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Preliminary Phytochemical Screening and Antipyretic Activity of *Carissa Spinarum* Root Extract

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Abstract

Carissa spinarum Linn. (Family: Apocynaceae) has been used traditionally for the treatment of inflammation-related disorders such as rheumatic pain and to relieve fever. In the present study the ethanolic extract of the roots of *Carissa spinarum* (ERCS) was evaluated for its phytochemical screening and antipyretic activity. Wistar albino rats were induced with Brewer's yeast (2 ml/kg, S.C.) for pyrexia and antipyretic activity was assessed with 100, 200 and 400 mg/kg, p.o. ethanolic extract. The ethanolic extract significantly (P <0.05) reduced the elevated body temperature in a dose dependent manner. The presence of wide varieties of phytoconstituents may attribute to the promising antipyretic activity of *Carissa spinarum* root extract.

Keywords: Antipyretic activity, Brewer's yeast, *Carissa spinarum*, Phytochemical screening

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INTRODUCTION

Carissa spinarum Linn. (*Carissa opaca* Stapf ex Haines, Family: Apocynaceae) is a thorny, evergreen shrub, widely distributed throughout the drier, sandy and rocky soils of India, Ceylon, Myanmar and Thailand. The roots of this plant has long been prescribed in the indigenous system of medicine as purgative, for the treatment of inflammation-related disorders such as rheumatism and pain, cleaning worm infested wounds of animals and in snake bite [1, 2]. In Chinese system of medicine the roots of the plant is known for the treatment of rheumatism and hepatitis [3]. Previous phytochemical investigations revealed the presence of caffeic acid [4],

ursolic acid, naringin [5], various cardiac glycosides [6], germacrane sesquiterpene and lignans [7]. Earlier studies have shown that the extract of the plant possesses cardiotonic [8], antibacterial [5] and potent antioxidant activity [6]. The roots of the plant is used by the tribal healers of Western Ghats region of Karnataka to treat intermittent fever and inflammatory conditions.

However, no scientific data are available regarding its usefulness as antipyretic agent. Keeping the above information in view, the present study was an endeavor to ratify the antipyretic activity of the ethanolic extract of the roots of *C. spinarum* (ERCS) on Brewer's yeast induced pyrexia.

MATERIALS AND METHODS

Plant material

The roots of *C. spinarum* were collected from Sirsi, Uttara Kannada District, Karnataka, India during May 2007. It was authenticated by Dr. Gopalakrishna Bhat, Department of Botany, Poorna Prajna College, Udupi, Karnataka, India. A voucher specimen no. 105b is deposited in the herbarium of our institute.

Preparation of extract

The collected roots of *C. spinarum* were washed; the bark was peeled off and then dried under shade. The coarse powder of the roots (500 g) was soaked in 1.5 L of 95% ethyl alcohol and extracted in the cold for 4 days with occasional shaking. After 4 days the ethanol layer was decanted off. The process was repeated for 4 times. The solvent from the total extract was filtered, the concentrate was evaporated to dryness under reduced pressure and low temperature (40^o C) on a rotary evaporator to give the ethanolic extract (13% w/w yield), which was stored at 4^o C until use. Suspension of the extract was prepared in 1 % Tween-80 and used to assess antipyretic activity.

Chemicals and drugs

All the chemicals and solvents were of analytical grade and were procured from Ranbaxy Fine Chemicals Ltd., Mumbai, India. Brewer's yeast was procured from Hi-media. Tween 80 from SD Fine Chemicals Pvt. Ltd, Bombay and paracetamol (PML) from Sigma-Aldrich, USA.

Experimental animals

Wistar albino rats of either sex, weighing about 150-180 g were used for experiments. Animals were maintained under standard conditions (12 h light / dark cycle; $25^o \pm 2^o$ C, 45-60 % RH) and were fed standard rat feed and water *ad libitum*. All the animals were acclimatized to laboratory conditions for a week before commencement of experiment. All experimental protocols were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) prior to the initiation of the experiment and the care of the laboratory animals was taken as per the CPCSEA regulations.

Preliminary phytochemical screening

Freshly prepared ethanolic extract of the roots of *C. spinarum* was subjected to preliminary phytochemical screening for detection of major secondary metabolites [9].

