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Research Article

Evaluation of anti-inflammatory and anti-pyretic activity of *Carissa carandas* L. leaf extract in rats

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ABSTRACT

Carissa carandas L. (Apocynaceae) is a medicinal plant and is widely distributed in tropical and subtropical regions of India. The present investigation attempted to find out the anti-inflammatory and anti-pyretic potentials of the methanol extract of *C. carandas* L. leaf. The extract was evaluated for phytochemical screening, which indicated the presence of steroids, glycosides, flavonoids, tannins, terpenoids and carbohydrates. The anti-inflammatory property was evaluated by using different models like carrageenan, histamine and dextran induced hind paw oedema in Wister Albino rats. The extract at the dose of 200 mg/kg body weight exhibited maximum inhibition of inflammation, *i.e.*, 72.10 %, 71.90 % and 71.80 % at the end of 3 hour with histamine, dextran and carrageenan induced rat paw oedema respectively. The anti-pyretic activity was evaluated by Brewer's yeast induced pyrexia in albino rats. The extract at the dose of 100 and 200 mg/kg p.o., showed the significant reduction in yeast induced elevated temperature in a dose depended manner and the effect also extended up to 4 hours after the drug administration. The results of this study indicated that the methanol extract from leaves of *C. carandas* L. possess significant anti-inflammatory and anti-pyretic activities in rodent models.

Key words: Anti-inflammatory; Anti-pyretic; *Carissa carandas* L.; Methanolic extract; Rat paw oedema

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INTRODUCTION

According to WHO (1993), 80 % of the world's population is dependent on the traditional medicines and a major part of the traditional therapies involves the use of plant extracts or their bioactive components [1]. Plant-based natural constituents can be derived from any part of the plant like bark, leaves, roots, fruits, seed, fruit rind, etc. [2]. Plants have been studied in detail for their anti-inflammatory and anti-pyretic properties; some secondary metabolites also have been found to be responsible for the same.

Carissa carandas L.is a species of flowering shrub in the dogbane family, Apocynaceae. It produces berry-sized fruits and is commonly known as Christ's thorn or Bengal Currant, 'Kalakke' in Tamil [3]. *C. carandas* L. traditionally used as stomachic, anti-diarrheal and anthelmintic. Stem is used to strengthen tendons and fruits are used in skin infections; whereas leaves are remedy for fevers, earache and syphilitic pain [4-7]. Alcoholic extract of root material decrease the blood pressure [8] and aqueous extract of root have been reported various pharmacological activities like histamine releasing,[9] anthelmintic, spasmolytic and cardiotonic [10]. Fruits have also been studied for its analgesic, anti-inflammatory [11] and lipase [12] activity. Carisone and carindone [13, 14], carinol, lignin [15], oderoside H [13] and 2-acetylphenol [10, 16] have been reported as chemical constituents present in roots. Fruits of this plant have been reported to contain a mixture of volatile principles [16, 17] like 2-phenyl ethanol, linalool, β-caryophyllene, isoamyl alcohol and benzyl acetate and a novel (Carissol) triterpenic alcohol [18]. The leaves are reported to have triterpene [19], tannins [20] and carissic acid [21]. However, no report is available on the anti-inflammatory and antipyretic potentials of the *C. carandas* L. leaf. Therefore in this current study, we made an attempt to evaluate the anti-inflammatory and antipyretic activity of methanolic extract of *C. carandas* L.

MATERIALS AND METHODS

Plant Material

The plant materials were collected from Boral in the Joinpur region of South 24-Pargans district of West Bengal (India) in December, 2001 and identified by the Central National Herbarium, Botanical Survey of India, Shibpur, Howrah, India. A voucher specimen [CNH/I-I (44) 2002-Tech. II/472] was deposited in the institution for further reference. The leaves were collected for experiment in 2012, shade dried and pulverized in a mechanical grinder, passed through 40 mesh sieve and stored in a closed container for further use.

