Exploring the anti-convulsant potential of cordia dichotoma leaf extract to combat seizures in swiss albino mice

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EXPLORING THE ANTI-CONVULSANT POTENTIAL OF CORDIA DICHOTOMA LEAF EXTRACTS TO COMBAT SEIZERS IN SWISS ALBINO MICE

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ABSTRACT

Many neuropsychiatric and neurodegenerative disorders, such as Alzheimer's disease, anxiety, cerebrovascular impairment, depression, seizures, and Parkinson's disease, are increasingly prevalent in the modern era, largely due to stressful lifestyles. Prolonged treatment of these disorders with synthetic drugs often leads to severe side effects. In recent years, scientific research has shifted focus toward phytochemical for the treatment of neurological disorders. Nootropic herbs refer to plants or plant parts that exert medicinal effects through their neuroprotective properties, attributed to active phytochemical such as alkaloids, steroids, terpenoids, saponins, phenolic, and flavonoids. These phytocompounds play a crucial role in maintaining the brain's chemical balance by modulating the function of receptors for major inhibitory neurotransmitters. In the present study, it was clearly demonstrated that Cordia dichotoma possesses antiepileptic activity, fulfilling the first objective of this investigation. It is well-known that herbal medicines generally have fewer side effects and lower risk of resistance. The plants used in this study are no exception, as toxicity studies revealed that Cordia dichotoma is relatively safe (LD50 of 19.7 g/kg, with no animal deaths observed even at a dose of 500 mg/kg). Thus, this study encourages further exploration of Ayurveda's untapped potential in treating epilepsy, as many plants remain unevaluated and may exhibit antiepileptic properties, which addresses the second objective of the research.

Key words:- Alzheimer's disease, Cordia dichotoma, phytocompounds, neurodegenerative disorders.

INTRODUCTION

The epilepsies are a group of common and potentially devastating disorders characterized by the periodic and abnormal discharge of neurons within the brain. Approximately 65 million people worldwide (~0.5% to 1% of general population) are diagnosed with epilepsy and present clinically with more than 40 distinct forms of the disorder. Epilepsy is diagnosed if a person has experienced two or more unprovoked seizures greater than 24 hours apart. A seizure is a transient alteration in behaviour resulting from the disordered, synchronous, and rhythmic firing of a population of neurons. The occurrence of a seizure during a person's lifetime may be as high as 10%; however, the occurrence of a single seizure will not result in the diagnosis of epilepsy. Many seizure disorders exist, each defined by factors including initiating event, seizure type, age of onset, and clinical manifestations. The signs and symptoms of these syndromes frequently overlap, and differential diagnosis of the form of epilepsy is sometimes difficult. Some patients prefer the term seizure disorder, due to the historically negative social stereotypes associated with the term epilepsy or epileptic. Epilepsy is most often treated with antiseizure or anticonvulsant drug (AED) therapy. [1-5] Epilepsy possess very complex pathophysiology involving neuronal plasticity, imbalance between gamma- amino butyric acid ergic (GABAergic) (inhibitory) and glutamatergic (excitatory) neurotransmitter system and ion exchange dysfunction.[6] Thus, a decrease in concentration of gamma- amino butyric acid (GABA) leads to many pathological processes in the brain which may be manifests into convulsive episodes.[6] Recurrent and spontaneous seizures may enhance reactive oxygen species (ROS) in the central nervous system.[7] Due to the high levels of polyunsaturated fatty acids, high rate of oxidative metabolism and lower levels of antioxidant defenses, the brain is more susceptible to oxidative stress.[8] Although a large number of anticonvulsant agents are present in the market, still a number of cases of drug resistance increases day by day. Available antiepileptic drugs are inadequate and not capable to control seizures in many patients (failure rate 30–40%).[9] Since the treatment duration is very long for epilepsy; therefore, the drugs produce a variety of side effects and other neuropsychological disorders.[10] It is important to note that currently available antiseizure drugs do not alter the underlying pathology or alter the disease progression; as such, this approach is not curative. Antiseizure drugs are also commonly used to treat non-seizure disorders, such as chronic pain, migraine, bipolar disorder, and depression. Interestingly, but perhaps not surprisingly, these disorders also display a high level of comorbidity with epilepsy [1-5].

