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Antiepileptic activity of leaves of *Leucas aspera.*

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Abstract

Leucas aspera (LA) is a plant that has been used in folk medicine to treat asthma, fever, skin diseases and has several pharmacological activities. The antiepileptic property of LA was not yet been studied. Aim: The present study was aimed to investigate the antiepileptic activity of ethanolic extract obtained from leaves of LA on pentylenetetrazole (PTZ) kindling seizures in mice. Method: The ethanolic extract 200mg/kg and 400mg/kg were evaluated for the antiepileptic activity against PTZ (40mg/kg IP) induced seizure method. Diazepam was used as the standard drug. Antiepileptic activity was evaluated by observing seizure intensity, motor coordination, depression and oxidative stress by malondialdehyde (MDA) content. Results: Treatment with extract was found to be in dose dependant manner with significant prolonged onset time, decreased duration and intensity of seizure when compared with vehicle treated group. Extract has even protected the animal from loss of motor coordination and depression. Conclusion: From the results of present study it can be concluded that, the ethanolic extract of leaves of LA protected the animals from seizure effect induced by PTZ and attenuated the oxidative stress induced by PTZ without producing loss of motor coordination and depression.

Keywords: *Leucas aspera*, Malondialdehyde, Pentylenetetrazole

Introduction

Epilepsy is the most common central nervous system disorder affecting more than 50 million people world wide [1]. It is characterized by recurrent spontaneous seizures and caused by sudden abnormal and recurrent electrical discharge from the affected brain cells. There are so many drugs available currently in the market to treat epilepsy, but none of them are free from side effects such as depression, ishchemia, impaired cognition and motor disability [2]. In recent years many research activities has been focused on screening of extracts obtained from herbs against epilepsy disorder. It was found that, great number of screened herbs are active against epilepsy with less side effects [3]. Therefore the present study was aimed to evaluate the protective ability of LA against epilepsy.

LA belonging to the family Lamiaceae is commonly known as Thumbai in Telugu. It is widely distributed throughout India. The plant is an annual erect, non aromatic herb. Stems are much branched, leaves are serrate, blunt tipped and the margins scalloped [4]. Traditionally, the decoction of whole plant and individual parts were used to treat asthama, fever, skin diseases, head ache, arthritic pain and snake bites [5]. The extract obtained from LA display a wide range of pharmacological activities such as antimicrobial [6], antinociceptive, antioxidant and cytotoxic activities [7]. Alchoholic extract of LA leaves showed significant free radical scavenging activity [8] and DNA protecting effect [9]. From this plant a large number of secondary metabolites such as aliphatic ketones, flavonoids, glycosides and terpenes have been isolated [5]. Since the LA has various secondary metabolites and pharmacological activities, the present investigation was focused to evaluate the protective effect of ethanolic extract of leaves of LA against PTZ induced convulsions in mice.

Materials and Methods

Pentylenetetrazole (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

Leaves from LA were collected from in and around agricultural lands surrounding Nalgonda district, Telangana, India. The leaves were shade dried and ground into fine powder. The powder was first defatted with petroleum ether at room temperature for 48H and then extracted with 70% ethanolic solution at room temperature for another 72H. The resultant ethanolic extract was concentrated under reduced pressure at room temperature using rotary vacuum evaporator.

Animals

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Male swiss albino mice weighing 25-30g were used in this study. All the animals were housed under 12H light and 12H 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/103/2013).

Experimental procedure

Extract was suspended in 0.2% carboxy methyl cellulose (CMC) suspension and given orally 1 hour before the administration of PTZ. Diazepam was used as standard and administered via IP route 30 minutes before the administration of PTZ. PTZ was dissolved in sterile saline solution and administered via IP route. Mice were randomly divided into 4 groups (n=6). All the animals in the group were treated as described below

Group 1: 0.2% CMC followed by PTZ (40mg/kg)
Group 2: Extract 200mg/kg followed by PTZ (40mg/kg)
Group 3: Extract 400mg/kg followed by PTZ (40mg/kg)
Group 4: Diazepam 2mg/kg followed by PTZ (40mg/kg)

Kindling was induced by a total of 6 treatments with PTZ on every 5 days (1, 5, 10, 15, 20 and 25). Vehicle, Extract and Diazepam were administered daily to the animals. On 25th day mice were observed for seizure activity and subjected to rotarod test and forced swimming test [10].

Seizure observation

The antiepileptic activity was assessed by observing onset of seizures, duration of seizure and intensity of seizure. Intensity of seizure was evaluated using the following score [10].

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

Motor coordination test

This was performed after observing seizure intensity using rotarod. All the animals were previously given the training on rotating rotarod with a speed of 10rpm for 5 minutes before commencements of treatment. The animals were placed on the rotating rotarod and latency to fall in seconds from the rotarod was noticed [11].

Forced swimming test

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

Malondialdehyde determination

The animals were sacrificed by decapitation at the end of experiments. The brains were homogenized with 10% w/v 0.1M 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 supernatant was mixed with 0.67%w/v of thio barbituric acid for 15min in boiling water bath. After cooling, the absorbance was measured at 532 nm. The results were expressed as nM/gm of protein in brain tissues based on standard graph which was plotted by using serial dilutions of 1, 1, 3, 3 tetramethoxy propane [10].

Statistical analysis

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

Results and Discussion

Repeated measures one way ANOVA test was conducted in order to test significance of results compared to control. The results of seizure activities were shown on table 1.

