Preliminary phytochemical screening and effect on insomnia in epileptic rats by using a herbal drug fennel plant.

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ABSTRACT:

Because of increased stress and changing living conditions, central nervous system disorders are a major concern in today's world. Because epilepsy is one of the most common CNS disorders, and because current antiepileptic drug treatments have a number of side effects, the current study was designed to evaluate the effect of *Foeniculum vulgare* on GTC seizures, as well as its effect on memory retention in seizure-induced rats, and the role of monoamines in seizure protection and memory impairment. To investigate the effect on GTC, an electro convulsiometer was used to administer a maximum electro shock (150mA intensity for 0.2 sec) after 14 days of treatment with *Foeniculum vulgare* (300 and 500 mg/kg). Before beginning treatment, rats were trained for conditioned avoidance responses, and the effect on memory was studied using an Actophotometer and rotarod. The percent reduction in time demonstrated that *Foeniculum vulgare* had potential antiepileptic and memory retentive effects.

Key words: Epilepsy, *Foeniculum vulgare*, Actophotometer, Roda rod.

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INTRODUCTION:

Epilepsy affects roughly 50 million people, 40 million of whom do not receive effective treatment, and is rapidly becoming a barrier to many lives. On the other hand, the numerous side effects of currently available antiepileptic medicines (AEDs) are a major source of concern for both patients and physicians. Traditional drugs are not recommended for long-term prophylaxis in the treatment of epilepsy because the unpredictability of seizure onset restricts their therapeutic efficacy[1]. Furthermore, cognitive flaws pose a severe concern in epileptic patients, and the deteriorating effects of the existing *Foeniculum vulgare* are anchoring epileptic patients' cognitive abnormalities. Although substantial studies on the neurobiological grounds of epilepsy have been conducted, studies demonstrating the involvement of brain catecholoamines and indoleamines in seizure reduction are scant[2]. However, a few studies have revealed that norepinephrine (NE) protects against electroshock-induced GTC seizures; shed some light on the role of neurotransmitters in seizure management; and the involvement of brain monoamines. Several publications have also offered information on seizures and their treatment; while there have been few attempts to research the effects of medications on memory following seizure induction, the role of monoamines in seizure prevention, and the effect of *Foeniculum vulgare* treatment on memory[3]. In light of these findings, we chose *Foeniculum vulgare*, which has been claimed to have efficacy in the

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treatment of epilepsy, enhancement of brain monoamine levels, and rhizomes being cited in the database for Indian medicinal plants in the treatment of epilepsy, for which no reported evidence was available. All of these factors prompted us to focus on the objectives of the current study, which were to evaluate: The protective effect of *Foeniculum vulgare* on the maximal electro shock (MES) induced seizures[2].

MATERIALS AND METHODS:

Drugs and chemicals

Methanol, ethanol, petroleum ether, and tween 80. Diazepam were brought from Cipla Pharmaceuticals Ltd., Mumbai, India.

Plant material

Botanical Survey of India Regional Centre, 10 Chatham Lines, Prayagraj, 211002 Dr. Arti Garg recognized the specimen of *Foeniculum vulgare* collected in April and May.

The Botanical Survey of India has received a specimen with the voucher number SIP/2022-23/173.

Animals housing and feeding conditions

Weighting 160–180 g, albino Wister rats were purchased from Saha Enterprises in Kolkatta, India. The animals were kept in propylene cages with rice husk bedding at 24 degree temperatures with 30–70 percent relative humidity. A 12:12 h light-dark cycle was maintained using the typical commercial pellet (M/s. Hindustan Lever Ltd., Mumbai, Maharashtra, India) and an endless supply of purified water. Following CPCSEA guidelines, the Animal Ethical Committee (SIP-IAEC/009/05/22) approved all experimental protocols and procedures.

Preparation of plant extracts

The bark of *Foeniculum vulgare* was machine-pulverized after being dried in the shade. The coarse powder was defatted with petroleum ether and then the methanol was extracted by cold maceration. The extract was concentrated using a rotating evaporator at low pressure. Phytochemical screening was done on them. They had been administered to the various groups in the right amounts based on their body weights after being dissolved in 0.5 percent carboxymethyl cellulose.

