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Study on Anti anxiety activity of ALERT – A Poly herbal formulation in Experimental animal models

Veeresh Babu D, Ramesh Kulkarni, Jerin George

Department of Pharmacology, Karavali College of pharmacy, Mangalore, India
***Corresponding Author**: **Veeresh Babu D**
Email id: veeresan111@gmail.com

ABSTRACT

The present study was performed to evaluate the anti anxiety activity of ALERT – a Poly herbal formulation in experimental animal model. Anti anxiety activity was determined in experimental Swiss Albino Mice. The test was carried out by Elevated plus maze, Light/dark exploration test, Open field tests, Rota-rod test and Hole board test. In the Elevated Plus maze the total number of arm entries and the time spent in open and enclosed arms were recorded. In Light and dark exploration tests, the total number of arm entries and the time spent in light and dark chambers were recorded. In Open field tests the number Ambulation, Rearing, Self grooming, activity in center were recorded. In Hole board test the number and duration of head-dippings were recorded. In Rota-rod tests, the loco motor activity with fall in time was recorded. The animals were treated with three doses of the Poly herbal formulation ALERT, of doses low (50mg/kg), moderate (150mg/kg) and high (200mg/kg). In Elevated Plus maze significant increase in the number of entries and duration of stay in open arm was observed. In Light/ Dark exploration tests, significant increase in the time spent and numbers of entries in light compartment were observed. In Open field tests, there was significant increase in the number of squares crossed, number of crossings and rearing. In hole board tests, has shown a significant reduction in locomotion. In Rota- rod tests, significantly decreased time spent on revolving Rota-rod. The study revealed that the Poly herbal formulation ALERT possess significant anti-anxiety effect.

Keyword: Anti anxiety activity of ALERT, Swiss Albino Mice.

INTRODUCTION

Anxiety disorder otherwise known as anxiety states, anxiety reaction anxiety disorder is one of the most common forms of neuroses consisting approximately about 30 to 40 per cent of all neurotic disorders. Anxiety is an internalized fear, aroused by an impulse to commit. It is a danger signal to the ego that dangerous impulse is about to break. It is, in fact, an unconscious reaction to depressed tendencies. Ross defines anxiety as a "series of symptoms which arise from faulty adaptation to the stresses and strains of life."An anxiety is a painful emotional experience produced by excitations in the internal organs of the body. In general, it is characterized by over concern which may turn over to panic or severe fear [1].

Physical Symptoms of Anxiety

When a person encounters dangerous situation or experiences anxiety, he is bothered with physical symptoms like heavy sweating, trembling of lips and hands, rapid breathing or breathing difficulty, rapid heartbeat, increased pulse rate, dryness of mouth and frequent urination etc. Also dizziness, muscular fatigue and tension are common symptoms [2, 3].

Psychological symptoms

Persons suffering from anxiety attacks are sensitive to criticism and are quickly discouraged. Tenseness, irritability, and fear arising out of fantasies or imagined danger, acute panic and loss of sleep, mild depression, lack of concentration and inability to make decision are other common psychological symptoms [2, 3].

ALERT is a licensed polyherbal formulation contains the extracts of medicinal plants. Here the present study is aimed to investigate anxiolytic activity of licensed polyherbal formulation "ALERT". The main aim of this work is to investigate the nootropic activity of polyherbal formulation and the evaluation will conducted using various models [4, 5].

The polyherbal formulation ALERT consisting of the plant ingredients like Each capsules contains

- *Jyotishmati (Celastruspaniculata)*
- *Tale A.B seed oil 75%*
- *Cow Ghee A.B 25 %*
- *Shankhapushpi (Convolvulus pluricaulis)*
- *Vacha(Acoruscalamus)*
- *Jatamamsi(Nardostachysjatamansi)*
- *Bringaraja(Eclipta alba)*

