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HONEY: A SWEET WAY TO CURE PSYCHOSIS

Milind Parle* and Arzoo

Pharmacology Division, Department of Pharm. Sciences, Guru Jambheshwar University of Science and Technology ('A' Grade NAAC Accredited, State Govt. University) Hisar -125001 (Haryana).

*** Corresponding Author: Prof. Dr. Milind Parle**
Pharmacology Division, Department of Pharm. Sciences, Guru Jambheshwar University of Science and Technology ('A' Grade NAAC Accredited, State Govt. University) Hisar -125001 (Haryana).

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ABSTRACT
Honey, a natural sweetener derived from insects is not only a sweetener but it's a unique gift from nature to mankind. Honey has been used since ancient times to fulfil medical and domestic needs. It contains proteins, enzymes, amino acids (glycine etc), minerals, vitamins, aromatic compounds and polyphenols, which contribute to its magical therapeutic power. Unfortunately, there are no reports available in literature about the neuropharmacological profile of Honey. Therefore, this study was undertaken to explore the anti-psychotic potential of Honey. Honey was administered orally chronically to rodents in two different concentrations 17.5ml/kg and 35ml/kg for 21 days. The anti-psychotic potential of Honey was assessed by using pharmacological models such as Ketamine-induced stereotypic behavior in mice, swim -induced grooming attempts in mice and pole climbing avoidance response in rats. Honey remarkably reduced the Ketamine induced falling, weaving, head-bobbing and turning behaviour in mice. It also reduced swim induced grooming attempts in mice. Furthermore, it reduced significantly the conditioned avoidance response in rats as reflected by reduction in time taken to climb the pole. Interestingly in our biochemical estimations, both, brain dopamine level and acetyl cholinesterase activity were remarkably reduced by Honey. Whereas, brain glutathione levels were remarkably increased by Honey, thereby suggesting enhanced scavenging of free radicals. These findings coupled together reveal the anti-psychotic potential of Honey.

KEYWORDS: Honey, Schizophrenia, Ketamine, Dopamine, Glutathione, Anti- oxidant.

INTRODUCTION

Schizophrenia continues to be a mysterious disease, fascinating the minds of psychiatrists, pharmacologists and neuroscientists all over the world for more than a century. The crucial welfare of the millions afflicted with schizophrenia is at stake. Schizophrenia is a disabling condition having onset earlier in men (15-25 yrs.) than in women (25-35yrs), with a lifetime global prevalence of 1%.[1] About half of all schizophrenics will attempt suicide at least once in their life time. A typical patient of schizophrenia experiences four types of clinical symptoms a) Apathetic attitude such as lack of emotions, flat affect, expressionless gaze b) Bizarre behaviour such as dis-organized conduct, hostility, unnecessary laughter or crying episodes, odd or irrational statements, extreme reaction to criticism, strange way of speaking, deterioration of personal hygiene, delusions, hallucinations, suspiciousness, c) Cognitive dysfunctions such as aphasia, irrelevant talk, dementia, loss of concentration, lack of judgement, d) Depressive symptoms such as lack of motivation, social withdrawal, anhedonia, insomnia, lack of interest in life, self harm etc. Honey is looked upon as a house-hold medicine, since ancient times. Honey comprises of amino acids (glycine etc), enzymes, minerals, vitamins, aromatic compounds and polyphenols, which contribute to its magical therapeutic powers.[2] Several studies are available in literature indicating wound healing, anti-inflammatory, anti-bacterial and anti-cancer properties of Honey.[3] However, there are no reports regarding anti-psychotic potential of Honey. Stress is found to be a powerful precipitating factor for the first episode of psychosis. Therefore, the present study was undertaken to explore anti-psychotic potential of Honey in rodents using various behavioural models of psychosis.

MATERIALS AND METHODS

Plant material

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

Experimental animals

A total of 66 adult Swiss mice divided in 11 groups weighing around 20-25g and 30 Wistar male rats divided in 5 groups weighing around 180-200g were procured from Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar. All

the animals were housed in Psychopharmacology laboratory under controlled conditions of temperature in a natural 12 h each light - dark cycle. 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 behavioural experiments. Experiments were carried out between 09:00 am to 5:00 pm. The experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC) and the care of animals was taken as per guidelines of CPCSEA, Ministry of Forests and Environment, Government of India (Registration number 0436). Each observation was recorded in a separate group of mice/rats. Each group consisted of six animals and each animals was used only once in the study.

Drug protocol

Haloperidol, 1 mg/kg, i.p. (RPG Science Pharmaceutical Pvt. Ltd), Olanzapine, 5 mg/kg, i.p. (Intas Pharmaceuticals Ltd) and Ketamine, 50 mg/kg, i.p. (Troikaa Pharmaceuticals Ltd) were administered daily for duration of 21 days to the animals. Saline was injected to control group for 21 consecutive days. Ketamine (50mg/kg, i.p) was injected for 21 consecutive days for inducing stereotypic behaviour.

