

ANALGESIC AND ANTIPYRETIC ACTIVITIES OF
AQUEOUS EXTRACT OF LEAVES OF
CARISSA CARANDAS LINN.

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Summary

Decoction of leaves of *Carissa carandas* is traditionally used to treat painful and inflammatory conditions. In the present study, analgesic and anti-pyretic activities of aqueous extract of *Carissa carandas* were studied using hot plate, acetic acid induced writhing and yeast induced hyperthermia method. Aqueous extract of *Carissa carandas* showed significant analgesic and anti-pyretic activities at all the doses used and in all models studied. Results support the traditional use of the plant in the treatment of pain and fever.

Keywords: *Carissa carandas*, analgesic, anti-pyretic, hot plate, acetic acid induced writhing.

Introduction

Carissa carandas Linn (syn. *C. carandas* Auct.) belong to the family *Apocynaceae*. It is called kerenda in Malaya, karaunda in India; Bengal currant or Christ's thorn in South India; namdaeng in Thailand; caramba, caranda, caraunda and perunkila in the Philippines [1]. The leaf decoction is valued in cases of intermittent fever, diarrhea, oral inflammation and earache [2, 3].

Material and Methods

Plant material

The leaves of *Carissa carandas* were collected from local area of Meerut district and identified and authenticated by Dr. Anjula Pandey, Principal Scientist, National Herbarium of Cultivated Plants, New Delhi. Voucher specimens (No. NHCP/NBPGR/2009-29/4813) have been kept in National Herbarium of Cultivated Plants, New Delhi and Department of Pharmaceutical Technology, MIET for future reference.

Plant extract

The leaves were dried under shade, reduced to moderately coarse powder and macerated with hot water for 48 hours to get aqueous extract. The aqueous extract was concentrated to dryness using Rotary evaporator, giving yield as 11.14% w/v and preserved in a refrigerator. Aliquot portions of the aqueous extract (AECC) of *Carissa carandas* were weighed and suspended in an appropriate volume of Tween 80 (2% v/v) for use on each day.

Acute toxicity study of the extract

Female Albino Wistar rats weighing 200-220 g were used in the study. Acute oral toxicity was performed as per OECD-423 guidelines [4]. The animals were fasted overnight with water *ad libitum*. The starting dose of 5 mg/kg of aqueous extract was administered orally to three animals in each group. If mortality was observed in two or three animals, then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again in three animals to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300, and 2000 mg/kg body weight. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days.

Preliminary Phytochemical Studies

The aqueous extract was then subjected to qualitative phytochemical screening for the identification of different phytoconstituents. Aqueous extract of leaves of *Carissa carandas* at a dose level of 100, 200 & 400 mg/kg b.w. were used for monitoring the analgesic and anti-pyretic activity.

Animals

Adult albino rats (200-250 gm b.w) and albino mice (20-25 gm b.w.) were kept in polypropylene cages at an ambient temperature of $25^{\circ} \pm 2^{\circ}\text{C}$ with 55-65% relative humidity and 12 h light/dark cycle. These animals had free access to water and normal laboratory diet (Lipton India Limited). The institutional animal ethics committee (IAEC) approved the use of animals for the present study, (Ethical clearance number: 711/02/a/CPCSEA).

Antipyretic Testing

Hyperthermia was induced in rats following the method of Teotino et al., 1963. Initial rectal temperatures of rats were recorded using a six channel tele-thermometer for 1 min. Rats were made hyperthermic by subcutaneous injection of 20% yeast suspension at a dose of 1 ml/100 gm body weight. When the temperature was at peak (18 hours after yeast injection) the rectal temperature were again recorded. Those animals that showed a rise in rectal temperature of more than 1.2°C were used [5]. Aqueous extract of *Carissa carandas* was given orally at a dose level of 100, 200 & 400 mg/kg as a suspension prepared in 2% Tween 80 solution. Animals were divided into five groups of six animals each. First group received 1 ml of 2% Tween 80 solution orally and served as control. Second, third, fourth and fifth groups received standard antipyretic agent i.e. paracetamol suspension (100 mg/kg) [6], aqueous extract (100 mg/kg), aqueous extract (200 mg/kg) and aqueous extract (400 mg/kg) respectively. The rectal temperatures of animals were recorded at 30 minutes intervals for 4 hours following the administration of Tween 80, standard drug and plant extracts [6].

