

Evaluation of Anti Epileptic Activity of Leaf Extract of *Cynodon Dactylon* (L.) Pers. in Validated Animal Models.

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Abstract

The main objective of the work is to evaluate the antiepileptic activity and to evaluate antiepileptic activity of alcoholic and aqueous extracts of leaf extracts of *Cynodon dactylon* in validated animal models. For assessing of anticonvulsant activity Pentylenetetrazole (PTZ), Maximal electro shock (MES), Strychnine and Picrotoxin induced convulsive models were used. Preliminary Phytochemical investigation of the Ethanolic extract of *Cynodon dactylon* (EECD) leaves reveals the presence of carbohydrates, Amino acids, Flavonoids, Tannins, Fixed oils, Fats, Glycosides, and saponins. However, the qualitative test for Proteins and Alkaloids yielded negative results. In PTZ, Strychnine and Picrotoxin induced convulsion models, medium and high doses (600 and 800mg/kg respectively), but not the low dose (400mg/kg) of EECD showed significant anti-convulsant activity by delaying the onset of convulsions and by prolong the onset of clonus and tonic-extensor convulsion. In MES induced convulsion model medium and high doses (600 and 800mg/kg respectively) but not the low dose (400mg/kg) of EECD had exhibited significant anticonvulsant effect by decreasing the duration of tonic-extensor phase and prolonging the onset of clonus convulsion. Concluded that the all models, convulsions induced by either chemicals or MES, the EECD exhibited a fairly good anticonvulsant effect.

Key Words

Cynodon dactylon, anticonvulsant and antiepileptic.

Introduction

An ideal antiepileptic drug should suppress all seizures without causing any unwanted effect. Unfortunately, the drugs available in the modern medicine not only fail to control the seizure activity in some patients, but quite frequently cause unwanted effects that range in severity from minimal impairment of the CNS to death from aplastic anemia or hepatic failure.¹ Rich floral biodiversity of India has provided herbal health practitioners and other traditional healers in the country with an impressive pool of 'natural pharmacy' from which plants are selected as ingredients to prepare herbal remedies and medicines (phytomedicine) for the treatment², management and control of a variety of human ailments. The plant possesses anti microbial³ and anti viral activity and has also been used to treat urinary tract infection, calculi and prostatitis. The plant aqueous extract is used as anti inflammatory,

diuretic, anti emetic and purifying agent. Leaves have been used as anti epileptic agent in traditional system of medicine in India⁴. But, its antiepileptic activity is not yet validated scientifically as on date. Hence in the current dissertation the antiepileptic activity of leaf extract of *Cynodon dactylon* in validated animal models is considered⁵.

Materials and Methods

Materials

Diazepam was a Gift sample from Ranbaxy Laboratories Ltd, Mumbai, India. Pentylenetetrazole was a Gift sample from Sigma-Aldrich, St.Louis, MO 63103 USA. Strychnine was a Gift sample from Sigma-Aldrich, St.Louis, MO 63103 USA. Picrotoxin was a Gift sample from Sigma-Aldrich, St.Louis, MO 63103 USA. Tween-80 was a Gift sample from S.D.fine Chem Ltd. Mumbai. All other chemicals and ingredients were used for study are of Analytical grade.

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Methodology^{6, 7, 8}

Leaves of *Cynodon dactylon* were collected in the month of August from the agricultural fields of J.P.Nagar, Dist. U.P. The plant was identified and authenticated by the renowned botanist Prof. Veda Vyas, Department of Plant Taxonomy, L.V.D. College, Raichur. The collected plant material was shade dried to retain its vital phytoconstituents and then subjected to size reduction for further extraction process.

Preparation of Different Extracts

Preparation of alcoholic extract

The powder of *Cynodon dactylon* leaves was charged in to the thimble of a Soxhlet apparatus and extracted using 95% alcohol for 18 hrs. Appearance of colourless solvent in the siphon tube was the indication of exhaustive extraction and based on that the further extraction was terminated. The extract was then transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50°C to get alcoholic extract. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated. The perfectly dried extract was then stored in an air tight container till used.

