International Journal of Biomedical Research
ISSN: 0976-9633 (Online); 2455-0566 (Print)
Journal DOI: https://doi.org/10.7439/ijbr
CODEN: IJBRFA

Original Research Article

The anti-anxiety activity of alcoholic extract of *Withania coagulans* fruits in Swiss albino mice by Light Dark Box Transition Test

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Abstract

Background: Numerous anti-anxiety medications used nowadays have many side effects. The *Withania coagulans* – an uncommon species, is not studied considerably for its activities on anxiety but in the late seventies where it was discovered for neuro-psycho-pharmacological activity. Hence, it was believed praiseworthy to explore anti-anxiety actions of the alcoholic excerpt of *Withania coagulans* fruits in Swiss albino mice using light-dark box transition test.
Objectives: To study the alcoholic excerpt of *Withania coagulans* fruits' anti-anxiety effect in Swiss albino mice using light-dark box transition test.
Methods: Light dark box transition experiment was used for assessing the anti-anxiety act of *Withania coagulans* fruits' alcoholic excerpt. If the extract had an anti-anxiety effect, then it was predictable that the period consumed in the lighted area would rise and on the other hand that of the dark area would fall. This rise in time in the lighted zone, if found statistically substantial, was measured for anti-anxiety effect.
Result: There was statistically very significant (p-value < 0.001) link observed between the alcoholic excerpt of *Withania coagulans* fruits with anti-anxiety effect in the Swiss albino mice by light-dark box transition test.
Conclusion: The alcoholic excerpt of *Withania coagulans* fruits attested the anti-anxiety effect in the Swiss albino mice through the light-dark box transition test.
Keywords: Anti-anxiety, Light Dark Box (LDB), Swiss Albino Mice (SAM), *Withania coagulans*.

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***Article History:**
Received: 08/05/2020
Revised: 30/05/2020
Accepted: 15/06/2020
DOI: https://doi.org/10.7439/ijbr.v11i6.5415

QR Code

How to cite: Gosavi D. D, Kamdi A. S, Kalambe S. M, Bohra P. N. The anti-anxiety activity of alcoholic extract of *Withania coagulans* fruits in Swiss albino mice by Light Dark Box Transition Test. *International Journal of Biomedical Research* 2020; 11(06): e5419. DOI: 10.7439/ijbr.v11i6.5415 Available from: https://ssjournals.com/index.php/ijbr/article/view/5415

1. Introduction

Anxiety is an emotional response comprising of the emotional state of fear, tension, nervousness, and concern, along with physiological stimulation[1]. The magnitude of the problem can be agreed from the statistics that prevalence of Anxiety disorder in Ageing population is 9% rendering to ICD-9 of World Health Organization[2,3]. Nonetheless, the occurrence of anxiety in paediatrics in India is smaller compared to the USA[4,5].

Though there are various allopathic medicines like Azapirones, Barbiturates, Benzodiazepines and Beta-blockers which act on different receptors, these drugs are not lacking adverse drug reactions. So, there is always a necessity to search for superior and harmless drugs.

Among the various behavioural models, the Light dark transition test is the best test to screen the anti-anxiety action in the mice [6]. This test deals with the bright space

anxiety and natural exploratory movement in a new atmosphere.[7] Rodents prefer the dark space (safe) as they have dislike for the brightly lit area (lightbox). A likely encounter occurs when the mouse is exposed to an uncommon neighbouring or novel object. This encounter is between the inclination to discover and the neo-phobia[8,9]. The rise in time spent in lighted box and number of crossings are indicators of anxiolytic effect[10]. More time spent by mice in the brightened area is comparable to the anti-anxiety action in human.

Withania coagulans also called as *Rishyagandha* in Sanskrit it is considered as 'vulnerable species'[11]. In 1977 Budhiraja *et al*, testified Central Nervous System (CNS) depressant effect of this herb characterized by a decrease in the spontaneous locomotor activity and potentiation of pentobarbitone induced hypnosis [12]. Nevertheless, a lot of work was done on diabetes and other diseases; this plant was not explored much for the CNS activity[13]. So, it was thought useful to search anxiolytic activities of an alcoholic excerpt of *W. coagulans*.

2. Methods

2.1 Control, Standard and Test drugs:

Distilled water was given as vehicle for control. Diazepam was used as the standard drug. The animals were treated 30 min before the experiment with the test drugs (WCFAlcE of 200 mg/kg, 500mg/kg and 1000 mg/kg doses p. o.). However, the test drug was given every day for 30 days throughout the experiment. The mice were observed for 5 min. 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 group.

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 the 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

The anxiolytic activity was assessed by the Light-Dark box (LDB) test/ Light-Dark box (LDB) transition test.

