Section: Miscellaneous

Original Research Article

ANTICONVULSANT ACTIVITY OF ETHANOLIC EXTRACT OF AEGLE MARMELOS (L.) CORRÊA LEAVES ALONE AND IN COMBINATION WITH VALPROIC ACID BY PENTYLENETETRAZOLE (PTZ) INDUCED SEIZURE MODEL IN ALBINO MICE

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ABSTRACT

Background: The aim is to evaluate the anticonvulsant activity of Aegle marmelos (L.) Corrêa leaves alone and in combination with valproic acid by pentylenetetrazole (PTZ) induced seizure model in albino mice. **Materials and Methods:** 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 valproic acid (therapeutic dose 100mg/kg, sub therapeutic dose (50mg/kg), combination of EEAM (200mg) and valproic acid (50mg/kg) and combination of EEAM (400mg/kg) and valproic acid (50mg/kg) were evaluated by pentylenetetrazole (PTZ) induced seizure model. After 60 minutes of oral administration of the test drug and standard drug, the albino mice were injected subcutaneously with PTZ (60mg/kg) to induce seizure. The onset and duration of clonic seizure with loss of righting reflex were observed in animals for 30 minutes. **Result:** EEAM exhibited significant anticonvulsant activity in PTZ model at higher test dose (400mg/kg) and in combination with valproic acid subtherapeutic dose (50mg/kg) when compared with control group while the lower test dose (200mg/kg) do not exhibit any anticonvulsant property. There was significant increase in the onset of clonic seizure ($p<0.05$) and significant decrease in the duration of clonic seizure ($p<0.05$) in all other groups except for lower test dose (200mg/kg) when compared to the control group. **Conclusion:** EEAM exhibited significant anticonvulsant activity only at higher dose of 400mg/kg and EEAM (both 200mg/kg and 400mg/kg) also potentiates the anticonvulsant activity of sub therapeutic dose of valproic acid when given in combination in the PTZ model.

INTRODUCTION

A seizure is a transient occurrence of signs or symptoms due to abnormal excessive or synchronous neuronal activity in the brain. Epilepsy describes a condition 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] Nearly 80 % of people with epilepsy belong to low- and middle-income countries and are more prone to physical as well as psychological comorbidities (like depression and anxiety).[3]

Etiology can be genetic, structural, metabolic, infectious, immune or unknown.[4] The underlying neuronal abnormality in epilepsy may be associated with enhanced excitatory amino acid (glutamate) transmission or impaired inhibitory (gamma amino butyric acid) transmission.[5] Regarding the former, genetic mutations or acquired functional alterations in Na+ and K+ channels have been identified. Regarding the latter, genetic mutations or acquired functional alterations leading to loss of function of GABAergic neurotransmission (alterations in GABA synthesis, release, or receptors) or gain of function of glutamatergic neurotransmission (alterations in glutamate receptors or reuptake) have been identified.[6]

The classification of seizures as revised by the International League against Epilepsy include.[4]

1. Focal onset seizures, when originating within networks limited to one hemisphere. It is further classified based on awareness (focal aware seizures and focal seizures with impaired awareness); based on the motor component (motor and non-motor seizures); based on clinical evolution (focal to bilateral tonic clonic seizures)

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2. Generalised onset, when originating at some point within, and rapidly engaging, bilaterally distributed networks. It is further classified based on motor component (tonic clonic and other types of motor seizures) and non-motor (example absence seizure)
3. Unknown onset

The aim of treatment is cessation of seizures without side effects. Both conventional and newer antiepileptic drugs (AEDs) treat various types of seizures.[7] However, the currently used AEDs not only fail to control seizure in some patients, but it frequently causes side effects which is an important reason of treatment failure with antiepileptic drugs.[8] Various herbs have been used as an alternative medicine for the treatment of epilepsy.[9] The use of both conventional drug therapies and herbal medicines is a very common practice among epileptic patients.[10]

Aegle marmelos (L.) Corrêa (Manipuri: Hari-khagok) commonly known as Bael belonging to the family Rutaceae, has been widely used in indigenous systems of Indian medicine due to its various medicinal properties. It is a medium to large sized deciduous glabrous, armed tree with the axillary and 2.5 cm long alternate trifoliate leaves, short flower and globular fruits.[1] Various pharmacological studies have shown antioxidant, 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 model.

