Analgesic and anti-Inflammatory effect of UP3005, a botanical composition Containing two standardized extracts of *Uncaria gambir* and *Morus alba*

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ABSTRACT

Background: Osteoarthritis (OA) is a chronic debilitating degenerative joint disease characterized by cartilage degradation and synovial inflammation exhibited by clinical symptoms such as joint swelling, synovitis, and inflammatory pain. 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 detrimental gastrointestinal and cardiovascular-related side-effects. As a result, the need for evidence based safer and efficacious alternatives from natural sources to overcome the most prominent and disabling symptoms of arthritis is a necessity. **Materials and Methods:** Describe the anti-inflammatory and analgesic effect of UP3005, a composition that contains a standardized blend of two extracts from the leaf of *Uncaria gambir* and the root bark of *Morus alba* in carrageenan-induced rat paw edema, abdominal constriction (writhing's) and ear swelling assays in mouse with oral dose ranges of 100–400 mg/kg. **Results:** *In vivo*, statistically significant improvement in pain resistance, and suppression of paw edema and ear thickness in animals treated with UP3005 were observed compared with vehicle-treated diseased rats and mice. Ibuprofen was used a reference compound in all the studies. *In vitro*, enzymatic inhibition activities of UP3005 were determined with IC50 values of 12.4 μg/ml, 39.8 μg/ml and 13.6 μg/ml in cyclooxygenase-2 (COX-1), COX-2 and lipoxygenase (5-LOX) enzyme activity assay, respectively. **Conclusions:** These data suggest that UP3005, analgesic and anti-inflammatory agent of botanical origin with balanced dual COX-LOX inhibition activity, could potentially be used for symptom management of OA.

Key words: Chronic pain, Cyclooxygenase-lipoxygenase dual inhibitor, *Morus alba*, Osteoarthritis, *Uncaria gambir*

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INTRODUCTION

Osteoarthritis (OA) is a chronic debilitating degenerative joint disease characterized by cartilage degradation and by synovial inflammation exhibited by clinical symptoms such as joint swelling, synovitis and inflammatory pain. Present day pharmacological or nonpharmacological management of arthritis are inadequate to address the underlying pathophysiological and biochemical mechanisms involved in cartilage degeneration and pain. Currently, pain relief therapeutics heavily relies on the use of prescription and over the counter nonsteroidal anti-inflammatory drugs (NSAIDs) as the first line of intervention where their long term usage causes detrimental gastrointestinal and cardiovascular-related side-effects. As a result, the need for evidence based safer and efficacious alternatives from natural sources to overcome the most prominent and disabling symptoms of arthritis is more urgent now than ever before.

Studies have showed OA as the whole joint disorder that affects all joint structures including the subchondral bone, cartilage and synovial membrane where each tissue interconnected at the cellular level by releasing and responding to inflammatory mediators. As the disease progresses,

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inflammation from the synovial membrane could acts as a trigger factor for several symptoms of OA by releasing pro-inflammatory factors that will increase and prolong cartilage damage. Inflammation plays a major role as a primary contributing factor for perpetuating cartilage degradation by promoting the destruction and impairing the ability of timely tissue repair, which leads to recurrent attack and persistence of chronic pain. Hence, intervening of the inflammatory process in the arthritis progression cascade may mitigate the severity of the disease and/or associated symptoms.

