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ORIGINAL ARTICLE

UP1306, a Botanical Composition with Analgesic and Anti-inflammatory Effect

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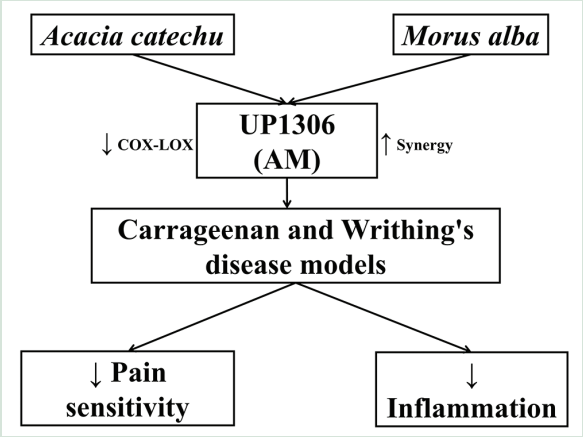
ABSTRACT

Background: Pain, one of the cardinal signs of inflammation, is the most common clinical manifestations of arthritis. Conventional pain relief therapy 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 deleterious gastrointestinal and cardiovascular-related side-effects. Hence, there is an equivocal need for evidence-based safer and efficacious alternatives from natural sources to overcome the most prominent and disabling symptoms of arthritis. **Materials and Methods:** Carrageenan-induced rat paw edema and abdominal constriction (writhing's) assays in mouse were used to evaluate the anti-inflammatory and analgesic effects of UP1306, a composition that contains a standardized blend of extracts from the heartwood of *Acacia catechu* and the root bark of *Morus alba* administered orally at dose ranges of 100–300 mg/kg. Cyclooxygenase (COX) and lipoxygenase (LOX) inhibition assays were carried out to determine the IC_{50} of *Acacia* and *Morus* extracts. The merit of combining these two extracts was also assessed. **Results:** Statistically significant improvement in pain resistance and suppression of edema were observed in animals treated with UP1306, when compared to vehicle-treated diseased rats and mice. Results from the high dose of UP1306 (300 mg/kg) were similar to those achieved by ibuprofen treatment at a dose of 200 mg/kg in early hours of treatment. *In vitro*, UP1306 showed dose-dependent inhibition of the enzymatic activities of COX and LO with IC_{50} values of 20.9 μg/mL, 49.2 μg/mL, and 11.1 μg/mL in COX-1, COX-2, and 5′-LO, respectively. **Conclusions:** These data suggest that UP1306, analgesic, and anti-inflammatory agent of botanical origin with dual COX-LO inhibition activity, could potentially be used to alleviate symptom associated to osteoarthritis.

Key words: *Acacia catechu*, cyclooxygenase-LO dual inhibition, inflammatory pain, *Morus alba*, osteoarthritis

SUMMARY

- Pain is the most common clinical manifestations of arthritis
- Carrageenan-induced rat paw edema and abdominal constriction (writhing's) assays in mouse are among the widely used models to evaluate the anti-inflammatory and analgesic effects of nutraceuticals



- Cyclooxygenase and lipoxygenase (LO) inhibition assays were carried out to determine the IC_{50} of *Acacia* and *Morus* extracts
- Efficacy of UP1306, a composition containing a blend of two standardized extracts from the heartwood of *Acacia catechu* and root bark of *Morus alba*, was evaluated in the above models
- UP1306 demonstrated its enhanced significance by improving the major cardinal signs of arthritis *in vivo* and inflammation markers *in vitro*
- UP1306 could potentially be considered as a dietary supplement product for the management of arthritis.

