



ANTI CONVULSANT EFFECT OF *CASSIA AURICULATA* SEED EXTRACT IN MICE

*Shravan Kumar Nanumala, T. Haripriya, S. Sanjay Singh, S. Shalini, N. Divya and B. Varsha Priyanka

Department of Pharmacology, Joginpally B.R. Pharmacy College, Hyderabad, India.

*Corresponding Author: Shravan Kumar Nanumala

Department of Pharmacology, Joginpally B.R. Pharmacy College, Hyderabad, India.

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ABSTRACT

Objective: The present study is to investigate anticonvulsant effect of *Cassia auriculata* on Maximal electroshock-induced seizures (MES) and Pentylentetrazole (PTZ) induced seizures. **Method:** The CSE (250, 500, 1000 mg/kg p.o.) doses were administered to mice and anti convulsant was observed by using MES and PTZ methods and the onset and duration of Hind Limb Tonic Extension were noted. **Result:** The CSE significantly abolished the HLTE in both the methods. The 1000mg/kg dose protected the animals more significantly ($p < 0.001$) from induced tonic convulsions. **Conclusion:** The data suggests that the ethanolic seed extracts of *Cassia auriculata* may produce its anticonvulsant effects via non-specific mechanisms since it abolished the hind limb tonic extension induced by MES as well as PTZ method.

KEYWORDS: *Cassia auriculata*, Pentylentetrazole, Maximal electroshock, Ethanolic extract, anti-epileptic.

INTRODUCTION

Epilepsy is defined as the repeated occurrence of sudden, excessive and/or synchronous discharges in cerebral cortical neuron resulting in disruption of consciousness^[1], disturbance of sensation, movements, and impairment of mental function or some combination of these signs. Therefore they are called ictal (Latin word meaning – strike) events.^[2] It involves reduction in inhibitory, i.e. GABA-mediated drive or increase in excitatory, i.e. glutamate mediated inputs and disturbances in the voltage dependent ion channels.^[3] Conventional drug therapy though effective is associated with various side effects, teratogenicity and chronic toxic effects.^[4] Hence there is a need for development of safe, effective herbal drugs. One of the herbal plants used as anti convulsant is *cassia auriculata* (fabaceae).^[5] It is also used for anemia, biliousness, febrifuge in splenic enlargements, cholera, jaundice, tumors, gout, bronchitis, probably in leprosy.^[6] detoxification ability^[7], anti inflammatory properties.^[8] The present study aims to evaluate the anti convulsant effect of *cassia auriculata* seed extract.

MATERIALS AND METHODS

Experimental Animals and Housing of Animals

Swiss Albino Mice (15-25g) of either sex were used for the study. Animals were obtained from in house facility and housed in the room in an artificial light/dark cycle (12/12 hr, light on from 7 a.m. to 7 p.m.), under standard conditions with free access to food and water. The study was performed in accordance with the guidelines issued by CPCSEA.

Plant material

The Seed of *Cassia auriculata* were collected from local areas of Rajendranagar, Hyderabad, Telangana, India and authenticated by Department of Pharmacognosy, Jawaharlal Nehru Technological University, Hyderabad.

Preparation of Extraction

Cassia auriculata ethanolic seed extract (CSE) was prepared by using ethanol, by maceration method for 72hrs at room temperature. The extract was concentrated by simple evaporation at room temperature. A suspension of CSE in water was prepared for oral administration.

Preliminary phytochemical studies

The result of preliminary phytochemical screening is presented by performing Qualitative phytochemical studies on *Cassia auriculata* ethanolic seed extract using suitable chemicals and reagents to confirm the presence of flavonoids, tannins, lipids, polyphenols, triterpenoids and steroids.

ANTICONVULSANT METHOD

Maximal Electroshock seizure (MES) model

The anti convulsant activity of *cassia auriculata* was evaluated using maximal electro shock model. MES of 50mA current for 0.2sec were administered through ear electrode to induce convulsion in the control and drug treated animals. The test animals were administered orally with the test doses 250, 500 and 1000 mg/kg of ethanolic extract of *cassia auriculata* seeds and the standard drug (25 mg/kg) is injected i.p and tested after

30mins for MES induced seizure response. All the tested animals were compared with the control mice.

Pentylentetrazole induced seizures (PTZ) model

PTZ at a dose of 60mg/kg i.p was administered 60 min after the i.p treatment with the drugs i.e., test drug doses 250, 500, 1000 mg/kg and standard (phenytoin-25 mg/kg) to induce clonic-tonic convulsions in mice. The incidence of hind limb tonic extension (HLTE) and duration of seizures were noted. The animals were considered protected if no HLTE has produced during the time limit.^[11]

Statistical analysis

The data were analyzed using One-way analysis of variance (ANOVA) followed by Dunnett's test. P values <0.05 were considered significant.

