

UP1304, a Botanical Composition Containing Two Standardized Extracts of *Curcuma longa* and *Morus alba*, Mitigates Pain and Inflammation in Adjuvant-induced Arthritic Rats

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ABSTRACT

Background: Though, the initial etiologies of arthritis are multifactorial, clinically, patients share pain as the prime complaints. Present day pain relief therapeutics heavily relies on the use of prescription and over the counter nonsteroidal anti-inflammatory drugs as the first line of defense where their long-term usage causes gastrointestinal and cardiovascular-related side effects. Hence, the need for evidence-based safer and efficacious alternatives from natural sources to overcome the most prominent and disabling symptoms of arthritis is an overdue. Here, we evaluated the anti-inflammatory and analgesic effect of UP1304, a composition that contains a standardized blend of two extracts from the rhizome of *Curcuma longa* and the root bark of *Morus alba* in adjuvant-induced arthritis models in rats. **Materials and Methods:** The anti-inflammatory and analgesic effects of the botanical composition were demonstrated in adjuvant-induced arthritis models in rats with oral dose ranges of 50–200 mg/kg. Ibuprofen at a dose of 100 mg/kg was used as a reference compound. *Ex vivo* sulfated glycosaminoglycan inhibition assays were performed. **Results:** Statistically significant improvements in pain resistance, suppression of paw edema and ankle thickness were observed in animals treated with UP1304 compared to vehicle-treated diseased rats. These results were similar to those achieved by ibuprofen treatment. Inhibitions of proteoglycan degradation were observed in a range of 37.5–61.7% for concentration of UP1304 at 50–200 µg/mL when compared to interleukin-1 α -exposed untreated explants. **Conclusions:** These data suggest that UP1304, for its analgesic and anti-inflammatory effects, could potentially be considered agent of botanical origin for the improvement of arthritis associated symptoms.

Key words: Anti-inflammatory, Arthritis, Bradykinin inhibition, Chronic pain, *Curcuma longa*, *Morus alba*

SUMMARY

- Pain is one of the cardinal signs of arthritis.
- Long term applications of commonly used non-steroidal anti-inflammatory drugs for pain relief are associated with cardiovascular and gastrointestinal side effects.
- Cartilage degradation evidenced as glycosaminoglycan loss from articular cartilage into the synovial fluid has been reported in arthritis patients.
- Adjuvant-induced arthritis model in rats are among the widely used models for efficacy evaluation of nutraceuticals.
- Efficacy of UP1304, a composition containing a blend of two standardized extracts from the rhizome of *Curcuma longa* and root bark of *Morus alba*, was evaluated in adjuvant-induced arthritis model in rats and in glycosaminoglycan releasing inhibition assays.
- UP1304 demonstrated its enhanced significance by improving the major cardinal signs of arthritis in vivo and ex vivo.
- UP1304 could potentially be considered as a dietary supplement product for the management of arthritis.

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic and systemic inflammatory autoimmune disease characterized by chronic inflammation of multiple joints with the subsequent erosive destruction of articular bone and cartilage.^[1] Though, the initial etiologies of the disease are multifactorial and difficult to define, clinically, patients share pain as the prime complaint.

Proinflammatory cytokines such as tumor necrosis factor-alpha (TNF- α), and interleukin (IL)-1, metabolic enzymes (e.g., cyclooxygenase [COX] and lipoxygenase [LOX]), bradykinin (BKB) BKB1 and BKB2, cellular component of immunology (T-cell), inducible nitric oxide (NO) synthase (iNOS), and activation of nuclear factor-kappa B (NF- κ B) are considered to be essential for initiation and progression of RA.^[2] The major active components curcumin from the rhizomes of *Curcuma longa*, and prenylated flavonoids and stilbenoids from the root bark of *Morus alba* L possess activities suggestive of benefits in the treatment of RA: (i) down-regulating the activity of COX-2, LOX, and iNOS enzymes, inhibition of the production of the inflammatory cytokines TNF- α , IL-1, -2, -6, -8, and -12, and suppression of NF- κ B activation

by curcumin;^[3-7] (ii) suppression of T-cell migration, and inhibition of CXCR-4-mediated chemotaxis and MEK/ERK pathways,^[8] proinflammatory mediator (e.g., COX-2, IL-1 β , and IL-6,^[9-11] NO) production, inducible NO synthase expression, prostaglandin E2 production, and activation of NF- κ B^[12] by prenylated flavonoids and stilbenoids from *M. alba* root bark extract have been reported. With these activities, a composition comprising the well-studied plant extracts at a specific ratio may provide a benefit in alleviating symptoms associated with RA or may slow down the progression of the disease.

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Currently, nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most frequently used prescribed or over the counter drugs and the first line of treatment in pain management. Nevertheless, this approach focuses mainly on relief of the disease-associated pain and is likely to mask the actual etiology leading to irreversible damage to the joint structure, which usually renders the treatment unsuccessful.

In the past years, significant progresses have been made in alleviating RA-associated symptoms by targeting specific pathways involved in the disease progression and maintenance. However, existing pharmaceutical drugs and nutraceuticals could not meet the rapidly increasing need of aging arthritic populations. Hence, the search for botanical alternatives that could provide a safe and effective solution to millions who suffers from chronic pains with progressive joint degeneration is still a demanding task.

