

# Curcumin-Galactomannoside Interaction Enhances the Bioavailability of Curcumin and Inhibits Inflammation and Oxidative Stress Associated with Experimental Arthritis

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## ABSTRACT

Curcumagalactomannosides (CGM) is a novel bio-available formulation. This formulation was made from curcumin (CM) and it comprises with fenugreek derived dietary fibre composed of galactose and mannose units. In the current study was designed to evaluate the anti-inflammatory action of CGM was compared with CM on carrageenan-induced acute model. The result indicated that the CGM showed the minimal dose with potent inhibitory effect than CM. CGM at 100 mg/kg bwt was found to be potent dose from carrageenan model. From the above data, we evaluated its anti-arthritis effect in adjuvant induced chronic arthritic model. Adult male wistar rats were divided into 4 groups: Normal control rats (NC); Adjuvant induced arthritic control rats (AA); AA+ CGM supplemented (100 mg/kg bwt); AA+ indomethacin administrated (IND 3 mg/kg bwt) for 30 days. The activities of inflammatory markers, antioxidant enzymes, histopathology and radiology of rat paw tissue were analyzed. The result indicated that there was a significant increment in the levels of antioxidant enzymes on CGM supplementation. The up-regulated gene level expressions of inflammatory markers were down regulated by supplementation of CGM. Supplementation of CGM to arthritic rats significantly improved the histological findings in paw as evident by reduced the massive influx of polymorphonuclear cells infiltration and also CGM protects against excess soft tissue volume, degenerative joint changes, sub-chondral erosion and joint space narrowing by radiological analysis. This current study shows that novel bio-available formulation of curcumin as CGM has an antioxidant, anti-inflammatory and anti-arthritis activity in experimental arthritic model.

**Keywords:** Antioxidants; Curcuminoids; Curcumingalactomannosides; Inflammation; Rheumatoid arthritis

## INTRODUCTION

Rheumatoid arthritis (RA) is a common and chronic inflammatory autoimmune disorder that can affect multiple joints that leads to pain, stiffness and progressive destruction of bone and cartilage, characterized by intense, destructive infiltration of synovial tissues produce a series of complex inflammatory cytokines and other soluble mediators in a pathogenic inflammatory cascade [1,2]. This disease affects about 1% of the human population and leads to reduced life expectancy [3]. In RA, activation of macrophages and the release of pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6), prostaglandin E2 (PGE2) and cyclooxygenase (COX) are highly expressed and play key roles in the pathogenesis of RA [4, 5]. PGE2, a bioactive lipid, plays a critical role in acute and chronic inflammation, infection and pain. Also the local and systemic inflammatory response also induce and activate oxidant generating

enzymes like NADPH oxidase, xanthine oxidase, myeloperoxidase, etc. Furthermore, these enzymes produce reactive oxygen (superoxide anion) and nitrogen species like nitric oxide which can exaggerate the pathogenesis through initiation of lipid peroxidation, alteration of antioxidant enzymes and depletion of glutathione [6]. Thus, it follows that suppression of these inflammatory mediators and oxidative stress can be used to treat RA.

Currently available anti-arthritis drug regimen mainly relies on non-steroidal anti-inflammatory drugs (NSAIDs) and disease modifying anti-rheumatic drugs (DMARDs), which can effectively reduce the symptoms of the disease but simultaneously pose potential side-effect [7]. So, there is a strong interest in the development of better anti-inflammatory therapeutic agents for RA that can prevent the progression of disease and confer safe prolonged treatment. The natural occurring phytonutrients in food has been extensively researched and the antioxidants, vitamins, minerals components

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are proven to be beneficial in prevention and therapy of many diseases such as cardiovascular diseases, inflammation and other health problems. Drugs derived from natural products, however are unique in that they have been used for thousands of years for a variety of diseases, but little is known either about the active component in them or the mechanism that has been used as manifest their effect.

