Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Influence of *Withania somnifera* on obsessive compulsive disorder in mice

Bhanu PS Kaurav[1], Manish M Wanjari[2], Amol Chandekar[1], Nagendra Singh Chauhan[3], Neeraj Upmanyu[1,3*]

[1]*Department of Pharmacology, R.K.D.F. College of Pharmacy, Bhopal M.P. India*
[2]*Department of Pharmacology, Central Research Institute (ay.) Amkho, Gwalior, M.P. India*
[3]*Department of Pharmaceutical Sciences, Doctor Hari Singh Gour Vishwavidyalaya Sagar- 470003 (M.P.) India*

ARTICLE INFO

Article history:
Received 1 September 2011
Received in revised form 27 January 2012
Accepted 15 March 2012
Available online 20 May 2012

Keywords:
Withania somnifera
Fluoxetine
Ritanserin
Parachlorophenylalanine
Obsessive compulsive behavior

ABSTRACT

Objective: To study the influence of methanolic and aqueous extract of *Withania somnifera* (*W. somnifera*) root on the marble-burying behavior of mice a well-accepted model of obsessive compulsive behavior. **Methods:** Mice were divided in different groups (n=6). Fluoxetine (5, 10, 15 mg/kg), (10, 25, 50, 100 mg/kg) and methanolic extract *W. somnifera* (MEWS) (10, 25, 50, 100 mg/kg) were administered *i.p.* 30 min. prior to the assessment of marble burying behavior and locomotor activity. The control group received vehicle of the extract. **Results:** Administration of aqueous extracts *W. somnifera* (AEWS) and MEWS (50 mg/kg) successively decreaesed the marble burying behavior activity without affecting motor activity. This effect of AEWS and MEWS was comparable to standard fluoxetine, ritanserin and parachlorophenylalanine. **Conclusions:** *W. somnifera* extract is effective in treating obsessive compulsive disorder.

1. Introduction

Obsessive compulsive disorder (OCD) is thought to be one of the most intractable and disabling anxiety related mental disorders in the community (characterized by persistent and distress causing thoughts (obsessions), which are ego-dystonic and seemingly purposeful behavior (compulsions) [1-4]. The brain areas like orbitofrontal cortex, the anterior cingulated cortex, the dorsolateral prefrontal cortex are involved in the pathophysiology of the OCD. The defects in serotonergic system has been implicated in OCD[5,6]. Some reports also indicated the involvement of the dopaminergic system in OCD[7]. Although OCD is classified as an anxiety disorder, practical anxiolytic agents are generally ineffective in reducing symptoms of OCD. At present, the most efficacious pharmacological treatments for OCD are antidepressants with serotonin (5-hydroxytryptamine) reuptake inhibition[5].

Withania somnifera (*W. somnifera*) (WS) or Ashwagandha, also known as "Winter Cherry" is the most famous Ayurvedic rejuvenative botanical (rasayana) that improves the body's ability to maintain physical effort and helps the body adapt to various types of stress. It is especially beneficial in stress related disorders such as arthritis, hypertension, diabetes, general debility, *etc*. The roots of *W. somnifera* are used to promote physical and mental health, *W. somnifera* has been used to stabilize mood in patients with behavioral disturbances[8]. Though animal experimentation supported the use of *W. somnifera* as a mood stabilizer in clinical conditions of anxiety and depression in Ayurveda, *W. somnifera* is found to decrease the degree of anxiety and depression and can be used as antidepressant. The anxiolytic and antidepressant actions of the bioactive glycowithanolides, isolated from *W. somnifera* roots, has been investigated in rats. The plants contain the alkaloids withanine and somniferine, which are used to treat nervous disorders[8].

Those plants which are used to treat anxiety and depression can be potential therapeutic strategy for treatment of OCD. Incidentally, it is found that *W. somnifera* possesses anxiolytic and antidepressant effect. Hence, it is contemplated that *W. somnifera* may be useful in the treatment of obsessive-compulsive disorder. Therefore, it was proposed to study the influence of methanolic and aqueous extract of *W. somnifera* root was investigated on the marble-burying behavior of mice-a well-accepted model

*Corresponding author: Dr. Neeraj Upmanyu, R.K.D.F. College of Pharmacy, Bhopal M.P. India.
Tel: +919425128642
E-mail: neerajupmanyu2007@gmail.com

of obsessive-compulsive behavior[9]. Further, the effect of *W. somnifera* root extracts is compared with the effect of fluoxetine a standard anti-OCD agent. To understand the involvement of serotonergic system, the effect of methanolic extract of *W. somnifera* is further studied in mice, pre-treated with either sub-effective dose of fluoxetine, or parachlorophenylalanine (PCPA)-a serotonin depleting agent.

