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ANTICONVULSANT ACTIVITY OF ETHANOLIC EXTRACT OF AEGLE MARMELOS (L.) CORRÊA ALONE AND IN COMBINATION WITH PHENYTOIN BY MAXIMAL ELECTROSHOCK INDUCED SEIZURE (MES) IN ALBINO MICE.

Pharma

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ABSTRACT

Aim: To evaluate the anticonvulsant activity of ethanolic extract *of Aegle marmelos* (L.) correa alone and in combination with phenytoin by maximal electroshock (MES) induced seizure in albino mice. **Method:** Ethanolic extract of *Aegle marmelos* (L.) correa (EEAM) was prepared using Soxhlet apparatus. The anticonvulsant activity of 2 doses of EEAM (200mg/kg and 400mg/kg), standard drug phenytoin (therapeutic dose 20mg/kg, sub therapeutic dose 10mg/kg), combination of EEAM (200mg) and phenytoin (10mg/kg) and combination of EEAM (400mg/kg) and phenytoin 10mg/kg were evaluated by maximal electroshock induced seizure (MES) model. After 1hr of oral administration of the test drug and standard drug, the pre-screened albino mice were subjected to MES seizures by electroconvulsiometer with a current of 45mA for 0.2 sec via transauricular electrodes. Duration of Tonic hind limb extension (THLE) was recorded and percentage protection calculated. **Results:** EEAM exhibited significant anticonvulsant activity in the MES model at doses 200mg/kg and 400mg/kg and also in combination with phenytoin subtherapeutic dose (10mg/kg) when compared with control group ($p < 0.001$). Combination of EEAM 400mg/kg and sub therapeutic dose of phenytoin 10mg/kg exhibited similar anticonvulsant effect when compared with standard therapeutic dose of phenytoin. **Conclusion:** EEAM exhibited significant anticonvulsant activity when given alone and it also potentiates the anticonvulsant activity of sub therapeutic dose of phenytoin when given in combination in the MES model.

KEYWORDS

Seizure, phenytoin, Maximal electroshock seizure, Aegle marmelos (L.) Correa

INTRODUCTION

A seizure is a paroxysmal event due to abnormal excessive or synchronous neuronal activity in the brain. Epilepsy is diagnosed when there are recurrent seizures due to a chronic, underlying process.[1] According to WHO, epilepsy is one of the most common neurological diseases globally with nearly around 50 million people suffering from it.[2] People with epilepsy are more prone to physical as well as psychological comorbidities (like depression and anxiety).[3] Fatal complication like Sudden Unexplained Death in Epilepsy Patients (SUDEP) might also occur in patients with uncontrolled generalised tonic clonic seizure.[4]

Etiology can be genetic, structural, metabolic, infectious, immune or unknown.[5] The underlying neuronal abnormality in epilepsy may be associated with enhanced excitatory amino acid (glutamate) transmission or impaired inhibitory (gamma amino butyric acid) transmission.[6]

Epilepsies may be primary (idiopathic) or secondary (due to trauma/surgery) .[7] The aim of treatment is cessation of seizures without side effects. Both conventional and newer antiepileptic drugs (AEDs) treat various types of seizures. However, the currently used AEDs frequently causes side effects which is an important reason of treatment failure with antiepileptic drugs.[8] Anti-epileptic drugs are also associated with drug interaction making it difficult to attain easy seizure control.[9] Various herbs have been used as an alternative medicine for the treatment of epilepsy.[10] The use of both conventional drug therapies and herbal medicines is a very common practice among epileptic patients.[11]

Aegle marmelos (L.) Correa (Manipuri: Hari-khagok) commonly known as Bael belonging to the family Rutaceae, has been widely used in indigenous Indian medicine due to its various medicinal properties. It is a medium to large sized deciduous tree with the axillary and 2.5 cm long alternate trifoliate leaves. Various pharmacological studies have shown antidepressant and anti-anxiety, anti-inflammatory, nephroprotective, hepatoprotective, antimicrobial, analgesic activities of *Aegle marmelos* leaves.[12] Therefore, the present study was undertaken to evaluate the anticonvulsant activity of *Aegle marmelos* (L.) Correa leaves in suitable experimental animal models.

