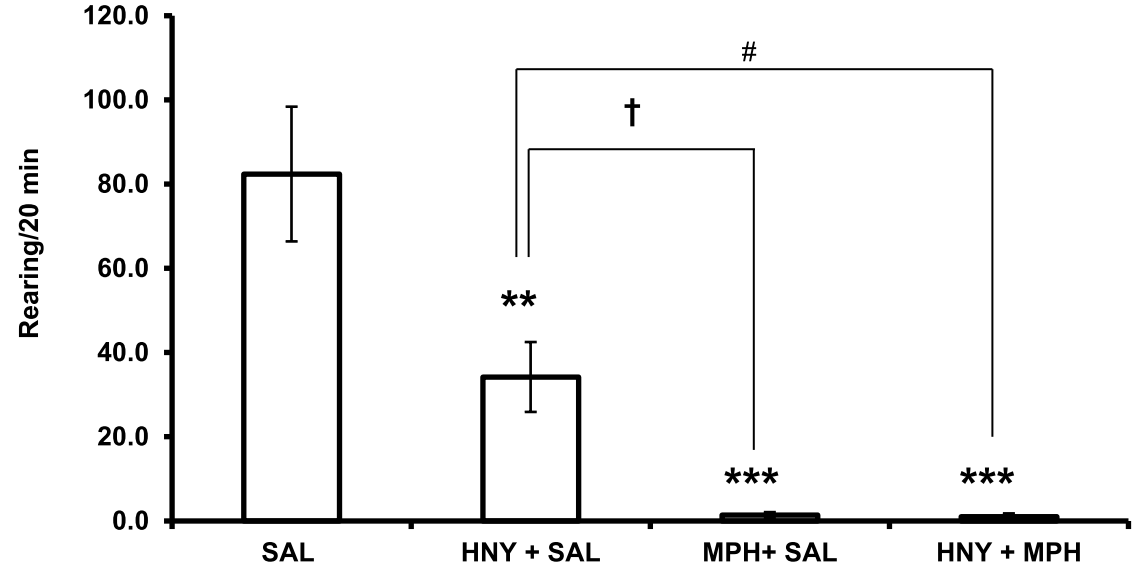

Figure 2b: Effects of morphine [MPH:10 mg/kg, i.p.] administered 45 min after treatment with honey [HNY:10 % v/v, p.o] or saline (SAL) on rearing activity in the open field test in mice.n=5; values are mean ± SEM; **p<0.01, ***p<0.001 when compared with SAL (control) group; #p<0.05 when compared with HNY + SAL treated group; †p<0.05 when compared with MPH + SAL treated group.

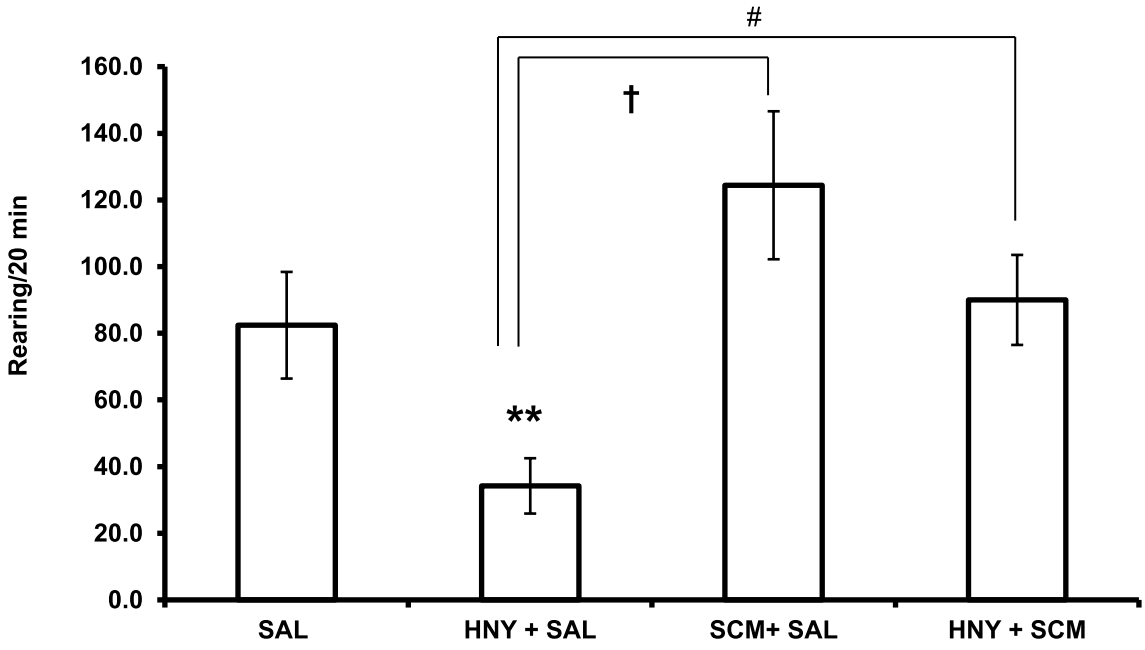

Figure 2c: Effects of scopolamine [1 mg/kg, i.p.] administered 45 min after treatment with honey [10 % v/v, p.o] or saline on rearing activity in the open field test in mice n=5; values are mean ± SEM; **p<0.01 when compared with (control) group; #p<0.05 when compared with HNY + SAL treated group; †p<0.05 when compared with SCM + SAL treated group.

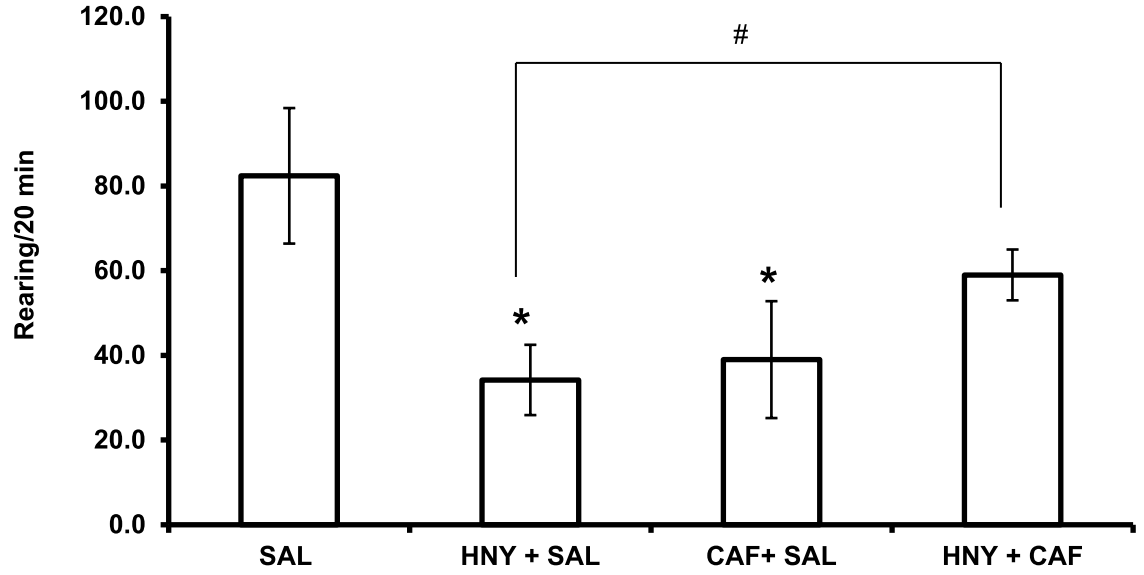

Figure 2d: Effects of caffeine :40 mg/kg, i.p] administered 45 min after each treatment of honey [10 % v/v, p. o] or saline on rearing activity in the open field test in mice, n=5; values are mean ± SEM; *p<0.05 when compared with SAL (control) group; #p<0.05 when compared with HNY + SAL treated group.

