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Amelioration of complete Freund's adjuvant-induced arthritis by *Calotropis procera* latex in rats

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Abstract

Background: Rheumatoid arthritis is the most common cause of disability, affecting 0.3–1% of the adult population worldwide. The latex of *Calotropis procera* possesses potent anti-inflammatory as well as analgesic properties. In light above facts, the present study was designed to evaluate anti-arthritic activity of *Calotropis procera* latex in complete Freund's adjuvant (CFA)-induced arthritis in *Wistar albino* rats. Complete Freund's adjuvant was injected into the left hind paw on day 0, and treatment of prednisolone and *Calotropis procera* latex was given from day 0 to 28. Various biochemical, hematological and functional parameters as well as radiological and histopathological changes of joint along with body weight and paw volume were measured.

Results: *Calotropis procera* treatment significantly lowered paw volume in CFA-induced arthritic rats. Significant improvement was observed in functional, biochemical and hematological parameters in *Calotropis procera*-treated rats. However, the body weight remained unaffected. Histological and radiographical examination of synovial joints in *Calotropis procera*-treated animals exhibited less synovial hyperplasia, infiltration and accumulation of inflammatory cell in synovial fluid, cartilage and bone erosion and joint space narrowing.

Conclusion: *Calotropis procera* latex possesses anti-arthritic activity, which is facilitated by modulation in the level of inflammatory mediators and oxidative stress. The improvement in hematological as well as biochemical parameters might be reflected on functional, histopathological, radiological changes and thereby disease progression.

Keywords: Latex, *Calotropis procera*, Rheumatoid arthritis, Complete Freund's adjuvant, Functional parameters, ELISA, Anti-arthritic activity, Radiology

Background

Rheumatoid arthritis (RA) is chronic inflammatory-erosive disorder of joints. The worldwide prevalence of the disease is 0.3–1%, and women are more prone to develop the disease than men [1]. It is characterized with swelling, pain and morning stiffness and predominantly involved small joints of the wrist, hands, feet and knee [2]. Disease progresses with joint erosion, muscle atrophy, loss of mobility and systemic manifestation, affecting individual's daily living activities as well as psychological health [3, 4].

It is well known that the pro-inflammatory cytokines like interleukins 1 (IL-1), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) are responsible for autoimmunity, chronic inflammation and joint damage [5]. Many pre-clinical and clinical studies revealed elevated level of these cytokines in the serum and synovial fluid in patients with RA [6–8]. The cytokines-mediated joint damage occurs by stimulation of mesenchymal cells like synovial fibroblasts, osteoclasts, chondrocytes and thereby release of tissue-destroying matrix metalloproteinases. The production of metalloproteinases

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tissue inhibitor by synovial fibroblast is also repressed by cytokines [9]. Along with this, nitric oxide (NO) mediates synovial inflammation by angiogenic cytokine production, matrix metalloproteinases activation and apoptosis [10]. Current medications such as disease-modifying anti-rheumatic drugs (DMARDs), non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and TNF inhibitor have high cost and serious adverse effects [11]. Moreover, 30% patients did not show significant improvement with these treatments [12]. Thus, alternative therapy with herbal medicine, effective in alleviating rheumatic pain with minimum side effects is required.

Calotropis procera is an Indian tropical plant with important medicinal properties like anti-diabetic [13], hepatoprotective [14], anti-oxidant [15], anti-pyretic [16] and anti-fertility [17] activity. Molecular docking of few phytochemicals present in the latex of *Calotropis procera* has been reported to exhibit potent anti-inflammatory and analgesic activity [18–20]. Also, the latex of *Calotropis procera* significantly reduces cell influx, inflammatory mediators, oxidative stress and thereby improves locomotor dysfunction in complete Freund's adjuvant (CFA)-induced arthritic rats [21, 22]. In light of these facts, the present study was carried out to evaluate the effect of dry latex of *Calotropis procera* in CFA-induced arthritis in rats.

Methods

Materials

Complete Freund's adjuvant was purchased from Sigma-Aldrich, USA (F5881), and prednisolone was purchased from Macleods Pharmaceuticals Ltd., Gujarat, India. Analytical grade chemicals and reagents were used throughout the experiment. Rheumatoid factor (RF) and C-reactive protein (CRP) were measured using diagnostic kits obtained from Beacon Diagnostics Pvt. Ltd., Gujarat, India. The plasma TNF-α and IL-6 were measured with rat ELISA kits purchased from Genxbio health sciences, Delhi, India.

Animals

The animals (procured from in-house animal facility) were kept in standard polypropylene cages (three rats/cage) and maintained at 22 ± 2 ○C temperature and 55 ± 5% humidity, with 12/12-h light and dark cycle. All the rats were fed with commercially available rat normal pellet diet (NPD) and given water ad libitum. The experimental protocol (KBIPER/17/596) was approved by the Institutional Animal Ethics Committee, Gujarat, India.