2.2 Light Dark Box Apparatus

The apparatus comprised of two boxes measuring 25 x 25 x 25 cm joined together with a common partition. One box was made dark by coating all the five sides and one lid (made up of plywood) with black paper, whereas the other box was kept open with a 40-W lamp illuminated 25 cm above the floor of it. Access between the light and dark area was provided by an 8 x 8 cm opening in the common partition.

2.3 Light Dark Box Procedure

The mice were placed individually in the centre of the lighted box with the head directed towards the dark box and observed for the next 5 minutes. The evaluation was done for the total time spent in the dark and lightbox as well as a number of crossings between dark to lightbox for five minutes. An entry is defined as the presence of all four paws in the respective box.

2.4 Statistical Analysis:

One way ANOVA was used for the statistical analysis.

3. Results

Table 1: Effect of oral administration of WCFAlcE on time spent (Mean±SD) in Lighted box (in seconds), Dark box (in seconds) and the number of crossings in the Light-Dark Box Test. (n = 6 in each group)

		Control	Standard	ALC-200	ALC-500	ALC-1000
Day 1	Light	83.98±52.76	115.49±17.52	110.84±47.84	89.43±31.36	133.24±22.52
	Dark	216.01±52.76	184.51±17.52	189.15±47.84	210.56±31.36	166.76±22.52
	NOC	3.50±0.92	4.14±0.69	3.83±0.75	4.50±1.19	4.16±0.75
Day 8	Light	67.72±38.92	186.81±39.74***	75.25±33.91	101.46±65.54	100.12±70.63
	Dark	232.25±38.93	113.53±39.73***	224.55±33.97	198.59±65.53	199.84±70.66
	NOC	4.12±1.12	4.57±0.97	4.83±1.16	5.50±1.41	5.00±0.89
Day 15	Light	53.89±30.48	132.91±23.16***	119.72±20.02**	136.69±37.84**	139.38±42.87**
	Dark	246.11±30.48	167.09±23.16***	180.28±20.02**	163.31±37.84**	160.62±42.87**
	NOC	3.25±1.03	5.71±1.79**	5±0.89**	6.25±2.25**	7.16±1.72***
Day 23	Light	45.91±23.33	106.95±29.54**	187.64±35.09***	192.32±56.25***	226.97±55.97***
	Dark	254.09±23.33	193.05±29.54**	112.35±35.09***	107.68±56.25***	73.03±55.97***
	NOC	3.37±1.06	6.71±1.97***	6.16±1.94**	7.25±2.65**	8±1.67***
Day 30	Light	48.42± 21.02	102.30± 15.46***	191.19± 35.33***	195.65± 85.66**	228.99± 53.85***
	Dark	251.57± 21.02	197.70± 15.46***	108.80± 35.33***	104.35± 85.69**	71.01± 53.85***
	NOC	3.62± 1.30	7.57± 1.71***	6.50± 2.07**	7.62± 2.38**	8.83± 1.16***

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

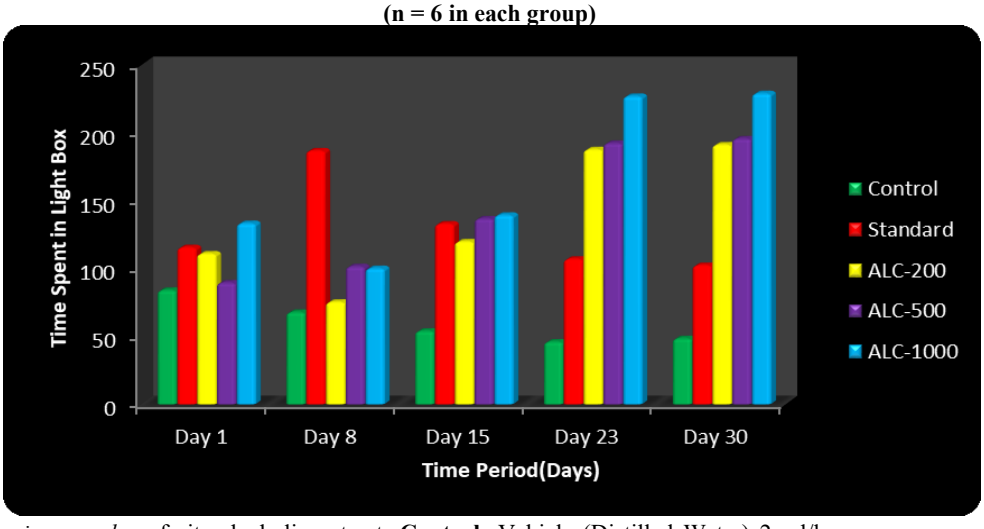
WCFAlcE: *Withania coagulans* fruits alcoholic extract.; **Light:** Time spent in the lighted box (in seconds); **Dark:** Time spent in the dark box (in seconds); **NOC:** Number of crossings between dark to the lighted box.; **Control:** Vehicle (Distilled Water) 2 ml/kg p. o. once a day for 30 days.; **Standard:** Standard drug (Diazepam) 5 mg/kg i. p. half an hour before the 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.

