National Journal of Physiology, Pharmacy and Pharmacology

RESEARCH ARTICLE

Alcoholic extract of *Azadirachta indica* (Neem) root has no anticonvulsant activity in rodents

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Received: March 28, 2018; **Accepted:** April 26, 2018

ABSTRACT

Background: Although roots of neem plant have established pharmacological activities such as analgesic, anti-inflammatory, antimicrobial, and anticarcinogenic, there are contradictory results as far as anticonvulsant activity is concerned. Hence, the present study was undertaken to explore the anticonvulsant activity of neem roots. **Objectives:** The objectives of this study were to study the anticonvulsant activity of alcoholic extract of neem root. **Materials and Methods:** In maximal electroshock method (maximal electroshock seizure), a total of 30 albino rats of either sex weighing 200–250 g were used and divided into six groups. The control group received 2 ml of distilled water orally daily for 15 days. The standard group received phenobarbitone 20 mg/kg i.p. The test groups received orally neem leaf extract at the dose of 200, 400, and 800 mg/kg for 15 days. Convulsions were produced by 150 mA current for 0.2 s on day 1, 3, 5, and 10 using electroconvulsiometer. Change in duration of hind limb extension was measured. In the pentylenetetrazole (PTZ) method, PTZ at a dose of 60 mg/kg i.p was given to produce convulsion in all mice. A total of 30 mice were divided into five groups of six animals. The control group received orally 2 ml of distilled water daily for 15 days. The standard group received diazepam 4 mg/kg i.p. daily for 15 days. The test group received orally NLE at the dose of 200, 400, and 800 mg/kg for 15 days. The onset of clonic convulsions and the total number of clonic convulsions in 20 min was observed. **Results:** The mean duration of hind limb extension in test group before the highest dose of neem extract (800 mg) administration was 8.7 ± 0.3 and after 15 days it was 8.8 ± 0.21. The mean onset of convulsions and mean number of convulsions in 20 min in the test group with highest dose of neem extract (800 mg) administration was 3.44 ± 0.24 and 42 ± 0.76, respectively, and there was no significant difference when compared to the control group. **Conclusion:** The alcoholic extract of *Azadirachta indica* (neem) root is not having any anticonvulsant activity in rodents.

KEY WORDS: Neem; Rats; Mice; Experimental Pharmacology; Epilepsy; Plant Extract

INTRODUCTION

Neem plant was tested for different activities. Isolation of active gradient from neem was started by Siddiqui in 1942. Then, more than 135 compounds were isolated from different parts of neem.[1] Different type of extracts from different part of plants was used for different activities, for example, aqueous extract - immunostimulant activity,[2] the ethanolic extract of the flowers[3] and methanolic leaf extracts - antipyretic activity,[4] chloroform extract of stem bark anti-inflammatory activity.[5] A massage oil to be used on epilepsy patients. It is prepared by cooking together the bark of the neem tree with the barks of several other trees in water and adding to three parts of that decoction one part sesame oil and one part goat's urine. Insanity due to epilepsy a massage with an oil composed of a decoction of many

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Website: www.njppp.com	Quick Response code
DOI: 10.5455/njppp.2018.8.0310626042018	

different herbs and the expressed juices of neem bark with cow's urine is the best medicine.[6]

Effects on Central Nervous System

Varying degrees of central nervous system depressant activity in mice were observed with the acetone leaf extract.[7] The anxiolytic activity of the fresh leaves was studied in rats and compared with that of diazepam using elevated plus maze and open field behavior test paradigms of anxiety. The extract was administered orally in various doses (10, 20, 50, 100, 200, 400, and 800 mg/kg) 45 min prior behavioral testing. The lower doses (up to 200 mg/kg) of the extract produced significant antianxiety effects in both the tests, however, the higher doses (400 and 800 mg/kg) it did not show any activity. The effects induced by low doses of extract were comparable to those induced by diazepam.[8]

Antiepileptic drugs in modern medicine are useful in epilepsy. However, on chronic use, they show side effects, for example, hirsutism. Antiepileptic drugs also require drug level monitoring because of their toxicity. No drug in modern medicine cures epilepsy. In phytopharmacology, there are contradictory studies of anticonvulsant activity of neem. Hence, in this study, we tried to evaluate whether neem root has anticonvulsant activity or not.

