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Original Article

Anticonvulsant Activity of Ethonolic Extract of *Nyctanthes arbor-tristis* in Albino Mice

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ABSTRACT

Objective: The main objective of the present study was to assess the anticonvulsant activity of ethonolic extract of *Nyctanthes arbor-tristis* (ENA) in albino mice. **Method:** The anticonvulsant activity of ethanolic extract of *Nyctanthes arbor-tristis* (200 and 400 mg/kg, P.O) was assessed in pentylenetetrazole (PTZ) and maximal electroshock (MES) induced convulsions in albino mice. Onset of convulsion, duration of convulsion, tonic extension phase, and mortality were noted in the present study. The antioxidant property was evaluated by measuring the lipid peroxidation (LPO) and reduced glutathione (GSH). **Results:** In the pentylenetetrazole induced convulsion, ethonolic extract of *Nyctanthes arbor-tristis* (400 mg/kg, P.O) significantly delayed the onset of convulsion, reduced the duration of convulsion ($p<0.05$) and reduced mortality. The ethonolic extract of *Nyctanthes arbor-tristis* at (400 mg/kg, P.O) reduced hind limb tonic extension phase in maximal electroshock induced convulsion in albino mice ($p<0.05$). The pre-treated ethonolic extract of *Nyctanthes arbor-tristis* showed significant antioxidant property by inhibition of lipid peroxidation and increases the reduced glutathione level in mice brain tissue ($p<0.001$). **Convulsions:** In conclusion the results indicated that *Nyctanthes arbor-tristis* possesses a significant dose dependent anticonvulsant activity.

Keywords: *Nyctanthes arbor-tristis*, Anticonvulsant, MES, PTZ, Epilepsy.

INTRODUCTION

Epilepsy is a common neurological abnormality manifested by recurrent unprovoked seizures affecting about 5 % of the world population. Seizure is abnormal function of ion channels and neural networks which results in rapid, synchronous, and uncontrolled spread of electrical activity in brain. Seizure symptoms vary according to the location of seizure activity and may include prominent motor symptoms and loss of consciousness, paroxysmal alterations in nonmotor functions, or changes in high-order functions. It has been shown to affect several brain activities and promote long-term changes in multiple neural systems. This disorder, if untreated, can lead to impaired intellectual function or death and is typically accompanied by psychopatho-logical consequences such as lose of selfesteem[1]. Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. This revival of interest in plant derived drugs is mainly due to the current widespread belief that "GREEN MEDICINE" is safe and more dependable than the costly synthetic drugs, many of which have adverse effects[2]. *Nyctanthes arbor-tristis* belongs to the family Oleaceae and has chemical constituents such as flavonoids, poly phenolic compounds, etc.; it is used for treatment of convulsions, oxidative stress, diabetes and inflammation in Indian indigenous medical system[3]. However, there is no scientific report available in support of anticonvulsant activity of *Nyctanthes arbor-tristis* in albino mice. Therefore, to justify the traditional claims we have assessed the anticonvulsant activity of *Nyctanthes arbor-tristis* in experimental mice.

MATERIALS AND METHODS

Plant materials

The mature green leaves of *Nyctanthes arbor-tristis* were collected from the Kadapa District, Andhra Pradesh, India. The plant was identified and authenticated by the Department of Botany SV University. A voucher specimen no.SV-3681 was deposited in the department of pharmacology, PRRM College of pharmacy, Kadapa.

Preparation of extract

Fresh leaves of *Nyctanthes arbor-tristis* were collected and shade dried at room temperature. Dried leaves were powdered mechanically through 40 mesh sieve. 100 g of freshly powdered leaves were evenly packed in soxhlet apparatus and the extraction was done with 95 % ethanol. Then solvent was evaporated at low temperature under reduced pressure. The dried extract thus obtained was kept in desiccators and was used for further experiments[4].

Experimental animals

Male wistar albino mice weighing 20-25 g were obtained from Raghavendra enterprises, Bangalore, India. They were kept in departmental animal house in polypropylene cages in an air-conditioned area at 25±2°C with 10:14 h light and dark cycle and maintained on balanced animal feed and water ad libitum. The experimental protocols were approved by the Animal Ethical Committee of PRRM College of pharmacy (1423/PO/a/11/CPCSEA).

