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# Investigation of the anxiolytic and antidepressant activity of ethanolic extract of whole plant of Ashwagandha (*Withania somnifera*), a popular medicinal plant of Indian subcontinent

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## Abstract

*Withania somnifera* is a very popular medicinal plant used in countries of Indian subcontinent especially in Bangladesh and India by local healers and manufacturers of traditional medicines. The whole plant or its parts are used in preparations for various treatment purposes, especially nervous system related disorders. Present study aimed to validate the common use like epilepsy, anxiety, high pulpitation, depression etc and/or, if possible, suggest the possible mode of action of this plant or plant products. An attempt was made to perform experiments to evaluate anxiolytic and antidepressant activity of the ethanolic extract of the whole plant of *W. somnifera*. The experiments were conducted at the Pharmacology lab, Dept. of Pharmacy, North South University during the period of July 2011 to December 2011. Hole board test, Elevated plus-maze test, Open field test, and Forced swimming test were conducted to determine the potential anxiolytic and antidepressant activity. The extract showed significant ($p \leq 0.05$) increase in the motor function and exploratoty behavior in the Hole board test, Elevated plus-maze test, and Open field test compared to the vehicle treated control group. In the forced swimming test, the extract showed significant ($p \leq 0.05$) decrease in the period of immobility compared to the control group. Compiling the results given by the extract in these different experiments, it can be reported that the ethanolic extract of *W. somnifera* have potential anxiolytic and/ or antidepressant activities.

## INTRODUCTION

*Withania somnifera*, commonly known as Ashwagandha, is a commonly used medicinal plant in Ayurvedic preparations[1]. It is found as a constituent in formulation prescribed for a wide range of musculoskeletal conditions such as arthritis, rheumatism etc. It is also used in preparations prescribed in alternative medicine system as a tonic to increase energy and develop health and longevity. *W. somnifera* also helps ameliorate degenerative conditions in athelets, elderly and during times of pregnancy. [2] [3]. Studies have shown that *W. somnifera* is capable to reduce the susceptibility of hippocampus and cerebral cortex to oxidative damage due to its antioxidative properties. [4]. It has also shown significant neuroprotective effects in 6-hydroxydopamine induced Parkinsonism in rats. [5]. Additionally it has shown to be effective in ameliorating Haloperidol-Induced Orofacial Dyskinesia in rat models. [6]. However, little scientific evidence is available on the acute neurological effects like anxiety suppression, depression, despair, exploratory behavior etc. Considering the wide variety of use of *W. somnifera* in traditional system of medicine and the neurological effects which *W. somnifera* is already shown to have, our current study would significantly add to the existing knowledge regarding the neuropharmacology of this plant. This would help to identify probable side effects of medicines having this plant as a constituent. This study even may lead to the new uses of *W. somnifera* in neurological disorders.

## MATERIALS AND METHODS

### Animals

Adult Swiss-Albino Mice, of either sex were procured from the Animal House of International Centre of Diarrheal Disease Research (ICDDR,B). The mice weighed around 20-25 g at the time of the experiment. The animals were placed in white plastic cages with a constant supply of food and water. The animals were provided with a 12/12 hrs day/night cycle. The procured animals were allowed to acclimatize for 1 week before the start of the experiment. Considering the possible neurological effects the drugs might have, each animal was used only once in an experiment. All experiments were conducted after receiving clearance (LSEC-15G-2012) from an ethical body of North South University following the international guidelines for laboratory animal welfare .

### Extract Preparation

*W. somnifera* (Ashwagandha) whole plant was collected from a local herb market in Dhaka. The purchased plant was identified by the National Herbarium at Mirpur, Dhaka and a sample specimen was deposited there. The collected plant was thoroughly washed with water to reduce the microbial load. The plant was then cleaned off all weeds and other unwanted debris. The whole plant was then air-dried in a hot oven at a temperature of 45˚C for 48 hours and was ground using mechanical grinder. The plant powdered was then soaked in ethanol, placed on an orbital shaker for 72 hours (rpm 175) and sieved using a clean cotton gauge followed by filtration by Whattman filter paper. The resulting ethanol solution was then evaporated at 60˚ C in a rotary evaporator till a concentrated jelly like liquid was obtained. This was then refrigerated at -10˚C for 7 days to gradually remove the rest of the ethanol, to obtain a gummy extract. This gummy extract further underwent freeze drying to obtain the final powdered extract.

