Research Article

Investigation of the Anti-obsessive Comulsive Activity of *Pyrus communis* Juice in Mice

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ABSTRACT

Obsessive compulsive disorder (OCD) is a disabling psychiatric condition. Obsessions are defined as recurrent, persistent, thoughts, images or impulses that are experienced as intrusive & inappropriate. Compulsions are repetitive behaviours or mental acts that the person feels driven to perform in response to an obsession. There are only potent selective serotonin reuptake inhibitors (SSRIs), which are consistently effective in patients of obsessive-compulsive disorder indicating that serotonin dysfunction is the underlying cause in OCD. An outgrowing research has been done in pharmacotherapy of OCD but research into effective herbal treatments for OCD has just started. The plants have antioxidant and anti-inflammatory property can be a potential therapeutic strategy for treatment of OCD. These evidences suggest that *pyrus communis* may found to be useful in the treatment of obsessive-compulsive disorder. To evaluate the anti-OCD effect of this fruit, we used flickering light induced OCD model and marble-burying behavior in mice. The results revealed that *pyrus communis* fresh juice (50% and 100%) reduced the marble burying behaviour in mice. The effect of pyrus communis fresh juice was found to be comparable to that of fluoxetine (15 mg/kg). We also found that serotonin level and GABA level was significantly increased. In conclusion, these findings taken together reveal the anti-obsessive compulsive potential of Honey.

Keywords: *pyrus communis* juice, anti-oxidant, obsessive compulsive disorder, fluoxetine.

INTRODUCTION

Pear is a gently sweet juicy fruit with glitter and buttery texture belongs to dicotyledonous plant species of genus *pyrus communis,* (family Rosaceae)[1]. In Sanskrit, it is named as 'Amritphale' because of its immense potential in human health care. Pear has unique phyto-constituents, which have numerous medicinal properties.

Pear fruit has high nutritional value and possesses multiple medicinal properties such as anti-inflammatory, sedative, anti-pyretic, anti-oxidant, hypolipidemic, hypoglycaemic, anti-aging, analgesic, spasmolytic, anti-tussive, anti-diarrheal, wound healing, anti-microbial and hepato-protective[2].

Pear contains arbutin, which is an excellent skin-whitening agent used in several cosmetic preparations. Eating a Pear before a big drinking session can significantly reduce your blood alcohol level and hangover symptoms. Pear influences certain enzymes in our body in such a way that alcohol is metabolized quickly. As pear has low acid content, it is recommended for weaning babies, because they aren't too harsh on a baby's digestive system. Pear provides energy to the body and boosts up immune system[1].

Obsessive compulsive disorder (OCD) is a disabling psychiatric condition. Obsessions are defined as recurrent, persistent, thoughts, images or impulses that are experienced as intrusive & inappropriate.

Compulsions are repetitive behaviours or mental acts that the person feels driven to perform in response to an obsession. Prevalence of paediatric obsessive compulsive disorder (mean age 7.5 and 12.5 years) is between 2% and 4%. Lifetime prevalence of adolescent obsessive compulsive disorder is 1.9%[3].

Co-morbid states are also frequently seen in obsessive compulsive disorder such as body dysmorphic disorder, anorexia nervosa, depersonalisation, hypochondriasis, tourette's syndrome, trichotillomania, autism, binge eating, compulsive buying, kleptomania, pathological gambling, self-injurious behaviour, sexual compulsions, borderline personality disorder, anti-social personality disorder etc. Imbalance of serotonin is the main cause of the obsessive–compulsive disorder.

Serotonin reuptake inhibitors are effective in alleviating obsessions and compulsions in patients. This led to the hypothesis that dysregulation of the serotonergic (5-HT) system is involved in the pathophysiology of obsessive – compulsive disorder.

Environment factors also play a role in the onset or enhancement of obsessive – compulsive disorder symptoms[4].

In some reports, it has shown that children are very much affected by environmental conditions and this can be the cause of some types of obsessive – compulsive disorder e.g. Divorce of parents, school related problems, traumatic injury and loss of loved ones like parents, grandparent, friend or pet.

Nutritional therapy is a complementary therapy based upon the assumption that plant foods are not only a source of nutrients and energy, but may additionally provide health benefits beyond basic nutritional functions. Therefore, this project was undertaken to

investigate anti-obsessive compulsive potential of Pear fruit juice in small laboratory animals.

MATERIALS AND METHODS

Plant Material

The fresh Pear fruit (*pyrus communis*) was collected from Hisar market and got authenticated from Department of Horticulture, Haryana University of Agriculture, Hisar (Ref. Hort. R-1740/ 2016-7). Pear juice was administered in different doses (50% v/v, 100% v/v, p.o) to mice and rats.

