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Evaluation of antiepileptic and anti-anxiety activities of Ethanolic extract of *Elephantopus scaber* leaves

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Abstract
The purpose of this study is to investigate the anticonvulsant and antianxiety effects of the ethanolic extract obtained from the leaves of *Elephantopus scaber* (ETES). In this study, rats were administered with doses of 100, 200, and 300 mg/kg of ETES and their antiepileptic and antianxiety effects were evaluated using experimental animal models. The antiepileptic activity was assessed through the maximal electroshock seizure (MES) test and the Pentyleneterazole (PTZ) induced convulsion assay. The calming effect was evaluated using the elevated plus maze (EPM) and open field test (opt) in rats. Result: in the MES method, the flexion and extensor phases were eliminated. The PTZ method, which involves delaying the onset of PTZ-induced myoclonic jerks, has been found to be effective in managing the condition. In the experiment, all groups that received treatment demonstrated a significant decrease in entries in both the open and closed arms. In option, the number of squares crossed by rats treated with extract decreased significantly. Conclusion: ethanolic extract of *Elephantopus scaber* (ETES) was found to possess antiepileptic activities however there is less anti-anxiety action in animal models.

Keywords: *Elephantopus scaber*, epilepsy, anti-anxiety, seizure

Introduction
Epilepsy and anxiety are separate but closely related neurological disorders that can have a profound effect on an individual's overall health and happiness. Epilepsy is a prevalent neurological disorder that impacts approximately 65 million individuals of all ages globally. People with epilepsy face a significantly higher risk of premature death, with the likelihood being up to three times greater than that of the general population. People with epilepsy frequently face stigma, discrimination, and violations of their human rights. (World Health Organization, 2019 [1]. Epilepsy is defined by recurring seizures, which are sudden and unexpected electrical disturbances in the brain. The manifestation and severity of these seizures can differ greatly, impacting individuals in various ways based on the type and location of the brain activity occurring. Anxiety is a common condition caused by excessive worry, fear, and anxiety. These emotions can be long-lasting and overwhelming, often causing individuals to avoid certain situations and hindering their ability to carry out daily tasks. Anxiety can take on different forms, including generalized anxiety disorder (gad), panic disorder, and social anxiety disorder. The connection between epilepsy and anxiety is intricate and reciprocal. Individuals with epilepsy frequently encounter heightened levels of anxiety as a result of the unpredictable nature of seizures, the fear of sustaining injuries during seizures, and the social stigma associated with the condition. Conversely, anxiety disorders can worsen epilepsy by raising stress levels, potentially leading to seizures in certain individuals [2]. Comprehending the relationship between epilepsy and anxiety is vital for successful management and treatment. Both conditions necessitate holistic care that takes into account not only the physical manifestations but also the emotional and psychological dimensions. Comprehensive treatment plans that encompass medication, therapy, lifestyle modifications, and a strong support system are crucial in enhancing outcomes and improving the overall quality of life for individuals living with epilepsy and anxiety [3]. The clinical effectiveness of anxiolytics and anticonvulsants can be hindered by several factors, such as side effects, slow onset of action, and issues related to patient adherence [4, 5, 6, 7].

To promote human welfare, it is essential to seek novel anxiolytic and antidepressant agents that can surpass the drawbacks of currently available treatments. There is interest in herbal treatments for anxiety and epilepsy, as many people turn to alternative medicine because of concern about side effects or needing further treatment.

Since ancient times, traditional medicinal plants have been crucial in treating various human ailments, often serving as a foundation for the development of new medications. According to the World Health Organization, 80% of individuals in developing countries depend on plant-based traditional medicines for their primary healthcare needs [8].

