Original article

Study of Antianxiety Property of Withania Somnifera Using Elevated Plus Maze in Albino Rats

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ABSTRACT:

Introduction: In the area of anxiolytic therapy, like any other, there is still the absence of a perfect drug. The use of barbiturates, with their inherent toxicity, low dose neuroleptics, with extrapyramidal side-effects potential, have given way to benzodiazepines and their over-usage for the past 25 years and more recently to buspirone with its delayed onset of action.

Materials and methods: All the experiments were performed between 0900-1400 hrs. Food but not water was with-held 12 hrs before experimentation. Institutional Animal Ethics Committee clearance was obtained before carrying out the study. The root powder of Withania somnifera was supplied by the Himalaya Drug Co., Bangalore. The powder was suspended in distilled water with the help of 2% gum acacia, which was used as the suspending agent. The dilutions were made depending on the doses used a stock solution of 10 mg/ml.

Results: The mean of total number of peeps out of closed arm Withania somnifera treated groups (III,IV & V) were 9.2±0. 70,10.0±0.68 and 10.5±0.62 respectively (P-value <0.01) indicating significant anxiolytic activity.

Conclusion: It was proved that the powdered root of Withania somnifera exerted an anxiolytic effect by the results of the elevated plus maze.

Keywords: Withania somnifera, Elevted plus maze, Anti anxiety property

INTRODUCTION

Anxiety is the most ubiquitous psychiatric symptom & one of the most common of all disorders that present to the general physician. It occurs as part of most psychiatric syndromes, particularly depressive ones.[1] It has been defined as “universal human experience, characterized by fearful anticipation of an unpleasant event in the future “, the exact cause of which, in contrast to fear; is vague.5 Anxiety accompanies at least some aspects of most normal lives, and ii can be an effective stimulus to improve performance. A little anxiety can improve performances, performance then plateaus as the anxiety increases, and eventually too much anxiety causes a decrease in the ability to function.[2]

In the area of anxiolytic therapy, like any other, there is still the absence of a perfect drug. The use of barbiturates, with their inherent toxicity, low dose neuroleptics, with extrapyramidal side-effects potential, have given way to benzodiazepines and their over-usage for the past 25 years and more recently to buspirone with its delayed onset of action. Benzodiazepines certainly provide rapid and effective relief from anxiety, but at the cost of disinhibition, ataxia, amnesia and drug dependence[3,4] It is clear therefore, that search for an ideal non-

sedating anxiolytic drug should continue to occupy an important position in central nervous system research. The challenge has been and indeed is still continuing, to develop an anxiolytic having efficacy yet lacking dependence potential excessive sedative properties and other unwanted effects.

Withania somnifera is commonly prescribed by ayurvedic physicians as a central nervous system sedative. Its root is known to possess central depressant properties.[5,6]

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MATERIALS AND METHODS

All the experiments were performed between 0900-1400 hrs. Food but not water was with-held 12 hrs before experimentation. Institutional Animal Ethics Committee clearance was obtained before carrying out the study.

The root powder of Withania somnifera was supplied by the Himalaya Drug Co., Bangalore. The powder was suspended in distilled water with the help of 2% gum acacia, which was used as the suspending agent. The dilutions were made depending on the doses used a stock solution of 10 mg/ml.

EQUIPMENTS:

Cook's Pole Climbing Apparatus, Elevated Plus Maze, Wooden Mouth Gag, Ryle's tube, Syringes Stop Watch, Hydrogen Peroxide, etc.,

Exclusion criteria:

1. Weight of the rat less than 100 gms. & more than 200 gms.
2. More than 4 months of age
3. Within 21 days of prior use for any experimental purposes
4. Any visible disease

METHODS:

Cook's Pole Climbing Apparatus (Plate-2)

The effect of drugs on memory by testing its effects on conditioned avoidance response (CAR) in experimental animals is a standard screening procedure for assessment of CNS activity. This effect was tested using the Cook's pole climbing apparatus which was first described by Cook & Weidley in 1957.
The apparatus consists of a wooden chamber with a metal grid floor through which an electric shock can be delivered to a rat placed on it. A wooden pole is provided in the chamber so that the rat can avoid the shock by climbing on to it. An external panel provides the timer, buzzer and connections for electrical stimulation. The animals are introduced into the cage via the front transparent sliding door from where they can be visualized.

