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Research Article

EVALUATION OF ANTI-COMPULSIVE EFFECT OF ETHANOLIC EXTRACT OF *CLITORIA TERNATEA* IN MICE

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ABSTRACT

Obsessive compulsive disorder (OCD) is a disabling psychiatric condition with limited treatment options. It is characterized by persistent thoughts (obsessions), which are ego-dystonic and associated with seemingly purposeful behaviors (compulsions). Only potent selective serotonin reuptake inhibitors (SSRIs) are consistently effective in patients of obsessive-compulsive disorder, 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. These evidences suggest that *Clitoria ternatea* may found to be useful in the treatment of obsessive-compulsive disorder. To evaluate this possibility, we studied the influence of ethanolic extract of *Clitorea ternatea* on marble-burying behavior in mice. The results revealed that ethanolic extract of *Clitorea ternatea* (EECT) (100, 200 and 400mg/kg) reduced the marble burying behavior in mice. The effects of EECT were comparable to that of fluoxetine (5, 10 and 15 mg/kg). We also studied the effect of subeffective dose of EECT (100 mg/kg) with the subeffective dose of fluoxetine (5 mg/kg), which significantly reduced the marble burying behavior in mice. In conclusion, the study indicates that EECT can modulate obsessive compulsive behavior and also potentiate the effect of fluoxetine, and could serve as an herbal treatment of OCD.

Keywords: *Clitoria ternatea*, Obsessive compulsive disorder, Fluoxetine, Selective serotonin reuptake inhibitor, Marble burying behavior.

INTRODUCTION

Medicinal plants, herbs, spices and herbal remedies are known to Ayurveda in India since long times. The use of the medicinal herbs for curing disease has been documented in history of all civilizations. With onset of scientific research in herbals, it is becoming clearer that the medicinal herbs have a potential in today's synthetic era, as numbers of medicines are becoming resistant. The efficacy of some herbs is beyond doubt, the example being *Clitoria ternatea Linn* (Family: *Fabaceae)*, is commonly known as ''Butterfly pea.'' The plant is a twining evergreen herb, which will grow up to 3 m (9 ft) high, climbing over any available prop. The stems are pubescent and spindly. The compound leaves are made up of three to nine oval or elliptical leaflets. The flowers are 2–4 cm long and in various shades of blue with a yellow throat or pure white with a big standard petal. The fruits are pods, resembling thin peas. Native to the island of Ternate in the Molluca archipelago, this species is now widely grown as ornamental, fodder or medicinal plant[1] The plant may start flowering 4 months after sowing. Roots, seeds and leaves of *C. ternatea* are commonly used in the Ayurvedic system of medicine. The roots and seeds have powerful laxative effects, the flowers are used to make collyrium and the leaves are used in Madagascar to relieve joint pain.[2, 3] The leaves are diuretic, anthelmintic and tonic and are useful in dementia, hemicranias, burning sensation, leprosy, inflammation, leucoderma bronchitis, asthma, pulmonary tuberculosis, ascites and fever.[4] *C. ternatea* is also reported as nootropic, anxiolytic, antistress, antidepressant and anticonvulsant,[5] and sedative,[6] using conventional animal models. These effects are reported to be mediated via serotonin (5HT), dopamine (DA), noradrenaline and acetylcholine.[5] It is also stated for enhancement of memory, increase in acetylcholine content and acetylcholinesterase activity in rats.[7, 8] It has been used as an ingredient in 'Medhya Rasayana' a rejuvenating recipe used for treatment of neurological disorders and considered to strengthen a person's intellect.[9]

Obsessive compulsive disorder (OCD) is characterized by persistent thoughts (obsessions), which are ego-dystonic and associated with seemingly purposeful behaviors (compulsions).[10, 11] Although OCD is an anxiety related disorder, patients with OCD demonstrate a high incidence of comorbid depression and its lifetime prevalence varies from 0.7% to 8.0%.[12]