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INTRODUCTION

Osteoarthritis (OA) is a disease of the whole joint, involving the articular cartilage, the subchondral bone, the synovial membrane, and other peri-articular structures[1] leading to pain, stiffness, and functional disability. The percentage of people affected by arthritis continues to grow as the world population ages. For example, due to its prevalence and consequential disability, arthritis impact is at the top for the middle-aged and older United States population rendering it as the leading cause of disability in the United States. In the United States, according to the 2010–2012 data from the National Health Interview Survey, an estimated 52.5 million (22.7%) of adults have self-reported and doctor-diagnosed arthritis; in the same survey, 22.7 million (9.8%) of all adults have been found to suffer from arthritis and arthritis-attributable activity limitation.[2] A study carried out to estimate the national and state-specific direct cost impact of arthritis and other rheumatic conditions (AORC) by the Center for Disease Control showed that, in 2003, the total cost of AORC in the United States was approximately $128 billion ($80.8 billion in direct and $47.0 billion in indirect costs).[3] These figures are expected to grow in the coming decades.

Unfortunately, there is no cure for this debilitating disease. When patients unable to perform their day to day activities due to the profound discomfort, pain, stiffness, and seek medical care, they face a very tough choice as current management of arthritis is inadequate due to the lack of primary therapies proven to be effective in hindering

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disease progression without significant side effects. Over the counter or prescribed nonsteroidal anti-inflammatory drugs (NSAIDs) that are primarily dispensed to curtail the symptoms of the disease are known to cause gastrointestinal (GI) and cardiovascular side effects when used for longer durations. As a result, there is an equivocal need for evidence-based safe and efficacious alternatives from natural sources.

As the current opinion inclined toward more to the reclassification of OA as a systemic musculoskeletal disease rather than a localized disorder of synovial joints,[4] it is imperative to acknowledge the prominent factor such as inflammation that sustain the perpetual attacks involving the cartilage, subchondral bone, synovial membrane, and the entire joint structure as a whole unit.

Mounting evidence have been documented through the years suggesting the key role of inflammation in OA pathophysiology and progression. Proinflammatory cytokines released by activated cells within and/or around articular structure are among the crucial mediators of the unbalanced processes involved in OA pathophysiology.[5] For instance, proinflammatory cytokines (in particular tumor necrosis factor alpha [TNF-α] and interleukin [IL-1]), which were shown to stimulate matrix metalloproteinases and a disintegrin and metalloproteinase with thrombospondin motif (ADAMTS) gene transcription were found at significantly elevated levels in the synovial fluid of patients with OA.[6-8] Similarly, associations of serum levels of TNF-α and IL-6 with cartilage loss as measured by joint space narrowing were also seen in older adults with radiographic evidence of OA.[9] These cytokines were also found as highly predictive of the rate of disease progression in OA.[10]

Despite the fact that the initiating cause of OA is not well-known and poorly understood, elevated levels of proinflammatory cytokines such as TNF-α, (IL-1 and IL-6), cellular immunity, inducible nitric oxide synthase (iNOS), and activation of nuclear factor-kappa B (NF-κB) are thought to be essential for disease initiation and progression.[11] The major flavan in *Acacia catechu*, catechin, and prenylated flavonoids and stilbenoids, from the root bark of *Morus alba* L., possess activities suggestive of benefits in OA including (i) inhibition of the activity of cyclooxygenase-2 (COX-2), lipoxygenase (5-LOX), platelets phospholipase A2, and proinflammatory cytokines such as TNF-α, ILs 1, 2, 6, 8, and 12[12,13] as a result of catechin; (ii) inhibition of inflammation activities;[14] (iii) suppression effect of T-cell migration; (iv) inhibition of CXCR-4-mediated chemotaxis and MEK/ERK pathway;[15] (v) inhibition of nitrogen oxide (NO) production, inducible NO synthase expression, prostaglandin E2 production, and activation of NF-κB;[16] and (vi) inhibition of proinflammatory mediators such as COX-2, IL-1β, and IL-6 and enhancing total antioxidant ability[17,18] as a result of prenylated flavonoids and stilbenoids from *M. alba* root bark extract have been reported.

Hence, in the present study, the hypothesis of formulating standardized extracts from these historically well-known plant materials into a well-defined composition designed as UP1306 for alleviation of OA associated symptoms has been evaluated in carrageenan-induced paw edema in rat and acetic acid-induced visceral pain in mouse models.

