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RESEARCH ARTICLE

Study on the Antiseizure Activities of Inner Bark of Guettarda Speciosa (L.)

S. ARUMUGAM, A. PALANIVELU, G. RETNASAMY and D. RAMAIYAN

For author affiliations, see end of text.

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ABSTRACT

This article reports the results of an investigation of antiepileptic activity of *Guettarda speciosa (L.) in rats.* The ethanolic (95%) extract of inner bark of *G. speciosa.* Linn (EEGS) was used for acute toxicity test and then it was screened for antiepileptic activity on Maximal Electroshock (MES)- and Pentylenetetrazole (PTZ)- induced seizures models in albino wistar rats. No toxicity was observed up to the recommended dose of 2000 mg/kg body weight orally as per OECD guidelines No. 423. Animals were pretreated with EEGS at doses of 200 and 400 mg/kg body weight. There was significant delay in clonic seizure induced by PTZ and a dose-dependent decrease in duration of hind leg extensor phase in MES model after treatment with the extract. In MES model, EEGS showed significant reduction in duration of hind leg extension with 200 mg/kg dose and effect was dramatically reduced with 400mg/kg. Similar dose-dependent delays on the onset of clonic convulsions were obtained with PTZ. The complete protective effect against mortality was reported in both models. This study predicted possible mechanism of the formulation mediated through chloride channel of the GABA or benzodiazepine receptor complex. The ethanol extract of the inner bark of *G. speciosa* (L.) deserve further investigation for detailed elucidation of active constituents and the mechanisms of action in the epilepsy treatment.

Keywords: *Antiepileptic activity, Traditional medicine, Guettarda Speciosa, MES, PTZ*

Epilepsy is one of the major neurological disorders where modern drug therapy is complicated by side-effects, teratogenic effects, and long-term toxicity. About 40% of the patients are refractory to therapeutic intervention and thus its effective and safe therapy remains a challenge [1-4]. 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 and can be invaluable sources of new antiepileptic compounds.

Guettarda speciosa Linn. (Family: Rubiaceae) is widely distributed from East Africa to India and through Malaysia to the South Pacific. This plant is common along the seashore, sea cliffs, beach thickets and low land forests. It is a spreading and much branched tree up to 20m height. Leaves are opposite, petiolate, leathery, oval with conspicuous striate veins, relatively large (to 20cm long). Flowers of this plant are white, tubular, fragment, borne in terminal clusters and the fruits are round and green syncarp with one seed per locule. Flowers and fruits are available throughout a year. In Fiji, the stem is used in a preparation utilized to promote menstruation and the plant is used to treat maternal postpartum infections. A decoction of the leaves is used to treat coughs, colds and sore throats. The inner bark is used in the treatment for conjunctivitis. In Tuvalu, the leaves are used for poultices. In Tonga, a tea made from the inner bark is used to treat epilepsy. In Tahiti, the plant has antidiarrheic, febrifugal and anticholinergic applications. In New Guinea, a preparation of the bark is used to cure dysentery. The native practitioners in and around Tirunelveli District, India, have claimed that the inner bark of this plant are being traditionally used in epilepsy [5-7]. Literature review showed that the plant contains loganic acid and secologanin [8, 9]. However there are no reports on the antiepileptic activity of the plant. Hence, the present study was designed to verify the claims of the native practitioners.

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 plant was dried in shade, separated and ground 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 with ethanol (95%v/v). 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 extract of *G. speciosa* was found to be 17.5 % w/w.

Preliminary phytochemical screening

The extract was screened for the various secondary metabolites like steroids, alkaloids, carbohydrates, proteins, flavonoids, tannins and glycosides using the standard methods [10]**.** Further investigation was carried out using the ethanol extract suspended in1% w/v Sodium carboxy methylcellulose (SCMC) for acute toxicity and antisiezure activity.

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).

Acute toxicity study

The acute toxicity of 95% ethanolic extract of the inner bark of *Guettarda speciosa* was determined as per the OECD guideline no. 423 (Acute Toxic Class Method) [11]. It was observed that the test extract was not lethal to the rats even at 2000mg/kg dose. Hence, $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of this dose were selected for further study.

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 95% ethanolic extract of the inner bark of *Guettarda speciosa* (L.) (EEGS) (200 and 400 mg/kg body weight) *p.o* respectively for 14 days. On the 14^{th} day, Seizures are induced to all the groups by using an Electro convulsiometer. 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° [12].

Effect on Pentylenetetrazole (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 90% ethanolic extract of the inner bark of *G.speciosa* (L.) (EEGS) (400 and 200 mg/kg/body weight) *p.o* respectively for 14 days. On the 14^{th} day, Pentylenetetrazole (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 [13].

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

Phytochemical screening

The results of preliminary phytochemical screening of the ethanolic extract of inner bark of *G.Speciosa.* Linn revealed that presence of alkaloids, flavonoids, carbohydrates, tannins, phenols, gums and mucilage and absence of saponins and steroids.

Effects of EEGS on MES-Induced Epilepsy

The duration of tonic hindleg extension in rats treated with vehicle was 12.33±0.76 seconds. The EEGS at doses of 200 mg/kg and 400 mg/kg protected the animals from seizures and significantly (p<0.001) reduced the duration of tonic hindleg extension to 6±0.58 and 4.17±0.30 seconds, respectively. The standard drug phenytoin abolished tonic hindleg extension in the animals. Phenytoin treated animals had 100% protection against MES induced seizures where as EEGS 200 mg/kg and 400 mg/kg had 51.35% and 66.21% protection, respectively (Table1 and Fig. 1).