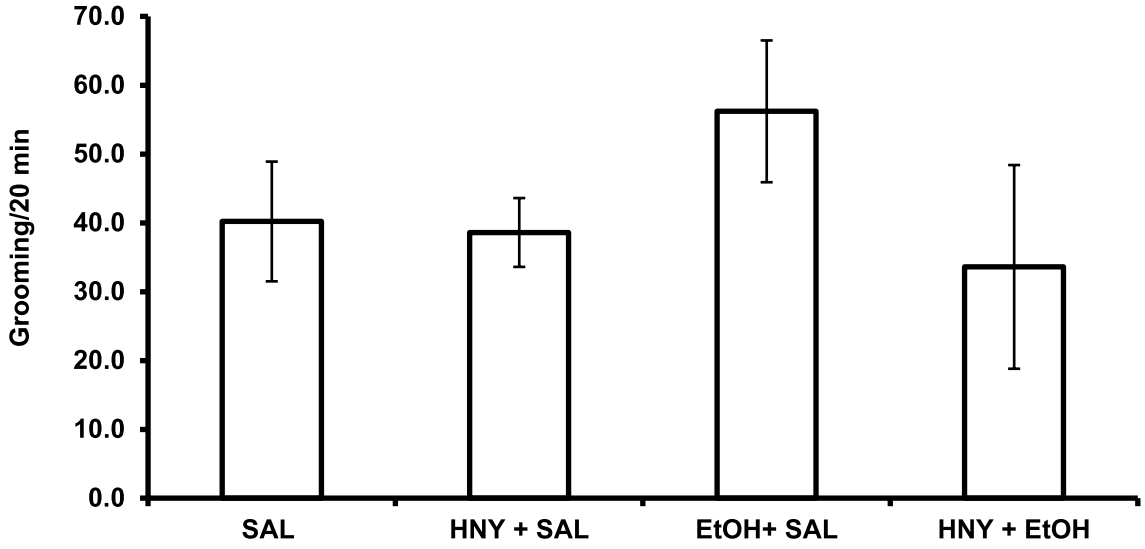

Figure 3a: Effects of ethanol [1.0 g/kg, i.p.] administered 45 min after treatment with honey [10 % v/v, p.o] or saline on grooming activity in the open field test in mice. n=5; values are mean ± SEM.

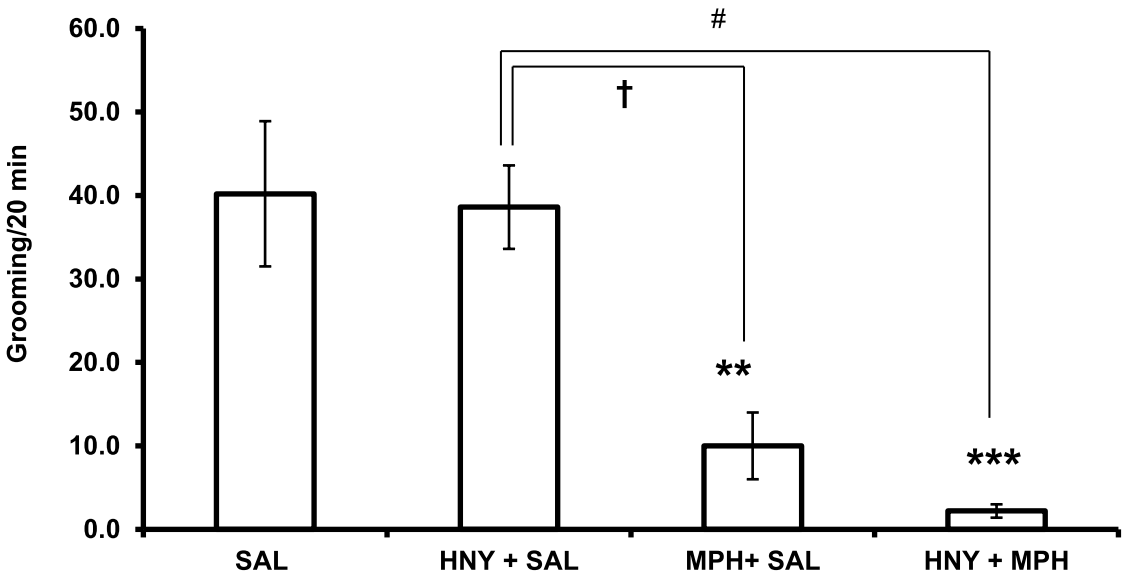

Figure 3b: Effects of morphine [10 mg/kg, i.p.] administered 45 min after each treatment of [10 % v/v, p.o] or saline (SAL) on grooming activity in the open field test in mice. n=5; mean ± SEM; **p<0.01, ***p<0.001 when compared with SAL (control) group; #p<0.05 when compared with HNY + SAL treated group; †p<0.05 when compared with MPH + SAL treated group.

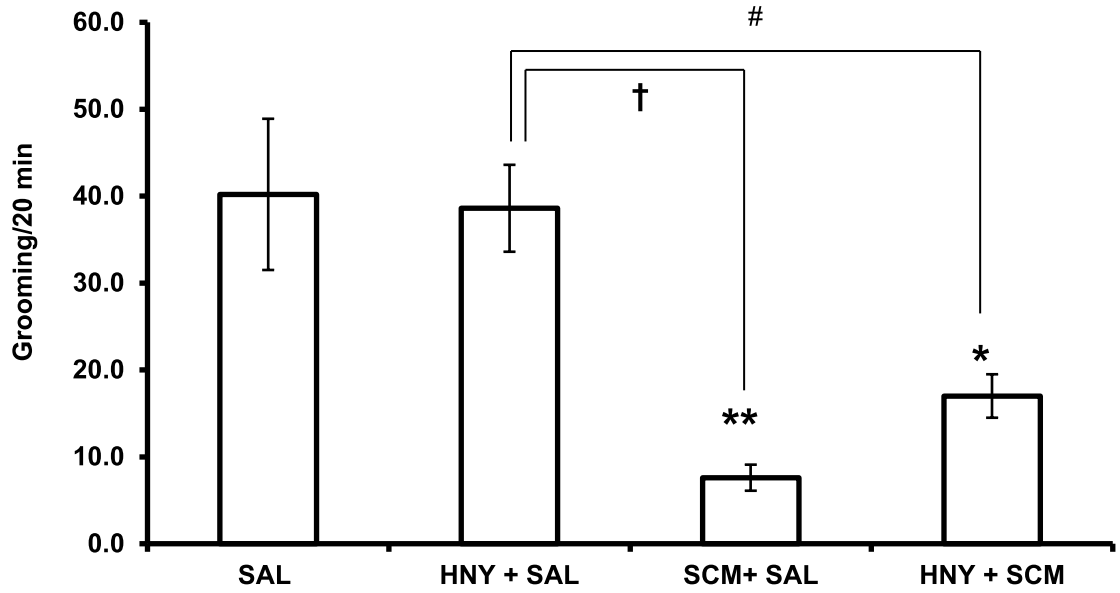

Figure 3c: Effects of scopolamine [1 mg/kg, i.p.] administered 45 min after treatment with honey [10 % v/v, p. o] or saline (SAL) on grooming activity in the open field test in mice. n=5; values are mean ± SEM; *p<0.05, **p<0.01 when compared with SAL (control) group; #p<0.05 when compared with HNY + SAL treated group; †p<0.05 when compared with SCM + SAL treated group.

