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Anti-inflammatory effect of Curcuma longa (turmeric) on collagen-induced arthritis: an anatomico-radiological study

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Abstract

Introduction and Objective. Curcuma longa (CL) or turmeric is an Ayurvedic herb that has been traditionally used to treat inflammatory conditions like rheumatoid arthritis (RA). Collagen-induced arthritis (CIA) is a well established experimental auto-immune mediated polyarthritis in susceptible strains of rodents. The main aim of the study was to observe the inflammatory, macroscopic and radiological changes in the arthritic ankle joints of experimentally collagen-induced arthritis animals treated with or without CL extract.

Materials and Methods. Thirty six male Sprague-Dawley (6-8 weeks-old, 150 ± 50) rats were equally divided into six groups. The first group served as a control while the rest five groups were immunized subdermally with 150 µg collagen type-II on day-0. All rats with established CIA with arthritis score (AS) exceeding 1 were treated orally with betamethasone (0.5 mg/ml/kg body weight) and varying doses of CL extract (30, 60 and 110 mg/ml/kg body weight) using olive oil as vehicle, daily for four weeks. Arthritic scoring (AS) of the paws, measurement of erythrocyte sedimentation rate (ESR) and paw thickness and radiological scoring were performed.

Results. Treatment with 110 mg/ml/kg CL showed significant mean difference in the ESR ($p<0.01$), AS ($p<0.05$) and radiological scores ($p<0.01$) on day-28 compared to the vehicle treated group. The mean difference for the ESR, AS and radiological scores of this highest CL dose group were found to be insignificant compared to the betamethasone treated group.

Conclusion. The administration of CL extract arrested the degenerative changes in the bone and joints of collagen-induced arthritic rats.
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Key words: *anatomy, arthritis, curcuma longa, experimental, joints, radiology, rats, turmeric*

Introduction

Curcuma longa (CL) which is also known as turmeric, is a rhizomatous herbaceous perennial plant of the ginger family Zingiberaceae. It is widely found in tropical South Asia. Its rhizomes are boiled for several hours and then dried in hot ovens, after which they are ground into a deep orange-yellow powder and commonly used as a spice in curries and other South East Asian cuisines, for dyeing and to impart colour to mustard condiments (1). Its active ingredient is curcumin and it has an earthy, bitter, peppery flavor (2).

Rheumatoid arthritis (RA) is a chronic autoimmune disease which causes chronic inflammation of the joints. It may involve any synovial joints particularly metacarpophalangeal and proximal interphalangeal joints, the wrist, shoulder and knee joints. In Malaysia, RA affects about 5 in 1000 people and 75% of the sufferers are women, according to the Arthritis Foundation of Malaysia, 2007 (3, 4). The inflammatory process causes oedema, pain, stiffness and redness (erythema) of the involved joints. The inflammation in RA causes damage to the synovial membrane, and periarticular cartilage and bone. This inflammation leads to destruction of the joints and results in disability of movements (5, 6).

Collagen-induced arthritis (CIA) is an experimental autoimmune mediated polyarthritis that is well established in susceptible strains of rodents by immunization with type-II collagen, the major constituent protein of articular cartilage. Compared to other experimental arthritis models, CIA has been shown to closely resemble that of human RA in terms of clinical, histological and immunological features as well as genetic linkage (7, 8). The CIA model was tested to be sensitive to betamethasone with expectation of the known anti-inflammatory response (9). Current conventional medications are reported to cause various types adverse effects (10, 11). Perhaps, this had lead to many patients trying on various alternative medicines and herbal products (12).

Various herbal extracts have been used to treat inflammatory arthritis. Recently, an important herb named *Justicia gendarussa* was tested on the CIA model and it was observed that this plant extract exhibited anti-arthritic properties (13, 14). Curcumin an important constituent of turmeric, has been reported to alter the nuclear factor (NF) kappaB transcription activity, inhibit prostaglandin E2 production and COX-2 expression, thereby acting as an efficient anti-inflammatory agent (15). A past study recruited eighteen patients with RA and observed the effect of curcumin on the symptoms of the RA (16). Interestingly, the same study found that treatment with curcumin for two weeks showed similar results compared to that of phenylbetamethasone treatment (16).

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No side effects were observed during the entire treatment with curcumin. Another recent study observed that in human chondrocytes, curcumin modulates the activation of NF-kappaB by inhibiting upstream kinases thereby acting as an effective anti-inflammatory agent for arthritis (17). Thus, anti-inflammatory and anti-arthritic properties of CL has been studied in human beings.

