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Original Article

ANTICONVULSANT ACTIVITY OF *PORTULACA OLERACEA* LINN. AND *EUPATORIUM BRIMANICUM* DC IN MES INDUCED SEIZURE: A COMPARATIVE STUDY

MAYANGLAMBAM MEDHABATI[1], LAISHRAM BABYCHA[2], ABHISHEK BHATTACHARJEE[3], NGANGOM GUNINDRO[4]

[1,3,4]Department of Pharmacology, Regional Institute of Medical Sciences, Imphal, India, [2]Department of Pharmacology, Jawaharlal Nehru Institute of Medical Sciences, Imphal, India
Email: babhishek637@gmail.com

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ABSTRACT

Objective: The study was aimed to evaluate and compare the anticonvulsant activity of aqueous leave extract of *Portulaca oleracea* Linn. and *Eupatorium brimanicum* DC in MES model in albino mice.

Methods: Aqueous Extracts were prepared by the soxhlet extraction method. MES model was chosen to evaluate anticonvulsant activity. 36 albino mice were selected and divided into 6 groups for this model. Group I received 2% gum acacia 1 ml/100 g orally. Group II received phenytoin-20 mg/kg orally. Group III and IV received 200 and 400 mg/kg of *Portulaca oleracea* Linn. Respectively. Group V and VI received 200 and 400 mg/kg of *Eupatorium brimanicum* DC respectively.

Results: The extracts didn't show any toxicity and significantly reduced hind limb tonic extension (HLTE) duration in MES model (50 mA, 0.2 sec) at higher doses.

Conclusion: The results suggest *Portulaca oleracea* Linn. and *Eupatorium brimanicum* DC extract possess anticonvulsant activity and justify their use in folk medicine.

Keywords: MES, Anticonvulsant, *Portulaca oleracea* Linn., *Eupatorium brimanicum* DC, Albino mice

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INTRODUCTION

Epilepsy is one of the most common neurological disorders which significantly affects the quality of life. It affects 70 million people in the world [1]. Epilepsy refers to a disorder of brain function characterized by the periodic and unpredictable occurrence of seizures. The underlying abnormality of epilepsy is poorly understood, but it may be associated with an imbalance between excitatory and inhibitory neurotransmitters 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-aminobutyric acid (GABA) receptor subunits and/or ion channel dysfunction [3]. Antiepileptic drug therapy is the mainstay of treatment for most of patients of epilepsy. The overall goal is to completely prevent seizure without causing any untoward side effects. Unfortunately, the drugs used currently not only fail to control seizure activity in approximately 30% of patients but frequently cause untoward effects like impairment of the CNS, aplastic anaemia, hepatic failure, etc [4, 5]. In order to overcome such drawbacks, there is a substantial need to develop novel antiepileptic drugs (AEDs) with more efficacy and safety. Moreover, herbal medicines are extensively used globally since ancient times due to their therapeutic efficiency and minimal side effects in nervous system. In recent years, several investigations for the search of novel and better tolerated antiepileptic drugs have progressed with promising results. Many plants are known to possess anticonvulsant property and are commonly used in traditional folklore. However, there is paucity of the anticonvulsant studies of the medicinal plants like *Portulaca oleracea* Linn. and *Eupatorium brimanicum* DC in animal models [6, 7]. Therefore, our study aimed to evaluate the anticonvulsant activity of these two medicinal plants in Maximal electroshock (MES) model of epilepsy in albino mice.

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, stopwatch, gum acacia, phenytoin Sodium (Knoll pharma, India), soxhlet apparatus, mixer grinder, evaporating dish, weighing machine, mortar and pestle.

Preparation of the extract

Fresh aerial parts of *Portulaca oleracea Linn. and Eupatorium Brimanicum DC* were collected from the Imphal, Manipur, identified and authenticated by Prof. P. K. Singh, Department of Life Sciences, Manipur University. The plant parts were cleansed, dried under shade. The leaves were separated and powdered by a mixer grinder. The powdered materials of these two plants were extracted separately with distilled water using a Soxhlet apparatus [8]. The extracts were evaporated, scraped out and stored in airtight container. The yields obtained were 35.2% and 37.13% for *Portulaca oleracea* Linn. and *Eupatorium brimanicum* DC respectively.

Phytochemical screening

The preliminary phytochemical studies of the extracts were done following standard procedures [9, 10]

Animals

Healthy albino mice of either sex weighing 25-30 g were procured from the central animal house of the institute. They were housed in standard polypropylene cages and acclimatized to the laboratory conditions for 7 d at room temperature with 12 hr light and dark cycle. The mice were given a standard laboratory diet and water *ad libitum*. Food was withdrawn 8 hr before and during experiments.

Acute toxicity study

Acute toxicity testing was carried out as per OECD guidelines 423 in albino mice [11]. Three animals were used for each step. The aqueous extracts of *Portulaca oleracea* Linn. and *Eupatorium brimanicum* DC were administered to the fasted mice at a dose of 300 mg/kg (p. o.) and observed once in every 30 min during the first

24 h and thereafter, daily for 14 d. As there was no mortality, the procedure was repeated with a higher dose of 2000 mg/kg, and the animals were observed for mortality and toxic symptoms. At the dose of 2000 mg/kg p. o. of the plant extracts, no mortality or toxic symptoms were detected in the tested animals and the dose was considered safe. Two doses of 200 mg/kg ($1/10^{th}$ of the maximum test dose) and 400 mg/kg ($1/5^{th}$ of the maximum test dose) of the plant extracts were selected as working doses for the experiment.