Curcuminoids, in turmeric (*Curcuma longa* L.), a traditional spice in south east Asia is known to possess a wide spectrum of beneficial pharmacological activities rendering it a potential therapeutic agent and/or functional food ingredient for the prevention, control and/or treatment of a variety of pro-inflammatory diseases [8, 9]. The unconjugated form of curcuminoids were shown to possess better antioxidant, anti-inflammatory, antiproliferative, neuroprotective, anti-amyloidic, and anti-neuroinflammatory potential [10,11]. However, its optimum potential is limited by its lack of solubility in aqueous solvents and poor oral bioavailability. This led us to investigate the benefits of complexing curcumin with other substances to increase systemic absorption and bioavailability. A novel enhanced bio available formulation of curcumin is formulated using fenugreek-derived dietary fibre for enhancing the therapeutic action of curcumin. Curcumin is complexed with fenugreek (*Trigonella foenum-graceum*) derived dietary fibre composed of galactose and mannose units to form Curcumin galactomannosides (hereinafter referred to as 'CGM', patented and registered formulation under the trademark, CurQfen®) [12].

In the current study, we focused mainly on the anti-inflammatory effect of bio-available formulation of Curcumin galactomannosides (CGM) complex and evaluate the anti-inflammatory activity of CGM in comparison with Curcumin and evaluate the molecular level action of CGM on experimental arthritic model.

## MATERIALS AND METHODS

### Materials

Freund's complete adjuvant was purchased from Sigma Aldrich Co., USA. Biochemical agents used in the study were purchased from Sigma Chemical Company, St. Louis, MO, USA and all other chemicals were of analytical grade and purchased from Merck, Mumbai, India. RNA isolation kit, RT-PCR kit, ELISA kits and antibodies were purchased from Eppendorf India Ltd, Chennai, India. Plasma CRP (C-reactive proteins) level was measured using the kit manufactured and supplied by M/s Diasys Diagnostics GmbH, Germany.

### Preparation of 'standard curcumin' (CM) and Curcuma galactomannosides (CGM)

Commercial grade natural unformulated curcumin (standard curcumin) with 95.1% purity was obtained from M/s Akay Flavours and Aromatics Pvt Ltd, Cochin, India. CGM containing 38.6% curcuminoids was prepared by following the procedure of [13].

### Animals

Adult male Wistar rats (weighing  $150 \pm 10$  g) bred in the host department animal facility were used for this study. They were kept in a controlled environment for temperature (24°C-26°C), humidity (55%-60%) and photoperiod (12:12 h light-dark cycle). A commercial laboratory balanced diet (Amrut Laboratory Animal Feeds, Maharashtra, India) and tap water were provided ad libitum. The animals received humane care, in compliance with

the host institutional animal ethics guidelines. All experiments were conducted as per the guidelines of the animal ethics committee CPCSEA (Registration CAF/Ethics/446) according to Government of India accepted principles for laboratory animals' use and care.

### Evaluation of anti-inflammatory effect of CM and CGM in carrageenan-induced rat paw edema (acute inflammatory model)

Anti-inflammatory activity was measured using carrageenan induced rat paw edema assay [14]. The different groups of rats were administered intraperitoneally with different doses of CM (25, 50, 100, 200 mg/kg.bwt) and CGM (25, 50, 100, 200 mg/kg.bwt) and standard drug diclofenac (DIC) (10 mg/kg.bwt). After 1h, 0.1 ml, 1% carrageenan (an edematogenic agent) suspension in 0.9% NaCl solution was injected into the sub-plantar tissue of the right hind paw. The paw volume was measured by a Plethysmometer. The measures were determined at 0 h (Vo: before edematogenic agent injection) and 1,2,3,4 and 5 hours intervals later (Vt). The difference between Vt and Vo was taken as the edema value.

### Experimental design for adjuvant-induced arthritis (chronic inflammatory model)

For immunizations, 0.1 mL of complete Freund's adjuvant containing heat killed mycobacteria in paraffin oil was injected to the right hind paw of animals.

