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Original Research Article

## The spontaneous motor activity of aqueous extract of *Withania coagulans* fruits in Swiss albino mice by actophotometer

Devesh D. Gosavi<sup>1</sup>, Amit S. Kamdi<sup>2\*</sup>, Suvarna M. Kalambe<sup>2</sup>, Pankaj N. Bohra<sup>3</sup>

<sup>1</sup>Department of Pharmacology, Mahatma Gandhi Institute of Medical Sciences, Sewagram, Maharashtra, India

<sup>2</sup>Department of Research and Development, Sanjeevani Multipurpose Society, Mul, Maharashtra, India

<sup>3</sup>Department of Paediatrics, SNEH Foundation, Chichwad, Pune, Maharashtra, India

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### \*Correspondence:

Dr. Amit S. Kamdi,

Email: [dramitkamdi99@gmail.com](mailto:dramitkamdi99@gmail.com)

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### ABSTRACT

**Background:** People spend about one third of their time in sleep every day. The various sedative and hypnotic medications used today have numerous side effects. In the late seventies a very little work was done on the *Withania coagulans* - a vulnerable species that is found scattered in the world. Therefore, it was essential to discover the CNS depressant activities of aqueous extract of *Withania coagulans* fruits in swiss albino mice by using actophotometer.

**Methods:** The spontaneous locomotor activity was evaluated by using the actophotometer. The CNS depressant drugs decrease the locomotor activity in mice as they impair the motor coordination so that mice stay at one place for the longer time. Therefore, there is less disruption of the beams of light. This spontaneous locomotor activity time is statistically correlated among the control, standard and the test drugs.

**Results:** There was statistically highly significant (p value <0.001) association observed between aqueous extract of *Withania coagulans* fruits with spontaneous locomotor activity in swiss albino mice on the actophotometer.

**Conclusions:** The aqueous extract of *Withania coagulans* fruits demonstrated the CNS depressant activity in swiss albino mice by actophotometer.

**Keywords:** CNS depressant, Actophotometer, Swiss albino mice, *Withania coagulans*

### INTRODUCTION

The importance of sleep can be understood from the fact that people spend about one third of their time in sleep every day. The prevalence of sleep disorders is about 15% in USA, and that in the adolescent is 6%.<sup>1,2</sup> However the prevalence in India is not clear.<sup>3</sup> Insomnia causes depression and vice versa.<sup>4</sup> Though benzodiazepines are most widely used for the treatment of insomnia among various medications prescribed, they are not devoid of the side effects. Therefore, there is always a need to invent new drugs.

The word *Solanaceae* means soothing. The *Solanaceae* family covers 84 genera comprising approximately 3,000 species. 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.<sup>5-7</sup> Out of all these species, the *W. coagulans* and *W. somnifera* are financially and curatively important.<sup>8-10</sup> As the name suggest the herb has milk coagulating property due to the presence of an enzyme, which is a plant hormone. The active principle named "Withanin" found in the seeds of the fruits is ferment like animal rennet. Therefore, it is also called as vegetable rennet or Indian rennet.<sup>11,12</sup> It is also used to

control the diabetes mellitus.<sup>13</sup> Spontaneous motor activity (SMA) is also referred as spontaneous locomotor activity (SLA) in mice was used to assess the central nervous system (CNS) depressant effect of *Withania coagulans*. This was assessed using actophotometer (photocell activity cage). It was found that if the drug had the sedative property then there was less disruption of the beams of light in the actophotometer as the rodents did not try to explore much because of the sedation.<sup>14</sup> On the other hand, stimulants had the opposite effect.<sup>15</sup> The *Withania coagulans* was not explored much for its central nervous system (CNS) depressant effect. Therefore, it was thought worthwhile to assess the CNS depressant action of the aqueous extract medicinal herb in swiss albino mice by actophotometer for spontaneous locomotor activity.

## METHODS

### Animals

The mice were procured after taking permission from Institutional Animal Ethical Committee (IAEC) of MGIMS. The animals were housed in polyvinyl wire web enclosures in the central animal room of institute approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Mice were grouped into 5 groups with 6 mice each.

### Preparation of aqueous extract of *Withania coagulans*

The plant was authenticated by the local botanist. The fruits of *Withania coagulans* were dried in shade for 15 days in closed room. Thereafter, they were powdered in an electric mixture, filtered and preserved in an airtight container for study.

