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Mucuna pruriens (Velvet bean) seed extract ameliorates epilepsy and anxiety against in vivo experimental models: A histopathological analysis

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Summary

Introduction: The plant *Mucuna pruriens* is traditionally used in Indian system of medicine for the therapy of several neurological disorders. Chemical investigations on the plant have established the presence of levodopa and tryptamine which are responsible for treating the neurological disorders like Parkinsonism. **Objective:** The objective of this research was to scientifically explore and ascertain its antiepileptic and anxiolytic activity in preclinical studies on Swiss albino mice. **Material and Method:** The antiepileptic and anxiolytic effect of ethanolic extract of *Mucuna pruriens* (EEMP) tested against maximal electroshock (MES) pentylenetetrazol (PTZ)-induced convulsions, Elevated Plus Maze, and light and dark arena. Actophotometer test performed to evaluate its potential locomotor activity deficit inducing action. **Result:** Treatment of mice with EEMP significantly upturned the MES-induced convulsions, which was reflected by diminution in the time interval (sec) of entire phases of MES-induced convulsions, with an improvement in GABA levels. In the PTZ model, pretreatment with EEMP delayed the latency and reduced the intensity to clonic convulsions ($p< 0.0001$), and also delayed the latency of tonic convulsions as well as decrease the mortality mice in the treated groups in

a dose-dependent manner. EEMP intervention dose dependently restored brain GABA levels. Assessment of GABA in mice's brain after administration of EEMP exhibits significant modulation of GABA levels. **Conclusion:** Overall, the findings suggest that *Mucuna pruriens* has anticonvulsant and anxiolytic properties that are mediated by positive GABAergic neurotransmission hence could be used to treat epileptic seizures, petitmal and grandmal epilepsy.

Keywords: Anticonvulsant; Anxiolytic; GABA estimation; Maximal electroshock (MES); *Mucuna pruriens*; Pentylenetetrazol (PTZ).

Resumen

extracto de semilla de *Mucuna pruriens* (frijol terciopelo) mejora la epilepsia y la ansiedad contra modelos experimentales in vivo: un análisis histopatológico

Introducción: La planta *Mucuna pruriens* se utiliza tradicionalmente en el sistema indio de medicina para la terapia de varios trastornos neurológicos. Las investigaciones químicas en la planta han establecido la presencia de levodopa y triptamina, que son responsables del tratamiento de trastornos neurológicos como el parkinsonismo. **Objetivo**: El objetivo de esta investigación fue explorar y determinar científicamente su actividad antiepiléptica y ansiolítica en estudios preclínicos en ratones albinos suizos. **Material y método**: El efecto antiepiléptico y ansiolítico del extracto etanólico de Mucuna pruriens (EMPE) se ensayó frente a convulsiones inducidas por electrochoque máximo (MES) pentilentetrazol (PTZ), Laberinto Elevado Plus y arena clara y oscura. Prueba de actofotómetro realizada para evaluar su potencial acción inductora de déficit de actividad locomotora. **Resultado**: El tratamiento de ratones con EEMP aumentó significativamente las convulsiones inducidas por MES, lo que se reflejó por la disminución en el intervalo de tiempo (s) de fases completas de convulsiones inducidas por MES, con una mejora en los niveles de GABA. En el modelo PTZ, el pretratamiento con EEMP retrasó la latencia y redujo la intensidad de las convulsiones clónicas ($p< 0,0001$), y también retrasó la latencia de las convulsiones tónicas, así como disminuyó la mortalidad de los ratones en los grupos tratados de una manera dependiente de la dosis. La intervención de EMPE restauró de forma dependiente de la dosis los niveles de GABA en el cerebro. La evaluación de GABA en el cerebro de ratones después de la administración de EEMP muestra una modulación significativa de los niveles de GABA. **Conclusión**: En general, los hallazgos sugieren que Mucuna pruriens tiene propiedades anticonvulsivas y ansio-

líticas que están mediadas por neurotransmisión GABAérgica positiva, por lo que podría usarse para tratar convulsiones epilépticas, epilepsia pequeña y granular.

Palabras clave: Anticonvulsivo; Ansiolítico; Estimador de GABA; electroshock máximo (MES); *Mucuna pruriens*; Pentilentetrazol (PTZ).

Resumo

Extrato de semente de *Mucuna pruriens* (feijão aveludado) melhora a epilepsia e a ansiedade contra modelos experimentais in vivo: uma análise histopatológica

Introdução: A planta *Mucuna pruriens* é tradicionalmente utilizada na medicina indiana para o tratamento de diversas desordens neurológicas. Investigações químicas na planta estabeleceram a presença de levodopa e triptamina, responsáveis pelo tratamento de distúrbios neurológicos como o parkinsonismo. **Objetivo:** O objetivo desta pesquisa foi explorar cientificamente e verificar sua atividade antiepiléptica e ansiolítica em estudos pré-clínicos em camundongos albinos suíços. **Material e Método:** O efeito antiepiléptico e ansiolítico do extrato etanólico de Mucuna pruriens (EEMP) testado contra convulsões induzidas por eletrochoque máximo (MES), pentilenotetrazol (PTZ), labirinto em cruz elevado e arena clara e escura. Teste de actofotômetro realizado para avaliar sua potencial ação indutora de déficit de atividade locomotora. **Resultado:** O tratamento de camundongos com EEMP aumentou significativamente as convulsões induzidas por MES, o que se refletiu na diminuição do intervalo de tempo (seg) de fases inteiras das convulsões induzidas por MES, com uma melhora nos níveis de GABA. No modelo PTZ, o pré-tratamento com EEMP retardou a latência e reduziu a intensidade das convulsões clônicas ($p < 0,0001$), e também retardou a latência das convulsões tônicas, bem como diminuiu a mortalidade dos camundongos nos grupos tratados de maneira dose-dependente. A intervenção do EEMP restaurou os níveis cerebrais de GABA dependentes da dose. A avaliação de GABA no cérebro de camundongos após a administração de EEMP exibe modulação significativa dos níveis de GABA. **Conclusão:** No geral, os achados sugerem que Mucuna pruriens tem propriedades anticonvulsivantes e ansiolíticas que são mediadas por neurotransmissão GABAérgica positiva, portanto, pode ser usado para tratar crises epilépticas, epilepsia petitmal e grandmal.

Palavras-chave: Anticonvulsivante; Ansiolítico; estimativa de GABA; Eletrochoque máximo (MES); *Mucuna pruriens*; Pentilenotetrazol (PTZ).