MATERIALS AND METHOD

Material selection

Animal selection

Swiss albino mice weighing 18-30 gm were used for the study. The mice were inbred in the central animal house of the Department of Pharmacology, Karavali College of Pharmacy, Mangalore, under suitable conditions of housing, temperature, ventilation and nutrition were used for antidepressant activity. They were kept in clean dry cages week before the beginning of the experiment to acclimatize with the experimental conditions. The animals were fed with standard pelleted diet (Lipton India Ltd., Mumbai) and distilled water *ad* libitum was maintained at 21^0C-23^0C under a constant 12hrs light and dark cycle. The animal care and experimental protocols were in accordance with CPCSEA /IAEC

Housing

Mice were housed in groups of six in each clean cage. The bedding material of the cages was removed and replaced thrice a week with fresh materials as often as necessary to keep the animals clean and dry. Bedding materials used in sufficient amount to keep animals dry between cage changes without coming into contact with watering tubes. Drinking tubes were examined routinely to ensure their proper function [6].

Chemicals used

- 1 % Tween 80
- Saline 1%
- Diazepam (1mg/kg)

Plant material and extraction of drug

The plants of *Adhatoda vasica*, *Glycyrrhiza glabra*, Piper Longum, Ocium sanctum, *Zingiber officinale*, *Azadirachta indica* were collected from Pune, Maharshtra, India and authentication of the plants was done by Dr. Rajesh Dabur, Regional Research Institute (AY.) Pune. Hydroalcoholic extracts of *Adhatoda vasica* (leaves), *Glycyrrhiza glabra* (bark), Piper Longum(fruit), Ocium sanctum(leaves), *Zingiber officinale* (rhizome), *Azadirachta indica* (leaves) were prepared separately by hot percolation method through soxhlet apparatus. There after extracts were dried by using rotary vacuum evaporator. The amount of extract were weighed and stored in amber colored airtight bottles.

Preliminary phytochemical screening of crude extracts [7]

The hydroalcoholic extracts of three plants material were subjected to qualitative tests in order to identify class of compound by using preliminary phytochemical test.

TLC profile of plant extracts [8]

TLC studies were performed for presence of principal constituents by using reported methods. The hydroalcoholic extracts of *Adhatoda vasica*, *Glycyrrhiza glabra*, Piper Longum, Ocium

sanctum, *Zingiber officinale*, *Azadirachta indica* were dissolved in 70% ethanol. The reported solvent systems used for establishing the profiles. Solvent system used for *Adhatoda vasica* was n-Butanol: Ethyl acetate: Water (4:1:5) for *Glycyrrhiza glabra*, Toluene: Ethyl acetate: Formic acid (5:4:1) and for O. sanctum Petroleum ether: Ethyl acetate: Formic acid. (9:0.5:0.5). *Zingiber officinale* Toluene: Ethyl acetate (5:5), *Azadirachta indica*, Toluene: Ethyl acetate (6:4). The Rf value of each resoluted compound was recorded.

Composition and preparation of tablet formulation [9]

Tablet formulation (400mg) was prepared which contain excipients viz, mannitol (84mg), Sodium starch glycolate (16mg), Magnesium stearate (8mg), Talc (20mg), Aerosil (12mg), Povidone (20mg) and extracts of *Adhatoda vasica* (leaves) (80 mg), bark of *Glycyrrhiza glabra* (bark), (80 mg), leaves of O. sanctum (80 mg). Piper Longum(fruit), (80mg), *Zingiber officinale* (rhizome), (80mg), *Azadirachta indica* (leaves) (80mg) extracts were passes through 40 mesh sieve. Mannitol and half quantity of sodium starch glycolate were passed through 40 mesh sieve and added to active drugs. Povidone was also dissolved in isopropyl alcohol. These were added to active drugs. Proper mixing of all above formed weight mass .This weight mass was passed through 10 mesh sieve to form granules. Granules were air dried at room temperature. After drying granules were passed through 20 mesh sieve. Talc, Aerosil was added to granules by passing through 60 mesh sieve and remaining half quantity of disintegrant was added. Magnesium stearate was passed through 60 mesh sieve and added at last. Compression of granules was done to form tablets by using tablet punching machine.

