■ *Original Article*

Evaluation of anxiolytic effect of elaeocarpus ganitrus in mice

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Abstract

Background: Antianxiety drugs are prescribed frequently and associated with several limitations factors that led to interest in the alternative remedies. Herbal drugs are used in developing and developed countries but there is lack of scientific evidence. Ayurvedic physician were used Elaeocarpus ganitrus for the treatment of mental calmness. **Objectives**: Evaluate the anxiolytic effect of Elaeocarpus ganitrus in mice. **Methods**: The experimental study was conducted with the extract of Elaeocarpus ganitrus in comparison with Diazepam 1mg/kg in 60 mice using open field test and passive avoidance apparatus in six experimental groups. The data were analyzed using Mann-Whitney test and $P<0.05$ was considered statistically significant. **Results:** There was a significant increase in number of square crossed, time spent in central square and rearing behavior of animal. There was also decreased significantly time prolongation in Step down latency and increase of attempts in step down errors as well as time spent in the shock zone**. Conclusion:** Elaeocarpus ganitrus showed anxiolytic effect but there is need to find out safety, efficacy and the exact mechanism of action of this herbal remedies.

Keywords: elaeocarpus ganitrus, anxiolytic

Introduction

Elaeocarpus ganitrus finds a prominent place in Hindu religion and in Ayurveda, the ancient Indian system of medicine. Fruits of the plant are worn by Hindu mystics as necklaces and various magical properties are attributed to them. Rosaries made from the fruits are commonly used by Hindu in their daily worship for purpose of counting prayers.

Ayurvedic physician, claim that used decoctions made from this fruits successfully in the treatment of mental disease reported by Chopra et al[1] and Nadkarni.[2]

Anti-anxiety drugs are frequently prescribed as treatment of anxiety. These drugs are associated with addiction with benzodiazepines, insomnia, decreased libido and ineffectiveness with fluoxetine, tachycardia with buspirone[3] are some of the limitation factors that led to interest in the alternative remedies.

Herbal drugs are used in rural areas in developing countries and developed countries where used of modern drugs predominant[4] use of traditional drugs is also growing in western world to treat illness.[5] The major hindrance in the amalgamation of herbal drugs into medical practices is the lack of scientific evidence and better understanding of efficacy and safety of the herbal products.[6] The historical use provides the source to study the specific plant species with potential to be used in a particular disease.

Herbal products may have complementary effects and often difficult to determine its biological activity in human. Herbs have been used for centuries to treat illness and health and still using more in medical treatment in the developing world. Regulation of herbal product in the terms of establishing safety

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and efficacy, consumers are unaware of the limited regulation of herbal products. Risk of combining herbal products with prescription drugs is mostly unknown. They are often perceived as safe because herbs are natural.[7] Adverse effect event reports of herbal drugs therapy may be very few and patients do not discuss with their physician[8] where standard therapies have failed to substantially improve patient out comes.

Herbal products like Elaeocarpus ganitrus which has anxiolytics effect, is used by the herbal practitioners for anxiety treatment is safe and has no adverse effect but there is no scientific evidence.

Hence the aim of the present study is to evaluate the anxiolytic effect of Elaeocarpus ganitrus in comparison with a standard drug like Diazepam in mice using open field test, activity monitoring and passive avoidance test.

Objective

Evaluate the anxiolytic effect of Elaeocarpus ganitrus in mice.

Methods

This animal experiment study was carried out to evaluate the anxiolytic effect of Elaeocarpus ganitrus in mice in the department of Clinical Pharmacology and Therapeutics after approval by the research committee of B.P. Koirala Institute of Health Sciences, Dharan, Nepal.

Maintenance of animals

Ethics of Animal Experiments

Animals were maintained as per standard guidelines of the Indian National Science Academy for the maintenance of laboratory animals. Locally inbred albino mice of either sex (20-30 g) were used. The mice were randomly assigned for experimental purpose. The mice were housed in groups of 10 mice same sex per cage in natural day and night cycle with maintenance of room temperature at near to 25° C by air-condition with free access to standard feed pellets, soaked Bengal gram and tap water. The animals were transferred to the laboratory at least one hour before the start of the experiment. The experiments were performed during day.

Preparation of drugs

Preparation of plant extract

Fresh fruits of Elaeocarpus ganitrus (EG) were washed and air-dried in the shade at room temperature (25°C). Dried fruits of EG were mechanically powdered and sieved. 1000 grams of the obtained fine powder of EG was macerated with 5000 ml of a mixture of water methanol (3/2 V/V) at room temperature by occasional shaking by mixer (Top tech Biomedical) for 48 hours.

