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Anti-inflammatory activity of quercetin-3-rhamnopyranosyl-(1-6) glucopyranoside isolated from *Delonix elata* against Freund's complete adjuvant induced inflammation in rats

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Delonix elata L. has been used for the treatment of pain and joint stiffness in the traditional medicines of Chitradurga District, Karnataka, India. In the present study, the anti-inflammatory activity of quercetin-3-rhamnopyranosyl-(1-6) glucopyranoside (QRPG) isolated from *D. elata* stem bark extract was carried out against Freund's complete adjuvant (FCA) induced inflammation in rats. The anti-inflammatory activity was evaluated by various methods such as radiographic analysis of hind paws, measurement of paw volume, joint diameter assessment, erythrocyte sedimentation rate, serum nitrites concentration, myeloperoxidase activity, the activity of lysosome enzymes (acid phosphatase, β-glucuronidases and collagenolytic enzymes) and histological observations. Indomethacin was used as the standard drug. QRPG showed significant attenuation in paw oedema of FCA-induced rats. Biochemical analysis in QRPG treated animals revealed a significant reduction in the levels of erythrocyte sedimentation rate, serum nitrites concentration, myeloperoxidase activity, lysosome enzyme activities as compared to control animals. Results obtained in biochemical assays were supported by the histological observations. The present investigation demonstrated promising anti-inflammatory activity of quercetin-3-rhamnopyranosyl-(1-6) glucopyranoside and it could be the principal compound in *D. elata* stem bark extract. Thus, this study provides the scientific basis for the ethnomedicinal uses of *D. elata* for joint problems.

Keywords: Anti-inflammation, *Delonix elata*, Freund's complete adjuvant, Indomethacin, Radiographic analysis.

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Introduction

Inflammation plays a significant role in the pathogenesis of various diseases like atherosclerosis, rheumatoid arthritis, asthma, and cancer. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used therapeutics for the treatment of inflammatory diseases. However, these NSAIDs exhibit various side effects like gastrointestinal ulcers, haemorrhage, and renal damage induced by long-duration administration which limits their usage for the treatment of chronic inflammation diseases[1]. Thus, the development of new anti-inflammatory agents with reduced side effects is a matter of pressing concern.

Medicinal plants have been the main remedy to treat various ailments for a long time and nowadays, many drugs have been developed from traditional medicine. Research into natural compounds found on their ethnopharmacological information has provided significant contributions to drug development and has paved the way for new pharmacological tools[2]. Studies using *in vivo* models of inflammation have led to the identification of a variety of natural extracts with proven anti-inflammatory activities[3-6]. Although the anti-inflammatory functions of natural extracts were initially described, it was the key role of follow-up phytochemical and pharmacological studies that led to the identification and characterization of a variety of natural active compounds.

Delonix elata L. belongs to the family Caesalpinaceae and is found in India's dry forests. Traditional medical practitioners residing in the villages of Chitradurga, Karnataka (India) have been using leaves and stem bark extracts of *D. elata* for curing pain and joint problems. In previous studies, authors reported the wound healing activity of *D.*

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elata stem bark extract and its constituent, quercetin-3-rhamnopyranosyl-(1-6) glucopyranoside[7]; antioxidant and hepatoprotective activity of stem bark extracts[8]; and significant antinociceptive activity of *D. elata* leaves extract[9]. Reports are available on preliminary investigations of the anti-inflammatory and anti-arthritic potential of *D. elata*[10-14]. However, the investigation on the anti-inflammatory activity of bioactive compounds from *D. elata* has not been carried out so far. Thus, the present investigation was undertaken to study the anti-inflammatory property of quercetin-3-rhamnopyranosyl-(1-6) glucopyranoside isolated from stem bark extract of *D. elata* against FCA-induced inflammation.

Materials and Methods

Plant sample collection and identification

The stem bark of *D. elata* was collected from Chitradurga, Karnataka (India) in November 2020. Taxonomic identification of the plant sample was done by Prof. V. Krishna and deposited at the Department of Biotechnology, Kuvempu University, Shankaraghatta-577451 (Voucher specimen no. KUBPHS201).

