

**Study on the Anti-Seizure Activity of Methanolic Extracts
of *Indigofera Tinctoria* (L.)**

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Summary

The present report is an investigation of anti seizure activity of *Indigofera tinctoria* (L.), is a well known plant which is being used in Indian Traditional Medicines for epilepsy, nervous disorders, bronchitis and liver ailments. The methanolic (90%) extract of *Indigofera tinctoria* (L.) (MEIT) was subjected to acute toxicity and then screened for antiepileptic activity on Maximal Electroshock (MES) and Pentylene tetrazole (PTZ) induced seizures models in albino wistar rats. Acute toxicity of extract was non toxic up to the recommended dose 2000mg/kg body weight orally as per OECD guidelines No.423. Animals were pretreated with MEIT at the doses of 200 and 400mg/kg body weight. The study reported the significant delay in clonic seizure induced by PTZ and dose dependent decrease in duration of hindleg extensor phase in MES model. In MES model, MEIT showed significant reduction in duration of hindleg extension with 200 mg/kg dose and effect was dramatically reduced with 400mg/kg. Similar dose dependent results were obtained in PTZ model by delayed the onset of clonic convulsions. The complete protective effect against mortality was reported in both the tests. This study predicted possible mechanism of the formulation mediated through chloride channel of the GABA or benzodiazepine receptor complex. However, the exact mechanism of action is not clear, but attributed to its antiepileptic effect. The methanolic extract of *Indigofera tinctoria* (L.) deserves further investigation for detailed elucidation of active constituents and the mechanisms of action.

Keywords: Antiseizure activity, Traditional Medicine, *Indigofera tinctoria*, Maximal Electroshock, Pentylene tetrazole

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Introduction

Epilepsy is among the most prevalent of the serious neurological disorders, affecting from 0.5 to 1.0% of the world's population ^[1]. In India, studies have reported the prevalence rate of epilepsy varying from 1710 to 9780 cases per million populations ^[2]. Despite the optimal use of available antiepileptic drugs (AEDs), many patients with epilepsy fail to experience seizure control. Moreover, many patients suffer with the strong side effects of chronic treatment, which may include chronic toxicity, cognitive impairment, sedation and teratogenesis^[3]. The increasing knowledge on the basic mechanisms of epilepsy, the most important convulsive disorder, has led to the rational development of compounds that block seizure onset or spread targeting specific neuronal substrates ^[4,5]. There is still a great demand for new anticonvulsant drugs, as the existing drugs fail to treat all types of convulsive disorders ^[6]. Medicinal plants used for the therapy of epilepsy in traditional medicine have been shown to possess promising anticonvulsant activities in animal models and can be invaluable sources of new antiepileptic compounds.

Indigofera tinctoria Linn. (Family: Fabaceae) was one of the original sources of indigo dye. It has been naturalized to tropical and temperate Asia, as well as parts of Africa, but its native habitat is unknown since it has been in cultivation worldwide for many centuries. It has light green pinnate leaves and sheafs of pink or violet flowers, cultivated extensively in Northern India, especially in Bengal, Bihar, Orissa, Sind and sothern India, Madras. The plant is a legume, so it is rotated into fields to improve the soil ^[7]. The plant has been extensively used in various folklore and traditional medicine systems for treatment of several disorders. In ayurveda & siddha used for Tikita rasam, katu rasam, ushna veeryam, katu, vipaka, anthelmintic, anti periodic. Roots used for anti poison, giddiness, colic, gonorrhoea, hair tonic. Leaves used for Jaundice, produce complexion, vatha fever, gout. In Unani used for haemostatic, sedative, piles, healer of ulcers, diuretic, dropsy. Decoction of the leaves used in blennorrhagia, roots in urinary complaints and hepatitis. Extract is used in the epilepsy and other nervous disorders, bronchitis and liver ailments ^[8]. The whole plant of *Indigofera tinctoria* Linn. contains glycoside indican, indigotine, indirubin, galactomannan composed of galactose and mannose, 2.5% of alkaloids, rotenoids and flavanoids ^[9] and their pharmacological activities hepatoprotective^[10], antidyslipidemic^[11], antiproliferative^[12], antileukaemia^[13] and plants as a Source of Anti-Cancer agents^[14] were reported. However there are no reports on the antiepileptic activity of the plant leaves. Hence, the present study was designed to verify the claims of the native practitioners.

