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Research Article

Anticonvulsant Activity Study of *Artemisia nilagirica*

*Santilna K.S.[1], Mahesh N.M.[2], Suresh J.[3]

[1]*Department of Pharmacology, J.S.S. College of Pharmacy, Ooty (A Constituent College of JSS University, Mysore, Karnataka)*
[2]*Department of Pharmacology, J.S.S. College of Pharmacy, Mysore (A Constituent College of JSS University, Mysore, Karnataka)*
[3]*Department of Pharmacognosy, J.S.S. College of Pharmacy, Mysore (A Constituent College of JSS University, Mysore, Karnataka)*

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ABSTRACT
Artemisia nilagirica is commonly called as Indian wormwood, which was used traditionally in the treatment of epilepsy. So in the present study the antiepileptic potential of the leaves of *Artemisia nilagirica* was evaluated. The leaves part of the plant was dried and powdered and subjected to maceration using solvents like diethylether, chloroform and ethanol (95%). All the three extracts were subjected to preliminary phytochemical tests and anticonvulsant activity (pentylenetetrazole induced convulsion). Results show that the alkaloids, flavonoids and terpenoids were identified to be present in all three solvents extracts. Among the extracts ethanolic extract (600 and 800mg/kg) produced better anticonvulsant activity than chloroform extract. Both the extracts were less potent than diazepam treated group. Diethylether extract did not produce anticonvulsant activity. The result obtained suggests that the ethanolic and chloroform extracts of *Artemisia nilagirica* may be beneficial in the treatment of epilepsy.

Keywords: *Artemisia nilagirica*; Pentylenetetrazole; Anticonvulsant; Chloroform extract; diethyether extract; ethanolic extract.

INTRODUCTION

Epilepsy is a common neurological disorder; characterized by periodic and unpredictable occurrence of seizures.[1] An imbalance between the excitatory and inhibitory neurotransmitters is responsible for seizures. Nearly about 50-80% of patients with epilepsy are controlled with currently available antiepileptic drugs. But these drugs cannot able to control seizures effectively in about 10-20% of the patients.[2]The treatments of epilepsy still remains inadequate eventhough new anticonvulsants are being developed. Furthermore, most of the marketed drugs used as antiepileptic agents have serious side effects; and even life-threatening conditions are experiencing by the epilepsy patients.[3],[4] Hence there is a need to discover an alternative agent from natural sources with good efficacy and minimum side effects.[5,6]

Artemisia nilagirica is an aromatic shrub, belongs to the family Asteraceae. It is commonly called as Indian wormwood. This is distributed throughout India, in hilly districts, upto 2400m elevation. It is erect, hairy and often half-woody. The stems are leafy and branched. Lower leaves are ovate in outline, deeply pinnatisect with small stipule like lobes at the base. Uppermost leaves are smaller, 3-fid and lanceolate. The leaves have tomentose beneath.[7] Artemisia nilagirica was reported for their medicinal activity in ancient time itself. The different extracts of leaves of *Artemisia nilagirica* were reported to show antimalarial, insecticidal, larvicidal, anticancer, antioxidant and antiulcer activities in *in vitro* and *in vivo* experimental models. *Artemisia nilagirica* was reported to contains l-linalool, p-cymene, camphor, α-thujone, borneol, terpinene-4-ol, α-pinene, linalyl acetate, α-farnesene etc.[8,9,10] Some Constituents like l-linalool, terpinene-4-ol, α-pinene were reported to possess anticonvulsant activity.[11,12] The aerial parts of *Artemisia nilagirica* are being used for treating the patients suffering from nervous diseases, kidney diseases (as diuretic), asthma, inflammation etc in Ayurvedic system of medicine.[13,10] However, there were no reports, in our knowledge, available about its anticonvulsant activity in animal models.

Due to the availability of the limited data about the *Artemisia nilagirica*, the present study was designed to extract the constituents present in the leaves of *Artemisia nilagirica* using ethanol, chloroform and diethyl ether and to assess the anticonvulsant activities possessed by these three extracts.

MATERIALS AND METHODS

Materials: Pentylenetetrazole was obtained from Sigma chemicals and Diazepam injection IP from Ranbaxy laboratories limited. All other chemicals used in the study were of analytical grade.

*Author for correspondence

Table 1: Phytochemical Analysis of *Artemisia nilagirica*

Phytochemical constituents	Diethyl ether extract	Chloroform extract	Ethanolic Extract
Alkaloids	+	+	+
Carbohydrates	-	-	-
Flavonoids	+	+	+
Glycosides	-	-	-
Tannins	+	-	+
Proteins	-	-	+
Amino acids	-	-	+
Steroids	-	+	+
Saponins		_	+
Terpenoids	+	+	+

Note: Positive and negative signs indicate presence and absence of the chemical constituents, respectively.

