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STUDIES ON PHYTOCHEMICAL AND ANTICONVULSANT PROPERTY OF *MARTYNIYA ANNUA LINN.*

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Abstract:

The present report is an investigation of anti seizure activity of *Martynia annua Linn*. is a well known plant which is being used in Indian traditional medicines for epilepsy, inflammation and tuberculosis. The methanolic extract of *Martynia annua Linn.* (MEMA) was subjected to acute toxicity and then screened for anticonvulsant activity on Maximal Electroshock (MES) and Pentylenetetrazole (PTZ) induced seizures models in albino wistar rats. Acute toxicity of extract was non toxic up to the recommended dose 2000 mg/kg body weight orally as per OECD guidelines No. 423. Animals were treated with MEMA at doses of 200 and 400 mg/kg body weight. These studies showed, the mean duration of extensor phase of test group reduced to significant level as compared to control group. In Pentylenetetrazol induced seizure test, onset of myoclonic spasm and clonic convulsion was delayed in the test group. MEMA showed anticonvulsant activity against MES and PTZ animal models. However, further studies still needed to be carried on exposure of the extract to humans.

Keywords: Anti seizure activity, *Martynia annua Linn.*, Maximal Electroshock (MES) , Pentylenetetrazole (PTZ).

Introduction:

Epilepsy is a common neurological disorder affecting more than 50 million persons worldwide. It is probable that the prevalence is higher in less developed countries because of higher incidence of antecedent factors such as brain infections, cranial and perinatal traumas parasitic infections (Sander and shorvon ,1987). In India, studies have reported the prevalence rate of epilepsy varying from 1710 from 9780 cases per million population (Gupta YK, 2000). The modern conventional antiepileptic drugs (AEDs) are effective in approximately 50% of patients, many cases still remain resistant to AED treatment (Hememann *et al*., 1994). These drugs are associated with vast array of side effects including chronic toxicity, teratogenecity, adverse effects on cognition and behavior among others (Raza *et al*., 2001). Thus, due to aforementioned reasons and others, it is pertinent to look for affordable and conventional alternative medicine with view to providing a better protection and activities- particularly medicinal plants. Moreover, medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects and have been used in the discovery and developed of new drugs (Farnsworth, 1994; cragg *et al*., 1997).

Martynia annua Linn. is commonly known in ayurveda kaakanassikaa belongs to family Martyniaceae. It small herb found in throughout India and it is native of Mexico. The leaves of this plant are used in epilepsy and also apply to tuberculous glands of the neck. Fruit is used for anti inflammatory. Ash of fruit, mixed with coconut oil applied on burns. Seed oil applied on abscesses and for treating itching and skin affections (Khare CP, 2007). The ayurvedic pharmacopoeia of India recommended the seed of *Martynia annua* for arresting of graying of hair. According to literature survey, the ethanol extract of *Martynia annua* root is shown antifertility effect on male

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rats and ethanol extract of *M. annua* seed have shown hypotensive action in cat and effects on respiration and nictitating membrane in rats (Mali PC *et al*., 2002).

Materials and Methods

Plant collection

The leaves of *Martynia annua Linn.* was collected from Tirupathi , Andhra Pradesh, India. It was identified and authenticated by Prof.Jayaraman, Plant Anatomy Research Centre (PARC), Chennai, Tamil Nadu. The voucher specimen **PARC / 2009 / 348** was preserved in our laboratory for future reference.

Preparation of extracts

The leaves of plants were dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (200gm) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus. Percentage yield of MEMA was found to be 19 % w/w.

Preliminary phytochemical screening:

The phytochemical examination of the methanolic extract of *Martynia annua Linn.* was performed by the standard methods **(**Harbone, 1973**).**

Animals used

Albino wistar rats (150-200g) of either sex were obtained from the animal house in Sree Vidyanikethan College of pharmacy, Tirupathi. The animals were maintained in a well- ventilated room with 12:12 hour light/dark cycle in polypropylene cages.

