Turmeric Extracts Containing Curcuminoids Prevent Experimental Rheumatoid Arthritis[#]

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Turmeric has been used for centuries in Ayurvedic medicine as a treatment for inflammatory disorders including arthritis. On the basis of this traditional usage, dietary supplements containing turmeric rhizome and turmeric extracts are also being used in the western world for arthritis treatment and prevention. However, to our knowledge, no data are available regarding antiarthritic efficacy of complex turmeric extracts similar in composition to those available for use as dietary supplements. Therefore, the studies described here were undertaken to determine the in vivo efficacy of well-characterized curcuminoid-containing turmeric extracts in the prevention or treatment of arthritis using streptococcal cell wall (SCW)-induced arthritis, a well-described animal model of rheumatoid arthritis (RA). Arthritic index, a clinical measure of joint swelling, was used as the primary endpoint for assessing the effect of extracts on joint inflammation. An essential oil-depleted turmeric fraction containing 41% of the three major curcuminoids was efficacious in preventing joint inflammation when treatment was started before, but not after, the onset of joint inflammation. A commercial sample containing 94% of the three major curcuminoids was more potent in preventing arthritis than the essential oil-depleted turmeric fraction when compared by total curcuminoid dose per body weight. In conclusion, these data (1) document the in vivo antiarthritic efficacy of an essential oil-depleted turmeric fraction and (2) suggest that the three major curcuminoids are responsible for this antiarthritic effect, while the remaining compounds in the crude turmeric extract may inhibit this protective effect.

Turmeric (*Curcuma longa* L. [Zingiberaceae]) is a perennial native to Asia, where its rhizome is used as a spice (being responsible for the yellow color of curry), a pigment dye for textiles or for skin in religious ceremonies, and a traditional medicine, primarily used for the treatment of inflammatory disorders.[1] Typical dietary intake of turmeric is estimated to be as high as 2.5 g/day (approximately 100 mg curcumin/day) in some countries,[2] while up to 8 g/day of curcumin, one of the three major curcuminoids (curcumin [**1**], demethoxycurcumin [**2**] and bis-demethoxycurcumin [**3**], see Figure 1) that constitute 3% of the total rhizome, is without evidence of side effects in phase I clinical trials.[3]

Organic extracts of the rhizomes of turmeric have previously been shown to have antiinflammatory effects in vitro.[4−8] However, analysis of these published reports is confounded by the use of extracts that are neither well characterized nor uniform in their composition. Most of these studies have evaluated the effects of various uncharacterized extracts designated as "curcumin" that inhibit production of TNF-α and IL-1β, as well as PGE₂.[4−8] The major site of action of these curcumin extracts is thought to be inhibition of transcription factor activation, including activation of NF-κB.[4,5,9] NF-κB activation during inflammation leads to increased gene expression of cytokines (TNF-α, IL-1β), chemokines (MCP-1), and other inflammatory proteins (COX-2) that are critical mediators of joint inflammation in rheumatoid arthritis (RA).[4,10−12]

Analysis of the scant number of publications in the literature investigating the antiarthritic efficacy of turmeric in animal or human studies is again confounded by the lack of characterization of the extracts tested, as well as the variety of models studied. In rodents, an uncharacterized curcumin extract (10−80 mg/kg orally)
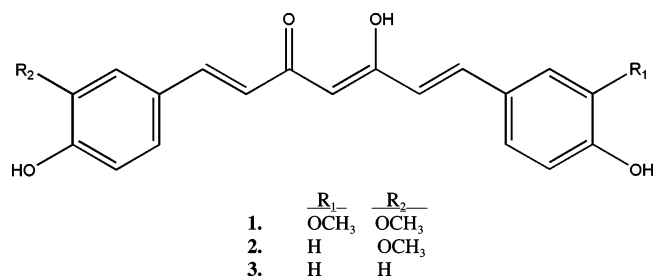

