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# Anti-convulsant activity of aqueous and alcohol extracts of roots and rhizomes of *Nymphoides indica* (L.) Kuntze in swiss albino mice

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**Abstract**

*Nymphoides indica* (L.) Kuntze (Menyanthaceae) is an aquatic herb used as an admixture along with *Nymphoides macrospermum* and as a substitute of Ayurvedic drug *Tagara* (*Valeriana jatamansi* Jones) in the treatment of various diseases like epilepsy, anemia, jaundice, tuberculosis to name a few. The present study has been undertaken to evaluate the anti-convulsant effect of aqueous and alcohol extract of roots and rhizomes of *Nymphoides indica* (L.) Kuntze using Maximum Electroshock Convulsion (MES) and Pentylenetetrazole (PTZ) induced convulsion methods in swiss albino mice. The oral administration of alcohol and aqueous extracts at the dose level of 300 and 600 mg/kg reduced duration of extensor phase (HLTE) and time taken for recovery in MES induced convulsion model. Similarly both extracts delayed onset of convulsions and reduced the time taken for recovery in PTZ induced convulsions. Alcohol extract was found to be more effective and effect of alcohol and aqueous extract on extensor phase (HLTE) (MES) and onset of convulsions (PTZ) was dose dependent. It is concluded that roots and rhizomes of *N. indica* exhibit anticonvulsant activity, justifying its use as a substitute of *Tagara* (*V. jatamansi*) in treatment of epilepsy.

**Key Words:** *Nymphoides indica*, Anti-convulsant activity, Maximum Electro Shock (MES), Pentylenetetrazole (PTZ), Phenytoin and Diazepam.

## 1. Introduction

*Tagara* is an important Ayurvedic drug used as one of the ingredients in many Ayurvedic preparations. The underground parts (rhizomes, roots, and stolon) are used in preparations like *Amrithamalka Taila, Bilwadi vati, Drakshadi choorna, Kalyana ghritha* which are efficacious in diseases like epilepsy, anaemia, jaundice, tuberculosis, mental disorders, fevers and also as a general and brain tonic. The accepted source of *Tagara* is *Valeriana jatamansi* Jones [1, 2]. In South India, a drug under the name *Granthika tagara*

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(kannada), botanically identified as *Nymphoides macrospermum* Vasudevan (Menyanthaceae) is used in place of Tagara for the same Ayurvedic preparations under the same formulations as it is claimed to possess similar therapeutic properties of *Tagara*. Moreover other species of *Nymphoides* Hill viz. *N. indica* (L.) Kuntze, *N. aurnaticum* (Dalz.) Kuntze, *N. hydrophylla* (Lour.) Kuntze, *N. parvifolium* (Griseb.) [3, 4, 5] Kuntze are also used as an admixture in *Granthika tagara* [6].

*N. macrospermum* possess anti-convulsant activity [7] while no work is carried out on *N. indica*. Hence the present study was undertaken to investigate anti-convulsant activity of aqueous and alcohol extract of rhizomes and roots of *Nymphoides indica* (L.) Kuntze against seizures induced by MES and PTZ models using swiss albino mice of either sex.

## 2. Material and Methods

### *2.1 Plant material*

Fresh roots and rhizomes were collected from Nagarcoil surroundings of Tirunelveli, Tamil Nadu during February 2007, identified and authenticated by Dr.S.N.Yoganarasimhan, Taxonomist and Research Co-ordinator, Department of Pharmacognosy, M. S. Ramaiah college of Pharmacy. The roots and rhizomes were washed thoroughly with tap water to remove extraneous matter, dried under shade, powdered and stored in air tight container. Voucher herbarium specimen (Shilpi Arora 024) has been deposited in the Herbarium of Department of Pharmacognosy of MSRCP, along with a sample of test drug for future reference.

### *2.2 Preliminary phytochemical study*

Preliminary phytochemical screening of aqueous and alcohol extracts of *N. indica* were carried out employing standard procedure [8].