The obtained crude preparation was centrifuge and filtered. The supernatant was concentrated under reduce pressure at 25° c and the extract obtained was stored in a refrigerator until use. The extract was dissolved in normal saline for intra-peritoneal injection.

Diazepam injection

Calmpose 10 mg per 2 ml (manufactured by Rambaxy India Limited) were procured from market which was used as standard drug.

Normal saline

Sodium chloride, 500 ml injection manufactured by Albert David Limited, Meerut, road, Ghaziabad, India was used as vehicle for the preparation of Elaeocarpus ganitrus.

On the study day drugs were prepared freshly in the early morning and were administered in a volume of 10 ml/kg intraperitoneally with the help of 26 gauge syringe.

Pattern of study

The behavioral tests were conducted. There were six treatment groups. Each group consists of ten animals of either sex. The mice were tested only once after the completion of the drug treatment schedule in the open field, passive avoidance apparatus and horizontal bar test.

The study was carried out in a sound proof room and observations were made through an inner circuit television to avoid disturbances to the animals during the behavioral studies.

Drug administration

Overnight fasted animals were selected randomly on the day of experiment for administration of Normal saline, Diazepam (standard drug) and study drug Elaeocarpus ganitrus.

The animal was acclimatized one hour before for behavioral tests.
One hour time interval between drug administration and behavioral tests was maintained.

Experimental procedures

Open field

This test utilizes behavioral changes in rodents exposed to novel environments and is used to detect angiogenic and anxiolytic activity under identical situations. Various types of open field apparatus have been used to test the mice.

An "Open field apparatus" suitable for mice were made comprising of a floor space of 40 cm x 4 cm and with 30 cm high walls. The floor was colored black and the floor area was divided into 9 (nine) equal squared by white lines. A mouse is placed at the center[9] of the field and is left for 2 minutes for acclimatization with the apparatus. Thereafter, for the next 5 minute the following parameters were noted:

a. Time spent in the central square
b. No. of squares crossed
c. Rearing (No. of times the animals stands on the near paws).

Passive avoidance

This test is done in a chamber of the size 34 cm x 34 cm x 20 cm with a grid floor through which electric shock of 20 mv were delivered. A shock free zone (SFZ) was provided in the centre of the chamber by placing an inverted Petri dish.

The mice were placed on the SFZ and when they try to get down from the SFZ and come in contact this grid floor. They were received electric shock. The mice gradually learn to avoid shock by staying on in the SFZ curbing their normal exploratory behavior. This was the principle of passive avoidance. The animal was initially trained till they avoid coming in contact with the shock zone passively sitting on the SFZ for at least a minimum of 60 sec. Those mice which do not learn in 5 training sessions were discarded. The parameters noted were:

1. Step down latency (duration for which animal stays in the SFZ)
2. Step down error (no. of attempts the animal makes to come to the shock zone)
3. Total time spent in the shock zone.

Horizontal bar test for muscle relaxant activity

Horizontal bar test was used to assess the sedative and/or ataxic properties, which could influence the open field and passive avoidance tests. After testing in open field / passive avoidance, mouse was put individually on horizontal bar (rod) suspended between two vertical stands. Animal that failed to hang on to the rod for 30 seconds were considered test positive indicating muscle relaxation in them.

Statistical analysis

The data obtained were analyzed using Mann-Whitney test. $P<0.05$ was considered statistically significant.

Results

Number of squared crossed

There was a significant increase in the number of square crossed in diazepam compare to control. There were also increase in number of square crossed in all treated groups which was increased significantly compared to standards (Table-1).

Table 1: Effect of drug observations in open field test

S N	Group	Mean ± (SD)			
		No. of Animal	No. of Square	Central time	No. of rearing
1.	Control Normal Saline 10 ml/kg	10	80.00± 2.98	5.80± 1.32	32.3 ± 1.89
2.	Diazepam 1 mg/kg	10	145.70±4.29	17.80±1.54	58.10±2.51
Diazepam vs control			< 0.001	< 0.001	< 0.001
3.	Test drug 50 mg/ kg	10	117.80±4.66	9.30±1.49	41.20±1.54
Diazepam vs T 50mg			< 0.001	< 0.001	< 0.001
4.	Test drug 100 mg/kg	10	113.30±1.88	14.00±2.16	44.90±2.60
Diazepam vs T 100 mg			< 0.06	< 0.001	< 0.001
5.	Test drug 50mg + Diazepam 1mg	10	163.40±9.14	20.00±2.10	60.20±2.39
Diazepam vs T50mg+ diazepam			<0.001	< 0.029	< 0.089
6.	Test drug 100mg + Diazepam 1mg	10	164.00±8.11	20.80±2.09	61.30±1.88
Diazepam vs T100mg + diazepam			< 0.001	< 0.004	< 0.007

Time spent in central square

Time spent in Central Square in the control and standard was 5.80 ± 1.32 and 17.80 ± 1.54 seconds respectively. There was a significant increase in diazepam groups compared to control. It was also increased significantly in test drug and combination of test drug plus standard drug (table-1).