Procedure: The effect of the drugs on learning and long term memory can be tested using this apparatus. This is an active avoidance test which involves two step procedure: Initial learning trials are followed by relearning trials, indicative of long term memory. On the day of training each rat is placed in the apparatus for 2 minutes. This is followed by a buzzer (50Hz) for 15 seconds. After this, foot shocks (80volts, 5 pulses/sec.) are delivered to the rats through the electric grid floor of the apparatus concurrently with the activation of the buzzer. Shock and buzzer are continued simultaneously till the rats climb the pole for 60 seconds lapse (to prevent harm to the animal).

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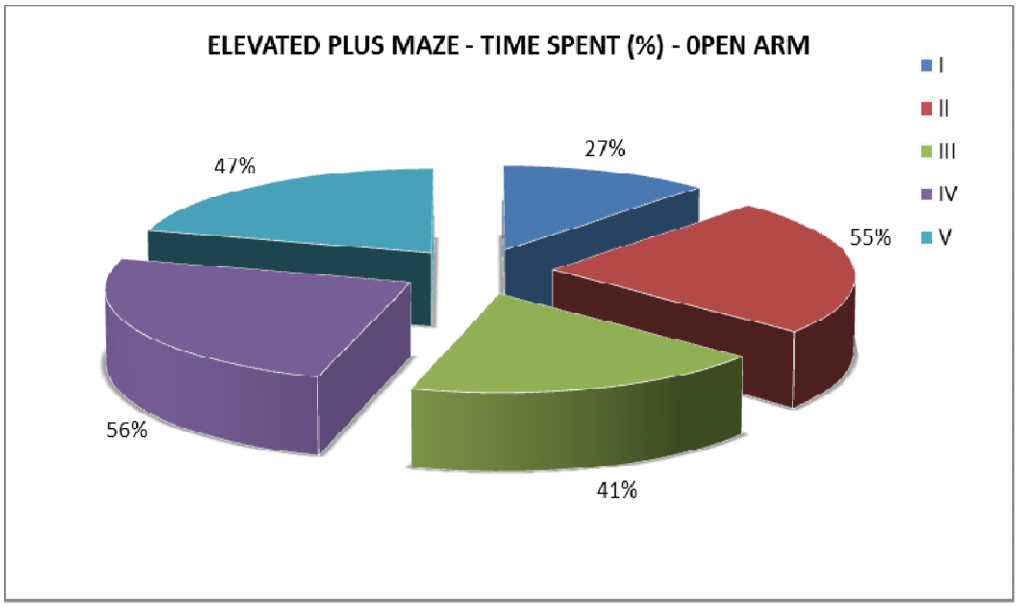
RESULTS

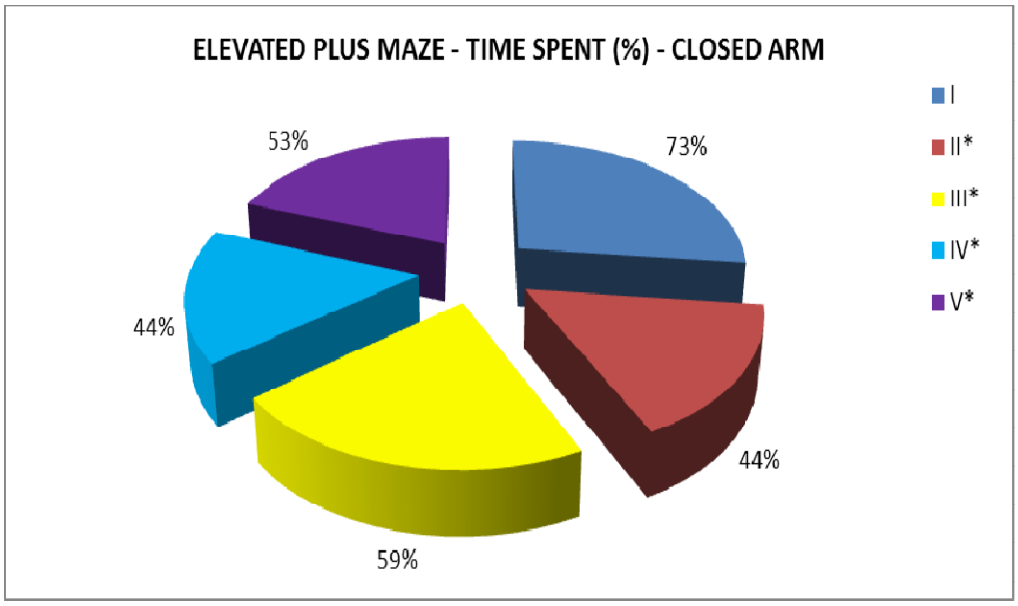
The study compound Withania somnifera was subjected to various tests for its nootropic and anxiolytic activities in comparison with the standard drugs

Effects on Anxiety by using Elevated plus maze.

