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Research Article

Anticonvulsant Activity of *Carissa carandas* Linn. Root Extract in Experimental Mice

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Abstract

Purpose: *The aim of the present study was to investigate anticonvulsant effect of the ethanolic extract of the roots of Carissa carandas (ERCC) on electrically and chemically induced seizures.*

Methods: *The ethanolic extract of the roots of C. carandas (100, 200 and 400 mg/kg, i.p.) was studied for its anticonvulsant effect on maximal electroshock-induced seizures and pentylenetetrazole-, picrotoxin-, bicuculline- and N-methyl-dl-aspartic acid-induced seizures in mice. The latency of tonic convulsions and the number of animals protected from tonic convulsions were noted.*

Results: *ERCC (100-400 mg/kg) significantly reduced the duration of seizures induced by maximal electroshock (MES). However, only 200 and 400mg/kg of the extract conferred protection (25 and 50%, respectively) on the mice. The same doses also protected animals from pentylenetetrazole-induced tonic seizures and significantly delayed the onset of tonic seizures produced by picrotoxin and N-methyl-dl-aspartic acid. The extract had no effect on bicuculline-induced seizures.*

Conclusion: *The data suggest that the ethanolic root extract of C. carandas may produce its anticonvulsant effects via non-specific mechanisms since it reduced the duration of seizures produced by maximal electroshock as well as delayed the latency of seizures produced by pentylenetetrazole and picrotoxin.*

Keywords: *Carissa carandas, Ethanol extract, Anticonvulsant activity, Convulsion, Seizures, Mice.*

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Introduction

Epilepsy is a major neurological disorder and up to 5% of the world population develops epilepsy in their lifetime[1]. The current therapy of epilepsy with modern antiepileptic drugs is associated with side effects, dose-related and chronic toxicity, as well as teratogenic effects, and approximately 30% of the patients continue to have seizures with current antiepileptic drugs therapy[2-4]. Traditional systems of medicine are popular in developing countries and up to 80% of the population relies on traditional medicines or folk remedies for their primary health care need[5]. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects[6]. Several plants used for the treatment of epilepsy in different systems of traditional medicine have shown activity when tested in modern bioassays for the detection of anticonvulsant activity[7] and many such plants are yet to be scientifically investigated.

Carissa carandas Linn. (Syn. *Carissa congesta* Wight) is a large dichotomously branched evergreen shrub with short stem and strong thorns in pairs, belonging to family Apocynaceae. The plant is native and common throughout much of India, Sri Lanka, Java, Malaysia, Myanmar and Pakistan. In traditional system of medicine, the plant is used as an anthelmintic, astringent, appetizer, antipyretic, in biliary, stomach disorders, rheumatism and disease of the brain[8]. Earlier studies have shown that the extract of the plant possesses cardiotonic, antipyretic and antiviral activity[9-11]. Various cardiac glycosides, a triterpenoidal constituent carissone and β-sitosterol were reported from the root extract of the plant[9,12]. In Western Ghats region of India, the decoctions and extracts of the roots of this plant are effective remedies in the management and/or control of convulsions and epilepsy. However, no scientific data are available to validate the folklore claim. The aim of the present study was, therefore, to evaluate the anticonvulsant potential of the ethanol extract of the roots of *C. carandas* in experimental animal models, with a view to providing a pharmacological justification (or otherwise) for the ethnomedical use of the plant's root in the management of convulsions and epilepsy in some rural communities of India.

Materials and Methods

Plant material

The roots of *C. carandas* were collected from Udupi, Karnataka, during April 2006. It was authenticated by Dr. Gopalakrishna Bhat, Department of Botany, Poorna Prajna College, Udupi, Karnataka, India. A voucher specimen (no. 105a) is deposited in the herbarium of our institute.

Preparation of extract

Fresh roots were collected and dried in the shade. The shade-dried roots of the plant (500 gm) were powdered and soaked in 1.5 L of 95% ethyl alcohol for 4 days and the liquid extract was decanted. The process of soaking and decanting was repeated for 4 times with fresh solvent. The solvent of the total liquid extract was evaporated by distillation to a concentrate over a water bath to a syrupy consistency and then evaporated to dryness under vacuum to give the dry extract (16% w/w yield). The extract was stored at 4 ºC until used as a suspension with 2% Tween 80/saline.

