Med & Health Dec 2020; 15(2): 26-36 https://doi.org/10.17576/MH.2020.1502.05

ORIGINAL ARTICLE

Curcumin Minimises Histopathological and Immunological Progression in the Ankle Joints of Collagen-Induced Arthritis Rats

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ABSTRAK

*Curcumin adalah satu rempah tradisional yang mempunyai potensi untuk menyembuhkan pelbagai jenis penyakit inflamatori, termasuk artritis. Kajian ini dijalankan untuk memerhatikan kesan-kesan curcumin ke atas perubahan histopatologi dan aras interleukin-1β (IL-1β) di dalam artritis aruhan kolagen (CIA). Tiga puluh ekor tikus jantan Sprague-Dawley (150±50 g) dibahagikan kepada lima kumpulan secara rawak. Satu kumpulan dijadikan kumpulan kawalan normal (CTRL), manakala selebihnya disuntik dengan 150 µg emulsi kolagen secara subkutan pada hari 0. Kumpulan CTRL dan CIA-Curcumin-d0 masing-masing diberi suplimentasi harian minyak zaitun oil (1 ml/kg) dan curcumin (110 mg/ml/kg) bermula pada hari 0. Kumpulan CIA-OV (kawalan negatif), CIA-Beta dan CIA-Curcumin-d14 pula, masing-masing diberi suplimentasi harian minyak zaitun (1 ml/kg), Betamethasone (0.5 mg/ml/kg), dan curcumin (110 mg/ml/kg) bermula pada hari 14. Suplimentasi harian tersebut diberi kepada tikus-tikus sehingga hari ke 42. Kajian ini menunjukkan bahawa kumpulan CIA-Beta (**P=0.00) dan CIA-Curcumin-d0 (**P=0.01) masing-masing mempamerkan purata skor histologi yang lebih rendah secara signifikan berbanding kumpulan CIA-OV. Aras IL-1β di dalam serum untuk kumpulan CIA-Beta dan CIA-Curcumin-d0 tidak meningkat secara signifikan pada hari ke 42 berbanding hari 0. Purata peningkatan aras IL-1β dari hari 0 ke hari 42 juga adalah rendah secara signifikan (**P≤0.01) untuk semua kumpulan CIA berbanding kumpulan CIA-OV. Tidak terdapat perbezaan yang signifikan dalan purata skor histologi dan aras IL-1β kumpulan of CIA-Curcumin-d0 berbanding kumpulan CIA-Beta. Kesimpulannya suplimentasi awal curcumin berpotensi untuk meminimakan perubahan yang disebabkan penyakit artritis aruhan kolagen pada tikus.*

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Kata kunci: arthritis, artritis reumatoid, Curcuma, Curcumin, ekstrak tumbuhan, Interleukin-1, pengkajian

ABSTRACT

Curcumin is a traditionally used spice with a potential to treat various inflammatory diseases including arthritis. This study was aimed at observing curcumin's effects on the histopathological progression and interleukin-1β (IL-1β) levels in collagen-induced arthritis (CIA). Thirty male Sprague-Dawley rats (150±50 g) were divided into five random groups. A group was assigned as the normal control (CTRL), while the remaining were subcutaneously immunised with 150 µg of collagen emulsion on day 0. CTRL and CIA-Curcumin-d0 groups were supplemented daily with olive oil (1 ml/kg) and curcumin (110 mg/ml/kg) from day 0, respectively. The CIA-OV (negative control), CIA-Beta and CIA-Curcumin-d14 groups were given daily supplementation of olive oil (1 ml/kg), Betamethasone (0.5 mg/ml/kg), and curcumin (110 mg/ml/kg) from day 14, respectively. The daily oral supplementations continued until day 42. The study showed that CIA-Beta (**P<0.05) and CIA-Curcumin-d0 (**P=0.01) groups had significantly lower mean histological scores compared to CIA-OV, respectively. Serum IL-1β levels for CIA-Beta and CIA-Curcumin-d0 were not significantly raised on day 42 as to compared to day 0, and the mean increment of IL-1β levels from day 0 to day 42 were significantly lower (**P≤0.01) for all the CIA groups compared to CIA-OV. There was no significant difference in both mean histological scores and IL-1β levels of CIA-Curcumin-d0 compared to CIA-Beta. Early supplementation of curcumin could potentially minimise disease progression of CIA in rats.

Keywords: arthritis, *Curcuma,* Curcumin, experimental, Interleukin-1, plant extract, rheumatoid arthritis

INTRODUCTION

Five out of 1000 Malaysian are affected by rheumatoid arthritis (RA), a chronic autoimmune joint disease (Arthritis Foundation of Malaysia). This disease affects more females than males in a ratio of approximately 8 to 1 and has a debilitating effect on the population as well as on health care costs.