In the current report, the analgesic and anti-inflammatory potential of UP1304, a botanical composition consists two standardized extracts from the rhizome of *C. longa*, Linn (Family: Zingiberaceae) and the root bark of *M. alba*, Linn (Family: Moraceae), have been evaluated in adjuvant-induced arthritis model in rats. Its *ex vivo* sulfated glycosaminoglycans (sGAG) inhibitions were also tested.

MATERIALS AND METHODS

UP1304

The detailed procedure for the preparation of the composition has been described in US patent # 20150072953.^[13] The composition contains a proprietary combination of two standardized ethanol extracts from root barks of *M. alba* and rhizomes of *C. longa* with not <10% curcumin and 2% mulberroside a in the final blend.

Glycosaminoglycan release inhibition assay

Articular cartilages from hock joints of rabbits (2.5 kg body weight) were removed immediately after each animal has been sacrificed. The articular cartilage explants were obtained by following the method described by Sandy *et al.*^[14] Briefly, after the articular surfaces were exposed surgically under sterile conditions, approximately 200–220 mg articular surfaces per joint were dissected and submerged into complete medium (Dulbecco's modified eagle medium [DMEM], supplemented with heat inactivated 5% fetal bovine serum (FBS); penicillin 100 U/mL; streptomycin 100 U/g/mL). They are then rinsed several times with the complete medium and incubated for 2 days at 37°C in a humidified 5% CO₂/95% air incubator for stabilization. The complete medium were replaced with a basal medium (DMEM, supplemented with heat-inactivated 1% FBS, 10 mM hydroxyethyl piperazineethanesulfonic acid, and penicillin 100 U/mL streptomycin 100 µg/mL). Approximately 30 mg cartilage pieces (2 mm × 3 mm × 0.35 mm/piece) were placed in 48-well plates and treated with given concentrations of test agents. After pretreatment for 1 h, 5 ng/mL of recombinant human interleukin-1α (rhIL-1α) was added to the culture medium and further incubated at 37°C in a humidified 5% CO₂/95% air incubator. The culture medium were collected 24 h later and the amount of sGAGs in the medium at the end of reaction reflecting the amount of articular cartilage degradation was determined by 1,9-dimethyl-methylene blue method using commercially available kit (The Blyscan proteoglycan and glycosaminoglycan assay) according to the instructions of the manufacturer.

Animals

Lewis rats purchased at the age of 8 weeks were acclimated upon arrival for a week before being assigned randomly to their respective groups. Rats (3/cage) were housed in a polypropylene cage and individually identified by numbers on their tail. Each cage was covered with wire bar lid and filtered top (Allentown, NJ). Individual cage was identified

with a cage card indicating project number, test article, dose level, group, and an animal number. The Harlan T7087 soft cob beddings was used and changed at least twice weekly. Animals were provided with fresh water and rodent chow diet # T2018 (Harlan Teklad, 370W, Kent, WA) *ad libitum* and were housed in a temperature controlled room (22.2°C) on a 12 h light-dark cycle. All animal experiments were conducted according to institutional guidelines congruent with a guide for the care and use of laboratory animals with approval reference #: UAS-CM1304.

Adjuvant-induced arthritis model

Adjuvant-induced rat arthritis model was developed to evaluate the analgesic and anti-inflammatory activity of composition UP1304 at doses of 200 mg/kg, 100 mg/kg or 50 mg/kg. Treatment was started a day before antigen inoculation. Animals (*n* = 10) were orally gavaged with a positive control ibuprofen (100 mg/kg), test articles: UP1304 (200 mg/kg, 100 mg/kg or 50 mg/kg) and vehicle control (propylene glycol). On the next day, arthritis was induced by sensitizing rats with an injection of complete Freund's adjuvant containing 5 mg/mL (W/V) suspension of heat killed mycobacterium tuberculosis in liquid paraffin into subplantar region of right hind paw of sedated rats^[15,16] an hour after the second dose of treatment. Allodynia was evaluated by responsiveness to pressure applied perpendicular to the central plantar surface of the right hind paw using Randall–Selitto. A positive response to the applied mechanical pressure, noted by the sharp withdrawal of the paw, was recorded automatically by an electronic Von Frey Anesthesiometer (2,390 series Electrovonfrey, IITC, Woodland Hills, CA).^[17] Mechanical allodynia is being evaluated before antigen, and day 3, 5, 7, 9 and 13 after antigen injection. Paw edema was measured with the use of plethysmometer (IITC, Woodland Hills, CA; Model 520) on day 1 (before antigen), day 3, 5, 7, 9, and 13 after antigen injection. Ankle diameter was measured using pocket thickness gage (7309, Mitutoyo corp. Japan) on day 1 (before antigen), day 3, 5, 7, 9, and 13 after antigen injection.