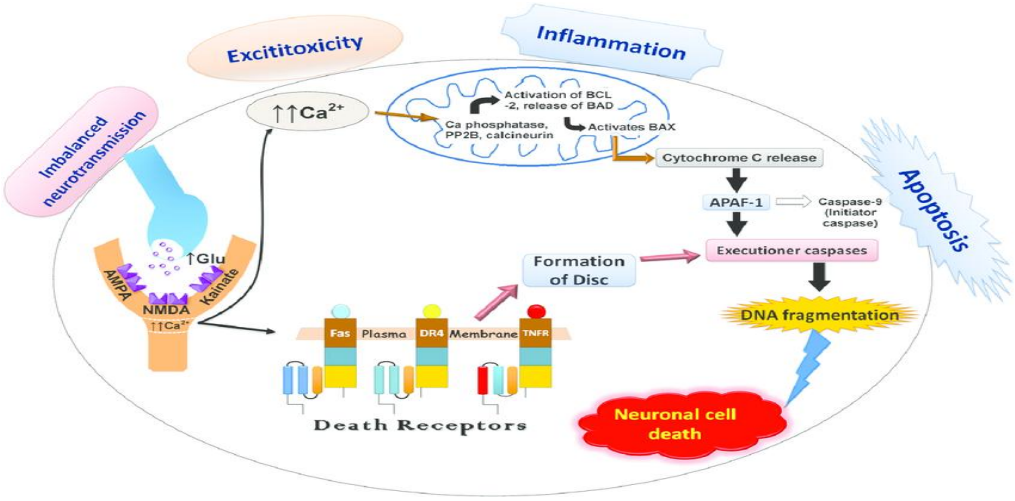


Figure 1: Basic cascade events in pathophysiology of epilepsy

Materials and Methods

Materials

The plant materials *C.dichotoma* (wholeplant,1part), were collected from the Hardi Forest in sarangarh, C.G, Bilaspur. The specimens were authenticated by Prof. Ashwini Kumar Dixit, Department of Botany, Guru Ghasidas Vishwavidyalaya, Chhattisgarh, India. The voucher specimens (Bot/GGV/2024/103) of plant materials were deposited in School Of Pharmacy, Campus, Chhattisgarh, Bilaspur, India, for further reference.

Preparation of formulation

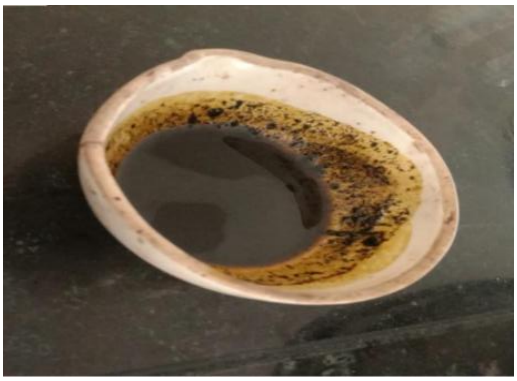
Leaves of *Cordia dichotoma*dried at room temperature (figure 2.1) and pulverized to form coarse powder. 500g of powdered leaves was placed in soxhlet apparatus (Perfit, India) and defatted with petroleum ether (60°-80°C) and extracted with Ethanol and water (figure 2.2). This extraction process was continued for about 48 hours or until methanol coming down the siphoning tube became colorless. Aqueous extract was obtained after macerating the powdered leaves (500gm) for 72 hours with occasional stirring (figure 2.3). Extracts so obtained were filtered and the filtrate was evaporated using vacuum evaporator (Perfit, India) under reduced pressure at ≤ 50° C. The crude extract obtained after evaporation were stored in desiccators and weighed. Physical nature and percentage yield of extract was recorded. The % yield of extracts was evaluated using the formula:

$$\% \text{ Yield} = \text{Weight of extract (g)} / \text{ Weight of dry powder (g)} \times 100$$

Figure 2.1: Leaves of *Cordia dichotoma* dried at room temperature

Figure 2.2.: Complete setup of Soxhlet apparatus carrying out the extraction of *Cordia dichotoma* leaves

Figure 2.2.2: Final obtained extract from Soxhlet process



Chemical

All the solvents used for the extraction process were of Laboratory grade and they were purchased from authorised local forms. Chemicals like PTZ, diazepam, and phenytoin were purchased from Gupta Medical Store, Chhattisgarh, India. For the estimation of GABA Transaminase (GABA‑T) activity, ELISA kit (MBS 939900; My Bio Source, Inc., San Diego, CA, USA) was used. All the reagents used in the study were of analytical grade.

