Research Article

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Evaluation of anticonvulsant activity of ethanolic extract of *Nymphaea alba* Linn. (white water Lily) and its comparison with phenytoin sodium and sodium valproate in albino rats

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ABSTRACT

Objective: The aim of the present work was to evaluate the anticonvulsant activity of ethanolic extract of Nymphaea alba Linn. in rats. **Materials and methods:** Anticonvulsant activity of ethanolic extract of Nymphaea alba Linn.(200 and 100mg/kg oral) is evaluated in albino rats both in MES induced artificial seizures by Electroconvulsiometer and Pentylene tetrazole (70mg/kg i.p)induced seizures. Phenytoin sodium and sodium valproate were used as standard drugs for these two methods respectively. All drugs were administered orally one hour before tests. **Results:** Oral administration of ethanolic extract of Nymphaea alba (200 and 100mg/kg) 1 hour before artificial induction of seizures significantly prevented Tonic hind limb extension (THLE) and also decreased the duration of seizures in MES models and was comparable to phenytoin sodium. In PTZ induced models it showed some protection compared to control but was statistically not significant. **Conclusion:** Nymphaea alba has significant anticonvulsant property in MES model at the dose of 100 and 200mg/kg and is comparable to phenytoin sodium. But Nymphaea alba did not show statistically significant anticonvulsant property in PTZ induced seizures.

KEY WORDS: Epilepsy, Nymphaea alba, protective, Electroconvulsiometer and Pentylene tetrazole.

INTRODUCTION

The term *epilepsy* refers to a disorder of brain function characterized by the periodic and unpredictable occurrence of seizures. The prevalence of epilepsy has been estimated at 5–10 persons per 1000. [1]

In most cases the initial combination therapy combines first-line drugs, i.e., carbamazepine, phenytoin, valproic acid, and lamotrigine. Overdose with phenytoin causes ataxia, diplopia, and stupor. The prolonged use of phenytoin often leads to Weight gain, menstrual irregularities, hirsutism, hypertrophy of gums, and coarsening of facial features in children. Chronic phenytoin use over several decades may occasionally be associated with peripheral neuropathy and probably with a form of cerebellar degeneration; an antifolate effect on blood and interference with vitamin K metabolism have also been reported. Carbamazepine causes Leukopenia and there have been instances of pancytopenia, hyponatremia, and diabetes insipidus as idiosyncratic reactions. Valproate commonly causes hepatotoxicity, weight gain, menstrual irregularities and polycystic ovarian syndrome.[2] The most common adverse effects Topiramate are somnolence, fatigue, weight loss, and nervousness. It can also precipitate renal calculi, which is most likely due to inhibition of carbonic anhydrase. Topiramate has been associated with cognitive impairment and angle closure glaucoma. [3, 4, 5, 6, 7, 8] At present the most commonly used antiepileptic drugs have severe and dose limiting adverse effects and hence search for better antiepileptic drug with less adverse effects continues.

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Nymphaea alba Linnaeus

Nymphaea alba Linnaeus (family Nymphaeaceae), commonly known as European White Water lily, White Lotus, or Nenuphar in English, kumuda/Naidile/utpala in Sanskrit, Nilofar in Unani, Alli in siddha/tamil, is an aquatic herb with perennial rhizomes or rootstocks anchored with mud. It grows in water from 30-150 centimeters deep and likes large ponds and lakes. The leaves may be up to thirty centimeters in diameter and they take up a spread of 150 centimeters per plant. The flowers are white and they have many small stamens inside. It is globally distributed in Europe, North Africa, Southwest Asia, India, China and Russia. All the parts of the plant have medicinal uses in traditional system of medicine. It is used as both an Aphrodisiac and anaphrodisiac- flower part is used as aphrodisiac and the root of the plant was used as an anaphrodisiac, being crushed and mixed with wine. It has astringent and cooling effect- filaments are used for bleeding piles and menorrhagia; produces calming and sedative effects upon the nervous system, and is useful in the treatment of insomnia, anxiety and epileptic disorders. Anti-oxidant, antiproliferative and anticarcinogenic action and inhibition of renal oxidative stress and hyperproliferative response were also reported. It also has anti-inflammatory, antimicrobial and antiseptic-used as a gargle for sore

throat, as a douche for leucorrhea and vaginitis, prostatitis; it is constipative- used in management of chronic diarrhea; it is hepatoprotective- the petroleum ether extract of plant given at a dose of 300mg/kg i.p. prevented necrosis of liver tissue and promoted liver regeneration in CCl4 induced toxicity. Seeds used in diabetes and cutaneous diseases. Other useful properties are Anodyne, Antiscrophulatic, Cardiotonic, Demulcent, and Spasmolytic. [9, 10, 11, 12]

