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Pharmacological Study

In vivo antiarthritic activity of *Rosa centifolia* L. flower extract

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Abstract

Introduction: *Rosa centifolia* L. (Rosaceae) have been used for the treatment of joint pain and rheumatoid arthritis (RA) in the traditional system of medicine. **Aim**: In this study, the antiarthritic activity of the alcoholic extract from the floral parts of *R. centifolia* was investigated. **Materials and Methods:** The anti-inflammatory and antiarthritic activity of *R. centifolia* alcoholic extract (RCAE: 32, 64, and 128 mg/kg) was evaluated using the carrageenan-induced paw edema and complete Freund's adjuvant (CFA) induced arthritis model. Serum from arthritic rats was collected for the estimation of pro-inflammatory cytokine levels. Further, the safety of RCAE was evaluated in an acute and sub-acute (28-day) oral toxicity study. **Results:** RCAE (64 and 128 mg/kg) significantly ($P < 0.01$) inhibited carrageenan-induced paw edema at 1, 3, and 6 h post carrageenan challenge and demonstrated significant ($P < 0.01$) antiarthritic activity on days 3, 7, 14, and 21 day following CFA immunization. Further, RCAE (128 mg/kg) treatment also produced a significant ($P < 0.01$) decrease in circulating pro-inflammatory cytokine levels as compared with control. Further, no toxicologically significant treatment-related effects were observed in the oral sub-acute toxicity study conducted with the extract. **Conclusion:** The result of study demonstrates the antiarthritic activity of *R. centifolia* and validates its traditional use for the treatment of RA.

Key words: Arthritis, carrageenan, inflammation, quercetin, *Rosa centifolia, Taruni*

Introduction

Rheumatoid arthritis (RA) is a progressive autoimmune inflammatory disease of synovial joints which causes the destruction of joint architecture and systemic abnormalities, resulting in physical disability, and early motility.[1] The exact etiology behind the pathogenesis of RA is unknown, but it is thought to be triggered by genetic and environmental factors.[2] The inflammatory environment in the synovium is primarily conditioned by T cells, B cells and macrophage-derived pro-inflammatory cytokines predominantly interleukin-1 (IL-1), IL-6 and tumor necrosis factor alpha (TNF-α) which results in synovial hyperplasia.[3]

Conventional drugs used for the treatment of RA include nonsteroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, IL-1 receptor antagonist, and anti-TNF-α drugs. Of these, the most effective in limiting the progression of the disease are glucocorticoids and cytokine antagonists. However, their use is also associated with a plethora of adverse effects including gastrointestinal ulcers, cardiovascular complications and emergence of opportunistic infections due to immune-suppression, which adds to the morbidity and mortality associated with RA.[4] Owing to these factors, patients with RA often seek alternative methods for symptomatic relief and are among the highest users of the complementary and alternative system of medicine.[5,6]

Rosa centifolia L. (Rosaceae) is a perennial plant commonly known as hundred-leaved rose or *Shatapatri* or *Taruni* and is available throughout India. It is a complex hybrid, bred from *Rosa gallica* L., *Rosa moschata* Herrm., *Rosa canina* L. and *Rosa damascene* Mill. It is used in the traditional systems of medicine for the management of inflammatory conditions including arthritis, cough, asthma, bronchitis, wounds, and ulcers.[7] Despite its widespread use in traditional medicine, only a few studies have evaluated the efficacy of this medicinal

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plant in experimental and clinical settings. The aqueous and alcoholic extract of this plant has shown to possess anticollagenase, antielastase and antioxidant activities in *in vitro* experiments.[8] Recently, the anti-inflammatory and antiarthritic activity of *R. centifolia* in adjuvant-induced arthritis model in 14 days treatment protocol is found reported.[9] These finding can only be used for anti-inflammatory activity, but not for antiarthritic activity. As immune mediated disease response is present in arthritic patients, the complete Freund's adjuvant (CFA) induced arthritis begins only after 13 days, which is characterized by immunological hyper-reactive state primarily by TNF-α, IL-6, IL-1 in synovium which fuels inflammatory process.[10,11] Therefore, the present study was thus designed to evaluate the antiarthritic efficacy of *R. centifolia* alcoholic extract (RCAE) in experimental models in rats with an aim to elucidate its probable mechanism of action in important pro-inflammatory cytokines which are important markers currently used in therapeutics.