	R₁	R₂
1.	OCH₃	OCH₃
2.	H	OCH₃
3.	H	H

Figure 1. Chemical structures of curcumin (**1**), demethoxycurcumin (**2**), and bis-demethoxycurcumin (**3**).

has been reported to reduce carrageenin-induced paw edema, a model of acute inflammation rather than actual destructive arthritis,[13] as well as paw swelling in adjuvant-induced arthritis (100−300 mg/kg/day orally).[14,15] In humans, one small double-blind crossover study evaluating a single dose of an uncharacterized curcumin extract (1.2 g/day) or phenylbutazone in rheumatoid arthritis (*n* = 18 patients) reported some success in decreasing disease symptoms.[16] In none of these reports was a chemical analysis of what one would assume to be curcumin-enriched extracts presented. In addition, to our knowledge, there are no reports on the antiarthritic effect of unpurified, chemically complex turmeric extracts more analogous in composition to dietary turmeric supplements available for over-the-counter use.

Previous evaluation by our group of randomly chosen, over-the-counter turmeric dietary supplements (*n* = 9) demonstrated that the majority of these extracts do not contain essential oils but are still very complex extracts that are composed of less than 50% curcuminoids (median value, 19.3%).[17] We therefore prepared, chemically characterized, and then tested the in vitro antiinflammatory efficacy, followed by the in vivo antiarthritic efficacy, of a complex turmeric extract depleted in essential oils and containing less than 50% curcuminoids, which we isolated from a commercial source of ground turmeric rhizome. The efficacy of this chemically complex turmeric extract was compared to that of a commercially available curcumin product (greater than 90% curcuminoids by

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Table 1. Chemical and Biological Characterization of Turmeric Samples

	curcuminoid content (% by weight)				
extract	total	curcumin (**1**)	demethoxy-curcumin (**2**)	bis-demethoxy-curcumin (**3**)	in vitro PGE$_2$ inhibition IC$_{50}$ (μg extract/mL)
crude turmeric extract	33.7	21.4	7.2	5.1	0.13
essential oil-depleted turmeric fraction	40.6	25.7	8.7	6.2	0.48
purified curcuminoids (commercial curcumin[a])	93.6	74.2	14.9	4.5	2.66

[a] Although labeled as "98+% curcumin", this product contained all three curcuminoids with total curcuminoid content < 98%.

HPLC analysis). The animal model used, streptococcal cell wall (SCW)-induced arthritis, is a well-characterized animal model of rheumatoid arthritis.[18−20] In this model, over a 28-day course, female Lewis rats develop an initial acute phase of joint swelling followed by a chronic phase of inflammation that is associated with actual joint destruction. Joint histopathology in this model is very similar to that seen in rheumatoid arthritis (RA), a disease typified by recurrent cycles of joint inflammation and gradual joint destruction.[21,22] In addition, a granulomatous inflammatory response, similar to that responsible for inactivating tuberculosis bacilli by walling off the invading bacteria, occurs in the liver and spleen of these animals at sites of SCW deposition.[18−20]

Results and Discussion

Chemical and in Vitro Biological Analysis of Turmeric. HPLC analysis of the essential oil-depleted turmeric extract (henceforth referred to as turmeric fraction) that was used in the in vivo arthritis model revealed a curcuminoid content of 41% and a negligible amount of essential oils (Table 1 and Figure S1B, Supporting Data). The chemical composition of this essential oil-depleted turmeric fraction can be compared to that of the crude turmeric extract in Table 1 and Figure S1A. The in vivo antiarthritic effect of the essential oil-depleted turmeric fraction was compared to that of a commercial curcumin product labeled as "98+% curcumin" (henceforth referred to as purified curcuminoids) that was found on HPLC analysis to be composed of a 94% mixture of three curcuminoids with no evidence of essential oils (Table 1 and Figure S1C). In vitro biological screening for antiinflammatory efficacy revealed a 10-fold lower IC$_{50}$ for the two chemically complex turmeric extracts as compared to the purified curcuminoids with respect to inhibition of PGE$_2$ production (Table 1), suggesting that the complex extracts could be more potent antiinflammatory agents.