Statistical analysis

Data were analyzed using Sigmaplot (Systat Software Inc., San Jose, CA) (version 11.0). The results are represented as mean ± one standard deviation. Statistical significance between groups was calculated by means of single factor analysis of variance (ANOVA) and by a *t*-test. *P* ≤ 0.05 were considered as significant. When normality test failed, for nonparametric analysis, data were subjected to Mann–Whitney sum ranks for *t*-test and Kruskal–Wallis one-way ANOVA on ranks for ANOVA.

RESULTS

Glycosaminoglycan

As seen in Figure 1, significant cartilage protection activity was observed by the composition UP1304. When the rabbit cartilage explants were treated with IL-1α, the amount of glycosaminoglycan (GAG) released into the culture medium increased significantly when compared to the vehicle group. UP1304 composition reduced IL-1α-mediated degradation of proteoglycan in a concentration-dependent manner (*P* < 0.05). Inhibitions of proteoglycan degradation were observed in a range of 37.5–61.7% for a concentration of UP1304 at 50–200 µg/mL when compared to IL-1α-exposed untreated explants. UP1304 at 200 µg/mL almost totally inhibited the rhIL-1α-induced proteoglycan degradation to the level of the normal control without rhIL-1α [Figure 1].

Adjuvant-induced arthritis model findings

Paw edema as a measure of inflammation

Adjuvant-induced experimental arthritis in rats was developed with a 100% rate of induction as a disease model of human RA. Cardinal signs of inflammation, hyperalgesia, swelling, and hyperemia were evident in all animals 24 h postpriming with an antigen. As seen in Figure 2, the positive control ibuprofen showed statistically significant reduction of 28.8%, 21.1%, 19.4%, 24.3% and 32.7% in paw edema on days 3, 5, 7, 9, and 13, respectively, as compared to vehicle control. Animals treated with an oral dose of 200 mg/kg UP1304 showed 24.0%, 32.1%, 30.6%, 38.5%, and 48.4% reduction, with an oral dose of 100 mg/kg showed 21.8%, 29.3%, 25.0%, 33.7% and 38.8% reduction and at a dose of 50 mg/kg showed 15.6%, 17.1%, 18.8%, 27.1%, and 38.2% reduction in paw edema on days 3, 5, 7, 9, and 13, respectively, when compared to the vehicle control animals [Figure 2]. These percentage reductions were statistically significant at each time point analyzed against vehicle control. As expected, the positive control showed greater inhibition in inflammation after 3 days of daily oral treatment when compared to any of the compositions. However, the degrees of inhibition were higher than ibuprofen for the compositions as treatment days progressed to day 13. These findings may highlight the presence of the accumulative effect of multiple compounds as a result of multiple administrations and lower doses of UP1304 are needed if it is used for chronic inflammation.

Pain sensitivity

Similarly, oral administration of composition UP1304 and ibuprofen showed a marked reduction in pain sensitivity. As depicted in Table 1,

statistically significant reduction in pain sensitivity was observed when rats were treated with 100 mg/kg of ibuprofen (31.3%, 39.5%, 48.8%, 52.5%, and 52.5% reductions on day 3, 5, 7, 9, and 13, respectively). The pain sensitivity inhibitions of orally gavaged UP1304 at a dose of 200 mg/kg were 27.1%, 38.2%, 51.6%, 52.8%, and 54.2%, at a dose of 100 mg/kg were 25.6%, 34.9%, 39.0%, 47.6%, and 46.2%, and at a dose of 50 mg/kg were 21.8%, 24.3%, 29.0%, 37.6%, and 40.8%, respectively [Table 1]. In these data, it was very obvious to address that ibuprofen showed the strongest analgesic activity than any of the UP1304 compositions on day 3 and 100 mg/kg or 50 mg/kg of UP1304 thereafter. Nevertheless, coinciding to the paw edema data, the activity of the composition at any of the doses administered were augmented as the treatment days were progressed to day 13.

Ankle diameter as a measure of inflammation

Furthermore, as compiled in Figure 3, stronger ankle diameter reduction data substantiate the harmonized effect of composition UP1304 in reducing inflamed joint at doses of 200, 100, and 50 mg/kg. Animals treated with an oral dose of 200 mg/kg of UP1304 showed 43.1%, 47.3%, 45.5%, 52.4 and 60.9% reductions in ankle diameter on days 3, 5, 7, 9, and 13, respectively, when compared to the vehicle control animals. These percentage reductions were statistically significant at each time point analyzed against vehicle control. The positive control ibuprofen showed statistically significant reductions of 37.2%, 34.8%, 36.8%, 33.5%, and 44.2% in ankle diameter on days 3, 5, 7, 9 and 13,