Preparation of extract and preliminary phytochemical screening

The powdered leaves were extracted with methanol in a soxhlet extractor. On evaporation of methanol under reduced pressure, a dark brown colored residue was obtained (yield 12.26 % w/w) and was stored in desiccators. For pharmacological experiments a weighed amount of the dried extract was dissolved in normal saline. The extract was subjected to qualitative chemical investigation for the identification of different phyto-constituents like sterols, glycosides, saponins, alkaloids, flavonoids, carbohydrates, tannins and proteins [22, 23].

Methanolic extract of *C. carandas* L. leaves was obtained (yield 12.26 % w/w) as a dark brown colored residue. The preliminary phytochemical studies of methanol extract of *C. carandas* L. leaves indicated the presence of glycosides, steroids, flavonoids, tanins and phenolic compound, terpenoids and carbohydrates.

Animals

Wister Albino rats of either sex weighing, 200-250 gram were used for the evaluation of anti-inflammatory and antipyretic activity. The animals were maintained on the suitable nutritional and environmental conditions throughout the experiment as per the rules and regulations of the Institutional Animal Ethics Committee. Experimental protocols for the pharmacological and toxicity studies were reviewed and approved by the Institutional Animal Ethical Committee (approval No. 787/PO/ac/03/CPCSEA).

Toxicity studies

An acute toxicity study was performed to determine LD_{50} using different doses of the extracts according to the method described under OECD guidelines [24]. The animals were kept fasting for overnight providing only water, after administration of methanol extract of *C. carandas* L. orally at doses of 5–2000 mg/kg body weight. Animals were then allowed free access to food and water and observed a period of 48 hours for signs of acute toxicity. The number of deaths within this period was recorded.

In acute toxicity study, the methanolic extract of *C. carandas* L. leaves did not shown lethality up to the dose level of 2000 mg/kg, which indicates as a safe drug.

Animal grouping for tests

Anti-inflammatory and anti-pyretic activities were determined in albino rats of either sex. Rats were divided into 4 groups of 6 animals in each group:

Group 1: Untreated control (normal saline, 1 ml)

Group 2: Test animals received test extract with 100 mg/kg body weight (25 mg) in 1 ml normal saline, p.o.

Group 3: Test animals received test extract with 200 mg/kg body weight (50 mg) in 1 ml normal saline, p.o.

Group 4: Standard group animals received diclofenac with 10 mg/kg p.o. (2.5 mg) in 1 ml normal saline for anti-inflammatory activity and paracetamol with 150 mg/kg p.o. (37.5 mg) in 1 ml normal saline for anti-pyretic activity.

Oral gastric gavage was used to administer the test, standard and control samples.

Evaluation of anti-inflammatory activity

Anti-inflammatory activity of methanolic extract of *C. carandas* L. leaves were evaluated by using histamine induced rat paw oedema, dextran induced rat paw oedema and carrageenan induced rat paw oedema models.

Histamine induced rat paw oedema

Oedema was induced by subplanter injection of 0.05 ml of 1 % freshly prepared histamine (Ranbaxy Fine Chemicals, India) solution [25], in to the right hind paws of the rats of 4 groups of 6 animals each. The volumes of the injected and contralateral paws were measured 1, 2 and 3 hours after induction of inflammation using a plethysmometer according to the method described by Winter *et al* with little modification [26]. The test groups received the extract (100 and 200 mg/kg p.o.), the standard group received diclofenac (10 mg/kg p.o.) and the control animals received the vehicle only (orally). All the treatments were given accordingly 30 min prior to the injection of histamine.

Dextran induced rat paw oedema

Oedema was induced by subplanter injection of 0.05 ml of 1 % freshly prepared dextran (Ranbaxy Fine Chemicals, India) solution in to the right hind paws of the rats of 4 groups of 6 animals each. The volume of the injected and contralateral paws were measured 1, 2 and 3 hours after induction of inflammation using a plethysmometer according to the method described by Maity*et al* with little

modification [25]. The test groups received the extract (100 and 200 mg/kg p.o.), the standard group received diclofenac (10 mg/kg p.o.) and the control animals received the vehicle only (orally).