Dose fixation [10]

A dose of 50mg/kg, 150mg/kg and 200mg/kg body weight

Antianxiety activity

The animals were selected in such a way that they were free from illness, injury, disease and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. Only those animals which are healthy having weights 18-30 g were selected and maintained at standard laboratory conditions.

Preparation and administration of doses [11]

All the doses were prepared in distilled water using 5% Tween 80 solution as suspending agent and administered orally. In all cases, the concentrations were prepared in 1 ml/100g of body weight. The test substances were administered in a single dose using a gastric intubation tube after fasting for 3 to 4 h.

Observations

Animals were observed initially after dosing at least once during the first 30 min, periodically during the first 24 h. additional observations like changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems and somato motor activity and behavioral pattern were also done. Attention was also given to observations of tremors and convulsions.

Elevated plus maze

The elevated plus maze apparatus consist of two open arm (16 X5cm) and two closed arm (16X5X12) having an open roof and is elevated to a height of 50cm from ground the arms are arranged around a central square. The animal were gently placed on the central square facing the open arm and the no of entries and timeSpent in open arm and no of entries and the time spent in closed arm for duration of 5min was noted on 1st, 5th and 10th day the entry into a arm is considered only when all the four paws of an animal are into the arm.

Statistical analysis

The results were expressed as Mean + SEM, Statistical significance was determined by one way ANOVA (Analysis of Variance) followed by t test by using the Graph pad Instant version and compared with control.

Light/dark exploration test

The apparatus consisted of two acrylic boxes. Two distinct chambers, a black chamber (20 x 30 x 30 cm) painted black and other open chamber made up transparent acrylic (30 x 30 x 30cm). The two chambers are connected through a small open doorway (8x 8 cm) situated on the floor level at the centre of the partition. One box was made dark by

covering its top with plywood and a 10W lamp illuminated the other box.

The light source was placed 25cm above the open box. The mice were placed individually in the center of the lit box and observed for the next 5 min for the time spent in lit and dark boxes. Each mouse was placed individually in the light compartment and observed or the next 5 minutes for the numbers of the crossing between two compartment and time spend in the light and dark compartment.

Statistical analysis

The results are expressed as Mean ± SEM and subjected to one way Analysis of Variance (ANOVA) followed by Tukey- Kramar multiple comparisons test using Graph pad prism software.

Rota rod model

Muscle relaxant activity is measured by using Rota rod apparatus initially animals were trained on the apparatus. The rota rod was set at 25rpm and animals which remained on the revolving rod for 3 min or more after low successive trials are included in the study. After 2 hrs of vehicle/drug administration the fall of time was noted on 1st, 5th, 10th day and is compared with the control group. The difference in the fall off time from rotating rod between the control and treated rat was taken as muscle relaxation index of the drug.

Statistical analysis

The results are presented as mean ± SEM. The difference between the groups was statistically analyzed using the unpaired Student's *t* test and analysis of variance (ANOVA) followed by Dunnett's test for all the treated groups. $P<0.05$ was considered statistically significant. The level of significance was noted and interpreted accordingly.

Open field test

Materials

Wooden box (100 x 100 x 30 cm)
Stopwatch

Procedure

This test utilizes behavioral changes in rodents exposed to novel environments and was used to confirm that the observed antidepressant effect was not due to stimulation of general motor activity. Various types of open field apparatus have been used to test the mice.

The open field test was carried out on the dark grey floor subdivided into 16 equal parts in wooden box (100 x 100 x 30 cm). A central square was drawn in the middle of the open field. The central square is used because some mice strains have high loco motor activity and cross the lines of the test chamber many times during a test session. Also, the central square has sufficient space surrounding it to give meaning to the central location as being distinct from the outer locations. The open field maze was cleaned between each mice using 70 % ethyl alcohol. Mice were carried to the test room in their home cages and were handled by the base of their tails at all times. Mice were placed into one of the four corners of the open field and allowed to explore the apparatus for 5 minutes. After the 5 minutes test, mice were returned in their home cages and open field was cleaned with 70 % ethyl alcohol and permitted to dry between tests. To assess the process of habituation to the novelty of the arena, mice were exposed to the apparatus for 5 minutes on 2 consecutive days. Parameters such as Activity in the centre, Number of squares crossed at periphery and Rearing (No. of times the animal stand on the rear paws).

