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Anticonvulsant activity of Rudraksha (*Elaeocarpus angustifolius* Blume) bark against picrotoxin-induced convulsions in rats

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Epilepsy is a neurological condition characterized by recurrent electrical activity in the brain's temporal lobe. It is a chronic disorder marked by an imbalance between excitatory and inhibitory neurotransmitters, resulting in uncontrolled excitability. Current antiepileptic drugs often have limited effectiveness and pose challenges in patient management. Literature surveys indicate that various species of *Elaeocarpus* have demonstrated antimicrobial, antioxidant, antidiabetic, antiasthmatic, antihypertensive, and antidepressant properties. Since Rudraksha (*Elaeocarpus angustifolius* Blume) is traditionally used to treat epilepsy and other brain-related disorders, the present study aimed to validate this claim by evaluating the anti-epileptic activity of the ethanolic bark extract of *E. angustifolius* on picrotoxin-induced convulsive rats. The extract at doses of 200 and 400 mg/kg BW was administered to rats for 14 days, followed by a single dose of picrotoxin (7.5 mg/kg *i.p.*) on the 14th day, with diazepam (12 mg/kg *i.p.*) used as a reference drug. Various oxidative parameters such as γ-aminobutyric acid (GABA), lactoperoxidase (LPO), superoxide dismutase (SOD), glutathione (GSH), and nitrate were estimated at the end of the treatment period. In the picrotoxin-induced convulsion model, treatment with ethanolic bark extract at 400 mg/kg caused a significant (p <0.01) decrease in LPO levels and a significant (p <0.001) increase in GABA levels in epileptic rats. The results concluded that the extract at a 400 mg/kg dose showed the most significant effect on picrotoxin-induced convulsions in rats.

Keywords: Anti-epileptic activity, Diazepam, GABA, Neurological disorders, Picrotoxin

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Introduction

Epilepsy, affecting individuals of all ages, stands as the prevalent chronic brain disorder globally. Surpassing 50 million cases worldwide, with almost 80% residing in low- and middle-income nations[1]. From ancient times, when epilepsy was attributed to curses or divine causes and treated with incantations, herbs, rituals, and magic, the understanding has evolved into a modern scientific perspective[2]. This entails recognizing the pivotal role of abnormal neuronal firing and excitatory neurotransmitters in precipitating seizures in patients[3]. Various agents, such as antiepileptic drugs, are accessible for treating different seizure types, aiming to diminish both the frequency and severity of seizures while maintaining acceptable levels of side effects. The perfect antiepileptic medication would effectively suppress all seizures without eliciting any adverse effects. Unfortunately, current drug options such as carbamazepine, phenytoin, valproic acid, and lamotrigine not only prove ineffective in controlling seizure activity for certain patients but also frequently result in side effects[4]. Approximately 90% of traditional medicines globally originate from medicinal plants such as Ginseng (*Panax ginseng*), Turmeric (*Curcuma longa*), St. John's Wort (*Hypericum perforatum*), and Ginger (*Zingiber officinale*), and are typically utilized in their crude forms[5]. One major benefit of herbal therapy is its potent therapeutic efficacy and minimal adverse effects. The remedial properties of medicinal plants can be attributed to secondary metabolites like alkaloids, flavonoids, steroids, and terpenoids[6].

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Furthermore, primary metabolites such as carbohydrates, proteins, vitamins, and lipids are pivotal for human health, particularly in muscle development and energy generation. Notably, *Securidaca longipedunculata* (Polygalaceae) has been documented to possess such properties, while *Mitragyna inermis* (Rubiaceae) contains alkaloids structurally resembling clinically effective anticonvulsants. Additionally, *Celtis integrefolia* (Ulmaceae) has been identified as containing γ-amino butyric acid (GABA), the deficiency of which could potentially result in seizures[7].

