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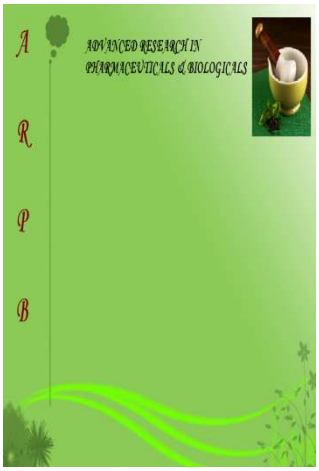

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ANTICONVULSANT ACTIVITY OF ETHANOLIC EXTRACT OF *ABRUS PRECATORIUS* LEAVES

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ABSTRACT:

Abrus precatorius which is commonly called as Indian licorice has been used for many centuries by traditional healers of Tanzania for the treatment of convulsions. The present work has been carried out to evaluate the anticonvulsant activity of the ethanolic extract (EE) of *A. precatorius* leaves against Pentylenetetrazole (PTZ), Picrotoxin and Maximal electroshock (MES) induced convulsions at the doses 100, 200, 400 mg/kg. Diazepam (5mg/kg, i.p.) and Phenytoin (25mg/kg i.p) act as reference standards. At the medium dose and high dose, *Abrus precatorius* extract significantly delayed the onset of clonic seizures induced by PTZ and Picrotoxin and reduced the duration of hind limb extension in MES induced convulsion test.
The phytochemical investigation of the leaves of *A.precatorius* revealed the presence of flavonoids, triterpenoids, saponins, reducing sugars, phenolic compounds and glycosides. The results obtained indicate that the ethanolic extract of *A. precatorius* may help to control grand mal and petitmal epilepsy.

Keywords: *Abrus precatorius*, Convulsions, PTZ, Picrotoxin, Maximal electroshock.

INTRODUCTION

The last ten years of the 20th century is called in neuroscience "decade of the brain"[1]. Epilepsy is a common neurological disorder characterized by paroxysmal dysrhythmia, seizure, with or without body convulsion and sensory or psychiatric phenomena[2]. There are many mechanisms by which seizures can develop in either normal or pathologic brains. Three common mechanisms include, 1) Diminition of inhibitory mechanism (especially synaptic inhibition due to GABA.

2) Enhancement of the excitatory synaptic mechanism (especially those mediated by NMDA).

3) Enhancement of endogenous neuronal burst firing (usually by enhancing voltage dependent calcium currents). Different forms of human epilepsy may be caused by any one or combination of the above mechanisms[3, 4].

Epilepsy is the second most common neurological disease in India. It affects an estimated 7 million people in India, and 50 million around the world. The prevalence of epilepsy is 0.7% in India, which is comparable to the statistics of U.S.A and other developing nations. The WHO has estimated approximately 80% people with epilepsy live in developing countries and most patients do not get adequate medical treatment[5]. It has been observed that the presently available antiepileptic drugs (AED's) are unable to control seizures effectively in as many as 25% of the patients[6,7].

The conventional AED's like Phenytoin, Carbamazeipine and Sodium Valproate carry with them several serious side effects notably neurotoxicity[8]. Moreover, approximately 30% of people with epilepsy have "intractable seizures" that do not respond to even the best available treatment[5]. Therefore there is indeed need for research in medicinal plants as herbal medicines have efficacy coupled with less side effects. This has accelerated the research regarding antiepileptic activity in plants which possess multiple mechanisms of action with least side effects.

Abrus precatorius (Indian licorice), is known as Ghumchi in India. In the Ayurvedic medicine, leaves of *Abrus precatorius* used as laxative, expectorant and aphrodisiac and in treatment of epilepsy. Seeds are found to be purgative, emetic, tonic, antiphlogistic and aphrodisiac[9]. *Abrus precatorius* is one of the 60 plants used by traditional healers of Tanzania for treatment of epilepsy[10].

MATERIALS AND METHODS

Drugs and chemicals: Pentylenetetrazole, Picrotoxin, Strychnine were obtained from Loba chemie, Mumbai. All other drugs and chemicals used in the study were of analytical grade obtained from various local chemical distributors.

Plant collection and authentication: The plant leaves were collected from Mangalore, Dakshina Kannada. The plant was authenticated by Dr. U. Srinivas, Professor and HOD Dept. of Pharmacognosy, Srinivas College of Pharmacy, Mangalore.

Preparation of extract and Phytochemical screening[11,12]: The leaves were shade dried for several days. The dried material was reduced to a coarse powder and was successively extracted by maceration using 70% ethanol. The extract was concentrated using a rotary evaporator at low temperature. It was preserved in airtight containers and kept at 4-5°C until further use. For the preparation of test sample the weighed amount of extract was dissolved in normal saline.

The preliminary phytochemical investigation was carried out for ethanolic extract of *A. precatorius* by standard methods.

