



Original Research Article

The motor coordination activity of alcoholic extract of *Withania coagulans* fruits in Swiss albino mice by rota rod test

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ABSTRACT

Background: The central nervous system (CNS) depressant effect is caused by the numerous sedative and hypnotic drugs that are currently used. Though in the late seventies some work was done on *Withania coagulans*, it is the vulnerable species not widely scattered. Therefore, it was worthy to investigate the CNS depressant activities of alcoholic extract of *Withania coagulans* fruits in Swiss albino mice by using rota rod test.

Aim: To study the motor coordination activity of alcoholic extract of *Withania coagulans* fruits in Swiss albino mice by Rota Rod Test.

Materials and Methods: Motor coordination was measured by using the Rota Rod Test. The CNS depressant drugs reduce the endurance time of mice on the rotating rod as they weaken the motor coordination so that mice fall early on the revolving rod. This endurance time is tested statistically among the control, standard and the test drugs.

Statistical Analysis: One way ANOVA was used for the statistical analysis.

Results: The endurance time in Swiss albino mice was statistically highly significantly (p -value < 0.001) associated with the alcoholic extract of *Withania coagulans* fruits on rota rod test.

Conclusion: The alcoholic extract of *Withania coagulans* fruits established the CNS depressant activity in Swiss albino mice by rota rod test.

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1. Introduction

The daytime impairment caused by insomnia is due to the individual experience of trouble with sleep initiation, extent, consolidation, quality, which occurs in spite of adequate opportunity for sleep, thus results in.¹ The prevalence of insomnia in India is 9% in the general population² compared to 10-30% in the world.³⁻⁵

There are various drugs like benzodiazepines and barbiturates used for the treatment of insomnia.⁶ However, they do not lack the side effects. The Rota rod test is widely used to evaluate the motor coordination in rodents.⁷ When a mouse is placed on a constant speed rotating rod after

ingestion of a central depressant, instead of walking on the rod the animal easily falls from it. This test was first introduced by Dunham and Miya for assaying the drug effects on the motor activity.⁸ Since then, the effects of various central depressants, investigated by this test have been reported.⁹⁻¹¹

The Solanaceae family covers 84 genera comprising approximately 3,000 species. These species are mainly annual shrubs distributed all over the world. The two genera namely *Withania* and *Physalis* play a vital role in the Unani and Ayurvedic systems in the South East Asia. The 23 acknowledged *Withania* species are extensively scattered in the drier portions of tropical and subtropical zones.¹²⁻¹⁴ Out of all these species, the *W. coagulans* and *W. somnifera* are financially and curatively important.¹⁵⁻¹⁷

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Withania coagulans is a rare species, not found widespread in the territory. This plant is primarily used for the milk coagulation. Though plenty of work has been done to study its antidiabetic activity, not much work is done to evaluate its action on the CNS.^{18–20} In 1977 Budhiraja et al. reported CNS depressant effect of this plant.²¹ Thereafter this plant was not much studied for the CNS activity. Thus, it was thought worthwhile to measure the CNS depressant action of this medicinal herb in Swiss albino mice by rota rod test.

2. Materials and Methods

2.1. Rota rod test apparatus

The Rota rod comprises of experimental partitions, with a common revolving rod of about 25 mm diameter with selectable speeds of around 5, 10, 15, 20 and 25 revolutions per minute. The interval counters are provided in each partition. The apparatus works on 220/30 Volts, single phase, 50 Hz, AC. On the floor of each partition there is a cantilever platform that is hinged at the back end.

2.2. Rota rod test procedure

The shaft's angular speed was adjusted by changing the location of the Drive Belt, from one pair corresponding grooves to another. The shaft revolved in anticlockwise direction and such the animal in general fell on the forward-facing side of the platform. In our experiment the shaft's angular velocity was adjusted to 25 revolutions per minute.

On switching on, the counter displayed some arbitrary reading as the counter was running i.e. counting. The counter was stopped by pressing the platform lightly till the switch below it activated by making a click sound. The platform below it was elevated lightly making certain that the switch below it was free. The device made ready to work.

Placing the mouse on the revolving shaft the RESET switch was concurrently pressed. On pressing the RESET switch counter started counting the time in seconds. Regardless of the preceding reading on the counter, the counter started calculating from 'zero' only. When animal fell off the revolving shaft on the platform, the platform was depressed down by the falling impact- stopping the counter. This disclosed the animal's endurance time in seconds. Endurance time is defined as the time period for which each mouse withstood on the revolving rod during test period.²²

To resume the counter again for the next trial, the platform was elevated gently, mouse was positioned on the revolving rod and the RESET switch was pressed, so that the counter started.

The Rota rod was designed for the normal rat of about 150 gm in weight. Since the mice weight was much lower than that of rats, the difference of weight was added to the platform by putting secured weight with the help of adhesive tape at the forward-facing end of the platform. All

the partitions were used concurrently.

2.3. Control, standard and test drugs

For control distilled water was given as vehicle. As the standard drug Diazepam was used. The test drugs (WCFAlcE of 200 mg/kg, 500mg/kg and 1000 mg/kg doses p. o.) were administered to the animals 30 min before the experiment. Throughout the period of experiment, the test drugs were given every day for 30 days. The mice were observed for 5 minutes. On Day 1, Day 8, Day 15, Day 23 and Day 30 recordings were done for all the groups. The recordings were taken half an hour after drug administration to the respective group.