MATERIALS AND METHODS

Plant extract preparation:

Aegle marmelos (L.) Correa leaves were collected from Imphal valley during the month of July 2020 and was authenticated by botany department, D.M. college, Imphal (acc no. DMH 12.2020). The leaves were cleansed, dried under shade, and powdered by mixer grinder. The ethanolic extract of *Aegle marmelos* (L.) Correa leaves were obtained by using Soxhlet apparatus as described by Knevel et al.[13] The percentage yield was 16%.

Phytochemical analysis

Phytochemicals analysis of the extract was carried out using standard procedure.[14-17] It revealed the presence of alkaloids, flavonoids, saponins and tannins.

Acute toxicity testing

Acute toxicity testing was done in healthy albino rats according to OECD guidelines 423.[18] Limit test with 2000 mg/kg was carried out with six animals (three animals per step). The extract was dissolved in 2% gum acacia and was given at 1mL/100 g of body weight in a single dose by gavage using a stomach tube. The animals were observed for 14 days. There was no mortality observed at the dose of 2000 mg/kg. Therefore 1/10th of maximum test dose i.e. 200 mg/kg and 400 mg/kg of EEAM were selected for the study as test 1 and test 2 respectively.

Prescreening:

Evaluation by this method was done as described by Swinyard et al.[19] Mice were procured from Animal house, RIMS and were pre-screened with a current of 45mA for 0.2 sec via transauricular ear clips using an

electro-convulsiometer. The occurrence of a hind limb tonic extension (HLTE) was taken as a positive response for MES. HLTE defined by hind limb extension more than 90˚ from the body and sustained for more than 3 sec following 10 sec after stimulation. 42 mice which showed hind limb tonic extension (HLTE) were selected for the study.

Experimental Animals

42 healthy adult albino mice weighing 20-30gm of either sex showing HLTE were used. Pregnant mice were excluded. Animals were housed into 7 polypropylene cages with 6 animals in each cages. They were maintained at a temperature of 22 ± 1˚ C in a 12 hr light/ dark cycle with free access to water and standard diet. All experimental procedures were conducted after getting the clearance from the Institutional Animal Ethics Committee (IEAC), RIMS, Imphal, Manipur (Registration no: 1596/GO/a/12/CPCSEA).

Maximal Electroshock Seizure Test

Table 1: Allotment of animals to different groups and their treatment

Groups (n=6)	Treatment given
I (control)	1% gum acacia
II (PHT10)	Phenytoin 10 mg/kg p.o.
III (PHT20)	Phenytoin 20 mg/kg p.o.
IV Test 1	EEAM 200 mg/kg p.o.
V Test 2	EEAM 400 mg/kg p.o.
VI (PHT10 +T1)	Phenytoin (10 mg/kg) + EEAM 200 mg/kg p.o.
VII (PHT10 + T2)	Phenytoin (10 mg/kg) + EEAM 400 mg/kg p.o.

Standard drug and plant extract were suspended in 1% gum acacia and administered orally with feeding tube at equal volumes of 1ml/100g body weight 60 minutes prior to MES. Electro-convulsive shock (current intensity—45mA duration—0.2 s) delivered via ear clip electrodes using electroconvulsiometer. The duration of tonic hind limb extension (THLE) recorded in seconds.

The percentage protection calculated as:

$$\frac{\text{mean duration of THLE in control} - \text{mean duration of THLE in test/standard}}{\text{mean duration of THLE in control}} \times 100$$

Statistical analysis:

The data were entered in Statistical Package for the Social Sciences (SPSS) version 21.0 and results were analysed for statistical significance using One-way Analysis of Variance (ANOVA) followed by Bonferroni test. P value less than 0.05 was considered significant.

RESULTS

Table 2: Effect of standard drug (phenytoin) and *Aegle marmelos* on duration of tonic hind limb extension and percentage protection

Groups	Duration of tonic hind limb extension(in seconds)	Percentage protection (%)
CONTROL	10.83 ± 0.48	0
PHT10 (10mg/kg)	5.83± 0.30*	46%
PHT20 (20mg/kg)	3.33± 0.33*††	69%
TEST 1 (200mg/Kg)	7.83± 0.48*†‡	27%
TEST 2 (400mg/Kg)	6.17± 0.30*‡	43%
PHT10 + TEST 1	5.67± 0.42*‡‡§§	47%
PHT 10 + TEST 2	3.83± 0.30*†§‖¶	64%