Graph -4



Graph -5



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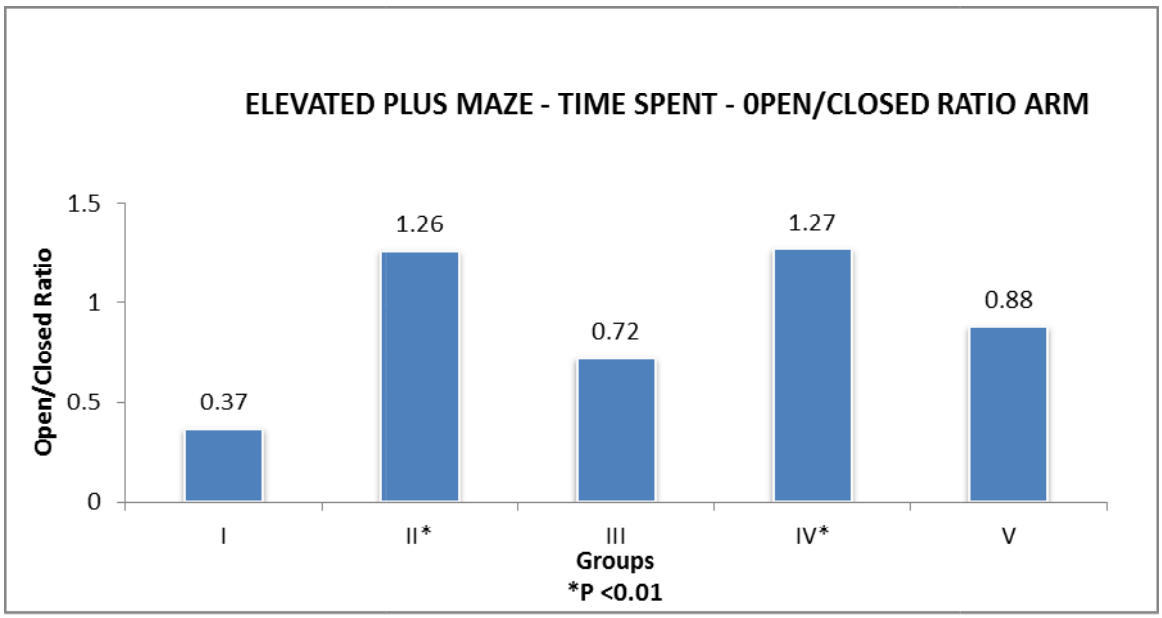
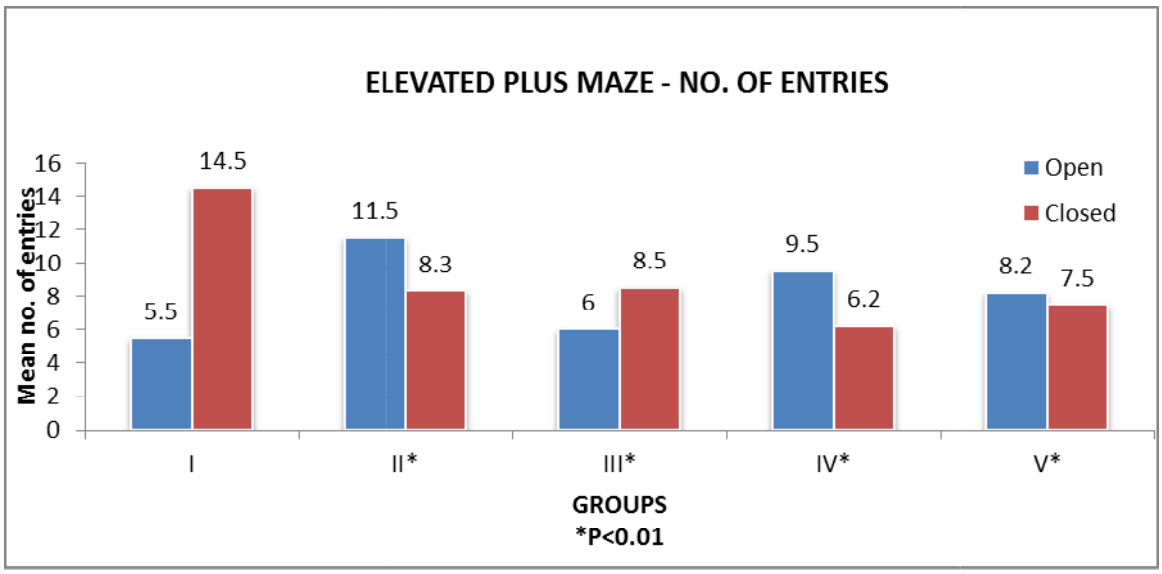
Graph -6



