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ANTICONVULSANT AND SEDATIVE ACTIVITIES OF EXTRACTS OF CARISSA CARANDAS LEAVES

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ABSTRACT

The present study was carried out to find out the preliminary phytochemical properties, anticonvulsant and sedative activities of the petroleum ether, ethyl acetate and ethanol extract of *Carissa carandas* leaves. The standard methods were used to screen the preliminary photochemicals present in petroleum ether, ethyl acetate and ethanol extracts of Carissa carandas leaves. The anticonvulsant efficacy of the extracts was determined using pentylenetetrazole (PTZ) in experimental animal models using diazepam as standard drug and the sedative effect was evaluated using pentobarbitone that induced sleep in mice. The efficacy of the extracts was compared against the standard drug Diazepam .The preliminary phytochemical investigation shows presence of alkaloids, glycoside, tannins, terpins . The crude extracts of ethanolic, ethyl acetate and petroleum ether at a dose 400 mg/kg has been found to significantly reduce the extensor and stupor and offer protection against convulsion induced by PTZ. The petroleum ether, ethyl acetate and ethanolic extract found to be non significant at a dose of 100 mg/kg in flexion. It is seen that the crude extracts of ethanolic, ethyl acetate and petroleum ether Of Carissa carandas leaves significantly ($p < 0.05$) prolonged the time of sodium pentobarbital-induced hypnosis. However, the animal study revealed that the anticonvulsant and sedative activities of Carissa carandas can be used in the treatment of epilepsy.

Keywords: *Carissa carandas*, anticonvulsant, pentobarbitone, hypnosis, epilepsy.

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INTRODUCTION

The various types of brain disorder like schizophrenia, anxiety, Huntington's disease, depression, Alzheimer's disease, Parkinson's disease, epilepsy, etc. The presently available Psychopharmacological agent does not properly meet the therapeutic possibilities for majority of patients with mental health problems but herbal medicines are the ultimate therapeutic remedies for such patients.

As synthetic drugs have lots of side effect here in many cases herbal remedy is being preferred in many of the brain disorders [1]. For the treatment of different alignments in human being many of the medicinal plants are being used either singly or in the combination. Even in today's trends the medical healers uses lots of medicinal plant to treat the different types of alignments and disease as because of their extremely lower side effects as compared to that of the traditional medicine or synthetic medicine. This use of medicinal plants by the medical healers is been practice for years together even today [2].

Carissa carandas commonly known as karvanda belonging to apocyanaceae family is rank-growing, straggly, woody, climbing shrub, usually growing to 10 or 15ft (3-5 m) high, sometimes ascending to the tops of tall trees; and rich in white, gummy latex. The branches, numerous and spreading, forming dense

masses, are set with sharp thorns, simple or forked, up to 2 in (5 cm) long, in pairs in the axils of the leaves.[3] Traditionally the plant is used as astringent, appetizer, antipyretic; lessen-thirst, biliousness and in diseases of the brain[4,5], studies have shown that the different parts of the plant Carissa carandas possess various activities like cardiotonic[6], anticonvulsant[7,] histamine releasing[8], neuropharmacological and diuretics[9], antipyretic [10], vanticancer[11], hepatoprotective[12] etc.

The present study was done to evaluate the anticonvulsant and sedative activity of various extracts of leaves of Carissa carandas in animal models (experimental) with a view to provide a pharmacological justification for the folkware use of the plants leaves in the management of brain diseases.

MATERIALS AND METHODS

Plant material;

The leaves of the plant Carissa carandas were collected from the places such as Parner, situated in Maharashtra, India. These leaves of the plant Carissa carandas were identified by Dr. S. Jayanthi, who is Joint Director, Botanical Survey of India; Pune with a voucher specimen (CCA01) has been kept in herbarium of botanical survey of India, Pune.

