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Research Article

"EVALUATION OF ANTICONVULSANT POTENTIAL OF METHANOLIC EXTRACT OF STEM BARK OF *BOMBAX CEIBA* IN ALBINO MICE"

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ABSTRACT

To evaluate anticonvulsant potential of Methanolic extract of stem bark of Bombax ceiba in albino mice. The Methanolic extract of stem bark of Bombax ceiba was studied for its anticonvulsant effect on maximal electroshock induced seizures and Pentylenete trazole induced seizures and Strychnine induced seizures in mice. The duration of hind limb tonic extension in case maximal electroshock induced seizures and onset of convulsions and percentage protection offered by the extract in case of Pentylene tetrazole induced seizures and Strychnine induced seizures were noted. The Methanolic extract of stem bark of Bombax ceiba significantly reduced the duration of seizures by maximal electroshock induced seizures and delayed the onset of tonic seizures produced by Pentylene tetrazole and Strychnine. The data suggest that the Methanolic extract of stem bark of Bombax ceiba will produce its anticonvulsive effect via non-specific mechanisms may be by acting on voltage dependent sodium ion channels since it reduced the duration of seizures produced by maximal electroshock, GABAergic system as well as glycinergic system as it delayed the latency of seizures produced by Pentylene tetrazole and Strychnine.

Keywords: *Bombax ceiba, Anticonvulsant activity; maximal electroshock seizure; Pentylene tetrazole; Strychnine.*

INTRODUCTION

Epilepsy is a common chronic neurological disorder characterized by recurrent unprovoked seizures. These seizures are transient signs and/or symptoms of abnormal, excessive or synchronous neuronal activity in the brain [1]. About 50 million people worldwide have epilepsy, with almost 90% of these people being in developing countries. The estimated proportion of the general population with active epilepsy (i.e. continuing seizures or with the need for treatment) at a given time is between 4 and 10 per 1000 people Epilepsy is more likely to occur in young children or people over the age of 65 years; however it can occur at any time [2].

A large number of agents called antiepileptic drugs are available to treat various types of seizures with the objective to reduce seizure frequency and severity with in a framework of acceptable level of side effects. The ideal antiseizure drug would suppress all seizures without causing any unwanted effect. Unfortunately drugs used currently not only fail to control seizures activity in some patients but they frequently cause side effects. In addition safety, tolerability, efficiency, expenses especially in long term therapy, serum drug monitoring etc. are other limitations with synthetic antiepileptic drugs. Further a large number of drug interactions seen with almost all current antiepileptic drugs make it more difficult to control seizures easily [3].

Traditional systems of medicine are popular in developing countries and up to 80% of the population relies on traditional medicines or

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folk remedies for their primary health care need [4]. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Several plants such as *With aniasomnifera (amhar), Ocimumsanctum, Brahmigrihta (bacopa), Catharanthusroseus, Caesalpinia crista, Citrus sinensis, Daturastramonium, Ricinuscommunis, Terminaliaglaucescens, Tetrapleuratetraptera, Sennasinguena, Jatrophagossypiifolia, Menthacardifolia*used for the treatment of epilepsy in different systems of traditional medicine have shown activity when tested in modern bioassays for the detection of anticonvulsant activity and many such plants are yet to be scientifically investigated[5].

The drugs which are extensively used in the treatment of epilepsy in ayurvedic and unani system of medicineare *Bacopamonnieri*[6], *Ficusplatyphylla* [7], *Viscumcapense*[8], *Clerodendruminfortunatum*[9], *Carissa carandas* [10], *Spondiasmombin* [11], *Boerhaaviadiffusa* [12] and *Opuntia vulgaris*[13]. The saponins which are present in these drugs are mainly thought to be responsible for their anti-epileptic activity. In the present study *Bombax ceiba* contains the saponin as its main chemical constituents so the scope of this plant to treat epilepsy will be evaluated because of the presence of saponins as their main chemical constituent.

