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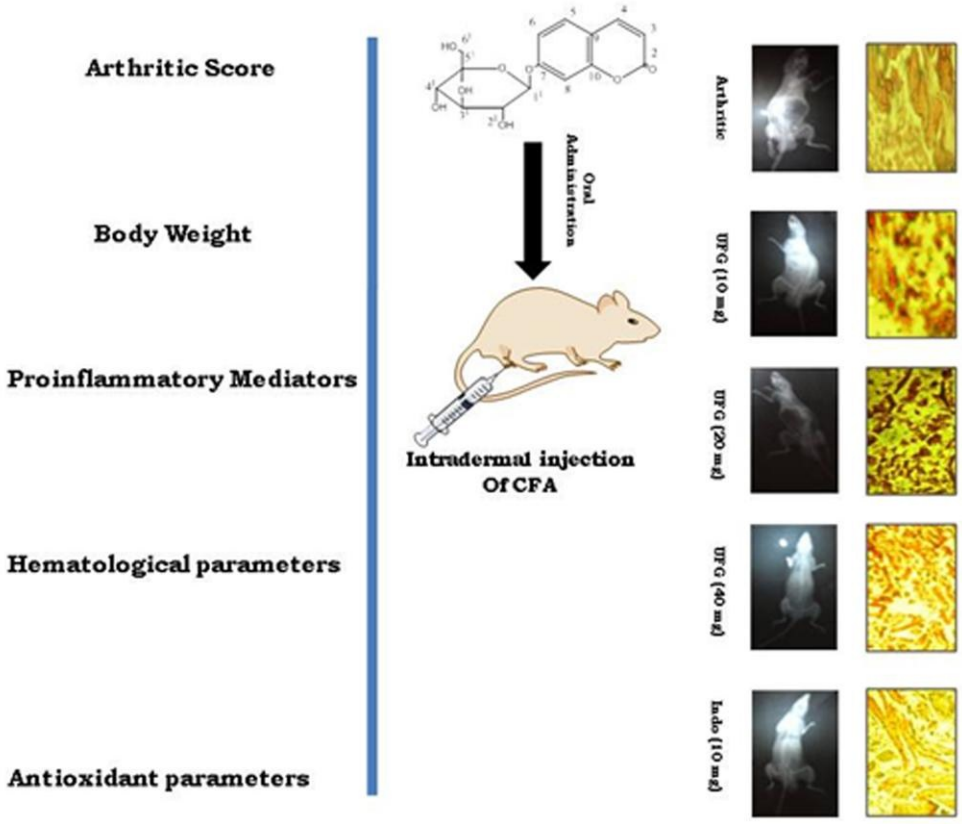

Graphical Abstract: Umbelliferone β-D-galactopyranoside Exerts Anti-inflammatory Effect by Attenuating COX-1 and COX-2

Umbelliferone β-D-galactopyranoside Exerts Anti-inflammatory Effect by Attenuating COX-1 and COX-2

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Abstract

Umbelliferone β-D-galactopyranoside (UFG) (benzopyrone) is a member of coumarin family, found in many plants exhibiting numerous pharmacological actions. The current experiment was carried out to exemplify the anti-inflammatory potential of UFG on chronic inflammation induced by complete freund adjuvant (CFA) (heat killed *Mycobacterium tuberculosis*) in experimental rats. Arthritis in rats were induced by intradermal administration of CFA (0.05 ml) in right hind paw which was confirmed by development of paw edema and arthritic index in comparison with normal control. The antiarthritic activity of UFG was determined by its ability to inhibit various biochemical markers, viz., proinflammatory, antioxidant enzymes, hematological parameters elevated upon administration of CFA. UFG was also tested for their inhibitory activity against cyclooxygenase-1 (COX-1) and COX-2 via enzyme inhibition assay and the results were monitored with the help of spectrophotometrically with a 96-well plate reader. The result of the study showed that UFG in a dose of 10, 20 and 40 mg/kg/day, p.o., secures rats against paw edema and arthritic score developed during arthritis. It markedly alters hematological and oxidative stress induced by the adjuvant. Moreover, the changes brought in inflammation/arthritis serum markers were reverted back to near normal level upon UFG treatment in dose dependent manner. Histopathological analysis of the joints of subjects showed UFG significantly decreases mononuclear infiltration and synovial hyperplasia which confirmed the utility of UFG as antiarthritic agents. In COX inhibition assay UFG found to act prominently to inhibit COX-2 then COX-1 which suggest as its plausible mechanism of action. The current

study clearly indicated that UFG possesses anti-inflammatory effect against CFA induced arthritis via suppressing COX-2 inhibition.

Keywords: Umbelliferone β-D-galactopyranoside, Antiarthritic, hematological parameters, Antioxidant.

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Introduction

Rheumatoid arthritis (RA) is one of the most common chronic autoimmune diseases that affect the cartilage destruction, synovial joints and typically producing the symmetrical arthritis, which leads the joint disability, joint destruction and deformity.[1-2] Rheumatoid arthritis condition start showing an upward curve with increasing sedentary lifestyle of the people with less movement of the body. Another reason for developing the arthritis is changing the lifestyle, food habit, chemical toxicity, insecticides, pollution and wide array of factors, which play a crucial role in the pathogenesis of crippling and painful syndrome of arthritis. In India approximately 13 million people are affected by some form of arthritis.[3] The currently available treatment for arthritis is DMARDs (disease modifying anti-rheumatic drugs) like azathioprine, methotrexate, leflunomide, sulphasalazine, hydroxychloroquine, NASIDs (non-steroidal anti-inflammatory drugs) like etoricoxib and corticosteroids like methylprednisolone and prednisolone. As above discussed treatment having the side affects such as NSAIDs causes gastric lesion, DMARDs causes hematological alteration and corticosteroids causes the serious health problems. The use of these drugs as an anti-arthritic agent not succeeded in all the cases. Therefore, new antiarthritic drug lacking those effects as being searched all over the world as alternates to DMARDs, NSAIDs and corticosteroids.[4]

Asian countries viz., India, China, Pakistan, Bangladesh, Nepal, Japan, Thailand and Srilanka are the gross place for the cultivation of medicinal plants, shrubs and herbs. In Ayurveda, *Aegle marmelos* Corr. (Rutaceae) extensively used plant to treat various diseases like diabetes, inflammation, asthma, indigestion, hypochondria, typhoid, melancholia, weakness, fever, hemorrhoids and heart palpitation etc. *Aegle marmelos* Corr. is a large tree, widely distributed in India, Pakistan, Bangladesh and China.[5-6] The different parts of *Aegle marmelos* Corr. used as an ethanomedicine including antibacterial and antifungal,[7] hypoglycemic (Ponnachan et al., 1993; Karunanayake et al., 1984; Das et al., 1996), diabetes,[8-10] anti-inflammatory,[11] regulation of thyroid hormone,[12] antifertility,[13] antihyperlipidemic[14] etc.

Umbelliferone β-D-galactopyranoside (fig. 1) is a benzopyrone in nature, a member of the coumarin family, found in many kinds of medicinal plants. Umbelliferone has been shown to diminish the lipid peroxidation and chemically induced colon carcinogenesis,[15] hepatic marker enzyme in Streptozotocin induced diabetes,[16] antioxidants and COX-2 inhibitory properties,[17] anti-inflammatory,[18] antidiabetic, antihyperlipidemic and antioxidant activity.[19-20] Presently,

there is no published source for the claim about the anti-arthritic and anti-inflammatory activities of UFG and its possible mechanism of action.

Material and methods

Umbelliferone β-D-galactopyranoside identification and characterization

The identification and characterization of Umbelliferone β-D-galactopyranoside (UFG) has been previously reported by Kumar et al.[20] The chemical structure is displayed in fig. 1.

Drugs and chemicals

Complete Freund's adjuvant (CFA) was purchased from Sigma Chemical Company, USA and Indomethacin (Micro Lab Pvt. Ltd., India) were purchased from the approved vender. Other chemicals like turpentine oil, formaldehyde, carboxyl methyl cellulose and reagents used for the study arose out of analytical grade and procured from an approved vender.

Animals and research protocol approval

Swiss albino Wistar rats (150-220 g) were used for the study. The animals were procured from the Central animal house, Siddhartha Institute of Pharmacy (SIP), Dehradun. The animals were housed under standard conditions of temperature (25 ± 1°C), relative humidity (55 ± 10%), and 12h light/dark cycles and received the standard pellet diet (Lipton rat feed, Ltd., Pune) with water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) constituted as per the rules of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India from the SIP (1435/PO/a/11/CPCSEA).