Analgesic Activity

Hot Plate Method

The hot plate method described by Turner (1965) was followed for the assessment of analgesic activity. Albino mice were introduced to a hot plate maintained at $55 \pm 0.5^\circ\text{C}$. The reaction time to the thermal stimulus was recorded as the time interval from introduction of the animal to the plate until the first lick of the limbs or the first jump of the animals. The test groups received aqueous extracts of *Carissa carandas* at 100, 200 & 400 mg/kg dose levels prepared as suspension in 2% Tween 80 orally, the standard group received Pentazocine (10mg/kg, i.p.) [7] and control group received only 1 ml of 2% Tween 80 solution. The reaction times were determined before and after 30 minutes, 1 hour, 2 hours and 3 hours period with reference to the control group receiving only vehicle.

Acetic Acid Induced Writhing

Acetic acid solution at a dose of 10ml/kg (0.6%) was injected i.p. and the number of writhes during the following 15 minutes period was observed. The test groups received aqueous extracts of *Carissa carandas* at 100, 200 & 400 mg/kg dose levels prepared as suspension in 2% Tween 80 orally, the standard group received Aspirin (10 mg/kg, orally) [7] and control group received only 1 ml of 2% Tween 80 solution. Significant reductions in number of writhes by drug treatment as compared to vehicle treatment animals were considered as a positive analgesic response. The percent inhibition of writhing was calculated [7].

$$\% \text{ Inhibition} = \frac{\text{WC} - \text{WT}}{\text{WC}} \times 100$$

Where,

WC = Mean number of writhes in control group.

WT = Number of writhes in test group.

Statistical Analysis [8]

All the results obtained from various activities, as described above, were analyzed statistically by using Student's t test and $p < 0.05$ were considered significant. The results are summarized in the tables given below.

Results

Acute toxicity studies

In the acute toxicity studies no signs of toxicity or mortality were observed at 2000 mg/kg dose level. Therefore we have taken 200 mg/kg as the therapeutic dose and made variations by taking 100 mg/kg as lower dose and 400 mg/kg as higher dose.

Phytochemical Screening

The aqueous extract showed positive test for proteins, phenols, terpenoids, flavonoids and glycosides.

Anti-pyretic Activity

The anti-pyretic activity of the aqueous extract of *Carissa carandas* have been shown in table 1, which showed significant activity at all dose levels. The results were comparable to that of Paracetamol, a prototype of anti-pyretic drug.

Table 1. Effect of different doses of Aqueous extract of *Carissa carandas* and Paracetamol on yeast induced hyperthermia in rats.

Groups	Rectal Temperature (°C)						
	Initial before yeast injection	18 Hrs. after Yeast injection	Time after drug administration (hrs)				
			0.5 hrs	1 hr	2 hrs	3 hrs	4 hrs
Control	36.10 ± 0.0966	37.46 ± 0.0881	38.51 ± 0.1195	38.73 ± 0.0919	39.25 ± 0.0846	39.35 ± 0.0763	39.21 ± 0.0703
Paracetamol (100 mg/kg)	36.01 ± 0.1194	37.35 ± 0.1335	37.03 ± 0.1115 ^a	36.66 ± 0.0918 ^a	36.15 ± 0.0670 ^a	35.91 ± 0.0600 ^a	35.88 ± 0.0600 ^a
AECC (100 mg/kg)	36.11 ± 0.1740	37.50 ± 0.1612	37.31 ± 0.1701 ^a	37.10 ± 0.1712 ^a	36.90 ± 0.1591 ^a	36.75 ± 0.1522 ^a	36.80 ± 0.1316 ^a
AECC (200 mg/kg)	36.06 ± 0.1256	37.45 ± 0.1335	37.21 ± 0.1681 ^a	36.80 ± 0.1390 ^a	36.51 ± 0.1579 ^a	36.23 ± 0.1542 ^a	36.18 ± 0.1514 ^a
AECC (400 mg/kg)	36.16 ± 0.1022	37.53 ± 0.1144	37.21 ± 0.1137 ^a	36.70 ± 0.0856 ^a	36.25 ± 0.0991 ^a	35.91 ± 0.0980 ^a	35.80 ± 0.0730 ^a

Values are expressed as mean ± S.E.M. (n=6);
Significance at ^ap< 0.001 as compared to control.
AECC: - Aqueous extract of *Carissa carandas*

Analgesic Activity

Hot Plate Method

The aqueous extract at all the dose levels has shown significant activity. Maximum activity has been shown by the extract at 400 mg/kg dose level.

Table 2: Effect of different doses of Aqueous extract of *Carissa carandas* by Hot Plate reaction time in mice.

