Analgesic, anti-inflammatory and antipyretic activities of *Pergularia daemia* and *Carissa carandas*

*[1]Bhaskar V.H., [2]Balakrishnan N.

[1]M.P.Patel College of Pharmacy, Kapadwanj, Gujarat-387620, India, [2]Department of Pharmacognosy, Technocrats Institute of Technology-Pharmacy, Bhopal-462021(MP), India.

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ABSTRACT

Background: The plant *Pergularia daemia* (Asclepiadaceae) and *Carissa carandas* (Apocynaceae) are traditionally used as a medicinal agent and they are widely distributed to tropical and subtropical region of India. In the present study the folklore uses of *P. daemia* and *C. carandas* was investigated.

Methods: The analgesic activity was studied in mice using hot plate and acetic acid induced writhing methods, while carrageenan induced paw edema was used to access anti-inflammatory activity. The antipyretic activity was evaluated by Brewer's yeast induced pyrexia in rats.

Results: The ethanol and aqueous extracts from roots of *P. daemia* and *C. carandas* exhibited significant ($p < 0.01$) analgesic, anti-inflammatory and antipyretic activities at the doses of 100 and 200 mg/kg body weight. In analgesic activity, the highest reaction time was observed (9.8 sec.) from ethanol extracts of *P.daemia* at a dose of 200 mg/kg body weight, while highest percentage of inhibition of abdominal constriction (72.67%) was observed for ethanol extracts of *C.carandas* at a dose of 100 mg/kg body weight. The ethanol and aqueous extracts of *P. daemia* and *C. carandas* were found to reduce significantly the formation of edema induced by carrageenan after 2 hrs. Both plants showed significantly competent on yeast induced hyperpyrexia in rats after 2 hrs.

Conclusion: The results of this study indicated that the ethanol and aqueous extracts from roots of *P. daemia* and *C. carandas* possess significant analgesic, anti-inflammatory and antipyretic activities in rodent models.

Keywords: Analgesic, Anti-inflammatory, Antipyretic, *Carissa carandas*, *Pergularia daemia*

INTRODUCTION

The roots of *Pergularia daemia* and *Carissa carandas* have been used to treat inflammation and pain and to reduce the fever by the folklore people of Salem, Dharmapuri and Coimbatore district, Tamilnadu state, India. Both plants are widely distributed to the Southern parts of India. *P.daemia* (Asclepiadaceae) is known as "Veliparuthi." in Tamil, "Uttaravaruni" in Sanskrit and "Utranajutuka" in Hindi. *C.carandas* belonging to the family of Apocynaceae is commonly known as Christ's thorn or Bengal Currant, 'Kalakke' in Tamil (1). Traditionally the plant *P.daemia* is used as anthelmintic, laxative, antipyretic and expectorant, and is also used to treat infantile diarrhoea and malarial intermittent fevers (2-4). Latex of this plant is used for toothache (5). Stem bark of this plant is remedy for cold (6) and fever (7). Aerial parts of this plant are reported to have various pharmacological activities like hepatoprotective (8), antifertility (9), anti-diabetic (10), analgesic, antipyretic and anti-inflammatory (11). Phytochemically the plant has been investigated for cardenolides, alkaloid and saponins (11) and it has been found that contains various triterpenes and steroidal compounds (12). *C.carandas* traditionally used as stomachic, antidiarrheal and anthelmintic; stem is used to strengthen tendons; fruits are used in skin infections and leaves are remedy for fevers, earache and syphilitic pain (1-4). Alcoholic extract of root material decrease the blood pressure (13) and aqueous extract of root have been reported various pharmacological activities like histamine releasing (14), anthelmintic, sapsmolytic and cardiotonic (15). Fruits have also been studied for its analgesic, anti-inflammatory (16) and lipase (17) activity. Carisone and carindone (18, 19), carinol, lignin (20), oderosideH (18) and 2-acetylphenol (15, 21) have been reported from root material. The leaves are reported to have triterpene (22), tannins (23) and carissic acid (24). Fruits of this plant have been reported to contain a mixture of volatile principles (21, 25) like 2-phenyl ethanol, linalool, β-caryophyllene, isoamyl alcohol and benzyl acetate and a novel (Carissol) triterpenic alcohol (26). The

Correspondence: pharmacobala@yahoo.co.in

present study was undertaken to determine the folklore uses of *P. daemia* and *C. carandas*.

