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Research Article

ANTI-OBSESSIVE–COMPULSIVE ACTIVITY OF HONEY

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ABSTRACT

Objective: The present study aim to investigate the anti-obsessive compulsive disorder potential of honey in mice.

Methods: The honey was given orally in two concentrations of 17.5 ml/kg and 35 ml/kg to mice for 21 days. The anti-obsessive compulsive activity was assessed on 21th day by in-vitro methods viz flickering-light induced obsessive- compulsive behaviour model developed in our laboratory (Patent No. 3087/DEL/2012) and marble-burying behaviour model. The biochemical estimation was also done on 21 days.

Results: When honey was administered chronically for 21 days significantly reduced gnawing behaviour and marble-burying behaviour of mice. Interestingly in our biochemical estimations, both, brain serotonin and GABA level were significantly increased by honey. The anti-Obsessive compulsive activity of honey may be due to the presence of Tryptophan, which is an important precursor of serotonin in the serotonergic neurons thereby enhancing the biosynthesis of serotonin to facilitate the anti- obsessive compulsive activity.

Conclusion: The present study revealed that honey possessed significant anti-OCD activity.

Keywords: Honey, Anti-obsessive–compulsive disorder, Antioxidant, Serotonin.

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INTRODUCTION

Honey is not only a sweetener but also it is a nature's gift to mankind. Honey has been used since long time medical and domestic needs. It contains proteins, enzymes, amino acids, minerals, trace elements, vitamins, aroma compounds, and polyphenols, which contribute to is its magical therapeutic power. The pure honey contains alkaloids, auterquinone, glycosides, cardiac glycosides, flavonoids, and reducing compounds. It has therapeutic, traditional, spiritual, nutritional, cosmetic, and industrial value. Honey is an excellent source of energy for athletes due to its high carbohydrate content. Fructose and glucose are the main carbohydrate constituents of honey, and its third greatest component is water. It possesses antimicrobial, antiviral, antiparasitory, anti-inflammatory, antioxidant, antimutagenic, antiseptic, sedative, antitoxic, laxative, antianemic, and anti-tumor effects [1]. Antioxidants acting as free radical scavengers may inhibit the cancer process *in vivo*. The antioxidant capacity of honey contributes to the prevention of several acute and chronic disorders such as diabetes, anti-inflammatory disorders, cardiovascular diseases, and cancer. Honey has action at neurological level and also cures Alzheimer's disease. From ancient time, honey has been using as traditional and safe remedy for diarrhea, cough, cold, wound, and infection, etc. [2].

Obsessive–compulsive disorder (OCD) is a disabling psychiatric condition with limited treatment options and is egodystonic and associated with seemingly purposeful behaviors (compulsions). Obsessions are defined as recurrent, persistent, thoughts, images, or impulses that are experienced as intrusive and inappropriate. Compulsions are repetitive behavior or mental acts that the person feels driven to perform in response to an obsession. Symptoms of OCD such as excessive hand washing, checking, praying, counting, aggressive sexual actions might produce shame, disrupted personality, and lack in confidence. Prevalence of pediatric OCD (mean age 7.5 and 12.5 years) is between 2% and 4%. Lifetime prevalence of adolescent OCD is 1.9% [3]. Comorbid states are also frequently seen in OCD such as body dysmorphic disorder, anorexia nervosa, depersonalization, hypochondriasis, Tourette's syndrome, trichotillomania, autism, binge eating, compulsive buying, kleptomania, pathological gambling, self-injurious behavior, sexual compulsions, borderline personality disorder, and antisocial personality disorder.

METHODS

Plant material

Honey (Patanjali) was purchased from local market of Hisar and was stored in refrigerator at 3-4°C in its dark colored bottle.

