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ANTI-EPILEPTIC ACTIVITY OF *MALACHRA CAPITATA L.* ON MAXIMAL ELECTROSHOCK (MES) AND PENTYLENETETRAZOLE (PTZ) INDUCED SEIZURES MODELS

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ABSTRACT

The present study is an investigation of anti-epileptic activity of *Malachra capitata L.* (Family- Malvaceae) is a well-known plant which is being used in Indian traditional medicines for treating epilepsy and inflammation. The aqueous extract of *Malachra capitata L.* (AMC) 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. p.o. Animals were treated with AMC at doses of 250 and 500 mg/kg body weight. Study results showed, the mean duration of extensor phase of treated groups reduced significant level than compared to control group. In Pentylenetetrazol induced seizure model, onset of myoclonic spasm and clonic convulsion was delayed in the AMC treated groups. AMC showed anti-epileptic activity against MES and PTZ animal models. However, further studies still needed to be carried on exposure of the extract to humans.

Keywords: Anti-epileptic activity, *Malachra capitata L.*, Maximal Electroshock (MES), Pentylenetetrazole (PTZ).

INTRODUCTION

Epilepsy (Greek - to seize) is a common chronic neurological disorder characterized by seizures. These seizures are transient signs and/or symptoms of abnormal, excessive or synchronous neuronal activity in the brain. People have seizures when the electrical signals in the brain misfire. The brain's normal electrical activity is disrupted by these overactive electrical discharges, causing a temporary communication problem between nerve cells. About 50 million people worldwide have epilepsy, and nearly two out of every three new cases are discovered in developing countries.

Epilepsy is more likely to occur in young

children or people over the age of 65 years, however, it can occur at any time. As a consequence of brain surgery, epileptic seizures may occur in recovering patients. Epilepsy is usually controlled, but cannot be cured with medication, although surgery may be considered in difficult cases. However, over 30% of people with epilepsy do not have seizure control even with the best available medications. Not all epilepsy syndromes are lifelong some forms are confined to particular stages of childhood. Epilepsy should not be understood as a single disorder, but rather as syndromic with vastly divergent symptoms but all involving episodic abnormal electrical activity in the brain [1].

Traditional medicinal practices have remained as a component of health care system of many societies in spite of the availability of well-established alternatives [2]. Epilepsy is a condition, which causes seizures to occur. It is one of the most common chronic diseases affecting human beings. According to several publications this can amount to 70% of the people with epilepsies, with a high prevalence of about 0.8% in children below the age of seven years [3]. These observations have led to a shift in focus to the use of herbal remedies in the management of epileptic seizures, probably because these measures fit into the cultures of people and are not usually as expensive as the more refined orthodox drugs. Besides, these orthodox drugs possess many side effects, contraindications and possible interactions with drugs used simultaneously.

The alternative drug therapy for the management of this disease can be by the use of medicinal plants and their active principles. In the present study plants from India with a traditional claim of anti-epileptic activity and neuro protective properties were selected and a poly herbal extract was prepared in aqueous medium.

Malachra capitata (L.) is a herb belongs to family: Malvaceae. Description: Mostly erect, coarse, annual or perennial herb 1-2 m tall, throughout densely whitish- or yellowish-tomentose with stellate hairs and usually also moderately to copiously hispid with simple or stellate hairs to 2 mm long; roots long-petioled; stipules lanceolate, 5-15 mm long; blades orbicular to ovate, 2-10 cm long, palmately sinuate to 3-, 5-, or 7-lobed, lobes mostly obtuse, crenate to serrate, the base obtuse or truncate; flowers in axillary, pedunculate, bracteate heads, bracts 1-2 cm long, stipitate and subtended by paired, filiform bracteoles, conduplicate, suborbicular to ovate, obtuse or acute, entire or once or twice dentate, obtuse to cordate at base, prominently veined and whitish basocentrally; involucre bracts wanting; calyx tubular-campanulate, 4-8 mm long, 5-lobed to below middle, lobes ovate-lanceolate, white with brownish or reddish nerves; petals yellow, obovate, 10-15 mm long, slightly exceeding staminal column; mericarps 3-3.5 mm long, muticous, reddish veined, puberulent; seed obovoid-cuneate, about 2.5 mm long, black, whitish-pubescent about hilum.

The root of the *Malachra capitata* (L.) is traditional remedies for the many disease condition such as pain, hepatic cirrhosis, inflammation, diarrhea, convulsion, dementia, pyrexia, ulcer, healing of wounds [4-7]. On the basis of the traditional use of the plant for treating convulsion, but no previous pharmacological (or) clinical study was carried out to test the antiepileptic activity of this plant. Since the antiepileptic effect of *Malachra capitata* has been experimentally not confirmed. Therefore, the aim of the present investigation was to evaluate the claimed antiepileptic activity of *Malachra capitata* L. in albino wistar rats.