Graph -7



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Graph -8

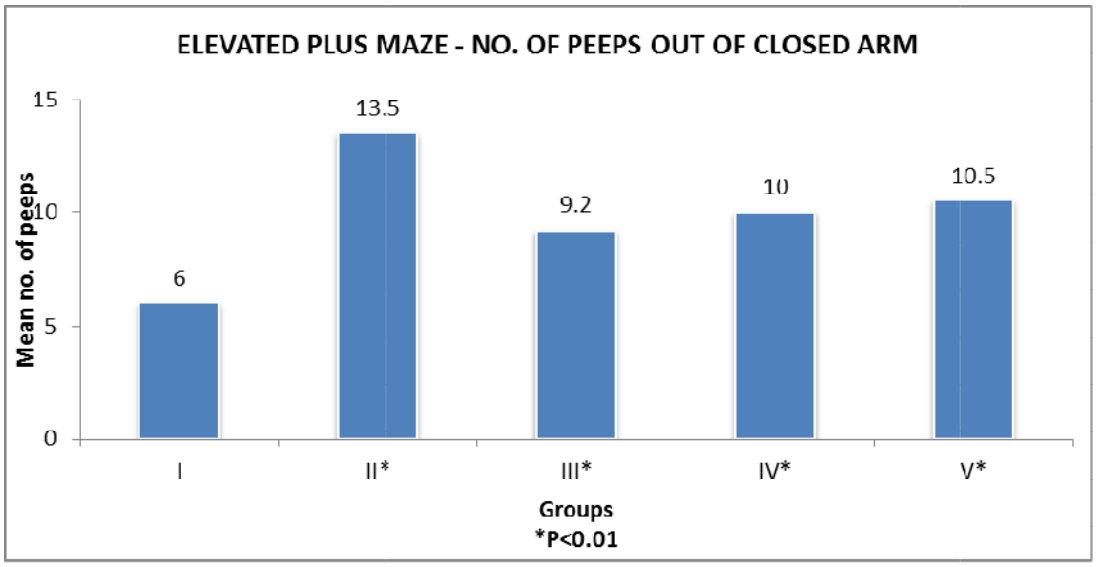

Table 8 Group I (Distilled water 2 ml/Orally)

Sl. No.	Time spent (sec)				Ratio Open/closed	No. of entries		No. of peeps	
	Open Arm	% Total	Closed Arm	% Total		Open Arm	Closed Arm	Ration Open/Closed	Out of closed arm
1	85	28.33	215	71.66	0.40	5	11	0.45	5
2	75	25.00	225	75.00	0.33	6	17	0.35	6
3	85	28.33	215	71.66	0.40	7	17	0.41	8
4	80	26.66	220	73.33	0.36	5	15	0.33	6
5	75	25.00	225	75.00	0.33	6	14	0.43	5
6	85	28.33	215	71.66	0.40	4	13	0.31	6
Mean	80.8	26.9	219.2	73.1	0.37	5.50	14.50	0.38	6.00

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Table 9 Group II (Diazepam 1 mg/kg BW - Orally)

Sl. No.	Time spent (sec)				Ratio Open/closed	No. of entries		No. of peeps	
	Open Arm	% Total	Closed Arm	% Total		Open Arm	Closed Arm	Ration Open/Closed	Out of closed arm
1	160	53.33	140	46.66	1.14	14	9	1.56	11
2	180	60.00	120	40.00	1.50	10	7	1.43	13
3	160	53.33	140	46.66	1.14	10	7	1.43	15
4	160	53.33	130	46.66	1.23	12	8	1.50	14
5	170	56.66	130	43.33	1.31	12	9	1.33	13
6	165	50.00	135	50.00	1.22	11	10	1.10	15
Mean	165.8	54.4	132.5	45.6	1.26	11.50	8.33	1.39	13.50