Macrophages play a central role in the pathogenesis of RA (Rodríguez-Ubreva et al. 2019). These mononucleocytes release key pro-inflammatory cytokines interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) into the synovium. Although shown to play a minor role in later stages of the disease, TNF- is crucial at the onset of arthritis and maintains a key role in regulating IL-1β expression (Alam et al. 2017). IL-1β induces cartilage destruction and a pivotal secondary mediator to TNF-

α in arthritis (McInnes et al. 2016). It is important in inducing rheumatoid arthritis-fibroblast like synoviocytes (RA-FLS) as well as osteoblasts and bone marrow stromal cells to express receptor activator of nuclear factor kappa-β ligand (RANKL), thus promoting osteoclastogenesis, joint inflammation propagation and concomitant cartilage and bone erosion (Wehmeyer et al. 2017). The imbalance between pro-inflammatory and anti-inflammatory cytokines in the synovium causes synovial hyperplasia, stimulates pannus formation and osteoclastic activity resulting in erosion of cartilage and subchondral bone (Molendijk et al. 2018).

The bane of RA transcends symptomology of the disease and involves the state of conventional treatment modalities. Current RA pharmacology, which includes non-steroidal anti-inflammatory drugs (NSAIDs) and disease-modifying anti-rheumatic drugs (DMARDs), focuses more on symptom control and starts to interfere more with the underlying immunological mechanism of rheumatoid arthritis using new approaches (Ferro et al. 2017). A systematic review of randomised placebo-controlled trials only showed the efficacy of nine agents in decreasing radiological progression in rheumatoid arthritis. These include infliximab, cyclosporin, sulphasalazine, leflunomide, methotrexate, parenteral gold, corticosteroids, auranofin and interleukin-1 receptor antagonists (Jones et al. 2003). TNF-α inhibitors have been proven to be effective in controlling the signs and symptoms of RA. However, efficacy of treatment and significant positive impact on the quality of life among RA patients are often impeded by adverse effects of hypersensitivities and infections (Papadopoulos et al. 2019; Singh et al. 2015), the cost of medication, including healthcare monitoring burdens as well as patient finances (Fazal et al. 2018) and the indirect costs to the society (Batko et al. 2019).

With growing interest in alternative treatments for RA, several studies have shown promising results in rat models (Taty Anna et al. 2011; Mateen et al. 2019). Curcumin is a yellow pigment and an active component from the perennial herb *Curcuma longa*. It has been extensively researched for its health benefits (Kocaadam & Sanlier 2017). The anti-inflammatory qualities of curcumin arise from its ability to inhibit cyclooxygenases II (Hu et al. 2017; Czekaj et al. 2016) as well as reduces the expression of cytokines including IL-1β and TNF-α (Benzer et al. 2018; Kim & Kim 2018). Rapidly metabolised in the circulation, curcumin causes no adverse effects even at doses reaching 1.3 g/kg body weight in rats (National Toxicology Program 1993). No toxicity was demonstrated in human trials using up to 8000 mg/day of curcumin for 3 months (Aggarwal & Harikumar 2009; Lao et al. 2006; Chainani-wu 2003).

Collagen-induced arthritis (CIA) is a widely studied model of rheumatoid arthritis (Grötsch et al. 2019). It utilises collagen type-II (CII), a major protein component of cartilage and a target protein in RA to induce an auto-immune polyarthritis in susceptible

strains of rodents. Aside from its rapid disease manifestation, CIA shares many of the histological, immunological and genetic features of RA (Trentham et al. 1977; Brand et al. 2007). In this study, the CIA model was used to compare the anti-arthritic effects of betamethasone and curcumin at various time points of supplementation by observing the histopathological and immunological progression of the disease in male Sprague-Dawley rats.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats were supplied by the Laboratory Animal Research Unit of Universiti Kebangsaan Malaysia (UKM), Kuala Lumpur. All rats were provided with a balanced diet and sufficient water. The rats were acclimated to their surroundings for 1 week in order to eliminate the effects of stress prior to initiation of the research. The research was approved by the Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC) (Ethics No.: FP/ANAT/2011/TATY/21-SEPTEMBER/394-OCTOBER-2011-JANUARY-2012-NAR-CAT2). The UKMAEC guidelines were followed throughout the study.

Oral Preparation

Curcumin C3 complex powder from Sabinsa Malaysia Sendirian Berhad (Malaysia) was dissolved in olive oil. Olive oil supplementation was from store bought olive oil (Bertolli, Italy). Betamethasone 21-phosphate disodium from Sigma Aldrich (MO, USA) was used.

