

## ANTICONVULSANT ACTIVITY OF ETHANOLIC EXTRACT OF FRUITS OF *Terminalia chebula* ON EXPERIMENTAL ANIMALS

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### ABSTRACT

**Objective:** To study the anticonvulsant activity of ethanolic extract of *Terminalia chebula* in albino mice.

**Methods:** The anticonvulsant activity of ethanolic extract of fruits of *Terminalia chebula* (200 and 500 mg/kg, p.o.) in mice was assessed by using maximum electroshock seizure (MES) test, Pentylenetetrazole (PTZ), and picrotoxin (PC) test.

**Results:** The ethanolic extract of *Terminalia chebula* significantly reduced the duration of seizures induced by maximal electroshock (MES). The ethanol extract in doses of 200 and 500 mg/kg conferred protection (17 and 50%, respectively) on the mice. The same doses also protected animals from pentylenetetrazole-induced tonic seizures and significantly delayed the onset of tonic seizures produced by picrotoxin.

**Conclusion:** The ethanolic extract of *Terminalia chebula* (EETC) possess anticonvulsant activity since it reduced the duration of seizures produced by maximal electroshock and delayed the latency of seizures produced by pentylenetetrazole and picrotoxin.

**Key words:** Anticonvulsant activity, *Terminalia chebula*, seizures, MES, PTZ, PC.

### 1. INTRODUCTION:

Epilepsy is a neurological disorder in which a person has two or more recurrent unprovoked seizures. Seizure is a paroxysmal event that is due to abnormal, excessive and hypersynchronous discharge from an aggregate of central nervous system neurons. Epilepsy is the second most common disorder of the central nervous system after stroke and up to 5% of world population develops epilepsy in their lifetime [1]. The current therapy of epilepsy with modern antiepileptic drugs is associated with side effects, dose-related and chronic toxicity, as well as teratogenic effects, and approximately 30% of the patients continue to have seizures with current antiepileptic drugs therapy. Traditional system is believed to be an important source of chemical substances with potential

therapeutic effects. Herbs may have antiepileptic effects in several ways. Some herbs may increase brain levels and/or the binding of nerve transmitter gamma aminobutyric acid (GABA), which quiets nerve activity [2, 3]. Concurrently, phytochemicals identified from traditional medicinal plants are presenting an exciting opportunity for the development of new types of therapeutics. This has accelerated the global effort to harness and harvest those medicinal plants that bear substantial amount of potential phytochemicals showing multiple beneficial effects in convulsion.

*Terminalia chebula* Retz. (Combretaceae) is one of the ingredients in a polyherbal formulation, "Geriforte" an Ayurvedic Rasayana that is known to promote physical and mental health and improve immune power of the

organism so that the body can tolerate any nature of stress [4, 5]. *Terminalia chebula* also possesses Laxative, Hypolipidemic, Antioxidant, Hepatoprotectant, Antiviral and Antibacterial activity [6, 7]. The plant is an important constituent of an herbal formulation contains the name TRIPHALA is very popular traditional medicine for chronic disorder like diabetes, nervine disorder & epilepsy [8, 9]. The aim of the present study was, therefore, to evaluate the anticonvulsant potential of the ethanol extract of fruits of *Terminalia chebula* (EETC) in experimental animal models, to provide a pharmacological justification for the traditional use of the plant's fruits in the management of epilepsy in some rural parts of India.

## **2. MATERIALS AND METHODS:**

### **Plant material**

The fruits of *Terminalia chebula* were collected from Nagaon, Assam and it was authenticated by Dr. Shiddamallayya N, Regional Research Institute (Ay.) Ashoka Pillar, Bangalore. The fruits of *Terminalia chebula* were separated and shade dried. The dried material was reduced to a coarse powder and was successively extracted in soxhlet apparatus using petroleum ether, chloroform and alcohol (according to their increasing polarity for 24 hrs). The solvents were redistilled and ethanolic extract was concentrated under reduced pressure and air dried. The yield of ethanolic extract was found to be 36%w/w. The extract was solubilized in aqueous solution.