Acute toxicity studies

Acute oral toxicity study of ethanolic extract of *Nyctanthes arbor-tristis* was carried out according to OECD guidelines 423.

Assessment of anticonvulsant activity

Pentylenetetrazole (PTZ) induced convulsion

Healthy adult male albino mice were divided into IV groups, containing 6 animals in each group. Group I received vehicle (1% CMC, P.O); group II animals were treated with standard drug diazepam (25 mg/kg, I.P.), group III, and IV (200 and 400 mg/kg, P.O) received ENA. Mice were administered extracts for seven days and on the experimental day, PTZ 65 mg/kg was injected intraperitoneally to mice after 30 min drug treatment. Immediately after PTZ administration mice were observed for (1) onset of convulsions, (2) duration of convulsion and (3) mortality for the duration of 30 min[5].

Maximum electro shock (MES) induced convulsions

The electrical shock applied through ear-clip electrodes separately to each mouse. The stimulus duration was 0.2 s and the current frequency 45 mA (60 Hz). Each group containing six mice which were administered with extract and standard drug (phenytoin 25 mg/kg I.P.) for seven days and on the experimental day, test was started 30 min after drug administration. The animals were observed for the occurrence of tonic hind limb extension and mortality for duration of 15 min[5]. The ENA was administered to Group III and IV (200 and 400 mg/kg body P.O) whereas group I and II received 1% of CMC and Phenytoin 25 mg/ kg I.P.., respectively.

Biochemical estimation

Tissue preparation

After observing onset of convulsions, duration of seizure following the administration of PTZ, the animals were sacrificed by cervical dislocation and brain was removed and washed in cooled 0.9% saline, kept on ice, blotted on filter paper, then weighed and homogenized in cold phosphate buffer (0.05 M, pH 7.4). The homogenates were centrifuged at 10000 rpm for 10 min at 4ºC (Remi, Model no: EELC-6087) and post-mitochondrial supernatant (PMS) was used for the estimation of lipid peroxidation. The supernatant was again centrifuged at 15000 rpm for 1 hour at 4ºC. The supernatant obtained was used for further estimation of GSH[6].

Lipid peroxidation

Two milliliter of suspension medium was taken from 10% of tissue homogenate. To this, 2 ml of 30% of trichloroacetic acid was added, followed by 2 ml of 0.8% thiobarbituric acid (TBA) reagent. The tubes were covered with aluminium foil and kept in shaking water bath for half an hour at 80 oC after half an hour; the tubes were taken out and kept in ice cold water for half an hour. There were then centrifuged at 3000 x *g* for 15 min. The absorbance of the supernatant was read at 535 nm at room temperature against appropriate blank. Blank consist of 2 ml distilled water, 2 ml of 30% TCA and 2 ml of 0.8% TBA[7]. The content of malonaldehyde (MDA), expressed as µ moles formed per milligram of protein in the tissue, was calculated using the formula:

Concentration = A x (V/E) x P

Where, A is the volume of solution, E is extinction coefficient (1.56 x 105m-1 cm-1) and P is the protein content of tissue calculated as milligram of protein per gram of tissue.

Reduced glutathione

To 2 ml of 10% of homogenate, which was prepared in sodium chloride solution, 2.5 ml of 0.02M EDTA was added and shaken vigorously. To 2 ml of this mixture 4 ml of cold distilled water and 1 ml of 50% trichloroacetic acid were added and

shaken for 10 min. Thereafter, the content were centrifuged at 3000 x *g* for 15min following centrifugation, 2 ml of the supernatant was mixed with 0.4M tris buffer (pH 8.9). The whole solution was mixed well and 0.1 ml of 0.01M DTNB was added, the absorbance was read within 5 min of addition of DTNB at 412 nm against reagent blank with no homogenate. For blank reading, the homogenate was substituted by 2 ml of distilled water[8]. The amount of glutathione in tissue was expressed as µmol/g of tissue.

µmol/mg wet tissue: [A/13600] x dilution factor x 1000

Statistical analysis

All the data were expressed in mean ± SEM. The significance of difference in means between control and treated animals was determined by One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Significance of difference between normal and control group were evaluated by un-paired student's *t*-test by using graph pad prism software version 5.0. $P<0.05$ was considered statistically significant[9].

