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Original Article

COMPARATIVE STUDY OF ANTICONVULSANT EFFECT OF THE LEAVES OF *SAPINDUS EMARGINATUS* AND *ACORUS CALAMUS* IN EXPERIMENTALLY INDUCED ANIMAL MODELS OF EPILEPSY

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ABSTRACT

Objective: To compare anticonvulsant activity of methanol extracts of *Sapindus emarginatus* (MESE) and *Acorus calamus* (MEAC) in experimental seizure models in mice.

Methods: Hind limb tonic extension (HLTE) in Maximal electroshock (MES) seizure and clonic seizure in Pentylenetetrazol (PTZ) seizure models were assessed. Group I (control) mice received 1% gum acacia in distilled water (1 ml/100 g). Topiramate (50 mg/kg) was administered in group II (standard) animals. Group III and IV mice were treated with 200 and 400 mg/kg of MESE, respectively. Mice in group V and VI were given MEAC at the dose of 200 and 400 mg/kg, respectively. Drugs were given orally suspended in 1% gum acacia suspension (1 ml/100 g) for 7 d. Next day after 1 h of drug administration, the seizure was induced for evaluation.

Results: Anticonvulsant property of both extracts was confirmed by reduction ($p<0.001$) in HLTE phase in MES model; delayed onset of the clonic seizure ($p<0.001$) and its shortened phase ($p<0.001$) in PTZ model when compared with the control. MESE-200 mg/kg produced significantly longer ($p<0.001$) HLTE phase with lower protection (40.34%) among the different doses of the extracts. Clonic seizure onsets and durations in PTZ model were comparable among the different extract-treated groups; however, mortality was higher (66.6%) with MESE-200 mg/kg.

Conclusion: Anticonvulsant activity of MESE and MEAC was evident; however, MESE at the dose of 200 mg/kg was less effective.

Keywords: Anticonvulsant, Maximal electric shock, Pentylenetetrazol, *Sapindus emarginatus, Acorus calamus*

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INTRODUCTION

Epilepsy is one of the common serious neurological disorders associated with substantial morbidity and mortality due to seizures and available medications [1]. The seizures arise from an excessively synchronous and sustained discharge of an aggregate of neurons in the brain [2]. The pathologies leading to epilepsy can occur anywhere in the circuit level in brain e. g. from abnormal synaptic connectivity to the receptor level, abnormality of γ-amino butyric acid (GABA) receptor subunits and/or ion channel dysfunction [3].

The antiepileptic drugs are often associated with debilitating adverse effects. The toxicity, intolerance and lack of efficacy are the limitations of the drugs. Therefore, the main goal in epilepsy research is the development of new agents which can overcome these limitations [4]. Many natural and synthetically modified natural products have been successfully developed to treat human diseases in almost all therapeutic areas [5]. The literature reveals that many medicinal plants are used in different systems of complementary and alternative medicines for the treatment of various ailments, including mood disorders and epilepsy [6]. *Sapindus emarginatus* Vahl. [7] and *Acorus calamus* Linn. [8] are among the medicinal plants reported to possess antiepileptic property. The present study attempts to compare the anticonvulsant activity of the two plants on experimental seizure models in mice, which are commonly used because of smaller size, the genetic, biological and behavioural characteristics closely resemble to human.

MATERIALS AND METHODS

The study was conducted in the Department of Pharmacology, Regional Institute of Medical Sciences (RIMS), Imphal after getting approval of the Institutional Animal Ethics Committee (No.1596/GO/a/12/CPCSEA).

Requirements

Albino mice, polypropylene cages, gavage feeding tube, electro-convulsiometer, ear clip electrodes, pentylenetetrazol, topiramate, stopwatch, markers, needles and syringes, distilled water, gum acacia (Hi Media Laboratories Pvt. Ltd, Mumbai, India), petroleum ether (Hi Media Laboratories Pvt. Ltd, Mumbai, India), methanol (Merk Specialities, Pvt. Ltd. Mumbai), soxhlet apparatus (Garg Process Glass India Pvt. Ltd. Malad, Mumbai), mixer grinder, evaporating dish, weighing machine (Shimadzu, corporation, Japan), mortar and pestle.

