



AN EXPERIMENTAL EVALUATION OF ANTICONVULSANT ACTIVITY OF *ASPARAGUS RACEMOSUS*

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ABSTRACT

Maximal electroshock seizures (MES) in Wistar albino rats and pentylenetetrazole (PTZ) induced seizures in albino mice were used to study anticonvulsant activity of ethanolic extract of *Asparagus racemosus* (EEAR). The EEAR was administered orally in graded doses (100, 200 and 400 mg/kg) in both the experimental models and the effects were compared with vehicle and standard drugs treated group (phenytoin sodium and valproic acid in MES and PTZ induced seizures respectively). Oral administration of two doses of EEAR 200 and 400 mg/kg, did show anticonvulsant activity by abolishing the extensor phase and animal protection in 33.34% and 66.67% of rats respectively to significant extent against maximal electroshock seizures (MES) and also it prolonged post-ictal depression significantly ($P < 0.05$) in a dose of 400 mg/kg in comparison to control, but not comparable to the standard phenytoin. The plant extract in the dose 400 mg/kg, showed 66.67% protection against clonic convulsions and 24h mortality which was statistically significant ($P < 0.05$) against control in PTZ model. It also decreased number of clonic convulsions and duration of clonic phase which was statistically ($P < 0.01$) significant in the above mentioned dose in comparison to control. However, the standard drug valproic acid provided 83.33% protection against PTZ induced convulsions which was statistically significant ($P < 0.001$) against control. It also provided statistically significant ($P < 0.01$) protection against 24h mortality in 83.34% animals as well as decreased number and duration of convulsions in comparison to control significantly ($P < 0.001$). The anticonvulsant activity of EEAR has not been found equi-effective or comparable with standard drugs in both models. These findings suggest that EEAR possesses anticonvulsant activity against both MES and PTZ induced convulsions. Further research is required to isolate the active principles and mechanisms involved in the anticonvulsant activity of this plant.

Keywords: *Asparagus racemosus*, Anticonvulsant activity, Maximal electroconvulsive shock, Pentylenetetrazole.

INTRODUCTION

Epilepsy, a brain disorder characterized by recurrent seizures, affects 1–2% of humans world-wide and shows its highest incidence in the first year of life. According to the WHO, 50 million people are affected world-wide; thereby accounting for 1% of global burden of disease.¹ In this modern era, it is the most frequent neurodegenerative disease. Epilepsy usually begins in childhood, potentially impeding education, employment, social relationships and development of a sense of self-worth. Epilepsy is among the disorders that are strongly associated with significant psychological and social consequences for everyday living.²

Epilepsy is a neurological disorder characterized by paroxysmal dysrhythmia, seizure, with or without bodily convulsions and sensory or psychiatric phenomena. There are many mechanisms by which seizures can develop in either normal or pathological brain. Three common mechanisms include, 1) diminution of inhibitory mechanisms (especially synaptic inhibition due to GABA). 2) Enhancement of excitatory synaptic transmission mechanisms (especially those mediated by NMDA). 3) Enhancement of endogenous neuronal burst firing (usually by enhancing voltage gated calcium currents). Different forms of human epilepsy may be caused by any one or combination of the above mechanisms.³ Antiepileptic drugs is used to prevent or interrupt seizures. Although 10 new antiepileptic drugs were made

available since the late 1980s, refractoriness to treatment is still an important issue in epilepsy care. Only two-thirds of patients are seizure-free under pharmacological treatment.⁴

The current therapy of epilepsy with modern antiepileptic drugs is associated with dose-related side effects, chronic toxicity, as well as teratogenic effects, and approximately 30% of the patients continue to have seizures with current antiepileptic drug therapy.⁵ Traditional systems of medicine are popular in developing countries and up to 80% of the population relies on these medicines or folk remedies for their primary health care need.⁶ Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects.⁷ Several plants used for the treatment of epilepsy in different systems of traditional medicine have shown activity when tested in modern bioassays for the detection of anticonvulsant activity and many such plants are yet to be scientifically investigated.