Regardless of the initial cause, elevated levels of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin (IL-1) and IL-6, cellular component of immunology, inducible nitric oxide synthase (iNOS) and activation of nuclear factor kappa-B (NF-κB) are considered to be essential to disease pathophysiology and progression of arthritis.[1] The major flavan in *Uncaria gambir*, catechin and prenylated flavonoids and stilbenoids, from the root bark of *Morus alba L* possess activities suggestive of benefits in arthritis including: (i) Inhibition of the activity of cyclooxygenase-2 (COX-2), lipoxygenase (5-LOX), and pro-inflammatory cytokines TNF-α, IL-1,-2,-6,-8, and-12[2,3] by catechin, (ii) anti-inflammatory activities,[4] (iii) suppression effect of T-cell migration, (iv) inhibition of CX chemokine receptor (CXCR-4)-mediated chemotaxis and extracellular signal-regulated kinases (MEK/ERK) pathway,[5] (v) inhibition of nitric oxide (NO) production, inducible NO synthase expression, prostaglandin E2 (PGE2) production, and activation of NF-κB[6] and (vi) inhibition of pro-inflammatory mediators such as COX-2, IL-1 β, and IL-6,[7,8] by prenylated flavonoids and stilbenoids from *M. alba* root bark extract have been reported. Therefore, a composition comprised of these well-studied plant extracts at a specific ratio may provide a benefit in alleviating symptoms associated with arthritis.

Through the years, significant numbers of animal models have been developed and utilized to study the anti-inflammatory and anti-nociception activity of plant extracts with diverse mechanisms of action in affecting pain perception and inflammation. Among these, carrageenan-induced rat paw edema, abdominal constriction (writhing's) tests and ear swelling assays in mouse are extensively used experimental animal models with applicable clinical and pathological features, which would help understand the anti-pain and anti-inflammatory activities of plant extracts.

Carrageenan inoculation into the intraplantar region of rat hind paw produces a classic model of hyperalgesia and edema. The hyperalgesia exhibited by the model is an essential feature of inflammatory pain, which consists of the action of COX mediated increase in prostaglandins which leads to peripherally and centrally mediated sensitization[9] accompanied by increased tissue fluid and plasma protein exudation forming a localized edema at the site of injection.[10] Once inoculated, it elicits two distinct phases of inflammatory reactions. The initial phase which lasts for 30–60 min is dominated by the release of histamine, serotonin and kinins, followed by prostaglandin and leukotrienes, which act relatively late in the development of inflammatory response. NSAIDs are known to prevent hyperalgesia of inflammation by blocking the prostaglandin pathway.[11]

Similarly, the inflammatory response observed during mouse ear swelling test is due to the formation of arachidonic acid (AA) metabolites mediated through both the COX and 5-LOX pathways. A single topical application of AA at 2 mg/ear to the mouse ear can result in an immediate vasodilatation and erythema followed by a progressive increase in edema formation that reached a plateau after 1 h. It's a rapid and short lived inflammatory response characterized by vasodilatation, tissue edema, protein leakage and inflammatory cell infiltration, signifies the advantage of the model for COX-LOX inhibitor screening.[12,13]

Behavioral response to visceral pain induced by an intraperitoneal administration of acetic acid has also long been used for screening of analgesics and NSAIDs like compounds for their anti-nociceptive benefits. The abdominal constrictions elicited by mice consist of contractions of the abdominal muscle that progress posteriorly and usually end with simultaneous flexor extension of both hind limbs with arching of the back. Upon injection of the irritant, behavioral response lasts for 30 min.[14,15]

Here, we evaluate the anti-inflammatory and analgesic effect of UP3005, a composition that contains a blend of two standardized extracts from the leaf of *U. gambir* and the root bark of *M. alba* in commonly used and well-accepted preclinical *in vivo* animal models.

MATERIALS AND METHODS

Individual materials

Uncaria gambir leaves were collected in Gunung malintang area of Sumatra, Indonesia. The dried leaves of *U. gambir* (2 kg) were extracted two times with 15-fold volume of water at 80°C for 7 h. The combined extraction solution was filtrated and dried under vacuum to afford 120 g of *U. gambir* extract. *M. alba* root barks were collected in Sichuan province of China. The dried root barks of *M. alba* (2 kg) were extracted 2 times with 7-fold volume of 70% aqueous ethanol at 80°C for 5 h. The combined extraction solution was filtrated and dried under vacuum to afford 294 g of ethanol extract powder.