MATERIALS AND METHODS

Plant collection

The Plant material of *Malachra capitata* (L.) roots was collected from Tirunelveli District, in the Month of August 2011. The plant was authenticated by Dr. V. Chelladurai, Research Officer Botany. C.C.R.A.S., Govt. of India. The voucher specimen of the plant was deposited at the college for further reference.

Preparation of plant extract

The roots of the *Malachra capitata* (L.) are properly washed in tap water and then rinsed in distilled water. The rinsed roots are dried in an oven at 35°C for 4 days. The dried roots of *Malachra capitata* was crushed to obtain powder. These powdered samples are then stored in airtight polythene bags protected from sunlight until use. The aqueous extract of each sample was prepared by soaking 10g of powdered sample in 200ml distilled water for 12h. The extracts are then filtered using Whatmann filter paper. Percentage yield of aqueous extract of *Malachra capitata* was found to be 10.5 % w/w.

Preliminary phytochemical screening

The phytochemical examination of aqueous extract of *Malachra capitata* (L.) roots was performed by the standard methods [8].

Animals used

Male albino rats (150-220g) were obtained from the animal house and maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given *ad libitum*. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA.

Acute Toxicity Study

The acute toxicity of aqueous extract of *Malachra capitata* 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 [9].

Anti-epileptic activity

Effect on Maximal electroshock (MES) induced seizures

Albino wistar rats of either sex weighing 160 to 220 gm were divided into four groups of six animals each. The first group received vehicle control (1% w/v SCMC, 1ml/100 g) whereas Group-II received standard drug (Phenytoin, 25mg/kg) intraperitoneally, Group-III and IV, received aqueous extract of *Malachra capitata* L. (AMC) (250 and 500 mg/kg body weight) *p.o* respectively for 20 days. On the 20th day, Seizures are induced to all the

groups by using an Electro convulsimeter. Maximal electroshock seizures were elicited by a 60 Hz alternating current of 150 mA intensity for 0.2 sec. A drop of electrolyte solution (0.9% NaCl) with lignocaine was applied to the corneal electrodes prior to application to the rats. This increases the contact and reduces the incidence of fatalities. The duration of various phases of epilepsy were observed. The percentage protection was estimated by observing the number of animals showing abolition of Hindleg Tonic Extension (or) extension not greater than 90° [10].

Effect on Pentylentetrazole (PTZ) induced seizures

Albino wistar rats of either sex weighing 160 to 220 gm were divided into four groups of six animals each. The first group received vehicle control (1% w/v SCMC, 1ml/100 g) whereas Group-II received standard drug (Diazepam, 4mg/kg) intraperitoneally, Group-III and IV, aqueous extract of *Malachra capitata* L. (AMC) (250 and 500 mg/kg/body weight) *p.o* respectively for 20 days. On the 20th day, Pentylentetrazole (PTZ) (90mg/kg body weight, *s.c*) was administered to all the groups to induce clonic convulsions. Animals were observed for a period of 30mins post – PTZ administration. The parameters noted were mean onset time of convulsions, duration of convulsion and recovery/Death (% recovery or % of survival) due to PTZ [11].

Statistical analysis

The data were expressed as Mean \pm S.E.M. and

statistically analyzed using one way ANOVA followed by Tukey-Kramer's Multiple comparison test, $p < 0.05$ was considered significant.

RESULTS

Phytochemical analysis

The aqueous extract of *Malachra capitata* L. revealed the presence of steroids, Alkaloids, Reducing sugars, tannins, gums, flavonoids.

Effects of AMC on MES Induced Epilepsy

The AMC at doses of 250 mg/kg and 500 mg/kg were protect animals from seizures and significantly ($p < 0.01$) reduced the duration of tonic hindleg extension. Whereas, the standard drug phenytoin treated animals exhibits abolished tonic hindleg extension. Phenytoin treated animals have shown 100% protection against MES induced seizures whereas AMC 250 mg/kg and 500 mg/kg have shown 64.65% and 82.01% protection respectively (Table-1).

Effect of AMC on PTZ Induced epilepsy

The AMC at doses of 250 mg/kg and 500 mg/kg significantly delayed the onset of clonic convulsions ($p < 0.01$) in dose dependent manner. Whereas, the standard drug diazepam (4mg/kg, *i.p*) delayed the onset of clonic convulsions. Diazepam treated animals have shown 100% protection against PTZ induced seizures whereas AMC 250 mg/kg and 500 mg/kg have shown 44.90% and 60.74% protection respectively (Table-2).

