

To Develop & Evaluate Poly Herbal Formulation for Anxiety Disorder

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ABSTRACT

The present study was designed to evaluate adaptogenic activity of poly herbal formulation (*Bacopa monnieri*, *Datura stramonium*, *Azadirachta indica*) in albino rats using different experimental models such as Light & dark model and plus maze model. The plant was subjected to preliminary phytochemical screening. The parameters like time spend in light and dark box, time spend in open and close arm were recorded. These activities are tested at oral doses of extract at 200 and 400 mg/kg and Diazepam 1mg/kg was used for comparison. Preliminary phytochemical screening revealed the presence of carbohydrates, glycoside, flavonoids, and tannins. Pretreatment with poly herbal formulation showed increase in anoxia stress tolerance time and swimming endurance time. The results from the study indicated that Poly herbal formulation possessed significant anti-anxiety activity.

Keywords: Antianxiety, Polyherbal, *Bacopa monnieri*, *Datura stramonium*, *Azadirachta indica*

INTRODUCTION

Anxiety describe as fluctuating mood, elevated attention, negative elucidation social phobia under the condition of potential threatening signs. Generalized disorder (GAD) , Social phobia, Specific phobia, Obsessive compulsive disorder (OCD), Post traumatic stress disorder, Separation anxiety disorder are different type of anxiety. It is an emotional state, unpleasant in nature, associated with uneasiness, discomfort and concern or fear about some defined or undefined future threat. Some degree of anxiety is a part of normal life. Treatment is needed when it is disproportionate to the situation and excessive. Some psychotics and depressed patients also exhibit pathology anxiety.

MATERIALS AND METHODS

Plant material :-

The plant of *Bacopa Monnieri*, seeds of *Datura Stramonium* and leaves of *Azadirachta Indica* were collected locally from Bhopal, Madhya Pradesh, India. The plant material was identified and authenticated by Dr. Saba Naaz (H.O.D., Department of Botany), Saifia Collage of Science Bhopal, Madhya Pradesh, India.

Extraction of plant: -

16.6gm of the dried powder of each plant was taken and cold maceration with hydro-ethanolic solvent with occasional stirring for 7 days. After 7 days, the suspension were filtered through the Whatman filter paper

No.1 and the filtered was evaporated to dryness at low temperature under reduce pressure in a rotatory evaporator and dried in desiccators.

Phytochemical screening of crude extracts: -

The crude extract obtained by solvent extraction was subjected to various qualitative tests with standard reported methods to detect the presence of common phytochemical constituents. All the chemicals and reagent used in phytochemical testing was of analytical grade. Tabel-1

Preparation of Oral formulation: -

A suspension formulation of ethanolic extract of Poly Herbal formulation in 0.5% Carboxy Methyl Cellulose solution was prepared for further *in-vivo* pharmacological study.

Selection and maintenance of animals: -

The animals were divided into four groups of six rats each:

Group-1 Control, received propylene glycol,

Group2- Standard (Diazepam 1mg/kg)

Group3-PHF at a dose of 200mg/kg

Group4-PHF at a dose of 400mg/kg

Elevated plus maze model: -

The elevated plus maze test is one of the most widely used test for measuring anxiety like behavior. The plus maze consists of two open arms, 50×10×40cm, and two enclosed arms, 50×10×40cm, with an open roof, arranged so that the two open arms are opposite to each other. The maze is elevated to a height of 50cm. The rats are housed in pairs for 10 days prior to testing in the apparatus. During this time the rats are handled by the investigator on the alternate days to reduce stress. Groups consist of 6 rats for each dose. 30 minutes after administration of the test drug or the standard, the rat is placed in the center of the maze, facing one of the enclosed arms. During a 5 minute test period the following measure article:

- (a) The time spent in the open arm (sec)
- (b) The time spent in the closed arm (sec)
- (c) Transfer latency (sec)
- (d) The no. of entries into open arm
- (e) The no. of entries into closed arm

Dark & Light Model

The testing apparatus consist of a light and dark chamber divided by photo cell equipped zone. A polypropylene cage, 44×21×21cm, is dark ended with black spray over one-third of its surface. A partition containing a 13cm long× 5cm high separates the dark one-third from the bright two-third of the cage. The cage rests on an animal activity monitor which count total locomotor activity. An electronic system using four sets of photocells across the partition and clocks the times spend in the light and dark compartment. Male rats are placed into the cage and treated 30 minutes before the experiment with the test drug and then the following parameters were observed for 5 minutes.

