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SHORT COMMUNICATION

EVALUATION OF ANTI-COMPULSIVE EFFECT OF METHANOLIC EXTRACT OF *BENINCASA HISPIDA* COGN. FRUIT IN MICE

SHIKHA GIRDHAR[1]*, MANISH M. WANJARI[2], SUNIL K. PRAJAPATI[3] and AMIT GIRDHAR[1]

[1]Seth G.L. Bihani S.D. College of Technical Education, Near Cihag Hospital, Gaganpath, Sriganganagar-335001 (Rajasthan), India
[2]Central Research Institute (Ayurveda), Opposite Jayarog Hospital, Aamkho, Lashkar, Gwalior – 474 009 (Madhya Pradesh), India
[3]Institute of Pharmacy, Bundelkhand University, Jhansi –284 128 (Uttar Pradesh), India

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Benincasa hispida Cogn. (Cucurbitaceae) commonly known as wax gourd (1) is an important ingredient of *kushmanda lehyam*, an Ayurvedic medicine widely used as rejuvenative agent (*Rasayana*) in treatment of epilepsy and other nervous disorders (2). In Ayurvedic system of medicine, *Benincasa hispida* fruits are used for treatment of schizophrenia and other psychologic disorders (3). Methanolic extract of *Benincasa hispida* fruit has been demonstrated to possess nootropic, antidepressant and anxiolytic like effect (4, 5), which suggests that *Benincasa hispida* influences various neurotransmitter systems including serotonergic system.

Obsessive-compulsive disorder (OCD) is characterized by persistent thoughts (obsessions), which are ego-dystonic and associated with seemingly purposeful behaviors (compulsions) (6). Its co-morbidity with major depression is often evident, and it is considered as an anxiety disorder (7). Only potent serotonin reuptake inhibitors (SSRIs) are consistently effective in patients of obsessive-compulsive disorder (8), 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. Incidentally, *Hypericum perforatum* (St. John's Wort), which possesses anxiolytic and antidepressant effect, has been found effective in treatment of OCD (9).

These evidences suggest that *Benincasa hispida* may be useful in the treatment of obsessive-compulsive disorder. Therefore, the influence of methanolic extract of *Benincasa hispida* fruit 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 (10). Further, the effect of methanolic extract of *Benincasa hispida* fruit was compared with the effect of fluoxetine – a standard anti-OCD agent. To understand the involvement of serotonergic system, the effect of methanolic extract of *Benincasa hispida* fruit was further studied in mice, pre-treated with either sub-effective dose of fluoxetine, or *p*-chlorophenylalanine (PCPA) – a serotonin depleting agent.

EXPERIMENTAL

Plant material

Benincasa hispida fruit, obtained from local market in the month of November 2006 was identified by Dr. N.K. Pandey, Research Officer (Botany) at Central Research Institute (Ayurveda), Gwalior, India. A voucher specimen (specimen no. CRI-GWL/F.B.10556) was submitted at the same institution.

Preparation of methanolic extract of *Benincasa hispida* fruit

Methanolic extract of *Benincasa hispida* fruit (MEBH) was prepared by simple maceration process as described previously (11). The fruit was

* Corresponding author: phone: +919461166961; fax: +91-0154-2468774, e-mail: rahejashikha@gmail.com

peeled off and seeds were removed. Pulp was mashed using an electric juicer to afford a soft mass and later on macerated with methanol (1 : 4) for seven days at room temperature with occasional stirring daily. On eighth day, the pulp mass was filtered and the filtrate was heated (below 55°C) and evaporated under reduced pressure till a strong brownish liquid was obtained (yield: 5% w/w). It was then stored at 2–4°C and protected from direct sunlight. The phytochemical screening of MEBH (12) revealed the presence of proteins, tryptophan, sterols, volatile oils, glycosides, phenolic compounds and absence of carbohydrates, flavonoids and alkaloids.

Drugs and chemicals

Fluoxetine HCl (Esteem Pharmaceuticals, Agra, India), was obtained as gift sample while *p*-chlorophenylalanine (PCPA) hydrochloride methyl ester was purchased from Sigma Aldrich, USA. All the drugs including MEBH were dissolved in 0.9% saline for pharmacological studies.

