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# *In vivo* anticonvulsant activity of *Aegle marmelos Corr.* Against pentylenetetrazole and electroconvulsive seizure in mice.

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## ABSTRACT

*Aegle marmelos* Corr. (Rutaceae) which has been widely used as an antiepileptic remedy in Indian traditional medicine. Different parts like leaves and ripe fruits have been used in the treatment of diarrhoeas, dysenteries, epilepsy and diabetes mellitus. We have taken aqueous extract isolated from the fruits of A. marmelos and studied their toxic effects. Acute, subacute and $LD_{50}$ values were determined in experimental rats. Phytochemical investigations revealed the presence of flavonoids, tannins and steroids. The anticonvulsant activity of aqueous extract of the fruits of *A. marmelos* was examined against experimental seizures induced by Pentylenetetrazole and Electroshock in mice at a dose level of 200 and 300 mg/kg. The aqueous extract of the fruits of *A. marmelos* protected mice against tonic convulsions induced by maximal electroshock and especially by pentylenetetrazole in mice. Data obtained from convulsion tests were expressed as mean ± SEM and were analyzed by one-way analysis of varience (ANOVA) followed by Dunnette's multiple comparison test.

***Keywords:*** *Aegle marmelos;* seizure; Maximal electroshock; Pentylenetetrazole

## INTRODUCTION

The plant *Aegle marmelos* Corr. (AM) belongs to the family Rutaceae and is known as vilvam in Tamil, baelin Hindi, bilwa or sripal in Sanskrit and bael tree in English[1]The various parts of this plant (mainly leaves and fruits) are widely used in traditional medicine for the treatment of various disorders. A decoction of plant leaves and fruit is used in remedies for dysentery, diarrhea, upper respiratory tract infections and heart ailments. [2, 3, 4]

Fresh aqueous and alcoholic extracts of this plant have been reported to have a stimulant effect on the heart and to decrease the requirement of circulatory stimulants. [5, 6]We have also proven its effects on myocardium. [7]Earlier studies have shown that the alcoholic extract of AM leaves protects against histamine-induced contractions in guinea pig ileum and tracheal chain [8] and serial extracts show anti-inflammatory, anti-pyretic and analgesic properties.[9]

In the present work, we have examined the possible protective effect of the aqueous extract of the fruits of *A. marmelos* against seizures induced by maximal electroshock (MES) or pentylenetetrazole (PTZ).

## MATERIALS AND METHODS

### Plant Materials

Fruits of *A. marmelos* were collected from the Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra (India) in July 2007. The plant sample was identified and authenticated by botanist, Botanical Survey of India, Pune, India.

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### Drugs

Alcohol and Tween 80 were obtained from Rajesh chemicals, Bombay. PTZ, Phenytoin and ethosuximide were purchased from sanjeevani agencies Ahmednagar. PTZ and ethosuximide were dissolved in saline solution (0.9%). Phenytoin sodium was dissolved in saline that was alkalinized slightly with 0.1 mM.potassium hydrochloride. The extract was dissolved in 5% v/v Tween 80 in distilled water. All drugs and extract were administered intraperitoneally (i.p.) in volume of 0.1 ml/10g of mice body weight.

### Extracts Preparation

The collected fruits of *A.marmelos* were washed, air-dried, powdered and macerated, in portions of 200g, with distilled water at room temperature for 3 days. After exhaustive extraction, the collected aqueous extract was dried and kept under refrigeration. The final weight of crude extract was 10.5 g. The extract was maintained at 4 $^0$ C throughout experiments.

### Animals

Male Swiss albino mice weighing 20-25g were used. The animals were obtained from National Toxicology Center, Pune. The animals were housed in standard cages with free access to food (standard laboratory rodent chow) and water ad-libitum. The animal house temperature was maintained at 23 ± 3.0 $^0$C with a 12-h light/dark cycle. The ethical guidelines for the investigation of experimental seizure in con-

**Table 1: Anticonvulsant activity against Maximal Electroshock induced Seizures in mice**

| Sr.No . | Group | Time (sec) in various phase of convulsion Tonic-flexion | Tonic-extensor | Clonic convulsion | stupor | Recovery /death | % inhibition |
|---|---|---|---|---|---|---|---|
| 1. | Control | 11.40±0.70 | 22.00±0.70 | 53.60±0.81 | 73.00±0.70 | Recovery | - |
| 2. | Phenytoin | 9.20± 0.37 | 3.40±0.50** | 16.20±0.37 | 44.80±0.58 | Recovery | 84.54 |
| 3. | Extract | 10.80±0.37 | 10.00±0.70* | 42.00±0.70 | 59.80±0.66 | Recovery | 54.54 |
| 4. | Extract | 10.00±0.31 | 7.10±0.50** | 35.00±1.14 | 49.20±0.86 | Recovery | 68.18 |

*Values are Mean ± SEM *=P<0.05, **= P<0.01.*

**Table 2: Anticonvulsant activity against pentylenetetrazole induced Seizures in mice.**

| Sr.No | Group | Time (sec) in various phase of convulsion Tonic-flexion | Tonic-extensor | Clonic convulsion | stupor | Recovery /death | % inhibition |
|---|---|---|---|---|---|---|---|
| 1. | Control | 11.00±0.60 | 24.00±0.54 | 55.60±0.86 | 72.00±0.78 | Recovery | - |
| 2. | Ethosuximide | 9.50± 0.47 | 3.10±0.43** | 15.20±0.39 | 41.80±0.54 | Recovery | 87.08 |
| 3. | Extract | 10.50±0.27 | 09.00±0.40* | 40.00±0.25 | 57.80±0.61 | Recovery | 62.50 |
| 4. | Extract | 10.00±0.36 | 06.52±0.56** | 33.00±1.14 | 45.20±0.46 | Recovery | 72.83 |

*Values are Mean ± SEM *=P<0.05, **= P<0.01.*

scious animals were followed in all tests. All efforts were made to minimize animal suffering and to reduce the number of animals used. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethical Committee and were in accordance with the guidelines of CPCSEA.