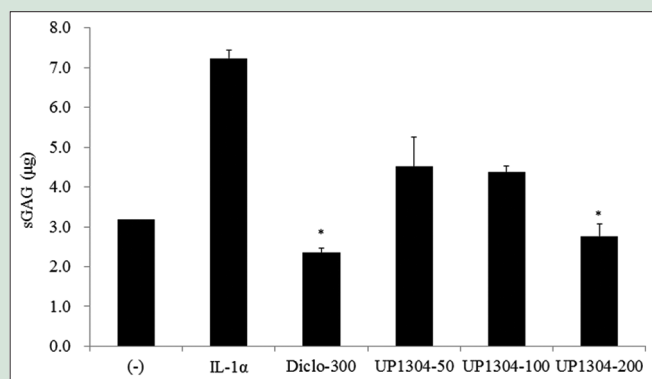


Figure 1: Effect of UP1304 on the glycosaminoglycan releasing assay. Cartilage explants were incubated for 24 h in Dulbecco's modified eagle medium with 1% heat-inactivated fetal bovine serum and 10 mM hydroxyethyl piperazineethanesulfonic acid. GAG releasing into the medium was significantly decreased by UP1304 treatment in a dose-dependent manner. UP1304 at concentration 200 µg/mL brought down the recombinant human interleukin-1alpha induced proteoglycan degradation to a normal level

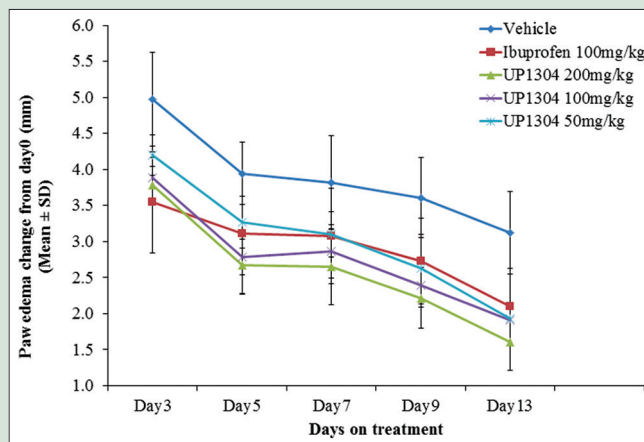


Figure 2: Anti-inflammatory effect of UP1304 as measured in paw edema of adjuvant-induced arthritis. Arthritis was induced by sensitizing rats with an injection of complete Freund's adjuvant containing 5 mg/mL (W/V) suspension of heat killed mycobacterium tuberculosis in liquid paraffin into subplantar region of right hind paw of sedated rats ($N = 10$). Data are expressed as mean \pm standard deviation. All treatment groups showed statistical significance at $P \leq 0.001$ versus vehicle at each time point

Table 1: Effect of UP1304 in pain sensitivity inhibition in AIA model

Groups	Dose (mg/kg)	n	Mean \pm SD				
			Day 3	Day 5	Day 7	Day 9	Day 13
Vehicle	0	10	98.2 \pm 6.3	90.7 \pm 5.4	84.0 \pm 2.9	81.6 \pm 3.6	69.3 \pm 3.5
Ibuprofen	100	10	67.4 \pm 5.6 [†]	54.8 \pm 2.6 [†]	43.0 \pm 4.6 [†]	38.8 \pm 2.3 [†]	32.9 \pm 2.6 [†]
UP1304	50	10	76.8 \pm 5.4 [†]	68.7 \pm 2.7 [†]	59.6 \pm 1.8 [†]	50.9 \pm 4.1 [†]	41.1 \pm 2.1 [†]
UP1304	100	10	73.1 \pm 3.7 [†]	59.1 \pm 2.6 [†]	51.2 \pm 3.6 [†]	42.8 \pm 3.2 [†]	37.3 \pm 1.0 [†]
UP1304	200	10	71.6 \pm 3.6 [†]	56.0 \pm 2.0 [†]	40.7 \pm 4.9 [†]	38.5 \pm 1.7 [†]	31.7 \pm 2.7 [†]

Arthritis was induced by sensitizing rats with an injection of complete Freund's adjuvant containing 5 mg/ml (w/v) suspension of heat-killed *Mycobacterium tuberculosis* in liquid paraffin into subplantar region of right hind paw of sedated rats ($n=10$). Rats treated with the test materials for 2 weeks. The study lasted for 2 weeks. [†] $P \leq 0.0001$ versus vehicle. SD: Standard deviation; AIA: Adjuvant-induced arthritis

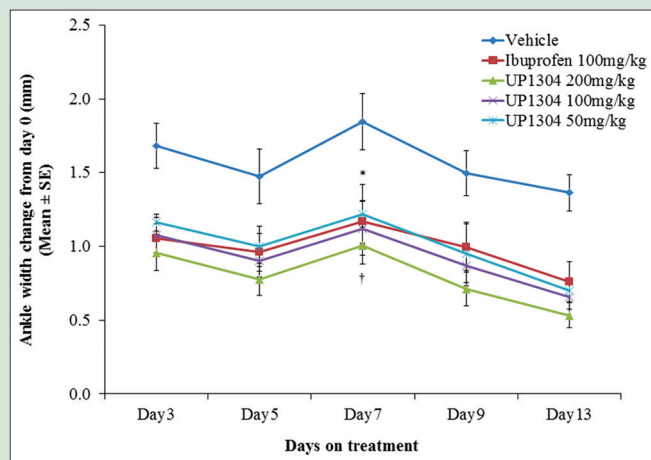


Figure 3: Anti-inflammatory effect of UP1304 as measured by ankle diameter in adjuvant-induced arthritis. Arthritis was induced by sensitizing rats with an injection of complete Freund's adjuvant containing 5 mg/mL (W/V) suspension of heat killed mycobacterium tuberculosis in liquid paraffin into subplantar region of right hind paw of sedated rats ($N = 10$). Data are expressed as mean \pm standard error. All treatment groups showed statistical significance at $P \leq 0.001$ versus vehicle at day 3, 5, 9, and 13. $^{\dagger}P \leq 0.001$ versus vehicle for the 200 mg/kg and 100 mg/kg UP1304; $^*P \leq 0.01$ versus vehicle for ibuprofen and 50 mg/kg UP1304

