International Journal of Phytomedicine 6 (2014) 59-62
http://www.arjournals.org/index.php/ijpm/index

Research Article

ISSN: 0975-0185

Anticonvulsant activity of leaf extracts of *Martynia annua* linn in experimental rats

Neeti Srivastav[1]*, Sarla Saklani[2], Vijay Juyal[3], Brijesh K Tiwari[1]

***Corresponding author:**

Neeti Srivastav

[1.]NKBR College of Pharmacy and Research Centre, Meerut (UP), India.
[2.]Department of Pharmaceutical Sciences, HNB Garhwal University, Srinagar. Uttarakhand. India.
[3]Department of Pharmaceutical Sciences, Bhimtal Campus. Uttarakhand, India.

Abstract

Martynia annua Linn (Martyniaceae) is a medicinal herb used for the treatment of epilepsy in the traditional health care system of Uttarakhand (India). The present study reports the anticonvulsant activities in the ethanolic and aqueous extracts of the leaves of *Martynia annua* Linn on the rats, induced both chemically and electrically. The models chosen for the activity were Maximal Electroshock (MES) and Pentylenetetrazole (PTZ) induced convulsions in rats. The test dose studied were 200 and 400 mg/kg body weight orally of ethanolic and aqueous extracts of *Martynia annua*. Acute toxicity studies of the extract shows that the extract was non toxic up to the recommended dose 2000mg/kg body weight orally as per OECD guideline no 423. In PTZ induced seizures, onset of clonic convulsions were studied while in MES model reduction in mean duration of extensor phase was noted. Both aqueous and ethanolic extracts showed anticonvulsant activities against MES and PTZ animal models.

Keywords: Martynia annua, Anticonvulsant activity, MES, PTZ.

.

Introduction

Epilepsy has been a serious disorder that accounts for about 1% of the world's burden of diseases. Various synthetic antiepileptic drugs are available, but their effectiveness vary with the entire range of population. They also possess drug interactions and side effects. The conventional antiepileptic drugs (AED) are effective in approximately 50% of the patients [1]. The side effects with these drugs commonly include: chronic toxicity, teratogenicity, adverse effects on cognition and behavior among others [2]. This may be one of the reasons that most of the scientific researches are inclined towards herbal medicines. Recently, medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects and have been used in discovery and development of new drugs [3]. Different types of human epilepsies have been characterized based on the classification of International League against epilepsy (ILAE). According to this classification, epilepsy has been divided into partial and generalized symptomatic and unclassified epilepsy. An imbalance between excitatory and inhibitory neurotransmitters is responsible for seizures. At neuronal level, seizures often occur when glutaminergic excitatory neurotransmitters overrides gamma amino butyric mediated inhibition [4].

The most popular and widely used animal seizure models are traditional PTZ and MES tests.

Prevention of seizures induced by PTZ in laboratory animals is the most commonly used preliminary screening test for characterizing potential anticonvulsant drugs. The MES test is considered to be the predictor of likely therapeutic efficacy against generalized tonic clonic seizure [5]. Generally compounds with anticonvulsant activity in petit mal are effective in PTZ induced seizure model. The MES model is used to identify compounds which prevent seizure spread [6].

The genus Martynia comprises of 7 species native to Mexico. Its common names are Devils claw (English), Bichu (Hindu), Kaakanassika (Sanskrit) and Vichchida (Gujarat). It belongs to the family Martyniaceae. The leaves and fruits are biologically active part of this plant. The leaves of this plant are used for treating epilepsy and also be applied to tuberculous glands of the neck [7, 8]. The leaves of Martynia were found to possess antibacterial properties [9]. Methanolic and aqueous extracts of leaves of *M. annua* possess antioxidant activities [10].

Fruits have been used as anti-inflammatory; seed oil applied on abscesses treats itching and skin infections [11]. *Martynia annua* fruits also showed analgesic and antipyretic activity [12]. It has been reported that ethanolic extract of *Martynia annua* root shows antifertility effect on male rats and hypotensive actions in cats and methanolic and aqueous extracts showed potent anthelmintic activity against earthworms [13, 14].