In Vivo Antiarthritic Efficacy of Intraperitoneal Turmeric Fraction Treatment. Daily intraperitoneal (ip) administration of the turmeric fraction (23 mg total curcuminoids/kg/day), beginning 4 days prior to SCW administration, significantly inhibited joint inflammation in both the acute inflammatory and chronic destructive phases of arthritis (64% and 72% inhibition, respectively; Figure S2A). In contrast, when the start of treatment was delayed until day 8, i.e., after the resolution of the acute phase but prior to initiation of the chronic phase of arthritis, this same dose of turmeric fraction was without a clinically significant effect on joint swelling (Figure S2B). Daily treatment with a lower dose of turmeric fraction (4 mg curcuminoids/kg/day) beginning 4 days prior to SCW injection was also without significant effect on joint swelling (Figure S2C). In all experiments, which also included vehicle- and turmeric fraction-treated nonarthritic animals ($n = 5−8$/group), there were no signs of toxicity as measured by weight gain, ALT, or creatinine in normal or SCW-treated animals receiving turmeric fraction treatment (data not shown). However, there was a 5−7% mortality in turmeric-treated normal and SCW animals (vs no deaths in vehicle-treated animals) of unknown etiology that was not statistical significant by Fisher's exact test (data not shown).

These results provide the first in vivo evidence of antiarthritic efficacy of a chemically complex turmeric product similar in chemical composition to commercially available dietary supple-

ments. Because pretreatment was required to prevent joint inflammation, these data support the use of curcuminoid-containing turmeric products only for arthritis prevention and not for disease treatment in the face of active inflammation. Mechanistic studies to be presented elsewhere suggest that early treatment with the curcuminoid-containing turmeric fraction may be needed to prevent activation of NF-κB, a transcription factor that induces the expression of key mediators of joint inflammation (Funk et al., unpublished data). It should also be stated, however, that while an 8-day delay before start of treatment with turmeric fraction was not efficacious, the possible effect of a somewhat earlier treatment (e.g., day 3 in the SCW model), as might occur clinically at the first signs of inflammation, was not yet examined.

In Vivo Antiarthritic Efficacy of Intraperitoneal Purified Curcuminoid Treatment. In contrast to the ineffectiveness of low-dose turmeric fraction on joint inflammation (Figure S2C), daily ip administration of the same low dose of purified curcuminoids (4 mg total curcuminoids/kg/day) beginning 4 days prior to SCW administration significantly inhibited joint inflammation in both the acute and chronic phases (75% and 68% inhibition, respectively; Figure 2A, circles). The degree of arthritis inhibition by the purified curcuminoids, as demonstrated by arthritic index (Figure 2A) or arthritis incidence (Figure 3), was similar to that caused by a 6-fold higher (by curcuminoid content) dose of turmeric fraction (Figures S2A and Figure 3). These results suggest that the remaining components of the turmeric fraction, other than the three major curcuminoids (compounds **1−3**), may actually inhibit the protective, antiarthritic effect of the curcuminoids, a finding that runs counter to the prevailing notion that complex botanical agents may provide increased efficacy for disease treatment. The identification of these other components is currently under investigation (Timmermann et al., unpublished data). Interestingly, pretreatment only with the purified curcuminoids for 5 days prior to SCW injection (Figure 2B) was sufficient to prevent joint inflammation over the subsequent 28-day course of the experiment (65% and 59% inhibition of acute and chronic inflammation, respectively), conferring a degree of protection similar to that seen with daily treatment (Figure 2A). This finding is particularly notable, as we are unaware of any other agents that have been shown in this model to have such lasting inhibitory effects on joint inflammation. Moreover, our demonstration of a prolonged protective effect from a brief period of pretreatment with either low-dose purified curcuminoids (Figure 2B) or high-dose turmeric fraction (data not shown) most definitely supports the possible use of curcuminoid-containing turmeric dietary supplements for arthritis prevention and also raises the question of whether continuous dosing would be needed and/or what effect less-than-perfect compliance might have on disease prevention.