Medications as treatment include selective serotonin reuptake inhibitors (SSRIs) such as paroxetine, sertraline, fluoxetine, and fenfluramine and the tricyclic antidepressants, in particular clomipramine.[11] When the therapeutic efficacy of the antidepressants drugs was analyzed; all treatments were of great benefit in pacifying obsessive-compulsive symptoms, associated anxiety and depression.[13] Those plants which are used to treat anxiety and depression can be a potential therapeutic strategy for treatment of OCD. *Hypericum perforatum* (St. John's Wort), and *Benincasa hispida* Cogn possess anxiolytic and antidepressant activity; have been found effective in treatment of OCD.[11, 14] These evidences suggest that *Clitoria ternatea* may be found useful in the treatment of obsessive-compulsive disorder. Therefore, the influence of ethanolic extract of *Clitorea ternatea* 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.

The present study was designed to investigate the effect of EECT as an anti-compulsive and to find whether it has any effect on locomotor activity, which was compared with the effect of fluoxetine as a standard anti-OCD agent.

MATERIALS AND METHODS

Plant Material

The aerial parts of plant were purchased from the local area of Pune. The plant material was authenticated at Botanical Survey of India. (V. No- HARCLIL2).

Preparation of extract

The dried aerial parts were powdered. The powdered material was extracted with ethanol (95%) by maceration extraction method. It was then filtered and concentrated by evaporation. The crude extract was weighed and percentage yield (5.23% w/w) was calculated. The dried extract was stored in refrigerator.

Drugs and Chemicals

Fluoxetine hydrochloride was gifted by Endoc Pharma, Rajkot, India. Solution of ethanolic extract of *Clitoria ternatea* (EECT) and Fluoxetine HCl were prepared in 0.9% saline. Fluoxetine HCl was

administered intraperitoneally. EECT and saline were administered orally. All drug solutions were prepared fresh.

Animals

Male albino swiss mice (22–25 g), were obtained from National Institute of Bioscience, Pune, grouped and housed (n=6), under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2°C, 55±2%). They received standard rodent chow (Nutrimix Laboratory Animals Diet) and water *ad libitum*. The experiments were carried between 9.00 to 14.00 h in a noise-free room. The animal studies were approved by Institutional Animal Ethics Committee (IAEC) constituted for the purpose of control and supervision of experiments on animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Treatments

Mice were divided into different groups (n=6). EECT (100, 200 & 400 mg/kg, p.o.) or fluoxetine (5, 10 & 15 mg/kg, i.p.) or sub-effective dose of EECT and fluoxetine were administered orally and i.p. respectively, prior to the assessment of marble-burying behavior and locomotor activity. The control groups received 0.9% saline (10 ml/kg, *p.o*). After 60 min of EECT administration and 30 min of fluoxetine administration, the marble-burying behavior and motor activity were assessed in separate groups. The doses of fluoxetine and EECT were based on our preliminary investigations and previous reports.

Assessment of obsessive-compulsive behavior

Marble-burying behavior model was used for studying the OCD in mice. Mice were individually placed in separate plastic cages (21 x 38 x 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.[15, 16]

Assessment of motor activity

As OCD is influenced by motor activity, the same was assessed by using Actophotometer (Dolphin) with rectangular arena, and equipped with four photo cells and receptors. Motor activity was assessed in terms of total number of counts of light beam interruptions in 10 min. An acquisition period of 5 min was given to each mouse before assessment of motor activity.

Statistical analysis

The data were analyzed by either one-way ANOVA followed by Newman-Keuls test, $p < 0.05$ was considered significant in all cases.

RESULT

Effect of EECT on marble-burying behavior and motor activity

For each treatment and test (MBB and MA), separate groups of mice were used. Mice were injected with saline (10 ml/kg, p.o.) or EECT (100, 200 and 400 mg/kg, p.o.) and 60 min thereafter, individual mouse was tested for marble-burying behavior and locomotor activity. One-way ANOVA indicated the significant influence of Ethanolic extract of *Clitoria ternatea* (EECT) (100, 200 and 400 mg/kg, p.o.) [F (3, 20) = 107.4, P<0.0001] dose dependently on OCD in mice. Newman-keuls test indicated that EECT (100, 200 and 400 mg/kg, p.o.) dose dependently decreased marble-burying behavior in mice. The effect of EECT was without any significant change (p>0.05) in motor activity [F (3, 20) = 0.2997, P=0.8252] when compared to the saline treated group. [Figure1 and Figure 2]

