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## ANTIEPILEPTIC ACTIVITY OF METHANOLIC EXTRACT OF *SYZYGIUM CUMINI* SEEDS IN ALBINO MICE

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### Abstract

#### Objectives:

To evaluate the antiepileptic activity of the methanolic extract of seeds of *Syzygium cumini* in experimentally induced antiepileptic animal models.

#### Methods:

Albino mice were divided into 8 groups of 6 mice each. Maximum electroshock induced seizures(MES) and Pentylentetrazole(PTZ) test were the animal models used to evaluate the antiepileptic activity. 4 groups were allocated to MES and 4 to PTZ. Control groups were administered gum acacia(1ml/mouse); Standard group – valproic acid (100mg/kg BW); T1 group – methanolic extract of *Syzygium cumini* seeds(200mg/kg BW); T2 group – methanolic extract of *Syzygium cumini* seeds(400mg/kg BW).

#### Result:

Methanolic extract of *Syzygium cumini* seeds produced significant antiepileptic effect shown by reduction in the duration of hind limb extensor phase in MES model( $2.36 \pm 0.36$ ,  $1.18 \pm 0.18$  at 200mg/kg and 400mg/kg respectively) and ability to delay the onset of myoclonic spasms and clonic convulsions in PTZ test when compared to Control.

**Keywords:** Antiepileptic, Electroshock induced seizures(MES), Pentylentetrazole(PTZ), *Syzygium cumini*

### Introduction

A seizure (from Latin “sacire”, to take possession of) is a paroxysmal event due to abnormal, excessive, hyper synchronous discharges from an aggregate of central nervous system neurons. Epilepsy describes a condition in which

a person has recurrent seizures. This definition implies that a person with a single seizure does not necessarily have epilepsy.<sup>1</sup> Epilepsy is the second most common neurological disorder after stroke.<sup>2</sup> About 50 million people worldwide have epilepsy, and nearly two out of every three new cases are discovered in developing countries. Epilepsy is more likely to occur in young children and elderly, however, it can occur at any time.<sup>3</sup>

Epilepsy if untreated can lead to impaired intellectual function or death.<sup>4</sup> It is a significant clinical problem due to inefficiency of current medication to cure seizures and subsequently side effects like drowsiness and cognitive impairment. Despite the massive scale of the problem and much research, epilepsy remains poorly understood. Presently available therapy is symptomatic, i.e. the drugs inhibit seizures, but whether any of these prevent the development of epilepsy (epileptogenesis) is uncertain. Insights that promise to provide molecular targets for both symptomatic and preventive therapies are being researched.<sup>5</sup>

The heterogeneity of the disease and our limited understanding of it makes discovery and development of anti-epileptic difficult.<sup>1</sup> Medicinal plants are in use since ages for neurological disorders. The added advantage of these would include its complementary nature to conventional treatment making them safer, well tolerated, economical and naturally accessible remedy.

*Syzygium cumini* Linn. (synonym *Eugenia jambolana* Linn.) is a very large evergreen tropical tree belonging to the family Myrtaceae, the plant is also mentioned in literature as Jamun, synonym as black plum or jambolan, the plant is very well known for their pharmacological properties since ancient ages.<sup>6</sup> The medicinal value is due to presence of malic acid, oxalic acid, gallic acid and tannins. Various works on tannin, flavonoids, essential oil and betulic acid was reported to have diverse pharmacological activities like gastroprotective, antiulcerogenic, antibacterial, anti-infective, antimalarial.<sup>7</sup> This study was designed to evaluate the antiepileptic activities of *Syzygium cumini* in order to scientifically justify its use in traditional medicine to treat epilepsy.

## **Material and Methods**

### **Collection and Extraction of plant material**

The fruits were collected locally. The fruit seeds were freed of pericarp, shade dried and powdered in a mixer and 100 gms of the seed powder was extracted with 70% methanol at 50 to 60 °C in a soxhlet apparatus for 72 hrs. The

methanol extract was then concentrated under reduced pressure at 40°C in a rotary evaporator to obtain a solid sample giving 15% yield.

### **Animals**

Swiss albino mice (48) weighing around 25g – 30g of either sex were randomly selected from central animal facility, J.S.S Medical College, Mysore. Animals were provided free access to tap water and commercial food and were maintained under standard laboratory conditions with a natural light and dark cycle under room temperature. The animals were acclimatized for 24 hours before the start of experimentation. All the drugs were administered orally to the animals. The study was conducted after obtaining institutional animal ethical committee clearance

### **Experimental protocol**

Animals were divided into 4 groups of 6 mice each. Group 1 (Control group) received 1ml gum acacia (vehicle), Group 2 (Standard group) received 100mg/kg Valproic acid, Group 3 - T1 received 200 mg/kg of methanolic extract of *Syzygium cumini* seeds, Group 4 – T2 received 400 mg/kg of methanolic extract of *Syzygium cumini* seeds. All the drugs were suspended in gum acacia and administered orally 1hr prior to induction of seizures. The anticonvulsant activity was screened using maximal electro shock (MES) model and pentylenetetrazole (PTZ) model.