In Vivo Antiarthritic Efficacy of Oral Purified Curcuminoid Treatment. Although dietary supplements are obviously taken orally, our initial experiments used an intraperitoneal mode of extract delivery in order to maximize delivery so that botanical bioactivity could be determined and compared independent of possible differential rates of gastrointestinal absorption and further metabolism. Subsequent examination of the effect of daily oral (po) treatment with a 30-fold higher dose of purified curcuminoids to account for a possible lower degree of gastrointestinal absorption
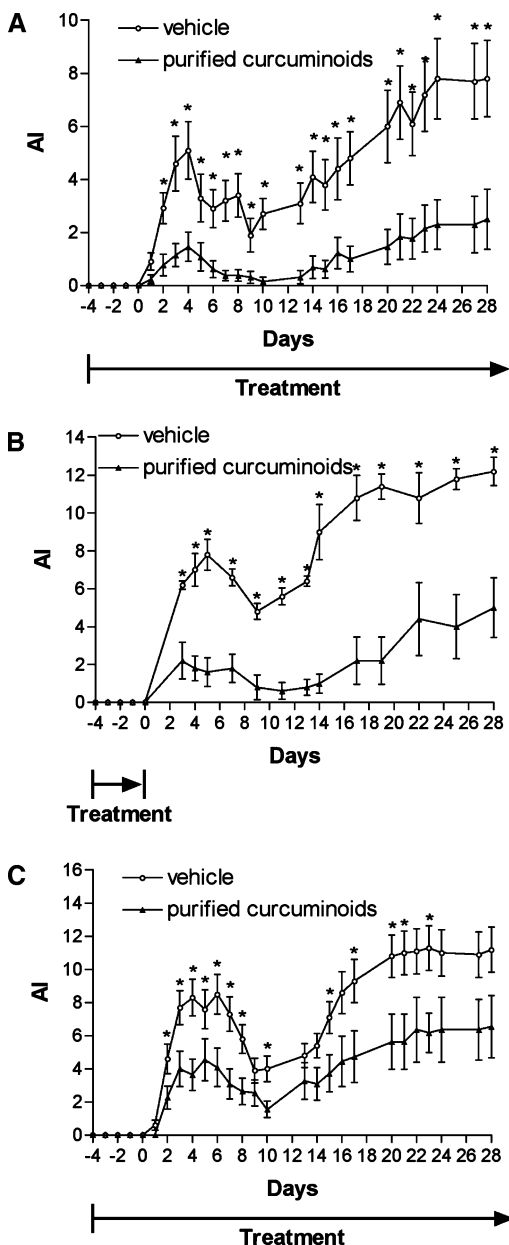
Figure 2. Effect of purified curcuminoids on joint inflammation. Female Lewis rats were injected on day 0 with SCW (25 μg/g) or vehicle. Joint swelling was assessed by daily calculation of the arthritic index (mean $\pm$ SEM) with statistical significance determined by Student's t test as described in methods. (A) Purified curcuminoids (4 mg curcuminoids/kg/day) or vehicle alone ip injections were begun 4 days prior to SCW administration ($n = 11$ animals/group) and continued on a daily basis until 10 days after SCW injection, at which time treatment frequency decreased to 5 days/week. *p < 0.02. (B) Purified curcuminoids (4 mg/kg/day) or vehicle alone ip injections were begun 4 days prior to SCW administration ($n = 5$ animals/group) and continued daily for only 5 days with last dose 6 h prior to SCW administration. *p < 0.01. (C) Purified curcuminoids (117 mg/kg/day) or vehicle alone was administered orally 4 days prior to SCW administration ($n = 10-11$ animals/group) and continued on a daily basis until 10 days after SCW injection, at which time treatment frequency decreased to 5 days/week. *p < 0.05.