Table 2: Pilot Study to Determine the Effective Dose Range for Ethanolic Extract

Sl no	Drug	Dose (mg/kg)	Onset of convulsion (seconds)	Duration of convulsion (seconds)	% mortality
1		100	42	829	100
2		200	42	825	100
3	Ethanolic extract	400	99	840	100
4		600	135	25	0
5		800	176	325	0
6		1000	180	522	0

Table 3: Pilot Study to Determine the Effective Dose Range for Chloroform Extract

Sl no	Drug	Dose (mg/kg)	Onset of convulsion (seconds)	Duration of convulsion (seconds)	% mortality
1		100	74	600	0
2		200	77	620	0
3	Chloroform	400	72	840	100
4	extract	600	74	815	100
5		800	75	736	100
6		1000	78	700	100

Table 4: Pilot Study to Determine the Effective Dose Range for Diethyether Extract

Sl no	Drug	Dose (mg/kg)	Onset of convulsion (seconds)	Duration of convulsion (seconds)	% mortality
1		100	32	520	100
2		200	30	410	100
3	Diethylether	400	83	936	100
4	extract	600	52	756	100
5		800	80	770	100
6		1000	89	876	100

Plant materials: The leaves of *Artemisia nilagirica* required for the study were collected from Kalpetta municipality of Wayanad, Kerala. The plant species was authenticated by Dr. K. N. Amruthesh, Principal investigator, UGC-Major Research Project, Department of Studies in Botany, University of Mysore. The leaves were dried under the shade and after optimum drying it was coarsely powdered and stored in air tight container till further use.

Method of extraction: The leaf powder was macerated for seven days individually using diethylether, chloroform and ethanol. The filtrate obtained was allowed to concentrate by evaporation by placing the marc on a boiling water bath. The dried marc was further kept in a desicator containing sodium sulphite. The percentage yield of corresponding extracts was determined.[14]

Preliminary Phytochemical analysis: The extracts were screened for the presence of alkaloids, carbohydrates, flavonoids, glycosides, tannins, proteins, aminoacids, steroids, saponins and terpenoids.[15]

Animals: Albino Swiss strain mice weighing 20-25gram of either sex were used. They were procured from registered breeder. The animals were acclimatized in animal quarantine one week before the experiment. They were housed in polypropylene cages. They were maintained at 25°C ± 2°C under 12 hours dark and light cycle. They were fed with standard animal feed and water ad libitum. Ethical clearance for handling the

Table 5: Anticonvulsant Effect of *Artemisia nilagirica* Extracts

Sl no	Drug	Dose (mg/kg)	Onset of convulsion (seconds)	Duration of convulsion (seconds)	% mortality
1	Tween80		60.666±4.889	612.666±24.493	50.00
2	Diazepam	4	No convulsion	Protected	Protected
3	Ethanolic extract	600	131.667±1.61[a]	67.5±16.89 [a]	Protected
		800	172.5±9.86 [a]	321±13.79 [a]	Protected
4	Chloroform extract	100	74±4.714	322.25±3.037 [a]	33.33
		200	79±5.859	631.66±19.220	50.00

[a]*P< 0.05 when compared to Tween80*

animals was obtained from the Institutional Animals Ethical Committee (042/2009).

Screening Anticonvulscant Activity by Pentylenetetrazole Induced Convulsive Method: A pilot study was done using three solvent extracts of *Artemisia nilagirica* in different concentration, to determine the effective dose range. Different doses (100, 200, 400, 600, 800 and 1000mg/kg) of these extracts were prepared in tween80 (5percent)[16] and were orally administered to each mice to confirm the anticonvulsant activity. Two effective doses were selected among these six concentrations of the different extracts to carry out further efficacy studies.

The extract was administered orally 1hr before the intraperitonial injection of pentylenetetrazole (80 mg/kg) and the mice were observed for the onset of convulsion and duration of clonic convulsions.[17,18] The animals were observed for 30 minutes after pentylenetetrazole administration.[19] After the pilot study animals were divided into six groups with six animals in each group. Tween80 (5percent) was used as negative control while diazepam was used as positive control (4mg/kg). Chloroform extract (100 mg/kg, 200 mg/kg) and ethanolic extract (600mg/kg, 800mg/kg) were used as test drugs. Diethylether extract was found to be not effective, hence not taken for further studies. The results obtained for the test were compared with control groups.

Statistical Analysis: All the results were expressed as mean ±SEM(n=6). Statistical analysis were performed with one way analysis of variance followed by Tukey's multiple comparison test by using graph paired prism5 demo software. P value $P < 0.05$ was considered to be statistically significant.

RESULTS

The yield of the extracts obtained was found to be 10.37 % for ethanolic extract, 9.41 % for chloroform extract and 3.53 % for diethyether extract.

