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; involucral 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, 1/10<sup>th</sup> (200mg/kg) and 1/5<sup>th</sup> (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 20<sup>th</sup> day, Seizures are induced to all the
groups by using an Electro convulsiometer. 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 Pentylenetetrazole (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, Pentylenetetrazole (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 ± 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 seizures in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Design of treatment</th>
<th>Flexion (sec.)</th>
<th>Extensor (sec.)</th>
<th>Clonus (sec.)</th>
<th>Stupor (sec.)</th>
<th>Recovery (sec.)</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle control</td>
<td>8.4±0.15</td>
<td>15.32±0.34</td>
<td>20.42±0.34</td>
<td>39.56±0.52</td>
<td>192.34</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>Phenytoin 25mg/kg,i.p.</td>
<td>3.1±0.22</td>
<td>0</td>
<td>8.79±0.55</td>
<td>15.49±0.25</td>
<td>95.28</td>
<td>100</td>
</tr>
<tr>
<td>III</td>
<td>AMC 250mg/kg,p.o</td>
<td>6.2±0.33</td>
<td>5.29±0.154</td>
<td>13.94±0.46</td>
<td>32.21±0.92</td>
<td>134.56</td>
<td>64.65</td>
</tr>
<tr>
<td>IV</td>
<td>AMC 500mg/kg,p.o</td>
<td>4.12±0.24</td>
<td>2.72±0.26</td>
<td>12.14±0.84</td>
<td>16.28±0.29</td>
<td>112.24</td>
<td>82.01</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± 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 seizures in rats

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

Values are expressed as mean ± 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 to 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 epileptiform 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 GABA 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
2. Oyeka IC. Intericiencia, 6, 1981, 156.


