

Evaluation of Anxiolytic effect of Polyherbal Formulation on Social Interaction, Light and Dark and Elevated Plus Maze in *Wistar albino* Rat Models.

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ABSTRACT

Background and Objective: Anxiety is a widespread mental illness that impacts millions of individuals globally. The investigation of safe and effective treatments for anxiety disorders is vital due to their substantial impact on world health. There is hope for the creation of new anxiolytic treatments through the use of traditional medicinal plants. The objective of this study was to assess the anxiolytic properties of a polyherbal formulation made up of nine medicinal plants that have been shown to have potential uses in the treatment of anxiety disorders. **Methods:** Wistar albino rats were given a polyherbal formulation that included *Withania somnifera*, *Bacopa monnieri*, *Nardostachys jatamansi*, *Centella asiatica*, *Matricaria recutita*, *Rhodiola rosea*, *Crocus sativus*, *Lavandula spp.*, and *Albizia julibrissin*. Different behavioral tests, including as the elevated plus maze, the light and dark box test, and the social interaction test, were used to evaluate the formulation's anxiolytic effects. Four groups of twenty-four healthy Wistar albino rats were given varying dosages of the test formulation. The formulation's anxiolytic activity was assessed by recording and analyzing behavioral reactions. **Result:** The results of the study showed that rats given the polyherbal formulation showed much better behavioral responses in every test. Strong anxiolytic effects were evident in the rats given the maximum dosage of the formulation, as they showed a significant increase in social contact, a preference for open arms in the elevated plus maze, and a decreased aversion to light areas in the light and dark box test. **Conclusion:** Anxiolytic activity of the polyherbal formulation in Wistar albino rats was positive, indicating that it may be useful in treating anxiety disorders. These results add to the increasing amount of data that supports the use of herbal remedies to treat anxiety and emphasize the need for more studies to fully investigate the therapeutic potential of formulations containing many herbs.

Keywords: Anxiety, Medicinal plants, Polyherbal formulation, Neurotransmitters, Brain, Wistar albino rats.

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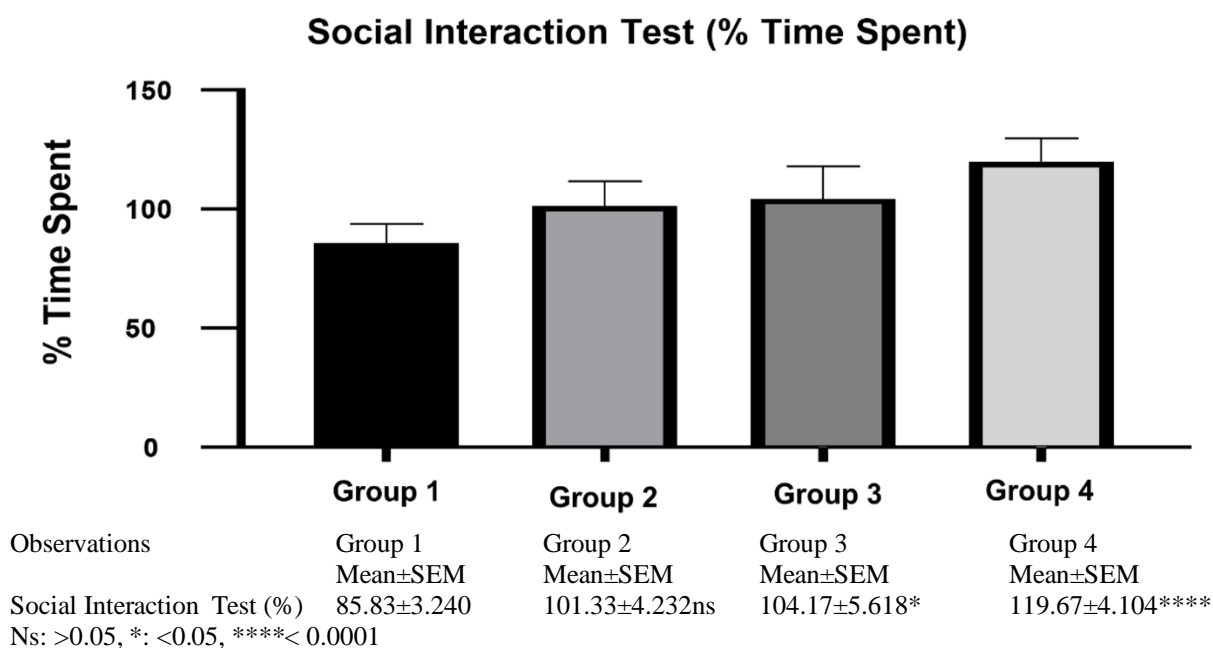
Conflict of interest: None