LABORATORY MODELS EMPLOYED FOR TESTING PSYCHOSIS

I) Ketamine- induced stereotypic behaviour in mice[4]

In this model, animals were divided into seven groups and each group consisted of six animals. The control group I received only saline (1ml/kg, i.p) and negative control group II received Ketamine (50mg/kg, i.p). The animals of groups III, IV received Haloperidol (1mg/kg, i.p) and Olanzapine (5mg/kg, i.p) respectively and after 30 min Ketamine (50mg/kg, i.p) was given, for 21 consecutive days. Group V received Honey only (35ml/kg, p.o.) for 21 days. The animals of test groups VI and VII received different concentrations of Honey (35ml/kg, 17.5 ml/kg, p.o) respectively and after 30 min Ketamine was given (50mg/kg, i.p) for 21 consecutive days. Each mouse was individually placed into a separate plastic cage ($37 \times 24 \times 30$ cm^{3}), which was divided into quadrants by lines on the floor and was allowed to acclimatize for at least 30 min before the experiments. Behavioural tests were performed between 9 am to 4 pm. The stereotypic behaviour was assessed by counting the number of turning, weaving, and head bobbing counts. Turning was measured by counting turn around attempt of each mouse every 10 min over 60 min period. Weaving and Head-bobbing counts were measured by counting its neck movements towards right and left and up and down every 10 min over 60 min. Ataxia was assessed by counting the number of falls every 10 min over 60 min.[5]

II) Pole climbing avoidance in rat[6]

In this model, animals were divided into five groups and each group consisted of six animals. The control group I received only saline (1ml/kg, i.p). The animals of groups II, III received Haloperidol (1mg/kg, i.p) and Olanzapine (5mg/kg, i.p) respectively, for 21 consecutive days. The animals of test groups IV and V received different concentrations of Honey (35ml/kg, 17.5 ml/kg, p.o) respectively, for 21 consecutive days. The pole-climb avoidance paradigm is an avoidance escape procedure used to separate neuroleptics from sedatives and anxiolytics. Whereas, sedative compounds suppress both avoidance and escape responding at approximately the same doses, neuroleptic drugs reduce avoidance at lower doses than those affecting escape responding. Male wistar rats weighing around 150 gm were used in the training and testing of rat was conducted in the Pole climbing apparatus, which has a floor that acts as a source of shock. At the centre of the instrument there is a wooden pole, which also serves as a shock free zone. The procedure and end-point observed in the present study was as described earlier.[6] Data were expressed in terms of the number of avoidance attempts and escape failures relative to the respective vehicle control group.

III) Swim induced grooming in mice[7]

In this model, animals were divided into four groups and each group consisted of six animals. The control group I received only saline (1ml/kg, i.p). The animals of group II received Haloperidol (1mg/kg, i.p), for 21 consecutive days. The animals of test groups III and IV received different concentrations of Honey (35ml/kg, 17.5 ml/kg, p.o) respectively, for 21 consecutive days. Sixty minutes after treatments, mice were placed individually in swimming cylinders (8x8x18cm high) filled with water (32°C) for three min. They were then removed and dried with towel for 30 seconds and placed immediately into Perspex boxes individually. The number and the total duration of grooming attempts were recorded over 15 min. period as described in literature.[7]

BIOCHEMICAL ESTIMATION

Estimation of brain neurotransmitter levels

i) Collection of brain samples

The animals were sacrificed by cervical decapitation under light anaesthesia on 22nd day 90 min after drugs administration. Immediately after 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 one liter *n*- butanol for spectroscopy) for 1 min in a cool environment. The sample was then centrifuged for 10 min at 2,000 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 min 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 estimation of Dopamine.[8]

II) Estimation of brain dopamine level

To 0.02 ml of aqueous, 0.05ml 0.4M EDTA and 0.01ml Sodium acetate buffer (pH 6.9) were added, followed by

0.01ml iodine solution (0.1M in ethanol) for oxidation. The reaction was stopped after two minutes by addition of 0.01ml Na_2SO_3 in 5ml NaOH. Acetic acid was added 1.5 minutes later. The solution was then heated to 100^oC for 6 minutes. When the sample again reached room temperature, excitation and emission spectra were read from the spectrofluorimeter at 330-375nm.[8] Tissue blanks was prepared by adding the reagents of the oxidation step in reversed order (sodium sulphite before iodine). Internal Standard was prepared by adding 500 µg/ml of dopamine in distilled water: HCl-butanol in 1:2 ratios and following the whole above mentioned procedure. Tissue blanks and internal reagent blank were prepared by adding the reagents of the oxidation step in reversed order (sodium sulphite before iodine).