Introduction

Anxiety and depression are dynamic and catastrophic complicated disorders, and it is now clear that providing suitable treatment options for patients without knowledge of both the clinical and molecular aspects of anxiety and depression is challenging. In recent decades, impressive research has been done on a wide range of neurological features of depression and anxiety [1]. Benzodiazepines are now the most often given drugs for anxiety disorders, but their therapeutic applicability as anxiolytics are limited by their undesirable side effects. As a result, research into novel pharmacological drugs derived from plants is highly encouraged [2]. Herbal treatments are increasingly being used by physicians in Asia and Europe, and researchers are looking into ancient therapies to discover a viable solution for these mind-affecting ailments.

The plant realm is an undeniably rich source of bioactive compounds [3]. The current emphasis of scientific study is on extracting, identifying, and characterizing the bioactive components of medicinal plants that might be employed as "lead" molecules in therapeutic drug discovery and development [4]. *Mucuna pruriens* var. utilis, an undervalued tropical legume, offers nutritional quality comparable to soya beans and other traditional legumes due to comparable protein, lipids, mineral, and other nutrient proportions. It traditionally has been consumed as a meal in a variety of nations, including India, Philippines, Nigeria, Ghana, Brazil, and Malawi [5]. In Ayurvedic medicine *Mucuna pruriens* Linn is a well-known medicinal plant used to cure diabetes, impotence, cancer, snake bite, and Parkinsonism. *Mucuna pruriens* endocarp is non-toxic and is 2 to 3 times more potent than levodopa in managing hyperprolactinemia [6] and motor symptoms of Parkinson's disease in animal models [7]. *Mucuna pruriens* has also been demonstrated to have neuroprotective effects in Parkinsonism animal models by increasing brain mitochondrial complex-I activity and considerably restoring dopamine and norepinephrine levels [8]. The presence of 5-indolic chemicals, particularly tryptamine and 5-hydroxytryptamine, were reported in phytochemical analysis of the seeds [9]. Alkaloids like Mucunadine, Mucunine, Prurienine and Prurine have been reported in seeds of M. pruriens which are used as drug component in Psychiatry [10]. Therefore, the present research was designed to assess the anxiolytic and anti-convulsant activity of an ethanolic seed extract of *Mucuna pruriens*.

Materials and methods

Drugs and reagents

Diazepam was procured from Ranbaxy Laboratories, New Delhi, India and Phenytoin, Pentylenetetrazol and GABA were purchased from Sigma Aldrich, Bangalore. All the other reagents and chemicals used in this study were of standard analytical grade.

Collection, authentication and extraction of plant material

The study was conducted using ethanolic extract of *M. pruriens* (EEMP) seeds, for which the seeds were purchased from local market of Prayagraj Uttar Pradesh, India in the month of November 2021 and authenticated by taxonomist Dr. Arti Garg at the Botanical Survey of India in Prayagraj. The voucher specimen has been deposited with accession number BSI/CRC/2021-22/405. The collected seeds (500 g) were cleaned, crushed, grind, and extracted with 99% ethanol using a Soxhlet apparatus for 72 h at 50°C. A rotary evaporator (Buchi, USA) was used to evaporate the solvent followed by lyophilization thus 50g of solid residue (10%w/w) was obtained.

Preliminary phytochemical screening of extract

The EEMP was examined qualitatively for the presence of various secondary metabolites as per standard procedures [11]. The presence of flavonoids, phenolics, glycosides, tannins, polysaccharides, saponins, and steroids were confirmed in the ethanol extract of *Mucuna pruriens*.

High Performance Liquid Chromatography

For qualitative estimation of Dopamine HPLC was performed as per earlier reported method by Rathod and Patel (2014) [12]. Chromatographic separation was performed with AGILENT HPLC (Model no. 1220 Infinity) equipped with binary pump and auto injector (20 µl). Kromasil100–5–C18 (250mm× 4.6mm × 5µ) column was used for analysis. Mobile phase used was Water: Methanol: Acetonitrile (100:60:40) (v/v) containing 0.2% Triethylamine, pH 3.3 filtered through 0.45 µ membrane filter (Millipore) and degassed by sonication**.** Flow rate 1 ml / min maintained throughout the run. OpenLabCDS Version A.04.06 chromatographic software was used for data acquisition. Column effluent was monitored at 280 nm with variable wavelength UV detector.

In vivo pharmacological assessment

Experimental Animals

Swiss Albino mice of either sex (25–35 g, 4–5 months aged) were procured from IIT-BHU Varanasi and kept in propylene cages under 12-hour cycle of light and darkness. All animals were accessed to chow and clean water *ad libitum.* This research approved by Institutional Animal Ethics Committee (IAEC) of United institute of Pharmacy with approval no. UIP/IAEC/Nov.-2021/14. The experiments were performed as per regulatory guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) Government of India.

Selection of Dose of EEMP

Earlier studies [13, 14] show that seed extract administration had no animal mortality at the dose of 2000 mg/kg. Whereas, Golbabapour *et al.* (2013) [15] confirms that at 5000 mg/kg b.w dose didn't show any histological sign of hepatic toxicity and renal toxicity including normal blood biochemistry analysis in animal models. On the basis of above cited literature two doses viz. 250 mg/kg and its double strength 500 mg/kg has been selected for pharmacological activity.

Assessment of anticonvulsant activity

Pentylenetetrazol induced convulsion

Animals were divided into five groups of six animals each (n = 6). For three days, the animals were treated as follows: Group 1: water, Group 2: diazepam (5 mg/kg), Group 3: EEMP (250 mg/kg) and Group 4: EEMP (500 mg/kg) orally. On the third day, 1 hour after the given treatment, convulsions have been induced chemically by administering PTZ (80 mg/kg) as per previous reported method of Showraki *et al.* (2016) [16] with slight modification. The animals were individually monitored in a plastic cage for 30 minutes, with the animals that survived being followed for up to 24 hours. Individual data such as Latency (onset of clonus), Onset of tonic convulsions, and Percentage protection were recorded and reported as percentage protection during the individual observation.

$$\%\ \boldsymbol{Protection} = \mathbf{100} - \frac{\boldsymbol{Number\ of\ animal\ showed\ seizures}}{\boldsymbol{Total\ number\ of\ animals\ used}} \times \mathbf{100}$$

Maximal Electroshock Shock Induced Convulsion

Animals were divided into five groups of six animals each (n = 6). For three days, the animals were treated as follows: Group 1: water, Group 2: phenytoin (25 mg/kg), Group 3: EEMP (250 mg/kg) and Group 4: EEMP (500 mg/kg) orally. On the third day, exactly one hour after the given treatment, convulsions were induced through 60A current given trans-auricularly for 0.2 seconds through tiny alligator clips affixed to each pinna using electro-convulsiometer as per previous reported method by Vogel and Vogel (2000) [17] with slight modification. The animals were monitored individually in a plastic cage for 30 min. During the observation, characteristics such as hind limb flexion, hind limb extensor, stupor, and death/survival were recorded, and the % protection was determined.