Elaeocarpus angustifolius Blume (Fig. 1) (synonyms *E. ganitrus* and *E. sphaericus*), also known as Rudraksha in Sanskrit and Rudraki in Hindi, belonging to the Elaeocarpaceae family, is typically cultivated in the Himalayan region of India. It is prized for both its appealing fruit stones and its therapeutic qualities[8]. The cultivation of Rudraksha extends from the Gangetic Plain at the base of the Himalayas to regions across Southeast Asia, including Indonesia, Guam, and Hawaii. These trees thrive in mountainous and hilly terrain, particularly in Nepal, Indonesia, Java, Sumatra, and Burma. Rudraksha is widespread along the foothills of all districts in Arunachal Pradesh, except Tawang and upper Subansiri, as well as some other high-altitude regions[9]. A total of 489 accepted species of *Elaeocarpus* are distributed across various regions worldwide, including Australia, East Asia, Malaysia, and the Pacific Islands. The native of this genus spans from the Western Indian Ocean to Tropical and Subtropical Asia, extending to the Pacific region[10]. *E. angustifolius* holds significant importance in ethnomedicine, with various parts displaying a range of pharmacological effects[11]. A thorough review of the literature reveals that *E. angustifolius* serves as a notable reservoir of numerous pharmacologically active compounds, including quercetin, geraniin, 3,4,5-trimethoxy geraniin, grandisines, rudrakine, gallic acid, ellagic acid, palmitic acid, and linoleic acid[12]. In folk medicine, it finds application in treating stress, anxiety, depression, palpitations, nerve pain, epilepsy, migraines, asthma, hypertension, arthritis, and liver diseases[13]. As per Ayurveda, wearing Rudraksha beads proves beneficial in regulating heartbeats and enhancing the nervous system[14]. The current study aimed to assess the anticonvulsant effects of ethanolic bark extracts of *E. angustifolius* (EBEEA) on seizures induced by picrotoxin in Wistar albino male rats.

Fig. 1 — *Elaeocarpus angustifolius* tree with a fruiting bud.

Materials and Methods

Plant material and preparation of extract

The bark of *E. angustifolius* was collected from Dehradun, Uttarakhand, in April 2021. The Botanical Survey of India, Dehradun, authenticated the plants with Accession No. 842. The bark, cleaned with plenty of distilled water, was air-dried for 12 days and powdered with the help of a mixer grinder. The coarse powder was extracted with 95% ethanol using a

Soxhlet apparatus. The extract was filtered and dried under reduced pressure. The total yield of the ethanol extract was found to be 30.32%. The extract was kept in an airtight container and stored at 4°C until further use.

Animals

Adult Wistar albino male rats weighing 200-250 g were utilized for the present study. They were housed in hygienic propylene cages and provided *ad libitum* access to food and water. The rats were grouped in sets of six per cage and maintained under standard conditions of room temperature (24±2°C), humidity (50-70%), and a 12-12 hour light-dark cycle. The experiment was conducted in a noise-free environment. Compliance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA) of the Government of India was ensured. Prior permission was obtained from the Institutional Animal Ethical Committee (Application No. CPCSEA/IAEC/SBS/ 2022/02, Protocol No. 273/PO/Re/S/2000/CPCSEA) for conducting animal experiments.

Acute toxicity study

The rats were divided into two groups of six each, regardless of sex, to evaluate acute toxicity following the guidelines of the Organization for Economic Co-operation and Development (OECD-TG 423)[15]. Group 1 served as the control and received a normal diet, while Group 2 received a single oral dose of EBEEA at 2000 mg/kg body weight, in addition to a normal diet. All rats were continuously monitored every hour on the first day and then daily for fourteen days to assess various parameters, including skin condition, fur appearance, eye health, respiratory pattern, salivation, diarrhoea, urination, tremors, ptosis, relaxation, gait, posture, lethargy, sleep, coma, and food/water intake, as well as any other behavioural changes. LD_{50} values were calculated, and a safe dose range was selected for the main study.