The plant *Asparagus racemosus* is native throughout tropical Africa, Java, Australia, India and Southern China and is a member of the family Liliaceae. It is an under shrub, climbing up to 1-3 metres, with a stout and creeping root-stock. The roots occur in clusters or fascicles at the base of the stem with succulent and tuberous rootlets.⁸ Roots of *Asparagus racemosus* locally known as "Satavari" have been advocated for ailments like dyspepsia, lactagogue, antidiarrheal, antiseptic,



antispasmodic, diuretic, aphrodisiac, nutritive tonic, demulcent, and for treatment of gonorrhoea, leprosy, hyperacidity, colic, haemorrhoids, hypertension, as abortifacient and for diseases of liver and kidney etc.⁹⁻¹⁰

Methanolic extract of fresh roots of *Asparagus racemosus* has ulceroprotective activity both in rats and patients of duodenal ulcers and antioxidant activity in rats. A rasayana akin to modern classification of adaptogens, has been reported to have potent adaptogenic activity.⁹

Several animal models of convulsions have been developed to evaluate antiseizure activity. The MES model is probably the best validated method for assessment of anti-epileptic drugs in generalised tonic-clonic and cortical seizures. The PTZ induced seizures are similar to the symptoms observed in the absence seizures. The objective of the present study was to investigate anticonvulsant activity of ethanolic extract of *A. racemosus* against the seizures induced by MES and PTZ.³

MATERIALS AND METHODS

Animal care and handling

This was done as per the guidelines set by the CPCSEA (committee for the purpose of control and supervision of experiments on animals). Male albino rats and mice (both Wistar strain) weight (125–160 g) and (25–35 g) were used to study the effect of test drug on MES and PTZ induced seizures respectively. A total of healthy 30 rats and 30 mice bred locally in the animal house of Kasturba Medical College, Manipal, and were selected for the study. Female animals were excluded because of the fact that estrus cycle influences the seizure threshold. They were housed under controlled conditions of temperature of $23 \pm 2^\circ\text{C}$, humidity of 50% and 10-14 h of light and dark cycles respectively. The animals were housed individually in polypropylene cages containing sterile paddy husk (procured locally) as bedding throughout the experiment and had free access to sterile food and water *ad libitum*. Animals were kept under fasting for overnight and weighed before the experiment. The study was undertaken after obtaining the approval of Institutional Animal Ethics Committee (IAEC approval letter no. IAEC/KMC/ 23/ 2010-2011).

Collection and preparation of ethanolic extract of *A. racemosus*

A. racemosus plants were procured locally in the month of April and authenticated by the professor of Botany, Mahatma Gandhi Memorial College, Udupi. After authentication, the tuberous roots were cleaned and shade dried and milled into coarse powder by a mechanical grinder. The shade dried roots were crushed into small pieces and powdered. The powder was loaded into Soxhlet extractor in 8 batches of 150 g each and was subjected to extraction for about 30-40 h with 95% ethanol. After extraction the solvent was distilled off and the extract was concentrated under reduced pressure on a water bath at a temperature below 50°C to a syrupy

consistency. Then it was dried in the desiccators. The yield was about 5.3 %.

Acute Toxicity Studies

Doses were selected and determined according to the previous acute toxicity studies of methanolic extract of *Asparagus racemosus*. Methanolic extract of *Asparagus racemosus* did not show any toxicity even at 5g/kg oral dose.⁹ We presumed that LD_{50} was beyond the dose of 5g/kg body weight. Hence in this study three doses 100, 200 and 400 mg/kg, body wt. were taken as lower, intermediate and higher dose respectively.

Drugs and chemicals

Test drugs: Ethanolic root extract of *Asparagus racemosus* (*EEAR*) was used for the present study in volume of 10 ml/kg, body wt., p.o., phenytoin sodium (Tab. Eptoin) (Abbott Pvt. Ltd. India), suspension was given orally (p.o.) in a volume of 10 ml/kg, body wt. in dose of 50 mg/kg, valproic acid (SUN PHARMA Pvt. Ltd. India) was dissolved in sterile saline and administered intraperitoneally (i.p.) in the dose of 300 mg/kg in volume of 10 ml/kg of body weight. Pentylentetrazole (PTZ) (Sigma Chemical Ltd, USA), the solution was prepared by taking the required amount of PTZ and dissolving it in 0.5% of normal saline in the dose of 80 mg/kg subcutaneously just before use. Carboxymethylcellulose (CMC) 0.5% prepared as suspension in volume of 10 ml/kg of body wt. and other chemicals used were of analytical grade. The suspension of all the doses of *EEAR* was made in 0.5% CMC.¹¹

Study Design and drug treatment

The animals were randomly allocated into five groups of six each. Rats and mice were used for the MES and PTZ experimental epileptic models respectively. Group-I received 2 ml of 0.5% CMC, p.o. and served as control. Group-II received phenytoin sodium (50 mg/kg, orally) and valproic acid (300 mg/kg, i.p.) as standard controls in MES method and PTZ induced seizures method respectively. Group- III, IV and V were administered three graded doses of test drug i.e. 100, 200 and 400 mg/kg, respectively, p.o. in both the experimental models.