Preparation of the drugs

Isolation and characterization of quercetin-3-rhamnopyranosyl-(1-6) glucopyranoside from stem bark extract of *D. elata* has been reported earlier[7]. Quercetin-3-rhamnopyranosyl-(1-6) glucopyranoside is abbreviated as QRPG (Fig. 1). For oral administration, QRPG at the concentration of 50 mg/kg was prepared with 1% (v/v) DMSO according to the acute toxicity study[7].

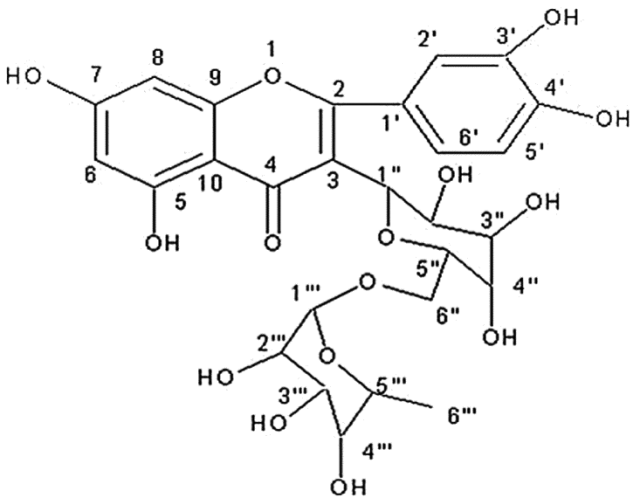

Fig. 1 — Quercetin-3-rhamnopyranosyl-(1-6)glucopyranoside.

Experimental design

Wistar albino rats of either sex, weighing about 180-200 g were used in the study. Animals were housed at 25 ±1 °C and with a relative humidity of 55-60%. They were fed with a standard commercial pellet diet (Champaka feeds and foods, Bangalore) and water *ad libitum* during the experiment. The Institutional Ethical Committee permitted the study (EC approval No. NCP/IAEC/CL/13/12/2010-11, Date: 28-11-2012).

Rats were separated into four groups consisting of six animals in each group. Group-1 (vehicle control) received 1% (v/v) DMSO (0.1 mL/Animal); group-2 (Inflammation control) inflammation was induced by intradermal injection of Freund's complete adjuvant (FCA) (0.1 mL) into the right hind paw[15]; group-3 received QRPG (50 mg/kg p.o) and group-4 received standard drug indomethacin (10 mg/kg p.o) once in a day. The treatment regimen of drugs (once daily orally) started on the 12th day of post-induction of arthritis and ended on the 28th day.

Paw volume and joint diameter assessment

The right hind paw volumes of all animals were measured using a plethysmometer just before FCA injection on day 0 and thereafter at different time intervals (4, 8, 12, 16, 21 and 28th day)[16]. The joint diameter of the right hind paw was measured on the above-mentioned testing days after induction of inflammation using a Vernier caliper[17].

Radiographic analysis

Anaesthetized rats were placed on a radiographic machine at a distance of 90 cm from the X-ray source. Radiographic analysis of hind paws was performed with a 40 kW exposition for 0.01s[18].

Biochemical parameters

On the 29th day, animals were sacrificed; blood was collected and used for biochemical tests. Erythrocyte sedimentation rate (ESR) was determined by the method described by Lewis[19]. Serum nitrites concentration was measured according to the procedure described by Miranda *et al.*[20]. Myeloperoxidase (MPO) activity was determined using the method of Bradley *et al.*[21]. The right hind paw of the sacrificed animals was amputated below the tibia, cut into pieces, and homogenized in 0.05 M tris buffer (pH 8), containing 0.15 M NaCl and 0-1% (v/v), Triton X-100, using 2 mL buffer for each paw. The resulting homogenate was centrifuged and the supernatant was used for the estimation of acid

phosphatase, β-glucuronidase and collagenolytic activities[22].