Experimental animals

Swiss mice of either sex, 8-10 weeks old, weighing about 25-30 g were used in experiments. Animals were housed in polypropylene cages maintained under standard condition (12 hours light / dark cycle; 25 ± 3 ^{0}C, 45-65% humidity) and had free access to standard rat feed (Hindustan Liver Ltd., India) and water *ad libitum*. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. All experimental protocols were reviewed and accepted by the Institutional

Animal Ethical Committee (IAEC) prior to the initiation of the experiment.

Drugs and chemicals

Pentylenetetrazole (PTZ; Sigma Chemical Co.), picrotoxin (PC; Sigma Chemical Co.), *N*-methyl-dl-aspartic acid (NMDLA; Sigma Chemical Co.), phenobarbitone (PHNB; Phenetone, Cipla, India) and 5,5-diphenylhydantoin sodium salt (Phenytoin, PHNY; Sigma Chemical Co.) were all dissolved in physiological saline. +Bicuculline (BC; Sigma Chemical Co.) was suspended in 0.5 ml of Tween 80 (Ranbaxy Laboratories Ltd) and adjusted to an appropriate volume with physiological saline. Diazepam (DZP; Calmpose, Ranbaxy Pharma, India) was also suspended in a minimum amount of polyethylene glycol 400 (Ranbaxy Laboratories Ltd) and adjusted to an appropriate volume with physiological saline. Fresh drug solutions were prepared on each day of the experiments. Drugs were administered intraperitoneally (i.p.) in a volume of 1 ml/100 g of animal. Control animals received equal volume of injections of the appropriate vehicle. The doses and pretreatment times of the extract of the roots of *C. carandas* and the standard antiepileptic drugs used were obtained from preliminary studies in our laboratory. The pretreatment dose and the times following the administration of either pentylenetetrazole (90 mg/kg, i.p.), picrotoxin (10 mg/kg, i.p.), bicuculline (40 mg/kg, i.p.) or NMDLA (400 mg/kg, i.p.) were *C. carandas* extract (100, 200 and 400 mg/kg, i.p., 30 min), diazepam (0.5 mg/kg, i.p., 20 min), phenobarbitone (10 mg/kg, i.p., 10 min) and phenytoin (25 mg/kg, i.p., 20 min).

Phytochemical screening

The freshly prepared extract of the roots of *C. carandas* (ERCC) was subjected to phytochemical screening tests for the detection of various constituents[13].

Acute toxicity study

The toxicity study was determined in mice by modified method of Lorke[14]. Mice fasted for 16 h were randomly divided into groups of 10 mice per group and were administered i.p. with the extract in doses ranging from 100-2000 mg/kg. The procedure described in detail earlier by Ojewole was followed for the determination of the acute toxicity of the plant extract[15].

Anticonvulsant activity

Electrically-induced seizures

In the electrically-induced seizure experiment, the maximal electroshock (MES) method described previously by Swinyard was employed[16]. In brief, tonic convulsions of the hind extremities of the mice were induced by passing alternating electrical current of 50 Hz and 150 mA for 0.2 sec through corneal electrodes. The animals were divided randomly into 7 groups containing 8 animals each. Group I served as vehicle control group treated with Tween-80 (0.25 ml, i.p., 30 min); groups II, III and IV served as test groups treated with the extract (100, 200 and 400 mg/kg, i.p., 30 min), respectively, and groups V, VI and VII served as reference groups treated with diazepam (0.5 mg/kg, i.p., 20min), phenobarbitone (10 mg/kg, i.p., 10 min) and phenytoin (25 mg/kg, i.p., 20 min), respectively, prior to the induction of convulsion. The number of animals protected from hind limb tonic extension seizure (HLTE) and the time spent in this position were determined for each dose group.

Chemically-induced seizures

The modified method of Vellucci and Webster[17] was used to assess the anticonvulsant effect of the extract. The animals were divided randomly into 7 groups containing 8 animals each and they were treated as described for electrically-induced seizure tests. Seizures were induced in mice with standard convulsing agents,

pentylenetetrazole (PTZ), picrotoxin (PC), bicuculline (BC) or N-methyl-dl-aspartic acid (NMDLA) and the animals were observed for 30 min for tonic convulsion episode. Hind limb extension was taken as tonic convulsion. The onset of tonic convulsion and the number of animals convulsing or not convulsing within the observation period were noted. The ability of the plant extract to prevent or delay the onset of the hind limb extension exhibited by the animals was taken as an indication of anticonvulsant activity[18].

Statistical analysis

The results of the duration of seizures in electrically-induced seizures and onset of seizures in chemically-induced seizures were analyzed using the paired Student's t-test, while the proportion of animals that exhibited tonic seizures in both cases was analyzed using Chi-squared test. A p value of <0.05 was considered as statistically significant.