Animals were grouped as follows:

Group I: Normal control rats (NC)

Group II: Adjuvant induced arthritic control rats (AA)

Group III: AA+ CGM (100mg/kg body weight in normal saline)

Group IV: AA+ indomethacin (IND 3 mg/kg body weight in normal saline).

Duration of experiment was 30 days. After overnight fasting, rats were sacrificed by euthanasia. For histological analysis, paw tissues were dissected, fixed in 10% buffered formalin and then decalcified for 7 days in 20% EDTA. The tissues were then processed and embedded in paraffin. Paw tissue and blood were also collected for various biochemical estimations. White blood cell (WBC) count was determined using a haematology analyser (Model-Diatron, Wein, Austria) and Plasma CRP level was measured by using Diasys Diagnostic kit (Germany). Serum was separated from the clotted blood sample by centrifuging at 5000 rpm for 10 min at 4°C and was stored at -20°C for analyses. Protein was assayed by [15].

### Measurement of oxidative stress in tissues

The supernatant obtained by centrifugation of the homogenate of the paw tissues was employed for the analysis of various endogenous antioxidants. Catalase (CAT) activity was determined by measuring the rate of decomposition of hydrogen peroxide at 240 nm and expressed in terms of units/mg protein [16]. The superoxide dismutase (SOD) activity was estimated by the nitro blue tetrazolium (NBT) reduction method [17]. Glutathione peroxidase (GPx) activity was followed by the method of [18] and other based on the oxidation of glutathione in the presence of H<sub>2</sub>O<sub>2</sub>. Glutathione (GSH) activity was measured by the method of [19] using 5, 5-dithiobis-2-nitrobenzoic acid (DTNB) reagent. Lipid peroxidation was estimated as thiobarbituric acid reactive substances (TBARS) and expressed as mmol of malondialdehyde

produced [20].

### Determination of NO concentrations in serum

NO was measured as its breakdown product of nitrite by using the Griess method. In the presence of H<sub>2</sub>O, NO is rapidly converted into nitrite and nitrate. Total production of NO therefore may be determined by measuring the stable NO metabolite nitrite (NO<sub>2</sub><sup>-</sup>). Equal volume of paw tissue supernatant and Griess reagent (1% sulphanilamide and 0.1% N-[naphthyl]ethylenediamine dihydrochloride:1:1) was mixed and absorbance was measured at 550 nm. The amount of nitrite was calculated from a NaNO<sub>2</sub> standard curve.

### Activity of cyclooxygenase (COX) in paw tissue

COX activity was assayed according to the method described by some author; Tissues were incubated with Tris-HCl buffer (pH 8), 5 mM glutathione, and 5 mM hemoglobin for 1 min at 25°C. The reaction was started by the addition of 200 µM arachidonic acid and followed by the incubation at 37°C for 20 min. The reaction was terminated after the addition of 10% trichloroacetic acid in 1 N hydrochloric acid. Following centrifugal separation and addition of 1% thiobarbiturate, COX activity was determined by reading absorbance at 530 nm.

### RT-PCR analysis of rat IL-6, TNF-α, iNOS and COX-2

Total RNA was isolated from paw tissues using RNA isolation kit (Sigma Aldrich) according to manufacturer's instructions. Primer pairs for rat IL-6, TNF-α, iNOS, COX-2 and GAPDH were as follows:

RT-PCR was performed in an Eppendorf thermocycler, using two step RT-PCR kit where reverse transcription and DNA amplification was done separately. 2 µg of total RNA was used as template in the first reaction (cDNA synthesis) that included dNTPs, oligo (dT) and reverse transcriptase enzyme. The second reaction included appropriate primers, PCR enzyme, cDNA formed in the first step and dNTPs. The PCR products were separated by electrophoresis on 1.5% agarose gel containing ethidium bromide and visualized by transillumination. The images were photographed and the intensities of bands were measured. The amounts of target PCR products were normalized against GAPDH PCR product in the corresponding samples.