MATERIAL AND METHODS

Plant Material

The plant materials were collected from Maruthamalai Hills, Coimbatore, India in the month of November 2006. Both plants were taxonomically identified by Dr P. Jayaraman, Plant Anatomy research Centre, Chennai, Tamil Nadu, India. The voucher specimen of *P. daemia* (PARC/2007/52) and *C. carandas* (PARC/2007/53) has been preserved in our laboratory for further collection and reference.

Preparation of extracts

Roots of *P. daemia* and *C. carandas* were dried under shade, powdered with a mechanical grinder and passed through sieve no 40. The sieved powder was stored in airtight container and kept at room temperature for further study. The dried powdered material (500 g) was extracted with 95 % ethanol using soxhlet apparatus for about 48 h. The aqueous extract was prepared by cold maceration (72 h.). Solvents were removed under reduced pressure by using rotary vacuum evaporator.

Phytochemiclal Analysis

The ethanol and aqueous extracts of *P. daemia* and *C. carandas* were subjected to phytochemical tests(27,28) to identify the nature of chemical constituents present in the plant material and it was found that various phytoconstituents like alkaloids, glycosides, tannins, flavonoids, steroids and terpenoids are present.

Animals

Wister albino rats of either sex weighing about 150-200 gm and adult albino mice of either sex weighing 25 -30 gm were employed for this study. The animals were maintained under standard conditions (temperature 23 ± 2 ° C, relative humidity 55 ± 10 % and 12 h. light : 12 h. dark cycle). Animals were allowed to take standard laboratory feed and tap water.

Toxicity Studies

Acute toxicity study of ethanol and aqueous extracts from roots of *P. daemia* and *C. carandas* was performed in albino rats according to OECD guidelines (29). The animals were kept fasting for overnight providing only water, after administration of ethanol and aqueous extracts of *P. daemia* and *C. carandas* orally at doses of 5– 2000 mg / kg. Animals were then allowed free access to food and water and observed a period of 48 hrs for signs of acute toxicity. The number of deaths within this period was recorded.

Animal groups and treatment

For analgesic, anti-inflammatory and antipyretic studies, animals were divided in to ten groups each of six animals (n=6). Group I served as control received 1% CMC solution, 10 ml/kg p. o., Group II received standard drug aspirin (150 mg/kg p. o with 1% CMC), and remaining groups received ethanol and aqueous extracts of *P. daemia* and C. *carandas* at doses of 100 and 200 mg/kg bw, p. o with 1% CMC respectively.

Analgesic activity by hot plate and acetic acid induced writhing method

The analgesic activity was evaluated by hot plate (Eddy's Hot Plate; Techno, India) method (30). In this method the reaction time in seconds was recorded at 0, 30, 60, 90 and 120 min and cut off period of 15 sec. The peripheral analgesic activity was evaluated by acetic acid induced writhing method (31). The percentage of inhibition of abdominal constrictions for the extract treated groups was compared with control group.

$$\% \text{ inhibition} = \frac{\text{Control - test}}{\text{Control}} \times 100$$

Evaluation of Anti-inflammatory Activity

The anti-inflammatory activity was evaluated by carrageenan induced rat paw edema model (32). The paw volume was measured at 0, 30, 60,120 and 180 min. respectively by using the plethysmograph (33). The percentage of inhibition was calculated by using the formula,

$$\% \text{ inhibition} = \frac{\text{Vc- Vt}}{\text{Vc}} \times 100$$

Where, Vc= Average paw volume of control; Vt = Average paw volume of test

Evaluation of Antipyretic Activity

The antipyretic activity was evaluated by Brewer's yeast induced pyrexia in rats (34). The rectal temperature of the rats was recorded at 0,30, 60, 120 and 180 min. by insertion of a clinical thermometer to a depth of 2 cm into rectum.

Statistical Significance

The results of the study were expressed as mean ± SEM ANOVA (35) was used to analyze and compare the data, followed by Dunnet's (36) test for multiple comparisons. $P < 0.01$ was considered significant in all experiments.

RESULTS

Phytochemical Screening

Preliminary phytochemical screening of various extracts from roots of *P. daemia* and *C.carandas* showed positive results for steroids, flavonoids, tannins, alkaloid and glycosides etc. (1)

Table 1. Phytochemical Screening of *P.daemia* and *C. carandas* root extracts.