Preparation of aqueous extract

About 100g of *Cynodon dactylon* leaves powder was taken in a round bottom flask (2000ml) and macerated in about 500 ml of distilled water and 10 ml of chloroform (preservative) for 24 hrs with shaking for every hour in a closed vessel. Then the marc was removed by filtering the extract, and then it was concentrated on a water bath at 50°C to get a semi solid mass. The extract was stored in an airtight container in a refrigerator below 10°C. The two extracts namely alcoholic and aqueous were examined for their color and consistency. Their percentage yield was calculated with reference to air-dried sample.

The alcoholic and aqueous extracts of *C.dactylon* leaves were subjected to the following investigations,

1. Preliminary phytochemical screening.
2. Pharmacological activities⁹
 - a. Determination of acute toxicity (LD₅₀)
 - b. Anti-convulsant activity

Preliminary phytochemical testing of extracts

The extracts were subjected to following chemical tests to detect the chemical constituents present in them.

0.5 gm of extract was dissolved in 5 ml of distilled water and filtered. The filtrate was used to determine the presence of various phytoconstituents as describe in the following Table No.2.

Animals

Albino mice of either sex weighing between 20-30g were procured from central animal house of N.E.T. Pharmacy College, Raichur for experimental purpose. The animals were acclimatized to laboratory conditions for 7 days. The animals were supplied with commercially available standard diet from Amrut laboratories and Pranav Agro industries Ltd. Sangali. Water was allowed *ad libitum* under hygienic conditions. All animal studies were performed in accordance to guideline of CPCSEA and Institutional Animal Ethical Committee (IAEC) of N.E.T. Pharmacy College, Raichur (Karnataka). (CPCSEA registration number. 576/2002/bc/IAEC/CPCSEA).

Determination of LD₅₀ of Leaf Extract of Cynodon Dactylon

The acute toxicity of leaf extracts of *C. dactylon* was determined by using albino mice of either sex weight between (20-25 g), maintained under standard conditions. The animals were be fasted for 3 hr prior to the experiments. Animals were administered with single dose of either alcoholic or aqueous leaf extract of *C. dactylon* and observed for its mortality up to 48 hr study period (short term toxicity). Based on the short-term toxicity profile, the next dose was decided as per OECD guidelines No 425. From the LD₅₀ dose 1/20, 1/10 and 1/5th doses were selected and considered as low, medium and high doses respectively.

Determination of anticonvulsant activity^{10, 11, 12}

PTZ (pentylenetetrazole) induced convulsions

Albino mice of either sex weighing between 22-25g were randomly selected and segregated in to five groups, each group consisting of six animals.

Group A -Normal control (2% w/v Gum acacia p.o.)

Group B- Standard (Diazepam 5mg/kg p.o)

Group C-Leaf extract of *C.dactylon* (400mg/kg p.o)

Group D-Leaf extract of *C.dactylon* (600 mg/kg p.o)

Group E-Leaf extract of *C.dactylon* (800 mg/kg p.o)

Experimental procedure

Albino mice of either sex with body weights between 22-25g were divided into five groups of 6 animals in each. Group A served as normal control and was administered with 2%w/v Gum acacia

suspension orally, Group B with diazepam (5mg/kg p.o.) and served as standard, Groups C, D and E with three different doses of leaf extracts (low, medium and high respectively) either alcoholic or aqueous of *C.dactylon* for seven consecutive days. On the eighth day one hour after the oral administration of either acacia suspension/standard drug/extracts respectively to different groups, PTZ 60 mg/kg was administered subcutaneously. Each animal was then placed into individual plastic cages and were observed initially for 30min and later up to 24 hrs. The following parameters were recorded during test session of initial 30min and up to 24 hrs respectively:

- Latency (onset of clonus)
- Onset of tonic-clonic convulsions
- Status of animal after 1hr
- Status of animal after 24 hrs

Percent protection

The values were expressed as mean \pm SEM from 6 animals. The results were subjected to statistical analysis by using ANOVA followed by Dennett's- t -test to calculate the significance difference if any among the groups. $p < 0.05$ was considered as statistically significant. The results are compiled in Table No. 3 and 4.

Maximal Electro Shock (Mes) Induced Convulsions

Albino mice of either sex weighing between 22-25g were divided into five groups each group was consisting of six animals.

