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EFFECT OF ETHANOLIC AND AQUEOUS EXTRACT OF *CENTELLA ASIATICA* ON MARBLE-BURYING BEHAVIOUR IN MICE

Neeraj Kumar*[1], Rajnish Kumar[2], Kamal Kishore Maheshwari[3]

[1,2]Shri Ram Murti Smarak college of engineering and technology, Bareilly-243202, UP, India.
[3]Department of Pharmacy, Rohilkhand University, Bareilly-243001, UP, India.

ABSTRACT

Obsessive compulsive disorder (OCD) is a wide spread disorder in about 2-3% of the population worldwide, 0.6% of Indians and the present effective treatment of OCD is selective serotonin reuptake inhibitor (SSRIs) demonstrate selective efficacy in any about 40-60 % of patient while 30 % of patients failing to respond to conventional treatment. The aim of study was the pre-clinical screening of anticompulsive effect by marble-burying model in mice. Study of marble-burying behaviour with locomotor activity in albino mice was carried out by specially designed model for anticompulsive activity and actophotometer for locomotor activity. For marble burying study, 7 groups (n=6) for control, test (50 and 100 mg/kg of aqueous and ethanolic extract) and standard drug (5 and 10 mg/kg of fluoxetine) while another 7 groups for control, test (50 and 100 mg/kg of aqueous and ethanolic extract) and standard drug (5 and 10 mg/kg of fluoxetine), of mice were used for locomotor activity. The data was analyzed by one way analysis of variance followed by Dunnett's test ($P < 0.01$). The ethanolic extract of *Centella asiatica* (50 and 100 mg/kg) was found significant in dose dependently ($p<0.01$), reduced the number of marbles buried without affecting the motor activity. The aqueous extract (100 mg/kg) also reduced ($P<0.01$) the number of marbles buried with slight reduction (13%) in locomotor activity when compared with fluoxetine. The finding of this study was suggested that ethanolic extract of *Centella asiatica* may significantly reduce anticompulsive behaviour comparable as fluoxetine.

Key words: Obsessive compulsive disorder, Fluoxetine, Marble-burying, Mandukaparni, Gotu kola.

INTRODUCTION

Centella asiatica (Linn.) (Family-*Apiaceae*) commonly known as 'Brahmi' in unani medicine, 'Mandukaparni' in Ayurveda and 'Gotu kola' in western world, consist of dried, fragmented aerial parts preferably leaves (Bhavna and Jyoti, 2011; Jamil *et al*., 2007). It is creeping plant, leaves with bigger and long petiolate, entire, crenate, orbicular and reniform, 1.5-6.5 cm of diameter, found in marshy areas of all over India, Sri Lanka, Madagascar and Africa upto an altitude of 650 m. The principle chemical constituents are reported from *Centella asiatica* are asiaticosides, madecassoide, madecassic acid, asiatic acid, brahmosides, brahmic acid, brahminosides, thankuniside, isothankunoside, centelloside, madasiatic acid, centic acid, cenellic acid, betulinic acid, indocentic acid, glucose, rhamanose, terpenoids (Kuroda *et al*., 2001), sitosterol, stigmasterol with calcium, iron and phosphate (Jiang *et al*., 2005). It is mainly used as brain tonic and claimed to possesses a wide range of pharmacological effects like wound healing (Suguna *et al*., 1996; Shetty *et al*., 2006), mental disorder (Appa Rao *et al*., 1977) like antidepressant (Qin *et al*., 1998), antitumour (Huang *et al*., 2004; Babu *et al*., 1995), atherosclerosis (Brinkhaus *et al*., 2000), fungicidal (Jagtap *et al*., 2009), antibacterial (Oyedeji *et al*., 2005), antioxidant (Jayashree *et al*., 2003; Zainol *et al*., 2003;), anticancer (Bunpo *et al*., 2004; Park *et al*., 2005),

Corresponding Author

Neeraj Kumar
Email: neerajsitm@yahoo.com

antinociceptive, ulcer (Shetty *et al*., 2006), anti-inflammatory (Somchit *et al*., 2004) and hepatoprotective activity (Antony *et al*., 2006). The triterpenoids present in this plant may change gene expression in human fibroblast cells and can be used in treatment of oedema with capillary filtration in venous hypertension (De Sanctis *et al*., 2001; Coldren *et al*., 2003). The study of Rao *et al*., 2005 was suggested that *Centella asiatica* treatment during postnatal period may enhance learning and memory in mice while its extract also helps in healing (Cheng *et al*., 2004)

Obsessive compulsive disorder (OCD) is a wide spread disorder in about 2-3% of the population worldwide and 0.6% of Indians (Doshi, 2009), characterized by recurring obsessions and compulsions that significantly interrupt the daily functioning of the patient (Matsushima *et al*., 2010) with co-morbidity and major depression considered as an anxiety disorder (Girdhar *et al*., 2010). Neuropsychological studies suggested that an imbalance in activity between the direct (excitatory) and the indirect (inhibitory) pathways within fronto-striatal circuitry leads to the development of OCD and cognitions (Saxena *et al*., 1998, 2001). Its life time prevalence rate is about 1-3 % (Prajapati *et al*., 2011).

