Investigation of the effect of Curcumin on Inflammatory Biomarkers in Arthritic Rats

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Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory disorder characterized by joint swelling, synovial inflammation and joint destruction. Curcumin (diferuolymethane) is the most active component of *Curcuma longa* L. Several clinical trials have indicated curcumin to be a notable anti-inflammatory and antioxidant compound. Therefore the aim of this study is investigating the effects of curcumin on levels of inflammation and inflammatory biomarkers in arthritic rats.

Arthritis was induced by subcutaneous injection of Freund's complete adjuvant (0.5 mL) into the right hind paw of Wistar male rats. Animals were divided into four groups (n=8). GroupI acted as control, group II arthritic rats received vehicle, group III arthritic rats were treated with curcumin (30 mg/kg, orally) and another group arthritic rats were treated with indomethacin (3 mg/kg, orally) seven days after injection of Freund's Complete Adjuvant for 15 days. The changes caused by chronic inflammation were evaluated by measurement of the ankle circumference three times per week. At the end of the experimental period, blood samples were collected by cardiac puncture to determine erythrocyte sedimentation rate, C.reactive protein levels and White blood cells count.

An increase in erythrocyte sedimentation rate, C. reactive protein concentrations, White blood cells count and ankle circumference was observed in arthritic rats compared with control rats ($p<0.05$). Curcumin significantly decreased inflammation and inflammatory biomarkers in arthritic rats ($p<0.05$).These results suggest that curcumin can possess beneficial effects in alleviating arthritic symptoms in Adjuvant-Induced Arthritis model.

Keywords: Curcumin, Freund's Complete Adjuvant, Rheumatoid arthritis, inflammation

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by joint swelling, synovial inflammation and cartilage destruction which commonly leads to significant disability and a consequent reduction in quality of life (Gabriel, 2001). Epidemiology of the arthritis in female: male is 3:1 and the prevalence is 0.5-1.0% of the world population (Gabriel, 2001; Narendhirakannan et al., 2007). RA caused by number of pro-inflammatory molecules released by macrophages (Henderson et al., 1987). These are including the reactive oxygen species and eicosanoids such as prostaglandins, leukotrienes and cytokines (interleukin-1, 6 [1L-1β, IL-6], and tumor necrosis factor α [TNFα]). The regulation of these mediators secreted by macrophages and other immune cells therefore may control the chronic inflammatory conditions (Joe and Lokesh, 2000).. The nuclear factor kappa B (NF-kB) and activator protein-1 (AP-1) mediated cytokine pathways and the cyclooxygenase-2 (COX-2) prostaglandin cascade are the most well studied pathways (Chun and Surh 2004).

The acute phase response develops in the setting of a wide range of acute and chronic inflammatory conditions severe bacterial, viral, or fungal infections; rheumatic and other inflammatory diseases. These conditions elicit a response in which IL-1, IL-6 and other cytokines trigger the synthesis by the liver of a variety of plasma proteins, including C-reactive protein (CRP) and fibrinogen. Because fibrinogen and certain other acute phase proteins (not including CRP) bind to erythrocytes and increase their sedimentation rate, the erythrocyte sedimentation rate (ESR) is a measure of the acute or chronic phase response. Monitoring CRP and ESR levels can provide useful information on the activity of diseases such as rheumatoid arthritis (Imboden et al., 2004).

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Recent evidence suggests that various herbal extracts including turmeric (*Curcuma longa* rhizomes) have potent anti-inflammatory activity in a variety of inflammatory diseases (Ammon and Wahl, 1991). Curcumin (difteruloylmethane) is the most active component of turmeric. It is believed that curcumin is a potent antioxidant and anti-inflammatory agent (Aggarwal et al., 2003), (Figure1). Some experimental studies indicate that curcumin has similar anti-inflammatory activity as some of the common nonsteroidal anti-inflammatory drugs (NSAIDs) like indomethacin, Vioxx, Celebrex, and ibuprofen, but it has no side effects, such as gastrointestinal distress and cardiovascular complications(Graumlich, 2001).

The molecular basis of the anti-inflammatory properties of curcumin is linked to its effects on several targets, including transcription factors, growth regulators, and cellular signaling molecules (Han et al., 2002). Curcumin is reported to directly influence the activity of various inflammatory regulators; it has been shown to reduce NF-kB activation, AP-1 binding to DNA, as well as to decrease the production of the enzyme COX-2, all of which play a pivotal role in the inflammatory cascade (Han et al., 2002). In addition, several studies have shown that curcumin can indirectly inhibit these inflammatory regulators through its ability to scavenge free radicals (Biswas et al., 2005).