Acute toxicity study

Acute toxicity study of ethanolic extract of the roots of *C. spinarum* was determined in Wistar albino rats (150-180 g) according to OECD guidelines No. 425 [10]. The animals were fasted overnight and the ethanolic extract was administered orally with a starting dose of 2000 mg/kg, to different groups of animals. Animals were observed continuously for first 3 h and monitored for 14 days for mortality and general behavior of animals, signs of discomfort and nervous manifestations.

Evaluation of antipyretic activity

The antipyretic activity of the ethanol extract was evaluated using Brewer's yeast induced pyrexia in Wistar rats [11]. Prior to the experiment, the rats were maintained in separate cages for 7 days and the animals with approximately constant rectal temperature (37 - 38 °C) were selected for the study. The animals were divided into five groups of six animals each. The 1st group served as control received vehicle. The 2nd group served as standard received paracetamol. The 3rd, 4th and 5th groups served as test groups and received the ethanolic extract 100, 200 and 400 mg/kg, p.o. respectively. Fever was induced by injecting subcutaneously, 2 ml/kg body weight of 20% w/v aqueous suspension of Brewer's yeast in normal saline and rectal temperature was recorded by thermal-probe. The experimental pyrexia rats showed a mean increase of 2 °C in rectal temperature at 18 h after Brewer's yeast injection. Rectal temperature was measured at 0, 1, 2 and 3 h after oral drug administration using a digital thermometer.

Statistical analysis

All values were expressed as mean ±SEM. The results were analyzed for statistical significance using one-way ANOVA followed by Dunnett's 't' test. A P value <0.05 was considered as significant.

RESULT

Preliminary phytochemical screening

Preliminary phytochemical investigation of the ethanolic extract of the roots of the plant led to the presence of glycosides, flavonoids, saponins, triterpenoids, steroids, phenolic compounds and tannins [Table 1].

Table 1: Phytochemical screening of roots of *C. spinarum*

Secondary metabolite	Result
Glycosides	Positive
Flavanoids	Positive
Saponins	Positive
Triterpenoids	Positive
Steroids	Positive
Tannins	Positive
Alkaloids	Negative

Acute toxicity study

There was no mortality amongst the graded dose groups of animals and they did not show any toxicity or behavioral changes at a dose level of 2000 mg/kg. This finding suggests that the ethanolic extract is safe in or non-toxic to rats and hence doses of 100, 200 and 400 mg/kg, p.o. were selected for the study.

Evaluation of antipyretic activity

The subcutaneous injection of yeast suspension markedly increased the rectal temperature 18 h of after the administration in all the groups of animals. The ethanolic extract at the doses of 100, 200 and 400 mg/kg showed a significant reduction ($P<0.05$) in elevated rectal temperature 2 h after the oral administration in a dose dependent manner [Table 2].

Table No. 2: Effect of ethanol extract of the roots of *C. spinarum* (ERCS) on Brewer's yeast induced pyrexia

Group	Dose (mg/ kg)	Basal temperature ^{o}c (mean ±SEM)	Rectal temperature in ^{o}c (mean ±SEM)			
			0 h	1 h	2 h	3h
Control	-	37.33 ±0.14	40.26 ±0.10	40.24 ±0.10	40.06 ±0.02	39.83 ±0.15
PML	200	37.30 ±0.02	40.23 ±0.45	39.71 ±0.09**	38.25 ±0.12**	37.70 ±0.09**
ERCS	100	37.25 ±0.09	40.22 ±0.18	40.14 ±0.09	39.81 ± 0.14*	38.97 ±0.05**
ERCS	200	37.35 ±0.02	40.17 ±0.14	40.01 ±0.03	39.08 ±0.08**	38.29 ±0.07**
ERCS	400	37.26±0.01	40.21 ±0.42	39.97 ±0.06	38.71 ±0.17**	37.99 ±0.03**

Values are mean ± SEM (n=6), Data were analyzed by One way ANOVA followed by Dunnett's 't' test. * $P<0.05$, ** $P<0.01$ when compared with vehicle treated control group.