MATERIALS AND METHODS

Animals

Albino rats of either sex weighing 200–250 g and albino mice of either sex weighing 30–50 g.

Plant Material

Neem roots were collected from neem tree in Navodaya Medical College campus. The roots were shade and were then powdered. Alcoholic extract was obtained by continuous extraction in percolator using 70% ethyl alcohol. Fresh solution was prepared by dissolving extracts in distilled water before each experimental procedure.

Chemicals

Pentylenetetrazol (PTZ), S.D. Fine Chem Ltd., Boisar.

Drugs

Inj. diazepam: 5 mg/ml (anoxol - sigma), Inj. phenobarbitone: 200 mg (Phenobarbitone - Samarth).

Instruments

Electroconvulsiometer - Inco (Ambala), Percolator - Maharashtra Emporium, Wardha.

Methods for Evaluating Anticonvulsant Activity

1. Maximal electroshock method: [9] 150 mA current for 0.2 s was used to produce convulsion in rats. The animals failed to produce convulsions were discarded from the study. Duration of hind limb extension was recorded as end point. All the 30 animals were divided into five groups of six animals each. The control group received 2 ml of distilled water orally daily for 15 days. The standard group received phenobarbitone 20mg/kg i.p. The test group received orally neem leaf extract at the dose of 200, 400, and 800 mg/kg for 15 days. Convulsions were produced on day 1, 3, 5, and 10. Change in duration of hind limb extension was measured.
2. PTZ convulsions: [10] PTZ at a dose of 60 mg/kg i.p was given to produce convulsion in all mice. All the 30 animals were divided into five groups of six animals each. The control group received orally 2 ml of distilled water daily for 15 days. The standard group received diazepam 4 mg/kg i.p. daily for 15 days. The test group received orally NLE at the dose of 200, 400, and 800 mg/kg for 15 days. Convulsions were produced on 15 days immediately after the last dose. The onset of clonic convulsions and the total number of clonic convulsions in 20 min was observed.

Statistical Analysis

The statistical analysis of data was done using Students *t*-test, one-way or two-way ANOVA followed by *post hoc* Dunnett's tests using SPSS. $P < 0.05$ was considered to be statistically significant.

RESULTS

The results obtained in the first method, i.e., maximal electroshock seizure (MES) method is shown in Table 1. The mean duration of hind limb extension in the control group was 8.6 ± 0.14 s, standard group was 8.7 ± 0.26 s, and in the test group was 8.9 ± 0.2 s, 9.6 ± 0.13 s, and 10 ± 0.1 s. Now, after 15 days, the mean duration of hind limb extension in standard group which received phenobarbitone 10 mg/kg was significantly ($P < 0.05$) increased to 10 ± 0.1 s. However, there was no such increase in mean duration of hind limb extension in the test groups which received 200, 400, and 800 mg/kg of neem root extract.

The results obtained in the second method, i.e., PTZ convulsion method is shown in Table 2. The mean onset of convulsions in control group was 3.36 ± 0.43 min and in standard group was 4.00 ± 0.45 min. The mean onset of convulsions in the test group was also very close to the control group. Now, the mean total number of convulsions in the control group was 41 ± 1.2, whereas in the standard group which received diazepam (20 mg/kg i.p) drastically and significantly ($P < 0.01$) reduced to 7.7 ± 0.67. However, there was no such decrease in mean total number of convulsions in

Table 1: Anticonvulsant effect of ***Azadirachta indica*** (neem) root in albino rats by maximum electroshock method