Qualitative phytochemical analysis

The extract obtained from the bark of *E. angustifolius* was subjected to various chemical tests to identify chemical constituents such as saponin, flavanoid, alkaloids, tannin, phenol, glycoside, steroids, and carbohydrates.

Quantitative phytochemical analysis

Estimation of alkaloid content

The bark extract (5 g) was mixed with 200 mL of 10% acetic acid in ethanol (purity 95%) and left to stand at room temperature for 4 hours. Subsequently, the mixture was filtered and concentrated to one-fourth of the total volume. Ammonium hydroxide was added drop by drop to the concentrated mixture until a precipitate formed. The precipitate was allowed to settle, filtered, and washed with dilute ammonium hydroxide. The resulting residue was weighed and recorded as the total alkaloid content[16]. The following formula was used to calculate the total alkaloid content.

$$\text{Alkaloid (\%)} = \frac{\text{(Weight of residue)}}{\text{(Weight of sample)}} \times 100$$

Estimation of flavonoid content

The aluminium chloride colourimetric assay was employed to estimate the total flavonoid content. A standard calibration curve of quercetin was created for estimation purposes. Stock solutions (1000 ppm) of quercetin and EBEEA were prepared and serially diluted using methanol to achieve 100 to 1000 µg/mL concentrations. One mL of various test solution concentrations was pipetted into test tubes. Subsequently, four mL of distilled water, followed by 0.3 mL each of 5% $NaNO_2$ and 10% $AlCl_3$, were added to each test tube. The samples were then incubated for 30 minutes at 25°C, after which 2 mL of 1M NaOH was added. Following this, the samples were allowed to stand until a yellowish-orange colour appeared, and finally, the volume of each test tube was adjusted to 10 mL with distilled water. The absorbance was measured at 510 nm using a colourimeter. The concentration of total flavonoid content was calculated from the calibration plot ($y = 0.0004x + 0.1994$; $R2 = 0.9983$) and expressed as mg quercetin equivalent (QE)/g[17].

Estimation of tannin content

A volume of 100 µL of a 10 mg/mL extract was dispensed into a test tube containing 7.5 mL of distilled water. The mixture was then vortexed after adding 0.5 mL of Folin-Ciocalteu reagent. Subsequently, 1 mL of a 35% sodium carbonate solution was added to the mixture, which was then transferred to a 10 mL volumetric flask. The volume of the mixture was adjusted to 10 mL with distilled water, and the mixture was shaken and left at room temperature for 30 minutes in the dark. Reference standard solutions (1-0.625 mg/mL) of gallic acid were prepared, and the absorbance of these solutions was measured against a blank prepared in the same manner as the test solution but without adding any

extract. Absorbance was measured at 725 nm using a UV-visible spectrophotometer. The standard calibration curve equation for gallic acid was determined to be y = 0.0101x + 0.2193, with an R2 value of 0.999. Tannin content was expressed as mg gallic acid equivalence per gram of extract (mg GAE/g extract)[18].

Estimation of saponin content

One gram of extract was added to 50 mL of 20% ethanol and heated for 4 hours at 55°C in a water bath with occasional stirring. The filtrate obtained after filtration was set aside, and the residue was then mixed with 50 mL of 20% ethanol, repeating the same process. The filtrates from both steps were combined and concentrated to 10 mL at 90°C. Next, 20 mL of diethyl ether was added and thoroughly mixed. The upper ether layer was discarded, while the lower portion was collected and mixed with 20 mL of n-butanol, followed by 5 mL of 5% aqueous NaCl. The upper butanol layer was separated and dried in an oven. The resulting residue was weighed and recorded as the total saponin content[19]. Total saponin content was calculated using the following formula.

$$\text{Saponin (\%)} = \frac{(\text{Weight of residue})}{(\text{Weight of sample})} \times 100$$