The composition

The composition material of UP3005 was prepared by

blending two standardized extracts including *U. gambir* leaf and *M. alba* root bark, respectively, with 1:1 ratio. The *U. gambir* leaf extract contains (+)-Catechin as the major component with a content of not <16%. The *M. alba* root bark extract contains stilbenoid, mulberroside A, not <4%. The content for each individual marker compound in UP3005 was determined and quantified by high performance liquid chromatography (HPLC) method using an agilent HPLC/photo-diode array (PDA) system with a Zorbax Eclipse XDB C-18 reversed-phase column. For the quantification of active marker, catechin standard was purchased from Sigma (Catalog number: 21510-4) and mulberroside A was from Chengdu Biopurify Phytochemicals (catalog number: BP0964).

High performance liquid chromatography analysis conditions

The following analytical method was used to determine the amount of catechin in the *U. gambir* leaf extracts, and mulberroside A in the *M. alba* root extracts. An agilent HPLC/PDA system with a C-18 reversed-phase column (Zorbax Eclipse XDB, 3.5 μm, 4.6 mm × 150 mm, Agilent) using a guard column of C-18 cartridge (4.0 mm × 3 mm, phenomenex) was used for the detection and quantitation of catechin and mulberroside A. Mobile phases consisted of 0.1% phosphoric acid in purified water (A), and methyl alcohol (B) the elution conditions are described as follows: Elution time, 0–15 min, 12% →18% B (v/v); 15–25 min, 18% B → 100% B (v/v) for cleaning with the injection volume of 10 μL. The flow rate was set to 1.0 ml/min passing through the Zorbax C-18 column with a column temperature of 35°C with the ultraviolet detection absorbance at 285 nm.

Animals

Animals were acclimated upon arrival for a week before being assigned randomly to their respective groups. CD-1 mice (5/cage) and Lewis 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 were 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 guide for the care and use of laboratory animals.

Carrageenan induced rat paw edema model

Local inflammation was induced by intraplantar injection of carrageenan λ (Sigma, St. Louis, MO; 100 μl of 1% [w/v] in saline; lot # 0001408463) into the plantar surface of right hind paw of sedated rat (with 2.5% isoflurane) at time 0 ($T = 0$).[10,16] Rats were acclimated in a procedure room for 20–30 min before each measurement was taken. Allodynia was evaluated by measuring responsiveness to a tip of Randell-Salitto applied perpendicular to the central plantar surface of the right hind paw. A positive response to the applied pressure, noted by sharp withdrawal of the paw, was recorded automatically by an electronic Von Frey Anesthesiometer (2390 series Electrovonfrey, IITC, Woodland Hills, CA).[17] Mechanical allodynia was evaluated before carrageenan inoculation, and thereafter 1 h, 2 h, 4 h and 6 h. Paw edema was measured with the use of Plethysmometer (IITC, Woodland Hills, CA; Model 520) at time 0 (before carrageenan), 1 h, 2 h, 4 h, and 6 h after carrageenan injection. Animals ($N = 5$/group) were orally gavaged with a positive control ibuprofen (Spectrum Chemical MFG, Gardena, CA; lot # ZG0097) (200 mg/kg); UP3005 at doses of 100, 200, or 300 mg/kg and vehicle control 1 h after carrageenan inoculation unless specified otherwise. Similarly, *U. gambir* and *M. alba* at doses of 150 or 300 mg/kg were administered to compare with 300 mg/kg of UP3005 for synergy, if any.

