Original Research Article

The anxiolytic action of alcoholic excerpt of Withania coagulans fruits in Swiss albino mice by Elevated Plus Maze

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A R T I C L E I N F O

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Withania coagulans

A B S T R A C T

Background: The various antianxiety drugs currently used cause numerous adverse drug reactions. Though in the late seventies some work was done on Withania coagulans, it is the vulnerable species not found plentiful. Therefore, it was worthy to investigate the anxiolytic actions of alcoholic extract of Withania coagulans fruits in Swiss albino mice by using Elevated Plus Maze (EPM) test.

Aim: To study the antianxiety activity of alcoholic extract of Withania coagulans fruits in Swiss albino mice by Elevated Plus Maze (EPM) test.

Materials and Methods: The antianxiety activity was measured using Elevated Plus Maze (EPM) test. The antianxiety drugs promote the mice to spend more time in open arm and less time in close arm. This increase in the time spent in the open space as well as number of entries from close to open space was correlated statistically with control and standard in the EPM test.

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

Results: Time spent in the open arm (Open) as well as number of entries into the open arm (EOA) by the Swiss albino mice was statistically highly significantly (p-value < 0.001) associated with the alcoholic extract of Withania coagulans fruits in Elevated Plus Maze (EPM) test.

Conclusion: The alcoholic extract of Withania coagulans fruits established the antianxiety activity in Swiss albino mice by Elevated Plus Maze (EPM) test.

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1977 Budhiraja et al. reported CNS depressant action of this herb. Afterwards this herb was not much explored for the CNS action, though lot of work was done on Diabetes and other diseases. Thus, it was thought worthwhile to investigate neuropsychopharmacological parameter like anti-anxiety action of Withania coagulans fruits alcoholic excerpt by means of Elevated Plus Maze (EPM). Mice have an aversion for high and open space and prefer enclosed space, thus spend more duration in the enclosed arm. The fall in duration consumed in open arms as well as less number of open arm entries are the indicators of high level of fear or anxiety. On the other hand, medications having anxiolytic action raise duration consumed in open arm and number of open arm entries.

2. Materials and Methods

2.1. Animals

Healthy male Swiss Albino mice weighing between 20-35 grams were chosen for anti-anxiety action. 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 CPCSEA. The animals were divided into 5 sets of 6 mice each. The rodents were kept under customary laboratory condition (12 hour light and dark cycle with light on at 6 a.m. and off at 6 p.m.) and temperature (22°C±3°C), humidity (60 ± 10%) with free access to food and water ad libitum according to OECD’s revised draft guidelines 425 and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The animals were allowed to adapt to the new surrounding by giving rest of one week prior subjecting them to experimentation. All the experimental mice were transferred to the behavior testing room 30 min prior to beginning the trial to accustom with the environment of the behavior testing room. Investigation was performed between 9:00 AM and 6:00 PM. Health of each animal monitored daily. Each mouse was used one time.

2.2. Collection of herb material

Since the Withania coagulans is a rare herb, and it is grown in the arid area of India, we purchased the whole herb from the market. The whole herb was authenticated by the Botanist. Afterwards the dried fruits were purchased from the market in bulk.

2.3. Preparation of excerpt

The fruits (with persistent calyx and pedicle) of Withania coagulans were dried in shade for 15 days in closed room at room temperature, powdered in an electric mixture, sieved and stored in an airtight container for trial. First the powder was tried for its solubility both in distilled water and ethanol and it was found that it had limited solubility. Afterwards the decision was taken to get the excerpt by soxhlet procedure. Thus, the alcoholic excerpt of the Withania coagulans fruits were prepared by means of Soxhlet apparatus. The excerpt dissolved in suitable vehicle was given orally.

2.4. Soxhlet alcoholic excerpt

The 30 g of powder material was charged inside the thimble of the primary chamber of the Soxhlet excerptor. The Soxhlet excerptor was positioned onto a flask comprising the excerptor liquid (100% distilled water). Afterwards 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 containing 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 repeated over hours to days. During each cycle, a part of the non-volatile compound dissolved in the ethanol or 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 excerption and based on that, further excerption was terminated. The non-soluble part of the excerpted solid left in the thimble was discarded. The excerpt 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 excerpt.

2.5. Preparation of medication formulation

The fresh solution was prepared by dissolving the excerpt in distilled water prior each experiment for oral administration.

2.6. Pilot test

A pilot study was done by means of the various dosages of the experimental medications i.e. 62.5 mg/kg, 100 mg/kg, 125 mg/kg, 200 mg/kg, 250 mg/kg, 500 mg/kg and 1000 mg/kg. The dosages 200 mg/kg, 500 mg/kg and 1000 mg/kg were selected as working dosages for all the experiments in present study.