Percentage protection of animals was recorded as percentage reduction in duration of seizures by considering control group as 100 %.

Experimental Epileptic Models

Two methods were employed to evaluate anticonvulsant activity in Wistar albino rats and mice.

A. Maximal electroshock induced seizures (MES) in rats

The animals were chosen by preliminary screening. Rats which showed extension of hind limb were included in the study. The seizures were induced by maximal electroshock in albino rats with the help of electroconvulsimeter by passing current of 50 mA for 0.2 second using ear clip electrodes. All drugs were given one hour prior to induction of convulsions. The animals were observed for the extensor phase as well as its duration



and post-ictal depression. The abolition of extensor (tonic phase) in drug treated group was taken as criteria for anticonvulsant activity.¹¹

B. Pentylenetetrazole (PTZ) induced seizures in mice

The albino mice were selected two weeks prior to conducting the experiment by injecting the pentylenetetrazole in a dose of 30 mg/kg subcutaneously in the scruff of neck. Only those mice which showed clonic convulsions within 30 minutes during preliminary examination were chosen for the present study. After one hour drug treatment, PTZ (80 mg/kg subcutaneously) was given in the scruff of neck. Animals were observed for clonic convulsions, number of convulsions in 30 minutes, duration of convulsions and 24 hour mortality. Absence of clonic convulsions in drug treated groups was taken as criteria for anticonvulsant activity.¹¹

Statistical analysis

The results were analysed for statistical significance using One way ANOVA, followed by Scheffe's test. A p-value <0.05 was considered significant. Computer statistical package SPSS (version 17) was used for analysis.

RESULTS

A. Anticonvulsant effect of EEAR on maximal electroshock (MES) induced seizures

Oral administration of two doses, 200 and 400 mg/kg of EEAR did show anticonvulsant activity by abolishing the extensor phase 33.34% and 66.67% of rats respectively to significant extent against maximal electroshock seizures

(MES) and also it prolonged post-ictal depression significantly ($P<0.05$) in a dose of 400 mg/kg in comparison to control. The standard drug phenytoin sodium showed 100% protection in animals against MES. It abolished the extensor phase completely ($P<0.001$), as well as prolonged post-ictal depression which was very significant in comparison to control as shown in Table 1. Similarly, 200 and 400 mg/kg doses of EEAR did show animal protection in 33.34% and 66.67% of rats respectively; however it was significant against control but not comparable to the standard phenytoin.

B. Anticonvulsant action of EEAR on pentylenetetrazole (PTZ) induced convulsions

The only dose of EEAR 400 mg/kg, showed 66.67% protection against clonic convulsions and 24h mortality which was statistically significant ($P<0.05$) against control. It also decreased number of clonic convulsions and duration of clonic phase which was statistically ($P<0.01$) significant in the above mentioned dose in comparison to control, however it was not comparable to the standard. Whereas, lower two doses of EEAR 100 and 200 mg/kg, failed to show any significant anticonvulsant activity. However, the standard drug valproic acid provided 83.34% protection against PTZ induced convulsions which was statistically significant ($P<0.001$). It also provided statistically significant ($P<0.01$) protection against 24-hrs mortality in 83.34% animals as well as decreased number and duration of convulsions in comparison to control significantly ($P<0.001$) as shown in Table 2.

Table 1: Effect of ethanolic extract *Asparagus racemosus* on MES induced seizures in rats

Group	Treatment	Dose (mg, ml /kg)	% of animals showing abolition of extensor phase	Duration of (Mean \pm S.E.M)		% of Animals protected
				Extensor phase (sec.)	P.I.D (min.)	
I	Control (0.5% CMC)	2	00.00	14.00 \pm 0.57	1.87 \pm 0.58	0
II	phenytoin sodium	50	100	0.00	3.88 \pm 0.36**	100
III-V	EEAR	100	00.00	13.16 \pm 0.40*	2.14 \pm 2.10	00
		200	33.34	3.83 \pm 0.47*	2.87 \pm 0.94	33.34
		400	66.67	1.66 \pm 0.33**	3.16 \pm 0.79*	66.67

EEAR = Ethanolic extract of *Asparagus racemosus*; Values are mean \pm SEM (n=6) statistical significance was determined by One-way ANOVA, followed by Scheffe's test. *p < 0.05 and **p < 0.001 Vs Control. PID-Post ictal depression.