Different phytochemicals present in N.alba and their biological activities

Phytochemical analysis has shown that it contains many active compounds. Different phytochemicals present are Active alkaloids like nupharine and nymphaeine having sedative and aphrodisiac property, Flavonoids-having sedative and anxiolytic property**;** Glycosides-responsible for cardiotonic action**;** Gallic acid has anti-inflammatory, immunomodulatory action**;** Tannic acid has antioxidant, antiviral and anticancer property; others being Sterols**,** Hydrolysable tannins and High molecular weight polyphenolic compounds.[11, 12]

Though there is mention in literature about antiepileptic activity of N.alba, there is no published scientific study on antiepileptic activity of N.alba. So we have taken this study to evaluate antiepileptic activity of N. alba and to compare this with standard antiepileptic drugs phenytoin sodium and sodium valproate.

MATERIALS AND METHODS

Animals

A sixty four (n=64) male, healthy albino rats were procured from the central animal house, department of Pharmacology, JJM Medical College, Davanagere inbred under suitable conditions of housing, temperature, ventilation and nutrition with a 12hour light: 12hour dark cycle. Rats with weight 100-200g, aged between 3-4months with normal behavior were included in this study.

Groups

Sixty four rats were divided into 8 groups i.e., Group A, B, C, D, E, F, G and H, each containing 8 rats.

MES induced seizure model: in this model various groups were; Group A served as Control group and received 10ml /kg of distilled water; Group B served as standard group received 400mg/kg phenytoin sodium diluted in distilled water; Group C and group D served as test groups and received 200 and 100mg/kg of ethanolic extract of *N.alba* suspended in 1% gum acacia in distilled water respectively.

PTZ induced seizure model: in this model various groups were; Group E served as Control group and received 10ml /kg of distilled water; Group F served as standard group received 2000mg/kg sodium valproate diluted in distilled water; Group G and group H served as test groups and received 200 and 100mg/kg of ethanolic extract of *N.alba* suspended in 1% gum acacia in distilled water respectively.

Acute oral toxicity study

A oral toxicity study of ethanolic extract of N.alba was done according to Organization for Economic Co-operation and Development (OECD) guidelines. The study was conducted with N.alba in different doses 5, 50, 300, 1000and 2000mg/kg administered orally to female rat and the animals were observed for signs of toxicity such as hyperactivity, grooming, convulsions, sedation, hypothermia, convulsions for 2hrs and for mortality upto 24hrs after administration of doses. Based on this study we have taken safe doses of Nymphaea alba.

Methods

MES induced seizures

A stimulus of 180 mA for 0.2s duration was given using the electroconvulsiometer and the responses were tested 1h after administration of standard or test drugs. Parameters observed were abolition of tonic hind limb extension (THLE), duration of THLE, and time taken to regain righting reflex. MES induced convulsions represents grand mal type epilepsy in humans and a substance is known to possess anticonvulsant property if it abolishes the extensor phase of MES convulsions. Hence THLE is taken as a major parameter.[13, 14, 15]

PTZ induced seizures

In the PTZ-induced seizure model, response to PTZ 40mg/kg i.p., was tested 1 h after administration of standard or test drug orally. The anti-epileptic effect was evaluated by the presence or absence of clonus seizures, time required for onset of seizures and total duration of seizure episode.[13, 15, 16]

Plant material

The entire plant was collected from Siddrabetta, National botanical garden, Tumkur, Karnataka during the month of February 2012. The plant was identified and authenticated by Professor Ramesh, Department of Botany, DRM science college, Davanagere. Flowers were separated, washed and dried in shade and powdered. Then the powder was filled in Whatman's filter paper number 1 in Soxhlet apparatus using ethanol for 24 hrs. The filtrate was collected in a petri dish and evaporated to dryness in vacuum desiccator. the yield of ethanolic extract of N. alba was found to be 11% w/w. The extract was stored in refrigerator for further pharmacological studies.