Elephantopus scaber belongs to the family Asteraceae and is a small plant native to Andaman Islands, Angola, Assam, Bangladesh, Cambodia, Cameroon, southcentral China, eastern China, Comoros, eastern Himalayas, Hainan, India, Java, Laos, Madagascar, Malaya, Mozambique, Myanmar, Southwestern Islands, Nepal, Nicobar Islands, Sri Lanka, Taiwan, Tanzania, Thailand, Vietnam, Zambia, Zai Er, Zimbabwe. The plant is commonly found in many parts of India, such as the Western Ghats, [9] and is widely distributed in the forests of Achanakma, Chhattisgarh. It is commonly called "Elephant's Foot" or "Elephant's Foot" (English). In India, it has many local names, such as Gojivha or Hastipadi (Sanskrit) and Gobhi (Hindi). It is known for its medicinal properties, like its fur, and is widely used by many indigenous communities to treat a variety of ailments, including rheumatism, dysentery, gout, eczema, gum disease, toothaches, and spider and snake bites [10], [11]. According to the original text, this plant is believed to have many therapeutic properties such as wound healing, [12] antioxidant, [13] antibacterial, apoptotic, [14] antipyretic, dysuria, hepatoprotective [15, 16], diuretic, etc. [17], anticancer, antidiabetic [18] and antiinflammatory activity [19]. This study aims to investigate the Antiepilepticand anxiolytic activities of the ethanolic extract of *Elephantopus scaber* leaves to improve the potential of this plant.

Materials and Methods

Preparation of extract

The *Elephantopus scaber* leaves were collected in the Uttar Pradesh region of India, close to Varanasi. The plant's botanical identification performed by Dr. Aswani Kumar Kushwaha assistant professor Department of Dravyaguna Faculty of Ayurveda, IMS, RGSC, BHU, Barkachha, Mirzapur (Ref. No. RGSC/ PID-AY/2021-01). The leaves were dried in the shade and milled into powder at room temperature. A 1:7 w/v ratio of milling power was percolated with 95 percent ethanol. The extract solvent was evaporated using a rotary evaporator under reduced pressure. The yield percentage was found to be 11.2 percent w/w. For additional investigation, a dried extract was employed, and several dosages were created.

Drugs and chemicals

Sisco Research Laboratories Pvt. Ltd. provided the pentyleneterazole (Sigma-Aldrich), phenytoin (Sigma-Aldrich), diazepam (Sigma-Aldrich), and organic solvents (ethanol, petroleum ether, and ethyl acetate). List of chemicals used were displayed in table 1.

Equipments

The list of equipments used were displayed in Table 2.

Animals

The animal house, SHEAT College of Pharmacy, Varanasi, provided the Charles Foster rats (150-200 g). Animals were maintained on a 12 h on and 12 h off light/dark schedule with free access to food and water. The animals were allowed to acclimatize to the environment of the laboratory for 7 days before the commencement of experiments. All of the experiments were approved by the Central Animal Ethical Committee of SHEAT College of Pharmacy (Reg. No. SHEAT|CPCSEA|MPH|002S.) by CPCSEA norms.

Evaluation of anticonvulsant activity

Maximal electroshock seizure (MES) Test

There was a total of 25 Charles Foster rats dispersed among five groups with five rats in each group (n=5). Group I (Control) served as an untreated group and received only carboxymethyl cellulose (0.5% CMC, p.o.). Group II (standard treated group) received a single dose of phenytoin (25 mg/kg i.p.),. whereas Groups III, IV, and V (EtES treated groups) received a single dose of *Elephantopus scaber* (EtES) leaves ethanolic extract 100, 200, and 300 mg/kg, po respectively. All the rats received electric shock stimulation 45 min after the treatment with standard drug and graded dose of EtES. To induce summarizing tonic-clonic seizures in animals, 150 mA of current was delivered through a corneal cathode (Electro-convulsometer, model no. 100-3) [20] Tonic flexion, tonic extensor stage, drowsiness, and recovery or death were among the readings. The criterion for the evaluation of anticonvulsant activity is the ability to prevent or reduction of the tonic extensor stage (E/F percentage) in summed tonic-clonic seizures [21]

Pentyleneterazole (PTZ) induced convulsions

In the PTZ-induced convulsion model, the rats were randomly divided into five groups with five animals in each group (n=5). Group I (normal control) received vehicle (0.5% CMC, p.o.) only. Group II received diazepam (4 mg/kg, i.p.), Groups III, IV, and V (EtES treated groups) received *Elephantopus scaber* (EtES) leaf ethanolic extract 100, 200, and 400 mg/kg, p.o., respectively. Convulsion was induced in all the rats (except Group I) by single-dose administration of PTZ (80 mg/kg, i.p.) after 30 min of the treatment with the EtES extract or standard drug. The duration of the jerk, the duration of the seizure, and the protection from PTZ-induced spasms were used to assess the anticonvulsant movement [22].