Groups of Animals

The animals were divided as follows.
Group I – Received 0.05ml/10g of Normal saline intra peritoneally.
Group II – Received Diazepam 1mg/kg intra peritoneally.
Group III – Received 50 mg/kg PHFR 50 orally.
Group IV – Received 100 mg/kg PHFR 100 orally.
Group V – Received 200 mg/kg PHFR 200 orally.

Statistical analysis

Results are prepared as Mean ± SEM. One way ANOVA was used for multiple comparison followed by Dunnett's multiple comparison tests. For all tests a "P" value of 0.05 or less was considered for statistical significance.

ANOVA (Analysis of variance)

In statistics, analysis of variance is a collection of statistical models and their associated procedures, in which the observed variance is partitioned into components due to different explanatory variables.

In its simplest form ANOVA gives a statistical test of whether the means of several groups are all equal and therefore generalize Dunnett's multiple comparison tests to more than two groups.

Hole board test

The loco motor activity was measured by using an actophotometer. It is equipped with 6 photo cells in the outer wall. Interruptions to the signals of photocell beam due to loco motor activity were recorded by means of a six digits counter. Each animal was observed for a period of 5 min. The animal was placed individually and the actophotometer was turned on .The animal was made familiarized with the instrument for 2 min then the basal activity score was noted as counts/5 min for next 5 min. The basal activity score was noted for all the animals before the administration of the drug on the first day and 60 min after administration of the drug on 1st, 5th and 10th day of treatment.

$$Percentagereductioninlocmotion = [no.\ of\ counts\ 5\ min, 60\text{min}\ after\ drug\ administration / no.\ of\ counts\ 5\ min, before\ drug\ administration] - 100$$

RESULTS

Table 1: Results of preliminary phytochemical screening

Sl. No.	Name of the Test	Observation [Ethanolic Extract]	Conclusion
I.	**Tests for Steroids**		Steroids were present in ethanolic extract.
	Salkowski reaction	+	
	Liebermann Burchard Liberman's reaction	+	
		+	
II.	**Tests for Saponins**		Saponins were present in ethanolic extract.
	Foam test	+	
	Haemolytic test	+	
III.	**Tests for Tannins and Phenolic** Compounds		Tannins were present in ethanolic extract.
	Lead acetate test	+	
	5% Fe Cl3 test	+	
	Bromine water test	+	
	Acetic acid solution test	+	
	Potassium dichromate test	+	
V.	**Tests for Flavonoids**		Flavanoids were present in ethanolic extract.
	Shinoda test	+	
	Lead acetate test	+	
	Alkaline solution	+	
	Ferric chloride test	+	
VI.	**Tests for Reducing Sugars**		
	Fehling's test	-	Reducing sugars were absent in ethanolic extract.
	Benedict's test	-	

ANTIANXIETY ACTIVITY OF ALERT

Anti anxiety property of ALERT – a polyherbal formulation in experimental animal models has been evaluated Elevated plus maze test, Light and dark exploration test, Rota rod, Hole Board Test and Open Field Test animal models the results obtained are as follows,

Elevated plus maze test

In this model number of entries and duration of stay in open arm and closed arm were measured.Diazepam 1mg/kg ($p<0.001$) and tablet formulation PHFA 200mg/kg ($p<0.01$) showed significantly increased the number of entries and duration of stay in open arm. While other groups

does not showed significant results. All treated groups were compared with control.

n=6, Values are expressed as Mean ± S.E.M. * = P<0.05, ** = p<0.01 and *** = p<0.001 Test drug treated groups were compared with Control Group. (Statistically analysed by one way ANOVA followed by Tuky- Kramar multiple comparisons test.). PHFA 50, PHFA 100 and PHFA 200 indicate polyherbal formulation at doses 50,100 and 200mg/kg body weight respectively.