Considering the above facts related to curcumin treatment, we embarked on the present study to determine the role of CL as an anti-inflammatory agent in RA. In this study, both macroscopic and radiological changes were observed, especially in the hind paws of the CIA rats treated with or without CL extract. An evaluation system with macroscopical and radiological parameters were employed and the underlying inflammatory changes that occurred in the blood were investigated in detail.

Materials and Methods

Animals

Thirty six male Sprague-Dawley rats aged 6-8 weeks (150 ± 50 g) were obtained from the Laboratory Animal Resource Unit, Universiti Kebangsaan Malaysia. Animals were housed one rat per cage, with food and water provided *ad libitum* and housed under standard laboratory conditions at room temperature (maintained at 20-24°C) with 12 hours light/dark cycle. All procedures of the experiment were approved by the Universiti Kebangsaan Malaysia Animal Ethic Committee (UKMAEC) (approval number: ANAT/2007/FAIZAH/10-JULY/197-JULY-2007 -JULY-2008).

CL extract, chemicals and reagents

Turmeric extract was procured from the Sabinsa Company, Malaysia. The content of total curcuminoids by HPLC was not less than 95% on dried basis. The purity of the curcuminoids by HPLC method showed bisdemethoxycurcumin (not less than 2.5% and not more than 6.5%), demethoxycurcumin (not less than 15% and not more than 25%) and curcumin (not less than 70% and not more than 80%). The olive oil (Bertolli®, Italy) was used as a vehicle for oral supplement. Betamethasone sodium phosphate powder was purchased from Sigma. Bovine type-II collagen in 0.1M acetic acid and incomplete Freund's adjuvant were purchased from Chondrex, USA. Other chemicals used in the experiments were of analytical grade from commercial sources.

Induction of arthritis

Bovine type-II collagen in acetic acid was emulsified with incomplete Freund's adjuvant based on method described by a previous study (13). Sprague-Dawley rats were divided equally into six different groups. Arthritis was induced systemically in five groups on day 0, by injecting 150 µg of bovine type-II collagen emulsified in incomplete Freund's adjuvant (IFA) subdermally at the base of the tail of each rat based on a modified method provided by Chondrex, USA and another previous study (13).

Oral supplement preparation

Both the turmeric extract and betamethasone were dissolved in olive oil according to the preferred doses. The prepared oral supplement solutions were stored in sealed bottles wrapped with aluminium foil and kept in a refrigerator (4°C). All supplement solutions were replenished weekly.

Treatment of animals

Oral supplement solutions of turmeric extract, betamethasone and the vehicle (olive oil) were given daily, initiated on the day after the onset of arthritis when the arthritis score exceeding 1 (on day 14) and were continued until day 28 of the experiment (14). Rats with established CIA were treated orally with betamethasone (0.5 mg/ml/kg body weight), olive oil (1.0 ml/kg body weight) and turmeric extracts (doses of 30, 60 and 110 mg/ml/kg body weight) (9).

Erythrocyte sedimentation rate (ESR) measurement

Blood samples were collected from the retro-orbital sinus on days 0, 14 and 28. ESR was determined by a modified method described by a study done previously (13). 120 µl of blood sample was taken directly and dropped into 30 µl of 0.109 mol/L sodium citrate, mixed well and then transferred into a 1.0 mm x 100 mm heparinised capillary tube. The tubes were held obliquely at an angle of 45° and the results were recorded after 15 minutes.

Arthritis assessment (macroscopic)

The rats were inspected daily for the onset of arthritis by oedema and/or erythema changes in the paws by two independent observers. The incidence and severity of arthritis were evaluated using a system of macroscopic arthritic scoring (AS) every 2 days, beginning on the day when arthritic signs were first visible (day 14). AS of the four paws of each rat were graded from 0 to 4 according to both oedema and erythema of the peri-articular tissues; 0, no erythema and swelling; 1, erythema and mild swelling confined to mid-foot or ankle joint; 2, erythema and moderate swelling extending from the ankle to the mid-foot; 3, erythema and severe swelling extending from the ankle to the metatarsal joints; 4, erythema and maximally inflamed ankle, foot and digits. The potential maximum of combined AS per animal was 16 (15). The hind paw swelling was measured using a dial calliper and expressed as the mean measurement of both hind paws of the rat (16). The body weight of the rats were monitored with a 0.5 g precision balance every 2 days.