Experimental Animals

A total of 48 adult Swiss mice divided in 8 groups weighing around 20-25g 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 hr each) in. 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. During study separate groups (6 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 (IAEC) and the care of animals was taken as per guidelines of CPCSEA, Ministry of Forests and Environment, Government of India (Registration number 0436).

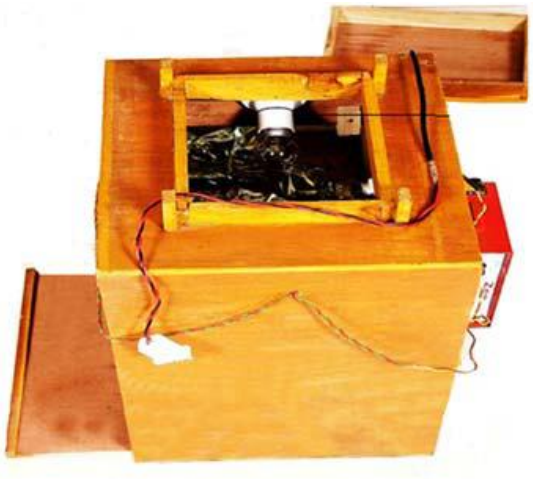
Drug Protocol

Fluoxetine (15 mg/ kg, i.p.) was administered daily for 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 Behaviour Model[5]

In this model, animals were divided into four groups and each group consisted of six animals. The control group received only saline (1ml/kg, i.p). The animals of group standard received Fluoxetine (15mg/kg, i.p) for 21 consecutive days. The animals of test groups received different concentrations of *pyrus communis* juice (50% v/v, 100% v/v, p.o) respectively, for 21 consecutive days. It was observed that when mice were exposed to flickering light continuously for a period of 1 hour they produced repetitive gnawing behaviour. This behaviour was correlated with compulsive action of patients suffering from Obsessive-compulsive disorder. 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 & flickering bulbs (15 watt) at the ceiling of the chamber. The dimensions of this unique plywood box were 36×30×45 cm^3. The thermocol pieces (4×3×1 cm3) 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 min. produced by four bulbs (15 watt) 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 & total number of gnawed pieces of thermocol was counted. 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 behaviour of mice was successfully reversed by established anti-Obsessive compulsive disorder medicines such as fluoxetine, venlafaxine, haloperidol & lorazepam. Furthermore, these animals behaved like normal mice after four days of the experiment. The validity of the flickering light induced obsessive-compulsive behaviour 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 behaviour model.



Flickering Light Induced Obsessive-Compulsive Behaviour Model

Marble-Burying Behavior Model[6]

In this model, animals were divided into four groups and each group consisted of six animals. The control group received only saline (1ml/kg, i.p). The animals of group standard received Fluoxetine (15mg/kg, i.p) for 21 consecutive days. The animals of test groups received different concentrations of *pyrus communis* juice (50% v/v, 100% v/v, p.o) respectively, for 21 consecutive days. Digging and burrowing are typical behaviour of mice species. Mice show digging behaviour in the response of novel environment. Marble-burying is a natural defence mechanism which appears in the state of stress. Marble-

burying helps in measuring the amount of digging. The Marble-burying behaviour model as describe earlier was employed in the present study. In this model, mice were individually placed in separate plastic cages (21×38×14 cm) containing 5 cm thick sawdust bedding. Twenty clean glasses marbles (diameter ~10 mm), were arranged evenly on the bedding. After 30 min exposure to the marbles, mice were removed, and unburied marbles were counted. A marble was considered buried, if its two-third size was covered with saw dust. The total number of marbles buried was considered as an index of obsessive–compulsive behaviour.

Marble-burying Behavior Model

Biochemical Estimation

Estimation of Brain Serotonin (5-Ht) Level[7]

The animals were sacrificed by cervical decapitation under light anaesthesia on the 10th day 90 min after drugs administration and 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 and 0.08 ml of supernatant phase was removed which 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. To 0.02 ml aqueous extract, 0.025 ml of OPT (O-phthaldialdehyde) reagent (20 mg in 100 ml conc. HCl) was added. The fluorophore was developed by heating to 100°C for 10 min. 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/mlm 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 conc. HCl without OPT was added.

Estimation of Brain Gaba Level[8]

Isolated brain was transferred to homogenization tube containing 5 ml of 0.01M hydrochloric acid and homogenized. Brain homogenate was transferred to bottle containing 8 ml of ice cold absolute alcohol and kept for 1 h at 0° C. The content was centrifuged for 10 min at 16000 rpm, supernatant was collected in petri-dish. Precipitate was washed with 5 ml of 75% alcohol for three times and washes were combined with supernatant. Contents in petridish 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 half an hour 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 1h at 90° C. Blue colour spot developed on paper was cut and heated with 2ml ninhydrin solution on water bath for 5 min. Water (5.0 ml) was added to solution and kept for 1h. Supernatant (2.0 ml) was decanted and absorbance was measured at 570 nm.