2.7. Control, standard and test medications

Distilled water was given as vehicle for control. Diazepam was used as the standard medication. The animals were treated 30 min prior the experiment with the test medications (WCFAlcE of 200 mg/kg, 500 mg/kg and 1000 mg/kg).
mg/kg dosages p. o.). Yet, the experimental medication was given every day for 30 days throughout the period of experiment. The mice were witnessed for 5 min. Recordings were done on Day 1, Day 8, Day 15, Day 23 and Day 30 for all the sets. The recordings were taken half an hour after medication administration to the respective set. After each trial the equipment was cleaned with super hypochlorous water to prevent the bias based on olfactory cues.

Medications were given in the following manner:

- Control: Vehicle (Distilled Water) 2 ml/kg p. o. O.D. for 30 days.
- Standard: Standard medication (Diazepam) 5mg/kg i. p. one time half an hour prior test.
- ALC-200: WCFAlcE 200 mg/kg p. o. O.D. for 30 days.
- ALC-500: WCFAlcE 500 mg/kg p. o. O.D. for 30 days.
- ALC-1000: WCFAlcE 1000 mg/kg p. o. O.D. for 30 days.

Where WCFAlcE = Withania coagulans fruits alcoholic extract.

2.8. Elevated plus maze apparatus

The EPM consisted of two open arms crossed with two closed arms making a sign of plus. The arms were connected together to form a Central Square. Maze made of powder coated mild steel and plexiglass. Open chamber was 30 cm in length and 8 cm in breadth. Enclosed chamber had opaque plexiglass walls of 15 cm height. The 16 character, 2 line display, showed the duration elapsed, duration in closed and open arms separately. The 9 pairs of infrared beams sensed animal movement. Variable timer for study duration from 1-99 minutes was available from the front panel and it was set at 5 minute duration. There was the separate control unit for minimizing human intervention. The apparatus was elevated to the height of 25 cm in a dimly illuminated room.

2.9. Elevated plus maze procedure

The mice were placed individually in the central square of the EPM with the head directed towards close arm and witnessed for the next 5 minutes. The evaluation was done for the duration consumed in the close arm, duration consumed in the open arm and number of entries into the open arm for five minutes. It was witnessed that animals consumed more duration in open arm as compared to closed arm if the particular medication has anxiolytic action. Number of entries into the open arm will be rose in the experimental set if the test medication has anxiolytic action as compared to control. An entry is defined as the presence of all four paws in the respective arm. After each trial the equipment was cleaned with super hypochlorous water to prevent the bias based on olfactory cues.

3. Results

As illustrated in the Table 1, on Day 1 and Day 8 there was no statistically significant difference in all the parameters like duration consumed in the open arm (Open), duration consumed in the closed arm (Close) and Entries into Open arm (EOA) for all the 3 dosages of 200 mg/kg, 500 mg/kg and 1000 mg/kg of WCFAlcE compared to control. Yet, on Day 15, Day 23 and Day 30 there were statistically significant differences in all the parameters like Open, Close and EOA for all the 3 dosages of 200 mg/kg, 500 mg/kg and 1000 mg/kg of WCFAlcE compared to control.

3.1. Action in the open arm

Further clarified from the Figure 1, duration consumed in the open arm rose significantly (p <0.001) for all the 3 dosages of WCFAlcE on days 15, 23 and 30 compared to control on those days. This rise was similar to that of standard diazepam. Furthermore, the dosage response relationship was witnessed with WCFAlcE for this parameter.

3.2. Action in the closed arm

Additionally, explained from the Figure 2, duration consumed in the closed arm fell significantly (p <0.001) for all the 3 dosages of WCFAlcE on days 15, 23 and 30 compared to control on those days. This fall was similar to that of standard diazepam. Moreover, the dosage response relationship was witnessed with WCFAlcE for this parameter.

3.3. Entries into open arm

Further, elucidated from the Figure 3, the mean entries into open arm rose significantly (p <0.001) for all the 3 dosages of WCFAlcE on days 15, 23 and 30 compared to control on those days. This rise was similar to that of standard diazepam. Additionally, the dosage response relationship was witnessed with WCFAlcE for this parameter.