2. Material and methods

2.1. Plant material

Dried root of *W. somnifera* were collected from the medicinal garden of Central Research Institute (Ay.), Aamkho, Gwalior, India. The identification of plant was done by Shri N.K. Pandey, Research Officer (Botany), Central Research Institute (Ay.), Aamkho, Gwalior, India. A voucher specimen (Field Book no. 10629) of the authenticated *W. somnifera* has been deposited in the herbarium of the Institute. The roots were coarsely powdered and subjected to defatting in Soxhlet apparatus using petroleum ether (60-80 ℃). The defatted air dried root powder was subjected to extraction in soxhlet apparatus using methanol. Concentrated extract was collected from the extraction-pot of soxhlet apparatus (MEWS). These air dried root powder was subjected to simple maceration process. The material was macerated with chloroform and water (1:4) for 48 h at room temperature with occasional stirring daily. The mixture was filtered and the filtrate was heated (below 55 ℃) and evaporated in rotary evaporator under reduced pressure till a strong brownish liquid was obtained (AEWS).

2.2. Animal

Swiss albino mice (22-25 g) of either sex were used. They were housed in groups (n=6) in polypropylene cages, under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2) ℃, (55±2)%. They received the standard rodent chow and water ad libitum. Drug administrations and experiments were carried between 9:00 am to 3:00 pm 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.

2.3. Drug

Fluoxetine (Esteem Pharmaceuticals, Agra), and ritanserin (Sun Pharma Advanced Research Center, Vadodara, India) were obtained as gift samples. Parachlorophenylalanine was purchased from Sigma Aldrich, USA. All the above drugs were dissolved in 0.9% saline. Drug solutions were freshly prepared and their doses are expressed in terms of their free bases.

2.4. Doses and treatments

Mice were divided in different groups (n=6). Fluoxetine (5, 10, 15 mg/kg), AEWS (10, 25, 50, 100 mg/kg) and MEWS (10, 25, 50, 100 mg/kg) were administered i.p. 30 min prior to the assessment of marble burying behavior and locomotor activity. The control group received 0.9% saline or vehicle of the extract. In another set of experiments, mice were pretreated with serotonergic neurotoxin, PCPA (300 mg/kg) for three consecutive day and 24 h, thereafter MEWS (50 mg/kg) or AEWS (50 mg/kg) or fluoxetine (15 mg/kg) were administered. After thirty minutes, the marble burying behavior and motor activity was assessed in separate groups. In another set of experiments mice were pretreated with fluoxetine (5 mg/kg) and after 30 min AEWS (10 mg/kg) or MEWS (10 mg/kg) were administered. After thirty minutes, the marble burying behavior and motor activity was assessed in separate groups. In another group, $5HT_{2A/2C}$ antagonist, ritanserin (10 mg/kg) was given 30 min prior to administration of MEWS (50 mg/kg), AEWS (50 mg/kg) and 30 min later the above behavioral parameters were observed. The doses of fluoxetine, PCPA and ritanserin were based on previous reports[10].

2.5. Marble-burying behaviour tests

Marble-burying model was used for studying the marble burying behaviour of mice[11]. Mice were individually placed in separate plastic cages (21 cm×38 cm×14 cm) containing 20 clean glass marbles (10 mm in 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 behaviour.

2.6. Locomotor activity tests

As OCD is influenced by motor activity, the same was assessed in separate groups of mice using actophotometer (Medicraft Electromedicals Pvt. Ltd., Lucknow) equipped with six infrared beams and photocells connected to the digital counter with rectangular arena of 30×30. Motor activity was assessed in terms of total number of counts of light beam interruption in 20 min.

2.7. Statistical analysis

The data were analyzed by one way ANOVA followed by Newman-Keul's test for multiple comparisons and two-way ANOVA followed by Bonferroni test for multiple comparisons, wherever applicable. The results are expressed as mean±SEM. A difference of P<0.05 was considered significant in all cases.