The 30 gm of powder material was charged inside the thimble of the primary chamber of the Soxhlet extractor.<sup>16</sup> The Soxhlet extractor was positioned onto a flask comprising the extraction liquid (100% distilled water). Thereafter a condenser (having continuous flow of tap water through inlet and outlet to condense the vapors) was attached to the top of the primary chamber. The solvent was heated to evaporate. The solvent vapors passed up through the distillation arm. The vapors were condensed, liquefied and the warm liquid was collected inside the chamber housing the thimble of powder. The certain amount of the desired dissolved in the warm solvent. When the Soxhlet chamber was almost full, it was automatically emptied by a siphon tube with the solvent passing back down to the flask. This cycle was allowed to repeat many times over hours to days. During each cycle, a part of the non-volatile compound dissolved in the water. After many cycles the desired compound was concentrated in the distillation flask. The appearance of colorless solvent in the siphon tube was the indication of exhaustive extraction and based on that, further extraction was terminated. The non-soluble part of the extracted solid left in the thimble was discarded. The

extract was again dried in the dark and closed room at room temperature. The percentage of yield was 7% after drying. Thus, we used 30 g of crude powder but we got 2 g of extract.

### Actophotometer apparatus

Actophotometer instrument was purchased from techno electronics, Lucknow, India. It was 30×30×30 cm in dimension. The two photocells were fixed 3 cm above the floor. Each wall of the cage had 3 equidistant holes in a horizontal array, 7 cm above the floor. Through the holes three light beams were passed from all sides of the apparatus. They crossed in the centre of the cage. There were the infra-red filters to minimize the effect of light on behaviour. The number of interruptions of the light beams were recorded on digital counters in the instrument automatically.

### Procedure

Each mouse was kept for 5 minutes in the activity cage. If mouse started the exploratory activity, then the beam of light was interrupted. Each interruption was picked up automatically by the digital recorder in the instrument and was counted. If the animal stopped the locomotor activity, the digital counter stopped recording. Again, when animal started walking, the activity was recorded. The "Walks" were defined as the number of times the mouse moved with all four feet in the space between two opposite walls of the cage. The total time spent in walking was recorded as above.

### Control, standard and test drugs

The fresh solution was prepared by dissolving the extract in distilled water before each experiment for oral administration. Injection diazepam was purchased from the market and then diluted in distilled water before administering mg/kg intraperitoneally to the mice.

Distilled water was given as vehicle for control. Diazepam was used as the standard drug. The animals were treated 30 minutes before the experiment with the test drugs (WCFAqE of 200 mg/kg, 500 mg/kg and 1000 mg/kg doses p.o.). However, the test drug was given every day for 30 days throughout the period of experiment. The mice were observed for 5 minutes. Recordings were done on day 1, day 8, day 15, day 23 and day 30 for all the groups. The recordings were taken half an hour after drug administration to the respective groups. After each trial the equipment was cleaned with super hypochlorous water to prevent the bias based on olfactory cues.

Drugs were given in the following manner.

**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.

**AQ-200:** WCFAqE 200 mg/kg p.o. once a day for 30 days.

**AQ-500:** WCFAqE 500 mg/kg p.o. once a day for 30 days.

**AQ-1000:** WCFAqE 1000 mg/kg p.o. once a day for 30 days.

Where, WCFAqE = *Withania coagulans* fruits aqueous extract.

## RESULTS

As shown in the Table 1 and further clarified from Figure 1, on day 1 to day 8 there was no statistically significant difference in the spontaneous locomotor activity by mice. However, on days 15, 23 and 30 spontaneous locomotor activity by mice in actophotometer decreased highly significantly ( $p < 0.001$ ) for all the three doses of 200 mg/kg, 500 mg/kg and 1000 mg/kg of WCFAqE compared to control. Furthermore, dose response relationship was observed for these doses. To conclude, this decrease in spontaneous locomotor activity by test drug was comparable to that of the standard diazepam.