The present effective treatment of OCD is selective serotonin reuptake inhibitor (SSRIs) demonstrate selective efficacy in any about 40-60 % of patient with 30 % of patients failing to respond to conventional treatment (Mishra *et al*., 2007). This data is suggests that herbal treatment of OCD may be useful so the influence of aqueous and ethanolic extract of *Centella asiatica* was investigated for the marble burying behaviour and actophotometric activity, methods commonly used for anticompulsive activity screening in mice (Mohandas *et al*., 2008).

MATERIAL AND METHODS

Plant material

Centella asiatica, whole plant obtained from herbal garden of department of pharmacy, Shri ram murti smarak college of engineering and technology (Pharmacy), Bareilly, UP, India in the month of February 2011 (Figure 1), was identified by its morphological characteristics (Zainol *et al*., 2008). A voucher specimen (srmscet/Pharma/11/063) was submitted in the pharmacognogy department of same institution.

Preparation of ethanolic and aqueous extract of Centella asiatica

Ethanolic and aqueous extract of *Centella asiatica* whole plant were prepared by hot soxhlet extraction method. The whole plant was collected, washed and shade dried then crushed using an electric grinder. The coarse powder was extracted with ethanol (EECA) and distilled water contains small amount of ethanol (AQCA).

Drugs and chemical

Fluoxetine, AQCA and EECA were dissolved in 0.9% saline for pharmacological studies.

Animals

Male Swiss albino mice (25-30 g) were used in this study was housed in poly propylene cages, under 12 hour light/dark cycle, controlled condition of temperature and humidity (25±2 ^{0}C, 55±2 % respectively). Mice received standard rodent chow and water *ad libitum.*

The experiments were carried between 9.00 AM to 03.00 PM in a noise free room. The animal studies were approved by institutional animal ethical committee constituted for the purpose of control and supervision of experiment on animals (715/02/C/CPCSEA).

Treatment

Mice were divided into four groups (n=6) for AQCA and EECA (50, 100 mg/kg), two groups for fluoxetine (5, 10 mg/kg) administered orally 30 min prior to the assessment of marble burying behaviour and same groups was also formed for locomotor activity study and one control group was formed to receive 0.9% normal saline.

Assessment of marble burying behaviour

Marble burying behaviour model was used for screening of obsessive compulsive disorder in mice (Njung'e *et al*., 1991). 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 (Figure 2, 3, 4). 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 (Girdhar *et al*., 2010).

Assessment of motor activity

As OCD is influenced by motor activity, the same was assessed by using actophotometer (Inco instruments and chemicals pvt. Ltd., India) with rectangular arena, and equipped with six photo cells and receptors. Motor activity was assessed in terms of total number of counts of light beam interruptions in 15 min. An acquisition period of 5 min was given to each mouse before assessment of motor activity (Girdhar *et al*., 2010).

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) followed by *Dennett's test*, wherever necessary $p < 0.05$ was considered significant in all cases. The groups treated with extracts and fluoxetine were compared with the respective control group.

RESULTS

Effect of AQCA, EECA and fluoxetine on marble-burying behaviour and motor activity

One-way ANOVA exhibited that EECA significantly influenced marble-burying behaviour ($p<0.01$) (Table 1) and the *Dennett's test* showed that EECA (50 and 100 mg/kg) was found significant in dose dependent manner ($p<0.01$) and reduced the number of marbles buried (Figure 5). The locomotor activity was not affected by EECA (50, 100 mg/kg) ($p<0.001$) (Figure 6) and similarly, fluoxetine significantly influenced marble-burying behaviour ($p<0.01$) and the *Dennett's test* showed that fluoxetine (5 and 10 mg/kg) dose dependently reduced ($p<0.01$) (Figure 5) marble burying behaviour in mice without any effect on motor activity (Figure 6). The AQCA (100 mg/kg) also reduced ($P<0.01$) (Figure 5) the number of marbles buried with slight reduction (13%) in locomotor activity (Figure 6).