3. Result

3.1. Effect of AEWS on marble-burying behavior and motor activity

One way ANOVA exhibited that AEWS significantly [F (4, 25) = 97.14, P<0.000 1] influenced marble burying behavior. The post hoc test showed that AEWS (25, 50 and 100 mg/kg) significantly reduced the no. of marbles buried (P<0.001) while the lower dose of AEWS (10 mg/kg) did not significantly reduced no. of marbles buried (P>0.05). Motor activity was

not affected by AEWS (10, 25 and 50 mg/kg) [F (4, 25) = 15.68, P>0.05]; however, AEWS (100 mg/kg) significantly suppressed the locomotor activity (P<0.001) and excluded from further study (Table 1).

3.2. Effect of MEWS on marble-burying behavior and motor activity

One way ANOVA exhibited that MEWS significantly [F (4, 25) = 78.22, P<0.000 1] influenced marble burying behavior. The post hoc test showed that MEWS (25, 50 and 100 mg/kg) significantly (P<0.001) reduced the no. of marbles buried while the lower dose of MEWS (10 mg/kg) did not significantly reduced no. of marbles buried (P>0.05). Motor activity was not affected by MEWS (10, 25 and 50 mg/kg) [F (4, 25) = 6.14, P>0.05]; however, MEWS (100 mg/kg) significantly suppressed the locomotor activity (P<0.01) and also excluded from further study (Table 2).

3.3. Effect of fluoxetine on marble-burying behavior and motor activity

One way ANOVA indicated that fluoxetine significantly [F (3, 20) =66.00, P<0.000 1] influenced marble burying behavior. The post hoc test showed that fluoxetine (10 and 15 mg/kg) dose dependently (P<0.001) reduced marble burying behavior in mice without any effect on motor activity [F (3, 20) =2.21, P>0.05] while the lower dose of fluoxetine (5 mg/kg) was found to be ineffective (P>0.05) (Table 3).

3.4. Effect of AEWS, MEWS and fluoxetine on marble-burying behavior and motor activity

One way ANOVA indicated that fluoxetine and AEWS or MEWS on combined administration has significant [F (5, 30) =88.80, P<0.000 1] influence on marble burying behavior. The post hoc test showed that co-administration of sub-effective dose of AEWS (50 mg/kg) with sub-effective dose of fluoxetine (5 mg/kg) significantly (P<0.001) attenuated marble burying behavior. Similarly, co-administration of sub-effective dose of MEWS (50 mg/kg) with sub-effective dose of fluoxetine (5 mg/kg) significantly (P<0.001) attenuated marble burying behavior. AEWS or MEWS along with fluoxetine has no effect on motor activity [F (5, 30) = 0.309, P=0.903 3] (Table 4).

3.5. Effect of AEWS and MEWS on marble-burying behavior and motor activity in PCPA treated mice

Two way ANOVA indicated that AEWS or MEWS and fluoxetine has significant interaction with PCPA and influenced: PCPA-drug treatment interaction [F (3, 40) = 28.85, P<0.000 1]; PCPA treatment [F (1, 40) = 149.4, P<0.000 1] and drug treatment interaction [F (3,40) = 89.54, P<0.000 1] the marble burying behavior. Post hoc test suggested pretreatment of mice with PCPA partially but significantly attenuated (P<0.001) the inhibitory effect AEWS and MEWS and completely eliminated (P<0.001) the effect of fluoxetine on the burying behavior with no per se effect (P>0.05). All these treatments did not influence the motor activity: PCPA-drug treatment interaction [F (3, 40) = 0.222, P=0.880 1]; PCPA treatment [F (1, 40) = 0.095, P=0.759] and drug treatment interaction [F (3, 40) = 1.526, P=0.222 5] (Table 5).

3.6. Effect of ritanserin and AEWS and MEWS on marble burying behavior and motor activity

Prior treatment of animals with ritanserin (10 mg/kg) significantly affected the inhibitory influence of AEWS (50 mg/kg) or MEWS (50 mg/kg) on marble burying behavior [F (5, 30) = 92.75, P<0.000 1]. Post hoc test showed that AEWS or MEWS showed reduction in inhibition of marble burying (P<0.001) in the presence of ritanserin and without any change in locomotor activity [F (5, 30) = 0.318, P=0.897 8]. There was no effect on no. of marbles buried when mice were treated with alone ritanserin (10 mg/kg) (P>0.05) (Table 6).