Assessment of anxiolytic activity

Elevated plus maze model

Two open arms (35 × 5 cm) and two closed arms (30 × 5 × 15 cm) extend from a shared center platform (5 × 5 cm) of the apparatus. The closed arms' floor and walls are black-painted wood. The entire maze is raised to a height of 50 cm above the floor. Mice weighing 18–22 g were selected, and were handled by the investigator on alternate days to minimize stress. The animals were divided into 4 groups of six animals each, and they were given treatment as follows: Group 1: water, Group 2: diazepam (5 mg/kg), Group 3: EEMP (250 mg/kg) and Group 4: EEMP (500 mg/kg) orally. Each animal was positioned in the center of the maze facing one of the enclosed arms 1 hour after the medication treatment was administered orally. The following parameters were recorded during a five-minute session: the number of entries into the open arm and the time spent in the open arm [18-20]. To eliminate any olfactory cues, the maze was cleaned down with 70% ethyl alcohol and left to dry between observations.

Light and dark arena

The apparatus has been set as per design of previously reported method by Crawley and Goodwin (1980) [21]. A wooden box with an open top and two separate compartments, one painted black and the other white, is used, each brightly lit by a 100 W white light source located 17 cm above the box. The two compartments are linked by a small open doorway (7.5 × 5cm) in the middle of the partition on the floor level. The animals were given treatment as follows: Group 1: water, Group 2: diazepam (5 mg/kg), Group 3: EEMP (250 mg/kg) and Group 4: EEMP (500 mg/kg) orally for 3 days. On the 3rd day, animals were placed initially in light compartment and parameters such as number of entries and time spent in light area were recorded in triplicate.

Locomotor activity

Locomotor activity has been performed as per standard protocol using actophotometer [22]. Mice were divided into groups of 5 with 6 animals in each group and given medications in the manner outlined for the elevated plus maze paradigm. Locomotor activity was assessed as a number of photocells counts by individually putting the animals in an actophotometer for 10 minutes before and exactly 60 minutes after the relevant treatment. The change in the locomotor activity was derived using photocell counts recorded before and after the assigned treatments, and reduction in locomotor activity is calculated using the following equation:

$$\%\ \textbf{Reduction in locomotor activity} = 100 - \frac{\textbf{Photocell counts after treatment}}{\textbf{Photocell couns before treatment}} \times 100$$

Biochemical Evaluation

Estimation of GABA in brain samples

Preparation of brain tissue samples

Mice were fasted up over night before being euthanized, and their brains were isolated and rinsed in cold 0.9 percent saline to remove attached tissues and blood clots. Using a tissue homogenizer, the brain tissues were homogenized with 0.1 N HCl in 80 percent ethanol (1 ml of ethanol for every 100 mg tissue). The homogenates were centrifuged for 20 minutes at 25° at 4500 rpm. The clean supernatants were then placed in microcentrifuge tubes and analyzed quantitatively through HPTLC [23].

Chromatographic conditions

Sample applicator- CAMAG Linomat 5; Scanner-CAMAG TLC scanner 3; Software-winCATS ver 1.14.26; Stationary phase-HPTLC plates silica gel 60 F 254; Mobile phase- n-butanol: glacial acetic acid: water (22:3:5 v/v/v); developing chamber- twin trough glass chamber (20 × 10 cm); developing mode-ascending mode; chamber saturation time-5 min; Detection reagent-0.2% w/v ninhydrin in acetone; Scanning wavelength-490 nm.

Histopathology

The isolated brain tissue was sliced and promptly fixed in 10% formalin solution for 24 hours. Dehydrated brain sections were paraffin-fixed, cut into 5 um thick slices, and stained with hematoxylin and eosin dye. A light microscope was used to examine the produced brain tissue slices [24].

Statistical analysis

The data was reported in the form of mean ± S.D. To examine statistical differences, one-way ANOVA was performed, followed by the post-Tukeys test. For all comparisons, $P<0.0001$ was considered statistically significant. All statistical analysis was performed using the Graph-Pad Prism 8.0.2 version.

Results

High Performance Liquid Chromatography

Mucuna pruriens ethanolic extract shows retention time at 2.503 mins along with other compounds at retention time of 3.75 and 5.07.

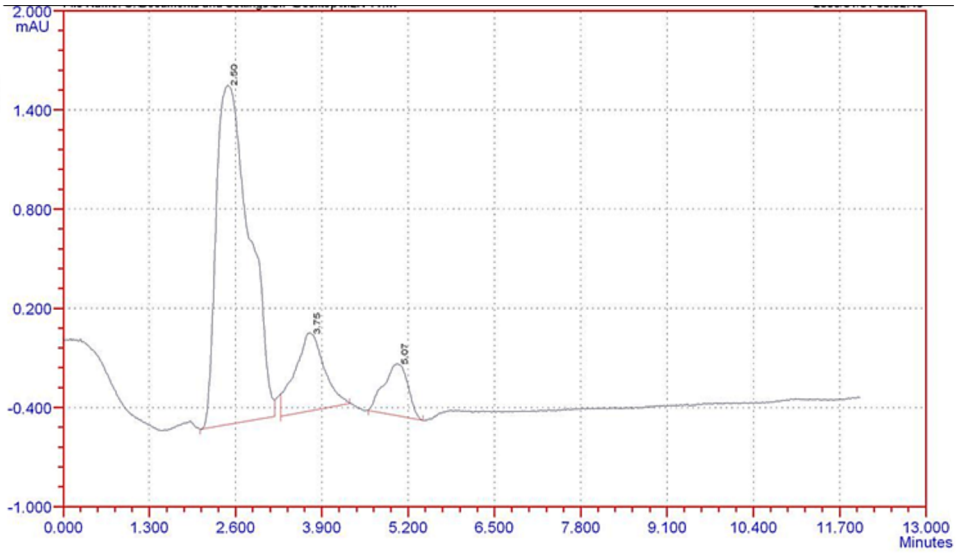

Figure 1. HPLC chromatogram of *Mucuna pruriens* ethanolic extract.

