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EFFECT OF *INDIGOFERA TINCTORIA* EXTRACTS ON NEUROTRANSMITTERS CONCENTRATIONS IN RAT BRAIN AFTER INDUCTION OF SEIZURE

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ABSTRACT

The leaves of *Indigofera tinctoria* Linn. (Family: Fabaceae) is traditionally used in the epilepsy and other nervous disorders, bronchitis and liver ailments. The purpose of the present study is to evaluate the effect of methanolic extract of *Indigofera tinctoria* (MEIT) on neurotransmitters concentrations in rat brain after induction of seizures by MES and PTZ. Our aim of study was relationship between seizure activities and altered the neurotransmitters such as noradrenaline, dopamine and serotonin in forebrain of rats in MES and PTZ seizure models. In MES model, MEIT (200 & 400 mg/kg) showed significantly restored the decreased levels of brain monoamines such as noradrenaline, dopamine and serotonin. Similarly in PTZ model, MEIT showed significantly increased the neurotransmitters in forebrain of rats. Thus, this study conclude that methanol extract of *Indigofera tinctoria* increased the neurotransmitters on rat brain, which may be decreased the susceptibility to MES and PTZ induced seizure in rats.

Keywords: Antiseizure activity, *Indigofera tinctoria*, Neurotransmitters, Noradrenaline, Dopamine and Serotonin.

Introduction

There is still a great demand for new anticonvulsant drugs, as the existing drugs fail to treat all types of convulsive disorders (Meldrum, 1997; Moldrich *et al*., 2003). The most important convulsive disorder, has led to the rational development of compounds that block seizure onset or spread targeting specific neurotransmitters (Dichter, 1997). However, most of the new rationally designed drugs are less efficient than conventional anticonvulsants but still induce strong side effects include chronic toxicity, cognitive impairment, sedation and teratogenesis (Moldrich *et al*., 2003). Medicinal plants used for the therapy of epilepsy in traditional medicine have been shown to possess promising anticonvulsant activities in animal models of anticonvulsant screening can be an invaluable source for search 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 (Annonymous 1). 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

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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 (Nadkarni, 1926). The whole plant of *Indigofera tinctoria* Linn. contains glycoside indican, indigotine, indiruben, galactomannan composed of galactose and mannose, 2.5% of alkaloids, rotenoids and flavanoids (Chopra *et al*., 1996) and their pharmacological activities Hepatoprotective (Singh *et al*., 2001), antidyslipidemic (Narender *et al*., 2006), antiproliferative (Kameswaran and Ramanibai, 2008) , and antileukaemia (Hoessel *et al*., 2009) were reported. In previous study, the methanoloic extract of *Indigofera tinctoria* Linn (MEIT) was subjected to acute toxicity and then screened for antiepileptic activity on Maximal Electroshock (MES) and Pentylenetetrazole (PTZ) induced seizures models in albino wistar rats was reported (Saravana Kumar *et al*., 2009). Therefore, the present study was performed to examine the effect of *Indigofera tinctoria* on biogenic amines concentrations in rat brain after induction of seizure by MES & PTZ model.

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.

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).

Experimental design

Albino wistar rats were divided into four groups of six animals each. Group I received 1% w/v SCMC, 1ml/100 g whereas Group-II received Phenytoin, 25mg/kg *i.p*, Group-III and IV, received methanoloic extract of *Indigofera tinctoria* (L.) (200 and 400 mg/kg b.w) *p.o* respectively for 14 days. On the 14th day, Seizures are induced to all the groups by using an Electro convulsiometer. The duration of various phases of epilepsy were observed.

Pentylenetetrazole (90mg/kg b.w, *s.c*) was administered to other groups to induce clonic convulsions after above respective treatment. Animals were observed for a period of 30mins post– PTZ administration.

Nor-Adrenaline and Dopamine Assay

The assay represents a miniaturization of the trihydroxide method. To 0.02ml of HCl phase, 0.05ml 0.4M and 0.01ml EDTA/Sodium acetate buffer (pH 6.9) were added, followed by 0.01ml iodine solution (0.1M in ethanol) for oxidation. The reaction was stored after two minutes by addition of 0.01ml Na_2SO_3 in 5m NaOH. Acetic acid was added 1.5 minutes later. The solution was then heated to 100 for 6 minutes. When the sample again reached room temperature, excitation and emission spectra were read in the microcuvette as with 5-HT: in some cases, the readings were limited to the excitation maxima. 395-485nm for NA and 330-375nm for Dopamine uncorrected instrument values (Schlumpf *et al*., 1974).

Serotonin Assay

As mentioned earlier some modifications in reagent concentration became necessary together with changes in the proportions of the solvent, in order to obtain in a good fluorescence yield with reduced volume for serotonin determination, the O-pthaldialdehyde (OPT) method was employed. From the OPT reagent 0.025ml were added to 0.02ml of the HCl extract. The fluorophore was developed by heating at 100°C for 10 min. After the samples reached equilibrium with the ambient temperature, intensity reading at 360-470 nm were taken in the micro cuvette (Schlumpf *et al*., 1974).

Statistical analysis

The data were expressed as mean ± 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

Effect of MEIT on monoamines levels in seizure induced rats by MES and PTZ:

Noradrenaline

In MES and PTZ models, Noradrenaline levels significantly ($p<0.01$) decreased in forebrain of epileptic control animals. MEIT at the doses of 200&400mg/kg, standard drugs phenytoin and diazepam treated animals showed a significantly ($p<0.01$) increased in Noradrenaline levels in forebrain of rats (Table 1 and 2).