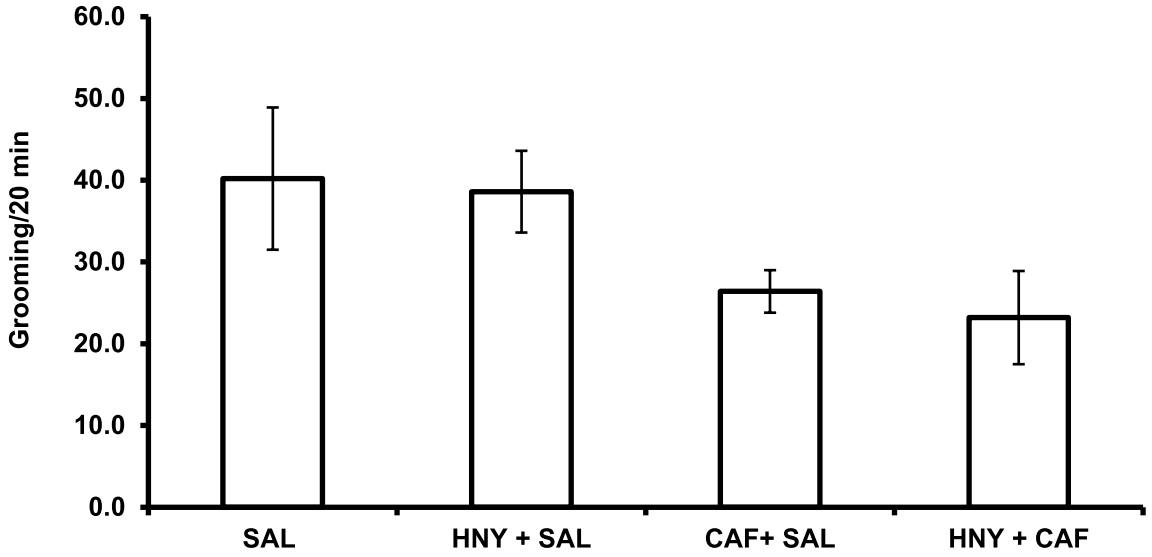

Figure3d: Effects of caffeine [40 mg/kg, i.p.] administered 45 min after treatment with honey [10 % v/v, p.o] or saline (SAL) on grooming activity in the open field test in mice. n=5; values are presented as mean ± SEM.

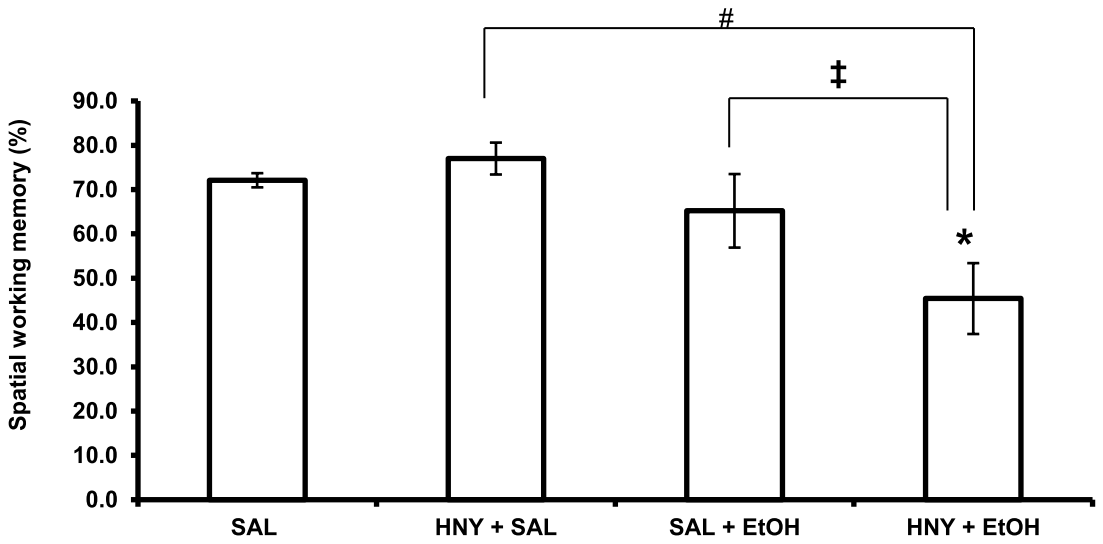

Fig.4a. Effects of ethanol [1.0 g/kg, i.p.] administered 45 min after treatment with honey [10 % v/v, p.o] or saline on spontaneous spatial working memory in Y-maze test in mice. n=5; values are mean ± SEM; *p<0.05 when compared with SAL (control) group; #p<0.05 when compared with HNY + SAL treated group; ‡p<0.05 when compared with EtOH +HNY treated group.

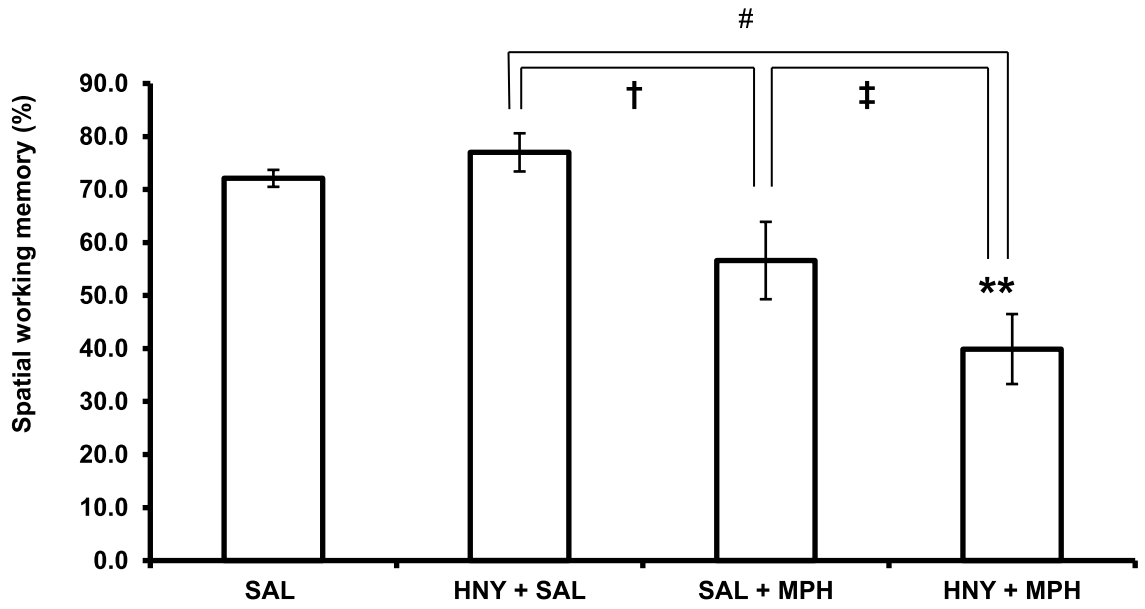

Figure 4b: Effects of morphine [10 mg/kg, i.p.] administered 45 min after treatment with honey [10 % v/v, p.o] or saline on spontaneous spatial working memory in Y-maze test in mice. n=5; mean ± SEM; **p<0.01 when compared with SAL (control) group; #p<0.05 when compared with HNY + SAL treated group; †p<0.05 when compared with MPH + SAL treated group; ‡p<0.05 when compared with MPH +HNY treated group.

