Obsessive-Compulsive Disorder (OCD) is characterized by absurd, recurrent thoughts (obsessions) followed by certain stereotyped actions (compulsions). 5-Hydroxytryptamine (5-HT) abnormalities may be involved in OCD, and further, cause changes in serotonergic transmission that may have direct or indirect effects on the neuronal firing of other neuromodulators affecting thoughts, feelings and behaviors. Serotonin-related genes that are found in OCD include those coding for the 5-HT transporter (5-HTT) and receptors (5-HT2A, 5-HT2B, 5-HT2C and 5-HT1B) as well the 5-HT enzyme tryptophan hydroxylase. OCD can impair all areas of brain functioning and produce devastating effects on patients and their families. Marble-burying behavior of mice has been employed to study anxiety disorders, including the OCD. The aim of this study was to test the efficacy of aripiprazole and alcohol per se and in combination on marble-burying behavior of mice. A total of 114 male Swiss mice divided in 19 groups were studied. Aripiprazole (0.1 mg/kg, i.p.) per se as well as ethanol (0.1% w/v) per se did not show any anti-compulsive activity. But the combination comprising of ineffective doses of aripiprazole (0.1 mg/kg, i.p.) and ethanol (0.1% w/v) showed significant anti-compulsive activity as reflected by inhibition of marble-burying behavior.

Keywords: aripiprazole, ethanol, marble-burying behavior

PHARMACOLOGY

COMBINATION OF ARIPIPRAZOLE AND ETHANOL ATTENUATES MARBLE-BURYING BEHAVIOR IN MICE

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Abstract: Obsessive-Compulsive Disorder (OCD) is characterized by absurd, recurrent thoughts (obsessions) followed by certain stereotyped actions (compulsions). 5-Hydroxytryptamine (5-HT) abnormalities may be involved in OCD, and further, cause changes in serotonergic transmission that may have direct or indirect effects on the neuronal firing of other neuromodulators affecting thoughts, feelings and behaviors. Serotonin-related genes that are found in OCD include those coding for the 5-HT transporter (5-HTT) and receptors (5-HT2A, 5-HT2B, 5-HT2C and 5-HT1B) as well the 5-HT enzyme tryptophan hydroxylase (1). OCD can impair all areas of brain functioning and produce devastating effects on patients and their families. Marble-burying behavior of mice has been employed to study anxiety disorders, including the OCD. The aim of this study was to test the efficacy of aripiprazole and alcohol per se and in combination on marble-burying behavior of mice. A total of 114 male Swiss mice divided in 19 groups were studied. Aripiprazole (0.1 mg/kg, i.p.) per se as well as ethanol (0.1% w/v) per se did not show any anti-compulsive activity. But the combination comprising of ineffective doses of aripiprazole (0.1 mg/kg, i.p.) and ethanol (0.1% w/v) showed significant anti-compulsive activity as reflected by inhibition of marble-burying behavior.

Keywords: aripiprazole, ethanol, marble-burying behavior

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in combination were able to inhibit marble-burying behavior of mice. The combination of aripiprazole and alcohol should evoke an absence of any toxicity, observed with the high doses of these drugs.

MATERIALS AND METHODS

Animals
All the experiments were carried out in adult male Swiss mice (22–25 g), housed under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25 ± 2°C, 55 ± 2%, respectively). They received standard rodent chow (Goldmohar brand, Lipton India Ltd.) and water ad libitum. Separate groups of mice were used for each set of experiments and each animal was used only once. The experimental protocol was approved by Institutional Animals Ethics Committee (IAEC). The care of animal was taken according to the guidelines of CPCSEA, Ministry of Environment and Forests, Government of India, New Delhi, India.

Marble-burying behavior model
The marble-burying behavior model, as described earlier (12), 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 glass marbles (diameter ~10 mm), were arranged evenly on the bedding. After 30 min exposure to the mar-

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![Figure 1](image1.png)

Figure 1. Effect of aripiprazole on marble-burying behavior of mice. Marble-burying behavior was tested in separate groups of mice. Each bar represents the mean ± SEM. *denotes p < 0.001 as compared to control group (one-way ANOVA followed by Tukey test for multiple comparisons). Ar 0.1 = aripiprazole 0.1 mg/kg, i.p., Ar 0.5 = aripiprazole 0.5 mg/kg, i.p., Ar 4 = aripiprazole 4 mg/kg, i.p., Ar 6 = aripiprazole 6 mg/kg, i.p.