Histopathology of paw tissue

Biopsies of the right hind paw of sacrificed animals were collected. Tissues were fixed in a fixative (10% formalin and 1% acetic acid) for 1 week at room temperature, dehydrated, embedded in paraffin, and sectioned into 4 µm. Tissue sections were stained with hematoxylin and eosin (H&E stain) and examined under a microscope for histological changes[23].

Statistical analysis

Results are expressed as mean±S.E.M. The statistical analysis was carried out using one-way ANOVA followed by Duncan's test. The differences in values at P <0.05 were considered statistically significant. Statistical analysis was performed by GraphPad Prism 5 software.

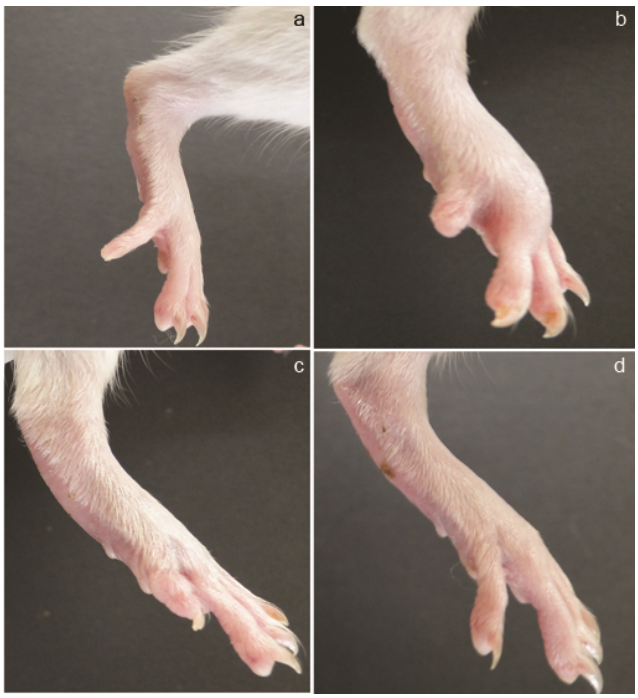

Fig. 2 — Hind paw of rat. a) Control, b) FCA treated, c) FCA + QRPG treated, d) FCA + Indomethacin treated.

Results

Paw volume and joint diameter assessment

Subplantar administration of FCA in the rat paw resulted in a significant increase in paw circumference, erythema, swelling, joint stiffness and hindrance in the movement was observed and it was steadily maintained up to 28 days (Fig. 2b) when compared to the vehicle control animals (Fig. 2a). The course of inflammation after adjuvant induction was measured by paw volume and ankle diameter and it increased at the injected right hind paw. The measurement of paw thickness of adjuvant-induced arthritic rats revealed a rapid increase in paw volume from day 4 and gradually increased further up to day 16. Observations on different days on paw volume and joint diameter are shown in Table 1 and 2 respectively. There was a significant reduction in the paw volume as well as ankle diameter observed in the paw oedema of rats treated with QRPG (Fig. 2c). Rats treated with indomethacin showed significant attenuation in paw volume and joint diameter from day 12 onwards as compared to control rats (Fig. 2d).

Radiographic analysis

X-ray images of normal animals showed an absence of soft tissue swelling and bone erosion (Fig. 3a). On contrary, radiographical analysis of the hind paw of FCA-induced rats showed swelling of the hind paw soft tissue, marginal bone erosion and narrowing of the ankle joint space (Fig. 3b). The X-ray studies of the hind paw of the QRPG treated animals showed a significant decrease in the soft tissue swelling and bone erosion (Fig. 3c) similar to that of indomethacin treated rats (Fig. 3d).

Determination of ESR, Serum nitrites and myeloperoxidase activity

In the present investigation, there was a significant increase in the level of ESR (8.1±0.2 mm), serum nitrites (49.85±1.35 µg/mL) and myeloperoxidase (119.05±0.85 IU) noticed in FCA induced rats. Rats administered with the compound QRPG showed significant depletion in levels of ESR (3.1±0.1 mm),