Drug solution

UFG and indomethacin were emulsified with 2% CMC (carboxyl methyl cellulose).

In vivo anti-inflammatory activity

Inflammation induced by inflammatory mediator

The antiinflammatory effect of UFG was evaluated against the various phlogistic or inflammatory mediators.[21] The particular potency of edemogens, the volume injected, and the time for estimation of edema are shows in parenthesis: turpentine oil (TO), arachidonic acid (AA) and prostaglandin (PGE_2). The inflammatory mediators were induced in the right hind paw of animals after 1 hr of tested and standard drug (indomethacin and aspirin) administration of fasted rats.[22] The joint diameter was estimated using the screw gauge micrometer.[5]

Complete fruend's adjuvant (CFA) induced arthritis in rats

The animals were divided into seven groups and six animals each. Group I (Non edema): served as vehicle control, 2% CMC, p.o; Group II (Non edema): treated with UFG 40 mg/kg, p.o.; Group III (edema control): 2% CMC, p.o.; Group IV (edema control): treated with UFG (10 mg/kg), p.o. ; Group V (edema control): treated with UFG (20 mg/kg), p.o.; Group VI (edema control): treated with UFG (40 mg/kg), p.o.; Group VII (edema control): treated with Indomethacin (10 mg/kg), p.o. Chronic arthritis was induced in all group rats by intradermal injection of CFA (0.5% w/v) into the all group animals except normal control group rats.[23] The edema of the hind paw was estimated by using the micrometer screw gauge.[5]

Arthritic index in CFA rats

Arthritic index was used for the estimation of chronic arthritis. CFA induced arthritic rats showed the clinical symptom of arthritis which was evaluated by using the visual scoring system on scale 0-4, no change: score 0, swelling and erythema of limb: score 1; mild swelling and erythema of limb: score 2; gross swelling and erythema of limb: score 3; gross deformity and inability of limb; score 4. A score of 4 limbs were counted and score more than 1 confirmed the arthritis and maximum score of arthritis is 16.[23,24]

Biochemical analysis

For estimation of biochemical parameters, blood sample was collected from all groups' rats by puncturing the retro-orbital under mild anesthesia condition and samples were collected into anticoagulation tubes. Different enzyme reagents were added to determined the White blood cells (WBC), Red blood cells (RBC),[25] Erythrocyte sedimentation rate (ESR)[26] and Hemoglobin (Hb).[27]

Measurement of Tissue Marker Enzymes

Alanine transaminases (ALT), alkaline phosphatase (ALP) and aspartate transaminase (AST) were estimated by using the reported method of King with minor modification.[28]

Antioxidant marker

All group rats were scarified at the end of the study using the excess of diethyl ether and the cartilage tissue was isolated for the estimation of Superoxide dismutase (SOD), Glutathione peroxidase (GPx),[29] Malondialdehyde (MDA) and Glutathione[30] were estimated by using the reported method with minor modification.

Inflammatory mediators

The cytokines inflammatory mediators like tumor necrosis factor – α (TNF-α) and Interleukin-6 (IL-6), Interleukin-2 (IL-2), Interleukin-1β (IL-1β) and Interleukin - 1α (IL-1α) was measured using the ELISA kits (Biosource Int., Camarillo, CA, USA) according to the instruction provided by the manufacture.[23]

Histopathological Examination of Joints

End of the experimental period, all group rats were killed by cervical dislocation. Hind paws of the all group rats were removed and fixed in the neutral buffered formalin (40%). The preserved hind paw of the rats were left for the 10 days for decalcification and embedded in paraffin for histological examination. The section of the hind paw was stained with the eosin and Hematoxylin.

In vitro COX and LOX assay

UFG and indomethacin both were examined for COX (COX-1 and COX-2) and 15-LOX inhibitory assay by using the COX and LOX inhibitory screening kit (Catalog No. 760111, 760700; Cayman Chemical, USA) following the manufacture's instruction.[23]

Statistical analysis

All the data were expressed as mean±SEM and an analysis of variance (ANOVA) was used for the statistical analysis using Graph Pad Prism version 5.0. The values were considered to be significant when the P value was $p<0.05$.

Result

Effect of UFG on phlogistic mediators

Oral administration of UFG significantly inhibited the paw edema at dose dependent manners as compared to untreated control; with a significant ($P<0.001$) inhibition of 14.53, 37.99 and 59.78% respectively for 10, 20 and 40 mg/kg b.w. dose of UFG, when the paw edema was estimated after 3 h of TO injection. On the contrary, oral administration of standard drug indomethacin (10 mg/kg) exhibited a relative inhibition of 57.82% (Figure 2A).

A considerably inhibition of paw edema was observed in UFG received rats in AA induced paw edema model. UFG showed the 19.30, 42.09 and 61.03% inhibition at UFG dose of 10, 20 and 40 mg/kg b.w. as compared to inflammatory control rats. Indomethacin showed the 59.38 % inhibition of paw edema in AA induced inflammatory rats (Figure 2B).

PGE_2 induced inflammation was significantly inhibited of 17.09, 28.78 and 64.29% respectively for 10, 20 and 40 mg/kg b.w. dose of UFG, when compared to the control rats. On the other hand, oral treatment of indomethacin revealed the 63.01% inhibition against the PGE_2 induced inflammation (Figure 2C).

Effect of UFG on CFA induce arthritis

CFA developed the redness and swelling over a 24 h period in the injected hind paw of rats and achieved utmost intensity on day 4 (developing the first swelling stage). Therefore, swelling and redness gradually increased until the 8^{th} day and after that increased the swelling again when circulated arthritis appeared and develops the secondary swelling stage, which was greater than the first swelling stage and reached at maximum level on day 21-25. The secondary stage was confirmed by increase the swelling in non-injected paw. Oral administration of UFG and indomethacin significantly ($P<0.001$) suppressed the swelling as well as minimized the arthritic index, which was occur due to form of polyarthritic, and inhibited the redness/paw edema in CFA induced arthritic rat hind paw (figure 3). Adjuvant induced arthritic rats showed the joint edema recovery 64.10, 79.49 and 88.89% of UFG dose 10, 20 and 40 mg/kg, respectively. Indomethacin treated rats showed the 84.62% recovery from the adjuvant induced disease (Figure 3).

Effect of UFG on body weight

As shown in figure 4A, normal control and normal control received UFG (40 mg/kg, b.w.) gradually gained the body weight at end of the experimental study. Adjuvant induced rats slightly gained body weight especially in the first 10 days as compared to normal rats. After that adjuvant induced rats showed the marked loss of body weight at end of the study as compared to normal rats. The body weight of normal control rats increased from 157.8±1.49 to 210.6±1.36 with growth gain rate of 1.89±0.23 per day and normal control received UFG 40 mg.kg b.w. increased body weight from 157±2.34 to 212.6±4.54 along with the weight grain rate of 1.99 per day. Adjuvant induced rat loss body weight form 161±3.18 to 139±4.92 with loss of body weight 0.78±0.09 per day. UFG treated rats showed the increased body weight with gain rate 1.54±0.98, 1.67±0.73 and 1.82±0.62 per day at dose of 10, 20 and 40 mg/kg b.w. and on the other hand indomethacin showed the increased body weight with gain rate 1.79±0.62 per day.

Effect of UFG on arthritic index

No sign and symptom of arthritic index was observed in normal control and normal control received UFG dose 40 mg/kg. Adjuvant induced rat start the showing the evidence of clinical inflammation in one or more hind paw of day 5. CFA induced rats rapidly increased the clinical sign of arthritis, which indicate the development of polyarthritic symptoms. CFA induced arthritic rat treated UFG and indomethacin showed the little or no effect till day 12 (Figure 4B). However, after this CFA induced arthritic development phase, the arthritic sign and symptom were starting to decrease and finally reached less than 60% in all groups as compared to the CFA induced arthritic control group rats.

Effect of UFG on hepatic marker in CFA rats

Adjuvant induced rats were showed the increase activity of membrane marker enzyme such as SGOT, SGPT and ALP and confirmed the affecting hepatic cells. Adjuvant induced rats received UFG showed the significantly ($P<0.001$) marked declined level of membrane marker enzymes at dose dependent manner (Figure 5A-C). The concentration of the acid phosphatase was higher as compared to the normal control and which is responsible for the cartilage destruction. The concentration of ACP was significantly ($P<0.001$) reduced by UFG and indomethacin received group rats as compared to adjuvant induced group rats (Figure 5D).

Effect of UFG on hematological alterations in CFA rats

Figure 5 displayed the CFA induced the hematological perturbations, including as decreased level of RBC, Hb and increased level of WBC, ESR as compared to normal control group were also positively altered by UFG treatment.