![Figure 2](image2.png)

Figure 2. Effect of aripiprazole (0.1, 0.5, 4 and 6 mg/kg, i.p.) on locomotor activity of mice using actophotometer. Motor activity was tested in separate groups of mice. Each bar represents the mean ± SEM. Bars marked as in Figure 1.
Synthesis and biologically active heterocycles from coumarin thiocyanate

Figure. 3. Effect of ethanol on marble-burying behavior of mice. Marble-burying behavior was tested in separate groups of mice. Each bar represents the mean ± SEM. *denotes p < 0.001 as compared to control group (one-way ANOVA followed by Tukey test for multiple comparisons). E 0.1 = ethanol 0.1% w/v, E 1.2 = ethanol 1.2% w/v, E 2.4 = ethanol 2.4% w/v, E 4.8 = ethanol 4.8% w/v

Figure. 4. Effect of ethanol (0.1, 1.2, 2.4 and 4.8% w/v) on locomotor activity of mice using actophotometer. Motor activity was tested in separate groups of mice. Each bar represents the mean ± SEM. Bars marked as in Figure 3

bles, 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 behavior.

Materials

Aripiprazole was gifted by Ind-Swift Laboratories Ltd., India. It was suspended in a vehicle consisting of Tween 5% in 0.9% saline. Ethanol was an analytical grade (99.9%, Changshu Yangyuan Chemical, China).

Treatments

Mice were divided into 19 groups and each group consisted of a minimum of six animals. Separate animals were used for each experiment.

Group I: Control group for young mice (n = 6).

Groups II, III, IV, V, VI, VII, VIII, IX: aripiprazole (0.1, 0.5, 4 and 6 mg/kg, i.p.) was injected to young male mice 30 min prior to the assessment of marble-burying behavior/locomotor activity.

Groups X, XI, XII, XIII, XIV, XV, XVI, XVII: ethanol (0.1, 1.2, 2.4 and 4.8% w/v) was injected to young male mice 30 min prior to the assessment of marble-burying behavior/locomotor activity.

Group XVIII, XIX: aripiprazole (0.1 mg/kg, i.p.) was injected 30 min prior to the administration of ethanol (0.1% w/v) for the assessment of marble-burying behavior/locomotor activity.

In the first set of experiments, aripiprazole (0.1, 0.5, 4 and 6 mg/kg, i.p.) and ethanol (0.1, 1.2, 2.4 and 4.8% w/v) were administered 30 min prior to the assessment of marble-burying behavior or
locomotor activity. In the second set of experiments, aripiprazole (0.1 mg/kg, i.p.) was injected 30 min prior to the administration of ethanol (0.1% w/v). Thirty minutes after the administration of ethanol, mice were subjected to above behavioral tests.

Actophotometer

Motor activity was assessed in separate groups of mice using actophotometer (Techno, Lukhnow), which had a circular arena of 40 cm, equipped with three infrared beams and photo-cells connected to digital counter. Motor activity was assessed in terms of total number of counts of light beam interruptions in 30 min.

Statistical analysis

The data were analyzed with one-way ANOVA followed by Tukey test for multiple comparisons. The values were expressed as the mean ± SEM; p < 0.05 was considered to be statistically significant in all cases.
RESULTS

Effect of aripiprazole on marble-burying behavior and motor activity in mice

Low dose of aripiprazole (0.1 mg/kg, i.p.) did not produce any significant effect on marble-burying behavior of mice, but high doses of aripiprazole (0.5, 4 and 6 mg/kg, i.p.) significantly reduced (p < 0.001) the number of marbles buried by mice (Fig. 1). Furthermore, aripiprazole (0.1 mg/kg, i.p.) did not show any significant effect on locomotor activity of mice, when measured using actophotometer (Fig. 2).

Effect of ethanol on marble-burying behavior and motor activity in mice

Low dose of ethanol (0.1% w/v) did not produce any effect on the marble-burying behavior of mice but ethanol in higher doses (1.2, 2.4 and 4.8% w/v) significantly inhibited (p < 0.001) the marble-burying behavior of mice (Fig. 3). Furthermore, ethanol (0.1% w/v) did not show any significant effect on locomotor activity of mice (Fig. 4).