Table 1 — Anti-inflammatory effect on paw oedema in FCA induced rats

Groups	Treatment	Dosage concentration	Paw volume (mL)					
			Day 4	Day 8	Day 12	Day 16	Day 21	Day 28
Group 1	Normal control	1% DMSO	0.17±0.01	0.18±0.1	0.19±0.05	0.21±0.02	0.23±0.02	0.21±0.01
Group 2	FCA induced	0.1 mL	1.69±0.06	1.65±0.02	1.75±0.05	1.88±0.02	1.8±0.02	1.76±0.03
Group 3	QRPG + FCA	50 mg/kg	1.85±0.15	1.8±0.18	1.86±0.1	1.65±0.06*	1.17±0.02*	0.9±0.01*
Group 4	Indomethacin+ FCA	10 mg/kg	1.75±0.07	1.75±0.01	1.69±0.01	1.34±0.11*	0.92±0.07*	0.57±0.01*

Values are the mean±S.E.M. of six rats. Symbols represent statistical significance. *P <0.05, as compared to FCA treated group.

Table 2 — Effect on reduction of paw oedema diameter of FCA treated rats

Groups	Treatment	Dosage concentration	Paw diameter (mm)					
			Day 4	Day 8	Day 12	Day 16	Day 21	Day 28
Group 1	Control	1% DMSO	3.76±0.21	3.69±0.1	3.71±0.13	3.74±0.18	3.72±0.16	3.35±0.12
Group 2	FCA	0.1 mL	5.23±0.02	5.22±0.02	5.85±0.07	5.86±0.01	5.8±0.02	5.68±0.01
Group 3	QRPG+FCA	50 mg/kg	5.56±0.2	5.38±0.15	6.1±0.04	5.64±0.08	5.18±0.17*	4.11±0.1*
Group 4	Indomethacin+FCA	10 mg/kg	5.2±0.07	5.11±0.11	5.88±0.1	4.46±0.09*	3.96±0.05*	3.62±0.02*

Values are the mean±S.E.M. of six rats. Symbols represent statistical significance. *P <0.05 as compared to FCA treated group.

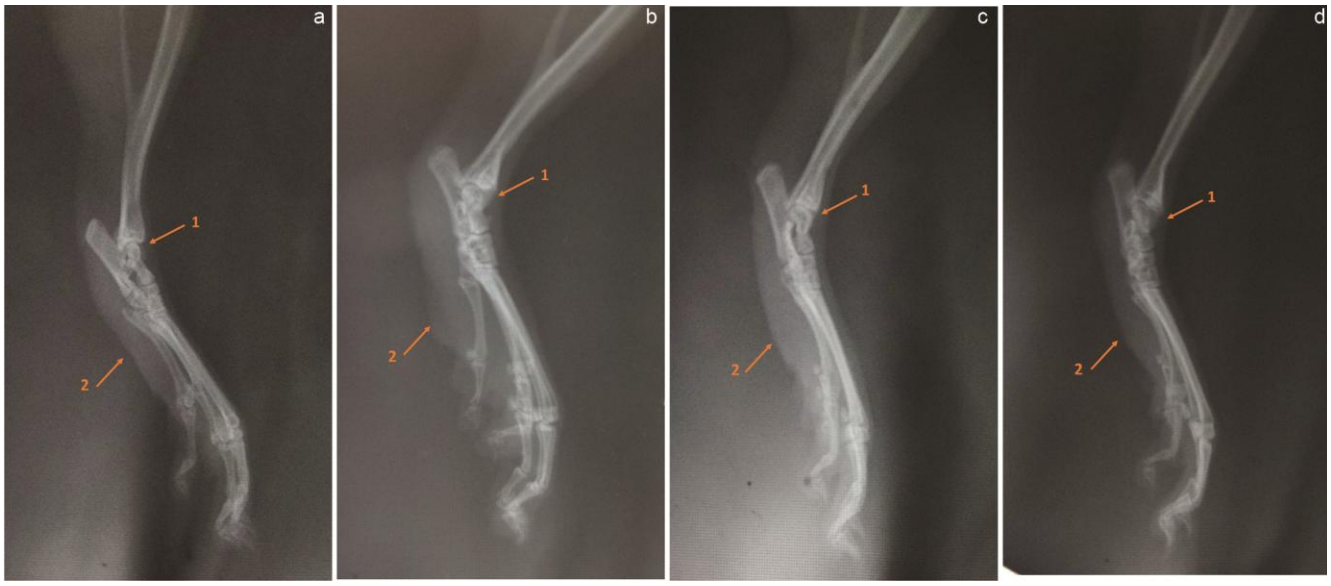

Fig. 3 — X Ray image of tibiotarsal joint of rat. a) Control, b) FCA treated, c) FCA + QRPG treated, d) FCA + Indomethacin treated. (1. Marginal joint erosion; 2. Soft tissue swelling)