Table 10 Group III (Withania Somnifera 70 mg/kg BW - Orally)

Sl. No.	Time spent (sec)				Ratio Open/closed	No. of entries		No. of peeps	
	Open Arm	% Total	Closed Arm	% Total		Open Arm	Closed Arm	Ration Open/Closed	Out of closed arm
1	120	40.00	180	60.00	0.60	8	8	1.00	12
2	115	38.33	185	61.66	0.62	5	7	0.71	9
3	125	41.66	175	58.33	0.71	5	7	O.71	7
4	120	40.00	180	60.00	0.67	5	9	0.56	8
5	115	38.33	185	61.66	0.62	7	10	0.70	10
6	150	50	150	50.00	1.00	6	10	0.60	9
Mean	124.2	41.0	175.8	59.0	0.72	6.0	8.50	0.71	9.17

Table 11 Group IV (Withania Somnifera 140 mg/kg BW - Orally)

Sl. No.	Time spent (sec)				Ratio Open/closed	No. of entries		No. of peeps	
	Open Arm	% Total	Closed Arm	% Total		Open Arm	Closed Arm	Ration Open/Closed	Out of closed arm
1	180	60.0	120	40.0	1.50	11	7	1.57	12
2	165	55.0	135	45.0	1.22	10	7	1.43	12
3	170	56.66	130	43.33	1.31	8	6	1.33	10
4	165	55.0	135	45.0	1.22	10	7	1.43	9
5	160	53.33	140	46.66	1.14	9	5	1.80	8
6	166	56.66	134	44.33	1.24	9	5	1.80	9
Mean	167.7	56.1	132.3	44.1	1.27	9.50	6.17	1.56	10.00

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Table 12 Group V (Withania Somnifera 280 mg/kg BW - Orally)

Sl. No.	Time spent (sec)				Ratio Open/closed	No. of entries		No. of peeps	
	Open Arm	% Total	Closed Arm	% Total		Open Arm	Closed Arm	Ration Open/Closed	Out of closed arm
1	140	46.66	160	53.33	0.88	10	9	1.11	12
2	130	43.33	170	56.66	0.76	9	7	1.29	11
3	150	50.0	150	50.0	1.00	8	7	1.14	12
4	145	48.33	155	51.66	0.94	7	6	1.17	8
5	130	43.33	170	56.66	0.76	7	7	1.00	10
6	145	48.33	155	51.66	0.94	8	9	0.89	10
Mean	140.0	46.7	160.0	53.3	0.88	8.17	7.50	1.10	10.50

Table 13 Effects of Withnia somnifera in the elevated plus-maze test in rats (Mean ±SEM)

Treatment Groups	n	Time spent (sec)				
		Open arm	% Total	Closed arm	% Total	Ratio Open/Closed
I Distilled water 2 ml	6	80.8 ± 2.01	27	219.2 ± 2.01	73	0.37 ± 0.01
II Diazepam 1 mg/kg BW	6	165.8 ± 3.27*	55	132.5 ± 3.10*	44	1.26 ± 0.05
III Withania somnifera 70 mg/kg	6	124.8 ± 5.39*	41	175.8 ± 5.39*	59	0.72 ± 0.06
IV Withania somnifera 140 mg/kg	6	167.8 ± 2.79*	56	132.3 ± 2.79*	44	1.27 ± 0.05
V Withania somnifera 280 mg/kg	6	140.0 ± 3.42*	47	160.0 ± 3.42*	53	0.88 ± 0.05
F-Value		100.7		104.6		64.8
P-Value		<0.001		<0.001		<0.001

One Factor ANOVA test

Studentized range test (Newman Kaul's range test)

*P-value <0.01: Significant

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Table 14 Effects of Withnia somnifera in the elevated plus-maze test in rats (Mean ±SEM)