Anticonvulsant activity

Pentylenetetrazol induced convulsion

PTZ caused hind and forelimb myoclonic jerks in all mice within 20–30 seconds of intraperitoneal administration. This was followed by a collapse on one side or the back, followed by generalized clonic discharges. All the treated groups were compared with the control group. Effect of EEMP on PTZ induced epilepsy is shown in (Fig. 2). In control group, onset of clonus and onset of tonic was at (38.8 ± 2.1) and (73.1 ± 5.2)

seconds respectively with survival of 1 out of 6 animals. In diazepam treated group, onset of clonus and onset of tonic was not detectable with survival of all 6 animals. In EEMP (250 mg/kg) treated group, onset of clonus and onset of tonic was at (153.1 ± 5.9, $p < 0.0001$) and (190.3 ± 8.1, $p < 0.0001$) seconds respectively with survival of 2 out of 6 animals. EEMP (500 mg/kg) treated group, onset of clonus and onset of tonic was at (194.8 ± 5.3, $p < 0.0001$) and (235.6 ± 8.8, $p < 0.0001$) seconds respectively with survival of 2 out of 6 animals. EEMP administration significantly reduced the latency to clonic and tonic convulsions in a dose-dependent manner. Also, the EEMP intervention significantly reduced the severity of clonic convulsions. Furthermore, Pretreatment with EEMP reduced mortality and offered protection against PTZ-induced seizures.

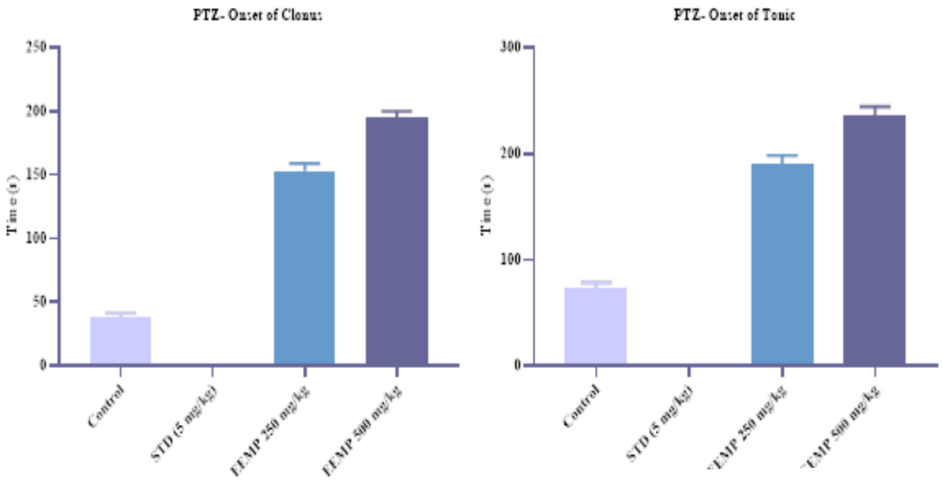

Figure 2. Effect of EEMP on PTZ-induced seizure activity in mice. Values are expressed as Mean ± SD for 6 animals per group.

Maximal electroshock induced convulsion

When compared to the control group, EEMP considerably reduced all convulsive phases induced by MES (tonic flexion phase, tonic extensor phase, clonic convulsion phase, and stupor). Similarly, mice administered with 25 mg/kg phenytoin were protected from MES-induced convulsions, as indicated by the absence of all convulsion phases. Effect of EEMP on MES induced convulsions is shown in (Fig. 3). The duration of HLTE (Hind Limb Tonic Extensor) and HLTF (Hind Limb Tonic Flexion) in control group was (18.1 ± 1.3) and (7.5 ± 0.9) seconds respectively with stupor at (128.5 ± 4.6) seconds. The duration of HLTE and HLTF in Phenytoin treated group was not detectable with stupor at (49.3 ± 2.7, $p < 0.0001$) seconds. In EEMP

(250 mg/kg), the duration of HLTE and HLTF was (5.3 ± 1.1, $p < 0.0001$) and (5.8 ± 0.8, $p < 0.0001$) seconds respectively with stupor at (88.3 ± 3.4, $p < 0.0001$) seconds. In EEMP (500 mg/kg), the duration of HLTE and HLTF was (4.8 ± 0.6, $p < 0.0001$) and (4.6 ± 0.7, $p < 0.0001$) seconds respectively with stupor at (67.5 ± 4.3, $p < 0.0001$) seconds. In the MES control group, no animals were recovered, whereas phenytoin administration resulted in complete recovery (100%) of all mice. At the same time, three-fourth of mice treated with EEMP recovered from MES.

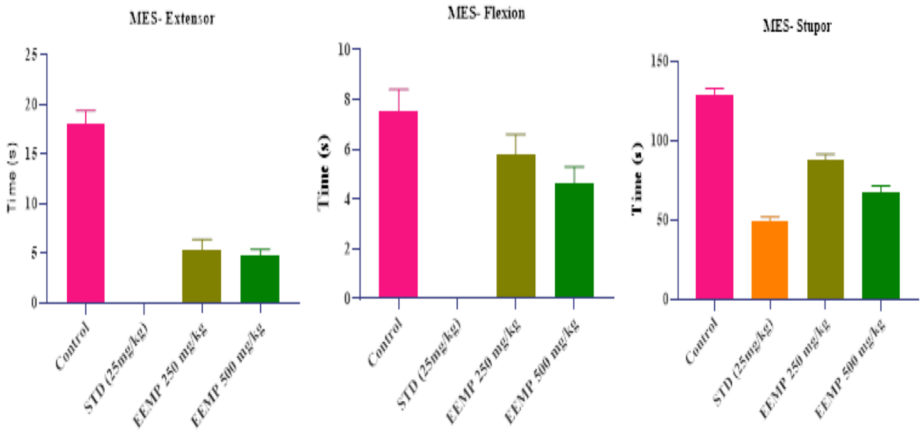

Figure 3. Effect of EEMP on MES-induced convulsions. Values are expressed as Mean ± SD for 6 animals per group.

Locomotor activity

As shown in Fig. 4, Control group didn't produce much change in locomotor activity (297.5 ± 10.2 - 283.5 ± 41.05). Both doses of EEMP 250 and 500 mg/kg exhibited less effectively reduction in locomotor activity (277.5 ± 61.5 - 261 ± 62.7, $p > 0.05$) and (296.1 ± 62.9 - 274.1 ± 37.9, $p > 0.05$) respectively. Diazepam (5 mg/kg, p.o) was used as a standard drug, which showed marked reduction in the locomotor activity from (250.1 ± 39.3, $p > 0.05$ - 126.6 ± 22.9, $p < 0.0001$). However, it can be concluded that EEMP did not severely affect the locomotor activity of mice after treatment.

