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EVALUATION OF ANTI-COMPULSIVE EFFECT OF ETHANOLIC EXTRACT OF BACOPA MONNIERI L. LEAVES IN MICE

Trupti A. Nimburkar¹, Anjali M. Wankhade[†], Manish M. Wanjari²

¹Vidyabharati College of Pharmacy, C.K. Naidu road, Camp, Amravati -444602 (Maharashtra) India.

²Central Research Institute (Ayurveda), Opposite Jayarog Hospital, Aamkho, Lashkar, Gwalior - 474 009 (Madhya Pradesh), India.

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ABSTRACT

Obsessive compulsive disorder (OCD) is a mental health condition where a person has obsessive thoughts and compulsive activity. OCD can impair all areas of brain functioning and produce devastating effects on patients and their families. Marble-burying behavior of mice is a well-accepted paradigm to screen anti-compulsive activity. The aim of present study was to evaluate the anti-compulsive effect of *Bacopa monnieri* extract in mice. The present investigation revealed a significant decrease in marble-burying behavior of mice after oral administration of EEBM (100, 200 and 400 mg/kg) and exhibit anti OCD like effect and the effect of EEBM was comparable to standard anti OCD drug Fluoxetine which also reduce the marble burying behavior in accordance with the previous findings. The maximum effective dose of EEBM was 200mg/kg, at which there was no change in the motor activity. The dose of 400mg/kg also exhibited inhibitory effect on marble burying behavior, but it also reduced the motor activity, hence it is not clear whether the effect of EEBM at 400mg/kg is perse effect or it is due to inhibition of motor activity. The experimental results of this study shows that ethanolic extract of *Bacopa monnieri* possess anticomulsive activities.

Corresponding author

ANJALI M. WANKHADE

Vidyabharati College of Pharmacy,
C.K. Naidu Road, Camp, Amravati -444602 (Maharashtra) India.
9422040625,
anju.wankhade@rediffmail.com

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INTRODUCTION

Bacopa monnieri L. (family *Scrophulariaceae*) is a traditional medicinal plant in India, commonly known as brahmi. Genus *Bacopa* include over 100 species of aquatic herbs distributed throughout the warmer regions of the world. [1] *Bacopa monnieri* has been used for centuries in Ayurvedic medicine, either alone or in combination with other herbs, as a memory and learning enhancer, sedative, anti-epileptic, antidepressant and antianxiety. [2] Animal studies of *Bacopa monnieri* whole plant or alcohol extracts have reported cognition enhancing effects including improved motor learning and acquisition, consolidation, and retention of memory. [3-4] which suggests that *Bacopa monnieri* influences various neurotransmitter systems including serotonergic system.

Obsessive-compulsive disorder (OCD) is characterized by persistent ritualistic thoughts (obsession) which are ego-diatomic and associated with seemingly purposeful behaviors (compulsions). [5] Its co-morbidity with major depression is often evident, and it is considered as an anxiety disorder. [6] Potent serotonin reuptake inhibitors (SSRIs) are consistently effective in patients of obsessive-compulsive disorder [7] which indicates 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. Those plants which are used to treat anxiety and depression can be a potential therapeutic strategy for treatment of OCD. [8] Evidence shows that *Bacopa monnieri* may be useful in the treatment of obsessive-compulsive disorder. Therefore, the influence of ethanolic extract of *Bacopa monnieri* was investigated on the marble-burying behavior of mice a well-accepted model of obsessive-compulsive behavior, due to its high face and predictive validity. [9]

MATERIALS AND METHODS

Collection and identification of plant materials:

The plant material was collected from the medicinal garden of Amravati, Dist-Amravati January 2017. The plant was identified by Taxonomist. The required plant part was separated for other extraneous matter and subjected to shade drying.

