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THE ANTIPSYCHOTIC ACTIVITY OF AQUEOUS EXTRACT OF WITHANIA COAGULANS FRUITS IN SWISS ALBINO MICE

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ABSTRACT

Background: Schizophrenia is one of the most devastating brain disorders. It is characterized by positive, negative and cognitive symptoms. The various antipsychotic drugs used today are not devoid of side effects. The Withania coagulans – a vulnerable species, is not studied much for its central nervous system effects except in late seventies. Therefore, it was thought worthwhile to investigate anti-psychotic activities of aqueous extract of Withania coagulans fruits.

Methods: Haloperidol induced catalepsy and Cook's Pole Climb Apparatus for conditioned avoidance response were used for testing the antipsychotic activity.

Results: There was statistically (p-value >0.05) no significant association between aqueous extract of Withania coagulans fruits with antipsychotic activity in Swiss albino mice.

Conclusion: Aqueous extract of Withania coagulans fruits did not demonstrate antipsychotic activity in Swiss albino mice.

KEYWORDS : Antipsychotic, Swiss Albino Mice (SAM), Aqueous Extract, Withania coagulans.

INTRODUCTION:

Schizophrenia is one of the most devastating brain disorders. The phenotype in schizophrenia is difficult to define because patients with this illness suffer from a wide variety of symptoms. The symptoms like poverty of speech, poor attention span, blunted affect and lack of motivation may reside for long periods of time. Such symptoms are called negative symptoms because they reflect the absence of normal interpersonal and social functions. The more overt and florid symptoms with abnormal manners and behaviors during an intense psychotic period are called positive symptoms like delusions and hallucinations (Andreasen, 1995). A third group of symptoms involves deficits in cognitive functions, i.e. dissociative thought disorders, such as tangentially, incoherence, looseness of associations, and impaired attention or information processing (Buchanan et al., 1994).

There are various antipsychotic drugs available for the treatment of schizophrenia. However, these drugs cause Parkinsonism like side effects in human. The cataleptic symptoms in rodents can be compared to the Parkinson-like extrapyramidal side effects in human seen clinically with administration of antipsychotic drugs (Duvoisin, 1976). Therefore, the phenomenon of catalepsy can be used for measuring the efficacy and the potential side effects of neuroleptics. Another screening method the conditioned avoidance response is also used for antipsychotic agent. Both these apparatus test the Dopamine hypothesis of schizophrenia.

Withania coagulans is a rare species. This plant is mainly used for the milk coagulation (I. Pandey & Nama, 2015). It is not commonly found and therefore it is categorized as *'vulnerable species'* (R. P. Pandey, Meena, Padhye, & Singhadiya, 2012). As a result, not much work is done on this plant to see the effect on Central Nervous System (CNS). In 1977 Budhiraja et.al. reported CNS depressant activity of this plant (Budhiraja, Bala, & Garg, 1977). Thereafter this plant was not much explored for the CNS activity, though lot of work was done on Diabetes and other diseases. Therefore, it was thought worthwhile to investigate anti-psychotic activities of Withania coagulans.

MATERIALS AND METHODS

1. Haloperidol induced Catalepsy

Catalepsy is defined as "failure of an animal to correct an externally imposed, unusual posture over a prolonged period," (Nair et al., 2007). Catalepsy was produced by injection Haloperidol 1 mg/kg intraperitoneally half an hour after the pretreatment with test drug or half an hour after pretreatment with vehicle (distilled water) for control. Both the front limbs of the mice were placed over 4.5 cm high wooden block and the time for which animal maintained the cataleptic posture was measured. The end point of catalepsy was considered when both front paws were removed from the bar or if the animal moved its head in an exploratory manner. First the time period for which mouse maintained the cataleptic posture was recorded. Later this period was converted into cataleptic scores by modified method of Costall and Naylor as follows: (Brenda Costall & Naylor, 1974)

Time	Score
0-10 seconds	0
11-30 seconds	1
31-60 seconds	2
1-2 minutes	3
> 2 minutes	4

Control, Standard and Test drugs:

Distilled water was given as vehicle for control. The animals were treated (30 min before haloperidol administration) 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. Recordings were done on Day 1, Day 15 and Day 30 for all the groups. The recordings were taken at 1/2 hour, 1 hour, 2 hours and 4 hours after haloperidol 1 mg/kg administration to the respective groups.