Materials and Methods

Animals

The experimental protocol was approved by the Institutional Animal Ethics Committee of All India Institute of Medical Sciences, New Delhi, India (No. 536/IAEC/09) and were carried out in accordance with the "Guidelines for Care and Use of Animals in Scientific Research" (Indian National Science Academy 2000). Adult male Wistar albino rats (150–180 g) from the institutional breeding stock were housed in groups of 3 and acclimatized to laboratory conditions for a period of 7 days (12/12 h of light and dark cycles and environmental temperature of 25 ± 2°C) before initiation of the experiments.

Plant material

R. centifolia flowers were procured in the month of December 2010 from the local market and were authenticated by Professor Mohammad Ali, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard. A voucher specimen (voucher no - PRL/JH/08/10) of the plant has been retained at the department herbarium for future reference.

Preparation of extract of *Rosa centifolia*

Fresh flower petals were cold macerated in absolute ethanol for 72 h with solvent change after every 24 h. The macerate was filtered through cotton wool, and the solvent was evaporated under reduced pressure till a semisolid residue was obtained. The yield of extract was 18% w/w with reference to the fresh petals. RCAE was further subjected to pharmacognostical standardization for detection of secondary plant metabolites[12] and was found to contain polyphenols and sugars.

Carrageenan induced paw edema

Five groups of male Wistar albino rats (n = 6) were used in this study. Animals were fasted overnight with free access to water before the experiment. On the day of the experiment, baseline paw volumes were recorded in cu cm by using a digital plethysmometer (Ugo Basile 7140). Thereafter, group I received 2 ml/kg 1% gum acacia suspension (vehicle control), group II received indomethacin (3 mg/kg) and groups III, IV and V received RCAE at doses of 32, 64, and 128 mg/kg respectively by gavage. Thirty minutes after administration of the vehicle/drug, paw inflammation was induced by the subcutaneous administration of 0.1 ml of 1% λ-carrageenan (freshly constituted in normal saline) into the subplantar surface of the left hind paw of the animal.[13,14] Paw volume was again measured at 1, 3, and 6 h post carrageenan administration.

Adjuvant induced arthritis

Grouping of animals and drug treatment was similar to that described earlier under "carrageenan-induced paw edema." On the day of the experiment baseline recording of the joint diameter was carried out by using a micrometer screw gauge and drug/vehicle was administered to the respective groups. Thirty minutes after administration of the vehicle/drug, arthritis was induced by a single injection of 0.1 ml of (CFA: 0.05% w/v *Mycobacterium butyricum* in mineral oil) into the subplantar surface of the hind paw of the animals. This was designated as day 0. All the groups were maintained on vehicle/drug treatment for 20 more days. Joint diameters were again measured on days 3, 7, 14, and 21. On day 21, terminal blood collection was carried out for estimation of serum cytokines as described earlier.[3] Briefly, serum TNF-α level was measured by using a commercial ELISA kit and IL-6 and IL-1β levels were estimated by dot-blot technique.

Dot blot for detection of serum cytokines

Serum (3 µl) was diluted in 7µl of PBS and manually blotted onto nitrocellulose membrane using a micropipette. Nonspecific sites were blocked by using 5% nonfat milk. Cytokine were detected by using cytokine specific primary antibodies (Santa Cruz Biotech Inc.,) and HRP conjugated secondary antibodies, followed by development with nickel-enhanced di-amino-benzidine substrate (Vector Laboratories, USA). Images were captured by using Alpha Imager EC Gel Doc system. Relative protein expression was expressed in terms of percentage integrated density value by using Alpha View Imaging software.[13]

Toxicity studies of the extracts

The toxicity profile of RCAE was evaluated according to the methodology described in earlier study.[3] Evaluation of acute oral toxicity of RCAE was carried out according to the Organization for Economic Cooperation and Development (OECD) guidelines for testing of chemicals – 425.[15] Briefly, a limit test (2 g/kg body weight) was performed using five male Wistar rats (150–180 g). All the animals were observed for mortality till 14 days after administration of the dose.