Effect of UFG on antioxidant enzyme in CFA induced arthritic rats

Figures 6 and 7 showed that the adjuvant induced rats significantly ($P<0.001$) increased the cellular toxicity (MDA level and ALP and SGPT activities), while the liver antioxidant enzymes such as SOD, GSH and GPx significantly ($P<0.001$) decreased as compared to normal control. Antioxidant enzyme alterations in CFA induced arthritic rats were normalized after treatment with UFG at dose dependent manner (Figure 7).

Effect of UFG on inflammatory mediators in CFA induced arthritic rats

Treatment with UFG exhibited momentous inhibition of proinflammatory cytokines such as the production of TNF–α on day 28, whereas UFG evidenced 16.67, 35.89 and 61.60 % inhibition at dose 10, 20 and 40 mg/kg and indomethacin 60.51% against the adjuvant induced arthritic rats

(Figure 8A). UFG showed the 16.5, 32.5 and 60.59% protection at a dose 10, 20 and 40 mg/kg against the proinflammatory cytokines such as IL-1 in adjuvant induced arthritic rats (Figure 8B). The result suggest that the antiinflammatory potential of UFG against the short and long term antiinflammatory condition induced by CFA, which is more akin to clinical inflammatory conditions. In view of the antiinflammatory effect of UFG on against the COX and LOX was considered worth appraising in order in further elucidate the possible mechanism of action (Figure 9).

Effect of UFG on histopathology in CFA induced arthritic rats

The histopathology study for each group showed in figure 11(A-F). Normal control group rats' histopathology study showed the clean articular with one to four layers of synovial cells. Normal control group rat histopathology showed the normal articular surface of joint which was very smooth with arrangement of loose connective tissue and contains the arrangement of fatty tissue below the synovial membrane (figure 11A). CFA induced arthritic rats showed the abnormal histology of joint with hyperplasia in articular capsule. The defect in synovial showed the deformity, pannus formation of cartilage and bone. The abnormability of joint showed the proliferation with enlarge sized and rebellious arrangement of synovial. The synovial membrane showed the loose arrangement of connective tissue which was causes the edema, hyperaemia and penetration with provocative cells (figure 11B). CFA induced arthritic rats treated with UFG; there were no sign of hyperaemia and only showing the inflammatory infiltration near the injected paw. All UFG received group rats showing the remarkable inhibition of all histology characteristic at dose dependent manner. By contrast, the high dose of UFG was showing the improvement of the histopathology as compared to other dose of UFG (figure 11C-E).

Discussion

Turpentine oil induced rat paw edema model widely used for the estimation of anti-inflammatory activity of a drug.[5] Turpentine oil induced inflammatory process involves three phases to induce the inflammation: initial phase releasing the histamine and serotonin: intermediate phase releasing the kinin like substance and later phase releasing the prostaglandins like substances.[27] Initial phase was characterized by increasing the vascular permeability; intermediate and later phase mediates the vascular response of inflammation.[27] UFG significantly ($P<0.001$) confirmed a prominent effect on this, suggesting the possible anti-inflammatory effect.[32] Following turpentine oil, UFG was evaluated against the AA induced inflammation in

rats. AA induced inflammation start form COX and LOX pathway and after that increase the inflammation through the proinflammatory mediator including leukotrienes and prostaglandins. UFG significantly inhibited the AA induced inflammation either COX or LOX or may be involved the both pathways. TO additional investigate, prostaglandin inflammatory mediator was used for induced the inflammation and UFG exhibited significant restrained of prostaglandin induced inflammation. PGE_2, which is known as powerful vasodilator depicts a synergistic action along with other inflammatory vasodilators viz. histamine and bradykinin. They exerts a powerful dilator action which is cumulative on precapillary arterioles which further cause the enhanced flow of blood in region of acute inflammation.[33] The permeability of post capillary venules has not been affected by PGE_2 though PGE_2 further augment the histamine and bradykinin induced permeability. By sensitizing afferent C fibers, PGE_2 further augment the effect of bradykinin which results in increment of pain.[21]

Taking into account the antiinflammatory activity of UFG and to scrutinize the long term antiinflammatory effect, UFG was further evaluated against the immunological chronic inflammation induced by CFA, which is more similar to clinical situations. CFA induced arthritis, it develop the redness and swelling over 24 h periods in the injected foot with irritation. CFA induced inflammatory reactions spread the arthritis in few days.[34,35] CFA increase the lymphocytes and monocytes/macrophage migration into the synovial cavity of inflamed area. Inflammatory mediators are the major contributors in the initiation and maintain the immune response.[37, 38] CFA induced arthritis develops in two reactions; primary reaction develops the swelling in hind paw and secondary reaction develops the swelling in front paw and nodules in ear and tails. CFA induced arthritic rats received UFG significantly ($P<0.001$) inhibited the humoral immune response, in fact due to its capability to restrain the acute inflammation via tumbling the vascular permeability and inhibiting the other inflammatory mediator such as PGE_2. UFG significantly ($P<0.001$) inhibited the secondary inflammation response, which was more effective than the indomethacin. The secondary lesion of the CFA, are the consequence of the delayed hypersensitivity reaction and UFG produced momentous effect on this as well, indicating that UFG might be effective in RA treatment.

The increased level of proinflammatory cytokines, like IL-1 and TNF-α, was observed in the serum sample and knee joint of RA patients.[38] Inflammatory molecules and numerous pro are used to determine the inflammation during the arthritis. TNF-α and IL-1, both are the

inflammatory mediator play an important role in the progression of the joint inflammation. TNF-α and IL-1 act collegial to enlist the leukocytes into the inflammatory joint. TNF-α (pleiotropic cytokines), which secretes from the macrophage/monocots and play an important role in both types of inflammation (acute and chronic). TNF-α found in the synovial fluid and sera of RA patients and its concentration has been associated with laboratory and clinical marker of RA. TNF-α blocking the signal by two pathways; firstly decline the severity of inflammation and secondary bone destruction in CFA induced arthritic rats. Another inflammatory mediator IL-1 destroyed the synovial cell by encouraging the prostaglandin (PGE_2) and producing synovial fibroblast.[39, 40] Our result exhibited that UFG had a compelling inhibitory effect on inflammatory mediators, which have already been shown to be convincing beneficial target in RA. The result also provides the evidence that the above discuses activities are mediated via restraint of AA pathway, which may be mediated via inhibition of COX/LOX pathway or both.

CFA induced arthritis rats showed the decline body weight at end of the study. CFA induced arthritic rats showed the decline body weight due to deficient absorption of glucose and leucine through the intestine.[34,41] CFA induced arthritic rats treated with UFG significantly ($P<0.001$) increased the body weight at dose dependent manner. The probable mechanism of action of UFG may be increase the glucose and leucine absorption through the intestine.

During arthritic condition, more important problem is anemia because the level of RBC and Hb decline and represents the abnormal storage of iron in reticuloendothelial system, synovial tissue, increase leukocyte count and failure of bone marrow. CFA induced arthritic rats showed the declined level of RBC and Hb and enhanced level of WBC and ESR represents the anemic condition. CFA induced arthritic rats treated with UFG significantly ($P<0.001$) decreased the leukocyte count by stimulated the immune system against invading antigens and claims the immune modulating effect.[27] ESR is equal to number of cells and concentration of proteins, especially fibrinogen, alpha and beta globins. ESR is an estimation tool for the determination of suspension stability of RBC's in plasma. CFA induced arthritic condition increased the amount of ESR which showed the elevation in the concentration of stress, inflammation, injury, surgery and tissue necrosis. The ESR counts significantly ($P<0.001$) decreased by UFG at dose dependent manner and justified the antiarthritic effect of drug. The level of WBC augment mild to moderate is very common feature of inflammatory reaction.[42] Irritating adjuvant released the IL-2β inflammatory cells and increased the level of WBC in inflamed area. IL-2β increase the

production of granulocyte and macrophage colony stimulating factor.[43] UFG significantly ($P<0.001$) decreased the level of WBC by decreased the level of inflammatory mediator and macrophages colony stimulator factors. The possible mechanism of action of UFG may be declined the production of inflammatory mediators.