Table 1
Effect of AEWS on marble burying behavior and motor activity.

Group	Number of marble buried	Number of locomotors count
Vehicle	12.12±0.73	495.50±15.42
AEWS 10 mg/kg	11.31±0.42	480.71±7.22
AEWS 25 mg/kg	4.22±0.56	550.42±20.12
AEWS 50 mg/kg	1.10±0.37*	540.32±22.46
AEWS 100 mg/kg	0.47±0.12*	280.24±20.46*

Results are expressed as mean ± SEM, *P<0.001 when compared to vehicle.

Table 2
Effect of MEWS on marble burying behavior and motor activity.

Group	Number of marble buried	Number of locomotors count
Vehicle	12.78±0.84	499.24±20.42
MEWS 10 mg/kg	13.12±0.96	492.66±15.24
MEWS 25 mg/kg	5.28±0.76*	520.22±20.46
MEWS 50 mg/kg	0.42±0.18*	522.28±18.42
MEWS 100 mg/kg	0.24±0.10*	340.22±16.98*

Results are expressed as mean ± SEM, *P<0.001 when compared to vehicle.

Table 3
Effect of fluoxetine on marble burying behavior and motor activity.

Group	Number of marble buried	Number of locomotors count
Vehicle	12.48±0.18	500.42±20.38
Fluoxetine 5 mg/kg	10.38±0.76	496.88±16.12
Fluoxetine 10 mg/kg	3.56±1.24*	460.38±10.22
Fluoxetine 15 mg/kg	0.58±0.12*	430.12±5.41

Results are expressed as mean ± SEM, *P<0.001 when compared to vehicle.

Table 4
Effect of AEWS, MEWS and fluoxetine on marble burying behavior and motor activity.

Group	Number of marble buried	Number of locomotors count
Vehicle	12.36±1.12	498.36±25.12
Fluoxetine 5 mg/kg	10.24±0.36	496.42±16.26
AEWS 10 mg/kg	11.46±0.56	490.24±10.68
MEWS 10 mg/kg	12.94±1.34	495.76±8.22
Fluoxetine 5 mg/kg + AEWS 10 mg/kg	0.00±0.00*	510.38±20.12
Fluoxetine 5 mg/kg + MEWS 10 mg/kg	0.00±0.00*	506.32±10.88

Results are expressed as mean ± SEM, *P<0.001 when compared to vehicle.

Table 5
Effect of PCPA treatment on marble burying behavior and locomotor activity.

Group	Number of marble buried	Number of locomotors count
Vehicle (10 mL/kg)	12.68±1.20	499.38±10.12
Vehicle (10 mL/kg)+Fluoxetine 15 mg/kg	0.12±0.06	502.76±9.82
Vehicle (10 mL/kg)+AEWS 50 mg/kg	1.20±0.32	500.12±8.43
Vehicle (10 mL/kg)+MEWS 50 mg/kg	0.32±0.12	510.68±4.40
Vehicle (10 mL/kg)+PCPA treatment	11.12±0.78	480.24±4.36
PCPA treatment +Fluoxetine 15 mg/kg	9.46±0.88	496.92±8.45
PCPA treatment + AEWS 50 mg/kg	7.32±1.42	504.36±7.38
PCPA treatment + MEWS 50 mg/kg	7.42±1.32	506.24±8.22

Results are expressed as mean ± SEM.

Table 6
Effect of ritanserin on marble burying behavior and locomotor activity.

Group	Number of marble buried	Number of locomotors count
Vehicle (10 mL/kg)	12.34±1.24	500.12±16.32
Ritanserin 10 mg/kg	1.32±0.76*	516.86±14.38
AEWS 50 mg/kg	0.78±0.32*	498.12±18.92
MEWS 50 mg/kg	5.82±1.20**	502.22±10.48
Ritanserin 10 mg/kg + AEWS 50 mg/kg	5.46±0.86**	508.78±8.32
Ritanserin 10 mg/kg + MEWS 50 mg/kg	11.32±1.42	501.12±6.46

Results are expressed as mean ± SEM, *P<0.001 when compared to vehicle, **P<0.001 when compared to respective drug control.