was also significantly protective when treatment was begun 4 days prior to SCW administration (Figure 2C). Statistically significant protection was demonstrated in this oral dosing study up until day 23 (48% inhibition on day 3 and 45% inhibition on day 23), after which time the lower degree of protection occurring in this study
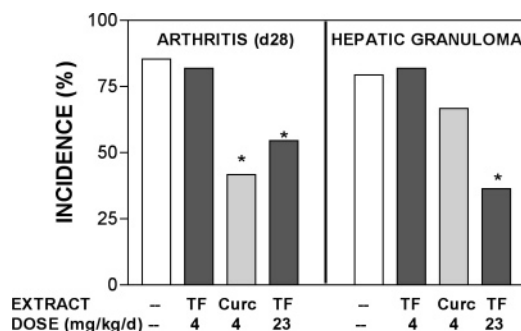

Figure 3. Comparison of effect of turmeric fraction (TF) vs purified curcuminoids (CURC) on incidence of arthritis vs hepatic granuloma formation. Female Lewis rats were injected on day 0 with SCW (25 μg/g) or vehicle. Botanical extracts or vehicle alone ip injections were begun 4 days prior to SCW administration and continued on a daily basis until 10 days after SCW injection, at which time treatment frequency decreased to 5 days/week. Incidence of joint swelling at day 28 was assessed by calculation of the arthritic index ($n = 11-34$ animals/group). The incidence of granuloma formation was assessed histologically as described in methods ($n = 11-23$ animals/group). Statistical significance was determined by Fisher's exact test. *p < 0.05 vs untreated. **p < 0.007 vs untreated.

was no longer statistically significant (42% inhibition on day 28, p = 0.06). This oral dose of curcuminoids corresponds to approximately 1 g curcuminoids/day in a 70 kg person after correcting for the surface area of rats versus humans,[23] a dose well below the 8 g/day curcumin dose previously reported to be without side effects in humans.[3] The effect of higher oral curcumin doses, or the addition of other botanically derived compounds, such as piperine, that can enhance curcumin absorption by 50% and 20-fold in rats and humans, respectively,[24] has not yet been tested in this arthritis model. In contrast to the increased mortality demonstrated with ip turmeric fraction treatment, there was no mortality, or other signs of toxicity, in normal or SCW animals treated ip or orally with purified curcuminoids (data not shown).

Effect of Turmeric Fraction or Purified Curcuminoids on Granuloma Formation. Dose-dependent prevention of granulomatous inflammation by the turmeric fraction paralleled the efficacy of this extract in preventing joint inflammation; high-dose turmeric fraction prevented hepatic granulomas, while low-dose did not (Figure 3). In contrast, there was a dissociation in the antiinflammatory effects of low-dose purified curcuminoids, as this extract prevented joint inflammation while having no effect on hepatic granuloma formation (Figure 3). The ability of the complex turmeric fraction, but not purified curcuminoids, to inhibit SCW-induced granuloma formation could be due to a requirement for (1) a higher dose of the three major curcuminoids for this antiinflammatory effect (i.e., 23 vs 4 mg total curcuminoids/kg/day) and/or (2) additional antiinflammatory effects of the remaining components of the turmeric fraction. In either case, these results demonstrate that a therapeutic window may exist for using purified curcuminoids for arthritis prevention without increasing the risk of reactivating latent granulomatous infections, such as tuberculosis, a disease made quiescent by granuloma formation around the invading microorganism. To state the converse, these results also suggest that antiarthritic doses of more complex turmeric extracts could increase the risk of reactivation of latent granulomatous infections, as has been reported in RA patients with latent tuberculosis infections when treated with TNF inhibitors, another potent antiinflammatory agent.[25,26]