Radiological assessment

At the end of the experiment on day 28, rats were anaesthetised by intramuscular injection of ketamine/xylazine (0.1 ml/100g body weight) and the hind paws were X-rayed (Proteus XR/a System; exposure: 45–48KVp/0.1 second) before being sacrificed using diethyl ether asphyxiation and immediate cervical dislocation. Radiographs of each rat were evaluated for soft tissue swelling and bone erosion, and were scored blindly by two independent observers on the scale of

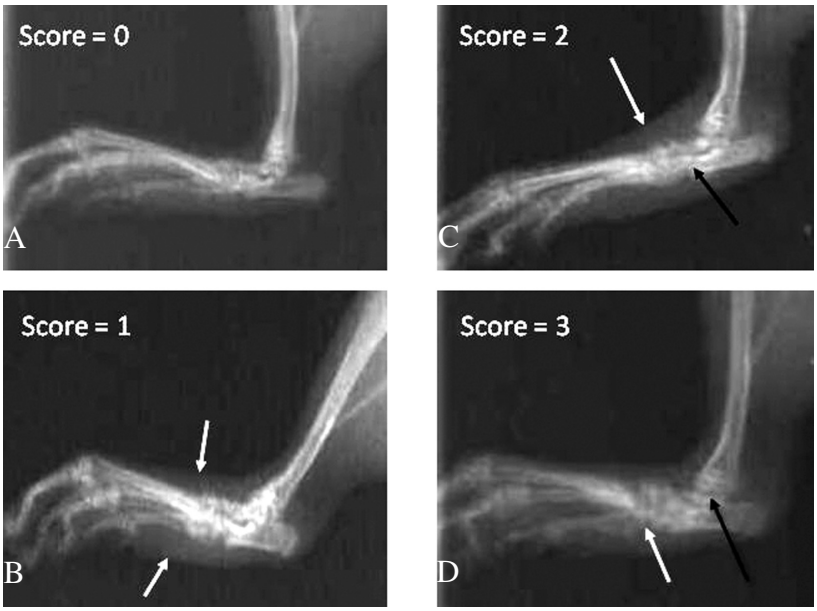


Fig. 1. The radiographs (A, B, C, D) referring to their respective radiological scores (0, 1, 2, 3) as mentioned in the method section. The arrows in B and C denote soft tissue swelling. The black arrows in C and D denote bone and/or joint erosion. In D, the white arrow denotes osteophyte formation.

0 (no bone changes), 1 (tissue swelling and oedema), 2 (bone and/or joint erosions) and 3 (bone erosion and osteophyte formation). The radiological scoring system and its respective radiographic appearance were denoted in Figure 1 (A, B, C, D). The total radiological scores were calculated from the sum of both hind paws, with a maximum possible score of 6 for each radiologic parameter per rat (14).

Statistical analysis

Data was expressed as means ± standard deviation (SD). Pearson's correlation test was used to measure the agreement of scores made by the two observers. One-way analysis of variance with post hoc LSD and Tukey tests were used to analyse differences between different groups. Statistical significance was accepted for $p \leq 0.05$.

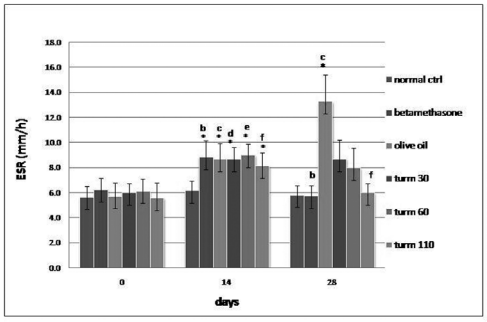
Results

Changes in ESR (Fig. 2)

The mean ESR values for the vehicle treated group showed continuous increase from day 0 to day 28. The contrary was observed in the ESR values for the betamethasone treated and 110 mg/ml/kg CL groups, which showed an increase from day 0 to day 14 and a decreased pattern from day 14 to day 28. The other CL treated groups showed a similar pattern of change but with lesser significance.