Carrageenan induced rat paw oedema

In this model, oedema of the right hind paws of the rats of 4 groups of 6 animals were induced by 0.1 ml of 1 % freshly prepared suspension of carrageenan (Sigma, USA), according Maity *et al* with little modification[25]. The paw was marked with ink at the level of lateral malleolus and immersed in the mercury column of a plethysmometer for measuring the paw volume. The paw volume was measured immediately before and 1, 2 and 3 hours after histamine, dextran and carrageenan injection. The percentage inhibition of inflammation was calculated by using the following formula:

Percentage inhibition of inflammation = $V_c - V_t / V_c \times 100$; where V_c is the average increase in paw volume of control rats and V_t is the average increase in paw volume of treated rats.

Evaluation of anti-pyretic activity in yeast induced elevated temperature

The antipyretic activity was evaluated by Brewer's yeast induced pyrexia in rats [27]. Only male rats were divided into 4 groups of 6 animals in each group. For the measurement of initial body temperature of each rat, a thermister probe was inserted 3-4 cm. in to the rectum and fastened to the tail by adhesive tape. Temperatures were recorded by a thermometer at predetermined time intervals after the administration of yeast extract, standard test drug, orally.

As the rats grouped earlier, fever was induced in each rat by the method described [27]. Each rat was given a subcutaneous injection of 10 ml/kg of 15 % w/v yeast extract suspension in normal saline. After 19th hours of yeast administration, the rectal temperature of each rat was recorded. At 19th hour the 4th group of animals received the standard drug, paracetamol at a dose of 150 mg/kg body weight p.o. and the methanolic extract of *C. carandas* L. leaf was administered orally at a dose of 100 and 200 mg/kg body weight to 2nd and 3rd group respectively. Then, the rats were restrained for recording of their rectal temperature at one hour's interval up to the 23th hour.

Statistical analysis

The data were analyzed for significance by using the unpaired two-tailed student's *t*-test. $P< 0.05$ was considered significant in all experiments. All other data was analyzed with simple statistics. The simple statistical analysis and paired samples t-test were conducted using MedCalc software version 11.6.1.0.

RESULTS AND DISCUSSION

The results of histamine, dextran and carrageenan induced rat paw edema in Wister Albino rats is presented in Table 1, 2 and 3, respectively. The results from these different models of anti-inflammatory activity showed significant anti-inflammatory activity of the methanolic extract of *C. carandas* L. leaves. However, the extract was compared to diclofenac, a non-steroidal anti-inflammatory drug. The extract at a dose of 200 mg/kg showed a maximum 72.1 % inhibition in histamine, 71.9 % inhibition in dextran and 71.8 % inhibition in carrageenan induced hind rat paw oedema after 3 hours.

The effect of yeast induced pyrexia and the anti-pyretic action of methanol extract of *C. carandas* L. leaves in rats were shown in Table 4. The result showed that the subcutaneous injection of yeast extract markedly elevated the rectal

Table 1: Effect of *C. carandas* L. leaf extract on histamine induced hind paw oedema in rats

Sl. No	Groups	Treatment	Paw volume increased after 3 hours (ml)	% Inhibition
1	Control	-	2.69 ± 0.53	-
2	Methanol extract	100 mg/kg p.o.	1.14 ± 0.18*	57.6
3	Methanol extract	200 mg/kg p.o.	0.75 ± 0.36*	72.1
4	Diclofenac	10 mg/kg p.o.	0.54 ± 0.18	79.9

*Values are expressed as mean ± SEM, n = 6; *p < 0.05 when compared to control*

Table 2: Effect of *C. carandas* L. leaf extract on dextran induced hind paw oedema in rats

Sl. No	Groups	Treatment	Paw volume increased after 3 hours (ml)	% Inhibition
1	Control	-	2.71 ± 0.53	1
2	Methanol extract	100mg/kg p.o.	1.16 ± 0.18*	2
3	Methanol extract	200mg/kg p.o.	0.76 ± 0.36*	3
4	Diclofenac	10 mg/kg p.o.	0.56 ± 0.19*	4