Evaluation of the 28 days oral toxicity of RCAE was carried out according to the OECD guidelines for testing of chemicals – 407.[4] Briefly, sixteen male Wistar rats (150–180 g) fromthe breeding stock were divided into two groups (n = 8). Group I received normal saline (1 mL/kg) and served as normal control and group II received RCAE at a dose of 640 mg/kg (5 times the maximum dose tested in antiarthritic study). Treatment was administered once daily for the duration of 28 days. After 28th day, blood and organs were collected after measurement of body weight, bleeding and clotting time. Hematological parameters (red blood cell, white blood cell and platelets) and biochemical (alanine transaminase, aspartate aminotransferase and serum creatinine) were studied to determine the effect of chronic administration of RCAE. To evaluate histopathological changes, H and E of heart, liver and kidney were carried out.

Statistical analysis

Statistical analysis of data was done using one-way ANOVA followed by Dunnette's Multiple Comparison (GraphPad InStat; Version 3.05, GraphPad Software, Inc., USA). $P < 0.05$ was considered to be significant.

Results

The anti-inflammatory activity of RCAE has been depicted in Figure 1. Following carrageenan administration, the maximum phlogistic response was observed at 6 h in all the groups. However, the RCAE (64 and 128 mg/kg) treated animals showed a marked decrease in paw edema at all-time points as compared to the control animals. The reference drug indomethacin (3 mg/kg) also significantly suppressed carrageenan-induced paw edema at 3 and 6 h. However, the maximum reduction in paw edema was produced by RCAE (128 mg/kg) at all-time points.

Administration of CFA produced an increase in the injected ankle joint diameter in all the animals [Figure 2]. Maximum joint swelling was observed on day 3 after, which there was a gradual reduction, except in group I (vehicle control), where there was a slight increase in joint diameter after day 14 [Figure 2]. RCAE and indomethacin treatment produced a significant reduction in joint swellings as compared to control. Further, the antiarthritic activity of RCAE (128 mg/kg) was comparable to that of indomethacin (3 mg/kg) throughout the observation period.

The serum TNF-α level in nonimmunized rats was below the detection limit of the kit used for this assay. Administration of CFA produced an increase in serum TNF-α level in all the tested animals [Figure 3] as compared to nonimmunized rats. This increase in serum TNF-α level was significantly inhibited by RCAE treatment at all dose levels. However, there was an approximately 2-fold increase in serum TNF-α level in the indomethacin treated animals as compared to control.

Figures 4 and 5 represent a comparison of serum IL-1β and IL-6 levels in the control, indomethacin and RCAE treated groups. A significant reduction in serum levels of both IL-1β and IL-6 was observed in the RCAE (128 mg/kg) treated group as compared to the control. However, indomethacin treatment only produced a significant reduction in serum IL-6 levels, while having no effects on serum IL-1β level.

Administration of RCAE at a dose of 2000 mg/kg body weight did not produce any mortality during the observation period of 14 days. The oral LD_{50} of RCAE was therefore established at >2000 mg/kg body weight in rats. Following repeated dose administration of RCAE at a dose of 640 mg/kg for 28 days, a statistically significant decrease in hepatic transaminases and an increase in WBCs were observed. However, these changes were within the physiological limits for rat and were therefore not considered to be toxicologically relevant. No other physiological, biochemical or histopathological changes were observed in the tested animals as compared to normal control (data not shown).