### *2.3 Preparation of plant extract*

#### *2.3.1 Alcohol extract*

The coarsely powdered roots and rhizomes (100g) were charged into a soxhlet's apparatus and successive hot extraction was carried out using ethanol (70% v/v) for 24 h. The liquid extract was concentrated in rotary flash evaporator at a temperature not exceeding 50º C to give a semisolid residue (yield 37 % w/w).

#### *2.3.2 Aqueous extract*

The aqueous extract was prepared by cold maceration with chloroform water for 24 h. The aqueous extract was concentrated to small volume, evaporated to dryness to give a semisolid residue (yield 45.6 % w/w).

### *2.4 Animals*

Swiss albino mice weighing 18-25 g of either sex were used for study. Animals were procured and housed in animal house of the M.S. Ramaiah College of Pharmacy Bangalore, at least 2 weeks prior to study, for acclimatization. Animals house was well maintained under standard hygienic conditions, at a temperature (25±1°c), room humidity (60%±10%) with 12 h day and night cycle, with diet of pellet chow (Sai Durga Feeds and foods, Bangalore) and water *ad libitum*. Cleaning and sanitation was carried out on alternate days. Paddy husk was provided as bedding material and changed everyday. All pharmacological experiments were carried out as per CPCSEA norms after obtaining approval of the Institutional Animal Ethics Committee of M. S. Ramaiah College of Pharmacy.

### *2.5 Drugs*

Pentylenetetrazole (PTZ) (Sigma, USA), Diazepam (Ranbaxy, India) and Phenytoin (Pfizer, India) were used in this study. The drugs were dissolved in water for injection and administered intraperitonially.

*2.6 Acute Toxicity studies*

Studies were carried out to evaluate acute toxicity and to determine minimum lethal dose of drug extracts. Swiss albino mice of either sex weighing between 20-30 g fasted overnight, were used for study. Each extract was orally administered at doses of 30, 100, 300, 1000 and 3000 mg/kg to separate groups of mice. Subsequent to administration of drug extracts, animals were observed closely for first three hours, for any toxic manifestations, like increased motor activity, salivation, clonic convulsions, coma and death. Subsequently observations were made at regular intervals for 24 h. The animals were under further investigation up to a period of 1 week [9].

*2.7 Assessment of anti-convulsant activity*

*2.7.1. Anti-convulsant activity against Maximal Elactro Shock (MES) induced convulsion in swiss albino mice*

Animals were randomly divided into 6 groups of 6 each. Group I served as control, received equivalent amount of respective vehicle p.o., group II received Phenytoin (25 mg/kg i.p.), served as reference standard; group III and IV received aqueous extract of roots and rhizomes at the dose of 300 and 600mg/kg p.o.; groups V and VI received alcohol extract of roots and rhizome at the dose of 300 and 600mg/kg p.o. respectively. The mice were subjected to MES at 150 mA for 0.2 sec through ear electrodes, 1 h after vehicle/drug administration and 30 min after phenytoin administration [10].

Animals were observed immediately for various parameters such as tonic flexon, hind limb extensor phase, clonic convulsions and stupor. Time taken for recovery or death (in sec) after electro-convulsive shock was also recorded. Values were expressed in terms of Mean ± SEM [11].

*2.7.2. Anti-convulsant activity against chemo shock (PTZ) induced convulsions in swiss albino mice*

Seizures were induced in mice with PTZ (70 mg/kg i.p.). Animals were randomly divided in to 6 groups of 6 each. Group I served as control and received equivalent amount of respective vehicle. Group II received Diazepam (4mg/kg i.p.), served as reference standard; group III and IV received aqueous extract of roots and rhizomes at the dose of 300 and 600mg/kg p.o.; groups V and VI received alcohol extract of roots and rhizome at the dose of 300 and 600mg/kg p.o. respectively. PTZ was injected intraperitoneally to extract treated and control groups after 1 h and to diazepam treated group after 30 min. Animals were individually placed in trays and observed for various symptoms of convulsions [12]. Latency and duration of myoclonic jerks as well as incidence of seizures were recorded along with time taken for recovery/death (in sec). Values were expressed in terms of Mean ± SEM [13, 14].