Dopamine

In MES and PTZ models, Dopamine levels significantly ($p<0.01$) decreased in forebrain of epileptic control animals. MEIT at the doses of 200&400mg/kg, standard drugs phenytoin and diazepam treated animals showed a significantly ($p<0.01$) increased in Dopamine levels in forebrain of rats (Table 1 and 2).

Serotonin

In MES and PTZ models, Serotonin levels significantly ($p<0.01$) decreased in forebrain of epileptic control animals were observed. MEIT at the doses of 200&400mg/kg, standard drugs phenytoin and diazepam treated animals showed a significantly ($p<0.01$) increased in Serotonin levels in forebrain of rats (Table 1 and 2).

Table: 1. Effect of MEIT on neurotransmitters levels in rat brain after MES induced epilepsy

Group	Design of Treatment	Noradrenaline	Dopamine	Serotonin
I	Vehicle Control(SCMC 1ml/100gm)	765±5.52	651.50±3.18	121±2.01
II	MES (SCMC 1ml/100gm)	448.3±15.38 [a **]	498.3±5.236 [a **]	83±1.80 [a **]
III	Phenytoin 25mg/kg,*i.p*	612.3±21.75 [b **]	878.7±2.679 [b **]	119.16±2.78 [b **]
IV	MEIT 400 mg/kg,*p.o*	595.3±23.12 [b **]	804.3±4.54 [b **]	107.33±0.88 [b**]
V	MEIT 200 mg/kg,*p.o*	548.7±18.01 [b **]	746.7±4.65 [b **]	98±0.81 [b*]

Values are expressed as mean ± SEM of six observations. Comparison between: **a**- Group I Vs Group II, **b**- Group III Vs Group IV and Group V. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's test *$p<0.05$;** $p<0.01$;
Units = pg/mg of wet tissue.

Table: 2. Effect of MEIT **on neurotransmitters levels in rat brain after PTZ induced epilepsy**

Group	Design of Treatment	Noradrenaline	Dopamine	Serotonin
I	Vehicle Control(SCMC 1ml/100gm)	775±5.52	831.50±3.18	141±2.01
II	MES (SCMC 1ml/100gm)	534.7±29.39[a**]	512.7±8.891[a**]	89.5±3 [a **]
III	Diazepam (4mg/kg), *p.o*	796.7±28.88[b**]	894.3±11.14[b**]	138.83±2.18 [b **]
IV	MEIT 400 mg/kg,*p.o*	758.7±30.54[b**]	844.7±8.073[b**]	126.50±1.08[b**]
V	MEIT 200 mg/kg,*p.o*	728±30.90[b**]	766.3±19.52[b**]	115.33±1.64 [b*]

Values are expressed as mean ± SEM of six observations. Comparison between: **a** - Group I Vs Group II, **b**- Group III Vs Group IV and Group V. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's test *$p<0.05$;** $p<0.01$;
Units = pg/mg of wet tissue.

DISCUSSIONS AND CONCLUSIONS

The role of neurotransmitters in epileptogenesis and in recurrent seizure activity is well-documented. Spontaneous and experimentally induced deficiencies in Noradrenaline, Dopamine and/or Serotonin (5-hydroxy-tryptamine or 5-HT). It has been implicated in the onset and perpetuation of many seizure disorders many experimental procedures designed to increase monoaminergic activity have proven antiepileptic properties (Applegate *et al*., 1986; Corcoran, 1988; McIntyre and Edson, 1989; Pelletier and Corcoran, 1993; Yan *et al*., 1995; Zis *et al*., 1992).

In Norepinephrine-lesioned rats showed a greater susceptibility to seizures induced by the chemoconvulsant (PTZ) and Maximal electroconvulsive shock (Mason and Corcoran, 1979). The antiepileptic role of endogenous Noradrenaline was inferred from studies that showed harmful effects of a damage of Noradrenaline on seizures induced by electrical stimulation or systemic administration of chemoconvulsants (Dailey and Jobe, 1986; Browning *et al*., 1989). In present study, MEIT significantly ($p<0.01$) increased the Noradrenaline in forebrain of rats and proves the antiepileptic activity of *Indigofera tinctoria* extract.

Chen *et al*. (Chen *et al*., 1954) demonstrated that pre-treatment with the monoamine-depleting agent reserpine decreased the epileptic threshold to PTZ and caffeine in mice. Reserpine lacks specificity, since this drug also depletes serotonin and dopamine, in addition to noradrenaline. Therefore, increased seizure susceptibility could be due to a multiple deficit of monoamines. Subsequent the present studies confirmed and extended these results. It became clear that MEIT significantly increased the serotonin, dopamine and noradrenaline. It produces significantly decreased the susceptibility to various epileptic stimuli.

In conclusion, specific neurotransmitters such as serotonin, dopamine and noradrenaline participate in the control of Maximal electroshock and pentylenetetrazole induced seizure in rat models. Our findings support the hypothesis that decreased the neurotransmitters levels in rat brain after induction of seizure. In *Indigofera tinctoria* extract treated rats, neurotransmitters such as serotonin, dopamine and noradrenaline levels significantly restored on forebrain. Thus MEIT increases the seizure threshold and decreased the susceptibility to MES and PTZ induced seizure in rats. Hence we suggest that methanol extract of *Indigofera tinctoria L.* possess antiseizure properties that may be due to restored the neurotransmitters in rat brain. These results support the ethnomedical uses of the plant in the treatment of convulsion. However more experimentation, detailed phytochemical and experimental analysis are required for a definitive conclusion.

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