Anxiolytic activity

Elevated plus maze model

The control animals preferred the closed (dark) arms and displayed anxiety-like signs such as immobility, freezing, and feces while entering the open arms. In control group, time spent and entries in open arm are (43.1 ± 4.9) (3 ± 0.8) respectively. In diazepam

treated group, time spent and entries in open arm are (239.8 ± 3.1, $p < 0.0001$) (12.5 ± 1.7, $p < 0.001$) respectively. The EEMP-treated animals (250 and 500 mg/kg, p.o) spent much more time, viz (177.5 ± 3.4, $p < 0.0001$) (6.1 ± 1.06, $p < 0.0001$) and (215.8 ± 3.4, $p < 0.0001$) (10.8 ± 1.06, $p < 0.0001$) (Fig. 5).

Light and Dark

Rodents in the light and dark arenas prefer to avoid entering and limit spontaneous exploratory behavior in the highly lighted region, which is a typical inclination when animal is introduced to an unusual environment [25]. Anxiety tends to show an increase in the number of entries and time spent bright arena (7.5 ± 0.95) (62.34 ± 10.66). The results given in fig. 6 indicate that the diazepam (5.0 mg/kg) treated mice showed a highly significant ($p< 0.0001$) increase in the number of bright chamber entries and time spent in bright arena compared to control group (10 ± 1.15) (120.50 ± 7.24). EEMP 250 mg/kg treated mice increased the number (8.16 ± 0.68, $p > 0.05$) and time spent (9.33 ± 0.94, $p < 0.0001$) in the bright arena. EEMP 500 mg/kg treated mice also significantly increased the number (107.90 ± 1.61, $p < 0.0001$) and time spent (116.20 ± 2.69, $p < 0.0001$) in the light compartment.

Brain GABA estimation

PTZ administration and MES model significantly reduced brain GABA levels in positive control mice compared to normal control animals. EEMP intervention dose-dependently restored brain GABA levels (Table 1) and Fig. 7.

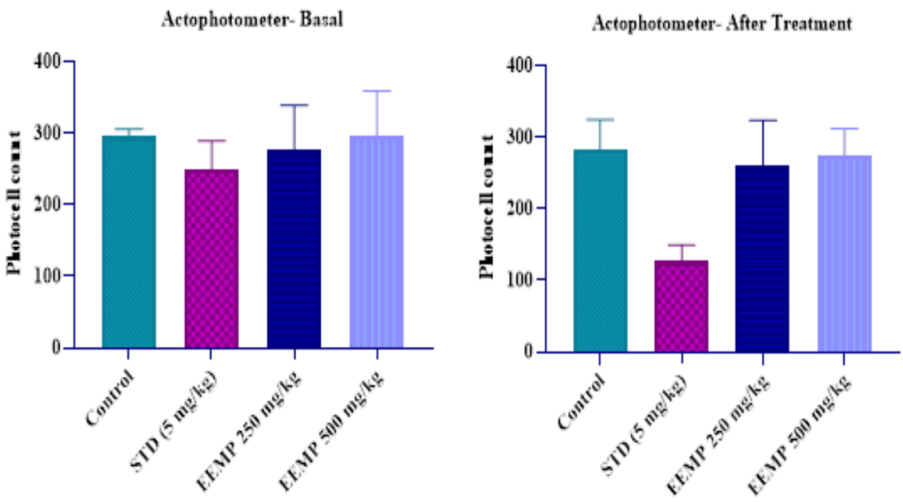

Figure 4. Effect of EEMP on locomotor activity (Actophotometer) in mice.

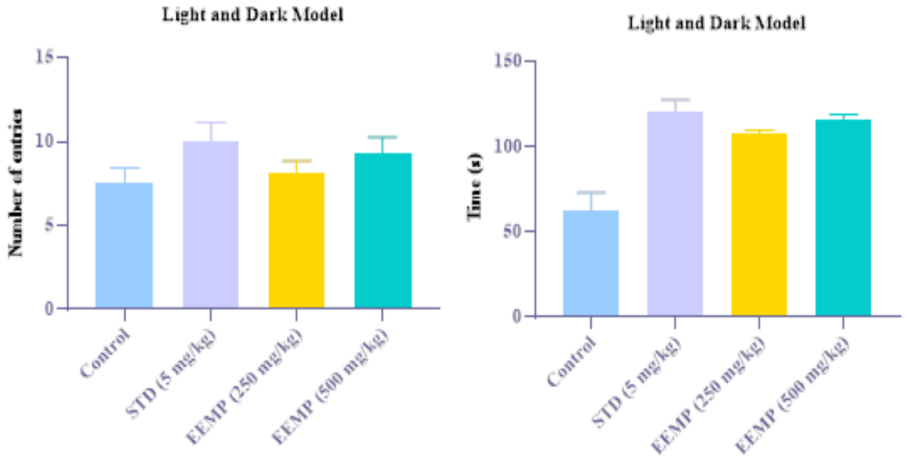

Figure 6. Effect of EEMP on light and dark model in mice.

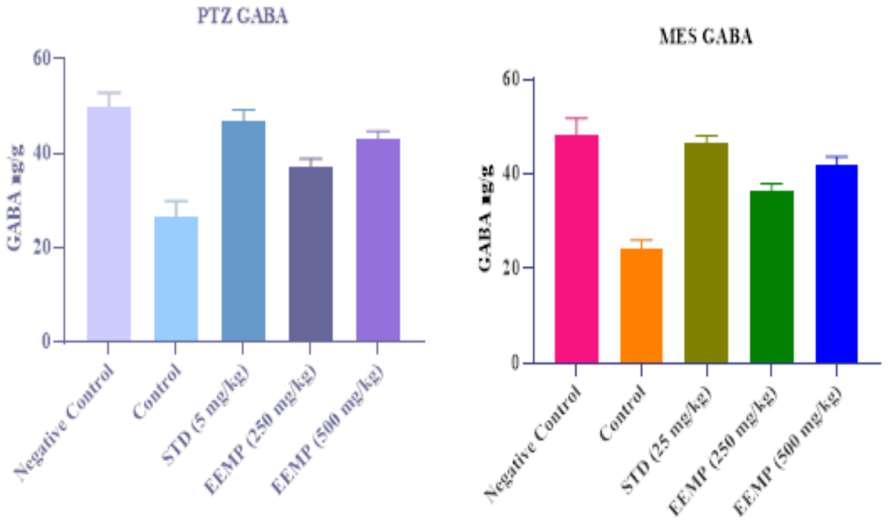

Figure 7. Effect of PTZ and MES on GABA.

Table 1. Effect of EEMP on GABA levels in mice brains.