Material and methods

Plant collection

The leaves of *Martynia annua* Linn were collected from field areas of Srinagar Garhwal, Uttarakhand, India. It were identified and authenticated by Dr Sarita Garg, NISCAIR, Delhi as *Martynia annua* Linn. The voucher specimen bearing No NISCAIR/RHMD/2013/2190/196/2 was deposited at the herbarium.

Preparation of the extract

The collected leaves were cleaned, shade dried, powdered and sieved. A weighed quantity of powder (500 gm) was subjected to successive hot percolation in soxhlet apparatus. Plant material was defatted by petroleum ether before extraction in ethanol and water. Ethanol was evaporated using rotary evaporator under reduced pressure. The extracts were concentrated under reduced pressure using rotary evaporator to obtain a dark bluish green coloured sticky residue.

Preliminary Phytochemical Studies

The phytochemical examination of ethanolic and aqueous extracts of *Martynia annua* Linn was performed by the standard methods [15].

Animals used

Wistar rats (150 – 250 gm) of either sex were obtained from the animal house of NKBR College, Meerut, India. The animals were maintained in a well ventilated room with 12: 12 hour light/ dark cycle in polypropylene cages. All the animals were allowed for free access to water and fed with standard commercial pelleted mice chaw. All the experimental procedures and protocols used were reviewed by Institutional animal ethical committee (IAEC).

Acute toxicity studies

The acute toxicity studies of ethanolic and aqueous extracts were determined in mice. The animals were fasted overnight prior to the experiment. The extracts were administered in doses 50, 300, 1000, 2000 p.o to different groups of mice each containing 6 animals and mortality was observed after 24 hrs. The ethanolic and aqueous extracts of *Martynia annua* leaves were devoid of mortality of animals at dose of 2000mg/kg in mice p.o and hence LD_{50} was selected as cut value. Subsequent to administration of drug extracts, animals were observed closely for three hours, for any toxic manifestations, like increased motor activity, salvation, clonic convulsion, coma and death. The animals were under further investigation up to period of one week. It was observed that the test extracts were not mortal even at 2000mg/ kg dose. This was as per OECD guideline no 423[16].

Maximal electro convulsive shock (MES)

Rats were divided into four groups of six animals each. The first group received vehicle control (1ml/100gm PEG 400 p.o), group II received standard drug (Phenytoin, 25 mg/ kg ip), group III and IV received ethanolic (EMA) and aqueous (AMA) extracts of *Martynia annua* 200 and 400 mg/kg b.w, p.o respectively. The time for the extracts to reach its maximum effect was determined 60 min after oral administration. The response of the anticonvulsant effect was abolition of hind limb extensor phase [17].

Pentylene tetrazole (PTZ) induced convulsions

Rats were divided into four groups of six animals each. The first group received vehicle control (1ml/100gm PEG 400 p.o) , group II received standard drug (Diazepam, 4 mg/ kg ip), group III and IV received ethanolic and aqueous extracts of *Martynia annua* 200 and 400 mg/kg bw, p.o. After 30 min of the dosage of standard and test extracts, PTZ (90 mg/kg b.w s.c) was given and response of time of onset of seizures (tonic clonic convulsions) and their duration were recorded [18, 19].

Statistical analysis

The data were expressed as mean ± standard error mean (SEM). The significance of differences among groups was assessed using one way analysis of variance (ANOVA). The test followed by Dun net's test and P value less than 0.05 were considered significant [20].

Results and Discussion

Phytochemical Screening

Ethanolic and aqueous extract of *Martynia annua* Linn reveals the presence of steroids, glycosides, tannins, carbohydrate, phenol, flavonoid, anthocyanin, oleic acid.