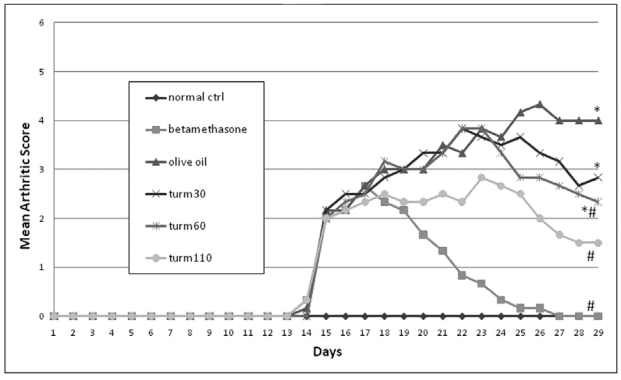
Changes in arthritic scores (AS) (Fig. 3)

The arthritic changes that occurred in all treatment groups showed similar pattern of increasing AS from day



Each bar represents mean value and standard deviation.
*** (p<0.05) significantly different compared to the normal control group on the same day.**
b, c, d, e, f (p<0.05) significantly different compared to the previous reading within the same group.

Fig. 2. Bar diagram showing the mean ESR values ± SD on days 0, 14 and 28, according to the different treatment groups.



Each point represents mean value (n = 6).
(p<0.05) significantly different compared to the olive oil treated group.
* (p<0.05) significantly different compared to the normal control group.

Fig. 3. Patterns of change in the mean arthritic scores (AS) from day-0 until day-28, according to the different treatment groups.

0 to day 14. The decreasing AS pattern after day 14 until day 28 was observed in all CL treated and positive control groups but with different degrees of change. The negative control group showed an increasing AS throughout the experiment.

Radiological observations

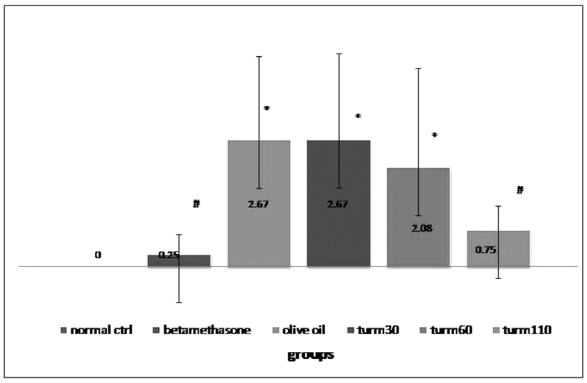
Radiological observations at the end of the experiment (i.e., on day 28) showed the changes that occurred at the ankle joint and bones of the CIA rats. The radiographs from the normal control group showed normal ankle joint images (Fig. 4A). The radiographs from betamethasone treated group appeared fairly normal and were are not significantly different when compared to the normal control group. No bone erosion and osteophyte formation were detected in the radiographs of this positive control group.

The negative control group that was only supplemented with olive oil and the turm30 group (CL dose 30 mg/kg) gave rise to radiographs that showed soft tissue swelling, bone erosion and osteophyte formation (Fig. 4B).

In the turm60 group, bone erosion was controlled and osteophyte formation did not occur. The CL dose of 110 mg/kg on the other hand, had successfully prevented bone erosion in the ankle joints, but the soft tissue swelling could still be seen on the radiographs (Fig. 4C).

Radiological scores from two independent observers were taken into account after correlation between the two was tested. The Pearson correlation value was found to be 0.928 (significant at $p<0.01$). The mean radiological score for the control group showed the normal characteristics of the foot joints (score 0). The other groups with CIA rats treated with various oral supplements showed increased mean radiological scores according to the supplement given (Fig. 5).

At day 28, radiological scoring of betamethasone treated group resulted in lowest or near normal average score of 0.25. It was followed by the turm110 group with mean radiological score of 0.75, which could be rounded up to 1.0 that represent radiological image of ankle joint with oedema and/or soft tissue swelling. These two low score groups were significantly different compared to the olive oil treated group.



Each bar represents mean value and standard deviation.
*** ($p<0.05$) significantly different compared to the betamethasone group.**
 # ($p<0.05$) significantly different compared to the olive oil group.

Fig. 5. Bar diagram showing the radiological scores ± SD on day-28, according to the different treatment groups.

On the other hand, both the olive oil and turm30 groups scored the highest (2.67), for which it meant they were soft tissue swelling, bone erosion and osteophyte formation that could be seen in the radiographs. Additionally, the turm60 group showed a lower radiological score of 2.08 compared to that of the former two groups. This intermediate CL dose group showed only soft tissue swelling and mild bone erosion without osteophyte formation. The radiological scores of these three high score groups were significantly different compared to the normal control group.

