NEUROPHARMACOLOGICAL AND DIURETIC ACTIVITIES OF *CARISSA CARANDAS* Linn. LEAF

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Summary

The crude methanolic extracts of leaves of *Carissa carandas* Linn. were evaluated for its neuropharmacological and diuretic activities. The extract of *Carissa carandas* leaves also potentiated the pentobarbital induced sleeping time in mice, and decreased the open field score in open field test, decreased the number of hole crossed from one chamber in the hole cross test and decreased the head dip responses in hole board test. Diuretic activity was proved by the electrolyte loss ratio (Na+/K+ excretion ratio was 1.46 and 1.43 at the doses of 200 and 400 mg/kg respectively) as that of the standard diuretic furosemide (1.48). The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

Key words: Neuropharmacological, Diuretic, *Carissa carandas,* Apocynaceae.

Introduction

Carissa carandas Linn. (syn. *Carissa congesta* Wight) is a large dichotomously branched evergreen shrub with short stem and strong thorns in pairs, belonging to the family Apocynaceae. The plant is mostly found in throughout of Bangladesh, India, Srilanka, Java, Malaysia, Myanmar, Nepal and Pakistan. The plant is cultivated as a hedge plant throughtout Bangladesh. In traditional system of medicine, the plant is generally used as an astringent, appetizer, antipyretic, in biliary disorders, stomach disorders, rheumatism and disease of the brain[1]. Previous studies have shown that the extract of the plant possesses cardiotonic, antipyretic and antiviral activities[2-4]. The root of this plant is also used as anthelmintic and antimicrobial purpose[5]. Unripe fruits are astringent and anti-scorbutic. Decoction of leaves is given at the commencement of remittent fever. The bark extract is also reported as cardiotonic[6,7]. Another study done by Sulaiman et al.[8] have shown that the chloroform extract of *Carissa carandas* leaves exhibited cytotoxic activity on human ovarian carcinoma cell line and unripe fruits also showed the activity against lung cancer cell line in human model.

The fruit part of this is also well known for its antifungal activities[9]. The alcoholic extract of the roots of *Carissa carandas* L. has been reported to possess cardiotonic activity and to produce a perceptible decrease in blood pressure in normal anaesthetized cats[10].

Phytochemical studies undertaken by different groups of workers of different parts of the plant have resulted in the isolation of various types of cardiac glycosides[11], fatty acids, terpenoids, flavonoids and phenolic acids. Carinol, a distinct type of terpenoid carindone was isolated from the roots of *Carissa carandas*[10]. Earlier studies have shown that the extract of the plant also possess cardiotonic, a triterpenoidal constituent carissone and β-sitosterol[1]. In Southern parts of Bangladesh, the decoctions and extracts of the leaves of this plant are effective remedies in management and/or control of different disease conditions. However, no research studies going on to validate the local claim. The aim of this present study was, therefore, to evaluate the neuropharmacological and diuretic potential of the methanol extract of the leaves of *Carissa carandas* in experimental animal models, with a view to provide a pharmacological justification (or otherwise) for the folklore use of the plants leaves in the management of neuropharmacological and diuretic disorders in some communities in Bangladesh.

Materials and Methods

Plant material collection and extraction

The leaves of *Carissa carandas* Linn. were collected from Naogaon District, Bangladesh in July 2007, and were taxonomically identified at the Bangladesh National Herbarium (accession no: 33967). The voucher specimens were deposited in the Bangladesh National Herbarium, Dhaka. About 400 g of powdered sample were taken in a clean, flat-bottomed glass container and soaked in 1,300 ml of 80% ethanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of cotton followed by a filtration through Whatmann filter paper and the filtrate thus obtained was concentrated using a rotary evaporator (Bibby RE200, Sterilin Ltd., U.K.) to get the crude extract.

Animals

Young Swiss-albino mice of either sex, weighing 20 - 25 g, purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used for the test. The animals were kept at animal house (Pharmacy Discipline, Khulna University) for adaptation after their purchase under standard laboratory conditions (relative humidity 55 - 65%, room temperature 25.0 ± 2.0°C and 12 h light-dark cycle) and fed with standard diets (ICDDR, B formulated) and had free access to tap water. The experimental met the national guidelines on the proper care and use of animals. The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol.

Drugs

Pentobarbital (Sigma Chemicals,U.S.A.), Furosemide (Square Pharmaceuticals Ltd, Bangladesh).

Chemical group test

The crude methanolic extract of leaves of *Carissa carandas* was tested for its different chemical groups as alkaloids, flavonoids, gums, reducing sugars, saponins, steroids and tannins[12]. In each test 10% (w/v) solution of the extract in methanol was taken unless otherwise mentioned in individual test.