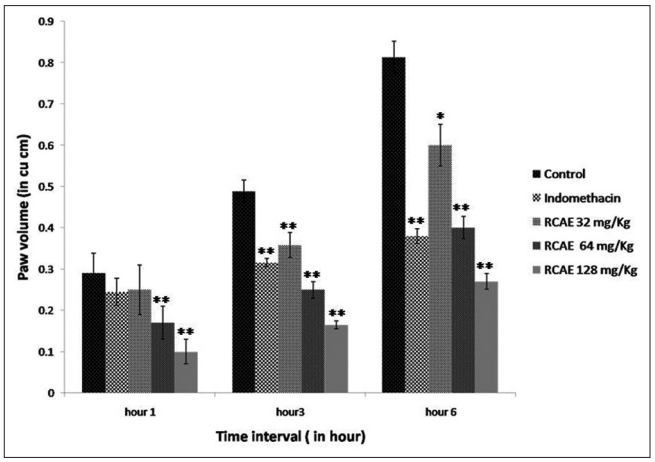

Figure 1: Effect of *Rosa centifolia* alcoholic extract on carrageenan induced paw edema in rats. RCAE: *Rosa centifolia* alcoholic extract. All values are mean ± standard error

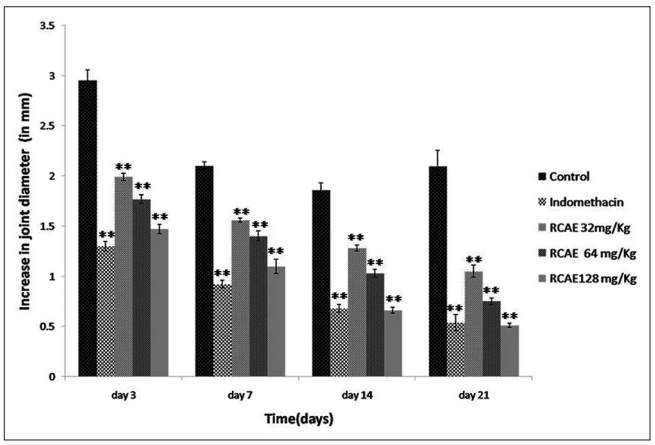

Figure 2: Effect of *Rosa centifolia* alcoholic extract on joint swelling in complete Freund's adjuvant induced arthritis in rats. RCAE: *Rosa centifolia alcoholic extract*. All values are mean ± standard error

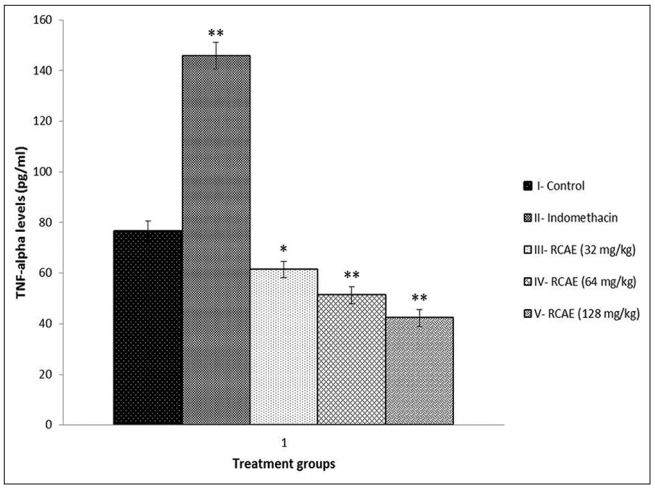

Figure 3: Effect of *Rosa centifolia* alcoholic extract on serum tumor necrosis factor alpha in complete Freund's adjuvant induces arthritis. RCAE: *Rosa centifolia* alcoholic extract. All values are mean ± standard error

Discussion

The carrageenan-induced paw edema produces a time-dependent triphasic response and is one of the most commonly used models for evaluation of anti-inflammatory activity as it highly correlated with the early exudative stage of inflammation in man. During the first phase which lasts