Materials and Methods

Plant collection

The leaves of *Indigofera tinctoria* Linn. was collected from abirami botanicals of Tuticorin, Tamilnadu, India. It was identified and authenticated by Prof. Jayaraman, Taxonomist, Tambaram, Chennai, Tamilnadu, India. The voucher specimen (IT-P-08-S5) of the plant was deposited at the college for further reference.

Preparation of extracts

The leaves of plants were dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (220gm) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. The methanolic extract of *Indigofera tinctoria* Linn. (MEIT) yielded thick violet semi-solid residues. Percentage yield of MEIT was found to be 18.9% w/w.

Preliminary phytochemical screening Phytochemical Screening

The phytochemical examination of the methanolic extract of *Indigofera tinctoria* was performed by the standard methods ^[15]. Further investigation was carried out using the ethanol extract suspended in 1% w/v Sodium carboxy methylcellulose (SCMC).

Animals used

Albino wistar rats (150-200g) of either sex were obtained from the animal house in C.L. Baid Metha College of Pharmacy, Chennai. The animals were 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 (Reference No: IAEC/XIII/06/CLBMCP/2008-2009 Dated on 4-09-2008. IAEC/XIII/10/CLBMCP/2008-2009 Dated on 11-12-2008).

Acute Toxicity Study

The acute toxicity of 90% methanolic extract of *Indigofera tinctoria* 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 ^[16].

Antiepileptic Activity

Effect on Maximal electroshock (MES) induced seizures

Albino wistar rats of either sex weighing 150 to 230 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 methanolic extract of the *Indigofera tinctoria* (L.) (MEIT) (200 and 400 mg/kg body weight) *p.o* respectively for 14 days. On the 14th 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° ^[17].

Effect on Pentylentetrazole (PTZ) induced seizures

Albino wistar rats of either sex weighing 150 to 230 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, received methanolic extract of *Indigofera tinctoria* (L.) (MEIT) (400 and 200 mg/kg/body weight) *p.o* respectively for 14 days. On the 14th 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 [18].

Statistical analysis

The data were expressed as mean \pm standard error mean (S.E.M).The Significance of differences among the groups was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test *P* values less than 0.05 were considered as significance.

Results

Phytochemical screening

The results of preliminary phytochemical screening of the methanolic extract of *Indigofera tinctoria* (L.) revealed that presence of alkaloids, flavonoids, carbohydrates, glycosides, tannins, terpenoids, phenols and absence of saponins and steroids.

Effects of MEIT on MES Induced Epilepsy

The duration of tonic hindleg extension in rats treated with vehicle was 13 ± 0.36 seconds. The MEIT at doses of 200 mg/kg and 400 mg/kg were protect animals from seizures and significantly ($p<0.001$) reduced the duration of tonic hindleg extension for 4 ± 0.36 and 2.67 ± 0.33 seconds respectively. Whereas, the standard drug phenytoin treated animals exhibits abolished tonic hindleg extension. Phenytoin treated animals have shown 100% protection against MES induced seizures where as MEIT 200 mg/kg and 400 mg/kg have shown 69.23 % and 79.48 % protection respectively (Table-1 and Figure-1).

Effect of MEIT on PTZ Induced epilepsy

In rats treated with vehicle, clonic convulsion appeared for 177.70 ± 2.86 seconds after PTZ and all rats died after seizures. The MEIT at doses of 200 mg/kg and 400 mg/kg significantly delayed the onset of clonic convulsions for 486.30 ± 4.91 ($p<0.001$) and 566.70 ± 3.67 ($p<0.001$) seconds respectively in dose dependent manner. Whereas, the standard drug diazepam (4mg/kg, *i.p*) delayed the onset of clonic convulsions for 695.30 ± 1.54 ($p<0.001$) seconds. Diazepam treated animals have shown 100% protection against PTZ induced seizures where as MEIT 200 mg/kg and 400 mg/kg have shown 69.97% and 81.50% protection of convulsion and 83.33% and 100% protection of mortality respectively (Table-2 and Figure 2).