Phytochemicals identified: The different chemical tests conducted have shown the presence of different phytochemical constituents in all the three extracts. Alkaloids, flavonoids and terpenoids were found in all the extracts. Sterols were found in chloroform and ethanolic extracts. Tannins were present in diethylether and ethanolic extracts [Table 1].

Anticonvulsant Activity: Six animals were used to assess the anticonvulsant activity using 100 to 1000mg/kg of each extract. Among the different concentrations of ethanolic extract (100 to 1000mg/kg), the duration and onset of convulsion was found to be increased at 600, 800, 1000mg/kg. There was no mortality in these three dose groups. In the animals treated with 100, 200, and 400mg per kg body weight of the extract, the onset was decreased and duration of convulsion was increased and there were no animal survived. Hence 600 and 800mg dose were found to be in the effective range [Table 2].

In the case of chloroform extract duration of convulsion was increased as the dose of chloroform extract was increased. There was no mortality in two doses (100 and 200mg/kg) groups. In the animals treated with 400, 600, 800 and 1000mg per kg body weight of the extract, the onset and duration of convulsion increased and there were no animal survived. Hence 100 and 200mg dose were found to be in the effective range [Table 3].

Among the different concentrations (100-1000mg/kg) of diethylether extract, the duration and onset of convulsion was found to be increased in all the concentrations. And there were no animal survived [Table 4]. Even the lower concentrations were also found to be ineffective.

In negative control treated groups, the onset and duration of convulsion were found to be 60 and 612 seconds respectively. But 50% of the animals tested died. There were no convulsions observed in positive control treated animal groups (diazepam 4 mg/kg). And all the animals were protected.

In ethanolic extract treated group, the onset and duration of convulsion of animals treated with 600mg/kg were significant ($P<0.05$) when compared to animals treated with 800mg/kg. However, at both doses all the animals were survived. The onset of convulsion of 600mg/kg and 800mg/kg of ethanolic extract was found to be significantly more ($P<0.05$) when compared to negative control group. Also the duration of convulsion was significantly less ($P<0.05$) when compared to negative control group.

In chloroform extract treated group the onset of convulsion was not significant to the animals treated with 100mg/kg when compared to 200mg/kg. And the mortality was found to be 33% in animals treated with 100mg/kg and 50% in animals treated with 200mg/kg.

The onset of convulsion in 100mg/kg and 200mg/kg were not significant (P<0.05) when
compared to negative control treated group. But the duration of convulsion was significantly less (P<0.05) in 100mg/kg of extract when compared to negative control treated group [Table 5].

DISCUSSION

A Number of medicinal plants were reported in our country. Traditionally, many of these plants were used in the treatment of epilepsy. But many of their pharmacological activity have not been investigated scientifically. Pentylenetetrazole was one of the most commonly used chemoconvulsant to identify anticonvulsant activity.[20] Generally, Pentylenetetrazole test is used for screening of drugs effective in petitmal epilepsy or absence seizure.[21]

Our present study reports that the ethanolic and chloroform extracts produced a significant effect in reducing the convulsion produced by pentylenetetrazole. Among the three extracts, ethanolic extract (600mg/kg and 800mg/kg) was found to be more effective against the pentylenetetrazole induced convulsion. It increased the onset and decreased the duration of convulsion when compared to negative control.

GABA is the major inhibitory neurotransmitter in the brain. The inhibition of GABA by the pentylenetetrazole elicits seizures. Standard antiepileptic drugs act by enhancing the inhibitory effect of GABA.[2] Therefore it is possible that the anticonvulsant activity of the extracts in the present study may be due to the activation of GABA.

Preliminary Phytochemical analysis revealed the presence of alkaloids, flavonoids, terpenoids and sterols in both chloroform and ethanolic extracts and saponins only in ethanolic extracts. From these identified chemical constituents, it is difficult to identify the constituent which is responsible for the anticonvulsant activity. However, some studies have reported that triterpenic steroids and triterpenoidal saponins possess anticonvulsant activity in some seizure models.[22,23] Similarly some monoterpenes and flavonoids have reported for their protective effect against pentylenetetrazole induced convulsion.[24,25,26]

The diethylether extract of *Artemisia nilagirica* was reported to contain thujones.[27] Studies were already reported that α-thujone was a GABA receptor antagonist and a toxic component, which enhance the convulsant effect. Our study is in compliance with the opinion of earlier study. [28]

CONCLUSION

Among the extracts of *Artemisia nilagirica*, ethanolic and chloroform extracts show anticonvulsant activity against pentylenetetrazole induced convulsions but less potent than diazepam. Further studies are needed to explore the mechanism as well as the active principle responsible for the anticonvulsant potential of *Artemisia nilagirica*.

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