Name of the Phytoconstituents	*Pergularia daemia*		*Carissa carandas*	
	Ethanol extract	Aqueous extract	Ethanol extract	Aqueous extract
Alkaloids	+	+	+	+
Glycosides	+	+	+	+
Steroids	+	+	+	+
Flavonoids	+	+	+	+
Tanins & Phenolic Compound	+	+	+	+
Terpenoids	+	+	+	+
Carbohydrates	+	+	+	+
Fixed oils & Fats	-	-	-	-
Proteins & Free amino acids	-	-	-	-
Gums & Mucilage	+	+	+	+
Saponins	+	+	+	+

+ = Positive; - = Negative

Table 2. Effects of ethanol and aqueous extracts of *P. daemia* and *C. carandas* on Latency in Mice Lick the Paw. (Hot Plate Method).

Time in min.	Reaction time in sec.									
			A		B		C		D	
	Control (10 ml/kg)	Standard (150 mg/kg)	100 mg/kg	200 mg/kg	100 mg/kg	200 mg/kg	100 mg/kg	200 mg/kg	100 mg/kg	200 mg/kg
0	3.73 ± 0.042	3.53 ± 0.033**	3.58 ± 0.016**	3.43 ± 0.021**	3.53 ± 0.021**	3.43 ± 0.033**	3.56 ± 0.021**	3.58 ± 0.016**	3.61 ± 0.040**	3.58 ± 0.0106**
30	3.73 ± 0.044	5.50 ± 0.129**	3.86 ± 0.033 NS	4.63 ± 0.076**	4.06 ± 0.190NS	4.48 ± 0.040**	5.05 ± 0.108**	4.65 ± 0.500**	4.46 ± 0.550**	4.51 ± 0.016**
60	3.73 ± 0.042	9.66 ± 0.004**	8.51 ± 0.016**	9.08 ± 0.040**	8.43 ± 0.033**	8.36 ± 0.108**	8.70 ± 0.068**	7.10± 0.060**	9.06 ± 0.098**	7.73 ± 0.060**
90	3.71 ± 0.040	8.56 ± 0.033**	7.86 ± 0.042**	8.31 ± 0.054**	7.50 ± 0.044**	7.50 ± 0.044**	7.83 ± 0.061**	6.38 ± 0.110**	7.63 ± 0.33**	6.63 ± 0.061**
120	3.73 ± 0.042	7.56 ± 0.049**	7.78 ± 0.101**	7.01 ± 0.016**	6.83 ± 0.033**	6.80 ± 0.051**	6.23 ± 0.061**	5.75 ± 0.102**	6.38 ± 0.040**	5.58 ± 0.127**

Each value presents the mean ± S.E.M. of six observations, NS *P > 0.05*, * *P < 0.05*, ** *P < 0.01* vs control

NS Not significant; A Ethanol extract of *P. daemia*; B Aqueous extract of *P. daemia*; C Ethanol extract of *C. carandas* ; D Aqueous extract of *C. carandas*

Hot plate test

In hot plate method both plants showed an increase in the animal reaction time to the heat stimulus. Values were found to be significant ($p < 0.01$) from 30 to 120 min after treatment with ethanol and aqueous extracts (Table 2). The highest reaction time was observed for ethanol extract of *P. daemia* (9.08 sec.) at a dose of 200 mg/kg. The highest reaction time in sec. was observed for ethanol and aqueous extracts of *C. carandas* at a dose of 100 mg/kg body weight 8.7 and 9.06 sec., respectively.

Acetic acid induced abdominal writhing

The ethanol and aqueous extracts of *C.carandas* and *P. daemia* decreased the number of acetic acid induced abdominal constrictions (Writhings) in mice, when compared to control ($p< 0.01$) as mentioned in the Table 3. The maximal percentage of inhibition of constriction of ethanol and aqueous extracts of *P. daemia* respectively at a dose of 100 mg/kg were 70.10% and 63.96% respectively. The maximal percentage of inhibition of constriction of ethanol and aqueous extracts of *C. carandas* at a dose of 100 mg/kg were 72.67% and 71.68% respectively.

Anti-inflammatory activity

The results of carrageenan induced rat paw edema is presented in Table 4.The highest percentage of inhibition was observed for ethanol extracts of *P. daemia* (61.24%) at a dose of 100 mg/kg , at 3hrs. The highest percentage of inhibition was observed for ethanol extracts of *C. carandas* (63.83%) at a dose of 100 mg/kg.