Pharmacological evaluation

Animals

In the experimental study, adult Swiss Albino Mice weighing 15 ± 20 g (obtained from School of Pharmacy, Chouksey Engineering College Animal House, Chhattisgarh, Bilaspur [Registration No. 1275/PO/Re/SPCSEA] were used. The animals were kept at a constant room temperature (22 ± 2°C) and maintained 12 h light and 12 h dark cycle. All the experiments were carried out according to protocol approved by the Institutional Animal Ethical Committee (SOP/IAEC/2024/452-07). The animals were fed ad libitum with standard pelleted feed and fresh water during the course of the study.

In vivo anticonvulsant

Maximal electric shock-induced seizures

The electrical stimulus (150 mA, 50 Hz, 0.2 s duration) was applied via ear clip electrodes using an electro-convulsio meter (Techno, India). Five groups (n = 6) of rats were divided as follows. Group I received the vehicle Stress+ Vehicle (0.9% Nacl, 10ml/kg p.o.). Group II received standard drug Stress+ Diazepam (2 mg/kg, o.p.). Group III, IV, and V received different doses (100, 200, and 400 mg/kg, p.o.) of CDE (*Cordia dichotoma)*, respectively. Thirty minutes after treatment, electrical stimulation was given to all the animals. Abolition of hind limb tonic extension within 2 min was the criterion in the model. [11]

Figure 3.1: Healthy Swiss albino mice chosen for the present study

Pentylenetetrazole ‐induced seizures

PTZ (100 mg/kg, i.p.) was used after 30 min of vehicle/drug treatment to induce clonic–tonic Convulsions in experimental Swiss Albino Mice. Six groups (n = 6) of Mice were divided as follows. Group I received the vehicle Stress+ Vehicle (0.9% Nacl, 10ml/kg p.o.). Group II received standard drug Stress+ Diazepam (2 mg/kg, o.p.). Group III, IV, and V received different doses (100, 200, and 400 mg/kg, p.o.) of CDE (*Cordia dichotoma)*, respectively. The onset time of clonic‐tonic, myoclonus, and tonic seizures has been then recorded. The animals were considered to be protected if no seizure has been observed during the observation period of 30 min. Finally, after 30 min observation, Mice were sacrificed by decapitation and the whole brain was dissected out for estimation of GABA‐T activity and *in vitro* antioxidant activity.[12]

Estimation of gamma‐ amino butyric acid‐ transaminase Activity

GABA‐T activity has been measured in the brain homogenate by ultraviolet/visible spectroscopic analysis [13] at 450 nm using ELISA kit (MBS939900; My Bio Source, Inc., San Diego, CA, USA). GABA‐T was estimated as pg/mg of protein. Four hundred milligram of each brain tissue was washed with phosphate buffer (PBS, pH 7.2–7.4) and it was then homogenized in 5.0 ml of PBS which was kept overnight at −20°C. The homogenates were centrifuged at 5000 × g for 5 min at the temperature 2–8°C. The supernatant was separated and evaluated for the estimation of GABA‐T activity using the user's manual provided by the company along with the kit and for estimation of oxidant stress markers.