INTRODUCTION

Anxiety is caused due to imbalance between excitatory and inhibitory neurotransmitters¹. People suffering from anxiety disorder worry excessively, carries along a fear, feeling restless and guilty or worthless which not only affects the mental health but at the same time affects the physical health as well. Anxiety comes in several forms: panic disorder, social anxiety, specific phobias, and generalized anxiety disorder. OCD and PTSD are two more illnesses that coexist with anxiety disorder². Anxiety leads to huge suffering and disability where there is associated avoidance behaviour and agoraphobia can make markedly disrupt family life as well as the life of sufferer³⁻⁴. Depending on the kind of anxiety illness a person has, different symptoms apply. Sweaty or cold hands are common signs of anxiety disorders. Parched mouth, palpitations in the heart, emesis, tingling or numbness in the hands or feet, Breathing difficulties, tense muscles, panic, fear, and unease, Frequent thoughts, nightmares, or flashbacks to unpleasant events, irrational, compulsive thoughts, etc. Natural medications are

becoming more and more popular in the current global environment since they are affordable, widely accessible, and generally free of adverse effects⁵. Traditional medical procedures are seen as an essential component of culture in the majority of developing nations. The antiviral, antibacterial, anticancer, anti-inflammatory, and postnatal problems like anxiety are treated with it in Ayurvedic and traditional Chinese medicine⁶. The selection of suitable plant for a pharmacological study is very important step, which involves study of its traditional use, chemical composition, and toxicity etc⁶⁻⁷. Numerous herbal remedies have also been the subject of pharmacological activity studies using experimental animal models⁸. Among the herbs utilized for their anxiolytic properties include *Rhodiola rosea*, *Crocus sativus*, *Bacopa monnieri*, *Centella asiatica*, *Matricaria recutita*, *Nardostachys jatamansi*, *Withania somnifera*, *Lavandula spp* and *Albizia julibrissin*⁹⁻¹⁰. Because of their effectiveness, safety, and lack of adverse effects, they are also much sought after in the industrialized world for a variety of health issues. The medical and therapeutic uses

Table 1: Social Interaction Test (% Time Spent)



of polyherbal formulations have led to their use worldwide¹¹⁻¹². Several herbs that are combined may be effective against different a target, which adds to the overall effect that is seen. Herbal remedies for mental illnesses mainly work by modulatingneural transmission through the binding of certain plant metabolites to neurotransmitter receptors, modifications to neurotransmitter production, and general function changes.¹³⁻¹⁴. Pharmacognosy is the study of natural products' morphological, chemical, and biologicalcharacteristics as well as their cultivation, history, collection, extraction, isolation, bioassay, quality control, and preparation¹⁵. Despite the fact that a large amount of research and studies have already been done on the use of herbal remedies for anxiety, there are still gaps in the literature. Taking these considerations into account, the current study aimed to create a polyherbal formulation for anxiety relief that required the fewest possible ingredients to ensure standardization.¹⁶

MATERIALS AND METHOD

Test sample: All the nine test sample (*Withania somnifera*, *Bacopa monnieri*, *Nardostachys jatamansi*, *Centella asiatica*, *Matricaria recutita*, *Rhodiola rosea*, *Crocus sativus*, *Lavandula spp*, *Albizia julibrissin*) were collected from a GMP certified Ayurvedic manufacturing unit Bilwal Medchem and Research laboratory pvt ltd, Reengus, Rajasthan identification and authentication was done by National Institute of Science Communication and InformationResources (NISCAIR), New Delhi.

Preparation of formulation: A proper amount of the crushed, air-dried medication was weighed out and then transferred to an extraction thimble. It was then continuously extracted for six hours using petroleum ether in a Soxhlet extractor. On a water bath, the solvent was removed from the extract after it had been quantitatively

filtered into a tared evaporating dish. Drying was done at 105° C for the residue. Before being used again, each dried extract was weighed and combined to make 100 grams.