Assessment of anti-anxiety activity

Elevated plus maze (EPM)

The effects of various anxiolytic and anxiogenic drug kinds on anxiety response are studied using EPM [23] two wide arms (48.5×10 cm) and two narrow arms (48.5×10×35.5 cm) make up the elevated plus maze, which has an open roof. The apparatus is raised to a 60.5 cm height above the ground. The rats are divided into five groups, each with five rats. Group I received a vehicle (0.5% CMC, p.o.) while group II received an intravenous dose of Diazepam 4 mg/kg,. *Elephantopus scaber* (EtES) leaf ethanolic extracts were administered to groups III, IV, and V at doses of 100, 200, and 300 mg/kg p.o. The testing began and lasted for 5 minutes after the rats had been administered the medications for 45 minutes.

Open field test

In an open-field experiment, rats were utilized to examine the extract's calming effects. An open field's floor was calculated to be 60×60 cm. The floor was covered with 15 by 15-centimetre squares of black and white. The open field was 60 centimetres high. The animal was placed in the center of the chamber and the group's activities were observed for 5 minutes. Throughout the experiment, it was recorded how many squares were crossed, how much time was spent in the middle, and how long the central rearing period lasted [24].

Statistical analysis

We computed the means and standard errors of the means (SEM) for each group. Analysis of variance (ANOVA) and the Dunnette's multiple comparisons test were used in the statistical analysis. The threshold for statistical significance was a p-value of less than 0.0001.

Result and Discussion

Assessment of antiepileptic activity

Maximal electroshock seizure (MES) test

In all treatment groups, *Elephantopus scaber* (EtES) leaves dramatically decreased the time that the hind limb was extended compared to the control group. All treatment groups experienced a decrease in clonus and the stupor phase, although none of them were statistically different from the control group (p>0.05), except the phenytoin group (p.05). In the phenytoin, 200, and 300 mg/kg EtES groups, extensor and flexion were completely eradicated (Figure 1).

Pentyleneterazole (PTZ) induced convulsions

In all treatment groups, an extract from the leaves of *Elephantopus scaber* (EtES) postponed the onset of PTZ-induced myoclonic jerks. The beginning of the convulsion was postponed by 180.80 seconds after receiving a dose of 300 mg/kg BW EtES, as opposed to 40 seconds in the control group. The length of the jerks was significantly shorter in all groups as compared to the control group (p 0.0001). While the other groups (phenytoin, 200 mg/kg, and 300 mg/kg) recovered and no clonus phase was observed, the beginning of clonus was delayed in the 100 mg/kg group compared to (p 0.001) in the control group. Except for the control group, none of the therapy groups had the start of an extensor found. No one died in either of the therapy groups, however the vehicle-treated control group experienced 50% mortality. (Figure 2).

Effect on Anxiety

Elevated plus maze

In the EPM test, all treatment groups showed significantly reduced entries in both the open and closed arms. The EtES-treated groups spent the same amount of time in the open arm as the control group, while the diazepam-treated group spent much more time in the open arm (Figure 3)

Open field test

The number of squares the EtES and diazepam-treated rat crossed decreased noticeably (p 0.001-0.01) when compared to the control groups. The number of central area rearing increased in all groups, but not substantially (p>0.05) except the diazepam group (p>0.01). None of the treatment groups' central region time varied significantly (p0.05), however, the diazepam group's central region time increased significantly (p0.001) when compared to the control group. (Figure 4).

The ethanolic extract of *Elephantopus scaber* leaves demonstrated antiepileptic and anxiolytic effects in rodent models. The leaves of EtES may be effective in treating seizures induced by MES and PTZ. In the case of MES-induced epilepsy, doses of 100 mg/Kg, 200 mg/Kg, and 300 mg/Kg significantly reduced or eliminated both extensor and flexion responses in the hind limbs, while also delaying the onset of seizures. Although the duration of the tonic phase of MES-induced seizures was reduced in the groups treated with EtES compared to the control group, this difference was not statistically significant, and the clonus phase remained unchanged. The positive control, phenytoin, effectively abolished the extensor and flexion phases of seizures and significantly reduced both clonus and stupor. The Extensor/Flexion ratio from the MES model served as a comprehensive measure for evaluating the antiepileptic potential of treatments effective against generalized tonic-clonic seizures. In subjects receiving EtES, the Extensor/Flexion ratio decreased. Antiepileptic drugs that inhibit voltage-gated Na+ channels are commonly used in the MES-induced epilepsy model.

