Pharmacognosy Communications
Volume 4 | Issue 4 | Oct–Dec 2014
www.phcogcommn.org

Research Article

Antiepileptic Activity of Whole Plant of Leucas Martinicensis

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ABSTRACT: Background: *Leucas martinicensis* (LM) is an herbal plant that has been used in folk medicine to treat malaria, kidney disorders, inflammation and rheumatism. **Objective**: The present study was aimed to investigate the antiepileptic activity of ethanolic extract of whole plant of LM against pentylenetetrazole (PTZ) induced seizures in mice. **Methods**: The extract was first examined by acute oral toxicity studies and then evaluated for antiepileptic activity against PTZ induced seizures. Diazepam was used as the standard drug. Antiepileptic activity was evaluated by observing various seizure activities such as onset of seizure, duration of seizure and intensity of seizure. Motor coordination effect was studied using rotarod. Depression was studied using the forced swim test. Oxidative stress was measured by estimating *Malondialdehyde* content in the brain at the end of experiments. **Results:** No abnormalities or toxic effects were observed in the animals during acute oral toxicity studies. The extract treated groups showed late on set of seizures, decreased duration and intensity of seizure when compared with vehicle treated group. The extract was determined to protect the animals from loss of motor coordination and depression. Following sacrifice, the *Malondialdehyde* content in the extract treated groups were found to be less thanin the vehicle treated group. **Conclusion:** From the results of our present study it can be concluded that, the ethanolic extract of whole plant of LM protected the animals from seizures and suppressed the oxidative stress induced by PTZ, without producing loss of motor coordination or depression.

KEYWORDS: Forced swim test, *Malondialdehyde*, Oxidative stress, Pentylenetetrazole, Rotarod test.

INTRODUCTION

Epilepsy is a neurological disorder characterized by recurrent, spontaneous brain seizures.[1] Epilepsy affects more than 2 million Americans and 50 million people worldwide.[2] It not only affects adults, but also children with the prevalence in children estimated to be 0.05–1% of the population worldwide.[3] From clinical investigations, it was found that nearly 55% of epileptic patients also suffered from depression.[4,5] Moreover, currently available antiepileptic drugs are not free from side effects such as depression, impaired cognition, ischemia and motor disability.[6] Therefore there is an urgent need to find new bioactive molecules that can attenuate and prevent epilepsy without inducing depression and other side effects. Drug molecules from herbs are more active against epilepsy.[7] in this study LM was selected to evaluate its ability to suppress seizures in a model of PTZ induced epilepsy in mice.

LM belongs to the family Lamiaceae, is an erect herb with angular stems and white flowers and found in waste lands of Tirumala hills, Andhra Pradesh, India.[8] In folk medicine, aqueous extract obtained from LM was used to treat malaria, kidney disorders, inflammation and rheumatism.[9–11] The plant extract was used by the tribes in Uganda as anti diarrhoea agent.[12] In Kenya's folk medicine, decoction of flowers and leaves of LM was used for the treatment of chronic asthama, fever and oedema.[13] From this plant, leucodin, theomine, glycine and leusin have been isolated.[14] Based on the facts that flavonoids can scavenge free radicals and Leucas species contain flavonoids, in our earlier work, we have screened the LM

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DOI: *10.5530/pc.2014.4.6*

and proved that ethanolic extract of whole plant of LM has flavonoid substances and possess free radical scavenging activity.[15]

MATERIALS AND METHODS

PTZ, 2- Thiobarbituric acid and 1, 1, 3, 3- Tetramethoxy propane were purchased from Sigma Aldrich, Saint Louis, MO, United States. All other reagents and solvents used were of analytical grade and were obtained from various other commercial sources.

Plant material and extraction

The whole plant of LM was collected from Tirumala hills, Andhra Pradesh, India and was authenticated by Dr. K. Madhava Shetty, Assistant Professor, Department of Botany, Srivenkateshwara University, Tirupathi, Andhra Pradesh, India. The LM specimen was prepared and submitted in the department of Botany under the voucher no. 1079. The entire plant was dried, powdered and defatted with petroleum ether at room temperature for 72 h. The defatted material was extracted with 70% ethanol at room temperature and was concentrated under reduced pressure using rotary vacuum evaporator.

Animals

Swiss albino mice weighing 25–28 g were used in this study. All the animals were housed under 12 h light and 12 h dark cycle and allowed for free access to standard pellet food and *ad libitum* except during experiments. All experiments were performed in accordance with ethical guidelines for care and use of laboratory animals approved by institutional animal ethical committee of Swami Ramananda Tirtha Institute of Pharmaceutical Sciences, Nalgonda, Telangana, India. (Ref No.SRTIPS/FM/1468/PO/a/11/CPCSEA/105/2013).

