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Evaluation of Antiepileptic activity of the Alcoholic extract of Adhatoda vasica leaves in rats

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ABSTRACT

The aim of the present study was to investigate anticonvulsant effect of ethanolic extract of the leaves of Adhatoda vasica on electrically and chemically induced seizures along with that, acute toxicity study was also carried out as per the OECD guidelines. The ethanol extract of the leaves of Adhatoda vasica (100 mg/kg, 150 mg/kg and 200 mg/kg) were studied for its anticonvulsant effect on maximal electroshock induced seizures and pentylenetetrazole induced seizures in rats. Ethanolic extract of Adhatoda vasica (100 mg/kg, 150 mg/kg and 200 mg/kg) significantly reduced the duration of seizures induced by maximal electroshock (MES) as well as protected animals from pentylenetetrazole induced tonic seizures. The results suggest that the ethanolic extract of the leaves of Adhatoda vasica may produce its anticonvulsant effects via non-specific mechanisms since it reduced the duration of seizures produced by maximal electroshock as well as delayed the latency of seizures produced by pentylenetetrazole.

Keywords: Adhatoda vasica, Antiepileptic activity, Seizures, rats.

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INTRODUCTION

Epilepsy is an important health problem. Around 30,000 people develop epilepsy every year and the condition will affect about one person in 20 at some time during their lives. There are around 20 to 70 new cases of epilepsy per 100,000 people per year [1]. There are many classes of antiepileptics that are of clinical usefulness with good prognosis for controlling seizures in most patients [2]. Despite this, many patients have seizures that are not adequately managed by the established antiepileptic drugs (AEDs) [3]. Moreover, the high incidence of detestable adverse effects from the use of AEDs is also a source of widespread concern in patients who use them chronically. So this treatment cost, have made traditional herbs and herbalists very useful and indispensable in the struggle for seizure management and future AED development. There is therefore need for research into medicinal plants with possible antiepileptic effects; Adhatoda vasica also known as malabar nut tree is part of the Acanthaceae plant family. It is a small evergreen, sub-herbaceous bush which grows commonly in open plains, especially in the lower Himalayas (up to 1300 meters above sea level), India, Sri Lanka, Burma and Malaysia. Adhatoda vasica possesses a wide-spectrum of medicinal properties, such as bronchodilator [4], antihistaminic [5], thrombopoeitic [6], antiasthamatic [7], pyorrhea [8] etc. The leaves of the plant contain an essential oil and alkaloids vasicine, N-oxides of vasine, vasicinone, deoxyvasicine and maiontone [9]. The roots are known to contain vasicinolone [10], vasicol [11], peganine [12] and 2-hydroxy-4-glucosyl-oxychalcone [13]. The flowers contain β-sitosterol-D-glucoside, kaempferol and its glycosides and quercetin [14].

Epilepsy is very common disorder affecting 0.5-1% of population. Epileptic seizures often cause transient impairment of consciousness, leaving the individual at risk of bodily harm and often interfering with education and employment. So the objective of the present study was to evaluate antiepileptic activity of Adhatoda vasica against seizures induced by MES (maximal electroshock) and PTZ (Pentylenetetrazol).

MATERIAL AND METHOD:

Preparation of extract:

The leaves of Adhatoda vasica were collected from commercial source and were authenticated by B.Usha Nalini, professor and HOD of Botany, Saint Agnes College, Mangalore. The leaves were dried under shade at room temperature and were crushed to a course powder and extracted with 70% v/v ethanol by cold maceration. This extract thus obtained was concentrated to dryness in a flash evaporator under reduced pressure and controlled temperature. The green coloured sticky semisolid extract obtained was used for antiepileptic activity.

Animals used:

Male albino Wistar rats (200-250g) were selected and housed under standard

conditions of temperature (25±1°C), relative humidity (30-60%) and 12 hours light/dark cycle. Animals were fed with standard pellet diet and water ad libitum. Animals had free access to food and water however water was withdrawn 8 hours before and during the experiment. The protocol of the experiment was approved by the Institutional ethical committee before the experiment.

Acute toxicity:

The alcoholic extract of Adhatoda vasica was administered to the animals in the doses of 600mg/kg, 800mg/kg, 1,000 mg/kg, intraperitonially (i.p) to different groups of mice, each containing six animals and mortality was observed after 24 hours. Acute study was carried out as per the OECD guidelines.

Assessment of anticonvulsant activity:

Maximum electroshock convulsions test (MES) [15]:

Five groups of adult rats, each group comprising of six animals are selected for the study. Group-I, Group-II, Group-III received extract at dose of 100mg/kg, 150mg/kg, & 200 mg/kg, respectively. Group IV received Phenytoin sodium (25 mg/kg) as a standard and group V served as control. A supra maximal electrical stimulus of 150mA was given to the animals for 0.2 seconds through ear clip electrodes. Animals were observed and various phases of maximal electroshock seizures viz; tonic hind limb flexion, tonic limb extension and tonic-clonic phases were noted. A reduction or complete abolition of tonic-extensor phase was taken as an index. E/F (extensor/flexor) was statistically analyzed.