*Values are expressed as mean ± SEM, n = 6; *p < 0.05 when compared to control*

Table 3: Effect of *C. carandas* L. leaf extract on carrageenan induced hind paw oedema in rats

Sl. No	Groups	Treatment	Paw volume increased after 3 hours (ml)	% Inhibition
1	Control	-	2.73 ± 0.54	-
2	Methanol extract	100mg/kg p.o.	1.18 ± 0.19*	56.0
3	Methanol extract	200mg/kg p.o.	0.77 ± 0.37*	71.8
4	Diclofenac	10 mg/kg p.o.	0.57 ± 0.19*	79.1

*Values are expressed as mean ± SEM, n = 6; *p < 0.05 when compared to control*

Table 4: Effect of methanol extract of *C. carandas* L. leaf on yeast induced pyrexia in rats

Sl. No	Groups	Treatment	Rectal temperature (ºC) after yeast injection at one hour time interval					
			0 hour	19 hours	20 hours	21 hours	22 hours	23 hours
1	Control (Normal saline)	5 ml/kg	37.40 ± 0.24	39.40 ± 0.42	39.40 ± 0.40	39.20 ± 0.18	39.00 ± 0.26	38.80 ± 0.26
2	Methanol extract	100 mg/kg p.o.	37.20 ± 0.09	39.40 ± 0.22	38.70 ± 0.26 *	38.00 ± 0.31	37.50 ± 0.13 *	37.30 ± 0.08*
3	Methanol extract	200 mg/kg p.o.	37.4 ± 0.11	39.7 ± 0.11	38.4 ± 0.17*	38.00 ± 0.17*	37.50 ± 0.14*	37.20 ± 0.09*
4	Paracetamol	150 mg/kg p.o.	37.50 ± 0.09	39.50 ± 0.26	38.30 ± 0.24*	37.60 ± 0.15	37.05 ± 0.14*	37.40 ± 0.11*

*Each value represents mean ± SEM, n= 6; *P < 0.05* (*test extracts compared with control value of corresponding hours*)

temperature after 19 hours of administration. The standard drug paracetamol at a dose of 150 mg/kg body weight p.o. significantly reduced the yeast-provoked elevation of body temperature. Treatment with the methanol extract of *C. carandas* L. leaves at the doses of 100 and 200 mg/kg p.o., decreased the rectal temperature in rats in a dose depended manner. The anti-pyretic effect started as early as 1 hr and the effect was maintained for 4 hours. The result showed significant ($p < 0.05$) antipyretic activity throughout the observation period up to 4 hours.

The anti-inflammatory and anti-pyretic activities of many plants have been attributed to their saponin [28], terpenoids, flavonoids and steroids contents [29]. Non-steroidal anti-inflammatory drugs (NSAID) such as diclofenac used in this study are known to inhibit cyclooxygenase enzymes I and II, which are implicated in the production of inflammation mediating agent prostaglandin (PGE_2) from arachidonic acid [30-32].The pattern of anti-inflammatory and anti-pyretic activities exhibited by this extract compared with the standard drug diclofenac and paracetamol, respectively. It is also believed, that the carrageenan induced edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of NSAID that is inhibition of cyclooxygenase in prostaglandin synthesis [33].

Dextran mediated inflammation was probably reduced as a result of antihistamine effects of the extract, as dextran is known to cause inflammation through both histamine and serotonin [34]. This suggests that the active principles like steroids or the combination of other constituents present in the extract shows the anti-inflammatory activity.

It is well known that paracetamol is a good and promptly acting antipyretic and is a poor inhibitor of prostaglandins synthesis in peripheral tissues, but more active on cyclooxygenase in brain [35]. The methanol extract of *C. carandas* L. leaves produced significant antipyretic activity in Brewer's yeast induced pyrexia in rats.

CONCLUSION

These experimental results have established a pharmacological evidence for the folklore claim about the usefulness of *C. carandas* L. leaves extract. Further, to study the possible mechanism of actions and isolation of active principle(s) responsible for such activity.

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