Bombax ceiba Linn.(Syn. *B. malabaricum* DC, *Salmaliamalabarica* Schott. &Endl.) Belonging to family Bombacaceae, known as Salmali in Ayurveda one of the important medicinal plants in tropical and subtropical India. It has number of traditional uses and its medicinal usage has been reported in the Indian traditional systems of medicine such as Ayurveda, Siddha and Unani. According to Ayurveda, it has stimulant, astringent, haemostatic, aphrodisiac, diuretic, antidiarrhoeal, cardiotonic, emtic, demulcent, antidysenteric, alterative, and antipyretic properties. It has been specifically proven for pharmacological activities like In-vitro Anti-inflammatory, Anti-diabetic, Anti-obesity, Hypotensive, Antioxidant, Antiangiogenic, Antimicrobial, Cytotoxicity, Aphrodisiac and Antipyretic [14]. Chemically, Bombaxceiba contains various biologically active phytoconstituents including saponins, tannins, gums, Lupeol, βsitosterol and βsitosterol-D-glucoside. Two compound lup-20 (29) en-3b-ol, named BC-1 and 2-hexyl-7, 8-dimethyl-1, 4-naphthaquinone, named ceibanaphthaquinone were isolated from the stem bark [15]. Considering the presence of saponins as one of its chief phytoconstituent the present study was carried out to evaluated the anticonvulsant potential of methanolic extract of stem bark of *Bombax ceiba* in albino mice

MATERIALS:

Plant materials

The stem bark of *Bombax ceibas were* collected from the medicinal plant supplier were authenticated by Dr. P. Veera Reddy, Professor, Government Ayurvedic College, Warangal, Telangana.

Preparation of plant extract:

The dried powder material (500 g) of the stem bark of *Bombax ceiba* was extracted with 2000 ml. of methanol in a soxhlet apparatus. The methanol extract was then distilled, evaporated and dried in vacuum. The resultant extract was brownish dried gum resin in nature which was then weighed; percentage yield of extract was calculated and as per requirement the extract was suspended in appropriate volume of 1% Tween 80 for the experiment.

Animals:

Albino mice of either sex, weighing about 25-30 grams were used in experiments. Animals were housed in polypropylene cages maintained under standard condition (12 hours light / dark cycle; 25± 3 ^{0} C) and had free access to standard rat/mice feed (Hindustan Lever Ltd., India) and water *ad libitum*. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. The experiments on animals were conducted in accordance with the IAEC and CPCSEA and our protocols were duly approved by the Institutional Ethical Committee.

Acute Toxicity Studies:

A preliminary pharmacological study was conducted to assess the gross behavioral

effects and safety effects of the drug. The acute toxicity study was carried on mice weighing about 20-25gm as per ICH guidelines [16].Overnight fasted mice received test extract at a dose of 100mg/kg intraperitoneally and mortality was observed for 14 days. If mortality was not observed for any animal then the procedure was repeated again with higher doses such as 300, 1000 and 2000 mg/kg.

The animals were observed continuously for 2 h for general behavioral, neurological, autonomic profiles and to find out percentage of mortality observations were tabulated according to Irwin's table [17]. For this the following check list was employed:

Stimulation: Hyperactivity, Piloerection, Twitching, Rigidity, Irritability, Jumping, Clonic convulsions, Tonic convulsions

Depression: Ptosis, Sedation, Loss of righting reflex (sleep), Loss of traction, Loss of Pinnal reflex, Catatonia, Ataxia, Loss of muscle rigidity, Analgesia.

Autonomic reflexes: Straub's tail, Laboured respiration, Cyanosis, Reddening, Abnormal secretions, balancing.

METHODS EMPLOYED IN SCREENING OF ANTICONVULSANT ACTIVITY:

Electrically Induced Seizures: [18]

Maximal electroshock test is the most widely used animal model in the antiepileptic drug discovery. In brief, tonic convulsions of the hind extremities of the mice was induced by passing alternating electrical current of 50 Hz and 150 mA for 0.2 sec through corneal electrodes. The animals were divided into three groups containing six animals each group. Group 1was treated as control and administered with 1ml of 1% Tween 80 (i.p), Group 2was treated with methanolic extract of *Bombax ceiba* (250mg/kg, i.p) and Group 3was treated with Phenytoin (25mg/kg, i.p)for 15 days 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.