Drugs were given in the following manner:

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

2. Cook's Pole Climb Avoidance

The experiment is based on following principle that when the sound of buzzer preceded the electroshock (punishment), animals learned to climb the wooden pole at the sound of buzzer only. The apparatus was based on the experimental technique originally described by Cook and Weidley and various modifications were incorporated after suggestions from various eminent users of the allied science. The present apparatus permitted the study of behavioral response developed in rodents to be employed in the evaluation of comparative effects of several CNS active pharmacological agents.

Apparatus:

Cook's Pole Climbing Apparatus consisted of an experimental chamber with floor-grid in a sound proof enclosure. The enclosure had a sliding door of dull-faced clear acrylic Perspex plastic for viewing the activity of the mice. The right-hand side comprised of the electronic controls which provided both types of stimuli viz. audible or electrical, which might be presented singly or simultaneously through control buttons. These buttons could be manipulated through a timer for approximately 30 seconds which could be terminated any time before 30 seconds. The magnitude of voltage in Cooke's Pole Climb apparatus was designed for the study of behavioral effects of antipsychotic agents on small animals like rat or mice. The pole was in two portions screwed in the small lid on the top of experimental chamber. The smaller portion worked as a handle and longer one served as a pole which hanged inside the experimental chamber. Also raising the front sliding door upwards allowed an animal to be introduced into or removed from the chamber. A sliding tray was provided beneath the floor grip, which could be pulled out for cleaning.

Procedure:

A group of approximately 100 mice were trained to provide a colony for experimental study. From this colony, we used 8 mice in each group to get better results (Holly, Ebrecht, & Prus, 2011). Every mouse in turn, was placed in the test chamber for a period of 30 seconds without any stimulus, to allow and accommodate to the situation. If it climbed the pole, it was placed back in the grid floor where it should remain. Then a series of shocks were delivered to the stainless steel grid floor continuously for 30 seconds or until the mice climbed to the safety area (Pole). After 2 or 3 exposures to this situation, they learned to climb the pole in respect to the buzzer only. When the latter response occurred on a stable basis, a conditioned avoidance response (CAR) is considered having developed. Prior to each experiment the CAR is reinforced a few times with the shock to increase the stability of the CAR throughout the day.

Control, Standard and Test drugs:

Distilled water was given as vehicle for control. Injection Haloperidol 0.1 mg/kg s.c. was used as the standard drug (Wadenberg, Browning, Young, & Hicks, 2001). The animals were treated 30 min 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. Recordings were done on Day 1, Day 15 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 (Haloperidol) 0.1 mg/kg s.c. 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

1. Haloperidol induced Catalepsy:

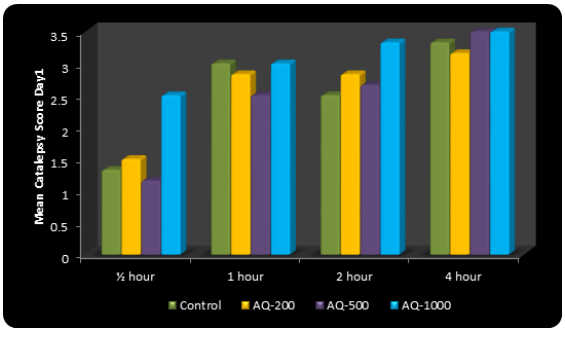
Table 1.1: Effect of oral administration of WCFAqE on Catalepsy scores (n = 6 in each group)

Treatment		Control	AQ-200	AQ-500	AQ-1000
Day 1	½ hour	1.33±0.51	1.50±0.54	1.16±0.75	2.50±1.20
	1 hour	3.00±0.63	2.83±1.16	2.50±1.04	3±0.89
	2 hours	2.50±1.04	2.83±0.75	2.66±1.03	3.33±0.81
	4 hours	3.33±1.21	3.16±1.16	3.50±0.54	3.50±0.54
Day 15	½ hour	1.00±0.89	2.00±0.89	2.33±1.36	2.16±1.47
	1 hour	3.16±0.75	2.83±1.47	2.83±1.16	2.50±1.37
	2 hours	2.16±0.98	3.16±0.98	2.83±1.16	2.00±0.89
	4 hours	2.50±1.37	2.16±1.16	2.50±1.04	2.33±1.21
Day 30	½ hour	2.00±1.09	2.00±1.09	1.66±1.03	2.16±1.16
	1 hour	3.33±1.21	3±1.26	2.83±1.16	3.16±1.16
	2 hours	2.83±1.16	3.16±1.16	3±1.26	3.16±1.16
	4 hours	3.00±1.26	3.00±1.26	3.33±1.21	3.00±1.26