Drugs and chemicals

Phenytoin sodium and sodium valproate were dissolved in distilled water and used as standard drugs for MES model and PTZ model respectively. These drugs were procured in pure powder form from Sun pharmaceuticals, Mumbai. Two different doses of N.alba (100 and 200mg/kg) were prepared separately by dissolving with 1% gum acacia in distilled water. All solutions were prepared freshly on the test days and administered orally.

The study was approved by Animal ethics Committee, JJM Medical College, Davanagere.

Statistical analysis

All the continuous variables were expressed as mean ± SD and oneway ANOVA was used to calculate mean and Standard deviation. Mean values were analyzed by independent T test for Probability (P) value. The percentage of animals protected from THLE was analyzed by Fisher's exact test for probability values.[13]

Results

MES induced seizures

In this model abolition for THLE was shown by both doses of N.alba. At the dose of 200mg/kg it was significant and at the dose of 100mg/kg it was highly significant. (P values 0.038 and 0.006 respectively) and also comparable to phenytoin sodium. The duration of THLE was also reduced in both N.alba groups and was highly significant (p values 0.007 and 0.003 respectively). The total duration of convulsion was reduced in both N.alba groups but was statistically significant in 100mg group (0.044). And the time taken to regain righting reflex was also reduced in both groups of N.alba. (Table 1, 2 and fig 1)

Though in minor parameters the difference was not significant, the major parameter i.e., abolition of THLE shows significant increase in abolition which is statistically significant which is a major parameter for assessment of antiepileptic activity of a drug.[14, 15]

PTZ induced seizures

In this model N.alba reduced the number of seizure attacks in 1 hour in both groups of N.alba. It was statistically significant in 200mg/kg group and highly significant in 100mg/kg group. The test drug also showed abolition of clonic convulsions in 100mg/kg group and increased time taken to onset of convulsions in N.alba 100mg/kg group. (Table 3, 4 and fig.2)

Table 1. Responses to ethanolic extract of *N. alba* in MES model

Group	Abolition of THLE (in %)	Duration of THLE phase (in seconds)	Total duration of convulsions (in seconds)	Time to regain righting reflex
Group A-Control	0	27.75±8.48	46.50±12.06	93.75±11.07
Group B-Phenytoin	100	0	28.25±4.89	76.62±8.42
Group C- N. alba 200mg/kg	50	15.75±2.75	39.87±7.29	87.12±12.93
Group D- N. alba 100mg/kg	75	15.5±2.12	38.50±5.98	86.87±9.20

**Values represent mean ± Standard deviation*

Table 2.Comparing the control group with standard and test groups in MES model

Group	Abolition of THLE (in %)	Duration of THLE phase (in seconds)	Total duration of convulsions	Time to regain righting reflex
Control with phenytoin sodium group	**P=0.0001	**P=0.0001	**P=0.0014	**P=0.0036
Control with *N. alba* 200mg/kg group	*P=0.038 (significant)	**P=0.007 (highly significant)	P=0.118	P=0.29
Control with N. alba 100mg/kg group	**P=0.006 (highly significant)	**P=0.003 (highly significant)	*P=0.044	P=0.198

**S=significant (P value less than 0.05 is significant); **S= highly significant (P value less than 0.01); NS=not significant*