### **Experimental animals**

Swiss mice of either sex, 8-10 weeks old, weighing about 25-30 g were used in experiments. Animals were housed in polypropylene cages maintained under standard condition (12 hours light / dark cycle; 25 ± 3°C, 45-65% humidity) and had free access to standard feed and water *ad libitum*. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. All experimental

protocols were reviewed and accepted by the Institutional Animal Ethical Committee (IAEC) prior to the initiation of the experiment.

### **Acute toxicity study**

The acute oral toxicity of ethanolic extract of *Terminalia chebula* (EETC) were determined in 3 hours fasted female albino mice by fixed dose method according to OECD guidelines No. 420 [10].

### **Assessment of Anticonvulsant activity**

#### **Maximal Electrical Shock (MES) -induced seizures:**

The animals were divided in four groups (n = 6). Group I served as vehicle control group. Groups III, and IV served as test groups treated with the extract (200 and 500 mg/kg, p.o., 60 min), respectively, and group II served as reference standard group received phenytoin (25 mg/kg, i.p., 20 min), prior to the induction of convulsion. The number of animals protected from hind limb tonic extension seizure (HLTE) and the time spent in this position were determined for each dose group [11].

#### **Pentylenetetrazole (PTZ) -induced seizures:**

The animals were divided in four groups (n = 6). Group I served as vehicle control group. Groups III, and IV served as test groups treated with the extract 200 and 500 mg/kg, p.o. The EETC was administered 60 min before the subcutaneous injection of PTZ (80 mg/kg). Group II received diazepam (2.0 mg/kg, i.p.) as a reference standard. The animals were observed for onset of convulsion upto 30 min after PTZ. Hind limb extension was taken as tonic convulsion. The onset of tonic convulsion and the number of animals convulsing or not convulsing within the observation period were noted. The ability of the plant extract to prevent or delay the onset of the hind limb extension exhibited by the animals was taken as an indication of anticonvulsant activity [12, 13].

#### **Picrotoxin (PC) -induced seizures:**

The animals were divided in four groups (n = 6). Group I served as vehicle control group. Groups III, and IV served as test groups treated with the extract 200 and 500 mg/kg, p.o. The EETC was administered 60 min before the subcutaneous injection of picrotoxin (10 mg/kg, i.p.). Group II received diazepam (2.0 mg/kg, i.p.) as a reference standard. The animals were observed for onset of convulsion upto 30 min after PC. Hind limb extension was taken as tonic convulsion. The onset of tonic convulsion and the number of animals convulsing or not convulsing within the observation period were noted. The ability of the plant extract to prevent or delay the onset of the hind limb extension exhibited by the animals was taken as an indication of anticonvulsant activity [11, 13].

#### **Statistical analysis**

All the values are expressed as mean  $\pm$  SEM. Statistical differences between means were determined by one-way ANOVA followed by Dunnett's post hoc test.  $p < 0.05$  was considered as significant.

### **3. RESULT:**

#### **Phytochemical screening**

The preliminary phytochemical screening of the ethanolic extract of *Terminalia chebula* revealed the presence of carbohydrates, anthraquinone glycosides, Saponin glycosides, flavanoids, tannins and polyphenols.

#### **Acute toxicity study**

Acute oral toxicity studies of the ethanolic extract of *Terminalia chebula* did not exhibit any sign of toxicity up to 2000 mg/kg body weight. Since there was no

mortality of the animals found at highest dose, hence 200 (1/10<sup>th</sup>) and 500 (1/4<sup>th</sup>) mg/kg, p.o. doses of extract were selected for evaluation of Anticonvulsant activity.

#### **Anticonvulsant activity**

Maximal electroshock produced hind limb tonic extension seizures (HLTE) in all the animals. The vehicle-treated mice showed tonic hind limb extension for duration of  $14.83 \pm 0.40$  sec. The EETC at doses of 200 and 500 mg/kg, respectively, protected 17% and 50% of mice and significantly reduced the duration of the seizures. However, phenytoin completely abolished the MES-induced tonic seizures in all the animals. (Table. 1).