III) Estimation of brain acetycholinesterase activity

Isolated brain was weighed and transferred to a glass homogenizer and homogenized in an ice bath after adding 10 volumes of sterile normal saline injection. The homogenate was centrifuged at 3000 rpm for 10 minutes and 0.4 ml of the resultant cloudy supernatant liquid was added to a test tube containing 2.6 ml of phosphate buffer. 0.1ml of DTNB was added to the above mixture and absorbance was noted at 412 nm. 0.02 ml of acetylcholine iodide solution was added and again absorbance was noted 15 min thereafter. Change in absorbance per min was calculated.[9]

IV) Estimation of brain glutathione level

Glutathione was measured spectrophotometrically. Briefly, brain tissues were homogenized with 10 times (w/v) 0.1M sodium phosphate buffer (pH 7.4). This homogenate was then centrifuged with 5% trichloroacetic acid to centrifuge out the proteins. To 0.1 ml of this supernatant, 2ml of phosphate buffer (pH 8.4), 0.5 ml of, 5,5'- dithiobis (2-nitrobenzoic acid) (DTNB) and 0.4 ml of double distilled water was added. The mixture was vortexed and the absorbance measured at 412 nm within 15 minutes. The results were expressed as µmol/g of tissue protein.[10]

STATISTICAL ANALYSIS

All values were expressed as mean ± S.E.M. The data were analyzed using one way ANOVA followed by Dunnett's t-test. $p<0.05$ was considered to be statistically significant.

RESULTS

A. Effect of Honey on Ketamine induced stereotypic behavior of mice

The stereotypic behavior was assessed by measuring the number of turning, weaving, falling and head-bobbing counts produced by mice. Turning behavior was measured by counting the turn-around of each mouse every 10 min over 60 min period. Weaving behavior was measured by counting its paw movements and standing on hind legs attempts every 10 min over 60 min period. Head- bobbing behavior was measured by counting its neck movements towards right / left and up/down every 10 min over 60 min period. Falling was assessed by counting the number of falls of each mouse on the floor of the cage every 10 min over 60 min period. Ketamine (50mg/kg, i.p) successfully evoked above described stereotypic behavior in mice. Honey, when administered at 17.5 ml/kg and 35 ml/kg concentrations orally for 21 successive days remarkably ($p<0.01$) decreased this stereotypic behavior of mice produced by Ketamine. Administration of Honey *per se* didn't evoke any stereotypic behaviour in mice. Animals pre-treated with Haloperidol (1mg/kg, i.p) and Olanzapine (5mg/kg, i.p) reduced the stereotypic behaviour induced by Ketamine. The effect of Honey was found to be comparable to that of Haloperidol and Olanzapine (Marketed anti-psychotic agents).

i) Effect of Honey on turning behavior of mice

Administration of Honey *per se* didn't evoke any turning behaviour in mice. Administration of Honey (p.o for 21 days) at the concentration of 17.5 ml/kg ($p<0.05$) and 35 ml/kg ($p<0.01$) dose dependently decreased the turning behavior of mice induced by Ketamine. Animals pre-treated with Haloperidol (1mg/kg, i.p) and Olanzapine (5mg/kg, i.p) also decreased the turning pattern of mice as expected **(See Fig 1).**

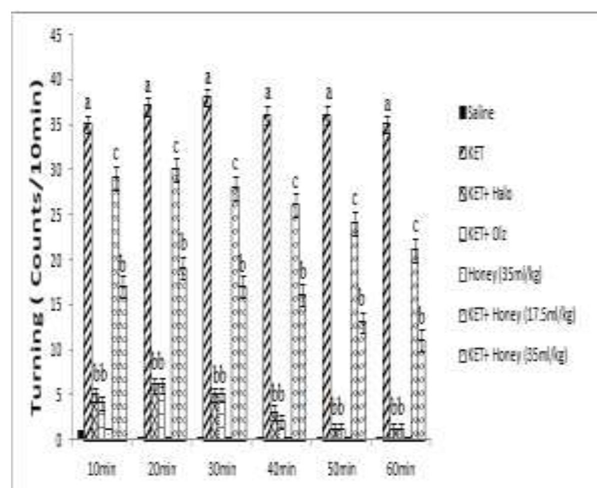

Fig 1. Effect of Honey on Ketamine induced turning behavior of mice, when observed every 10 min. over 60 min. period.

Values are in mean ± SEM (n = 6).

a denotes $p<0.01$ as compared to control group.

b denotes $p<0.01$ as compared to Ketamine group.

c denotes $p<0.05$ as compared to Ketamine group.

KET = Ketamine, Halo = Haloperidol, Olz = Olanzapine

Honey was administered at 17.5 ml/kg and 35 ml/kg per orally for 21 days.