Group A -Normal control (2% gum acacia p.o)

Group B- Standard (Phenytoin 25mg/kg p.o)

Group C-Leaf extract of *C.dactylon* (400 mg/kg p.o)

Group D-Leaf extract of *C.dactylon* (600 mg/kg p.o)

Group E-Leaf extract of *C.dactylon* (800 mg/kg p.o)

Experimental procedure

Albino mice of either sex with a body weight between 22-25g were divided into five groups of 6 animals in each. Group A served as control and was administered with 2% gum acacia suspension, Group B with phenytoin (25mg/kg p.o.) and served as standard. Group C, D and E with three different doses of either alcoholic or aqueous leaf extracts of *C. dactylon* (low, medium and high respectively) for seven consecutive days. On the eighth day one hour after oral administration of acacia suspension/standard drug/different extracts to respective groups, MES seizures were induced by

electroconvulsimeter. A 60 mA current was delivered transauricularly for 0.2sec in mice. This current intensity elicited complete tonic extension of the hind limbs in control mice. For recording various parameters, mice were placed in clear rectangular plastic cages with an open top, permitting full view of the animal's motor responses to seizure. In the pilot study various phases of convulsions, viz., tonic flexion, extension, clonus, stupor and mortality due to convulsions were selected as the parameters. Phenytoin (25mg/kg p.o.) used as standard drug.

Strychnine Induced Convulsions

Albino mice of either sex weighing between 22-25g were randomly selected and segregated in to five groups, each group consisting of six animals.

Group A -Normal control (2%gum acacia p.o.)

Group B- Standard (Diazepam 5mg/kg p.o)

Group C-Leaf extract of *C.dactylon* (400 mg/kg p.o)

Group D-Leaf extract of *C.dactylon* (600 mg/kg p.o)

Group E-Leaf extract of *C.dactylon* (800 mg/kg p.o)

Experimental procedure

Albino mice of either sex with body weights between 22-25g were divided into five groups of 6 animals in each. Group A served as normal control and was administered with 2%w/v Gum acacia suspension orally, Group B with diazepam (5mg/kg p.o.) and served as standard, Groups C, D and E with three different doses of leaf extracts either alcoholic or aqueous of *C.dactylon* (low, medium and high respectively) for seven consecutive days. On the eighth day one hour after the oral administration of either acacia suspension/standard drug/extracts respectively to different groups, Strychnine nitrate 2 mg/kg was administered subcutaneously. Each animal was then placed into individual plastic cages and were observed initially for 30min and later up to 24 hrs. The following parameters were recorded during test session of initial 30min and up to 24 hrs.

- Latency (onset of tonic)
- Status of animal after 30 minutes
- Status of animal after 24 hr.
- Percent protection

Picrotoxin Induced Convulsions

Albino mice of either sex weighing between 22-25g were randomly selected and segregated in to five groups, each group consisting of six animals.

- Group A -Normal control (2% gum acacia p.o.)
- Group B- Standard (Diazepam 5mg/kg p.o)
- Group C-Leaf extract of *C.dactylon* (400 mg/kg p.o)
- Group D-Leaf extract of *C.dactylon* (600 mg/kg p.o)
- Group E-Leaf extract of *C.dactylon* (800 mg/kg p.o)

Experimental procedure

Albino mice of either sex with body weights between 22-25g were divided into five groups of 6 animals in each. Group A served as normal control and was administered with 2%w/v Gum acacia suspension orally, Group B with diazepam (5mg/kg p.o.) and served as standard, Groups C, D and E with three different doses of leaf extracts either alcoholic or aqueous of *C.dactylon* (low, medium and high respectively) for seven consecutive days. On the eighth day one hour after the oral administration of either acacia suspension/standard drug/extracts respectively to different groups, Picrotoxin 3.5mg/kg was administered subcutaneously. Each animal was then placed into individual plastic cages and were observed initially for 30min and later up to 24 hrs. The following parameters were recorded during test session of initial 30min and up to 24 hrs respectively:

- Latency (onset of tonic-extensor convulsion)
- Status of animal after 1hr
- Status of animal after 24 hr
- Percent protection

The values were expressed as mean \pm SEM from 6 animals. The results were subjected to statistical analysis by using ANOVA followed by Dennett's- t -test to calculate the significance difference if any among the groups. $p < 0.05$ was considered as statistically significant. The results are compiled in Table No. 5 and 6.

Results and Discussion

Preliminary phytochemical testing of extracts

The results of preliminary qualitative phytochemical examination are shown in Table No.2.

Assessment of anti convulsant activity of ethanolic extract of *Cynodon dactylon* leaves.

