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Bacopa monnieri Inhibit the Ethanol-Withdrawal Syndrome on Marble-Burying Behavior in C-57/Mice

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ABSTRACT

Obsessive–compulsive disorder is an anxiety disorder, characterized by persistent thoughts (obsessions) that are ego-dystonic and associated with apparently purposeful behaviors (compulsions). Obsessive–compulsive disorder is often evident in alcohol dependent patients, particularly in state of abstinence. The obsessive thoughts about cleanliness, exactness and household tools responsible for anxiety are apparently neutralized by repetitive rituals such as excessive and repetitive cleaning, arranging, checking and rechecking. Moreover, both the conditions are associated with similar neurohumoral changes; e.g., serotonin dysfunction, hyperactivity of dopamine and glutamate. Alterations in the serotonergic system have been implicated in OCD, although the precise mechanisms underlying these abnormalities have not been identified. Only potent serotonin reuptake inhibitors (SSRIs) are consistently effective in patients of OCD. Bacopa monnieri (Brahmi, Family: Scrophulariaceae), a traditional Ayurvedic medicinal plant is extensively used for centuries for the treatment of epilepsy, insomnia and anxiety and also as a mild sedative and memory enhancer. Marble-burying behavior of mice simulates some aspects of obsessive–compulsive behavior; therefore, it is often used to screen anti-compulsive drugs due to high predictive and good face validity. In this study after BM treatment, we found more significant upregulation in the synthesis of 5-HT, alternation in the ACh level and reduction in DA level. The prominent 5-HT level probably activates their receptor, which make easy the release of Ach.

Keywords: *Obsessive–Compulsive Disorder, Bacopa monnieri, Sedative, Marble-Burying Behavior, Serotonin.*

INTRODUCTION

Obsessive–compulsive disorder is an anxiety disorder, characterized by persistent thoughts (obsessions) that are ego-dystonic and associated with apparently purposeful behaviors (compulsions) [1,2]. Obsessive–compulsive disorder is often evident in alcohol dependent patients [3], particularly in state of abstinence [4].

It is suggested that the characteristic behavior of chronic alcohol users may derive at least in part, from the co-morbidities existing obsessive–compulsive behavior [5]. The obsessive thoughts about cleanliness, exactness and household tools responsible for anxiety are apparently neutralized by repetitive rituals such as excessive and repetitive cleaning, arranging, checking and rechecking [6]. Moreover, both the conditions are associated with similar neurohumoral changes; *e.g.,* serotonin dysfunction, hyperactivity of dopamine and glutamate [7-10].

Alterations in the serotonergic system have been implicated in OCD, although the precise mechanisms underlying these abnormalities have not been identified. Only potent serotonin reuptake inhibitors

(SSRIs) are consistently effective in patients of OCD [11]. It has been reported that the marble burying behavior is inhibited by anxiolytic several selective serotonin reuptake inhibitors (SSRIs), fluvoxamine, sertraline and paroxetine [12].

Bacopa monnieri (Brahmi, Family: Scrophulariaceae), a traditional Ayurvedic medicinal plant is extensively used for centuries for the treatment of epilepsy, insomnia and anxiety and also as a mild sedative and memory enhancer [13, 14, 15].

Bacopa monnieri (BM) has been in use since time immemorial as a nerve tonic for improvement of memory. Besides, *BM* displays antioxidant, antistress, anxiolytic properties in experimental animals [16, 17]. In previous study, they were analyzed how *BM* regulates memory and learning through the GABA receptor in the stratum. It is currently recognized as being effective in the treatment of mental illness[18].

Marble-burying behavior of mice simulates some aspects of obsessive–compulsive behavior; therefore, it is often used to screen anti-compulsive drugs due to high predictive and good face validity [19]. Therefore, the effect of *BM* inhibits ethanol-withdrawal syndrome was investigated on marble-burying behavior in C57/mice.

MATERIALS AND METHODS

Animals

Adult male albino Swiss mice (22–25 g) were group housed (n=6–10) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Mice were purchased from National Institute of Nutrition, Hyderabad, India. The animal studies were approved by the Institutional Animal Ethics Committee (Reg. No. 831/BC/04/CPCSEA), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India. Animals were naive to drug treatment and experimentation at the beginning of all studies. Each experimental group was comprised of six mice. Testing was carried out in a counterbalanced order with respect to the treatment conditions in the noise free room.

Drug and Chemicals

Bacopa monnieri (Brahmi capsule, 250 mg per capsule) was purchased from Himalaya Herbal Health Care, Bengaluru, India. Fluoxetine (Cadila, India) used in the present study was dissolved in 0.9% saline. Other chemicals used in the present investigation were of analytical grade.