Visceral pain perception model (writhing's test)

Mice ($N = 6$/group) were habituated under an inverted Plexiglas observation chamber for 30 min to allow them to acclimatize to their surroundings. Animals were treated orally with treatment articles at different doses, 100 mg/kg of ibuprofen or vehicle control (propylene glycol) 30 min before intraperitoneal administration of freshly made acetic acid solution (0.7% in 0.9% NaCl) at 10 ml/kg using 26 gauge needle syringes. The experiment was carried out in room temperature. After the challenge, each animal was placed back into its own individual section of the observation chamber and the number of constriction of the abdominal muscle together with stretching were counted cumulatively over a period of 30 min.[14]

Mouse ear swelling test

On the test day, once body weights were taken, animals were randomized to UP3005 and Ibuprofen groups ($N = 10$/group) and T0 measurements of ear thicknesses were taken using micrometer clipper. Then mice were gavaged with the respective treatment dosages orally at 100 mg/kg or 200 mg/kg UP3005. Ibuprofen was used as a positive control at a dose of 100 mg/kg. The vehicle treated group received propylene glycol only. One hour after treatment, AA suspended in ethanol was applied topically at a dose of 2 mg/20 μl/ear on the right ear.[18] Ethanol was applied on the left ear as a control. Two hours after induction (i.e. 3 h after treatment), ear swelling measurements were taken again. Data are reported as mean ± standard deviation (SD) and percent change of vehicle.

Cyclooxygenase-lipoxygenase assays

Cyclooxygenase-2 inhibition effect was tested by

commercial colorimetric COX (ovine) inhibitor screening assay kit (Cayman Chem., Co., Cat# 760111). In briefly, 150 µl of assay buffer, 10 µl of heme, 10 µl of COX-1 or COX-2 enzyme and 20 µl of test materials were added into 96-well plate. The plate was shaken carefully for a few second and incubated at 25°C for 5 min. A volume of 20 µl of colorimetric substrate solution and AA were added to initiate the reaction. After shaken carefully, it was incubated for 10 min at 25°C and the absorbance of each well was measured at 590 nm using a plate reader. Similarly, 5-LOX inhibition effect was tested by commercial 5-LOX (5-LOX: Potato, Cat# 60401) inhibitor screening assay kit (Cayman Chem., Co., Cat# 760700). In briefly, 90 µl of 5-LOX enzyme and 10 µl of test materials were added into 96-well plate and the plate was shaken carefully for a few second. 10 µl of substrate (linoleic acid) was added to initiate the reaction and the plate was placed on a shaker for 5 min. Hundred microliter of chromogen was added to each well to stop enzyme catalysis and develop the reaction. The plate was placed on a shaker for 5 min and the absorbance of each well was measured at 490 nm using a plate reader.

Statistical analysis

Data were analyzed using Sigmaplot (Version 11.0) (Systat Software, Inc., San Jose, CA). The results are represented as mean ± one S.D. Statistical significance between groups was calculated by means of a single factor analysis of variance, followed by a paired *t*-test. $P \leq 0.05$ 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 analysis of variance on ranks for analysis of variance.

RESULTS

Carrageenan induced rat paw edema model was developed and applied to assess analgesic and anti-inflammatory activity of composition UP3005. Upon intra-articular injection, cardinal signs of inflammation such as swelling and hyperalgesia were evident in all the rats. Statistically significant inhibition in pain sensitivity and inflammation were observed for all the doses of orally administered UP3005 at which the highest being in the 300 mg/kg and the lowest in 100 mg/kg. As shown in Figure 1, when rats were treated with UP3005 at the highest dose (300 mg/kg), 53.7%, 55.3%, and 48.8% reduction in inflammation and 56.1%, 53.3% and 44.8% reductions in pain sensitivity were observed at 1 h, 3 h and 5 h after treatment, respectively. Statistical significance reductions in pain sensitivity and inflammatory paw edema were also observed in rats treated even with the lower dose of UP3005 (100 mg/kg) such as 31.4%, 33.0% and 26.6% reduction in inflammation; 33.6%, 30.3%, and 28.3% reductions in pain sensitivity at 1 h, 3 h and 5 h after treatment [Figures 1 and 2]. These percent reductions were very comparable to the inhibitions observed for the positive control ibuprofen such as 52.5%, 62.9% and 51.6% reductions in inflammation and 58.6%, 64.0% and 50.7% reductions in pain sensitivity, respectively [Figures 1 and 2]. Despite the fact that compositions UP3005 excelled in efficacy as analgesic and anti-inflammatory agents than individual components (gambir or morus) at a dose of 300 mg/kg, data in Table 1 shows the unexpected synergistic activity of components when formulated together at a specific ratios of 1:1. When rats were given the composition UP3005 at a dose of 300 mg/kg, the observed results were greater than the theoretically calculated values both in inflammation and pain sensitivity at each time points analyzed (1, 3 or 5 h after treatment) [Table 1].