Adjuvant-Induced Arthritis (AIA) is the most widely used model for studying the pathogenesis of RA and for screening the new drugs for treatment of rheumatoid disease, which shares some features with human RA, such as swelling, cartilage degradation and loss of joint function. It has been previously reported that administration of Freund's Complete Adjuvant (FCA) increased ankle circumference, CRP, ESR levels and White blood cells count (WBC) in arthritic rats (Simoes et al., 2005; Cai et al., 2006; Funk et al., 2006 a). Therefore, the present study, using this model, is designed to investigate the effects of curcumin on inflammation, plasma CRP, ESR levels and WBC count in comparison with indomethacin which has provided experimental evidence for its therapeutic efficacy in the treatment of RA.

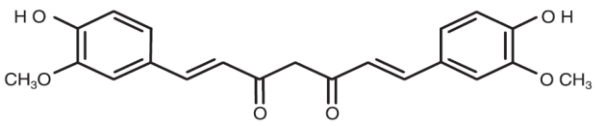

Figure 1. Chemical structure of curcumin (Wang et al., 2008)

Methods and Materials

Materials: Curcumin, Freund's Complete Adjuvant (FCA), Indomethacin were purchased from Sigma–Aldrich, Ether was purchased from Merck. Ketamin (Germany Rotex Medica), Xylasin (Holland Alfasan Woerden).

Animals: 32 adult male Wistar albino rats (180-220 g, from Laboratory Animals Unit, Faculty of Science, Urmia University) were used in the experiment. The rats were housed under standard conditions and received food and water *ad libitum*; the temperature was maintained at 22±2ºC and relative humidity (40-60%) with 12h light/ dark cycle (in the departmental animal house). Rats were acclimated to their surroundings over 1 week to eliminate the effect of stress prior to initiation of the experiments**.** In addition, this research was approved by the animal experiment Ethics Committee of this University.

Induction of arthritis: The method described by Newbould in 1963 was employed with some modifications (Newbould, 1963). To induce adjuvant arthritis, rats were anesthetized with Ketamin (70mg/kg intraperitoneally [I.P]) and Xylazin (5 mg/kg I.P) and adjuvant arthritis was induced by subcutaneous injection of 0.5 ml FCA (suspension of heat-killed *Mycobacterium tuberculosis* in mineral oil) into sub plantar tissue of the right hind paw of each rat in the test groups using a 1 ml glass syringe with a 21 GA needle, while, the control rats were injected with 0.5 ml of normal saline.

Experimental setup: Animals were divided into four groups of eight animals in each group as follows:

Group I: Control rats (untreated)

Group II: Adjuvant induced arthritic (AIA) rats (0.2 ml normal saline)

Group III: Arthritis treated with curcumin (30 mg/kg/day, orally) for 15 days.

Group IV: Arthritis treated with indomethacin (3 mg/kg/day, orally, as reference drug) for 15 days by gavage starting 7 days after adjuvant injection. At the end of the experimental period (on day 22nd), rats were fasted overnight and killed after general anesthesia by inhalation of ether. Blood samples were collected by cardiac puncture to determine ESR, CRP and WBC count.

Measurement of ankle circumference: Ankle circumference (mm) was measured for paws with a flexible strip three times per week.

Hematological examination: CRP factor was measured by means of an antibody to purified CRP by latex slide test in serum by use of a kit (Holland Medco-ERP Ltd). ESR was determined by a modified method based on ICSH (International Council for Standardization in Hematology)

selected methods (Bull et al., 1993). Briefly, 120µL of blood sample was taken directly and dropped into 30µl of 0.109 mol/L sodium citrate mixed well, and then transferred into a 1.0 mm×100 mm capillary tube. The tubes were held obliquely at an angle of 45ºc and the results were recorded at 15 min. Leukocytes count was determined with a picoscale hematological analyzer.

Statistical analysis: All the results were expressed as mean ± standard error (S.E.M). Data were analyzed using One-way ANOVA followed by Tukey test .$p<0.05$ was considered as statistically significant.

Figure 2 depicts the anti-inflammatory effect of curcumin and indomethacin on the changes in ankle edema of control and experimental animals. Swelling and redness developed over a 24 h period in the foot injected with adjuvant. The finding of this study showed that on 7th day after FCA injection, the ankle circumference was significantly increased compared to the day one in the RA groups ($p<0.05$). The increasing of circumference was significant till the end of study (day 21) ($p<0.05$). Rats injected with saline did not show any joint swelling at any point in the study. Upon curcumin and indomethacin administration, the inflammation was started to decrease significantly when compared by AIA rats ($p<0.05$).