serum nitrites (34.05±0.15 µg/mL), and myeloperoxidase (61.8±1.2 IU) which is on par with the indomethacin treated rats (Table 3).

Lysosomal enzymes estimation in paw homogenate

FCA induced rats showed elevation in the activity levels of acid phosphatase (3.1 ± 0.2 IU), β-glucuronidases (8.42 ± 0.23 IU) and collagenolytic enzymes (347 ± 2.6 IU) in paw tissue as compared with the vehicle control rats. Table 4 presents the effect of QRPG on the attenuation of elevated levels of lysosomal enzyme activities in paw tissue of FCA-treated rats. The effect of QRPG on acid phosphatase, *β*-Glucuronidase and collagenolytic enzyme activities in the paw tissue of experimental animals was significantly reduced to a normal level and the result was nearer to the effect of indomethacin.

Histopathology of paw tissue

Paw tissue histology of normal animals showed the absence of infiltration of cells and the presence of a compact arrangement of connective tissue (Fig. 4a). Histology of paw tissue of FCA-treated animals showed intense inflammation with significant infiltration of leukocytes and neutrophils in the connective tissues. The infiltrates accumulated between collagen fibres and into intercellular spaces (Fig. 4b). The histopathological observation of the hind paws of rats administered with the QRPG (Fig. 4c) showed a remarkable decrease in the number of inflammatory cells. Substantial reduction in the infiltration of inflammatory cells was observed in indomethacin treated animal group (Fig. 4d).

Discussion

Adjuvant arthritis in rats is a widely used experimental model sharing several features with rheumatoid arthritis in humans[24]. In this regard, the present study demonstrated the effect of quercetin-3-rhamnopyranosyl-(1-6) glucopyranoside on FCA induced arthritis in rats. FCA is the water-in-oil emulsion containing heat-killed *Mycobacteria tuberculosis* and it is an effective means of potentiating cellular and humoral antibody response to immunogens. Its pathophysiology involves initial

Table 3 — Anti-inflammatory effect on reduction of ESR, serum nitrites and myeloperoxidase levels

Groups	Treatment	Dosage concentration	ESR (mm)	Serum nitrites (μg/mL)	Myeloperoxidase (IU)
Group 1	Vehicle control	1% DMSO	2.75±0.25	27.05±0.25	44.6±3.8
Group 2	FCA	0.1 mL	8.1±0.2	49.85±1.35	119.05±0.85
Group 3	QRPG+FCA	50 mg/kg	3.1±0.1*	34.05±0.15*	61.8±1.2*
Group 4	Indomethacin+FCA	10 mg/kg	3.2±0.2*	30.7±0.51*	64.2±2.4*

Values are the mean±S.E.M. of six rats. Symbols represent statistical significance. *P <0.05 as compared to FCA treated group.

Table 4 — Estimation of lysosomal enzyme activities in hind paw homogenates of rats

Groups	Treatment	Dosage concentration	Lysosomal enzymes activity		
			Acid phosphatase (IU)	β-Glucuronidase (IU)	Collagenolytic enzyme (IU)
Group 1	Control	1% DMSO	0.32±0.2	1.61±0.26	133±1.1
Group 2	FCA	0.1 mL	3.1±0.2	8.42±0.23	347±2.6
Group 3	QRPG + FCA	50 mg/kg	1.12±0.03*	3.21±0.21*	193±0.8*
Group 4	Indomethacin+FCA	10 mg/kg	0.97±0.2*	3.08±0.34*	187±2.4*

Values are the mean±S.E.M. of six rats. Symbols represent statistical significance. *P <0.05, as compared to FCA treated group.

