



Evaluation of Anxiolytic Activity of *Eulophia Nuda* (Tubers) In Swiss Albino Mice

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ABSTRACT

Alternative medicine and drugs made from plants that have "mind-altering" effects are becoming more popular. The elevated plus maze and the light and dark test were used as various animal models in this study to investigate the anti-anxiety activity of methanolic extract of *Eulophia nuda* (Lind. The dose of methanolic extract of *E. nuda* tubers (50, 100, and 200 mg/kg) was chosen in accordance with OECD guidelines, with diazepam (1 mg/kg) serving as the standard of reference. According to the results, *E. nuda* extract at doses of 100 and 200 mg/kg showed anti-anxiety effects that were nearly comparable to those of diazepam, while the dose of 50 mg/kg did not exhibit anti-anxiety activity on any of the paradigms used. The anxiolytic mechanism(s) and phytoconstituents behind the reported central effects of the methanolic extract of *E. nuda* require further investigation.

KEY WORDS: *Eulophia nuda* (Lind.), anxiety, anxiolytics, elevated plus maze, light and dark test.

INTRODUCTION

Anxiety disorder is among the most prevalent and severe psychiatric illnesses. To evaluate drugs for their ability to reduce anxiety, numerous animal models have been created. The mechanism underlying fear behaviour in animals has been greatly clarified through basic research. Despite significant scientific progress, there haven't been any novel drugs or agents for the treatment of anxiety in more than a decade. Patients with anxiety disorders face a considerable lot of bodily and psychological distress, and their rates of substance addiction and health issues are also noticeably greater. Even though pulmonary arterial hypertension patients frequently experience anxiety and sadness, only around one-fourth of them receive effective treatment. Our objective is to review the literature on the incidence, consequences, and management of anxiety and depression. Anxiety and sadness are prevalent psychiatric conditions, as well as frequently co-occurring with other neurologic illnesses. Early detection and treatment of these co-morbidities are crucial for improving patients' health outcomes. [1,2,3] One-eighth of the world's population suffers from anxiety, which has grown significantly in importance as a research topic in psychopharmacology during the past ten years. Alternative medicine and drugs made from plants that have "mind-altering" effects are becoming more popular. Motor tension, sympathetic hyperactivity, apprehension, and vigilance syndromes are all signs of anxiety, which is a condition of extreme fear. Despite the significant negative side effects they cause, such as sedation, muscle relaxation, ataxia, amnesia, ethanol and barbiturate potentiation and tolerance, benzodiazepines remain the most frequently prescribed treatment for anxiety. [4] In various regions of the world, numerous herbal medications have been utilised as anxiolytics. One of the most well-liked alternative therapies, which also include massage therapy, megavitamins, and homoeopathy, is the self-administration of herbal medications. Disorders of the central nervous system (CNS) have traditionally been treated using plants. Folk remedies have particular benefits, such as those provided by herbs like *Passiflora coerulea*, *Valeriana officinalis*, *Matricaria recutita*, *Jatropha cillata*, *Salvia guaranitica*, *Tilia tormentosa*, and *Tilia europeae* that "calm down," "tranquilly," and "raise mood." It is important to understand that the complex neuropsychopathology of humans cannot ever be completely replicated in animal models. However, they are fundamentally important to the research of neuropsychiatric disorders because of the substantial physiological and anatomical parallels between humans and animals. As a result, research involving animal models is generally motivated by similarities in human structure, physiology, and genetics. Animal models also provide a number of benefits over clinical research, including being easier to obtain, maintain, and handle as well as more cheap and repeatable. These advantages have led to the development of multiple rodent models of anxiety disorders, which are commonly used to study how animals' behaviour changes in response to various types of stress. The most often utilised models in neuropsychiatric research are rodents (rat and mouse). [5,6]

MATERIALS AND METHODS

Animals

Swiss albino mice (20–25 g) were used in the present study. The animals were procured from Disease Free Animal House, Vidyabharati College of Pharmacy, Amravati, Maharashtra, India. They were provided pellet and tap water ad libitum and were exposed to 12-h light and 12-h dark cycle. The animals were acclimatized to the laboratory conditions before experiments. Experimental protocol was approved by Institutional Animal Ethics Committee. Care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. Experiment protocol was approved by Institutional Animal Ethics Committee.