Pentylentetrazole produced tonic seizures in all the animals used. The EETC, in doses of 200 and 500 mg/kg, respectively, protected 33% and 83% of mice against seizures, and significantly delayed the latency of the seizures. The standard antiepileptic drug, diazepam inhibited seizures completely. (Table. 2).

Picrotoxin produced tonic seizures in all the animals. The EETC (200 and 500 mg/kg) did not affect the incidence of seizures, but significantly prolonged latency of seizures. The standard antiepileptic drug, diazepam protected 83% the animals from convulsions. (Table. 3).

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**Table 1:** Effect of ethanol extract of the fruits of *T. chebula* (EETC) on maximal electroshock (MES) - induced seizures in mice.

Treatment	Dose	No. of animal convulsed/No. of animal used	% protection	Duration of HLTE (sec) Mean ± SEM
Control	---	6/6	00	14.83 ± 0.40
Standard (phenytoin)	25 mg/kg, .i.p.	0/6	100	---
Low Dose of EETC	200 mg/kg, p.o.	5/6	17	12.50** ± 0.56
High Dose of EETC	500 mg/kg, p.o.	3/6	50	9.33** ± 0.21

\* Results are expressed as Mean ± SEM; (n=6). Significance at  $P<0.05^*$ ,  $P<0.01^{**}$  as compared to control.

**Table 2:** Effect of ethanol extract of the fruits of *T. chebula* (EETC) on Pentylenetetrazole (PTZ) induced seizures in mice.

Treatment	Dose	No. of animal convulsed/No. of animal used	% protection	Latency of tonic convulsion (min) Mean ± SEM
Control	---	6/6	00	5.53 ± 0.40
Standard (diazepam)	2.0 mg/kg, .i.p.	0/6	100	---
Low Dose of EETC	200 mg/kg, p.o.	4/6	33	12.80** ± 0.47
High Dose of EETC	500 mg/kg, p.o.	1/6	83	15.33

\* Results are expressed as Mean ± SEM; (n=6). Significance at  $P<0.05^*$ ,  $P<0.01^{**}$  as compared to control.

**Table 3:** Effect of ethanol extract of the fruits of *T. chebula* (EETC) on Picrotoxin - induced seizures in mice.

Treatment	Dose	No. of animal convulsed/No. of animal used	% protection	Latency of tonic convulsion (min) Mean ± SEM
Control	---	6/6	00	14.13 ± 0.57
Standard (diazepam)	2.0 mg/kg, .i.p.	1/6	83	21.85
Low Dose of EETC	200 mg/kg, p.o.	6/6	00	15.40 ± 0.42
High Dose of EETC	500 mg/kg, p.o.	6/6	00	19.12* ± 0.31

\* Results are expressed as Mean ± SEM; (n=6). Significance at  $P<0.05^*$ ,  $P<0.01^{**}$  as compared to control.

#### 4. DISCUSSION:

The observation of present study indicates that ethanol extract of *T. Chebula* (EETC) possesses anticonvulsant activity in mice. GABA is the major inhibitory neurotransmitter in the brain while glutamic acid is an excitatory neurotransmitter in the brain. The inhibition of GABA neurotransmitter and the enhancement of the

action of glutamic acid have been shown to be the underlying factors in epilepsy. The present study shows that the ethanol extract of fruits of *T. Chebula* protected some of the animals against seizures induced by maximal electroshock. Antiepileptic drugs which inhibit voltage-dependent Na<sup>+</sup> channels, such as phenytoin can prevent MES-induced tonic extension

