

## RESEARCH ARTICLE

## OPEN ACCESS

## Anxiolytic activity of methanolic extract of *Erythrina variegata* Linn. leaves in Wistar rats

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### Abstract

**Ethnopharmacological relevance:** *Erythrina variegata* (Fabaceae) is widely used as a tranquilizer and/or sedative in Traditional system of medicine.

**Aim of study:** The present study was aimed to investigate anxiolytic effects of methanolic extract of leaves of *Erythrina variegata*.

**Materials and methods:** The anxiolytic activity of methanolic extract of *Erythrina variegata* leaves at doses (100 and 200 mg/kg; p.o) was studied by elevated plus maze model and Rota rod test in rats. **Results:** In elevated plus maze test, methanolic extract of *Erythrina variegata* produced significant reduction in onset of anxiety and the effect was comparable to that produced by diazepam. In the Rota rod test in rats, *Erythrina variegata* (200 mg/kg) showed significant muscle relaxant property. The effect of the extract was comparable to that of the standard drug diazepam (1 mg/kg). The results of the present study specified that the methanolic extract of *Erythrina variegata* leaves keeps significant anxiolytic like activity. **Conclusion:** The results recommended that chronic administration of the methanolic extract of the leaves of *Erythrina variegata* exerts an anxiolytic-like effect on rats, and it may serve as a new approach for the treatment of anxiety.

**Keywords:** *Erythrina variegata* (Fabaceae), anxiolytic activity, diazepam, elevated plus maze test, Rota rod test.

## INTRODUCTION

Actually, in the world around 40% of the individuals are suffering from central nervous system disorders develop the symptoms of anxiety [1] and suffer from insomnia. Statistically, 10% of the world population suffering from several forms of anxiety [2] and 30% of the adult population has insomnia [3]. Even today, traditional medicine is still the predominant means of health care in developing countries, where around 80% of their total population depends on it for their wellbeing [4]. Anxiety disorders are normally treated by benzodiazepines, buspirone, and antidepressant drugs; although these drugs are clinically effective, but are associated with several side effects [5, 6]. For example, in spite of their relative safety, benzodiazepines can lead to disturbing effects such as amnesia, dependence liability, and sedation, which cause considerable concern. Thus, there is a need for the development of safer anxiolytic drugs.

In Ayurvedic system of medicine, the *Erythrina* species (Family-Fabaceae), including *Erythrina variegata* Linn. are frequently used as anxiolytic, anthelmintics, carminatives, febrifuge, diuretics, expectorant and in rheumatism and skin diseases. *Erythrina variegata* Linn. (Family-Fabaceae)

commonly known as Mandar, is an erect, deciduous tree with dense branches and black spine. Bark is yellowish-grey and smooth. Leaves are trifoliate, leaflets are broadly ovate, acute and smooth. Flowers are pea shaped and red in colour [7]. Phytochemical screening of three extracts (petroleum ether, methanol and aqueous) of leaves showed presence of alkaloid, flavonoids, saponin glycosides and steroidal compounds. Literature survey revealed that *Erythrina variegata* have antioxidant, anti-hyperlipidemic [8], anti-inflammatory [9], antibacterial and osteoprotective activity[10], which have scientific justified data; however there are stem bark of *Erythrina variegata* reported about the anxiolytic activity but there is no activity on leaves of this plant.

The present study was aimed at evaluating the anxiolytic (elevated plus-maze) and sedative (spontaneous locomotor activity by Rota rod apparatus), effects of methanolic extracts of leaves of *Erythrina variegata* in rat models. The effects were compared to those of standard drug generally administered for the treatment of anxiety (diazepam).

## MATERIALS AND METHODS

### Collection and authentication of plant material

The leaves of *Erythrina variegata* were collected from a commercial supplier and were authenticated by Dr. H.B. Singh, Principal Scientist, National Institute of Sciences Communication and Information Resources (NISCAIR), New Delhi, India. A voucher specimen has been deposited at the NISCAIR Herbarium (NISCAIR/RHMD/consult/2009-10/1307/110 dated November 06, 2009)

### Preparation of Extract

Powdered plant material (100 g) was extracted with 95% methanol using Soxhlet extraction apparatus. The solvent was removed under reduced pressure till the semi solid mass was obtained. The extract was stored in the refrigerator and a weighed amount was suspended in dimethylsulphoxide (DMSO) prior to administration.