Table No 1: Effect of ethanolic extract of *Leucas aspera* and diazepam on PTZ induced seizure in mice

Group	Dose	Onset of seizure(sec)	Duration of seizure (sec)	Intensity of seizure (sec)
Group 1	-	20.83±3.962	147.0±12.45	4.667±0.210
Group 2	200mg/kg	46.33±5.69**	123.3±8.89^{ns}	4.00±0.258^{ns}
Group 3	400mg/kg	87.83±8.408***	77.00±6.923****	3.00±0.258****
Group 4	2mg/kg	1706±17.70****	19.17±2.926****	1±0.0****

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 No 1: Effect of ethanolic extract of *Leucas aspera* and diazepam on PTZ induced seizure in mice

Extract significantly prolonged the onset of seizure at a 200 mg/kg and 400 mg/kg and one way ANOVA comparison was found to be significant (F (1.322, 6.611) = 6.082, P<0.0001). Extract at dose of 400 mg/kg significantly decreased the duration of seizure and the comparison was found to be significant (F (3, 20) = 43.76, P<0.0001). Extract at a dose of 400mg/kg protected animals very effectively against PTZ induced seizure which was evident by the seizure score 3 and the comparison was found to be significant (F (3, 20) = 57.50, P<0.0001). The protective effect of extract on rotarod test was shown on Table 2. Mice treated with extract at a dose of 400 mg/kg were stayed on rotarod more time when compare to extract at a dose of 200 mg/kg but not the diazepam treated group. The extract protected the animals against neurotoxicity induced by PTZ and the comparison between the group was found to significant (F (3, 20) = 23.14, P<0.0001).

The protective effect of extract on immobility time was shown in Table 2.

Table No 2: Effects of ethanolic extract of *Leucas aspera* 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	11.00±1.826	220.3±13.79	0.8380±0.05
Group 2	49.50±6.286**	91.17±7.547****	0.8163±0.072ns
Group 3	95.50±13.97***	49.83±8.28****	0.6070±0.061*
Group 4	22.50±2.432ns	197.8±8.719ns	0.415±0.024**

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 No 2: Effects of ethanolic extract of *Leucas aspera* and Diazepam on rotarod performance, forced swim test and MDA content

Extract significantly decreased immobility time at 200 mg/kg and 400mg/kg. Diazepam induced depression in animals which was clearly evident from its immobility time and was found to be significant (F 3, 20) = 69.15, P<0.0001). The effect of extract on lipid peroxidation was shown in table 2. Extract at a dose of 400mg/kg decreased MDA content when compared to vehicle treated group but not at a dose of 200 mg/kg. This clearly indicates that extract protect the animals against lipid peroxidation induced by PTZ and the comparison between the group was found to be significant (F (3, 8) = 13.07. P<0.0019).

Epilepsy is a neurological disorder caused by several mechanisms including genetic factor, mitochondrial dysfunction, modified cytokine levels, oxidative stress and environmental factors. Among these oxidative stress is considered as the most probable causative mechanism. The brain is particularly more susceptible to oxidative stress since it utilises the highest amount of oxygen compared with other body organs [13]. Oxidative stress is quantified by biochemical parameter such as MDA. Highest amount of MDA content in brain tissue indicates the oxidative stress [10].

PTZ is a non competitive antagonist of the GABA $_A$ receptor and widely used as an experimental animal model for evaluating the effectiveness of antiepileptic drugs. Repeated administration of (chronic model) PTZ in animal induces seizure through excess generation of free radicals [14].

From the literature survey, it was found that LA has shown very good free radical scavenging and antioxidant activity. In addition, it was reported that flavonoid such as Leucasin isolated from alcoholic extract of leaves of LA has antioxidant activity [8]. In recent years, there are so many scientific studies revealed and confirmed that flavonoids or flavonoid containing extracts can attenuate the oxidative stress by scavenging free radicals induced by PTZ [11, 15]. Therefore in our study, ethanolic extract of leaves of LA was selected since it has antioxidant flavonoid such as Leucasin.

In our study, it was observed that, ethanolic extract of LA at 400mg/kg increased the onset time of seizure, shortened the duration of seizure and decreased the intensity of seizure when compared to vehicle. Rotarod test clearly indicated that, extract has protected the animal from neorotoxic effect induced by PTZ. The protective effect of extract was further confirmed by the low levels MDA content in the brain of animal when compared with the vehicle treated group. The protection may be due to its antioxidant property.

Forced swimming test is the most widely used animal model for evaluating depression activity of biochemical compounds since depression is the most reported side effect of antiepileptic drugs and psychiatric disorder in patients with epilepsy. Drugs or herbal compounds having antiepileptic activity without causing depression are more interested in research [3]. In our study, it was observed that diazepam caused depression since diazepam increased immobility time in forced swimming test but not by extract.

Conclusion

The present study demonstrated that ethanolic extract of leaves of LA significantly suppressed seizures induced by PTZ without producing depression. The protective effect of extract may be due to its antioxidant activity which is evident by the decreased MDA content. However further research is required to identify and isolate the responsible compounds for its antiepileptic activity.

Author's contribution

Ramalingam Ramani conceived the present study, carried out interpretation of statistical data, drafted the manuscript and assisted in pharmacological screening.

Bindu Madhavi Boddupalli has carried out the extraction process, assisted in data collection and drafting the manuscript.

Ramya Miryala carried out the pharmacological screenings

Ravinder Nath Anisetti revised the manuscript for its intellectual content and given final approval.
Nagulu Malotu supervised and guided the animal's experiments.
Deepthi Balla collected the plant material and helped in statistical treatment of results.

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