Preparation of plant extract

The collected leaves of *Sapindus emarginatus* Vahl. and *Acorus calamus* Linn. from the Imphal valley were identified and authenticated by Prof. P. K. Singh, Department of Life Sciences, Manipur University (Acc. no. MUMP-0197 and 1423). The plant extraction was done following principles adopted by Lin J *et al.* [9]. The shade dried powdered leaves were defatted with petroleum ether and extracted with methanol using soxhlet apparatus separately. The extracts obtained were evaporated and stored in airtight containers. The yield obtained was 10% and 6% for *Sapindus emarginatus* and *Acorus calamus,* respectively.

Phytochemical screening

The preliminary phytochemical studies of the extracts were done using standard procedures [10, 11].

Acute toxicity testing

The acute toxicity testing was done as per OECD guidelines 423 [12] in albino mice. Three animals were used in each step. The methanol extracts of leaves of *Sapindus emarginatus* (MESE) and *Acorus calamus* (MEAC) were administered to the fasted mice at a dose of 300 mg/kg orally (p. o.) followed by observation once in every 30

min during the first 24 h, and then daily for 14 d. As there was no mortality, the same procedure was repeated with the higher dose of 2000 mg/kg. No mortality or toxic symptom was detected in the animals. The doses of 200 and 400 mg/kg of the extracts were chosen for the study.

Experimental animals

Healthy albino mice of either sex weighing 25-30 g were obtained from the animal house, RIMS, Imphal. The mice were housed in polypropylene cages and acclimatized to the laboratory conditions for 7 d at room temperature with natural light and dark cycle. They were maintained on standard diet with water *ad libitum.*

Experimental design

For both the MES and PTZ seizure models, selected mice were divided into six groups (I, II, III, IV, V and VI) of 6 mice in each. The mice in group I (Control) received 1% gum acacia in distilled water (DW) at the dose of 1 ml/100 g orally. The group II (Standard) animals were administered Topiramate-50 mg/kg (Sun Pharma Laboratories Ltd. East Sikkim, B. No BSR 0857). The mice in group III and IV were treated with 200 and 400 mg/kg of MESE, respectively. The group V and VI mice were given 200 and 400 mg/kg of MEAC, respectively. The standard drug and the plant extracts were suspended in 1% gum acacia in DW and administered at the dose of 1 ml/100g/day orally for 7 consecutive days. The next day after 1h of drug administration, seizure was induced for evaluation. Each mouse was tested only once.

Maximal electroshock (MES) seizure model

Selection of animals

Electroconvulsiometer (Techno Electronics, Lucknow) was used for seizure induction. The mice showing hind limb tonic extension (HLTE) at a current of 50 mA for 0.2 sec via a pair of ear clip electrodes were selected. HLTE was defined by extension of hind limb more than 90° from the body and sustained for more than 3 seconds [13]. A recovery period of 5 d was given before the main test.

Procedure

The mice were subjected to an electrical stimulus of 50 mA of alternating current from the electroconvulsiometer for 0.2 sec via ear clip electrodes. The resulting seizure passed through different phases-tonic flexion, tonic extension, clonic convulsions, stupor, and recovery or death [14]. The reduction or complete of abolition of HLTE phase was considered as protection action against convulsion [15]. The HLTE phase and its percentage protection were assessed. The percentage of protection was calculated using the following equation [16].

$$\% \text{ Protection} = \frac{\text{Duration of HLTE in control} - \text{Duration of HLTE in test or standard}}{\text{Duration of HLTE in control}} \times 100$$

Pentylenetetrazol (PTZ) seizure model

The mice in the different groups were treated as per the experimental design for 7 consecutive days. The next day after 1h of the drug treatment, the animals were injected 1% PTZ (HI Media Lab. Pvt. Ltd. Batch No. GRM1742-25G) in DW at the dose of 100 mg/kg subcutaneously in the scruff of the neck and observed. There was a sequence of excitement, myoclonic jerks, clonic seizures, one or more maximal tonic seizure followed by recovery or death. The development of clonic seizure with loss of righting reflex was taken as a positive seizure response [15] and its abolition or suppression was taken as protection against PTZ seizures. The mortality, time of onset of clonic seizure (seizure latency) and its duration in the different groups were recorded. The percentage reduction of the clonic phase [16] was calculated as follows.

$$\% \text{ reduction of clonic phase} = \frac{\text{Clonic phase in control} - \text{Clonic phase in test or standard}}{\text{Clonic phase in control}} \text{x100}$$

Analysis of results

The results obtained were expressed as mean±standard deviation (SD) and analysed by One-way analysis of variance (ANOVA) followed by Bonferroni test. P<0.05 was considered significant. IBM SPSS statistics version 21 was used for data analysis.