### Animals

Wistar rats (160-240 g) and mice (25-35 g) of either sex were obtained from Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India. The animals were housed in polypropylene cage under standard conditions ( $25 \pm 2$  °C, 12 h light and dark cycle) fed with standard pellet feed (Ashirwad Industries, Mohali, Punjab, India) and water *ad libitum*. All the experimental procedures and protocols involving animals were reviewed by the Institutional Animal Ethical Committee (Registration number: 1279/ac/09/CPCSEA) and were in accordance with the guidelines of CPCSEA.

### Phytochemical testing

Preliminary phytochemical screening of the methanolic extract of *Erythrina variegata* was carried out to test the presence of the active chemical constituents such as carbohydrate, alkaloids, glycosides, flavonoids, tannins, protein, triterpenoids, and saponin [11].

### Acute toxicity study

The methanolic extract of *Erythrina variegata* leaves (MEEV) was administered orally in doses of 10, 20, 40, 80, 100, 200 and 400 mg/kg to the groups of mice (n = 6) and percentage mortality was noted 24 h later.

### Behavioral studies

In the acute experiments, each animal was subjected to only one behavioral test, whereas in the chronic experiments, the same animal was subjected to the elevated plus-maze and Rota rod apparatus test with an interval of 24 h.

### Elevated plus maze (EPM) test in rats

The EPM experiments were carried out in a sound attenuated, temperature controlled ( $23 \pm 1$ °C) room. The environment was illuminated by two 40-W fluorescent lights placed 1.3 m away from the EPM. The plus shaped maze consisted of two opposite open arms (50 × 10 cm), crossed at a right angle by two arms of the same dimensions enclosed by 50-cm high walls with no roof. The maze was located 50 cm above the floor. Rats naturally avoid the open arms of the elevated plus maze and anxiolytic compounds typically increase the exploration of these arms without changing the

number of enclosed arm entries [12]. Twenty-four rats were divided into four groups each group containing six rats. The first group received normal saline 5 ml/kg body weight i.p, the second group was injected with diazepam 1 mg/kg i.p, third and fourth groups received 100 and 200 mg/kg of methanolic extract of leaves of *Erythrina variegata*, respectively. The parameters observed were number of entries in the open and closed arms and time of permanence in the open arms. The first and the last parameters were expressed in percentage. A rat was considered to have entered an arm when all four legs were on the arm. The number of entries in the closed arms was considered as the locomotor activity index and the percentage of the time spent and percentage of entries on the open arms as the anxiety index [13, 14, 15].

### Rota-rod test in rats

The rats were preselected one day before the test on the rotating rod (3 cm in diameter, 20 r.p.m.). The animals that held onto the rotating rod for 2 minutes were placed again on the same rotating rod on the next day and were observed for 2 minutes. The number of animals falling off the Rota-rod within 2 minutes was recorded [16].

### Statistical analysis

The data were analyzed by one-way ANOVA followed by Dunnett's post hoc test using Graph Pad Prism 5 software. The difference of  $p < 0.05$  was considered significant.

## RESULTS

### Yield of plant extract

100 g of powdered leaves of *Erythrina variegata* was taken for extraction with petroleum ether, methanol and water. The yield of petroleum ether, methanol and aqueous extract was found to be 9.83, 24.1 and 7.77 % w/w respectively.

### Phytochemical testing

Phytochemical testing showed that petroleum ether, methanol and aqueous extracts of *Erythrina variegata* contain alkaloid, glycosides, saponins, flavonoids and steroidal compounds.

### Acute toxicity study

The result of the acute oral administration of methanolic extract of *Erythrina variegata* in various doses of 10, 20, 40, 80, 100, 200 and 400 mg/kg indicated no mortality up to 7 days after treatment.

### Behavior studies

#### Elevated Plus Maze Test

The results of the elevated plus-maze test are shown in Table 1. One-way ANOVA indicated a significant difference in number of entries into open arms by the *Erythrina variegata* (200 mg/kg) and diazepam treated groups in comparison to the control. In the treatment, both diazepam and the extract (200 mg/kg) also increased the percentage time spent in the open arm when compared with vehicle-treated rats.