Animals: Swiss mice of either sex, 8-10 weeks old, weighing about 25-30 g were used in experiments. Animals were housed in polypropylene cages maintained under standard condition (12 hours light / dark cycle; 25 ± 30C, 45-65% humidity) and had free access to standard feed and water *ad libitum*. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. All experimental protocols were reviewed and accepted by the Institutional Animal Ethical Committee (IAEC) prior to the initiation of the experiment.

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Acute Toxicity Studies: The mice were fasted for 3 hr prior to the experiment. Animals were observed for its mortality up to 48 hr study period following the administration as per OECD guidelines No 423. From the LD50 dose $1/20^{th}$, $1/10^{th}$ and $1/5^{th}$ doses were selected and considered as low, medium and high doses respectively[13].

Assessment of Anticonvulsant activity

Pentylenetetrazole induced seizures: Mice of either sex weighing 18- 22g were randomly divided into 5 groups of 6 each. Group I served as control and was given only PTZ. Second group of animals were given the reference drug (diazepam 5mg/kg, i.p). The rest of three groups were given test compound of low (100mg/kg), medium (200mg/kg), and

high dose (400 mg/kg) given through oral route. The seizures were induced in all the animals by intraperitoneal injection of Pentylenetetrazole (80 mg/kg), 30 minutes after administering the test drug. Animals were placed in an individual cage and were observed for 1 hour. Seizures and tonic-clonic convulsions were recorded[14]. The anticonvulsant property of the plant extract was assessed by its ability to prevent or delay the onset of clonic and tonic convulsions. Onset time and % mortality against PTZ induced convulsions were measured.

Picrotoxin induced convulsions: Mice of either sex weighing 18- 22g were randomly divided into 5 groups of 6 each. Group I served as control and was given only PTZ. Second group of animals were given the reference drug (diazepam 5mg/kg, i.p). The rest of three groups were given test compound of low (100mg/kg), medium (200mg/kg), and high dose (400 mg/kg) given through oral route. The clonic convulsions were induced in all the animals by intraperitoneal injection of Picrotoxin (3.5mg/kg), 30 minutes after administering the test drug. Animals were placed in an individual cage and were observed for 1 hour[14]. Onset of clonic convulsions was noted in all groups.

Maximal electro shock induced convulsions: Mice of either sex weighing 18- 22g were randomly divided into 5 groups of 6 each. After 30 minutes of treatment, the maximal electro shock in animals was induced by a electrical stimulus (50 mA; 50 Hz; 1sec duration), applied through ear-clip electrodes using a stimulator apparatus. Group I served as control and animals were treated with saline. Group II animals were treated with standard drug Phenytoin (25mg/kg). Rest three groups of mice were pre-treated with the test extract at low (100mg/kg), medium (200mg/kg), and high dose (400 mg/kg) given through oral route. The criterion for the anticonvulsant effect was considered the absence or decrease in the duration of hind limb tonic extensor within 10s after delivery of the electroshock[14].

Statistical analysis

Data were expressed as Mean ± SEM and were analyzed by the one-way ANOVA followed by the Dunnett's test.

RESULTS AND DISCUSSIONS

Phytochemical screening: The preliminary phytochemical screening of the ethanolic extract of *Abrus precatorius* revealed the presence of flavonoids, triterpenoids, saponins, reducing sugars, phenolic compounds and glycosides.

Acute Toxicity Studies: Ethanolic extract of *A. precatorius* leaves did not exhibit any signs of toxicity or mortality when given through oral route up to a dose of 2000mg/kg. Hence $1/20^{th}$, $1/10^{th}$ and $1/5^{th}$ of LD_{50} dose namely 100 mg/kg, 200 mg/kg, 400 mg/kg were selected as low, medium and high doses respectively for evaluation of anticonvulsant activity calculated according to the OECD guidelines 423.

Anticonvulsant activity

Pentylenetetrazole induced seizures: Pretreatment with extract of *A.precatorius* at medium and high doses (200 and 400mg/kg) showed significant alteration in the onset of tonic-clonic seizures and increased the threshold for clonic and tonic convulsions compared to control group. Also extract treatment showed protection against PTZ induced mortality in a dose dependent manner. Standard drug diazepam (5mg/kg) had abolished the clonic and tonic seizures and offered 100% protection (Table 1).

Table 1: Effect of *Abrus precatorius* extract on PTZ induced seizures.

Drugs	Dose (mg/kg)	Onset time in secs (mean±sem)		% protection
		Clonic	Tonic	
Control	-	53.67±3.029	389.8±44.93	0
Diazepam	5	0.00±0.0	0.00±0.0	100
A. precatorius extract	100	109.5±11.63*	458±39.83	16.66
A. precatorius extract	200	145.33±18.472***	523±26.1*	50
A. precatorius extract	400	212.8±21.10***	678±156.2***	66.66

*Results are expressed as Mean ± SEM; (n=6). The values were found to be significant at ***P<.0001 when compared with the control.

Picrotoxin induced seizures

Picrotoxin produced tonic seizures in all the animals except diazepam treated animals. The *A. precatorius* extract did not affect the incidence of seizures but there was significant decrease in number of deaths and increase in the duration of death time at the high dose (400 mg/kg) as shown in Table 2.