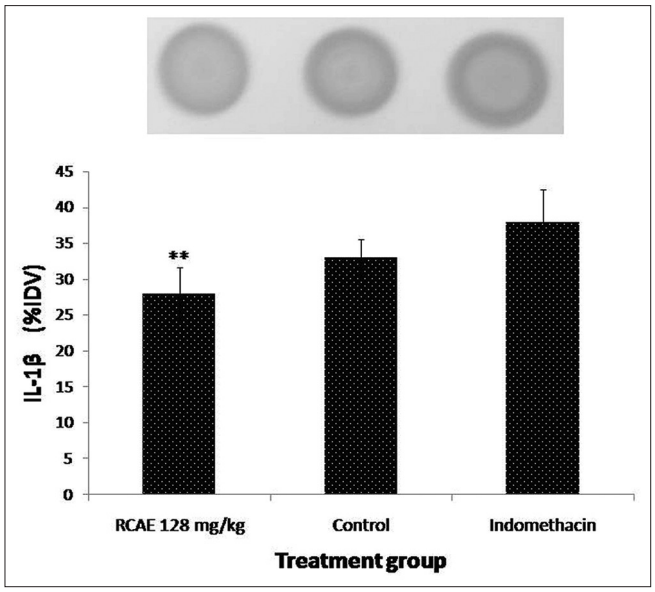

Figure 4: Effect of *Rosa centifolia* alcoholic extract on serum interleukin-1β in complete Freund's adjuvant induces arthritis. RCAE: *Rosa centifolia* alcoholic extract. All values are mean ± standard error

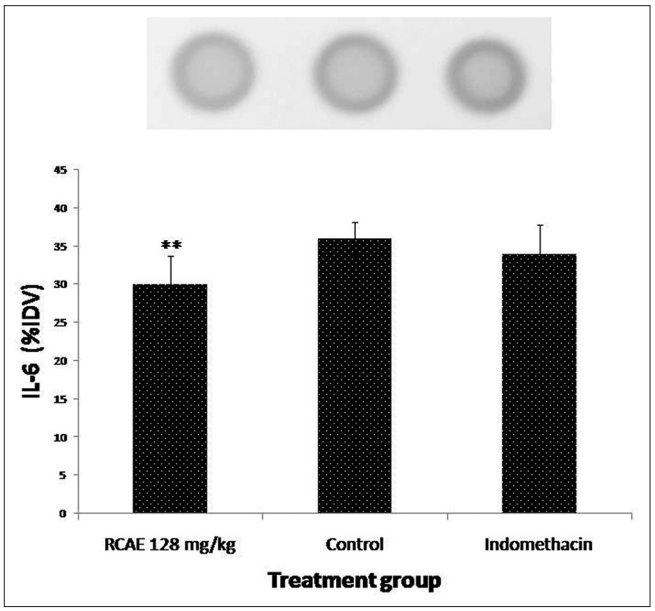

Figure 5: Effect of *Rosa centifolia* alcoholic extract on serum interleukin-6 in complete Freund's adjuvant induces arthritis. RCAE: *Rosa centifolia* alcoholic extract. All values are mean ± standard error

for approximately 1 h following carrageenan administration there is a sudden increase in paw volume which correlates with increased vascular permeability induced by the action of histamine, the second phase which lasts from approximately 2 to 3 h post carrageenan administration is attributed to serotonin and kinins generation. The paw edema gradually elevates to a peak during 4–6 h after carrageenan administration. This is the third phase and is attributed to the generation of prostaglandins and leukotrienes.[13,16] In this study, indomethacin only produced inhibition of inflammation at 3 and 6 h post carrageenan administration, owing to its primary activity against prostaglandin synthesis. However, as compared to control, RCAE treatment produced a significant inhibition of inflammation at all observation points, suggesting that RCAE possesses inhibitory activity against multiple autacoids mediators. The anti-inflammatory effect observed in the present study is similar to the effects reported with other *Rosa* species.[17,18]

To evaluate the antiarthritic activity of the RCAE, the CFA induced arthritis model was used. CFA induced arthritis is widely used for the pharmacological evaluation of antiarthritic agents because of the high degree of similarity it has with the human disease.[10,19] In the present study, there was a dose-dependent inhibition of joint swelling in the RCAE treated groups as compared to control. Also, the delayed increase in joint diameter (indicative of cell-mediated immunity) was also not observed in the test drug-treated group.

