ORIGINAL ARTICLE

Screening of *Careya arborea* Roxb for their anticonvulsant properties in experimental animals

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Background: Bark of *Careya arborea* Roxb are traditionally used in the ayurvedic system of medicine for the treatment of epilepsy. **Aims**: The aim of the present study was to evaluate anticonvulsant activity of *C. arborea* Linn. bark against experimental induced seizures. **Settings and Design:** Convulsion was induced by maximal electroshock seizures (MES), pentylenetetrazol (PTZ) and PTZ-induced kindling model. **Materials and Methods:** Petroleum ether (PE), chloroform (CH), methanol (ME) and aqueous (AQ) extract of *C. arborea* bark at 150 and 300 mg/kg b.w. were administered in all models. **Statistical Analysis:** Mean values and standard error mean was determined for all models and data was analyzed by one-way ANOVA, followed by Dunnett's test. **Results and Conclusion:** The ME and AQ extract of *C. arborea* bark at 300 mg/kg b.w. p.o. showed the most significant ($P < 0.01$) anticonvulsant effect by decreasing the duration of hind limb extension (extensor phase), clonus and also the duration of stupor phase, as compared with control in MES and PTZ and the extracts also inhibited seizure score in PTZ-induced kindling model.

Key words: *Careya arborea* Roxb, maximum electroshock, pentylenetetrazol, pentylenetetrazol kindling

INTRODUCTION

Epilepsy is a very common disorder, characterized by seizures which take various forms and result from episodic neuronal discharges, the form of the seizure depending on the part of the brain affected. In many patients, presently available antiepileptic drugs (AED) such as phenobarbital, phenytoin, benzodiazepines, sodium valproate, etc., are unable to control seizures efficiently. Furthermore, the dose-related neurotoxicity and other side effects associated with established AEDs limit their clinical use. The newer AEDs like oxocarbazepine, gabapentin, felbamate, etc., represent a real progress in the treatment of non-responders or refractory patients. However, the problem of adverse effects has also not been circumvented completely.[1]

In the Ayurvedic system of medicine, the bark of *C. arborea* Roxb (Lecythidaceae), have been in clinical use for centuries. *C. arborea* have reported antioxidant and hepatoprotective,[2] antitumor,[3] antileishmanial,[4] and antidiarrhoeal activity;[5] it also used in the treatment of expectorant, antipyretic and epileptic fits.[6,7]

However, the anticonvulsant activity of *C. arborea* bark has not been scientifically investigated. Hence, the present study was undertaken to evaluate its traditionally claimed anticonvulsant activity on Wistar albino rats against seizures induced by maximal electroshock seizures (MES), pentylenetetrazol (PTZ) and PTZ kindling model.

Access this article online	
Quick Response Code:	Website: www.greenpharmacy.info
	DOI: 10.4103/0973-8258.111604

MATERIALS AND METHODS

Plant Material

In the present study, *C. arborea* bark was collected in the month of August 2009 from the local areas of Jambhoti (Western Ghat), Belgaum, Karnataka. The plant material was authenticated by Dr. Harsha Hegde, Research Officer, Regional Medical Research Centre (RMRC), Indian Council for Medical Research (ICMR), Belgaum. The herbariums of plant have been deposited at ICMR, Belgaum, India with voucher specimen number-RMRC-477.

Drugs

PTZ, Diazepam I. *P* (Sigma Pharmaceutical Ltd), Phenytoin I.P (Eptoin, Acme formulation Pvt., Ltd.) and Sodium valproate (Son Pharmaceutical Ind. Ltd.)

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Received: 20-04-2012; **Accepted:** 02-01-2013

Preparation of Extracts

The fresh bark was cleaned; air dried and powdered. The coarse powder 200 g was subjected to successive extraction in soxhlet extractor with petroleum ether (PE), chloroform (CH) and methanol (ME). Before extracting with the next solvent, the powdered material was dried in hot air oven below 50°C. Another set of powder was macerated with CH water for seven days to obtain the aqueous (AQ) extract.[8]

Animals

Male albino-Wistar rats weighing 150-180 g were used for anticonvulsant screening and mice weighing 25-30 g were used for acute toxicity study and they were housed in standard laboratory conditions of temperature (25 ± 2°C), 12 h light and dark places and with food and water *ad libitum*.

Preparation of Dosage Form

The emulsions of extracts were prepared by triturating the accurately weighed quantity of extracts with 2% acacia in a glass mortar, with gradual addition of normal saline, to make up the required volume. Vehicle, to be administered to respective control group, was prepared using the same procedure without the addition of extracts.