Table 1. Effect of methanolic extract of *Malachra capitata* L. (AMC) On MES induced seizers in rats

Group	Design of treatment	Flexion	Extensor	Clonus	Stupor	Recovery	% protection
I	Vehicle control	8.4 \pm 0.15	15.32 \pm 0.34	20.42 \pm 0.34	39.56 \pm 0.52	192.34	0
II	Phenytoin 25mg/kg,i.p.	3.1 \pm 0.22	0**	8.79 \pm 0.55**	15.49 \pm 0.25**	95.28	100
III	AMC 250mg/kg,p.o	6.2 \pm 0.33*	5.29 \pm 0.1.54**	13.94 \pm 0.46	32.21 \pm 0.92	134.56	64.65
IV	AMC 500mg/kg,p.o	4.12 \pm 0.24**	2.72 \pm 0.2 6**	12.14 \pm 0.84*	16.28 \pm 0.29**	112.24	82.01

Values are expressed as mean \pm SEM of six observations. Comparison between Group I Vs Group II, Group II Vs Group III & Group IV. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's test.

* $p < 0.05$; ** $p < 0.01$; ns-non significant

Table 2. Effect of methanolic extract of *Malachra capitata* L. (AMC) On PTZ induced seizers in rats

Group	Design of Treatment	Onset of convulsions (sec.)	Duration of convulsion (sec.)	Protection mortality %
I	Vehicle control	165.29 \pm 0.68	59.19 \pm 0.18	50
II	Diazepam(4mg/kg)	569.19 \pm 1.14**	22.14 \pm 0.12**	100
III	AMC 250	423.24 \pm 1.32**	42.17 \pm 0.52*	83.33
IV	AMC 500	529.21 \pm 1.52**	29.36 \pm 0.27**	100

Values are expressed as mean \pm SEM of six observations. Comparison between Group I Vs Group II, Group II Vs Group III & Group IV. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test.

* $p < 0.05$; ** $p < 0.01$; ns-non significant

DISCUSSIONS AND CONCLUSION

In India, studies have reported the prevalence rate of epilepsy varying from 1710 from 9780 cases per million populations. The modern conventional antiepileptic drugs (AEDs) are effective in approximately 50% of patients, many cases still remain resistant to AED treatment [12]. These drugs are associated with vast array of side effects including chronic toxicity, teratogenicity, adverse effects on cognition and behavior among others [13]. 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.

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" [14,15]. This model based on observation of the stimulation by repeated electrical pulses induce in different neuronal structures one characteristic standard of epileptic activity [16]. In our present study, it is found that treatment with AMC 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 [17,18]. 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 [19,20]. Since, AMC significantly inhibited

generalized tonic-clonic seizures in MES test; it suggests the presence of anticonvulsant compounds.

We found that treatment with AMC 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 GABAA receptor complex [21].

Since PTZ has been shown to interact with the GABA neurotransmission [22] 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 [23-28], the antagonism of PTZ- induced seizures suggests the interaction of the AMC with the GABA-ergic neurotransmission.

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

REFERENCES

1. Blume W, Lüders H, Mizrahi E, Tassinari C, van Emde Boas W, Engel J. Glossary of descriptive terminology for ictal semiology: report of the ILAE task force on classification and terminology. *Epilepsia*, 42, 2001, 1212-1218.
2. Oyeka IC. *Interciencia*, 6, 1981, 156.
3. Ndoye NF. *Eur. J. Epilepsy*, 14, 2005, 7.
4. Ames BN, Cathcart R, Schwiers E, Hochstein P. Uric acid provides as antioxidant defense in humans against oxidant and radical caused aging and cancer: a hypothesis. *Proc Natl Acad Sci*, 78, 1981, 8658-62.
5. Rice-Evans CA, Miller NJ and Paganga G. Antioxidant properties of phenolic compounds. *Trends Plant Sci*, 2, 1997, 152-159.
6. Jigna, P, Rathish, N and Sumitra P. Preliminary screening of some folklore medicinal plants from western India for potential antimicrobial activity. *Indian J. Pharmac*, 37 (6), 2005, 408-409.
7. Okwu DE. Evaluation of chemical composition of indigenous species and flavoring agents. *Global J. Pure & Appl. Sci*, 7 (3), 2001, 455-459.
8. Harbone JP. Phytochemical methods, a guide to modern technique of plant analysis (*Chapmann and Hall, London*), 1973, 1-271.
9. OECD 2002. Acute oral toxicity. Acute oral toxic class method guideline 423 adopted in: Eleventh Addendum to the OECD, guidelines for the testing of chemicals organisation for economical co-operation and development.
10. Balakrishnan S, Pandhi P, Bhargava VK. Effects of Nimodipine on the efficacy of commonly used anti-epileptic drugs in rats. *Ind J Exp Biol*, 36, 1998, 51-54.
11. Kulkarni SK and George B. Significance of long term potentiation in cognitive functions and epilepsy. *Ind J Pharmacol*, 31, 1999, 14-22.
12. Heinemann UE, Draguhn E, FickernJ, Stabel and Zhang CL. Strategis for the development of drugs for pharmacological resistant epilepsies. *Epilepsia*, 35, 1994, S10- S21.
13. Raza MF, Shaheen MI, Choudhary A, Suria AU, Rahman S, Sombati and Delorenzo RJ. Anticonvulsant activities of the FS-1 Sub-fraction isolated from roots of *Delphinium denudatum*. *Phytother. Res*, 15, 2001, 426-430.

