PHARMACOLOGICAL EVALUATION OF ALCOHOLIC EXTRACT OF STEM BARK OF *ERYTHRINA VARIEGATA* FOR ANXIOLYTIC AND ANTICONVULSANT ACTIVITY IN MICE

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Summary

The objective of the present study was to evaluate the anxiolytic and anticonvulsant activity of alcoholic extract of stem bark of *Erythrina variegata* (ALEEV) in mice. Preliminary phytochemical studies of the extract revealed the presence of alkaloids, flavanoids, tannins and phenolic compounds, carbohydrates, proteins, saponins and triterpenoids. Acute oral toxicity was performed in albino mice as per OECD guidelines no.425 and it was found to be that the extract was safe till 2000 mg/kg. Anxiolytic activity of ALEEV was assessed using elevated plus- maze test (EPM), Open field test (OFT) and light-dark transition (LDT) models and anticonvulsant activity was assessed by using pentylene tetrazole (PTZ) induced convulsion models and maximal electroshock (MES) induced convulsion models. Pretreatment with ALEEV (400 and 800 mg/kg, po), increased the number of entries into the open arm and the time spent in the open arms in EPM; Increased total locomotion, time spent in central compartment, ambulation in OFT and Increased the latency to enter into the dark compartment, increased the time spent in the light compartment and increased number crossings between light and dark compartments in LDT indicating that ALEEV at these dose levels possess anxiolytic activity. Similarly in anticonvulsant models pretreatment with ALEEV (400 and 800 mg/kg, po) delayed the onset of clonic convulsions in PTZ induced convulsion model and duration of extensor phase in MES induced convulsions indicating that this extract possess anticonvulsant activity. These results suggested that ALEEV at doses of 400 and 800 mg/kg exhibited both anxiolytic and anticonvulsant activities but the effect was significant with high dose (800 mg/kg) of ALEEV.

Key words: Anxiolytic, Anticonvulsant, *Erythrina variegate*.

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Introduction

Anxiety affects one-eighth of total population of the world and become a very important area of research interest in psychopharmacology during this decade [1]. Currently, the most widely prescribed medications for anxiety disorders are benzodiazepines. However, the clinical uses of benzodiazepines are limited by their side effects such as psychomotor impairment, potentiation of other central depressant drugs and dependence liability [2]. Therefore, the development of new medications processing anxiolytic effect without the complications of benzodiazepines would be of great importance in the treatment of anxiety related disorders. Epilepsy is the most common neurological disorder of the brain and is characterized by recurrent unprovoked seizures. The established antiepileptic drugs produce adverse effects such as ataxia, hepatotoxicity and megaloblastic anaemia [3]. The use of herbal medicines by physicians in Europe and Asia, exploring their traditional remedies to find a suitable cure of these 'mind affecting diseases' and herbal medicines are often considered to be gentle and safe alternative to synthetic drugs[4,5]. *Erythrina variaegata* belongs to the plant family Fabaceae. A wide spectrum of biological activities has been reported for different parts of the plant. Further folkore medicine suggests that *Erythrina variegata* barks acts on the central nervous system so as diminish or abolish its function [6]. However there was lack of scientific data regarding its effect on the central nervous system. Hence the present study was designed to evaluate the anxiolytic and anticonvulsant activity of alcoholic extract of stem bark of *Erythrina variegata* in mice.

Materials and Methods

Drugs and chemicals

Phenytoin (Sun Pharmaceuticals India.Ltd, Halol, India). Diazepam (Ranbaxy Laboratories Ltd, New Delhi, India), Pentylene tetrazole (Sigma-Aldrich, St.Louis, USA) were used for the study. All the solvents used for the extraction process are of Laboratory grade and they are purchased from local firms.

Preparation of extract and its phytochemical investigation:

Dried powdered stem bark was successively extracted with petroleum ether and 70 % ethanol using soxhlet apparatus for 18 hours. The solvents were dried under vaccum and the alcoholic extract of *Erythrina variegata* (ALEEV) was used for further study.

ALEEV was subjected to phytochemical investigation [7] to determine the secondary metabolites. ALEEV showed presence of alkaloids, flavanoids, phenolics, tannins, carbohydrates, proteins, saponins and triterpenoids.