Animals

Male Swiss albino mice (22–25 g) were used. They were housed in groups in polypropylene cages, under 12 h light/dark cycle and controlled conditions of temperature and humidity (25 ± 2°C, 55 ± 2%, respectively). They received the standard rodent chow and water *ad libitum*. The experiments were carried between 9.00 to 15.00 h in a noise free room. The animal studies were approved by Institutional Animal Ethics Committee constituted for the purpose of control and supervision of experiments on animals.

Treatments

Mice were divided into different groups (n = 6). MEBH (200, 400, 600 mg/kg) or fluoxetine (5, 10, 15 mg/kg) or sub-effective dose of MEBH 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, *ip*). After 30 min, the marble-burying behavior and motor activity were assessed in separate groups.

In another set of experiments, mice were pretreated with PCPA (300 mg/kg, *ip*) for 3 consecutive days and 24 h thereafter MEBH (600 mg/kg, *ip*) or fluoxetine (15 mg/kg, *ip*) were administered. Thirty minutes thereafter, marble-burying behavior and motor activity were assessed in separate groups. The doses of fluoxetine and MEBH were based on our preliminary investigations and previous reports (5, 13).

Assessment of marble-burying behavior

Marble-burying behavior model was used for studying the OCD in mice (14). 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.

Assessment of motor activity

As OCD is influenced by motor activity, the same was assessed by using Actophotometer (Biocraft Scientific Systems Pvt. Ltd., India) 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 or two-way ANOVA followed by Bonferroni test for multiple comparisons, wherever necessary; $p < 0.05$ was considered significant in all cases.

RESULTS

Effect of MEBH and fluoxetine on marble-burying behavior and motor activity

One-way ANOVA exhibited that MEBH significantly influenced marble-burying behavior [F (3, 20) = 57.76, ($p < 0.0001$)] (Fig. 1A). The *post hoc* test showed that MEBH (400 and 600 mg/kg) significantly dose dependently ($p < 0.001$) reduced the number of marbles buried while the lower dose of MEBH (200 mg/kg) did not show significant reduction in the number of marbles buried ($p > 0.05$). Motor activity was not affected by MEBH (200, 400, 600 mg/kg) [F (3, 20) = 0.36, $p = 0.7807$] (Fig. 1A).

Similarly, fluoxetine significantly influenced marble-burying behavior [F (3, 20) = 36.15, $p < 0.0001$] (Fig. 1B). The *post hoc* test showed that fluoxetine (10 and 15 mg/kg) dose dependently reduced ($p < 0.01$ and $p < 0.001$, respectively) marble burying behavior in mice without any effect on motor activity [F (3, 20) = 0.52, $p = 0.6711$] while the lower dose of fluoxetine (5 mg/kg) was found ineffective ($p > 0.05$) (Fig. 1B).

Further, one-way ANOVA indicated that fluoxetine and MEBH combined administration in sub-