Group	Drug and doses	Duration of hind limb extension (in seconds) (mean±S.E.M.)				
		Before drug administration	After drug administration			
			48 h	5 days	10 days	15 days
Control	Distilled water	8.6±0.14	8.7±0.26	8.6±0.2	8.6±0.19	8.6±0.25
Standard	Phenobarbitone (20 mg/kg i.p)	8.2±3	8.8±0.15*	8.9±0.2*	9.6±0.13*	10±0.1*
Test I	NRE (200 mg/kg orally)	8.8±0.25	9.4±0.36	8.8±0.43	8.3±0.24	8.2±0.23
Test II	NRE (400 mg/kg orally)	8.7±0.31	8.8±0.22	8.2±0.27	8.3±0.51	8.3±0.39
Test III	NRE (800 mg/kg orally)	8.7±0.3	8.6±0.37	9±0.35	8.2±0.26	8.8±0.21

*P<0.05. S.E.M: Standard error of the mean

Table 2: Anticonvulsant effect of ***Azadirachta indica*** (neem) root in albino rats by PTZ convulsion method

Groups	Drugs and dose	Onset of convulsions (in minutes)	Number of convulsions (in 20 min)
Control	Distilled water	3.36±0.43	41±1.2
Standard	Diazepam (20 mg/kg i.p.)	4.00±0.45*	7.7±0.67*
Test I	NRE (200 mg/kg orally)	3.48±0.45	41±1.2
Test II	NRE (400 mg/kg orally)	3.29±0.49	45±1.1
Test III	NRE (800 mg/kg orally)	3.44±0.24	42±0.76

*P<0.01. PTZ: Pentylenetetrazole

the test groups which received 200, 400, and 800 mg/kg of neem root extract.

DISCUSSION

The MES- and PTZ-induced convulsions are the basic and preliminary studies for establishing anticonvulsant activity to any substance. In our study, we compared the neem root extract with that of a standard drug phenobarbitone and diazepam in MES- and PTZ-induced convulsions, respectively. In MES method, the mean duration of hind limb extension in the test groups which received 200, 400, and 800 mg/kg of neem root extract was 8.2 ± 0.23 s, 8.3 ± 0.39 s, and 8.8 ± 0.21 s, respectively. There was no significant difference when compared to the control group (8.6 ± 0.25). In PTZ-induced convulsions model, the mean onset of convulsions in the test groups (3.48 ± 0.45, 3.29 ± 0.49, and 3.44 ± 0.24 min) was very close to the control group (3.36 ± 0.43 min). The mean total number of convulsions in the test groups which received 200, 400, and 800 mg/kg of neem root extract was 41 ± 1.2, 45 ± 1.1, and 42 ± 0.76, respectively. There was no significant difference when compared to the control group (41 ± 1.2). Phenobarbitone significantly decreased duration of hind limb extension. Diazepam significantly delayed the onset and decreased number of PTZ convulsion. Neem root extract failed to decrease duration of hind limb extension at any dose and also failed to delay the onset and decreased number of PTZ convulsion at any dose as done by standard drug phenobarbitone and diazepam. This clearly suggests that neem root has no anticonvulsant activity in MES as well as PTZ-induced convulsions method.

Numerous claims and studies have contradictory statement about neem and its anticonvulsant effect. In one ayurvedic claim, taking neem leaves along with ajwain in water for 3 months cures epilepsy.[11] Neem is also sold as capsules for the cure of epilepsy by online herbal pharmacy.[12] One research concludes that neem contains more protective effect as compared to valproic acid on PTZ developed chemical kindling.[13] Neem plant preparations are used by various natural healers for epilepsy.[14] Singh *et al*., in 1987, showed fractions of acetone extract of leaf varying degrees of central nervous system depressant activity in mice with neem leaf extract.[15] By all these reports, we can tell that neem is having anticonvulsant activity, but on the contrary, Singh *et al*., 1990, showed that propylene glycol extract of neem leaf has no anticonvulsant activity.[16] Considering these studies, we tried to evaluate whether ethanol extract of neem root has any anticonvulsant action.

We tried to study the anticonvulsant activity with only alcoholic extract of neem root. Further, detailed investigations are required with various parts of neem plant, especially seed extract and leaf extract before the scientific community finally rejects the anticonvulsant activity of neem plant.

CONCLUSION

Since maximum electroshock convulsions (MES) are a model of grand mal epilepsy and PTZ convulsion is a model for petit mal epilepsy,[17] we can conclude that the ethanolic extract of *Azadirachta indica* (neem) root is not having any anticonvulsant activity in rodents.