Plant materials and drugs

The identification and authentication of *C. procera* was done by Pharmacognosy Department of KBIPER, and voucher specimen was preserved. The fresh latex was collected from the aerial parts of the plant growing in the wild. Thereafter, the latex was lyophilized, triturated with gum acacia, filtered and administered.

Experimental design

The adult female Wistar rats weighing 200–250 g were randomized (based on body weight) into four groups (n = 6).

Group I—Normal control (NC) group
Group II—CFA-induced arthritic (DC) group
Group III—Prednisolone-treated (PS10) group (10 mg/kg, *p.o.*)
Group IV—Dry latex of *C. procera* (DL250)-treated group (250 mg/kg, *p.o.*)

Arthritis was induced in rats as per the procedure described by Gohil et al. [23]. Briefly, for induction of arthritis, 0.2 ml CFA (containing 10 mg/ml of heat-killed *M. tuberculosis*) was injected into left hind paw of all the animals except group I. One hour before the injection of CFA, group III and group IV animals were administered prednisolone and *C. procera* treatment, respectively, from day 0 and continued till day 28, whereas normal control and disease control animals received 0.1 ml vehicle only.

Paw volume

The left hind paw volume was measured weekly from day 0 to day 28 using digital plethysmometer (Orchid Scientific & Innovative India Pvt Ltd., Maharashtra, India) from all groups.

Blood collection, hematological and biochemical estimation

At the end of study, blood samples were collected under pentobarbital (60 mg/kg, *i.p.*) anesthesia into EDTA-coated tubes and used for measurement of hemoglobin (Hb), total white blood cell (WBC) count, red blood cell (RBC) count and erythrocyte sedimentation rate (ESR).

Thereafter, blood was centrifuged at 5,000 RPM, 4°C for 10 min (REMI, Germany) and separated plasma was used for biochemical estimation like rheumatoid factor (RF), C-reactive protein (CRP), TNF-α and IL-6. Nitrite level was measured by Griess reagent system [24].

Joint stiffness

Joint stiffness was measured as per score described by Nagakura et al. [25] at the end of the study. The rat was held from back with left palm, and the bending and extension of limbs in each direction were performed within the limits of motion with right fingers. Score 2:

restriction of both bending and extension movement of ankle, Score 1: restriction of either bending or extension movement of ankle score 0: no restriction of ankle movement (Table 1).

Mobility test

On day 28, mobility score was performed as mentioned below—Score 6: animal walks normally, score 5: ipsilateral hind paw of the animal fully the floor, score 4: only toe of the ipsilateral hind paw of the animal touches the floor, score 3: fully touches the contralateral hind paw and fully touches the floor, score 2: only toe of the contralateral hind paw of the animal touches the floor, score 1: animal only crawls with fore limbs, score 0: animal does not move [25].

Gait test

At the end of the study, rats from each group were allowed to move freely on a table top for gait test. Score 2: creeping behavior (moving on the two fore limbs, dragging the two hind limbs), score 1: inactive use of paw to support the body, score 0: active use [25]. Adjuvant-treated paw was overlooked for gait score because swelling and abscess formation were observed in several animals (Table 2).

Arthritic index

Two observers blinded with study were observed ears, nose, tail, forepaws and hind paws for the presence of inflammation and/or nodules. Arthritic index was calculated according to the scoring system of Bartlett and Schleyerbach, 1985 [26] (Table 1), and results are given in Table 3.

Visual weight-bearing test

For visual weight-bearing test, both paws of rats were dipped in ink and their walkway pattern on paper was scored [27]. The scoring system is shown in Table 2 and the results are shown in Table 4.

Histopathology and X-ray analysis of left hind limb joint

On day 28, rats were anesthetized using pentobarbital (60 mg/kg, *i.p.*) and subjected to X-ray examination of the left hind legs. Thereafter, the rats were killed; left hind limb joints were collected and stored in 10% formalin. Formalin-fixed tissue was dehydrated, embedded

Table 1 Scoring system for arthritic index

Lesion site	Nature of lesion	Score
Ears	Absence of nodules	0
	Presence of nodules	1
Nose	Absence of swelling of connective tissue	0
	Presence of swelling of connective tissue	1
Tail	Absence of nodules	0
	Presence of nodules	1
Fore paws	Absence of inflammation	0
	Presence of inflammation at least in one joint	1
Hind paws	Absence of inflammation	0
	Slight inflammation	1
	Moderate inflammation	2
	Marked/severe inflammation	3

Table 3 Effect of *C. procera* on functional parameters of CFA-induced arthritic rats

Groups	Gait test	Mobility test	Joint stiffness	Visual weight-bearing test
NC	0 ± 0.00	7.00 ± 0.00	0 ± 0.00	0 ± 0.00
DC	1.83 ± 0.54*	3.53 ± 1.43*	1.83 ± 0.68*	1.75 ± 0.55*
PS10	0.17 ± 0.06#	6.66 ± 2.73#	0.33 ± 0.13#	0 ± 0.00#
DL250	0.33 ± 0.13#	6.50 ± 2.66#	0.4 ± 0.16#	0.16 ± 0.18#