PTZ (Pentylentetrazole) induced convulsions

EECD leaves were screened for anticonvulsant activity using PTZ induced convulsion model in mice. Chronic study was conducted using low, medium and high doses of EECD (400, 600 & 800mg/kg respectively). The above mentioned doses were administered daily once for a period of 7 consecutive days. It was observed that lower dose

(400mg/kg) of EECD did not produce significant anticonvulsant effect as compared to control. But medium and high doses (600 & 800mg/kg respectively) exhibited a significant anti convulsant effect by increasing onset time of seizures and reducing the duration of tonic-clonic seizures. The lower, medium and higher doses of EECD offered a protective effect of 33.33%, 83.33% and 83.33% up to 1hr interval respectively. The standard drug diazepam (5mg/kg) exhibited a significant anticonvulsant activity and offered 100% protection.

MES induced convulsions

EECD leaves were screened for anticonvulsant activity using MES induced convulsion model in mice. Chronic study was conducted using low, medium and high doses of EECD (400, 600 & 800mg/kg) respectively. The above mentioned doses were administered daily once for a period of 7 consecutive days. It was observed that lower dose (400mg/kg) of EECD did not produce significant anticonvulsant effect as compared to control. But medium and high doses (600 & 800mg/kg respectively) exhibited a significant anticonvulsant effect by reduced the duration of tonic extensor phase and tonic-clonic seizures. The lower, medium and higher doses of EECD offered a protective effect of 33.33%, 66.66% and 83.33% up to 1hr interval respectively. The standard drug phenytoin (25mg/kg p.o.) exhibited a significant anticonvulsant activity and offered 100% protection.

Strychnine induced convulsions

EECD leaves were screened for anticonvulsant activity using strychnine induced convulsion model in mice. Chronic study was conducted using low, medium and high doses of EECD (400, 600 & 800mg/kg respectively). The above mentioned doses were administered daily once for a period of 7 consecutive days. It was observed that lower dose (400mg/kg) of EECD did not produce significant anti convulsant effect as compared to control. But medium and high doses (600 & 800mg/kg respectively) exhibited a significant anticonvulsant effect by increasing onset time of seizures and reducing the duration of tonic-clonic seizures. The lower, medium and higher doses of EECD offered a protective effect of 0%, 66.66% and 83.33% up to 1hr interval respectively. The standard drug diazepam (5mg/kg) exhibited a significant anticonvulsant activity and offered 100% protection.

Picrotoxin induced convulsions

EECD leaves were screened for anticonvulsant activity using picrotoxin induced convulsion model in mice. Chronic study was conducted using low, medium and high doses of EECD (400, 600 & 800mg/kg respectively). The above mentioned doses were administered daily once for a period of 7 consecutive days. It was observed that lower dose (400mg/kg) of EECD did not produce significant anticonvulsant effect as compared to control. But medium and high doses (600 & 800mg/kg respectively) exhibited a significant anticonvulsant effect by increasing onset time of seizures and reducing the duration of tonic seizures. The lower, medium and higher doses of EECD offered a protective effect of 33.33%, 66.66% and 83.33% up to 1hr interval respectively. The standard drug diazepam (5mg/kg) exhibited a significant anticonvulsant activity and offered 100% protection.

Assessments of Anti Convulsant Activity of Aqueous Extract of Cynodon Dactylon Leaves

PTZ (Pentylentetrazole) induced convulsions

AECD leaves were screened for anticonvulsant activity using PTZ induced convulsion model in mice. Chronic study was conducted using low, medium and high doses of AECD (400, 600 and 800mg/kg respectively). The above mentioned doses were administered daily once for a period of 7 consecutive days. It was observed that, none of the doses i.e. lower, medium or high doses of aqueous extract of *Cynodon dactylon* did not exhibited anticonvulsant activity. Further, the percentage protection against PTZ induced convulsions was both clinically and statistically insignificant, compared to the standard drug diazepam (5mg/kg p.o.).

MES induced convulsions

AECD leaves were screened for anticonvulsant activity using PTZ induced convulsion model in mice. Chronic study was conducted using low, medium and high doses of AECD (400, 600 & 800mg/kg respectively). The above mentioned doses were administered daily once for a period of 7 consecutive days. It was observed that, none of the doses i.e. lower, medium or high doses of aqueous extract of *Cynodon dactylon* did not exhibited anticonvulsant activity. Further, the percentage protection against MES induced convulsions was both clinically and statistically insignificant,

compared to the standard drug phenytoin (25mg/kg p.o.).