Experimental design

The animals were divided into four groups, each consisting of six rats. The first group was the normal control and received normal saline (5 mL/kg, *p.o.*). The second group received diazepam (12 mg/kg, *i.p.*) on the 14th day and served as the standard group. Groups 3 and 4 were treated as test groups and received EBEEA at doses of 200 and 400 mg/kg, *p.o.*, respectively, for 14 days. The doses were calculated based on LD_{50} value, i.e. 2000 mg/kg. On the 14th day, all groups received picrotoxin (7.5 mg/kg, *i.p.*) 45 minutes after administration of the vehicle or extract and 30 minutes after the standard[20]. Subsequently, behavioural parameters such as the onset of clonic convulsion, duration of convulsion, and mortality were noted over the next 30 minutes.

Biochemical examination and histology of brain

At the conclusion of the 14th day, the rats from each group were euthanized using cervical dislocation, and their brains were carefully extracted for the examination of biochemical parameters and histology. One-half of each brain was diced into small pieces after being rinsed in ice-cold normal saline, and the tissue was homogenized with Tris buffer (10% w/v) at 10,000 rpm for 10 minutes. The resulting supernatant was then separated and utilized as brain homogenate for the estimation of GABA, LPO, SOD, GSH, and Nitrate levels[21-25]. All reagents used for the biochemical parameters were kept at cold temperatures. The remaining half of each brain was rinsed with normal saline and preserved in a 5% formalin solution at room temperature for subsequent histological examination.

Estimation of LPO

The brain homogenate (2 mL) was mixed with 2 mL of 20% trichloroacetic acid and centrifuged at 2,000 rpm for 10 minutes. After centrifugation, the supernatant (3 mL) was separated and combined with 2 mL of thiobarbituric acid (0.067% in 1M Tris hydrochloride, pH 7), and the absorbance was measured at 535 nm. Distilled water was used instead of brain homogenate for the blank. The equation derived from the standard curve of lipid peroxidation was Y = 0.0113X – 1.0061, where Y represents the absorbance of the test sample and X indicates the concentration of malondialdehyde in the test sample, calculated as X = Y + 1.0061/0.0113 nmol/mL.

Estimation of SOD

Brain homogenate (0.5 mL) was diluted with 0.5 mL of distilled water and combined with 0.25 mL of ethanol and 0.15 mL of chloroform. The mixture was shaken and then centrifuged at 2,000 rpm for 10 minutes, after which the supernatant was separated. Next, the supernatant (0.5 mL) was mixed with 0.5 mL of carbonate buffer, 0.5 mL of EDTA, and 0.4 mL of epinephrine, and the absorbance was measured at 480 nm at intervals from zero to 3 minutes, with 0.5-minute intervals. A blank was prepared without adding brain homogenate and epinephrine. SOD activity was calculated using the standard curve (-0.008X - 0.487) in EU/dL, where X represents the final absorbance (at 3 min) minus the initial absorbance (at 0 min).

Estimation of GSH

The homogenate (1 mL) was combined with 1 mL of 10% trichloroacetic acid and centrifuged for 10 minutes at 2,000 rpm to separate the supernatant. Subsequently, the supernatant was mixed with 4 mL of DTNB reagent and 1.5 mL of phosphate buffer. The mixture was left at room temperature for 5 minutes, after which the absorbance was measured at

412 nm. GSH was quantified in mg of GSH per gram of tissue using the equation derived from the standard curve of reduced glutathione (Y = 0.006X + 0.144), where Y represents the absorbance of the test sample, and X indicates the concentration of reduced glutathione in the test sample.

Estimation of nitrate

The brain homogenate (2 mL) was diluted with 2 mL of distilled water and centrifuged at 2,000 rpm for 5 minutes to separate the supernatant. Next, the supernatant (1 mL) was combined with 1 mL of Griess reagent, and the mixture was incubated for 10 minutes at room temperature in the dark. The absorbance was measured at 540 nm against the blank. The concentration of nitrate/nitrite in the supernatant was determined in µmol/L using a sodium nitrite standard curve (Y = 0.005X - 0.012), where Y represents the absorbance of the test sample and X indicates the concentration of nitrate/nitrite in the test sample.