Table 2: Effect of EE of *Abrus precatorius* on Picrotoxin induced seizures

Drugs	Dose (mg/kg)	Onset time in secs (mean±sem)		Duration of death time	% mortality
Control	0	307.67±3.029	467.8±44.93	710±42.04	100%
Standard diazepam	5	0.00±0.0	0.00±0.0	0.00±0.0	0%
A. precatorius extract	100	410.5±11.63	816.2±39.83	973±78.33	100%
A. precatorius extract	200	588.33±18.472***	1090±99.1***	1275±134.1	83.33%
A. precatorius extract	400	760.8±21.10***	1610±156.2***	1910±111.1	33.33%

* Results are expressed as Mean ± SEM; (n=6). The values were found to be significant at ***P<.0001 when compared with the control.

MES induced convulsions

Ethanolic extract (200 and 400 mg/kg) of *Abrus precatorius* significantly increased the onset of clonus and decreased the duration of extensor phase along with abolition of flexion phase of grandmal seizures. Extract also showed protection against mortality in a dose dependent manner. Low dose (100 mg/kg) had no significant effect on these parameters (Table 3).

Table 3: Effect of EE of *Abrus precatorius* on MES induced seizures.

Drugs	Dose mg/kg	Duration of tonic flexion sec/30min)	Duration Of tonic Extensor (sec/30 sec)	Latency (onset of clonus)	% Protection
Control		Not observed	15.35±0.60	2.18±0.47	0
Phenytoin	25	6.83±0.79***	Not observed***	13.17±1.29***	100
Abrus precatorius extract	100	Not observed	13.17±1.10	4.18±0.29	33.33
Abrus precatorius extract	200	Not observed	9.67±1.04**	8.22±1.49***	50
Abrus precatorius extract	400	Not observed	7.77±1.08***	9.16±1.01***	83.33

* Results are expressed as Mean ± SEM; (n=6). The values were found to be significant at ***P<.001 when compared with the control. Ns= not significant.

DISCUSSION

There are a number of synthetic anticonvulsant drugs currently available for use in the management and treatment of individuals with epilepsy. However, most of the synthetic drugs are not only unaffordable but also possess many toxic adverse effects. Therefore, the development of cheap, effective and safe anticonvulsant agents from plants and other sources is the need of the hour.

In most common forms of epileptic seizures, effective drugs appear to work either by promoting the inactivated state of voltage-activated Na+ channels or enhance GABA- mediated synaptic inhibition[15].

Prevention of PTZ-induced seizures in laboratory animals is the most commonly used preliminary screening test for characterizing potential anti-convulsive drugs. The test is assumed to identify anticonvulsant drugs effective against generalized clonic seizures as PTZ produces clonic and tonic convulsions. The antiepileptic drug should abolish or increase the threshold for clonic and tonic convulsions. The mechanism by which PTZ exert its convulsive action is by acting as an antagonist at the GABA-

A receptor complex. Drugs that offer protection against tonic–clonic seizures induced by PTZ are considered to be useful to control myoclonic and absence seizures in humans[16,17].

Diazepam acts through the activation of GABA-A receptors and facilitate the GABA mediated opening of chloride channels[18,19]. PTZ induced seizures suggest that, the extract *A.precatorius* might affect GABA-ergic neurotransmission as PTZ has been shown to interact with the GABA neurotransmitter.

Picrotoxin, on the other hand, is a selective non-competitive antagonist of gamma amino butyric acid (GABA) at GABA-A receptor, there by blocking the chloride channels linked to GABA-A receptor[20]. Ethanolic extract of *A. precatorius* at medium and high doses significantly delayed the onset of tonic convulsions and also increased the duration of death time. So the anticonvulsant effect produced might be through suppression of the action of Picrotoxin on chloride channels linked to GABA-A receptor. This process will in turn inhibit GABA neurotransmission and activity in the brain. So anticonvulsant effect produced by the extract might be through blocking of the chloride ion channel linked to GABA-A-receptors.

MES is also one of the commonly used models for preliminary testing of anticonvulsant drugs that produces generalized tonic-clonic seizures namely hind limb tonic extensor, tonic flexion and clonic convulsions. In untreated animals a single MES produced an immediate tonic hind limb extension for 5- 10 sec duration followed by clonic seizures. It has often been stated that antiepileptic drugs that block MES induced tonic extension act by blocking seizure spread, moreover MES induced tonic extension can be prevented either by drugs that inhibit voltage dependant Na+ channels (Phenytoin, Valproate) or by drugs that block glutaminergic excitation mediated by the N-methyl- D-aspartate (NMDA) receptor (felbamate)[21,22].

CONCLUSION

The results obtained from these experimental models clearly confirmed the anticonvulsant activity of ethanolic extract of *A. precatrorius* in mice. Phytoconstituents like flavonoids and saponins present in ethanolic extract of *Abrus precatorius* leaves may be responsible for anticonvulsant effect.

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