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# EVALUATION OF ANXIOLYTIC AND ANTIDEPRESSANT ACTIVITIES OF ASHWAGANDHA CHURNA IN MICE

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## ABSTRACT

The current study was explored to evaluate the anxiolytic and antidepressant activities of Ashwagandha churna in mice using various animal models of anxiety and depression. The anxiolytic activity was evaluated in elevated plus maze (EPM) and Actophotometer test and the anti-depressant activity was evaluated in forced swimming test (FST) and Tail suspension test (TST). The efficacy of ashwagandha churna was compared with the standard anti-anxiety (diazepam 2 mg/kg,p.o) and anti-depressant (imipramine – 5 mg/kg, p.o) drugs. Result shown that the ashwagandha churna at the dose of 400 mg/kg on 5$^{th}$ day of drug treatment is as effective as standard drugs used in anti-anxiety and anti-depressant activities in mice by increased time spent and entries to open arm in EPM model, decreased locomotor score (Actophotometer test) and decreased immobility time in FST and TST model respectively. Hence it can be concluded that ashwagandha churna may be used as a potent therapeutic agent for treating anxiety and depressive disorders.

**KEYWORDS:** Anxiolytic, Antidepressant, Ashwagandha churna.

## INTRODUCTION

Anxiety and depressive disorders are the most common mental, emotional, and behavioral problems affecting one-eighth of the total population worldwide, and have become a very important area of research interest in psycho-pharmacology. Stress has significant impact on causation of these two diseases. Though several drugs are available for treatment of anxiety and depression, many are associated with some limitations and also drugs having properties to combat both anxiety and depression are very few.[1] These conditions create an opportunity for alternative treatment of neuropsychiatric disorders by use of medicinal plants or by plant-based formulations.

Despite numerous studies that report the efficacy of Ashwagandha (*Withaniasomnifera* [WS]) in the treatment of various diseases like, The root extract of WS has anti-inflammatory,[2] antioxidant,[3] anti-diabetic,[4] adaptogenic activity against stress,[5] spermatogenic activity,[6] anti-anxiety and anti-depressant effects[7] and the leaf extract of WS showed anticancer activity,[8] But there is no scientific evidence and lacking critical data on safety and tolerability of Ashwagandha formulation for treating anxiety and depression.

Hence, the present study was aimed to investigate the usefulness of the formulation, Ashwagandha churna as a suitable herbal supplement in treating anxiety and depression. Ashwagandha churna is a compound of Ayurvedic formulation for the treatment of various psychiatric illness. This formulation contains plants like *Withaniasomnifera*, *Zingiberofficinalis*, *Piper longum*, *Piper nigrum*, *Elettariacardamomum*, *Mesuaferrea*, *syzygiumaromaticum.* Despite, only limited literature available on this components. There is no evidence regarding the pharmacological evaluation of Anti-anxiety and anti-depressant activities for this formulation. Thus the study was planned to evaluate the effects of Ashwagandha churna for both anxiety and depression in experimental animal models.

## MATERIALS AND METHODS

### 1. Animals

Swiss albino mice of either sex (20 to 25gm body weight) were used in this study. All animals were procured from Mother Theresa post Graduate and Research Institute of Health Sciences, Puducherry. Experimental protocol was approved by their Institutional Animal Ethics Committee. The animal had access to food and water ad libitum was provided with alternate light and dark cycles of 12hours each. The

animals were acclimatized to the laboratory conditions before experiments.

**2. Drugs**

**Test Drug**: Ashwagandha churna (obtained from Ayurvedic Pharmacy, puducherry, Manufactured by AryaVaidhyaShala, Kottakkal, Kerala,India)

**Standard drug:** Diazepam (obtained from Ray chemicals, Pvt, Ltd., Bangalore) and Imipramine (obtained from Gen pharma, Pvt, Ltd., Pune) was used respectively as a standard antianxiety and antidepressant drug. Distilled water was used as a vehicle.

All drugs were prepared freshly on the test day and administered orally (p.o) 1 hr prior to the experiment as a single dose for 5days.