Number of rearing

There was significant increase in the rearing behavior of animals with diazepam in comparison to control group. There was also increased rearing of number in test drug plus diazepam group (Table-1).

Step down latency

There was significant decreased time prolongation in step down latency in standard group as compared to control group. There was also significant decreased time in other treatment group.

Step down error

A significant increase in step down error in standard group was observed as compared to the control group. It was also statistically increased in other treatment groups compared to standard group (Table-2).

Table 2: Effect of drug observations in passive avoidance test

S N	Group	Mean ± (SD)			
		No. of Animal	Step down latency (second)	Step down error	Time in shock zone
1.	Control Normal Saline 10 ml/kg	10	222.30±8.22	2.20± 0.42	30.70± 2.11
2.	Diazepam 1mg / kg	10	90.10±4.38	7.70±0.82	152.60±3.13
Diazepam vs Control			< 0.001	< 0.001	< 0.001
3.	Test drug 50 mg/ kg	10	87.40±5.73	4.10±1.19	118±5.23
Diazepam vs T50mg			< 0.190	< 0.001	< 0.001
4.	Test drug 100 mg/kg	10	86.60±4.14	4.50±1.58	128.60±4.62
Diazepam vs T100mg			< 0.063	< 0.001	< 0.001
5.	Test drug 50 mg/kg + Diazepam 1 mg/kg	10	76.10±4.77	3.10±1.37	147.60±2.06
Diazepam vs T50mg + Diazepam			<0.001	< 0.001	< 0.002
6.	Test drug 100 mg/kg + Diazepam 1 mg/kg	10	83.20±6.47	3.90±1.28	144.80±3.79
Diazepam vs T100mg +Diazepam			< 0.019	< 0.001	< 0.001

Time spent in the shock zone

There was a significant increase in the time spent in the shock zone with standard treatment group compared to control as well as other treatment groups (Table-2).

Horizontal bar test

All these animals used in the control, standard and test drug were tested on the horizontal bar indicating that there were no significant muscle relaxant properties.

Discussion

The incidence of pathologic anxiety in the community is very high and is associated with lot of morbidity. Life time prevalence anxiety in women is 30.5% and male is 19.2%[10]. Hence, it is very important to address this problem of anxiety and find effective remedies. Though several drugs are available, all are associated with some limitations.

Benzodiazepines which are the standard antianxeity drugs are associated with sedation and addition. Buspirone, the non-sedating anxiolytic agent, which gave rise to much optimism, is slowing acting and not effective in a high percentage of patients.[2] It is also associated with several side effects such as tachycardia, gastric discomfort etc.[2] Though selective serotonin reuptake inhibitors are considered as one of the important group of anxiolytic agents, they are also associated with significant side-effects such as insomnia, decreased libido, sexual dysfunction and are only effective in approximately 50% to 60% of patients.[11]

An anxious animal shows reduced ambulation associated with periodic freeze or immobility and reduction in normal behavior. Such as rearing and grooming. Anxiety is also associated with augmented autonomic activity resulting in increased defecation

and urination. All these effects are accentuated by anxiogenic drugs and attenuated by anxiolytics.

Standard screening procedures such as locomotor activity, open field method, and passive avoidance are used to screen the anxiolytics effect of Elaeocarpus ganitrus in comparison with a standard like diazepam.[12] Open field model was used in our study that is more sensitive to anxiolytic effect produced by diazepam and is effective in screening different classes of anxiogenic and anxiolytic drugs.

There is a need of alternative medicine for anxiety in refractory cases which has safe and no adverse effects. Elaeocarpus ganitrus (EG) is used in mental disease, epilepsy has reported.[1,2,13] Herbal practioner is using EG to soothes the anxiety, calm the brain, harmonizing the physical emotion. According to the Ayurveda, Elaeocarpus ganitrus ® is used in disease of the head which is supported by Bhattacharya et al.[14]

In our study different dose of EG produce anxiolytic effect as indicated by significantly increase in number of square crossing and rearing as well as time spent in central square by animals and its was also seen that there was significant decrease in step down latency as well as significant increase in step down error and time spent in shock zone by animals. It shows Elaeocarpus ganitrus has central nervous system effect and our finding is indicative of anxiolytics effect which supports the Bhattacharya et al.[14] Other species of Elaeocarpus also shows central nervous system effect reported by Singh RK.[15]

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Further detailed Pharmacological studies are needed to find out biological activity and the exact mechanism of action of Elaeocarpus ganitrus.

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