EtES significantly delayed the onset of PTZ-induced spasms compared to the control group, and the treatment groups exhibited notably shorter durations of jerking. The animals were treated with EtES at doses of 200 and 300 mg/kg BW, with no instances of clonus observed. In contrast to a 50% mortality rate in the control group, there were no fatalities among the animals receiving EtES treatment. These results indicate that EtES was effective in managing PTZ-induced seizures.

An open area was employed to examine the behavioral responses of animal test subjects under conditions of anxiety. Through the use of an open-field test, the emotionality of rodents was assessed in a controlled manner. To evaluate the locomotor activity of the animals, the total number of crossings within the central zone of the test was calculated. Notably, the diazepam group spent more time in the center compared to the control group; however, there were no significant differences in the time spent in the central area among the EtES-treated groups. Despite this, the overall number of crossings made by the animals was significantly reduced across all groups. The lack of any substantial variation in the time spent in the central region indicated that the EtES treatment did not have a meaningful impact on anxiety levels.

Table 1: List of chemicals and drugs

Material	Source
Elephantopus scaber Linn. leaves	Varanasi, Uttar Pradesh
Ethanol	Sigma Aldrich, USA
Petroleum ether	HimediaLaboratories
Ethyl acetate	LobaChemie, Mumbai
Methanol	SD fine chemicals
Carboxy methyl cellulose	HimediaLaboratories
Pentyleneterazole	Sigma Aldrich, USA
Phenytoin	Sigma Aldrich, USA
Diazepam	Sigma Aldrich, USA

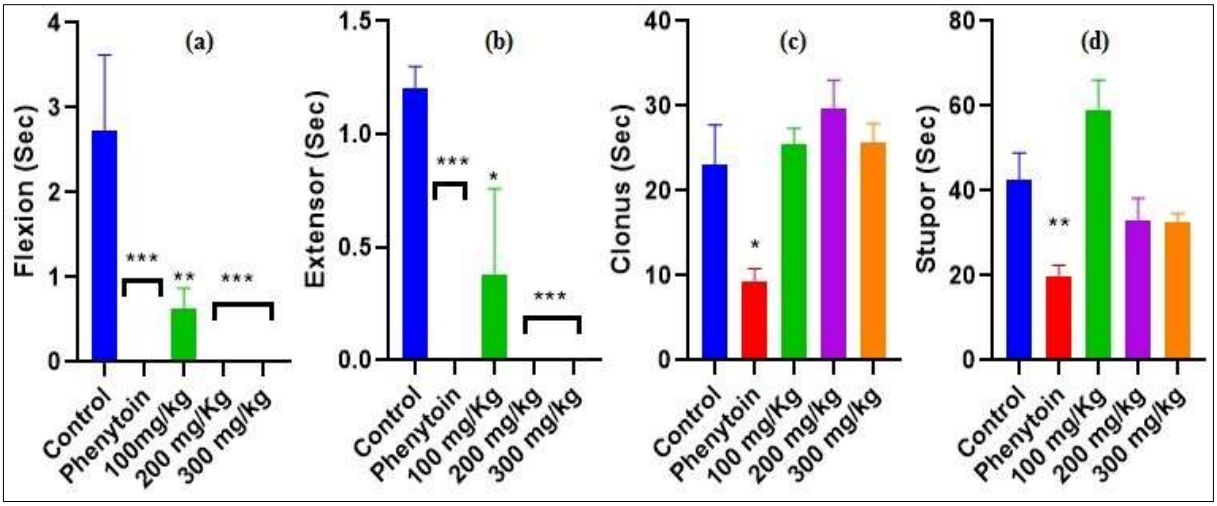


Fig 1: Effect of EtES leaves on maximum electroshock seizure: (a) Flexion was eliminated by all dosages of EtES compared to the control group (b) Extensor phage was also decreased at all dosages of EtES (c) Clonus phase did not significantly change (d) stupor phase did not significantly differ from the control group. (****=P<0.0001, ***= P<0.001, **= P<0.01, *= P<0.05)