Strychnine induced convulsions

AECD leaves were screened for anticonvulsant activity using Strychnine induced convulsion model in mice. Chronic study was conducted using low, medium and high doses of AECD (400, 600 & 800mg/kg respectively). The above mentioned doses were administered daily once for a period of 7 consecutive days. It was observed that, none of the doses i.e. lower, medium or high doses of aqueous extract of *Cynodon dactylon* did not exhibited anticonvulsant activity. Further, the percentage protection against Strychnine induced convulsions was both clinically and statistically insignificant, compared to the standard drug diazepam (5mg/kg p.o.).

Picrotoxin induced convulsions

AECD leaves were screened for anticonvulsant activity using Picrotoxin induced convulsion model in mice. Chronic study was conducted using low, medium and high doses of AECD (400, 600 & 800mg/kg respectively). The above mentioned doses were administered daily once for a period of 7 consecutive days. It was observed that, none of the doses i.e. lower, medium or high doses of aqueous extract of *Cynodon dactylon* did not exhibited anticonvulsant activity. Further, the percentage protection against Picrotoxin induced convulsions was both clinically and statistically insignificant, compared to the standard drug diazepam (5mg/kg p.o.)

Anticonvulsant Activity

There are a number of synthetic anticonvulsant drugs currently available for use in the management, control and treatment of individuals with epilepsy. However, most of the synthetic drugs are not only inaccessible and unaffordable, but also possess many toxic adverse effects. Therefore, there is a great need for the development of cheap, effective and safe anticonvulsant agents from plants and other sources. Prevention of PTZ induced seizures in laboratory animals is the most commonly used preliminary screening test for characterizing potential anticonvulsant drugs. The test is assumed to identify anticonvulsant drugs effective against generalized clonic seizures, as PTZ produces clonic and tonic convulsions. It has been demonstrated that, a neural pathway sub serving clonic PTZ convulsions is located in the forebrain while the brain stem is

involved in the network of tonic PTZ induced convulsions. The antiepileptic drug should abolish or increase the threshold for clonic and tonic convulsions. The mechanism by which PTZ exert its convulsant action is by acting as an antagonist at the GABAA receptor complex. Drugs offer protections against tonic-clonic seizures induced by PTZ are considered to be useful to control myoclonic and absence seizures in humans. Various factors like age, sex, species, diet, water, day/light cycle, temperature, preparation dose and route of administration are known to affect the response of the animal to PTZ induced seizures. Increase in the threshold for clonic and tonic convulsions by EECD after PTZ induced seizure suggest that, the extract might have affecting GABA-ergic neurotransmission as PTZ has been shown to interact with the GABA neurotransmitter. MES is also one of the commonly used models for preliminary testing of anticonvulsant drugs that produces generalized tonic-clonic seizures i.e. hind limb tonic extensor (HLTE) and clonic convulsions. It has often been stated that antiepileptic drugs that block MES induced tonic extension act by blocking seizure spread, moreover MES induced tonic extension can be prevented either by drugs that inhibit voltage dependant Na^+ channels (phenytoin, valproate) or by drugs that block glutaminergic excitation mediated by the N-methyl- D-aspartate (NMDA) receptor. EECD at medium and high doses (600 and 800mg/kg respectively) but not lower dose (400mg/kg) had significantly increased the duration of tonic extensor phase and onset of clonus as compared to control and thus exhibited anticonvulsant effect and the percent protection was 66.66%, 83.33% and 33.33% respectively. Standard drug (phenytoin 25mg/kg) had abolished the tonic extensor phase and showed 100% anticonvulsant effect by preventing seizure spread. The percentage protection (Anticonvulsant effect) was found to be increased dose dependent. Hence, the anticonvulsant activity of EECD against MES induced convulsions involve blockade of seizure spread, which perhaps occurred by inhibiting Voltage dependant Na^+ channels.

Strychnine is another convulsion producing drug used for testing anticonvulsant activity. Glycine is an inhibitory neurotransmitter in the CNS and strychnine is a competitive antagonist of the glycine receptor. Strychnine produces convulsion by

antagonizing the inhibitory spinal cord and brainstem reflexes of glycine and anticonvulsant drugs should delay the seizure produced by strychnine. Strychnine produces convulsion by antagonizing the inhibitory reflexes of glycine in spinal cord and brainstem, so anticonvulsant effect produced by EECD might be through suppression of the action of strychnine on glycine inhibitory mechanisms.