Plant Material

Tubers of *Eulophia nuda* were collected from Shree Kshetra Bhimashankar Ayurved, Mumbai. and were authenticated at the Department of Botany, Vidyabharati Mahavidyalaya.

Preparation of Methanolic Extract

Tubers of *E. nuda* were collected, thoroughly washed, cut into pieces, shade dried for a week and ground to fine powder with mixer. The powder obtained was successively extracted with methanol using a Soxhlet apparatus. The extract was evaporated to dryness. The yield of methanolic extract was obtained 14.18%.

Preliminary Phytochemical Screening

The methanolic extract of tubers of *E. nuda* undergoes different phytochemical tests for identification of different phytochemicals such as Flavonoids, Alkaloids, Tannins, Steroid, etc. [7]

Drugs

Diazepam 10mg/2ml ampules purchased from Matoshree Medical Stores, Valgaon, Amravati, was used as a reference drug. It was diluted with distilled water to the required strength before use. Different concentrations of the *E. nuda* extract were prepared in distilled water. All the solutions were prepared freshly on test days and administered orally.

Elevated Plus Maze

The plus maze apparatus consisting of two open arms and two closed arms with an open roof which was 50 cm elevated from the floor used to observe anxiolytic behavior in animals. Each new cohort of the mouse placed on the elevated plus-maze apparatus 30 min after the administration of the dose. Each mouse placed in the center of the elevated plus-maze with its head facing the open arms. The behavioral effects of the mouse observed for 5 min with a different kind of parameter (time spent in open arms and the number of the entry in the open arms). [8] In EPM test, the percent time spent on the open arms was determined as follows:

$$\% = 100 \times \text{Number of seconds spent on open arms} / 300 \text{ total seconds (5 min observation time).}$$

Light and Dark Box Test

This is another behavioral test used to evaluate the anxiolytic effect in rodents. It has an advantage over other paradigms that it does not require prior training and easy to use and quick process. In the light/dark test, drug-induced increase in behaviours in the white part of a two-compartment box, in which a large white compartment is illuminated and a small black compartment is darkened, is suggested as an index of anxiolytic activity. An increase in transitions without an increase in spontaneous locomotion is considered to reflect anxiolytic activity. [9] An animal was placed in the center of the light compartment and was observed for 5 min for the time spent in open (white/light) compartment. Time spent in the light compartment was determined.

Statistical Analysis

Results are expressed as mean \pm SEM. Data were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test with $p < 0.05$ as the criteria for significance.

RESULTS

Extraction

The yield of methanolic extract was obtained 14.18%. The extract showed the presence of alkaloids, flavonoids, saponins, glycosides, steroids, carbohydrates, protein, and phenols.

Elevated Plus Maze

Administration of diazepam (1mg/kg) significantly increased the percentage time spent in the open arms and the number of open arm entries ($P < 0.001$) compared to saline-treated group [Table 1]. Methanolic extract of *E. nuda* tubers at 100 mg/kg ($P < 0.01$) and 200 mg/kg ($P < 0.05$) significantly increased

the percentage time spent in the open arms. Entries in the open arms increased significantly at 100 mg/kg ($P < 0.05$) and 200 mg/kg ($P < 0.01$). Plant extract at 50 mg/kg had no significant effects on any of the parameters that were measured on the EPM.