respectively, compared to vehicle control [Figure 3]. In this particular case, the composition UP1304 (at 200 mg/kg) showed greater inhibition in ankle width than any of the treatment groups, including ibuprofen, for all the time points monitored. Actually, rats treated with an oral dose of ibuprofen at 100 mg/kg and composition UP1304 at 50 mg/kg showed a very similar reaction to the treatment.

DISCUSSIONS

Pain, one of the cardinal signs of inflammation, is the most common clinical manifestations of arthritis. Inflammations serve as a link for articular cartilage, subchondral bone and synovial membrane at the time of arthritis disease progression. During the course of arthritis progression, inflamed synovium alters the dynamics of cartilage matrix degradation and repair, leading to excess production of the proteolytic enzymes accountable for cartilage degradation. Cartilage breakdown in turn amplifies synovial inflammation leading to a perpetual circle.^[18] As a result, inflammation of the synovial membrane could be considered as the inflammatory liaison for surrounding joint structures where targeted intervention could help alleviate the symptoms of the disease and perhaps also prevent from further structural damage. The inconvenient truth is, despite the advances in therapeutic discovery, there is still a major challenge in the development of a safe, effective and economical therapy for managing chronic inflammatory pain in arthritis. In particular, the adverse cardiovascular and gastrointestinal side effects associated with long-term use of selective or nonselective NSAIDs reinforce the need to develop botanical alternatives with anti-inflammatory and analgesic activities without accompanied side-effects.

The major bioactive component of turmeric plant, curcumin, from the rhizomes of *C. longa* L, and prenylated flavonoids and stilbenoids from the root bark of *M. alba* L possess activities suggestive of benefits in chronic pain management of arthritis. For instance, curcumin has been shown to have potent anti-inflammatory and anti-catabolic effects through their effects on the NF- κ B pathway^[19,20] and transcription activation factor-1 (AP-1),^[21] which include reducing the IL-1 β -mediated up-regulation of NF- κ B targets, such as matrix

metalloproteinase (MMP)-1, MMP-3, MMP-9, and COX-2, as well as reducing chondrocyte apoptosis. Besides, curcumin may also simultaneously support anabolism by reversing the cytokine-induced suppression of collagen type II and β 1-integrin synthesis.^[22] Curcumin also showed inhibitory effects on the JNK mitogen-activated protein kinase (MAPK) pathway leading to suppression of MMP-3 and MMP-13 mRNA up-regulation in human osteoarthritis (OA) and bovine articular chondrocytes;^[21] decreased the IL-1 β -stimulated production of NO and prostaglandin E₂ (PGE₂) in chondrocytes through signal transduction pathways involving p38 MAPK, JNK, NF- κ B, and AP-1;^[23] and inhibited IL-1 β -stimulated pro-inflammatory mediators, NO, PGE₂, IL-6, IL-8, and MMP-3 production in human articular chondrocytes.^[24]

Similarly, a variety of bioactive compounds from *M. alba* root bark have showed *in vivo* or *in vitro* anti-inflammatory activity. For example, while oxysresveratrol showed suppression of T-cell migration and inhibition of CXCR-4-mediated chemotaxis and MEK/ERK pathways,^[8] *M. alba* extract showed inhibition of a disintegrin and metalloprotease with thrombospondin type I motifs-1 (ADAMTS1).^[25]

Hence, the collective pharmacological activities of *C. longa* and *M. alba* implies their wide array of applications for arthritis treatments. As such, taking these well established and historically sound indications of these plant materials, we hypothesized to achieve significant anti-inflammatory and analgesic efficacy by formulating two standardized extracts from *C. longa* and *M. alba* at a specific ratio and yielding a composition designated as UP1304. Given the above facts, it is presumed that the composition UP1304 would possess all the qualities contributed by the individual components and could be used for symptom relief and/or as disease modifying agent of arthritis. We further proceeded to demonstrate the hypotheses using established adjuvant-induced arthritis animal models.