Table 1.Effect of AQCA, EECA and Fluoxetine on marble-burying behaviour and locomotor activity

Groups	Dose(ip) mg/kg	Marble burying behaviour	Locomotor activity observed for 15 min		
			Before dosing	After dosing	% Change in activity
Control (0.9 % saline)	10 mL/kg	19.0±1.67	402.0±33.81	394.80±40.11	1
AQCA	50	11.8±1.72	391.5±13.45	380.83±18.04	2
	100	07.5±1.04*	385.1±34.34	333.83±39.00	13
EECA	50	08.6±1.03	389.0±29.84	370.16±35.90	4
	100	04.5±1.04*	410.0±11.04	393.83±26.26	4
Fluoxetine	05	05.5±1.04*	387.8±28.16	382.33±28.97	1
	10	03.1±0.75*	398.0±05.40	369.00±06.26	7

(n=6, $p< 0.05$ considered as significant, *$p< 0.01$)

Figure 1. *Centella asiatica* plant

Figure 2. Marble burying model

Figure 3. Marble buried after fluoxetine treatment

Figure 4. Marble buried after *Centella asiatica* treatment

Figure 5. Effect of *Centella asiatica* and fluoxetine treatment on marble burying

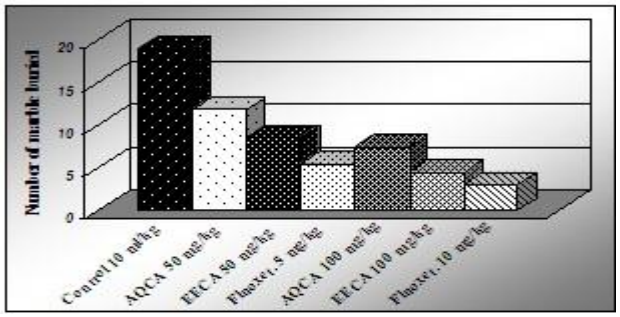
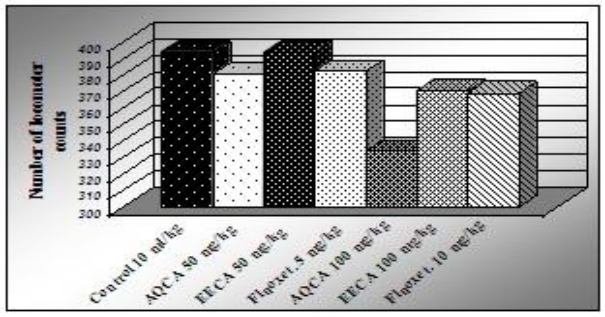


Figure 6. Effect of *Centella asiatica* and fluoxetine treatment on locomotor counts



DISCUSSION

The present study was designed to investigate the effect of EECA on marble burying behaviour as compare with the effect of fluoxetine. The results were revealed that EECA exhibited anticompulsive effect by inhibiting marble burying behaviour and it was comparable to the inhibiting effect of fluoxetine which is used in OCD pharmacotherapy (Table 1). The dose of fluoxetine was set based on the finding of Girdhar *et al.*, 2010. The EECA at a dose of 50 mg/kg showed a trend toward inhibited marble burying behaviour while a dose 100 mg/kg significantly reduce the number of buried marbles (Figure 5) with very less reduction (4%) in locomotor activity when compared with control. The AQCA at a dose 100 mg/kg was also reduced the marble burying behaviour with 13% reduction in locomotor activity. The fluoxetine at a dose of 5 mg/kg significantly reduced marble burying behaviour without reduction (1%) in locomotor activity and at a dose of 10 mg/kg was highly significant for reduction in marble burying behaviour with non significant reduction (7%) in locomotor activity (Figure 6). Four plants were screened for anticompulsive behaviour which have active ingredient that have been found to modulate serotonergic activity were located in *Hypericum perfirmatum, Silybum morianum* (Prajapati *et al*., 2011) *Benicasa hipsida* (Girdhar *et al*., 2010), *Psilocybe argentipes* (Matsushima *et al*., 2009) and *Lagenaria siceraria* (Prajapati *et al*., 2011) as described in literature. Mechanism of action associated with active constituents of these plants may includes monoamine oxidase, serotonin inhibition, nor-epinephrine and dopamine reuptake (Girdhar *et al*., 2010).

CONCLUSION

The *Centella asiatica* significantly reduce marble burying behaviour in mice, the animal model of anticompulsive activity without adversely affecting locomotor activity almost same effectively as authentic fluoxetine so these finding suggests that *Centella asiatica* may exhibit anticompulsive activity in clinical use.

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