Preparation of plant extract:

The extraction was performed by the process of maceration. About 250 g of powdered material was soaked in 800 ml ethanol at 25 ± 2 °C for 72 h in a beaker and mixture needs to be stirred every 18 h using a sterile glass rod. Filtrate was obtained 3 times with the help of Whatman No. 1 filter paper and sterilized cotton filter. The solvent was removed by rotary evaporator and the yield of the extract was obtained. [10]

Drugs and administrations

Fluoxetine hydrochloride was gifted by Cadila, Ahmedabad, India. Ethanolic extract of *Bacopa monnieri* (EEBM) and Fluoxetine HCl were prepared in 0.9% saline. Fluoxetine HCl was administered intraperitoneally. EEBM and saline were administered orally. All drug solutions were prepared fresh.

Animals

The Swiss mice (weighing 20-25g), were obtained from the animal house of department of Pharmacology, Vidyabharti college of Pharmacy, Amravati. All the animals were acclimatized to the animal house prior to use. They were kept in cages in animal house with a 12hr light: 12hr dark cycle at temperature (25 ± 1 °C) with $50 \pm 55\%$ of relative humidity. Animals were fed on pellets and tap water *ad libitum*. The care and handling of mice were in accordance with the internationally accepted standard guideline for use of animals (CPCSEA). Experiments were performed in accordance with the committee for the purpose of control and supervision of experimental animals (CPCSEA) (1504/PO/Re/S/11/CPCSEA dated 9/8/16) guidelines after the approval of the experimental protocol by the Institutional animals ethical committee (IAEC).

Phytochemical screening:

The crude extracts of *Bacopa monnieri* L. were subjected to preliminary phytochemical screening for their presence and absence of active phytoconstituents. [11]

Experimental design:

Mice were divided into different groups (n=6). EEBM (100, 200, 400 mg/kg) po, fluoxetine (15 mg/kg). EEBM were administered orally (po) and fluoxetine were administered intraperitoneally (ip) 30 min prior to the assessment of marble-burying behavior and locomotor activity. The control group received 0.9% saline (10 mL/kg, po). After 30 min, the marble-burying behavior and motor activity were assessed in separate groups.

Evaluation of Anticompulsive activity:**Marble burying behavior**

Marble-burying behavior model was used for studying the OCD in mice. Mice were individually placed in separate plastic cages (21 × 38 × 14 cm) containing 20 clean glass marbles (10 mm diameter) evenly spaced on 5 cm deep saw dust. After 30 min exposure to the marbles, mice were removed and results were expressed as number of marbles buried at least two-third in saw dust. The total number of marbles buried was considered as an index of obsessive-compulsive behavior.^[12]

Motor activity by actophotometer

The locomotor activity was measured using an actophotometer. The movement of the animal cuts off a beam light falling on the photocell and a count was recorded and displayed digitally. Motor activity was assessed in terms of total number of counts of light beam inter-ruptions in 10 min. An acquisition period of 5 min was given to each mouse before assessment of motor activity.^[13]

Statistical analysis:

The data were expressed as a mean ± SEM. Results were analyzed statistically by one way ANOVA followed by Dunnet test. Statistical significant was considered as P < 0.05 in all cases.

RESULTS**Preliminary Phytochemical Screening**

Phytochemical test of *Bacopa monnieri* revealed the presence of tannin, saponine, flavonoid, steroid, terpenoids.

Table no.1: Phytochemical investigation of bacopa monnieri.

Sr no.	Natural product	Inference
1	Alkaloids	Negative
2	Saponine	Positive
3	carbohydrate	Negative
4	Protein	Negative
5	Flavonoid	Positive
6	Steroid	Positive
7	Amino acid	Negative
8	Tannin	Positive
9	Glycosides	Negative
10	Terpenoid	Positive

Effect of EEBM on marble burying behavior and motor activity:

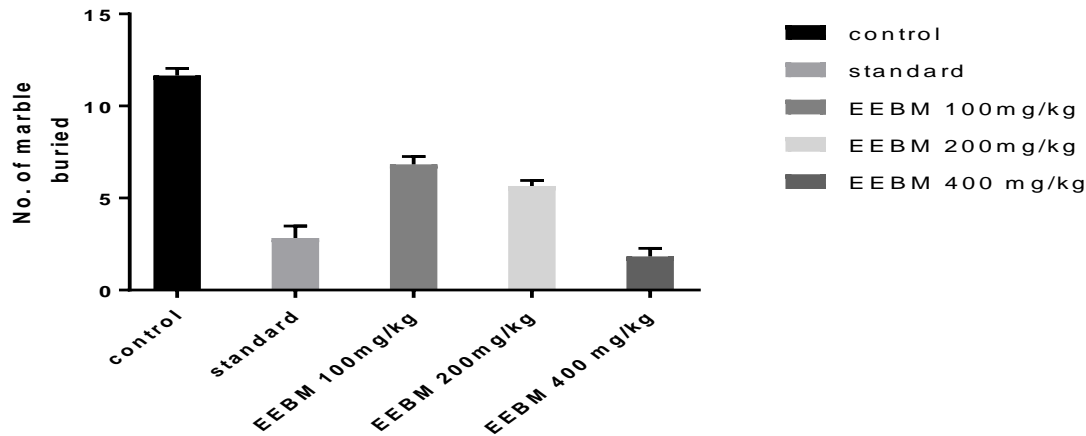
One way ANOVA exhibited that EEBM significantly influenced marble burying behavior. The Dunnett's test showed that the EEBM 100, 200 and 400 mg/kg significantly reduced the number of marbles buried (Table no.2 and fig no. 1) Motor activity was not affected by EEBM at the dose of 100 and 200 mg/kg, however EEBM at a dose of 400mg/kg significantly suppressed the locomotor activity (Table no.3 and fig no. 2).

Marble burying behavior**Table no.2: Effect of ethanolic extract of bacopa monnieri on marble burying behavior in mice.**

Treatment	Dose	No. of marble buried Mean ± SEM
Control	10 ml/kg	11.66 ± 0.38
Standard (Fluoxetine)	15 mg/kg	1.66 ± 0.20**
EEBM	100 mg/kg	6.83 ± 0.43**
EEBM	200 mg/kg	5.66 ± 0.30**
EEBM	400 mg/kg	1.83 ± 0.44**

Each value represents the mean ± S.E.M (n=6). *p<0.05, **p<0.01 compared with control and standard group.

Effect of ethanolic extract of bacopa monnieri on marble burying behavior in mice.



Each value represents the mean \pm S.E.M (n=6). *p<0.05, **p<0.01 compared with control and standard group

Figure 1. Effect of ethanolic extract of bacopa monnieri on marble burying behavior in mice.

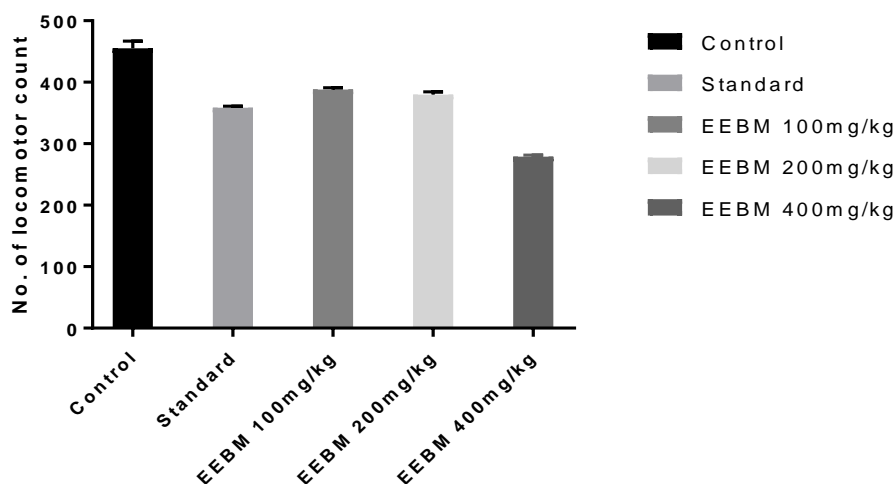
Motor activity

Table no.3: Effect of ethanolic extract of bacopa monnieri on motor activity in mice.