MATERIALS AND METHODS

UP1306

Detailed procedure for the preparation of the composition has been described in the United States patent #20150072953.[19] The composition contains a proprietary combination of two standardized ethanol extracts from *M. alba* and *A. catechu.* Dried *M. alba* root barks were cut, crushed, and then extracted with approximately seven-fold volume of 70% ethyl alcohol in water (v/v); the extraction was carried out at 100°C for 4 h 3 times. The concentrated solution was dried by vacuum freeze-drying to obtain *M. alba* 70% EtOH extract powder with a yield of 19.6% (w/w). The major active components mulberroside A, kuwanon G, albanin G, and morusin in the *Morus* extracts were quantified with a Luna C18 reversed-phase column (Phenomenex, 10 μm, 250 mm × 4.6 mm) in a Hitachi high-performance liquid chromatography (HPLC) system at 325 nm. The standardized *Morus* extracts contain number <4% mulberroside A and no <3% of total bioflavonoids including kuwanon G, albanin G, and morusin. Catechins enriched *Acacia* extract was obtained by repeated crystallization from the heartwoods aqueous extract of an India medicinal plant, *A. catechu*. (+)-Catechin was identified as the major active flavan in the *A. catechu* extract. The *Acacia* extract was standardized as no <65% of catechin and a minor enantiomer epicatechin based on quantification by HPLC.

Compositions UP1306 was prepared by mixing the standardized extract (not less than 65% catechins) from heartwood of *A. catechu* and *M. alba* root bark ethanol extract (not less than 7% stilbenes and bioflavonoids) at a ratio of 1:2 by weight. The active contents in UP1306 are no <15% catechins and no <2% stilbenes and bioflavonoids.

Cyclooxygenase-LOX enzyme inhibition assay

COX inhibition effect was tested by commercial colorimetric COX (ovine) inhibitor screening assay kit (Cayman Chem., Co., Cat# 760111). In brief, 150 μL of assay buffer, 10 μL of heme, 10 μL of COX-1 or COX-2 enzyme and 20 μL of UP1306 at concentration of 10, 20, 50, and 100 μg/mL were added into a 96-well plate. The plate was shaken carefully for a few second and incubated at 25°C for 5 min. Colorimetric substrate solution (20 μL) and arachidonic acid 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 (VICTOR™ X3 PerkinElemr, Waltham, MA). Similarly, 5-LOX inhibition activity was tested by commercial 5-LOX (potato, Cat# 60401) inhibitor screening assay kit (Cayman Chem., Co., Cat# 760700). In brief, 90 μL of 5-LOX enzyme and 10 μL of UP1306 at concentration of 10, 25, and 50 μg/mL were added into 96-well plate, and the plate was shaken carefully for a few second. Substrate (linoleic acid 10 μL) was added to initiate the reaction, and the plate was placed on a shaker for 5 min. Chromogen (100 μL) was added to each well to stop enzyme catalysis and develop the reaction. The 96-well plate was placed on a shaker for 5 min and the absorbance of each triplicate well was measured at 490 nm using a plate reader.

Animals

Lewis rats and CD-1 mice 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) and mice (5/cage) were housed in a polypropylene cage and individually identified by numbers on their tails. Each cage was covered with wire bar lid and filtered top (Allentown, NJ, USA). 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, USA) *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 the institutional guidelines congruent with guide for the care and use of laboratory animals.

Carrageenan-induced rat paw edema

Inflammation and pain sensitivity was induced by intraplantar injection of 100 μL of 1% (w/v) carrageenan λ (Sigma, St. Louis, MO;