Experimental animals

A total of 48 adult Swiss mice divided into eight groups weighing around 20-25 g were procured from the Disease Free Small Animal House, Chaudhary Charan Singh Haryana Agriculture University of Veterinary Sciences, Hisar. All the animals were housed in Psychopharmacology Laboratory under controlled conditions of temperature in a natural light-dark cycle (12 hrs each). Water-boiled wheat porridge (Dalia) was given to the animals as food. The animals were acclimatized for at least 5 days to the laboratory conditions before behavioral experiments. Experiments were carried out between 09:00 am and 5:00 pm. During the study, separate groups (six animals) of mice and rats were made so that each animal was used only once. The experimental protocol was approved by the Institutional Animals Ethical Committee, and the care of animals was taken as per guidelines of CPCSEA, Ministry of Forests and Environment, Government of India (Registration number 0436).

Drug protocol

Fluoxetine (15 mg/kg, i.p.) was administered daily for the duration of 21 days to the animals. Saline was injected to control group for 21 consecutive days.

Laboratory models employed for testing OCD

Flickering light-induced obsessive–compulsive behavior model [4]

It was observed that when mice were exposed to flickering light

continuously for a period of 1 h, they produced repetitive gnawing behavior. This behavior was correlated with compulsive action of patients suffering from OCD. It is possible that mice experienced abnormal situation when they were exposed to mild aversive environment such as flickering light in the present model leading to continuous biting of objects present in their surroundings. We provided small pieces of thermocol, which were wrapped with glazed paper as novel objects. A mouse was kept in the unique chamber consisting of mirror on its four walls and flickering bulbs (15 W) at the ceiling of the chamber. The dimensions of this unique plywood box were 36 cm × 30 cm × 45 cm. The thermocol pieces (4 cm × 3 cm × 1 cm) wrapped with glazed paper were placed at the floor of the chamber uniformly. Then, this mouse was exposed to flickering light for a period of 60 minutes. Produced by four bulbs (15 W) each fixed at the ceiling of the chamber to which animals had no access. All the thermocol pieces were removed from the unique chamber at the end of the experiment, and a total number of gnawed pieces of thermocol was counted (Parle and Rana 2012). It was observed that there was a significant increase in the number of gnawed pieces of thermocol, when mouse was exposed to flickering light in the unique chamber from where there was no escape. This repetitive gnawing behavior of mice was successfully reversed by established anti-OCD medicines such as fluoxetine, venlafaxine, haloperidol, and lorazepam.

Furthermore, these animals behaved such as normal mice after four days of the experiment. The validity of the flickering light-induced obsessive–compulsive behavior model was studied using different categories of drugs such as fluoxetine, olanzapine, quetiapine, and risperidone. The findings were highly promising and confirmed the usefulness of the flickering light-induced obsessive–compulsive behavior model.

Marble-burying behavior model [5]

Digging and burrowing are typical behaviors of mice species (Deacon, 2006). Mice show digging behavior in the response of novel environment. Marble-burying is a natural defense mechanism which appears in the state of stress. Marble-burying helps in measuring the amount of digging. The marble-burying behavior model as described earlier was employed in the present study (Njung'e and Handley, 1991). In this model, mice were individually placed in separate plastic cages (21 cm × 38 cm × 14 cm) containing 5 cm thick sawdust bedding. 20 clean glass marbles (diameter ~10 mm) were arranged evenly on the bedding. After 30 minutes exposure to the marbles, mice were removed, and unburied marbles were counted (Shende *et al*., 2012). A marble was considered buried, if its two-third size was covered with sawdust.

The total number of marbles buried was considered as an index of obsessive–compulsive behavior.