Acute oral toxicity

Extract of LM in 0.2% Carboxyl Methyl Cellulose (CMC) was subjected to acute oral toxicity studies and was performed according to OECD guidelines number 423 using female non pregnant Swiss albino mice weighing 25-28 g. Mice were divided into 2 groups of 3 each. First group was considered as vehicle control group receiving 0.2% CMC and second group was administered with 400 mg/kg of extract of LM. The dose was fixed based on a literature survey.[16–19] Immediately after the dose the mice were observed for first 4 hours and then for14 days to record mortality. Mice were also observed for the presence of any toxic symptoms such as weakness, aggressiveness, refusal of food, loss of weight, diarrhoea and discharge from eyes and ears.

Convulsions induced by PTZ

A total of 24 male mice were divided into 4 groups each containing 6 mice. The first group was considered as the vehicle control and received 0.2% CMC. The second and third groups were considered as extract treated groups and received aqueous ethanolic extract of LM in 0.2% CMC at doses of 200 mg/kg PO and 400 mg / kg PO respectively. The fourth group was considered as the standard group and received diazepam at a dose of 2 mg / kg IP. One hour after treatment, mice in all the groups except the diazepam treated group were treated with PTZ (40 mg / kg IP). For the diazepam group, PTZ was injected after 30 minutes. Vehicle, diazepam or extract was administered daily to the animals (single dose) but PTZ was given to the animals on 1, 5, 10, 15, 20 and 25th days. On 25th day, after the last PTZ dose was administered, the mice were observed over a period of 60 minutes for onset of seizures, duration and intensity of seizures. The intensity of seizure was measured as per score given below:[20]

0: No response, 1: Ear and facial twitching, 2: Convulsive waves axially through the body, 3: Myoclonic body jerks, 4: Generalized clonic convulsions and turn over into side position, 5: Generalized convulsions with tonic extension episode and status epiletpticus, 6: Mortality.

Rotarod test

The rotarod test was performed after observing seizure intensity. All the animals were previously given the training on rotating rotarod with a speed of 10 rpm for 5 min before commencement of treatment. The animals were placed on the rotating rotarod and latency to fall from the rotarodin seconds was noticed.[21]

Forced swim test

Following the rotarod test, the animals were subjected for forced swim test to assess depressive behavior. In this test, the animals were placed individually in a glass cylinder (25 X 12 X 25 cm) containing water at room temperature up to a level of 15 cm for 5 min and total immobility period in seconds was noted. The animals were judged to be immobilized when they stopped struggling and remained floating motionless in water making only those movements necessary to keep their head above water.[22]

Malondialdehyde determination

The animals were sacrificed by decapitation at the end of experiments. The brains were homogenized with 10% (w/v) 0.1 M phosphate buffer (pH 7.4). The homogenized tissue was mixed with 2 volumes of cold 10% w/v trichloro acetic acid to precipitate proteins. The precipitate was centrifuged, pelleted and an aliquot of super-

natant was mixed with 0.67% (w/v) of thiobarbituric acid for 15min in boiling water bath. After cooling, the absorbance was measured at 532 nm. The results were expressed as nm/mg of protein in brain tissues based on standard graph which was plotted by using serial dilutions of 1, 1, 3, 3 tetramethoxy propane.[20]

Statistical analysis

Results were expressed as mean ± SEM and the data was analyzed using one way analysis of variance (ANOVA) by using the software Graph Pad Prism version 6.03. In all the analysis, extract and diazepam treated groups were compared with vehicle treated group.

RESULTS

Acute oral toxicity study

All the animals were closely observed for up to 4 hours and found to be normal in behaviourand without any apparent toxic symptoms or mortality. All the animals survived upto 14 days and no death occurred.

Effect of extract on PTZ induced convulsions

One way ANNOVA analysis was conducted in order to test significance of results compared to control group (Table 1). Animals treated with extract (200 mg / kg and 400 mg / kg) produced significantly delayed onsets of seizure compared to control. The same delayed onset effect was observed for the animals treated with diazepam (standard). One way ANNOVA comparison for onset of seizure was found to be significant (F (1.086, 5.428)= 6209), p<0.0001). Extract at doses of 200 mg / kg and 400 mg / kg significantly decreased the duration of seizure. One way ANNOVA comparison for duration of seizure was found to be significant (F (1.838, 9.189)=116.0, p<0.0001). Animals treated with vehicle were affected with very severe convulsions, such as generalized convulsions with toxic extension episode and also status epilepticus. None of the animals treated with extract (400 mg / kg) showed the severe form of seizure and they were limited only to myoclonic body jerks. Mice treated with diazepam showed only ear and facial twitching.One way ANNOVA comparison for intensity of seizure was found to be significant (F (2.441, 12.21)= 99.37, p<0.0001).

Effect of extract on rotarod test

The effects of the extract on the rotarod test were shown in (Table 2). Mice treated with extract at both the doses managed to stay on the rotarod for more time when compared with the vehicle treated group. The extract protected the animals significantly against neurotoxicity induced by PTZ and the comparison was found to be significant (F (1.371, 6.856)=69.40, p<0.0001).