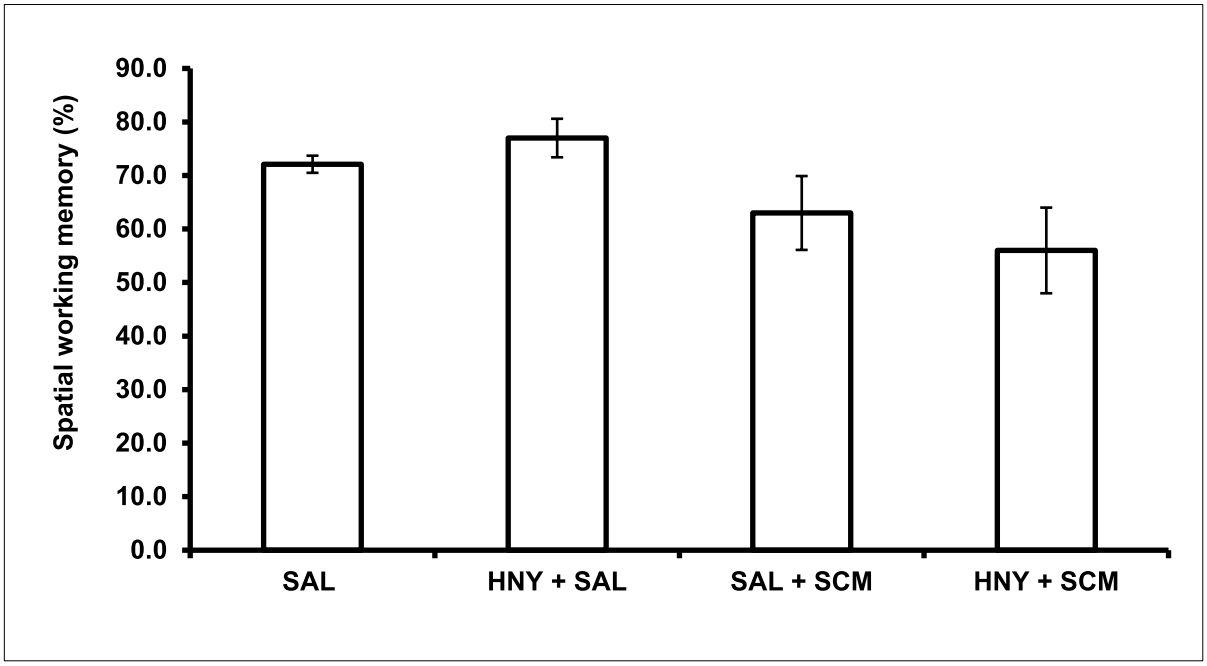

Figure 4c: Effects of scopolamine [1 mg/kg, i.p.] administered 45 min after treatment with honey [10 % v/v, p.o] or saline on spontaneous spatial working memory in Y-maze test in mice. n=5; values are mean ± SEM.

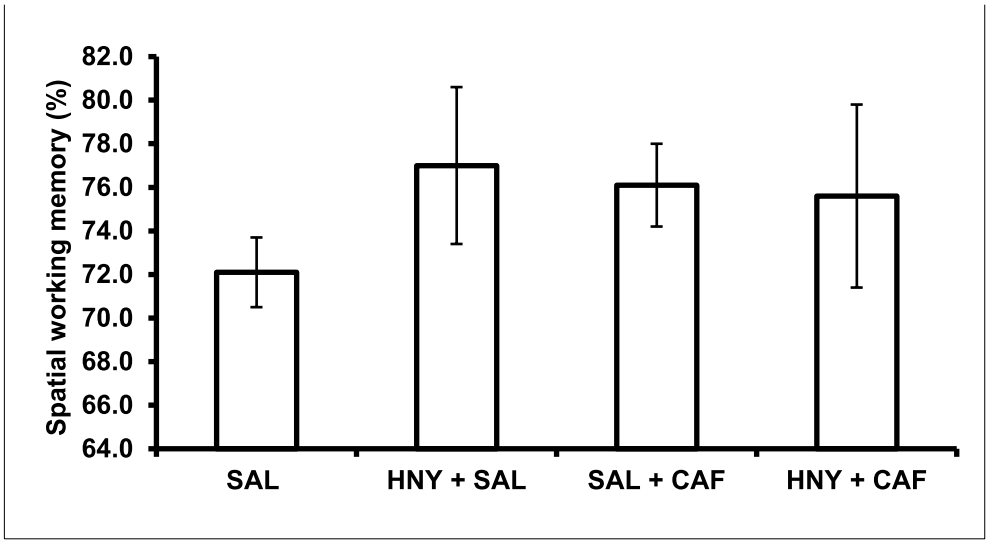

Figure 4d: Effects of caffeine [40 mg/kg, i.p] administered 45 min after treatment with honey [10 % v/v, p.o] or saline on spontaneous spatial working memory in Y-maze test in mice. n=5; values are mean ± SEM.

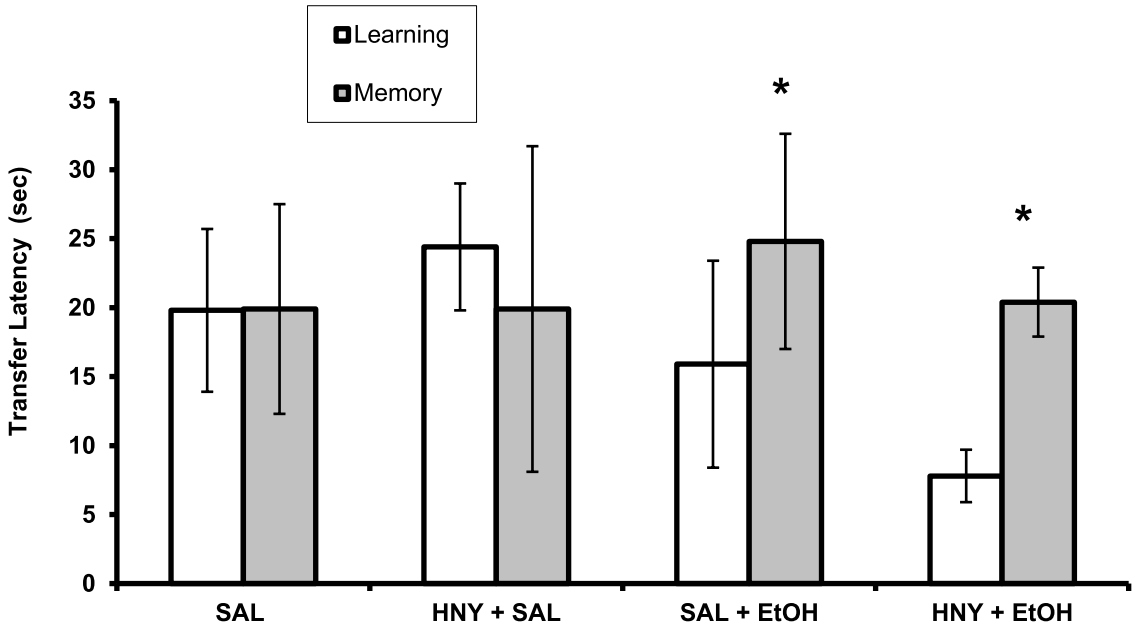

Figure 5a: Effects of ethanol [(EtOH:1.0 g/kg, i.p.] administered 45 min after treatment with [HNY:10 % v/v, p.o] or saline (SAL) on the transfer latency to the enclosed arm in the acquisition trial [Learning: TL1] and retention trial [memory:TL2] using the Elevated Plus maze test in mice. Each mouse was tested after the administration of ethanol on the first day (TL1), and 24 h later on the second day (TL2), n=5; values are mean ± SEM; *p<0.05 when compared with corresponding TL1 group.