Biochemical estimation

Estimation of brain neurotransmitter levels

i. Estimation of brain serotonin (5-HT) level

The animals of group were sacrificed by cervical decapitation under light anesthesia on the 22nd day 90 minutes after drugs administration. Immediately after the decapitation, the whole brain was dissected out. Weighed quantity of tissue was homogenized in 0.1 ml hydrochloric acid - butanol (0.85 ml of 37% hydrochloric acid in 1 L *n*-butanol for spectroscopy) for 1 minute in a cool environment. The sample was then centrifuged for 10 minutes at 2000 rpm. 0.08 ml of supernatant phase was removed and added to an Eppendorf reagent tube containing 0.2 ml of heptane (for spectroscopy) and 0.025 ml of 0.1 M hydrochloric acid. After 10 minutes of vigorous shaking, the tube was centrifuged under same conditions to separate two phases. Upper organic phase was discarded, and the aqueous phase (0.02 ml) was used for the estimation of serotonin. To 0.02 ml aqueous extract, 0.025 ml of O-phthaldialdehyde (OPT) reagent (20 mg in 100 ml concentrated HCl) was added. The fluorophore was developed by heating to 100°C for 10 minutes. After the samples reached equilibrium with the ambient temperature, readings were taken at 360/470 nm in the spectrofluorimeter. Internal standard was prepared by adding 500 µg/ml of serotonin in distilled water: HCl-butanol in 1:2 ratios and following the whole above-mentioned procedure. For serotonin tissue blank and internal reagent blank, 0.025 ml concentrated HCI without OPT was added [6].

ii. Estimation of brain gamma-aminobutyric acid (GABA) level

Isolated brain was transferred to homogenization tube containing 5 ml of 0.01M hydrochloric acid and homogenized. Brain homogenate was transferred to a bottle containing 8 ml of ice cold absolute alcohol and kept for 1 h at 0°C. The content was centrifuged for 10 minute**s** at 16,000 rp; supernatant was collected in Petri dish. Precipitate was washed with 5 ml of 75% alcohol for 3 times and washes were combined with supernatant. Contents in Petri dish were evaporated to dryness at 70°C on water bath under stream of air. To the dry mass, 1 ml water and 2 ml chloroform were added and centrifuged at 2000 rpm. Upper phase containing GABA (2.0 ml) was separated, and 10 µl of it was applied as spot on Whatman paper (No.41). The mobile phase consisted of n-butanol (50 ml) acetic acid (12 ml) and water (60 ml). The chamber was saturated for ½ hr with mobile phase. The paper chromatogram was developed with ascending technique. The paper was dried in hot air and then spread with 0.5% ninhydrin solution in 95% ethanol. The paper was dried for 1 hr at 90°C. Blue color spot developed on paper was cut and heated with 2 ml ninhydrin solution on water bath for 5 minutes. Water (5.0 ml) was added to solution and kept for 1 hr. Supernatant (2.0 ml) was decanted and absorbance was measured at 570 nm [7].

RESULTS

Effect of honey on gnawing behavior of mice using flickering light-induced obsessive–compulsive behavior model

Honey at the concentrations of 17.5 and 35 ml/kg, when administered (p.o) for 21 consecutive days showed remarkable ($p<0.01$) reduction in gnawing behavior in mice as compared to control group. Fluoxetine (15 mg/kg; i.p.) used as a standard drug remarkably reduced gnawing behavior in mice (Fig. 1).

Effect of honey on marble-burying behavior of mice using marble-burying behavior model

Administration of honey at the concentration of 17.5 and 35 ml/kg (p.o) for 21 consecutive days showed remarkable ($p<0.01$) reduction in marble-burying behavior as compared to control group. Fluoxetine (15 mg/kg; i.p.) used as a standard drug remarkably reduced marble-burying behavior in mice (Fig. 2).

Effect of honey on brain serotonin level

Administration of honey (p.o) at the concentration of 35 ml/kg for 21 consecutive days showed significantly ($p<0.05$) increase in brain serotonin level in mice as compared to control group (Fig. 3).

Effect of honey on brain GABA level

Administration of honey (p.o) at the concentration of 35 ml/kg for 21 consecutive days showed significantly ($p<0.05$) increase in brain GABA level in rodents as compared to control group (Fig. 4).