Treatment Groups	GABA levels (ng/g of brain tissue)	
	PTZ	MES
Negative Control	48.43 ± 3.36	49.91 ± 2.96
Positive Control	24.33 ± 1.76	26.4 ± 3.29
STD (5 mg/ kg) Diazepam	46.37 ± 1.72	-
STD (25 mg/ kg) Phenytoin	-	46.97 ± 2.29
EEMP (250 mg/kg)	36.25 ± 1.72	37.11 ± 1.78
EEMP (500 mg/kg)	42.03 ± 1.66	43.04 ± 1.66

Histopathology

Figure 8 shows photomicrographs of section of brain of PTZ induced convulsion in mice. In negative control mice brain section, normal cells were noticed with natural architecture (8a). However, brain tissue from the positive control group showed neuronal loss after PTZ injection, with the histological variations observed like degeneration of pyramidal cells, necrosis and pyknosis, and degeneration of neurons (8b). Animal groups treated with standard drug (8c) and EEMP 500 mg/kg (8e) exhibits significant neuroprotective action as confirmed by protected neuronal structures and normal architecture with high neuronal density while in case of animal group treated with EEMP 250 mg/kg, generation of glial cells along with mild infiltration of pyramidal cells have been observed (8d).

Figure 9 illustrates the photomicrographs of the transverse sections of brain of MES induced convulsion in mice. Negative control group animal brains showed normal hilar neuronal cells without edema or cerebral congestion (9a). In the positive control group MES (50 mA, 0.20 s) delivered through auricular electrodes results a massive neuronal injury in all hippocampal segments showed neuronal loss along with marked cerebral congestion, and moderate infiltration (9b). Brain tissue in animals treated with standard drugs (9c) and EEMP 500 mg/kg (9e) exhibits gliosis having round nuclei and prominent nucleoli. There was no cerebral congestion or edema observed. Brain section from animals treated with EEMP 250 mg/kg exhibited trivial cerebral congestion or cerebral edema. The shrunken, argyrophilic neurons with glial cells are intermixed with apparently normal cells (9d).

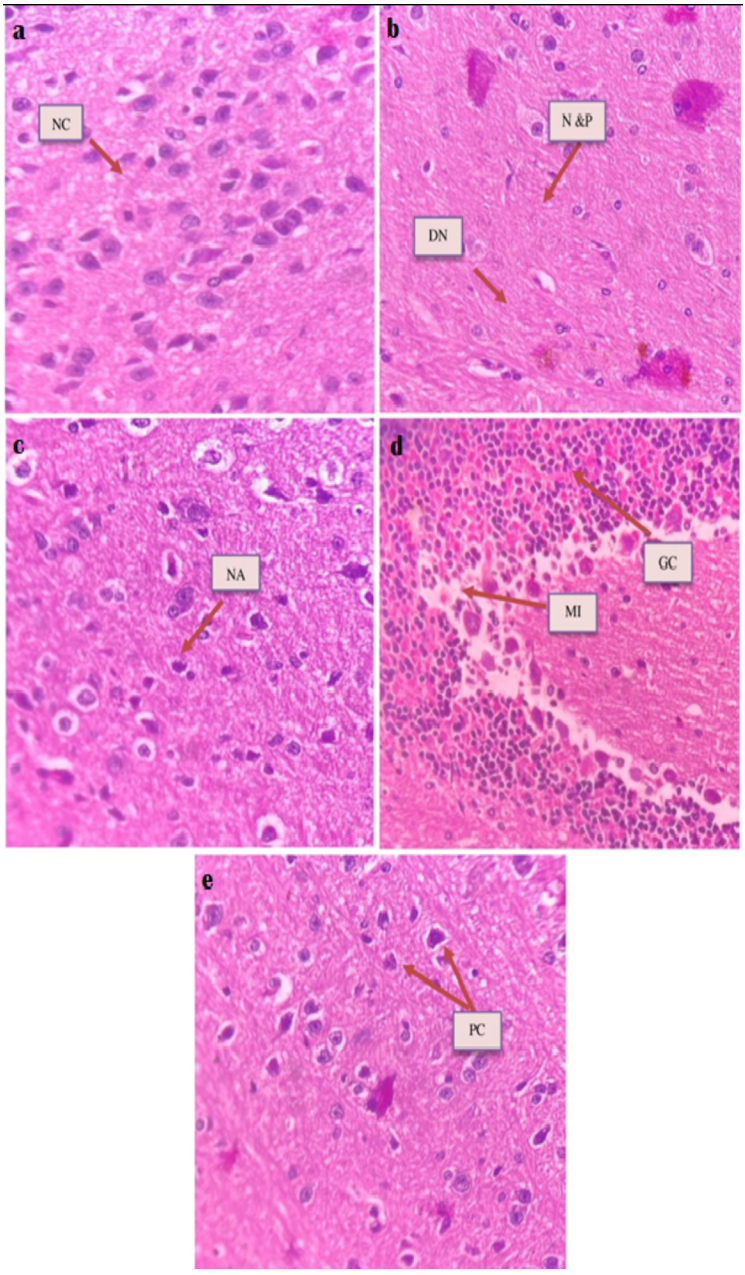

Figure 8. Photomicrographs of mice brain tissue (40x) in PTZ-model; a) Negative control; b) Positive control; c) Standard (5 mg/kg); d) EEMP 250 mg/Kg; e) EEMP 500 mg/Kg. Abbreviations: NA (Normal architecture); NC (Normal cells); DN (Disruption of neurons); MI (Moderate infiltration); GC (Glial cells); N & P (Necrosis & Pyknosis); PC (Pyramidal cells).

