Therapeutic potential of russelioside B as anti-arthritic agent in Freund’s adjuvant-induced arthritis in rats

Riham A. El-Shiekh a, Sahar El-Mekkawy b, Samar M. Mouneir c, Azza Hassan d, Essam Abdel-Sattar a, *

a Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo, 11562, Egypt
b Department of Chemistry of Natural Compounds, National Research Centre, Dokki, Giza, 12622, Egypt
c Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Egypt
d Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Egypt

ARTICLE INFO

Keywords:
Antirheumatic
Russelioside B
Caralluma
Pregnane glycosides
Rheumatoid markers
Osteoclastogenesis

ABSTRACT

Ethnopharmacological relevance: Caralluma species are traditional edible herbs used in folkloric medicine as antidiabetic, antioxidant, antipyretic, antirheumatic, anti-inflammatory and anthelmintic agents. C. quadrangula was selected in this study to document the traditional use of the genus as anti-rheumatic treatment and the possible mechanisms of action.

Aim of the study: The higher mortality rates and shorter survival among the patients suffering from rheumatoid arthritis (RA) led to the increased interest on searching for new treatments for RA. Russelioside B (RB), a major pregnane glycoside found in C. quadrangula, was evaluated as a new anti-rheumatic agent.

Materials and methods: The n-butanol fraction of C. quadrangula was chromatographed on a silica gel column to isolate RB. The adjuvant-induced arthritis (AIA) model was established in rats by intradermal injection of complete Freund’s adjuvant (CFA) to evaluate its anti-arthritic effect. Ibuprofen was used as a reference drug. Forty rats were randomly divided into 5 groups (n = 8): normal (NOR); CFA model (CFA); ibuprofen, 5 mg/kg; RB, 25 mg/kg and RB, 50 mg/kg. The treatments were initiated from day 16 when AIA model was established and continued up to day 40. Serum diagnostic rheumatoid markers, inflammatory cytokines, oxidative stress biomarkers, cartilage and bone degeneration enzymes were assessed.

Results: RB at 50 mg/kg b. wt., showed significant decreases in the activities of hyaluronidase and β-glucuronidase enzymes as well significant decreases in the levels of proinflammatory cytokines as nuclear factor-kappa-β (NF-κB), tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-1β (IL-1β) compared to the CFA group; 11.04 ± 0.61 pg/mg protein, 4.35 ± 0.25 pg/mg protein, 3.32 ± 0.13 pg/mg protein & 2.75 ± 0.14 pg/mg protein for RB, 50 mg/kg b. wt. group vs. 25.33 ± 2.13 pg/mg protein, 25.65 ± 2.1 pg/mg protein, 22.20 ± 1.34 pg/mg protein & 13.27 ± 1.40 pg/mg protein for the arthritic group, respectively. The total antioxidant capacity (TAC) was significantly restored to normal values in RB, 50 mg/kg treated rats (4.01 ± 0.09 nmol/mL vs. 3.71 ± 0.27 nmol/mL) and the levels of myeloperoxidase (MPO) reduced by 10-folds of the CFA arthritic group. Bone histomorphometry revealed that RB treatment significantly attenuated the CFA-induced bone loss in a dose-dependent manner.

Conclusions: These findings suggested that the anti-arthritic effect of RB was mediated through the reduction of the rheumatoid markers, anti-inflammatory and antioxidant action, inhibition of cartilage and bone degenerative enzymes as well as attenuation of bone loss and osteoclastogenesis.