Experimental animals:

Swiss albino mice of either sex (18-25g) procured from BioNeeds, Bangalore and all were acclimatized for 7 days in the animal house of P.E.S. College of Pharmacy, Bangalore. All the animals were maintained under standard conditions, i.e.; room temperature of 24± 1^0 C, relative humidity (45-55 %) and a 12:12h light/dark cycle. The animals had free access to standard rat pellet, with water supplied *ad libitum* under strict hygienic conditions. The animals were habituated to laboratory conditions 48 h before the start of the experiment to minimize any of non specific stress. All the experiments were carried out between 08:00 and 13:00 h, after obtaining permission from the Institutional Animal Ethics Committee (IAEC) of PES College of Pharmacy, Bangalore. All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute oral toxicity:

Acute oral toxicity of aqueous extracts of stem bark of *Erythrina variegata* was determined by using female, nulliparous and non pregnant mice weighing 18-22 g. The animals were fasted for 3 hrs prior to the experiment. Up and down procedure OECD guideline no. 425 [8] was adopted for toxicity studies. Animals were administered with single dose of extract and observed for their mortality during 48 hours study period (short term) toxicity.

PHARMACOLOGICAL EXPERIMENTS

Assessment of anxiolytic activity

(i) Elevated Plus Maze model:

The Elevated plus-maze test is described elsewhere. The apparatus comprises of two open arms (35 cm ×5 cm) and two closed arms (30 cm × 5 cm ×15cm) that extend from a common central platform (5 cm ×5 cm). The floor and walls of the closed arms is wooden and painted black. The entire maze is elevated to height of 50 cm above the floor level. Mice of 18-22 were housed in pair for 10 days prior to testing in the apparatus. During this time the mice are handled by the investigator on alternate days to reduce stress. A group consists of 6 mice for each dose.

One hour after oral administration of the test drug or the standard, each mouse was placed in the center of the maze facing one of the open arms. During a five minutes test period the following measures were taken: the number of entries into and the time spent in the open and the closed arms, time spent in central zone and rearing. The procedure was conducted preferably in a sound attenuated room[9,10].

(ii) Open Field:

Spontaneous motor activity was evaluated in open field test [11]. The open field apparatus is made up of plywood and consisted of square 56 cm x 56 cm. The entire apparatus was painted black and 6mm thick white lines divided the floor into 16 square of identical dimension. Open field was lighted by 40 W bulb focusing on to the field from the height of about 100cm. The entire room, except the open field was kept dark during the experiment. Each animal was placed at one corner of the apparatus and the following behavioral aspects were noted in the next 5min.

a) Latency: Time taken by animal to leave square in which it was placed.
b) Time taken to enter central compartment.
c) Total locomotion and central locomotion.
d) Ambulation: Number of squares passed by animal.
e) Rearing: Number of times animal stood on its hind leg.

(iii) Light-Dark model:

Natural aversion of animal for brightly lit places was evaluated in light-dark transition model. Light dark box is a rectangular box of 46 x 27x 30cm (l x b x h), which is divided into 2 compartments. $1/3^{rd}$ is for the dark compartment and $2/3^{rd}$ is served as light compartment. Extract/vehicle or standard drug was administered orally. 60 min after oral administration, the mouse were placed individually in the light compartment and observe for a period of 5 min. Parameters like total locomotion in light zone, number of rearing, time spent in light and dark zones and number of crossings/entries in the each zone were observed during this period [12].

Anticonvulsant activity:

(i) PTZ (Pentylene tetrazole) induced convulsions:

Effect of ALEEV was tested against chemically induced convulsions. Male albino mice weighing between 20-25g were divided into five groups each comprising of six animals. One hour after vehicle/standard/extract treatment PTZ (pentylenetetrazole 80 mg/kg s.c.) was administered to all groups of mice. Each animal was placed in to individual plastic cage and

were observed initially for 30min and later up to 24 hrs. The following parameters were recorded during test session of initial 30min and at the end of 24 hrs; Latency (onset of clonus), Onset of tonic convulsions and status of animal after 24 hrs [13].