Free radical, reactive oxygen species (ROS)/reactive nitrogen species (RNS) are capable to destroy the protein, DNA, membrane lipids and cartilage. Adjuvant activates the inflammatory mediators and generates the free radical, ROS in large amount of surrounding tissue of inflamed area and starts the damaging of tissue area.[44,45] The increase level of ROS in inflamed area alter the activity of endogenous antioxidant defense and impair the destruction of affected joints, such as cartilage, synovial fluid and other articular constituents.[46] Arthritis condition, granulocytes and macrophages start the accumulation in damage area of synovial fluid and start the production of O_2^- and H_2O_2.[47] Enhance level of free radical decline the level of endogenous antioxidant marker and start the cell damage, and increase the lipid peroxidation.[48] Free radicals are important mediator to provoke the inflammation and subsequently minimize the endogenous antioxidant marker enhance inflammation.[49] Antioxidant marker such as SOD, GPx and GSH plays an important role in the detoxification of superoxide anion and H_2O_2, respectively. Superoxide anion and H_2O_2 decline by enzyme GPx, but alternatively, may react again with superoxide and form the hydroxyl free radical, which have the greater tendency to producing the greater toxicity and having a longer half-life than superoxide anions.[50] Another antioxidant enzyme SOD along with GPx reduces the formation of hydroxyl free radical and decline the half life of hydroxyl free radical. CFA induced arthritic showed the decreased level of GSH as an upshot of their utilization during oxidative stress and cellular lysis.[51,52] Oxidative condition, GSH is oxidize to disulfide (glutathione, GSSG) and these oxidize form can be exported out the cells and causing the accumulation of cellular glutathione. GSH play a crucial role against LPO, which donate the electron to GPx in the diminution of H_2O_2 and act as a nucleophilic co substrate to glutathione transferases mainly in the detoxification of xenobiotics.[53] For GSH cycle, GR is necessary for upholds the sufficient level of cellular GSH. Phagocytic cells in the inflamed area initialize the production of uncontrolled ROS, which start the consumption of the endogenous antioxidant (CAT and SOD) along with the GSH during the oxidative stress, cell lyses and showed the effect on the lysosomal destruction and membrane LPO.[48, 54] Adjuvant induced arthritic rats showed the increased level of ALP, ALAT and LPO, which confirmed the decrease

lysosomal stability. Adjuvant induced rat showed the increased level of ALP, due to increased peiarticular ostopenia and bone erosion, which are release into the circulation during the bone formation and restoration. The considerably increase/decrease level of antioxidant (GSH, SOD and CAT) and cellular toxicity markers (ALAT, ALP and LPO), respectively that have been shown in adjuvant induced arthritic rats that treated with UFG or indomethacin, especially from the start of arthritis. This in turn highlight that the role of the UFG in preventing bone and organs damage in CFA induced arthritic model through scavenge the free radical.

Many evidences showed that the single molecule and single target drug development have been less relevant in treatment of the many chronic disease including arthritis. Since, chronic diseases involve the inter-reliant etiological factors and multiple organs systems. Now, the drug discovery main stream set the multiple target approaches to produce the effect of the chronic diseases.[55] As manifest from the results of the present investigation, a plant derived coumarin; UFG appears to exert potential effect on the multiple pathological manifestations of adjuvant induced arthritis in animals (Figure 12). Consequently UFG may be confirming to be a clinical value if systematically scrutinized further.

Conclusion

Thus, it can be concluded that Umbelliferone β-D-galactopyranoside (UFG) confirms the considerably antiinflammatory effect mediated through the inhibition of AA pathway. UFG exhibits the inhibition of COX pathway with noticeable inhibition of COX-2 could be the underlying mechanism for the antiinflammatory effect observed.

Conflict of interest

The authors declare that there are no conflicts of interest.

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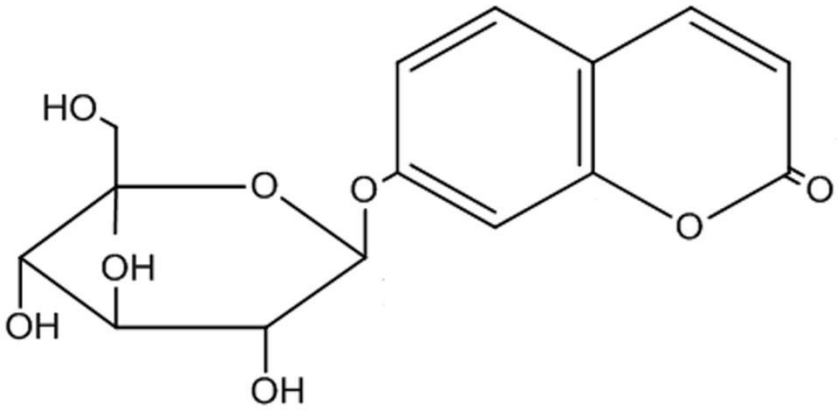

Figure 1: Structure of as Umbelliferone β-D-galactopyranoside (UFG)

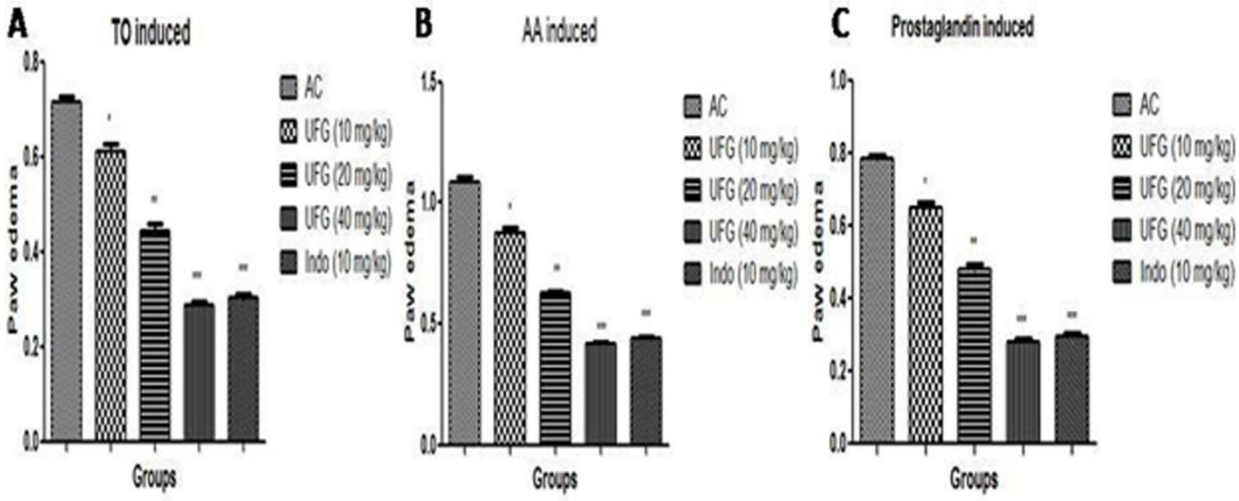

Figure 2: Effect of UFG on paw edema induced by the various phlogistic in Wistar rats. The data are expressed in mean ± SEM) (n = number of animals in each group = 6). NC= Normal Control, AC= Arthritic Control, UFG= Umbelliferone β-D-galactopyranoside, Indo=Indomethacin, TO= Turpentine oil, AA= Arachidonic acid; The comparisons were made by ANOVA followed by Dunnett's test. ns=non-significant, *P < 0.05 is considered as significant, **P < 0.01 is considered as very significant, ***P < 0.001 is considered as extremely significant.

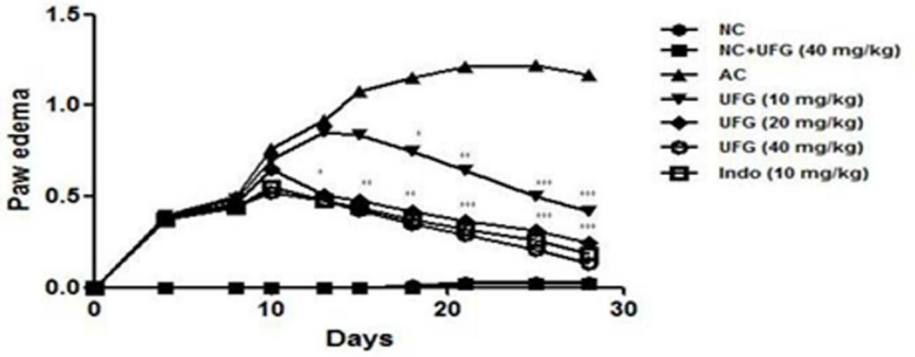

Figure 3: The modulatory effects of UFG and indomethacin on hind paw edema of adjuvant induced arthritic rats. NC= Normal Control, AC= Arthritic Control, UFG= Umbelliferone β-D-galactopyranoside, Indo=Indomethacin; The comparisons were made by ANOVA followed by Dunnett's test. ns=non-significant, *P < 0.05 is considered as significant, **P < 0.01 is considered as very significant, ***P < 0.001 is considered as extremely significant.