Conclusions. The results of these translational studies provide compelling evidence to support further clinical testing of curcuminoid-containing turmeric dietary supplements for the prevention of rheumatoid arthritis or RA flares. Moreover, these studies suggest that purified curcuminoids may be safer and more efficacious for

this use than complex curcuminoid-containing turmeric extracts. However, several caveats should be mentioned when extrapolating these results to clinical use. To our knowledge, no clinical trials in RA patients have yet been done to document the safety or antiarthritic efficacy of specific turmeric products available for over-the-counter use. We are also not aware of any studies in animals or humans investigating the use of turmeric products for the treatment or prevention of other types of arthritis, such as osteoarthritis. At the same time, the studies presented here do provide strong evidence supporting the need for well-designed in vivo preclinical trials and clinical trials when testing specific antiinflammatory effects of well-characterized botanical extracts. For example, our in vitro biological screening PGE_2 assay suggested that purified curcuminoids might be 10-fold less potent antiinflammatory agents, while our in vivo studies demonstrated the exact opposite with respect to antiarthritic efficacy. In short, the chemical complexity of bioactive botanical extracts perhaps serves as a mirror for the complexity that may also be associated with their rational clinical use.

Experimental Section

General Experimental Procedures. Turmeric rhizome powder was purchased from San Francisco Herb and Natural Food (San Francisco, CA). Commercial curcumin was obtained from Fisher Scientific (ACROS Organics, curcumin 98+%, # 218580100, Lot A017528901) and subjected to quantitative HPLC analysis before use in vitro or in vivo. Extract analyses were performed with an Agilent 1100 series HPLC system with quaternary pump, degasser, thermostated column compartment, thermostated autosampler, diode array detector, and ChemStation for LC 3D, Rev. A.09.03 (1417) software for system control and data acquisition (Agilent Technologies Inc., Palo Alto, CA).

Sample Preparation. The crude turmeric extract was prepared by mechanically stirring turmeric powder (500 g) with methanol (1.8 L) at 25 °C for 24 h. The mixture was filtered through a fritted funnel, and after washing the marc with fresh methanol, the solvent from the combined filtrate and washings was stripped off under vacuum using a 20l Laborota 20 rotary evaporator. The resulting residue, after leaving under vacuum for 48 h, weighed 47.5 g (9.5%). The curcuminoid-containing turmeric fraction was prepared by mechanically stirring turmeric powder (1 kg) with *n*-hexane (3.5 L) at 25 °C for 24 h. The mixture was filtered as above and the marc washed thoroughly with fresh *n*-hexane. The hexane-washed marc, after drying under the hood overnight, was extracted with methanol (3.5 L) by stirring mechanically at 25 °C for 24 h and filtered, and the marc was washed with methanol. The solvent from the combined filtrate and washings was stripped off, and the residue was left under vacuum overnight as described above. The residue was redissolved in methanol and the solvent stripped off. The resulting residue, after leaving under vacuum overnight, was powdered in a mortar and left in a vacuum oven set at 45 °C for a minimum of 48 h. The yield of turmeric fraction was 31.3 g (3.1%).

Chemical and Biological Analyses. For HPLC analyses, triplicates of approximately 1 mg of sample were weighed using an analytical balance, dissolved in 5 mL of methanol, sonicated for 1 min, and diluted with HPLC grade methanol in a 10 mL volumetric flask. One milliliter from each solution was transferred into an amber autosampler vial, and $3 \times 10 \mu L$ were injected onto the HPLC column. Stock solutions of pure curcumin, demethoxycurcumin, and bis-demethoxycurcumin were prepared individually, and their purity was confirmed by HPLC, MS, NMR, and elemental analysis. Samples were injected onto a Synergy, 4 μm, Hydro-RP 250 $\times$ 4.6 mm column coupled with SecurityGuard AJO-4287 guard column (Phenomenex, Torrance, CA). The mobile phases used were as follows: (A) 500 μL of acetic acid/L of Nanopure water; (B) 500 μL of acetic acid/L of acetonitrile. The organic mobile phase was filtered through a 0.2 μm PTFE membrane filter, and the water-based mobile phase was filtered through a 0.2 μm cellulose nitrate filter. The mobile phases were thoroughly degassed before analysis and throughout the analysis. The eluent was monitored at 425, 370, 280, and 250 nm; the flow rate was 1 mL/min. In vitro screening for antiinflammatory activity of extracts, as determined by inhibition of prostaglandin E_2 (PGE_2) production, was conducted before use in animals to ensure reproducibility of extract preparation. Briefly, U937 cells (ATCC, CRL-1593.2), cultured in RPMI 1640 with 25 mM