The results were expressed in mean ± SEM.
*p< 0.001 when other groups compared with control group.
††p<0.005, † P<0.05 when subtherapeutic dose (PHT_{10}) compared to PHT_{20}, test 1, test 2, PHT_{10} + test 1, PHT_{10}+ test 2
‡p<0.001, ‡‡p<0.005, when standard therapeutic dose (PHT_{20}) compared to test 1, test 2, PHT_{10}+ test 1, PHT_{10}+ test 2
§p<0.001, §§p<0.005, when test 1 compared to test 2, PHT_{10}+ test 1, PHT_{10}+ test 2
‖p<0.005 when test 2 compared to PHT_10+ test 1, PHT_{10}+ test 2.
¶p<0.05 when PHT_{10}+ test 1 compared to PHT_{10}+ test 2.
(One way ANOVA followed by Bonferroni test).

There were significant decreases in duration of tonic hind limb extension (THLE) in all the groups when compared to control group.

When standard therapeutic dose of phenytoin (PHT_{20}) compared with other groups, the group receiving (PHT_{10}+ test 2) show comparable result.

When test 2 (400mg/kg) was compared with (PHT_{10} + test 1) and (PHT_{10} + test 2) group, there was significant decrease in THLE in (PHT_{10}+ test 2) group.

It can also be seen that there was a significant decrease in duration of THLE in test 2 group receiving higher dose of extract at 400mg/kg when compared with test 1 group receiving extract dose at 200mg/kg showing the dose dependent activity of the extract.

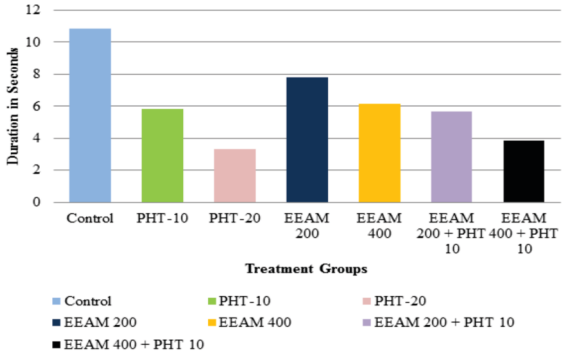

Figure 1: Effect of EEAM depicting the duration of tonic hind limb extension (in seconds) in different treatment groups of MES model

DISCUSSION

Efficacy of a test drug in the MES model is determined by the suppression of tonic hind limb extension (THLE). It exerts its convulsion effect by inhibiting the activity of inhibitory neurotransmitter gamma amino butyric acid (GABA) at GABA-A receptors. Several cellular mechanisms are implicated through which drugs can elevate seizure threshold. One such mechanism is the facilitation of entry of Na+ and Ca2+ ions into the cell thus prolonging the duration of convulsion. Drugs that block these ions entry such as phenytoin, carbamazepine, felbamate, valproate, etc can inhibit MES induced THLE.[20] In the present study, phenytoin was used as a standard drug for the MES model.

Phenytoin prolongs the inactivated state of voltage sensitive neuronal Na+ channel at therapeutic concentration. At higher concentration, it also depresses the presynaptic release of excitatory glutamate neurotransmitter, facilitates the inhibitory GABA release and reduces Ca2+ influx.[7]

The MES model was evaluated as described by Swinyard et al.[19] It was seen that there was significant decrease in duration of tonic hind limb extension (THLE) in all other groups when compared to control group. When therapeutic phenytoin group i.e PHT_{20} was compared with all other groups there was significant decrease in duration of THLE (3.33± 0.33 sec) while it shows comparable result with PHT_{10}+ test 2 group (3.83± 0.30 sec). It can also be seen that there was a significant decrease in duration of THLE in test 2 group receiving higher dose of extract at 400mg/kg (3.83± 0.30 sec) when compared with test 1 group receiving extract dose at 200mg/kg (5.67± 0.42 sec) showing the dose dependent activity of the extract. The findings are in accordance with previous studies conducted by Sharma and kausik et al[21] and Shinde et al[22].

CONCLUSION

The ethanolic extract of *Aegle marmelos* (L.) correa produced significant anticonvulsant effect against MES induced seizure in mice. Further study is required to elucidate its specific mechanism of action and active principles responsible for potential anticonvulsant property.

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