- (a) Time spent in light box (sec)
- (b) Time spent in dark box (sec)
- (c) Transfer latency (Sec)
- (d) No. of crossing

Statistical analysis: -

All data represent mean +_ S.E.M. value. The data were analyzed by means of analysis of variance (ANOVA). Whenever ANOVA was significant, further multiple comparisons were made using Bonferroni's test as the post hoc test. All analysis was performed using the spss statistical software. The levels of statistical significance ranged from $p < 0.05$ to $p < 0.001$

RESULTS

Table-1. Preliminary phytochemical screening of crude extracts

Serial no.	Test	(+) indicate presence	(-) Indicate absence
1	Carbohydrates	+	
2	Proteins		-
3	Glycosides	+	
4	Flavonoids	+	
5	Steroids		-
6	Phenolic/tannins	+	

Acute oral toxicity

Acute oral toxicity study was evaluated as per OECD guidelines (425) on albino rats. Animals were provided by Sagar Institute of Research and Technology -Pharmacy, Bhopal (M.P.) and experiment was done in the lab. Before experimentation rats were fasted overnight with water *ad libitum*. Group-1 Six animals were selected which receives dose of 2000mg/kg, and Group-2 six animals were selected which receives dose of -5000mg/kg. All twelve animals were received dose of 2000mg/kg, 5000mg/kg body weight of ethanolic extract of Poly Herbal Extract by gavage using oral cannula(limit test).Animals were observed individually for any toxicity sign of gross changes like convulsion, tremor, circling, depression, and mortality after dosing for 24 hours, with special attention given during the first 4 hours, and thereafter, 24 hours, Administered dose was found tolerable (as no death found). Therefore, two dose levels 200 mg/kg & 400 mg/kg was selected for anti-anxiety activity.

Table -2 Result of Elevated plus maze model

Serial No.	Group	Time spent in open arm(sec)	Time spent in closed arm(sec)	No. of entries in open arm(sec)	No. of entries in closed arm(sec)
		Mean \pm S.E.M.	Mean \pm S.E.M.	Mean \pm S.E.M.	Mean \pm S.E.M.
1.	Control (Propylene glycol)	72.83 \pm 8.78	210.40 \pm 1.24	6.22 \pm 1.23	13.83. \pm 0.5
2.	Standard (Diazepam)	120.38 \pm 7.78	175.5. \pm 11.5	16.10 \pm 0.52	7.66 \pm 0.51
3.	PHF 200mg/kg	98.32 \pm 6.76	168.5 \pm 6.73	11.33 \pm 1.33	10.0.9 \pm 1.10
4.	PHF 400mg/kg	110.23 \pm 5.29	171.8 \pm 3.24	14.05 \pm 0.54	8.07 \pm 0.63

n=6

Value are expressed as mean \pm S.E.M. *= P <0.05 **= P <0.01 and ***= P <0.001. Test drugs treated group compare with control group (Statistical analysis by one way ANOVA)