As shown in Table 1, from Day 1 to Day 8 the parameters like time spent in the lighted box (Light), time spent in the dark box (Dark) and the number of crossings in between Dark to the lighted box (NOC) did not show any statistically significant difference for all the three doses of 200 mg/kg, 500 mg/kg and 1000 mg/kg of WCFAlcE compared to control.

However, the parameters like Light, Dark and NOC displayed statistically significant differences for all the three doses of 200 mg/kg, 500 mg/kg and 1000 mg/kg of WCFAlcE compared to control on Day 15, Day 23 and Day 30.

3.1 Activity in the Lighted Box:

Figure 1.1: Effect of oral administration of WCFAlcE on time spent in the Lighted box (in seconds) in the Light-Dark Box Test. (n = 6 in each group)

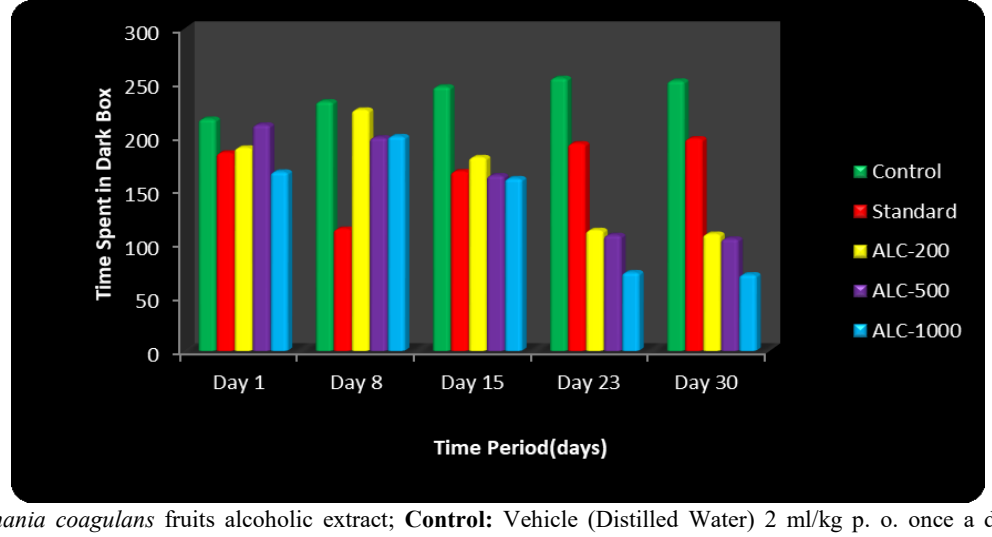


WCFAlcE: *Withania coagulans* fruits alcoholic extract; **Control:** Vehicle (Distilled Water) 2 ml/kg p. o. once a day for 30 days.; **Standard:** Standard drug (Diazepam) 5 mg/kg i. p. half an hour before the test.; **ALC- 200:** WCFAlcE 200 mg/kg body weight p. o. once a day for 30 days.; **ALC- 500:** 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.

Further elucidated from Figure 1.1; statistically, no significant difference was observed in the time consumed by mice in the lighted box on days 1 and 8 for any of the doses. Yet, time consumed by mice in the lighted box raised very significantly ($p < 0.001$) for all the three treatments of WCFAlcE on days 15, 23 and 30 compared to control. This surge was equivalent to that of the standard drug diazepam. Furthermore, there was a dose-response relationship witnessed for this parameter with WCFAlcE.

3.2 Activity in the Dark Box:

Figure 1.2: Effect of oral administration of WCFAlcE on time spent in the Dark box (in seconds) in the Light-Dark Box Test. (n = 6 in each group)