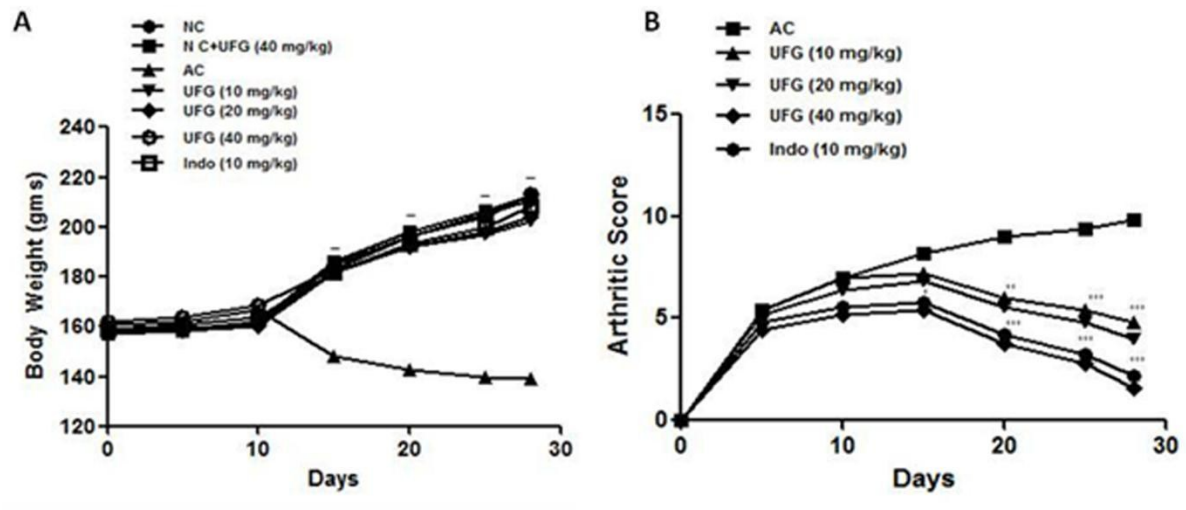

Figure 4: A: The modulatory effects of UFG and indomethacin on the body weight of the CFA induced arthritic rats. **B:** The modulatory effects of UFG and indomethacin on the arthritic index of CFA induced arthritic rats. NC= Normal Control, AC= Arthritic Control, UFG= Umbelliferone β-D-galactopyranoside, Indo=Indomethacin; The comparisons were made by ANOVA followed by Dunnett's test. ns=non-significant; *P < 0.05 is considered as significant, **P < 0.01 is considered as very significant, ***P < 0.001 is considered as extremely significant.

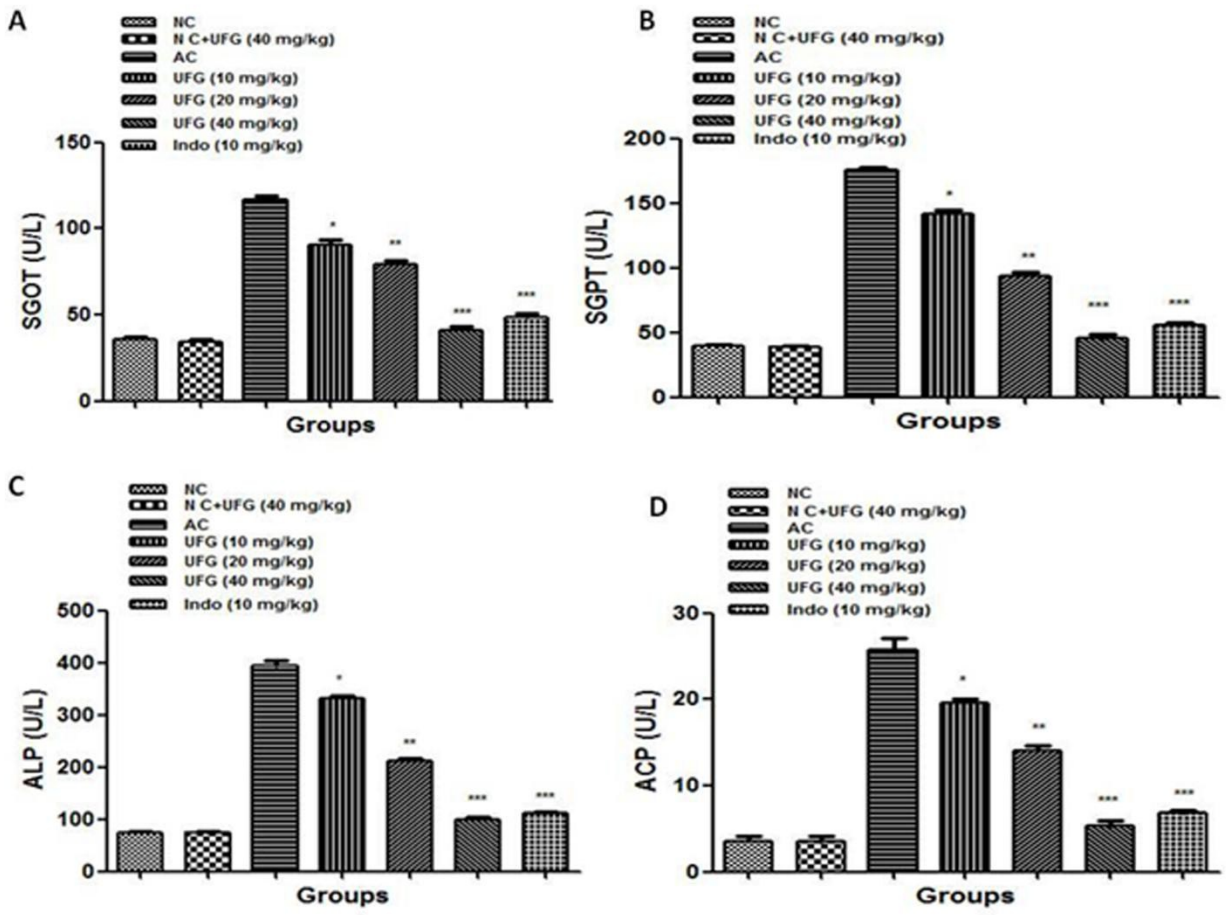

Figure 5: A: The modulatory effects of UFG and indomethacin on the SGOT level of the CFA induced arthritic rats. **B:** The modulatory effects of UFG and indomethacin on the SGPT level of the CFA induced arthritic rats. **C:** The modulatory effects of UFG and indomethacin on the ALP level of the CFA induced arthritic rats. **D:** The modulatory effects of UFG and indomethacin on the ACP level of the CFA induced arthritic rats. NC= Normal Control, AC= Arthritic Control, UFG= Umbelliferone β-D-galactopyranoside, Indo=Indomethacin, SGOP= Serum glutamic oxaloacetic transaminase, SGPT= Serum glutamate-pyruvate transaminase, ALP= Alkaline phosphatase, ACP= Acid phosphatase; The comparisons were made by ANOVA followed by Dunnett's test. ns=non-significant, *P < 0.05 is considered as significant, **P < 0.01 is considered as very significant, ***P < 0.001 is considered as extremely significant.