Assessment of anticonvulsant activity by MES

In MES model, the duration of tonic extension of hind limb is used as an end point i.e. the protective action. The results of anticonvulsant effects of *M. annua* plant against MES induced convulsion are shown in table 1. The data shows that both the extracts reduce the hind limb extension in a dose dependent manner. Ethanolic extracts of 200 and 400 mg/kg decreases the duration of hind limb extensor in 3.45±0.194 and 2.06±0.071sec respectively which is most significant ($p<0.01$) as compared to control 11.13±0.388 sec. Aqueous extract of 200 and 400 mg/kg decreases the duration of hind limb extensor in 5.01±0.215 and 2.2±0.194 sec respectively which is significant ($p<0.01$) as compared to control 11.13±0.388 sec.

Table 1: Effect of *Martynia annua* extract on MES.

Treatment	Flexon	Extensor	Clonus	Stupor	% Protection
Control	8.36±0.162	11.13±0.388	21.31±0.273	37.45±0.274	0
Standard	3.2±0.167**	0.55±0.25**	8.5±1.732**	8.43±0.185**	100
EMA 200	6.7±0.229**	3.45±0.194**	10.41±3.301**	27.4±1.52**	66
EMA 400	3.93±0.189**	2.06±0.071**	9.63±3.062*	14.15±1.00**	83
AMA 200	7.41±0.193**	5.01±0.215**	13.66±2.746**	33.53±0.452**	50
AMA 400	5.58±0.252**	2.2±0.194**	15.05±0.176*	14.83±0.281**	66

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.

Assessment of anticonvulsant activity by PTZ

The anticonvulsant property of different extracts of *M.annua* is assessed by its ability to delay the onset of myoclonic spasm and clonic convulsion. The results of anticonvulsant effects of *M.annua* plant against PTZ induced convulsion are shown in table 2. Ethanolic extracts of *M.annua* at doses of 200 and 400 mg/kg shows onset of convulsions after 431.66±8.019 and 534.33± 7.994 sec respectively which is significant (p<0 .01) when compared to control 184.66± 2.906 sec. Aqueous extract of the same plant at test doses of 200 and 400 mg/kg shows onset of convulsions after 223.33±7.71 and 310.83±2.762sec respectively which is most significant (p<0 .01) as compared to control 184.66± 2.906 sec.

Table 2: Effect of *Martynia annua* extract on PTZ

Treatment	Onset of convulsions	Duration of convulsions	% Protection
Control	184.66± 2.906	80.66± 2.06	33
Standard	691.66 ± 5.998**	11.16 ± 0.872**	100
EMA 200	431.66 ± 8.019**	33 ± 1.51**	67
EMA 400	534.33± 7.994**	27.66 ± 1.892**	83
AMA 200	223.33 ± 7.71**	70.33 ± 1.45**	50
AMA 400	310.83 ± 2.762**	54.66 ± 1.926**	66

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.

Discussion

As indicated from fig 1, the maximum reduction of hind limb extensor was observed in EMA 400mg/kg b.w (2.06±0.071sec) followed by AMA 400mg/kg b.w (2.2±0.194sec) which were far better than the control (11.13±0.388 sec). The % protection in EMA and AMA higher doses were 83 and 66% respectively whereas in control the % protection was nil. Fig 2 shows that onset of convulsions were delayed maximum in EMA 400mg/kg having values of 534.33± 7.994sec followed by AMA 400 (310.83± 2.762). The results were much potent as compared to the control 184.66± 2.906.

Conclusion

In the present study anticonvulsant activity of ethanolic and aqueous extract of *Martynia annua* leaves were investigated by means of PTZ and MES models. The oral administration of both the extracts of *Martynia annua* showed delayed onset of convulsions in PTZ and reduction in tonic hind limb extension in MES model, indicating its anticonvulsant activity. Higher protection was observed with higher dose i.e. 400mg/kg. The percentage protection of ethanolic and aqueous extracts of *M. annua* at a dose of 400 mg/kg orally was found to be 83 and 66% respectively in both the models as compared to 100% in case of standard. The extracts showed dose dependent protection in rats. The results demonstrate the broad and potent anticonvulsant activity in rats (both MES and PTZ models) of the compounds in ethanolic and aqueous extract of the leaves of *Martynia annua.* Development of anticonvulsants from ethanolic and aqueous extract may produce natural antiepileptic drugs.