RESULTS

Effect of *Pyrus Communis* Juice on Gnawing Behaviour of Mice using Flickering-Light Induced Obsessive-Compulsive Disorder

Pyrus communis juice at the concentrations of 50% v/v and 100% v/v, when administered (p.o) for 21 consecutive days, showed dose dependably ($p<0.05$, $p<0.01$) reduction in gnawing behaviour of mice as compared to control group. Fluoxetine (15mg/kg; i.p.) used as a standard drug remarkably reduced gnawing behaviour in mice.

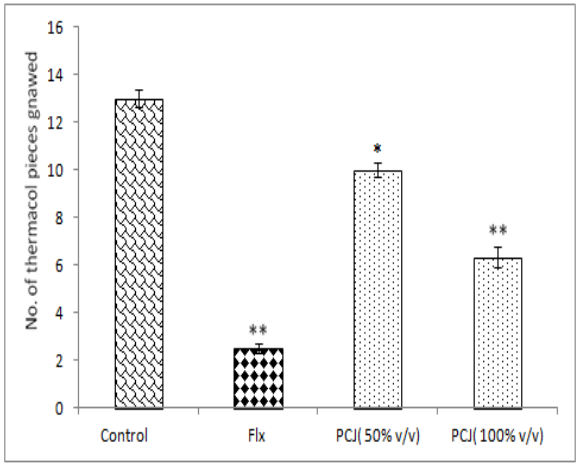


Figure 1: Effect of *pyrus communis* juice (pear juice) on gnawing behaviour of mice

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.; Flx = Fluoxetine (15mg/kg, i.p), PCJ = *Pyrus communis* juice; *Pyrus communis* juice was administered at 50% v/v and 100 % v/v per orally for 21 days.; Statistical analysis was carried out by one way ANOVA followed by Dunnett's t-test.

Effect of *Pyrus Communis* Juice on Marble-Burying Behaviour of Mice

Administration of *pyrus communis* juice at the concentration of 50% v/v and 100% v/v (p.o), for 21 consecutive days showed dose dependably ($p<0.05$, $p<0.01$) reduction in marble-burying behaviour as compared to control group. Fluoxetine (15 mg/kg; i.p.) used as a standard drug remarkably reduced marble-burying behaviour of mice.

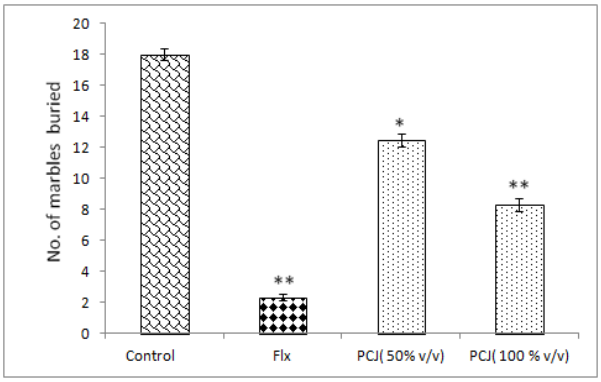


Figure 2: Effect of *pyrus communis* juice (pear juice) on marble-burying behaviour of mice using marble-burying behaviour model

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.; Flx = Fluoxetine (15mg/kg, i.p), PCJ = *Pyrus communis* juice; *Pyrus communis* juice was administered at 50% v/v and 100 % v/v per orally for 21 days.; Statistical analysis was carried out by one way ANOVA followed by Dunnett's t-test.

Effect of *Pyrus Communis* Juice on Brain Serotonin Level

Administration of *pyrus communis* juice (p.o) at the concentration of 100% v/v for 21 consecutive days showed significantly ($p<0.05$) increase in brain serotonin level in mice as compared to control group.

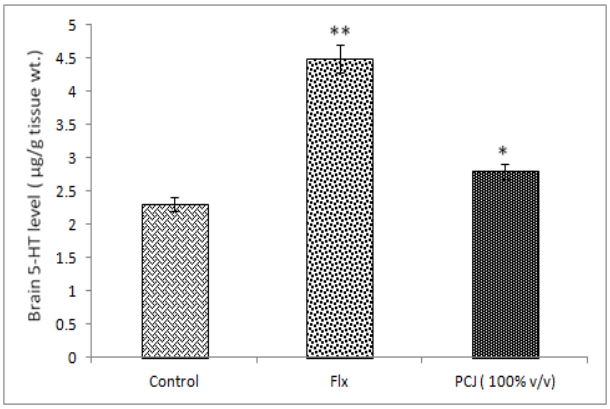


Figure 3: Effect of *pyrus communis* juice (pear juice) on brain 5-HT level in mice

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.; Flx = Fluoxetine (15mg/kg, i.p), PCJ = *Pyrus communis* juice; *Pyrus communis* was administered at 100% v/v per orally for 21 days.; Statistical analysis was carried out by one way ANOVA followed by Dunnett's t-test.