1. Introduction

RA is a systemic autoimmune disease characterized by chronic inflammation of the joints and surrounding tissues with hyperproliferation of the synovial lining (Riehemann et al., 1999). Uncontrolled active RA causes an abundant decline in the quality of life of patients and increases their mortality. The prevalence of RA varies between 0.3% and 1% and is more common in women and in developed
countries (WHO, 2018). The goal of treatment is to stop or minimize joint damage, maintain normal joint functions and alleviate pain (Mahdi et al., 2018). Chronic diseases such as arthritis need lifelong treatment. Medications such as disease-modifying anti-rheumatoid drugs (DMARDs), non-steroidal anti-inflammatory drugs (NSAIDs), and corticosteroids are common therapy used for arthritis (Scott et al., 2010). Approximately 30% of the patients failed to respond to previous ordinary treatments besides their well-documented side effects and prohibitive cost (Perumal et al., 2017). These have made natural products more desirable in the treatment of arthritis because of reduced toxicity, lower costs and fewer side effects than synthetic drugs. Dietary supplements and herbal remedies such as omega-3 fatty acids, curcumin, resin and resveratrol have been used for centuries to reduce pain and inflammation (Maroon et al., 2010). A number of natural compounds have been reported to exhibit anti-arthritis potential including anthraquinone, withanolides, terpenes, polyphenols, and stilbene (Gupta, 2017).

Genus Caralluma R. Br. belongs to the Apocynaceae family with approximately 120 species. They are succulent perennial edible herbs with several uses in traditional medicine as a powerful treatment for kidney pain, inflammation, diabetes, rheumatism, fever, ulcer, wounds and cuts (Adnan et al., 2014; Bin-Jumah, 2019). Pregnan glycosides, terpenoids, flavone glycosides and sterols are the major classes of compounds reported in Caralluma species and all of these verify their medicinal significance. Caralluma quadrangula (Forsk.) is a succulent shrub in this genus, traditionally used for ulcer, diabetes and RA treatments (Adnan et al., 2014; Bin-Jumah, 2019). Russelioside B (RB) is the major pregnane glycoside isolated by our group from C. quadrangula (Al-Yahya et al., 2000). No study was done to evaluate its potential for treatment of rheumatism. Accordingly, in this study we evaluated RB as a major compound from C. quadrangula for its anti-arthritis effect in AIA model to authenticate its traditional use for RA and to illustrate its mechanisms of action.

2. Materials and methods

2.1. Plant materials

Aerial parts of Caralluma quadrangula (Forsk.) N. E. Br. (syn. Stapelia quadrangula Forsk.) were collected from Al-Taif Governorate, Saudi Arabia (April 2018) and dried. Plant authentication was done by a staff member at the Taxonomy Department at the Faculty of Science in King Abdulaziz University and a specimen (CQ 1027-B) was deposited in the herbarium of College of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia.

2.2. Isolation of russelioside B

The air-dried powdered of Caralluma quadrangula aerial parts (480 g) were extracted with methanol and the methanolic extract was evaporated until dryness to yield a brown residue (82 g). The methanolic extract (65 g) was fractionated into chloroform fraction (8.8 g), and n-butanol fraction (35.8 g). The n-butanol fraction was chromatographed on a silica gel column according to the procedures described previously to isolate russelioside B (calogenin 20-O-β-D-glucopyranosyl-3-O-[(β-D-glucopyranosyl)-1→4]-β-D-(3-O-methyl-6-deoxy) galactoside) (Al-Yahya et al., 2000) in amount of 1.7g. The structure of RB (Fig. 1) was confirmed by superimposed IR and by comparison of its 1H-NMR and 13C-NMR (Suppl. Figs. S1 and S2) with those reported in the literature (Al-Yahya et al., 2000).

2.3. Biological assays

2.3.1. Animals

Male Wistar rats, with a weight of 150–170 g, were purchased from the Laboratory animal colony, Helwan (Cairo, Egypt). All animals were housed in a temperature-controlled room (22 ± 2 °C) and allowed free access to standard pelleted forage and tap water. All rats were adaptively fed for 7 days before experiments. All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH) and were reviewed and approved by the Institutional Research Ethics Committee (MP-2319) at the Faculty of Pharmacy, Cairo University, Egypt.

2.3.2. Induction of arthritis

AIA rat model was established by intradermal injection of a single dose of 0.1 mL CFA into the left hind footpad (day 1), except for the control group. CFA contains 10 mg/mL heat-killed Mycobacterium tuberculosis. The injection of CFA produced definite edema within 24 h with progressive arthritis by day 15 after inoculation. Treatments were initiated from day 16 when AIA model was established and continued up to day 40.