Acute toxicity studies-

The 20–25 g healthy Swiss albino mice were placed randomly into one of five (n = 3) groups after being given only water for 3–4 hours. They received extracts orally along with a 0.5 percent CMC control at doses of 5, 50, 300, and 2000 mg/kg b.w. post-esophageal (p.o.). The study was conducted in accordance with OECD recommendations (423: acute toxic class method). The animals were monitored for signs of toxicity, morbidity, and death for the first 24 hours, with particular focus on the first 4 hours, and their behavioural, neurological, and autonomic profiles were assessed. They were also observed for the following 72 hours and for the remaining 14 days. The test dose was determined.[5]

Induction of maximal electroshock seizures (MES).

An electro convulsiometer was used to induce GTC seizures (Inco Co., Ambala, India). Maximal seizures were induced using corneal electrodes and a 60 Hz alternating current of 150 mA intensity for 0.2 seconds. A drop of 0.9

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percent sodium chloride electrolyte solution with lignocaine was applied to the corneal electrodes, ensuring greater contact and lowering the death rate to zero. The percentage of protection from seizures provided by medicines was calculated by inhibiting complete hind limb tonic extension (HLTE) or HLTE that did not exceed a 90° angle with the plane of the body[6]. The durations of 1) tonic flexion, 2) tonic extension, 3) clonic convulsions, and 4) righting reflex were also measured.

Behavioral test

Test for locomotor activity:

Each mouse's spontaneous locomotor activity was recorded for 10 minutes using an actophotometer. Methanol and aqueous extracts of Flaveria trinervia wholeplant were delivered 60 minutes before the test, as was the usual medication, chloropromazine hydrochloride. The control group was given DMSO orally 60 minutes before the test[7][6].

Muscle co-ordination test:

The rotarod device was used for this test. The rotarod device consists of a rubber-coated metal rod (3 cm in diameter) coupled to a motor with a speed set to 20 spins per minute. The rod was 45 cm long and was separated into three portions by iron discs, allowing three mice to be tested at the same time. To dissuade the animals from jumping off the roller, the rod was positioned roughly 50 cm above the table top. Cages beneath the portion serve to limit the animals' movements when they fall off the roller. The device was tested on Swiss albino mice. The test only included animals that had proven their capacity to stay on the rotating rod (20 rpm) for 5 minutes.[6]

Statistical analysis:

The results were presented as mean SEM. All data obtained were statistically analysed using a one-way ANOVA followed by Dunnett's post-hoc multiple comparison test. Graph Pad Prism software was used to create graphs from all of the results (v.5). p values less than 0.05 were considered statistically significant.

RESULTS:

EXTRACTION-

Table 1: ***The extraction value of Foeniculum vulgare (M.) seed powder by hot extraction method.***

S.N.	Nature of extract	Values (% w/w) by hot extraction
01	Petroleum ether	3.70
02	Chloroform	2.69
03	Ethanol	10.65
04	Diethyl ether	3.65
05	Aqueous	6.20

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PHYTOCHEMICAL SCREENING:

Phytochemical screening of the extract showed the presence of sterols, terpenoids, flavones, tannins and glycosides.

GENERAL PHARMACOLOGICAL OBSERVATION:

Mice orally treated with the *Flaveria trinervia* whole plant ethanol extract (300 and 500 mg/kg) and were submitted to the general observations, which did not show any difference in their behavioral patterns as determined during the observation periods. They were alert with normal grooming, touch response and pain response. Alertness, limb tone and grip strength were normal and the animals did not show staggering gait or contractions.

TEST FOR LOCOMOTOR ACTIVITY:

Test for locomotor activity The actophotometer performance of F. trinervia ethanol (300 and 500 mg/kg) revealed a considerable effect on locomotor activity. However, as compared to Diazepam, the ethanol extract of F. trinervia (High Dose) exhibited a substantial effect (27.17 percent) (21.06 percent). These extracts also revealed the onset and duration of locomotor activity decreases. However, as compared to the control group (4.02 percent), this minimal percentage observed in the control group may be attributed to the effect of the 2% DMSO therapy.(Table1).