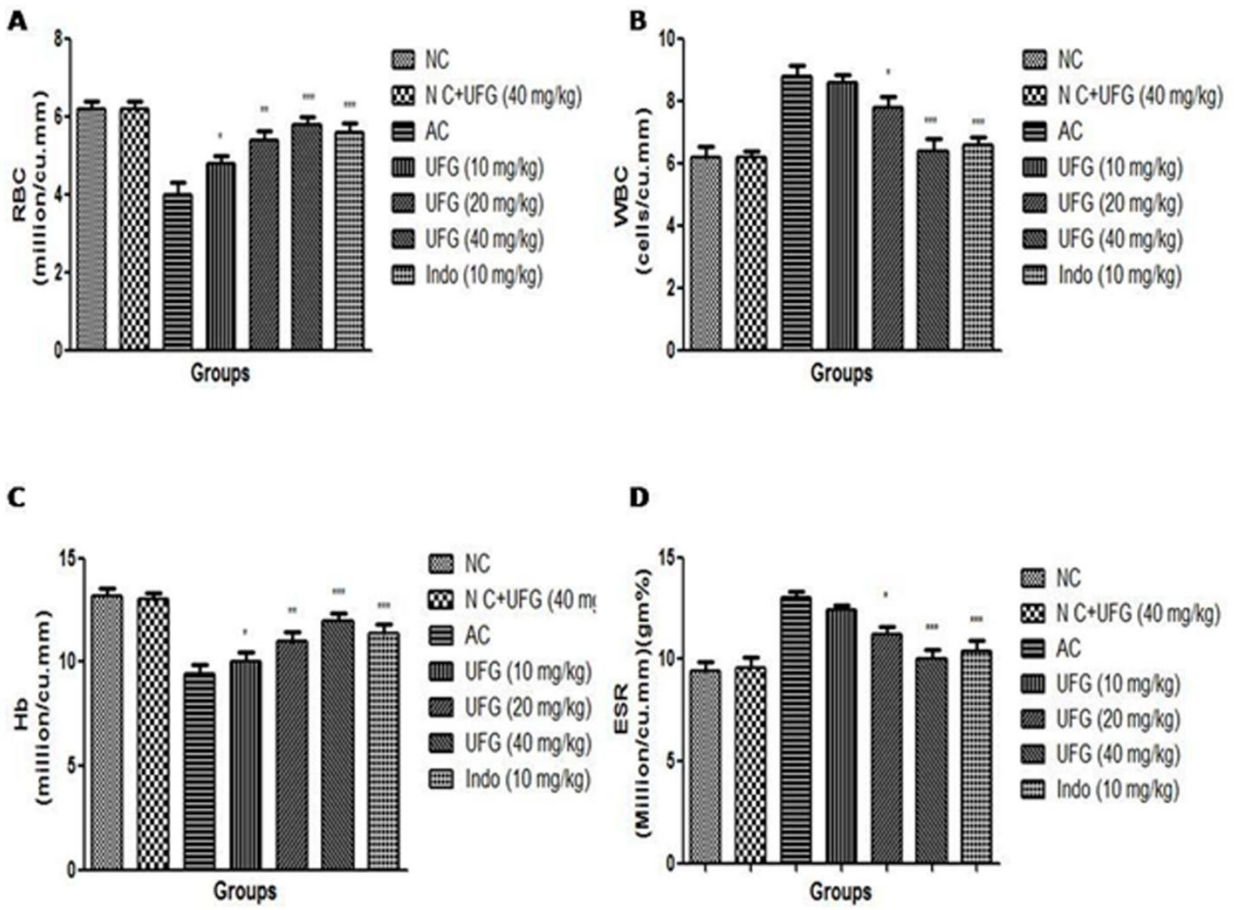

Figure 6: A: The modulatory effects of UFG and indomethacin on the RBC counts of the CFA induced arthritic rats. **B:** The modulatory effects of UFG and indomethacin on the WBC count of CFA induced arthritic rats. **C:** The modulatory effects of UFG and indomethacin on the Hb level of the CFA induced arthritic rats. **D:** The modulatory effects of UFG and indomethacin on the ESR of CFA induced arthritic rats. NC= Normal Control, AC= Arthritic Control, UFG= Umbelliferone β-D-galactopyranoside, Indo=Indomethacin, RBC= Red blood cells, WBC= White blood cells, Hb= Hemoglobin, ESR= Erythrocytes sedimentation rate; The comparisons were made by ANOVA followed by Dunnett's test. ns=non-significant, *P < 0.05 is considered as significant, **P < 0.01 is considered as very significant, ***P < 0.001 is considered as extremely significant.

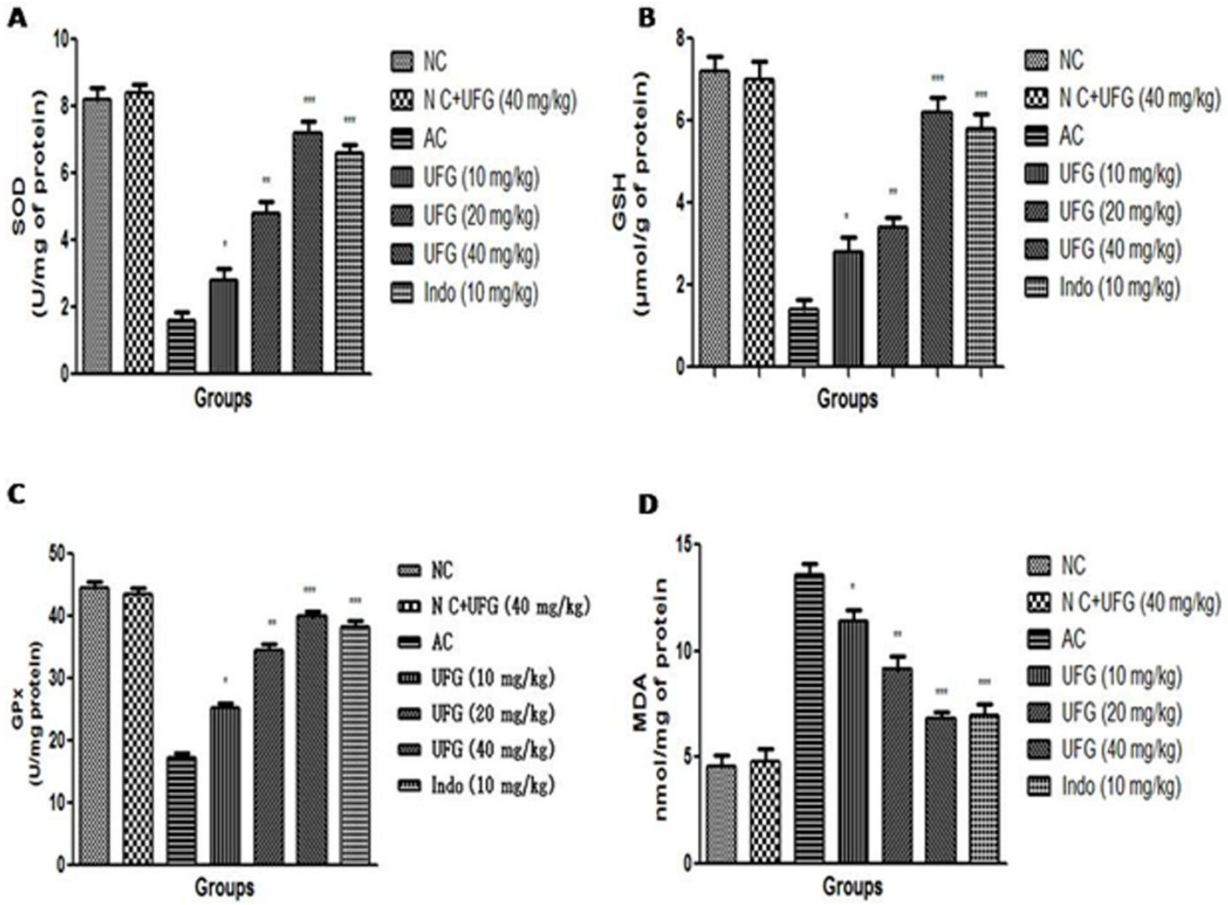

Figure 7: A: The modulatory effects of UFG and indomethacin on the SOD level of CFA induced arthritic rats. **B:** The modulatory effects of UFG and indomethacin on the GSH level of CFA induced arthritic rats. **C:** The modulatory effects of UFG and indomethacin on the GPx level of CFA induced arthritic rats. **D:** The modulatory effects of UFG and indomethacin on the MDA level of CFA induced arthritic rats. NC= Normal Control, AC= Arthritic Control, UFG= Umbelliferone β-D-galactopyranoside, Indo=Indomethacin, SOD= Superoxide dismutase, GSH= Glutathione, GPx= Glutathione peroxidase, MDA= Malonaldehyde,; The comparisons were made by ANOVA followed by Dunnett's test. ns=non-significant, *P < 0.05 is considered as significant, **P < 0.01 is considered as very significant, ***P < 0.001 is considered as extremely significant.