**3. Acute toxicity**

Acute toxicity was generally carried out for the determination of $LD_{50}$ value in experimental animals. This study was performed in mice as per the OECD 423 guidelines. The aim of acute toxicity study was to establish therapeutic index of a particular drug and to ensure safety-in-vivo. In this study, all animals were fasted overnight before the treatment of Ashwagandha churna at various doses and general behavioral sign of the treated mice were monitored individually for 30 minutes after dosing, periodically during 24 hrs. From this study, the result showed that there was no mortality and sign of toxicity observed at 2gm/kg of animal. Hence, $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400 mg/kg) of these doses were selected for further studies.[9]

**4. Experimental Design**

All the animals preferred in this study were divided randomly based on their body weight into four groups (n=4), Treatment schedule was 5days and the groups were as follows:

**Group 1:** Control group received distilled water (1ml, p.o).

**Group 2: (a)Positive control-1:** Standard group received Diazepam (2mg/kg, p.o). [10] (For Anti-anxiety activity).

**(b) Positive control-2:** Standard group received Imipramine (5mg/kg,p.o).[11] (For Antidepressant activity).

**Group 3:** Test group -1, received Ashwagandha churna (ASWC, 200mg/kg, p.o).

**Group 4:** Test group -2, received Ashwagandha churna (ASWC, 400mg/kg, p.o).

**5. Experimental Methods**

**A. Anti-Anxiety Activity**

**a) Elevated Plus Maze Model (EPM)[12]**

The anti-anxiety activity was evaluated using EPM model in *Swiss albino* mice of either sex (weight about 25-35g). The EPM apparatus, consisting two open arms (16cm x 5cm) and two closed arms (16cm x 5cm) extended from a common central platform (5cm x 5cm). The entire maze is elevated (25cm) from the floor having an open roof. After drug treatment, each mouse was placed at the center of the maze with its head facing the open arm and the following parameters were noted for 5 minutes. (a) First preference of mice to open and closed arm. (b) Number of entries in open and closed arms (an arm entry defined as the entry of four paws into the arm) (c) Average time each animal spends in each arm (average time = total duration in the arm/number of entries). The open-arm entries and open-arm time were used as indices of anxiety. The animal activities was noted, 60min after administration of the drug on $1^{st}$ and $5^{th}$ day of treatment.

**b) Actophotometer Model[12,13]**

The locomotor activity was measured by using an actophotometer. It contains 6 photo cells in the outer wall. Interruptions to the signals of photocell beam due to locomotor activity were recorded by means of a six digits counter. The animal was placed individually and the actophotomer was turned on. The animal was made familiarized with the instrument before experimentation and then the basal activity score was noted as counts for 10min. The basal activity score was noted for all the animals before the administration of the drug on the first day and 60min after administration of the drug on $1^{st}$ and $5^{th}$ day of treatment. Percentage decrease in motor activity was also calculated by using the formula;

**[(1-T/C) ×100]** {**T**-Test; **C**-control}

**B. Antidepressant Activity**

**a) Forced Swim Test apparatus [FST][14]**

FST is the most frequently used behavioral model for screening antidepressant like activity in rodents. The mice were individually forced to swim in open glass chamber (25×15×25cm) containing fresh water to a height of 15cm and maintained at 26°±1°C. At this height of water, animals were not able to support themselves by touching the bottom or the side walls of the chamber with their hind-paws or tail. Each animal showed vigorous movement during initial 2min period of the test. The duration of the immobility was manually recorded during the next 4min of the total 6min testing period. Mice were considered to be immobile when they ceased struggling and remained floating motionless in water. Following swimming session, mice were towel dried and returned to their housing conditions. This activity was performed after 60min of drug administration on $1^{st}$ and $5^{th}$ day of drug treatment.

**b) Tail Suspension Test [TST][15]**

TST is the most commonly employed behavior model for screening antidepressant activity in mice. The mice were suspended individually 5 cm above the floor by an adhesive tape placed approximately 1cm from the tip of the tail. Testing was carried out in a darkened room with minimal background noise. Mice were considered to be immobile only when they hung passively or remained

completely motionless. The total duration of immobility was quantified during a test period of 6min on both the 1st and 5th day of drug treatment. The test was conducted in a dim lighted room and each mouse was used only once in the test.

## RESULT

### A. Anti-anxiety Activity

**Table 1: Effect of ASWC on Elevated plus Maze Test in mice (open arm).**

| Groups | Treatment | No. of Entries in open arm(count) [Mean ± SEM] | | Average Time (sec) Spent in open arm [Mean ± SEM] | |
|---|---|---|---|---|---|
| | | Day-1 | Day-5 | Day-1 | Day-5 |
| I | Control (Vehicle) | 1.250 ± 0.250 | 1.500 ± 0.288 | 11.00 ± 1.683 | 11.75 ± 1.702 |
| II | Standard (Diazepam, 2mg/kg,p.o) | 5.000 ± 0.408* | 6.250 ± 0.478** | 62.58 ± 1.102** | 68.15 ± 2.950** |
| III | Test-1 (ASWC, 200mg/kg.p.o) | 3.000 ± 0.707# | 4.000 ± 0.408* | 47.63 ± 1.772# | 54.25 ± 2.839* |
| IV | Test-2 (ASWC, 400mg/kg,p.o) | 3.250 ± 0.250# | 5.250 ± 0.250** | 57.05 ± 2.216* | 66.81 ± 1.801** |