Statistical analysis was carried out by one way ANOVA followed by Dunnett's t-test.

ii) Effect of Honey on weaving behavior of mice

Administration of Honey *per se* didn't evoke any weaving behaviour in mice. Administration of Honey

(p.o for 21 days) at the concentration of 17.5 ml/kg ($p<0.05$) and 35 ml/kg ($p<0.01$) dose dependently decreased the weaving behavior of mice induced by Ketamine. Animals pre-treated with Haloperidol (1mg/kg, i.p) and Olanzapine (5mg/kg, i.p) also decreased the weaving pattern exhibited by mice **(See Fig 2).**

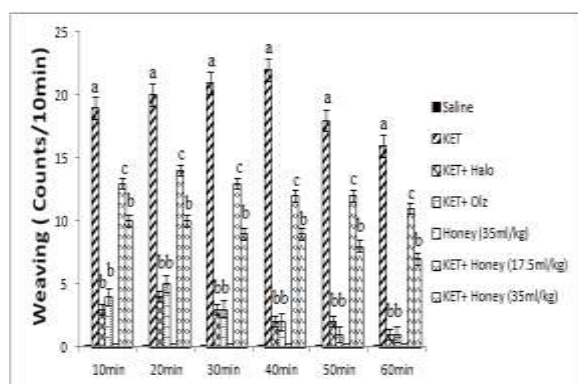

Fig 2. Effect of Honey on Ketamine induced weaving behavior of mice, when observed every 10 min. over 60 min. period.
Values are in mean ± SEM (n = 6).
a denotes $p<0.01$ as compared to control group.
b denotes $p<0.01$ as compared to Ketamine group.
c denotes $p<0.05$ as compared to Ketamine group.

KET = Ketamine, Halo = Haloperidol, Olz = Olanzapine
Honey was administered at 17.5 ml/kg and 35 ml/kg per orally for 21 days.
Statistical analysis was carried out by one way ANOVA followed by Dunnett's t-test.

iii) Effect of Honey on head-bobbing behavior of mice
Administration of Honey *per se* didn't evoke any head-bobbing behaviour in mice. Administration of Honey (p.o for 21 days) at the concentration of 17.5 ml/kg ($p<0.05$) and 35 ml/kg ($p<0.01$) dose dependently decreased the head-bobbing behavior of mice induced by Ketamine. Animals pre-treated with Haloperidol (1mg/kg, i.p) and Olanzapine (5mg/kg, i.p) also decreased the head-bobbing counts in mice **(See Fig 3).**

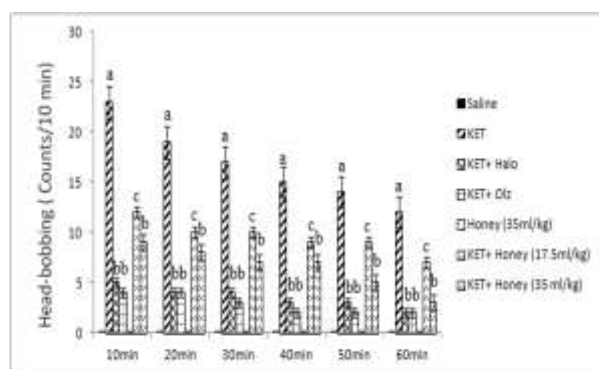

Fig 3. Effect of Honey on Ketamine induced head-bobbing behavior of mice, when observed every 10 min. over 60 min. period.
Values are in mean ± SEM (n = 6).
a denotes $p<0.01$ as compared to control group.
b denotes $p<0.01$ as compared to Ketamine group.
c denotes $p<0.05$ as compared to Ketamine group.

KET = Ketamine, Halo = Haloperidol, Olz = Olanzapine
Honey was administered at 17.5 ml/kg and 35 ml/kg per orally for 21 days.
Statistical analysis was carried out by one way ANOVA followed by Dunnett's t-test.

iv) Effect of Honey on falling behavior of mice
Administration of Honey *per se* didn't evoke any falling behaviour in mice. Administration of Honey (p.o for 21 days) at the concentration of 17.5 ml/kg ($p<0.05$) and 35 ml/kg ($p<0.01$) dose dependently decreased the falling behavior of mice induced by Ketamine. Animals pre-treated with Haloperidol (1mg/kg, i.p) and Olanzapine (5mg/kg, i.p) also decreased the falling behaviour **(See Fig 4).**

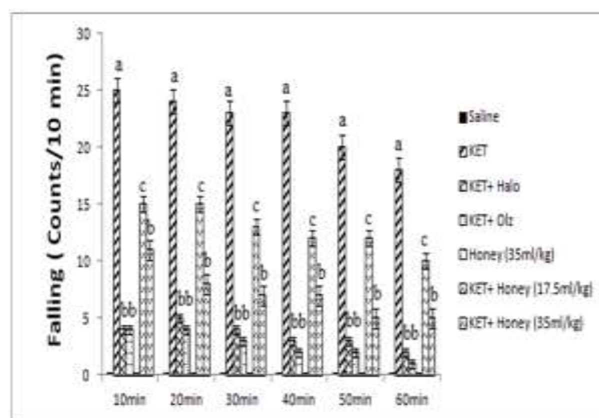

Fig 4. Effect of Honey on Ketamine induced falling behavior of mice, when observed every 10 min. over 60 min. period.
Values are in mean ± SEM (n = 6).
a denotes $p<0.01$ as compared to control group.
b denotes $p<0.01$ as compared to Ketamine group.
c denotes $p<0.05$ as compared to Ketamine group.