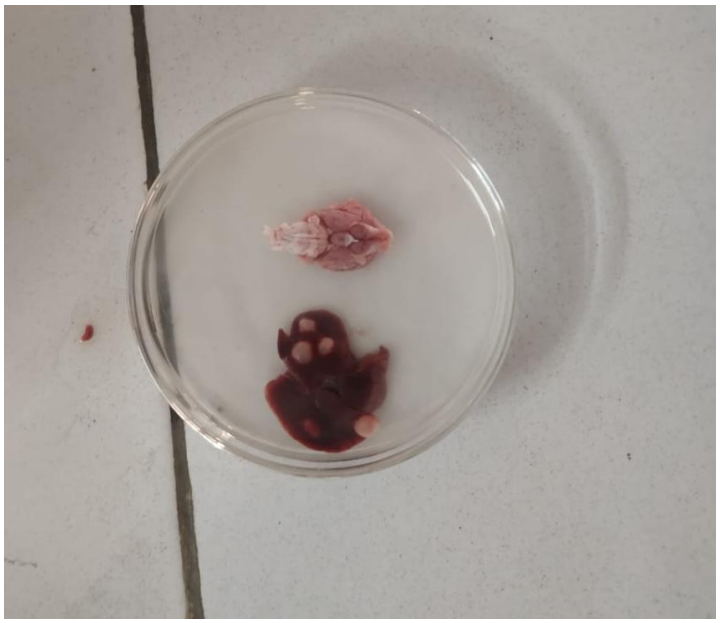


Figure 5.1: The extracted brain sample from the experimental mice

Evaluation of Anti-Oxidant Activity

Total phenol and total flavonoids content

Phenolic and flavonoids compounds are widely distributed in plants, where they act as antioxidants and free radical scavenging agents. Phenolic compounds are most abundant class of secondary metabolites in plants. Total phenolic content was measured using the Folin–Ciocalteu reagent in the extract and expressed as Gallic acid equivalent (GAE), shown in Table 6.1 and Figure 6.2. Total flavonoids content in plant extract was determined using aluminum chloride colorimetric method and total flavonoids were expressed as mg of total Catechins content/g of sample, shown in Table 6.2 and Figure 6.3. The total phenolic content in hydro-alcoholic extract was found to be 64.65 and total flavonoids content of hydro-alcoholic extract was found to be 46.43, as compiled in Table 6.3.

Concentration (µg/ml)	**Absorbance**
0	0
100	0.112
200	0.252
300	0.387
400	0.502
500	0.681

Table 6.1: Calibration curve of standard Catechins for estimation of total flavonoids

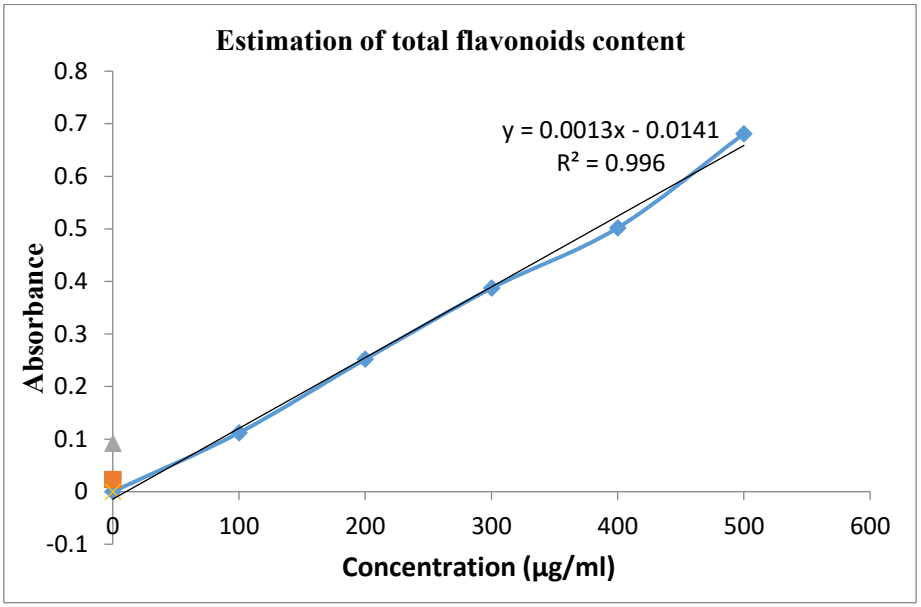


Figure 6.2: Standard Calibration Curve for estimation of Total Flavonoids

Table 6.3: Total phenolic content and total flavonoids content of hydro-alcoholic extract

Extracts	Total Phenolic Content (mg of GAE/g)	Total Flavanoid Content (mg of Catechin /g)
C.dichotoma	64.65 ± 1.2	46.43 ±1.32

Values represent mean ± SD (n=6)

