# ANTIARTHRITIC ACTIVITY OF *CARISSA SPINARUM* ROOT EXTRACT IN FREUND'S ADJUVANT INDUCED POLYARTHRITIS IN RATS

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**Summary**

The plant *Carissa spinarum* used locally in Indian and Chinese system of medicines for various painful inflammatory and arthritic conditions was assessed for its anti-arthritic effect using the Freund's adjuvant induced-polyarthritis in rats. The ethanolic extract of the roots of *C. spinarum* (ERCS) at the dose range of 100, 200 and 400 mg/kg, p.o. and phenylbutazone showed significant ($P<0.05$), dose dependent anti-arthritic properties by reducing the arthritic edema in the adjuvant-induced established and non-established arthritis in rats. The triterpenoids, flavonoids, tannins and other chemical compounds present in ERCS are speculated to account for the observed pharmacological effects of the plant's extract in the experimental animal paradigms used. The findings of this experimental animal study indicate that ethanol extract of the roots of *C. spinarum* possesses anti-arthritic properties; and thus lend pharmacological credence to the folkloric and ethnomedical uses of the plant in the treatment arthritic conditions.

**Key words:** *Carissa spinarum*, Freund's adjuvant induced-arthritis, Phenylbutazone, Root extract

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## Introduction

Rheumatoid arthritis is a major ailment among the rheumatic disorders. It is a chronic condition with multiple causation and affects the people in their most active period of life. Freund's adjuvant-induced arthritis have been used as a model of chronic inflammation and is of considerable relevance for the study of pathophysiological and pharmacological control of inflammatory processes, as well as the evaluation of analgesic potential or anti-inflammatory effects of drugs (1). Adjuvant-induced arthritis in rats is characterized by infiltration of the synovial membrane and associated with destruction of the joints, resembles rheumatoid arthritis in humans in terms of immunological and biochemical features (2). The inflammatory response has received a great deal of interest in the field of medical research because inflammation and pain underlie almost every disease process (3).

*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 (4). The roots of this plant has long been prescribed in the indigenous system of medicine as purgative, for the treatment of rheumatism, cleaning worm infested wounds of animals and in snake bite (4, 5). In Chinese system of medicine the roots of the plant is well known for the treatment of hepatitis and rheumatoid arthritis (6). Previous phytochemical investigations revealed the presence of caffeic acid (7), ursolic acid, naringin (8), various cardiac glycosides (9), germacrane sesquiterpene and lignans (10). Earlier studies have shown that the extract of the plant possesses cardiotonic (11), antipyretic (12) and potent antioxidant activity (9).

In the Western Ghats region of India, the roots of this plant are being used by the traditional practitioners for relieving pain, inflammation and chronic rheumatic disorders (4). However, no sufficient data are available to prove its folklore claim as chronic anti-inflammatory and anti-arthritic agent. Therefore, the present study reports on the anti-arthritic effect of the ethanol extract of the roots of *C. spinarum* in Freund's adjuvant-induced non-established and established polyarthritis in rats. This would help provide scientific basis for the use of this plant locally for treating arthritic conditions.

## Materials and Methods

### Plant materials and preparation of the extract

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. Fresh roots were collected and dried by means of shade drying. The shade dried roots of the plant (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 pharmacological activities.

**Chemicals and drugs**

All the chemicals and solvents were of analytical grade and were procured from Ranbaxy Fine Chemicals Ltd., Mumbai, India. Freund's complete adjuvant (FCA), phenylbutazone (PB) was procured 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 (Kamadenu Agencies, Bangalore, India) 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.

**Acute toxicity study**

Acute toxicity study of ethanolic extract of the roots of *C. spinarum* (ERCS) was determined in Wistar albino rats (150-180 g) according to OECD guidelines No. 425 (13). 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.

**Phytochemical screening**

Freshly prepared ethanolic extract of the roots of *C. spinarum* (ERCS) was subjected to preliminary phytochemical screening for detection of major chemical constituents (14).

**Anti-arthritic activity**

***Freund's adjuvant induced arthritis***

This model was employed to study the anti-inflammatory effect of ethanol extract in chronic inflammation. The rats were divided into nine groups of six animals each. The animals of all the groups were injected with 0.1 ml of Freund's complete adjuvant into the sub-plantar region of the right hind paw (15). The rats of group I served as control and treated with vehicle throughout the experimental period. The rats of group II and group III, IV and V were treated with phenylbutazone (100 mg/kg, i.m.) and ERCS (100, 200 and 400 mg/kg, p.o.) respectively daily from day 1 to day 30 of the experiment. To study the effect of ERCS on completely established arthritis, the rats of group VI and group VII, VIII and IX were treated with phenylbutazone and ERCS respectively, starting from $16^{th}$ day of experiment for 30 days. After the treatment schedules, the paw volume was measured and compared with that of non-affected joint. Secondary lesions developed in the ears, forelimbs, hind limbs and tails were scored (16).