DISCUSSION

OCD is characterized by persistent thoughts (obsessions) which are egodystonic and associated with seemingly purposeful behavior (compulsions). OCD may be a consequence of decreased serotonin levels and enhanced dopamine levels in brains of patients suffering with OCD. Only potent serotonin reuptake inhibitors (SRIs) were consistently found to be effective in patients of OCD [4]. The dopaminergic system may also be involved in the etiology of OCD. This is suggested by pharmacological data showing that administration of dopamine antagonists is effective in certain forms of OCD, with and without comorbid tics resistant to SRIs alone. The dopamine system includes five subtypes of dopamine receptors (D1-D5). There have been positive

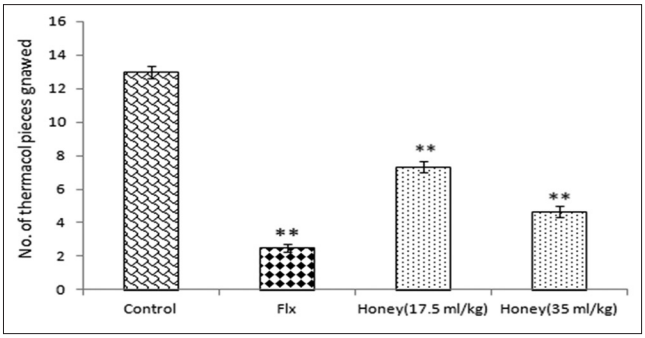

Fig. 1: Effect of honey gnawing behavior of mice using flickering light-induced obsessive–compulsive behavior model. Values are in mean±standard error of mean (n=6). **Denotes p<0.01 as compared to control group. Flx=Fluoxetine. Honey was administered at 17.5 ml/kg and 35 ml/kg per orally for 21 days. Statistically analysis work was carried out by one-way ANOVA followed by Dunnett's t-test

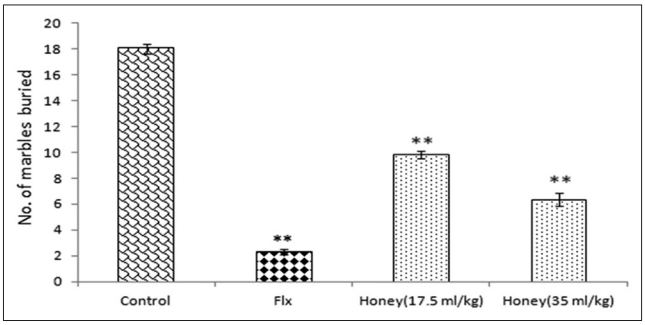

Fig. 2: Effect of honey on marble-burying behavior of mice using marble-burying behavior model. Values are in mean±standard error of mean (n=6). **Denotes p<0.01 as compared to control group. Flx=Fluoxetine. Honey was administered at 17.5 and 35 ml/kg per orally for 21 days. Statistically analysis work was carried out by one-way ANOVA followed by Dunnett's t-test

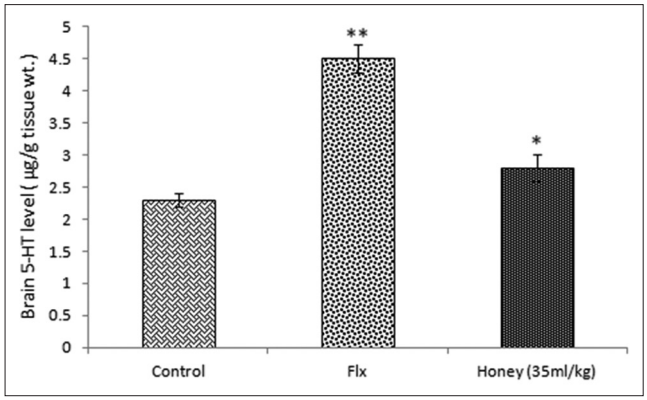

Fig. 3: Effect of honey on brain 5-HT levels. Values are in mean±standard error of mean (n=6). *Denotes p<0.05 as compared to control group. **Denotes p<0.01 as compared to control group. Flx=Fluoxetine. Honey was administered at 35 ml/kg per orally for 21 days. Statistically analysis work was carried out by one-way ANOVA followed by Dunnett's t-test