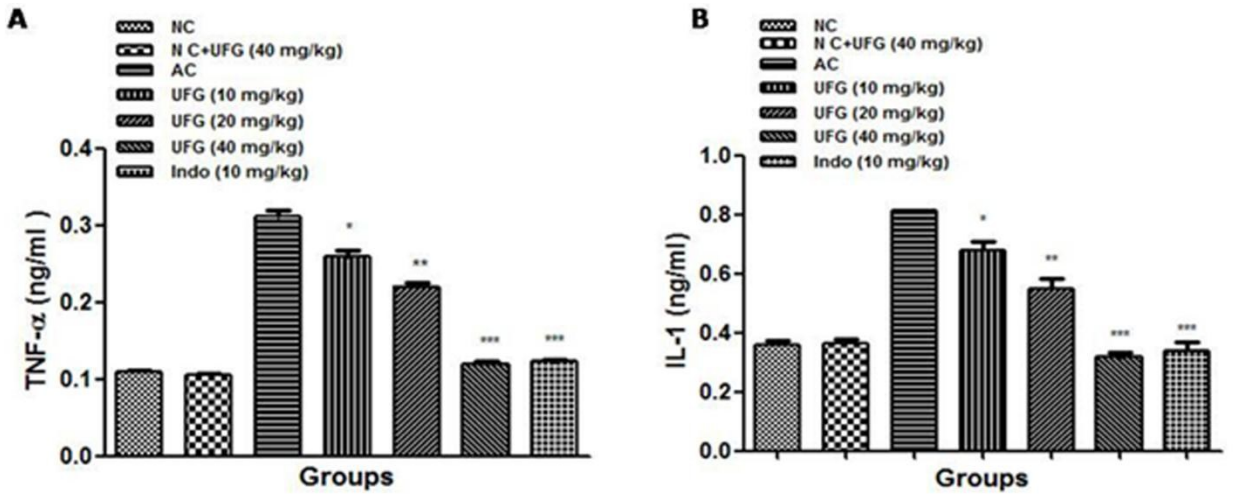

Figure 8: A: The modulatory effects of UFG and indomethacin on the TNF-α level of CFA induced arthritic rats. **B:** The modulatory effects of UFG and indomethacin on the IL-1 level of CFA induced arthritic rats. NC= Normal Control, AC= Arthritic Control, UFG= Umbelliferone β-D-galactopyranoside, Indo=Indomethacin, TNF-α= Tumor necrosis factor-α, IL-1= Interlukin-1; The comparisons were made by ANOVA followed by Dunnett's test. ns=non-significant, *P < 0.05 is considered as significant, **P < 0.01 is considered as very significant, ***P < 0.001 is considered as extremely significant.

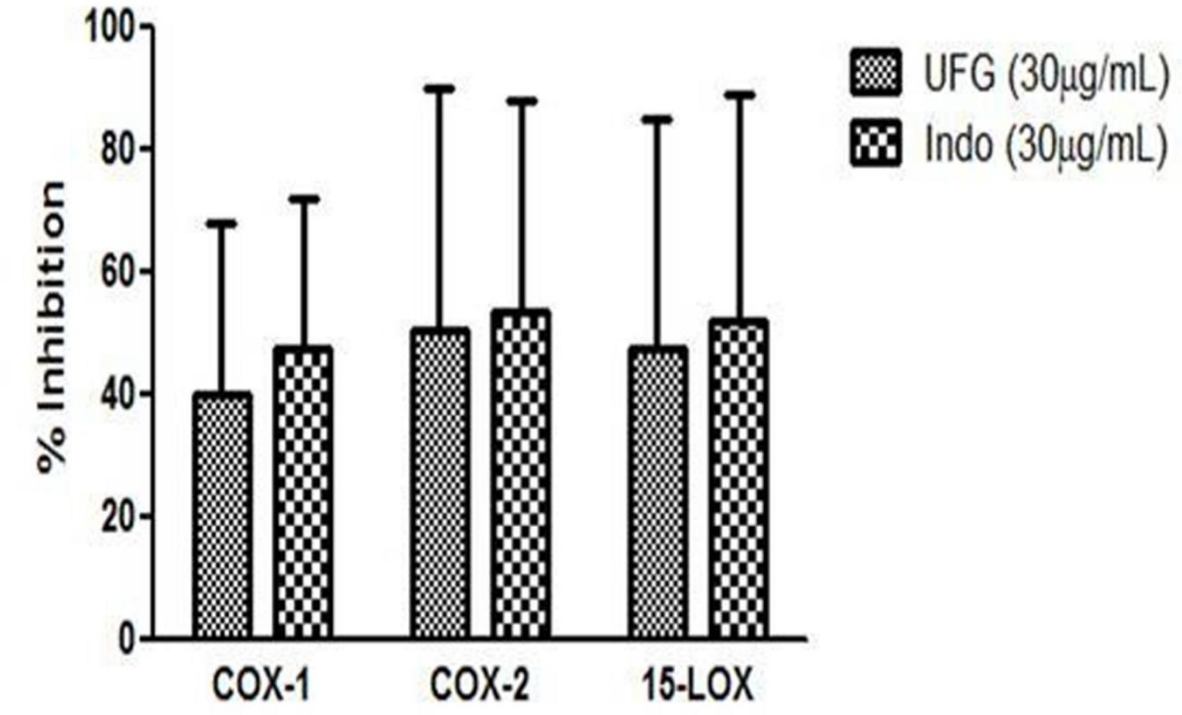

Figure 9: Invitro cyclooxygenase and lipoxygenase inhibitory activity of UFG and indomethacin. Values are represented as mean ± SD. UFG= Umbelliferone β-D-galactopyranoside, Indo=Indomethacin, COX= Cyclooxygenase, LOX=Lipoxygenase.

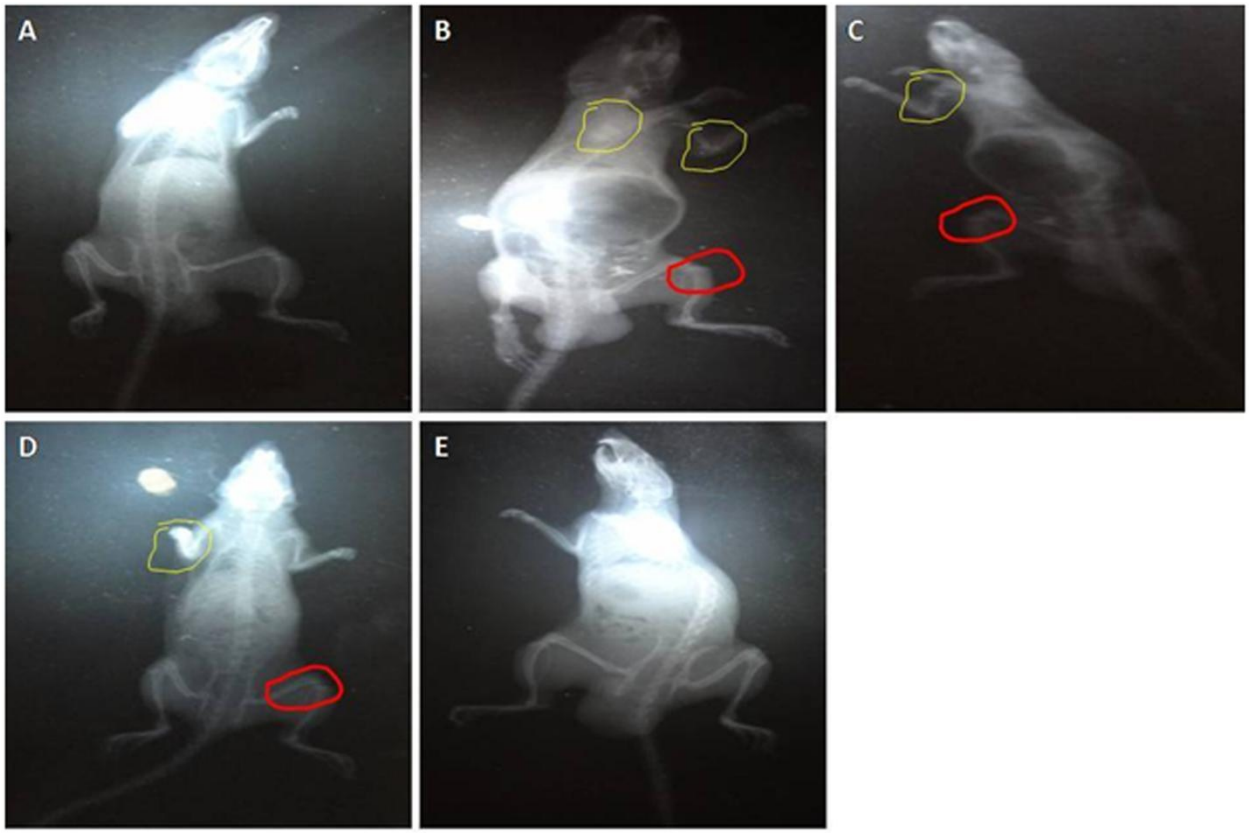

Figure 10: Effect of UFG in CFA induced arthritic rats (X-rays photograph). **A:** Arthritic Control; **B:** UFG (10 mg/kg), **C:** UFG (20 mg/kg), **D:** UFG (40 mg/kg), **E:** Indomethacin 10 mg/kg.