### Drugs and Other reagents

Pharmaceutical grade Diazepam and Desipramine was procured from Square Pharmaceutical, to be used as a positive control. All other chemicals were procured from Sigma-Aldrich unless otherwise mentioned.

### Drug doses and Route of Administration

*W. somnifera* lyophilized powder was suspended in Normal saline (0.9% NaCl) with Tween 80 using an ultrasonicator. Concentration of 125mg/kg, 250mg/kg and 500mg/kg of the extracts were used to find out the dose dependant activity (if any). Diazepam was administered at a dose of 1mg/kg and Desipramine at a dose of 60mg/kg. All doses were given per orally (p.o.). The positive control drugs were also suspended in Tween 80. The behavioral tests were carried out in a sound-proof roof between 09:00am--1:00pm to minimize the confounding influence on the animals of various times of the day[7]. The mice were fed with the extracts, the positive control drug and the vehicle 120 minutes before the start of every experiment.

### Procedures

### Hole board test

The Hole board test was carried out using a slightly modified method as described by Silvestre et al. [8]. Mice in groups of 10 each were placed individually in the centre of the apparatus and allowed to explore the apparatus for 5 minutes. The behavior parameters that were measured were, Ambulation (sections entered), complete head dips (completely placing the head inside the hole, till the ears), rearing (standing of the rats on its hind legs), crossing (diagonal movement of the rats in the apparatus through the centre) and defecation (the total number of fecal boli). Readings were taken at 120 minutes after the drug treatment (Vehicle, Diazepam 1mg/Kg, and Extract at doses of 125mg/Kg, 250mg/Kg, and 500mg/Kg; p.o.).

### Elevated plus-maze test

This experiment was carried out following the method described by Pellow et al. [9] the subjects were placed individually in the centre of the maze facing an open arm. The parameters that were observed for the next 5 minutes were the following: time spends in the open (a) and closed (b) arms and the number of entries in open (a) and closed (b) arms and total entries (activity). An arm entry was considered only when all four feet entered into that arm. Readings were taken at 120 minutes after the drug treatment (Vehicle, Diazepam 1mg/Kg, and Extract at doses of 125mg/Kg, 250mg/Kg, and 500mg/Kg; p.o.).

### Open field test

A slightly altered method as described by E Schiorring was used in this experiment. [10]. The open field consisted of a rectangular base (300cm×350cm). The floor was made from acryl of black and white color and the walls were of clear plexi-glass 75 cm high. The floor of the cage was divided into black and white squares (50cm×50cm) to make a total of 42 squares. The squares were numbered according to a chessboard (one side was letter as A, B, C.and the other side was numbered as 1, 2, 3 …8). This helped us to describe the route of movement. The letters and the numbers were not painted on the board rather learnt by the observer. The behavioral parameters that were noted were: Forward locomotion (FL), Rearing on the wall (RW), and number of fecal bolus (FB) during the 5 minute period of the experiment. Readings were taken at 120 minute after the drug treatment (Vehicle, Diazepam 1mg/Kg, and Extract at doses of 125mg/Kg, 250mg/Kg, and 500mg/Kg; p.o.).

### Forced swimming test

Forced Swimming test was conducted using a slightly modified method from the one described by Alexandre et al. [11]. Each animal was placed in a rectangular glass tub measuring 30cm×25cm×20cm filled with water at a height of 15cm. The water temperature was maintained at 23±1˚C. The animals were forced to swim for 15 minutes on the first day and were allowed to return to their cage. On the second day, each of the mice was placed again into the water and was forced to swim for 7 minutes. The experimental sessions were videoed and the period of immobility during the last 5 minutes were timed. The animals were considered as immobile as it ceased struggling and moved only to keep afloat in the water keeping its head above the water-line. Readings were taken 120 minutes after the drug treatment (Vehicle, Diazepam 1mg/Kg, and Extract at doses of 125mg/Kg, 250mg/Kg, and 500mg/Kg; p.o.).

### Statistical Analysis

Results were expressed as mean ± SEM (standard error of mean) of responses of n number of animals. All tests were done using SPSS Software Ver. 20. Statistical significance was determined by One-way Analysis of Variance (ANOVA) followed by post hoc Dunnett test. The *P* values less than 0.05 were considered to be significant.