Table: 1. Effect of methanolic extract of *Indigofera tinctoria* (L.) (MEIT) on Maximum electroconvulsive shock (MES)-induced seizures in rats.

Group	Design of Treatment	Flexion (seconds)	Extensor (seconds)	Clonus (seconds)	Stupor (seconds)	Recovery (seconds)	% Protection
I	Vehicle control (SCMC,1ml/100g)	8.50±0.22	13±0.36	19.83±0.30	40 ±0.73	174.6	0
II	Phenytoin 25mg/kg, <i>i.p</i>	4.17±0.30**	0	9.17±0.40**	16.17±0.79**	92.6	100
III	MEIT 200mg/kg, <i>p.o</i>	6.67±0.49 ^{ns}	4 ±0.36***	15 ±0.68*	31.83±1.53*	137.32	69.23
IV	MEIT400 mg/kg, <i>p.o</i>	5.17±0.30**	2.67±0.33***	13 ±1.03***	17 ±0.44***	112.43	79.48
d.f=3, 20. F=		5.00	104.63	3.33	5.178	-	-

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; ***p<0.001; ns-non significant.

Table: 2. Effect of methanolic extract of *Indigofera tinctoria* (L.) (MEIT) on Pentylentetrazole (PTZ) induced seizures in rats.

Group	Design of Treatment	Onset of clonic convulsions (seconds)	Duration of convulsion (Seconds)	Protection convulsion %	% Protection mortality
I	Vehicle control (SCMC,1ml/100g)	177.70±2.86	72.17±1.17	0	50
II	Diazepam (4mg/kg, <i>i.p</i>)	695.30±1.54***	11.50±0.81***	100	100
III	MEIT (200 mg/kg, <i>p.o</i>)	486.30±4.91***	39.83±1.33***	69.97	83.33
IV	MEIT (400mg/kg, <i>p.o</i>)	566.70±3.67***	29.83±1.27***	81.50	100
df=3,20,23 F=		2238.62	104.32	-	

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; ***p<0.001; ns-non significant.

Figure-1. Effect of methanolic extract of *Indigofera tinctoria* (L.) (MEIT) on Maximum electroconvulsive shock (MES) - induced seizures in rats.