### Histopathological analysis of paw tissue

The entire paw tissue sections (5 µm) fixed by immersion at room temperature in 10% formalin solution. For histopathological examinations, paraffin-embedded paw tissue sections were stained with hematoxylin-eosin (H and E) followed by examination and photographed under a light microscope for observation of structural abnormality. The severity of paw tissue inflammation was judged by two independent observers blinded to the experimental protocol.

### Radiological analysis

Before sacrificing the animals, X-rays were taken at the joints of the hind paw of the animals for evaluating the bone damage. Radiographs were taken using X-ray apparatus and industrial X-ray film (Fuji photo film, Japan). The X-ray apparatus was operated at 220 V with a 40 V peak, 0.2 second exposure time, and a 60 cm tube-to film distance for anterior-posterior projection.

### Statistical analysis

The results were analyzed using a statistical program SPSS/PC+, version 11.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA was employed for comparison test of significant differences among groups. Pair fed comparisons between the groups was made by Duncan's multiple range tests. P<0.05 was considered as significant.

## RESULTS

### A comparative effect of CM and CGM on carrageenan-induced paw edema rats (dose response study)

To investigate, the comparative effect of anti-inflammatory activity of the CM and CGM was evaluated by using carrageenan-induced paw edema method. Carrageenan induced paw edema model was treated with the different concentration of CM (25, 50, 100, 200 mg/kg bwt) and CGM (25, 50, 100, 200 mg/kg bwt). At the dose of 100 mg/kg bwt CGM showed 82% of the paw edema inhibition as compared with the different doses of CM treated rats. From this result, CGM showed a minimal dose with potent inhibitory effect than CM on carrageenan induced paw edema and as compared to the reference drug.

### The percentage inhibition of paw volume by CGM (adjuvant model)

The percentage of inhibition of paw volume by CGM at a dose of 100 mg/kg bwt showed higher inhibitory effect on adjuvant-induced rats than indomethacin treated rats. As shown in Table 1, 82% of inhibition by CGM compared with standard drug indomethacin group on the 21st day.

**Table 1:** The percentage inhibition of paw volume by CGM (adjuvant model) Values expressed as average of six rats in each group.

Groups	% Inhibition of paw volume		
	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day
Adjuvant induced	0	0	0
CGM treated (100 mg/kg)	62 <sup>a</sup>	70 <sup>a</sup>	82 <sup>a</sup>
Indomethacin (3 mg/kg)	33 <sup>a</sup>	52 <sup>a</sup>	72 <sup>a</sup>

Note: aStatistical difference with adjuvant-induced group at P<0.05.

### Effect of CGM on inflammatory markers in experimental rats

The levels of CRP, total WBC count, TBARS, nitrate and total COX were increased significantly (P<0.05) in adjuvant-induced arthritic rats. Administration of CGM was reduced markedly (P<0.05) as compared with indomethacin treated rats and hereby, decreased the CRP level in plasma, total WBC count, TBARS, nitrate and total COX levels.

### Effect of CGM on the activity of SOD, CAT, GPx and GSH level in adjuvant-induced arthritic rats

Adjuvant-induced arthritis creates an oxidative stress in the paw homogenates in the form of significant (P<0.05) reduction in SOD, GPx activities, CAT and the GSH level in comparison with the normal control group (P<0.05). These deleterious effects associated with adjuvant-induced arthritic condition were improved by CGM administration, the level of antioxidants and GSH levels



significantly increased ( $P < 0.05$ ) as compared with indomethacin-induced rats.

### Expression of the pro-inflammatory markers like COX-2, iNOS and cytokines like IL-6, TNF- $\alpha$ by RT-PCR

The gene expression of COX-2, iNOS was upregulated in adjuvant-induced arthritic rats. Cytokines like IL-6, TNF- $\alpha$  also be increased significantly in AA rats. Administration with CGM, down regulated the gene expression of pro-inflammatory markers like COX-2, iNOS and also cytokines like TNF- $\alpha$ , IL-6 as compared with indomethacin treated rats.