Ethanol-Withdrawal State

The ethanol-withdrawal state was produced in experimental (ethanol diet) group as described previously. In brief, on day 1, all groups received a nutritionally balanced liquid diet containing sucrose (9.68% w/v) and vitamin supplement in 100 ml calibrated drinking bottle.

From day 2, (8.00 h) till day 9 (8.00 h), the experimental group received a liquid diet containing ethanol (5.96% w/v) instead of sucrose; while the control group was pair-fed sucrose and vitamin containing liquid diet. On day 9 (8.00 h), ethanol was replaced with sucrose (ethanol-withdrawal) [20].

Assessment of Marble-Burying Behavior and Locomotor Activity

Marble-burying behavior of mice was studied as described previously [21]. In brief, each mouse was individually placed in a plastic cage (21×38×14 cm), containing 5 cm thick sawdust bedding,

and three photo cells connected to digital counter. Twenty small glass marbles (diameter 10–12 mm) were arranged on the bedding evenly spaced in four rows.

After 30min exposure, the number of unburied marbles was counted. A marble covered at least two-third of its size by seeing dust was considered as 'buried'. Total number of light beam interruptions in 30min were considered as an index of locomotor activity.

Influence of Ethanol-Withdrawal on Marble-Burying Behavior in Mice

Marble-burying behavior was measured at 0, 6, 24, 48, and 96 h time interval after ethanol withdrawal of mice. The time interval at which animal buried maximum marble was recorded in experimental (ethanol diet) group. The locomotor activity was recorded simultaneously by the using of the same animals.

Effect of Acute Treatment with BM or Fluoxetine on Marble-Burying Behavior after Ethanol-Withdrawal

The experiment shows that experimental (ethanol diet) group exhibited maximum marble-burying at 24 h time interval after the withdrawal of ethanol.

Therefore, the experimental group was treated with BM (0.05 and 0.1%, p.o.) or fluoxetine (10 and 30mg/kg, p.o.) or vehicle (10ml/kg, p.o.), 30 min prior to the recording of marble-burying behavior and locomotor activity at 24 h time interval after ethanol-withdrawal. For each of the above treatment and dose, separate group (n=10) of mice was employed.

Effect of Chronic Treatment with BM Or Fluoxetine on Marble-Burying Behavior After Ethanol-Withdrawal

In another set of experiments, the experimental (ethanol diet) group was treated twice daily (8.00 h and 20.00 h) with BM (0.05 and 0.1%, p.o.) or fluoxetine (10 and 30mg/kg, p.o.) or vehicle (10 ml/kg, p.o.) whereas the control (sucrose diet) group daily received saline (10 ml/kg, p.o.).

The above drug treatment was terminated on day 9 at 08.00 h *i.e.*, a time at which ethanol was withdrawn. Marble-burying behavior and locomotor activity was recorded at 0, 6, 24, 48, and 96 h following ethanol-withdrawal.

Statistical Analysis

The results are expressed as mean ± S.E.M. Data from chronic BM /fluoxetine treated group were analyzed using two-way ANOVA followed by Bonferroni test. Data from acute BM/fluoxetine treated group were analyzed by one way ANOVA followed by Newman–Keuls test for multiple comparisons. $p<0.05$ was considered statistically significant in all the cases. The statistical difference was regarded as p value < 0.05.

RESULTS

Influence of Ethanol-Withdrawal on Marble-Burying Behavior in Mice

Two-way ANOVA followed by Bonferroni test revealed that in the ethanol-withdrawal state, the marble-burying behavior was significantly higher at 6, 24, 48 and 96 h time interval compared to control (sucrose diet) group with its peak at 24 h time interval (Fig.1). Two-way ANOVA revealed a significant ethanol-withdrawal effect [$F(4, 10) = 64.07$, $p<0.0001$].

However, locomotor activity in the ethanol - withdrawal state was unaffected [$F(4, 10) = 3.29$, $p = 0.5938$].