Results

Phytochemical screening

Phytochemical screening of the extract (ERCC) showed that the crude extract contained small quantities of alkaloids, flavonoids, saponins and large amounts of cardiac glycosides, triterpenoids, phenolic compounds and tannins.

Acute toxicity study

There was no mortality amongst the graded dose groups of mice up to a dose of 2000 mg/kg for duration of 72 h. This finding probably suggests that the ethanol extract is relatively safe or non-toxic in mice at the doses used for this study.

Anticonvulsant assessment

Maximal electroshock produced hind limb tonic extension seizures (HLTE) in all the animals used. The vehicle-treated mice showed tonic hind limb extension for a duration of 15.15 ± 0.19 sec. ERCC (100 mg/kg) significantly reduced the latency, but did not alter the incidence of seizures elicited by maximal electroshock to any significant extent. ERCC at doses of 200 and 400 mg/kg, respectively, protected 25% and 50% of mice and significantly reduced the duration of the seizures. The standard antiepileptic drug, diazepam protected 50% of mice against seizures and significantly reduced the duration of the seizures, while phenobarbitone significantly protected 87.5 % of mice and reduced the duration of the seizures. However, phenytoin completely inhibited the MES-induced tonic seizures in all the animals used (Table 1).

Pentylenetetrazole produced tonic seizures in all the animals used. A dose of 100 mg/kg of ERCC protected 25% of animals against seizures and did not affect the onset of seizures to any significant extent. ERCC, in doses of 200 and 400 mg/kg, respectively, protected 50% and 62.5% of mice against seizures, and significantly delayed the latency of the seizures. The standard antiepileptic drugs, diazepam and phenobarbitone, completely protected the animals from seizures. Phenytoin neither affected the onset nor the incidence of convulsion to any significant extent, as shown in Table 2.

Picrotoxin produced tonic seizures in all the animals. ERCC (200 and 400 mg/kg) did not affect the incidence of seizures, but significantly prolonged latency of seizures. The standard antiepileptic drugs, diazepam significantly protected the animals from convulsions and prolonged the latency of seizures, while phenobarbitone did not alter the incidence, but significantly delayed the onset of seizures. Phenytoin neither affected the onset nor the incidence of convulsion to any significant extent (Table 3).

Bicuculline induced tonic seizures in all the mice used. However, all the doses of ERCC (100, 200 and 400 mg/kg) did not alter the incidence of seizures significantly. Besides, 400 mg/kg of ERCC significantly shortened

Table 1: Effect of ethanol extract of the roots of *C. carandas* (ERCC) on maximal electroshock (MES) - induced seizures in mice

Dose (mg/kg)					No. of animals convulsed/ No. used	Animals protected (%)	Duration of HLTE (sec) Mean ± SEM
Tween 80	ERCC	DZP	PHNB	PHNY			
0.25 ml	-	-	-	-	8/8	0	15.15 ± 0.19
-	100	-	-	-	8/8	0	12.93 ± 0.15*
-	200	-	-	-	6/8	25	9.62 ± 0.13**
-	400	-	-	-	4/8	50	6.73 ± 1.03**
-	-	0.5	-	-	4/8	50	5.06 ± 0.93**
-	-	-	10	-	1/8†	87.5	4.66 ± 0.23**
-	-	-	-	25	0/8††	100	0

* $p < 0.05$, ** $p < 0.01$, vs. Tween 80 treated group (0.25 ml, i.p.); Student's t-test.
† $p < 0.01$, †† $p < 0.001$ vs. Tween 80 treated group (0.25 ml, i.p.); Chi-squared test.
DZP- Diazepam; PHNB- Phenobarbitone; PHNY- Phenytoin; HLTE- Hind limb tonic extension seizure.

Table 2: Effect of ethanol extract of the roots of *C. carandas* (ERCC) on pentylenetetrazole (PTZ) – induced seizures in mice

	Dose (mg/kg)					No. of animals convulsed/ No. used	Animals protected (%)	Latency of tonic convulsion (min) Mean ± SEM
PTZ	Tween 80	ERCC	DZP	PHNB	PHNY			
90	0.25 ml	-	-	-	-	8/8	0	6.34 ± 0.95
90	-	100	-	-	-	6/8	25	9.61 ± 1.78
90	-	200	-	-	-	4/8	50	12.29 ± 2.26*
90	-	400	-	-	-	3/8	62.5	16.20 ± 1.63**
90	-	-	0.5	-	-	0/8†	100	0
90	-	-	-	10	-	0/8†	100	0
90	-	-	-	-	25	8/8	0	6.65 ± 1.09

* $p < 0.05$, ** $p < 0.025$ vs. Tween 80 treated group (0.25 ml, i.p.); Student's t-test.
† $p < 0.001$ vs. Tween 80 treated group (0.25 ml, i.p.); Chi-squared test.
DZP- Diazepam; PHNB- Phenobarbitone; PHNY- Phenytoin.