REFERENCES

1. Biswas K, Chattopadhayay I, Banerjee RK, Bandyopadhyay U. Biological activities and medicinal properties of neem

(*Azadirachta indica*). Curr Sci 2002;82:1336-45.
2. Sen P, Mediratta PK, Ray A. Effect of *Azadirachta indica* on some biochemical, immunological and visceral parameters in normal and stressed rats. Indian J Exp Biol 1992;30:1170-5.
3. Al-Hashemi ZS, Hossain MA. Biological activities of different neem leaf crude extracts used locally in Ayurvedic medicine. Pac Sci Rev Nat Sci Eng 2016;18:128-31.
4. Okpanyi SN, Ezeukwv GC. Anti-inflammatory antipyretic activities of *Azadirachta indica*. Planta Med 1981;41:34-9.
5. Selvi PT, Kumar MS, Yaswanth T, Adiyaman E, Anusha PT. Central nervous system depressant activity of aqueous extract of leaves of *Azadirachta indica* Linn in Mice. Asian J Pharm Res 2012;2:97-9.
6. Mohanty I, Senapati MR, Jena D, Palai S. Diversified uses of cow urine. Int J Pharm Pharm Sci 2014;6:20-2.
7. Patil P, Patil S, Mane A, Verma S. Antidiabetic activity of alcoholic extract of Neem (*Azadirachta indica*) root bark. Natl J Physiol Pharm Pharmacol 2013;3:142-6.
8. Jaiswal AK, Bhattacharya SK, Acharya SB. Anxiolytic activity of *Azadirachta indica* leaf extracts in rats. Indian J Exp Biol 1994;32:489-91.
9. Castel-Branco MM, Alves GL, Figueiredo IV, Falcão AC, Caramona MM. The maximal electroshock seizure (MES) model in the preclinical assessment of potential new antiepileptic drugs. Methods Find Exp Clin Pharmacol 2009;31:101-6.
10. Kandratavicius L, Balista PA, Lopes-Aguiar C, Ruggiero RN, Umeoka EH, Garcia-Cairasco N, *et al*. Animal models of epilepsy: Use and limitations. Neuropsychiatr Dis Treat 2014;10:1693-705.
11. Shop for Ayurvedic and Herbal Medicine. Available from: http://www.myyog.com/epilepsy/145-epilepsy/230-Nature-care-for-Epilepsy.html.; http://www.myyog.com. [Last accessed on 2018 Feb 13].
12. Neem "The village Pharmacy. Available from: http://www.netowne.com/alt-healing/ayurveda/index.htm; http://www.netowne.com. [Last accessed on 2018 Feb 13].
13. Gaurav K, Rai DV. Effect of valproic acid and *Azadirachta indica* on behavioural alterations and antioxidative stress in pentylenetetrazol- induced kindling in rats. Ann General Psychiatry 2008;7:S257.
14. Tiwari R, Verma AK, Chakraborty S, Dhama K, Singh SV. Neem (*Azadirachta indica*) and its potential for safeguarding health of animals and humans: A review. J Biol Sci 2014;14:110-23.
15. Singh PP, Junnarkar AY, Reddy GS, Singh KV. *Azadirachta indica*: Nueropsychopharmacological and antimicrobial studies. Fitoterapia 1987;58:235-8.
16. Singh PP, Junnarkar AY, Thomas GP, Tripathi RM, Verma RK. Pharmacological study of *Azadirachta indica*. Fitoterapia 1990;61:164-8.
17. Gupta YK, Jatinder M, George B, Kulkarni SK. Methods and considerations for experimental evaluation of antiepileptic drugs. Indian J Physiol Pharmacol 1999;43:25-43.

How to cite this article: Dixit R, Patil PR. Alcoholic extract of *Azadirachta indica* (Neem) root has no anticonvulsant activity in rodents. Natl J Physiol Pharm Pharmacol 2018;8(8):1171-1174.

Source of Support: Nil, **Conflict of Interest:** None declared.