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.

Experimental Animals:42 healthy adult albino mice weighing 20-30gm of either sex were used. Pregnant mice were excluded. Animals were housed into 7 polypropylene cages with 6 animals in each cage. 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).

Pentylenetetrazole model (PTZ model)

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

Groups (n=6)	Treatment given
I (Control)	1% Gum acacia
II (VPA50)	Valproic acid 50 mg/kg p.o.
III (VPA 100)	Valproic acid 100 mg/kg p.o.
IV (Test 1)	Dose 200 mg/kg p.o.
V (Test 2)	Dose 400 mg/kg p.o.
VI (VPA 50 + T1)	Valproic acid (50 mg/kg) + dose 200 mg/kg p.o.
VII (VPA 50+T2)	Valproic acid (50 mg/kg) + dose 400 mg/kg p.o.

Valproic acid (VPA) and plant extract were suspended in 1% gum acacia and administered per oral (p.o) with feeding tube at uniform volumes of 1ml/100g body weight as shown in the above table 60 minutes prior to subcutaneous PTZ (60mg/kg) injection. The onset and duration of clonic seizure with loss of righting reflex were observed in animals for 30 minutes.

The percentage protection was calculated as[19]

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

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.

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RESULTS

Table 2: Effect of standard drug (valproic acid) and Aegle marmelos on onset and duration of clonic seizure.

Groups	Onset (in secs)	Duration of clonic seizure (in secs)	Percentage protection (%)
Control	190 ± 4.45	556 ± 15.36	0
VPA 50 (50mg/kg)	303 ± 12.91 *	447 ± 13.59**	20
VPA 100 (100mg/kg)	419 ± 23.71 **†	303 ± 9.96**††	45
TEST 1 (200mg/kg)	205 ± 33.96 ††‡	514 ± 19.26*†‡	8
TEST 2 (400mg/kg)	302 ± 16.29 *‡‡§§	447 ± 13.49**‡§§	20
VPA 50 + TEST 1	338 ± 17.27 **§§	410 ± 12.36**‡§	26
VPA 50 + TEST 2	399 ± 17.75 **†§‖	360 ± 24.17**†§‖	35

The results were expressed in mean ± SEM, p< 0.05 was considered significant.

** p< 0.001, *p<0.05 when other groups compared with control group.

††p<0.001, † p<0.05 when subtherapeutic dose (VPA50) compared to VPA100, test 1, test 2, VPA50+ test 1, VPA50+ test 2

‡p<0.001, ‡‡p<0.05, when standard therapeutic dose (VPA100) compared to test 1, test 2, VPA50+ test 1, VPA50+ test 2

§p<0.001, §§p<0.05, when test 1 compared to test 2, VPA50+ test 1, VPA50+ test 2

‖p<0.05 when test 2 compared to VPA50+ test 1, VPA50+ test 2.

(One way ANOVA followed by Bonferroni test).

1. When compared to control group

a) **Onset of clonic seizure:** There was significant increase in the onset of clonic seizure (p<0.05) in all other groups except for test 1 (200mg/kg).

b) **Duration of clonic seizure:** there was significant decrease in the duration of clonic seizure (p<0.05) in all other groups except for test 1 (200mg/kg) when compared to the control group.

2. When compared to standard subtherapeutic VPA50 group

a) **Onset of clonic seizure:** there were significant increase in onset of clonic seizure in VPA100 and VPA50+ test 2 group (p<0.05) but test 1 showed significant decrease (p<0.05)

b) **Duration of clonic seizure:** there were significant decrease in duration of clonic seizure in VPA100 (p<0.001) and VPA50+ test 2 (p<0.005) but test 1 (p<0.05) showed increase in duration of clonic seizure.