Pharmacological studies

Neuropharmacological activity

i) Pentobarbital induced hypnosis

Pentobarbital induced hypnosis test was carried out by the method of Williamson *et al.*[13]. The test animals were divided into three groups consisting of seven mice in each group. Group I was the control group and group II and III were the experimental groups. The experimental groups were administered with the methanolic extract of *Carissa carandas* at dose of 250 and 500 mg/kg body weight intra-peritoneally (i.p.), while the animals of group I (control) were supplied with distilled water containing 0.1% (v/v) tween-80 (i.p.)at the dose of 10 ml/kg of body weight. The total sleeping time were recorded for both control as well as for treated groups.

ii) Exploratory behaviour

This experiment was performed by (i) Open field test[14] (ii) Hole cross test[15] and (iii) Hole board test[16]. The test animals were divided into three groups consisting of seven mice in each group. Group I was the control group and group II and III were the experimental groups. The experimental groups were administered with the methanolic extract of *Carissa carandas* (prepared by distilled water and tween-80) at dose of 250 and 500 mg/kg of body weight intra-peritoneally (i.p.), while the animals of group I (control) were supplied with 0.1% (v/v) tween-80 (i.p.) at the dose of 10 ml/kg of body weight. The observations were made on 0 min before injection and 30, 60, 120 and 240 min after injections of the test samples and control.

Diuretic activity

Diuretic activity of the extract was investigated using the method as described by Lipschitz et al.[17] The test animals were randomly chosen and divided into five groups having ten mice in each. Twenty-four hours prior to the experiment, the test animals were placed in to metabolic cages with the withdrawal of food and water. Group-1 or the control group received vehicle (1% Tween 80 in water) at a dose of 10 ml/kg body weight orally. Group-2 was provided with urea solution at a dose of 500 mg/kg. Group-3 was provided with standard diuretic drug furosemide at a dose of 0.5 mg/kg.Group-4 and group-5, the test groups were treated with the methanol extract of MP at the doses of 200 and 400 mg/kg respectively. From the graduated urine chamber of metabolic cage, the urinary output of each group was recorded 5 h after the above treatments. Collected urine was centrifuged and then estimated for sodium and potassium by using digital flame photometer (Elico Pvt. Ltd., model CL 22D). Chloride was estimated by the Schales and Schales method reproduced by Godkar [18].

Statistical analysis:

Student's *t*-test was used to determine a significant difference between the control group and experimental groups.

Results

Preliminary phytochemical analysis

Results of different chemical tests on the methanol extract of *Carissa carandas* Linn. showed the presence of alkaloids, glycosides, steroids, flavonoids, and tannins (Table 1).

Table 1. Phytochemical properties of *Carissa carandas* crude leaf extract

Compound	Alkaloids	Glycosides	Steroids	Gums	Flavonoids	Saponins	Reducing sugars	Tannins
Observation	+ve	+ve	+ve	- ve	+ ve	-ve	-ve	+ve

Key: +ve = Presence -ve = Absence

Neuropharmacological activity

i) Pentobarbital induced hypnosis Test

Table 2 showed the effect of *Carissa carandas* on pentobarbital induced hypnosis in mice. The total sleeping time was about 68 and 91 min at dose of 250 and 500 mg/kg of body weight respectively where as in control group it was about 32 min.

Table 2. Effect of methanolic extract of *Carissa carandas* on pentobarbital induced hypnosis in mice

Animal group	*Treatment*	*Time of onset of sleep (min)*	*Total sleeping time (min)*
I (Control)	0.1% Tween 80 solution	7.315±0.133	32.45±2.03
II (Test group-I)	Me. Extract 250 mg/kg.	7.14±0.0578*	68.57±2.39**
III (Test group-II)	Me. Extract 500 mg/kg	7.39±0.114*	91.86±7.93**

Values are Mean ± SEM; *, $P<0.05$; **, $P<0.001$ vs. control, Student's t-test; Me.= Methanol

Table 3: Effect of *Carissa carandas* on exploratory behavior in mice

Group	Response at				
	0 min	30 min	60 min	120 min	240 min
Effect on Open Field Test					
I (Control)	86.26±2.07	92.73±1.85	97.57±1.84	105.25±1.33	105.84±1.47
II (Me. Ext.) 250 mg/kg	85.84±1.66*	78.55±1.15*	70.25±1.55*	63.57±1.88*	61.93±3.24*
III (Me. Ext.) 500 mg/kg	86.67±2.04*	68±2.39*	55±2.79*	45.31±3.05*	42.56±3.37*
Effect on Hole Cross Test					
I (Control)	8.52±0.65	8.87±0.44	8±0.48	9.34±0.78	8.36±0.56
II (Me. Ext.) 250 mg/kg	6.17±0.68*	6.13±0.54*	3.97±0.47*	3.66±0.36*	1.98±0.19*
III (Me. Ext.) 500 mg/kg	8.32±0.66*	5.90±0.37*	3.89±0.26*	2.18±0.24*	1.17±0.18*
Effect on Hole Board Test (Head dipping)					
I (Control)	14.55±0.65	20.14±1.14	22.56±1.08	18.83±0.72	15.0±0.83
II (Me. Ext.) 250 mg/kg	12.98±0.53*	11.16±0.36*	10.43±0.52*	8.306±0.40*	5.14±0.32*
III (Me. Ext.) 500 mg/kg	14.23±0.83*	10.85±0.68*	8.15±0.65*	5.17±0.56*	2.46±0.57*

Values are expressed as mean ± S.E.M.(n=7); Me., methanolic. *, $P<0.001$ vs. control.

ii) Exploratory behavior Test

Test for exploratory behavior in mice was performed by (i) Open field test (ii) Hole cross test and (iii) Hole board test. It was observed that the extract decreased the number of open field score (Table 3), caused decrease in the number of hole crossed from one chamber to another chamber (Table 3), and also decreased head dip responses (Table 3) in mice at dose of 250 and 500 mg/kg of body weight from 30 min to 240 min.