PHFA-Polyherbal formulation ALERT

Table 2: Anti anxiety of Polyherbal formulation - ALERT (PHFA) and Diazepam in mice by Elevated plus maze test

Group	Dose/day (mg/kg)	Time spent in open arm(s)	Time spent in closed arm(s)	No of entries in open arm(s)	No of entries in closed arm(s)
Control (Saline 1%)	1%	73.13 ± 4.91	205.50 ± 5.11	4.11 ± 0.13	15.15 ± 0.21
STANDARD (Diazepam)	1	120.13 ±5.73**	163.33±3.26**	20.56 ±0.19**	8.91±0.30**
PHFA 50	50	85 ± 5.16	197.50 ± 10.32	9.13 ± 1.24	12.12 ± 1.17
PHFA100	100	105.67 ± 5.23	179.17 ± 5.26	12.23 ± 1.53	9.16 ± 1.10
PHFA 200	200	116.17 ± 6.27*	170.50 ± 6.59*	14.17 ± 1.34*	8.22 ± 1.52**

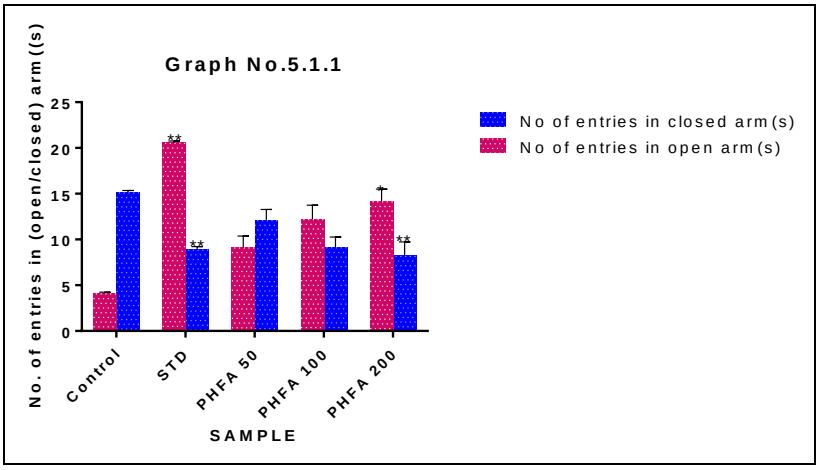

Fig.1: Anti anxiety of Polyherbal formulation - ALERT (PHFA) and Diazepam in mice by Elevated plus maze test

Light/dark exploration test

In this model the time spent and number of entries in light compartment were measured results obtained are in the (Table No. 5.1.2, Graph No. 5.1.2).Diazepam 1mg/kg (p<0.001) and tablet formulation PHFA 200mg/kg (p<0.01) showed significantly increased the time spent and numbers of entries in light compartment while other groups does not showed significant results. All treated groups were compared with control.

Table 3: Anti anxiety of Polyherbal formulation of ALERT (PHFA) and Diazepam in mice by Light/dark exploration test

Group	Dose/day (mg/kg)	Time spent in light area(sec) Mean ± S.E.M.	No of entries in open arm(s)
Control (Saline 1%)	1%	71.33 ± 4.67	6.35 ± 0.67
STANDARD (Diazepam)	1	141.83 ±6.73***	14.5 ±1.60***
PHFA 50	50	88 ± 6.79	7.50 ± 1.10
PHFA 100	100	99.67 ± 5.24	12.12 ± 1.43
PHFA 200	200	111.17 ± 6.27**	13 ± 1.24**

n=6, Values are expressed as Mean ± S.E.M. * = P<0.05, ** = P<0.01 and *** = P<0.001 Test drug treated groups were compared with Control Group. (Statistically analysed by one way ANOVA followed by Tuky- Kramar multiple comparisons test.). PHFA 50, PHFA 100 and PHFA 200 indicate polyherbal formulation at doses 50,100 and 200mg/kg body weight respectively.