Effect of extract on forced swim test

The extract significantly decreased immobility time at a dose of 200 mg / kg and 400 mg / kg (Table 2). Diazepam treated mice showed greater immobility in water and the comparison was found to be significant (F(2.203, 11.02) = 123.4, p< 0.0001).

Effect of extract on MDA content

The effect of the extract on MDA content was shown in (Table 2). Extract at a dose of 400 mg / kg and 200 mg / kg non significantly decreased MDA content when compared to vehicle treated group and the comparison was found to be (F(1.451, 2.901)= 17.31, p=0.0255).

Table 1: Effect of ethanolic extract of LM and diazepam on PTZ induced seizure in mice

Group	Dose	Onset of seizure (sec)	Duration of seizure (sec)	Intensity of seizure (Score)
Group 1	-	16.83±3.208	193.7±6.216	5
Group 2	200mg/kg	21.00±0.966^{ns}	152.2±6.877*	3.5±0.223**
Group 3	400mg/kg	50.00±2.569**	94.17±7.472***	2.667±0.210***
Group 4	2mg/kg	1744±21.60****	27.33±2.333****	0.667±0.210****

All the values are represented as mean ± SEM where n=6, Symbols represent statistical significance as ns P>0.05, * P <0.05, ** P <0.01, *** P<0.001 and **** P<0.0001 vs control group

Table 2: Effects of ethanolic extract of LM and Diazepam on rotarod performance, forced swim test and MDA content

Group	Rotarod test (sec)	Forced swim test (sec)	MDA content (nM/mg protein) (n= 3)
Group 1	8.5±0.991	280.7±10.86	0.797±0.039
Group 2	61.50±8.378**	130.3±11.69***	0.668±0.033ns
Group 3	158.50±9.266****	63.67±6.642***	0.572±0.034^{ns}
Group 4	47.33±5.057**	316.2±11.76ns	0.502±0.011*

All the values are represented as mean ± SEM where n=6, Symbols represent statistical significance as ns P>0.05, * P <0.05, ** P <0.01, *** P<0.001 and **** P<0.0001 vs control group

DISCUSSION

In our earlier studies, we reported that ethanolic extract of whole plant of LM contains phenolic substances (38.624 ± 14.171 GAE/g) and flavonoids (42.281 ± 8.880 RE/g). Ethanolic extract of LM also showed significant free radical scavenging activity and IC_{50} values were determined for super oxide radical (55.00 ± 8.11µg / ml), hydroxyl radical (226.95 ± 75.79 µg / ml) and nitric oxide (54.89 ± 1.85 µg / ml).[15] In this present study, LM was evaluated for its ability to protect the animals against oxidative stress induced by PTZ based on its content of flavonoid substances.

Oxidative stress is an underlying mechanism in the development and progression of epilepsy.[23] Oxidative stress is caused by excessive production of reactive oxygen species such as hydroxyl, super oxide anion radical and nitric oxide. Furthermore, seizure activity during epilepsy can increase the amount of free radicals and decrease the antioxidant defence mechanism in the brain which further induces oxidative stress.[24] The present study was focused on treating epilepsy by suppressing oxidative stress. The PTZ induced model was selected to induce seizures in animals since PTZ induced seizure activity mimics the increased oxidative stress in the brain by altering membrane phospholipid metabolism and ultimately results in the release of free radicals which is evident by elevated MDA content in the brain.[20] In the present study, it was observed that ethanolic extract of LM delayed the onset of seizure, shortened the duration of seizure and decreased the intensity of seizure when compared to vehicle treated animals. The rotarod test results indicate that the extract protected the animals from neurotoxic effect induced by PTZ. The protective effect of LM may be attributed to its free radical scavenging ability since the MDA content of extract treated group is less than vehicle treated group.The forced swim test is awidely used animal model for evaluating the depression caused by drug molecules.[25] Since depression is the most reported side effect of antiepileptic drugs and psychiatric disorder in patients with epilepsy, herbal compounds or drugs having antiepileptic activity without inducing depression are the most interesting in drug development.[7] In the forced swim test, it was observed that diazepam and the vehicle treated group showed depressive behavior which was evident from increased immobility time. In the extract treated group, the immobility was less than in the vehicle treated group,indicating that the extract does not induced depression.In recent years herbs rich in phenolic and flavonoid contents were given the prime importance because of their free radical scavenging ability.[26–28] In addition, flavonoid such as rutin[29] or flavonoid rich extract[25] was shown to provide significant protective effects against seizure induced by PTZ. These protective effects of LM may be due to its flavonoid contents. In this study, LM showed dose dependent effects. These positive results encourage the future studies for isolation of responsible compound and its characterization.

CONCLUSION

The present study demonstrated that ethanolic extract of LM significantly suppressed the seizures induced by PTZ without inducing depression in mice. This protective effect of LM may be due to its free radical scavenging ability which is evident by the decreased MDA content.

However further research is required to identify and isolate the responsible compounds for its antiepileptic effect.

CONFLICT OF INTEREST

No conflict of interest.

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