RESULTS

Acute toxicity studies

There was neither change in behavioral pattern nor any sign of toxicity during the observations up to 24 h for mortality. The extracts were safe up to a maximum dose of 4000 mg/kg. The biological evaluation was carried out at doses of 200 and 400 mg/kg.

Anticonvulsant activity

Effect of ENA on PTZ induced convulsions

PTZ produces convulsions in all groups. The group pre-treated with ENA at the dose of 200 mg/kg P.O. did not showed significant delay the onset of convulsion and duration of seizures in mice and reduced mortality to 33.33%. Similarly the group received ENA at a dose of 400 mg/kg P.O. showed significantly delay the onset of convulsion ($p<0.05$), reduced the duration of convulsion ($p<0.05$) and not found any mortality. The standard anti-epileptic drug, diazepam (5 mg/kg) blocked the clonic convulsions and mortality in mice against pentylenetetrazole induced convulsions (Table 1).

Effect of ENA on MES-induced convulsions

ENA showed significant anticonvulsant activity by significantly lowering the duration of hind limb tonic extension (HLTE) induced by maximal electroshock. ENA at a dose of 200 mg/kg P.O. not showed significant reduction HLTE and reduced the mortality to 33.33%. ENA dose of 400 mg/kg P.O. significantly reduced the duration of HLTE ($p<0.05$) and no mortality was found. Phenytoin (25 mg/kg I.P.) significantly reduced the duration of MES-induced HLTE ($p<0.01$) and completely prevented the various phases of convulsion induced by MES (Table 2).

Biochemical estimation

Effect on lipid peroxidation (LPO)

The brain homogenate showed significantly increase in LPO in control group .The effect of extract was showed significant inhibition of LPO in mice brain tissue as compared to control group. The ethanolic extract (200 and 400 mg/kg P.O.) showed a significant protection by reducing the elevated levels of LPO ($P<0.001$) compared to control group (Fig 1).

Effect on reduced glutathione (GSH)

The ethanol extract of *Nyctanthesarbor-tristis*200 and 400 mg/kg dose showed significant ($p<0.05$ and $p<0.001$) increases in brain GSH level compared to control (Fig 2).

DISCUSSION

The observations emanated in the present study indicated that the ENA was without any lethal effect in a dose up to 4000 g/kg and possessed significant anticonvulsant activity against convulsions induced by PTZ and MES.PTZ is a powerful CNS stimulant and a convulsant drug. It induces convulsions by inhibiting the activity of GABA at GABA-A receptors and direct depolarization of CNS neurons[10]. GABA is a major inhibitory neurotransmitter in the brain, and the inhibition of its neurotransmission has been thought to be the underlying factor in epilepsy[11]. The enhancement of the GABAergic neurotransmission is reported to antagonize seizures, while the inhibition of the neurotransmission promotes seizures[12]. As, it has been noted that the drug which are effective in petitmal seizures are effective in preventing PTZ induced clonic convulsions. Again, diazepam facilitates GABAergic transmission is the drug of choice for PTZ poisoning.

ENA dose of 200 and 400 mg/kg significantly delayed the onset of convulsion, reduced the duration of convulsion and no mortality was found in mice. The standard anti-epileptic drug diazepam (5 mg/kg) completely antagonized the seizures. The findings of the present study, therefore, tend to suggest that ethanolic extract of *Nyctanthes arbor-tristis* has anticonvulsant activity might have inhibited and/or attenuated PTZ -induced seizures of the mice used by enhancing, or in some ways interfering with GABAergic neurotransmission.

The maximal electroshock-induced convulsion in animals represents grandmal type of epilepsy. The tonic extensor phase is selectively abolished by the drugs effective in generalized tonic clonic seizure. The most outstanding action of phenytoin showed abolition of tonic extensor phase of MES seizure many drugs that increase the brain content of Gama amino butyric acid (GABA) have exhibited anticonvulsant activity against seizures induced by MES[13].

The ethanol extract caused significant decrease in the duration of hind limb tonic extension (HLTE) induced by maximal electroshock. ENA dose of 200 and 400 mg/kg significantly reduced the duration of HLTE and reduced the mortality. The standard antiepileptic drug phenytoin (25 mg/kg I.P.) significantly reduced the duration of HLTE of MES-induced convulsion and completely abolished the various phases of convulsion. Reduction in the duration of tonic hind limb extension of MES induced convulsion indicated ENA has anticonvulsant activity.