Effect of *Pyrus Communis* Juice on Brain Gaba Level

Administration of *pyrus communis* juice (p.o) at the concentration of 100% v/v for 21 consecutive days showed significantly ($p<0.05$) increase in brain GABA level in rodents as compared to control group.

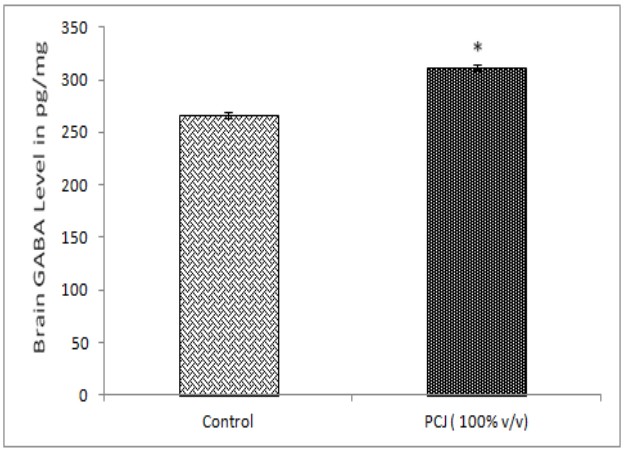


Figure 4: Effect of *pyrus communis* juice (Pear juice) on brain GABA level in mice

Values are in mean ± SEM (n = 6).; *denotes $p<0.05$ as compared to control group.; PCJ = *Pyrus communis* juice; Pyrus communis was administered at 100% v/v per orally for 21 days.; Statistical analysis was carried out by one way ANOVA followed by Dunnett's t-test.

DISCUSSION

Obsessive–compulsive disorder (OCD) is a mental disorder characterized by absurd, recurrent and uncontrollable thoughts (obsessions) that produce anxiety, which are followed by repetitive behaviours (compulsions) aimed at reducing anxiety. Obsessions are defined as recurrent, persistent, thoughts, images or impulses that are experienced as intrusive & inappropriate. Compulsions are repetitive behaviours or mental acts that the person feels driven to perform in response to an obsession. Serotonin seemly plays a more important role than dopamine in the onset as serotonin levels decreased where as dopamine increased. Serotonin is a neurotransmitter which involved in mood, appetite, sleep, pain control and cerebral activation[3]. Only potent serotonin reuptake inhibitors were consistently found to be effective in patients of obsessive compulsive disorder.

Marble-burying behaviour of mice has been used to model obsessive compulsive disorder due to the excessive nature of the behaviour and due to pharmacological effects of clinical standards.

In this model mice bury the unpleasant object (marble), which cause aversion and fearful thoughts. It is a well-accepted paradigm to screen anti-compulsive activity, as it is based on the principle that burying behaviour is an unconditioned defensive reaction in rodents[9].

When *Pyrus communis* juice was orally administration for 21 days, it significantly reduced marble-burying behaviour in mice.

The newly developed device (Patent No. 3087/DEL/2012) flickering light model was designed in our laboratory comprised of 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 behaviour.

In the standard group, there was remarkable decrease in number of thermocol pieces gnawed behaviour after administration of Fluoxetine, thereby confirming the validity of these models[5].

Furthermore, oral administration of *Pyrus communis* juice for 21 days significantly reduced the number of thermocol pieces gnawed.

Pyrus communis juice 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.

Therefore, it appears that this nutrient act through influence on serotonergic systems as there was remarkable increase in serotonin levels via increased biosynthesis of serotonin due to the presence of tryptophan[10].

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 hyper-activity and obsessive- compulsive behaviour 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 *pyrus communis* juice.

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. Oxidative stress is one of the mechanisms involved in neuronal damage induced by free radicals.

The enhanced oxidative stress can lead to modification of cellular components and induce cell damage and death.

A particularly destructive aspect of oxidative stress is the production of reactive oxygen species, which include free radicals and peroxides.

To overcome neuronal damage, the brain needs a sufficient supply of anti-oxidants which can be increases due to the supplementation of higher amounts of a greater variety of anti-oxidants. Fortunately, *pyrus communis* juice contains natural anti-oxidants like flavonoids, polyphenols, quercetin and vitamin C etc.

Thence, finding indicated that *pyrus communis* juice enhanced scavenging of free radicals in the brain, thereby preventing occurrence of obsessive-compulsive behaviour.

Therefore, all these findings reflect the anti-obsessive compulsive potential of *pyrus communis* juice.

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