Discussion

The arthritic score took into account the physical changes observed especially at the region of the paws. Inflammation and erythema of the ankle joint were scored depending on the severity of the observed symptoms. The control group had maintained its normal score of 0 throughout the experiment. Perhaps, this was due to the control group not being immunized with collagen emulsion.

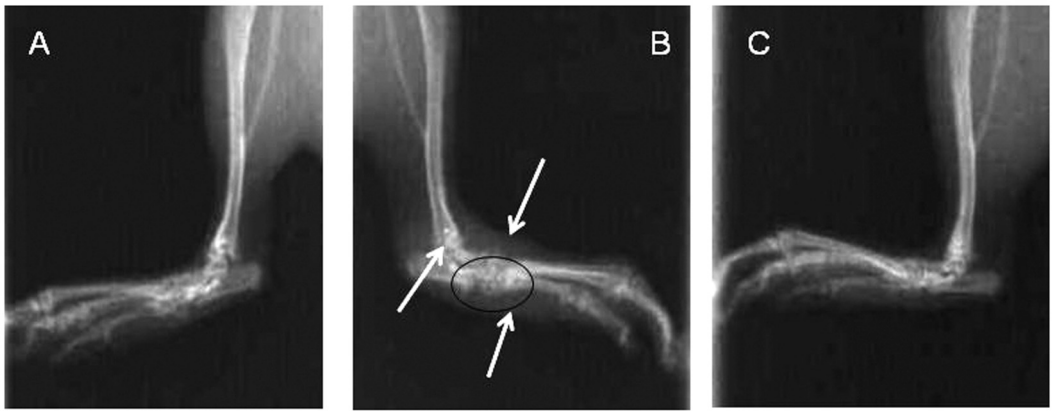


Fig. 4. The radiographs show, a normal rat ankle joint from normal control group (A) and a rat ankle joint from the negative control group (fed with olive oil) (B),with soft tissue inflammation and joint erosion (shown with arrows) and osteophyte formation (circled area). Radiograph C shows a rat ankle joint from the CL treated group (highest dose of 110mg/kg body weight), with a nearly-normal ankle joint appearance.

The negative control group showed a continuous increase of AS beginning on day 14 after immunization with collagen emulsion through to day 28 of the experiment. This vehicle supplemented group suffered progressive arthritic symptoms due to no anti-inflammatory action that could prevent or slow down the ongoing inflammation process (5). Therefore, the joints were severely inflamed with erythema at the end of experiment.

On the other hand, the positive control group that received betamethasone, showed changes that showed evidence of an anti-inflammatory effect throughout its healing process. There was an initial increase in AS in this group, but declined after day 17. The decrease in AS continued until day 28 during which the AS of 0 was achieved. These findings supported the anti-inflammatory effect of betamethasone in the treatment of arthritic symptoms as reported by Larsson et al. (9). Additionally, glucocorticoids are also reported to reduce the edema and inflammation in rats with collagen-induced arthritis (CIA) (17).

Similarly, the CL treated groups showed a dose dependent pattern of arthritic score (AS). The highest CL dose of 110 mg/kg (turm110) showed the most significant decrease in AS, followed by the 60 mg/kg (turm60) and 30 mg/kg (turm30) groups. The decrease in AS showed that CL supplement had successfully reduced the inflammation and erythema in the ankle joints of the CIA rats. The decrease may be due to CL supplementation that had caused a reduction in the formation of new blood capillaries (angiogenesis), which commonly occur in untreated arthritic joints (18, 19). It is postulated that angiogenesis process may worsen the inflammatory process. Fluid from the blood vessels may ooze out into the affected tissue area thus causes oedema. This process is very common in an inflammatory reaction, including the inflammation that occurred in the ankle joints of the CIA rats.

Previous studies also reported that curcumin could reduce the expression of angiogenesis-linked genes, vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9) (20, 21). Additionally, Jackson et al. and Funk et al. reported that curcumin prevented angiogenesis as well as inhibited neutrophil activation and synoviocyte proliferation (22, 23). In general sense, curcumin or turmeric was also reported to have protective and suppressive effect against arthritis, that may largely attributed to its good antioxidant activity against the high oxidative stress condition (24, 25).

ESR is considered to be an indicator of inflammatory reaction within the circulatory system. It involves the rate of production of a few types of proteins that influence the blood fluidity that changes in diseased conditions. Inflammatory reaction occurring in RA will increase the ESR due to the increase in the production rate of those inflammatory mediator proteins in the blood (26, 27).