Adjuvant-induced arthritis in rats is one of the most widely used experimental animal models of inflammatory polyarthritis with clinical and pathological features shared by many of the higher animals. It is characterized by chronic inflammation of multiple joints associated with subsequent progressive, erosive destruction of articular bone and cartilage, mononuclear cell infiltration, pannus formation and functional impairment.^[26] It has been reported previously that when a complete adjuvant was used as an antigen to induce a disease model of arthritis in rats, it elicits two intertwined phases of inflammation. The primary reaction is an acute inflammation mediated partially through the BKB, COX-LOX pathways (on day 0 through day 8) at the site of inoculation followed by a more delayed and complex secondary systemic reaction as a result of generalized immunologic burst (on days 9 through 14) to constituents of antigen that triggers both cellular and humoral response in association with TNF- α , IL1-beta and NF- κ B.^[16] Pain sensitivity inhibition activity of curcumin was previously observed in the early phase of adjuvant-induced arthritis model. In this model, the antihyperalgesic activity in terms of paw withdrawal latency by a hot plate test was noted as early as 6 h and lasted for 48 h when curcumin was administered concurrently with the adjuvant at a dose of 100 mg/kg. There were also decrease in oxidative stress markers, and down-regulation of tissue level of TNF- α , IL1 and IL6 suggesting the potential antihyperalgesic mechanisms.^[27] In a more prolonged study that lasted for 35 days, using adjuvant-induced arthritis as a chronic model of inflammation, IL-1 β increased to 2-folds on day 21 and 10-folds on day 35 which were significantly brought down by curcumin administered orally at a dose of 100 mg/kg for the entire duration of study.^[28] Mirroring these findings, in our study, 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 UP1304 was administered orally at a dose as low as 50 mg/kg.

Cartilage degradation occurs as a result of an imbalance in the homeostasis of two fundamental matrix components such as GAGs and

type II collagen.^[29] This pathogenesis is triggered in part by the action of inflammatory cytokines, primarily IL-1,^[30,31] that also mediate the production of proinflammatory mediators (including NO and PGE₂) and matrix degrading enzymes. While the catabolic enzymes, MMPs disrupt collagen fibers,^[32] members of the ADAMTS family degrade aggrecan and both cases result in the release of GAGs.^[33] GAGs, besides serving as a building block for cartilage, they exert specific pharmacologic effects such as decreasing IL1-induced gene expressions by inhibiting the cytokine intracellular signaling cascade and NF-κB activation.^[34] GAG loss from articular cartilage into the synovial fluid in human rheumatoid (RA) and OA patients has been reported.^[35] In fact, a direct strong correlation were observed between synovial GAGs level and patients with osteoarthritis ($[r = 0.65]$ and RA $[r = 0.95]$).^[36] This clearly implies that the reductions in sGAG *ex vivo* assay observed as a result of UP1304 treatment could have a potential therapeutic advantage in maintaining structural integrity of articular cartilage in both OA and RA conditions beyond curtailing associated pain. In support of our study, previously prenylated flavonoids^[11] extracted from *M. alba* have shown inhibition of the catabolic enzyme a disintegrin and metalloprotease with thrombospondin type I motifs-1 (ADAMTS1)^[25] which otherwise could have caused degradation of cartilage. Similarly, curcumin also showed suppression of catabolic enzymes MMP-3 and MMP-13 mRNA upregulation in human OA chondrocytes through inhibitory effects on the JNK MAPK pathway.^[21]

Complementing previously reported data, we have documented statistically significant improvement in pain resistance, and suppression of paw edema and ankle thickness in animals orally treated with UP1304 compared to vehicle-treated diseased rats. These marked inhibitions in pain and swelling were observed in all the models evaluated when UP1304 was administered orally at a dose as low as 50 mg/kg. To substantiate our findings, oxyresveratrol and mulberroside A from the root bark of *M. alba* have been reported with anti-inflammatory effect on carrageenan-induced paw edema model in rats at a dosage of 7.5 mg/kg and 50 mg/kg respectively.^[9] Similarly, another report showed inhibition of PGE₂ and suppression of COX-2 mRNA in carrageenan-induced paw edema and peritonitis in mice treated with Morus extract.^[37] In a similar study, when curcumin was administered orally for 2 consecutive weeks at a dose of 50 mg/kg to rats that were subjected to carrageenan-induced inflammation after the last dose, statistically significant suppression in inflammatory paw edema (69%) and tissue TNF-α (32%) were observed.^[38] In another study, when rats provided with dietary curcumin at 0.2% daily for 10 weeks, a 12% improvement in paw edema as a measure of inflammation and significantly decreased activity of 5'-LOX activity in the polymorphonuclear lymphocytes were observed in carrageenan-induced rat paw edema model.^[39]

Due to the novelty, we were unable to compare the findings documented here against previously published data. To the best of our knowledge, extracts from the rhizome of *C. longa* and root bark of *M. alba* were never been tested in combination for the indications described above before. However, previously, when the merit of formulating these two plant materials were determined using the commonly used equation (Colby's equation) on data obtained from carrageenan-induced rat paw edema model, clearly interesting yet, an unexpected synergy was observed from the combination of these extracts that the beneficial effects seen with the composition treatment exceeded the predicted based on simply summing the effects observed for each of its constituents at the given ratio. Moreover, compared at an equivalent dosage, the composition UP1304 excelled in performance than its individual components (J Integr Med, 2015). Therefore, we strongly believe that putting these traditionally well-known folk medicinal plants into a standardize blend provides a novelty to the composition as demonstrated its efficacy in multiple animal models and biomarkers.