Data represented as mean ± SEM; *$p < 0.05$ compared to NC group and #$p < 0.05$ compared to DC group (one-way ANOVA followed by Tukey's multiple comparison test). *NC* normal control group, *DC* CFA-induced arthritic group, *PS10* prednisolone-treated group, *DL250* dry latex of *C. procera*-treated group

Table 2 Scoring system for visual weight-bearing test

Score	Criteria
0	Normal paw pressure, equal weight on both hind paws
0.5	Normal paw pressure, paw is completely on the floor, but toes are unequal to control hind paw
1	Slightly reduced paw pressure, paw is completely on the floor, but toes are not spread
1.5	Reduced paw pressure, intermediate between categories 1 and 2
2	Slight reduced paw pressure, paw curled with only some parts of the hind paw lightly touching the floor
2.25	Moderately reduced paw pressure, paw curled with toes only lightly and occasionally touching the floor
2.5	Slightly reduced paw pressure, paw curled with toes only lightly and occasionally touching the floor
2.75	Moderately reduced paw pressure, paw curled with toes only lightly and occasionally touching the floor
3	Severely reduced paw pressure, paw completely elevated

Table 4 Effect of *C. procera* on biochemical parameters of CFA-induced arthritic rats

Groups	IL-6 (ng/mL)	TNF-α (ng/L)	RF factor (IU/mL)	CRP (mg/L)	Nitrite (μM)
NC	1.78 ± 0.54	105.98 ± 12.62	1.03 ± 0.47	2.10 ± 0.97	1.59 ± 0.18
DC	3.39 ± 0.32*	154.90 ± 25.88*	10.13 ± 0.28*	8.68 ± 1.53*	5.87 ± 0.28*
PS10	1.00 ± 0.43#	108.62 ± 15.86#	6.20 ± 0.04#	4.11 ± 1.18#	2.88 ± 0.25#
DL250	1.33 ± 0.58#	155.20 ± 15.95	7.38 ± 0.12#	4.98 ± 0.73#	3.33 ± 0.32#

Data represented as mean ± SEM; *$p < 0.05$ compared to NC group and #$p < 0.05$ compared to DC group (one-way ANOVA followed by Tukey's multiple comparison test). *NC* normal control group, *DC* CFA-induced arthritic group, *PS10* prednisolone-treated group, *DL250* dry latex of *C. procera*-treated group

in paraffin wax and sectioned. The sections were stained with hematoxylin–eosin and examined for cartilage destruction, synovial space, hyperplasia of tissue and bone erosion.

Statistical analysis

All data are expressed as mean ± SEM. The data were subjected for statistical analysis using GraphPad Prism 5.0 (CA, USA). The statistical significance of difference between various groups was tested by one-factor analysis of variance (ANOVA) followed by Tukey's multiple comparison test. The results of body weight and paw edema were assessed by two-way ANOVA and Bonferroni's post hoc test. In each test, $p < 0.05$ was considered statistically significant.

Results

Effect of *C. procera* on body weight

Compared to control rats, CFA-induced arthritic rats showed a significant decrease in body weight. Prednisolone treatment and *C. procera* treatment did not show significant change in body weight as compared to CFA-induced arthritic rats. However, the body weight was slightly higher in *C. procera*-treated rats than prednisolone-treated rats (Fig. 1).

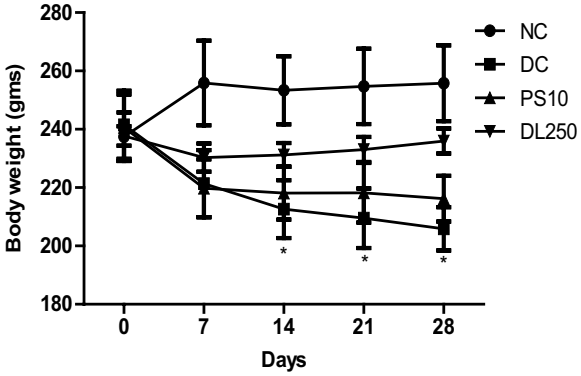

Fig. 1 Effect of *C. procera* on body weight of CFA-induced arthritic rats. Data represented as mean ± SEM; *$p < 0.05$ compared to NC group (two-way ANOVA followed by Bonferroni's post hoc test). *NC* normal control group, *DC* CFA-induced arthritic group, *PS10* prednisolone-treated group, *DL250* dry latex of *C. procera*-treated group

Effect of *C. procera* on paw volume

A linear increase in paw volume was observed in CFA-induced arthritic rats compared to control rats throughout the experiment. Both prednisolone treatment and *C. procera* treatment showed significantly lower paw volume compared to CFA-induced arthritic rats. Moreover, the paw volume was similar in prednisolone-treated rats and *C. procera*-treated rats (Fig. 2).