Treatment Groups	n	No. of entries		Ratio Open/Closed	No. of peeps out of closed arm	
		Open arm	Closed arm		Range	Mean ±SEM
I Distilled water 2 ml	6	5.5 ± 0.43	14.5 ± 0.96	0.38 ± 0.02	5-8	6.0 ± 0.45
II Diazepam 1 mg/kg BW	6	11.5 ± 0.62*	8.3 ± 0.49*	1.39 ± 0.07*	11-15	13.5 ± 0.62*
III Withania somnifera 70 mg/kg	6	6.0 ± 0.5	8.5 ± 0.56*	0.7 ± 0.06	7-12	9.2 ± 0.70*
IV Withania somnifera 140 mg/kg	6	9.5 ± 0.43*	6.2 ± 0.40	1.56 ± 0.08*	8-12	10.0 ± 0.68*
V Withania somnifera 280 mg/kg	6	8.2 ± 0.48*	7.5 ± 0.5*	1.10 ± 0.06*	8-12	10.5 ± 0.62*
F-Value		24.8	27.3	62.3	64.8	
P-Value		<0.001	<0.001	<0.001	<0.001	

One Factor ANOVA test

Studentized range test (Newman Kaul's range test)

*P-value <0.01: Significant

Effects on **Anxiety using Elevated plus Maze:** Results from tables 8,9,10,11 &12 show the mean value of control group (I) & standard drug diazepam treated group (II) with that of test compound Withania somnifera treated groups (III,IV&V). The Withania somnifera treated groups (III, IV &V) spent more time in open arm 124.2±5.39(41%), 167.7±2.79(56%) & 140.0±3.42(47%) respectively. Ratios of time spent in open to closed arm were 0.72±0.06, 1.27±0.05 and 0.88±0.55 respectively. The mean number of entries into open & closed arm were 6.0±0.52, 8.5±0.56, 9.5±0.43 & 6.2±0.40 and 8.2±0.48 & 7.5±0.5 respectively. The corresponding ratio of number of entries into open to closed arms were 0. 71±0.06, 1.56±0.08 & 1.10±0.06 respectively.[7,8,9]

Results summarized from tables (13,14) and Graphs (4,5,6,7,8) show comparative analysis of Withania somnifera treated groups (III, IV & V) with control group(I) and standard treated Diazepam treated group(1I). The test compound Withania somnifera treated groups (III, IV & V) spent greater time in the open arm 124.2±5.39(41%), 167.7±2,79(56%) & 140.0±3.42(47%) respectively (P-value <0.01) indicating significantly anxiolytic activity. The corresponding mean ratios of time spent in open arm to closed arm were 0.72±0.06, 1.27±0.05 & 0.88±+0. 05 respectively (P-value <0.01). The corresponding mean of number of entries into open & closed arm with their ratios were 6.0±0.52, 8.5±0.56, 0.71±0.06, 9.5±0.43, 6.2±0.40 & 1.56±0.08 and 8.2±0.48, 7.5±0.50 & 1.10±0.06 respectively (P-value <0.01) indicating significant anxiolytic activity.

The mean of total number of peeps out of closed arm Withania somnifera treated groups (III,IV & V) were 9.2±0. 70,10.0±0.68 and 10.5±0.62 respectively (P-value <0.01) indicating significant anxiolytic activity.

DISCUSSION

Anxiety is symptom associated with nearly all types of Psychiatric illness and a certain degree of anxiety has been a consistent factor in human behaviour as a means of survival in a hostile environment increasing the general level of awareness. Pharmacotherapy of Anxiety is recommended Jbr a maximum of several weeks. The standard anxiolytic drugs benzodiazepines although provide rapid & effective relief from the anxiety. The long term treatment can lead to disinhibition & pharmacological dependence and other side effects. Withania

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somnifera is used in Ayurvedic Medicine as central nervous system depressant. Some of its central effects include antistress, anti anxiety & central nervous system inhibitory properties. Hence, the present study was undertaken to evaluate its effect of Anxiety.

Results summarized from tables 13, 14 shows that Withania somnifera treated groups ('III, IV & 1') had a significant effect on exploratory behaviour based tests using elevated plus more which is evidenced by greater amount time spent in open arm (P-value <0.01) and an increase in number of entries into open arm (P <0.01) and ratio of number of entries into open to closed arms (P-value <0.01) indicating significant anxiolytic activity. Also, the number of peeps out of closed arm were more as compared to the control group (P-value <0.01) indicating significant anxiolytic activity.

Further, the effects of Withania somnifera on Exploratory behaviour based tests were not dose-dependent the maxima/response seen with the dose of 140 mg/kg BW[10]

The test compound has shown good safety profile till the dose of 280 mg/kg BW. There were no sedation, convulsion, tremors, etc.

CONCLUSION

It was proved that the powdered root of Withania somnifera exerted an anxiolytic effect by the results of the elevated plus maze.

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