14. 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. *Epilepsy Res*, 2, 1988, 145-181.
15. Oliveira FA, Almeida RN, Sousa MFV, Barbosa-Filho JM, Diniz SA, Medeiros IA. Anticonvulsant properties of *N*-salicyloyltryptamine in mice. *Pharmacol Biochem Behav*, 68, 2001, 199-202.
16. Quintans-Júnior LJ, Almeida RN, Falcão ACGM, Agra MF, Sousa MFV, Barbosa-Filho JM. Avaliação da Atividade anticonvulsivante de plantas do Nordeste Brasileiro. *Acta Farm Bonaerense*, 21, 2002, 179-184.
17. Stables JP, Kupferberg HJ. The NIH Anticonvulsant Drug Development (ADD) Program: Preclinical Anticonvulsant Screening project. In: *Antiepileptic Drugs*, 4th edn. Ed. Levy RH, Mattson RH, Meldrum BS, Raven Press, New York. 1995, 4-17.
18. Kupferberg HJ. Antiepileptic drug development program: a cooperative effort of government and industry. *Epilepsia*, 30 (Suppl 1), 1989, S51-S56.
19. Macdonald RL and Kelly KM. Antiepileptic drug mechanisms of action. *Epilapsia*, 36, 1995, S2-S12.
20. White HS. Clinical significance of animal seizure models and mechanism of action studies of potential antiepileptic drugs. *Epilepsia*, 38 (Suppl. 1), 1997, 9.
21. Ramanjaneyulu R, Ticku MK. Interactions of pentamethylenetetrazole and tetrazole analogues with the picrotoxinin site of the benzodiazepine- GABA receptor-ionophore complex. *Eur. J. Pharmacol*, 98, 1984, 337-345.
22. De Deyn PP, D'Hooge R, Marescau B and Pei YQ. Chemical model of epilepsy with some reference to their applicability in the development of anticonvulsant. *Epilepsy Res*, 12, 1992, 87-110.
23. Coulter DA, Huganard JR and Prince DA. Characterization of the ethosuximide reduction of low-threshold calcium current in thalamic neurons. *Ann. Neurol*, 25, 1989, 582-593.
24. Harish Babu B, Mohana Lakshmi S, Saravana Kumar A. A review on traditional system of medicine for treats Epilepsy. *International Journal of Biological & Pharmaceutical Research*, 1(1), 2010, 1-6.
25. Senthil Kumar KK and Raj Kapoor B. Study on phytochemical profile and anti-epileptic activity of *oxalis corniculata* L. *International Journal of Biological & Pharmaceutical Research*, 1(1), 2010, 33-36.
26. E. Madhan Mohan, Ch. Krishna Mohan, P. Amudha. Effect of *indigofera tinctoria* extracts on Neurotransmitters concentrations in rat brain after Induction of seizure. *International Journal of Phytopharmacology*, 1(1), 2010, 23-27.
27. Amritpal Singh, Sanjiv Duggal, Asish Suttee, Aswinder Singh, Shankar Katekhaye. *Eclpita alba* linn. - ancient remedy with therapeutic Potential. *International Journal of Phytopharmacology*, 1(2), 2010, 57-63.
28. Harish Babu .B, Mohana Lakshmi S, Saravana Kumar A. Studies on phytochemical and anticonvulsant Property of *martyniya annua* linn. *International Journal of Phytopharmacology*, 1(2), 2010, 82-86.