Experimental Setup

A total of 30 male rats (150±50 g) were evenly divided into five random groups. CTRL group, which was the normal control, was given daily supplementation of 1.0 ml/kg/day olive oil from day 0. Rats in the remaining groups underwent CIA induction on day 0 via subcutaneous immunisation with 150 µg collagen II emulsions on day-0. CIA-OV group, which acted as the negative control, was treated with 1.0 ml/kg/day of olive oil from day 14. The group CIA-Beta was the positive control group that was treated with 0.5 mg/kg/day betamethasone from day 14. CIA-Curcumin-d0 group received daily supplementation of 110 mg/kg/day curcumin from day 0. The group CIA-Curcumin-d14 received a daily treatment of 110 mg/kg/day curcumin from day 14. Treatment for all groups was continued daily up to day 42 and was administered via oral gavage. The rats were sacrificed on day 42 of the study and their ankle joints were harvested.

Induction of CIA

Arthritis was induced in male Sprague-Dawley rats using a method adapted from Brand et al. (2007) and as recommended by the manufacturer, Chondrex Inc (WA,USA). An amount of 150 µg collagen II was emulsified in an incomplete Freund's adjuvant and was injected subcutaneously at the base of the tail on day 0.

Histological Scoring Assessment

Both right and left hind paws of each rat were examined daily. The rats were sacrificed on day 42; their hind paws harvested up to the knees and were fixed in 10% formalin. Decalcification was carried out with 8% formic acid for two months. The tissue was then embedded in paraffin wax, longitudinally cut into 5 µm sections and stained with haematoxylin and eosin (H&E). All slides were assessed by two blinded observers and scoring was done using a modified version of the system adopted from Zhu et al. 2005. The ankle joint slides were scored using a scale ranging from 0 (normal joint), 1 (normal synovium with occasional mononuclear cells), 2 (definite arthritis, a few layers of flat to rounded synovial lining cells and scattered mononuclear cells), 3 (clear hyperplasia of the synovium with three or more layers of loosely arranged lining cells and dense infiltration with mononuclear cells), and 4 (severe synovitis with pannus and erosions of articular cartilage and subchondral bone).

Serum IL-1β Level Measurement

Blood sample of the rats was taken from the retro-orbital sinus on day 0 and day 42. IL-1β levels were measured using commercially available enzyme-linked immunosorbent assays (ELISA) kits according to recommendations by the manufacturer.

Statistical Analysis

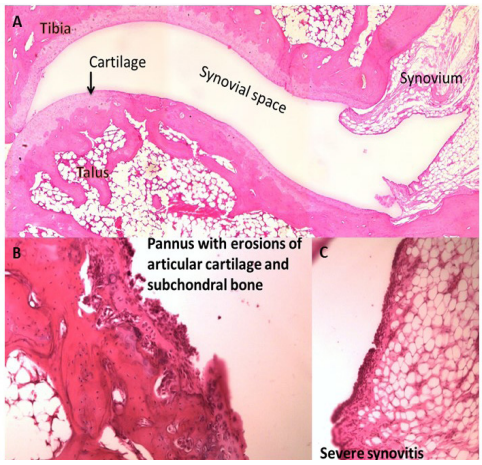

Figure 1: Representative histological features of a normal synovial joint (A), and the arthritic changes in the synovial joints (B and C).

All data were expressed as the mean ± standard error of the mean (SEM). Histological scores were subjected to Pearson's correlation. The data were then analysed by one-way ANOVA to compare the differences between groups using Statistical Package of Social Sciences (SPSS) for Windows Version 25.0 (IBM Corp., NY, USA). Statistical significance was accepted for $P<0.05$.

RESULTS

Following collagen immunisation, all CIA rats developed arthritic symptoms on day 14 of the study. All CIA groups showed increased mean histological scores and mean serum IL-1β levels on day 42 when compared with day 0. The study showed that both CIA-Cucumin-d0 and CIA-Curcumin-d14 had lower histological scores and IL-1β level compared to CIA-OV.