### **Acute toxicity test:**

The acute toxicity of methanol extract of *Syzygium cumini* seeds were determined in albino mice. The extract was administered in doses of 50, 300, 1000 and 2000 mg/kg to different groups of mice and mortality was observed after 24 hrs

The extract was devoid of mortality of animal at the dose of 200 mg/kg and hence >200mg/kg was taken as LD<sub>50</sub> cut off value.

### **Experimental design**

The anticonvulsant activity of methanolic extract of *Syzygium cumini* seeds was evaluated for maximum electroshock induced seizure (MES) in mice and pentylenetetrazole (PTZ) model

#### 1. Maximal electroshock induced seizures:-<sup>[8]</sup>

Swiss albino mice weighing 25-30gm were used. Electrical stimulation was applied via corneal or ear electrode with a current strength of 50 mA for 0.2 sec. The resultant seizure passes through various phases; phase of tonic hind limb

flexion, phase of tonic hind limb extension and finally followed by variable short clonic interval which may lead to asphyxial death in some animals. 24 hours before testing of anticonvulsants (to avoid any possible kindling effect) the animals were pre-screened for their ability to develop full tonic extension in the maximal electroshock test. Only those animals showing hind limb tonic extension response was chosen for maximal electroshock test.

Evaluation- Decrease in duration of tonic hind limb extension was considered as protection action this test.

## 2. Pentylenetetrazole (PTZ) Induced Convulsion:<sup>[8]</sup>

Pentylenetetrazole is a central nervous system stimulant. The convulsive effect is analogous to petit mal type of convulsions in man. The test compound and the reference drug was given orally to the respective groups of albino mice. Another group of same number of mice serves as control. Sixty minutes after oral administration of drugs, 70 mg/kg Pentylenetetrazole was injected intraperitoneally. Each animal was placed in an individual plastic cage for observation lasting 30 minutes. Within 30 minutes they developed a sequence of excitement, myoclonic jerks, clonic seizures, one or more maximal tonic seizures and few deaths.

Evaluation:- The anticonvulsant property was assessed by its ability to delay the onset of Myoclonic spasms and clonic convulsions.

### Statistical Analysis

Results were presented as Mean  $\pm$  SEM. One way ANOVA was used for multiple comparisons followed by Dunnet's "t" test. For all the tests a 'P' value of 0.05 or less was considered as statistical significant.

### Result

The methanolic extract of SC seeds, both 200mg/kg and 400mg/kg exhibited a dose dependent significant (P<0.01 and P<0.001) reduction in the duration of hind limb extensor phase in MES model.

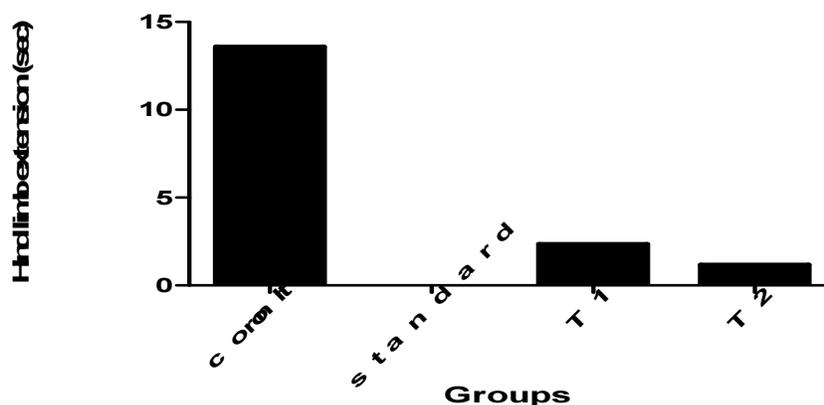
**Table 1- Duration of hind limb extensor phase (sec) in MES model**

GROUPS	Hind limb Tonic Flexion (sec)	Hind limb Tonic Extension(sec)	Clonus (sec)	Postical Depression (sec)
Control	---	13.6 $\pm$ 0.889	13.8 $\pm$ 1.65	5.86 $\pm$ 1.08

Standard	3.6 ± 0.58***	0	8.8 ± 1.69***	1.18 ± 0.68***
T1	3.36 ± 0.36***	2.36 ± 0.36***	5.35 ± 0.35**	2.5 ± 1.76
T2	2.36 ± 0.26***	1.18 ± 0.18***	4.86 ± 0.98***	1.9 ± 0.59**

Values represent mean of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dunnet’s “t” test. \*\*P< 0. 01 \*\*\*P < 0. 001.