4. Discussion

In pharmacological investigations, the possible influence of extracts of *W. somnifera* was studied on marble burying behavior in mice. Results of the investigations revealed AEWS and MEWS reduced the marble burying behavior and exhibited anti-OCD like effect. This effect of AEWS and MEWS was comparable to standard, anti-OCD drug, fluoxetine which also reduced the marble burying behavior in concordance with the previous findings[12]. The maximum effective dose of AEWS and MEWS was 50 mg/kg, at which there was no change in the motor activity. The dose of 100 mg/kg also exhibited inhibitory effect on MBB, but it also reduced the motor activity. Hence, it is not clear whether the effect of AEWS or MEWS at 100 mg/kg is per se effect or it is due to inhibition of motor activity. The present study is the first to investigate the influence of *W. somnifera* extracts on marble burying behavior. This indicates that the AEWS and MEWS both have anti-OCD like effect. The anti-OCD like effect of AEWS and MEWS was further substantiated by the observation that the sub-effective dose of AEWS and MEWS potentiated the effect of sub-effective dose of fluoxetine and exhibited the significant marble burying behavior. This effect of AEWS and MEWS to potentiate the action of fluoxetine differentiates it from anti-anxiety to anti-OCD drug.

Although the exact mechanism of action of AEWS or MEWS to exhibit anti-obsessive activity was not elucidated in the present study, it appears the extracts act as like fluoxetine *i.e.* through serotonergic system. The involvement of serotonergic system was substantiated by the fact that the pretreatment of mice with PCPA partially but significantly

attenuated the inhibitory effect of AEWS and MEWS and completely eliminated the effect of fluoxetine on the burying behavior. However, it is not clear by what mechanism of AEWS and MEWS influences serotonergic system. Previous study has shown that *Hypericum perforatum* (*H. perforatum*) (St. John's Wort), which is known to possess antidepressant and anxiolytic action[13,14] 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 probably the major serotonergic component of *H. perforatum* that contributed to effect of *H. perforatum* on marble burying[15]. Therefore, it is also possible that AEWS and MEWS might have influence on 5-HT reuptake. The phytochemical data on *W. somnifera* revealed the presence of tryptophan (Dr. Duke's Phytochemical and Ethnobotnical Databases), which is an important precursor of 5HT in the serotonergic neurons. The preliminary phytochemical studies on of AEWS and MEWS revealed the presence amino acids in the extract and tryptophan may be enhancing the biosynthesis of serotonin and facilitating the anti-obsessive effect of AEWS and MEWS. As $5HT_{2A/2C}$ is the important postsynaptic receptor involved in the action of standard anti-OCD agents, it was also observed whether AEWS and MEWS treatment influences $5HT_{2A/2C}$ in exhibiting anti-obsessive effect. It was observed that prior treatment with $5HT_{2A/2C}$ antagonist, ritanserin attenuated the effect of AEWS and MEWS on marble burying behavior. This indicates the involvement of post synaptic $5HT_{2A/2C}$ receptors in the action of AEWS and MEWS. This also suggests that AEWS or MEWS show the action through enhanced serotonergic transmission at the level of post synaptic $5HT_{2A/2C}$ receptors.

Concludingly, it is clear from the present investigations that root extracts of *W. somnifera* exhibit significant anti-obsessive effect in marble burying behavior test in mice and the effect may be attributed to enhanced serotonergic function and facilitation of serotonergic transmission.

The plant may exhibit various pharmacological activities. The previous literature has well documented the several effect of *W. somnifera*[16,17]. It has been observed that steroidal lactone (steroids) is believed to be responsible for its various pharmacological action e.q. anxiolytics, antidepressant, anticonvulsant, anti-inflammatory, antioxidant, antitumor, immunomodulatory. Hence, *W. somnifera* may exhibit very potent activity and effect in variety of CNS disorders. As mentioned earlier, *W. somnifera* exhibit anxiolytic and antidepressant effect and such plants can also have influence on obsessive-compulsive behavior, an anxiety related disorder. Therefore, the effect of *W. somnifera* on Obsessive-compulsive behavior needs to be evaluated.

Conflict of interest statement

We declare that we have no conflict of interest.

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