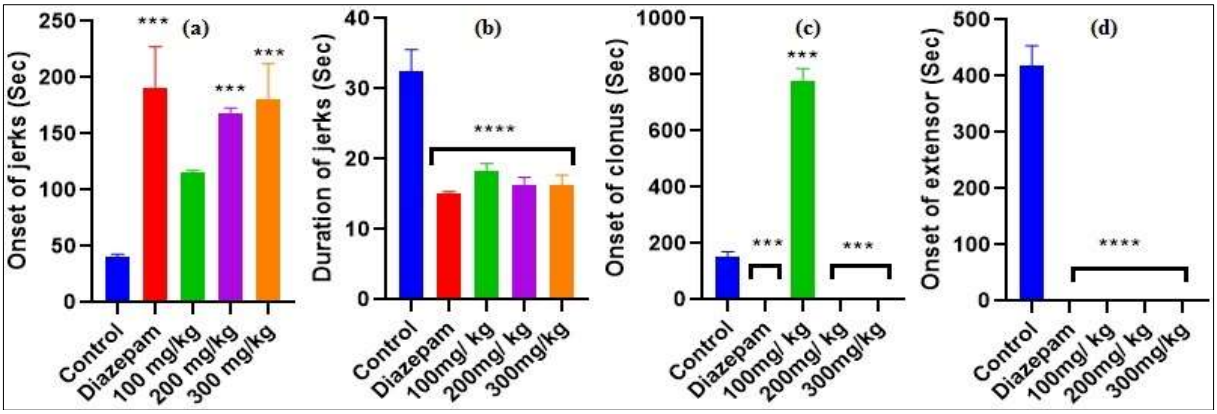
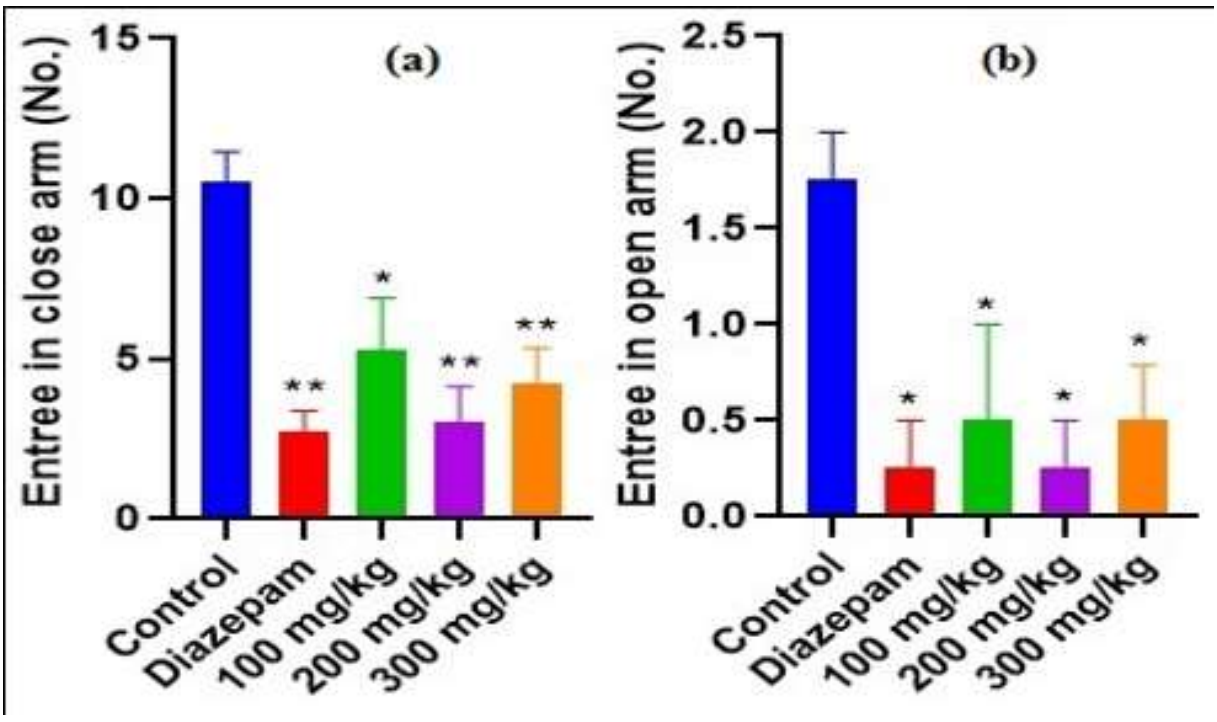