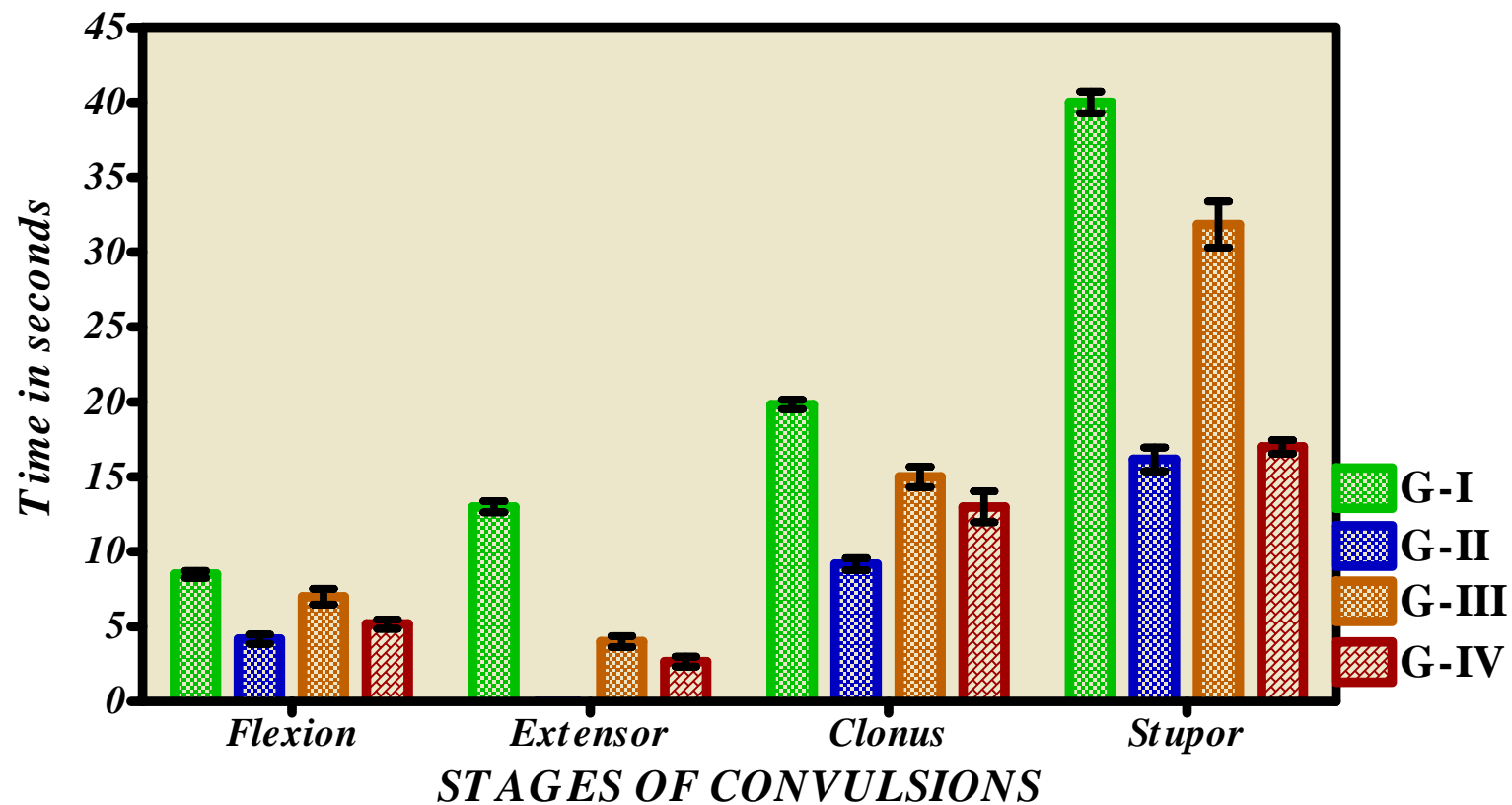
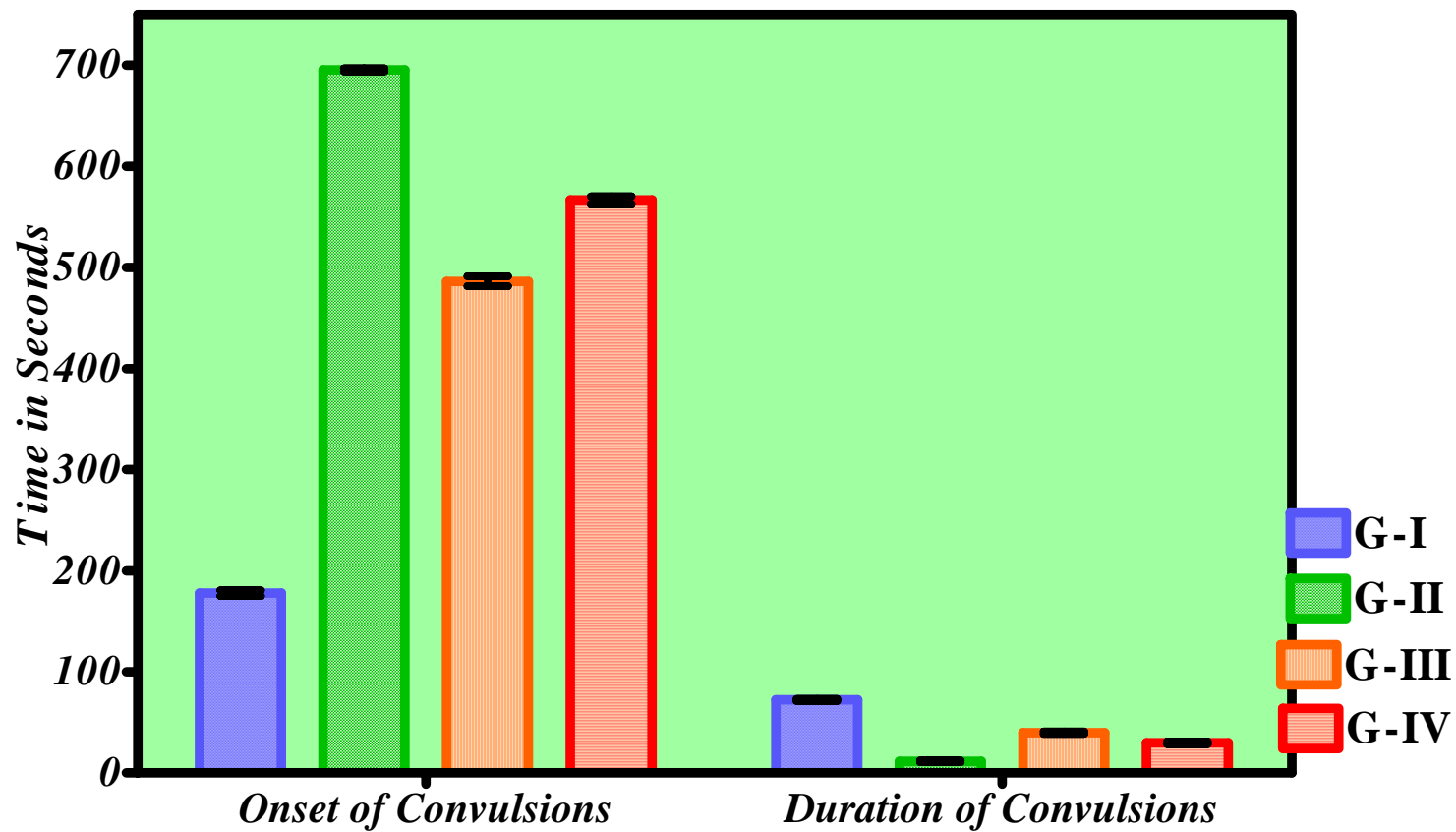