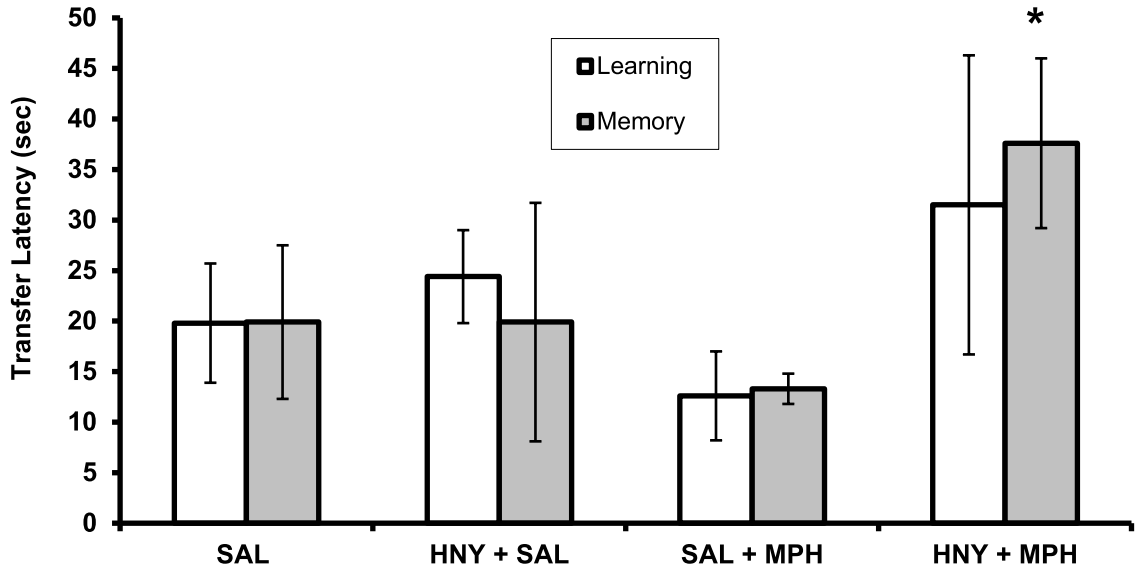

Figure 5b: Effects of morphine [MPH:10 mg/kg, i.p.] administered 45 min after treatment with honey [HNY:10 % v/v, p.o] or saline (SAL) on the transfer latency to the enclosed arm in the acquisition trial [Learning: TL1] and retention trial [memory:TL2] using the Elevated Plus maze test in mice. Each mouse was tested after the administration of ethanol on the first day (TL1), and 24 h on the second day (TL2), n=5; values are mean ± SEM; *p<0.05 when compared with corresponding TL1 group.

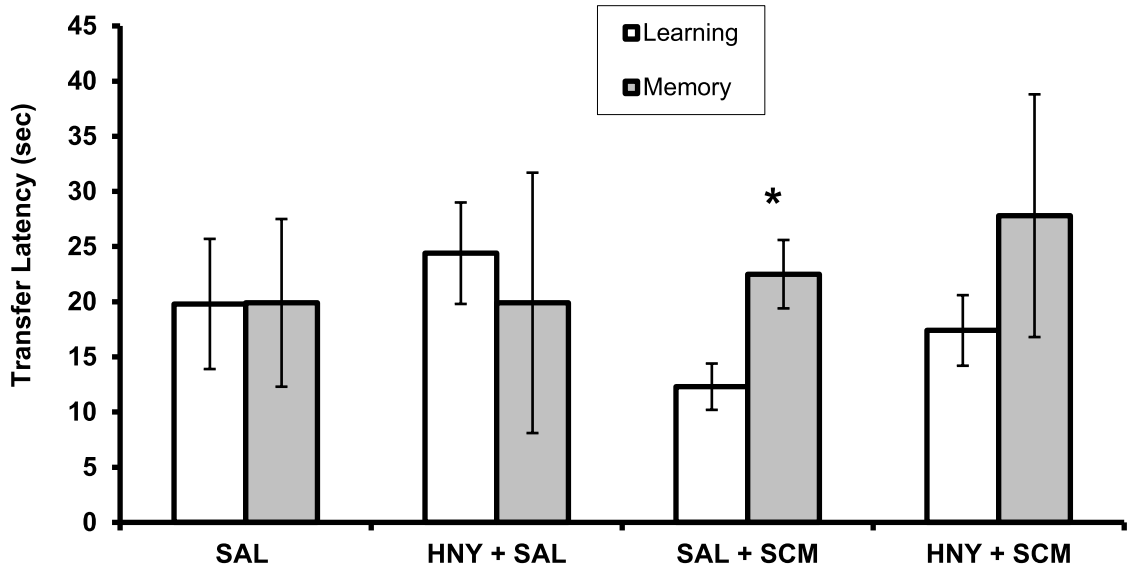

Figure 5c: Effects of scopolamine [SCM:1 mg/kg, i.p.] administered 45 min after treatment with honey [HNY:10 % v/v, p.o] or saline (SAL) on the transfer latency to the enclosed arm in the acquisition trial [Learning: TL1] and retention trial [memory:TL2] using the Elevated Plus maze test in mice. Each mouse was tested after the administration of ethanol on the first day (TL1), and 24 h later on the second day (TL2), n=5; values are mean ± SEM; *p<0.05 when compared with corresponding TL1 group.

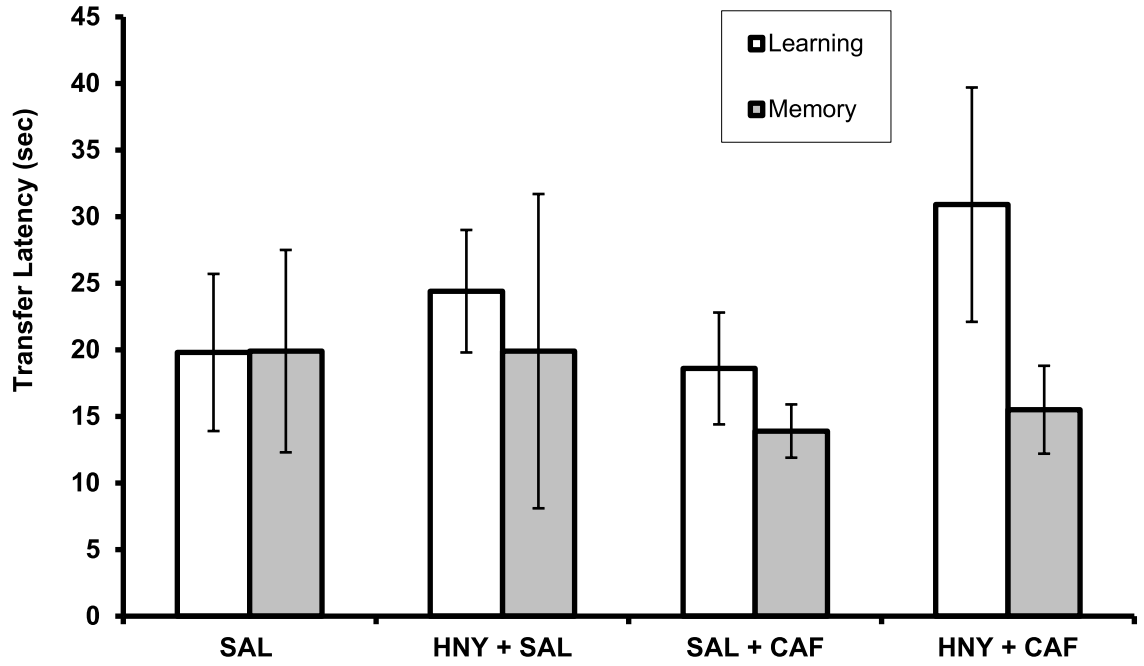

Figure 5d: Effects of caffeine [CAF:40 mg/kg, i.p.] administered 45 min after treatment with honey [HNY:10 % v/v, p.o] or saline (SAL) on the transfer latency to the enclosed arm in the acquisition trial [Learning: TL1] and retention trial [memory:TL2] using the Elevated Plus maze test in mice. Each mouse was tested after the administration of ethanol on the first day (TL1), and 24 h on the second day (TL2), n=5; values are mean ± SEM.