Pentylenetetrazole (Metrazol) Induced convulsions (PTZ) [16]:

Five groups of adult rats, each group comprising of six animals are selected for the study. The Groups-I, Groups-II, Groups-III received the extracts at a dose of 100 mg/kg, 150 mg/kg and 200 mg/kg, respectively (i.p). Group IV received sodium valproate (380 mg/kg) as standard and Group V served as control. After 45 minutes, pentylenetetrazole was administered by i.p route in a dose of 80 mg/kg of the body weight. The animals were kept in individual plastic cages to observe the onset of clonic convulsion up to 30 minutes after PTZ administration.

Statistical Analysis:

The data are presented as mean ± SEM for PTZ and MES were analyzed by one way analysis of variance (ANOVA) followed by Dunnett's test. Differences were considered to be statistically significant when $P<0.001$.

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RESULTS AND DISCUSSION

Acute Toxicity:

The alcoholic extract of Adhatoda vasica was found to be safe upto 800 mg/kg. The mortality was observed at a dose of 1g/kg i.p. The results are shown in Table 1.

Table 1. Results of acute toxicity study

Groups	Dose mg/kg	No. of animals	Dose difference (a)	Dead	Mean mortality (b)	Product (a x b)
1	800	6	-	0	-	-
2	850	6	50	1	0.5	25
3	900	6	50	2	1.5	75
4	950	6	50	4	3	150
5	1000	6	50	6	5.5	275

Anticonvulsant Activity,

The alcoholic extract of Adhatoda vasica in doses of 100 mg/kg, 150 mg/kg, 200 mg/kg protected animals from seizures and duration of tonic hind leg extension was reduced. The duration of tonic hind leg extension in rats treated with vehicle (control) was 11.83 ± 0.307 seconds whereas rats treated with the extract (100, 150, 200 mg/kg) exhibited hind leg extension for 8.16±0.307, 6.16 ± 307 and 3.66± 0.759 seconds respectively. Comparison of control and test groups by Dunnet's, where $P<0.001$ indicating the activity is very highly significant. The data are given in Table. 2.

Table 2. Effect of alcoholic extract of *Adhatoda vasica* on maximum electroshock (MES) induced seizures in rats

Groups	Duration in seconds (Mean ± S.E.M)			E/F
	Flexor (F)	Extensor (E)	Tonic-clonic)	
Group-I	2.66±0.210	8.16±0.307	2.66±0.210	3.61±0.281*
Group-II	2.66±0.333	6.16±0.307	2.33±0.210	2.48±0.317*
Group-III.	2.5±0.233	3.66±0.759	2.5±0.223	1.88±0.218*
Group IV.	2.0± 0.258	2.16±0.307	2.83±0.30	1.66±0.210*
Group V	2.33±0.21	11.83±0.307	2.33±0.210	5.33±0.379

The results are represented as Mean ± S.E.M (n=6). The data was analyzed by one way ANOVA followed by Dunnet Test where * p value is < 0.001 when compared with the control group

The alcoholic extract of Adhatoda vasica significantly delayed the onset of convulsions at doses of 100 mg/kg and 200 mg/kg. In animals treated with vehicle, clonic convulsions appeared 73 ± 3.61 sec, and all the animals died after seizures. Vasaka extract in doses of 100 mg/kg, 150 mg/kg, and 200 mg/kg delayed the onset of clonic convulsions up to 1060 ± 52.90, 1360±106.93 and 1870±56.73 seconds

respectively. The test group is compared with control group P<0.001 indicating the results are highly significant. The data is given in Table 3. So in conclusion, the alcoholic extract has given maximum protection against MES induced seizures and PTZ induced convulsions, which is almost comparable to Phenytoin sodium and sodium valproate. In both the cases (MES and PTZ induced convulsions), as the dose is increased, the protection is also increased. The phytochemical study of extracts revealed the presence of alkaloids, tannins, triterpenes and steroids. The phytochemicals such as tannins, triterpenes and steroids were reported as active substances for anticonvulsant activity [17]. Hence, these phytochemicals might be contributing to the anticonvulsant activity of alcoholic extract of Adhatoda vasica. Though the extract showed significant activity against the seizures it is also necessary to determine the exact compound responsible for this activity. Further work is needed to determine the mechanism of antiepileptic activity.

Table 3. Effect of alcoholic extract of *Adhatoda vasica* on seizures induced by Pentylenetetrazole (PTZ) in rats

Groups	Onset of clonic convulsions after PTZ administrations in seconds
Group-I	1060 ± 52.90*
Group-II	1360±106.93*
Group-III.	1870±56.73*
Group IV.	2240 ± 73.7*
Group V	73 ± 3.61

The results are represented as Mean ± S.E.M (n=6). The data was analyzed by one way ANOVA followed by Dunnet Test where * p value is < 0.001 when compared with the control group

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