Similarly, visceral pain perceptions were attenuated by UP3005 administered orally. Behavioral responses observed for the duration of 30 min were reduced to 42.2 ± 24.0, 50.5 ± 17.6 and 65.7 ± 17.2 for UP3005 doses of 400, 300 and 200 mg/kg, respectively, to that of the vehicle control, that is 77.4 ± 18.7 [Table 2]. The positive control ibuprofen showed 25.3 ± 14.3 [Table 2]. These reductions in pain sensitivity were statistically significant for both ibuprofen and UP3005 (at doses of 400 mg/kg and 300 mg/kg) when compared to vehicle control.

Moreover, as shown in Table 3, after 2 h of topical AA application, the highest ear thicknesses were observed when mice were treated with the vehicle control (0.27 mm ± 0.02 mm), which indicates the induction of inflammation. However, thicknesses of edematous ear

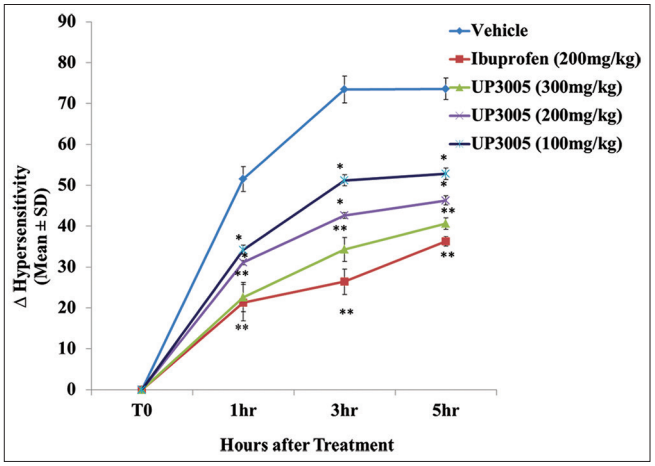

Figure 1: Dose correlated analgesic activity of composition UP3005 in carrageenan induced rat paw edema model. Female Lewis rats ($N = 5$) were treated with ibuprofen (200 mg/kg) and UP3005 (300 mg/kg, 200 mg/kg, and 100 mg/kg), or vehicle an hour after intraplantar carrageenan inoculation in rat paw edema model (**$P \leq 0.0001$ versus vehicle. *$P \leq 0.001$ versus vehicle)

were significantly reduced to 0.15 mm ± 0.02 mm (or 43.9% inhibition) by oral treatment of mice with 100 mg/kg ibuprofen [Table 3]. The composition UP3005 showed dose correlated inhibition of AA induced mouse ear swelling with percentage inhibitions of 45.8% and 59.8% at doses of 100 mg/kg and 200 mg/kg, respectively [Table 3]. At least in this assay, the potency from UP3005 either at 100 mg/kg or 200 mg/kg was higher than the positive control Ibuprofen.

In vitro, enzymatic inhibition activities of UP3005 were determined with IC50 values of 12.4 μg/ml, 39.8 μg/ml and 13.6 μg/ml in COX-1, COX-2 and 5-LOX enzyme activity assay, respectively [Table 4].