PHFA- Polyherbal formulation ALERT

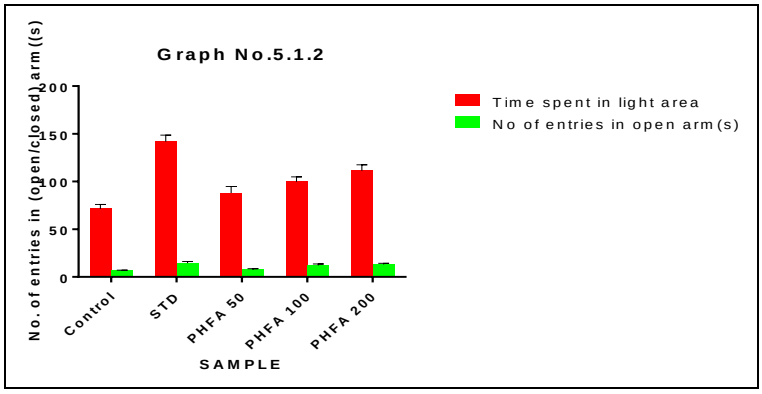

Fig. 2: Anti anxiety of Polyherbal formulation of ALERT (PHFA) and Diazepam in mice by Light/dark exploration test.

Rota rod test

Diazepam 1mg/kg (p<0.001) and tablet formulation PHFA 200mg/kg (p<0.01) showed significantly decreased time spent on revolving Rota rod. All treated groups were compared with control.

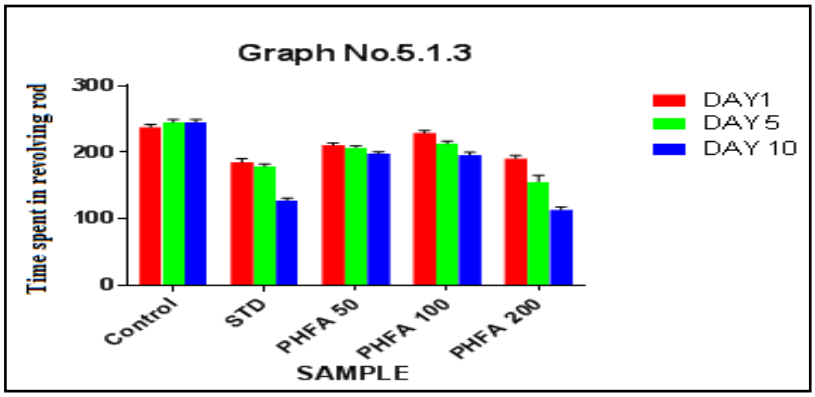

Fig. 3: Anti anxiety of Polyherbal formulation of ALERT (PHFA) and Diazepam in mice by Rota rod test

Table 4: Anti anxiety of Polyherbal formulation of ALERT (PHFA) and Diazepam in mice by Rota rod test

Group	Dose/day (mg/kg)	Time spent on revolving rod (sec)		
		DAY 1	DAY 5	DAY 10
Control (Saline 1%)	1%	238±4.442	245.5±4.357	245.5±4.357
STANDARD (Diazepam)	1	185.6±5.031**	178.8±3.772**	128±3.055**
PHFA 50	50	211 ± 3.18	207.50 ± 2.32	198 ± 3.24
PHFA100	100	229.1±4.053	213.3±3.73**	196.5±3.757**
PHFA 200	200	191±4.42**	156±9.879**	113.8±4.143**

n=6, Values are expressed as Mean ± S.E.M. * = P<0.05, ** = P<0.01 and *** = P<0.001 Test drug treated groups were compared with Control Group. (Statistically analysed by one way ANOVA followed by Turkey- Kramar multiple comparisons test.). PHFA 50, PHFA 100 and PHFA 200 indicate polyherbal formulation at doses 50,100 and 200mg/kg body weight respectively.

PHFA- Polyherbal formulation ALERT

Open field test

The open field test was performed to confirm the property to alter general motor activity by **PHFA-** Polyherbal formulation ALERT, because any alteration in general motor activity may give false positive/ negative results in forced swim test. There was a slight increase in the number of squares crossed (peripheral) by mice in PHFA treated groups but it was statistically significant compared to control. There was a significant increase in no. of crossings in diazepam group as compared to control group. There was significant increase in the rearing of animals with diazepam in comparison to the control group. There was increase in number of rearing in PHFA drug treated groups.