References

[1]. Heinemann UE, Draghun E, Fickerer J, Stabel and Zhang CL. Strategies for the development of drugs for pharmacological resistant epilepsies. Epilepsia. 1994; 10-21.

[2]. Raza MF, Shaheen MI, Choudhary A, Suria AU, Rahman S, Sombati and Delorenzo RJ. Anticonvulsant activities of the FS 1 subfraction isolated from roots of *Delphinium denudatum.* Phytotherapy Research. 2001; 15: 426-430.

[3]. Farnsworth NR. Ethno pharmacology and drug development. In: Ethnobotany and the search for new drugs. Foundation symposium, Prance GT and J Marshal (Eds). John Wiley and Sons: Chichester, 185, 1994, 42-59.

[4]. Balakrishnan N, Sanit K, Balasubramanium A, Sangameswaran B, Chaurey M. Antiepileptic activity of *Alangium salvifolium* leaf extracts. Herbal Tech Industry. 2010; 20-23.

[5]. Loscher W, Schmidt D. Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical consideration. Epilepsia Research.1988; 2: 145-181.

[6]. Kupferberg HJ. Antiepileptic drug development program: a co-operative effort of government and industry. Epilepsia.1989; 30 (1): S 51- S56.

[7]. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants. National Institute of Science Communication. New Delhi.1996.

[8]. Satyavati GV, Gupta AK, Tandon N. Medicinal plants of India.vol II. Indian Council of medicinal research. New Delhi. 1987.

[9]. Sermakkani M, Thangapandian V. Phytochemical and antibacterial activity of *M.annua* L against different pathogenic bacteria. J of herbal medicine and toxicology. 2010; 4(2): 221-224.

[10]. Nagda D, Saluja A, Nagda C. Antioxidant activities of methanolic and aqueous extracts from leaves of *M. annua* Linn. Pharmacognosy Journal.2009; 1(4): 288-297.

[11]. Khare CP. Indian Medicinal Plants an illustrated dictionary. Springer publications. 2007, 399-400.

[12]. Kar DM, Nanda BK, Pradhan D, Sahu SK, Dash GK. Analgesic and antipyretic activity of fruits of *M annua* Linn. Hamdard medicine. 2004; 47(1):32-35.

[13]. Mali PC, Ansari AS, Chaturvedi M. Antifertility effect of chronically administered *M.annua* root extract on male rats. J of ethnopharmacology. 2002; 82(2-3): 61-67.

[14]. Nirmal SK, Nikalye AG, Jadav RS, Tambe VD. Anthelmintic activity of *M.annua* roots. Indian drugs. 2007; 44(10):772-773.

[15]. Harbone JP. Phytochemical methods, a guide to modern technique of plant analysis. Chapman and Hall, London. 1973. 1-271.

[16]. OECD 2002. Acute oral toxicity. Acute oral toxic class method guidelines 423 adopted 23.03.1996. In: Eleventh Addendum to the OECD guidelines for the testing of chemical organization for economical co-operation and development, Paris. June 2000.

[17]. Hosseinzadeh H, Khosravan V. Anticonvulsant effects of aqueous and ethanolic extracts of *Crocus sativus* L stigmas in mice. Arch International Med. 2002; 5:44-47.

[18]. Sayyah M, Valizadeh J, Kamalinejad M. Anticonvulsant activity of leaf essential oils of *Laurus mobilis* against MES and PTZ. Phytomedicine. 2002; 9: 212-216.

[19]. Sonavane GS, Palekar RC, Kasture VS, Kasture SB. Anticonvulsant and behavioral actions of *Myristica fragrans* seeds. Indian Journal of Pharmacology. 2002; 34: 332-338.

[20]. Kulkarni SK. Experiment on intact preparation :Pharmacology of CNS. Handbook of experimental pharmacology. 3rd edition. Mumbai: Vallabh Prakashan. 1999; 131-134.