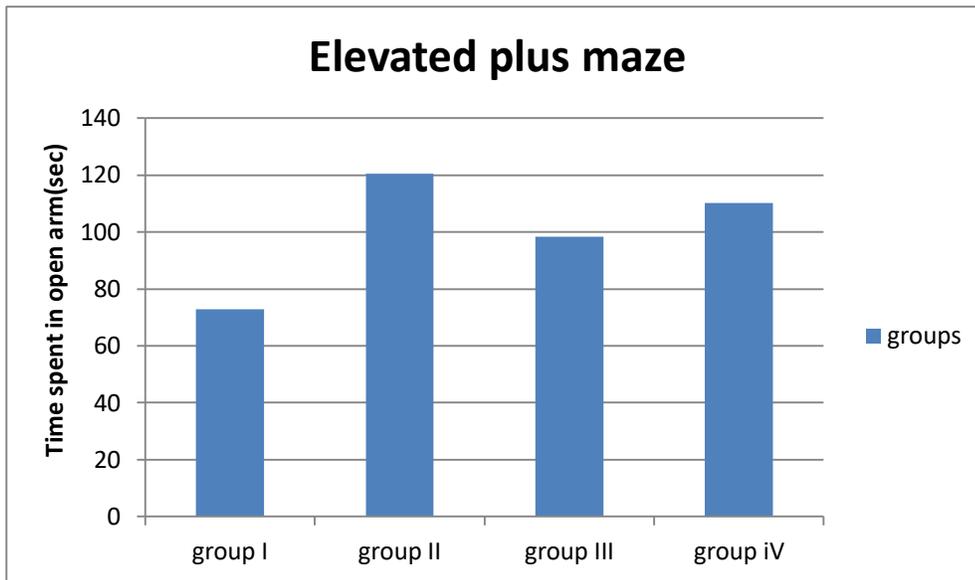


Figure 1 Elevated plus maze model: Time spent in open arm

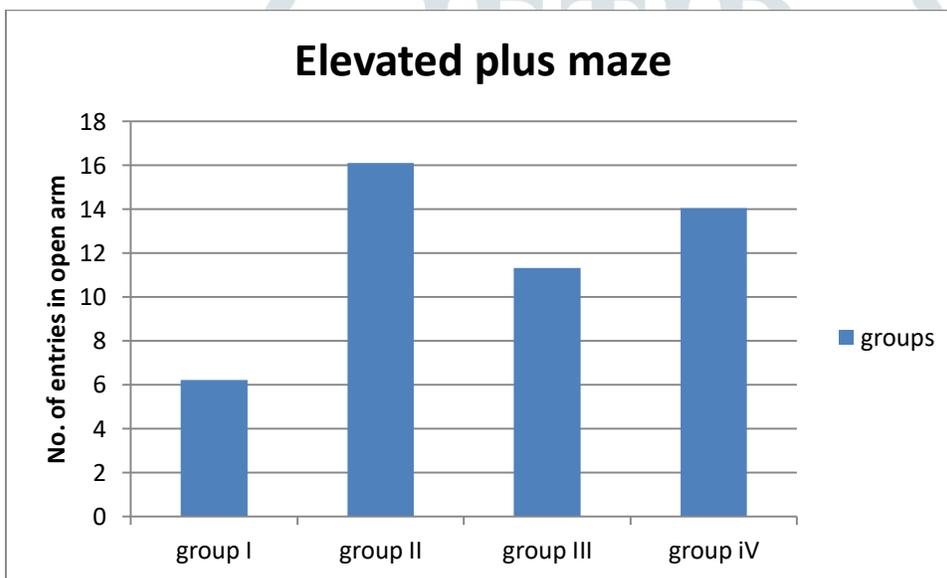


Figure 2 Elevated plus model: No. of entries in open arm (sec)

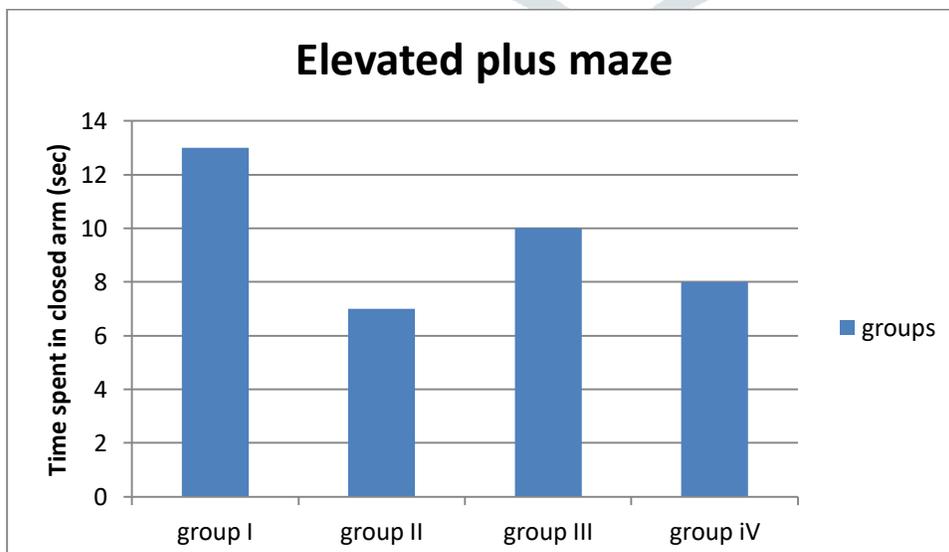


Figure 3 Elevated plus model: Time spent in closed arm (sec)