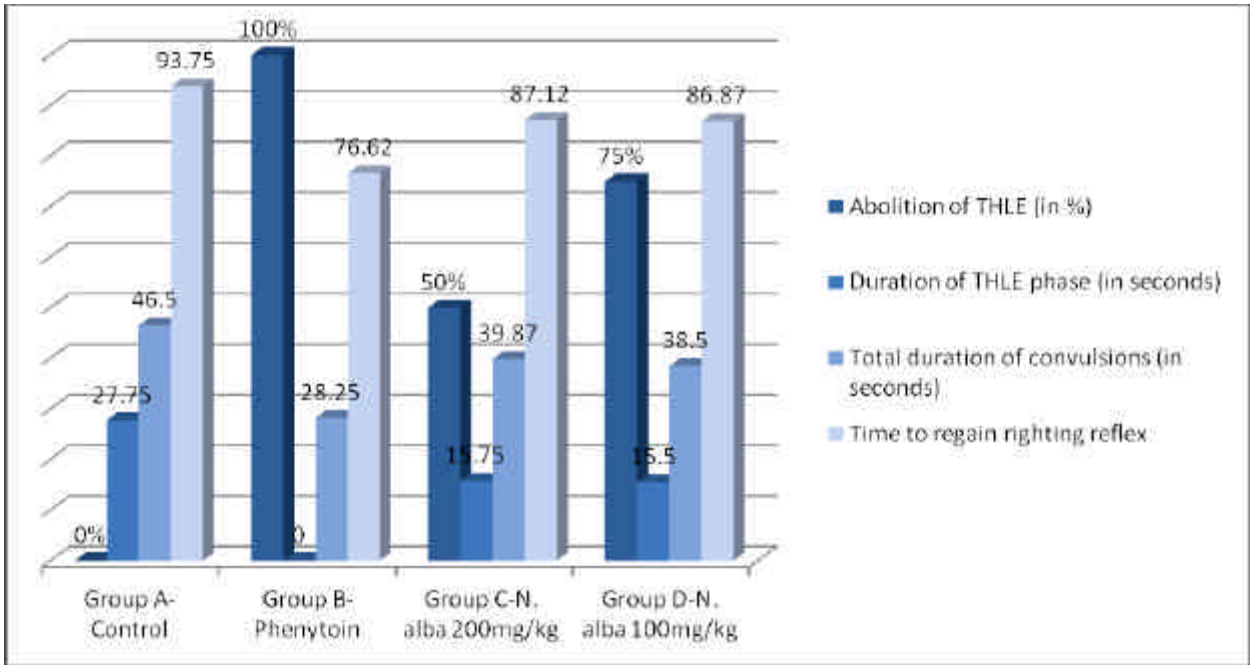

Fig. 1. Bar diagram showing four parameters results in all the four groups in MES model

PTZ induced seizures

Table 3. Responses to ethanolic extract of N. alba in PTZ model

Group	Abolition of clonic convulsions (in %)	Onset of convulsions (in seconds)	Number of seizure attacks in 1 hour
Group E-Control	0	74.37±20.36	3.37±1.19
Group F-Sodium valproate	100	—	0
Group G- *N. alba* 200mg/kg	0	90.50±8.33	2.25±1.04
Group H- *N. alba* 100mg/kg	37.5	90.75±6.70	1.60±0.55

**Values represent mean+/- Standard deviation*

Table 4.Comparing the control group with standard and test groups in PTZ model

Group	Abolition of clonic convulsions (in %)	Onset of convulsions (in seconds)	Number of seizure attacks in 1 hour
Control with sodium valproate group	**P=0.00015	**P=0.0001	**P=0.0001
Control with N. alba 200mg/kg group	P=1.00	*P=0.028	*P= 0.031(significant)
Control with N. alba 100mg/kg group	P=0.19	P=0.07	**P=0.010(highly significant)

S=significant (P value less than 0.05 is significant); **S= highly significant (P value less than 0.01); NS=not significant.