Effect of aripiprazole plus ethanol on marble-burying behavior and motor activity in mice

The combination of low doses of aripiprazole (0.1 mg/kg, i.p.) and ethanol (0.1% w/v) significantly (p < 0.001) inhibited the number of marbles buried by mice (Fig. 5). Furthermore, this combination did not exhibit any significant effect on the locomotor function of mice (Fig. 6).

DISCUSSION

OCD may be defined as the irruption in the mind of uncontrollable, egodystonic and recurrent thoughts, impulses or images. It is characterized by unconscious conflicts, which were defensive and punitive (13). Marble-burying by mice has been used to model anxiety disorders including OCD due to the excessive nature of the behavior, as well as pharmacological effects of clinical standards (14). LHRH antagonist attenuates the effect of fluoxetine on marble-burying behavior of mice (12).

In the present study, aripiprazole (0.1 mg/kg, i.p.) and ethanol (0.1% w/v) independently did not produce any significant effect on marble-burying behavior of mice (15, 16) but, the combination of aripiprazole (0.1 mg/kg, i.p.) and ethanol (0.1% w/v) attenuated their marble-burying behavior. However, aripiprazole at higher doses (0.5, 4 and 6 mg/kg, i.p.) did inhibit marble-burying behavior of mice. Also ethanol at higher doses (1.2, 2.4 and 4.8% w/v) inhibited marble-burying behavior of mice. However, this inhibition of marble-burying behavior of mice can be attributed to the anxiolytic effect of alcohol at higher doses. Further, ethanol (2.4 and 4.8% w/v) showed significant enhancement in locomotor function of mice.

In view of above, we had selected such low doses of aripiprazole (0.1 mg/kg, i.p.) and ethanol (0.1% w/v) which did not produce any effect on marble-burying behavior on their own. Thus, it appears that at these low doses, aripiprazole and ethanol when administered in combination may be acting synergistically to produce anti-compulsive effect. Another advantage of this combination would be the absence of any toxicity, observed with higher doses of these compounds. The combination used did not exhibit any significant effect on the locomotor function of mice. Aripiprazole is the first next-generation atypical antipsychotic that is active against both positive and negative symptoms of schizophrenia, has a low propensity for extrapyramidal side effects, causes minimal weight gain or sedation (9). Aripiprazole is a dopamine system stabilizer with potent partial agonist activity at 5-HT1A receptors and antagonist activity at 5-HT2A receptors (17, 18). Ethanol is a sedative-hypnotic substance that has motor stimulant effects at low to moderate doses, and sedative or ataxic effects at higher doses (10, 11). At low or moderate doses, alcohol primarily acts as an unselective γ-aminobutyric acid (GABA) agonist. One of the classical properties of single-dose administered ethanol is the reduction of anxiety (19). It has been demonstrated in humans and rodents that, at moderate doses, ethanol has anxiolytic effects in a variety of experimental paradigms (20, 21). 5-Hydroxytryptamine (5-HT) abnormalities may be involved in OCD and, further, cause changes in serotonergic transmission that may have direct or indirect effects on the neuronal firing of other neuromodulators affecting thoughts, feelings and behaviors. An antidepressant mirtazapine, which is a 5-HT2A antagonist, has shown to be of benefit to OCD patients (22). The 5-HT2A receptors are located on GABA-containing interneurons, which are known as modulatory interneurons present in the deep layers of the cerebral cortex. These modulatory interneurons are a potential site of the anxiolytics and antidepressants actions of 5-HT2A antagonists, as well as very low levels in basal ganglia and thalamus (23). The 5-HT2A receptor antagonists have been found to be anxiolytic in some animal models (24). Activation of 5-HT2A receptor releases serotonin, which appears to modulate the stress activated hypothalamic-pituitary-adrenal (HPA) axis hormonal response by facilitating the
release of stress hormones like adrenocorticotrophic hormone (ACTH), oxytocin, prolactin, and corticosterone, whereas 5-HT\textsubscript{2A/C} antagonists tend to block the increase in corticosterone levels (25).

The combination of aripiprazole (0.1 mg/kg, i.p.) and ethanol (0.1% w/v) attenuated marble-burying behavior in mice, thereby suggesting that this combination had anti-compulsive effect. This may be due to synergistic action, which strongly potentiates anxiolytic action of aripiprazole via blocking of 5-HT\textsubscript{2A} receptor and weak agonistic action on the GABA\textsubscript{A} receptor.

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