daily for 30 days.[35] This gives the notion that the results observed in the present study in alleviating RA-associated symptoms could be partially attributed to anti-TNF-α activity of baicalin and catechin in UP446.

Over the years, augmented reports have been generated to support involvement of chemokines and their receptors in the pathogenesis of RA. As chemokines play a pivotal role in for the recruitment, localization, and retention of inflammatory cells in inflamed synovium and hence result in bone and cartilage destruction, preventing this phenomenon could cause moderation of the inflammatory process. Studies have shown that baicalin can inhibit the binding of chemokines to leukocytes or cells transfected to express specific chemokine receptors.[36-38] Therefore, another possibility for UP446 to reduced severity of arthritis could be by binding to chemokines and limit their biological function which would have otherwise assisted in more inflammatory cells to migrate to the synovium and cause serious damage to the already inflamed joint.

Moreover, irrefutable evidences have been documented that, NF-κB plays a major role in the regulation of inflammatory genes. In RA, active NF-κB plays a pivotal role in both, at the initiation and maintenance of chronic inflammation. Some have suggested that suppression of activation of NF-κB or targeting NF-κB inhibitors to specific tissues or cell-type could mitigate severity of bone and cartilage destruction.[39-42] For instance, previously Xue *et al.*,[43] have showed that when rats with acute pancreatitis were treated with 50 mg/kg of baicalin intraperitoneal, a significant inhibition of activation of NF-κB was observed. Similarly, a significant inhibition in activation of NF-κB was also reported when IL-1β or TNF-α activated human mast cell line-1 (HMC-1) were treated with baicalein (metabolite of baicalin) at concentration of 30 μM.[44,45] On the other hand, an increase in anti-inflammatory cytokine IL-10 level was found significantly increased when a polyseptic C57BL/6J mice were treated with baicalin at 100 mg/kg intraperitoneally.[46] Substantiating these findings, blunting[11] and normalization of expression[10] of NF-κB were reported as a result of administrating UP446. Thus, arthritis mitigation observed in the present study could partially be explained by the ability of UP446 to inhibit cellular gene expression regulated by transcription factor NF-κB.

Knowing the fact that UP446 is active in inhibiting arachidonic acid metabolism, COX, LOX, iNOS, cytokines (ILs and TNF), and NF-κB;[9-10] it was not surprising to see significant progressive decreases in thickness of both ankles, improved pain resistance, and suppression of paw edema in animals treated with UP446 compared to vehicle-treated diseased rats. These marked inhibitions in pain and swelling were observed both in the primary and the secondary inflammatory reactions in the course of adjuvant-induced arthritis pathology when UP446 was administered orally at a dose of 50 mg. In support of our data, Krakauer *et al.*,[47] have demonstrated that, besides reducing mRNA and protein expression of IL-1β, IL-6, and TNF-α, when baicalin was incubated at 100 μg/ml with human peripheral blood mononuclear cells, it showed a 98% inhibition in staphylococcal exotoxins-stimulated proliferation of T-cells. This may further justify disease modifying activity of UP446 in the second phase of immunological reaction that occurred after day-9 of treatment.

Similarly, Kubo *et al.*,[48] have showed that baicalin suppresses the secondary lesion in adjuvant-induced arthritis in rats. In their study, rats were given 100 mg/kg of baicalin orally for 27 days where inhibition of edema was observed after day-11 of treatment. The authors presumed that the anti-inflammatory activity of baicalin could be attributed to inhibition of delayed-type allergic reaction or activation of components. In a similar study, when catechin (as low as 60 mg/kg) was given orally to adjuvant-induced Sprague-Dawley rats, a significant suppression in secondary inflammatory paw edema, hypersensitivity, and polyarthritis index as well as inhibition in production of IL-1, TNF-α, and prostaglandin E2 was observed.[49]

CONCLUSION

To sum up, in addition to its dual COX-LOX inhibition activity, various reports have shown impact of UP446 to decrease expression of pro-inflammatory cytokines TNF-α and IL-1β and/or inhibiting activation of transcription factor NF-κB. In the present study, UP446, a defined bioflavonoid composition of primarily baicalin and catechin, has showed a significant improvement in the major cardinal signs of arthritis which includes reduction in pain sensitivity, paw edema and ankle diameter. Though specificity, potency, and long-term use needs further clinical evidence; UP446, analgesic and anti-inflammatory agent of botanical origin, could potentially be used as medical foods and dietary supplements to manage the symptoms associated with RA.

AUTHOR CONTRIBUTIONS

MY conceived and designed, carried out study, data calculation, statistical analysis, data interpretation, and drafted/edited the manuscript. MP assisted in conducting the study. QJ and LB conceived the study, participated in its design, interpreted data, and edited the manuscript. All authors read and approved the final manuscript.

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