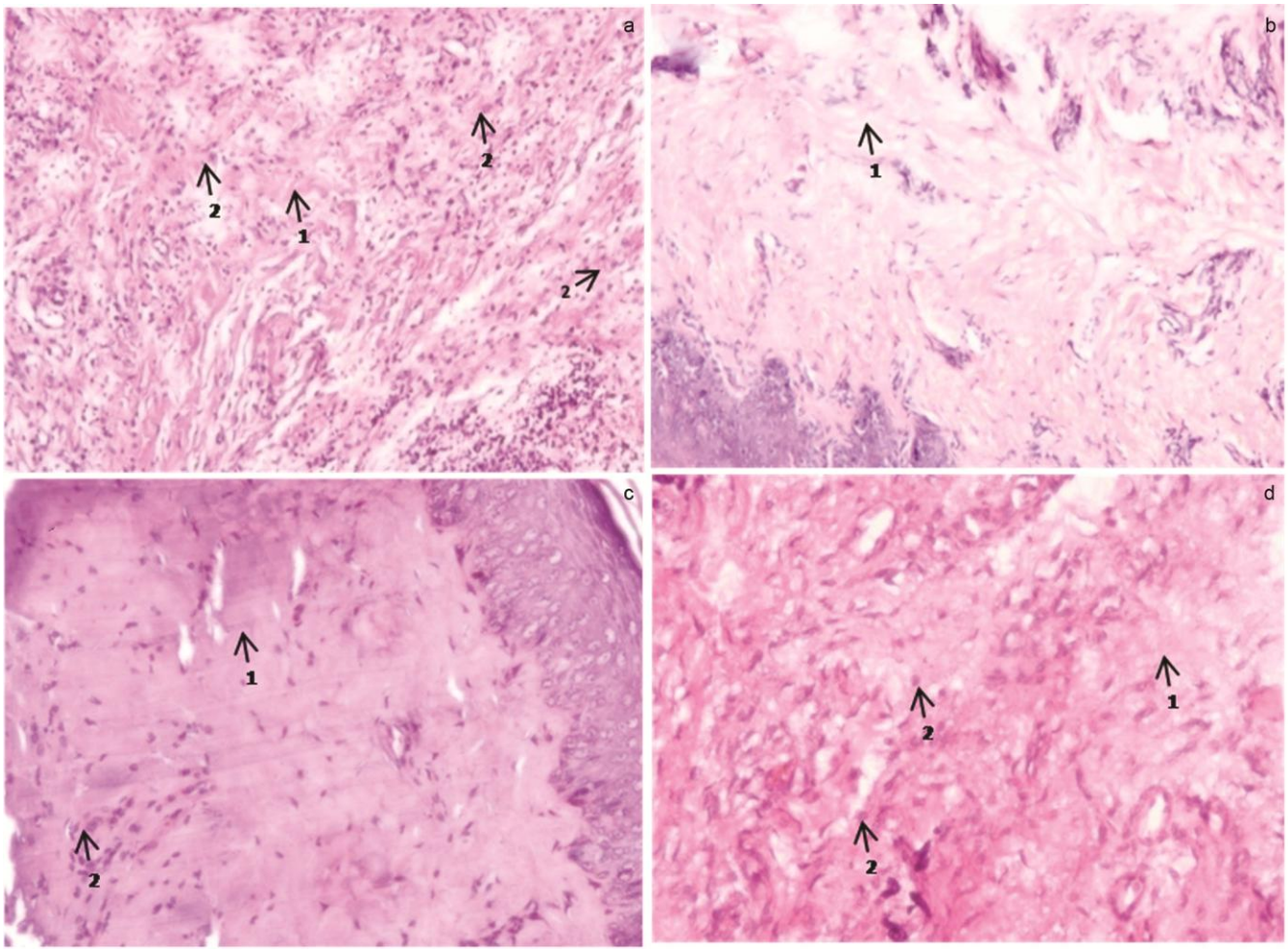

Fig. 4 — Histology of hind footpad of rat. a) Control, b) FCA treated, c) FCA + QRPG treated, d) FCA + Indomethacin treated. (1. Collagen tissue; 2. Lymphocytic infiltration).