Acute Toxicity

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD), revised draft guidelines 423, received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Assessment of Anticonvulsant Activity in Rats

Maximal electroshock seizures

The method used for screening is according to Gupta and Pandhare,[9,10] with minor modifications. Wistar albino rats were divided into ten groups of six rats in each group. Ear clip electrodes were applied after 30 min of administration of normal saline and standard drug and 60 min after the extract administration. The incidence and duration of extensor tonus was noted. A complete abolition of hind limb tonic extension was considered as 100% protection.

Group I: Control, received normal saline (1 ml/rat p.o), Group II: Standard, received phenytoin (25 mg/kg b.w i.p), Group III: PE-300 mg/kg b.w. p.o), Group IV: PE-150 mg/kg b.w. p.o, Group V: CH-300 mg/kg b.w. p.o, Group VI: CH-150 mg/kg b.w. p.o, Group VII: ME-300 mg/kg b.w. p.o, Group VIII: ME-150 mg/kg b.w. p.o, Group IX: AQ-300 mg/kg b.w. p.o, Group X: AQ-150 mg/kg b.w. p.o.

Chemically-induced convulsion PTZ

The method used for screening PTZ model was according to Patil and Duraiswami,[11,12] with minor modifications. The rats were divided into ten groups (Group I–X) ($n = 6$) same as above. All the groups were treated with PTZ (80 mg/kg, b.w. i.p.) 45 min after administration of standard drug and 60 min after oral administration of extracts. The animals were observed for onset, presence or absence of clonic convulsions and mortality.

PTZ-induced kindling in rats

The method used for screening PTZ-kindling model was according to Gupta.[9] The anticonvulsant activity in this model was assessed by its ability to protect against PTZ-induced kindling seizures. Male Wistar rats were first weighed and were selected for the experiment depending on their weight.

Establishment of PTZ kindled seizures in rats

The ME extracts showed potent activity hence it was used for the kindled model. Kindled seizures were induced by intraperitoneal injection of subconvulsant doses of PTZ-30 mg/kg, in rats, on alternate days, three times a week, nearly 14 injections. The rats were observed for a period of 30 min. After subconvulsant PTZ and seizure activity was scored using a scoring system which ranges from 0 to 5.

Scoring system for PTZ kindled seizures

Stage	Symptoms
0	No change
1	Hyper activity, restlessness, vibrissae twitching
2	Head nodding, head clonus, myoclonus jerks
3	Unilateral or bilateral limb clonus
4	Fore limb clonic seizures
5	Generalized clonic seizures, with loss of righting reflex

Animals showing five-stage seizures were considered to be kindled after which, the PTZ treatment was stopped. To ascertain whether the increased sensitivity to PTZ is persistent, the rats were rechallenged with subconvulsant PTZ (30 mg/kg, i.p), on 3rd and 10th day after PTZ treatment had ended. Only rats which had stage five seizures on both the days were used for experiments. The selected rats were divided into six groups of five rats each. On the 11th day the rats were used for activity.

Group I: Control, received normal saline (1 ml/rat p.o), Group II: Standard, received sodium valproate-300 mg/kg b.w i.p + diazepam-4 mg/kg b.w i.p, Group III: CAME (*C. arborea* methanol extract)-300 mg/kg b.w. p.o, Group IV: CAME-150mg/kgb.w.p.o,GroupV:Sodiumvalproate-300mg/kg b.w i.p + CAME-300 mg/kg b.w. p.o, Group VI: Sodium valproate-300 mg/kg b.w i.p + CAME-150 mg/kg b.w. p.o.

subconvulsant dose of PTZ-30 mg/kg i.p was administered to control and treated animals, which produced different stages of seizures.

The effect of drugs on seizure was assessed by the presence or absence of five stages of seizures in each rat, which was confirmed by observing each rat for 30 min after PTZ administration. All rats were treated with PTZ, 15 min after i.p. administration of sodium valproate, 60 min after i.p. administration of standard drug and 45 min after oral administration of the extracts.

Statistical Analysis

The data are presented as mean ± standard error mean (SEM) and analyzed by one-way ANOVA, followed by Dunnett's test. Data were considered statistically significant if $P < 0.05$.[13]

RESULTS

Acute Toxicity

The extracts were found to be safe in the dose used and there was no mortality up to a dose of 3000 mg/kg, b.w. as per the OECD Guide Line No. 423. Hence 1/10th and 1/20th dose i.e., 300 mg/kg b.w. and 150 mg/kg b.w. were selected for the activity.

