EFFECT OF *GUETTARDA SPECIOSA* EXTRACTS ON BIOGENIC AMINES CONCENTRATIONS IN RAT BRAIN AFTER INDUCTION OF SEIZURE

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ABSTRACT

The inner bark of *Guettarda speciosa* is used traditional Indian medicine to treat epilepsy. Previous studies have demonstrated that extracts of these plants 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. The purpose of the present study is to investigate the effect of ethanolic (95%) extract of *Guettarda speciosa* (EEGS) on biogenic amines concentrations in rat brain after induction of seizures by MES and PTZ. Our aim of study was relationship between seizure activities and altered the monoamines such as noradrenaline (NA), dopamine (DA), serotonin (5-HT) and Gamma amino butyric acid (GABA) in forebrain of rats in MES and PTZ seizure models. In MES model, EEGS (200 & 400 mg/kg) showed significantly restored the decreased levels of brain monoamines such as NA, DA, 5-HT and GABA. Similarly in PTZ model, EEGS showed significantly increased the monoamines in forebrain of rats. Thus, this study suggests that ethanol extract of *Guettarda speciosa* increased the monoamines on rat brain*,* which may be decreased the susceptibility to MES and PTZ induced seizure in rats.

Keywords: Antiepileptic Activity, Traditional Medicine, *Guettarda Speciosa*, Biogenic Amines, NA, DA, 5-HT and GABA

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]. Interestingly, the prevalence of epilepsy in developing countries is generally higher than in developed countries[2]. Epileptic seizures are paroxysmal clinic events arising from neuronal hyperexcitability and hypersynchrony of the cerebral cortex, either locally (partial seizures) or diffusely in both hemispheres (generalised seizures). The agitated neuronal activity that occurs during a seizure is caused by a sudden imbalance between the inhibitory and excitatory signals in the brain with δ-aminobutyric acid (GABA), noradrenaline, serotonin, and dopamine respectively, being the most important neurotransmitters involved[3].

The role of dopamine and serotonin in epilepsy remains controversial, but both have convincingly been implicated in the pathophysiology of seizures[4,5]. All the currently available antiepileptic drugs are synthetic molecules. 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. In previous study, the ethanolic (95%) extract of inner bark of *Guettarda speciosa.* Linn (EEGS) 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 [6]. Therefore, the present study was performed to examine the effect of *Guettarda speciosa* on biogenic amines concentrations in rat brain after induction of seizure by MES & PTZ model.

Guettarda speciosa **Linn** (Family: Rubiaceae) is widely distributed from East Africa to India and throughout to Malaysia into the South Pacific. A decoction of the leaves is used to treat coughs, colds and sore throats. The native practitioners in and around Tirunelveli District, India, have claimed that the inner bark of this plant are being traditionally used in epilepsy[7,8,9]. Upon literature review it was found that the plant contains loganic acid and secologanin[10,11]. Anti epileptic and antidiarrhoeal activity of Guettarda specoisa was reported[6,12]. Therefore, the present study was performed to verify the effect of *Guettarda speciosa* on biogenic amines levels in rat brain after induction of seizure by MES and PTZ model.

MATERIALS AND METHODS

Plant collection

The Plant material of *Guettarda Speciosa* used for investigation was collected from Tirunelveli District, in the Month of August 2007. The plant was authenticated by Dr.V.Chelladurai, Research Officer Botany. C.C.R.A.S., Govt. of India. The voucher specimen (CHE-SA-GS-01) of the plant was deposited at the college for further reference.

Preparation of extracts

Inner bark of the whole plants were dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (60gm) 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. Percentage yield of ethanolic (95%) extract of *G. Speciosa* was found to be 17.5 % w/w.

Animals used

Albino wistar rats (150-230g) 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 (Ref No. IAEC / XIII / 01 / CLBMCP / 2007 - 2008 dt.24-07-2007).

Experimental design

Albino wistar rats were divided into four groups of six animals each. Group I received vehicle control (1% w/v SCMC, 1ml/100 g) whereas Group-II received standard drug (Phenytoin, 25mg/kg) *i.p*, Group-III and IV, received 95% ethanolic extract of the inner bark of *Guettarda speciosa* (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.