4. Discussion

In our study, as illustrated in the Table 1 and further clarified in Figures 1, 2 and 3, on Day 1 to Day 8 there was no statistically significant difference in all the parameters like duration consumed in the open arm (Open), duration consumed in the closed arm (Close) and Entries into Open arm (EOA) for all the 3 dosages of 200 mg/kg, 500 mg/kg and 1000 mg/kg of WCFAlcE compared to control. Yet, on Day 15, Day 23 and Day 30 there were statistically highly significant rise (p <0.001) in the parameters like Open and EOA for the above dosages of WCFAlcE compared to control. In contrast, highly significant fall (p <0.001) was witnessed for the Close. This rise or fall was similar to that of the standard diazepam. It is expected that the
Table 1: Effect of oral administration of WCFAlcE on duration consumed (mean±SD) in Open arm (in seconds), Closed arm (in seconds) and Entries into open arm in Elevated plus maze apparatus. (n = 6 in each set)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Standard</th>
<th>ALC-200</th>
<th>ALC-500</th>
<th>ALC-1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>55.16± 18.34</td>
<td>60.83± 22.78</td>
<td>50.16± 47.23</td>
<td>36±29.55</td>
<td>40.66± 19.36</td>
</tr>
<tr>
<td>Close</td>
<td>213.83± 11.95</td>
<td>207±21.50</td>
<td>218.83± 44.93</td>
<td>203.66± 43.38</td>
<td>215.16± 30.89</td>
</tr>
<tr>
<td>EOA</td>
<td>2.50± 2.16</td>
<td>2.16± 1.72</td>
<td>2.16± 1.47</td>
<td>2.33± 1.63</td>
<td>2.66± 1.21</td>
</tr>
<tr>
<td>Open</td>
<td>41±17.22</td>
<td>53.16± 8.23</td>
<td>37±10.03</td>
<td>44.66± 25.38</td>
<td>39.33± 20.94</td>
</tr>
<tr>
<td>Day 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Close</td>
<td>223.16± 31.26</td>
<td>202.83± 19.56</td>
<td>227.50± 23.76</td>
<td>221.66± 26.71</td>
<td>225.50± 26.65</td>
</tr>
<tr>
<td>EOA</td>
<td>2.83± 1.94</td>
<td>2.50± 2.07</td>
<td>2.66± 1.63</td>
<td>2.50± 1.64</td>
<td>2.83± 1.16</td>
</tr>
<tr>
<td>Open</td>
<td>24.66± 7.60</td>
<td>66.66±</td>
<td>74.83±</td>
<td>91.33±</td>
<td>100.33±</td>
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<tr>
<td>Day 15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Close</td>
<td>248.83± 13.36</td>
<td>153.66±</td>
<td>173.83±</td>
<td>165.66±</td>
<td>147±37.46***</td>
</tr>
<tr>
<td>EOA</td>
<td>2.00± 1.26</td>
<td>4.50± 0.54***</td>
<td>5.33± 1.21***</td>
<td>6.16± 1.16***</td>
<td>7±1.26***</td>
</tr>
<tr>
<td>Open</td>
<td>21.50± 10.91</td>
<td>97.66±</td>
<td>108.16±</td>
<td>135.66±</td>
<td>144.66±</td>
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<tr>
<td>Day 23</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Close</td>
<td>250.83± 18.86</td>
<td>143±32.98***</td>
<td>131.33±</td>
<td>119.83±</td>
<td>94.66±24.72***</td>
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<tr>
<td>EOA</td>
<td>2.16± 0.75</td>
<td>5.50± 1.04***</td>
<td>6.33± 1.36***</td>
<td>7±1.26***</td>
<td>8.16± 0.75***</td>
</tr>
<tr>
<td>Open</td>
<td>17± 10.13</td>
<td>148.16±</td>
<td>169±32.67***</td>
<td>193.50±</td>
<td>210.83±</td>
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<tr>
<td>Day 30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Close</td>
<td>250.83± 23.14</td>
<td>101.66±</td>
<td>97.16±</td>
<td>66.83±</td>
<td>47.66±29.04***</td>
</tr>
<tr>
<td>EOA</td>
<td>2.50± 0.83</td>
<td>7.16± 1.32***</td>
<td>6.83± 1.47***</td>
<td>7.83± 1.16***</td>
<td>9.33± 1.03***</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01 and ***p < 0.001 when compared to control set

WCFAlcE: Withania coagulans fruits alcoholic excerpt
Open: Duration consumed in Open arm (in seconds)
Close: Duration consumed in closed arm (in seconds)
EOA: Entries into Open arm
Control: Vehicle (Distilled Water) 2 ml/kg p. o. O.D. for 30 days.
Standard: Standard medication (Diazepam) 5 mg/kg i. p. half an hour prior experiment.
ALC-200: WCFAlcE 200 mg/kg body mass p. o. O.D. for 30 days.
ALC-500: WCFAlcE 500 mg/kg body mass p. o. O.D. for 30 days.
ALC-1000: WCFAlcE 1000 mg/kg body mass p. o. O.D. for 30 days.