[14, 15]. Pentylenetetrazole may elicit seizures by blocking GABA/Cl<sup>-</sup> channel complex. Picrotoxin induces seizure, by blocking the chloride channels linked to GABA-A receptor. Diazepam, a standard antiepileptic drug is believed to produce their effects by enhancing GABA mediated opening of chloride channel on GABA-A receptor leading to more chloride ion entering the neuron which in turn decreases the neuronal activity in the brain [16]. In the present study diazepam shown to antagonize the seizure induced pentylenetetrazole and picrotoxin. The extract was also shown to delay the latency of pentylenetetrazole and picrotoxin induced seizures, suggesting that the extract exhibiting anticonvulsant affect, probably by opening the chloride channels associated with GABA receptors. Thus, in conclusion, *T. Chebula* possesses anticonvulsant property against the MES, PTZ and PC induced seizures. From the above result, further investigation is warranted in order to isolate and identify the specific molecules which are responsible for the anticonvulsant activity.

#### 5. BIBLIOGRAPHY:

- 1) Rao S, Subbalakshmi K. An experimental study of the anticonvulsant effect of amlodipine in mice. *Singapore Med J* 2010; 51(5): 424-8.
- 2) Visweswari G, Shiva Prasad K, Loknath V. The antiepileptic effect of centella asiatica on the activities of Na<sup>+</sup>/K<sup>+</sup>, Mg<sup>2+</sup> and Ca<sup>2+</sup>-ATPases in rat brain during Pentylenetetrazole-induced epilepsy. *Ind J Pharmacol* 2010; 42(2): 82-6.
- 3) Mansoorkhani M J K, Moein M, Oveisi N. Anticonvulsant Activity of Teucrium polium Against Seizure Induced by PTZ and MES in Mice. *Iran J Pharm Res* 2010, 9 (4): 395-401.
- 4) Singh N, Nath R, Mishra N, Kohli RP. An experimental evaluation of Anti-stress effects of Geriforte. *J. Crude Drug Res* 1978; 3: 125-32.
- 5) Rege NN, Thatte UM, Dahanukar SA. Adaptogenic properties of six rasayanas herbs used in ayurvedic medicine. *Phytother Res* 1999; 13(4): 275-91.

- 6) Kokate CK, Purohit AP, Gokhale SB. *Pharmacognosy*. Nirali Prakashan. Pune 2001; 12<sup>th</sup> ed: 216-7.
- 7) Sukh Deb. *A Selection of Prime Ayurvedic Plant Drugs*. Anamaya Publishers, New Delhi, 2006: 410-6.
- 8) *Indian Herbal Pharmacopoeia revised new 2<sup>nd</sup> Edition*, 2002: 439-48.
- 9) Chattopadhyay R.R., Bhattacharyya S.K. *Plant Review Terminalia chebula: An update*. *Pharmacog Rev* 2007; 1(1): 151-6.
- 10) Mrs. Prema Veeraraghavan. *Expert consultant, CPCSEA, OECD guide line No. 420; Oct 2000*.
- 11) Hegde K, Thakkar SP, Joshi AB, Sharstry CS, Chandrashekar KS. Anticonvulsant activity of Carissa carandas Linn. Root extract in experimental mice. *Trop J Pharm Res* 2009; 8(2): 117-25.
- 12) Shirish DA, Veena SK, Sajjay BK. Anticonvulsant activity of roots and rhizomes of Glycyrrhiza glabra. *Ind J of Pharmacol* 2002; 34: 251-5.
- 13) Amabeoku GJ, Chikuni O. Cimetidine-induced seizures in mice. *Biochem Pharmacol* 1993; 46(12): 2171-5.
- 14) Westmoreland BF, Benarroch EE, Dube JR, Regan TJ, Sandok BA. *Medicinal neurosciences*, Rochester: Margo Foundation, 1994, pp 307- 12.
- 15) Rang HP, Dale MM, Ritter JM, Moore PK. *Pharmacology*. India: Churchill Livingstone, 2005; 5<sup>th</sup> ed: 456-73.
- 16) McDonald RL, Kelly KM. Antiepileptic drugs: Mechanisms of action. *Epilepsia* 1993; 34:S1-S8.

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