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

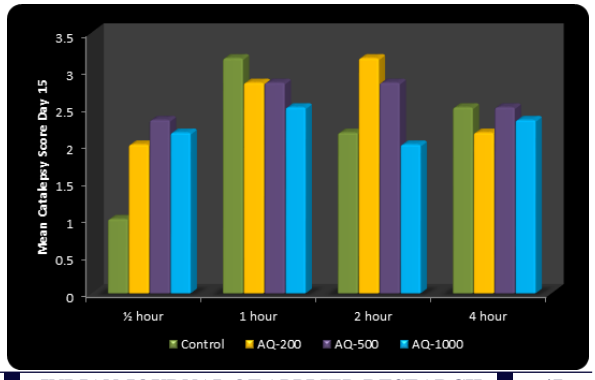
WCFAqE: Withania coagulans fruits aqueous extract.
Control: Vehicle (Distilled Water) 2 ml/kg p. o. once a day for 30 days.
AQ-200: WCFAqE 200 mg/kg body weight p. o. once a day for 30 days.
AQ-500: WCFAqE 500 mg/kg body weight p. o. once a day for 30 days.
AQ-1000: WCFAqE 1000 mg/kg body weight p. o. once a day for 30 days.
As observed in Table 1.1 and further explained from the figures 1.1.1,1.1.2 and 1.1.3, there was statistically no significant difference in the Catalepsy scores on ½ hour, 1 hour, 2 hour, 4 hour with three doses of 200 mg/kg, 500 mg/kg and 1000 mg/kg of WCFAqE compared to control on Day1, Day15 and Day 30.

Figure 1.1.1: Effect of oral administration of WCFAqE on Catalepsy scores on Day1 (n = 6 in each group)



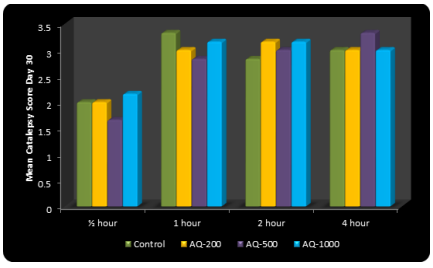
WCFAqE: Withania coagulans fruits aqueous extract.
Control: Vehicle (Distilled Water) 2 ml/kg p. o. once a day for 30 days.
AQ-200: WCFAqE 200 mg/kg body weight p. o. once a day for 30 days.
AQ-500: WCFAqE 500 mg/kg body weight p. o. once a day for 30 days.
AQ-1000: WCFAqE 1000 mg/kg body weight p. o. once a day for 30 days.

Figure 1.1.2: Effect of oral administration of WCFAqE on Catalepsy scores on Day15 (n = 6 in each group)



WCFAqE: Withania coagulans fruits aqueous extract.
Control: Vehicle (Distilled Water) 2 ml/kg p. o. once a day for 30 days.
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 1.1.3: Effect of oral administration of WCFAqE on Catalepsy scores on Day30 (n = 6 in each group)



WCFAqE: Withania coagulans fruits aqueous extract.
Control: Vehicle (Distilled Water) 2 ml/kg p. o. once a day for 30 days.
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.

2. Conditioned Avoidance Response by Cook's Pole Climb Avoidance:

Table 2.1: Effect of oral administration of WCFAqE on blockage of CAR in mice (n = 8 in each group)

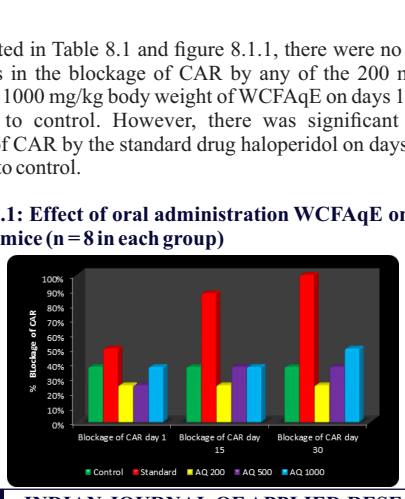
Group	Control	Standard	AQ-200	AQ-500	AQ-1000
Blockage of CAR Day 1	37.50%	50%	25%	25%	37.50%
Blockage of CAR Day 15	37.50%	87.50%***	25%	37.50%	37.50%
Blockage of CAR Day 30	37.50%	100%***	25%	37.50%	50%

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

CAR: Conditioned Avoidance Response.
WCFAqE: Withania coagulans fruits aqueous extract.
Control: Vehicle (Distilled Water) 2 ml/kg p. o. once a day for 30 days.
Standard: Standard Drug (Haloperidol) 0.1 mg/kg s.c. half an hour before test.
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.