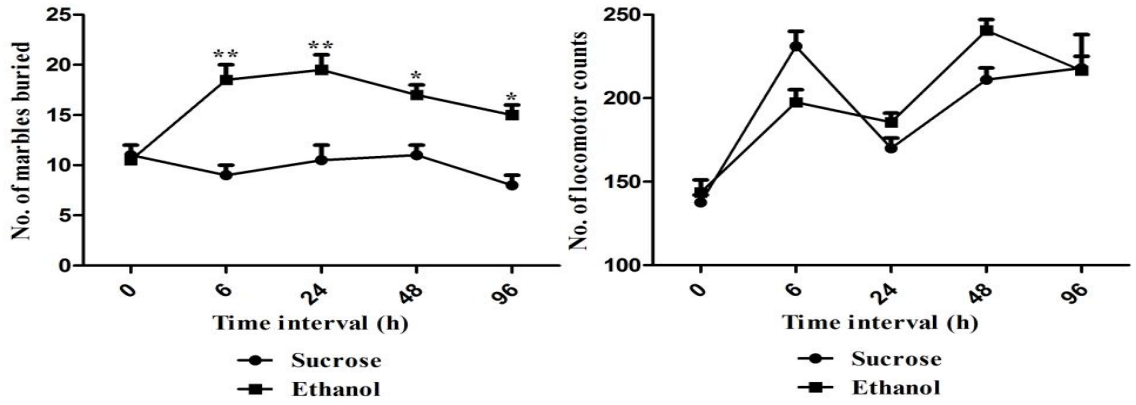

*Fig. 1: Influence of ethanol-withdrawal on marble-burying behavior in mice: On day 1, control (sucrose diet) and experimental (ethanol diet) groups received sucrose (9.68%w/v) and vitamins containing liquid diet. From day 2 (8.00 h) to day 9 (8.00 h), ethanol (5.96% w/v) was added to a liquid diet of the experimental group. Control group was pair-fed isocaloric liquid diet. On day 9, ethanol was withdrawn and the marble-burying behavior along with locomotor activity was assessed at 0, 6, 24, 48, and 96 h intervals. Values are expressed as mean±S.E.M (n = 10). Values are statistically significant at *p <0.001 vs. respective control group (Two-way ANOVA followed by Bonferroni test).*

Effect of Acute Treatment with BM or Fluoxetine on Marble-Burying Behavior after Ethanol-Withdrawal

One-way ANOVA followed by Newman–Keuls test revealed that acute treatment with BM (0.05 and 0.1%, p.o.), dose dependently inhibited the peak increase in the marble-burying behavior in ethanol-withdrawal state [F(2,15)=56.81, p<0.0001] as shown in Fig. 2. Fluoxetine (10 and 30 mg/kg, p.o.) had a similar effect [F(2,15)=52.77, p<0.0001]. However, BM [F(2,15)=0.2823, p=0.7580] and Fluoxetine [F(2,15)=0.3114, p=0.7371] did not show any effect on locomotor activity in the ethanol withdrawal state.

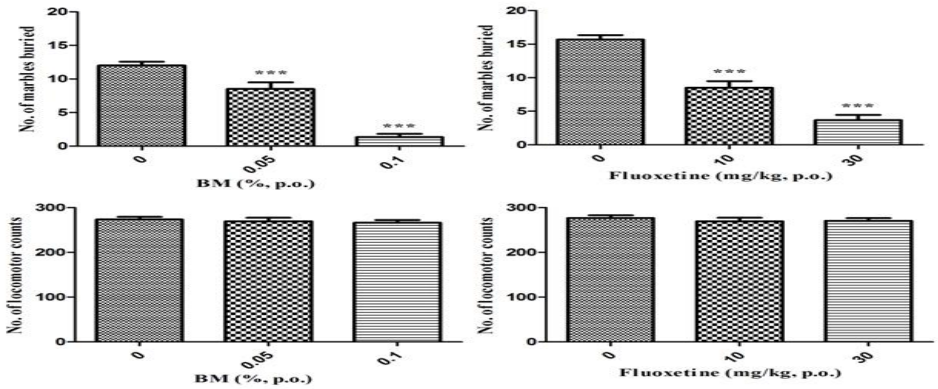

Fig. 2*: Effect of acute treatment with BM or fluoxetine on marble-burying behavior after ethanol withdrawal: On day 10, 24 h after ethanol-withdrawal, experimental (ethanol diet) groups were treated with BM (0.05 and 0.1%, p.o.) or fluoxetine (10 and 30 mg/kg, p.o.) or vehicle (10 ml/kg, p.o.), and after 30 min, marble-burying behavior and locomotor activity of individual mouse was assessed. Values are expressed as mean±S.E.M (n = 10). Values are statistically significant at *p <0.001 vs. respective control group (One-way ANOVA followed by Newman–Keuls post hoc test).*

Effect of Chronic Treatment with BM or Fluoxetine on Marble-Burying Behavior after Ethanol-Withdrawal

Two-way ANOVA followed by Bonferroni test revealed that chronic treatment with BM (0.05 and 0.1%, p.o.), dose dependently inhibited the peak increase in the marble-burying behavior in ethanol withdrawal state [F(3,20)=119.38, p<0.0001] as shown in Fig. 3. Fluoxetine (10 and 30 mg/kg, p.o.) had a similar effect [F(3,20)=123.58, p<0.0001].

Chronic treatment with BM (0.05 and 0.1%, p.o.) [F(3,20)=1.26, p=0.3147], and fluoxetine (10 and 30 mg/kg, p.o.) [F(3,20)=1.05, p=0.3930] was shows not significant effect on the locomotive activity of ethanol treated groups of mice.