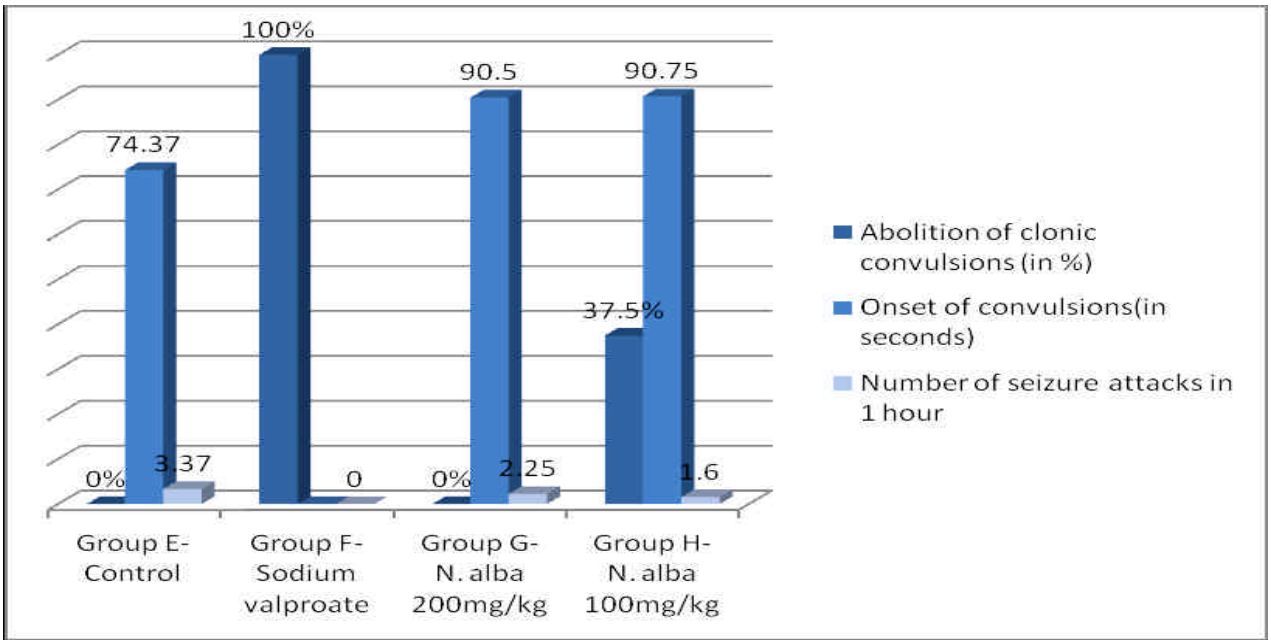

Fig. 2. Bar diagram showing all the three parameters in PTZ model

DISCUSSION

The maximal electro shock (MES) induced convulsions in animals represent grand mal type of epilepsy in humans. Similarly chemo convulsions due to Pentylenetetrazole which produce clonic type of convulsions resemble petit mal type of convulsions in man. The protection by a drug for convulsions in these animal models represents protection for respective type of epilepsies in humans.[15]

In present study the results *N.alba* shows a significant antiepileptic activity in MES model. It has showed significant protection for THLE. It decreased duration of THLE and total duration of convulsions which may signify reducing the severity of grand mal epilepsy. It also reduced time taken to regain righting reflex which may signify recovery from an epileptic attack.

In PTZ model N.alba showed highly significant reduction in number of seizure attacks in 1 hour which may signify reducing the severity of petit mal epilepsy. It also showed protection for clonic convulsions at 100mg/kg dose.

Another finding observed from the results is 100mg/kg *N.alba* has better antiepileptic activity than 200mg/kg group. And this appears like therapeutic window phenomenon and needs to be evaluated more in further studies with various doses. Another study done by us on anxiolytic activity of N.alba (which is not published yet) also showed the same pattern of results i.e., 100mg/kg group showed more anxiolytic activity than 200mg/kg group.

Probable mechanism of anticonvulsant action of *N.alba*: It is an established fact that the anxiolytic, anticonvulsant, muscle relaxant, and sedative-hypnotic actions of the BZDs is due to binding and modulating the GABA-BZD receptor-chloride channel complex. Phy

tochemical tests of *N. alba* revealed the presence of flavonoids, tannins, and saponins. The anticonvulsants action of *N. alba* could be due to the binding of any of these phytochemicals to the $GABA_A$-BZD complex. In support of this, it has been found that flavones bind with high affinity to the BZD site of the $GABA_A$ receptor. A study done by Tippeswamy B. S. et al. on anxiolytic activity of *N. alba* on albino mice showed significant anxiolytic activity and the mode of action of drug explained was flavonoids present in N.alba act on GABA-BZD complex and enhance GABAergic action. [11]

The antiepileptic activity of barbiturates, benzodiazepines (diazepam, clonazepam), vigabatrin, tiagabine, valproic acid is through enhancement of GABAergic activity. Benzodiazepines increase the frequency while phenobarbital increases the duration of the opening of Chloride channel. Vigabatrin inhibit the enzyme GABA transaminase, which is responsible to metabolise GABA, and thus increase the neuronal concentration of GABA. Tiagabine and valproate rather inhibit GABA uptake by inhibiting the GABA uptake transporter (GAT) in neurons as well as in glia. Gabapentin and pregabalin are GABA analogues and increase brain GABA concentrations. Topiramate also act by activation of $GABA_A$ receptors. It enhances the availability as well as the inhibitory actions of GABA at postsynaptic GABA A receptors. Valproic acid, also activates glutamic acid decarboxylase (GAD) and thus increases the synthesis of GABA.[8]

Results of this study indicate ethanolic extract of *Nymphaea alba* has got antiepileptic potential. In conclusion this study suggests that Nymphaea alba has more beneficial effect in grand mal type and also has benefit in petitmal type of epileptic disorders. Thus it holds the scope for a new generation of antiepileptic drug; however there is need for further studies on other experimental animals and human being, to establish its usefulness, exact mode of action and toxicity data.

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