Preparation of extracts;

Preparation of the extracts; the leaves of the plant Carissa carandas were collected and then these leaves were air dried under shade and then coarsely powdered. This was done with the help of mechanical grinder. These powered leaves was then extracted using various solvents ranging from nonpolar to polar such as petroleum ether, ethyl acetate, chloroform, ethanol and aqueous successively.

Preliminary phytochemical evaluation;

The preliminary phytochemical screening of petroleum ether, ethyl acetate and ethanolic extracts of Carissa carandas leaves were carried out for qualitative identification of type of phytoconstituents present [13]

Selection of Animals;

The Healthy mice weighing 20±5 gm of either sex were used for the study and housed individually under standard condition of temperature (25± 1ºC), 12 h light/dark cycle and fed with standard pellet diet and water ad libitium. The experiment has been approved by the institutional animal ethical committee and as per CPCSEA guidelines ref: (approval no. 1211/ac/08/CPCSEA).

Acute toxicity studies;

Swiss albino mice weighing 18-22gm of either sex. These mice were used for acute oral toxicity studies. The study was carried out as per to the standards set by OECD and animals were observed to study the mortality and behavioural changes occurring in animals.

Drugs and solvents;

Solvents - Petroleum ether, chloroform, ethyl acetate Ethanol (Loba Chemicals, Mumbai)

Drugs- Pentylenetetrazole (PTZ, Sigma Aldrich, USA), Diazepam (Ranbaxy Laboratory limited.), pentobarbitone (Sigma Aldrich, USA)

Evaluation of anticonvulsant activity;

Pentylenetetrazole (PTZ) induced convulsion;

Here albino mice weighing 18-22gm of either sex were taken and are were divided into eleven different groups.

Group I was served as control and was treated with PTZ 80 mg/kg, intraperitonially,

Group II was treated with diazepam (4mg/kg i.p).

Groups III, IV, V, VI, VII, VIII, IX, X and XI, were treated with three different doses of petroleum ether, ethyl acetate and ethanolic leaves extracts of *C.carandas*, (100,200 & 400 mg/kg). This was done for seven consecutive days.

On the eighth day of treatment the animals were administered with PTZ 80 mg/kg intraperitonially. This was done one hr after the oral administration of control, standard and extracts in their respective groups.

Each animal in the group were observed initially for 30min and then later up to 24 hrs.

The parameters such as onset of clonus, flexion, stupor, recovery, and death and % protection were recorded. The values were expressed as mean ± SEM from 6 animals. The statistical analysis was done by using ANOVA followed by Dunnett's- t -test to calculate the significance difference if any among the groups. $P<0.05$ was considered significant. Those Mice that did not convulse 30 min even after pentylenetetrazole administration were considered to be protected [14.]

Pentobarbitone-induced sleep;

Here again the Mice were divided into eleven groups, (n=6). Animals belonging to

Group I considered as control and were administered vehicle (distilled water 10 ml/kg,p.o.).

Animals from Group II were served as standard and were administered diazepam 4 mg/kg i.p.

Animals from Group III, IV and V were received 100, 200 and 400 mg/kg of petroleum ether extract of *Carissa carandas* leaves respectively.

Group VI, VII, VIII were administered 100, 200 and 400 mg/kg of ethyl acetate extract of *Carissa carandas* leaves respectively.

Animals belonging to Group IX, X and XI were administered 100, 200 and 400 mg/kg of ethanol extract of *Carissa carandas* leaves respectively.

In all the above group Pentobarbitone 85 mg/kg was injected i.p. 30min after administration of their respective doses.

The time interval between disappearance and reappearance of righting reflex of the animal was considered to be the Hypnotic sleeping time for the animals [15]

Stastiscal analysis;

All the result was statistically analyzed by one way analysis of variance (ANOVA) followed by Dunnett's t test. The results were expressed as mean ± standard error mean (SEM). P values of < 0.05 and < 0.01 were considered as significant, compared to control.