DISCUSSION

The present work assessed the ameliorating effects of honey on scopolamine, morphine, caffeine and ethanol-induced novelty- induced behavior (NIB) and memory impairment in male mice using open field test (NIB), spontaneous alternation testing (Y-maze) and elevated plus maze test (learning and memory test).

In this study, locomotor and rearing activities were significantly affected by administration of honey in male mice while the grooming was not different from the normal saline (control). This is consistent with the previous study conducted by Akanmu *et al*.[19]. Locomotor and rearing activities are indicative of explorative activity in rodent which is considered to be centrally excitatory[27].

Ethanol administered with honey increased the locomotor activity when compared with honey alone and decreased the rearing and grooming activities when compared with either ethanol or honey alone. The results showed a synergistic effect between honey and ethanol on NIB at the dose of ethanol tested. Frye and Breese[28] and Acevedo *et al*.[29] reported that low dose of ethanol increases locomotor activity while high dose reduces locomotor activity. Grooming is an expression of anxious behaviour,[30] decrease in rearing and grooming in this study indicates CNS depression[20]. Ethanol has been shown to increase the frequency of $GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs) that are action potential dependent or independent in hippocampal CA1 pyramidal neurons, the amygdale and spinal motor neuron[31-34]. In the central amydgala, inhibitory GABAergic transmission has been suggested to play a role in the expression of emotionality, including behavioral states of fear and anxiety as well as in mediating the behavioral effects of acute and chronic consumption of ethanol[35]. Locomotor activity is believed to be a central excitatory activity. The major excitatory neurotransmitter is glutamate whose activities can be mediated through any of four sub-types of excitatory amino acid receptors namely; N-methyl-D-aspartate (NMDA) receptors, α-amino-β-hydroxy-thydroxy-5-methyl-4-180-azopropionate (AMPA) receptors, kainite receptors and metabotropic receptors[36]. Other neurotransmitters believed to have central excitatory activity via activation of the excitatory receptors include the histaminergic system which has direct stimulatory

effect on the NMDA receptors. Also, the central adrenoceptors when stimulated will increase the excitatory activity[37]. The reduction of locomotor activity of ethanol is mediated by inhibitory action on the neurotransmitter mentioned above[32,33]. However, administration of honey along with ethanol increased the locomotor activity which suggests that honey has significant effect on ethanol's effect. Rearing activity is also a central excitatory activity mediated via excitatory neurotransmitter in the brain. The same receptors mediating locomotor activity could therefore be involved in rearing activity. These include: the glutamatergic, serotonergic, histaminergic, adrenergic and Dopamine$(D)_2$ receptors in the brain[36]. Co-administration of honey and ethanol decreased rearing activity, suggesting possible potentiation effect with honey. It has been reported that D_1 and D_3 receptors are involved in induction of grooming activity or it could be the stimulation of the D_2 receptor which is involved in reduction of grooming activity[38,39]. Ethanol increased the grooming activity, thus suggesting that ethanol has activity on dopaminergic system in the brain. Co-administration of honey and ethanol decreased grooming which indicates possible interactive effect of honey on ethanol pharmacological effect on the dopaminergic system.

Ethanol administered with honey decreased spontaneous spatial working memory in Y-maze when compared with normal saline, honey alone and ethanol alone. Ethanol administered with honey decreased transfer latency to the enclosed arm in the acquisition trial [learning: TL1] using the elevated plus maze test in mice when compared with normal saline, honey alone and ethanol but increased transfer latency to the enclosed arm in the retention trial [memory: TL2] when compared with learning TL1 in elevated plus maze. Ethanol has been reported to impair learning across a number of tasks in both rats and mice[40,41]. This showed that honey could not reverse ethanol-induced learning deficits after 24 hours of administration but reversed the short-term effect on co-administration of honey and ethanol. Therefore, more research needs to be done on learning and memory with daily co-administration of honey and ethanol.

Morphine is a μ-opioid receptor agonist which is widely distributed within the mammalian brain[42]. Morphine administered with honey significantly decreased the locomotor, rearing and grooming activities in mice when compared with honey alone, normal saline and morphine alone. This is contrary to the previous study done by Bardo *et al*.[43] that novelty-induced behavior in rats was unaffected by acute administration of morphine. In the current study, co-administration of honey and morphine reduced novelty-induced behavior in mice suggesting synergism between honey with morphine. Morphine administered with honey decreased spontaneous spatial working memory in Y-maze test in mice and increased transfer latency to the enclosed arm in the acquisition trial [learning: TL1] using the elevated plus maze test in mice. It has been reported that morphine induces deficits in learning and memory[44] through increase in dopamine release in the ventral tegmental area[45] and dopamine receptors are widely dispersed within the mammalian brain with dose dependent effects on learning and memory. Since honey co-administration with morphine increased transfer latency in Elevated plus maize, therefore honey can alleviate the memory impairment of morphine suggesting it effects on dopaminergic system.

From this study, scopolamine increased locomotor and rearing activities butreduced grooming activity. This is consistent with previous study conducted by Poorheidari *et al*.[46] and Rojas-Carvajal *et al*.[47]. Cholinergic signaling in the hippocampus, striatum and/or frontal cortex has been reported to correlate positively with scopolamine-induced hyperactivity[46] and central infusion of scopolamine in the amygdale was found to increase locomotor activity[48]. Generally, cholinergic muscarinic antagonist such as scopolamine is found to increase locomotor activity[49]. However, honey reduced the locomotor and rearing activities induced by scopolamine but increased grooming activity when compared with scopolamine alone. The results showed that honey significantly inhibited scopolamine induced excitatory effect in mice suggesting its effects on the cholinergic system. Scopolamine administered with honey decreased spontaneous spatial working memory in mice when compared with normal saline, either honey or scopolamine alone. Scopolamine administered with honey increased transfer latency to the enclosed arm in the acquisition trial [learning: TL1] using the elevated plus maze test in mice when compared with scopolamine alone but a decrease was observed when compared with normal saline and honey alone. Scopolamine administered with honey increased transfer latency to the enclosed arm in the acquisition trial [memory: TL2] using the elevated plus maze test in mice when compared with normal saline, honey alone and scopolamine alone. It has been reported that scopolamine consistently increased general non-cognitive performance measures, such as response latency and the number of missed trials and is effective in impairing acquisition/learning and short-term and working memory[50].

The results showed that a single dose of honey could not revert the effect of scopolamine on memory impairment despite its involvement in the cholinergic system in elevated plus maze.

From the study, caffeine administered with honey increased the locomotor and rearing activities while caffeine administered with honey decreased the grooming activity when compared with normal saline, honey alone and caffeine alone. The altered locomotor activity of rats treated with caffeine has already been shown to be environmentally determined: the drug elicits stimulatory effects in a familiar environment and suppressive effects in a novel environment[51]. This study collaborated the work done by Marin *et al.*[52] that caffeine induced locomotor stimulation at doses between 30 and 60 mg/kg in adolescent rat. Caffeine's effects on locomotor activity were found to be via blockade of adenosine A_1 and A_{2A} receptors[53]. This means that honey can ameliorate the effect of caffeine on adenosine receptors suggesting its involvement in antagonizing caffeine at adenosine A_1 and A_{2A} receptors. Caffeine administered with honey decreased spontaneous spatial working memory in Y-maze test in mice when compared with honey alone and caffeine alone. However, an increase was seen when compared with normal saline. Caffeine administered with honey increased transfer latency to the enclosed arm in the acquisition trial [learning: TL1] using the elevated plus maze test in mice when compared with normal saline, honey alone and caffeine alone. Caffeine administered with honey decreased transfer latency to the enclosed arm in the acquisition trial [memory: TL2] using the elevated plus maze test in mice when compared with normal saline, honey alone and the same effect was observed when compared with caffeine alone. The results indicate that a single dose of honey enhanced the activity of caffeine on memory in elevated plus maze.