## RESULT

### Hole Board Test

TABLE 1 shows that 125mg/Kg and 250mg/Kg dose of *W. somnifera* extract showed tendency to increase the ambulation, head dips, and rearing than the vehicle treated control group, although the result was not statistically significant. The 500mg/Kg dose of *W. somnifera* extract produced significant ($p \leq 0.05$) increase in the ambulation (76.51%), head dips (77.28%), and rearing (39.16%). Diazepam produced more profound activities: 104.02%, 92.94%, and 56.26% increase in ambulation, head dips, and rearing respectively. Both the extracts and the Diazepam failed to make any impact on the other parameters.

### Elevated Plus-maze test

*W. somnifera* increased, in a dose dependent manner, the total time spent in open arms, entry into the open arms, and activity (total number of entry) (TABLE 2). The highest activity was found with 500mg/Kg dose of the extract with 24.43% ($p \leq 0.05$) and 46.36% ($p \leq 0.05$) increase in the total time spent in open arms and number of entry into the open arms respectively. Diazepam showed similar type of activity but with greater extent ($p \leq 0.01$).

### Open field test

The Forward locomotion and Rearing on the wall were increased significantly ($p \leq 0.05$) by the treatment of *W. somnifera* 500mg/Kg dose (50.35% and 46.18% respectively) and Diazepam 1mg/Kg dose (65.39% and 59.02% respectively). The other two doses of *W. somnifera* (125mg/Kg and 250mg/Kg) showed tendency to be effective but was not statistically significant (TABLE 3).

### Forced swimming test

TABLE 4 shows that the period of immobility was tremendously reduced by treatment of both the *W. somnifera* 500mg/Kg dose ($p \leq 0.05$) and Desipramine 60mg/Kg dose ($p \leq 0.01$) with a reduction of 33.32% and 51.22% respectively compared to the vehicle treated control group.

## DISCUSSION

In the Hole board test, increased number of ambulation, head

**Table 1.** Effects of *W. somnifera* on rats in the Hole board test.

| Group | n | Ambulation | Head dip | Crossing | Rearing | Defecation |
|---|---|---|---|---|---|---|
| Vehicle | 12 | 16.22±2.12 | 15.01±3.22 | 2.34±0.81 | 19.41±2.78 | 3.10±0.61 |
| *W. somnifera* 125mg/Kg | 12 | 20.17±3.21 | 18.91±2.11 | 2.99±0.70 | 23.0±2.68 | 2.98±0.90 |
| *W. somnifera* 250mg/Kg | 12 | 23.23±4.23 | 20.37±3.13 | 3.01±0.92 | 24.01±3.0 | 3.40±0.72 |
| *W. somnifera* 500mg/Kg | 12 | 28.63±1.90* | 26.61±2.62* | 3.89±0.88 | 27.02±1.91* | 3.45±0.81 |
| Diazepam 1mg/Kg | 12 | 33.10±3.17‡ | 28.96±2.43* | 5.42±1.11 | 30.33±2.30* | 5.23±1.05 |

i) Values are expressed as Mean±S.E.M of 12 mice. Differences between groups are determined by One-Way ANOVA followed by post hoc Dunnett test.
ii) *p<0.05 and ‡p<0.01 compared to the control group.

**Table 2.** Effects of *W. somnifera* on rats in the Elevated plus-maze test.

| Group | n | TSOA | %TSOA | NEOA | %NEOA | Activity |
|---|---|---|---|---|---|---|
| Vehicle | 10 | 46.30±9.21 | 15.43% | 4.60±1.10 | 89.84% | 9.23±1.74 |
| *W. somnifera* 125mg/Kg | 10 | 49.90±11.30 | 16.63% | 4.80±1.62 | 47.52% | 10.10±2.11 |
| *W. somnifera* 250mg/Kg | 10 | 59.81±10.22 | 19.94% | 5.74±1.32 | 43.75% | 13.12±2.30 |
| *W. somnifera* 500mg/Kg | 10 | 73.30±14.12* | 24.43% | 9.10±0.81* | 46.36% | 19.63±1.98* |
| Diazepam 1mg/Kg | 10 | 86.91±11.28‡ | 28.97% | 10.22±1.21‡ | 42.60% | 23.99±2.05‡ |

i) Values are expressed as Mean±S.E.M of 10 mice. Differences between groups are determined by One-Way ANOVA followed by post hoc Dunnett test.
ii) *p<0.05 and ‡p<0.01 compared to the control group.
iii) TSOA= Total Spent time in Open Arm, NEOA= Number of Entries into Open Arm.