**Acute Toxicity Study**

The acute toxicity study of the extracts of A. marmelos was performed . [10] Adult Swiss albino mice weighing 20-25g of both sex were divided into four groups, each containing four animals for the purpose of determining the $LD_{50}$ value of a single extract. Each group was caged separately. Four different doses of 1000, 1250, 1500 and 2000 mg/kg body wt were employed for each test drug. Each animal in every group was administered with an extract of a pre-determined dose intraperitoneally. About 24 h later, the number of dead animals in a group was recorded. The data were tabulated. The toxicological effect was assessed on the basis of mortality, which was expressed as an $LD_{50}$ value.

**Phytochemical Screening**

The extract were also screened with the help of chemical test like Liberman Buchard, ferric chloride , Copo of magnesium, Vanillinesulphuric acid tests for the presence of sterols ,phenolic compounds,flavonoids and saponins respectively.[11]

**Anticonvulsant Activity**

**MES-Induced Seizures**

Electroconvulsive shock, inducing Hind Limb Tonic Extension (HLTE) in 99.9% of animals. [12] The electrical stimulus (50 mA, 50 Hz, 1 sec duration) was applied through corneal electrodes. Animals were divided into four groups each consisting of six animals, administered with aqueous extract (100,200 mg/kg), phenytoin (25 mg/kg, as positive control), saline (10 ml/kg, as control), and after 30 minutes various phases of convulsion i.e. tonic flexion, tonic extensor, clonic convulsion, stupor and recovery were studied by applying electrical stimulus of 50 mA 50 Hz, 1 s duration with cornel electrode and noted time spent in each phase. The criterion for anticonvulsant effect was abolition of HLTE within 10 s after delivery of the electroshock.

**PTZ-Induced Seizures**

Minimal i.p. dose of PTZ at which 99.9% of animals showed HLTE [13] was determined. This dose of (100 mg/kg) was then given to four groups each consisting of six animals, administered with aqueous extract (100,200 mg/kg), ethosuximide (150 mg/kg, as positive control), saline (10 ml/kg, as control). The time of peak effect of ethosuximide (30 min after administration) was previously established. [14] The time for the extract to reach its maximum effect was determined as 30 min after i.p. injection. If no HLTE occurred during a 30-min period of observation, the animals were considered protected.

**Data Analysis**

The statistical analysis of data obtained from convulsion tests were carried out using one-way analysis of varience (ANOVA) followed by Dunnette's multiple comparison test. All the results obtained in the study were compared with the vehicle control group. P values < 0.05 were considered to be statistically significant.

**RESULTS**

**Acute Toxicity**

The results showed that $LD_{50}$ value of aqueous extract of Fruits of *A. marmelos* is 1660 mg/kg..

**Phytochemical Analysis**

The phytochemical analysis of aqueous extract of Fruits of *A. marmelos* reveal a high content of flavonoids , tannins and steroids; moreover, there have also been reports of the presence of Aegeline, imperatorin, xanthotoxol [15],coumarins [16],essential oils [17],minerals like vanadium, zinc, chromium ,copper, iron, potassium, sodium, and nickel [18].

**Anticonvulsant Activity**

**MES-Induced Seizures**

The aqueous extract produced dose-dependent anticonvulsant ef-

fect against with an $ED_{50}$ of 100, 200 mg/kg (Table 1) when compared with control.

**PTZ-Induced Seizures**

The aqueous extract prevented PTZ-induced seizures in a dose-dependent manner (Table 2) when compared with control.

**DISCUSSION AND CONCLUSION**

Current available anticonvulsant drugs are able to efficiently control epileptic seizures in about 50% of the patients; another 25% may show improvements whereas the remainder does not benefit significantly. [19] Furthermore, undesirable side effects from the drugs used clinically often render treatment difficult; so that a demand for new type of anticonvulsants exists. One of the approaches to search for new antiepileptic drugs is the investigation of naturally occurring compounds, which may belong to new structural classes. In traditional medicine, *A. marmelos* is used in treatment of diarrhea, dyspepsia, dysentery and mental diseases. In the present study we have evaluated the effect of leaves and fruit alcoholic extract of *A marmelos.* against seizures induced by maximal electroshock (MES) or pentylenetetrazole (PTZ) in mice. The results indicate that the aqueous extract produced dose-dependent anticonvulsant effect against MES and PTZ-induced seizure with an $ED_{50}$ of 100, 200 mg/kg (Table 1and 2) respectively.

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-dependent Na+ channels, such as phenytoin, valproate, felmamate and lamotrigine.

On the other hand drugs that reduce T-type $Ca^{2+}$ currents, such as ethosuximide can prevent seizures induced by PTZ. This type of seizures can also be prevented by drugs that enhance gamma amino butyric acid type A ($GABA_A$) receptor mediated inhibitory neurotransmission, such as benzodiazepines and Phenobarbital and perhaps valproate and felbamate. [20]

The leaves and fruit aqueous extract of *A.marmelos* possesses protective effect against experimental seizures induced by PTZ and MES. The result obtained is more significant in PTZ-induced seizures. However, the exact mechanism and the active compounds involved in these effects need to be clarified in future studies.

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