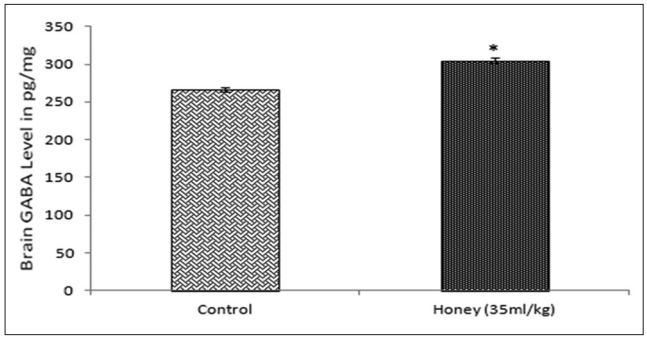

Fig. 4: Effect of honey on brain gamma-aminobutyric acid levels. Values are in mean±standard error of mean (n=6). *Denotes p<0.05 as compared to control group. Halo=Haloperidol, Olz=Olanzapine. Honey was administered at 35 ml/kg per orally for 21 days. Statistically analysis work was carried out by one-way ANOVA followed by Dunnett's t-test

results with the association between polymorphisms in the gene coding for D4 dopamine receptor and OCD, but no significant role of D2 and D3 receptors has been found [8]. Marble-burying behavior of mice has been used to model OCD due to the excessive nature of the behavior and due to pharmacological effects of clinical standards. The mice bury the unpleasant object (marble), which cause aversion and fearful thoughts in marble burying model. It is a well-accepted paradigm to screen anti-compulsive activity, as it is based on the principle that burying behavior is an unconditioned defensive reaction in rodents, which is species-specific, not associated with physical danger and does not habituate upon repeated testing [9]. The newly developed device (Patent No. 3087/DEL/2012) in our laboratory comprised a peculiar chamber, which has mirrors on its all four walls, thermocol pieces wrapped with glazed paper at the floor of the chamber, and four flickering bulbs at the ceiling of the chamber. The thermocol pieces wrapped with glaze paper were provided at the floor of the chamber for quantifying the gnawing behavior. In both of these models, namely, the flickering light model and marble-burying model, there was a remarkable decrease in a number of thermocol pieces gnawed and decrease in marble-burying behavior after administration of fluoxetine, thereby confirming the validity of these models. Furthermore, oral administration of honey for 21 days, in the present study, also reduced significantly marble-burying behavior and the number of thermocol pieces gnawed. Honey, contains tryptophan, an important precursor of serotonin. This fact may be lead to enhancement in the biosynthesis of serotonin, thereby facilitating the anti-compulsive effect [10]. Therefore, it appears that this nutrient acts through influence on serotonergic and dopaminergic systems as there was a remarkable increase in serotonin levels through increased biosynthesis of serotonin due to the presence of tryptophan in this nutrient and diminished levels of dopamine in the brains of rodents. These findings revealed anti-obsessive–compulsive potential of honey. In addition to above, GABA, an inhibitory neurotransmitter, may also be playing an important role in the pathogenesis of OCD. It has been reported that GABA decreased the hyperactivity and obsessive–compulsive behavior in laboratory animals [11]. In the present study, there was a remarkable increase in the levels of GABA, an inhibitory neurotransmitter, which might have further helped in anti-obsessive–compulsive effect of honey.