2.3.3. Drug administration

After adaptive feeding, 40 rats were randomly divided into 5 groups with 8 each: normal (NOR), CFA model (CFA), ibuprofen, RB at a dose of 25 mg/kg b. wt. (RB, 25 mg/kg) and russelioside B at a dose of 50 mg/kg b. wt. (RB, 50 mg/kg). Ibuprofen as reference drug for anti-arthritis was prepared in a saline solution and used at a dose of 5 mg/kg. Meanwhile, rats in the NOR group and CFA group were received saline. All the animals were injected (i.p.) at the same dosing volume of 0.5 mL/100g.

2.3.4. Assessment of the arthritis

The hind paw swelling (Δ mm) every 4 days was evaluated in terms of the volume of the left hind paw with PV-200 plethysmometer (Tai-meng Technology Co., Ltd., Chengdu, China), using the following equation:

\[
\text{Paw edema (％)} = \frac{(V_t-V_0)/V_0 \times 100}{\%
\]

Where \(V_0\) and \(V_t\) were the volume of left hind paw before and after induction, respectively. Original weight and paw volume were determined before modeling.

2.3.5. Evaluation of arthritis index (AI)

The severity of arthritis was evaluated by five-grade scoring scale every 4 days from day 12: 4 = swelling from ankle joints to the entire paw, 3 = swelling from toes to ankle joints, 2 = swelling of toes and toe joints, 1 = erythema or swelling of toe joints, 0 = no erythema or swelling. The AI score of each rat was the sum of the four limbs and the maximum arthritis score was 16.

2.3.6. Blood and tissue sampling

Blood was collected from retro-orbital plexus 1 h after the last administration under thiopental sodium anesthesia in a dose of 40 mg/kg b. wt. Blood samples were allowed for coagulation at room temperature and then centrifuged at 3500 rpm for 10 min. Serum was then separated and stored at −80 °C. The left hind paw ankle joints were collected from all animals and stored at −80 °C for the assessment of cartilage degradation [Hyaluronidase (EC 3.2.1.35)] and bone
degeneration enzymes [\(\beta\)-glucuronidase (\(\beta\)-GLU, EC 3.2.1.23)] in the ankle joint homogenate of all experimental animals.

2.3.7. Biochemical analysis

Using ELISA kits and according to the manufacturer’s instructions, the following parameters were measured; RF (CUSBIO Biotech, Hubei, China), anti-cyclic citrullinated peptides (Anti-CCP) and high sensitivity C-reactive protein (hr-CRP) (ElAab Science, Wuhan, China), nuclear factor-kappa-B (NF-κB) (Dokki, Cairo, Egypt), Myeloperoxidase (MPO, ELISAab Science, Wuhan, China), anti-oxidant capacity (TAC) was determined using kits from Bio-diagnostic (Dokki, Cairo, Egypt).

2.3.8. Histopathological examination

Rats were rapidly sacrificed by intraperitoneal injection of thiopental sodium in a dose of 100 mg/kg.b.wt. then cervical dislocation after blood sampling. Paws at the level of the ankle and knee joints were removed and fixed in 10% neutral buffered formalin (pH 7.0) for 3 days. The paw tissues were then dehydrated for approximately 2 weeks in 5% EDTA in buffered formic acid solution, accompanied by embedding in paraffin. Finally, sections (5 μm) were stained with Hematoxylin and Eosin (H&E) for histopathological examination. For the assessment of tissue damage, histopathological analysis was scored from 0 (no damage) to 5 (drastic severe damage) according to the method of Pettit et al. (2001) and Pritzker et al. (2006), with some modifications. The pathological parameters used for these evaluations were inflammation, cartilage destruction, bone erosion and pannus formation.

2.3.9. Bone histomorphometry of the tibia

A standard histomorphometric examination of the tibial metaphysis was carried out in order to evaluate the secondary effects of CFA on bone resorption and the potential protective effects of RB on bone loss. All histomorphometrical parameters were performed according to standard procedures of Parfitt et al. (1987), with some modifications.