RESULTS

Phytochemical screening

The qualitative phytochemical analysis of MESE and MEAC detected the presence of alkaloids, flavonoids, saponins and tannins. In addition, the MESE and MEAC revealed the presence of protein and starch, respectively.

MES seizure model

The HLTE phase (sec) in the groups receiving 1% gum acacia (control), Topiramate-50 mg/kg (standard), MESE-200 mg/kg, MESE-400 mg/kg, MEAC-200 mg/kg and MEAC-400 mg/kg were 21.32±1.8, 7.55±1, 12.72±0.4, 8.13±0.7, 8.28±0.7 and 8.26±0.6 respectively. The HLTE phases were reduced (P<0.001) in all the drug-treated groups when compared with the control group. The HLTE durations among the standard, MEAC and MESE-400 mg/kg treated groups were not significantly different. But MESE-200 mg/kg showed significantly longer (P<0.001) HLTE phase than all the groups except the control group. The percentage protection of HLTE (table 1) was 64.58 (standard), 40.34 (MESE-200 mg/kg), 61.86 (MESE-400 mg/kg), 61.16 (MEAC-200 mg/kg) and 61.25 (MEAC-400 mg/kg).

Table 1: Effect on HLTE in maximal electroshock seizure model

Treatment group	HLTE (sec)	% protection
I. Control-1%gum acacia in DW.	21.32±1.84	-
II. Topiramate-50 mg/kg	7.55±0.99*†	64.58
III. MESE-200 mg/kg	12.72±0.44*	40.34
V. MESE-400 mg/kg	8.13±0.69*‡	61.86
V. MEAC-200 mg/kg	8.28±0.67*‡	61.16
VI. MEAC-400 mg/kg	8.26±0.59*‡	61.25
One way ANOVA F	177	
df	35	
P	<0.001	

Values are mean±SD. n=6, *P<0.001 when compared with control, †P<0.001 when compared with MESE-200 mg/kg, ‡P<0.001 when compared with MESE-200 mg/kg. (One way ANOVA followed by Bonferroni test).

Pentylenetetrazol (PTZ) seizure model

The onset of clonic seizure (sec) in the control, Topiramate, MESE-200 mg/kg, MESE-400 mg/kg, MEAC-200 mg/kg and MEAC-400 mg/kg groups were 119.3±6.3, 390±30, 237±1.4, 239±2.4, 232±2.0 and 232±2.3 respectively. There was significant (P<0.001) delay in the onset in the Topiramate, MESE and MEAC treated groups when compared with the control group; however, it was not significantly different among the extract-treated groups. The clonic phases (sec) in the control, standard, MESE-200 mg/kg, MESE-400 mg/kg, MEAC-200 mg/kg and MEAC-400 mg/kg groups were 21.36±2.1, 5.08±4.1, 10.58±5.2, 8.46±0.8, 8.20±3.4 and 6.90±6.1 respectively. It was shortened (P<0.001) in the Topiramate, MESE and MEAC treated groups when compared with the control group, but the durations were comparable in the Topiramate and extract-treated groups.

The percentage reduction of clonic phase in the different groups was 76.22 (standard), 50.46 (MESE-200 mg/kg), 60.39 (MESE-400 mg/kg), 61.61 (MEAC-200 mg/kg) and 67.70 (MEAC-400 mg/kg). Topiramate provided a maximum reduction of the clonic phase with least mortality (16.6%) among the groups, while MESE-200 mg/kg showed highest (66.6%) mortality (table 2).