Fig. 1: WCFAlcE: Withania coagulans fruits alcoholic excerpt Control: Vehicle (Distilled Water) 2 ml/kg p. o. O.D. for 30 days.
Standard: Standard medication (Diazepam) 5 mg/kg i. p. half an hour prior experiment.
ALC-200: WCFAlcE 200 mg/kg body mass p. o. O.D. for 30 days.
ALC-500: WCFAlcE 500 mg/kg body mass p. o. O.D. for 30 days.
ALC-1000: WCFAlcE 1000 mg/kg body mass p. o. O.D. for 30 days.
Fig. 2: Effect of oral administration of WCFAlcE on duration consumed in the Close Arm (in seconds) in the Elevated Plus Maze Apparatus. (n = 6 in each set)

(WCFAlcE: Withania coagulans fruits alcoholic excerpt
Control: Vehicle (Distilled Water) 2 ml/kg p. o. O.D. for 30 days.
Standard: Standard medication (Diazepam) 5 mg/kg i. p. half an hour prior experiment.
ALC-200: WCFAlcE 200 mg/kg body mass p. o. O.D. for 30 days.
ALC-500: WCFAlcE 500 mg/kg body mass p. o. O.D. for 30 days.
ALC-1000: WCFAlcE 1000 mg/kg body mass p. o. O.D. for 30 days.)

Fig. 3: Effect of oral administration of WCFAlcE on Entries into Open Arm in the Elevated plus maze apparatus. (n = 6 in each set)

(WCFAlcE: Withania coagulans fruits alcoholic excerpt. EOA: Entries into Open Arm
Control: Vehicle (Distilled Water) 2 ml/kg p. o. O.D. for 30 days.
Standard: Standard medication (Diazepam) 5 mg/kg i. p. half an hour prior experiment.
ALC-200: WCFAlcE 200 mg/kg body mass p. o. O.D. for 30 days.
ALC-500: WCFAlcE 500 mg/kg body mass p. o. O.D. for 30 days.
ALC-1000: WCFAlcE 1000 mg/kg body mass p. o. O.D. for 30 days.)
substance having anxiolytic action would significantly raise the duration consumed by mice in the Open arm as well as entries into open arm compared to that of control.\textsuperscript{15,16} In contrast, duration consumed by mice in the closed arm would significantly fall. Thus, our results demonstrate the anxiolytic action for all 3 dosages of WCFAlcE similar to diazepam in the Elevated Plus Maze (EPM).

Our research is exceptional one as not one person has used the Elevated Plus Maze to test the anxiolytic action of the alcoholic excerpt of Withania coagulans fruits. Yet, this method was used to assess the anxiolytic action in Withania somnifera which closely resembles Withania coagulans. Bhattacharya et al. in 2000 demonstrated that the glycowithanolides obtained from the roots of Withania somnifera had significant (p<0.05) anxiolytic action at the dosages of 20 mg/kg and 50 mg/kg p. o. Both the duration consumed in open arm and the number of entries into the open arm were rose compared to control and same as that of standard lorazepam. In contrast duration consumed in the closed arm and number of entries into the closed arm were decreased.\textsuperscript{20} Similarly in another study, both the duration consumed in open arm and the number of entries into open arm were rise significantly (p<0.05) in middle cerebral artery occluded (MCAO) rodents treated with Withania somnifera root excerpt compared with MCAO rodents alone.\textsuperscript{21} If we look toward herb species used in folk medicine such as Withania somnifera (Ashwagandha) which closely resembles Withania coagulans, it showed that withanolides work as anxiolytic agent.\textsuperscript{22} Both Withania coagulans and Withania somnifera have abundant withanolides. Thus anxiolytic action of withania coagulans may be because of withanolides. It was found that 3β-hydroxy-2, 3-dihydrowithanolide F is responsible for the anxiolytic as well as CNS depressant action of the herb.\textsuperscript{23} It is the key challenge to understand the exact mechanism of action of withanolides for the anxiolytic action.

Maguire JL et al.\textsuperscript{24} and Koonce CJ et al.\textsuperscript{18} confirmed that estrous cycle changes in female mice can considerably modify stage of anxiety during ovarian cycle. Thus, in our experiment we used only male mice to avoid impact of hormonal alterations over result. After each trial the equipment was cleaned with super hypochlorous water to prevent the bias based on olfactory cues.\textsuperscript{25} The elevated plus maze apparatus is the most famous test among all existing animal models of anxiety.\textsuperscript{15} It has a robust predictive validity for screening anxiolytic medications, thus it has been used for drug discovery.\textsuperscript{16,26}

There is a need of much more work to be done to understand the exact mechanism of action of withanolides. Results obtained in this study demonstrate potent anxiolytic action of Withania coagulans fruits alcoholic excerpt.

5. Conflict of Interest

There is no conflict of interest among authors.

6. Source of Funding

Mahatma Gandhi Institute of Medical Sciences (MGIMS), Sevagram.

7. Ethical Approval

Research was approved by Institutional as well as the Animal Ethics Committee of MGIMS, Sevagram.

References


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