Fig 2: Effect of EtES leaves on PTZ-induced convulsion: (a) Onset of jerk was delayed in EtES-treated groups (b) Duration of jerk decreased in EtES groups (c) Onset of clonus was delayed in the 100 mg/Kg group and did not occur in higher dosage of EtES group. (d) Onset of extensor. (****= p<0.0001, ***= p<0.001, **= p<0.01, *= p<0.05)



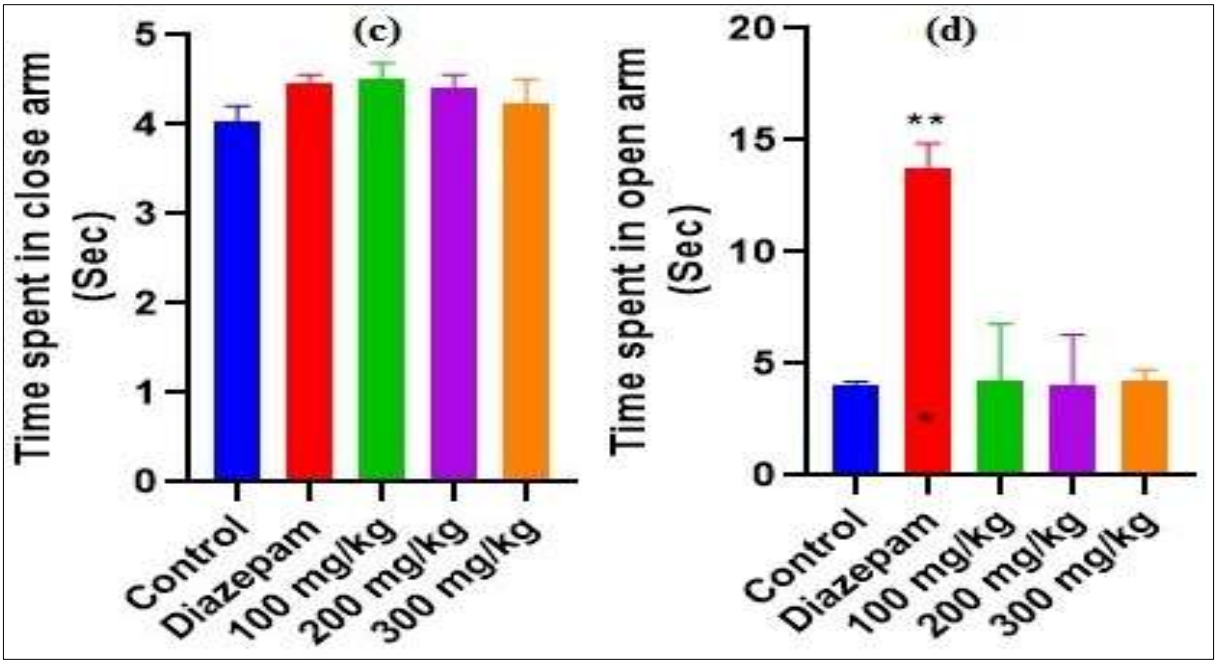


Fig 3: Elevated plus maze Test: Time spent in open and closed arm decreased (a&b) significantly (c) Time spent in the close arm did not change significantly (d) Time spent in the open arm did not change significantly compared to control group. (****= $p<0.0001$, ***= $p<0.001$, **= $p<0.01$, *= $p<0.05$)