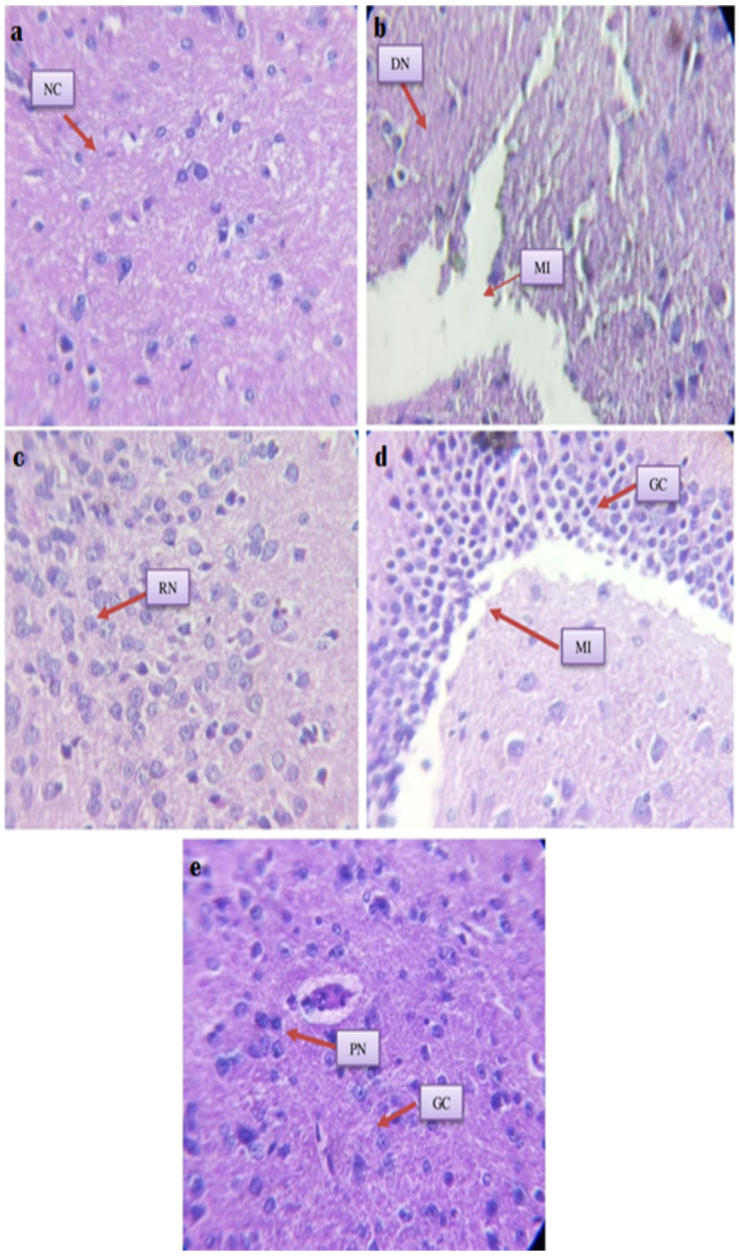

Figure 9. Photomicrographs of mice brain tissue (40x) in MES-model; a) Negative control; b) Positive control; c) Standard (25 mg/kg); d) EEMP 250 mg/Kg; e) EEMP 500 mg/Kg. Abbreviations: NC (Normal cells); DN (Disruption of neurons); MI (Moderate infiltration); GC (Glial cells), RN (Round nuclei); PN (Prominent nucleoli)

Discussion

Fear and anxiety are described as a victim's reaction to actual or specific dangers that may potentially harm its homeostasis. This reaction might be physiological or behavioral in nature. A few basic anxiety animal models, such as the elevated plus maze and the light dark model, have been used to measure anxiety-like behavior in mice. All of these therapies rely on subjecting the patient to unexpectedly unpleasant surroundings [26]. Epilepsy is one of the most common and serious brain illnesses. The term "seizure" refers to a brief change in behavior induced by chaotic, coordinated, and rhythmic firing of groups of brain neurons [27]; epidemiologic studies reveal that the prevalence and significance of epilepsy cannot be overstated. Most studies, however, have concluded that the average incidence of epilepsy in affluent countries is around 50 cases per 100,000 individuals per year, and that it rises rapidly with age [28]. The majority of anticonvulsant action in mice has been assessed using a few traditional animal models, such as PTZ-induced convulsions and MES-induced convulsions [29, 30].

Drugs that enhance brain GABA content, as well as the administration of centrally active GABA mimic agents, have been proven in studies to be a successful therapeutic method for the treatment of epilepsy. As a result, to determine the effect of the extract on GABA levels in the brain, the animals were treated with the extracts and GABA levels were determined using the HPTLC technique [24]. The ethanolic extract of seeds of *Mucuna pruriens* was tested for anxiolytic and anticonvulsant activity in the current study utilizing experimental models such as elevated plus maze test, light and dark test, locomotor activity by actophotometer, and anticonvulsant activity was studied using pentylenetetrazol induced convulsions and MES produced convulsions, followed by GABA estimate in mice brain tissues. The raised plus maze is presently one of the most extensively used animal anxiety models [18-20]. The test is essentially based on the observation that exposing an animal to an elevated maze array results in a considerably larger approach-avoidance conflict than exposing the animal to an open maze array. When exposed to a new environment, animals shun open entrance and prefer to remain in closed arms. The EEMP significantly increased the time spent and number of admissions into the open arm of our study, indicating that the test drugs may reduce fear and anxiety in mice. A considerable increase in efforts to enter the bright arena by EEMP treated mice in a dosage dependent manner may reflect an attempt to overcome the inhibition that is experienced under normal conditions.

In the pentylenetetrazol induced convulsions model, EEMP (250 and 500 mg/kg) significantly increased the onset of clonus, onset of tonus, and percentage protection when compared to the control group, whereas in the MES induced convulsions model,

EEMP (250 and 500 mg/kg) significantly decreased the duration of tonic extensor. GABA appears to be important in the pathogenesis of several neuropsychiatric disorders. Many traditional drugs used to treat mental illnesses are known to work in part by enhancing GABA activity, whilst some novel substances may have therapeutic advantages entirely through GABAergic actions. In this current study, three days of EEMP (250 and 500 mg/kg) treatment followed by GABA quantification demonstrated a significant rise in GABA levels in the brain that upturned the symptoms produced by MES and PTZ when compared to the control group. Histopathological studies also justify the antiepileptic and anxiolytic effect of EEMP as seen in Fig. 8 and Fig. 9 that in extract treated group of both the models of PTZ and MES, the neuronal loss was not present as compared to positive control group.

Conclusion

Mucuna pruriens may offer adequate protection against epileptic seizures, implying that it might be utilized to treat petitmal and grandmal epilepsy. This gives justification for its usage in traditional medicine to treat epilepsy and anxiety. Our findings are preliminary on the basis of preclinical studies, and further exhaustive research is required on mechanistic basis in future.

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Conflict of interest

None to declare

Ethics approval

This research approved by Institutional Animal Ethics Committee (IAEC) of United institute of Pharmacy with approval no. UIP/IAEC/Nov.-2021/14.

References

1. V.D. Thakur, S.A. Mengi, Neuropharmacological profile of *Eclipta alba* (Linn.) Hassk, *J. Ethnopharmacol.*, **102**(1), 23-31 (2005).

2. M. Emamghoreishi, M. Khasaki, M.F. Aazam, *Coriandrum sativum*: Evaluation of its anxiolytic effect in the elevated plus-maze, *J. Ethnopharmacol.*, **96**(3), 365-370 (2005).