DISCUSSION

Clinical manifestations of symptoms of OA are associated with alterations in the articular cartilage interconnected with synovial membrane inflammation. Proinflammatory mediators primarily of cytokines, COX-LOX enzymes, NO, and PGE2 are byproducts of inflamed synovium, which changes 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, generating a perpetual circle. 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 curve further structural damage. However, despite the extraordinary advances in therapeutic discovery, development of a safe, effective and economical therapy for managing chronic inflammatory pain in arthritis still encounters a major challenge. In particular, the adverse cardiovascular and gastrointestinal side-effects associated with long term use of selective or non-selective NSAIDs have emphasized the need to develop natural alternatives with anti-inflammatory and analgesic activities devoid of such associated untoward effects.

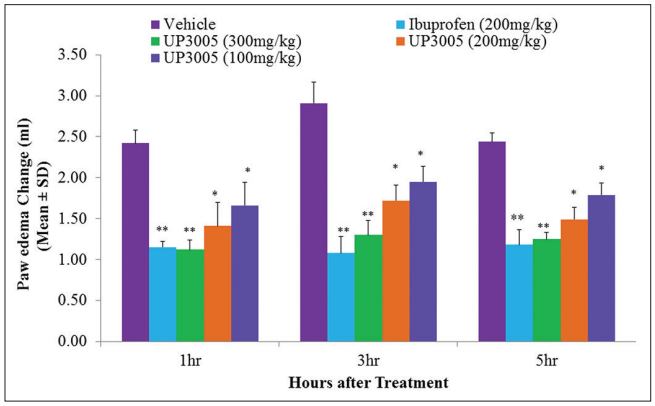

Figure 2: Dose correlated anti-inflammatory activity of composition UP3005 in carrageenan induced rat paw edema model. Female Lewis rats ($N = 5$) were treated with ibuprofen (200 mg/kg) and UP3005 (300 mg/kg, 200 mg/kg, and 100 mg/kg), or vehicle an hour after intraplantar carrageenan inoculation in rat paw edema model (**$P \leq 0.0001$ versus vehicle. *$P \leq 0.001$ versus vehicle)

While traditional NSAIDs were prescribed to control joint pain and treat inflammatory conditions such as rheumatoid arthritis and OA through their anti-inflammatory and analgesic effects by nonselective inhibition of COX activity, the therapeutic approach to inhibit the progression of OA by dietary supplements, partially depends on decreasing inflammatory activity by inhibiting inflammatory stimuli (iNOs, NO), enzymes (COX-2), inflammatory mediators (PGE2), and inflammatory cytokines (IL-1 β, IL-6, IL-8 and TNF-α).[19]

The major flavans, including catechin and epicatechin in *U. gambir*, 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, catechin has shown to inhibit the activity of COX-2, 5-LOX, phospholipase A2, NO production, NF-κB activation and pro-inflammatory cytokines such as TNF-α, and multiple interleukins that is IL-1,-2,-6,-8,

Table 1: Unexpectedly enhanced Analgesic and anti-inflammatory activity of composition UP3005 in carrageenan induced rat paw edema model

Composition	Compound	Dose mg/kg	*n*	Percentage change of vehicle					
				Paw edema			Pain sensitivity		
				1-h	3 h	5 h	1-h	3 h	5 h
UP3005	*U. gambir*	150	5	25.6*	26.1*	23.8*	27.4*	30.0*	22.4*
	M. alba	150	5	30.6*	29.6*	24.2*	30.1*	31.5*	23.0*
	Expected**	-	-	48.4	48.0	42.2	49.2	52.0	40.2
	Observed¥	300	5	53.7□	55.3□	48.8□	56.1□	53.3□	44.8□
	U. gambir	300	5	39.0*	28.6*	19.2	36.9*	32.4*	19.6
	M. alba	300	5	45.1□	38.3*	31.3*	44.6□	39.2*	32.5*