RESULT

Phytochemical screening;

Table no .1 indicates the Preliminary phytochemical investigation of the extracts.

Anticonvulsant activity;

Table 2 indicates PTZ induced convulsion,

It was seen that the Diazepam treated group shows significant ($p < 0.05$) reduction in the mean time of tonic hind limb flexion and clonus, whereas in control group the animals shows the development of tonic hind limb extension and stupor.

The Petroleum ether, ethyl acetate and ethanolic extract of Carissa carandas at all doses show no significant decrease in the mean time of tonic hind limb flexion as compared to that of control group. The result analysis when compared with control suggests that there is a decrease in the mean time of clonus, stupor and extension in petroleum ether, ethyl acetate and ethanolic extract of Carissa carandas leaves.

Table 1: Phytochemical Analysis of Carissa Carandas Leaf Extract

Sr.No.	Phytochemical Constituents	Pet.ether extract	Ethyl acetate extract	Ethanol extract
1	Steroids	+	+	+
2	Saponins	+	+	+
3	Tannins	+	-	+
4	Alkoloids	+	-	+
5	Carbohydrates	-	+	+
6	Proteins	+	+	-
7	Amino acids	-	-	+
8	Flavonoids	-	+	+
9	Diterpenes	-	-	+
10	Phenols	-	+	+

Table 2: Anticonvulsant activities of Extracts against PTZ induced convulsions in mice

Group	Groups	Treatment	Time spent in Various phases of convulsion (Sec)					
			Flexion	Extension	Clonus	Stupor	R/ D	% Protection
I	Control	D/W10ml/kg, p.o.	5.16±0.30	9.83±0.30	43.83±0.87	182.17±2.94	R	0
II	Standard	Diazepam 4 mg/kg ,i.p.	5.16±0.30ns	3.00±0.36**	16.50±0.76**	81.66±2.06**	R	69.48
III	Petroleum ether extract	100 mg/kg, p.o.	5.66±0.21ns	7.83±0.30**	39.00±1.46ns	137.00±2.88**	R	20.34
IV	Petroleum ether extract	200 mg/kg, p.o.	6.16±0.30ns	5.66±0.21**	38.16±1.77ns	131.83±2.52**	R	42.42
V	Petroleum ether extract	400 mg/kg, p.o.	5.33±0.33ns	5.00±0.25**	30.16±2.61**	117.67±2.09**	R	49.13
VI	Ethyl acetate extract	100 mg/kg, p.o.	5.83±0.47ns	7.50±0.42**	40.00±1.57ns	140.17±2.12**	R	23.7
VII	Ethyl acetate extract	200 mg/kg, p.o.	6.33±0.21ns	6.66±0.33**	33.16±2.52**	127.67±2.04**	R	32.24
VIII	Ethyl acetate extract	400 mg/kg, p.o.	5.16±0.30ns	5.50±0.42**	29.83±1.99**	116.17±2.71**	R	44.04
IX	Ethanol extract	100 mg/kg, p.o.	5.16±0.30ns	7.33±0.33**	42.33±3.20ns	139.00±2.73**	R	25.43
X	Ethanol extract	200 mg/kg, p.o.	6.33±0.21ns	6.50±0.42**	33.16±1.62**	127.83±2.12**	R	33.87
XI	Ethanol extract	400 mg/kg, p.o.	6.00±0.44ns	5.16±0.30**	28.16±0.94**	118.33±2.63**	R	47.5

ns-nonsignificant ,* P< 0.05, **P<0.01 Values are Mean± SEM , n=6,when compared with control by using one way ANOVA followed by Dunnette's multiple comparison test

Pentobarbital-induced sleeping time;

As shown in Table 3 the petroleum ether extract at all the doses significantly ($p<0.05$) prolonged the sleeping time induced by sodium pentobarbitone in a dose related manner. Whereas the ethyl acetate and ethanol extract when given in the dose of 100 mg/kg does not show significant effect on the sleeping time.