Table 2: Effect of ethanolic extract *Asparagus racemosus* on PTZ induced seizures in mice

Treatment (Groups)	Dose (mg, ml /kg)	% of animals showing abolition of clonic phase	% of animals protected from mortality in 24 hrs	No. of clonic convulsions (in 30mins)	Duration of clonic phase (in sec)
Control, 0.5% CMC (I)	2	0.00	0.00	7.50 \pm 0.428	18.0633 \pm 0.428
valproic acid (II)	300	83.34**	83.34**	0.66 \pm 0.210***	01.5800 \pm .21***
EEAR (III-V)	100	0.00	16.67	7.00 \pm 0.258	17.6933 \pm 0.258
	200	0.00	16.67	6.66 \pm 0.22	12.8333 \pm 0.210*
	400	66.67*	66.67**	2.83 \pm 0.307**	7.7733 \pm 0.307**

EEAR = Ethanolic extract of *Asparagus racemosus*; Values are mean \pm SEM (n=6). Statistical significance was determined by One-way ANOVA, followed by Scheffe's test. *p < 0.05; **p < 0.01 Vs Control and ***p < 0.001 Vs Control.



DISCUSSION

The observations emanated from the present study indicated that the *EEAR* was without any lethal effect in a dose up to 5g/kg and possessed anticonvulsant activity against seizures induced by MES and PTZ in a dose dependent way and also it significantly increased post-ictal depression, thereby indicating its central nervous system depressant action. It is well known fact that drugs which provide protection against seizures induced by maximal electroshock are generally predicted to be effective against generalized tonic clonic seizures and cortical focal seizures.¹²⁻¹⁴ MES induced convulsions can be prevented effectively by drugs that inhibit voltage dependent Na⁺ channels, such as phenytoin, valproate and lamotrigine etc.¹⁵ Phenytoin showed a total abolition of the extensor phase of MES seizure.

The higher dose of *EEAR* showed significant protection against pentylenetetrazole induced convulsions as well as 24-hr mortality in mice. The seizures induced by chemoconvulsant (pentylenetetrazole), is useful in identifying drugs that are effective against absence seizures.¹² Hence, *EEAR* may be useful in absence seizures in higher dose. Two mechanisms have been put forth for PTZ induced convulsions, either by inhibiting gamma amino butyric acid (GABA) pathway in CNS or by increasing the central noradrenergic activity.¹⁶ Moreover, *EEAR* showed significant protection, particularly against PTZ induced convulsions in present study. Therefore, it is likely that *EEAR* might possibly be producing anticonvulsant action by increasing level of (GABA), an inhibitory neurotransmitter in the central nervous system. However, several mechanisms are involved in anticonvulsant activity; it is very premature at this stage of the study to say that anticonvulsant action appears to be due to increased level of GABA. This could be a possible hypothesis but certainly needs further investigation in future.

The multiplicity of mechanisms of actions and the broad spectrum of anticonvulsant activity of *Asparagus racemosus* might be due to the presence of different active components. According to the previous study, phytochemical screening the methanolic extract showed presence of flavonoids, steroidal glycosides, racemosol, terpenoids and saponins. The mechanisms will be better understood once the anticonvulsant active components of *Asparagus racemosus* are identified.⁹

In conclusion, these findings suggest that *EEAR* possesses anticonvulsant activity against both MES and PTZ induced seizures in rats and mice respectively. It is well known that very few drugs available like valproic acid which is effective against grandmal and petitmal epilepsy, however it is contraindicated in children (<3y) and pregnant woman because of its high hepatotoxic and teratogenic activities respectively. In Ayurveda, root of *A. racemosus* has been considered and described as absolutely safer for long term use even during pregnancy and lactation. Systemic administration of very high doses

of its extract didn't produce any abnormality in behavioral pattern in mice and rats. LD₅₀ of the plant root product could not be assessed since it did not show mortality even up to the oral dosage of 64g/kg. This plant extract was remarkably safer; no adverse reactions have been reported in the doses of root powder used clinically.⁹ Hence, it is prudent to know at this stage that *EEAR* having similar properties could be another substitute to the therapy. However, its efficacy and adverse profile has to be screened thoroughly. This could explain the basis for the use of *Asparagus racemosus* in traditional medicine for the management of epilepsy. Further research is required to isolate the active principles and elucidate the actual mechanisms involved in the anticonvulsant activity of this plant.

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