HEPES and 10% FBS were plated, differentiated with 10 nM PMA (Sigma) for 24 h, washed with media, and then treated with LPS (1 μg/mL) with or without varying concentrations of curcuminoid-containing extracts for an additional 24 h. Culture supernatants were collected and stored at −80 °C prior to PGE_2 immunoassay (R & D Systems, Minneapolis, MN).

Animal Procedures. Following standard protocols[18−20] conducted in accordance with institutional guidelines, female Lewis rats (Harlan, Indianapolis, IN) were administered a single ip injection of vehicle (normal saline) or peptidoglycan-polysaccharide polymers (25 μg rhamnose/g body weight) isolated from the sonicated cell wall of Group A *Streptococcus pyogenes* (Lee Laboratories, Grayson, GA). At the indicated times, control and SCW-treated animals received an intraperitoneal (ip) injection of botanical sample or vehicle (0.5−1 μL/g DMSO) or oral delivery of botanical sample or vehicle (0.5 μL/g 8% saccharine in DMSO). Intraperitoneal treatments with turmeric fraction or purified curcuminoids were administered either beginning 4 days prior to SCW administration (pretreatment protocols) or 8 days after SCW administration (delayed treatment) and were continued on a daily or 5$\times$ weekly basis as indicated. For oral dosing studies, purified curcuminoids were administered orally by syringe beginning 4 days prior to SCW administration and were continued on a daily basis until day 14, at which time dosing was switched to 5$\times$ weekly. For comparison of antiarthritic response to the two samples, dosing was normalized and expressed as mg total curcuminoids/g body weight. Joint inflammation was determined in a blinded fashion by daily assessment of arthritic index (AI) in each distal limb using standard criteria (0 = normal; 1 = slight erythema and edema; 2 = increased edema with loss of landmarks; 3 = marked edema; 4 = marked edema with ankylosis on flexion).[18−20] Where indicated, inhibitory effects of treatments on AI were calculated on day 3 for the acute phase or day 28 for the chronic phase of joint swelling. To monitor for possible toxic effects of treatments in normal or SCW-treated animals, daily weights were recorded, and serum creatinine and alamine aminotransferase (ALT) levels in blood samples obtained 28 days after injection of SCW (or vehicle) were determined using a Hemagen Diagnostics Endocheck Plus Chemistry analyzer to monitor for possible renal- or hepato-toxicity, respectively.

Histology. Liver specimens obtained 28 days after SCW injection were fixed in 10% formalin, and tissues were embedded in paraffin. Granuloma formation was assessed in hematoxylin and eosin (H&E) stained sections of liver using standard criteria.[18−20]

Statistical Analyses. Values are presented as mean ± the SEM with statistical significance determined by ANOVA with post hoc testing or by Fishers Exact Test, as appropriate, using Instat software (Graphpad, San Diego, CA).

Supporting Information Available: HPLC analyses of the turmeric extracts used for the in vivo studies as well as data demonstrating the in vivo antiarthritic efficacy of the turmeric fraction are available in Figures S1 (A, B, and C) and S2 (A, B, and C), respectively. This material is available free of charge via the Internet at http://pubs.acs.org.

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