Pentylenetetrazole Induced Seizures:[19]

Pentylenetetrazole is a Chemo-convulsant agent which exerts its anticonvulsant effect by inhibiting the activity of GABA at GABAA receptors. The animals were randomly divided into three groups containing six animals each. Group 1was treated as control and administered with 1ml of 1% Tween 80 (i.p), Group 2 was treated with methanolic extract of *Bombax ceiba* (250mg/kg, i.p) and Group 3was treated with diazepam (5mg/kg, i.p). Seizures were induced in mice with standard convulsing agents, pentylenetetrazole (60 mg/kg., s.c,) after 30 min of drug treatment and the animals were observed for one hour for tonic convulsion episode. 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 of 24 hrswas 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.

Strychnine Induced Seizures: [20]

The convulsing action of strychnine is due to interference with post synaptic inhibition mediated by glycine. The animals were randomly divided into five groups containing six animals each. Group 1was treated as control and administered with 1ml of 1% Tween 80 (i.p), Group 2 was treated with methanolic extract of *Bombax ceiba* (250mg/kg, i.p) and Group 3was treated with diazepam (5mg/kg, i.p). 30 min after drug treatment tonic convulsion were induced in mice by strychnine (2 mg/kg, i.p). The latency to the onset of tonic convulsion and the lethality during the following 24 hour was recorded. 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.

STATISTICAL ANALYSIS: [21]

The results for electrically induced seizures, pentylenetetrazole induced seizures and strychnine induced seizures were expressed as Mean ± Standard Error of Mean. The Significance of differences among the group

was assessed using one way analysis of variance (ANOVA). The test followed by Dunnet's test p values less than 0.05 were considered as statistically significant.

RESULT:

The percentage yield of methanolic extract of *Bombax ceiba was found to be* 14.5w/v.

Acute Toxicity Studies:

The extract was found to be safe at 2000mg/kg body weight by intraperitoneal route. After 48 hrs mice was found to be well tolerated. There was no mortality and no signs of toxicity. The stimulatory depressive and autonomic profile was found to be normal.

Assessment of Anticonvulsant Activity of methanolic extract of *Bombax ceiba* by:

Electrically Induced Seizures:

MES produced hind limb tonic extension seizures in all the animals used. The control mice showed tonic limb extension for the duration of 19.63±0.29 sec. Methanolic extract of *Bombax ceiba* at the dose of 250 mg/kg protected 83.33 % of mice and alter the incidence of seizures elicited by MES to a significant extent. The standard antiepileptic drug, Phenytoin (25mg/kg) profoundly antagonized the seizures produced by MES. The results are given in table 1.

Table 1: Effect of Extract on Maximal Electroshock Induced Seizures in Mice

S.no	Treatments	Dose, i.p	Duration of HLTE(sec)	Quantal protection	% protection
1	Control	1ml of 1% Tween 80	19.63±0.29	4/6	66.67
2	***Bombax ceiba***	250 mg/kg	13.39 ± 0.38*	5/6	88.33
3	Phenytoin	25 mg/kg	8.41±0.31**	6/6	100

Values are mean ± S.E.M. (n=6) *P < 0.05 and **P < 0.01 when compared to controlusing One way ANOVA followed by Dunnet's test.

Pentylenetetrazole Induced Seizures:

Intraperitoneal administration of Pentylenetetrazole induced tonic convulsions with 16.67% protection in the control group. Diazepam at 4 mg/kg protected the animals from Pentylenetetrazole induced convulsions with no tonic convulsions occurring within the period of observation. Methanolic extract of *Bombax ceiba* at the dose of 250 mg/kg protected 83.33% of mice and significantly delayed the latency of seizures. The above results are tabulated in table 2.

Table 2: Effect of Extract on Pentylenetetrazole Induced Seizures in Mice

S.no	Treatments	Dose, i.p	Onset of seizures(min)	Quantal protection	% protection
1	Control	1ml of 1%	3.64 ± 0.37	1/6	16.67
2	*Bombax ceiba*	250 mg/kg	6.59 ± 0.42**	5/6	83.33
3	Diazepam	5 mg/kg	12.48 ± 0.54***	6/6	100

Values are mean ± S.E.M. (n=6) *P < 0.05 and **P < 0.01 when compared to controlusing One way ANOVA followed by Dunnet's test.

Strychnine Induced Seizures:

Strychnine (2 mg/kg) elicited tonic seizures in all the animals used. A dose of 250 mg/kg of methanolic extract of stem bark of *Bombax ceiba* significantly prolonged the latency of seizures produced by strychnine and provided 83.33% of protection to the animals. The standard anti-epileptic drug diazepam significantly delayed the latency of seizures and totally protected the animals.