Result

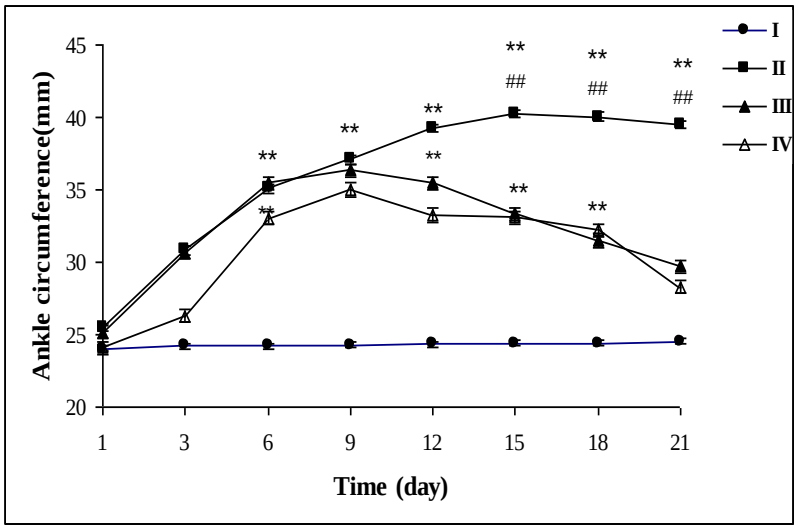

Figure 2. Ankle Circumference changes in arthritic rats. I: control group (-♦-), II: AIA rats (-■-), III: arthritic rats treated with 30 mg/kg curcumin (-▲-) and IV: arthritic rats treated with 3 mg/kg indomethacin (-Δ-). Values represent means ± standard error of the mean (S.E.M). ## $p<0.05$ compared with arthritic rat treated with drugs, ** $p<0.05$ compared with control rats (n=8).

Serum CRP and ESR levels increased by FCA administration (Figure 3A, 3B) in arthritic rats. In comparison with control values, CRP and ESR were raised in arthritic groups ($p<0.05$). These factors decreased significantly in arthritic rats after treated with curcumin and indomethacin ($p<0.05$).

Figure 3C shows that total leukocyte count was significantly increased in the arthritic rats as compared with the control rats ($p<0.05$) and in treated animals with curcumin and indomethacin was significantly decreased when compared with AIA group (II) ($p<0.05$).

