International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.1, pp 640-643, Jan-Mar 2010

Anticonvulsant Activity of Ethanolic Extract of *Aegle marmelos* (Leaves) in Mice

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ABSTRACT: The anticonvulsant effect of Ethanolic extract from the leaves of *Aegle marmelos* on maximal electroshock (MES) or pentylenetetrazole (PTZ) in male mice examined in this study. This medicinal plant belongs to the Rutaceae family and the leaves are popularly used in the treatment of inflammation, asthma, hypoglycemia, febrifuge, hepatitis and analgesic. The extract of *Aegle marmelos* (orally) was administered in mice at the doses of100 and 200 mg/kg. The Extract suppressed hind limb tonic extensions (HLTE) induced by MES and also exhibited protector effect in PTZ-induced seizures, at 200 mg/kg dose. Since the ethanolic extract of *Aegle marmelos* delayed the occurrence of MES and PTZ convulsions, it is concluded that it interfere with gabaergic mechanism(s) to exert their anticonvulsant effect in addition it reveals the presence of flavonoid attributed to their anti-convulsant action. The activity reported was dose dependent.
Keywords: *Aegle marmelos*, Anticonvulsant effects, Maximal electroshock, Pentylenetetrazole.

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 per 100, 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 [1].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.

Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin. Over 4000 different naturally occurring flavonoids have been described. The flavonoids show various biological activities including antioxidant, anti-inflammatory activity, activity on coronary heart diseases and cytotoxic-antitumor activity [5]. The plants containing saponins or flavonoids exhibit anticonvulsant activity [6,7]. However, the anxiolytic properties of these flavonoids have been rarely investigated. Previously, the anxiolytic-like effects of simple flavones, chrysin (5,7-dihydroyflovone) have been reported, which behaves as a competitive ligand of the benzodiazepine receptors[8] . Apigenin has been reported to show similar activity in mice with only slight sedative effect [9]. Therefore, in view of the above observations, we

planned to study the anti-convulsant property of *Aegle marmelos*.

MATERIALS AND METHODS

PREPARATION OF EXTRACT

Aegle marmelos leaves were collected from Chennai, Tamil nadu,India in the month of February 2009.The leaf was authentified by Prof. Dr. P. Jayaraman Ph.D.,The Director, Plant Anatomy Research Centre(PARC), Pharmacognosy Institute, West Tambaram, Chennai and the authentication number is PARC/2009/301.The powdered dried leaves were defatted by extraction with petroleum ether (60-80°C). The defatted material was then extracted with ethanol (95%) for 72 hrs by hot percolation method and subjected to vacuum distillation. The final product (8.5gms) was then freeze-dried and stored in the refrigerator. Preliminary phytochemical investigations of the extract were conducted as per the procedures described by Kokate[10] revealed the presence of flavanoids, saponins, carbohydrates, phenolic compounds and alkaloids.

DRUGS

PTZ and Diazepam were purchased from Sigma Chemical Co (Hyderabad, INDIA). Different concentrations of the drugs were prepared freshly by suspending in gum acacia in water. The solvents used were of analytical grade. Ethanol, Petroleum ether (BDH, Mumbai, India) and Gum acacia in water (M/S Hi-media, Mumbai, India) used as solvent and vehicle respectively.

ANIMALS

Albino mice weighing between 18-22 g were used. The animals were procured from 'The Animal house'of SRM College of Pharmacy. They were placed in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2°C and relative humidity of 30-70%. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat/mice chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC) and were in accordance with the guidelines of the IAEC. (Regn.No.56/2009/CPCSEA).

MAXIMUM ELECTROSHOCK-INDUCED SEIZURE MODEL

Electroconvulsive shock (50 mA for 0.2 sec) was delivered through ear electrodes to induce hind limb tonic extensions (HLTE) in mice. The extract was administered orally at the doses of 100 and 200mg/kg into test groups. Gum acacia in water and Diazepam (4mg/kg) were administered orally into two groups of animals as control and positive control groups, respectively. Electroconvulsive shock was delivered 60 min after the administration of drugs. Occurrence of HLTE and duration of seizures were noted closely for 2 min. The animals that did not exhibit HLTE were considered protected .Percentage of inhibition of seizures relative to controls was calculated[11].