Determination of GAMA butyric acid and glutamate in brain homogenate

Homogenization of brain tissues was carried out in 0.1 M Per chloric acid containing 4 mM sodium meta bi sulphite solution (30 µL per 10 mg of tissue).[14] The homogenate was then centrifuged at 10,000 rpm at 4°C for 15 min, and the resultant pellet was then separated from the supernatant. Pellet and filtered supernatant were then stored separately at −80°C. Concentration of GABA and glutamate was estimated using pre column derivatization with 0-phthalaldehyde (OPA) and detection of fluorescence has been done.[15] the procedure of derivatization includes mixing of 20 µL filtered supernatant with 6 µL OPA, and after 2 min, this mixture was then injected into the solvent stream of the HPLC system. OPA‑GABA and OPA-glutamate were separated out on a reversed phase 3.9 mm × 150 mm column (nova-pack, 4 µm, C18, waters) using a binary gradient system of mobile Phase A (38.74 mM sodium acetate dissolved in 90% milli-Q water and 10% methanol, pH 5.75;) and mobile Phase B (buffer containing 20% solution A and 80% methanol, pH 6.75) at 35°C at a flow rate of 0.5 mL/min. Fluorometric detection was performed at 360 and 450 nm, respectively, with a fluorescence detector (waters, 474). External standards (Sigma, USA) were used to quantify the GABA and glutamate by linear regression in this method and expressed in term of µg/mg protein.

Statistical analysis

Statistical analysis was performed using Graph Pad Prism Software Version 5.01, Inc. Fay Avenue, La Jolla, CA, USA. All data were expressed as a mean ± standard error of the mean. One‑way ANOVA followed by the Tukey's multiple comparison tests was used for comparisons of different groups of animals. Values of $P < 0.05$ were considered statistically significant.

Result and Discussion

The aim of the present work was to explore the protective role of CDE against convulsion induced by physical (MES) and chemical (PTZ) injury in experimental rats [Figure 1]. This study provided evidence that CDE is capable of blocking generalized seizures induced by MES test in treated rats. Inhibition of inducible MES seizures by a CDE might support the continuation of the traditional use of this formulation in the convulsive disorders [Figure 2]. Moreover, CDE also attenuated the PTZ increase in the activity of GABA‑T, GABA, and glutamate level & decrease in GSH levels in the Mice brain. Maximal electric shock (MES) and PTZ are most commonly used to produce acute seizure in experimental animals.[16] Electroshock produces generalized electrical stimulation of the large portions of the brain; it provokes the neurons to fire repetitively, which is the characteristic

Table 1: Effect of present CDE on PTZ-induced seizures

Name of The Drug used for Convulsion	Number of animals convulsed/used	Protection (%)	Latency period (min)
PTZ treated (100 mg/kg)	**0/6**	**0.00**	**11.62±0.57**
Diazepam	**4/6**	**100**	**no convulsion formed**
CDE 100	**3/6**	**42.01**	**12.48±0.51[b]**
CDE 200	**5/6**	**63.66**	**15.17±0.48[ab]**
CDE 400	**4/6**	**73.33**	**11.67±0.61[ab]**

Values are expressed as mean± SEM (n=6). Statistical comparison was analyzed by one‑way ANOVA followed by Tukey's multiple comparison test. aP<0.05, statistically significant as compared to negative control, b$P < 0.05$, statistically significant as compared to diazepam (2 mg/kg, i.p.). CDE: *Cordia. Dichotoma* ethanol, PTZ: Pentylenetetrazole, SEM: Standard error of mean

