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ANTICONVULSANT OF ACTIVITY OF *SORGHUM VULGARE* L. ON MAXIMAL ELECTROSHOCK AND PENTYLENETETRAZOLE INDUCED SEIZURE IN ALBINO WISTAR RATS

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ABSTRACT

The present study is an investigation of anticonvulsant activity of the methanol leaves extract of *Sorghum vulgare* L. in rats and in order to verify the traditional use of the plant in the treatment of epilepsy. The maximal electroshock seizure (MES) and the pentyleneterazole (PTZ) models were used for assessing the anticonvulsant effects of the methanol leaves extract in rats. The methanol extract of *Sorghum vulgare* L. (250 & 500 mg/kg p.o) of that produced significant protection against MES & PTZ-induced convulsion and onset of seizures compared with the control group in rats. The results obtained from this study indicate that the methanol leaves extract of *Sorghum vulgare* L. may be beneficial in both absence and tonic clonic seizures.

Keywords: *Sorghum vulgare* L., Rats, Anticonvulsant, Pentylenetetrazole, MES.

INTRODUCTION

The plant *Sorghum vulgare* L., known as Millet or Guinea Corn. Sorghum is generally classified under two varieties, saccharine and non-saccharine. The saccharine sorghums are not used for producing sugar owing to the difficulty of crystallization. The plant *Sorghum vulgare* L., (cv. Cholam), a grass species is widely cultivated for its edible grains across northern part of Tamil Nadu. It can grow in prolonged drought hit and arid soils with more root-to-leaf area. It belongs to Poaceae family. On the basis of the traditional use of the plant for treating convulsion, but no previous pharmacological (or) clinical study was carried out to test the anticonvulsant activity of this plant [1]. Since the anticonvulsant effect of *Sorghum vulgare* L. has been experimentally not confirmed. Therefore, the present study was performed to verify the anticonvulsant of effect of *Sorghum vulgare* L. on MES and PTZ induced seizure in rats.

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MATERIALS AND METHODS

Plant collection

The Plant material of *Sorghum vulgare* L. used for investigation was collected from Tirunelveli District, in the Month of August 2014. The plant was authenticated by Dr.V.Chelladurai, Research Officer Botany. C.C.R.A.S., Govt. of India. The voucher specimen of the plant was deposited at the college for further reference.

Preparation of extracts

The leaves of *Sorghum vulgare* L. was dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (100gm) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. Percentage yield of methanolic extract of *Sorghum vulgare* L. was found to be 11.5 % w/w.

Preliminary phytochemical screening

The phytochemical examination of methanol extract of leaves of *Sorghum vulgare* L. was performed by the standard methods [2].

Experimental Animals

Wister albino rats weighing between 180-250gm each maintained in a 12 h light/dark cycle at a constant temperature 25 ºC with free access to feed (Sai durga feeds and foods, Bangalore) and water. All animals were fasted prior to all assays and were allocated to different experimental groups each of 6 rats. Moreover the animals were kept in specially constructed cages to prevent coprophagia during the experiment. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA.

Acute toxicity study

Acute toxicity study of methanol extract of *Sorghum vulgare L.* was determined by acute toxic class method of OECD guidelines. In acute oral toxicity study mortality was not observed up to 2000mg/kg body weight [3].

Anti-seizure activity

Effect on Maximal electroshock (MES) induced seizures

Albino wistar rats of either sex weighing 160 to 220 gm were divided into four groups of six animals each. The first group received vehicle control (1% w/v SCMC, 1ml/100 g) whereas Group-II received standard drug (Phenytoin, 25mg/kg) intraperitoneally, Group-III and IV, received methanol extract of *Sorghum vulgare L.* (MESV) (250 and 500 mg/kg body weight) *p.o* respectively for 20 days. On the 20th day, Seizures are induced to all the groups by using an Electro convulsiometer. Maximal electroshock seizures were elicited by a 60 Hz alternating current of 150 mA intensity for 0.2 sec. A drop of electrolyte solution (0.9% NaCl) with lignocaine was applied to the corneal electrodes prior to application to the rats. This increases the contact and reduces the incidence of fatalities. The duration of various phases of epilepsy were observed. The percentage protection was estimated by observing the number of animals showing abolition of Hindleg Tonic Extension (or) extension not greater than 90° [4].

Effect on Pentylenetetrazole (PTZ) induced seizures

Albino wistar rats of either sex weighing 160 to 220 gm were divided into four groups of six animals each. The first group received vehicle control (1% w/v SCMC, 1ml/100 g) whereas Group-II received standard drug (Diazepam, 4mg/kg) intraperitoneally, Group-III and IV, methanol extract of *Sorghum vulgare L.* (MESV) (250 and 500 mg/kg/body weight) *p.o* respectively for 20 days. On the 20th day, Pentylenetetrazole (PTZ) (90mg/kg body weight, *s.c*) was administered to all the groups to induce clonic convulsions. Animals were observed for a period of 30mins post – PTZ administration. The parameters noted were mean onset time of convulsions, duration of convulsion and recovery/Death (% recovery or % of survival) due to PTZ [5].

Statistical analysis

The data were expressed as Mean ± S.E.M. and statistically analyzed using one way ANOVA followed by Dunnett's test, $p<0.05$ was considered significant.

RESULTS

The results of preliminary phytochemical screening of the methanol extract of leaves of *Sorghum vulgare* L. revealed that presence of alkaloids, flavonoids, glycosides, tannins, saponins, terpeniods and absence of steroids.