lot # 1408463V) into the plantar surface of right hind paw of sedated rat (with 2.5% isoflurane from Piramal healthcare Lot: A19E14A) at time 0 (t = 0).[20,21] 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 (IITC, Woodland Hills, CA; model #2888) 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, USA).[22] Mechanical allodynia was evaluated before carrageenan inoculation, and thereafter 1, 2, 4, and 6 h. Paw edema was measured with the use of Plethysmometer (IITC, Woodland Hills, CA; Model 520) at time 0 (before carrageenan), 1, 2, 4, and 6 h after carrageenan injection).[20-22] Animals (n = 5 per group) were orally gavaged with a positive control ibuprofen at 200 mg/kg (Spectrum Chemical MFG, Gardena, CA; lot # ZG0097); UP1306 at doses of 100, 200, and 300 mg/kg or vehicle control (0.5% carboxymethyl cellulose, CMC) 1 h after carrageenan inoculation. Dose-correlated efficacy and comparison study against individual components were assessed at T0 (before induction), and 1, 2, 4, and 6 h after induction. The merit of combining extracts of *A. catechu* and *M. alba* was also evaluated in this model using the Colby's equation.[23] In this method, for a formulation of two or more materials together will presume to have unexpected synergy, if the observed value of a certain end point measurement is greater or equal to the hypothetically calculated values.[23] Paw edema and pain sensitivity percent change values of *A. catechu* extract (100 mg/kg) and *M. alba* extract (200 mg/kg) at 1, 3, and 5 h after treatment were used to determine the calculated efficacy values and compared to the observed percent change values of the composition UP1306 (300 mg/kg) at the specified time points.

Visceral pain perception model (Writhing's test)

Mice (n = 6/group) were habituated under an inverted plexiglass observation chamber for 30 min to allow them to acclimatize to their surroundings. Animals were treated orally with UP1306 at oral doses of 300 mg/kg, 200 mg/kg, and 100 mg/kg, ibuprofen at an oral dose of 200 mg/kg 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.[24]

Statistical analysis

Data were analyzed using SigmaPlot (Version 11.0, Systat Software, Inc., San Jose, CA, USA). The results are represented as mean ± standard deviation. Statistical significance among groups was calculated by means of single factor analysis of variance (ANOVA) and by t-test. $P \leq 0.05$ ($P \leq 0.05$) were considered statistically 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

Cyclooxygenase-LOX Inhibitions

As shown in Table 1, UP1306 dose-dependently inhibited COX-1 and COX-2 enzymes, with IC_{50} 20.9 μg/mL and 49.2 μg/mL, respectively. Similarly, an IC^{50} of 11.1 μg/mL was observed for the 5-LOX enzyme.

Table 1: Concentrations dependent UP1306 effect on cyclooxygenase-1/-2 and 5-lipoxygenase enzyme activity inhibition

Dose (μg/mL)	Inhibition rates (%)			COX-1/COX-2
	COX-1	COX-2	5-LOX	
UP1306 10	38.1±0.67	23.7±1.51	41.4±1.25	1.6
UP1306 25	54.1±0.74	46.0±1.15	78.6±0.40	1.2
UP1306 50	72.3±0.64	51.9±0.47	93.4±0.20	1.4
UP1306 100	81.0±0.35	72.7±0.70	-	1.1

COX: Cyclooxygenase; 5-LOX: 5-Lipoxygenase

Composition UP1306 versus extracts from *Acacia catechu or Morus alba*

Analgesic and anti-inflammatory activity of composition UP1306 at a dose of 300 mg/kg were tested in rat with carrageenan-induced paw edema. For comparison, each individual extract (*A. catechu* or *M. alba*) was tested at the same dose of 300 mg/kg administered orally an hour after edema induction. Upon intraplantar injection of carrageenan to the hind paw, cardinal signs of inflammation such as swelling and hyperalgesia were evident in all the rats. As seen in Table 2, composition UP1306 showed a statistically significant higher inhibition in pain sensitivity and inflammation compared to either *A. catechu* or *M. alba* extract given alone. These changes in the magnitude of inhibitions were determined as increases with a range of 56.1–148.6% and 12.1–45.0% in inflammation and 46.9–106.8% and 19.2–32.1% in pain sensitivity against extracts from *Acacia* and *Morus* plants, respectively, at durations of 1–5 h of treatment.

