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Research Article

Evaluation of antiepileptic activity of leaf extract of *Cynodon dactylon* in validated animal models

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ABSTRACT

Ethanolic extract of leaves of Cynodon dactylon were prepared. The phytoconstituents such as alkaloids, carbohydrates, glycosides, flavonoids, saponins, sterols and tannins were identified by standard procedure. Anti convulsant activity was studied against maximal electroshock (MES) and Pentylenetetrazole induced convulsions in mice. Ethanolic extract of Cynodon dactylon in the dose of 600 and 800mg/kg respectively protected 66.66% and 83.33% of animals used and significantly ($p<0.05$) delayed pentylenetetrazole (60mg/kg s.c.) induced tonic seizures. Similarly, the same dose of Ethanolic extract of Cynodon dactylon significantly ($p<0.05$) reduce the duration of tonic extensor seizure induced by MES. The data obtained suggest that Ethanolic extract of Cynodon dactylon have anticonvulsant property and may probably be affecting both GABA-aminergic and glycine inhibitory mechanism.

Key words: *Cynodon dactylon, anti convulsant, MES, Pentylenetetrazole, mice*

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INTRODUCTION

Epilepsy is a neurological disorder that affects a wide range of people throughout the world. It is a disorder of brain characterize by unpredictable and periodic occurrence of a transient alteration of behavior due to the disordered, synchronous and rhythmic firing of populations of brain neurons.[1] Incidence of epilepsy in developed countries is approximately 50 per 100,000 while that of developing country is 100 per100,000.[2] It has been observed that the presently available antiepileptic drugs are unable to control seizures effectively in as many as 25% of the patients.[3] The conventional antiepileptic agents like phenytoin, carbamazeipine and sodium valporate carry with them several serious side effects notably neurotoxicity.[4] As majority of antiepileptic drugs are consumed life long, concomitant administration of other drugs predisposes to the risk of drug interaction. However, newer antiepileptics like gabapentin, vigabatrin, lamotrigine, etc are used supplemental to the conventional agents. Thus, it is necessary to

investigate for an antiepileptic agent that is highly efficacious as well as safe in items of drug related toxicity. The aim of treating an epileptic is not only to abolish the occurrence of seizures but also to lead a self sustained life.

Cynodon dactylon (L.) Pers. (Family: Poaceae) commonly known as "Doob" in India, is a weed and has been regarded to possess various medicinal properties. The plant posses antimicrobial and antiviral activity [5] and has also been used to treat urinary tract infection, calculi and prostatitis. The aqueous plant extract is used as anti-inflammatory, diuretic, anti-emetic and purifying agent.[6] It also has significant application in treating dysentery, dropsy and secondary syphilis.[7] *Cynodon dactylon* has been used as an antiepileptic agent in traditional system of medicine in India.[8] The objective of this investigation was to ascertain the scientific basis of its use in treatment of epilepsy. The present investigation reports antiepileptic activity of the ethanolic extract of *Cynodon dactylon* on which no previous data available.

MATERIALS AND METHODS

Collection and identification of plant material

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 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^{o}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.

Experimental 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).

Acute toxicity study

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 LD_{50} dose 1/20, 1/10 and $1/5^{th}$ doses were selected and considered as low, medium and high doses respectively.

Assessment of anticonvulsant activity [9]

The animals were divided into five groups of six each. Group I received 2% Gum acacia p.o., Group II received Phenytoin (25 mg/kg p.o.)/ diazepam (5.0 mg/kg p.o.), Group III received ethanolic extract of *C.dactylon* (400 mg/kg p.o.), Group IV received ethanolic extract of *C.dactylon* (600 mg/kg p.o.) and Group V received ethanolic extract of *C.dactylon* (800 mg/kg p.o.).

Maximal electroshock induced seizures

60 min after drug administration maximal electro shock seizures are elicited by the application of electric shock (12 mA, 50 Hz for 0.2 sec) using corneal electrodes. Abolition of the hind limb tonic extensor spasm was recorded as a measure of anticonvulsant activity. Phenytoin (25 mg/kg p.o.) was used as reference standard.

Pentylenetetrazol-induced seizures

60 min after drug administration, seizure was induced by subcutaneous injection of PTZ (60 mg/kg) and the mice were observed for onset of myoclonic spasm and clonic convulsions. Diazepam (5mg/kg, p.o.) was included as a reference standard. The animals were observed for onset of convulsion up to 30min after PTZ administration.

Statistical analysis:

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet‟s„t‟- test. P values <0.05 were considered significant.