(ii) *Maximal Electro Shock (MES) induced convulsions:*

Effect of ALEEV was tested against electrically induced convulsions. Briefly, a 60mA current was delivered transauricularly for 0.2 sec in mice via small alligator clips attached to each pine by electroconvulsometer. This current intensity elicited complete tonic extension of the hind limbs in control mice. For recording various parameters, mice were placed in a clear rectangular plastic cage with an open top, permitting full view of the animal's motor responses to seizure in the pilot study of various phases of convulsions, viz. tonic flexion, extension, stupor and mortality due to convulsions are selected as the parameters [14].

Results

Acute oral toxicity:

No mortality was recorded up to 2000 mg/kg with alcoholic extract of *Erythrina variegata*, hence the extract was found to be safe up to a dose level of 2000 mg/kg.

ASSESSMENT OF ANXIOLYTIC ACTIVITY

Elevated Plus-Maze Test:

The classic anxiolytic benzodiazepine; diazepam has long been reported for its anxiolytic activity in mice with EPM. In our study also, a significant anxiolytic effect was recorded with diazepam (2 mg/kg. po) as it increased the number of entries and the time spent in open arms along with significant decrease in time spent in closed arms and neutral zone.

ALEEV at doses of 400 mg/kg, 800 mg/kg showed significant anxiolytic activity by increasing the number of entries in open arms along with time spent in open arms and significant reduction in time spent in closed arms and neutral zone. However, the effect of ALEEV (400, 800 mg/kg) on number of entries in closed arms and rearing was insignificant. The anxiolytic activity shown at higher dose of ALEEV (800 mg/kg) was comparable with Diazepam 2 mg/kg. p.o. The results are shown in the table no.1

Open-Field Test (OFT):

ALEEV was subjected to evaluate the effect on exploratory behavior and emotionality in open field test. Significant increase in total locomotion was recorded along with increased central locomotion and ambulation i.e. number of squares crossed by animal in central compartment as well as in peripheral compartment with ALEEV, but effect was significant at high dose (800 mg/kg) of ALEEV. ALEEV also decreased the time spent in peripheral square where it was placed and time taken by animal to enter in central compartment as compared to control group, which shows decreased fear of animal indicates anxiolytic activity of alcoholic extract of stem bark of *Erythrina variegata*. The results are shown in the table no.2.

Light-Dark Transition (LDT) model:

Different doses of ALEEV (400 and 800 mg/kg) were administered for screening anxiolytic activity in light-dark transition model. Both doses of ALEEV had increased the time spent in light zone, total locomotion in light zone and number of crossings between the light and dark zone along with decreased time spent in dark zone compared to control but the effect was significant in case of 800 mg/kg dose. ALEEV also increased the latency i.e; time taken to enter dark zone which showed decrease in fear of animal indicating significant anxiolytic activity of the stem bark of alcoholic extract of *Erythrina variegata.* The results are shown in the table no.3.

ANTICONVULSANT ACTIVITY

PTZ (Pentylene tetrazole) induced convulsions:

Standard diazepam (5 mg/kg) abolished seizures completely and also showed 100 percent protection against seizures and mortality. Both the doses (400 and 800 mg/kg) of ALEEV showed significant anticonvlsant activity by delaying the onset of tonic-clonic seizures. ALEEV also exhibited protection against mortality in dose dependent manner i.e; 16.66% animals protected with 400 mg/kg where as 50% animals protected with 800 mg/kg dose but the extract was failed to abolish seizures completely. The results are shown in the table no.4.

MES (Maximal Electro Shock) induced convulsions:

ALEEV showed anticonvulsant activity against MES induced convulsions by increasing the time of onset of clonus and decreasing the duration of extensor phase, but the effect was significant at 800 mg/kg dose of ALEEV. ALEEV showed protection against MES induced mortality in a dose dependent manner. 33.33% animals were protected with 400 mg/kg where as 66.66% with 800 mg/kg. In this study, phenytoin 25 mg/kg had prevented extensor phase and also showed 100% protection against mortality. The results are shown in the table no.5.