Data are presented as percentage change of vehicle. Rats (n=5) were gavaged with composition UP3005 (300 mg/kg), *gambir* (150 or 300 mg/kg) and *morus* extract (150 or 300 mg/kg), and vehicle 1-h after carrageenan induced paw edema induction. **Expected - Calculated value according to Colby's equation[32]=A-B i.e., A= (G+M), B= (GM)/100. ¥Observed-data observed when a composition was orally administered at 300 mg/kg. *P≤0.05; □P≤0.001. *U. gambir=Uncaria gambir*; *M. alba=Morus alba*

Table 2: Anti-nociceptive effect of UP3005 in CD-1 mice visceral pain model

Group	Dose (mg/kg)	Mean abdominal constriction/30 min±SD	Percentage change	*P*
Vehicle	0	80.4±18.8	-	-
Ibuprofen	200	25.3±14.3	68.6	0.0002
UP3005	400	42.2±23.9	47.6	0.0118
UP3005	300	50.5±17.6	37.2	0.0174
UP3005	200	65.7±17.2	18.3	0.1866

CD-1 mice (*n*=6) were treated with UP3005 (400 mg/kg, 300 mg/kg or 200 mg/kg) 30 min before intraperitoneal administration of acetic acid solution (0.7% in 0.9% NaCl) at 10 ml/kg. The number of constriction of the abdominal muscle together with stretching were counted cumulatively over a period of 30 min. SD=Standard deviation

Table 3: Anti-inflammatory activity of UP3005 in AA induced mouse ear swelling test

Group	Dose (mg/kg)	ΔMean ear thickness±SD	Percentage change	*P*
Vehicle	0	0.27±0.02	-	-
Ibuprofen	100	0.15±0.02	43.9	0.00001
UP3005	100	0.15±0.04	45.8	0.00001
UP3005	200	0.11±0.02	59.8	0.00001

CD-1 mice (*n*=10) were treated with UP3005 (100 mg/kg or 200 mg/kg) an hour before topical application of AA. Induction lasted for 2 h. Right ear measurements were taken before treatment and 3 h after treatment (i.e., 2 h after AA). SD=Standard deviation; AA=Arachidonic acid

Table 4: IC_{50} values of UP3005 in COX and LOX enzyme activity inhibition assay

COX-1	COX-2	COX-1/COX-2	LOX
12.4 μg/ml	39.8 μg/ml	0.31	13.6 μg/ml

LOX=Lipoxygenase; COX=Cyclooxygenase; IC_{50}=Inhibitory concentration 50%

and-12.[2,3,20,21] These compounds are the primary biomarkers frequently isolated in patients experiencing long term arthritis. The main proinflammatory cytokines involved in the pathogenesis of arthritis are TNF-α and IL-1 β. It has been documented that TNF-α has an early and crucial role in the cascade of proinflammatory cytokine production and subsequent inflammatory process.[22] It activates IL-1 β and IL-6 and thereby causes induction of hyperalgesia, which is mediated through downstream COX products like prostaglandin.[22,23] Therefore, the anti-inflammatory and analgesic activities observed in the present study could be partially explained by the anti-TNF-α activity of catechin in UP3005.

Similarly, a variety of bioactive compounds from *M. alba* root bark have showed *in vivo* or *in vitro* anti-inflammatory activity. For example, suppression of T-cell migration, inhibition of CXCR-4-mediated chemotaxis and MEK/ERK pathway,[5] inhibition of NO production, reduction of inducible NO synthase expression, inhibition of PGE2 production, and suppression of activation of NF-κB by oxyresveratrol,[6] inhibition of both NO production and iNOS, as well as reduction of pro-inflammatory mediators such as COX-2, IL-1 β, IL-6 by total flavonoids from the root bark[7] and by prenylated flavonoids[8] as well as inhibition of A disintegrin and metalloprotease with thrombospondin type I motifs-1[24] from *M. alba* extract were reported. Hence, these collective pharmacological activities of *M. alba* implies its wide array of application for arthritis treatments.