PTZ-INDUCED SEIZURES

PTZ at the dose of 80 mg/kg (minimal dose needed to induce convulsions) was injected i.p. to induce clonic-tonic convulsions in mice. Doses of 100 and 200mg/kg of the extract were administered orally into test groups. Gum acacia in water and Diazepam (4 mg/kg) were administered orally into two groups of animals as control and positive control groups, respectively. PTZ was injected i.p. 60 min after the administration of drugs. Occurrence of HLTE and duration of seizures were noted. If no HLTE occurred during the time limit, the animals were considered protected. Percentage of inhibition of seizures relative to controls was calculated[11].

STATISTICAL ANALYSIS

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

RESULTS

MES-INDUCED SEIZURES

Albino mice pretreated with the ethanolic extract have been significantly protected from convulsions induced by electroshock one hour post-dosing. The percentage inhibition achieved at the doses 100 and 200mg/kg were 46% ($p<0.001$) and 69% ($p<0.001$) respectively. Extract at both the doses, prolonged the onset of convulsions in the extract treated group compared to vehicle treated control group (Table 1).

PTZ-INDUCED SEIZURES

Animals treated with methanolic extract at a dose of 200mg/kg showed alteration in the occurrence of HLTE and duration of seizures significantly as related to controls in the model of convulsion induced by pentylenetetrazole in mice but did not alter significantly at 100mg/kg. Percentage of inhibition of seizures for 200 mg/kg relative to controls was 44.34% (Table 2).

DISCUSSION

Data from this study show that *Aegle marmelos* significantly increases the onset time and decreases the duration of seizures by electroconvulsive shock. The study also revealed that the onset of tonic convulsion produced by PTZ was significantly delayed and also duration of seizures was prolonged.

MES and PTZ may be exerting their convulsant effects by inhibiting the activity of gamma amino butyric acid (GABA) at GABA-A receptors [12]. Gamma amino

butyric acid is the major inhibitory neurotransmitter which is implicated in epilepsy. The enhancement and inhibition of the neurotransmission of GABA will attenuate and enhance convulsion, respectively [13].

Diazepams a standard antiepileptic drug has been shown to exert their antiepileptic effects by enhancing GABA-mediated inhibition in the brain[14] . It is possible that Diazepam antagonize MES and PTZ convulsions in this study by enhancing GABA neurotransmission.Since the methanolic extract of *Aegle marmelos* delayed the occurrence of MES and PTZconvulsions, it is probable that it may be interfering with gabaergic mechanism(s) to exert their anticonvulsant effect.

Phytochemical tests carried out in the present study show that the extract contains saponins, tannins and flavonoids. The plants containing saponins or flavonoids exhibit anticonvulsant activity[6, 7]. As the saponins and flavonoids present in *Aegle marmelos* might contribute to the anticonvulsant activity of the plant. Further research is in progress to isolate the compound responsible for their anticonvulsant activity.

Table. 1. Effect of Ethanolic extract of *Aegle marmelos* (EEAM) on tonic seizures induced by maximal electroshock in mice

Treatment Group	Dose mg/kg (p.o)	Onset time (Sec)	Duration of HLTE (Sec)	Percentage inhibition of convulsions
Control (Group-I)	1ml/kg	2.16±0.48	109.5±2.63	_
Diazepam (Group-II)	4	0	0	100
EEAM (Group-III)	100	6.67±0.49*	63.16±1.6*	42.3
EEAM (Group-IV)	200	11.09±0.86*	30.46±4.04*	68.57

Values are given as mean±SEM for six rats in each group. Results are statistically significant at *P<0.001 as compared with control.

Table. 2. Effect of Ethanolic extract of *Aegle marmelos* (EEAM) on pentylenetetrazoleinduced Seizures in mice

Treatment Group	Dose mg/kg (p.o)	Onset time (Sec)	Duration of HLTE (Sec)	Percentage inhibition of convulsions
Control (Group-I)	1ml/kg	61.33±2.23	46.16±3.02	_
Diazepam (Group-II)	4	0	0	100
EEAM (Group-III)	100	83.33±2.01*	31.33±1.73*	31.89
EEAM (Group-IV)	200	95±1.67*	19±1.53*	58.84

Values are given as mean±SEM for six rats in each group. Results are statistically significant at *P<0.001 as compared with control.

ACKNOWLEDGEMENT
Authors are grateful to Dr. K.S Lakshmi, Dean & Dr. K. Ilango, Vice Principal, College of Pharmacy & SRM University, for providing necessary facilities to carry out this work.

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