Diuretic activity

The effect of the methanolic extract of *Carissa carandas* Linn. on the urination of mice was observed for 5 h which revealed that the extract has a marked diuretic effect in the test animals. This was comparable to that of standard drug furosemide and diuretic agent urea. Electrolyte loss showed similar ratio (Na+/K+ excretion ratio was 1.46 and 1.43 at the doses of 200 and 400 mg/kg respectively) as that of the loop diuretic furosemide (1.48) (Table 4).

Table 4. Effect of methanolic extract of *Carissa carandas* Linn. on urine excretion parameters in mice

Treatment	Dose (mg/kg; p.o.)	Volume of urine (ml)[b]	Concentrations of ions (m.eq.l^{-1})			
			Na^+	K^+	Cl^-	Na^+/K^+
Group-1(Control)	-	2.54 ± 0.07	74.67 ± 1.23	47.74 ± 1.17	78.55 ± 1.25	1.52
Group-2(Urea)	500	3.73 ± 0.06	113.66 ± 1.36**	75.57 ± 1.28**	88.75 ± 1.37*	1.48
Group-3(Furosemide)	0.5	4.15 ± 0.12	121.85 ± 1.75**	85.47 ± 1.66**	93.37 ± 1.59*	1.48
Group-4(ME)	200	4.25 ± 0.08	117.55 ± 1.18**	79.35 ± 1.86**	91.73 ± 1.58*	1.46
Group-5(ME)	400	4.95 ± 0.06	132.76 ± 1.54**	92.24 ± 1.81**	97.60 ± 1.87*	1.43

ME: Methanolic extract of Carissa carandas Linn.; Values are expressed as mean ± SEM (Number of animals, n = 10); *indicates P<0.01, **indicates P<0.001 vs. control; [b]Collected for 5 hours after treatment.

Discussion

Central depressants elicit their effect by interfering with the functions of the cerebral cortex. A most important method of investigating the probable cortical manifestation of a drug is to check its effect on the pentobarbital narcosis as pentobarbital has multifarious effects on the cerebral cortex[19]. The pentobarbital sleeping time test was performed performed to find out whether the water extract of the plants have any effect on the cerebral cortex. Pentobarbital shorten the onset of sleep and increases sleep duration. The methanolic extract of *Carissa carandas* Linn reduced the onset of sleep and potentiated the pentobarbital induced sleeping time in mice, which suggests its central depressant activity[20] thus, suggesting the probable tranquilizing action[21].

It has been experimentally proven that, in the absence of a special task to perform, the behaviour of a given animal tend to maintain that inner activation level that is, at times, inconsistent with the actual level of activation of the animals. In order to get as accurate a picture as possible, on the effect of the drug on exploration, the open field test was performed. The extract also made mice to reduce their behavioural exploration, which further support the central sedative properties of the extract. The overall results tend to predict the CNS depressant action of the extract.

Diuretic activity may be very useful in a number of conditions like hypertension, hypercalciuria, cirrhosis of liver. Furosemide, used as the standard drug in this experiment belongs to the loop or high-ceiling diuretics, which act by inhibiting Na+/ K+/Cl^- co-transport of the luminal membrane in the ascending limb of the loop of Henle and have the highest efficacy in mobilizing Na^+ and Cl^- from the body. The extract was able to increase the volume of urine with statistical significance along with a considerable Na+ and Cl^- load which was comparable to that of furosemide. The diuretic action of the extract may be due to its action on the kidney. The extract may also contain a high proportion of osmotically active compounds or their metabolites that lead to an increased urine volume. There was an increase

in the ratio of concentration of excreted sodium and potassium ions after plant extract treatment. This also indicates that the extract increases sodium ion excretion to a greater extent than potassium, which is a very essential quality of a good diuretic with lesser hyperkalaemic side effect. This observed diuretic effect may be due to the effect of extract on the glomerular filtration rate and the direct inhibitory effect on the reabsorption mechanism of salt. Further studies may be carried out to identify whether these actions are associated with the same agent or a number of agents that are responsible for such activities.

Conclusion

Thus, in the present investigation, it could be suggested that the methanol extract of *Carissa carandas* Linn leaves part showed potent neuropharmacological and diuretic activities. These facts indicate the scientific basis of *Carissa carandas* Linn. being used as a traditional medicine. However, further experiments may help to determine the pharmaceutical potentialities of the plant as a medicine.

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