*p<0.01, **P<0.001, #P<0.05 Vs control

**Table 2: Effect of ASWC on Elevated plus Maze Test in mice (closed arm).**

| Groups | Treatment | No. of Entries in closed arm (count) [Mean ± SEM] | | Average Time (sec) Spent in closed arm [Mean ± SEM] | |
|---|---|---|---|---|---|
| | | Day-1 | Day-5 | Day-1 | Day-5 |
| I | Control (Vehicle) | 5.000 ± 0.9129 | 4.500 ± 0.6455 | 47.25 ± 1.215 | 46.79 ± 0.736 |
| II | Standard (Diazepam, 2mg/kg, p.o) | 1.500 ±0.2887* | 1.500 ± 0.2887* | 43.57 ± 0.781# | 35.25 ± 0.876* |
| III | Test-1 (ASWC, 200mg/kg, p.o) | 2.250 ± 0.4787# | 2.000 ± 0.4082# | 41.92 ± 0.877* | 40.42 ± 1.055# |
| IV | Test-2 (ASWC, 400mg/kg, p.o) | 2.000 ± 0.4082# | 2.000 ± 0.5774* | 41.46 ± 1.031# | 38.75 ± 1.132* |

*p<0.01, #P<0.05 Vs control

From the **Table: 1 & 2**, the results showed that, The test drug, Ashwagandha churna (ASWC) at a dose of 200mg/kg and 400mg/kg significantly increased (**P<0.001, #P<0.05) the number of entries and average time spent in the open arms while decreased the number of entries and duration of time spent in closed arm when compared to the control group. On 5th day of drug treatment showed more significant effect as well as produced almost similar effect to that of diazepam treated group (**P<0.001), when compared with the 1st day drug treatment.

**Table 3: Effect of ASWC on Locomotor activity (Actophotometer) in mice.**

| Groups | Treatment | Locomotor activity (score) in 10 minutes [Mean ± SEM] | | % change in Activity | |
|---|---|---|---|---|---|
| | | Day-1 | Day-5 | Day-1 | Day-5 |
| I | Control (Vehicle) | 87.00 ± 2.380 | 88.25 ± 3.119 | NA | NA |
| II | Standard (Diazepam, 2mg/kg,p.o) | 40.75 ± 5.648* | 39.25 ± 1.493* | 53.2(↓) | 55.5(↓) |
| III | Test-1 (ASWC, 200mg/kg.p.o) | 58.00 ± 3.367# | 52.00 ± 2.799* | 33.3(↓) | 41.1(↓) |
| IV | Test-2 (ASWC, 400mg/kg,p.o) | 48.50 ± 1.848* | 41.25± 3.146** | 44.3(↓) | 53.3(↓) |

**NA**-Not Applicable, **(↓)** -% decrease in activity. *p<0.01, **P<0.001, #P<0.05 Vs control

From the Table: 3 showed that the basal activity score in four groups recorded for 10minutes. Locomotor activity is considered as an index of alertness and the spontaneous decrease in basal activity score implicates the reduction of anxiety. From the observation, there is significant decrease (*p<0.01, **P<0.001) in the locomotor score in case of animals treated with ASWC at lower and higher dose when compared to control groups. But when compared to the standard treated group, the test drug does not showed significant effect.

### B. Anti-depressant Activity

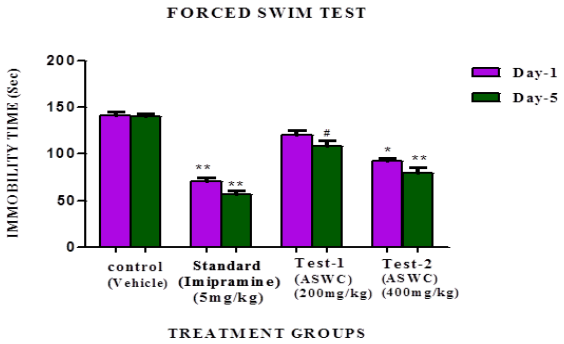


**Figure 1: Effect of ASWC on Forced Swim Test in mice.**

*p<0.01, **P<0.001, #P<0.05 Vs control.