RESULTS AND DISCUSSION

In pentelenetetrazole induced seizure model, EECD 600 and 800mg/kg produced significant ($p<0.01$) reduction in duration of convulsion and was comparable to that produced by diazepam 5mg/kg, but EECD 400mg/kg did not exhibit anticonvulsant effect. In the maximal electro shock induced seizure model, EECD 600 and 800mg/kg and diazepam 5mg/kg showed significant ($p<0.01$) reduction in duration of convulsion, but EECD 400mg/kg did not exhibit anticonvulsant effect. The anticonvulsant activity of at various dose levels viz, 400, 600 and 800mg/kg p.o. were studied by the pentelenetetrazole and maximal electro shock induced seizure models. The most popular and widely used animal seizure models are maximal electro shock and pentelenetetrazole induced seizure. Prevention of seizures induced by pentelenetetrazole in laboratory animals is the most commonly used preliminary screening test for characterizing potential anticonvulsant drugs. The maximal electro shock-induced seizure test is considered to be a predictor of

likely therapeutic efficacy against generalized tonic-clonic seizures. By contrast, the pentelenetetrazole test represents a valid model for human generalized myoclonic and also absence seizures. Other chemoconvulsant models for primary generalized seizures include by bicuculine ($GABA_A$ receptor antagonized), strychnine (glycine receptor antagonist) and aminophylline (adenosine-receptor antagonist). The pentelenetetrazol eassay has been used primarily to evaluate antiepileptic drugs. However, it has been shown that, most anxiolytic agents are also able to prevent or antagonize metrazole-induced convulsion. Generally, compounds with anticonvulsant activity in the petit mal epilepsy are effective in pentelenetetrazole-induced seizure model.[10]

Data from the study showed that the tonic convulsion produced by pentelenetetrazole was significantly delayed by EECD. The data also show that diazepam antagonize the pentelenetetrazole-induced convulsion. According to be Sarro et al, pentelenetetrazole may be exerting its convulsive effect by inhibiting the activity of gamma amino butyric acid (GABA) at $GABA_A$ receptors[11] the major inhibitory neurotransmitter which is implicated in epilepsy. The enhancement and inhibition of the neurotransmission of GABA will attenuate and enhance convulsion respectively.[12-14] Phenobarbitone and diazepam have been shown to exert their antiepileptic effects by enhancing the GABA mediated inhibition in the brain.[15]

It is possible that diazepam and EECD antagonize pentelenetetrazole convulsion in this study by enhancing GABA neurotransmission. Since the EECD delayed the occurrence of pentelenetetrazole induced convulsion, it is probable that it may be interfering with GABA-aminergic mechanism to exert its anticonvulsant effect. The maximal electro shock test is the most widely used animal model in antiepileptic drug discovery, because seizure induction is simple and the predictive value for detecting clinically effective antiepileptic is high. [16]

The maximal electro shock test identifies agents with activity against generalized tonic clonic seizures using clinically established antiepileptic drugs. The pharmacology of acute maximal electroshock dose not differs from the pharmacology of generalized tonic-clonic seizures in genetic models with chronic epilepsy, eg. audiogenic- seizure susceptible mice and rats or epileptic gerbils.[17] In addition to identifying drug activity against generalized tonic-clonic seizures, it has often been proposed that the maximal electroshock test predicts anticonvulsant drug effects against partial seizures. The anticonvulsant activity of EECD 800mg/kg, in maximal electro shock model indicates that EECD might be precipitate the tonic and clonic seizures. From the above study it was concluded that the EECD flavonoids exhibits anticonvulsant activity and the probable mode of action may be due to GABA-aminergic mediation, glycine inhibitory mechanism and inhibit the electrical kindling effect.

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Fig.-1. Anti convulsant activity of eecd against mes indused convulsions in mice