3. When standard therapeutic VPA100 group was compared with test 1, test 2, VPA50+ test 1 and VPA50 + test 2

a) **Onset of clonic seizure:** there were significant decrease in onset of clonic seizure in test 1 (p<0.001) and test 2 (p< 0.005)

b) **Duration of clonic seizure:** there were significant increase in duration of clonic seizure in test 1, test 2, VPA50+ test 1 (p<0.001).

4. When test 1 (200 mg/kg) was compared with test 2, VPA50+ test 1 and VPA50 + test 2

a) **onset of clonic seizure:** there were significant increase in onset of clonic seizure in test 2 (p<0.05), VPA50+ test 1 (p<0.005) and VPA50 + test 2 (p<0.001)

b) **duration of clonic seizure:** there were significant decrease in duration of clonic seizure in test 2 (p<0.05), VPA50+ test 1 and VPA50 + test 2 (p<0.001).

5. When test 2 (400mg/kg) was compared with VPA50+ test 1 and VPA50 + test 2

a) onset of clonic seizure: there was significant increase in onset of clonic seizure in VPA50 + test 2 (p<0.05)

b) duration of clonic seizure: there was significant decrease in duration of clonic seizure in VPA50 + test 2 (p<0.005)

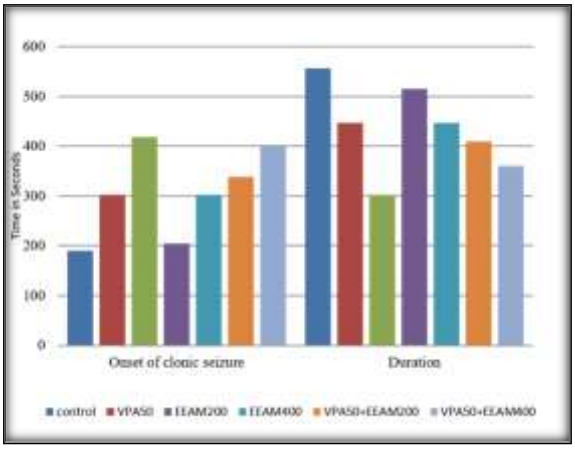

Figure 1: Effect of EEAM in different treatment groups of PTZ model showing onset and duration of clonic seizure (in seconds)

DISCUSSION

In PTZ induced seizure model, efficacy of a test drug as an anticonvulsant is measured by determining its ability to suppress clonic seizure. Pentylenetetrazole is a tetrazole derivative that exerts its convulsive effect by antagonising the inhibitory GABA neurotransmitter. In the present study, valproic acid was used as a standard drug for the PTZ induced seizure model.[20]

Valproic acid has a broad-spectrum anticonvulsant action. It acts by several mechanisms which include prolongation of Na+ channel inactivation, weak attenuation of Ca2+ mediated T current, enhance release of inhibitory GABA neurotransmitter and blockage of excitatory glutamate neurotransmitter. It is effective in partial seizure and GTCS as well as in absence, myoclonic and atonic seizures.[21]

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The PTZ model was evaluated as described by Vogel and Vogel.[22] There were significant increase in the onset of clonic seizure ($p<0.05$) and significant decrease in the duration of clonic seizure ($p<0.05$) in all other groups except for test 1 (200mg/kg) which show comparable result to the control group. This shows that the lower extract dose at 200mg/kg does not possess any anticonvulsant effect.

When the onset of seizure for standard therapeutic VPA100 group (419 ± 23.71sec) was compared with other groups, the onset of clonic seizure in VPA50+ test 1 (338 ± 17.2 sec) and VPA50 + test 2 (399 ± 17.75 sec) show comparable result. Similarly, when the duration of clonic seizure for therapeutic VPA100 group (303±9.96) was compared to all other groups, group VPA50 + test 2 exhibit comparable result (360 ± 24.17). This shows that co administration of extract (dose at 200mg/kg and 400mg/kg) with sub therapeutic dose of valproic acid enhanced the anticonvulsant activity. This study finding is similar with previous study as conducted by Reeta et al.[23]