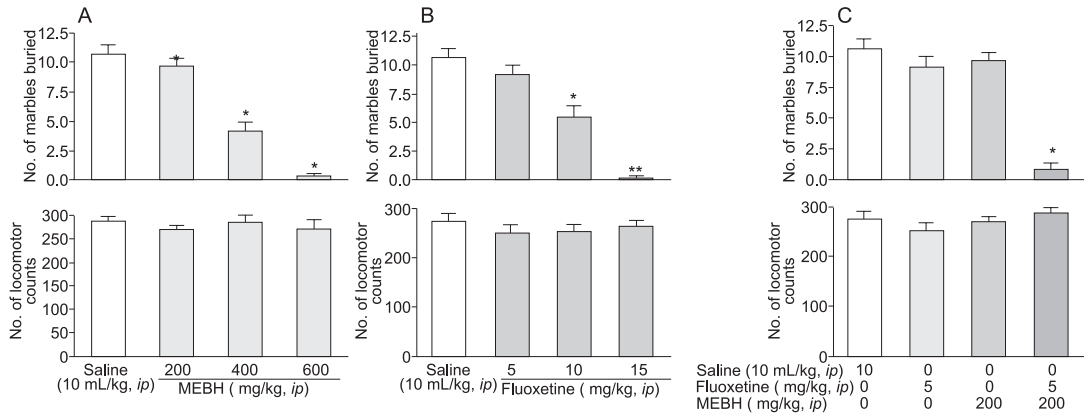

Figure 1. Influence of various drug-treatments on marble-burying behavior and locomotor activity in mice [(A) MEBH, (B) fluoxetine, and (C) MEBH plus fluoxetine]. Separate groups of mice were injected with saline (10 mL/kg, *ip*) or fluoxetine (5–15 mg/kg, *ip*) or increasing doses of MEBH (200–600 mg/kg, *ip*), and 30 min thereafter, individual mouse was tested for marble-burying behavior and locomotor activity. For co-administration studies, separate groups of mice were injected with saline (10 mL/kg, *ip*) or fluoxetine (5 mg/kg, *ip*) and 5 min later either saline (10 mL/kg, *ip*) or MEBH (200 mg/mouse, *ip*), and 30 min thereafter, individual mouse was tested for marble-burying behavior and locomotor activity. Marble-burying behavior and locomotor activity was tested in separate groups of mice. Each bar represents the mean ± SEM of data from 6 mice. *$p < 0.001$ *vs.* respective saline control and fluoxetine control or MEBH control (one-way ANOVA followed by Newman–Keuls *post hoc* test)

effective doses had significant [F (3, 20) = 38.50, $p < 0.0001$] (Fig. 1C) influence on marble-burying behavior. The *post hoc* test showed that co-administration of sub-effective dose of MEBH (200 mg/kg) and sub-effective dose of fluoxetine (5 mg/kg) significantly ($p < 0.001$) attenuated marble-burying behavior without affecting the motor activity [F (3, 20) = 1.21, p = 0.32] (Fig. 1C).

Effect of PCPA pre-treatment on the influence of MEBH and fluoxetine on marble-burying behavior and motor activity

Two-way ANOVA indicated that MEBH and fluoxetine had significant interaction with PCPA [PCPA-Drug treatment interaction (F (2, 30) = 44.82, $p < 0.0001$); PCPA treatment effect (F (1, 30) = 124.7, $p < 0.0001$) and drug treatment effect (F (2, 30) = 139.6, $p < 0.0001$)] and influenced the marble-burying behavior. *Post hoc* test suggested that pre-treatment of mice with PCPA partially but significantly attenuated ($p < 0.001$) the inhibitory effect of MEBH, whereas completely eliminated ($p < 0.001$) the effect of fluoxetine on the burying behavior. PCPA pre-treatment *per se* did not affect the marble-burying behavior ($p > 0.05$) (Fig. 2). All these treatments did not influence the motor activity [PCPA-Drug treatment interaction (F (2, 30) = 1.293, p= 0.2893); PCPA treatment (F (1, 30) = 1.988, p = 0.1689) and drug treatment interaction (F (2, 30) = 139.6, p= 0.1003)] (Fig. 2).

DISCUSSION AND CONCLUSION

The results of the present investigations revealed that methanolic extract of *Benincasa hispida* fruit exhibited anti-compulsive effect by inhibiting marble-burying behavior and it was comparable to that of fluoxetine. Effect of fluoxetine on marble-burying behavior is in concordance with the previous findings (13). Various reports suggested that marble-burying behavior may be more related to OCD (15, 16) or the compulsive behavior (14, 17, 18) and does not model anxiety.

The anti-compulsive effect of MEBH was further substantiated by the observation that the sub-effective dose of MEBH potentiated the effect of sub-effective dose of fluoxetine and exhibited the significant inhibition of marble burying behavior. This effect of MEBH to potentiate the action of fluoxetine strongly differentiates it from anti-anxiety to anti-OCD drug.