### Effect of CGM on paw histopathological picture in adjuvant-induced arthritic rats

Histopathological studies indicated that inflammatory cell infiltration, proliferated epithelium, proliferated collagen, epidermal edema were markedly suppressed in CGM administrated rats.

### Effect of CGM by radiological analysis

Radiological study showed that arthritic rats suffered serious bone resorption and joint narrowing; treatment with CGM suppressed joint pathology as well as joint swelling in the rat hind paw.

## DISCUSSION

Concerns about the safety and efficacy of many drugs used for the treatment of autoimmune diseases have persisted for many years and researchers put high efforts to find the new drugs with fewer side effects. Rheumatoid arthritis is a chronic, systemic disorder with symmetrical, inflammatory polyarthritis that may produce progressive joint damage. The lack of a potent, reliable treatment for early RA is a major problem in modern medicine. According to the older treatment strategy of the treatment pyramid, treatment of newly diagnosed RA was to be started with NSAIDs and if the treatment failed to alleviate the symptoms, more potent DMARDs were to be tried. Recently, it was reported that natural product might be good treatment candidates that satisfy these requirements and it could provide a safer alternative because generally their use is devoid of adverse effects. Curcumin is one such natural product and is traditionally used to cure number of inflammatory diseases. Curcumin is rapidly metabolize, conjugation in liver and excreted in the feces. This clearly states the poor systemic bioavailability of curcumin. Because of the poor bioavailability and rapid plasma clearance and metabolism of curcumin, its therapeutic applications are limited. This led us to investigate the benefits of complexing curcumin with other substances to increase systemic absorption and bioavailability. The present study aims to evaluate the anti-inflammatory activity of novel formulation of curcumin as curcumingalactomannosides (CGM) with enhanced curcumin bioavailability would significantly in an adjuvant induced arthritic rat's model. A novel enhanced bio available formulation of curcumin is formulated using fenugreek-derived dietary fibre for enhancing the therapeutic action of curcumin. Curcumin is complexed with fenugreek (*Trigonella foenum-graceum*) derived dietary fibre composed of galactose and mannose units to form Curcumin galactomannosides-(CGM) [13].

Paw swelling is one of the major factors in assessing the degree of inflammation and curative efficacy of drugs. Here, the CGM treated rats showed paw edema inhibition of 82% in the

carrageenan model as compared with CM treated rats showed paw edema inhibition of 72%. By the comparison of CGM and CM, the minimal dose with potent inhibitory effect was seen in CGM at 100 mg/kg bwt has 82%. CGM administration provided enhancement in free curcuminoids bioavailability with improved pharmacokinetics when compared to CM.

Increase in WBC count has been suggested to be one of the characteristic diagnoses of arthritis. In our present study, AA induced arthritic animals showed elevated WBC level. CGM treatment significantly decreased WBC, revealing its beneficial role against arthritis. CRP is an important biomarker for various inflammatory diseases. CRP is secreted by the liver in response to a variety of inflammatory cytokines. An elevated CRP level can provide support for the presence of an inflammatory disease, like rheumatoid arthritis. Our findings represent increased CRP in AA rat leads to the release of acute phase protein into the blood. Administration of CGM decreased the CRP in plasma and the level was lower than that of indomethacin treated rats.

Prostaglandin is formed by the interaction of two distinct but related enzymes, COX-1 and COX-2. The constitutive enzyme COX-1 is responsible for maintain normal renal function, gastric mucosa integrity and hemostasis and the inducible isoform COX-2 is unregulated in inflamed tissue and is therefore thought to be responsible for the enhanced production of prostaglandins. In the present study, administration of CGM showed significant inhibition of paw edema volume. Also the activity of total COX and COX-2 expression was decreased in CGM treated groups. Reduction of paw swelling and decreased expression of COX-2 showed the immunological protection rendered by the CGM.