Picrotoxin induced convulsion, is another model for the testing of chemically induced convulsion in mice. Post synaptic GABA_A- receptors are functionally linked to BDZ, barbiturate receptors and chloride-ion channels to form GABA-chloride ionophore complex, which is intimately involved in the modulation of GABAergic neurotransmission. Picrotoxin a GABA-receptor antagonist produces seizures by blocking the chloride ion channel linked to GABA_A-receptors, thus preventing the entry of chloride ions in to the brain. This process will in turn inhibit GABA neurotransmission and activity in the brain. Phenobarbitone and diazepam are believed to enhance GABAergic neurotransmission by increasing chloride ion flux through chloride ion channel at GABA-receptor sites. This hypothesis may explain the observed protective effects and/or antagonistic actions, of phenobarbitone and diazepam against picrotoxin (PCT)-induced seizures in mice.

EECD at medium and high doses (600 and 800mg/kg respectively) but not lower dose (400mg/kg) had significantly delayed the latency onset of convulsions and latency onset of tonic-extensor convulsion and offered 66.66%, 83.33% and 33.33% protection respectively. The anticonvulsant effect (increased percent protection) was found to be dose dependent i.e. from low dose to high dose. Standard drug diazepam (5mg/kg) had abolished the tonic convulsions and offered 100% protection.

Picrotoxin produces convulsion by blocking the chloride ion channel linked to GABA_A-receptors, thus preventing the entry of chloride ions in to the brain. This process will in turn inhibit GABA neurotransmission and activity in the brain. The anticonvulsant effect produced by EECD might be through interfering with its chloride ion channel linked to GABA_A-receptors. On the contrary, the aqueous extract of leaves of *Cynodon dactylon* at doses 400, 600 and 800 mg/kg, which are designated

as low, medium and high doses respectively, did not exhibited any anticonvulsant activity against pentylenetetrazole, strychnine, picrotoxin and MES induced convulsions. On observation and reference to reported data, it was clear that, the EECD showed the presence of saponins and flavonoids. However, these two phytoconstituents were absent aqueous extract of the same leaf. Hence it is concluded that, the EECD leaves possesses significant anticonvulsant activity against pentylenetetrazole, strychnine, picrotoxin and MES induced convulsions.

Conclusion

In the present study we have selected a plant *Cynodon dactylon* and ethanolic and aqueous extract were prepared from the dried leaves and tested for its anticonvulsant activity in validated animal models. In the all models, convulsions induced by either chemicals or MES, the EECD exhibited a fairly good anticonvulsant effect. Retrospective reports reveal that the phytoconstituents such as flavonoids and saponins are responsible moieties in most of the plants for their anticonvulsant activities. Indeed, the ethanolic extract of leaf of *Cynodon dactylon* has shown the positive test for its presence of flavonoids and saponins. Hence these moieties in the leaf extract of *Cynodon dactylon* responsible for the exhibited anticonvulsant activity of the plant. Thus our study supports the folklore application of *Cynodon dactylon* leaves in the treatment of convulsive disorders in the light of modern science.

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Table 1: Nature and Percentage yield of the extracts.

S.No	Name of the Extract	Nature	Colour	%Yield (w/w) in gm (%)
1.	Alcohol	Sticky	Dark green	2.50
2.	Aqueous	Sticky	Dark brown	1.00

Table 2: Qualitative chemical examinations of extracts.

Phytoconstituents	Alcohol extract	Aqueous extract
Carbohydrates	Present	Present
Proteins	Absent	Absent
Amino acids	Present	Present
Flavonoids	Present	Absent
Tannins	Present	Absent
Saponins	Present	Absent
Fixed oils and Fats	Present	Present
Alkaloids	Absent	Present
Glycosides	Present	Present
Sterols	Present	Absent

Table 3: Effect of EECD leaves on PTZ (60mg/kg) induced convulsion in mice.