RESULTS

The phytochemical analysis reveals that the ethanolic seed extract of *Cassia auriculata* consists of flavonoids,

alkaloids, triterpenoids, tannins, saponins and large amounts of cardiac glycosides. The anticonvulsant activity of ethanolic extract at various doses (250, 500, 1000 mg/kg. p. o.) was studied by the maximum electroshock-induced and PTZ seizure models. The anticonvulsant activity induced by MES model of the ethanolic extract of *Cassia auriculata* is shown in Table 1, at dose level of 500 mg/kg elicits significant activity, though lesser comparable to that of Phenytoin (standard) and it also showed potent activity at 250 & 1000mg/kg. In PTZ induced seizures, the administration of *Cassia auriculata* ethanolic seed extract at doses of 250 and 1000 mg/kg b.w. 1 hr prior to the injection of PTZ, significantly ($p < 0.01$) delayed the on-set of convulsions as shown in Table 2.

MAXIMAL ELECTROSHOCK-INDUCED SEIZURES MODEL

Table 1: Effect of ethanolic extract of *Cassia auriculata* seed on MES induced convulsions in mice.

S. No.	GROUP	DOSE (mg/kg)	DURATION OF HLTE (sec)
1	Control	2% gum acacia p.o.	13.1±0.41
2	CSE	250 mg/kg	8.2±0.25*
3	CSE	500 mg/kg	6.2±0.54**
4	CSE	1000 mg/kg	3.10±0.20**
5	Phenytoin	25 mg/kg	2.14 ±0.42**

Values are expressed as mean ± S.E.M; (n =6) * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

The duration of HLTE in control group was found to be 13.1±0.41 seconds. In mice treated with the test drug doses 250, 500, 1000 mg/kg the duration of HLTE was found to be 8.2±0.25, 6.2±0.54 and 3.1±0.20 seconds respectively. The HLTE duration for the mice treated with phenytoin (25mg/kg) was found to be 2.14±0.42.

The duration of HLTE decreased significantly in a dose dependent manner in test drug treated group. The duration of HLTE decreased was moderately significant ($p < 0.05$) whereas it was significant ($p < 0.01$) for the test doses 200 and 400 mg/kg and the phenytoin treated (25 mg/kg) groups.

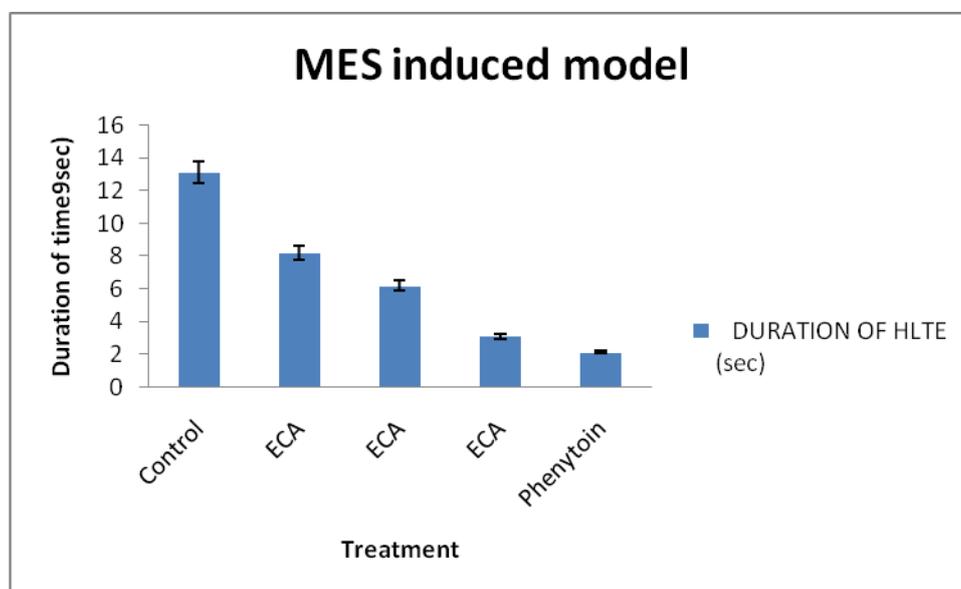


Fig. 1: Effect of ethanolic seed extract of *Cassia auriculata* on MES induced convulsions in mice.

PENTYLENETETRAZOLE (PTZ) INDUCED SEIZURES MODEL**Table 2: Effect of ethanolic extract of *Cassia auriculata* seed on PTZ induced convulsions in mice.**

S. No.	GROUP	DOSE(mg/kg)	ONSET TIME (sec)	DURATION OF HLTE (sec)
1	Control	2% gum acacia p.o.	61.0±5.14	36.1±0.75
2	CSE	250 mg/kg p.o.	52.1±1.44*	31.14±0.65*
3	CSE	500 mg/kg p.o.	48.6±1.25*	28.2±0.75**
4	CSE	1000 mg/kg p.o.	43.1±1.02**	26.12±0.25**
5	Phenytoin	25 mg/kg p.o.	0.31±0.56**	0.14±0.46*