Conclusion

In conclusion, the result indicates that honey has central activity and can significantly interfere with the pharmacological effects of ethanol, morphine, scopolamine and caffeine.

Authors Contribution

Akanmu MOA was responsible for the design of the study, Akanmu OA, Oluwole O, Adeyemi IO and Akanmu MOA performed the experiment and the statistical analysis and wrote the manuscript. Adeyemi IO contributed to the conception and design of study. Balogun ST, Sodipo OA and Paul LM contributed to the results discussion. All authors participated in the preparation of the manuscript.

Conflict of Interest

The author declared no conflict of interest

REFERENCES

1. Greenish HG (1999). Materia Medica. 3thEdn. Scientific Publishers, Jodhpur (India) pp:522-523.
2. Bagde AB, Sawant RS, Bingare SD, Sawai RV and Nikumbh MB (2013) Therapeutic and nutritional values of honey [Madhu]. *International Research Journal of Pharmacy* 4(3):19-22. https://doi.org/10.7897/2230-8407.04305
3. Amudha K and Sunil G (2013) Potential benefits of honey in type 2 diabetes mellitus: A review. *International Journal of Collaborative Research on Internal Medicine & Public Health* **5**(4): 199-216. https://www.iomcworld.org/articles/potential-benefits-of-honey-in-type-2-diabetes-mellitus-areview.pdf
4. Ahmed R, Khan NA and Waseem M (2017) Phytochemistry and Medicinal Importance of Honey - A Review. *Journal of Integrated Community Health* **6**(3&4): 31-34. https://journals.indexcopernicus.com/api/file/viewByFileId/213935.pdf
5. Khan IU, Dubey W and Gupta V (2014) Medicinal Properties of Honey: A Review. *International Journal of Pure and Applied Bioscience* **2**(5):149-156.
6. Laichena SF (1998) Antimicrobial Food Additives. 2ndEdn. Springer-Verlag Berlin Heidel berg New York, pp: 7.
7. Miguel MG, Antunes MD, & Faleiro ML (2017) Honey as a Complementary Medicine. *Integrative Medicine Insights 12*, 1178633717702869. https://doi.org/10.1177/1178633717702869
8. Vallianou NG, Gounari P, Skourtis A, Panagos J and Kazazis C (2014) Honey and its Anti-Inflammatory, Anti-Bacterial and Anti-Oxidant Properties. *General Medicine (Los Angel), an open access Journal* 2(2): 1-5.DOI: 10.4172/2327-5146.1000132
9. Wilson JI, George BO and Umukoro GE (2011) Effects of honey on the histology of liver in adult wistar rats. *Biology and Medicine* 3 (1): 1-5. Doi: 10.15406/mojbm.2017.02.00046.

10. Purbafranil A, Hashemi SAG, Bayyenat S, Moghaddam HT and Saeidi M (2014) The Benefits of Honey in Holy Quran. *International Journal of Paediatrics* **2**(9):67-72. DOI:10.22038/ijp.2014.3417
11. Manyi-Loh CE, Anna M, Clarke AM and Ndip RN (2011) An overview of honey: Therapeutic properties and contribution in nutrition and human health. *African Journal of Microbiology Research* 5(8):844-852. http://www.academicjournals.org/ajmr DOI: 10.5897/AJMR10.008
12. Ali M (2015) Text Book of Pharmacognosy, 2nd Edn. Satish Kumar Jain for CBS,Publishers and distribution Pvt, Limited. Pp. 65-67.
13. Azman KF, Zakaria R, Othman Z, & Abdul Aziz CB (2018) Neuroprotective effects of Tualang honey against oxidative stress and memory decline in young and aged rats exposed to noise stress. *Journal of Taibah University for Science 12*(3), 273–284. https://doi.org/10.1080/16583655.2018.1465275
14. Kljakic O, Janickova H, Prado VF, Prado MAM (2017) Cholinergic/glutamatergic co-transmission in striatal cholinergic interneurons: new mechanisms regulating striatal computation. *Journal of Neurochemistry 142 Suppl 2*, 90–102. https://doi.org/10.1111/jnc.14003
15. de Leon AS and Tadi P (2022) Biochemistry, Gamma Aminobutyric Acid. [Updated 2022 May 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.
16. Qaid EYA, Zakaria R, Mohd Yusof NA, Sulaiman SF, Shafin N, Othman Z, Ahmad AH, Abd Aziz CB, Muthuraju S (2020) Tualang Honey Ameliorates Hypoxia-induced Memory Deficits by Reducing Neuronal Damage in the Hippocampus of Adult Male Sprague Dawley Rats. *Turkish journal of Pharmaceutical Sciences 17*(5):555–564. https://doi.org/10.4274/tjps.galenos.2019.32704
17. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th edition. Washington (DC): National Academies Press (US); 2011. Available from: https://www.ncbi.nlm.nih.gov/books/NBK54050 / doi: 10.17226/12910
18. Gomes PB, Noronha EC, de Melo CT, Bezerra JN, Neto MA, Lino CS, Vasconcelos, SM, Viana GS, de Sousa FC (2008) Central effects of isolated fractions from the root of *Petiveria alliacea* L. (tipi) in mice. *Journal of Ethnopharmacology 120*(2):209–214. https://doi.org/10.1016/j.jep.2008.08.012
19. Akanmu MA, Olowookere TA, Atunwa SA, Ibrahim BO, Lamidi OF, Adams PA, Ajimuda BO and Adeyemo LE (2011) Neuropharmacological effects of Nigerian honey in mice. *African Journal of Traditional Complementary Alternative Medicines*, **8**(3):230-249. https://doi.org/10.4314/ajtcam.v8i3.65285
20. Akanmu AO, Sodipo OA, Sandabe UK, Shamaki BU, Balogun ST and Jubrin J (2021) Novelty-induced behavior and memory enhancing activities of aqueous and ethanol extracts of *Solanum incanum* Linn. fruits in mice. *African Journal of Pharmacy and Pharmacology* **15**(2):33-42. https://doi.org/10.5897/AJPP2020.5210
21. Krebs-Kraft DL, Rauw G, Baker GB, Parent MB (2009) Zero net flux estimates of septal extracellular glucose levels and the effects of glucose on septal extracellular GABA levels. *European Journal of Pharmacology 611*(1-3):44–52. https://doi.org/10.1016/j.ejphar.2009.03.055
22. Nasri S, Roghani M, Baluchnejadmojarad T, Balvardi M, Rabani T (2012) Chronic cyanidin-3-glucoside administration improves short-term spatial recognition memory but not passive avoidance learning and memory in streptozotocin-diabetic rats. *Phytotherapy Research 26*(8):1205–1210. https://doi.org/10.1002/ptr.3702
23. Mamiya T, Asanuma T, Kise M, Ito Y, Mizukuchi A, Aoto H, Ukai M (2004) Effects of pre-germinated brown rice on beta-amyloid protein-induced learning and memory deficits in mice. *Biological and Pharmaceutical Bulletin* 27(7):1041–1045. https://doi.org/10.1248/bpb.27.1041
24. Joshi H and Megeri K (2008) Antiamnesic evaluation of *Clerodendrum phlomidis* Linn. bark extract in mice. *Revista Brasileira de Ciências Farmacêuticas* **44**(4):717-725. https://doi.org/10.1590/S1516-