The ethanolic extract of *Nyctanthes arbor-tristis* significantly reversed these toxic effects of PTZ, revealing the antioxidant activity of the extract. Decreased LPO and increased levels GSH in both doses of ENA has offered protection against oxidative stress. Inhibition of oxidative stress may be one of the possible mechanisms for the antiepileptic property of *Nyctanthes arbor-tristis*. Several studies also indicate that on medicinal plants antioxidant activities are due to the presence of polyphenols and flavonoids[14-16].

The presence of flavonoids, poly phenolic compounds in *Nyctanthes arbor-tristis* extract probably responsible for anticonvulsant activity. Flavonoids have been reported to process significant anticonvulsant activity in various plants[17]. Taken together, our results strongly support the anticonvulsant potential of the plant *Nyctanthes arbor-tristis* and its use in traditional medicine.

CONCLUSION

From the experimental study it can be concluded that ethanolic extract of *Nyctanthes arbor-tristis* had exhibit

significant anticonvulsant activity against pentylenetetrazole and electroshock-induced seizure in albino mice.

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Table 1. Anticonvulsant activity of *Nyctanthes arbor-tristis* on PTZ induced convulsion in albino mice

Groups	Treatment	Onset of clonic convulsions (min)	Durations of convulsion (min)	Mortality/used (%)
Control	1% CMC (2mL/kg, P.O)	1.05±0.09	5.09±1.04	3/6 (50%)
Standard	Diazepam (5.0 mg/kg, I.P.)	0.00±0.00***	0.00±0.00***	0/6 (0%)
ENA	200 mg/kg, P.O	1.79±0.45	2.50±0.65	2/6 (33.33%)
ENA	400 mg/kg, P.O	3.56±0.60*	1.85±0.54*	0/6 (0%)

All the results were expressed as mean ± SEM (n=6), for each experimental group. The statistical analysis was carried out using one way ANOVA method. Significant after analysis of variance (ANOVA) followed by Tukey test. $^{*}P<0.05$ and $^{***}P<0.001$; when compared to control group.

Table 2. Anticonvulsant activity of *Nyctanthes arbor-tristis* on MES induced convulsion in albino mice

Groups	Treatment	Times in seconds of various phases (mean ± SEM)				
		Flexion	Extension	Stupor	Recovery	Mortality/used (%)
Control	1% CMC (2mL/kg, P.O)	18.35±2.7	14.68±1.50	145.5±7.60	190.0±8.00	5/6 (83.33%)
Standard	Phenytoin sodium (25 mg/kg, I.P.)	9.02±1.45**	-----	-----	12.60±1.18	0/6 (0.0%)
ENA	200 mg/kg, P.O	8.95±0.89**	9.62±0.76	130±9.62	170.0±6.40	2/6 (33.33%)
ENA	400 mg/kg, P.O	8.76±1.67**	8.32±1.02*	62.50±8.04*	120.40±9.67*	0/6 (0.0%)

All the results were expressed as mean ± SEM (n=6), for each experimental group. The statistical analysis was carried out using one way ANOVA method. Significant after analysis of variance (ANOVA) followed by Tukey test. *P<0.05, and *P<0.01; when compared to control group.