Table 1: Effect of ALEEV on Elevated plus maze test in mice

TREATMENTS	Elevated plus maze model					
	NUMBER OF ENTRIES (COUNTS/5MIN)		TIME SPENT IN (SEC/5MIN)		TIME SPENT IN NEUTRAL ZONE (COUNTS/5MIN)	REARING (Counts/5min)
	OPEN ARM	CLOSED ARM	OPEN ARM	CLOSED ARM		
Control (3%tween 80)	3.66±0.55	13.16±1.22	29.16±7.94	218.16±14.28	52.66±7.67	4.83±0.47
Diazepam (2 mg/kg)	12.16±1.04***	15.16±1.04	137.16±9.33***	142.83±7.71***	20.0±3.49**	7.33±0.55
ALEEV (400 mg/kg)	7.66±0.84*	12.0±0.93	89.83±12.13**	170.66±8.55*	39.5±3.98	4.33±0.66
ALEEV (800 mg/kg)	11.16±0.79***	13.5±1.05	131.16±16.19***	150.83±10.44***	19.16±7.07**	6.16±1.13

Values are expressed as mean±SEM from 6 mice. P<0.05 *, <0.01 ** and <0.001 *** as compared to control using One way ANOVA followed by Tukey Kramer's test.

Table 2: Effect of ALEEV on open field test.

Treatments	Time spent in square where it is placed (sec/5min)	Time taken to enter central compartment (sec/5min)	Total locomotion (sec/5min)	Central locomotion (sec/5 min)	Ambulation (Counts/5min)	Rearing (Counts/min)
Control (3%tween 80)	9.33±1.78	44.50±8.08	64.16±19.52	11.16±2.52	61.83±6.95	7.33±1.60
Diazepam (2 mg/kg)	3.83±0.79*	24.16±4.23	271.66±8.77***	44.16±6.43**	173.33±16.20***	14.66±1.54*
ALEEV (400 mg/kg)	5.50±0.88	30.16±7.22	229.16±19.90*	28.83±4.06	129.33±17.97	8.66±0.71
ALEEV (800 mg/kg)	4.83±1.70	25.83±4.84	260.33±15.12**	38.16±7.71*	159.83±14.89**	9.83±1.22

Values are expressed as mean±SEM from 6 mice. P<0.05 *, <0.01 ** and <0.001 *** as compared to control using one way ANOVA followed by Tukey Kramer's test.

Table 3: Effect of ALEEV on light-dark transition model.

Treatments	**Latency (sec)**	**Time spent in dark zone (sec/5 min)**	**Time spent in light zone (sec/5 min)**	**No. of crossings (Counts/5min)**	**Total locomotion time in light zone (sec/5min)**	**Rearing (Counts/5min)**
Control (3%tween 80)	10.16±1.86	06.83±9.30	93.16±9.30	4.83±0.70	60.33±6.31	7.66±1.40
Diazepam (2 mg/kg)	19.16±3.13	107.83±8.91***	192.16±8.91***	11.33±0.98*	174.33±10.51***	9.66±0.95
ALEEV (400 mg/kg)	15.66±3.31	57.66±9.60	130.66±11.01	8.66±1.20	107.33±12.17	7.83±0.94
ALEEV (800 mg/kg)	22.16±4.45	124.33±12.40	169.33±13.41***	10.83±0.90*	151.16±12.91***	8.83±1.32

Values are expressed as mean±SEM from 6 mice. $P<0.05$ *and <0.001 *** as compared to control using one way ANOVA followed by Tukey Kramer's test.

Table 4: Anticonvulsant activity of ALEEV against PTZ-induced convulsions in mice

TREATMENTS	LATENCY (Onset of clonus) (Sec/30min)	ONSET OF TONIC (Sec/30min)	% PROTECTION AGAINST SEIZURES	% PROTECTION AGAINST MORTALITY
Control (3%tween 80)	51.83±5.48	369.16±23.64	0	0
Diazepam (5 mg/kg)	No clonus	No tonus	100	100
ALEEV (400 mg/kg)	128.66±9.02**	451.66±30.03	0	16.66
ALEEV (800 mg/kg)	170.33±14.10***	521.50±16.68*	0	50

Values are expressed as mean±SEM from 6 mice. P<0.05 *, <0.01 ** and <0.001 *** as compared to control using one way ANOVA followed by Tukey Kramer's test.

Table 5: Anticonvulsant activity of ALEEV against maximal electric shock induced convulsions.