CONCLUSIONS

To sum up, the clinical application of UP1304 could be rationalized by the fact that the primary biomarkers frequently isolated in patients experiencing long-term arthritis seemed to be modulated by active components of the composition, from curcumin and/or morus separately. In addition to the GAG releasing inhibitions observed in the current study, various reports previously have shown curcumin and prenylated flavonoids to decrease expression of proinflammatory cytokines TNF-α and IL-1 β, NO, iNOS and/or inhibiting activation of transcription factor NF-κB, as well as hindering catabolic enzymes MMPs and ADAMTS1. In the present study, UP1304, a composition containing a blend of two standardized extracts from the rhizome of *C. longa* and root bark of *M. alba*, has demonstrated its enhanced significance by improving the major cardinal signs of arthritis. Therefore, UP1304, an analgesic and anti-inflammatory agent of botanical origin, could potentially be considered as a dietary supplement product for the management of arthritis.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Matsui K, Tsutsui H, Nakanishi K. Pathophysiological roles for IL-18 in inflammatory arthritis. *Expert Opin Ther Targets* 2003;7:701-24.
- Goldring MB, Otero M. Inflammation in osteoarthritis. *Curr Opin Rheumatol* 2011;23:471-8.
- Abe Y, Hashimoto S, Horie T. Curcumin inhibition of inflammatory cytokine production by human peripheral blood monocytes and alveolar macrophages. *Pharmacol Res* 1999;39:41-7.
- Surh YJ, Chun KS, Cha HH, Han SS, Keum YS, Park KK, *et al.* Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: Down-regulation of COX-2 and iNOS through suppression of NF-κB activation. *Mutat Res* 2001;480-481:243-68.
- Jobin C, Bradham CA, Russo MP, Juma B, Narula AS, Brenner DA, *et al.* Curcumin blocks cytokine-mediated NF-κB activation and proinflammatory gene expression by inhibiting inhibitory factor I-κB kinase activity. *J Immunol* 1999;163:3474-83.
- Huang MT, Lysz T, Ferraro T, Abidi TF, Laskin JD, Conney AH. Inhibitory effects of curcumin on *in vitro* lipoxygenase and cyclooxygenase activities in mouse epidermis. *Cancer Res* 1991;51:813-9.
- Bennett A. The production of prostanoids in human cancers, and their implications for tumor progression. *Prog Lipid Res* 1986;25:539-42.
- Chen YC, Tien YJ, Chen CH, Beltran FN, Amor EC, Wang RJ, *et al.* *Morus alba* and active compound oxyresveratrol exert anti-inflammatory activity via inhibition of leukocyte migration involving MEK/ERK signaling. *BMC Complement Altern Med* 2013;13:45.
- Zhang DD, Ling S, Zhang HP, Shi HX, Xue YL, Yang XL, *et al.* Effects of total flavones from *Morus alba* L. on inflammation reaction of macrophages. *Shizhen Guoyi Guoyao* 2010;21:2787-90.
- Cheon BS, Kim YH, Son KS, Chang HW, Kang SS, Kim HP. Effects of prenylated