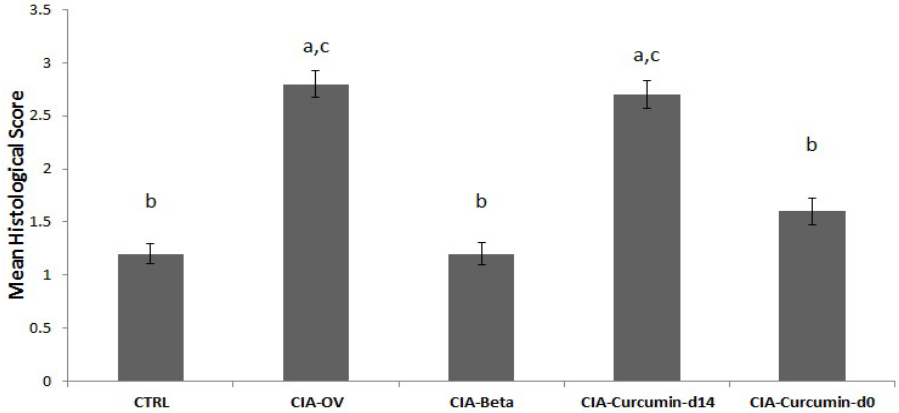

Figure 2: Mean histological scores in different groups of rats. a - significantly higher (*P<0.05) compared to control (CTRL), b - significantly lower (*P<0.05) compared to the negative control (CIA-OV), c - significantly higher (*P<0.05) compared to betamethasone (CIA-Beta).

Histological Scores

Figure 1 shows the representative histological features of the normal and arthritic synovial joints. The mean histological scores of the control and experimental animal groups were shown in Figure 2 where CIA-OV and CIA-Curcumin-d14 had a mean histological score that was significantly higher (*P<0.05) when compared to the control group. Interestingly, groups treated with betamethasone and curcumin on day 0 had mean histological scores that were not significantly different from the normal control, CTRL. The CTRL, CIA-Beta and CIA-Curcumin-d0 groups had significantly lower (*P<0.05) histological scores when compared to the CIA-OV group. On the other hand, the rats treated with curcumin on day 14 and olive oil on day 14 demonstrated significantly higher

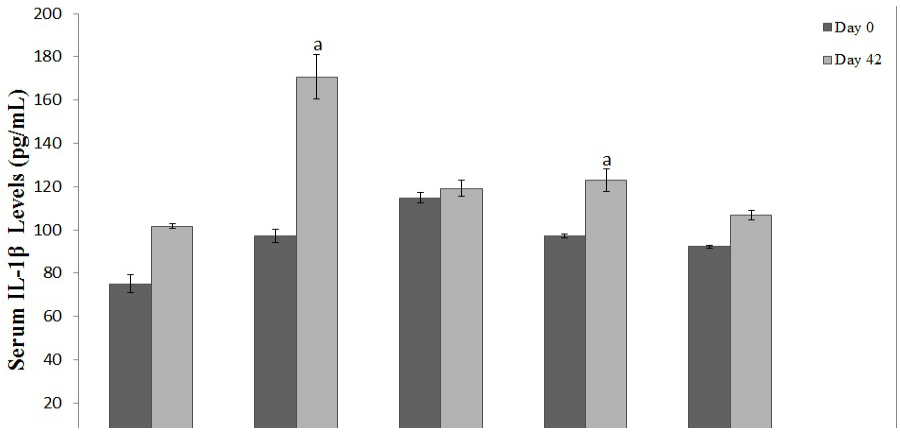

Figure 3: Mean serum 1L-1β levels in different groups of rats on days 0 and 42. a - Serum IL-1β level significantly raised (*P<0.05) on day 42 compared to day 0.

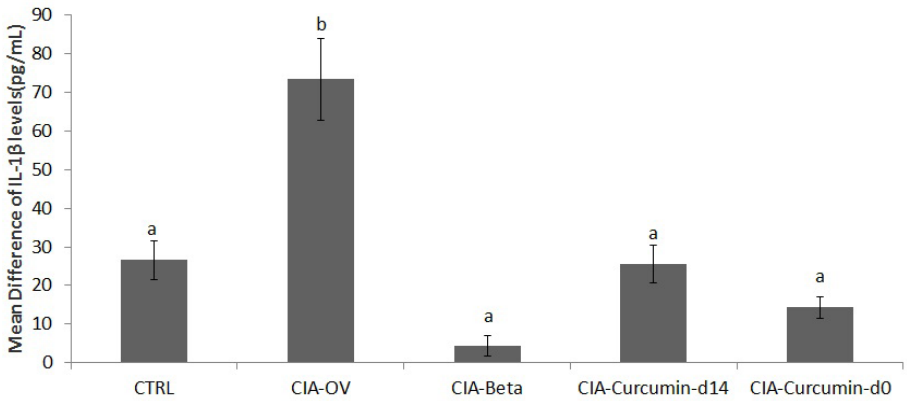

Figure 4: Mean difference of serum 1L-1β levels in different groups of rats on day 42 compared to day 0. a - Significantly lower (*P<0.05) compared to CIA-OV, b - Significantly higher (*P<0.05) compared to CIA-Beta

(*P<0.05) arthritic score compared to rats treated with betamethasone.