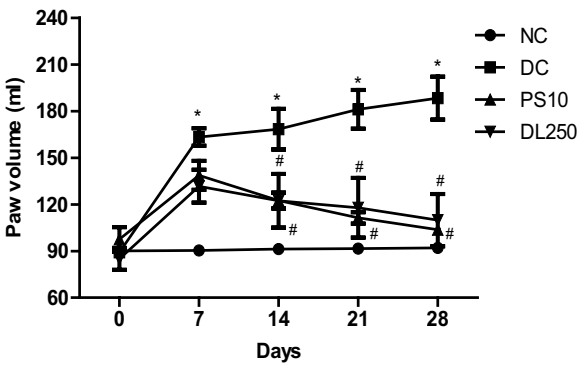

Fig. 2 Effect of *C. procera* on left hind paw volume of CFA-induced arthritic rats. Data represented as mean ± SEM; *$p < 0.05$ compared to NC group; #$p < 0.05$ compared to DC group (two-way ANOVA followed by Bonferroni's post hoc test). *NC* normal control group, *DC* CFA-induced arthritic group, *PS10* prednisolone-treated group, *DL250* dry latex of *C. procera*-treated group

Effect of *C. procera* on gait test, mobility test, joint stiffness and visual weight-bearing test

Functional parameters like gait test, mobility test, joint stiffness and visual weight-bearing test significantly differ between CFA-induced arthritic rats and control rats. Further, in prednisolone-treated rats as well as in *C. procera*-treated rats, significant improvement was observed in all these parameters (Table 3).

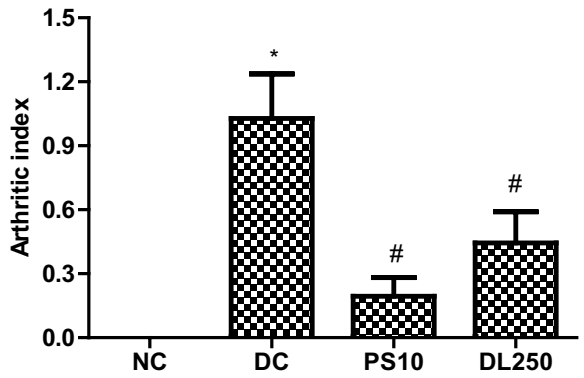

Fig. 3 Effect of *C. procera* on the arthritic index of CFA-induced arthritic rats. Data represented as mean ± SEM; *$p < 0.05$ compared to NC group; #$p < 0.05$ compared to DC group (two-way ANOVA followed by Bonferroni's post hoc test). *NC* normal control group, *DC* CFA-induced arthritic group, *PS10* prednisolone-treated group, *DL250* dry latex of *C. procera*-treated group

Table 5 Effect of *C. procera* on hematological parameters of CFA-induced arthritic rats

Groups	Hb (gm%)	ESR (mm/h)	Total WBC (thousand/cmm)	RBC (millions/cmm)
NC	15.16 ± 0.93	5.16 ± 1.81	6.25 ± 0.87	7.37 ± 0.61
DC	9.23 ± 0.50*	13.16 ± 2.18*	12.45 ± 0.67*	3.56 ± 0.67*
PS10	13.22 ± 0.50#	6.57 ± 2.54#	7.89 ± 0.44#	5.23 ± 0.34#
DL250	12.11 ± 1.04#	8.21 ± 1.65#	9.34 ± 0.89#	5.76 ± 0.67#

Data represented as mean ± SEM; *$p < 0.05$ compared to NC group and #$p < 0.05$ compared to DC group (one-way ANOVA followed by Tukey's multiple comparison test). *NC* normal control group, *DC* CFA-induced arthritic group, *PS10* prednisolone-treated group, *DL250* dry latex of *C. procera*-treated group

Effect of *C. procera* on arthritic index

There was a significant increase in the arthritic index after CFA administration. Prednisolone-treated rats, as well as *C. procera*-treated rats, showed significantly lower arthritic index compared with CFA-induced arthritic rats (Fig. 3).

Effect of *C. procera* on biochemical parameters

In comparison with control rats, biochemical parameters like IL-6, TNF-α, RF factor, CRP and nitrite levels were significantly elevated in CFA-induced arthritic rats. The treatment with prednisolone significantly restores all biochemical parameters. *C. procera* treatment significantly lowered IL-6, RF factor, CRP and nitrite levels without altering TNF-α level (Table 4).

Effect of *C. procera* on hematological parameters

In CFA-induced arthritic rats, significantly lower Hb level and RBC count, as well as higher ESR and total WBC count, was observed in comparison with control rats. Both prednisolone and *C. procera* significantly restored all these hematological changes (Table 5).