From the figure 1, the result showed that, compared with the control group, ASWC at a dose of 400mg/kg significantly decreased the duration of immobility (*p<0.01, **P<0.001) on both day of treatment, on the 5th day of test drug treatment showed more significant effect when compared to control group. Whereas, at a dose of 200mg/kg (day-1) showed no statsistically significant reduction in the duration of immobility. Additionally, Imipramine, a classical antidepressant agent, caused a significant reduction in the immobility time (**P<0.001) in the FST.

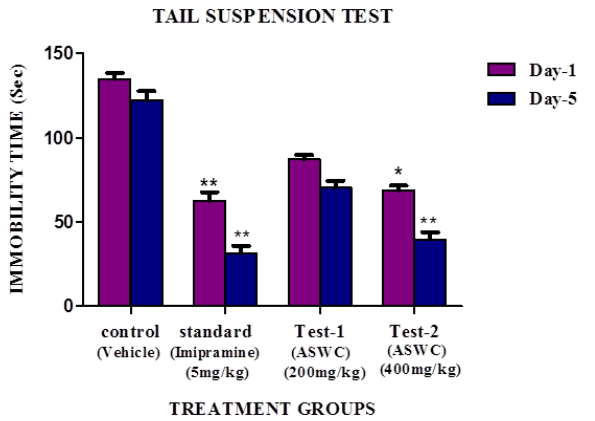


**Figure 2: Effect of ASWC on Tail Suspension Test in mice.**
*p<0.05, **P<0.01 Vs control.

From the figure: 2, the result showed that, when compared with the control group, ASWC at a dose of 400mg/kg (*p<0.05, **P<0.01) and 5mg/kg of imipramine significantly (**P<0.01) shortened the duration of immobility in the tail suspention test (TST). The antidepressant effect of ASWC at the highest dose was similar to that of imipramine. Hence, ASWC produced dose dependent action. However the difference from the control group with the lowest dose of ASWC (200mg/kg) was not statistically significant.

## DISCUSSION

The present work has evaluated the anxiolytic and antidepressant activities of Ashwagandha churna in mice. Generally, Anxiety and depression are caused due to imbalance between excitatory and inhibitory neurotransmitter. Both Benzodiazepines and Tricyclic antidepressants have been extensively used for last 40years to treat several forms of anxiety and depression. But, due to their unwanted side effects, alternative treatment strategies were preferred. In the search of alternatives, we preferred ashwagandha churna, one of the ayurvedic formulation for our study in various non-conditioned behavioral animal model for anxiety (Elevated Plus Maze test and Actophotometer test) and for depression (Forced Swimming test and Tail Suspension test). These tests are classic and standard models for screening of anxiety and antidepressant.

In Elevated Plus maze (EPM), as expected, the standard drug (Diazepam) and test drug (ASWC-200mg/kg and 400mg/kg) produced significant increase in the number of entries and average time spent in open arm and decreases number of entries and time spent in a closed arm when compared with control group. The test dose effect is almost comparable with standard group. Hence, the results suggest that the test drug may possess a positive modulation effect on $GABA_A$-chloride channels and thereby may cause a rise in GABA levels in brain.

From Actophotometer test, There is a significant decrease in the locomotor score in case of animal treated with ASWC on 5th day treatment compared to control group. This is also comparable with the effect of standard drug (diazepam). But on the 1st day of treatment (both doses) showed minimal changes in comparison with standard and control. Hence the given test drug has an anxiolytic effect.

The results obtained from FST and TST demonstrates that oral administration of test drug at higher dose (400mg/kg) for 5 consecutive days produce a significant reduction in the duration of immobility as comparable with the control group and standard group (imipramine). This observation suggests that the ASWC has antidepressant effect. This may be caused by preservation of monoamine neurotransmitters.

Our data showed that, ASWC exerted both anxiolytic and Antidepressant activity in several classical animal models which is almost comparable with the standards. Being the formulation, the observed activity profile may be attributed to one or more bioactive principles in it. Because, from our literature survey, it was reported that, the root extract of ashwagandha contains (glycowithanolides) produced antianxiety and antidepressant activity [16]. Due to the presence of glycowithanolides, ashwagandha churna might produce these activities.

## CONCLUSION

Our present study suggest that, the anxiety and depression show important overlaps and even anxiety may be an early maintenance of depression as suggested by analyzing the obtained experimental results that the mice treated with ashwagandha churna at low dose exhibited anxiolytic activity and at high dose exhibited antidepressant activity. This activity may be produced due to the active constituents (withanone or Glycowithanolides) present in Ashwagandha. However further studies are needed to clarify the exact mechanism of action and to determine if administration of ashwagandha churna is beneficial for patients with anxiety and depression.

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