As illustrated in Table 8.1 and figure 8.1.1, there were no significant differences in the blockage of CAR by any of the 200 mg/kg, 500 mg/kg and 1000 mg/kg body weight of WCFAqE on days 1, 15 and 30 compared to control. However, there was significant ($p<0.001$) blockage of CAR by the standard drug haloperidol on days 15 and 30 compared to control.

Figure 2.1.1: Effect of oral administration WCFAqE on blockage of CAR in mice (n = 8 in each group)



CAR: Conditioned Avoidance Response.
WCFAqE: Withania coagulans fruits aqueous extract.
Control: Vehicle (Distilled Water) 2 ml/kg p. o. once a day for 30 days.
Standard: Standard Drug (Haloperidol) 0.1 mg/kg s.c. half an hour before test.
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.

DISCUSSION

1. Haloperidol Induced Catalepsy (HIC):

Catalepsy is defined as a nervous condition having persistent rigidity of the limbs, mutism, complete inactivity, fixed posture and decreased sensitivity to pain regardless of outside stimuli and failure to correct such externally imposed posture (MedicineNet, 2016). The cataleptic symptoms in rodents can be compared to the Parkinson-like extrapyramidal side effects in human seen clinically with administration of antipsychotic drugs (Duvoisin, 1976). The phenomenon of catalepsy can be used for measuring the efficacy and the potential side effects of neuroleptics.

As observed in Table 1.1 and further explained from the figures 1.1.1,1.1.2 and 1.1.3 there was statistically no significant difference in the Catalepsy scores on ½ hour, 1 hour, 2 hour, 4 hour with three doses of 200 mg/kg, 500 mg/kg and 1000 mg/kg of WCFAqE compared to control on Day1, Day15 and Day 30. Neuroleptics which have an inhibitory action on the nigrostriatal dopamine system induce catalepsy (Chermat & Simon, 1975; Brenda Costall & Naylor, 1974), while neuroleptics with little or no nigrostriatal blockade produce relatively little or no cataleptic behavior (Honma & Fukushima, 1976). The agents which increase the dopamine transmission will inhibit the haloperidol induced catalepsy. In contrast, the agent which have anti-dopaminergic activity will potentiate the haloperidol induced catalepsy. Our test drug neither increased nor decreased the neuroleptic induced catalepsy, thus we can conclude that our test drug do not have any activity on the dopamine receptors. This was further confirmed by the conditioned avoidance response test. There are no previous articles reported which studied the Withania coagulans effect on the haloperidol induced catalepsy in rodents. However, Kumar and Kulkarni showed that the polyherbal formulation of Withania somnifera (similar species as that of Withania coagulans) significantly ($p<0.05$) blocked the haloperidol induced catalepsy in mice (Kumar & Kulkarni, 2006).

2. Cook's Pole Climb Avoidance (CPCA):

Conditioned Avoidance Response (CAR) is a dopaminergic mediated response. Blockade of this response is attributed to blockade of postsynaptic dopaminergic receptors in nigrostriatal and mesolimbic dopaminergic systems as evidenced by the fact that haloperidol, a dopamine receptor antagonist blocks the CAR learning (Sutton & Beninger, 1999). This test was used in the screening of antipsychotic agents as phenothiazines block them.

As elucidated from Table 2.1 as well as figure 2.1.1, our study showed no blockage of conditioned avoidance response (CAR) for both the WCFAqE as the results were not statistically different from that of control. From both the above tests (HIC and CPCA) it is obvious that WCFAqE did not display the antipsychotic activity. It means they may not act on the dopaminergic receptors. Nevertheless the exact mechanism of action of these test drugs is still a mystery and more behavioural tests based on the mechanism of action needs to be done.

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