Table 3: Hypnotic activities of Extracts against Pentobarbitone induced sleeping in mice

Group	Groups	Treatment	Carissa carandas	
			Onset of sleeping	Duration of sleeping
I	Control	D/W10ml/kg, p.o.	7.50±0.42	40.50±1.17
II	Standard	Diazepam 4 mg/kg ,i.p.	4.50±0.83**	80.00±1.46**
III	Petroleum ether extract	100 mg/kg, p.o.	7.16±0.30ns	49.50±1.38**
IV	Petroleum ether extract	200 mg/kg, p.o.	6.00±0.25*	60.66±1.14**
V	Petroleum ether extract	400 mg/kg, p.o.	5.00±0.25**	72.16±1.16**
VI	Ethyl acetate extract	100 mg/kg, p.o.	7.83±0.30ns	40.33±1.33ns
VII	Ethyl acetate extract	200 mg/kg, p.o.	5.66±0.21**	61.83±1.32**
VIII	Ethyl acetate extract	400 mg/kg, p.o.	5.50±0.22**	72.33±1.38**
IX	Ethanol extract	100 mg/kg, p.o.	7.50±0.34ns	40.50±0.76ns
X	Ethanol extract	200 mg/kg, p.o.	6.33±0.42ns	61.50±1.68**
XI	Ethanol extract	400 mg/kg, p.o.	5.83±0.60*	73.83±0.94**

ns-nonsignificant ,* P< 0.05, **P<0.01 Values are Mean± SEM , n=6,when compared with control by using one way ANOVA followed by Dunnette's multiple comparison test

DISCUSSION

It is seen that the Pentylenetetrazole-induced convulsion decapitates a valid model for human generalized and absence seizure [16]. Pentylenetetrazole has been used experimentally to identify and study the pharmaceuticals that may control the seizure susceptibility.

The exact mechanism of epileptogenic action of pentylenetetrazole is still unclear, but it has been reported that PTZ produces seizures by inhibiting gamma-amino butyric acid (GABA) activity [17] .Due to the enhancement of GABAergic neurotransmission it has been shown to inhibit or attenuate seizure. Whereas the inhibition of GABAergic neurotransmission or activity is known to promote or facilitate seizure [18]. Many of the Anticonvulsant agents like diazepam and pentobarbitone by enhancing the action of GABA-A receptor inhibit pentylene-tetrazole-induced seizure, and there by facilitates the opening of chloride-ion channels [19]. Thus the crude petroleum ether, ethyl acetate and ethanolic extract may produce their anticonvulsant activity by enhancing GABA ergic neurotransmission. The crude petroleum ether, ethyl acetate and ethanolic extract of the plant was found to show the sedative effect, as shown by its abilities to prolong pentobarbitone-induced sleeping time.It is also known that drugs having the sedative properties prolonged the sleep time as that produced by barbiturates [20]. Phytochemical Retrospective reports reveal that the triterpenoids and saponins (phytoconstituents) present in most of the plants are responsible for their anticonvulsant activity. The Sedative and hypnotic effect in the plant was found due to the compounds such as flavonoids, terpenes and saponins.

The pharmacological effects of petroleum ether, ethyl acetate and ethanolic extract observed in this study are likely due to the phytoconstituents such as flavonoids, terpines, tannins or alkaloids.

CONCLUSION

From the present study it was observed that the leaves of *Carissa carandas* possess anticonvulsant and sedative action .This action may be due to presence of various phytoconstituents such as flavonoids, terpines, alkaloids etc. Further the isolation and detection of phytoconstituents is essential.