2.4. Statistical analysis

Data are presented as the mean ± SD and were analyzed by one-way analysis of variance (ANOVA) followed by Tukey’s test for multiple comparisons and using the Kruskal-Wallis test followed by Dunn’s post hoc test for immunohistochemistry data. The Statistical Package for Social Science version 15 (SPSS, Chicago, IL, USA) and a trial version of Graph Pad Prism were used to perform the statistical analysis. Differences were considered to be significant at p < 0.05.

3. Results

3.1. Effect of russelioside B on complete blood count (CBC)

The blood count of some parameters such as haemoglobin (Hgb) level, red blood cell (RBC) count, white blood cell (WBC), mean platelet volume (MPV) and haematocrit (HCT) were measured (Table 1). The CFA arthritic groups treated with ibuprofen and RB (25 and 50 mg/kg b. wt.) showed a decrease in WBC count as compared to the CFA arthritic group. Hgb, RBC, MPV and HCT values of CFA arthritic groups treated with ibuprofen and RB (25 and 50 mg/kg b. wt.) were significantly higher than those of the CFA arthritic group.

3.2. Effect of russelioside B on body weight, paw volume, paw swelling and arthritic index

The CFA arthritic animals showed significant body weight loss compared to body weight gained in the normal rats; 194.83 ± 13.98 g vs. 257.51 ± 5.3 g. The CFA groups treated with RB showed a significant reduction in body weight (Fig. 2a) due to its appetite suppression effects by 4.4% and 11.62% at doses of 25 and 50 mg/kg b. wt., respectively.

The CFA arthritic animals showed a significant increase in paw volume by 102.70% compared with the normal rats. However, the paw volumes of CFA groups treated with ibuprofen and RB (50 mg/kg b. wt.) showed no significant difference when compared with the animals in the normal group (Fig. 2b). Additionally, the CFA groups treated with ibuprofen, RB 25 and 50 mg/kg b. wt., showed a significant decrease in the paw-swelling percentage and arthritic index compared to the CFA group (Fig. 2c and d).

3.3. Effect of russelioside B on rheumatoid biomarkers

The treated CFA arthritic animals with RB (25 and 50 mg/kg b. wt.) showed significant decrease in RF, hr-CRP and Anti-CCP close to the values of the positive control (ibuprofen). The decrease in RF, hr-CRP and Anti-CCP count was 67.98%, 84.47%, and 83.4% relative to their values of the positive control (ibuprofen). The decrease in RF, hr-CRP and Anti-CCP count was 67.98%, 84.47%, and 83.4% relative to their values of the positive control (ibuprofen).

3.4. Effect of russelioside B on inflammatory cytokines

The treatment of the CFA arthritic group with RB at a dose of 50 mg/kg b. wt. showed significant anti-inflammatory action by decreasing the levels of NF-κB, TNF-α, IL-6, and IL-1β by 60%, 83%, 85%, and 79%, respectively, as compared to their elevated levels in the CFA arthritic group (Fig. 4).

3.5. Effect of russelioside B on oxidative stress biomarkers

The serum level of TAC of animals of RB-treated groups (25 and 50 mg/kg b. wt.) showed significant increase compared to the normal group (Fig. 3). The serum level of TAC of animals of RB-treated groups (25 and 50 mg/kg b. wt.) showed significant increases compared to the normal group (Fig. 3). The serum level of TAC of animals of RB-treated groups (25 and 50 mg/kg b. wt.) showed significant increases compared to the normal group (Fig. 3).

3.6. Effect of russelioside B on hyaluronidase and β-glucuronidase enzymes activity

The CFA arthritic animals treated with RB (50 mg/kg b. wt.) showed significant decreases in the activity of both lysosomal enzymes