Estimation of GABA

The brain homogenate obtained after homogenization was transferred to a container containing 8 mL of chilled absolute alcohol and placed in a refrigerator at 0°C for 1 hour. Afterwards, it was centrifuged at 16,000 rpm for 10 minutes, and the supernatant was collected in a Petri dish and washed with alcohol. The collected mass was then dried in a water bath at 70°C, mixed with 1 mL of water and 2 mL of chloroform, and centrifuged at 2,000 rpm for 5 minutes. The upper layer containing GABA was isolated and spotted onto Whatman filter paper for paper chromatography, using a mixture of n-butanol (50 mL), acetic acid (12 mL), and water (60 mL) as the mobile phase. After drying the paper with hot air, a 0.5% ninhydrin solution in 95% ethanol was spread onto it and dried again. The blue-coloured spot on the paper was then cut and heated with 2 mL of ninhydrin solution in a water bath for 5 minutes, then adding 5 mL of water and allowing it to stand for 1 hour. The supernatant was removed, and the absorbance was measured at 570 nm. A standard curve for GABA was prepared from a stock solution (1 mg/mL in 0.01N HCl). The standard equation for GABA was found to be Y = 0.0055X + 0.2816.

Statistical analysis

The statistical analysis of the data was conducted using GraphPad 9.0 software. All values were presented as mean±SEM. One-way ANOVA, followed by Dunnett's multiple comparison test, was applied for statistical analysis. A *p*-value of less than 0.001 was considered highly significant, less than 0.01 was considered moderately significant, while greater than 0.05 was considered not significant.

Results

Phytochemical analysis

The qualitative phytochemical analysis of the ethanolic bark extract of *E. angustifolius* indicated the presence of alkaloids, tannins, saponins, and flavonoids. Subsequently, based on the preliminary phytochemical analysis, quantitative analysis was conducted to determine the total alkaloid, saponin, flavonoid, and tannin contents. The quantities of these constituents present in the dry mass of the bark are presented in Table 1.

Acute toxicity test

All the rats treated with a single dose of EBEEA at 2000 mg/kg exhibited normal behavioural, motor, and neuronal functions without any mortality during the 14-day study period. The condition of the skin, fur, eyes, respiratory pattern, as well as autonomic nervous system (ANS) and central nervous system (CNS) characteristics in the treated rats remained unaffected. Moreover, the water and feed intake patterns of the rats were regular and consistent throughout the study period. The selection of dose levels was based on OECD-TG 423[15], which determined 200 and 400 mg/kg doses.

Effect on picrotoxin-induced convulsions

The impact of EBEEA on the onset and duration of convulsions is detailed in Table 2. The findings revealed that the treatment offered partial protection against picrotoxin-induced convulsions but did not entirely suppress them. Additionally, the results indicated that the group pre-treated with diazepam showed no signs of convulsions. EBEEA at a dosage of 400 mg/kg conferred highly significant protection (p <0.001) against the onset of clonic convulsions and moderate protection (p <0.01) against the duration of convulsions in rats.

Table 1 — Total alkaloid, flavonoid, saponin and tannin contents present in the bark of *Elaeocarpus angustifolius*

S. No.	Content	Availability
1	Total alkaloid content (%)	48.8±0.61
2	Total flavonoid content (mg QE/g)	34±1.44
3	Total saponin content (%)	5.03±0.05
4	Total tannin content (mg GAE/g)	15.27±0.630

Effect on antioxidant enzymes

The impact of EBEEA on various enzyme levels in the brain is outlined in Table 3. The results indicated that administration of the extract at a dosage of 400 mg/kg for 14 days had a significant (p <0.001) effect on reducing nitrate levels and a moderately significant (p <0.01) effect on reducing LPO levels in epileptic rats compared to the control group. At a dosage of 200 mg/kg, it demonstrated a moderately significant (p <0.01) reduction in nitrate levels, whereas the reduction in LPO levels was the least significant (p <0.05). A significant (p <0.001) increase was observed in the GSH and SOD levels of rats receiving the 400 mg/kg extract. However, this effect was moderate at 200 mg/kg compared to the control group. Overall, higher doses of the extract increased GSH and SOD levels and decreased LPO and nitrate levels.