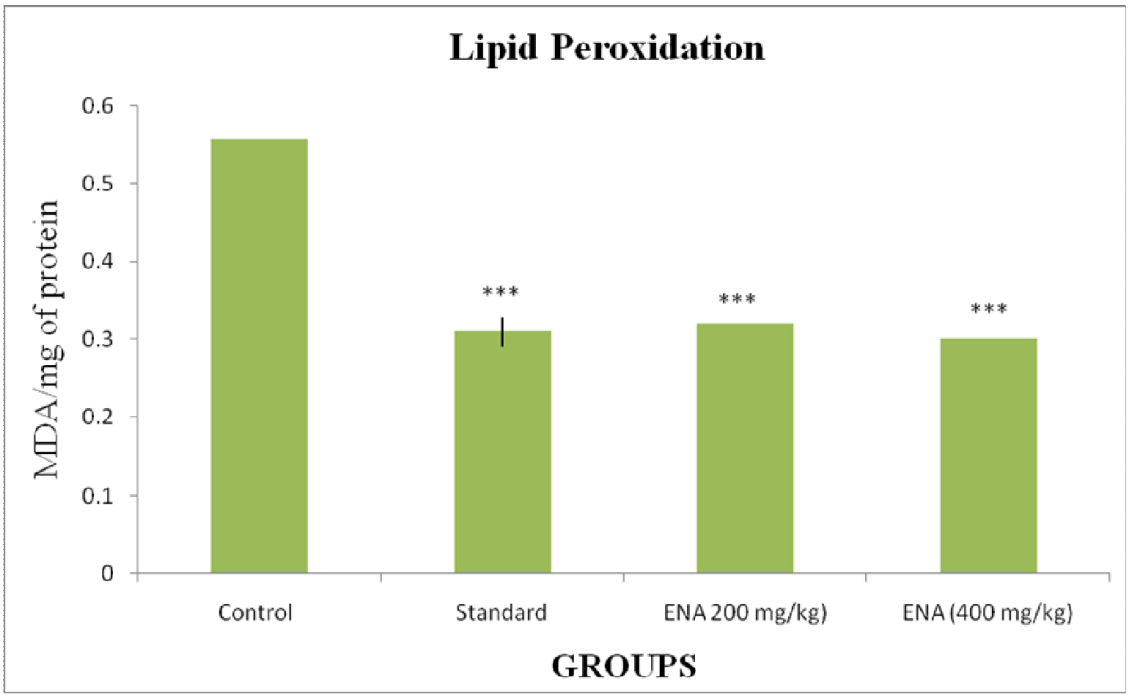

Figure 1. Effect of ENA on lipid peroxidation

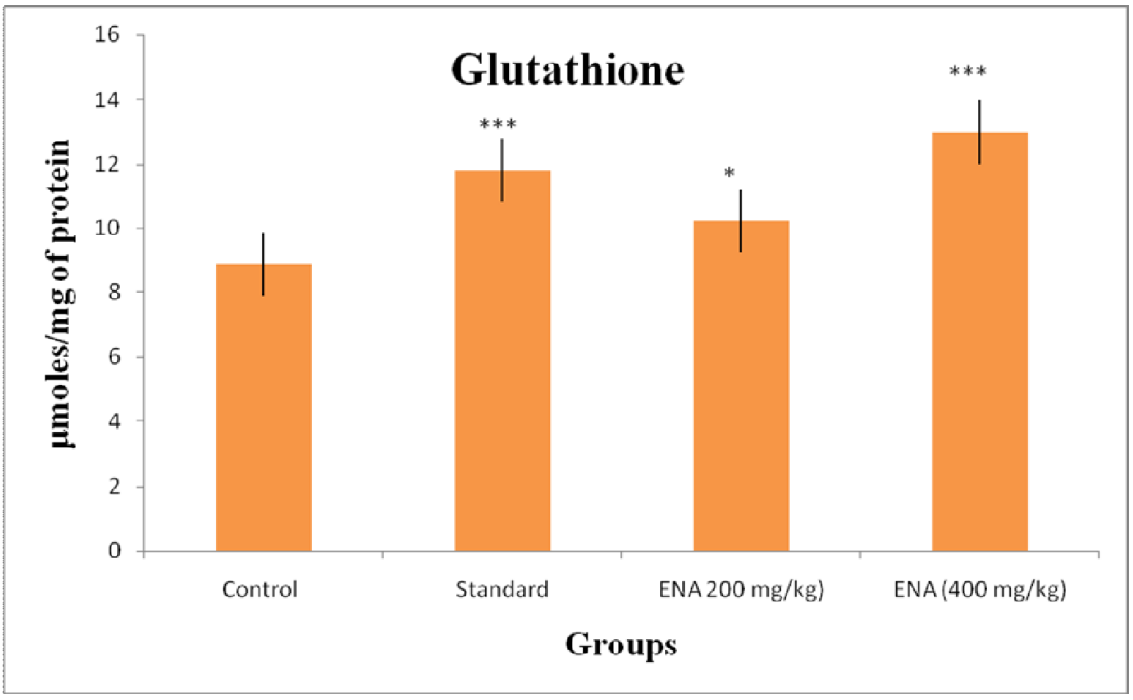

Figure 2. Effect of ENA on Lipid Peroxidation