KET = Ketamine, Halo = Haloperidol, Olz = Olanzapine
Honey was administered at 17.5 ml/kg and 35 ml/kg per orally for 21 days.
Statistical analysis was carried out by one way ANOVA followed by Dunnett's t-test.

B. Effect of Honey on pole climb avoidance in rats
Administration of Honey (p.o) at the concentration of 17.5ml/kg and 35ml/kg for 21 successive days remarkably ($p<0.01$) inhibited the conditioned avoidance response in rats as indicated by increased time spent on the grid floor of the chamber. The effect of Honey was found to be comparable to that of Haloperidol (1 mg/kg, i.p.) and Olanzapine (5 mg/kg, i.p.) (Antipsychotic agents) **(See Fig 5).**

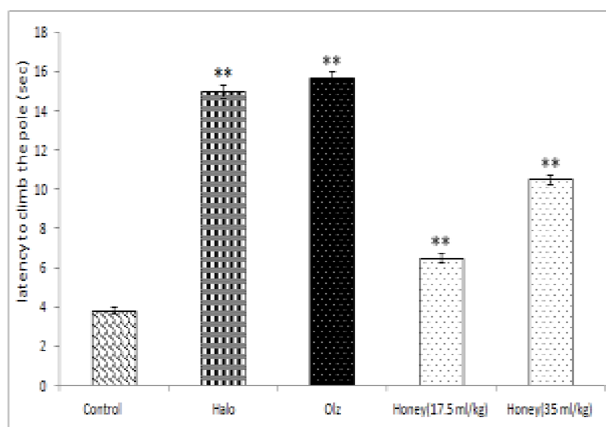

Fig 5. Effect of Honey on pole climb avoidance in rats
Values are in mean ± SEM (n = 6).
** denotes p<0.01 as compared to control group.

Halo = Haloperidol, Olz = Olanzapine
Honey was administered at 17.5 ml/kg and 35 ml/kg per orally for 21 days.
Statistical analysis was carried out by one way ANOVA followed by Dunnett's t-test.

C. Effect of Honey on swim induced grooming in mice
Honey, at the concentration of 17.5ml/kg (p.o for 21 days) significantly (p<0.05) decreased the number of grooming attempts and remarkably (p<0.01) reduced the duration of swim- induced grooming behaviour in mice as compared to the control group. However, at the concentration of 35ml/kg (p.o for 21 days), Honey remarkably (p<0.01) reduced both, the duration of swim-induced grooming behaviour **(See Fig 6)** and number of swim- induced grooming attempts **(See Fig 7)** of mice as compared to the control group, when administered for 21 consecutive days.

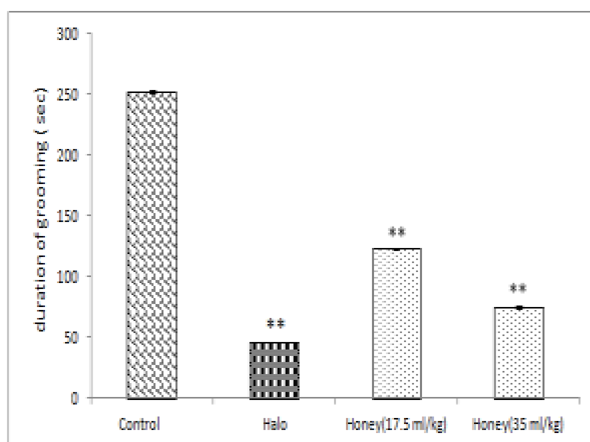

Fig 6. Effect of Honey on duration of grooming of mice in swim induced grooming.
Values are in mean ± SEM (n = 6).
** denotes p<0.01 as compared to control group.

Halo = Haloperidol
Honey was administered at 17.5 ml/kg and 35 ml/kg per orally for 21 days.
Statistical analysis was carried out by one way ANOVA followed by Dunnett's t-test.

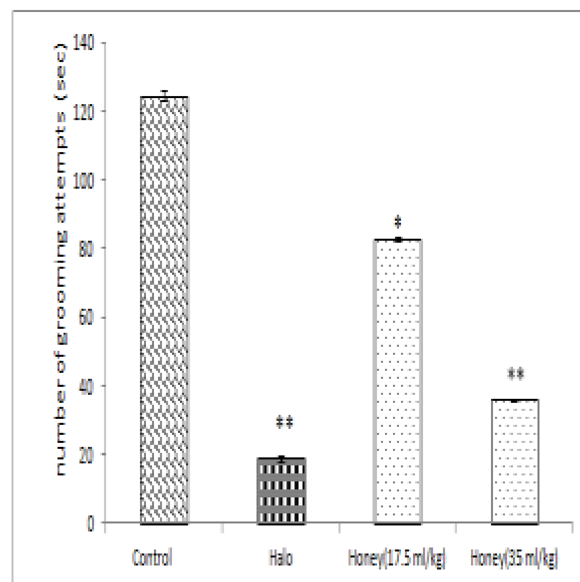

Fig 7. Effect of Honey on number of grooming attempts of mice in swim induced grooming.
Values are in mean ± SEM (n = 6).
* denotes p<0.05 as compared to control group.
** denotes p<0.01 as compared to control group.