Maximal Electroshock Seizures

MES produced various phases of convulsion, i.e., flexon, extension, clonus and stupor. The duration of tonic extension of the hind limb was used as end point, i.e., prevention or decrease in the duration of hind limb extension was considered as a protective action. The result of the extracts is compared with the result produced by control. The data resulted from anticonvulsant effect of different extracts showed that the ME extract 300 mg/kg b.w. decreased the duration of hind limb extension (7.17 ± 0.27 s), which is most significant ($P < 0.01$) when compared with control (14.28 ± 0.16 s) and the effects produced by ME extract-150 mg/kg b.w. (8.80 ± 0.2375 s) and AQ extract 300 mg/kg b.w. (11.24 ± 0.56 s).

In other words the ME extract was able to decrease the duration of hind limb extension (extensor phase), which indicated the extract at 300 mg/kg b.w. dose possesses potent anticonvulsant activity against generalized tonic–clonic seizure (grand mal) as compared with control. Results are shown in Table 1.

PTZ-induced Seizure

The standard drug diazepam in a dose of 4 mg/kg b.w. given i.p. provided 100% protection. The results of anticonvulsant effect of CA against PTZ induced seizures are shown in Table 2.

The statistical data obtained from the anticonvulsant effect of CA against PTZ-induced seizure revealed that ME extract at both 300 and 150 mg/kg and AQ extract at 300 mg/kg showed significant (277.70 ± 6.972, 280.60 ± 23.780 and 162.00 ± 3.282 s) activity when compared with the effect produced by control (115.50 ± 2.499 s) and other extracts of CA. There was no incidence of mortality in the group of animals treated with extracts.

Overall the ME extract of *C. arborea* bark was able to show potent activity against MES and PTZ-induced seizures.

PTZ-kindled Seizures in Rats

In this model, the reduction in number of scores or abolition in scores is considered for the evaluation of anticonvulsant activity of drugs in all groups. All the animals in the control group showed all the stages of seizures from 0 to 5. The animals that received sodium valproate (300 mg/kg b.w. i.p.) and diazepam (4 mg/kg b.w. ip) i.e., 15 min and 60 min before subconvulsant dose of PTZ (30 mg/kg i.p.) showed 100% reduction in the seizure score[3-5] whereas 60% and 80% reduction in scores 1 and 2 stage, respectively.

Table 1: Effect of *Careya arborea* bark extract against maximal electroshock seizures induced convulsions

Group	Treatment	Time(s) in various phase of convulsion (mean±SEM)				Recovery (R) or death (D)
		Flexon	Extensor	Clonus	Stupor	
1	Normal saline	3.878±0.07	14.28±0.16	10.48±0.17	117.1±0.76	R
2	Phenytoin	1.747±0.08**	0.00**	8.35±0.26**	31.01±0.26**	R
3	PE 300	4.705±0.21	15.02±0.83	11.17±0.32	122.1±1.04	R
4	PE 150	4.693±0.24	14.05±0.43	11.56±0.40	123.3±1.30	R
5	CH 300	4.27±0.38	15.8±0.98	11.59±0.45	123.2±3.63	R
6	CH150	3.88±0.08	14.82±0.72	11.56±0.35	123.4±1.67	R
7	ME 300	1.422±0.13**	7.17±0.27**	8.52±0.17**	43.61±1.46**	R
8	ME 150	1.75±0.10**	8.8±0.23**	13.19±0.27**	81.87±1.50**	R
9	AQ 300	4.97±0.28**	11.24±0.56**	13.21±0.21**	92.37±2.41**	R
10	AQ 150	5.04±0.50*	14.5±0.25	11.35±0.34	107.5±3.45*	R

PE – Pet. Ether; CH – Chloroform; ME – Methanol; AQ – Aqueous; *n*=6, *P<0.05, **P<0.01 compared with control group, One way ANOVA, followed by Dennett's test; SEM – Standard error mean

The synergistic effect of ME extract-300 mg/kg and sodium valproate-300 mg/kg., i.p. exhibited further reduction in seizure scores up to 60% and 80% in seizure score 1 and 2 and 100% in seizure score 3, 4 and 5. Thus, the extracts with sodium valproate showed marked reduction in the seizure scores, the synergistic effect of ME extract [(150 mg/kg b.w. p.o.) and sodium valproate (300 mg/kg. b.w. i.p.)]. Combination with sodium valproate, the extracts exhibited further reduction in seizure scores 60% and 80% in seizure score (1 and 3) 100% in seizure score (2, 4 and 5). Thus, the extracts with sodium valproate showed marked reduction in the seizure scores. Results are shown in Table 3.