Serum IL-1β levels

CIA-OV and CIA-Curcumin-d14 possessed significantly raised serum IL-1β (*P<0.05) on day 42 compared to day 0 (Figure 3). There were no significant increases in the serum IL-1β levels for CIA-Beta and CIA-Curcumin-d0 at the end of the experiment.

Figure 4 shows the mean difference in serum IL-1β levels between days 0 and 42. This study demonstrated that all treatment forms were proven to be significantly better (*P<0.05) compared to the negative control group (CIA-OV). It also showed that only CIA-OV had a significantly higher mean difference of IL-1β level between the two time points compared to betamethasone treated group (CIA-Beta).

DISCUSSION

In RA, the expression of TNF-α within the synovium leads to a cascade of pro-inflammatory cytokines that is responsible for the onset and progression of joint destruction. Vascular endothelial growth factor (VEGF) is responsible for angiogenesis and is a marked feature in RA (Ding et al. 2019) while matrix metalloproteinases (MMP) stimulates osteoclastic bone and cartilage erosion (Samarpita et al. 2018). Clinical trials of anti-TNF drugs have shown normalisation of these cytokines in the blood and presumably the joint, resulting in a delayed joint destruction in addition to symptom relief (Vomero et al. 2019).

Extensive research on curcumin has elucidated a myriad of anti-inflammatory activities. Its actions on the inflammatory pathway include inhibiting reactive oxygen species (ROS) production and suppressing the activity of lipid peroxides, nitric oxide synthase, lipoxygenase, and cyclooxygenase (Banji et al. 2011; Menon & Sudheer 2007; Kang et al. 2004; Das & Das 2002). A recent study has also demonstrated curcumin's ability to suppress the increased levels

of TNF-α, IL1-β as well as other MMPs (Dai et al. 2018).

From this study, curcumin supplementation at the day of CIA induction proved to be as effective as betamethasone treatment in reducing the histological and immunological progression of CIA. This is consistent with previous and more recent studies with curcumin on the CIA rat model either as a sole supplement or in a combinational therapy (Wang et al. 2019; Taty Anna et al. 2011). Curcumin acts by suppressing TNF-α which is both important for the onset of disease (Lubberts & van den Berg 2000) and responsible for the regulation of IL-1β as well as other cytokines that propagate the disease process (Zheng et al. 2015). This study suggests that early supplementation was effective in delaying the onset and suppressing the progression of CIA in rats resulting in comparable efficacies between curcumin and betamethasone treatment.

The supplementation of curcumin on day 14 of the study significantly reduced serum levels of IL-1β but did little to suppress the histological progression of CIA in this study. The heterogeneous nature of the disease prevents the isolation of a single factor that may lead to joint destruction. Disparities in structural and serological effects of RA treatment have been reported in a previous study (Eberhardt et al. 1990). Regardless, the importance of early treatment to prevent irreversible joint destruction has also been emphasised in previous studies (Kolarz et al. 2018; Quinn 2005; Goekoop-Ruiteman et al. 2005). Failure in the suppression of pro-inflammatory cytokines at the initial onset of arthritis allows propagation of the disease via cytokine cascade resulting in bone erosion and systemic osteoporosis (Jung et al. 2019).

Early treatment of RA has been a subject of debate. Conventional therapies such as anti-TNF-α therapies bear the burden of weighing in potential benefits of preventing long-term irreversible joint damage against the high costs and adverse effects associated with immediate and long-term use (Siebert et al. 2015). This study suggests that curcumin, with its favourable safety profile and better availability, is able to provide similar benefits compared to betamethasone treatment. Further research in human models will provide a better understanding of the benefits of curcumin in treating RA.

CONCLUSION

The study demonstrated that early curcumin supplementation resulted in a significant delay in the histological and immunological progression of CIA in rats with a reduced histological score of the ankle joints and lower serum IL-1β levels. Betamethasone treatment from day 14 and curcumin supplementation from the day of immunisation was of similar effectiveness. The study also showed that curcumin supplementation, regardless of the time of commencement, also suppressed the immunological progression of CIA in rats.

ACKNOWLEDGEMENT

This research was a special study project by medical undergraduate students and supported by a research grant from the Faculty of Medicine, UKM with the project code of FF-063-2012. The authors acknowledge the help received from the staff of the Histology Laboratory, Anatomy Department, Faculty of Medicine, UKM.

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Received: 26 Dec 2019
Accepted: 10 Jul 2020

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