Table 3: Effect of ethanol extract of the roots of *C. carandas* (ERCC) on picrotoxin (PC) - induced seizures in mice

	Dose (mg/kg)					No. of animals convulsed/ No. used	Animals protected (%)	Latency of tonic convulsion (min) Mean ± SEM
PC	Tween 80	ERCC	DZP	PHNB	PHNY			
10	0.25 ml	-	-	-	-	8/8	0	14.15 ± 0.60
10	-	100	-	-	-	8/8	0	15.39 ± 0.76
10	-	200	-	-	-	8/8	0	17.05 ± 0.97*
10	-	400	-	-	-	8/8	0	19.32 ± 1.30*
10	-	-	0.5	-	-	1/8†	87.5	22.60 ± 1.70**
10	-	-	-	10	-	8/8	0	24.36 ± 1.35**
10	-	-	-	-	25	8/8	0	14.60 ± 1.91

* $p < 0.05$, ** $p < 0.025$ vs. Tween 80 treated group (0.25 ml, i.p.); Student's t-test.
† $p < 0.01$ vs. Tween 80 treated group (0.25 ml, i.p.); Chi-squared test.
DZP- Diazepam; PHNB- Phenobarbitone; PHNY- Phenytoin.

the latency of seizures in mice. The standard antiepileptic drugs, diazepam and phenobarbitone, completely protected the animals from seizures. Phenytoin neither affected the onset nor the incidence of convulsion to any significant extent (Table 4).

NMDLA induced tonic seizures in all the mice used. ERCC (200 and 400 mg/kg) significantly prolonged the latency of the seizures. However, only the higher dose (400 mg/kg) protected 25% of the animals from convulsions. The standard antiepileptic drugs, diazepam, phenobarbitone and phenytoin did not significantly affect the incidence or the onset of seizures (see Table 5).

Discussion

The results of the present study indicate that ethanol extract of the roots of *C. carandas* (ERCC) possesses anticonvulsant activity in mice. GABA is the major inhibitory neurotransmitter in the brain while glutamic acid is an excitatory neurotransmitter in the brain. The inhibition of GABA neurotransmitter and the enhancement of the action of glutamic acid have been shown to be the underlying factors in epilepsy[19,20]. Our study shows that the ethanol extract of the roots of *C. carandas* protected some of the animals against seizures induced by maximal electroshock, pentylenetetrazole, picrotoxin and NMDLA and also delayed the latency of the seizures.

In the present study maximal electroshock produced seizures in all the animals used. Antiepileptic drugs that block MES-induced tonic extension are known to act by blocking seizure spread[21]. Moreover, drugs that inhibit voltage-dependent Na+ channels, such as phenytoin can prevent MES-induced tonic extension[21,22]. However, phenobarbitone is as effective against electrically-induced convulsion as it is against pentylenetetrazole-induced convulsions in mice and phenobarbitone is known to reduce the electrical activity of neurons within a chemically-induced epileptic focus in the cortex, while diazepam does not suppress the focal activity but prevents it from spreading[23,24]. Diazepam had anticonvulsant effect on both PTZ-induced seizures and maximal electroshock-induced seizures, in which diazepam effect on the former (100% protection) is better than the latter (50% protection). This is consistent with the report that benzodiazepine (BDZ) agonists such as diazepam, clonazepam, etc, are more potent in the prevention of PTZ-induced seizures than in that of MES-induced tonic seizures[25].

Pentylenetetrazole induced seizures in all the mice used. Pentylenetetrazole may elicit seizures by inhibiting gabaergic mechanisms[26]. Standard antiepileptic drugs, diazepam and phenobarbitone, are believed to produce their effects by enhancing GABA-mediated inhibition in the brain[20]. It is, therefore, possible that the anticonvulsant effects shown in this study by the drugs against seizures produced by PTZ might be due to the activation of GABA neurotransmission. Since the extract similarly antagonized seizures elicited by pentylenetetrazole in mice, it is probable, therefore, that it may also be exerting its anticonvulsant effects by affecting gabaergic mechanisms.