DISCUSSION

Various extracts were screened anticonvulsant activity using established models like MES, PTZ-induced and PTZ-Kindling convulsion in Wistar albino rats. It has often been stated that AED that block MES-induced tonic extension act by blocking seizure spread. Moreover, MES-induced tonic extension can be prevented either by drugs that inhibit voltage-dependent Na channels, such as phenytoin, valproate, felbamate and lamotrigine, or by drugs that block glutamatergic excitation mediated by the N-methyl-Daspartate receptor, such as felbamate.[14]

The anticonvulsant activity of ME and AQ extracts may be due to the presence of tannins[15,16] and flavonoids[17] since, they possess the action on central nervous system.[18]

The ME and AQ extracts were able to minimize the duration of various phases of seizure in MES induced convulsion, but prevention or decrease in the duration of hind limb extension was considered as a protective action, considerably minimized the duration of hind limb extension (extensor phase of seizure).

In PTZ-induced seizures, the ME and AQ extracts showed significant activity as compared with control, since it was able to delay the onset of action of seizure. Moreover, there was no mortality in the group of animals treated with ME extract.

In PTZ-kindled seizures, ME and AQ extracts showed significant activity, since it was able to reduce seizure score. The results of this study and findings suggest that ME and AQ extracts are beneficial in treatment of seizures. There was no significant difference in the result of both the doses of extracts of both the drugs selected for the study. Hence, the results are not dose dependent.

Table 2: Effect of *Careya arborea* bark extract against pentylenetetrazol induced convulsions

Group	Treatment	Onset of action of convulsions in sec. (mean±SEM)	% of mortality
1	Control (normal saline+PTZ)	115.50±2.499	100.0
2	Standard (diazepam+PTZ)	0000	00.0
3	PE 300+PTZ	82.01±6.963	85.0
4	PE 150+PTZ	82.46±1.544	100.0
5	CH 300+PTZ	77.27±6.944*	68.0
6	CH150+PTZ	81.69±6.310	50.0
7	ME 300+PTZ	277.70±6.972**	00.0
8	ME 150+PTZ	280.60±23.780**	00.0
9	AQ 300+PTZ	162.00±3.282**	17.0
10	AQ 150+PTZ	81.24±3.038	68.0

PE – Pet. Ether; CH – Chloroform; ME – Methanol; AQ – Aqueous; *n*=6, **P*<0.05, ***P*<0.01 compared with control group, One way ANOVA, followed by Dennett's test; SEM – Standard error mean; PTZ – Pentylenetetrazol

CONCLUSION

From the obtained results we demonstrate that the ME and AQ extracts of *C. arborea* bark possesses protective effects against experimental seizures induced by MES, PTZ and PTZ kindling. However, the exact mechanism(s) and the active compound(s) involved in these effects need to be clarified in future studies.

ACKNOWLEDGMENT

Authors are thankful to Principal, KLE University's College of Pharmacy, Belgaum for providing necessary facilities to carry out this work.

Table 3: Pentylenetetrazol-kindling seizure model for *Careya arborea* bark extract

Seizure score	Control		CAME-300		CAME-150		SOD. VALP+DIAZ		SOD. VALP+CAME-300		SOD. VALP+CAME-150	
	A	B	A	B	A	B	A	B	A	B	A	B
1	5	0	1	80	2	60	2	60	2	60	2	60
2	5	0	3	40	3	40	1	80	1	80	0	100
3	5	0	0	100	2	80	0	100	0	100	1	80
4	5	0	0	100	0	100	0	100	0	100	0	100
5	5	0	0	100	0	100	0	100	0	100	0	100

A – No. of Wistar albino rats showed seizure score; B – % reduction in seizure score; *n*=Total no of Wistar albino rats (5); CAME – *C. arborea* methanol extract; SOD. VALP – Sodium valproate; DIAZ – Diazepam

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How to cite this article: Shinde GS, Karadi RV, Khedkar AS, Dere PJ, Mandavkar YD, Khalure PR. Screening of *Careya arborea* Roxb for their anticonvulsant properties in experimental animals. Int J Green Pharm 2013;7:29-33.

Source of Support: Nil, **Conflict of Interest:** None declared.