2.8 Statistical analysis

Data are expressed as Mean ± SEM (Standard error of mean). Statistical evaluation was done using One Way Analysis of Variance (ANOVA) followed by Tukey Kramer multiple comparison test.

## 3. Results

*3.1. Preliminary Phytochemical Study*

It revealed the presence of carbohydrates, glycosides, phenolic compounds, flavonoids, saponins, tannins, gums and mucilage and phytosterols in both alcohol and aqueous extract.

*3.2. Acute Toxicity studies*

No toxic symptoms or death were observed in any of the animals up to the dose of 3000 mg/kg body weight, with both extracts up to one week.

*3.3. Anti-convulsant activity*

*3.3.1. Maximum electroshock (MES) induced convulsion model*

In MES induced convulsions, aqueous extract of rhizomes and roots of *Nymphoides indica* reduced duration of extensor phase significantly ($p<0.05$ and $p<0.01$ respectively) for doses 300 and 600 mg/kg. At the same time, alcohol extract also at doses 300 and 600 mg/kg significantly ($p<0.01$ and $p<0.001$ respectively) reduced duration of extensor phase. Effect of alcohol extract at 600 mg/kg was comparable with that of standard drug phenytoin. The effect of both extracts on extensor phase was dose dependent in comparison with control group. Both extracts and the standard drug significantly reduced time taken for recovery ($p<0.001$), when compared with control group. No mortality was observed in any group. The results are presented in Table 1.

*3.3.2. Pentylenetetrazole (PTZ) induced convulsions model*

In PTZ induced convulsions, the latency period was not significant with aqueous extract of roots and rhizomes of *Nymphoides indica* at 300 mg/kg whereas it was significant ($p<0.05$) at 600 mg/kg. Alcohol extract significantly delayed the onset of convulsions. The protective effect of alcohol extract on latency period was dose dependent ($p<0.01$ for 600 mg/kg and $p<0.05$ for 300 mg/kg). Both aqueous and alcohol extracts significantly ($p<0.01$ and $p<0.001$ respectively) decreased time taken for recovery. Diazepam at a dose of 4 mg/kg, totally abolished episodes of convulsions. The results are presented in Table 2.

## 4. Discussion and conclusions

Epilepsy is one of the major disorders for which Tagara is used in Ayurveda. It is a common disorder affecting 0.5-1% of population. Usually there is no recognizable cause, although often develops after brain damage, such as trauma, infection or tumor growth or other type of neurological growth. Medicinal plants used for therapy of epilepsy in traditional medicine have shown to possess promising anticonvulsant activities in animal models and can be an invaluable source for search of new anticonvulsant compounds [15].

**Table 1.** Effect of aqueous and alcohol extracts of roots and rhizomes of *N. indica* on extensor phase and time taken for recovery (MES induced seizures).

| Treatment | Dose | Time (in sec) | |
|---|---|---|---|
| | | Extensor phase | Time taken for recovery |
| Control (distilled water) | - | 18.1 ± 1.5 | 566.7 ± 29.0 |
| Phenytoin | 25 mg/kg | 0.6 ± 2.0*** | 28.3 ± 3.80*** |
| Aqueous extract | 300 mg/kg | 9.8 ± 1.2* | 225.8 ± 62.4*** |
| Aqueous extract | 600 mg/kg | 7.8 ± 2.2** | 208.3 ± 46.0*** |
| Alcohol extract | 300 mg/kg | 7.5 ± 2.5** | 174.1 ± 42.5*** |
| Alcohol extract | 600 mg/kg | 6.1 ± 2.0*** | 143.3 ± 48.0*** |