3. M.G. Magaji, A.H. Yaro, A.M. Musa, J.A. Anuka, I. Abdu-Aguye, I.M. Hussaini, Central depressant activity of butanol fraction of *Securinega virosa* root bark in mice, *J. Ethnopharmacol.*, **141**(1), 128-133 (2012).

4. J.S.S. Quintans, A.R. Antoniolli, J.R.G.S. Almeida, V.J. Santana-Filho, L.J. Quintans-Júnior, Natural products evaluated in neuropathic pain models - a systematic review, *Basic Clin. Pharmacol. Toxicol.*, **114**(6), 442-450 (2014).

5. M. Pugalenthi, V. Vadivel, P. Siddhuraju, Alternative food/feed perspectives of an underutilized legume *Mucuna pruriens* var. utilis--a review, *Plant Foods Hum. Nutr.*, **60**(4), 201-218 (2005).

6. R.A. Vaidya, A.R. Sheth, S.D. Aloorkar, N.R. Rege, V.N. Bagadia, P.K. Devi, *et al.*, The inhibitory effect of the cowhage plant-*Mucuna pruriens*-and L-dopa on chlorpromazine-induced hyperprolactinaemia in man, Neurol. India, **26**(4), 177-178 (1978).

7. G. Hussian, B.V. Manyam, *Mucuna pruriens* proves more effective thanl-DOPA in Parkinson's disease animal model, *Phytother. Res.*, **11**(6), 419-423 (1997).

8. B.V. Manyam, M. Dhanasekaran, T.A. Hare, Neuroprotective effects of the anti-parkinson drug *Mucuna pruriens*, *Phytother. Res.*, **18**(9), 706-712 (2004).

9. L. Sathiyanarayanan, S. Arulmozhi, *Mucuna pruriens* Linn. - A comprehensive review, *Pharmacognosy Reviews*, **1**(1), 157-162 (2007).

10. M.C. Pant, L.D. Joshi, Identification of pharmacologically active substances in the seeds of Mucuna pruriens (Abs), In: *Proceedings of Annual Conference of Indian Pharmacological Society*, Lucknow, India, 1970, p. 24.

11. C.K. Kokate, *Practical Pharmacognosy*, Vallabh Prakashan, New Delhi, India, 1994, pp 107-109.

12. B.G. Rathod, N.M. Patel, Development of validated RP-HPLC method for the estimation of L-Dopa from *Mucuna pruriens*, its extracts and in Aphrodisiac formulation, *Int. J. Pharma Sci. Res.*, **5**(8), 508-513 (2014).

13. A.H. Gavishiddappa, S.D. Desai, P. Biradar, H.M. Nanjappaiah, H. Shivakumar, B.B. Ishwar, **Evaluation of acute oral toxicity and phytoconstituents of methanolic extract of** *Mucuna pruriens***,** *J. Pharm. Sci. & Res.*, **7**(1), 33-36 (2015).

14. A.B. Krishna, H.K. Manikyam, V.K. Sharma, N. Sharma, Acute oral toxicity study in rats with *Mucuna pruriens* seed extract, *Asian J. Plant Sci. Res.*, **6**(2), 1-5 (2016).

15. S. Golbabapour, M. Hajrezaie, P. Hassandarvish, N.A. Majid, A.H.A. Hadi, N. Nordin, *et al.*, Acute toxicity and gastroprotective role of *M. pruriens* in ethanol-induced gastric mucosal injuries in rats, *Biomed. Res. Int.*, **2013**, 974185 (2013).

16. A. Showraki, M. Emamghoreishi, S. Oftadegan, Anticonvulsant effect of the aqueous extract and essential oil of *Carum carvi* L. seeds in a pentylenetetrazol model of seizure in mice, *Iran. J. Med. Sci.*, **41**(3), 200-208 (2016).

17. H.G. Vogel, W.H. Vogel, *Drug Discovery and Evaluation: Pharmacological Assays*, Springer, 2000, pp. 230-244.

18. S. Pellow, S.E. File, Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: A novel test of anxiety in the rat, *Pharmacol. Biochem. Behav.*, **24**(3), 525-529 (1986).

19. S. Hogg, A review of the validity and variability of the elevated plus-maze as an animal model of anxiety, *Pharmacol. Biochem. Behav.*, **54**(1), 21-30 (1996).

20. R.J. Rodgers, N.J. Johnson, Behaviorally selective effects of neuroactive steroids on plus-maze anxiety in mice, *Pharmacol. Biochem. Behav.*, **59**(1), 221-232 (1998).

21. J. Crawley, F.K. Goodwin, Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines, *Pharmacol. Biochem. Behav.*, **13**(2), 167-170 (1980).

22. J.S. Sancheti, M.F. Shaikh, P.F. Khatwani, S.R. Kulkarni, S. Sathaye, Development and validation of a HPTLC method for simultaneous estimation of L-glutamic acid and γ-aminobutyric acid in mice brain, *Indian J. Pharm. Sci.*, **75**(6), 716-721 (2013).

23. Z. Kazmi, S. Zeeshan, A. Khan, S. Malik, A. Shehzad, E.K. Seo, *et al.*, Anti-epileptic activity of daidzin in PTZ-induced mice model by targeting oxidative stress and BDNF/VEGF signaling, *Neurotoxicology*, **79**, 150-163 (2020).

24. A.E. Arrant, N.L. Schramm-Sapyta, C.M. Kuhn, Use of the light/dark test for anxiety in adult and adolescent male rats, *Behav. Brain Res.*, **256**, 119-127 (2013).

25. R.S. Fisher, W. van Emde Boas, W. Blume, C. Elger, P. Genton, P. Lee, *et al.*, Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE), *Epilepsia*, **46**(4), 470-472 (2005).

26. C. Belzung, G. Griebel, Measuring normal and pathological anxiety-like behaviour in mice: a review, *Behav. Brain Res.*, **125**(1-2), 141-149 (2001).

27. A.E. Cavanna, Epilepsy and disorders of consciousness, *Behav. Neurol.*, **24**(1), 1 (2011).

28. E. Beghi, The epidemiology of epilepsy, *Neuroepidemiology*, **54**(2):185-191 (2020).

29. V. Bhosle, Anticonvulsant and antioxidant activity of aqueous leaves extract of *Desmodium triflorum* in mice against pentylenetetrazole and maximal electroshock induced convulsion, *Rev. Bras. Farmacogn.*, **23**(4), 692-698 (2013).

30. A.M. Alshabi, I.A. Shaikh, S.M.B. Asdaq, The antiepileptic potential of *Vateria indica* Linn in experimental animal models: Effect on brain GABA levels and molecular mechanisms, *Saudi J. Biol. Sci.*, **29**(5), 3600-3609 (2022).

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