Free radicals are highly reactive molecules generated predominantly during cellular respiration and normal metabolism. Imbalance between cellular production of free radicals and ability of cells to defend against them is referred to as oxidative stress [12]. The enhanced oxidative stress can lead to modification of cellular components and induce cell damage and death. By inducing cellular metabolic stress, these factors appear to increase the possibility of neuronal damage [13]. To overcome role of oxidative damage, the brain needs a sufficient supply of antioxidants. Thus, as and when, antioxidant activity increases due to the supplementation of higher amounts of a greater variety of antioxidants, cellular damage lessens, and health improves. This finding indicated that honey enhanced scavenging of free radicals in the brain, thereby preventing the occurrence of obsessive–compulsive behavior.

CONCLUSION

Honey has been used since ancient times to fulfill medical and domestic needs. In the present study, chronic administration of honey for 21 days to rodents exhibited powerful anti-OCD activity when tested using flickering light-induced obsessive–compulsive behavior model developed in our laboratory (Patent No. 3087/DEL/2012) and marble-burying behavior model. Administration of honey to separate groups of mice for 21 days significantly reduced gnawing behavior and marble-burying behavior of mice. The anti-obsessive–compulsive activity of honey may be due to the presence of tryptophan, which is an important precursor of serotonin in the serotonergic neurons, thereby enhancing the biosynthesis of serotonin to facilitate the anti-obsessive–compulsive activity.

These findings taken together reveal the anti-obsessive–compulsive potential of honey.

REFERENCES

1. Bogdanov S, Jurendic T, Sieber R, Gallmann P. Honey for nutrition and health: A review. J Am Coll Nutr 2008;27(6):677-89.
2. Singh MP, Chourasia HR, Agarwal M, Malhotra A, Sharma S, Sharma D, *et al*. Honey as complementary medicine: A review. Int J Pharm Bio Sci 2012;3(2):12-31.
3. Boileau B. A review of obsessive-compulsive disorder in children and adolescents. Dialogues Clin Neurosci 2011;13(4):401-11.
4. Parle M, Rana T. How to test medicines of obsessive – Compulsive disorder? Int Res J Pharm 2012;3:1-6.
5. Deacon RM. Digging and marble burying in mice: Simple methods for *in vivo* identification of biological impacts. Nat Protoc 2006;1(1):122-4.
6. Schlumpf M, Lichtensteiger W, Langemann H, Waser PG, Hefti F. A fluorometric micromethod for the simultaneous determination of serotonin, noradrenaline and dopamine in milligram amounts of brain tissue. Biochem Pharmacol 1974;23(17):2437-46.
7. Tamboli AM, Rub RA, Ghosh P, Bodhankar SL. Antiepileptic activity of lobeline isolated from the leaf of *Lobelia nicotianaefolia* and its effect on brain GABA level in mice. Asian Pac J Trop Biomed 2012;2(7):537-42.
8. Parle M, Gaikwad U. Clinical features of obsessive-compulsive disorder. Int Res J Pharm 2009;2:84-6.
9. Girdhar S, Wanjari MM, Prajapati SK, Girdhar A. Evaluation of anti-compulsive effect of methanolic extract of *Benincasa hispida* Cogn. Fruit in mice. Acta Pol Pharm 2010;67(4):417-21.
10. Madhura TK. Role of oxidative stress in the pathogenesis of OCD. Biochem Anal Biochem 2015;4:1-7.
11. Fava L, Bellantuono S, Bizzi A, Cesario ML, Costa B, De Simoni E, *et al*. Review of obsessive compulsive disorders theories. Glob J Epidemiol Public Health 2014;1:1-13.
12. Hamid AA, Aiyelaagbe OO, Usman LA, Ameen OM, Lawal A. Antioxidants: Its medicinal and pharmacological applications. Afr J Pure Appl Chem 2010;4(8):142-51.
13. Kale MA, Bindu M, Khadkikar P. Role of antioxidants and nutrition in oxidative stree: a review. Int J App Pharm, 2015;7: 1-4.