Table 5: Effect of PHFA on open field test in mice

Group	Dose/day (mg/kg)	No. of squares crossed (Mean ± SEM)		No of Rearings (Mean±SEM)
		Centre	Periphery	
Control (Saline 1%)	0.05ml/10g	11.40± 0.07	79.11± 0.11	3.33± 0.55
STANDARD (Diazepam)	1	29.28± 0.11***	111.37±0.15***	8.48± 0.06***
PHFR 50	50	12.28± 0.11	55.18±0.66	12.48± 0.05
PHFR100	100	19.28± 0.11*	71.37±0.16*	10.48± 0.06*
PHFR 200	200	25.18± 0.23***	99.26±0.15***	7.48± 0.16***

Values were mean ± S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis test. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control

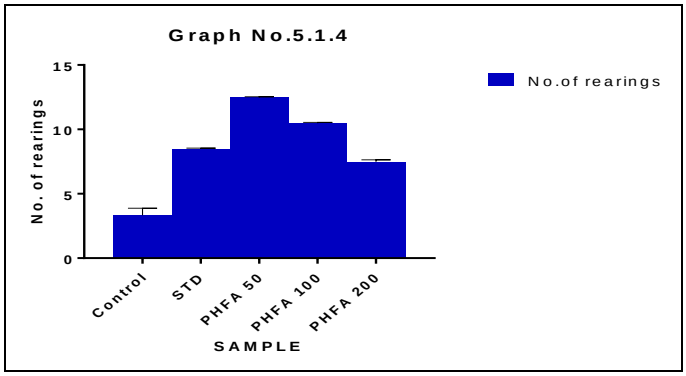

Fig.4: Effect of PHFA on open field test in mice

Hole board test

The results obtained were presented in **fig. 5** and it indicates that PHFA 50,100, 200 has shown a significant reduction in locomotion on 1,5,10 day of the treatment. It also shows significant percentage reduction in locomotion. Results of diazepam (1mg/kg), PHFA (50,100 and 200mg/kg) were compared with control group it showed a significant effect with P<0.01.

Table 6: Effect of PHFA on Hole board test in mice

Group	Dose/day (mg/kg)	No of count/5min [Mean±SEM]			
		Before treatment	After 60 min of treatment		
			Day 1	Day 5	Day 10
Control (Saline 1%)	0.05ml /10g	363.1± 5.618	365.7± 4.5	351±4.161	359.5±4.365
Standard (Diazepam)	1	236.5± 5.69	282.6± 3.9**	261.1± 5.36**	196.3± 2.917**
PHFA 50	50	240± 3.72	349.6± 9.1	315± 9.87	264± 6.89
PHFA100	100	231± 6.83	329± 6.9	270.3± 3.35	220± 3.18
PHFA 200	200	219± 4.12	299± 3.1**	250± 1.22**	187± 0.12**

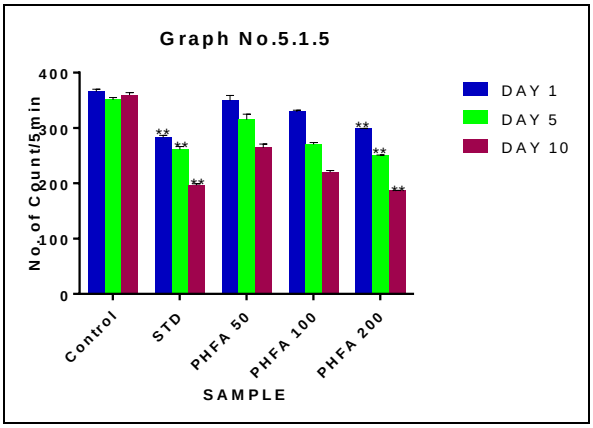

Fig. 5: Effect of PHFA on hole board test in mice

Polyherbal formulation- ALERT (PHFA) was subjected to qualitative tests it was found to contain steroids, saponins, tannins, phenolic compounds and flavonoids.