- flavonoids and biflavonoids on lipopolysaccharide-induced nitric oxide production from the mouse macrophage cell line RAW 264.7. *Planta Med* 2000;66:596-600.
11. Hošek J, Bartos M, Chudik S, Dall'Acqua S, Innocenti G, Kartal M, *et al.* Prenylated flavonoids from *Morus alba* L. cause inhibition of G1/S transition in THP-1 human leukemia cells and prevent the lipopolysaccharide-induced inflammatory response. *J Nat Prod* 2011;74:614-9.
 12. Chung KO, Kim BY, Lee MH, Kim YR, Chung HY, Park JH, *et al.* *In-vitro* and *in-vivo* anti-inflammatory effect of oxyresveratrol from *Morus alba* L. *J Pharm Pharmacol* 2003;55:1695-700.
 13. Yimam M, Jia Q, Jiao P, Brownell LA, Chu M, Hong MF, *et al.* Compositions and Methods for Joint Health. US Patent# 20150072953; Issued March 12, 2015.
 14. Sandy JD, Brown HL, Lowther DA. Degradation of proteoglycan in articular cartilage. *Biochim Biophys Acta* 1978;543:536-44.
 15. Singh S, Khajuria A, Taneja SC, Khajuria RK, Singh J, Qazi GN. Boswellic acids and glucosamine show synergistic effect in preclinical anti-inflammatory study in rats. *Bioorg Med Chem Lett* 2007;17:3706-11.
 16. Newbould BB. Chemotherapy of arthritis induced in rats by mycobacterial adjuvant. *Br J Pharmacol Chemother* 1963;21:127-36.
 17. Vivancos GG, Verri WA Jr, Cunha TM, Schivo IR, Parada CA, Cunha FQ, *et al.* An electronic pressure-meter nociception paw test for rats. *Braz J Med Biol Res* 2004;37:391-9.
 18. Guidelli GM, Barskova T, Brizi MG, Lepri G, Parma A, Talarico R, *et al.* One year in review: Novelties in the treatment of rheumatoid arthritis. *Clin Exp Rheumatol* 2015;33:102-8.
 19. Singh S, Aggarwal BB. Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane) [corrected]. *J Biol Chem* 1995;270:24995-5000.
 20. Brouet I, Ohshima H. Curcumin, an anti-tumour promoter and anti-inflammatory agent, inhibits induction of nitric oxide synthase in activated macrophages. *Biochem Biophys Res Commun* 1995;206:533-40.
 21. Liacini A, Sylvester J, Li WQ, Zafarullah M. Inhibition of interleukin-1-stimulated MAP kinases, activating protein-1 (AP-1) and nuclear factor kappa B (NF-kappa B) transcription factors down-regulates matrix metalloproteinase gene expression in articular chondrocytes. *Matrix Biol* 2002;21:251-62.
 22. Shakibaei M, John T, Schulze-Tanzil G, Lehmann I, Mobasher A. Suppression of NF-kappaB activation by curcumin leads to inhibition of expression of cyclo-oxygenase-2 and matrix metalloproteinase-9 in human articular chondrocytes: Implications for the treatment of osteoarthritis. *Biochem Pharmacol* 2007;73:1434-45.
 23. Chowdhury TT, Salter DM, Bader DL, Lee DA. Signal transduction pathways involving p38 MAPK, JNK, NFkappaB and AP-1 influences the response of chondrocytes cultured in agarose constructs to IL1beta and dynamic compression. *Inflamm Res* 2008;57:306-13.
 24. Mathy-Hartert M, Jacquemond-Collet I, Priem F, Sanchez C, Lambert C, Henrotin Y. Curcumin inhibits pro-inflammatory mediators and metalloproteinase-3 production by chondrocytes. *Inflamm Res* 2009;58:899-908.
 25. Peng J, Gong L, Si K, Bai X, Du G. Fluorescence resonance energy transfer assay for high-throughput screening of ADAMTS1 inhibitors. *Molecules* 2011;16:10709-21.
 26. Bolon B, Stolina M, King C, Middleton S, Gasser J, Zack D, *et al.* Rodent preclinical models for developing novel antiarthritic molecules: Comparative biology and preferred methods for evaluating efficacy. *J Biomed Biotechnol* 2011;2011:569068.
 27. Singh AK, Vinayak M. Curcumin attenuates CFA induced thermal hyperalgesia by modulation of antioxidant enzymes and down regulation of TNF α , IL-1 β and IL-6. *Neurochem Res* 2015;40:463-72.
 28. Banerjee M, Tripathi LM, Srivastava VM, Puri A, Shukla R. Modulation of inflammatory mediators by ibuprofen and curcumin treatment during chronic inflammation in rat. *Immunopharmacol Immunotoxicol* 2003;25:213-24.
 29. Bijlsma JW, Berenbaum F, Lafeber FP. Osteoarthritis: An update with relevance for clinical practice. *Lancet* 2011;377:2115-26.
 30. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nat Rev Rheumatol* 2011;7:33-42.
 31. Sandell LJ, Xing X, Franz C, Davies S, Chang LW, Patra D. Exuberant expression of chemokine genes by adult human articular chondrocytes in response to IL-1beta. *Osteoarthritis Cartilage* 2008;16:1560-71.
 32. Hollander AP, Heathfield TF, Webber C, Iwata Y, Bourne R, Rorabeck C, *et al.* Increased damage to type II collagen in osteoarthritic articular cartilage detected by a new immunoassay. *J Clin Invest* 1994;93:1722-32.
 33. Bondeson J, Wainwright S, Hughes C, Caterson B. The regulation of the ADAMTS4 and ADAMTS5 aggrecanases in osteoarthritis: A review. *Clin Exp Rheumatol* 2008;26:139-45.
 34. Rovati LC, Girolami F, Persiani S. Crystalline glucosamine sulfate in the management of knee osteoarthritis: Efficacy, safety, and pharmacokinetic properties. *Ther Adv Musculoskelet Dis* 2012;4:167-80.
 35. Dingle JT, Page Thomas DP, Hazleman B. The role of cytokines in arthritic diseases: *In vitro* and *in vivo* measurements of cartilage degradation. *Int J Tissue React* 1987;9:349-54.
 36. Carroll G. Measurement of sulphated glycosaminoglycans and proteoglycan fragments in arthritic synovial fluid. *Ann Rheum Dis* 1989;48:17-24.
 37. Hassimotto NM, Moreira V, do Nascimento NG, Souto PC, Teixeira C, Lajolo FM. Inhibition of carrageenan-induced acute inflammation in mice by oral administration of anthocyanin mixture from wild mulberry and cyanidin-3-glucoside. *Biomed Res Int* 2013;2013:146716.
 38. Heeba GH, Mahmoud ME, El Hanafy AA. Anti-inflammatory potential of curcumin and quercetin in rats: Role of oxidative stress, heme oxygenase-1 and TNF- α . *Toxicol Ind Health* 2014;30:551-60.
 39. Manjunatha H, Srinivasan K. Protective effect of dietary curcumin and capsaicin on induced oxidation of low-density lipoprotein, iron-induced hepatotoxicity and carrageenan-induced inflammation in experimental rats. *FEBS J* 2006;273:4528-37.

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