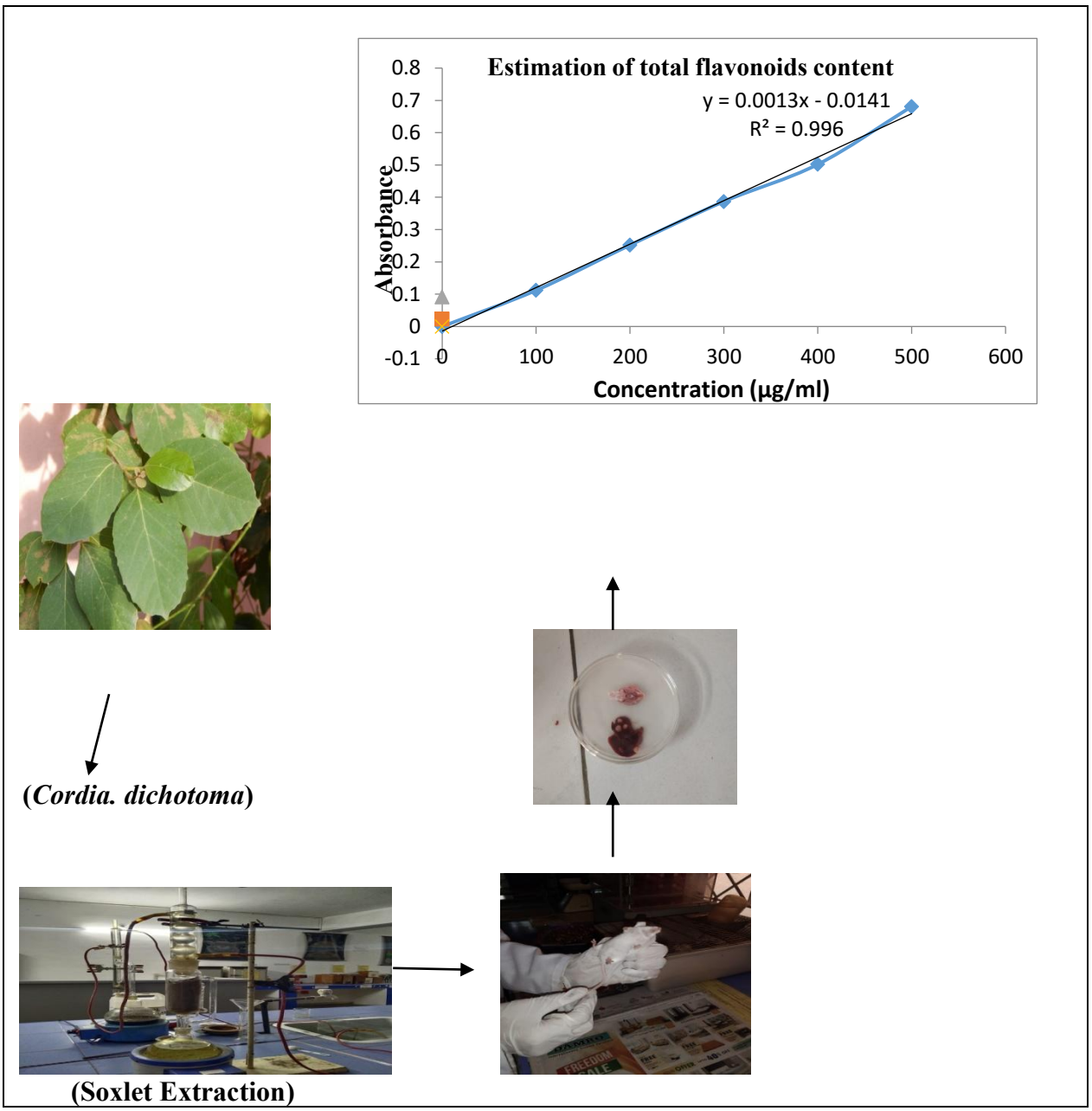


Figure1:- Graphical abstract

Conclusion

From this study it is clearly understood that alcoholic extract of *Cordia dichotoma* was highly effective in all type of animal models. It suggests a different mechanism attributed by different animal models were probably similar with that of chemical constituents present in the extracts as results of standard and test groups were significant. Therefore, it can be concluded that anticonvulsant activity of extract of *Cordia dichotoma* may be due to the blocking of seizure spread by Na+ glutamatergic excitation and NMDA receptor mediation. This study also suggests that extract of *Cordia dichotoma* was also effective against tonic clonic seizures. Thus the plants Shorearobusta and Sterculiaguttata possess anticonvulsant activity against MES, PTZ, Strychnine and Picrotoxine animal models. Further studies are, however, needed to isolate the active principle(s) of the plant and to enlighten the mechanism underlying its anticonvulsant effect. To this end we have to perform studies that will elucidate the exact mechanism of action of these active principle(s) before recommending these extracts for clinical application. The objective of study was to report antiepileptic activities of medicinal plant selected. *Cordia dichotoma* showed significant result in 300 mg/kg and 500 mg/kg dose of different extracts with $p<0.05$. Many parameters of animal models were positively modified by plant's extract like, onset of tonic and clonic convulsions were delayed while the duration of epileptic phases were reduced, as revealed from various behavioural analysis. At dose of 500 mg/kg of CDE in PTZ model showed highly significantly result. It is clearly concluded that *Cordia dichotoma* possess antiepileptic activity, this fulfil first objective of study. It is a well-known fact that herbal medicines have lesser side effects, lesser resistance, the plants used in the study are also not an exception as the toxicity studies revealed that *Cordia dichotoma* is less toxic (LD50 = 19.7 gm/kg and even at dose of 5000mg/kg no animal was killed), thus, the study motivates to explore the hidden potency of Ayurveda in the treatment of epilepsy as ample number of plant are yet to be evaluate and many of them may have antiepileptic activity, this explores second objective of study. Hence, *Cordia dichotoma* may offer a therapeutic avenue for addressing cognitive dysfunction in convulsion, a pressing issue with limited treatment option. The outcomes included improved cognitive performance, modulation of biochemical markers, and preservation of neuronal integrity in epileptic mice. Dose-dependent effects of *Cordia dichotoma* on cognitive function and associated parameters was seen, potentially identifying optimal therapeutic doses. The study's findings hold implications for advancing therapeutic interventions and understanding the neuroprotective potential of natural compounds like *Cordia dichotoma* in epileptic management