Note: All the mice under study recovered, there was no death. All values are Mean ± SEM. One Way ANOVA (One Way Analysis of Variance), P< 0.0001 is considered extremely significant. Tukey Kramer comparison test *P<0.05, **P<0.01, ***P<0.001 vs. Control n = 6. (n=number of animals)

**Table 2.** Effect of aqueous and alcohol extracts of roots and rhizomes of *N. indica* on onset of convulsions and time taken for recovery (PTZ induced seizures).

| Group | Treatment (mg/kg b.w) | Time (in sec) | | |
|---|---|---|---|---|
| | | Onset of Convulsions | Time taken for Recovery | Lethality |
| 1. | Control (PTZ) | 81.0 ± 15.0 | 1455 ± 67.0 | 2/6 |
| 2. | Diazepam (4 ) + PTZ (70) | 0.0 ± 0.0*** | 0.0 ± 0.0*** | 0/6 |
| 3. | Aqueous extract (300) + PTZ (70) | 119.0 ± 16.0 | 822.1 ± 115** | 2/6 |
| 4. | Aqueous extract (600) + PTZ (70) | 144.1 ± 10.7* | 816.0 ± 134** | 0/6 |
| 5. | Alcohol extract (300) + PTZ (70) | 143.3 ± 23.1* | 672.5 ± 92.6*** | 2/6 |
| 6. | Alcohol extract (600) + PTZ (70) | 161.3 ± 4.7** | 627.5 ± 113*** | 0/6 |

All values are Mean ± SEM. One Way ANOVA (One Way Analysis of Variance) P< 0.0001 is considered extremely significant. Tukey Kramer comparison test *P<0.05, **P<0.01, ***P<0.001 vs. Control n = 6 (n=number of animals).

MES induced convulsions and PTZ induced convulsions are analogous to grandmal type of and petitmal type of convulsions respectively. *Nymphoides indica* extracts exhibited significant anticonvulsant activity against both the models. Any compound effective against these experimentally induced convulsion models, are effective against petitmal and grandmal type of epilepsy.

The oral administration of alcohol and aqueous extract at the dose level of 300 and 600 mg/kg reduced the duration of extensor phase and time taken for recovery in MES induced convulsion model. Similarly both extracts delayed the onset of convulsions and reduced the time taken for recovery in PTZ induced convulsions. The alcohol extract was found to be more effective and the effects of alcohol and aqueous extract on extensor phase and onset of convulsion was dose dependent. Drugs that are effective against grandmal seizures, blocking sustained high frequency repetitive firing (SRF) like phenytoins, carbamazepine, have anticonvulsant property against MES- induced convulsions. Drugs that are effective against petitmal seizures reduce T-type calcium currents, and these type of seizures can also be prevented by drugs that enhance $GABA_A$ – BZD receptor-mediated neurotransmission, such as benzodiazepines and phenobarbitone. Studies have shown activation of N-methyl D-aspartate (NMDA) receptor is also involved in initiation and generalization of PTZ- induced convulsions. Drugs that block glutamatergic excitation mediated by NMDA receptor, such as felbamate, have an anticonvulsant property against PTZ- induced convulsions [7, 16]. Anticonvulsant activity of *N. indica* may be attributed to one or more of the above mechanisms.

In conclusion, observations suggest that the aqueous and alcohol extracts of *N. indica* possess anti-convulsant activity which may be due to presence of glycoside, phenolic compound, flavonoids, saponins, tannins and phytosterols which are present in root and rhizome extracts (as revealed by preliminary phytochemical investigations) and are also reported to be present in *N. macrospermum* [17]. Thus *N. indica* may serve as a potential additional source of Tagara like *N. macrospermum* in the treatment of epilepsy.

Further studies are required to identify the bioactive compound(s) responsible for the anticonvulsant effect and to understand the mechanism of action involved.

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