Treatment	Dose	No. of locomotor counts Mean \pm SEM.
Control	10 ml/kg	455.16 \pm 11.80
Standard(Fluoxetine)	15 mg/kg	348.33 \pm 1.95**
EEBM	100 mg/kg	388 \pm 2.92**
EEBM	200 mg/kg	379.83 \pm 4.47**
EEBM	400 mg/kg	279.16 \pm 2.41**

Each value represents the mean \pm S.E.M (n=6). *p<0.05, **p<0.01 compared with control and standard group

Effect of ethanolic extract of bacopa monnieri on motor activity in mice.



Each value represents the mean \pm S.E.M (n=6). *p<0.05, **p<0.01 compared with control and standard group

Figure 2: Effect of ethanolic extract of bacopa monnieri on motor activity in mice.

DISCUSSION

The results of the present investigations revealed that ethanolic extract of *Bacopa monnieri* exhibited anti-compulsive effect by inhibiting marble-burying behavior and it was comparable to that of fluoxetine. The present investigation revealed a significant decrease in marble-burying behavior of mice after oral administration of EEBM (100, 200 and 400 mg/kg) and exhibit anti OCD like effect and the effect of EEBM was comparable to standard anti OCD drug Fluoxetine (15 mg/kg) which also reduce the marble burying behavior in accordance with the previous findings. The maximum effective dose of EEBM was 200mg/kg, at which there was no change in the motor activity. The dose of 400mg/kg also exhibited inhibitory effect on marble burying behavior, but it also reduced the motor activity, hence it is not clear whether the effect of EEBM at 400mg/kg is perse effect or it due to inhibition of motor activity. The result of this study shows that ethanolic extract of *Bacopa monnieri* has positive effect on obsession and compulsion and generalized anxiety in mice.

OCD has been linked to abnormalities with the neurotransmitter serotonin, although it could be either a cause or an effect of these abnormalities. Serotonin is thought to have a role in regulating anxiety. It is hypothesized that the serotonin receptors of OCD sufferers may be relatively understimulated. OCD patients benefit from the use of SSRIs, a class of antidepressant medications that allow for more serotonin to be readily available to other nerve cells.^[14] So, it can be assumed that ethanolic extract of *Bacopa monnieri* may have an identical effect to SSRI or some facilitatory effect on serotonergic neurotransmission. In the present study, phytochemical screening of ethanolic extract of *Bacopa monnieri* revealed the presence of flavonoids, steroids, saponins, tannins, terpenoids and phytosterols, so it is possible that the mechanism of anti-compulsive action of *Bacopa monnieri* may be due to involvement of any of these phytoconstituents in serotonergic neurotransmission. Moreover, triterpenoids (steroidal compounds), which are able to cross blood brain barrier (BBB) due to their lipophilic nature, are present in *Bacopa monnieri*. So, it can be assumed that such compounds might also be responsible to elicit anti-compulsive activity at a molecular level in central nervous system.^[15]

CONCLUSION

Ethanolic extract of *Bacopa monnieri* dose dependently attenuated marble-burying behavior in mice, and the effect was comparable to that shown by fluoxetine, a reference standard drug. The present study concludes that the ethanolic extract of *Bacopa monnieri* shows anti-compulsive (anti-OCD) effect in dose-dependent manner. Phytochemical screening of the extract has shown the presence of flavonoids, saponins and steroids, which may account for biological activities. In the same way, identification and isolation of major constituents responsible for the activity and precise anti-compulsive mechanisms need to be identified.

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