CIA suffered by the rats after being immunized with collagen emulsion in this experiment caused the systemic inflammatory reaction to directly influence the ESR (14). Generally, an increase in ESR indicates that there is an ongoing disease process in the body (5). This experiment observed that ESR for the negative control group had continuously and significantly increased from the beginning till the end of the experiment.

The betamethasone or CL treated groups on the other hand showed significant decrease in ESR compared to the control group at the end of experiment. The decrease in ESR for the CL treated groups was dose-dependent. Treatment with betamethasone successfully reduced the ESR to the normal level similar to that of the control group. An increase of ESR as stated earlier was due to the inflammatory reaction, in which the rats' immune system was interacting with the type-II collagen molecules injected subdermally into the tail and entered the circulatory system. The injection of collagen emulsion may have induced the rats' immune system to produce antibody molecules against the type-II bovine collagen thereby causing an inflammatory reaction against collagen emulsion and articular cartilage systemically. The inflammatory reaction may also cause other changes in blood, such as an increase in the level of C-reactive proteins and inflammation of the joints that will progress into joint damage (28).

Decrease in ESR after the supplementation of betamethasone and CL extract suggested that those supplements have an anti-inflammatory effect towards CIA. These findings was supported by a research performed by Oelzner et al., who stated that treatment with glucocorticoid reduced the ESR significantly (29). However, the exact mechanism involved in this anti-inflammatory effect that caused a decrease in ESR was not understood properly. The decrease may be attributed to the direct effect of the decrease in the disease processes as a whole, after the supplementation of CL or betamethasone orally.

Physical changes were detected following radiological observations result from the reactions of different supplements given to the rats from day-14 until the end of the experiment (day-28). Betamethasone supplementation had successfully suppressed the inflammatory reaction as observed in the rats' foot.

The negative control group supplemented with only olive oil, and the turm30 group (CL dose 30 mg/kg) showed soft tissue swelling, bone erosion and osteophyte formation. Those changes may have occurred due to ongoing inflammatory reactions that were not effectively suppressed. These findings suggested that olive oil cannot prevent the inflammatory reactions that had finally caused the bone erosion and osteophyte formation. Supplementation with low dose of CL was also shown to be incapable in halting the inflammatory process due to CIA effectively.

In the turm60 group, bone erosion was controlled and osteophyte formation did not occur. Increasing the CL dose to 110 mg/kg on the other hand, had successfully prevented bone erosion in the ankle joints. However, the soft tissue swelling could still be observed. This observation showed that the increment of CL dose resulted in better anti-inflammatory effect in suppressing the radiological changes that occurred in CIA rats.

Bone erosions that occurred in CIA were due to the changes in the normal activity of osteoblasts and osteoclasts that play an important role in the dynamic new bone tissue formation process (30). The increased production of inflammatory reaction mediators, such as IL-1, TNF-α and other factors in CIA promotes the maturation and activation process of the osteoclasts. This causes bone resorption to occur in an increased rate, and finally it results in bone erosion (31).

Those pro-inflammatory cytokines could also increase the production of receptor activator for nuclear factor-kappaB ligand (RANKL) that activates the osteoclast cells responsible in the bone erosion process (32). Additionally, activated synovial fibroblasts and T cells are also the potent factors in the osteoclasts' differentiation process (33). Synovial fluid macrophages in rheumatoid arthritis joints reported to be capable in differentiating into osteoclasts that may cause lacunar bone resorption (34). These synovial macrophages may also release prostaglandin and tissue proteases which would promote lacunar bone resorption activity by the osteoclasts (35).

Osteophyte formation occurred when the inflammatory reaction were left alone without any treatment or control. Disturbances in the balanced osteoblasts and osteoclasts functions will in turn cause the new bone tissue formation to occur without proper control. This will cause the newly formed bone tissue to take a form that is structurally different from the former and the normal functions of the bones will also be affected (22). Therefore, the supplementation of betamethasone and CL at a high dose had successfully halted the inflammatory process that occurred due to CIA. The findings give evidence of the protective effect of CL in preventing the diseases like arthritis. Additionally, CL has also been reported to suppress the production of pro-inflammatory cytokines (especially IL-1 and TNF-α), and therefore prevent or suppress the progression of bone erosion process (36).

In conclusion, the oral administration of CL extract was found to be beneficial in arresting the inflammatory and degenerative changes in the bone and joints of the CIA rats observed both macroscopically and radiologically. CL could be tried as an effective supplement in RA. Further studies may be needed to corroborate such fact.

Acknowledgement

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