Synergistic effect of *Acacia* and *Morus* extracts

Carrageenan-induced paw edema was utilized to evaluate a possible synergy or unexpected effect of extracts from *Acacia* and *Morus* when formulated together in a specific 1:2 ratio using Colby's method.[23] When rats were given UP1306 composition at a dose of 300 mg/kg, the observed results were greater than the theoretically calculated values both in inhibitions of inflammation and pain sensitivity at each time points analyzed (1, 3, or 5 h after treatment) [Table 3]. These findings may suggest that formulating two standardized extracts from *Acacia* and *Morus* at a 1:2 ratio has a far greater benefit than using either *Acacia* or *Morus* extract alone.

Dose-correlated activity of UP1306

As disclosed above, compositions UP1306 showed superior anti-inflammatory and analgesic activities than individual extract from *A. catechu* or *M. alba* at a dose of 300 mg/kg. To determine the optimum dosage of the composition UP1306 at which it would result in significant inhibition in pain and inflammation, doses of 300, 200, and 100 mg/kg were administered orally an hour postmodel induction to rats with carrageenan-induced paw edema. As seen in Figures 1 and 2, a clear dose-correlated, statistically significant inhibition in hypersensitivity and inflammation was observed for all the doses tested when compared to vehicle control. As expected, the positive control ibuprofen showed statistically significant inhibitions both in pain sensitivity and paw edema at each time points measured. When inhibitions observed from UP1306 treatment at the highest dose tested (300 mg/kg) were compared to ibuprofen, P = 0.10, 0.001 and 0.001 in pain sensitivity; 0.77, 0.05, and 0.29 in paw edema inhibitions at 1 h, 3 h, and 5 h, respectively, were found. At lease at this dose, the composition UP1306 showed similar efficacy to that of the positive control at early hours of treatment.

Table 2: Analgesic and anti-inflammatory activity comparison of UP1304 against extracts from *Morus* or *Acacia* in carrageenan-induced rat paw edema model

Group	Dose (mg/kg)	*n*	Percent change of vehicle (%)					
			Inflammation			Pain sensitivity		
			1 h	3 h	5 h	1 h	3 h	5 h
Ibuprofen	200	5	53.0±0.6**	60.1±0.4**	51.3±0.5**	52.4±3.4**	59.7±4.0**	43.3±2.0**
UP1306	300	5	55.4±0.1**	49.2±0.4**	43.0±0.3**	51.4±3.3**	48.4±3.0**	36.6±3.1**
Acacia	300	5	35.5±0.1**	29.3±0.3**	17.3±0.1**	35.0±1.1**	31.1±2.4**	17.7±2.1**
Morus	300	5	38.2±0.1**	43.9±0.2**	31.4±0.1**	38.9±1.2**	37.1±1.1**	30.7±2.1**

All treatment groups showed statistical significance at $^{**}P \leq 0.001$ when compared to vehicle control at the time when percent changes were computed; Rats were treated orally with ibuprofen (200 mg/kg), UP1306 (300 mg/kg), *Morus* (300 mg/kg), *Acacia* (300 mg/kg), or vehicle 1 h after carrageenan inoculation; Hypersensitivity threshold and paw edema was determined by subtracting 1, 2, 4, and 6 h individual values from their respective T0 value; Data are expressed as mean of percent reduction relative to vehicle with SD and reported as 1, 3, and 5 h after treatment. SD: Standard deviation

Table 3: Synergistically enhanced analgesic and anti-inflammatory activity of UP1306 in carrageenan-induced rat paw edema model

Composition	Extract	Dose (mg/kg)	*n*	Percentage change of vehicle					
				Inflammation			Pain sensitivity		
				1 h	3 h	5 h	1 h	3 h	5 h
UP1306	*Acacia*	100	5	21.9±0.2	23.0±0.1	17.6±0.1	23.9±2.1	27.5±0.9	22.6±2.1
	Morus	200	5	32.2±0.2	33.0±0.1	27.9±0.2	33.8±2.7	34.3±2.9	25.7±1.8
	Expected**	-	-	47.1±0.4	48.4±0.2	40.6±0.4	49.6±4.8	52.4±3.8	42.4±5.1
	Observed¥	300	5	53.3±0.2	54.3±0.1	46.3±0.3	54.8±3.3	53.8±3.0	43.6±3.1