Effect of fluoxetine on marble-burying behavior and motor activity

Mice were injected with saline (10 ml/kg, i.p.) or fluoxetine (5, 10 and 15 mg/kg, i.p.) and 30 min thereafter, individual mouse was tested for marble-burying behavior and locomotor activity. One-way ANOVA indicated the dose dependently influence of fluoxetine (10 and 15 mg/kg, i.p.) [F (3, 20) = 43.42, P<0.0001] on OCD in mice. Newman-keuls test indicated that fluoxetine (5, 10 and 15 mg/kg, i.p.) dose dependently decreased marble-burying behavior (Table 5, Fig.12) in mice. Fluoxetine (5 mg/kg, i.p) did not produce any significant effect on MBB (p>0.05) when compared to the saline treated group. The effect of fluoxetine was without any significant change (p>0.05) in motor activity [F (3, 20) = 0.6100, P=0.6163] when compared to the saline treated group. [Figure 3 and Figure 4]

Effect of sub effective dose of Fluoxetine with the sub effective dose of EECT on marble-burying behavior and motor activity

Separate groups of mice were injected with saline (10 ml/kg, p.o.), EECT (100mg/kg, p.o.), fluoxetine (5 mg/kg, i.p.) and EECT plus fluoxetine (100mg/kg, p.o. plus 5 mg/kg, i.p.). After 60 min of administration, individual mouse was tested for marble-burying behavior and locomotor activity, except fluoxetine (5 mg/kg i.p.) treated group were assessed after 30 min. One-way ANOVA indicated the significant influence of sub effective dose of EECT (100 mg/kg, p.o.) with the sub effective dose of Fluoxetine (5 mg/kg, i.p.) [F (3, 20) = 170.8, P<0.0001] on OCB in mice. Newman-keuls test indicated that sub effective dose of EECT (100 mg/kg, p.o.) with the sub effective dose of Fluoxetine (5 mg/kg, i.p) decreased obsessive-compulsive behavior in mice as indicated by decrease in the no. of marbles buried when compared to the saline treated group. The effect of sub effective dose of EECT with the sub effective dose of fluoxetine in MBB was without significant change (P>0.05) in motor activity [F (3, 20) = 0.4670, P=0.7086] when compared to the saline treated group. [Figure 5 and Figure 6]

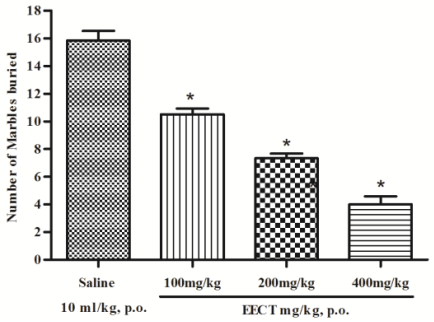

Fig 1: Results are expressed as mean ± SEM (n=6). *p<0.001 when compared to saline.

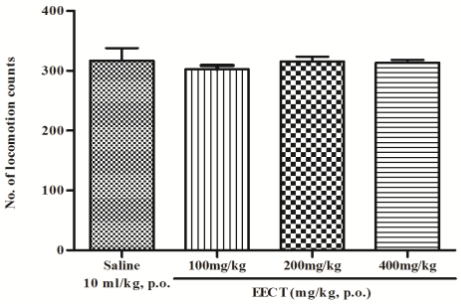

Fig 2: Results are expressed as mean ± SEM (n=6)

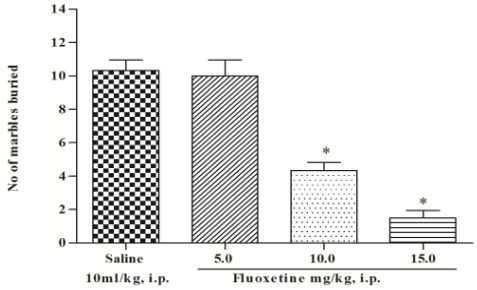

Fig 3: Results are expressed as mean ± SEM (n=6). *p<0.001 when compared to saline.