Table 3: Effect of Extract on Strychnine Induced Seizures in Mice

S.no	Treatments	Dose, i.p	Onset of seizures(Mins)	Quantal protection	% protection
1	Control	1ml of 1% Tween	4.16 ± 0.46	1/6	16.67
2	*Bombax ceiba*	250 mg/kg	7.60 ± 0.33 **	5/6	83.33
3	Diazepam	5 mg/kg	10.95 ± 0.44 ***	6/6	100

Values are mean ± S.E.M. (n=6) *P < 0.05 and **P < 0.01 when compared to controlusing One way ANOVA followed by Dunnet's test.

DISCUSSION:

This study has been carried out to establish the antiepileptic effect of methanolic extract of stem bark of *Bombax ceiba*. Assessment of Electrically Induced Seizures, Pentylenetetrazole Induced Seizures and Strychnine Induced Seizures were performed for screening of antiepileptic effect of extract.

Epilepsy is "an episodic disorder of the nervous system arising from the excessive synchronous and sustained discharge of a group of neurons". A seizure results when a sudden imbalance occurs between the excitatory and inhibitory forces with in the network of cortical neurons. Excitatory and inhibitory currents are primarily mediated by different channels including voltage- and ligand-gated channels. Voltage-gated channels include sodium and calcium channels. Ligand-gated channels include GABA and glutamate channels. Most of the pharmacologic agents used in treating epilepsy target these different channels [22].

MES produces convulsions mainly by opening the voltage dependent sodium ion channels thereby causing the repetitive firing of action potential. It mainly identifies the agents with the activity against generalized tonic clonic seizures. It is generally useful for screening of drugs such as phenytoin which blocks the frequency of voltage dependent sodium channel opening. [23]The result of present study showed that the methanolic extract of stem bark of *Bombax ceiba* decreased the duration of tonic hind leg extension in maximal electroshock-induced seizures may be by preventing the repetitive firing of action potential by blocking sodium ion conductance.

Pentylenetetrazole exerts its convulsing effect by inhibiting the activity of GABA at $GABA_A$ receptors. GABA is a major inhibitory neurotransmitter which is implicated in epilepsy. The enhancement and inhibition of GABA will attenuate and enhance convulsions, respectively. GABA inhibits the neuronal responsiveness (excitability) and by increasing the chloride ion conductance through opening of the chloride-ion channel [24]. The drugs which inhibit the neuronal activity by enhancing the GABA such as diazepam which causes the hyperpolarization of cell thereby increasing the chloride channels conductance are mainly screened by this method. Methanolic extract of stem bark of *Bombax ceiba* increased the latency of convulsion and decrease the seizure threshold by acting on the GABAergic system

The convulsing action of strychnine is due to interference with post synaptic inhibition mediated by glycine. It directly antagonizes the inhibitory spinal cord and brainstem reflexes of glycine and thus increasing the spinal reflexes. This glycine potentiates the convulsive effect of strychnine by acting on Gly2 receptors which are functionally linked to NMDA receptors present in the central nervous system and that interaction of glycine with this receptor leads to excitatory rather than inhibitory effects [25]. Strychnine induced

convulsions is used for screening of the drugs which acts on the glutaminergic system and decrease the level of excitatory amino acids. It serves as a model of therapy resistant seizures arising from the lower brainstem and spinal cord [172]. There was increased time for onset of convulsions In this study, Methanolic extract of stem bark of *Bombax ceiba* showed protection increase the latency of convulsion against *strychnine induced convulsions* probably act on glycinergic transmission.

CONCLUSION:

The present study was conducted to evaluate the anticonvulsant potential of Methanolic extract of stem bark of *Bombax ceiba* in experimental mice by Maximal electroshock method, Pentylenetetrazole induced seizures and Strychnine induced seizures. The extract may exert it effect by blocking high frequency firing of neurons through action on voltage gated Sodium ion channels, Potentiates $GABA_A$ responses and decreasing synaptic release of glutamate. "So the present study indicates the Methanolic extract of stem bark of *Bombax ceiba* possess the anti-epileptic activity by different mechanisms" However, the exact mechanism and the active principle by which this extract exert its action remain unclear. Further studies are required to study precise mechanism of actions.

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