Acute Toxicity Study

The acute toxicity of MEMA was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not mortal even at 2000mg/kg dose. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study.(OECD, 2002)

Antiepileptic Activity

Effect on Maximal electroshock (MES) induced seizures

Albino wistar rats of either sex weighing 150 to 230 gm were divided into four groups of six animals each. The first group received vehicle control (1% w/v SCMC, lml/100 g) whereas Group-II received standard drug (Phenytoin, 25mg/kg) intraperitoneally, Group III and IV, received methanolic extract of *Martynia annua Linn.*(MEMA) (200 and 400 mg/kg body weight) p.o respectively for 14 days. On the 14th day, Seizures are induced to all the groups by using an Electro convulsiometer (Balakrishnan S *et al*., 1998).

Effect on Pentylenetetrazole (PTZ) induced seizures

Albino wistar rats of either sex weighing 150 to 230 gm were divided into four groups of six animals each. The first group received vehicle control (1% w/v SCMC, lml/100 g) whereas Group-II received standard drug (Diazepam, 4mg/kg) intraperitoneally, GroupIII and IV, received methanolic extract of *Martynia annua Linn*. (MEMA) (200 and 400 mg/kg/body weight) p.o respectively for 14 days. On the 14th day, Pentylenetetrazole (PTZ) (90mg/kg body weight, s. c) was administered to all the groups to induce clonic convulsions (Kulkarni SK *et al*., 2006).

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the groups was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test *P* values less than 0.05 were considered as significance.

Results

Phytochemical screening

The results of preliminary phytochemical screening of the methanolic extract of *Martynia annua Linn.* revealed that presence of steroids.

Effects of MEMA on MES Induced Epilepsy

The duration of tonic hindleg extension in rats treated with vehicle was 12.21±0.03seconds. The MEMA at doses of 200 mg/kg and 400 mg/kg were protect animals from seizures and significantly ($p<0.001$) reduced the duration of tonic hindleg extension for 3.92±0.1.61 and 2.12±0.31 seconds respectively. Whereas, the standard drug phenytoin treated animals exhibits abolished tonic hindleg extension. Phenytoin treated animals have shown 100% protection against MES induced seizures where as MEMA 200 mg/kg and 400 mg/kg have shown 66.31 % and 82.731 % protection respectively (Table-1).

Effect of MEMA on PTZ Induced epilepsy

In rats treated with vehicle, clonic convulsion appeared for 187.75±0.668 seconds after PTZ and all rats died after seizures. The MEMA at doses of 200 mg/kg and 400 mg/kg significantly delayed the onset of clonic convulsions for 495.83±1.511 ($p<0.001$) and 584±1.22 ($p<0.001$) seconds respectively in dose dependent manner. Whereas, the standard drug diazepam (4mg/kg, *i.p*) delayed the onset of clonic convulsions for 705.31±1.22. ($p<0.001$) seconds. Diazepam treated animals have shown 100% protection against PTZ induced seizures where as MEMA 200 mg/kg and 400 mg/kg have shown 70.33% and 82.88% protection of convulsion and 83.33% and 100% protection of mortality respectively (Table-2).

Table 1. Effect of methanolic extract of *Martynia annua Linn.* (MEMA) On MES induced seizers in rats.

Group	Design of treatment	Flexion	Extensor	Clonus	Stupor	Recovery	% protection
I	Vehicle control	8.9±0.32	12.21±0.03	18.78±0.32	38.2±0.58	189.2	0
II	Phenytoin 25mg/kg,i.p.	3.9±0.33**	0**	8.91±0.54**	16.29±0.35*	93.5	100
III	MEMA 200mg/kg,p.o	6.72±0.51*	3.92±0.1.61**	14.81±0.36*	31.58±0.95	141.79	66.31
IV	MEMA 400mg/kg,p.o	4.09±0.22**	2.12±0.31**	12.82±0.85**	16.23±0.69*	113.51	82.731

Values are expressed as mean ± SEM of six observations.
*p<0.05; ** p<0.01.Comparison between Group I *Vs* Group II, Group III &Group IV
Statistical significant test for comparison was done by ANOVA, followed by Dunnet's test

Figure 1: Effect of methanolic extract of *Martynia annua Linn.*(MEMA) on Maximal electroconvulsion shock (MES) induced seizurs in rats.