Effects of MESV on MES Induced Seizure

The duration of tonic hindleg extension in rats treated with vehicle was 18.22±0.48 seconds. The MESV at doses of 250 mg/kg and 500 mg/kg were protect animals from seizures and significantly ($p<0.01$) reduced the duration of tonic hindleg extension for 7.14±0.0.67 and 3.56±0.52 seconds respectively. Whereas, the standard drug phenytoin treated animals exhibits abolished tonic hindleg extension. Phenytoin treated animals have shown 100% protection against MES induced seizures whereas MESV 250 mg/kg and 500 mg/kg have shown 60.81% and 80.46% protection respectively (Table 1).

Effect of MESV on PTZ Induced Seizure

In rats treated with vehicle, clonic convulsion appeared for 152.33±3.28 seconds after PTZ and all rats died after seizures. The MESV at doses of 250 mg/kg and 500 mg/kg significantly delayed the onset of clonic convulsions for 421.56±2.19 ($p<0.01$) and 564.21±3.17 ($p<0.01$) seconds respectively in dose dependent manner. Whereas, the standard drug diazepam (4mg/kg, *i.p*) delayed the onset of clonic convulsions for 728.64±2.21 seconds. Diazepam treated animals have shown 83.31% protection against PTZ induced seizures whereas MESV 250 mg/kg and 500 mg/kg have shown 58.06% and 68.73% protection respectively (Table 2).

DISCUSION AND CONCLUSION

It was found from the above observations that *Sorghum vulgare L.* has shown anticonvulsant activity against seizures induced by MES & PTZ. It was effective against MES induced seizures, since inhibition of the MES test predicts activity against generalized tonic-clonic seizure and coritical focal seizures [6].

Table 1. Effect of methanolic extract of *Sorghum vulgare L.* (MESV) On MES induced Seizures in rats

Group	Design of treatment	Flexion	Extensor	Stupor	Recovery	% protection
I	Vehicle control	9.15±0.42	18.22±0.48	40.21±0.42	187.22	0
II	Phenytoin 25mg/kg, i.p.	3.27±0.46**	0**	14.22±0.37**	90.37	100
III	MESV 250mg/kg, p.o	4.85±0.33*	7.14±0.0.67**	31.21±0.28**	140.42	60.81
IV	MESV 500mg/kg, p.o	3.72±0.29**	3.56±0.52**	18.41±0.42*	105.69	80.46

Values are expressed as mean ± SEM of six observations. Comparison between Group I *Vs* Group II, Group II *Vs* Group III &Group IV. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. *p<0.05;** p<0.01; ns-non significant.

Table 2. Effect of methanolic extract of *Sorghum vulgare L.* (MESV) On PTZ induced Seizures in rats

Group	Design of Treatment	Onset of convulsions (sec.)	Duration of convulsion (sec)	Protection convulsion%	Protection mortality %
I	Vehicle control	152.33±3.28	74.61±1.52	0	50
II	Diazepam(4mg/kg)	728.64±2.21**	12.45±0.67**	83.31	100
III	MESV 250	421.56±2.19**	31.29±0.19*	58.06	83.33
IV	MESV 500	564.21±3.17**	23.33±0.49**	68.73	100

Values are expressed as mean ± SEM of six observations. Comparison between Group I *Vs* Group II, Group II *Vs* Group III &Group IV. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. *p<0.05;** p<0.01; ns-non significant.

The MES test is the most frequently used as an animal model for identification of anticonvulsant activity of drugs for the generalized tonic-clonic seizures "grand mal" [7,8]. This model based on observation of the stimulation by repeated electrical pulses induce in different neuronal structures one characteristic standard of seizure activity [9]. In our present study, it is found that treatment with MESV on rats significantly reduces in tonic hindleg extensor stage in MES induced epilepsy. The MES model – to identify compounds which prevent seizure spread, corresponding to generalized tonic-clonic seizures in humans [10]. Currently used anticonvulsant drugs (e.g. phenytoin, carbamazepines) effective in therapy of generalized tonic-clonic and partial seizures have been found to show strong anticonvulsant action in MES test [11]. Since, MESV significantly inhibited generalized tonic-clonic seizures in MES test; it suggests the presence of anticonvulsant compounds.The administration of PTZ in the present study induced Straub's tail phenomenon, followed by jerky movements of the whole body and convulsions in PTZ treated control group animals along with an increase on the percentage mortality of rats. Clonic seizures induced by PTZ are blocked by drugs that reduce T-type calcium currents (Ethosuximide) and drugs that enhance inhibitory Neuro-transmission by GABA receptors. The results obtained from the study suggest that the methanol extract of *Sorghum vulgare L.* leaves have anti-convulsant property and the results verify its traditional use in epilepsy [12,13].

The results of this study shows that the methanol extract of *Sorghum vulgare L.* possess anticonvulsant properties which are possibly mediated partly via facilitation of GABA transmission. These results suggest that the leaves of *Sorghum vulgare L.* will be beneficial in the management of absence and tonic-clonic seizures. The present study is a preliminary attempt in evaluating the anti-convulsants activity of *Sorghum vulgare L.* leaves.

However, more precise mechanisms of MESV anticonvulsant activity and the relationship between the seizure and $GABA_A$ receptor subunits and the other neurotransmitter systems which may explain how MESV produce anticonvulsant effect must be investigated further.

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