Picrotoxin also produced seizures in all the mice used. Picrotoxin is known to elicit seizures, by antagonizing the effect of GABA via blocking of the chloride channels linked to $GABA_A$-receptor[19,20]. In this study, diazepam and phenobarbitone were shown to antagonize the effect of picrotoxin while the extract was also shown to delay the latency of picrotoxin–induced seizures, suggesting that the extract may be affecting gabaergic mechanisms, probably by opening the chloride channels associated with GABA-receptors.

Bicuculline is a selective antagonist of GABA at the $GABA_A$-receptors[20]. The fact that the extract did not affect the seizures induced by bicuculline, suggests that its effect on gabaergic mechanisms may not be via the stimulation of $GABA_A$-receptors. NMDLA was

Table 4: Effect of ethanol extract of the roots of *C. carandas* (ERCC) on bicuculline (BC) - induced seizures in mice

Dose (mg/kg)						No. of animals convulsed/ No. used	Animals protected (%)	Latency of tonic convulsion (min) Mean ± SEM
BC	Tween 80	ERCC	DZP	PHNB	PHNY			
40	0.25 ml	-	-	-	-	8/8	0	10.80 ± 0.49
40	-	100	-	-	-	8/8	0	9.78 ± 0.71
40	-	200	-	-	-	8/8	0	10.28 ± 0.74
40	-	400	-	-	-	8/8	0	5.93 ± 0.33*
40	-	-	0.5	-	-	0/8†	100	0
40	-	-	-	10	-	0/8†	100	0
40	-	-	-	-	25	8/8	0	10.13 ± 0.52

* $p < 0.025$, vs. Tween 80 treated group (0.25 ml, i.p.); Student's t-test.
† $p < 0.001$ vs. Tween 80 treated group (0.25 ml, i.p.); Chi-squared test.
DZP- Diazepam; PHNB- Phenobarbitone; PHNY- Phenytoin.

Table 5: Effect of ethanol extract of the roots of *C. carandas* (ERCC) on N-methyl-dl-aspartic acid (NMDLA) - induced seizures in mice

Dose (mg/kg)						No. of animals convulsed/ No. used	Animals protected (%)	Latency of tonic convulsion (min) Mean ± SEM
NMDLA	Tween 80	ERCC	DZP	PHNB	PHNY			
400	0.25 ml	-	-	-	-	8/8	0	3.56 ± 0.54
400	-	100	-	-	-	8/8	0	4.58 ± 0.36
400	-	200	-	-	-	8/8	0	5.79 ± 0.39*
400	-	400	-	-	-	6/8	25	8.75 ± 1.41*
400	-	-	0.5	-	-	8/8	0	4.02 ± 0.53
400	-	-	-	10	-	8/8	0	2.35 ± 0.29
400	-	-	-	-	25	8/8	0	3.93 ± 0.43

* $p < 0.05$ vs. Tween 80 treated group (0.25 ml, i.p.); Student's t-test.
DZP- Diazepam; PHNB- Phenobarbitone; PHNY- Phenytoin.

also shown to elicit seizures in all the mice used. NMDLA, a specific agonist at the NMDA receptors, mimics the action of glutamic acid and thus induces seizures by enhancing the glutaminergic system[20]. It is not surprising that the standard drugs, diazepam and phenobarbitone, did not alter NMDLA-induced seizures to any significant extent. In this study, the extract was shown to delay the latency of seizures induced by NMDLA. It may, therefore, be exerting its anticonvulsant effect partly by affecting glutaminergic mechanisms.

The phytochemical screening of the extract revealed the presence of small quantities of alkaloids, flavonoids, saponins and large amounts of cardiac glycosides, triterpenoids, phenolic compounds and tannins. Based on the present state of knowledge of the chemical constituents of the extract, it is not possible to attribute with certainty its anticonvulsant effect to one or several active principles among those detected in the screening. However, triterpenic steroids and triterpenoidal saponins are reported to possess anticonvulsant activity in some experimental seizure models such as MES and PTZ[27,28]. Some alkaloids, monoterpenes,

flavonoids also have protective effects against PTZ, picrotoxin and NMDLA-induced convulsions[29-32]. It is worthwhile to isolate the bioactive principles, which are responsible for these activities; the process has commenced in our laboratory. These findings justify the traditional use of this plant in the control and/or treatment of convulsions and epilepsy.

Conclusion

It can be concluded from the study that the anticonvulsant effects of the ethanolic root extract of *C. carandas* may be via non-specific mechanisms. However, extensive studies are needed to evaluate the precise mechanism(s), active principles, and the safety profile of the plant as a medicinal remedy for convulsive disorders.

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