CONCLUSION

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

REFERENCES

1. Lowenstein DH. Seizures and epilepsy. In: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson J, Loscalzo J, editors. Harrison's Principles of Internal Medicine. New York: McGraw-Hill; 2018. p. 3050-68.
2. WHO. Epilepsy. [internet] 2019 Jun [cited on 2020 Nov 11]. Available from https://www.who.int/news-room/fact-sheets/detail/epilepsy.
3. Trinka E, Kwan P, Lee B, Dash A. Epilepsy in Asia: Disease burden, management barriers, and challenges. Epilepsia. 2019 Mar; 60 Suppl 1:7-21.
4. Fisher RS, Cross JH, French JA, Higurashi N, Hirsch E, Jansen FE et al. Operational classification of seizure types by the international league against Epilepsy: Position paper of the ILAE commission for classification and terminology. Epilepsia. 2017 Apr; 58(4):522-30.
5. Rang HP, Dale MM, Ritter JM, Flower RJ, editors. Rang and Dale's Pharmacology. 6th ed. London: Churchill Livingstone; 2007.
6. Simonato M. Epilepsy an update on disease mechanisms: The potential role of microRNAs. Front Neurol. 2018 Mar 19; 9:176.
7. Smith D, Chadwick D. The management of epilepsy. J Neurol Neurosurg Psychiatry. 2001 Jun; 70 Suppl 2: II15-21
8. Perucca P, Gilliam FG. Adverse effects of antiepileptic drugs. Lancet Neurol. 2012 Sep; 11(9):792-802.
9. Schachter SC. Botanicals and herbs: A traditional approach to treating epilepsy. Neurotherapeutics. 2009 Apr; 6(2):415-20.
10. Tyagi A, Delanty N. Herbal remedies, dietary supplements and seizures. Epilepsia. 2003 Feb; 44(2):228-35.
11. Rahman S, Parvin R. Therapeutic potential of Aegle marmelos (L.)-An overview. Asian Pac J Trop Dis. 2014 Feb; 4(1):71–7.
12. Patel PK, Sahu J, Sahu L, Prajapati NK, Dubey BK. Aegle marmelos: a review on its medicinal properties. Int J Pharm Phytopharm Res. 2012 Mar 1; 1(5):332-41.
13. Knevel AM, Digangi FE. Jenkins' Quantitative pharmaceutical chemistry. 7th ed. New York: McGraw Hill Book Co.; 1977.
14. Kumar U, Kumar B, Bhandari A, Kumar Y. Phytochemical investigation and comparison of antimicrobial screening of clove and cardamom. Int J Pharm Sci Res. 2010; 1(12):138-47.
15. Shah B, Seth AK. Textbook of pharmacognosy and phytochemistry. 2nd ed. New Delhi: Elsevier; 2014.
16. Dhanasekaran M, Abraham GC, Mohan S. Preliminary phytochemical and histochemical investigation on Kigelia pinnata DC. Int J Pharm Sci Res. 2014; 5(7):413-19.
17. Bargah RK. Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of Moringa pterygosperma. J Pharmacogn Phytochem. 2015; 4(1):7-9.
18. OECD. OECD guideline for testing of chemicals 423: Acute oral toxicity-acute toxic class [Internet]. 2001 [Cited 2019 July 20]. Available from: http://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl423.pdf.
19. Swinyard EA, Brown WC, Goodman LS. Comparative assays of antiepileptic drugs in mice and rats. J Pharmacol Exp Ther. 1952 Nov 1; 106(3):319-30.
20. Gupta SK. Drug screening methods. 3rd ed. New Delhi: Jaypee Brothers Medical Publishers; 2016.
21. Tripathi KD. Essentials of medical pharmacology. 8th ed. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd; 2019.
22. Vogel GH, Vogel WH, editors. Drug discovery and evaluation: pharmacological assay. 2nd ed. Heidelberg: Verlag; 1997.
23. Reeta KH, Mehla J, Pahuja M, Gupta YK. Pharmacokinetic and pharmacodynamic interactions of valproate, phenytoin, phenobarbitone and carbamazepine with curcumin in experimental models of epilepsy in rats. Pharmacol Biochem Behav. 2011 Sep; 99(3):399-407.

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