Figure-2. Effect of methanolic extract of *Indigofera tinctoria* (L.) (MEIT) on Pentylenetetrazole (PTZ) induced seizures in rats.



Discussions and Conclusions

The most popular and widely used animal seizure models are the traditional MES and PTZ tests. 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"^[19,20]. This model based on observation of the stimulation by repeated electrical pulses induce in different neuronal structures one characteristic standard of epileptic activity ^[21]. PTZ-induced seizures test is considered as an experimental model for the "generalized absence seizures" ^[20] and also a valid model for human generalized myoclonic seizures and generalized seizures of the petitmal type ^[19].

In our present study, it is found that treatment with MEIT on rats significantly reduces in tonic hindleg extensor stage in MES induced epilepsy. The MES test – to identify compounds which prevent seizure spread, corresponding to generalized tonic-clonic seizures in humans ^[22,23]. 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 ^[24,25]. Since, MEIT significantly inhibited generalized tonic-clonic seizures in MES test; it suggests the presence of anticonvulsant compounds.

Similarly, we found that treatment with MEIT on PTZ induced rats significantly reduce the duration of convulsion and delayed the onset of clonic convulsion. PTZ may cause seizures by inhibiting chloride ion channel associated with GABA_A receptors ^[19,26,27]. Since PTZ has been shown to interact with the GABA neurotransmission ^[19,28] 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 ^[29,31], the antagonism of PTZ- induced seizures suggests the interaction of the methanolic extract of *Indigofera tinctoria* (L.) with the GABA-ergic neurotransmission. The effect of the MEIT in the PTZ test could therefore suggest antiepileptic efficacy against the above mentioned seizures type in man.

Preliminary phytochemical analysis performed in this study shows that alkaloids and flavonoids are the major components of the MEIT. Hence, these properties could be mediated by several compounds present in the extract and could explain the use of this plant in traditional medicine in the treatment of epilepsy.

The study concluded with significant antiepileptic activity of methanolic extract of *Indigofera tinctoria* (L.) against various models of epilepsy but was unable to reveal the exact mechanism of its action.

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