A fluorimetric micromethod for the simultaneous determination of serotonin, noradrenaline and dopamine

On the 14th day after observed the convulsion all groups rats were sacrificed, whole brain was dissected out and separated the forebrain. Weighed quantity of tissue and was homogenized in 0.1 mL hydrochloric acid -

butanol, (0.85 ml of 37% hydrochloric acid in one liter *n*- butanol for spectroscopy) for 1 min in a cool environment. The sample was then centrifuged for 10 min at 2,000 rpm. 0.08 mL of supernatant phase was removed and added to an Eppendorf reagent tube containing 0.2 mL of heptane (for spectroscopy) and 0.025 mL 0.1 M hydrochloric acid. After 10 min of vigorous shaking, the tube was centrifuged under same conditions to separate two phases. Upper organic phase was discarded and the aqueous phase (0.02 mL) was used for estimation of Serotonin, Nor Adrenaline and Dopamine assay[13].

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 DA uncorrected instrument values[13].

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 5-HT 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, excitation / estimation spectra or intensity reading at 360-470 nm were taken in the micro cuvette[13].

Estimation of brain GABA content

The brain gamma amino butyric acid (GABA content was estimated according to the method of Lowe et al., (1958)[14] Animals were sacrificed by decapitation and brains were rapidly removed, and separated forebrain region. It was blotted, weighed and placed in 5ml of ice-cold trichloroaceticacid (10% w/v), then homogenized and centrifuged at 10,000rpm for 10min at 0°C. A sample (0.1ml) of tissue extract was placed in 0.2ml of 0.14 M ninhydrin solution in 0.5M corbonate-bicorbonate 1 buffer (pH9.95), kept in a water bath at 60°C for 30min, then cooled and treated with 5ml of copper tartrate reagent (0.16% disodium carbonate, 0.03% copper sulphate and 0.0329% tartaric acid). After 10min fluorescence at 377/455nm in a spectofluorimeter was recorded.

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the group 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 EEGS 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. EEGS at the doses of 200&400mg/kg, standard drugs phenytoin and diazepam treated

animals showed a significantly (p<0.05 & 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. EEGS at the doses of 200&400mg/kg, standard drugs phenytoin and diazepam treated animals showed a significantly (p<0.05 & 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. EEGS at the doses of 200&400mg/kg, standard drugs phenytoin and diazepam treated animals showed a significantly (p<0.05 & p<0.01) increased in Serotonin levels in forebrain of rats. **Table 1 and 2.**

Gamma amino butyric acid

In MES and PTZ models, GABA levels significantly (p<0.01) decreased in forebrain of epileptic control animals were observed. EEGS at the doses of 200&400mg/kg, standard drugs phenytoin and diazepam treated animals showed a significantly (p<0.05 & p<0.01) increased in GABA levels in forebrain of rats. **Table 1 and 2.**

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

Group	Design of Treatment	Noradrenaline	Dopamine	Serotonin	GARA
I	Vehicle Control(SCMC 1ml/100gm)	765±5.52	651.50±3.18	191±2.01	285±1.49
II	MES (SCMC 1ml/100gm)	433.66±2.38 ^{a**}	481.66±4.49^{a**}	73±1.80 ^{a**}	232.16±2.18 ^{a**}
III	Phenytoin 25mg/kg,*i.p*	589±2.17 ^{b**}	698.50±3.59^{b**}	99.16±2.78^{b**}	289.83±1.38 ^{b**}
IV	EEGS 400 mg/kg,*p.o*	573.16±4.99^{b**}	656±1.77^{b**}	89.33±0.88^{b**}	278.5±1.17^{b**}
V	EEGS 200 mg/kg,*p.o*	749.33±4.16^{b*}	582.83±4.3^{b*}	83±0.81^{b*}	272.66±2.3^{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 't' test *p<0.05;** p<0.01; Units = pg/mg of wet tissue.