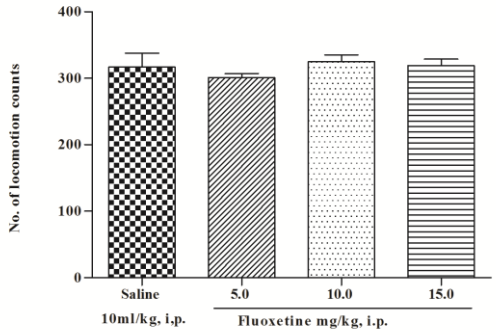

Fig 4: Results are expressed as mean ± SEM (n=6)

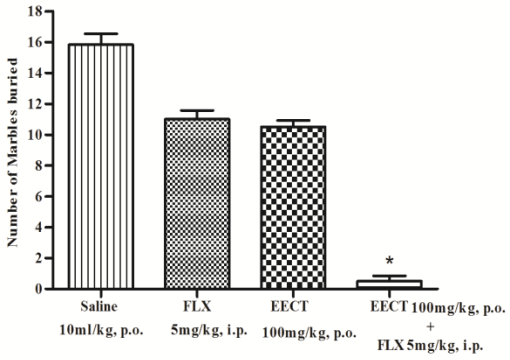

Fig 5: Results are expressed as mean ± SEM (n=6). *p<0.001 when compared to saline.

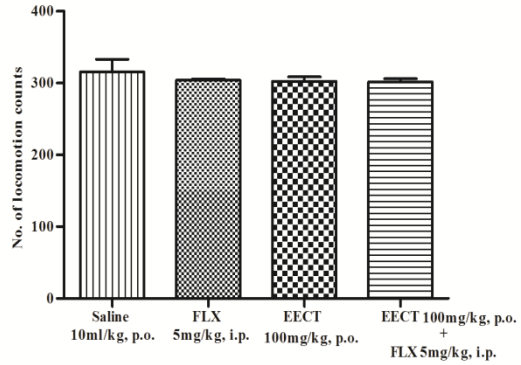

Fig 6: Results are expressed as mean ± SEM (n=6)

DISCUSSION

The present investigation revealed a significant decrease in marble-burying behavior of mice after oral administration of EECT (100, 200 and 400 mg/kg) without any noticeable change in locomotor activity. In addition, EECT did not produce sedation in selected dose range but in higher dose it was sedative, which is also reported in previous studies.[6] The co-administration of sub effective dose of EECT (100mg/kg, p.o) with the sub effective dose of selective serotonin reuptake inhibitor (Fluoxetine) significantly decreased marble-burying behavior of mice. It indicates that sub effective dose of EECT potentiated the effect of sub effective dose of fluoxetine.

The same animals were used for testing and treatment of OCD and no any adaptation to testing conditions were observed. Earlier reports also suggest no adaptation to marble-burying behavior on repeated testing. Marble-burying behavior of mice is a well-accepted paradigm to screen anti-compulsive activity, as it is based on the principle that burying behavior is an unconditioned defensive reaction in rodents, species-specific, not associated with physical danger and does not habituate upon repeated testing.[8,14,15] Incidentally, *Hypericum perforatum* (St. John's Wort) and *Benincasa hispida,* which possess anxiolytic and antidepressant activity, have been found effective in treatment of OCD.[11,14] Hence, *Clitoria ternatea* is also reported for its anxiolytic and antidepressant activity,[5] and based on this evidence, it is evaluated for its effect in OCD.

As previous reports suggest that marble-burying does not model anxiety but it is related to compulsive behavior.[17, 18, 19, 20] In present study, the observed effect of EECT is may be due to its anti-compulsive rather than its anxiolytic effect.

From results, it is clear that EECT exhibits significant anti-compulsive effect in marble-burying behavior test in mice and the effect may be attributed to enhanced serotonergic function and might have influence on 5-HT reuptake. However the mechanisms of this effect and therapeutic efficacy of ethanolic extract of *Clitoria ternatea* in OCD require further study.

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