Table 1. Effects of ethanol extracts of *Foeniculum vulgare (M.)* on locomotor activity by actophotometer.

Groups	Test Drug	Dose(mg/kg)	Mean reaction before drug administration (sec)	Mean reaction time after drug administration (sec)	% Decrease intime
Group I	Normal	2	410 ± 12.75	393.67 ± 17.38	4.14%
Group II	Standard (Diazepam)	2	394.17 ± 17.67	311.33 ± 6.37 **	21%
Group III	EEFV	300	410 ± 40.10	250 ± 17.10 **	39.17%
Group IV	EEFV	500	413.17 ± 42.81	300.33 ± 15.5 *	27.06%

EEFV- Ethenolic Extracts of Foeniculum vulgare.

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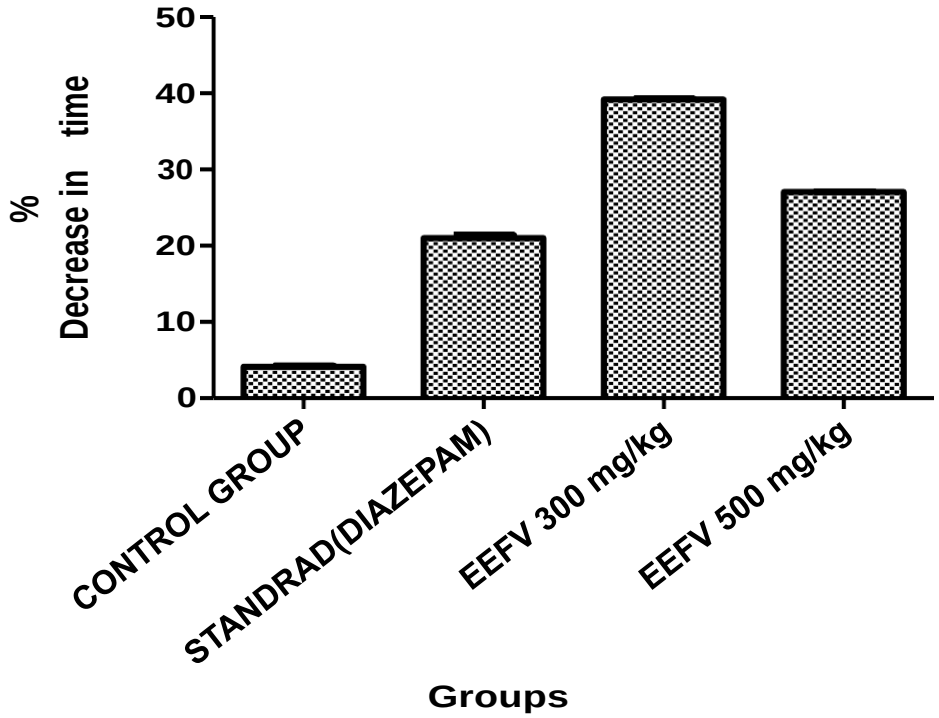

Fig1-Effects of ethanol extracts of *Foeniculum vulgare (M.)* on locomotor activity by actophotometer.

Muscle co-ordination test:

Flaveria trinervia extracts had a substantial influence on motor coordination as measured by rotarod performance, revealing a significant decrease in spontaneous motor activity in mice. This impact was detected 1 hour after the medication was administered. The results of the motor coordination test demonstrated that both methanol and aqueous extracts significantly reduced motor coordination in mice following oral administration. However, ethenol extracts (22.71 percent) had a substantial effect. However, when compared to the control group, the diazepam-treated group showed a statistically significant drop in motor coordination activity (11.56 percent) (1.11 percent). This small proportion found in the control group could be attributed to the effect of the 2% DMSO therapy (Table 2).

Table 2. Effects of ethanol extracts of *Foeniculum vulgare (M*) on muscle coordination activity by rotarod.