In addition, the serum levels of three pro-inflammatory cytokines (viz. TNF-α, IL-6 and IL-1β) were also evaluated, which are predominantly secreted by macrophages and are found to be overexpressed in RA.[20,21] RCAE treatment produced a significant reduction in serum levels of all the three pro-inflammatory cytokines as compared to control animals. On the other hand, indomethacin significantly decreased the serum levels of IL-6, and significantly increased the serum levels of TNF-α while having no effect on circulating IL-1β levels. The effect of indomethacin treatment on serum pro-inflammatory cytokine levels is similar to the results reported earlier.[3,4,22] This difference in effect on the cytokine profile suggests that RCAE may be having different or additional mechanisms (in comparison to indomethacin) which are responsible for its antiarthritic effect.

The important phytochemical principles present in RC are polyphenols, *viz.* gallic acid, rutin, quercitrin, myricetin, quercetin, and kaempferol.[23] Of these, quercetin has been the most widely evaluated. Quercetin has been shown to inhibit the production of TNF-α, NO, IL-1β and MCP-1; which are important inflammatory mediators derived from macrophages.[24] In addition quercetin and kaempferol have also been shown to inhibit inducible nitric oxide synthase, cyclooxygenase-2 and C-reactive protein, and down-regulate nuclear factor-kappa B pathway.[25] However, due to the presence of multiple flavonoids principles in RCAE, the observed antiarthritic activity cannot solely be attributed to quercetin and kaempferol.

Based on an LD_{50} value of >2000 mg/kg bodyweight, RCAE can be considered to be of low acute oral toxicity. Further, long-term administration of RCAE at a high dose level of 640 mg/kg bodyweight also did not produce any overt pathological changes, thus demonstrating a favorable toxicity profile.

Conclusion

Based on the results obtained in the study, RCAE could be explored further as a potentially safer alternative for the treatment of RA.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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हिन्दी सारांश

तरूणी (रोजा सेंटीफोलिया) अर्क का गठिया विरोधी प्रभाव

रोहित कुमार, विनोद नायर, सुरेंदर सिंह, योगेन्द्र कुमार गुप्ता

तरुणी (रोजा सेंटीफोलिया एल.) को जोड़ के दर्द तथा गठिया (रिहयूमेटोइड अर्थ्राइटीस) के इलाज के लिए चिकित्सा की पारंपरिक पद्धति के अनेक जड़ीबूटी वाले सूत्रों में एक घटक के तौर पर इस्तेमाल किया जाता है। इस अध्ययन में गुलाब के फूल के भागों से अल्कोहोलिक अर्क (आर.सी.ए.ई.) की गठिया रोधी गतिविधि की जांच की गई । तरुणी फूल के अर्क की प्रज्जवलन रोधी गतिविधि (रेसः ३२, ६४ और १२.८ मि.ग्रा.) का मूल्यांकन केराजीनान से उद्दीपित पंजे में शोथ के मॉडल का उपयोग करते हुए किया गया। गठिया से पीड़ित चूहों को आंकलन के लिए जमा किया गया। पुनः आर.सी.ए.ई. की निरापदता का मूल्यांकन तीव्र और अर्धजीर्ण (२८ दिन) मौखिक विषाक्तता के अध्ययन में किया। आर.सी.ए.ई. उपचार (६४ और १२८ मि.ग्रा./कि.ग्रा.) से केराजीनान से उद्दीपित पंजे की शोथ में केराजीनान चुनौती के १, ३ और ६ घंटे बाद उल्लेखनीय कमी आयी और सी.एफ .ए. टीकाकरण के बाद दिन ३, ७, १४ और २१ पर गठिया रोधी गतिविधि में उल्लेखनीय कमी दर्शायी गई। पुनः आर.सी.ए.ई. (१२८ मि.ग्रा./कि.ग्रा.) उपचार से कंट्रोल की तुलना में परिचालित प्रज्जवलन उन्मुख साइटोकाइन स्तरों में भी उल्लेखनीय कमी उत्पन्न हुई। अध्ययन के परिणाम स्वरूप तरुणी फूल के अर्क की गठिया रोधी गतिविधि के साथ इसके उपचार में पारंपरिक चिकित्सा में उपयोग का सत्यापन होता है और इसमें विषाक्तता प्रभाव भी नहीं पाई गई ।