Light and Dark Box

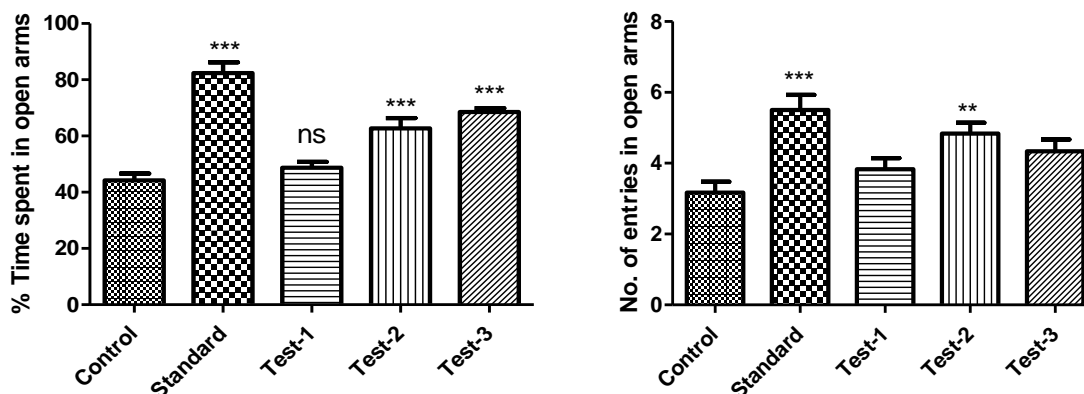
Test Diazepam (1mg/kg) significantly increased the time spent in light compartment ($P < 0.001$) compared to saline-treated group [Table 2]. Significant increase in the time spent in the light compartment ($P < 0.05$) was seen with administration of 100 and 200 mg/kg of methanolic extract of *E.nuda* tubers compared to saline-treated group [Table 2]. Plant extract at 50 mg/kg did not produce any significant effects that were measured by light and dark box test.

Table 1:

Effect of different treatments on the time spent by mice behavior in elevated plus maze

Treatment	Dose	% Time spends in open arms	% Entry into open arms
Saline	1 ml/kg	44.16 ± 2.42	3.16 ± 0.30
Diazepam	1 mg/kg	82.33 ± 3.90***	5.5 ± 0.42***
<i>E .nuda</i>	50 mg/kg	48.66 ± 2.15	3.83 ± 0.30
<i>E .nuda</i>	100 mg/kg	62.66 ± 3.74***	4.83 ± 0.30
<i>E .nuda</i>	200 mg/kg	68.5 ± 1.40***	4.33 ± 0.33

Each value is presented as the mean ± SEM (n = 6), MEEN = Methanolic extract of *Eulophia nuda* tubers. “*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ vs control group (Dunnnett’s test)”.



Graph 1. % Time spent in open arms.

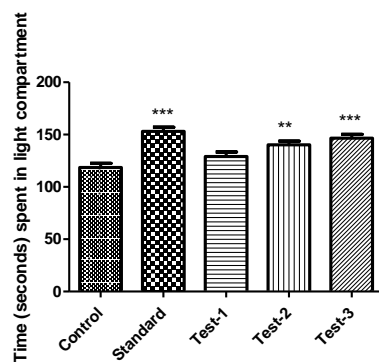
Graph 2. Number of entries in open arms.

Table 2:

Effect of different treatments on the time spent by mice behavior in light and dark test

Treatment	Dose	Time (second) spent in light compartment.
Saline	1 ml/kg	118.5 ± 4.04
Diazepam	1 mg/kg	153 ± 4.14***
<i>E .nuda</i>	50 mg/kg	129 ± 4.34
<i>E .nuda</i>	100 mg/kg	140.16 ± 3.58
<i>E .nuda</i>	200 mg/kg	146.5 ± 3.76***

Each value is presented as the mean ± SEM (n = 6), MEEN = Methanolic extract of *Eulophia nuda* tubers. “*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ vs control group (Dunnnett’s test)”.



Graph 3. Time (second) spent in light compartment.