Effect of *C. procera* on histopathological changes of synovial joint

The histopathological changes of tarsotibial joints from different animal groups are shown in Fig. 4. In control rats, synovial lining showed a thin layer of flat and quiescent cells and normal synovial space. No leucocyte infiltration or bone erosion was observed. Complete Freud's adjuvant-induced arthritic rats showed proliferation of synovial membrane cells which formed a thick and multilayered synovial cell lining and reduced synovial space. In addition, diffused infiltration of synovial inflammatory cells, pannus formation in focal areas and new blood vessels formation in inflammatory synovial tissue were also observed. Prednisolone-treated and *C. procera*-treated group exhibited less synovial hyperplasia, infiltration and accumulation of inflammatory cell in synovial fluid, cartilage and bone erosion and joint space narrowing in comparison with CFA-induced arthritic group.

Effect of *C. procera* on X-ray radiographic changes of synovial joint

As shown in Fig. 5, X-ray radiography of tarsotibial joints of left hind legs was performed to evaluate the effect of *C. procera* on CFA-induced arthritic rats. The control rats demonstrated the normal structure of joints and bone. Complete Freud's adjuvant-induced rats displayed arthritic changes like excessive soft tissue swelling, joint space narrowing and bone erosion. The bones were seen unprotected by cartilage. Prednisolone treatment and *C. procera* treatment exhibited an inhibitory effect on swelling and bone erosion.

Discussion

Rheumatoid arthritis affects billions of people worldwide and, thus, the most common cause of disability. The potential side effects of presently available treatment enforce to identify more effective therapy that will benefit a wide range of patients. From last few years, herbal products are gaining more attention for the prevention and treatment of many diseases. The reports suggesting anti-inflammatory and analgesic activities of *C. procera* were considered for the present to investigate its effect in arthritic rats.

Complete Freund's adjuvant-induced arthritis is commonly used murine model of chronic polyarthritis. The pathophysiological changes like synovial hyperplasia and cartilage degradation observed in this model are similar to clinical arthritis [28]. More importantly, this model is

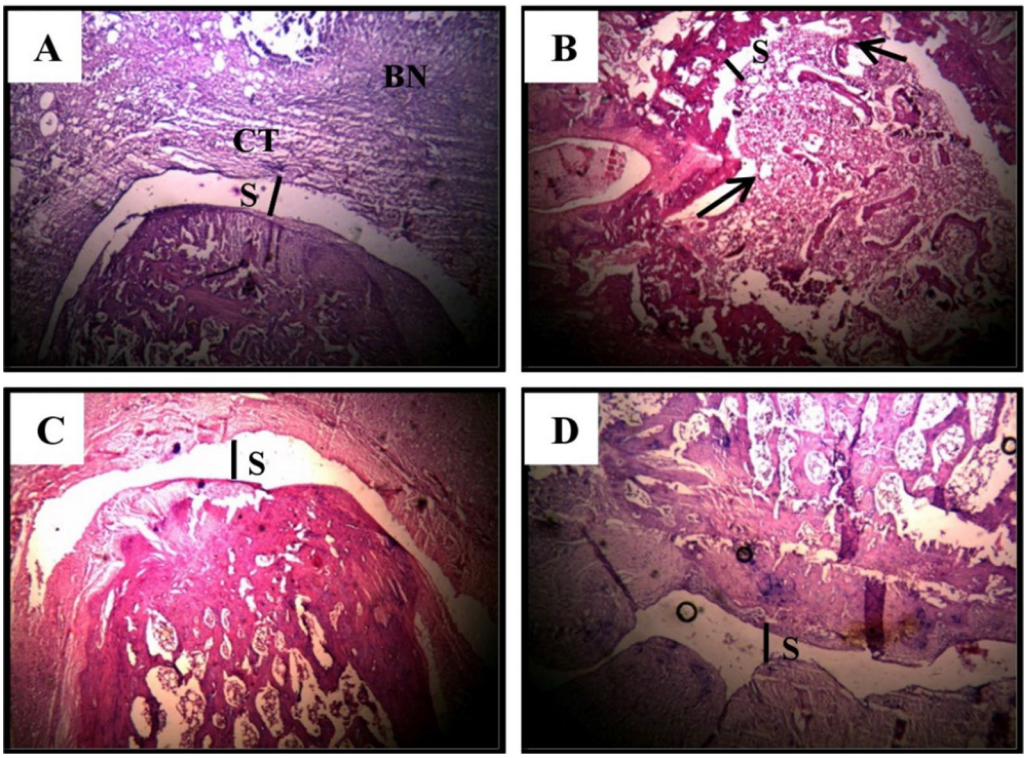

Fig. 4 Histological sections (40 ×) of tarsotibial joints of left hind paw form different animal groups. **A** Control group showed normal synovial space (S), articular cartilage (CT), bone structure (BN) and absence of inflammatory cells; **B** CFA-induced arthritic group showed marked infiltration of inflammatory cells, synovial hyperplasia and marked reduction in synovial joint (shown by arrow); **C** prednisolone-treated group and **D** dry latex of C. procera-treated group showed less inflammatory cell infiltration, minimal synovial hyperplasia and modest reduction in joint space