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REFERENCES

1. Sandhya S, Vinod KR , Sravan Kumar. Herbs Used for Brain Disorders. Hygeia. J. D. Med, 2010; 2(1):38-45.
2. Ross-Ibarra J, Molina-Cruz A. The ethnobotany of Chaya: a nutritious Maya vegetable. Economic Botany. 2002; 56:350-365.
3. Kirtikar KR, Basu BD. Indian medicinal plant. Volume II, Lalit Mohan Basu, Allahabad, 2003, pp 1546.
4. Anonymous (1978), The Ayurvedic Formulary of India, Ministry of Health and Family Planning, Govt. of India, New Delhi, Part I, PP. 149.
5. Vohra MM, De NN. Comparative cardiotonic activity of Carissa carandas {L}.and Carissa spinarum {A}. Indian journal of medical research. 1963; 51(5): 937-940.
6. Hegde K, Thakker SP, Joshi AB, Shastry CS, Chandrashekhar KS. Anticonvulsant activity of *Carissa carandas Linn.* root extract in experimental mice. Tropical Journal of Pharmaceutical Research 2009; 8(2):117-125.
7. Rajasekaran A, Jeyasudha V, Kalpana B, Jayakar B. Preliminary phytochemical and antipyretic evaluation of *Carissa carandas*. Indian J Nat Prod 1999; 15(1):27-29.
8. Joglekar SN and Gaitonde BB. Histamine releasing activity of Carissa Carandas roots (apocyaneceae). Japanese Journal of Pharmacy.1970; 20:367-372.
9. Saha R, Hossain L , Bose U , Rahman A. Neuropharmacological and diuretic activities of Carissa carandas Linn leaf. Pharmacologyonline. 2010; 2:320-327.
10. Balakrishnan N, Bhashkar VH. Analgesic, anti-inflammatory and antipyretic activities of *Pergulariadaemia and Carissa carandas*. DARU. 2009; 17(3):168-174.
11. Sulaiman SF, Wong ST, Ooi KL, Yusof SR, Tengku M, Tengku S. Anticancer Study of *Carissa carandas* extracts. Project Report. USM, 2008.
12. Hegde K, Joshi AB. Hepatoprotective effect of *Carissa carandas* Linn. Root Extract against CCL4 and Paracetamol induced Hepatic Oxidative Stress. Indian Journal of Experimental Biology. 2009; 47:660-667.
13. Kokate CK. Practical Pharmacognosy. 4th ed. New Delhi: Vallabh Prakashan; 1994.
14. Griebel G, Perrault G, Tan S, Schoemaker S, Sanger D. Pharmacological studies on synthetic flavonoids: comparision with diazepam. Neuropharmacol. 1999; 38:965-977.
15. Aziz A, Khan I. Pharmacological evaluation of Sedative and Hypnotic activities of methanolic extract of *Lycopus europaeus* in mice. The Journal of Phytopharmacology. 2013; 2(4):8-12.
16. Vijayalakshmi A, Ravichandiran V, Anbu J, Velraj M, Jayakumari S. Anticonvulsant and neurotoxicity profile of the rhizome of *Smilax china* Linn. in mice. Indian J Pharmacol. 2011; 43(1):27-30.
17. Rocha L. Subchronic treatment with antiepileptic drugs modifies pentylenetetrazol-induced seizures in mice: Its correlation with benzodiazepine receptor binding. Neuropsychiatr Dis Treat. 2008; 4(3):619-625.
18. Shin EJ, Bach JH, Nguyen TTL, Jung BD, Oh KW, Kim MJ, *et al*. *Gastrodia Elata* Bl attenuates cocaine-induced conditioned place preference and convulsion, but not behavioral sensitization in mice: importance of GABAA receptors. Curr Neuropharmacol. 2011; 9(1):26-29.
19. Praveen KU, Naga PK, Murali KB, Swarnalatha M. Evaluation of antiepileptic activity of methanolic extract of *Brassica nigra* sseeds in mice. International Journal of Pharmaceutical Innovations 2013; 3(2):73-84
20. Dehar N, Walia R, Ratol S. Potentiation of thiopentone sodium induced hypnosis by BerberisAristata in rodents. Asian J Pharm Clin Res. 2012; 5(1):131-133.