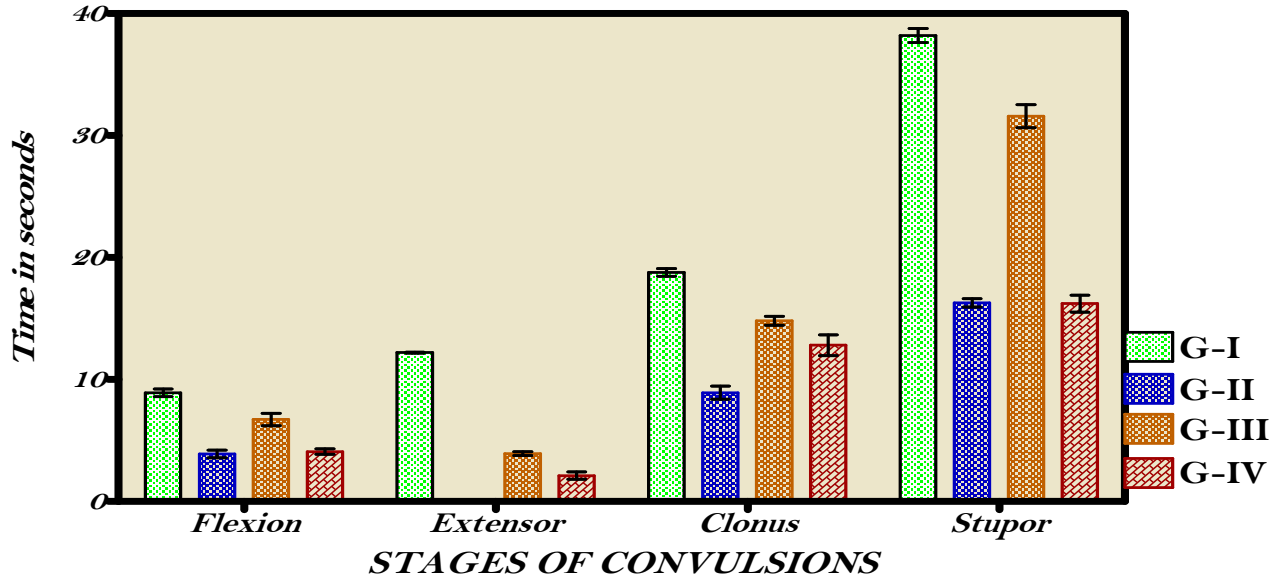
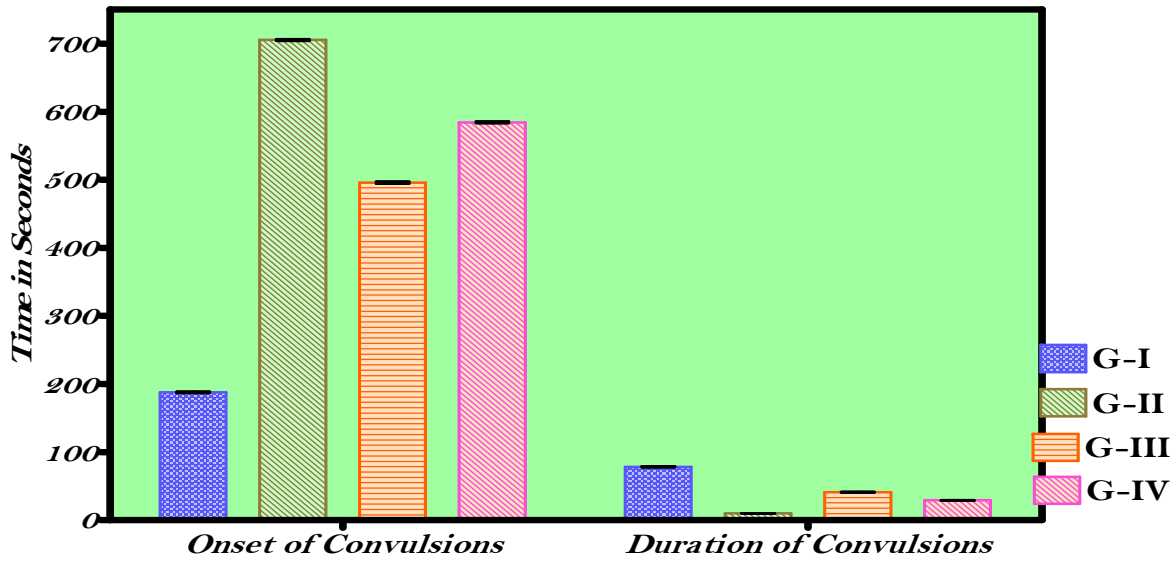