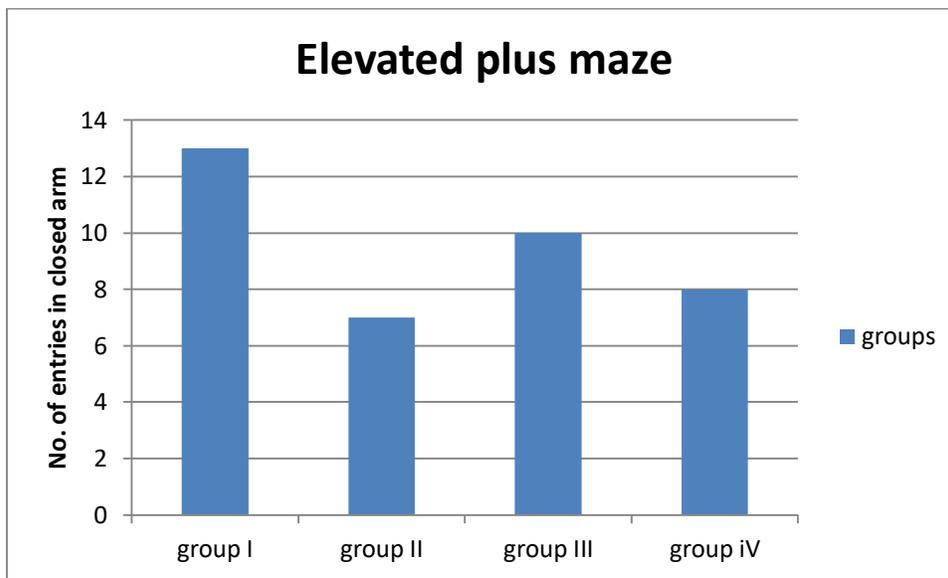


Figure 4 Elevated plus model: No. of entries in closed arm (sec)

Table -3 Result of Light and dark model

Serial No.	Group	Time spent in light box(sec) Mean±S.E.M.	Time spent in dark box(sec) Mean±S.E.M.
1.	Control (Propylene glycol)	78.33 ± 6.22	74.16 ± 0.5
2.	Standard(Diazepam)	116.67 ± 0.5	85.33 ± 1.6
3.	PHF 200mg/kg	98.16 ± 7.37	80.16 ± 4.6
4.	PHF 400mg/kg	112.83 ± 3.79	82.26 ± 2.4

n=6

Value are expressed as mean ±S.E.M. *=P<0.05 **=P<0.01 and ***=P<0.001 Test drug treated group compare with control group (Statistical analysis by one way ANOVA)

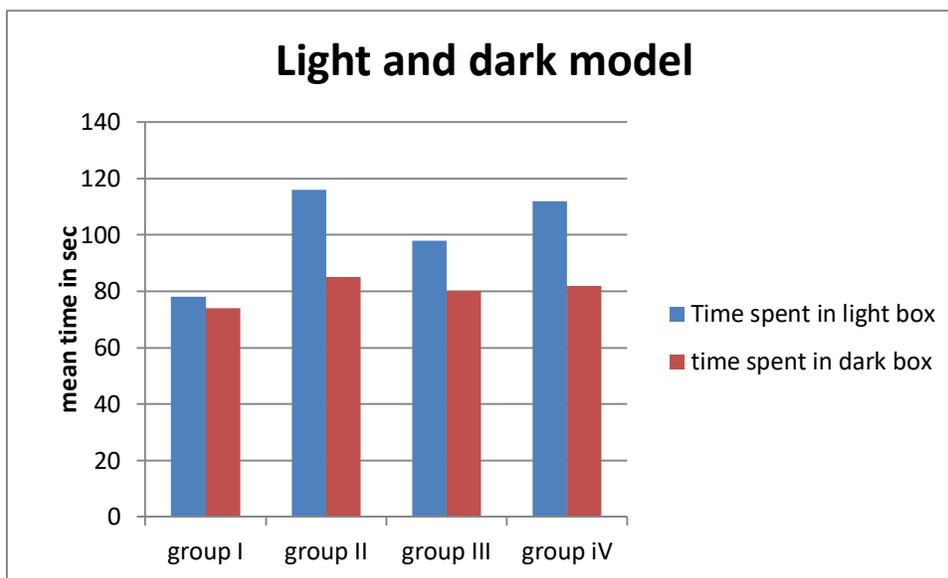


Figure 5 Light and Dark Model: Time spent in light and dark model

DISCUSSION

So the present study was aimed was an attempt to develop synergistic polyherbal formulation for anxiety management from plants used in the Indian System of Medicine (ISM) and which are scientifically proved as potential anxiolytic agent. Qualitative phytochemical tests of hydroalcoholic extract of (*Bacopa monnieri*, *Datura stramonium*, *Azadirachta indica*) revealed the presence of carbohydrates, glycoside, flavonoids, and phenolic tannins these compounds are reported for potent anxiolytic action. In both models diazepam 1mg/kg and oral formulations 200mg/kg, 400mg/kg showed significant anxiolytic property

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