more relevant for the evaluation of anti-inflammatory as well as the analgesic potential of drugs [29]. Though the female rats show variability in disease onset and severity in CFA-induced arthritis [30], female rats are more susceptible for development of disease as compared to male rats [31]. Hence, CFA-induced arthritic model in female rats was used in the present study. In CFA-induced arthritic rats, T cells are infiltrated from spleen, Peyer's patches and lymph nodes in the inflamed joints. This immune response was induced by specific antigen heat-shock protein (Hsp65). Along with this, various cytokines like IL-17, IFN and TNF-α seem to be expressed during early phase of inflammation. As the inflammation progresses, increased levels of IL-4, IL-6, monocyte chemotactic protein 1 and TGF-β can also be detected.

Rheumatoid cachexia is characterized by reduced muscle mass and weakness due to RA. About two-thirds of people with RA suffer from this complication. TNF-α and IL-1 are the key inflammatory cytokines involved in the pathogenesis of rheumatoid cachexia [32]. This cytokines are also found to be associated with higher rates of protein breakdown in RA patients as compared to healthy subjects [33]. Likewise, progressive decrease in body weight was also observed in CFA-induced arthritic rats [23, 34]. Similar results for body weight reduction in CFA-induced arthritic rats were also observed in the present study. Both prednisolone treatment, as well as *C. procera* treatment to arthritic rats, did not show improvement in the body weight. However, many researchers reported improvement in the body weight after prednisolone treatment [35, 36]. Contrary to this, other researcher reported a decrease in body weight after prednisolone treatment [37].

Swelling of paw is a simple and sensitive parameter for assessment of therapeutic efficacy of various anti-inflammatory drugs [38]. Paw volume was increased progressively in CFA-induced arthritic rats throughout the experiment, whereas it was reduced after 14 days in prednisolone-treated group and *C. procera*-treated group. Along with severe inflammation, arthritic animals also showed ulcer, pus formation and severe deformities in injected paw. As both treatments reduce inflammation, no such signs were observed in drug-treated animals (Fig. 6). It is believed that the change in paw volume is associated with activation of monocytes. These activated monocytes produce various pro-inflammatory cytokines

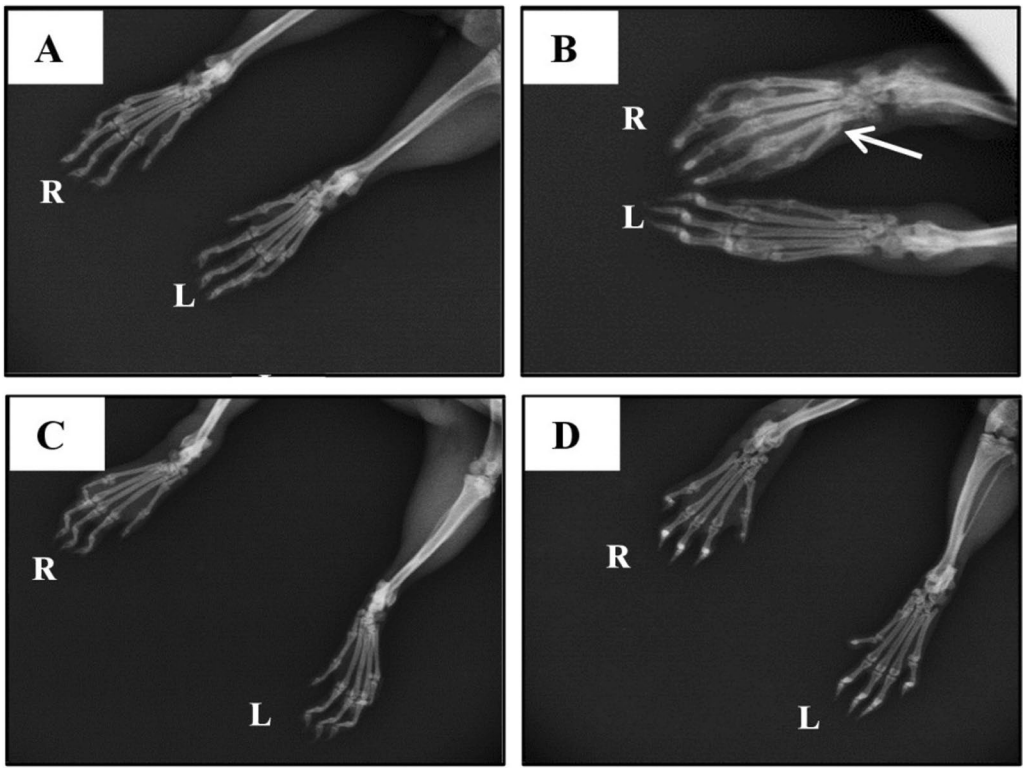

Fig. 5 X-ray radiographic of tarsotibial joints of hind legs from different animal groups. **A** Control rats show a normal structure of joint; **B** CFA-induced arthritic rats show swelling of soft tissue on left hind paw (arrow), joint destruction, bone erosion and deformities; **C** prednisolone-treated group **D** and dry latex of *C. procera*-treated group show less soft tissue swelling, reduced bone destruction with normal space in joint