Halo = Haloperidol
Honey was administered at 17.5 ml/kg and 35 ml/kg per orally for 21 days.
Statistical analysis was carried out by one way ANOVA followed by Dunnett's t-test.

D. Effect of Honey on brain dopamine level
Administration of Honey (p.o) at the concentration of 35ml/kg for 21 consecutive days showed remarkable (p<0.01) decrease in brain dopamine levels of rodents as compared to the control group **(See Fig 8).**

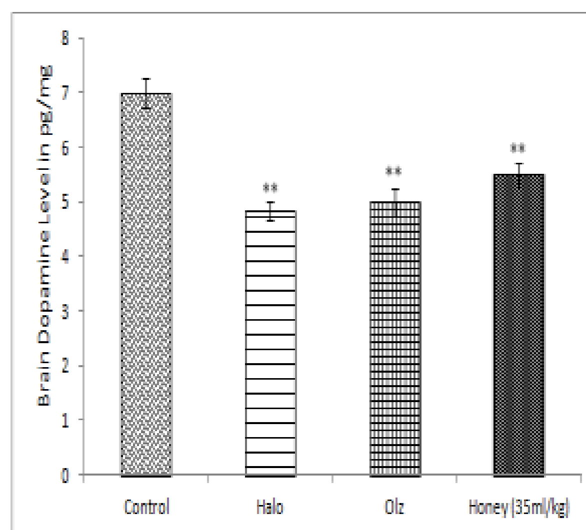

Fig 8. Effect of Honey on brain Dopamine levels
Values are in mean ± SEM (n = 6).

** denotes p<0.01 as compared to control group.
Halo = Haloperidol, Olz = Olanzapine
Honey was administered at 35 ml/kg per orally for 21 days.
Statistical analysis was carried out by one way ANOVA followed by Dunnett's t-test.

E. Effect of Honey on brain acetylcholinesterase activity

Administration of Honey (p.o) at the concentration of 35ml/kg for 21 consecutive days showed remarkable (p<0.01) decrease in brain Acetyl cholinesterase activity in rodents as compared to control group **(See Fig 9).**

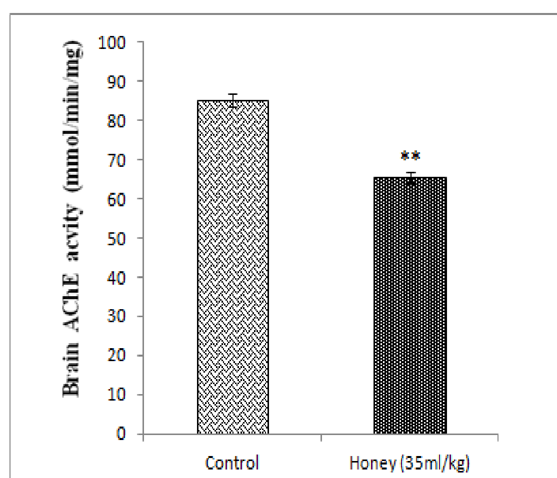

Fig 9. Effect of Honey on brain AchE levels

Values are in mean ± SEM (n = 6).
** denotes p<0.01 as compared to control group.

Honey was administered at 35 ml/kg (p.o) for 21 days.
Statistical analysis was carried out by one way ANOVA followed by Dunnett's t-test.

F. Effect of Honey on brain glutathione level

Administration of Honey (p.o) at the concentration of 35ml/kg for 21 consecutive days showed remarkable (p<0.01) increase in brain Glutathione level in rodents as compared to control group **(See Fig 10).**

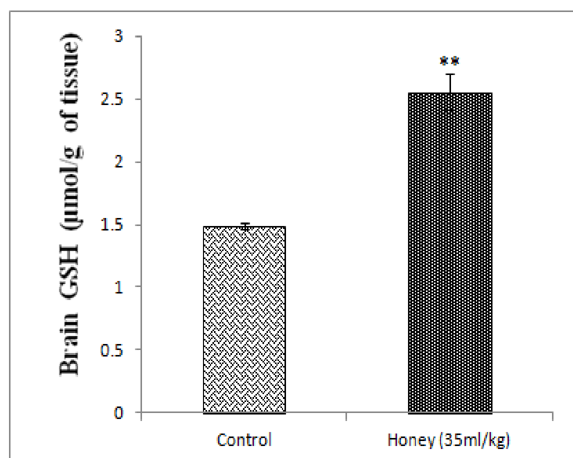

Fig 10. Effect of Honey on brain GSH levels

Values are in mean ± SEM (n = 6).
** denotes p<0.01 as compared to control group.

Honey was administered at 35 ml/kg per orally for 21 days.
Statistical analysis was carried out by one way ANOVA followed by Dunnett's t-test.