#### Rota rod test

The effect of *Erythrina variegata* on the fall off time in Rota rod test is presented in Table 2. ANOVA showed a significant difference in the effects of methanolic extract of *Erythrina variegata* (200 mg/kg) and diazepam when compared with vehicle.

## DISCUSSION

Anxiety induces a particular form of behavioural inhibition, which happens in response to environmental events that are novel, non-rewarding (under conditions where reward is expected) or punishing. In animals, this behavioural inhibition may take the form of immobility, or suppression of a behavioural response. Development of new anxiolytic drugs requires animal testing that give a good guide to activity in humans, and much ingenuity has gone into developing and validating such tests. For example, a rat placed in an unfamiliar environment normally responds by remaining immobile, though alert behavioural suppression for a time which may represent 'anxiety' produced by the weird environment. This immobility is reduced if anxiolytic drugs are administered. The 'elevated plus-maze' is a widely used test model for anxiolytic activity. Two arms of the raised horizontal cross are closed in, and the others are open. Normally rats spend most of their time in the closed arms and avoid the open arms. Administration of anxiolytic drugs increases the time spent in the open arms and also increases the mobility of the rats, as judged by the frequency of crossing the intersection [17]. The results of the present study indicated that the methanolic extract of *Erythrina variegata* leaves possesses significant anxiolytic activity as evidenced by the increased in number of entries and time spend in open arms as compared to the control. The activity was found to be comparable to the standard drug.

On the other hand, loss of coordinated motor movement is one of the pharmacological effects of anxiolytics drugs. The effect of *Erythrina variegata* methanolic extract on coordinated motor movement was assessed using rota rod test. The latency (in seconds) to drop off the rota rod was recorded up to a limit 120 second [18,19]. The present study, statistical analysis of the latency to fall from the rota rod revealed that oral administration of *Erythrina variegata* leaves at 200 mg/kg and diazepam produced significant motor incoordination compared to vehicle control. The phytochemical tests of methanolic extract of *Erythrina variegata* leaves showed the presence of various phytoconstituents viz. alkaloid, glycosides, saponins, terpenoids, flavonoids and steroidal compounds. Flavonoids and terpenoids contribute synergistically to the neuroprotection mainly via antioxidant activity [20, 21]. *Erythrina variegata* contains rich in phenolics and flavonoids compounds and used as a therapeutic agent against neurodegenerative diseases, cancer, diabetes, cardiovascular dysfunctions, inflammatory diseases and aging. It is reported that flavonoids and terpenoids compounds have anxiolytic property, which partly explains the reason for such activity of methanolic extract of leaves of *Erythrina variegata*. Further studies are acceptable to isolate the anxiolytic compound and to elucidate their exact mechanism of action.

## CONCLUSION

It may be concluded, that the methanolic extract of leaves of *Erythrina variegata* has an anxiolytic effect and this extract has potential for clinical use in the treatment of anxiety which support its traditional uses.

## Conflict of interest statement

The authors have declared that there is no conflict of interest.

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**Table1: Effect of drugs on behaviour of rats in elevated plus maze paradigm.**

Treatment	Dose	Number of entries into open arms	% of time spent into open arm
Control	5 ml/kg	3.667 ± 0.3333	51.50 ± 8.857
Standard	1 mg/kg	6.667 ± 0.3333 <sup>a</sup>	110.30 ± 20.770 <sup>a</sup>
MEEV	100 mg/kg	5.000 ± 0.8563	67.50 ± 5.065
MEEV	200 mg/kg	5.833 ± 0.3073 <sup>a</sup>	98.67 ± 11.670 <sup>a</sup>

Data represent the mean ± SEM (n = 6).

<sup>a</sup>p < 0.01 compared to control.

**Table 2: Effect of drugs on number of fall off time in Rota rod test.**

Treatment	Dose	Number of fall off time
Control	5 ml/kg	4.333 ± 1.211
Standard	1 mg/kg	11.330 ± 1.862 <sup>a</sup>
MEEV	200 mg/kg	8.000 ± 3.464 <sup>a</sup>

Data represent the mean ± SEM (n = 6).

<sup>a</sup>p < 0.01 compared to control.

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