Groups	Test Drug	Dose(mg/kg)	Mean reaction before drug administration (sec)	Mean reaction time after drug administration (sec)	% Decrease intime
Group I	Control	2%	755.5 ± 12.75	747.67 ± 17.38	1.02%

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Group II	Standard (Diazepam)	2	729.17 ± 17.67	646.33 ± 6.37 **	11.69%
Group III	EEFV	300	868 ± 40.10	570 ± 17.10 **	34.17%
Group IV	EEFV	500	836.17 ± 42.81	650.33 ± 65.5 *	22.06%

EEFV- Ethenolic Extracts of Foeniculum vulgare.

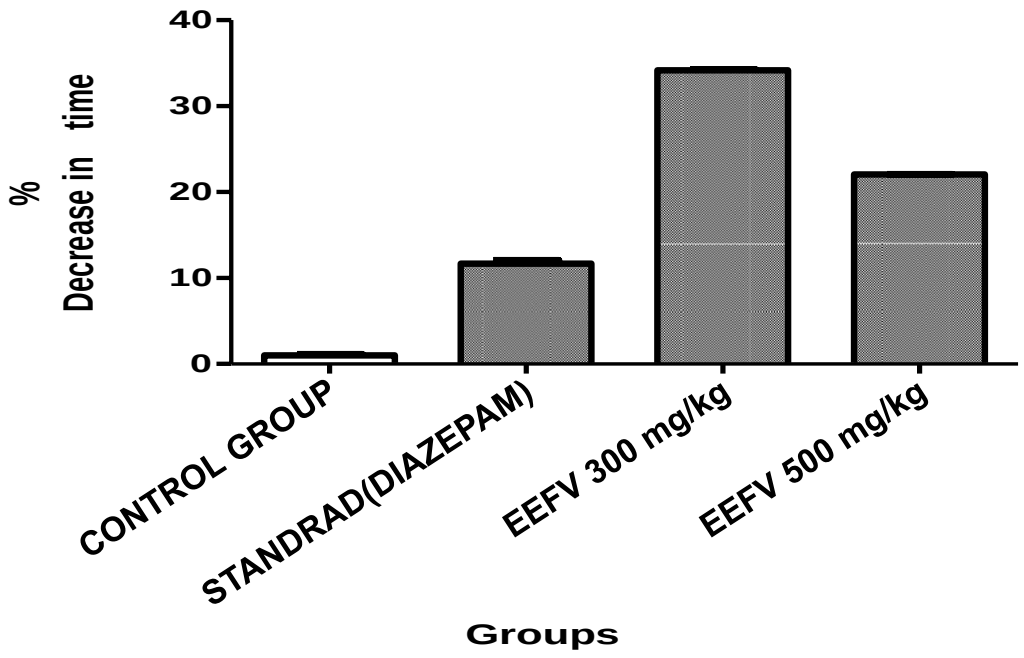

Fig2-Effects of ethanol extracts of *Foeniculum vulgare (M* **) on muscle coordination activity by rotarod.**