**Graph 1: Comparison of hind limb tonic extension (HLTE) between different groups in MES model.**

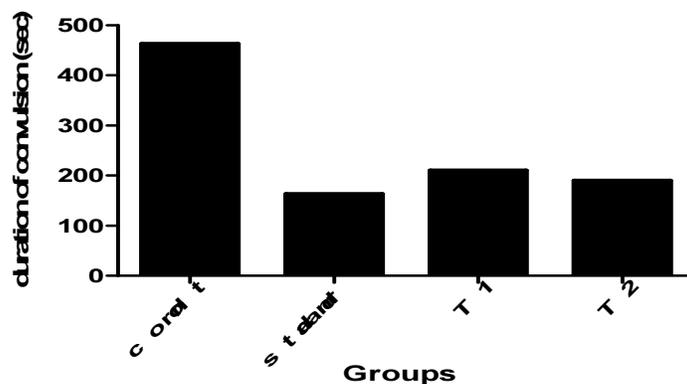


**PTZ MODEL:** The methanolic extract of SC seeds significantly (P<0.01 and P<0.001) delayed the onset of seizure induced by PTZ when compared to control.

**Table 2 – Duration of convulsion in PTZ model**

GROUPS	Onset of convulsions (sec)	Duration of convulsion (sec)
Control	60.20 ± 1.25	463.48 ± 11.39
Standard	79.20 ± 1.56	163.44 ± 17.04**
T1	70.39 ± 2.49	210.59 ± 13.49
T2	75.20 ± 2.55***	190.37 ± 17.49**

Values represent mean of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dunnet’s “t” test. \*\*P< 0. 01 \*\*\*P < 0. 001.

**Graph 2: Comparison of duration of convulsions (sec) between different groups in PTZ model.**

### Phytochemical Screening

The phytochemical screening of the extract revealed the presence of small quantities of alkaloids, flavonoids and saponins. However the triterpenic steroids, flavonoids and saponins are reported to possess anticonvulsant activity in some experimental seizure models such as MES and PTZ.

### Discussion

Epilepsy is the third most common neurological disorder affecting the worldwide population.<sup>9</sup> The high incidence of adverse effects from the use of antiepileptic drugs is a source of widespread concern in patients who use them chronically. Hence the need for development of a potent antiepileptic with lesser side effects. Indigeneous plants have shown to have effect on CNS activity. The methanolic extract of *Syzygium cumini* contains flavonoids and saponins. Flavonoids have been reported to possess significant anticonvulsant activity in various plants.<sup>4</sup> Saponins are known to have antagonistic activity against amphetamine and sedative property.<sup>10</sup> The maximal electroshock-induced convulsion in animals represents grandmal type of epilepsy. Protection against electroshock induced seizures in mice and rats is used as an indication for compounds which may prove effective in grandmal epilepsy. Methanolic extract of *Syzygium cumini* seeds on single administration, dose dependently reduced/ abolished the tonic hind limb extension phase in MES induced seizures in albino mice. The duration of tonic hind limb extension with methanolic extract was  $2.36 \pm 0.36$  at a dose 200 mg/kg which is statistically significant when compared to the control. Protection against hind limb extensor phase shows anticonvulsant property of methanolic extract of *Syzygium cumini* seeds. Drugs that are used in

the treatment of tonic clonic and partial seizures suppress tonic hind limb extension in maximal electroshock- induced convulsion.

The Pentylenetetrazole (PTZ) Induced Convulsion test is indicative of anticonvulsant activity of drugs against petit mal seizures. The methanolic extract of *Szygium cumini* seeds at a dose of 400mg/kg showed anticonvulsant activity in the PTZ model by increasing the seizure threshold and decreasing the duration of convulsion.

Decrease in duration of tonic hind limb extension in MES model, increased latency of onset and decreased duration of seizures in PTZ induced seizures model reveals depression effect on CNS. The CNS depressant activity may be due to the increase in the concentration of GABA in the brain.<sup>10</sup>

### Conclusion

From the above study it is concluded that the methanolic extract of *Syzygium cumini* seeds may be valuable in the treatment of convulsive disorders especially grand mal epilepsy as an adjuvant. Further research is needed to isolate the bioactive compound responsible for the anticonvulsant activity and confirm the mechanism of action.

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