Table 2: Effect on clonic seizure in pentylenetetrazol seizure model

Treatment group	Clonic seizure onset (sec)	Duration of clonic seizure (sec)	% reduction of clonic phase	% mortality
I. Control-1% gum acacia in DW	119±6.38	21.36±2.12	-	100
II. Topiramate-50 mg/kg	390±30.02*†	5.07±4.08*	76.22	16.6
III. MESE-200 mg/kg	237±1.47*	10.58±5.19*	50.46	66.6
IV. MESE-400 mg/kg	239±2.42*	8.46±0.76*	60.39	33.3
V. MEAC-200 mg/kg	232±2.04*	8.20±0.76*	61.61	50
VI. MEAC-400 mg/kg	232±2.33*	6.90±3.44*	67.70	50
One way ANOVA F	278.8	19.8		
df	35	35		
P	<0.001	<0.001		

Values are mean±SD. n=6, *P<0.001 when compared with control, †P<0.001 when compared with MESE and MEAC. (One way ANOVA followed by Bonferroni test).

DISCUSSION

The suitable animal models are used to study various human disorders including seizures and behavioural disorders. Mice have commonly used rodents because of small size, ease of housing and maintenance. Interestingly, the genetic, biological and behavioural characteristics of the rodents closely resemble those of humans [17]. The MES and PTZ induced seizure models are considered gold standards in the evaluation of anticonvulsant activity in the early stages of testing [4]. For predictability of the results, it is important to obtain coherent findings in the different models, and at least two methods are often recommended as congruent findings are not always obtained between the methods [18]. The MES model identifies agents with activity against generalized tonic-clonic seizures, whereas the PTZ model identifies compounds that are efficacious against generalized absence and myoclonic seizures [19, 20].

Topiramate acts through multiple mechanisms such as inhibition of voltage-dependent sodium and calcium currents, potentiation of GABA-mediated events, blockade of glutamatergic neurotransmission and enhancement of potassium currents [21]. The observation of the already proven effect of topiramate as an anticonvulsant drug proved the validity of the experimental design followed.

The reduction of HLTE phase in the MES model; significantly delayed onset and shortened duration of clonic seizure in PTZ model revealed the anticonvulsant properties of MESE and MEAC. The methanol extract of leaves of *Sapindus emarginatus* at the dose of 200 mg/kg was less effective as evident by the lower percentage of protection of THLE in MES model and higher percentage mortality the PTZ seizure model.

It is known that the MES induced seizures are abolished by the drugs that block voltage-gated Na^+channels [22] or by the drugs that block the glutamatergic receptor [23]. The PTZ induced seizures are prevented by drugs that reduce T-type Ca^{2+} currents and also by drugs that enhance gamma-aminobutyric acid (GABA) mediated inhibitory neurotransmission [20, 22].

Literature review reveals that various classes of the phytochemicals such as saponins [24], flavonoids [24], terpenes, alkaloids, lactones and coumarins [25] exhibit anticonvulsant property. Therefore, the observed anticonvulsant effects of the two plants could be attributed to the alkaloids, flavonoids, saponins and tannins present in the extract. The prevention of the MES and PTZ induced seizures suggests that the methanol extract of *Sapindus emarginatus* and *Acorus calamus* act by multiple mechanisms, possibly by blocking of voltage-gated Na^+channels or glutamatergic receptors, and/or enhancement of GABAergic neurotransmission or reduction of T-type Ca^{2+}currents in the brain.

CONCLUSION

The findings of our study reveal that the methanol extracts of *Sapindus emarginatus* and *Acorus calamus* leaves act by multiple mechanisms as anticonvulsants. The *Sapindus emarginatus* leaf extract is associated with a weaker anticonvulsant effect at 200 mg/kg dose. Further studies with isolated biologically active principles of the plants in the genetic models of epilepsy will be more meaningful and interesting.

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Nil

AUTHORS CONTRIBUTIONS

Sanjenbam Rita and Ngangom Gunindro designed the experiment and involved in the interpretation of data. Priyadarshini Shougrakpam and Abhishek Bhattacharjee carried out the experimental work, analysis of data and manuscript preparation. All the authors went through the finale manuscript.

CONFLICTS OF INTERESTS

Declared none

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