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of russelioside B (25 and 50 mg/kg) on complete blood count (CBC) in CFA-induced arthritic rats.</td>
</tr>
<tr>
<td>Groups</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>i- Normal</td>
</tr>
<tr>
<td>ii- CFA</td>
</tr>
<tr>
<td>iii- CFA +</td>
</tr>
<tr>
<td>iv- CFA +</td>
</tr>
<tr>
<td>Russelioside B, 25 mg/kg b.wt.</td>
</tr>
<tr>
<td>Russelioside B, 50 mg/kg b.wt.</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± SD. 
**Statistical analysis was carried out by one-way ANOVA followed by Tukey’s multiple comparison test. 
*Significant difference from normal group at p < 0.05. 
**Significant difference from normal group and CFA-induced group at p < 0.05. 
α: CFA: complete Freund’s adjuvant-induced arthritic rats; WBC: white blood cells; Hgb: haemoglobin; RBC: red blood cells; WBC: white blood cells; MPV: mean platelet volume; HCT: haematocrit.
compared to CFA arthritic rats; 24.37 ± 0.76 ng/mg protein vs. 73.43 ± 1.4 ng/mg protein for hyaluronidase activity, respectively, and 1.48 ± 0.05 ng/mg protein vs. 3.47 ± 0.04 ng/mg protein, respectively, for β-glucuronidase activity (Fig. 6).

3.7. Histopathological examination

No histological abnormalities were demonstrated in the joints of normal rats in which the cartilage revealed smooth articular surface (Fig. 7a) with normal chondrocytes embedded within the normal cartilage matrix and normal subchondral bone (Fig. 7b). Meanwhile, joint of osteoarthritic rats (CFA) revealed pronounced destructive arthritis represented by severe deterioration of cartilage and subchondral bone which was surrounded by numerous osteoclasts with marked distortion of the cortical surface (Fig. 7c and d) in addition to accelerating pannus formation of massive synovial inflammation with intense lymphoplasmacytic infiltrates. Massive edema associated with massive diffuse infiltration of the joint capsule and lymphoplasmacytic infiltrates was frequently demonstrated in this group. Conversely, ibuprofen treatment significantly decreased joint swelling with impressive diminution of cartilage degeneration, pannus formation and osteophytes formation, in addition to a significant decrease in adherent osteoclasts (Fig. 7e and f). Similarly, the treatment with RB revealed significant improvement, with pronounced regression of the pathological alterations shown in CFA arthritic group, in a dose-relevant manner. The articular cartilage of CFA treated with RB (25 mg/kg) group, maintained its smooth surface with focal loss of cartilaginous matrix (Fig. 7g and h). While the animals of the CFA arthritic group treated with RB (50 mg/kg), apparently showed normal articular cartilage with necrosis of individual chondrocytes within the normal cartilage matrix (Fig. 7i and j). There was a significant increase of the pathologic score of cartilage destruction, bone erosion, inflammation and pannus formation in the CFA group. A significant decrease of the aforementioned parameters was recorded in CFA treated with RB (25 mg/kg b. wt.), compared to the CFA group. Non-significant difference was recorded between CFA treated with RB (50 mg/kg b. wt.) and CFA treated with Ibuprofen (5 mg/kg b. wt.) (Fig. 8a–d).

3.8. Bone histomorphometry

From bone histomorphometry, trabecular thickness and trabecular number were significantly diminished in CFA group compared to the
normal rats, concurrently with a marked increase of trabecular separation and osteoclast number per bone surface (Fig. 9). A significant increase of trabecular thickness and trabecular number was recorded in CFA group treated with ibuprofen. Additionally, ibuprofen markedly decreased trabecular separation and osteoclast number per bone surface. On the other hand, RB treatment significantly attenuated the CFA-induced bone loss in a dose-dependent manner, represented by increase in both trabecular thickness and trabecular number as well as decrease in trabecular separation and osteoclast number per bone surface (Fig. 9).

4. Discussion

The model of arthritis was established by the injection of CFA in rats, which showed a significant increase in paw edema volume and loss of appetite that expressed as loss in body weight appeared in rats on day 12, where the symptoms of inflammation gradually aggravated on day 16. This observation was in agreement with the results reported in the literature for arthritic animals, due to the increase in leptin level (Kadhem, 2016; Aloke et al., 2019). On the other hand, RB treatment significantly attenuated the CFA-induced bone loss in a dose-dependent manner, represented by increase in both trabecular thickness and trabecular number as well as decrease in trabecular separation and osteoclast number per bone surface (Fig. 9).