DISCUSSION

Presence of vitamin C in Honey, which is a powerful anti-oxidant helps in reducing certain symptoms of schizophrenia. Antioxidant molecules are those, which delay or prevent the oxidative damage to the target organ. 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.[11] The brain is quite vulnerable to reactive oxygen species (ROS) damage, because of its low antioxidant levels. To overcome ROS damage, the brain needs a sufficient supply of antioxidants. Therefore, ROS pathways could be beneficially modulated so as to get neuro-protective effect. Decreased levels of anti-oxidants seem to be the cause of the brain being susceptible to schizophrenic attack. Increased GSH levels correspond to enhanced free radical scavenging effect resulting in neuroprotection. Ascorbic acid or "vitamin C" is a monosaccharide antioxidant found in both animals and plants. As one of the enzymes needed to make ascorbic acid has been lost by mutation during human evolution, it must be obtained from the diet. In cells, it is maintained in its reduced form by reaction with glutathione, which can be catalyzed by protein disulfide isomerase and glutaredoxins. Ascorbic acid is a reducing agent and can reduce and thereby neutralize, reactive oxygen species such as hydrogen peroxide. Glutathione is a cysteine-containing peptide found in most forms of aerobic life. It is not required in the diet and is instead synthesized in cells from its constituent amino acids. Glutathione has antioxidant properties, since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. In cells, glutathione is maintained in the reduced form by the enzyme glutathione reductase and in turn reduces other metabolites and enzyme systems, such as ascorbate in the glutathione-ascorbate cycle, glutathione peroxidises and gluta-redoxins, as well as reacting directly with oxidants[12], [13] Due to its high concentration and its central role in maintaining the cell's redox state, glutathione is one of the most important cellular anti-oxidants. Oxidative stress occurs, where the balance between antioxidant and ROS are disrupted because of depletion of glutathione. ROS is produced by living organisms as a result of normal cellular metabolism in normal to moderate concentrations. They help in healing and protective physiological processes. But at high levels, they adversely modify cell components such as lipid protein and DNA. This shift in equilibrium from healing physiological state to pathological state is termed

as Oxidative Stress. Oxidative Stress is found to play an important role in the development of chronic disorders like cancer, Alzheimer's disease, diabetes and schizophrenia etc. Human beings have integrated anti-oxidant system built in their bodies, inclusive of enzymatic and non enzymatic pathways, which are usually effective in blocking harmful effects of ROS.[14]

ROS can be divided into 2 groups: free radicals and non radicals. Molecules containing one or more unpaired electrons and thus giving reactivity to the molecule are called free radicals. When 2 free radicals share their unpaired electrons, non radical forms are created. The 3 major ROS that are of physiological significance are superoxide anion, hydroxyl radical and hydrogen peroxide. ROS can lead to DNA modifications in several ways, which involves degradation of bases, single- or double stranded DNA breaks, purine, pyrimidine or sugar-bound modifications, mutations, deletions, translocations, and cross-linking with proteins. Most of these DNA modifications are highly relevant to carcinogenesis, ageing, neurodegenerative, cardiovascular, and autoimmune diseases. The enhanced oxidative stress can lead to modification of cellular components and induce cell damage and death. The process by which different anti-oxidants disperse through the bloodstream to protect the cells at different sites is referred to in science as "anti-oxidant synergy." When a specific anti-oxidant meets a free radical in the bloodstream at its appropriate activity site, it naturally combines with it and converts the free radical into harmless water and oxygen. Thus, as and when, anti-oxidant activity increases due to the supplementation of higher amounts of a greater variety of anti-oxidants, cellular damage lessens and health improves. The brain is uniquely vulnerable to oxidative injury, due to its high metabolic rate and elevated levels of polyunsaturated lipids. Oxidative Stress has been implicated as a potential contributor to acute central nervous system (CNS) injury by ischemic or hemorrhagic stroke or trauma.[14] The plasma levels of anti-oxidants have been shown to decrease with age. It has been shown in literature that schizophrenic patients show high level of free- radical generation leading to abnormal behaviour. Furthermore, stress is an important precipitating factor for evoking the first episode of psychosis. Stress stimulates production of free radicals forcing the individual into abnormal behaviour. GSH is highly abundant in all cell compartments and is the major soluble anti-oxidant. Ratio between oxidized glutathione and reduced glutathione is one of the important determinants of oxidative stress in the body. In the present study, Honey, when administered for 21 days significantly enhanced reduced glutathione levels in the brains of mice. This finding indicated that honey enhanced scavenging of free radicals in the brain, thereby preventing occurrence of psychotic attack. Honey is a rich source of Vitamin C also, a powerful antioxidant, which might be contributing favourably in reversing stereotypic behaviour induced by Ketamine and thereby, protect the brain from psychotic episodes. The presence of kaempferol and quercetin in honey further help in restoring cerebral blood flow and protect the neurons against inflammatory processes leading to cell injury.[15]