TREATMENTS	DURATION OF TONIC FLEXION (Sec/30min)	DURATION OF TONIC EXTENSOR (Sec/30min)	LATENCY (ONSET OF CLONUS) (Sec/30min)	% PROTECTION AGAINST MORTALITY (24h)
Control (3%tween 80)	Not observed	15.33±0.55	2.16±0.47	0
(Phenytoin 25 mg/kg)	6.83±0.79***	Not observed***	13.16±1.35***	100
ALEEV (400 mg/kg)	Not observed	11.83±0.60	5.83±0.87	33.33
ALEEV (800 mg/kg)	Not observed	8.83±0.70***	9.0±0.85***	66.66

Values are expressed as mean±SEM from 6 mice. $P<0.001$ *** as compared to control using One way ANOVA followed by Tukey Kramer's test.

Discussion and Conclusion

Diazepam, a standard anxiolytic used clinically and is also employed in behavioral pharmacology as a reference compound for inducing anxiolytic-like effects, even when the compound being screened does not act via benzodiazepine receptors[15]. In our study, the anxiolytic effect of alcoholic extract of stem bark of *Erythrina variegata* was studied in elevated plus-maze, open field test and light-dark transition models. The elevated plus-maze is one of the most widely used model of animal anxiety, having been employed by many research laboratories in the past 6 years and has been extensively validated for use with rats and mice [16,17]. The test is principally based on the observations of Montgomery showing that exposure of animals to an elevated maze alley evokes an approach-avoidance conflict that is considerably stronger than that evoked by exposure to an open maze allay. In our study, ALEEV at doses of 400 mg/kg, 800 mg/kg altered the time spent and arm entries in open arms and decreased the time spent in closed arms compared to control. The effect was insignificant with low dose (200 mg/kg). The time spent in neutral zone is also reduced by both the extracts compared to control group. Decreased aversion to open arms compared to control group indicates the anxiolytic activity of stem bark of *Erythrina variegata* and the magnitude of anxiolytic effect of 800 mg/kg of alcoholic extract of *Erythrina variegata* was comparable to that of standard drug diazepam 2 mg/kg p.o. The open field test is now one of the most popular procedure in animal psychology and used to study emotionality in animals. The procedure consists of subjecting an animal usually a rodent to an unknown environment from which escape is prevented by surrounding walls. In fact, anxiety behavior in the open field triggered by two factors: individual testing (the animal is separated from its social group) and agoraphobia (as the arena is very large relative to animal's natural environment) [18]. ALEEV at a dose of 800 mg/kg had exhibited a significant anxiolytic effect by increasing total locomotion, central locomotion and ambulation with subsequent decrease in immobility time. Low dose (400 mg/kg) of alcoholic extract showed significant effect on total locomotion without significant effect on other parameters. Light-dark transition model based on the natural aversion of mice for brightly lit places. In a two compartment box, one dark and one brightly lit, the total activity, the time spent in the light compartment and the crossings between the light and dark compartment provides information about the preference of the animal for the dark compartment.

As anxiolytics should reduce the natural aversion to light, the essential feature of this model is that anxiolytic drugs increase the number of crossings and/or the time spent in the light compartment.

The later parameter is generally considered to be the most relevant one [19]. In our present study, high dose (800 mg/kg) of ALEEV significantly increased the time spent in light zone and total locomotion compared to control. The other important parameter i.e. number of crossings between light and dark zone was also significantly altered by higher dose (800 mg/kg) of ALEEV, Other parameters like latency, rearing also altered by alcoholic extract of *E.variegata* but the effect was insignificant.

The experimental models used to evaluate the anticonvulsant activity, MES and PTZ tests, are assumed to identify anticonvulsant drugs effective against generalized tonic-clonic partial seizures and generalized clonic seizures respectively [20]. In our present investigation with PTZ induced convulsion model, pretreatment with Alcoholic extract (400 and 800 mg/kg) of *E.variegata* significantly delayed the onset of seizures by delaying the onset of clonus as well as tonic phases and also the extract offered protection against mortality in dose dependent manner.

In MES induced convulsion model, reference drug; Phenytoin (25 mg/kg) completely abolished the important tonic extensor phase of the tonic-clonc seizures and offered 100% protection and produced tonic flexon convulsions, where as ALEEV (800 mg/kg) significantly altered extensor phase duration and onset of clonus phase. Where as low dose of ALEEV (400 mg/kg) altered extensor phase duration and onset of clonus but the effect was insignificant. Both the doses of ALEEV completely abolished flexon convulsions and offered protection against mortality in dose dependent manner.

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