Table 3. Analgesic effects of ethanol and aqueous extracts of *P. daemia* and *C. carandas* on Acetic acid induced abdominal constriction in mice.

No. of writhing 10 min ./ % of Inhibition									
Control (10 ml/kg)	Standard (150 mg/kg)	A		B		C		D	
		100 mg/kg	200 mg/kg	100 mg/kg	200 mg/kg	100 mg/kg	200 mg/kg	100 mg/kg	200 mg/kg
84.76 ± 1.249	23.33 ± 0.666**	25.16 ± 0.166**	33.83 ± 0.166**	30.33 ± 0.210**	35.66 ± 0.210**	23.00 ± 0.930**	32.00 ± 0.516**	23.83 ± 0.833**	35.00 ± 0.632**
-	72.27	70.10	59.80	63.96	57.62	72.67	61.97	71.68	58.41

Each value presents the mean ± S.E.M. of six observations, ^{NS} *P > 0.05*, * *P < 0.05*, ** *P < 0.01* Vs control
^{NS} Not significant; ^{A} Ethanol extract of *P. daemia*; ^{B} Aqueous extract of *P. daemia*; ^{C} Ethanol extract of *C. carandas* ; ^{D} Aqueous extract of *C. carandas*

Table 4. Effects of ethanol and aqueous extracts of *P. daemia* and *C. carandas* on Carrageenan induced rat paw.

Time in min.	Paw volume (ml)									
	Control (10 ml/kg)	Standard (150 mg/kg)	A		B		C		D	
			100 mg/kg	200 mg/kg	100 mg/kg	200 mg/kg	100 mg/kg	200 mg/kg	100 mg/kg	200 mg/kg
0	1.23 ± 0.021	1.00 ± 0.036**	1.25 ± 0.022^{NS}	1.26 ± 0.011^{NS}	1.17 ± 0.011^{NS}	1.16 ± 0.020^{NS}	1.13 ± 0.021*	1.15 ± 0.012^{NS}	1.08 ± 0.038**	1.18 ± 0.010^{NS}
30	1.61 ± 0.030	0.816± 0.040**	1.30 ± 0.027**	1.33 ± 0.016**	1.24 ± 0.020**	1.27 ± 0.030**	1.30 ± 0.051**	1.28 ± 0.047**	1.38 ± 0.087**	1.40 ± 0.025**
60	2.06 ± 0.033	0.683 ± 0.010**	1.33 ± 0.016**	1.17 ± 0.011**	1.09 ± 0.020**	1.10 ± 0.034**	0.733 ± 0.021**	0.766 ± 0.033**	0.825 ± 0.027**	0.833 ± 0.021**
120	2.48 ± 0.307	0.733± 0.010**	0.900 ± 0.012**	0.950 ± 0.018**	0.875 ± 0.025**	0.920 ± 0.025**	0.857 ± 0.034**	0.800 ± 0.036**	0.836 ± 0.070**	1.02 ± 0.017**
180	2.58 ± 0.030	1.21± 0.040**	1.00 ± 0.040**	1.05 ± 0.027**	1.01 ± 0.027**	1.05 ± 0.045**	0.933 ± 0.021**	1.05 ± 0.034**	1.30 ± 0.044**	1.22 ± 0.028**

Each value presents the mean ± S.E.M. of six observations, ^{NS} *P > 0.05*, * *P < 0.05*, ** *P < 0.01* vs control
^{NS} Not significant; ^{A} Ethanol extract of *P. daemia*; ^{B} Aqueous extract of *P. daemia*; ^{C} Ethanol extract of *C. carandas* ; ^{D} Aqueous extract of *C. carandas*.

Antipyretic activity

The ethanol and aqueous extracts of *P. daemia* and *C. carandas* (100 & 200 mg/kg) produced significant antipyretic activity at 60 min. Both plants showed significant ($p< 0.01$) antipyretic activity throughout the observation period up to 3hrs. (Table 5).

DISCUSSION

Phytochemically, the ethanol and aqueous extracts of root of *Pergularia daemia* and *Carissa carandas* showed the maximum phytochemicals like alkaloids, glycosides, steroids, flavonoids, saponin, tannin & phenolic compounds, terpenoids, carbohydrates, gums and mucilage. The anti-inflammatory, analgesic and antipyretic activities of many plants have been attributed to their saponin (37), terpenoids, flavonoids and steroids contents (38). 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 (PGE_2) from arachidonic acid (39-41).The pattern of anti-inflammatory, analgesic and antipyretic activities exhibited by these extracts were similar to that of aspirin in which suggests that the plant's activity may be mediated by cyclooxygenase I and II inhibition.