Schizophrenia is a common and highly disabling mental disorder without clear pathophysiology. A number of putative mechanisms have been proposed to explain the aetiology and pathogenesis of schizophrenia, which includes abnormal neuronal development, impaired neuronal transmission[16], viral infections and auto-immune dysfunction. It was observed that glutamate concentrations were reduced in the CSF of patients suffering with schizophrenia.[17] Furthermore, it was proposed that decreased glutamatergic activity may be an etiologic factor in the disorder.[18] In addition, the concentration of *N*-acetyl-aspartyl glutamate (NAAG), an acidic dipeptide, which acts as an antagonist at NMDA receptors[19] was elevated in hippocampus and the activity of glutamate carboxypeptidase II (GCP II), the enzyme that cleaves NAAG to produce glutamate and *N*-acetyl aspartate (NAA), was selectively reduced in the frontal cortex, temporal cortex and hippocampus of schizophrenic brains. Much of the transmission of excitatory information in the brain occurs via the binding of glutamate to its receptors and directly or indirectly, the activity of most neurons in the brain is influenced by this excitatory amino acid. The blockade of one specific glutamate receptor, viz. the NMDA receptor, which plays a critical role in the plasticity of nervous connections associated with learning and memory, appears to mimic certain symptoms of schizophrenia. Two of the more popular psychoactive drugs of the 1970's, phencyclidine ('Angel Dust') and Ketamine ('Special K') specifically block NMDA receptors and cause hallucinations in humans, as well as stereotyped behavior and social withdrawal in both rats and humans, thereby reproducing the symptoms of schizophrenia. Ketamine, a dissociative anesthetic, causes a schizophrenia-like psychosis in healthy individuals and exacerbates the psychotic symptoms in schizophrenic patients. In the light of these observations, it appears that schizophrenia results from hypoactivity of glutaminergic transmission in the brain. Ketamine (an established NMDA antagonist) induced stereotypic behaviour such as falling, head-bobbing, weaving and turning counts were remarkably diminished, when the animals were pre-treated with honey for 21 days. Honey is a rich source of glycine, which is an agonist for NMDA receptors. Therefore, it appears that Honey could reverse the stereotypic behaviour induced by Ketamine by displacing Ketamine from NMDA receptors. Furthermore, Honey is a rich source of glutamic acid and glutamine as well[20], which promote the synthesis of glutamate in mesolimbic pathway. This endogenous glutamate might have activated NMDA receptors, which appear to be interlinked with GABA-ergic neurons resulting in enhanced levels of GABA, decreasing release of dopamine thereby showing anti-psychotic effect. Hyperactivity of dopamine within mesolimbic

system causes bizarre symptoms (hallucinations, hostile behavior and delusions) and cognitive dysfunction. In the present study, Honey significantly decreased brain dopamine levels. This effect of Honey may be responsible for decreasing stereotypic behaviour observed in mice, corresponding to bizarre behaviour observed in humans.

There is substantial evidence showing the role of cholinergic system in the pathogenesis of schizophrenia. Post- mortem and neuro-imaging studies showed reduced number of M1 and M4 muscarinic receptors in schizophrenic patients in several key loci, including caudate nucleus, putamen, hippocampus, cingular cortex and prefrontal cortex, leading to decreased cholinergic activity. On the other hand, cholinergic drugs and anti-cholinesterase agents improved the cognitive functions of the patients suffering with schizophrenia.[21]

Honey contains fairly good amount of choline, which in turn serves as a precursor for the synthesis of acetylcholine, thereby enhancing cholinergic transmission in brain.[2] It is noteworthy to mention that choline is essential for maintaining and improving overall brain function. Furthermore, inhibition of AChE activity by Honey observed in the present study also leads to enhanced cholinergic transmission desired for improving cognitive function.

CONCLUSION

Honey, a natural sweetener derived from insects is not only a sweetener but it's a unique gift from nature to mankind. Honey has been used since ancient times to fulfil medical and domestic needs. Unfortunately, there are no reports available in literature about the neuropharmacological profile of Honey. Honey contains proteins, enzymes, amino acids, minerals, trace elements, vitamins, aromatic compounds and polyphenols, which contribute to its magical therapeutic power. In the present study, chronic administration of Honey to rodents exhibited powerful anti-psychotic activity when tested using pharmacological models such as Ketamine-induced stereotypic behaviour in mice, swim -induced grooming attempts in mice and pole climbing avoidance response in rats. Honey remarkably reduced the Ketamine induced falling, weaving, head-bobbing and turning behaviour in mice. It also reduced swim induced grooming attempts in mice. Furthermore, it reduced significantly the conditioned avoidance response in rats as reflected by reduction in time taken to climb the pole. Interestingly in our biochemical estimations, both, brain dopamine level and acetyl cholinesterase activity were significantly reduced by Honey. Whereas, brain glutathione levels were significantly increased by Honey, thereby suggesting enhanced scavenging of free radicals. These findings coupled together reveal the anti-psychotic potential of Honey. This is the first report to the best of our knowledge documenting the anti-psychotic potential of Honey in rodents. In the light of these promising pre-clinical findings, clinical trials may be carried out to establish anti-psychotic potential of Honey in patients suffering with psychosis.

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