Procedure of experimental and ethical clearance:

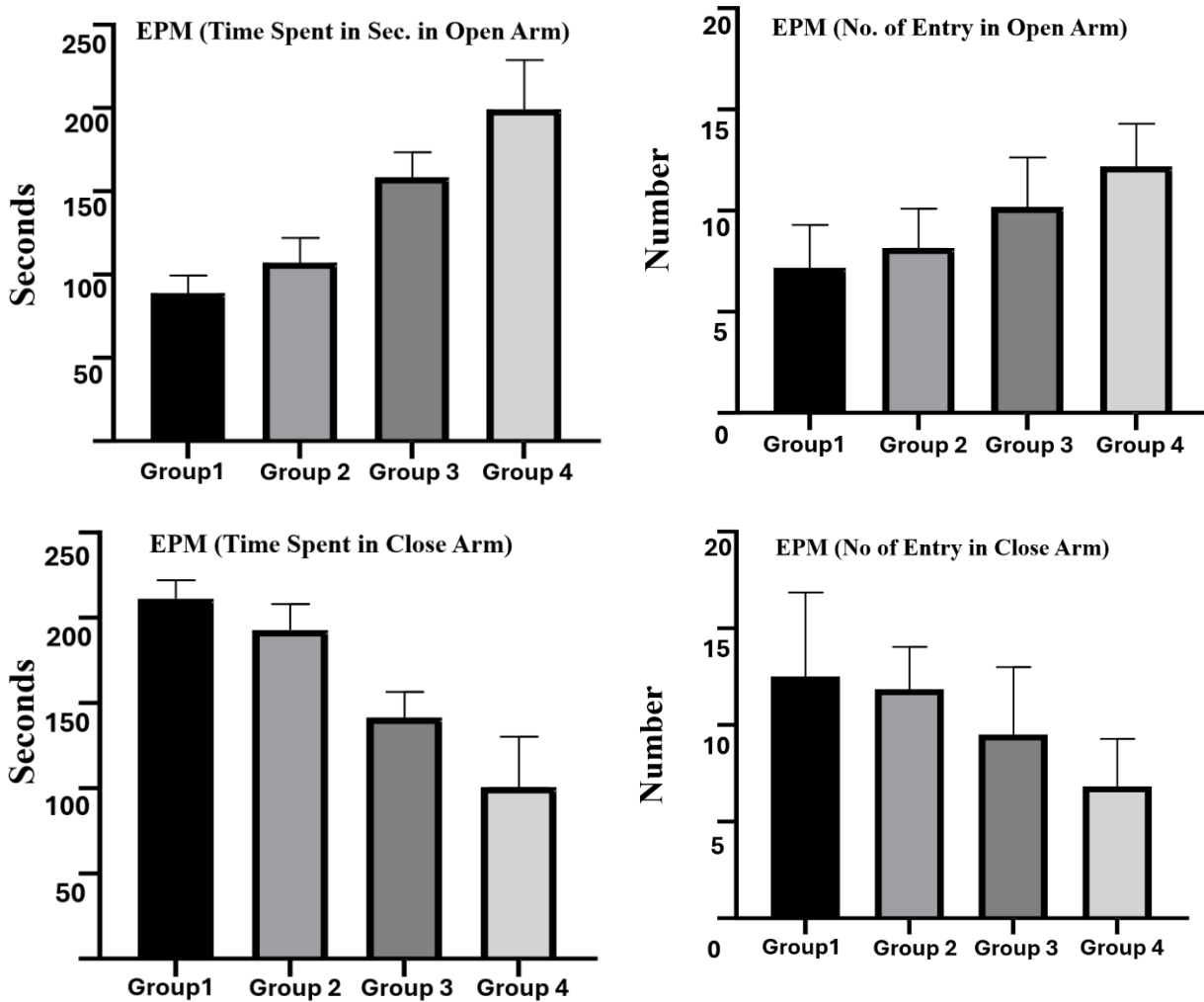
Twenty four healthy Wistar albino rats was Collected from CPCSEA registered Animal house of Bilwal Medchem and research laboratory pvt ltd against form C after Institutional ethical approval (IAEC approval no-BMRL/AD/CPCSEA/IAEC/2023/01/04). Animals were housed at temperatures ranging from 22-250C and humidity ranging from 30-70%. They were kept in Twelve hour dark and twelve hour light environment.

Experimental Procedure: Twenty-four Wistar albino rats both male and female i.e. 12 maleand 12 female of weight around 140 gram to 180 gram and of age 12 to 16 weeks were taken.The animals were divided into four groups each consisting of six rats in each group. Group 1 received tween solutions of 5 mg/kg, Group 2,3 and 4th received test formulation 100,200, 400 mg/kg orally for thirty days and evaluation of their social interaction, Elevated plus maze and Light and Dark responses was done.

Social Interaction Test: The tool used to identify shifts in social and exploratory nature consists of Perspex box measuring 51 x 51 cm and 20 cm high, with floor markings measuring 17 x 17 cm. Two rats from different cages are given an oral treatment with the test chemical one hour before the test. They are put in the box, which has 60 W of strong light, and their activity is monitored by remote video recording for ten minutes.