Effect on GABA level

The results in Fig. 2 demonstrate a significant (p <0.001) increase in GABA levels in epileptic rats treated with 400 mg/kg of EBEEA for 14 days. This activity was comparable to the standard group's (p <0.001). However, at a dose of 200 mg/kg, the extract exhibited a moderately significant (p <0.05) increase in GABA levels in experimental rats.

Table 2 — Effect of bark extract of *Elaeocarpus angustifolius* on picrotoxin induced convulsions in rats

Group	Onset of clonic convulsions (min)	Duration of convulsion (min)	Mortality (%)
Control (normal saline)	1.45±4.21	11.53±1.06	100%
Standard (Diazepam)	28.20±2.05***	1.25±1.770***	0%
Test 1 (200 mg/kg)	14.39±2.99*	7.02±1.39ns	33.33%
Test 2 (400 mg/kg)	20.58±2.65***	3.09±2.01**	0%

Values expressed as mean±SEM for n =6; *p <0.05, **p <0.01, ***p <0.001, ^{ns}p >0.05; results compared with the control group

Effect on histology of brain

The histology of the brains from all groups was conducted to observe any changes occurring after treatment (Fig. 3). The brain histology of the rats in the control group (Fig. 2a) revealed brain parenchyma with glial cells in a nondescript pattern within a fibrillary matrix, gemistocytes, prominent chronic inflammatory infiltrate, prominently congested blood vessels, fibrinous exudates, significant oedema, and microcystic changes with granulation tissue. The standard group (Fig. 2b) exhibited brain parenchyma with glial cells in a nondescript pattern within the fibrillary matrix, mild chronic inflammatory infiltrate, and mild oedema. In the group treated with 200 mg/kg of EBEEA (Fig. 2c), the brain parenchyma displayed various regions with glial cells, few gemistocytes noted, significant chronic inflammatory infiltrate, many congested blood vessels, oedema, and microcystic changes. However, the group treated with 400 mg/kg (Fig. 2d) showed gemistocytes distributed variably,

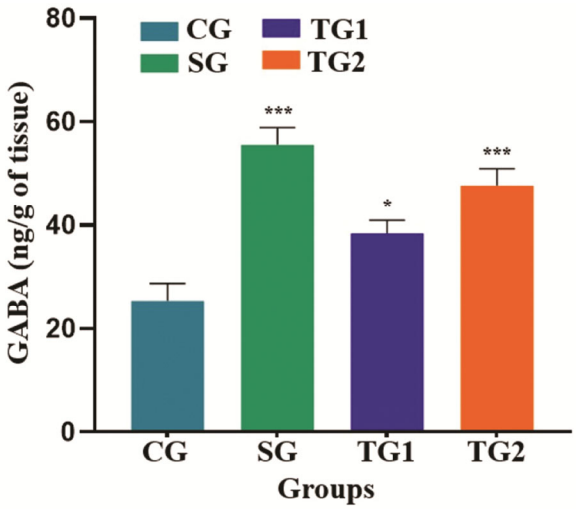

Fig. 2 — Effect of bark extract of *Elaeocarpus angustifolius* on GABA level in convulsive rats. Results expressed as mean±SEM for n = 6; *p <0.05, ***p <0.001; results compared with the control group; CG = Control group, SG = Standard group, TG1 = Test group 1 and TG2 = Test group 2.

