INTRODUCTION
Honey is not only a sweeter 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 its magical therapeutic power. The pure honey contains alkaloids, aurantquinone, 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, antidepressant, sedative, 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 used 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
It was observed that when mice were exposed to flickering light
Estimation of brain neurotransmitter levels

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

readsings were taken at 360/470 nm in the spectrophotometer. 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 HCl 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 minutes at 16,000 rpm; 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

Adminstration 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

Adminstration 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

Adminstration 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
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 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 serotnin in this nutrient and diminished levels of dopamine in the brains of rodents. These findings revealed anti-obessive-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-obessive-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