Table 2. Effect of methanolic extract of *Martynia annua Linn.* (MEMA) On PTZ induced seizers in rats.

Group	Design of Treatment	Onset of convulsions(sec.)	Duration of convulsion(sec.)	Protection convulsion%	Protection mortality %
I	Vehicle control	187.75±0.66	78.4±0.72	0	50
II	Diazepam(4mg/kg	705.31±1.13**	10.2±0.14**	100	100
III	MEMA 200	495.83±1.51**	41.25±0.60**	70.33	83.33
IV	MEMA 400	584.36±1.22**	29.15±0.28**	82.88	100

Values are expressed as mean ± SEM of six observations.
*p<0.05; ** p<0.01.Comparison between Group I *Vs* Group II, Group III &Group IV
Statistical significant test for comparison was done by ANOVA, followed by Dunnet's test

Figure 2: Effect of methanolic extract of *Martynia annua Linn.* (MEMA) On PTZ induced seizers in rats.



Discussions and Conclusions:

The MES test is the most frequently used as an animal model for identification of anticonvulsant activity of drugs for the generalized tonic-clonic seizures "grand mal" (Loscher and Schmidt, 1988; Oliveira *et al*., 2001)**.** This model based on observation of the stimulation by repeated electrical pulses induce in different neuronal structures one characteristic standard of epileptic activity (Quintans-Júnior *et al*., 2002)**.** In our present study, it is found that treatment with MEMA on rats significantly reduces in tonic hindleg extensor stage in MES induced epilepsy. The MES model – to identify compounds which prevent seizure spread, corresponding to generalized tonic-clonic seizures in humans (Kupferberg, 1989; Stables and Kupferberg, 1995)**.** Currently used anticonvulsant drugs (e.g. phenytoin, carbamazepines) effective in therapy of generalized tonic-clonic and partial seizures have been found to show strong anticonvulsant action in MES test (White, 1997; McDonald and Kelly, 1993)**.** Since, MEMA significantly inhibited generalized tonic-clonic seizures in MES test; it suggests the presence of anticonvulsant compounds.

We found that treatment with MEMA on PTZ induced rats significantly reduce the duration of convulsion and delayed the onset of clonic convulsion. Although animal models based on pentylenetetrazole (e.g. pentylenetetrazole threshold, and acute convulsions) have still been widely used for drug screening, the mechanism by which pentylenetetrazole elicits its action has not been completely understood. One generally accepted mechanism by which pentylenetetrazole exerts its action is by acting as an antagonist at the picrotoxin sensitive site of the $GABA_A$ receptor complex (Ramanjaneyulu and Ticku, 1984).

Since PTZ has been shown to interact with the GABA neurotransmission (Loscher and Schmidt, 1988; De Deyn *et al*., 1992) and PTZ induced seizures can be prevented by drugs that enhance gamma amino butyric acid type A ($GABA_A$) receptor-mediated inhibitory neurotransmission such as benzodiazepines and phenobarbital (Coulter *et al*., 1989; McDonald and Kelly, 1995)**,** the antagonism of PTZ- induced seizures suggests the interaction of the MEMA with the GABA-ergic neurotransmission.

The study concluded MEMA possesses an anticonvulsant effect which results from the potentiation of the activity of GABA. However, more precise mechanisms of MEMA anticonvulsant activity and the relationship between the seizure and $GABA_A$ receptor subunits and the other neurotransmitter systems which may explain how MEMA produce anticonvulsant effect must be investigated further.

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