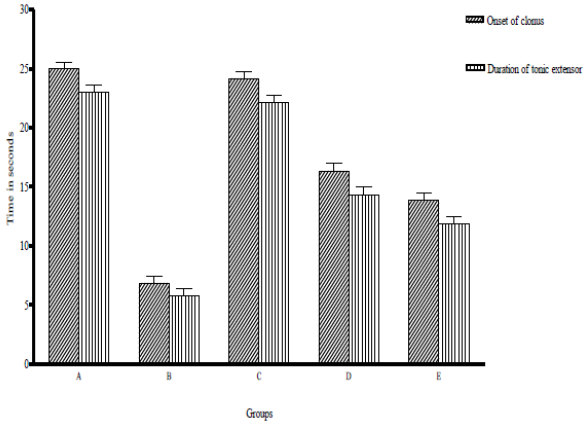
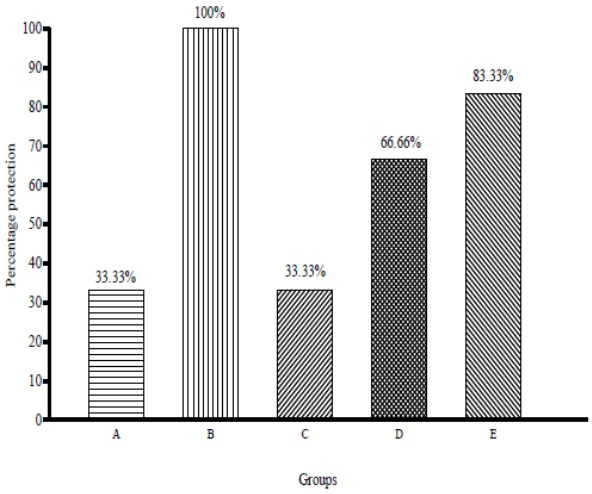


Fig.-2. Percentage convulsion protection activity of eecd against mes induced convulsions in mice



A: 2% gum acacia p.o., B: Diazepam 5mg/kg p.o., C: EECD 400mg/kg p.o., D: EECD 600mg/kg p.o. E: EECD 800mg/kg p.o.

Fig.-3. Anticonvulsant activity of eecd against ptz induced convulsions in mice

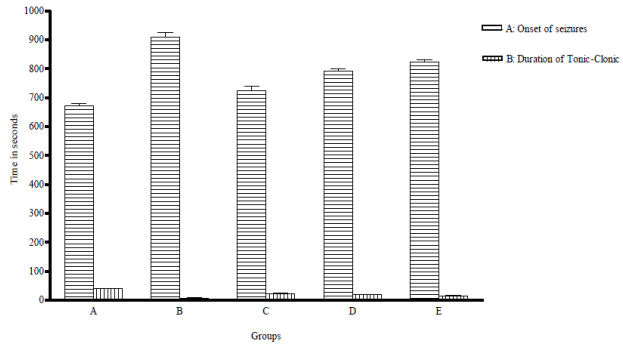
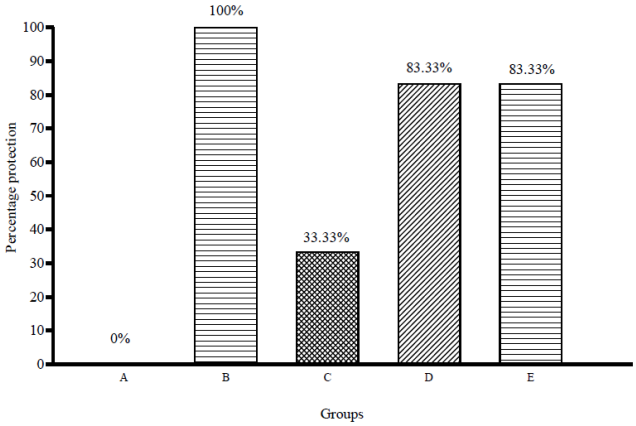


Fig.-4. Percentage convulsion protection activity of eecd against ptz induced convulsions in mice.



A: 2% gum acacia p.o., B: Diazepam 5mg/kg p.o., C: EECD 400mg/kg p.o.. D: EECD 600mg/kg p.o. E: EECD 800mg/kg p.o..

Table 1: Effect of EECD leaves on MES induced convulsions in mice.

Treatment	Dose	Onset of clonus convulsion (sec)	Duration of extensor convulsion (sec)
Control	2% gum acacia	25 ± 0.57	23 ± 0.57
Phenytoin	25mg/kg p.o.	6.83 ± 0.60	5.8 ± 0.60
EECD	400mg/kg p.o.	24.16 ± 0.60	22.16 ± 0.60
EECD	600mg/kg p.o.	16.33 ± 0.66**	14.33 ± 0.66**
EECD	800mg/kg p.o.	13.84 ± 0.60**	11.83 ± 0.60**

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

Table 2: Effect of EECD leaves on PTZ (60mg/kg) induced convulsion in mice

Treatment	Dose	Onset of convulsion (sec)	Duration of convulsion (sec)
Control	2% gum acacia	670.5 ±10.135	39.66 ± 0.66
Diazepam	5mg/kg p.o.	908.83 ±15.39	6.83 ± 0.71
EECD	400mg/kg p.o.	724.5 ±15.23*	23 ± 0.96
EECD	600mg/kg p.o.	791.16 ±9.02**	19.33 ± 0.91**
EECD	800mg/kg p.o.	823.33 ±7.81**	15.33 ± 0.49**

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