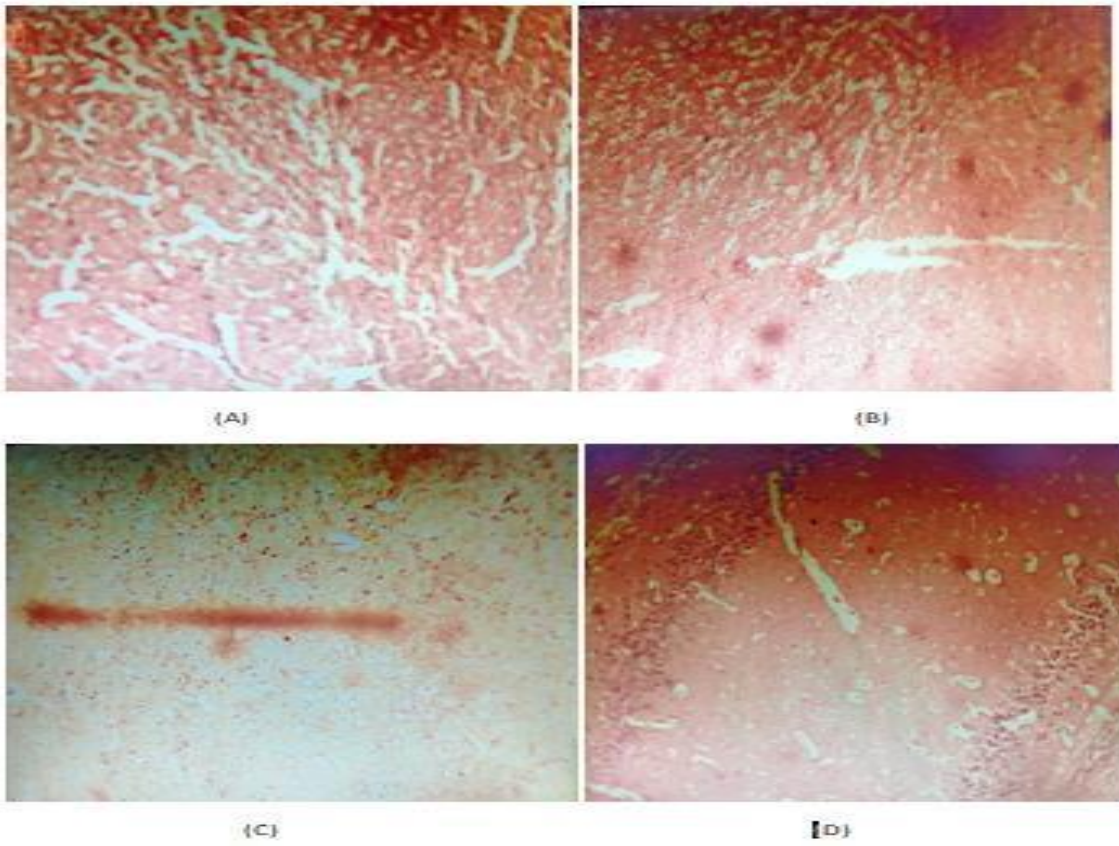

***Fig 3*- Histopathological outcomes: (A)Normal group with distinct sinusoidal space and central vein architecture.(B) Typical diazepam (2 mg/kg) the treated group had fewer disorganised as well as significant regeneration activity.(C) EEFV low dose (300mg/kg) treated group with less disarrangement.(D) Normal architecture with regeneration activity after a high dose of EEFV (500mg/kg).**

Discussion:

Anxiety and depression are prevalent in society, and they are directly or indirectly linked to morbidity and, to a lesser extent, mortality. As a result, it is critical to pay urgent attention to these issues and identify effective solutions[8]. Despite the fact that various pharmaceutical industries have developed several drugs to treat these disorders, these synthetic drugs are usually associated with some limitations, and there is an urgent need for alternative medications to combat these disorders[1]. The forced swimming test, actophotometer-based locomotion tests, and rotarod-based muscle co-ordination tests are the most commonly used animal models for CNS depressant screening. These tests are extremely sensitive and specific to all the main types of CNS depressants. The CNS depressive effect of F. trinervia methanol and aqueous extracts demonstrated a substantial depression pattern in the forced swim test, locomotor activity test, and muscle co-ordination test in mice[7]. The actophotometer-measured decrease in locomotor activity and the rotarod-measured decrease in grip were shown to be extract-dependent. A decrease in locomotion shows a CNS depressive effect. The CNS depressing

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activity could be attributed to an increase in GABA concentration in the brain. Methanolic and aqueous extracts (50 mg/kg and 100 mg/kg p.o.) supplied to mice demonstrated considerable CNS depressing effects in the current investigation[9]. According to the results, the administration of methanol and aqueous extracts reduced the immobility period of mice subjected to the forced swimming test. Both extracts were shown to be less effective than the usual drug diazepam (2 mg/kg, p.o.) at spontaneously depressing rats in locomotor and muscular coordination tests. F. trinervia extracts significantly reduced immobility, locomotor activity, and muscle coordination activity in mice, indicating a central nervous system depressive action. Based on the findings of this study, we may conclude that F. trinervia extracts exhibit neuropharmacological action, as evidenced by considerable reductions in immobility time, motor activity, and muscle coordination[3]. It stands to reason that it could be useful as a CNS depressant in clinical settings. The current work was a preliminary attempt that will necessitate further extensive examination, including identification of active compounds, as well as preformulation studies for the development of a possible dosage form[4].

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