ANTIANXIETY ACTIVITY OF ALERT

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Elevated plus maze test

Diazepam 1mg/kg ($p<0.001$) and tablet formulation PHFA 200mg/kg ($p<0.01$) showed significantly increased the number of entries and duration of stay in open arm. While other groups does not showed significant results. All treated groups were compared with control.

Light/dark exploration test

Diazepam 1mg/kg ($p<0.001$) and tablet formulation PHFA 200mg/kg ($p<0.01$) showed significantly increased the time spent and numbers of entries in light compartment while other groups does not showed significant results. All treated groups were compared with control.

Rota rod test

Diazepam 1mg/kg ($P<0.001$) and tablet formulation PHFA 200mg/kg ($p<0.01$) showed significantly decreased time spent on revolving Rota rod. All treated groups were compared with control.

Open field test

The open field test was performed to confirm the property to alter general motor activity by PHFA- Polyherbal formulation ALERT, because any alteration in general motor activity may give false positive/ negative results in forced swim test. There was a slight increase in the number of squares crossed (peripheral) by mice in PHFA treated groups but it was statistically significant compared to control. There was a significant increase in no. of crossings in diazepam group as compared to control group. There was significant increase in the rearing of animals with diazepam in comparison to the control group. There was increase in number of rearing in PHFA drug treated groups.

Hole board test

The results indicated that PHFA 50,100, 200 has shown a significant reduction in locomotion on 1,5,10 day of the treatment. It also shows significant percentage reduction in locomotion. Results of diazepam (1mg/kg), PHFA (50,100 and 200mg/kg) were compared with control group it showed a significant effect with $P<0.01$. Anxiolytic effect of any drug can be evaluated by using elevated plus maze, the height and open spaces of the maze induces fear in animals. Rodents generally, have a tendency to prefer closed and dark places. When an animal is kept on the elevated plus maze facing the open arm it showed reduced number of movements by spending most of the time in closed arm. When treated with the anxiolytic drug the number of entries and time spent in open arm was significantly increased and the number of entries and time spent in closed arm was decreased and the results were comparable with the standard drug diazepam (1mg and 2mg/kg) and PHFA (100mg and 200mg/kg). The open arm-closed arm approach for anxiolytic effect has worked well in identifying the anxiolytic drugs having potential effect on benzodiazepine/GABAA receptor. Hence the results suggest that the test drug may possess a positive modulation effect on GABAA-chloride channels and thereby may cause a rise in GABA levels in brain.

Test drug (PHFA) (100mg and 200mg/kg) has shown a significant percentage reduction in locomotion and the results where comparable with standard drug diazepam (1mg and 2mg/kg) and Polyherbal formulation PHFA (100mg and 200mg/kg). This action was possibly due to sedative effect of the drug by acting on benzodiazepine/GABA receptor complex. PHFA has also shown a significant reduction in time spent on revolving rod indicating the presence of muscle relaxant effect and the results where comparable with the standard drug diazepam (1mg and 2mg/kg) and control.

CONCLUSION

The study was taken up to evaluate polyherbal formulations of ALERT for anxiolytic

activities.The acute toxicity study conducted for polyherbal formulations indicated that they are safe up to 2000 mg/kg body weight. Polyherbal formulations of ALERT (PHFA) produced significant antianxiety activity comparable to the standard Diazepam (1mg/kg) by Elevated plus maze test, Light/dark exploration test, rota rod test, open field test and Hole Board Test. It was evident from the results that ALERT (PHFA) produced significant anti anxiety activity in all the five models.

From the results obtained it can be concluded that ALERT has antianxiety activity. Results obtained in the present study suggest that the polyherbal formulation ALERT has a dose dependent effect. These results also suggest a probable involvement of benzodiazepines/GABA receptor along with 5-HT receptor and provide the evidence that ALERT can be used as a potent anxiolytic drug. However further studies are required at molecular level to evaluate its exact mechanism of action.

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