93322008000400019

25. Parle M, Dhingra D, Kulkarni SK (2004) Memory-strengthening activity of Glycyrrhiza glabra in exteroceptive and interoceptive behavioral models. *Journal of Medicinal Food* 7(4):462–466. https://doi.org/10.1089/jmf.2004.7.462
26. GraphPad Prism (2007). GraphPad Software Inc., San Diego CA, www.graphpad.com.
27. Ajayi AA and Ukponmwan OE (1994) Possible evidence of angiotensin II and endogenous opioid modulation of novelty-induced rearing in the rat. *African Journal of Medicine and Medical Sciences 23*(3):287–290. https://pubmed.ncbi.nlm.nih.gov/7604756/
28. Frye GD, Breese GR (1981) An evaluation of the locomotor stimulating action of ethanol in rats and mice. *Psychopharmacology75*(4):372–379. https://doi.org/10.1007/BF00435856
29. Acevedo MB, Nizhnikov ME, Spear NE, Molina JC, Pautassi RM (2013) Ethanol-induced locomotor activity in adolescent rats and the relationship with ethanol-induced conditioned place preference and conditioned taste aversion. *Developmental Psychobiology* 55(4):429–442. https://doi.org/10.1002/dev.21048.
30. Pelloux Y, Costentin J, Duterte-Boucher D (2015) Differential involvement of anxiety and novelty preference levels on oral ethanol consumption in rats. *Psychopharmacology 232*(15):2711–2721. https://doi.org/10.1007/s00213-015-3910-5
31. Ziskind-Conhaim L, Gao BX, Hinckley C (2003) Ethanol dual modulatory actions on spontaneous postsynaptic currents in spinal motoneurons. *Journal of Neurophysiology* 89(2):806–813. https://doi.org/10.1152/jn.00614.2002
32. Ariwodola OJ, Weiner JL (2004) Ethanol potentiation of GABAergic synaptic transmission may be self-limiting: role of presynaptic GABA(B) receptors. *The Journal of Neuroscience* 24(47):10679–10686. https://doi.org/10.1523/JNEUROSCI.1768-04.2004
33. Carta M, Mameli M, Valenzuela CF (2004) Alcohol enhances GABAergic transmission to cerebellar granule cells via an increase in Golgi cell excitability. *The Journal of Neuroscience* 24(15):3746–3751. https://doi.org/10.1523/JNEUROSCI.0067-04.2004.
34. Roberto M, Gilpin NW, Siggins GR (2012). The central amygdala and alcohol: role of γ-aminobutyric acid, glutamate, and neuropeptides. *Cold Spring Harbor Perspectives in Medicine* 2(12), a012195. https://doi.org/10.1101/cshperspect.a012195
35. Roberto M, Kirson D, Khom S (2021) The Role of the Central Amygdala in Alcohol Dependence. *Cold Spring Harbor Perspectives in Medicine*, 11(2):a039339. https://doi.org/10.1101/cshperspect.a039339
36. Tatarczyńska E, Klodzińska A, Chojnacka-Wójcik E, Palucha A, Gasparini F, Kuhn R, Pilc A (2001) Potential anxiolytic- and antidepressant-like effects of MPEP, a potent, selective and systemically active mGlu5 receptor antagonist. *British Journal of Pharmacology* 132(7):1423–1430. https://doi.org/10.1038/sj.bjp.0703923
37. Rang H, Dale M, Ritter J and Moore P (2003) Pharmacology. Edinburgh, New York: Churchill Livingston.
38. Matell MS, Berridge KC, Aldridge JW (2006) Dopamine D1 activation shortens the duration of phases in stereotyped grooming sequences. *Behavioural Processes*, 71(2-3), 241–249. https://doi.org/10.1016/j.beproc.2005.09.008
39. Taylor JL, Rajbhandari AK, Berridge KC, Aldridge JW (2010) Dopamine receptor modulation of repetitive grooming actions in the rat: potential relevance for Tourette syndrome. *Brain Research* 1322:92-101. doi: 10.1016/j.brainres.2010.01.052.
40. Sircar R, Basak AK, Sircar D (2009) Repeated ethanol exposure affects the acquisition of spatial memory in adolescent female rats. *Behavioural Brain Research* 202(2):225–231. https://doi.org/10.1016/j.bbr.2009.03.036
41. Sey NYA, Gómez-A A, Madayag AC, Boettiger CA, Robinson DL (2019) Adolescent intermittent ethanol impairs behavioral flexibility in a rat foraging task in adulthood. *Behavioural Brain Research*, *373*, 112085. https://doi.org/10.1016/j.bbr.2019.112085
42. Shiigi Y, Takahashi M, Kaneto H (1990) Facilitation of memory retrieval by pretest morphine mediated by mu but not delta and kappa opioid receptors. *Psychopharmacology*

102(3):329–332. https://doi.org/10.1007/BF02244099

43. Bardo MT, Neisewander JL, Pierce RC (1989) Novelty-induced place preference behavior in rats: effects of opiate and dopaminergic drugs. *Pharmacology, Biochemistry and Behavior* 32(3):683–689. https://doi.org/10.1016/0091-3057(89)90018-x

44. Kitanaka J, Kitanaka N, Hall FS, Fujii M, Goto A, Kanda Y, Koizumi A, Kuroiwa H, Mibayashi S, Muranishi Y, Otaki S, Sumikawa M, Tanaka K, Nishiyama N, Uhl GR and Takemura M (2015) Memory Impairment and Reduced Exploratory Behavior in Mice after Administration of Systemic Morphine. *Journal of Experimental Neuroscience* 9: 27–35. https://doi.org/10.4137/jen.s25057

45. Pothos E, Rada P, Mark GP, Hoebel BG (1991) Dopamine microdialysis in the nucleus accumbens during acute and chronic morphine, naloxone-precipitated withdrawal and clonidine treatment. *Brain Research* 566(1-2):348–350. https://doi.org/10.1016/0006-8993(91)91724-f

46. Poorheidari G, Pratt J and Dehghani N (2002) Effects of low-dose scopolamine on locomotor activity: No dissociation between cognitive and non-effects. *Neuroscience Research Communications* 31(3):165 – 174. https://doi.org/10.1002/nrc.10049

47. Rojas-Carvajal M, Chinchilla-Alvarado J, Brenes JC (2022) Muscarinic regulation of self-grooming behavior and ultrasonic vocalizations in the context of open-field habituation in rats. *Behavioural Brain Research* 418:113641. https://doi.org/10.1016/j.bbr.2021.113641

48. Nomura Y, Nishiyama N, Saito H, Matsuki N (1994) Role of cholinergic neurotransmission in the amygdala on performances of passive avoidance learning in mice. *Biological & Pharmaceutical Bulletin* 17(4):490–494. https://doi.org/10.1248/bpb.17.490

49. Pelsőczi P, Lévay G (2017) Effect of Scopolamine on Mice Motor Activity, Lick Behavior and Reversal Learning in the IntelliCage. *Neurochemical Research* 42(12), 3597–3602. https://doi.org/10.1007/s11064-017-2408-4

50. Alshabi AM, Shaikh I.A, Asdaq SMB (2022) The antiepileptic potential of *Vateria indica* Linn in experimental animal models: Effect on brain GABA levels and molecular mechanisms. *Saudi Journal of Biological Sciences* 29(5):3600–3609. https://doi.org/10.1016/j.sjbs.2022.02.059

51. Britton DR, Indyk E (1990) Central effects of corticotropin releasing factor (CRF): evidence for similar interactions with environmental novelty and with caffeine. *Psychopharmacology 101*(3):366–370. https://doi.org/10.1007/BF02244055

52. Marin MT, Zancheta R, Paro AH, Possi AP, Cruz FC, Planeta CS (2011) Comparison of caffeine-induced locomotor activity between adolescent and adult rats. *European Journal of Pharmacology* 660(2-3), 363–367. https://doi.org/10.1016/j.ejphar.2011.03.052

53. Fisone G, Borgkvist A and Usiello A (2004). Caffeine as a psychomotor stimulant: mechanism of action. *Cellular and Molecular Life Sciences: CMLS*, 61(7-8), 857–872. https://doi.org/10.1007/s00018-003-3269-3