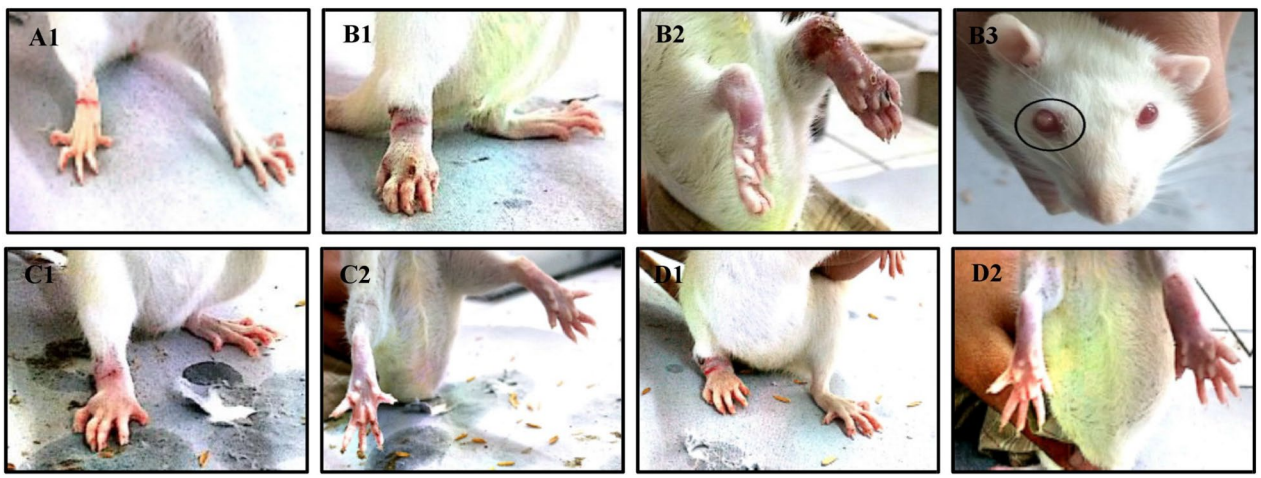

Fig. 6 Representative photographs of CFA-induced arthritic rats from different animal groups. **A1** Control rats show both paws normal; **B1** front view and **B2** back view of left hind paw show severe inflammation, ulcer formation, pus formation and even deformities. **B3** nodule in the eye (circle) indicates the extra-articular symptoms in CFA-induced arthritic rats; **C1** front view and **C2** back view of prednisolone-treated rats as well as (**D1**, **D2**) *C. procera*-treated rats show less inflammation and also no ulcer or pus formation

including IL-1, IL-6, TNF-α and trigger the production of matrix metalloproteinases and acute phase protein. This may lead to the development of specific cellular and humoral immune responses [39–41]. An elevated level of inflammatory cytokines was observed in RA patients [42] as well as in experimentally induced arthritic rats [43]. Various in vivo and in vitro studies reported reduction in tissue TNF-α level [22, 44, 45]. Prednisolone treatment reduced both TNF-α and IL-6 levels in CFA-induced arthritic rats, whereas *C. procera* treatment affects only IL-6 level in CFA-induced arthritic rats.

Rheumatoid arthritis is a connective tissue disorder affecting para-articular structures and causes pain, stiffness and swelling of joints. This physical impairment can be evaluated by various functional parameters like gait test, mobility test, joint stiffness and visual weight-bearing test. As the disease progresses, it also involves extra-articular tissues like skin, eye, lung, heart, kidney, blood vessels and many more. In the present study, nodule formation in the eye of CFA-induced arthritic rats was observed (Fig. 6). The arthritic index is a secondary immune response which involves inflammation, nodule formation and dissemination of disease at non-injected sites [46]. It is used to evaluate disease progression and severity. The arthritic index was higher in CFA-induced arthritic rats. Likewise, other functional parameters like gait test, mobility test, joint stiffness and visual weight-bearing test were also altered in CFA-induced arthritic rats. Both prednisolone-treated group and *C. procera*-treated group showed improvement in all these functional parameters.

Hematological alterations like anemia of chronic disease, leukocytosis and rise in ESR exhibited by patients with active RA [47]. A moderate rise in the WBC count is mediated by IL-1β [48]. Studies also revealed that anemia occurs in chronic arthritic patients due to reduction in erythropoietin levels, a diminished response of the bone marrow erythropoietin and premature destruction of RBCs. Addition to this, higher ESR is associated with increase production of endogenous proteins such as fibrinogen and α/β globulin [49]. CFA-induced arthritic rats exhibited lower RBC count and Hb content, whereas higher WBC counts and ESR. Similar hematological changes in arthritic rats were reported by many researchers [50, 51]. *C. procera* treatment, as well as reference drug prednisolone treatment, exhibited a potent inhibitory effect on all hematological changes induced by CFA administration. The results exhibiting a beneficial effect of prednisolone in experimentally induced RA were supported by previous findings [52, 53].