DISCUSSION

Since the last 40 to 50 years, benzodiazepines have been widely used to treat a variety of anxiety disorders. However, because of their unfavorable side effects, alternative treatment options, particularly in primary care settings, are being investigated. Finding novel treatments for certain diseases can often be found by looking to medicinal plants. Research on natural anxiolytic medications has been done in the search for an alternative, more focused, and maybe cost-free therapy.

In order to identify botanicals and pharmaceuticals with favourable effects in the treatment of various CNS illnesses, various pharmacological models have been used over time to evaluate medicinal plants for neuropharmacological activity. In some cases, the choice of test techniques also reveals information about the test substance's mechanism(s), in addition to determining effectiveness. In this study, the elevated plus maze and light/dark exploration tests were used to investigate the anxiolytic effect of methanolic extract of *Eulophia nuda* tubers.

The results of the present study showed that the administration of *Eulophia nuda* extract by oral route in mice produced a significant anxiolytic effect in two well-consolidated anxiety animal models. Elevated plus maize, in this animal model, the anxiety-related behaviours in mice were significantly decreased indicates that anxiety in mice was relieved after treatment with extract. In the present study, administration of *Eulophia nuda* extract has produced an increase in time spent by mice in the illuminated side indicating an anxiolytic effect of plant, which was confirmed by the increase in time spent in the EPM.

One of the most frequently utilized validated models to examine anxiety-reducing medications is the Elevated Plus Maze test. This test is based on the inherent tension between the desire to explore new environments and avoiding potentially unsafe areas. It is used to assess mice's and rats' emotional states and psychomotor abilities. Results of our study on the elevated plus maze following treatment with methanolic extract of *Eulophia nuda* showed that the anxiolytic activity, most characteristic indices of anxiolytic activity, was significantly attenuated in the EPM. Total arm entries are metric reflecting variations in anxiety or general activity, and time spent on the central platform appears to be associated to decision-making and/or risk evaluation.

The light/dark test is based on mice' intrinsic aversion to brightly lighted places as well as their spontaneous exploratory behaviour in response to moderate stressors such as unfamiliar environment and light. As a result, this test may be beneficial for predicting anxiolytic or anxiogenic activity in mice. Transitions have been claimed to be an indication of activity-exploration due to habituation over time, and time spent in each compartment to be a reflection of aversion. In this study, *E. nuda* (50-200 mg/kg) significantly increased the latency of entry into the dark compartment and time spent in the light box. Diazepam also significantly increased the time spent in the light box and reduced the duration of stay in the dark section. The effects seen with *E. nuda* during the light/dark exploration test point to the presence of anxiolytic activity, with peak response being evoked at dose of 200 mg/kg.

Earlier reports on the chemical constituents of plants and their pharmacology suggest that plants containing flavonoids, alkaloids and phenols possess activity against many CNS disorders.

Investigations on the phytochemical screening of *Eulophia nuda* revealed the presence of alkaloids, glycosides, steroids, saponins, carbohydrate, phenols, protein compounds and flavonoids. It is possible that the mechanism of anxiolytic action of title plant could be mediated by these phytochemicals. Anxiolytic activity of *E. nuda* is likely to be associated with its phenolic content and flavonoids.

CONCLUSION

In conclusion, the methanolic extract of *Eulophia nuda* tubers considerably reduced anxiety caused by the Elevated plus maize method and the Light and dark box. According to the findings of this investigation, the tubers of *E. nuda* have anti-anxiety properties.

The results obtained in this study suggest that the methanolic extract of *Eulophia nuda* tubers possesses anxiolytic activity due to one or a combination of phytoconstituents identified in the extract, probably due to the presence of phytoconstituents like flavonoids and phenolic compounds. However, further studies are needed to isolate the bioactive compound(s) and demonstrate the precise molecular mechanisms responsible for the pharmacological actions of methanolic extract of *Eulophia nuda*.

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