Elevated Plus Maze: The EPM's structure consists of two open arms that are 50 × 10 × 40 cm in size and two enclosed arms that are the same size and have an open roof. The open arms are positioned opposite one another. The maze is raised to a 50-centimeter height. The rat is put in the middle of the maze, facing one of the enclosed arms

Table 2: Elevated Plus Maze



Groups	Open Arm		Close Arm		Total Time Spent Mean±SEM
	Total Time Spent Mean±SEM	No of Entry Mean±SEM	No of Entry Mean±SEM		
Group 1	88.83±4.408	7.17±0.872	12.50±1.784		211.17±4.408
Group 2	107.17±6.172ns	8.17±0.792ns	11.83±0.910ns		192.83±6.172ns
Group 3	158.50±6.190****	10.17±1.014ns	9.50±1.432ns		141.50±6.190****
Group 4	199.33±12.101****	12.17±0.872**	6.83±1.014*		100.67±12.101****

ns: >0.05, *: <0.05, **: <0.01 ****< 0.0001

one hour after the test sample is administered orally.

Light and Dark: The model is composed of a zone fitted with photocells dividing a chamber that is light and one that is dark. A third of the surface of an animal cage made of polypropylene, measuring 44 × 21 x 21 cm, was covered in black spray paint. The cage has two sections: the

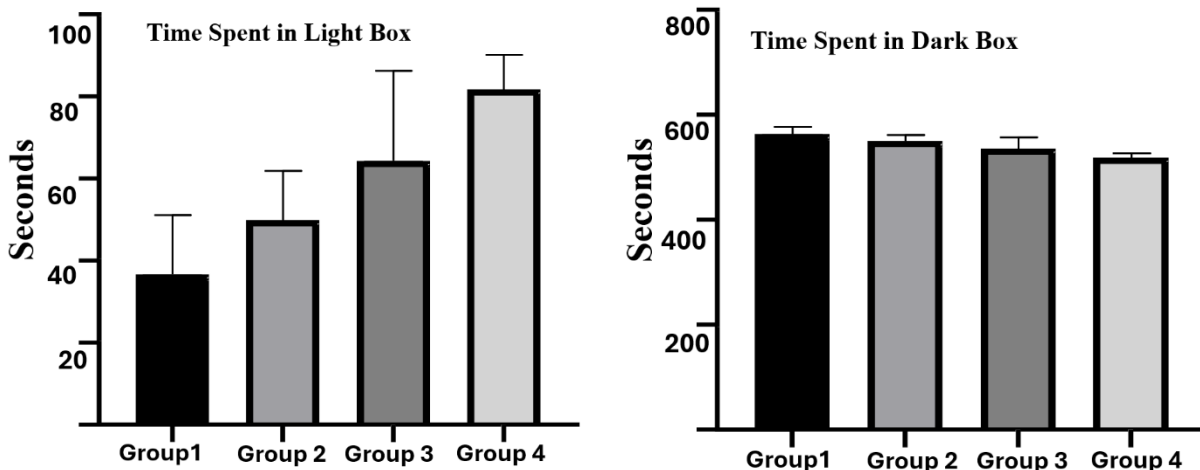
dark and light which are separated by a barrier with an entrance measuring 13 cm long by 5 cm high. This activity was monitored an hour after the test sample is administered orally.

RESULTS AND DISCUSSION

The social interaction examination consisted of four different groups, Group 1, Group 2, Group 3, and Group 4. The table no 1 above shows the results from the social

interaction test and the mean values with SEM for each group. The social interaction test is a measure of social behavior. In this test, the rats spend time with others or investigate others' interactions, particularly in bright or unfamiliar settings. The results indicate that Group 1 performed at 85.83% in the social interaction test, while Group 2 performed at 101.33%, Group 3 at 104.17%, and Group 4 at 119.67%. These values suggest that there may be variations in the social behavior or anxiolytic activity among the different groups. However, without additional context or specific points from the discussion, it is difficult to provide a comprehensive interpretation of these results. From the results it can be interpreted that the one-way ANOVA followed by Dunnett's multiple comparisons test as shown in the table, the mean differences between the groups and the 95% CI of these

Table 3: Light and Dark (Time Spent (Sec) in Light Box and Dark Box



Groups	Light Box Mean±SEM	Dark Box Mean±SEM
Group 1	36.67±5.909	563.33±5.909
Group 2	49.83±4.902ns	550.17±4.902ns
Group 3	64.33±8.950*	535.67±8.950*
Group 4	81.67±3.490***	518.33±3.490***

ns: >0.05, *: <0.05, ***: <0.001

differences are presented. It also shows that the differences are Statistically significant, and gives an adjusted p-value. Therefore, the groups are compared and table no shows the mean differences between Group 1 and each of the other groups. The mean difference between group 1 and group 2 is -15.50, 95% CI: -31.24 and 0.2445. “No” and “ns” (not significant) mean the result is not significant. The adjusted p-value for the comparison is 0.0542.