Previously involvement of NF-κB in OA pathogenesis has been reported in a way that when stimulated by pro-inflammatory cytokines, chemokines, stress-related factors and extracellular matrix degradation products, it will trigger the expression of multiple genes which induce destruction of the articular joint, leading to OA onset and progression.[25,26] As a result, the compounds that likely intervene a targeted NF-κB signaling pathway could provide potential benefits in arthritis management.[27] Thus, symptomatic mitigation observed in the present study could partially be explained by the ability of UP3005 to inhibit cellular gene expression regulated by transcription factor NF-κB.

It is also a common phenomenon to find correlation of increased level of NO and pain associated with regional hyperthermia in arthritic joints. This could be caused by vasodilation induced by extravascular NO production with osteoblasts, chondrocytes and macrophages due to mechanical stimulation of endothelial cells or by stimulated neurons.[28] In fact, both catechin and *M. alba* have shown to inhibit production of NO or expression of iNOS which suggest their relevance in alleviating pain in OA.

In support of previously reported data we have showed statistically significant improvement in pain resistance, and suppression of paw edema and ear thickness in animals treated with UP3005 compared to vehicle-treated diseased rats and mice. These marked inhibitions in pain and swelling were observed in all the models evaluated when UP3005 was administered orally at a dose as low as 100 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.[6] Similarly, another report showed inhibition of PGE2 and suppression of COX-2 messenger RNA in carrageenan induced paw edema and peritonitis in mice treated with morus.[29] 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 PGE2 was observed.[30] In additional note, the composition UP3005 showed statistical significance inhibition in pain sensitivity and inflammation in both the carrageen induced paw edema and mouse ear swelling tests at a dose as low as 100 mg/kg; However, the minimum efficacious dosage in the visceral pain was 200 mg/kg. These variances in efficacies could be as a result of differences in animal models where the visceral pain model is considered as a nonspecific model at which it requires a higher dosage to show a change in pain resistance.

Therefore, OA as characterized by degeneration of articular cartilage, the changes in subchondral bone and intra-articular inflammation with synovitis, proinflammatory cytokines from the inflamed synovial membrane are the major indications of inflammatory responses leading to inflammatory and destructive responses.[31,32] Hence, the hypothesis of formulating *U. gambir* and *M. alba* with their historically well-known anti-inflammatory and analgesic activity into a well-defined specific composition to hinder inflammatory process of OA has an attractive application in curtailing associated symptoms or disease progression.

CONCLUSION

Overall, in addition to the balanced dual COX-LOX inhibition activity observed, various reports have shown catechin and prenylated flavonoids extracted from leaf of *U. gambir* and root bark of *M. alba* to decrease expression of pro-inflammatory cytokines TNF-α and IL-1 β, NO, iNOS and/or inhibiting activation of transcription factor NF-κB. In the present study, UP3005, a composition contains a proprietary blend of two standardized extracts from the leaf of *U. gambir* and root bark of *M. alba,* has shown a significant improvement in the major cardinal signs of arthritis which includes reduction in pain sensitivity, and swelling. Moreover, it could also be easily inferred from these data that a significant therapeutic threshold could be achieved by administering the composition UP3005 than delivering either of its components (morus or gambir) alone. Though the long-term use indications require further clinical evidence; UP3005, an analgesic and anti-inflammatory agent of botanical origin, could potentially be used as medical foods and dietary supplements to manage the symptoms of OA.

Authors' contributions

MY and YCL conceived and designed, carried out study, data calculation, statistical analysis, data interpretation, and drafted/edited the manuscript. BM, TWK, HJK, JSO assisted in conducting the *in vivo* study. PJ, MH, JBN, MRK and SC conducted structure elucidations, identification, material sourcing and extractions. QJ, EJH and MC 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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