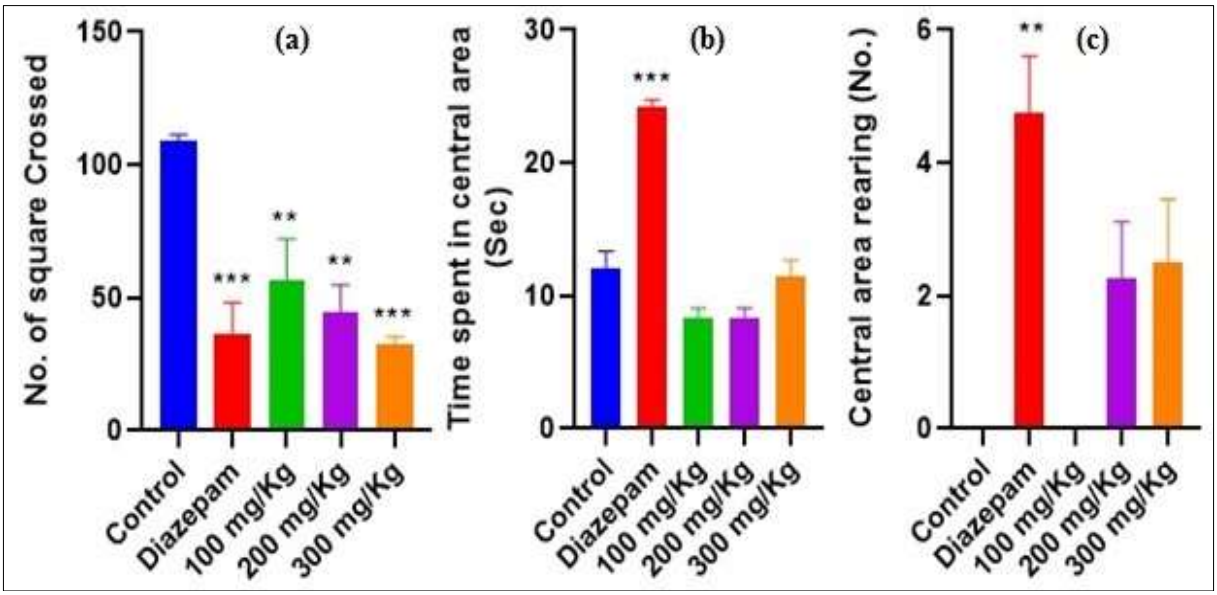


Fig 4: Open field test: (a) The number of squares crossed by rats decreased significantly compared to the control group. (b)Time spent in the central arm by the EtES group did not change significantly. (c) The number of central areas rearing by the EtES group did not change significantly. (****= $p<0.0001$, ***= $p<0.001$, **= $p<0.01$, *= $p<0.05$)

Table 2: List of Equipments

Equipment	Company/Supplier
Electro-convulsometer	Techno, model no. 100-3
Rota Evaporator	Buchi India Pvt Ltd
Weighing Balance	Mettler Toledo
pH Meter	Eutech Instruments
Milli-Q-Water Apparatus	Milli pore
Vortex	Techno, Ambala
Micropipettes	Thermo Scientific
Centrifuge	Remi
Ultrasonicator	Shimadzu
Autoclave	Techno, Ambala
Eppendorf Tubes	Thermo Scientific
Elevated plus maze apparatus	In- House
Open field test apparatus	In- House

Conclusion

The ethanolic extract of *Elephantopus scaber* leaves exhibits promising antiepileptic and anti-anxiety effects. Experimental results indicate that the extract significantly reduces the frequency and severity of seizures in animal models, suggesting a potential role in the management of epilepsy. Additionally, the extract demonstrates fewer anxiolytic properties, as evidenced by anxiety-related behavioral tests.

These findings support the traditional use of *Elephantopus scaber* in managing neurological and psychological conditions. However, further studies, including clinical trials, are needed to confirm its efficacy and safety in humans. The extract's precise mechanism of action should also be elucidated to better understand how it affects neurological and anxiety pathways.

Overall, *Elephantopus scaber* presents as a potential therapeutic candidate for epilepsy and anxiety, warranting more extensive research to explore its full potential and applicability in clinical settings.

Data Availability: The data used to support the findings of this study are included within the article.

Conflicts of Interest: The author declares that there are no conflicts of interest.

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