Production of NO in appropriate magnitude serves as a key-signaling model in various physiological processes. But at the same time NOS has been found to cause pathological conditions in chronic inflammation. Our study showed that nitrite production in CGM treated animals was significantly lower as compared to adjuvant treated rat, suggests that decreased progression of chronic inflammation in animals might have been a decreased cellular production of NO by inhibiting iNOS activity. CGM also significantly suppresses the iNOS expression. Therefore, the inhibitory activity of CGM may be due to their anti-inflammatory properties and ability to counteract NO induced oxidative damage, which eventually helps in re-modelling of cells.

The reactive oxygen species degrade synovial fluid and induce depolymerization of hyaluronic acid which in turn leads to a loss of viscosity in the joints. The cartilage destruction by ROS in arthritic rats treated with CGM has strongly inhibited through increase in the levels of endogenous enzymatic antioxidants, such as SOD, CAT, GPx and non-enzymatic antioxidant GSH. It is known that oxidative damage induced by ROS is an important mechanism that underlies destructive and proliferative synovitis and articular degradation and a remarkable increase in ROS and H<sub>2</sub>O<sub>2</sub> in arthritic rats is observed. High lipid peroxidation levels were an indicator of reduced antioxidant capacity and increased oxidative stress in RA, where CGM supplemented animals showed great reduction in TBARS values as compared to indomethacin induced group, though TBARS was significantly high among AA-treated animals as compared to the normal control group. It is thus observed that CGM has an enhanced ability to increase the antioxidant status and thereby to reduce the oxidative stress in RA, as compared to the normal rats.

Cytokines have a fundamental role in causing inflammation and now it has become clear that cytokines are the prime suspects in articular destruction. The rheumatoid joints have infiltrating macrophages that can secrete chemokines and cytokines of the innate and adaptive immune system. The recruitment of activated T and B cells involves secretion of chemokines by antigen presenting cells and synovial endothelium. Activated T cells produce cytokines in the joints further amplifying the inflammatory cascade. TNF- $\alpha$  has been reported to be involved in early joint swelling as well as in chronic joint inflammation and the concomitant erosive changes in cartilage and bone. IL-6 is a pleiotypic cytokine with a wide range of biological activities in immune regulation, hematopoiesis, inflammation and oncogenesis. IL-6 is reported to play devastating role in cartilage and bone degradation during arthritis. In the present study, the gene expression of IL-6 was found elevated in arthritic rats. In the present study, CGM supplementation significantly down regulated the expression of IL-6 in paw tissue of arthritic rats, thereby reducing disease severity. It is also known to be responsible for the increase of serum g-globulin and the emergence of rheumatoid factors. High levels of IL-6 have been observed in both sera and synovial fluids from the affected joints of patients with RA. CGM significantly reduced the production of TNF- $\alpha$  and IL-6. These data indicate that CGM may have the potential to regulate pro-inflammatory cytokines in arthritic condition.

From the above results were supported by histopathological and radiological analysis. Histopathological studies indicated that inflammatory cell infiltration, proliferated epithelium, proliferated collagen, epidermal edema were markedly suppressed in CGM administrated rats. Radiological study showed that arthritic rats suffered serious bone resorption and joint narrowing; treatment with CGM suppressed joint pathology as well as joint swelling in the rat hind paw.

## CONCLUSION

From the present experimental findings of both pharmacological and biochemical parameters observed from the current investigation, it is concluded that at the dose of 100 mg/kg bwt of novel bioavailable formulation of curcumin as curcumingalactomannosides (CGM) possesses potentially useful anti-inflammatory and anti-arthritis activity since it gives a positive result in controlling inflammation in adjuvant induced arthritic model in rats. The drug is a promising anti-inflammatory as well as anti-arthritis agent of food supplement in the treatment of inflammatory disorders.

## CONFLICT OF INTEREST

Authors disclose the conflict of interest. It is the patented curcumin formulation of M/s Akay Flavours & Aromatics Pvt Ltd, Cochin, India, trademarked as CurQfen®.

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