Values are expressed as mean ± S.E.M ; (n =6) *P<0.05, **P<0.01, ***P<0.001

The onset time of action for control, test (250, 500 and 1000 mg/kg) and Phenytoin (25 mg/kg) treated mice was 61.0±5.14, 52.1±1.44, 48.6±1.25, 43.1±1.02 and 0.31±0.56 seconds respectively. Whereas the duration of HLTE for control, test (100, 200 and 400 mg/kg) and phenytoin (25 mg/kg) treated mice was found to be 36.1±0.75, 31.14±0.65, 28.2±0.75, 26.12±0.25 and 0.14±0.46 seconds respectively. The onset of action was

moderately significant (p<0.05) for the test doses 250 and 500 mg/kg and significant (p<0.01) for the test dose of 1000 mg/kg and phenytoin (25mg/kg). On the other hand the duration of HLTE was moderately significant (p<0.05) for the test dose 250 mg/kg and phenytoin (25mg/kg) and significant (p<0.01) for the test doses 500 and 1000 mg/kg.

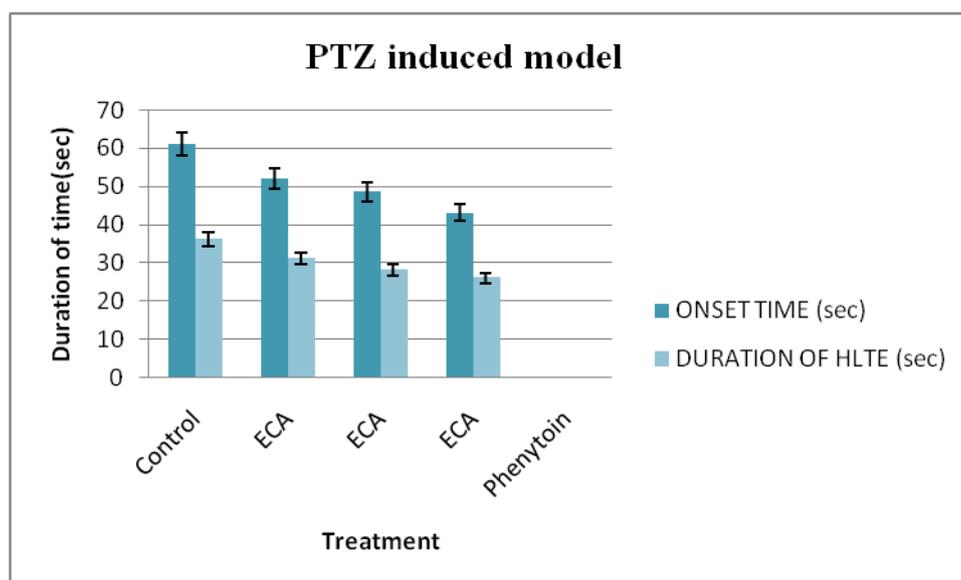


Fig 2: Effect of ethanolic extract of *Cassia auriculata* seed on PTZ (60mg/kg s.c.) induced convulsions in mice.

DISCUSSION

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.

Neurological and behavioral disorders are one of the most common problems now a day's throughout the world are associated with costly treatment. Dependence, addiction and habituation are the major drawbacks of modern allopathic drugs used to treat psychological problems. Pharmacological evaluation of the anticonvulsant properties of the CSE against PTZ & MES induced seizure revealed that the extract exhibited statistically significant & dose dependent delay in the onset of seizure and also reduction in the duration of

HLTE. The convulsant activity of MES and PTZ may be by inhibition of gamma amino butyric acid (GABA) at GABA-A receptors. GABA is an inhibitory neurotransmitter. The enhancement and inhibition of the neurotransmission of GABA will attenuate and enhance convulsion, respectively.^[13] Pentylenetetrazole is an antagonist of GABA-A receptor which is widely implicated in epilepsy.^[14] Further-more; drugs which protect animals against the generalized clonic seizure induced by PTZ are effective in protection and management of petit mal epilepsy.^[15] voltage dependent sodium channel blockers used as antiepileptic drugs, such as Phenytoin or glutaminergic blockers mediated by NMDA receptors such as felbamate can prevent tonic extension induced by MES.

The seizures elicited by pentylenetetrazole may be by blocking chlorine channel complex or GABA which denotes that the anticonvulsive activity of *Cassia auriculata* may involve glutaminergic excitation and

GABAergic inhibition mechanisms or voltage gated sodium channel inhibition. Upon phytochemical screening of the plant it was found to contain flavonoids, sterols, alkaloids, glycosides, saponins and triterpenoids, which may be the reason for anticonvulsant action of *Cassia auriculata* seeds.

CONCLUSION

It may be concluded that the *Cassia auriculata* seed extract have potent anticonvulsant effect against both MEZ and PTZ induced seizures which may be due to presence of Phytochemicals present in it. The anticonvulsant action present for the drug may be due to glutaminergic transmission or GABAergic transmission or sodium channel blockage. Further studies are to be conducted to evaluate the active principle, precise mechanism(s) and safety profile of the plant as medicinal agent to treat convulsions.

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