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCE

1. Potnis VV, Albhar KG, Nanaware PA, Pote VS. A review on epilepsy and its management. J. Drug Deliv. Ther. 2020 May 1;10(3):273-9.
2. Khanna L, Agarwal N, Kandel S. Concepts in Periodic Discharges: A Descriptive Study. International Journal of Epilepsy. 2018 Oct;5(02):068-74.
3. Costello DJ, Matthews E, Aurangzeb S, Doran E, Stack J, Wesselingh R, Dugan P, Choi H, Depondt C, Devinsky O, Doherty C. Clinical outcomes among initial survivors of cryptogenic new-onset refractory status epilepsy (NORSE). Epilepsia. 2024 Jun;65(6):1581-8.
4. Liu WK, Kothare S, Jain S. Sleep and epilepsy. InSeminars in Pediatric Neurology 2023 Dec 1 (Vol. 48, p. 101087). WB Saunders.
5. Yücel Y, Sidow NO. A General Overview of Epilepsy: its Classification, and Management. Current Researches in Health Sciences-II. 2023;161.
6. Henschel O, Gipson KE, Bordey A. GABAA receptors, anesthetics and anticonvulsants in brain development. CNS Neurol Disorder Drug Targets 2008;7:211‑24
7. Martinc B, Grabnar I, Vovk T. The role of reactive species in epileptogenesis and influence of antiepileptic drug therapy on oxidative stress. Current Neuro pharmacol 2012;10:328‑43
8. Patel M. Mitochondrial dysfunction and oxidative stress: Cause and consequence of epileptic seizures. Free Radic Biol Med 2004;37:1951‑62.
9. Goldenberg MM. Overview of drugs used for epilepsy and seizures: Etiology, diagnosis, and treatment. *P* T 2010;35:392‑415.
10. Lee SK. Old versus new: Why do we need new antiepileptic drugs? JEpilepsy Res 2014;4:39‑44.
11. Bergen DC, Silberberg D. Nervous system disorders: a global epidemic. Archives of neurology. 2002 Jul 1;59(7):1194-6.

12. Feigin VL, Vos T, Nichols E, Owolabi MO, Carroll WM, Dichgans M, Deuschl G, Parmar P, Brainin M, Murray C. The global burden of neurological disorders: translating evidence into policy. The Lancet Neurology. 2020 Mar 1;19(3):255-65.

13. Peat MA, Gibb JW. High‐performance liquid chromatographic determination of indoleamines, dopamine, and norepinephrine in rat brain with fluorometric detection. Anal Biochem 1983;128:275‐80.

14. Kendrick KM, Keverne EB, Chapman C, Baldwin BA. Microdialysis measurement of oxytocin, aspartate, gamma‐aminobutyric acid and glutamate release from the olfactory bulb of the sheep during vagino cervical stimulation. Brain Res 1988;442:171‐4.

15. Löscher W. Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs. Seizure 2011;20:359‐68.

16. Löscher W. Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs. Seizure 2011;20:359‐68.