Groups	Dose (mg/kg)	Reaction Time (Seconds)				
		Initial	Time after drug administration (Hrs)			
			0.5 hrs	1 hr	2 hrs	3 hrs
Control		5.13± 0.092 6	5.14 ± 0.0928	5.15 ± 0.0947	5.14 ± 0.0913	5.15 ± 0.0758
Pentazocine	10	5.09 ± 0.070 0	7.72 ± 0.0675 b	8.83 ± 0.0792 b	9.68 ± 0.0623 b	9.62± 0.0583 b
AECC	100	5.11± 0.034 7	5.26 ± 0.0571	5.72 ± 0.0764 b	6.48 ± 0.0725 b	6.36 ± 0.0681 b
AECC	200	5.15 ± 0.069 4	5.56 ± 0.0909 a	6.39 ± 0.0804 b	7.66 ± 0.0503 b	7.56 ± 0.0432 b
AECC	400	5.13 ± 0.084 5	5.75 ± 0.0700 b	6.66 ± 0.0657 b	8.51 ± 0.0545 b	8.44 ± 0.0553 b

Values are expressed as mean ± S.E.M. (n=6); significance at ^ap<0.01, ^bp<0.001 as compared to control.

AECC: - Aqueous extract of *Carissa carandas*

Acetic Acid Induced Writhing

The aqueous extracts at dose levels of 100, 200 and 400 mg/kg exhibited 47.70, 64.02 & 72.80% inhibition of writhing as compared to that of 76.14% inhibition shown by Aspirin. It is quite evident from the result that the extract at 400 mg/kg showed comparable activity to that of Aspirin.

Table 3: Effect of different doses of Aqueous extract of *Carissa carandas* on Acetic acid induced writhing in mice.

S. No.	Groups	Dose (mg/kg)	No. of Writhing (Mean \pm SEM)	% Inhibition
1	Control		39.83 \pm 1.2496	-
2	Aspirin	10	9.5 \pm 0.6192 ^a	76.14
3	AECC	100	20.83 \pm 1.7403 ^a	47.70
4	AECC	200	14.33 \pm 1.2020 ^a	64.02
5	AECC	400	10.83 \pm 0.7924 ^a	72.80

Values are expressed as mean \pm S.E.M. (n=6); significance at ^ap< 0.001 as compared to control.

AECC: - Aqueous extract of *Carissa carandas*

Discussion

The analgesic and antipyretic activities of many plants have been attributed to their terpenoids and flavonoids contents [9]. The present study establishes the anti-pyretic and analgesic activities of the aqueous extract of *Carissa carandas* in the models used. Since antipyretic and analgesic activities are commonly mentioned as characteristic of drugs or compounds which have an inhibitory effect on prostaglandin biosynthesis [10], the yeast induced hyperthermia in rat model was, therefore, employed to investigate the antipyretic activity of this plant. It was found that aqueous extract at the dose of

100, 200 & 400 mg/kg showed a significant decrease in rectal temperature similar to that shown by the standard drug, paracetamol. This result seems to support the view that the extracts have some influence on prostaglandin biosynthesis because prostaglandin is believed to be a regulator of body temperature [11].

Likewise, the analgesic activity of aqueous extract of the plant was evaluated using the hot plate method and writhing test in mice. The hot plate method is useful in detecting centrally acting analgesics [12] whereas acetic acid induced writhing method is useful to detect peripheral analgesic effects. Acetic acid, which is used as an inducer for writhing syndrome, causes algesia by liberation of endogenous substances, which then excite the pain nerve endings [5]. The fact that the aqueous extract of *Carissa carandas* showed analgesic activity in both the models studied, indicate that this effect could be due to the presence of two components; one acting centrally and the other via peripheral route [12].

NSAID such as aspirin used in this study are known to inhibit cyclooxygenase enzymes I and II which are implicated in the production of inflammation mediating agent prostaglandin (PGE₂) from arachidonic acid [13, 14, 15]. The pattern of analgesic activity exhibited by the extract was similar to that of aspirin which suggests that the plant's activity may be mediated by cyclooxygenase I and II inhibition.

Conclusion

From the above results, it can be deduced that the aqueous extract has shown dose dependent significant activity in all the models used. Therefore the present study had verified the traditional use of *Carissa carandas* in fever and pain. As the phytochemical screening has shown the presence of flavonoids, terpenoids and glycosides in the aqueous extract, its potent activity may be attributed to the presence of these phytoconstituents. More detailed phytochemical studies are, however, necessary to identify the active principle(s) and exact mechanism of action.

Acknowledgement

The authors are thankful to Dr. Anjula Pandey, Principal Scientist, National Herbarium of Cultivated Plants, National Bureau of Plant Genetic and Resources, New Delhi for identification and authentication of the plant and also to the Department of Pharmaceutical Technology, MIET, Meerut for providing research facilities to carry out the work.

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