**Expected: Calculated value according to Colby's method[23]=A-B, i.e., A=(percentage change value of *Acacia* at 100 mg/kg + percentage change value of *Morus* at 200 mg/kg); B=(percentage change value of UP1306 at 300 mg/kg)/100. ¥Observed: Data observed when rats was orally administered with UP1306 at 300 mg/kg. Data are presented as mean of percent change relative of vehicle control with SD. Rats (*n*=5) were gavaged with composition UP1306 (300 mg/kg), *Acacia* extract (100 mg/kg) and *Morus* extract (200 mg/kg), and vehicle 1 h after carrageenan induced paw edema induction. SD: Standard deviation

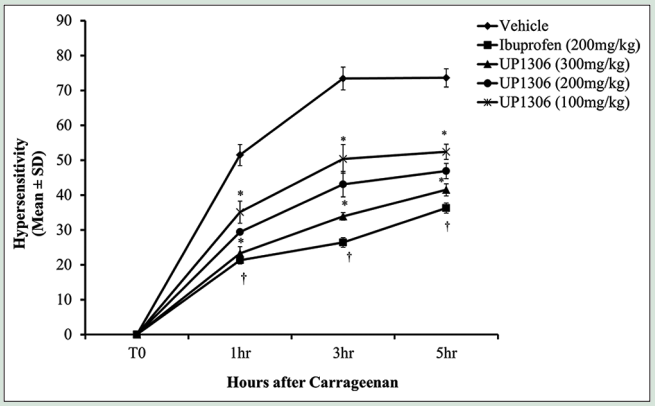

Figure 1: Dose correlated analgesic activity of UP1306. Rats (n = 5) were gavaged with composition UP1306, ibuprofen or vehicle 1 h after carrageenan-induced paw edema induction. Pain sensitivity data are expressed as mean ± standard deviation $^{\dagger}P \leq 0.0001$, versus vehicle; $^{*}P \leq 0.001$, versus vehicle

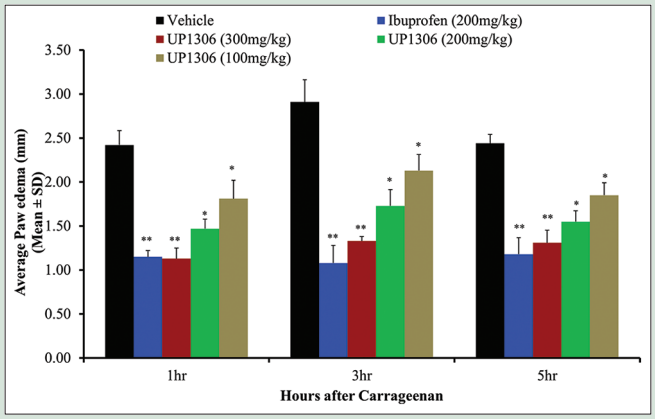

Figure 2: Dose-correlated anti-inflammatory activity of UP1306. Rats (n = 5) were gavaged with composition UP1306, ibuprofen or vehicle 1 h after carrageenan-induced paw edema induction. Inflammation data are expressed as mean ± standard deviation $^{**}P \leq 0.0001$, versus vehicle; $^{*}P \leq 0.001$, versus vehicle

Visceral pain perception

Composition UP1306 was tested at doses of 300 mg/kg, 200 mg/kg, and 100 mg/kg to alleviate a visceral pain inflicted by the intraperitoneal administration of freshly prepared 0.7% acetic acid in CD-1 mice at a volume of 10 mL/kg. Immediately after injection of the irritant, animals showed abdominal constrictions consisting of contractions of the abdominal muscle which progressed posteriorly and ended with simultaneous flexor extension of both hind limbs with an arching of the back. These behavioral responses observed for the duration of 30 min were reduced to 50.8 ± 17.2 (34.4% decrease compared to vehicle), 54.3 ± 15.5 (29.9% decrease compared to vehicle), and 64.0 ± 11.5 (17.4% decrease compared to vehicle) for UP1306 doses of 300, 200, and 100 mg/kg, respectively, compared to that of the vehicle control, 77.5 ± 16.1 [Figure 3]. The positive control ibuprofen showed 41.8 ± 12.6. These reductions in pain sensitivity were statistically significant for both ibuprofen and UP1306 (at doses of 300 mg/kg and 200 mg/kg) when compared to the vehicle control.