Table 1. Effect of ethanolic extract of inner bark of *Guettarda Speciosa Linn. (*EEGS) on Maximum electroconvulsive shock (MES)-induced seizures in rats

Group	Design of Treatment	Flexion (seconds)	Extensor (seconds)	Clonus (seconds)	Stupor (seconds)	Recovery (seconds)	% Protec-tion
I	Vehicle control (SCMC,1ml/100g)	5.67±0.33	12.33±0.76	8.83±0.48	14±0.36	180.52	0
II	Phenytoin 25mg/kg,*i.p*	4.17±0.30 *	0	7±0.37 *	11.17±0.60**	104.24	100
III	EEGS 200mg/kg,*p.o*	5.17±0.30 ns	6±0.58 ***	8±0.44 ns	13.67±0.56ns	137.32	51.35
IV	EEGS 400 mg/kg,*p.o*	4.67±0.21ns	4.17±0.30 ***	7.17±0.54 *	12.67±0.67ns	112.43	66.21
	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 ethanolic extract of inner bark of Guettarda Speciosa Linn. (EEGS) on Pentylenetetrazole (PTZ) induced seizures in rats

Group	Design of Treatment	Onset of clonic convulsions (seconds)	Duration of convulsion (Seconds)	Protection convulsion %
I	Vehicle control (SCMC,1ml/100g)	91±1.36	72.29±8.83	0
II	Diazepam (4mg/kg,*i.p)*	765.33 ±9.06***	6.58±2.62***	100
III	EEGS (400 mg/kg,*p.o)*	496.83 ±3.70***	22.14±3.30***	69.65
IV	EEGS (200mg/kg,*p.o)*	300.83 ±5.79***	31.87±1.33***	55.91
	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.

Effect of EEGS on PTZ-Induced epilepsy

In rats treated with vehicle, clonic convulsion appeared for 91±1.36 seconds after PTZ and all rats died after seizures. The EEGS at doses of 200 mg/kg and 400 mg/kg significantly delayed the onset of clonic convulsions for 300.83±5.79 ($p<0.001$) and 496.83±3.70 ($p<0.001$) seconds, respectively in a dose dependent manner. Whereas, the standard drug diazepam (4mg/kg, *i.p*) delayed the onset of clonic convulsions for 765.33±9.06 seconds. Diazepam treated animals showed 100% protection against PTZ induced seizures where as EEGS 200 mg/kg and 400 mg/kg showed 55.91% and 69.65% protection, respectively (Table 2 and Fig. 2).

DISCUSSION

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 ("grand mal") tonic-clonic seizures [14, 15]. This model is based on observation of the stimulation by repeated electrical pulses induce in different neuronal structures one characteristic standard of epileptic activity [16]. PTZ-induced seizures test is considered as an experimental model for the "generalized absence seizures" [15] and also a valid model for human generalized myoclonic seizures and generalized seizures of the petitmal type [14].

In our present study, we found that treatment with EEGS on rats significantly reduced tonic hind leg extension in MES induced epilepsy. The MES test serves to identify compounds which prevent seizure spread, corresponding to generalized tonic-clonic seizures in humans [17, 18]. 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 [19, 20]. Since, EEGS significantly inhibited generalized tonic-clonic seizures in MES test; it suggests the presence of anticonvulsant compounds.

Similarly, we found that treatment with EEGS in PTZ-induced seizure in rats significantly reduce the duration of convulsion and delayed the onset of clonic convulsion. PTZ may cause seizures by inhibiting chloride ion channels associated with $GABA_A$ receptors [14, 21, 22]. Since PTZ has been shown to interact with the GABA neurotransmition [14, 23] and PTZ-induced seizures can be prevented by drugs that enhance $GABA_A$-receptor-mediated inhibitory neurotransmission such as benzodiazepines and phenobarbital [24-26], the antagonism of PTZ-induced seizures suggests the interaction of the ethanolic extract of the inner bark of *Guettarda speciosa* (L.) with the GABAergic neurotransmission. The effect of the EEGS in the PTZ test could therefore suggest antiepileptic efficacy against the above-mentioned seizures type in man.

The study concludes that the inner bark of *Guettarda speciosa* (L.) has significant antiseizure activity against various models of epilepsy, although the exact mechanism of its action was not investigated.

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CURRENT AUTHOR ADDRESSES

S. Arumugam, Department of Pharmacology C.L. Baid Metha College of PharmacyThoraippakkam, Chennai Tamilnadu, India. E-mail: sarganjune1@gmail.com (Corresponding author)

A. Palanivelu, Department of Pharmacology C.L. Baid Metha College of PharmacyThoraippakkam, Chennai, Tamilnadu, India.

G. Retnasamy, Department of Pharmaceutical Chemistry, St. Peter's Institute of Pharmaceutical Sciences, Vidhya Nagar, Hanamakonda, Warangal, Andhra Pradesh, India.

D. Ramaiyan, Department of Pharmaceutics, Kakatiya Institute of Pharmaceutical Sciences, Pembarthi (V), Hasanparthy (M), Warangal, Andhra Pradesh, India.