Table 2 : Effect of EEGS on neurotransmitters levels in rat brain after PTZ induced epilepsy

Group	Design of Treatment	Noradrenaline	Dopamine	Serotonin	GARA
I	Vehicle Control(SCMC 1ml/100gm)	765±5.52	851.50±3.18	191±2.01	291.83±1.3
II	MES (SCMC 1ml/100gm)	528.16±2.37 ^{a**}	576.83±4.65 ^{a**}	95.5±3 ^{a**}	205.16±1.64^{a**}
III	Diazepam (4mg/kg), *p.o*	609±2.3 ^{b**}	899.16±5.43 ^{b**}	132.83±2.18 ^{b**}	292.33±1.22^{b**}
IV	EEGS 400 mg/kg,*p.o*	748.83±4.64^{b*}	895.66±2.44^{b**}	127.50±1.08^{b**}	281±1.35^{b**}
V	EEGS 200 mg/kg,*p.o*	767.16±4.22^{bns}	771.50±4.35^{b**}	116.33±1.64 ^{b*}	276.66±1.52^{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 't' test *p<0.05;** p<0.01; Units = pg/mg of wet tissue.

DISCUSSIONS AND CONCLUSIONS

The role of biogenic amines in epileptogenesis and in recurrent seizure activity is well-documented. Spontaneous and experimentally induced deficiencies in gamma amino butyric acid (GABA), noradrenaline (NA), dopamine (DA) 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[15, 16, 17, 18, 19, 20].

In present study, the established antiepileptic drugs such as phenytoin and diazepam restored the monoamine levels on brain [21]. Similarly EEGS significantly ($p < 0.05$ & $p < 0.01$) increased monoamines levels in forebrain of rats. Many drugs that increase the brain contents of GABA have exhibited anticonvulsant activity against seizures induced by MES and PTZ[22]. MES is probably the best validated method for assessment of anti-epileptic drugs in generalized tonic-clonic seizures [23].

GABA is a major inhibitory neurotransmitter of CNS and increase in its level in brain has variety of CNS dependent effects including anticonvulsant effect [24]. In addition to the GABA binding site, the GABA$_A$ receptor complex appears to have distinct allosteric binding sites for benzodiazepines, barbiturates, ethanol etc [25]. We therefore studied the effect of *Guettarda speciosa* extract on brain GABA content. *Guettarda speciosa* extract showed significant ($p < 0.05$ & $p < 0.01$) increased GABA content in brain dose dependently. This suggests that the anticonvulsant activity of *Guettarda speciosa* extract is probably through elevation of brain GABA content.

In Norepinephrine-lesioned rats showed a greater susceptibility to seizures induced by the chemoconvulsant PTZ and electroconvulsive shock [26]. The antiepileptic role of endogenous Norepinephrine was inferred from studies that showed harmful effects of a damage of Norepinephrine system on seizures induced by electrical stimulation or systemic administration of chemoconvulsants [27, 28]. In present study, EEGS significantly ($p < 0.05$ & $p < 0.01$) increased the NA in forebrain of rats and proves the antiepileptic activity of *Guettarda speciosa* extract.

Chen et al [29] 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 (5-HT) and DA, in addition to NE. Therefore, increased seizure susceptibility could be due to a multiple deficit of monoamines[30]. Subsequent the present studies confirmed and extended these results. It became clear that EEGS significantly increased the serotonin (5-HT) and DA and NA. It produces significantly decreased the susceptibility to various epileptic stimuli.

In conclusion biogenic amines participate in the control of Maximal electroshock and pentylenetetrazole induced seizure in rat models. Our findings support the hypothesis that decreased the monoamines levels in rat brain after induction of seizure. In *Guettarda speciosa* extract treated rats, monoamines such as NA, DA, 5-HT and GABA levels significantly restored on forebrain. Thus EEGS increases the seizure threshold and decreased the susceptibility to MES and PTZ induced seizure in rats. Hence we suggest that ethanol extract of inner bark of *Guettarda speciosa L.* possess antiepileptic properties

that may be due to restored the biogenic amines in rat brain. These results support the ethnomedical uses of the plant in the treatment of epilepsy. However more experimentation, detailed phytochemical and experimental analysis are required for a definitive conclusion.

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