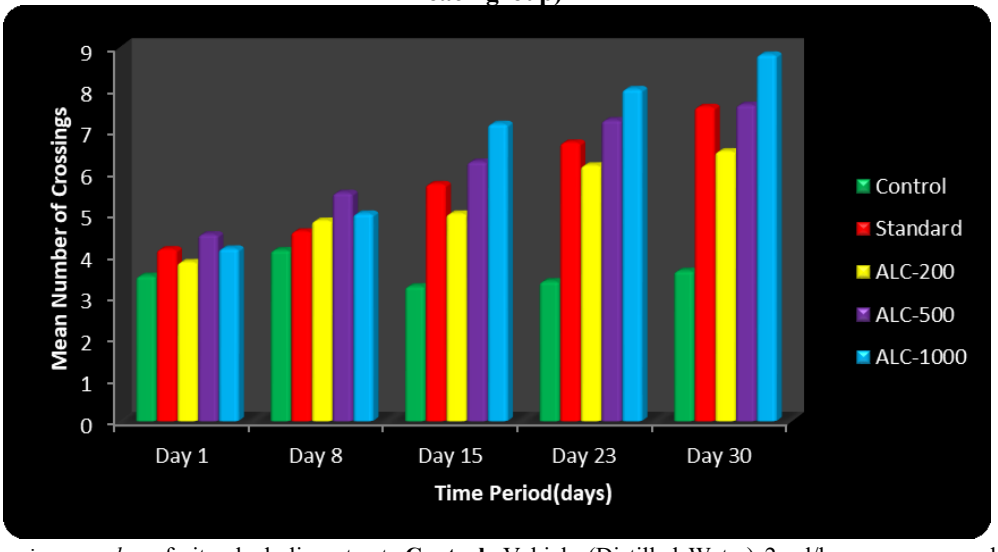


WCFAlcE: *Withania coagulans* fruits alcoholic extract; **Control:** Vehicle (Distilled Water) 2 ml/kg p. o. once a day for 30 days.; **Standard:** Standard drug (Diazepam) 5 mg/kg i. p. half an hour before the test.; **ALC- 200:** WCFAlcE 200 mg/kg body weight p. o. once a day for 30 days.; **ALC- 500:** 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.

Further elucidated from Figure 1.2, there was statistically no substantial difference in the time consumed by mice in the dark box on days 1 and 8. But, time consumed by mice in the dark box reduced very substantially ($p < 0.001$) for all the three doses of WCFAlcE on the respective days of 15, 23 and 30 compared to control. Furthermore, the dose-response relationship observed for this parameter with WCFAlcE for the doses of 200 mg/kg, 500 mg/kg and 1000 mg/kg.

3.3 Number of Crossings:

Figure 1.3: Effect of oral administration of WCFAlcE on Number of Crossings in the Light-Dark Box Test. (n = 6 in each group)



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 the 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.

As explained from Figure 1.3, on days 1 and 8, there was statistically no substantial difference in the number of crossings by mice in between the light and the dark boxes. Still, the number of crossings raised considerably ($p<0.001$) for all the three doses of WCFAlcE on the days 15, 23 and 30 compared to control. Additionally, the dose-response relationship was detected for three doses of WCFAlcE for this parameter.

4. Discussion

As demonstrated in the Table 1 and further explained in Figures 1.1, 1.2 and 1.3, on Day 1 to Day 8 there was no statistically substantial difference in all the factors like time consumed in the lighted box (Light), time consumed in the dark box (Dark) and the number of crossings between Dark to the lighted box (NOC) for all the three doses 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 was the statistically very significant rise ($p <0.001$) in the parameters like Light and NOC for all the three doses of 200 mg/kg, 500 mg/kg and 1000 mg/kg of WCFAlcE compared to control. On the other hand, a very significant fall ($p <0.001$) was witnessed for the Dark. This rise or fall was comparable to that of the standard diazepam. Predictably, the drug having anxiolytic action would significantly increase the time consumed by mice in the lighted box along with the number of crossings between dark to lighted box compared to that of control[8,9]. On the other hand, the time consumed by mice in the dark box would significantly drop.

Our experiment is an exceptional one as nobody has used the light-dark transition model to test the anxiolytic action of an alcoholic excerpt of *Withania coagulans* fruits earlier. There are the plant species used in traditional medicine such as *Withania somnifera* (Ashwagandha) which closely resembles *Withania coagulans*. This study shows that withanolides work as an anxiolytic agent[14]. Both *W. coagulans* and *W. somnifera* have ample withanolides. So, the anxiolytic effect of *Withania coagulans* maybe because of withanolides. It was found that 3β-hydroxy-2, 3-dihydrowithanolide F is responsible for the anxiolytic along with central nervous system depressant effect of the shrub[15]. It is the crucial test to comprehend the precise mechanism of action of withanolides for the anxiolytic effect.

Maguire *et al*.[16] and Koonce *et al*.[17] studied oestrous cycle changes in female mice modifying the stage of anxiety during the ovarian cycle. As a result, in this study, we used only male mice to avoid the influence of hormonal changes on the results. The equipment was cleaned with super hypochlorous water after each trial to prevent the bias based on olfactory cues, [18].

More work is required To recognize the precise mechanism of action of withanolides. Our results prove strong anxiolytic action of *Withania coagulans* fruits alcoholic extract for all the three doses of 200 mg/kg, 500 mg/kg and 1000 mg/kg.

Conflict of Interest:

There is no conflict of interest among authors.

Source of Support:

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

Ethical Approval:

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

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