Rheumatoid factor is an autoantibody, directed against the Fc region of IgG. The complex formation between RF and IgG is responsible for the development of RA [54]. Rheumatoid factor was found to be positive in approximately 80% of RA patients [55]. Similar to RF, CRP level is also recognized as an important non-specific inflammatory marker for RA. In this way, CRP level and RF measurement are valuable to understand the severity and disease progression in RA patients [56]. Contrary to prednisolone treatment and *C. procera* treatment group, higher CRP and RF level was observed in CFA-administered arthritic rats. A similar effect on CRP and RF levels after prednisolone treatment was also reported in arthritic rats [52] as well as in RA patients [57].

Further, there is growing evidence suggesting the role of NO in the pathogenesis of many autoimmune diseases including RA [58]. Preclinical studies reported a profound increase in NO level and restoration by selective inhibitors in experimentally induced arthritic animals [59, 60]. Further, the beneficial effects of NO synthesis inhibition inferred indirectly, with use of glucocorticoids, salicylates, indomethacin and methotrexate in RA patients [61, 62]. On the other hand, due to the short half-life and high reactivity, the routine assessment of NO levels in biological fluids is difficult. Therefore, NO concentration was measured indirectly in terms of nitrite [63]. In the present study, CFA-induced arthritic rats showed higher plasma nitrite level. The prednisolone and *C. procera* treatment significantly prevented the rise in nitrite level. One of the researchers reported that the treatment of non-dialyzable proteins of *C. procera* latex significantly decreases tissue level of nitrite [64].

Elevation of above biochemical markers reflected in histological changes of joints also. Control animals show the tarsotibial joints with no infiltration and bone erosion. However, the proliferation of synovial cell lining, reduced synovial space, pannus formation and new blood vessel formation ensued in CFA-induced arthritic rats. These histopathological changes in tarsotibial joints resemble with earlier studies [28, 65]. Prednisolone and *C. procera* treatment retards inflammatory cell infiltration and synovial hyperplasia. Thus, less bone destruction and joint space narrowing were observed as compared to CFA-induced arthritic rats. Earlier studies reported diminishing joint destruction by prednisolone treatment [52, 66] as well as *C. procera* treatment [21, 22] in arthritic rats.

Radiographic evaluation of joints is a useful diagnostic measure to indicate the severity of the disease. Primary radiographic change in RA is soft tissue swelling; thereafter, bone erosion and joint space narrowing were observed in a more advanced stage of arthritis [67]. Bone destruction, characterized by soft tissue swelling and loss of articular cartilage, was observed in CFA-induced arthritic rats, whereas control rats showed normal bony structure. These changes in arthritic rats were similar

to previously reported radiographic changes [50, 68]. The standard drug prednisolone-treated group and *C. procera*-treated group showed delayed soft tissue swelling and cartilage destruction. Therefore, bony erosion in both of these treatment groups was less compared to the arthritic group. Combination of DMARD and low-dose prednisolone reduces disease progression in RA patients [69].

Conclusion

Calotropis procera latex possesses anti-arthritic activity, which is facilitated by modulation in the level of inflammatory mediators and oxidative stress. Moreover, the improvement was also observed in hematological parameters like Hb level, ESR, WBC and RBC count with the treatment that may be reflected in functional, histopathological, radiological changes and thereby disease progression. However, isolation of constituent of *C. procera* latex is required to understand its mechanism of anti-arthritic activity and to establish a more convincing rationale for clinical trials [70].

Abbreviations

CFA: Complete Freund's adjuvant; CRP: C-reactive protein; DMARDs: Disease-modifying anti-rheumatic drugs; ELISA: Enzyme-linked immunosorbent assay; ESR: Erythrocyte sedimentation rate; IL: Interleukin; NO: Nitric oxide; NPD: Normal pellet diet; NSAIDs: Non-steroidal anti-inflammatory drugs; RA: Rheumatoid arthritis; RBC: Red blood cell; RF: Rheumatoid arthritis; TNF: Tumor necrosis factor; WBC: White blood cell.

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Authors' contributions

RP designed the study, analyzed the data, wrote the manuscript; SK executed the experiment and collected and analyzed the data; PG helped in study designing, manuscript editing, co-ordination; SD supervised the study; GS conceptualized and supervised the study. The author read and approved the final manuscript.

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Availability of data and materials

Data and material are available upon request.

Declarations

Ethics approval and consent to participate

The experimental protocol (KBIPER/17/596) was approved by the Institutional Animal Ethics Committee of K. B. Institute of Pharmaceutical Education and Research (KBIPER).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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