The observation that both plants increased pain threshold of animals could be due to inhibition of sensitization of pain receptors by prostaglandins at the inflammation site (42). After administration of acetic acid several mediators such as cytokines, eicosanoids and arachidonic acid are liberated from membrane after phospolipaseA_2 activity leading to production of prostaglandins and leukotrienes (43). The analgesic activity of the ethanol and aqueous extracts of *P. daemia* and *C. carandas* may be due to inhibition of phospolipase A_2 or even blocking cyclooxygenase (COX-1 and/or COX-2).

Carrageenan induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The

Table 5. Effect on ethanol and aqueous extracts of *P. daemia* and *C. carandas* on Brewer's yeast induced pyrexia in rats.

Time in min.	Temperature (° C)									
	Control (10 ml/kg)	Standard (150 mg/kg)	A		B		C		D	
			100 mg/kg	200 mg/kg	100 mg/kg	200 mg/kg	100 mg/kg	200 mg/kg	100 mg/kg	200 mg/kg
0	35.60 ± 0.051	34.63 ± 0.021**	35.03 ± 0.021**	34.70 ± 0.044**	35.05 ± 0.022**	34.51 ± 0.040**	35.25 ± 0.034**	34.93 ± 0.021**	35.36 ± 0.021**	34.73 ± 0.042**
30	36.30 ± 0.044	34.78 ± 0.016**	35.31 ± 0.054**	34.98 ± 0.030**	35.28 ± 0.016**	34.73 ± 0.042**	35.71 ± 0.054**	35.13 ± 0.049**	35.56 ± 0.033**	34.93 ± 0.042**
60	36.53 ± 0.042	35.01 ± 0.016**	35.73 ± 0.042**	35.45 ± 0.022**	35.48 ± 0.104**	35.05 ± 0.022**	35.86 ± 0.021**	35.53 ± 0.042**	35.86 ± 0.042**	35.18 ± 0.060**
120	37.06 ± 0.042	35.86 ± 0.042**	36.06 ± 0.021**	35.73 ± 0.042**	35.76 ± 0.088**	35.50 ± 0.044**	36.43 ± 0.021**	36.26 ± 0.084**	36.43 ± 0.021**	35.80 ± 0.100**
180	37.70 ± 0.068	36.66 ± 0.061**	36.86 ± 0.021**	36.26 ± 0.042**	36.13 ± 0.042**	36.33 ± 0.042**	37.25 ± 0.071**	37.16 ± 0.021**	37.03 ± 0.021**	36.13 ± 0.042**

Each value presents the mean ± S.E.M. of six observations, ^{NS} $P > 0.05$, * $P < 0.05$, ** $P < 0.01$ vs control
^{NS} Not significant; ^{A} Ethanol extract of *P. daemia*; ^{B} Aqueous extract of *P. daemia*; ^{C} Ethanol extract of *C. carandas* ; ^{D} Aqueous extract of *C. carandas.*

first phase is due to release of histamine and serotonin. The second phase is caused by the release of bradykinin, proteases, prostaglandins and lysosomes (44). Prostaglandins play a major role in development of second phase of reaction that is measured at 3 hrs. These mediators take part in the inflammatory response and are able to stimulate nociceptors and thus induce pain (45). Carrageenin induced edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non steroidal anti-inflammatory agents that is inhibition of cyclooxygenase in prostaglandin synthesis (46). Based on these reports it may be concluded that the inhibitory effect of the ethanol and aqueos extracts of *P. daemia* and *C. carandas* (100 and 200 mg/kg, b. wt) on carrageenin induced inflammation in rats could be due to inhibition of the cyclooxygenase in prostaglandin synthesis.

It is well known that most of the non steroidal anti-inflammatory drugs possess antipyretic activity through inhibition of prostaglandins synthesis in hypothalamamus (47). Both plants produced significant antipyretic activity in Brewer's yeast induced pyrexia in rats and in this situation both extracts could inhibit the prostaglandins synthesis in hypothalamamus.

CONCLUSION

These experimental results have established a pharmacological evidence for the folklore claim of the drugs to be used as an analgesic, anti-inflammatory and antipyretic agent. Further studies on possible mechanism of actions and isolation of active principle(s) responsible for such activity are currently under progress in our laboratory

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