**Table 1: Effect of oral administration of WCFAqE on spontaneous locomotor activity of mice in seconds (mean±SD) in actophotometer.**

Days	Control	Standard	AQ-200	AQ-500	AQ-1000
<b>Day 1</b>	254.50±15.63	258±30.35	245.83±30.20	239.50±31.48	255.50±32.92
<b>Day 8</b>	270.33±82.41	231.33±20.14	243±32.23	230±71.16	242.83±39.21
<b>Day 15</b>	263.50±7.55	176.66±40.91***	174±23.06***	158.66±17.55***	137.33±12.69***
<b>Day 23</b>	259.66±7.28	158.50±27.34***	151±24.24***	134.33±22.31***	121.16±21.81***
<b>Day 30</b>	251.66±6.43	128.50±26.28***	120.66±27.63***	103.83±15.51***	91.83±15.21***

\* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$  when compared to control group.

**WCFAqE:** *Withania coagulans* fruits aqueous 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.

**AQ-200:** WCFAqE 200 mg/kg body weight p.o. once a day for 30 days.

**AQ-1000:** WCFAqE 1000 mg/kg body weight p.o. once a day for 30 days.

**WCFAqE:** *Withania coagulans* fruits aqueous extract.

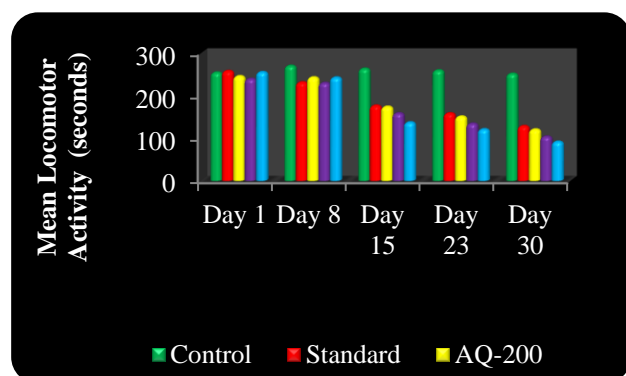
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**AQ-200:** WCFAqE 200 mg/kg body weight p.o. once a day for 30 days.

**AQ-500:** WCFAqE 500 mg/kg body weight p.o. once a day for 30 days.

**AQ-1000:** WCFAqE 1000 mg/kg body weight p.o. once a day for 30 days.



**Figure 1: Effect of oral administration of WCFAqE on spontaneous locomotor activity of mice in seconds (mean ±SD) in actophotometer.**

**AQ-500:** WCFAqE 500 mg/kg body weight p.o. once a day for 30 days.

## DISCUSSION

As shown in the Table 1 and Figure 1, on day 1 to day 8 there was no statistically significant difference in the spontaneous locomotor activity by mice. However, on days 15, 23 and 30 spontaneous locomotor activity by mice in actophotometer decreased highly significantly ( $p < 0.001$ ) for all the three doses of 200 mg/kg, 500 mg/kg and 1000 mg/kg of WCFAqE compared to control. The dose response relationship was also observed.

Our study is unique one as there are no other studies reported which have tested the effect of any part of *Withania coagulans* on locomotor activity of mice using actophotometer. However, in 1977 Budhiraja et al reported the significant ( $p < 0.001$ ) decrease in spontaneous locomotor activity at the doses of 1 g/kg, 200-400 mg/kg and 5 ml/100 g of the aqueous extracts of *Withania coagulans*.<sup>17</sup> He further concluded the CNS depressant activity based on the pentobarbitone sleeping time.<sup>17</sup> In another study, at the doses of 100 mg/kg and 200 mg/kg intraperitoneal, leaf aqueous extract of the *Withania Somnifera* (a similar species as that of *Withania coagulans*) significantly ( $p < 0.001$ ) decreased the locomotor activity and dose response relationship was also observed.

It was used to record the spontaneous locomotor activity of mice. In this activity “walks” and “rears” can be measured.<sup>18</sup> However, this instrument does not measure the “grooming” and “washing” by rodents.<sup>18</sup> In our experiment we focused on the “walk” part to measure the locomotor activity. Since, it took 15 days for the effect to appear, the results cannot be extrapolated in acute condition, rather the test drug can be used in chronic conditions.

The stimulants increase the spontaneous locomotor activity in contrast to the depressants which decrease the same.<sup>19</sup> Therefore the test substance might have CNS depressant activity. Hence it can be concluded that similar Withanolides in both the species might be responsible for this activity.

## CONCLUSION

To conclude, our test herb *W. coagulans* demonstrated the CNS depressant activity in the swiss albino mice for the three doses of WCFAqE by actophotometer. However, the further research is warranted to understand *W. coagulans* better at the receptor level.

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