Table 3 — Effect of bark extract of *Elaeocarpus angustifolius* on LPO, Nitrate, GSH and SOD levels in picrotoxin-induced convulsive rats

Group	LPO (nmol/mL)	Nitrate (µmol/L)	GSH (mg GSH/g)	SOD (EU/dL)
Control (normal saline)	98.53±2.73	140.73±4.65	67.04±3.76	43.41±3.47
Standard (Diazepam)	79.22±2.75***	41.68±4.89***	118.76±3.98***	90.90±2.89***
Test 1 (200 mg/kg)	89.23±2.39*	115.16±4.19**	85.78±3.12**	63.21±3.22**
Test 2 (400 mg/kg)	84.42±2.08**	61.61±4.85***	103.52±3.89***	81.05±2.19***

Values expressed as mean±SEM for n = 6; *p <0.05, **p <0.01, ***p <0.001; results compared with the control group

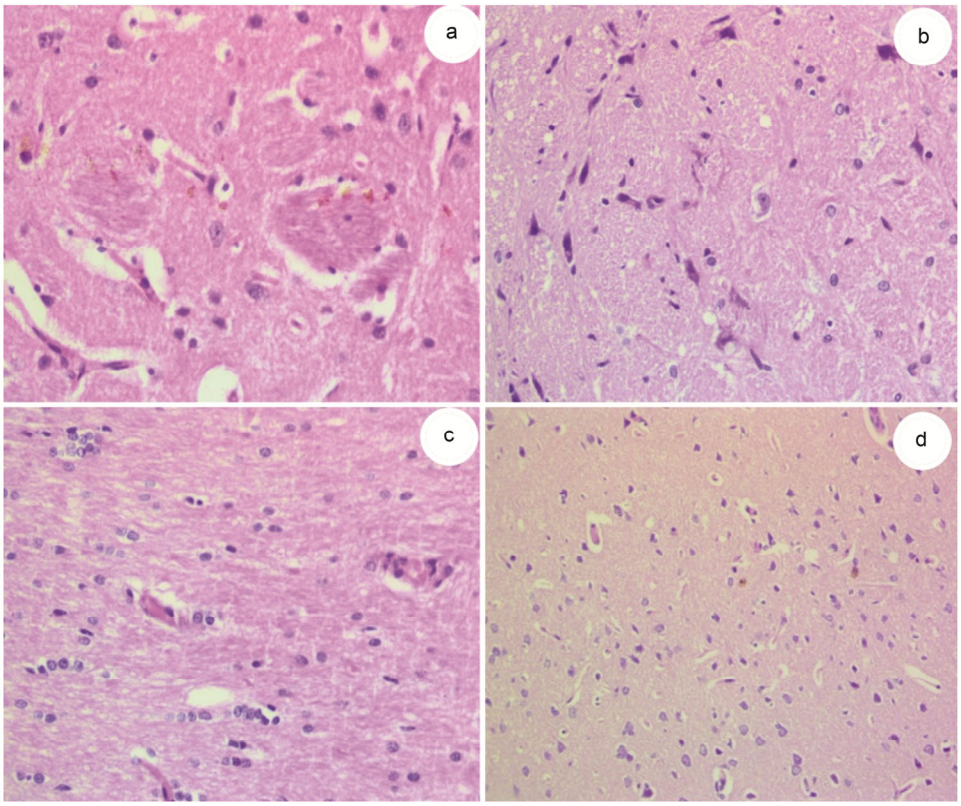

Fig. 3 — Effect of bark extract of *Elaeocarpus angustifolius* on histology of brain in picrotoxin-induced convulsive rats. a) Control group; b) Standard group; c) Test group 1 (200 mg/kg); and d) Test group 2 (400 mg/kg).

moderate chronic inflammatory infiltrate, minimal oedema, and occasional microcystic changes.