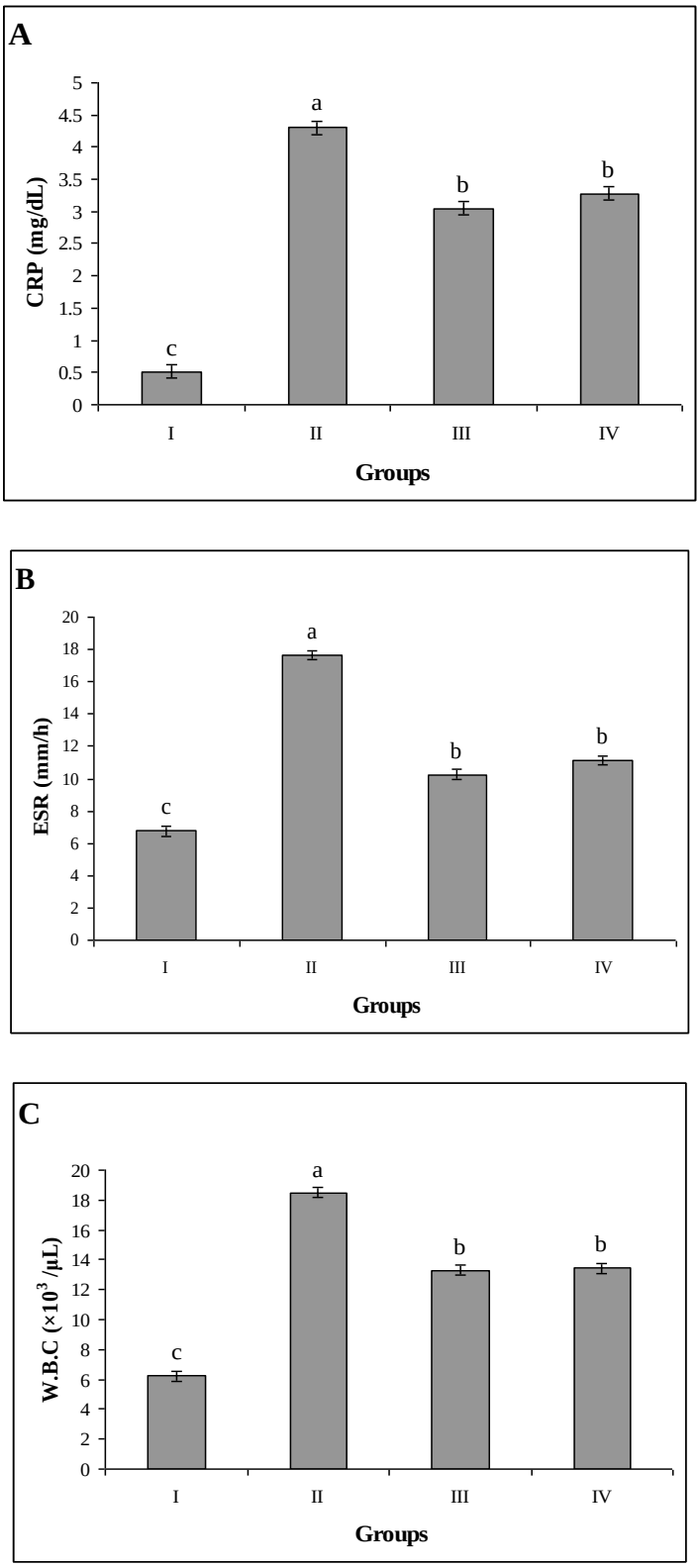

Figure 3. Effect of curcumin and indomethacin on serum CRP (A), ESR (B) and WBC(C) in arthritic rats. I: control group, II: AIA rats, III: arthritic rats treated with 30 mg/kg curcumin and IV: arthritic rats treated with 3 mg/kg indomethacin. Values represent means ± the S.E.M. Same words aren't significant in $p<0.05$ in Tukey test (n=8).

Discussion

Freund's complete adjuvant is generally used to induce arthritis in animal models. Adjuvant arthritis in rat shows several clinical and histological similarities to human RA. In the present study, following a single injection of FCA at the plantar surface, rats developed pronounced arthritis in the paws and ankles, showing 100% incidence. FCA injection resulted in a significant increase in ankle circumference with in 24h. The results of this study indicated that the curcumin exhibits anti-inflammatory properties in adjuvant-induced arthritic rats. There are several similar reports, for example, oral administration of curcumin has been shown to decrease elevated levels of the glycoprotein Gp A72, with concomitant lowering of paw inflammation in arthritic rats (Joe et al., 1997). Funk et al. determined the in vivo efficacy of curcumin in the prevention or treatment of arthritis using streptococcal cell well-induced arthritis, a model of RA (Funk et al., 2006 b). In this model, curcumin prevented joint inflammation when treatment was started before, but not after, the onset of joint inflammation. Results of this study supported the anti-inflammatory effect of curcumin. The molecular basis of the anti-inflammatory properties of curcumin has been attributed to its effects on several targets including transcription factors, enzymes, and cellular signaling molecules, including NF-kB, AP-1, and COX-2. Curcumin has been shown to directly inhibit activation of transcription factors NF-kB and AP-1 (Han et al., 2002;Kang et al., 2004). Curcumin has also been reported to suppress COX-2, the key enzyme in the formation of prostaglandins, a family of compounds derived from arachidonic acid through the COX pathway (Kang et al., 2004; Chun et al., 2003). Prostaglandins are potent mediators in the inflammatory response.

In this AIA model, both ESR and CRP were found to be markedly associated with the development of the disease, significantly elevated ESR and CRP levels were observed in arthritic rats in comparison with the control rats. Administration of curcumin similar to indomethacin 7 days after arthritis induction exhibited an inhibition on over produced plasma CRP and ESR levels, and statistically significant differences were indicated only in the arthritic group (II). IL-1β, IL-6 and TNFα are pro-inflammatory cytokines released from activated macrophages at the site of inflammation, and influencing hepatic metabolism by up regulating acute phase protein gene expression (Baumann and Gauldie, 1994). Elevated IL-1 and IL-6 levels have been reported in isolated spleen cells from rats having elevated CRP levels following the induction of CFA arthritis (Giffen et al., 2003). This kinetic change of the serum IL-1β and IL-6 levels was associated with the elevated ESR and CRP levels. Curcumin can decrease the expression and activity of these cytokines (Biswas et al., 2005), thus can decreased CRP and ESR levels in the blood. In all arthritic groups we observed a markedly higher leukocyte count as compared with the healthy rats. Franch *et al.* and Carlson *et al.* reported that leukocytosis, neutrophilia and an increase in the number of lymphocytes were observed after 21 and 49 days of adjuvant-induced arthritis in rats (Franchand et al., 1994; Calosn et al., 1985). These changes agree with our results in this study. In the present study, leukocytosis was significantly inhibited by curcumin ($p<0.05$). There was no significant difference between treated animals with curcumin and indomethacin concomitantly. However, Funk *et al.* reported that curcumin decreased WBC count in SCW-induced arthritis (Funk et al., 2006 a), which agrees with our results in this study.

In conclusion, these results suggest that curcumin can possess beneficial effects in alleviating arthritic symptoms in AIA model.

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