The doses in this paper were selected based on the previous study of RB as appetite suppressant, where 25 and 50 mg/kg b. wt. showed a dose-dependent manner and the higher dose significantly alleviated all worse symptoms of obesity (Abdel-Sattar et al., 2018; EL-Maraghy et al., 2016). The treatment with RB significantly (p < 0.05) suppressed inflammation by inhibiting paw volume, paw swelling and reducing AI, especially at the higher dose (50 mg/kg), compared with arthritic rats. This effect was in line with the results reported for carumbelloside-II and -IV isolated from Caralluma umbellate (Asclepiadaceae), both showed anti-inflammatory activity in carrageenan induced left hind paw edema model by reduction of edema volume by 60% at dose of 40 mg/kg b. wt. (Ray et al., 2011, 2012).

The elevated rheumatoid markers in serum such as RF and anti-CCP are highly observed in rheumatic patients, also hr-CRP levels are elevated as a sign for inflammation (Taylor et al., 2011). Treatment with RB, especially at the higher dose (50 mg/kg), significantly suppressed their levels compared to CFA arthritic rats, with no significant difference to the normal and ibuprofen treated groups.

In most cases, chronic inflammation may lead to changes in blood parameters such as; decrease in RBC count, HCT, Hgb levels (Choudhary et al., 2014) and increases in WBC count (Zhang et al., 2014; Kisacik et al., 2008). Therefore, the blood picture provides additional information about inflammation in RA. Treatment with ibuprofen and higher dose of RB (50 mg/kg) showed a normalization of blood count (decrease in WBC count and increase levels of Hgb, RBC, MPV and HCT)
comparable to the normal values (Kisacik et al., 2008).
Although the serum concentrations of NF-kB, TNF-α, IL-6, and IL-1β of AIA rats were significantly 2–6 folds higher than normal group (p < 0.01); both RB and ibuprofen treatment significantly down regulated their levels in serum (p < 0.01). The dramatic decrease in the levels of NF-kB, TNF-α, IL-6 and IL-1β of the CFA arthritic group treated with RB at a dose of 50 mg/kg b. wt. indicating the high efficacy of RB for treating chronic arthritis (which are also aggravating factors in the case of Covid-19 infection). These cytokines play a major role through intracellular pathways in the pathogenesis of arthritis-associated bone loss by initiating osteoclast differentiation and activation (Braun and Zwerina, 2011). It is worth to note that RB by inhibiting these inflammatory cytokines would inhibit osteoclastogenesis and bone resorption occurred in RA.

In this concern, previous studies noted that some pregnane glycosides isolated from the stems of Hoya kerrii exhibited potent anti-inflammatory activity by down-regulation of mRNA expression of iNOS and COX-2 (Seeka et al., 2017). Another explanation of the anti-inflammatory activity of pregnane glycosides that they interfere with steroidogenic enzymes to down-regulate corticosteroid production (Komarnytsky et al., 2013) through strong inhibition of 11β-hydroxylase and steroid 17-alpha-monooxygenase, and weak inhibition of cytochrome P450 side chain cleavage enzyme and 21β-hydroxylase.

The depletion of TAC and elevation of MPO enzyme lead to destructive action on the tissue components and the pathogenesis of RA (El-Hawary et al., 2016). Supplementation of RB efficiently alleviated MPO. Also, it protected cartilage and bone degeneration by diminishing the elevated levels of cartilage-degrading enzymes such as hyaluronidase and activities of degenerative lysosomal enzymes like β-glucuronidases (β-GLU). The levels of these enzymes were increased in the synovial joint of arthritic animals lead to labilizing effects on the lysosomal membrane and therefore, increase the release of these degenerative enzymes and bone resorption in arthritic patient (El-Hawary et al., 2016).

To evaluate inflammation and bone lesions induced by AIA, hematoxylin and eosin (H & E) staining was subsequently performed. There were no pathological findings of arthritis were observed in normal joints. In contrary, model slices in the CFA group exhibited severe synovitis, with synovial hyperplasia, inflammatory cells’ infiltration into the joint cavity and erosion of bone and cartilage. The treatment with
ibuprofen and the higher dose of RB, showed a decrease in synovial hyperplasia, cartilage surface erosion, and joint degradation noticeably, with substantially reduced infiltrated inflammatory cells. The suppression effect of RB on AIA rats was further confirmed by bone histomorphometry. Significant increases in trabecular thickness and trabecular number, in addition to decreases of trabecular separation and osteoclast number per bone surface revealed the potential protective effects of RB on bone loss resulting from the AIA model.