acute periarticular inflammation with synovial mononuclear infiltration, followed gradually by synovial hyperplasia and damage to periarticular bone and cartilage just as in the case of arthritis in human[25]. In agreement with this, in the present study, intradermal administration of FCA in the rat paw resulted in a significant increase in paw circumference, swelling, joint stiffness and level of lysosomal enzymes (acid phosphatase, *β*-glucuronidases and collagenolytic enzymes), myeloperoxidase, serum nitrites and ESR. The above findings in arthritic rats were well supported by radiographical analysis of hind paw and histological observations of paw tissue with marginal bone erosion, narrowing of the ankle joint space, infiltration of leukocytes, and neutrophils in the connective tissues.

During arthritis conditions, the damaged tissue releases prostaglandins which send messages to trigger inflammation resulting in pain and swelling. The non-steroidal anti-inflammatory drugs such as indomethacin, block prostaglandins by blocking Cox-1 and Cox-2 enzymes. This decreases inflammation and reduces pain and stiffness[26]. In inflammations, erosions and joint space narrowing are more common during the early stages of the disease with further progression as the disease advances, subluxations, malalignment and ankylosis are more apparent in the later stages of the disease[27]. The X-ray studies of the hind paw in QRPG treated animals showed a considerable and significant decrease in the soft tissue swelling and bone erosion similar to that of standard drug indomethacin treated rats. These anti-inflammatory effects may be due to the blocking of excessive production of RNS and ROS during the inflammatory phase[28,8].

Many investigators provide evidence and suggested the role of oxidative stress in the pathogenesis of inflammation[29]. Various forms of antioxidant therapy showed promising results in experimental arthritis models[28].

Depletion in the levels of ESR, Serum nitrites, and MPO was observed in the QRPG treated rats. During inflammation processes, tissue breakdown increases the amount of fibrinogen and globulins in the bloodstream. In many inflammatory diseases like rheumatoid arthritis, chronic infections collagen disease, and neoplastic disease these proteins will interact with the red blood cells and cause the elevation in the ESR[30]. Serum nitrite has been proposed as an index of immune system activation[31]. Serum nitrite is increased in rats with inflammation. In inflammation, MPO may play a role in the pathogenesis and severity. MPO involves in oxidant production by neutrophils. It uses superoxide and hydrogen peroxide to catalyse the generation of hypochlorous acid and free radicals[32].

Evidence is increasing that lysosomal enzymes play an important role in inflammation and its activities have also been detected in certain experimental inflammations in animals, including rat paws made edematous by FCA, formalin, serotonin, and dextran. The extensive tissue breakdown in adjuvant arthritis is due to the release and degradative action of lysosomal enzymes in the connective tissue components[33]. The suppression of lysosomal enzyme release (acid phosphatase, *β*-gucuronidase and collagenolytic enzymes) observed in QRPG treated rats might be due to its stabilizing effect on lysosomal membranes, as rupture of these membrane release glycohydrolases that destroy the organic cartilage matrix[34]. The membrane-stabilizing property of QRPG observed in the present study could be due to its antioxidant effect.

Histology of paw tissue of control animals showed inflammation with a substantial infiltration of neutrophils and leukocytes in the connective tissues. Neutrophils play important role in tissue damage in chronic disease processes, such as rheumatoid arthritis, chronic obstructive pulmonary disease (COPD), and asthma[35]. In the present study, histopathological examination further confirmed the anti-inflammatory effect of QRPG by the alleviation of neutrophils and leukocytes infiltration.

Conclusion

The present study showed that QRPG isolated from *Delonix elata* possess significant anti-inflammatory properties against FCA-induced inflammation in rats by increasing the antioxidant levels and reducing the inflammation in rats. This study supports the traditional use of *D. elata* to treat inflammatory conditions. However, the precise mechanism of action remains to be further investigated.

Acknowledgement

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Conflict of interest

The authors declare that there are no conflicts of interest associated with this publication.

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