Likely the mean difference between Group 1 and Group 3 is -18.33 with 95% CI (-34.08 to -2.589). “Yes” “*” The result is significant. This comparison has the adjusted p-value of 0.0206. The mean difference between Group 1 and Group 4 is -33.83 with 95%CI ranging from -49.58 to -18.09. Yes, “*” means that there is a significant result. This comparison has the adjusted p-value of <0.0001. The mean difference between Group 1 and Group 4 is -33.83 with 95%CI ranging from -49.58 to -18.09. Yes, “****” means that there is a significant result. This comparison has the adjusted p-value of <0.0001. This implies that the social behaviors and anxiolytic activities are different from one group to another. Nonetheless, without more discussion or particular points, it becomes very difficult to make a complete interpretation. The groups comprising Group 1, Group 2, Group 3, and Group 4 underwent EPM test. As mentioned in table no 2 illustrates different parameters such as time spent in open arms and closed arms, total time spent, and entered by each of the rats in each group. Each parameter's mean value is shown with its standard error of the mean (SEM) in the "Mean±SEM" values. Group 1 data shows that the average amount of time spent in the open arm is 88.83 seconds, with a standard deviation of 4.408. Likewise, the closed arm's mean number of entries is 7.17, with a standard error of 0.872. It is evident from the results that

Group 1 and Groups 3 and 4 differ significantly in terms of social behavior or anxiolytic activity, but not Group 1 and Group 2. The table shown indicates that there are statistically significant differences between Group 1 and Groups 3 and 4 in terms of the amount of time spent in the open arm and closed arm of the elevated plus maze test, as determined by the results of the One-way ANOVA and Dunnett's multiple comparisons test. However, for both measures, there's no significant distinction between Groups 1 and 2. Furthermore, significant variations exist between Group 1 and Group 4 concerning the quantity of entries in both the open and closed arms. Significant differences are not evident in the other comparisons. These findings indicate that social behavior or anxiolytic activity differs between Groups 1 and 3, but not between group 1 and 2.

Results of light and dark tests conducted on 4 groups

Group 1, 2, 3 and 4. It involves the time each group spends in a light box and a dark box. The mean time spent in each box is shown, along with the SEM for each group. Group 1 spent on average 36.67 seconds (±5.909 SEM), Group 2 spent 49.83 seconds (±4.902 SEM), Group 3 spent 64.33 seconds (±8.950 SEM). The average time spent in the dark box by group 1 was 563.33(±5.909 SEM), group 2 was 550.17(±4.902 SEM), group 3 was 535.67(±8.950 SEM). This showed that group 1 spent considerably more time in the light box and less in the dark box. Group four had the longest time in both boxes and then the time of group three and group two. The results of the Dunnett's multiple comparisons test indicate the mean differences and their 95% confidence intervals between Group 1 and other groups for the variables Time Spent in Light Box and in Dark Box. Light and dark tests on table no 3 show the outcomes for groups 1, 2, 3, and 4. Each group has to remain inside a light box and a dark box for a specific duration that is

being timed. The difference in the mean time spent in the light box between Groups 1 and 2 was not significant. Nevertheless, Group 1 spent less time than both Group three (MD = -27.67), and Group four (MD = -45.00); these are statistically significant ($p < 0.05$). Time Spent in Dark Box is more by group one than Group two (13.17 mean difference), without significance. However, Group 1 spent more time than either Group 3 (mean difference = 27.67) and Group 4 (mean difference = 45.00), and these were significant, $p < 0$.

CONCLUSION

The comprehensive assessment of the polyherbal formulation's anxiolytic effects on social interaction, light and dark, and elevated plus maze tests in Wistar albino rat models leads to the conclusion that the formulation has promising potential for use as an effective anxiety treatment. The efficacy of the formulation is indicated by the study's findings, which show a significant change in the groups' social conduct and anxiolytic activity. These results add to the increasing amount of research that supports the use of herbal medicines to treat anxiety. The study is an important contribution to the field of herbal medicine and anxiety treatment because of its meticulous approach, comprehensive results, and perceptive discussion, which offer unique insights into the anxiolytic characteristics of the polyherbal formulation.

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