Although the exact mechanism of action of MEBH to exhibit anti-compulsive activity was not elucidated in the present study, it appears the MEBH acts through its influence on serotonergic system. The involvement of serotonergic system

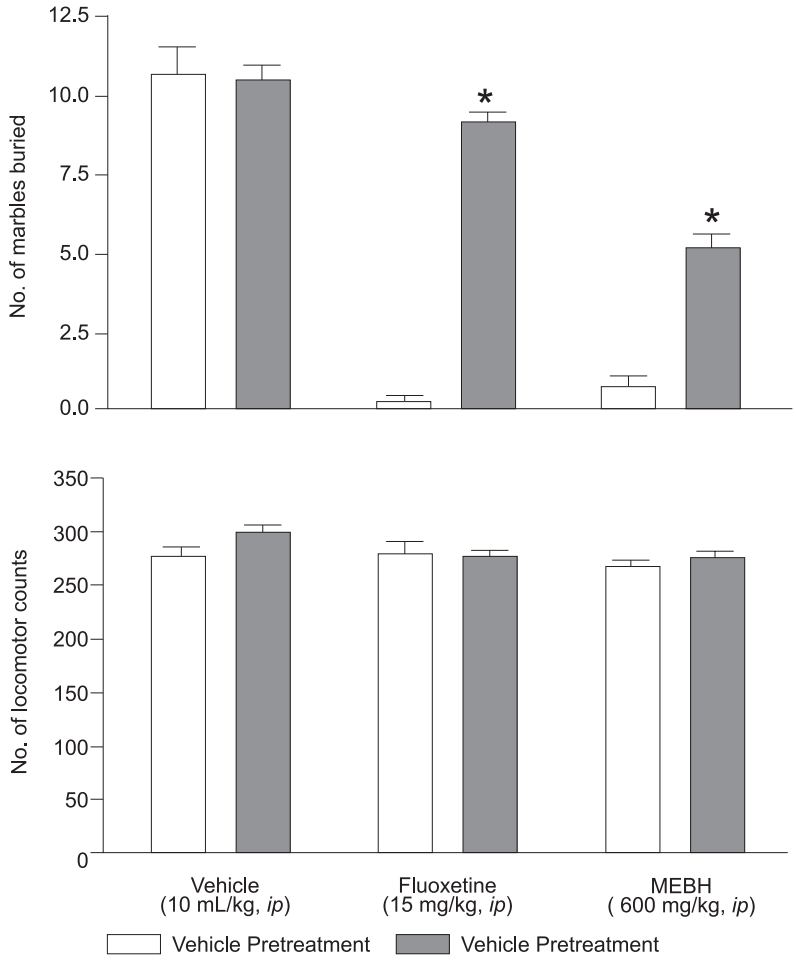

Figure 2. Effect of PCPA pre-treatment on the anti-compulsive effect of fluoxetine and MEBH in mice. Separate groups of mice were injected with PCPA [300 mg/kg, *ip* (×3 days)] or saline [10 mL/kg, *ip* (×3 days], and 24 h after last dose, saline (10 mL/kg, *ip*) or fluoxetine (15 mg/kg, *ip*) or MEBH (600 mg/kg, *ip*) were administered, and 30 min thereafter, individual mouse was tested for marble-burying behavior and locomotor activity. Marble-burying behavior and locomotor activity was tested in separate groups of mice. Each bar represents the mean ± SEM of data from 6 mice. *p < 0.001 *vs*. respective fluoxetine and MEBH control. (Two-way ANOVA followed by Bonferroni *post hoc* test)

was substantiated by the fact that pretreatment of mice with PCPA partially and significantly attenuated the inhibitory effect of MEBH and completely eliminated the effect of fluoxetine on the burying behavior. However, it is not clear by what mechanism MEBH influences serotonergic system. Previous study has shown that *Hypericum perforatum (*St. John's Wort), which is known to possess antidepressant and anxiolytic action (19, 20), has showed putative anti-obsessive effect and it was speculated the anti-obsessive effect could be related to the inhibition of 5-HT reuptake by *H. perforatum*. Hyperforin was thought to be the major serotonergic component of *H. perforatum* that contributed to effect of *H. perforatum* on marble burying (9). It is also possible that MEBH might have influence on 5-HT reuptake.

The preliminary phytochemical studies on MEBH also revealed the presence of tryptophan in the extract (data not shown). Tryptophan is an important precursor of serotonin in the serotonergic neurons and may be enhancing the biosynthesis of

serotonin to facilitate the anti-compulsive effect of MEBH.

In conclusion, it is clear that MEBH exhibits significant anti-compulsive effect in marble-burying behavior test in mice and the effect may be attributed to enhanced serotonergic function.

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