S.No	Treatment	Avg.Wt (g)	Avg.Vol.Of Dose (ml)	Onset Of Seizure (Sec) Mean ± SEM	Duration Of Tonic Clonic Seizure (Sec) Mean± SEM	Status Of Animal (No. of animals Alive)	% Protection (1hr)
1	Control (2% Gum acacia p.o.)	21.33	0.21	670.5 ±10.135	39.66 ± 0.66	0	0
2	Diazepam (5mg/kg p.o.)	24.33	0.24	908.83 ±15.39	6.83 ± 0.71	ALL	100
3	EECD (400mg/kg p.o.)	24.33	0.25	724.5 ±15.23*	23 ± 0.96	2	33.33
4	EECD (600mg/kg p.o.)	21.33	0.22	791.16 ±9.02**	19.33 ± 0.91**	5	83.33
5	EECD (800mg/kg p.o.)	21.00	0.21	823.33 ±7.81**	15.33 ± 0.49**	5	83.33

Values are mean ± SEM; n=6; One way analysis of variance (ANOVA) followed by Dunnett's' test. Where, **represents highly significant at p<0.01, EECD: Ethanolic extract of *Cynodon dactylon*. , PTZ: Pentylenetetrazole.

Table 4: Effect of EECD leaves on MES induced convulsions in mice.

S.No	Treatment	Avg.Wt (g)	Avg.Vol.Of Dose (ml)	Onset Of Seizure (Sec) Mean ± SEM	Duration Of Tonic Extensor (Sec) Mean ± SEM	% Protección (1hr)
1	Control (2%gum acacia p.o.)	24.6	0.25	25 ± 0.57	23 ± 0.57	33.33
2	Standard Phenytoin (25mg/kg)	23.8	0.23	6.83 ± 0.60	5.8 ± 0.60	100
3	EECD (400mg/kg p.o.)	23	0.23	24.16 ± 0.60 ^{ns}	22.16 ± 0.60 ^{ns}	33.33
4	EECD (600mg/kg p.o.)	22	0.22	16.33 ± 0.66**	14.33 ± 0.66**	66.66
5	EECD (800mg/kg p.o.)	24	0.24	13.84 ± 0.60**	11.83 ± 0.60**	83.33

Values are mean ± SEM; n=6; One way analysis of variance (ANOVA) followed by Dunnett's' test, Where, **represents highly significant at p<0.01, EECD: Ethanolic extract of *Cynodon dactylon*, MES: Maximal electro shock.

Table 5: Effect of AECD leaves on Strychnine (2mg/kg s.c.) induced convulsion in mice.

S.No	Treatment	Avg. Wt (g)	Avg. Vol. Of Dose (ml)	Onset Of Tonic Clonic Convulsions Mean ± SEM	Status Of Animal (1hr) (No. of animals Alive)	% Protection
1	Control (2%gum acacia)	22	0.22	272.16 ± 10.91	0	0
2	Diazepam (5mg/kg p.o.)	22	0.22	459 ± 10.76	ALL	100
3	AECD (400mg/kg p.o.)	21	0.21	283 ± 7.35	0	0
4	AECD (600mg/kg p.o.)	23	0.23	299.16 ± 6.59	1	16.66
5	AECD (800mg/kg p.o.)	24	0.24	336.5 ± 11.67	2	33.33

Values are mean ± SEM; n=6; One way analysis of variance (ANOVA) followed by Dunnett's test, AECD: Aqueous extract of *Cynodon dactylon*.

Table 6: Effect of AECD leaves on Picrotoxin (3.5mg/kg s.c.) induced convulsions in mice.

S.No	Treatment	Avg. Wt (g)	Avg. Vol. Of Dose (ml)	Onset Of Tonic Clonic Convulsions Mean ± SEM	Onset Of Tonic Convulsion mean ± SEM	Status Of Animal (No. of animals Alive)	% Protection
1	Control (2%gum acacia p.o.)	20.67	0.21	664.16 ± 7.53	681.66 ± 9.27	2	33.33
2	Diazepam (5mg/kg p.o.)	22.00	0.22	841.5 ± 10.02	852.5 ± 10.18	ALL	100
3	AECD (400mg/kg p.o.)	22.22	0.22	670 ± 9.69	681.83 ± 8.86	2	33.33
4	AECD (600mg/kg p.o.)	21.34	0.22	682.16 ± 9.89	694.66 ± 9.54	2	33.33
5	AECD (800mg/kg p.o.)	20.43	0.21	697 ± 12.97	707 ± 13.20	3	50

Values are mean ± SEM; n=6; One way analysis of variance (ANOVA) followed by Dunnett's test. AECD: Aqueous extract of *Cynodon dactylon*.