Drugs were given as follow:

Control: Vehicle (Distilled Water) 2 ml/kg p. o. once a day for 30 days.

Standard: Standard drug (Diazepam) 5mg/kg i. p. once half an hour before test.

ALC-200: WCFAlcE 200 mg/kg p. o. once a day for 30 days.

ALC-500: WCFAlcE 500 mg/kg p. o. once a day for 30 days.

ALC-1000: WCFAlcE 1000 mg/kg p. o. once a day for 30 days.

Where WCFAlcE = *Withania coagulans* fruits alcoholic extract

Animals: Swiss albino mice of either sex were used for this study.

3. Results

As observed in the Table 1, on Day 1 to Day 8 there was no statistically significant difference in endurance time by mice. However, on days 15, 23 and 30 endurance time by mice on rota rod apparatus reduced highly significantly ($p < 0.001$) for all the three doses of 200 mg/kg, 500 mg/kg and 1000 mg/kg of WCFAlcE compared to control. Furthermore, dose response relationship was observed for this reduction for all the three doses. To conclude, this reduction in endurance time by test drug was comparable to that of standard drug diazepam.

4. Discussion

As observed in Table 1, on days 15, 23 and 30 endurance time by mice on rota rod apparatus 'decreased' highly significantly ($p < 0.001$) for all the three doses of 200 mg/kg, 500 mg/kg and 1000 mg/kg of WCFAlcE compared to control. Furthermore, dose response relationship was observed for this 'decrease'.

Our study is extraordinary one as there are no other articles reported which have verified the effect of any of the extract of *Withania coagulans* on mice using Rota rod apparatus. Yet, *Withania somnifera* (WS) which is a related species to *Withania coagulans* (WC) having same

Table 1: Effect of oral administration of WCFAlcE on endurance time (mean±SD) of mice in seconds in Rota rod test.

| | Control | Standard | ALC-200 | ALC-500 | ALC-1000 |
|--------|-------------|--------------|---------------|--------------|--------------|
| Day 1 | 18.66±9.11 | 17.16±8.08 | 16.33±11.21 | 17.66±14.37 | 20.50±11.97 |
| Day 8 | 17.83±12.49 | 12.50±5.82 | 17.50±11.48 | 18.33±10.44 | 19.50±10.31 |
| Day 15 | 23.16±3.86 | 8.66±2.58*** | 11.50±2.42*** | 9.50±3.27*** | 8.33±1.63*** |
| Day 23 | 21.33±2.42 | 5.83±1.94*** | 9.83±1.94*** | 7.66±1.86*** | 5.66±2.06*** |
| Day 30 | 19.50±3.72 | 3.50±1.04*** | 7.16±2.78*** | 5.66±1.50*** | 3.83±1.16*** |

Where * means p value < 0.5, ** means p value < 0.01, *** means p value < 0.001

WCFAlcE : Withania coagulans fruits alcoholic extract

Control: Vehicle (Distilled Water) 2 ml/kg p. o. once a day for 30 days.

Standard: Standard drug (Diazepam) 5 mg/kg i. p. half an hour before test.

ALC- 200: WCFAlcE 200 mg/kg body weight p. o. once a day for 30 days.

ALC- 500: WCFAlcE 500 mg/kg body weight p. o. once a day for 30 days.

ALC- 1000: WCFAlcE 1000 mg/kg body weight p. o. once a day for 30 days.

genus, had been tested for the motor coordination using Rota rod apparatus in middle cerebral artery occluded (MCAO) rodents. It was found that WS had significantly ($p < 0.05$) 'increased' the performance of MCAO rodents treated with WS root extract on Rota rod apparatus.²³ Hence it can be concluded that the different Withanoloides in both the species might be responsible for this activity. The term "Withanolide" composed of "withan" from the genus Withania plus "olide" is biochemical term for a lactone. Today about 400 Withanolides discovered from fifty eight solanaceous species belonging to twenty two genera. Structurally they are steroids built on an ergostane skeleton.²⁴

There are two varieties of rota rod test. First is the constant speed rota rod, and second is accelerating Rota rod.²⁵ In our test we used the constant speed Rota rod. But it has few disadvantages. Rod's diameter can affect the performance of mice,²⁶ therefore we used Rota rod of 25 mm diameter. There can be impairment of memory of mice rather than the motor coordination by the test drug,²⁷ to eliminate this bias we trained the mice robustly before test. The constant speed rota rod consumes lot of time,²⁷ because the time limit needs to be set which declines the sensitivity of this test.²⁷ To eradicate this bias, we used the maximum velocity of 25 revolutions per minute on the Rota rod test which had a range of 5 to 25 revolutions per minute. The maximum endurance time of mouse on this apparatus was 38 seconds. This test can estimate the muscle relaxant property of any compound but does not really distinguish between anxiolytics and neuroleptics. Typically, the central depressant medicines have muscle relaxant action. Hence, this test can be valuable to measure the central depressive effect of any medication.⁸

Though stimulants rise the performance of mice on the rota rod test so that mice take longer time to fall from the rotating rod,¹¹ the depressants drop the endurance time of mice on Rota rod so that mice fall early on revolving rod.²⁸ To conclude, our test drug WCFAlcEs might have depressant action on mice. Since the effect appeared after 2 weeks, it cannot be used in the acute settings. Therefore it is

more suitable for the chronic settings.

5. Source of Funding

None

6. Conflict of Interest

None.

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