dips, and rearing by the treatment of the *W. somnifera* extract is an indication of the decrease and increase of the anxiety and exploratory behavior of the animals respectively. A dose dependent decrease in the number of rears after the drug administration is consistent with anxiogenic action while an increase indicates anxiolytic action[12]. Head dipping is considered by many investigators as an indication of neophilia (attraction to new surrounding), which can be translated as an increased exploratory behavior and anxiolytic activity in the extract[13]. Increased ambulation and rearing can also be interpreted as indications of anxiolytic action of the extract[12]. A decrease in the number of rears after administration of ketamine, an anesthetic agent, in the holeboard has been described previously[14].

*W. somnifera* extract increased the percentage of time spent in open arms and the number of entries into open arms in the elevated plus maze. It has been reported that increased time spending in the open arms is an indication of anxiolytic activity[15]. The increased number of entry into open arms and the total entry can be translated as the anxiolytic activity of the extract [14].

**Table 3.** Effects of *W. somnifera* on rats in the Open field test.

| Group | n | FL | RW | FB |
|---|---|---|---|---|
| Vehicle | 10 | 180.31±10.30 | 19.62±4.51 | 8.42±2.41 |
| *W. somnifera 125mg/Kg* | 10 | 189.11±10.10 | 20.92±3.31 | 7.62±1.93 |
| *W. somnifera 250mg/Kg* | 10 | 235.20±11.30 | 23.88±4.44 | 7.92±0.89 |
| *W. somnifera 500mg/Kg* | 10 | 271.10±13.62* | 28.68±5.40* | 7.33±1.21 |
| Diazepam 1mg/Kg | 10 | 298.21±16.23‡ | 31.20±6.11* | 6.13±1.87 |

i) Values are expressed as Mean±S.E.M of 10 mice. Differences between groups are determined by One-Way ANOVA followed by post hoc Dunnett test.
ii) *p<0.05 and ‡p<0.01 compared to the control group.
iii) FL= Forward Locomotion, RW= Rearing in the wall, FB= Fecal bolus

**Table 4.** Effects of W. somnifera on mice in the forced swimming test.

| Group | n | Period of immobility (second) |
|---|---|---|
| Vehicle | 10 | 205.03±14.21 |
| *W. somnifera* 125mg/Kg | 10 | 183.91±13.10 |
| *W. somnifera* 250mg/Kg | 10 | 161.21±12.98 |
| *W. somnifera* 500mg/Kg | 10 | 136.72±13.63* |
| Desipramine 60mg/Kg | 10 | 100.01±10.09‡ |

i) Values are expressed as Mean±S.E.M of 10 mice. Differences between groups are determined by One-Way ANOVA followed by post hoc Dunnett test.
ii) *p<0.05 and ‡p<0.01 compared to the control group

In the open field experiment the test animals exhibited a significantly increased forward locomotion and Rearing on the wall on administration of the *W. somnifera* extract. Some investigators have reported that increased activity like locomotion, rearing, grooming is an indication of the increased motor function which might occur due to the anxiolytic activity of the treatment [[10]]. So, it can be suggested that *W. somnifera* has anxiolytic activity.

In the forced swimming test, the extract treated test animals showed a significant reduction in the total immobility period. This essentially indicated a marked antidepressant activity of the test compounds [[16]].

## CONCLUSION

*W. somnifera* extract was tested for anxiolytic and antidepressant activity using four suitable animal models. From the above observations we can safely suggest that *W. somnifera* (Ashwagandha) contains some potential molecules that have anxiolytic and/or antidepressant activity. Our findings essentially corroborate the pervious works indicating that *W. somnifera* has marked neuropharmacological effect. The novelty of our findings lie in the newer dimension of neurological effects *W. somnifera* might have. Currently we are doing further studies on chemical fractions of this crude extract which eventually might lead us to the isolation and identification of active compounds responsible for shown anxiolytic and antidepressant action. Further studies on receptor level interaction might enable us to better understand the mechanism of action of this plant's neuropharmacological action.

## CONFLICT OF INTEREST

The authors have no competing interest to declare.

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