DISCUSSION

Appearance of pyrexia or fever comes in first line of defense against infection. Fever may be a result of infection or one of the sequelae of tissue damage, inflammation, graft rejection or other diseases. The local inflammatory response is accompanied by a systemic response known as auto phase response. This response is marked by induction of fever, increased production of leukocytes and production of large number of acute phase protein in liver [12]. Many systemic acute phase responses are due to the combined action of IL-2, IL-6, TNF-α, leukotriens, each of this cytokine acts on hypothalamus to induce fever response [13]. When body temperature elevates, the temperature regulatory system dilates the blood vessels and increases sweating to reduce the temperature by nervous feedback mechanism [14]. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained.

Antipyretic drugs reduce elevated body temperature. Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PGE_2 biosynthesis. These synthetic agents irreversibly inhibit COX-2 with a high selectivity and are toxic to the hepatic cells, glomeruli, cortex of brains and heart muscles. Natural COX-2 inhibitors have lower selectivity with fewer side effects [15].

The result of the present study indicates that both the standard drug and the ethanolic extract of the roots of *C. spinarum* treated groups of animals were compared with the control group and found to possess significant reduction in the Brewer's yeast induced pyrexia in a dose dependent manner. The phytochemical investigation of the ethanolic extract of the roots of the plant revealed the presence of glycosides, flavonoids, saponins, triterpenoids, steroids, phenolic compounds and tannins. Moreover, the flavonoids, tannins and triterpenoids are known to inhibit prostaglandin synthesis [16]. It is not unreasonable, therefore to speculate that the flavonoids, triterpenoids, tannins and other chemical compounds present in the plant's extract are responsible for the observed antipyretic effect. It is worthwhile to isolate the bioactive principles which are responsible for this activity; the process has commenced in our laboratory. These findings justify the traditional use of this plant in the treatment of pyretic conditions and validate its claim of being used for the said purpose in folklore medicine.

CONCLUSION

It can be concluded from the study that the ethanolic extract of the roots of *C. spinarum* possesses significant antipyretic property, which are probably mediated via inhibition of prostaglandin synthesis as well as COX-2 inhibitory mechanisms. However, extensive studies are needed to evaluate precise mechanism(s) and active principles of the plant as a medicinal remedy for pyrexia conditions.

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REFERENCES

[1] Kirtikar KR, Basu BD, Indian Medicinal Plants, Vol. II, Lalit Mohan Basu: Allahabad; **2003**; pp. 1548-1549.
[2] Chopra RN, Nayar SL, Chopra IC, Glossary of Indian Medicinal Plants, CSIR: New Delhi; **1956**, pp. 52.
[3] http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200018364.
[4] Raina MK, Bhatnagar JK, Atal CK, *The Indian J Pharmacy*, **1971**; 33:76-77.
[5] Mathuram V, Brahmadhayalaselvam A, Hussain AJ, Rao RB, Patra A, *J Indian Chem Soc*, **1998**; 75:262-264.
[6] Rastogi RC, Kulshreshtha DK, Rastogi RP, *Indian J Chem*, **1969**; 7:1102-1104.
[7] Rao RJ, Kumar US, Reddy SV, Tiwari AK, Rao JM, *Nat Prod Res*, **2005**; 19:763-769.
[8] Vohra MM, De NN, *Indian J Med Res*, **1963**; 51:937-940.
[9] Harborne JB, Phytochemical methods, Chapman and Hall: London; **1984**, pp. 84.
[10] OECD Guideline For The Testing of Chemicals: No. 425, **2001**; 1.
[11] Loux JJ, Deplama PD, Yankel SL, *Toxicol Appl Pharmacol*, **1972**; 22:672-675.
[12] Gopta MB, Nath R, Srivastava N, *Internat J Immunopharmacol*, **1996**; 18:696-700.
[13] Kuby Imunology, In: Thomas J. Kindt, Richard A. Goldsby, Barbara Anne Osborne, Janis Kuby. Leukocyte migration and inflammation, **2007**. pp. 384-385.
[14] Chattopadhyaya D, Ghoshal G, *J Pharm Pharmaceutics Sci*, **2005**; 8:558-564.
[15] Cheng L, Ming-Ling H, Lars B, *Acta Pharmacologica Sinica*, **2005**; 26:926-933.

[16] Mohammad Sayyah, Naghmeh Hadidi, Mohammad Kamalinejad, *J Ethnopharmacol* **2004**; 92:325-329.