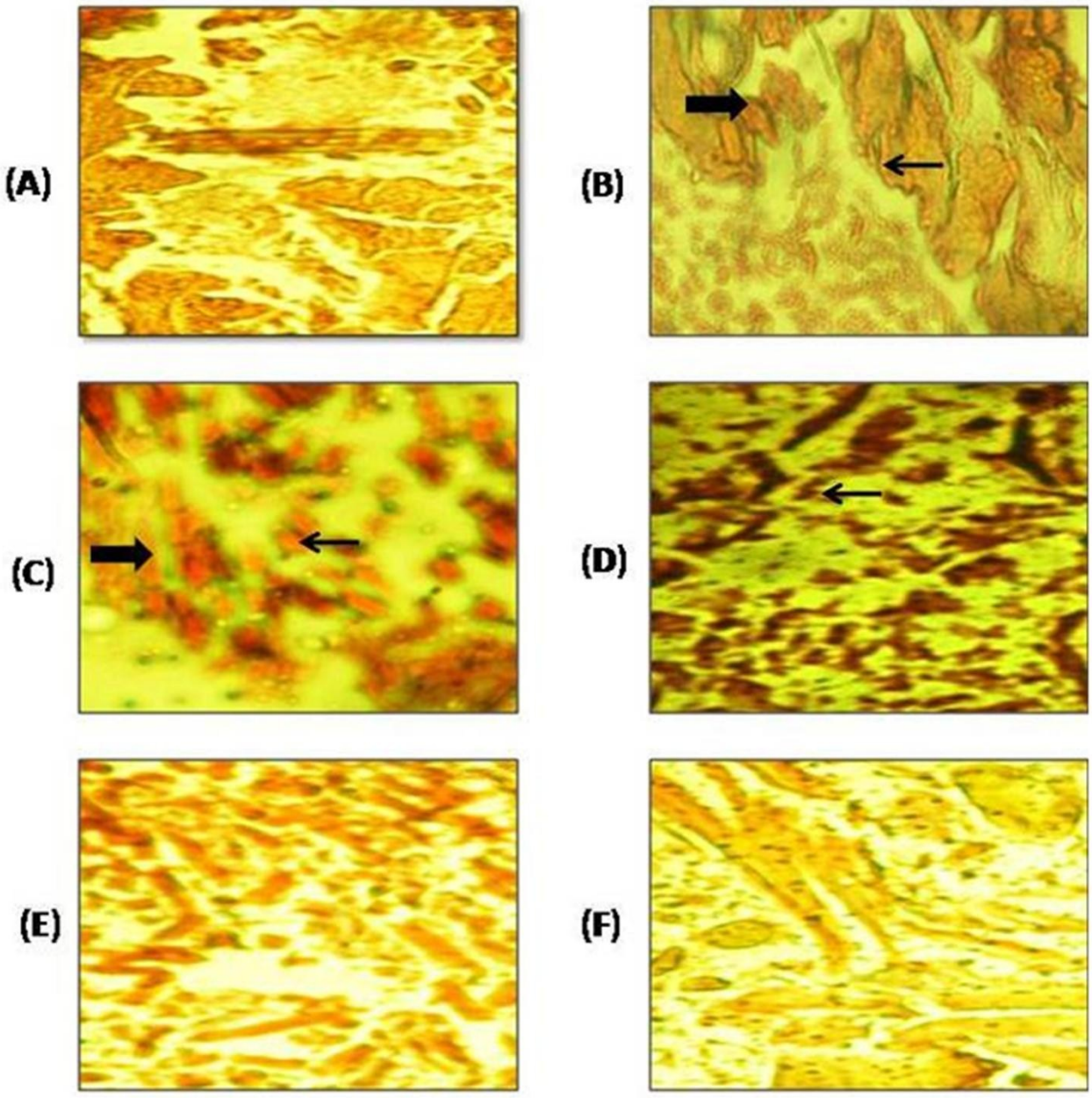

Figure 11: The influence of UFG on the histopathological change of ankle joint in CFA induced arthritic rats. Representative pictures are shown. (A): Normal group; (B): CFA induced arthritic control showed the leukocytes (arrow) and synovitis (arrow head); (C): CFA induced rats treated with UFG (10 mg/kg) showed the leukocytes (arrow) and synovitis (arrow head); (D): CFA induced rats treated with UFG (20 mg/kg) showed the leukocytes (arrow); (E): CFA induced rats treated with UFG (40 mg/kg) showed the improved histopathological profile towards normal architecture with less deformity of the bone, less leukocytes infiltration, synovitis and cartilage; (D): CFA induced rats treated with indomethacin (10 mg/kg) showed the improved histopathological profile towards normal architecture with less deformity of the bone and cartilage. Hematoxylin and eosin staining of tissue specimens from the left ankle joints of different group rats.

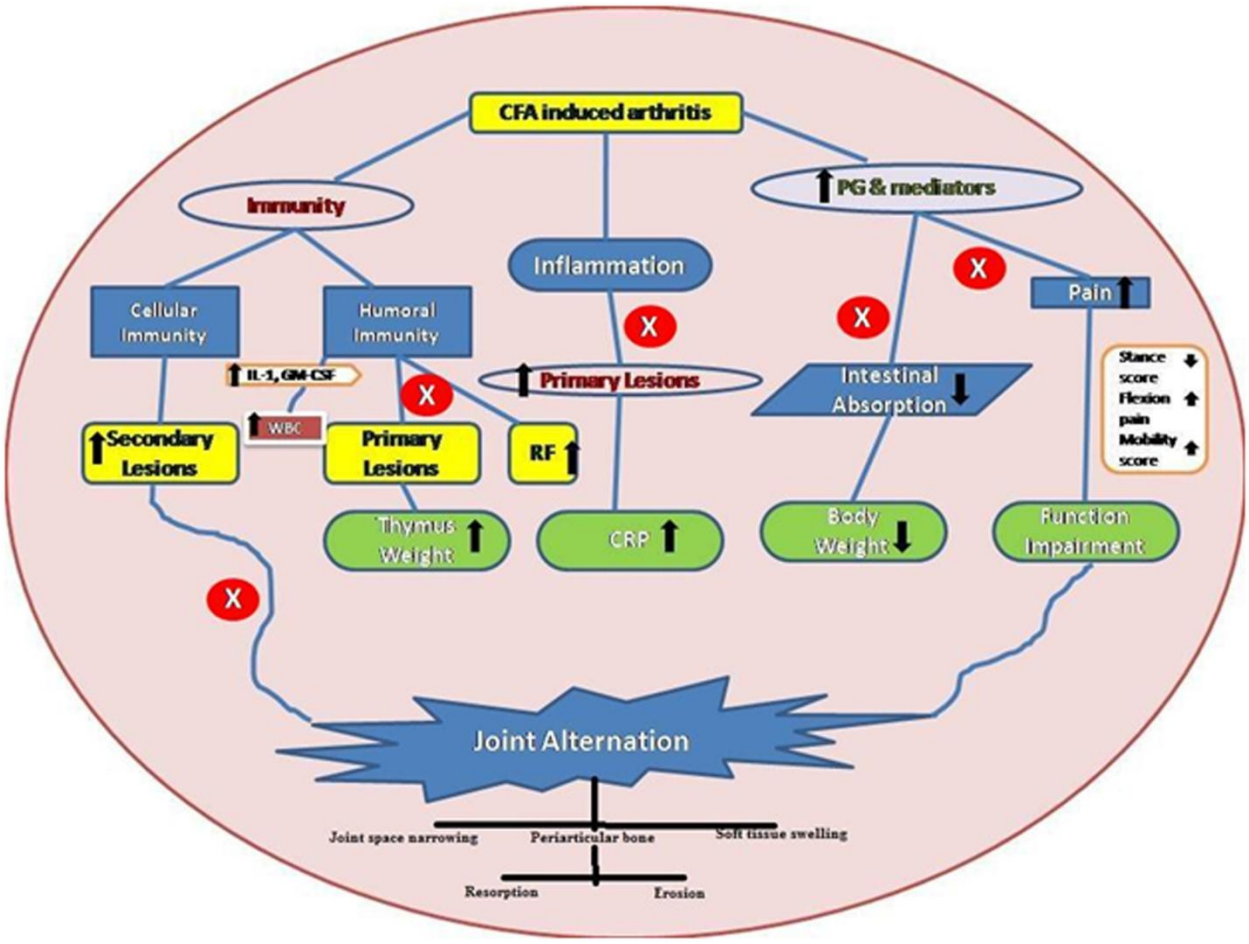

Figure 12: Antiarthritic effect of UFG in CFA induced arthritic rats. Cross mark sign indicates the inhibition of CFA induced arthritic induced pathological changes in Wistar rats by UFG. CRP- C-reactive protein, IL-1- Interlukin-1, GM-CSF- Granulocyte macrophage colony stimulating factor, WBC-White blood cell.