Discussion

Earlier reports based on traditional knowledge have indicated that Rudraksha (*E. angustifolius*) is utilized to treat neurological disorders, including epilepsy. Hence, the present study was designed to validate the traditional claim regarding the plant's efficacy against epilepsy. It is well-established that recurrent and spontaneous epileptiform discharges in the brain are characteristic of epilepsy. These abnormal discharges result from an imbalance between excitatory and inhibitory inputs, leading to over-excitation and synchronization of cortical neuronal networks[26]. This hyperactivity can induce epileptic seizures and contribute to epileptogenesis, the progressive conversion of a healthy brain into an epileptic one[27].

Presently, researchers are exploring plant-derived drugs due to their specific therapeutic properties and relatively low adverse effects. Currently, available antiepileptic drugs exhibit limited efficacy and pose challenges in patient management. Medicinal plants represent the oldest source of medicine in human history, leading to the identification, characterization, and isolation of active chemical constituents with therapeutic potential, which serve as the basis for synthetic drugs.

The pathophysiology of degenerative and chronic neurological disorders like epilepsy involves oxidative stress, manifested by convulsive episodes. Reactive oxygen or nitrogen species and reduced enzymatic or non-enzymatic defences affect cellular processes essential for brain function, leading to mitochondrial dysfunction, DNA damage, altered neural signalling, and inhibition of neurogenesis. Inflammation is also implicated in seizure activity, capable of initiating or sustaining seizures[28].

The brain is particularly susceptible to oxidative damage due to its high aerobic metabolic demand and iron load[29]. Prolonged seizures result in increased cellular superoxide production, overwhelming endogenous antioxidant defences. SOD converts superoxide to less harmful species, thereby mitigating oxidative stress[30]. The present study revealed that EBEEA significantly increased SOD levels in rats, suggesting its potential as an antioxidant.

Additionally, the extract reduced MDA levels, indicative of decreased lipid peroxidation associated with epilepsy. NO is implicated in epileptiform hyperactivity, produced by neuronal nitric oxide synthase (nNOS) activation following NMDA receptor stimulation. Aberrant NMDA receptor activation contributes to epileptogenesis[31]. The study demonstrated a significant reduction in nitrate levels in convulsive rats following EBEEA treatment, suggesting its ability to alleviate oxidative stress.

GABA is the primary inhibitory neurotransmitter in the central nervous system (CNS), modulating neuronal excitability. Enhanced GABA levels exert an anticonvulsant effect via GABAergic pathways[32]. The extract significantly increased GABA levels in the brain, potentially contributing to its anticonvulsant properties. Previous research has highlighted the role of phytoconstituents like alkaloids, flavonoids, tannins, and saponins in anti-epileptic activity[33]. The bark extract of *E. angustifolius* contains similar constituents, potentially responsible for elevating GABA levels in the brain.

Conclusion

In conclusion, this study explored the potential anticonvulsant effects of *E. angustifolius* ethanolic bark extract (EBEEA) in a rat model of picrotoxin-induced epilepsy. The phytochemical analysis revealed the presence of alkaloids, tannins, saponins, and flavonoids in the bark extract. EBEEA exhibited no adverse effects at a dosage of 2000 mg/kg over a 14-day period, indicating its safety profile. Notably, EBEEA demonstrated a significant delay in convulsion onset and reduction in duration, particularly evident at 400 mg/kg. Furthermore, treatment with EBEEA resulted in decreased nitrate and lipid peroxidation levels, coupled with increased glutathione and superoxide dismutase levels, indicating antioxidant activity. Additionally, EBEEA administration led to elevated GABA levels in epileptic rats, especially notable at the 400 mg/kg dosage. Histological analysis revealed varying degrees of inflammation and oedema, with fewer pathological changes observed in the 400 mg/kg group. These findings suggest that EBEEA possesses anticonvulsant properties, possibly mediated through its antioxidant effects and modulation of GABA levels. Further research, including extract purification and mechanistic elucidation, is necessary to fully understand its therapeutic potential in managing epilepsy.

Conflict of interest

The authors declare that they have no conflicts of interest.

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