**Statistical Analysis**

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

## Results

**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 ERCS is safe in or non-toxic to rats and hence doses of 100, 200 and 400 mg/kg, po were selected for the study.

**Phytochemical Screening:** Preliminary phytochemical investigation of the ERCS led to the presence of glycosides, flavonoids, saponins, triterpenoids, steroids, other phenolic compounds and tannins.

**Freund's adjuvant induced arthritis:** The effect of ERCS against adjuvant induced polyarthritis is reported in Table 1. The ethanolic extract significantly (P<0.05) reduced the edema of affected joints by 36.23, 49.27 and 66.66 % in non-established arthritis and 30.43, 44.92 and 57.97 % in established arthritis at a dose range of 100, 200 and 400 mg/kg, p.o. as compared to the untreated control animals. The cardinal signs of chronic inflammatory reactions like redness, swelling and immobility of the affected joints were significantly less in ERCS treated animals than untreated control group. It also inhibited established polyarthritis to a significant extent, increased the mobility of the affected joints and inhibited the development of secondary lesions in a dose dependent manner.

**Table 1. Effect of ethanol extract of the roots of *C. spinarum* (ERCS) on Freund's adjuvant induced polyarthritis in rats**

| Group | Treatment | Dose (mg/kg) | Paw Edema volume (ml) | % inhibition | Secondary lesions |
|---|---|---|---|---|---|
| I | Control | - | 0.69±0.010 | - | Moderately severe |
| II | Phenylbutazone | 100(0-30 days) | 0.14±0.004*** | 81.15 | Absent |
| III | ERCS | 100(0-30 days) | 0.44±0.006* | 36.23 | Moderately mild |
| IV | ERCS | 200(0-30 days) | 0.35±0.008* | 49.27 | Mild |
| V | ERCS | 400(0-30 days) | 0.23±0.004** | 66.66 | Mild |
| VI | Phenylbutazone | 100(15-45 days) | 0.19±0.004*** | 72.46 | Mild |
| VII | ERCS | 100(15-45 days) | 0.48 ±0.009* | 30.43 | Moderately mild |
| VIII | ERCS | 200(15-45 days) | 0.38±0.006* | 44.92 | Moderately mild |
| IX | ERCS | 400(15-45 days) | 0.29±0.005** | 57.97 | Mild |

n=6 in each group; *P<0.05, **P<0.01, ***P<0.001 compared to control.

## Discussion

The model of adjuvant-induced arthritis in rats has been extensively used in the study of inflammatory processes and validated as a model of chronic pain (17). This fact is corroborated by the evidence of spontaneous pain behaviors in arthritic rats, such as reduced locomotor activity, increased scratching or itching of affected joints, formation of secondary lesions (18) and an attempt to protect the affected paws (19).

Most of the investigators have reported that inhibition of adjuvant-induced polyarthritis in rats is one of the most suitable models to screen antiarthritic agents since it closely resembles human arthritis. A number of natural products are used in various traditional medical systems to treat pain and inflammation. Our present observations indicate that administration of ethanolic extract of the plant to arthritic animals resulted in the suppression of generalized immunological response to the constituents of the Freund's complete adjuvant. The action of ERCCS against both non-established and established arthritis confirms the effectiveness of this plant against arthritic conditions.

Phytochemical investigation of ERCS revealed the presence of glycosides, flavonoids, saponins, triterpenoids, steroids, tannins and other phenolic compounds. A number of investigators have shown that flavonoids, triterpenoids, tannins, polyphenolic compounds and a host of other secondary plant metabolites all exhibits anti-arthritic properties (20, 21) It is not unreasonable, therefore to speculate that the chemical compounds present in the plant's extract are responsible for the observed antiarthritic activity. It is worthwhile to isolate the bioactive principles, which are responsible for these activities, which is in process. These findings justify the traditional use of this plant in the treatment inflammatory conditions and validate its claim of being used for the said purpose in folklore medicine. It can be concluded that ethanol extract of the roots of *C. spinarum* possesses significant, dose dependent antiarthritic properties.

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