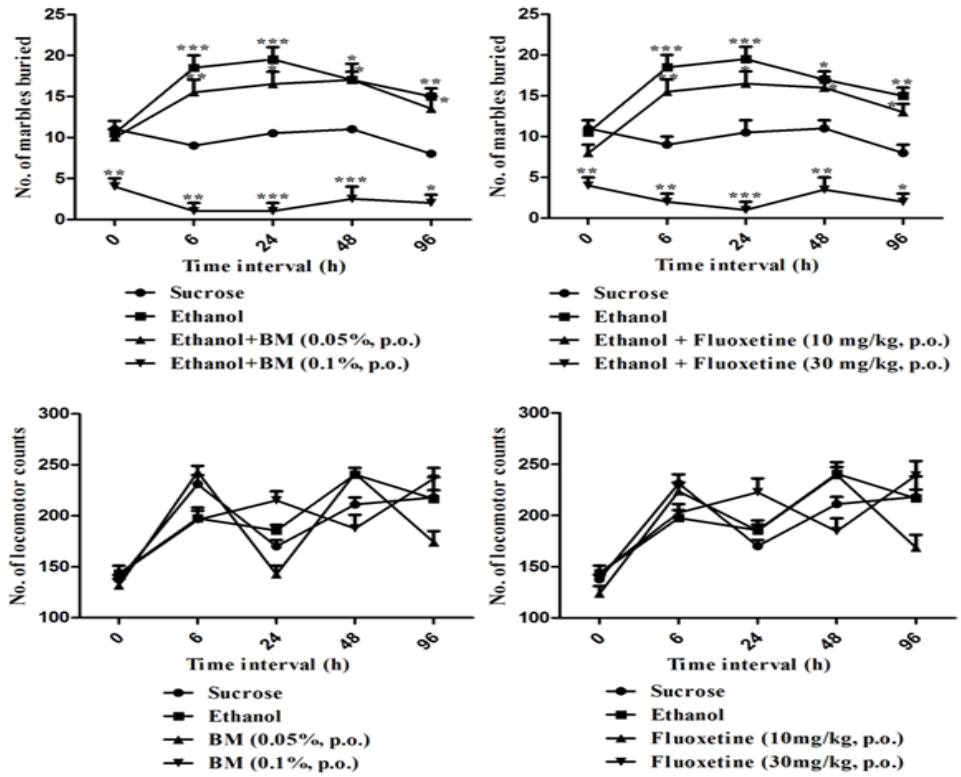

*Fig. 3: Effect of chronic treatment with BM or fluoxetine on marble-burying behavior after ethanol-withdrawal: Experimental (ethanol diet) groups were treated with BM (0.05 and 0.1%, p.o.) or fluoxetine (10 and 30 mg/ kg, p.o.) or vehicle (10 ml/kg, p.o.) twice daily (8.00 h and 20.00 h). Control (sucrose diet) group was treated daily with saline (10 ml/kg, p.o.). On the 9th day, ethanol was withdrawn; marble-burying behavior and locomotor activity of individual groups of mouse was examined at 0, 6, 24, 48, and 96 h time intervals. Values are expressed as mean±S.E.M (n = 10). Values are statistically significant at *p <0.05 vs. respective control group (saline treated), p<0.05 vs. respective vehicle treated experimental group (Two-way ANOVA followed by Bonferroni test).*

DISCUSSION

To investigate the effect of BM on neurotransmitter mediated learning and memory, we provide BM (40 mg/kg) extract orally toThe balanced function of various neurotransmitters such as acetylcholine, serotonin, catecholamine [22], GABA [23] and glutamate [24] were all reported to involve in the regulation of memory formation. The have been reported that the Bacopa monnieri treatment increased the 5-HT level in the hippocampus, hypothalamus and cerebral cortex [25], and it may also modify the Ach

concentration directly or indirectly through other neurotransmitter systems.

In this study after BM treatment, we found more significant upregulation in the synthesis of 5-HT, alternation in the ACh level and a reduction in DA level. The prominent 5-HT level probably activates their receptor, which make easy the release of Ach [26]. Remarkably on the other hand the inhibitory effects of cholinesterase activity of Bacopa monnieri also alter the ACh level and enhance memory [27, 28]. The decreased cholinesterase activity may reduce the DA level and excess acetylcholine turnover, which in addition boost the memory [29]. In the previous study B. monniera has been documented to provide neuroprotection against cigarette smoke induced apoptosis [30,31, 32, 33, 34] and aluminium induced oxidative stress [35]. In this study, B. monniera reduced the infarct size similar to sodium selenite thereby validating the neuroprotective effects of this drug. These observations clearly suggested that BM treatment enhances the learning ability and memory, possibly through modulating the 5-HT synthesis and its transportation.

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