Assessment of anticonvulsant effect

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 an extension of hind limb more than 90° from the body and sustained for more than 3 seconds [12]. A recovery period of 5 d was given before the main test.

Procedure

The animals were divided into 6 (six) groups. Each group consisted of 6 (six) animals. The drug treatment schedule of the different groups was demonstrated in table 1.

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 1h after drug administration. The resulting seizure passed through different phases-tonic flexion, tonic extension, clonic convulsions, stupor, and recovery or death [13]. The HLTE phase was recorded and assessed. The reduction or complete of abolition of HLTE phase was considered as protection against convulsion [14].

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

Group	Treatment
I (Control)	2% Gum acacia, p. o.
II (Standard)	Phenytoin Sodium 20 mg/kg, p. o. dissolved in 2% Gum acacia
III	200 mg/kg of *Portulaca oleracea*, p. o. dissolved in2% Gum acacia
IV	400 mg/kg of *Portulaca oleracea*, p. o, dissolved in 2% Gum acacia
V	200 mg/kg of *Eupatorium brimanicum*, p. o, dissolved in 2% Gum acacia
VI	400 mg/kg of *Eupatorium brimanicum*, p. o, dissolved in 2% Gum acacia

Standard drug and the plant extracts were suspended in 2% gum acacia in DW and administered at the dose of 1 ml/100g orally.

Table 2: Effects of aqueous extract of *Portulaca oleracea* linn. and *Eupatorium brimanicum* DC on MES induced seizure in mice

Treatment group	Duration of HLTE(Sec)
I. Control-2% gum acacia in DW	15.52±0.37
II. (Standard) Phenytoin Sodium 20 mg/kg	----------
III. 200 mg/kg of *Portulaca oleracea*	13.50±0.37
IV. 400 mg/kg of *Portulaca oleracea*	10.92±0.91*†
V. 200 mg/kg of *Eupatorium brimanicum*	13.23±0.22
VI. 400 mg/kg of *Eupatorium brimanicum*	10.22±0.32*†‡
One way ANOVA	
F	16.06
df	4
p	<0.001

n=6 in each group, values are mean±SEM, * p<0.001, compared to control, † p<0.05 compared to group III, ‡ p<0.05 compared to group V.

Statistical analysis

The results were analyzed for statistical significance using one-way 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 *Portulaca oleracea* Linn. and *Eupatorium brimanicum* DC revealed the presence of alkaloids, flavonoids, saponins and tannins.

Anti-convulsant effects of aqueous extract of *Portulaca oleracea* Linn. and *Eupatorium brimanicum* DC on MES induced seizure in mice are shown in table 2.

DISCUSSION

Research into epilepsy and development of anti-epileptic drugs relies on studies in experimental animals. Mice are commonly used rodents because of smaller size, ease of housing and maintenance. Interestingly, the genetic, biological, and behavioural characteristics of the rodents closely resemble to those of humans [15]. Maximal electroshock (MES) seizure is the mainstay of any antiepileptic drug screening. In this present study, the anticonvulsant activity of *Portulaca oleracea* Linn. and *Eupatorium brimanicum* DC were evaluated by maximal electroshock seizure (MES) in albino mice. The MES is probably the best-validated method for the assessment of anti-epileptic drugs in generalized tonic-clonic seizure [16]. Efficacy in this model was determined by decreased in duration of hind limb tonic extension (HLTE). MES causes several changes at cellular level, which can disrupt the signal transduction in the neurons. One of the most important mechanism by which it causes cellular damage is facilitation of ca^{2+}entry into the cell in large amount and thus, prolonging the duration of convulsion [17]. Apart from ca^{2+}ions MES also facilitates the entry of Na^{+}ion. Blockade of these ions can prevent the MES-induced tonic extension [18]. The aqueous extract of *Portulaca oleracea* Linn. and *Eupatorium brimanicum* DC were found to possess significant anti-convulsant activity in albino mice at the dose of 400 mg/kg, p. o against MES induced seizure. However, the extract of *Eupatorium brimanicum* revealed maximum anticonvulsant potential. Mice treated with phenytoin 20 mg/kg didn't exhibit any HLTE for which the possible anticonvulsant mechanism is inhibition of voltage-sensitive Na^{+}channels in the neurons.

The preliminary phytochemical analysis of *Portulaca oleracea* Linn. and *Eupatorium brimanicum* DC showed the presence of flavonoids, alkaloids, saponins and tannins. Several reports suggest that flavonoids and alkaloids have potent anticonvulsant activity in various seizure models [19-21]. Therefore, the presence of such compounds in both the extracts may be responsible for their anticonvulsant potential. This study provides experimental support for the traditional use of these plants for the treatment of epilepsy.

CONCLUSION

Results of the present study show that the aqueous extract of *Portulaca oleracea* linn. and *Eupatorium brimanicum* DC leaves (400 mg/kg) produced a significant anticonvulsant effect against MES induced seizure in mice. These observations suggest that aqueous extract of *Portulaca oleracea* Linn. and *Eupatorium brimanicum* DC possibly act by modulating ion channels in CNS to exert their anticonvulsant effect. However, further research is required to elucidate its specific mechanism of action and active principles responsible for potential anticonvalsant property.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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