Pregnane glycosides attracted the attention in the recent years due to their unique structural features and significant diverse bioactivities which have been reported such as; anti-epileptic, anti-hyperglycemic, cytotoxic, immunosuppressive, neuroprotective, and anti-inflammatory activities. Accordingly, they have great potential as leads in medicine (Shao et al., 2018). In addition to anti-arthritic activity concluded from our study, the immunosuppressive action of pregnanes reported from other studies, could be one of their potential mechanisms to be used for the treatment of RA (Qin et al., 2018; Feng et al., 2008; Li et al., 2006; Ye et al., 2006). In addition, their anti-inflammatory (Zhang et al., 2016; Itthiarbha et al., 2012; Chen et al., 2017), antioxidant effects (Abdel-Sattar et al., 2018; Rehman et al., 2014), are also supported their anti-arthritic activity. Finally, our study could substantiate the traditional uses of genus *Caralluma* for the treatment of arthritis due to their high dominance of pregnane contents.

**Conclusion**

It was proved that RB demonstrated a potential anti-arthritic activity in AIA model, mainly by reducing the rheumatoid markers, cartilage and bone degenerative enzymes in addition to its anti-inflammatory and antioxidant action. Also, RB inhibited bone erosion, cartilage destruction, pannus formation and inflammation-induced bone loss, which are characteristic signs of arthritis. Therefore, RB may have therapeutic value in preventing or delaying the progression of RA. However, further research is required to investigate its efficacy, safety, drug interaction, and its pharmacokinetic properties before considering it as drug lead for the treatment of RA.

**Declaration of competing interest**

None.
Fig. 7. Photomicrograph (H&E, scale bar, 100 μm) of ankle joint of; (a & b) Normal group, (c&d) CFA group, (e & f) CFA + Ibuprofen group, (g & h) CFA + Russelioside B group (25 mg/kg b. wt), (i & j) CFA + Russelioside B group (50 mg/kg b. wt.) (n = 8 rats per group). (a) normal articular cartilage with smooth surface, (b) normal chondrocytes embedded within normal cartilage matrix, (c) deterioration of cartilage and subchondral bone, (d) numerous osteoclasts (arrows), (e) smooth articular surface, (f) necrosis of chondrocytes (arrows), (g) smooth articular surface, (h) focal loss of cartilaginous matrix (arrow), (i) normal cartilage matrix, (j) necrosis of individual chondrocytes (arrow).
CFA: complete Freund’s adjuvant-induced arthritic rats; IBU: ibuprofen treated group; NOR: normal group; RB: russelioside B treated groups.

Fig. 8. The mean score of histopathological parameters: (a) cartilage damage, (b) bone erosion, (c) inflammation and (d) pannus formation recorded in the different groups (n = 8 rats per group).
CFA: complete Freund’s adjuvant-induced arthritic rats. Values are means ± SD. Statistical analysis was performed using the Kruskal-Wallis test followed by Dunn’s post hoc test.
Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2021.113779.

Authors’ contribution


Funding

This work is funded by the Academy of Scientific Research and Technology, Egypt, under the “Egypt Knowledge and Technology Alliances (EG-KTA) Program” (No.: KTA-C2-2.10).

References


Fig. 9. The mean scores of bone histomorphometry parameters: (a) Trabecular thickness (Tb.Th., μm), (b) Trabecular number (Tb.N., mm⁻¹), (c) Trabecular separation (Tb.Sp., mm), and (d) Number of osteoclasts per bone surface (N.Oc/Bs, mm⁻¹) recorded in the different groups (n = 8 rats per group). CFA: complete Freund’s adjuvant-induced arthritic rats. Values are means ± SD. Statistical analysis was performed using the Kruskal-Wallis test followed by Dunn’s post hoc test.