DISCUSSION

Magnifying OA as the big picture, loss of articular cushion (cartilage degradation and/or wear and tear) and chronic inflammatory pain are the "two sides of the same coin." Pain, one of the cardinal signs of inflammation, is the most common clinical manifestations of arthritis. In relation to early changes in joints of both arthritic patients and animal models, currently, it is recognized that influx of inflammatory cells into the articular region leading to hyperplasia and activation of

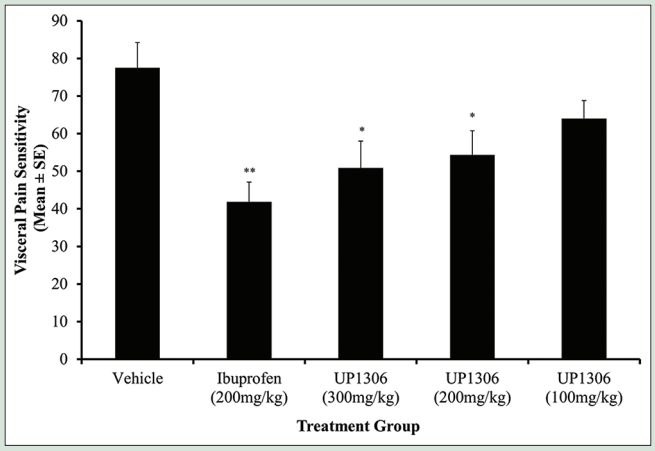

Figure 3: Anti-nociceptive effect of UP1306 in CD-1 mice visceral pain model. CD-1 mice (n = 6) were treated with UP1306 (300 mg/kg, 200 mg/kg and 100 mg/kg) and ibuprofen (200 mg/kg) 30 min before intraperitoneal administration of acetic acid solution (0.7% in 0.9% NaCl) at 10 mL/kg. The numbers of abdominal muscle constriction together with stretch were counted cumulatively over the period of 30 min. Data are expressed as mean ± standard deviation

resident inflammatory cells are the basis of arthritis pathophysiology.[25] Proinflammatory mediators primarily of cytokines, COX-LOX enzymes, NO, and predominant prostaglandin E2 (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 such, inflammation could be considered as the core for the central network of articular cartilage degradation, subchondral bone remodeling, and synovitis at the time of arthritis progression.

As of to date, despite the extraordinary advances in drug development arena; there still is a gap in the development of a safe, effective, and economical therapy for managing chronic inflammatory pain in arthritis. In particular, the adverse cardiovascular and GI side effects associated with long-term use of selective or nonselective NSAIDs underline the need to develop botanical alternatives with anti-inflammatory and analgesic activities without accompanied side effects. Here, we formulated two standardized extracts from historically well-known plant materials into a well-defined composition designed as UP1306 and evaluated their application in alleviating symptoms associated with OA in carrageenan-induced paw edema in rat and acetic acid-induced visceral pain in mouse models.

Previously, the major flavans, including catechin and epicatechin extracted from heartwood of *A. catechu*, prenylated flavonoids and stilbenoids extracted from the root bark of *M. alba* L. possessing activities suggestive of benefits in chronic pain management of arthritis has been reported. 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 ILs that is IL-1, -2, -6, -8, and -12.[12,13,26,27] These compounds are the primary biomarkers frequently associated with patients experiencing chronic arthritis. The main proinflammatory cytokines involved in the pathogenesis of arthritis are TNF-α and IL-1β. It has been documented that TNF-α has an early and crucial role in the cascade of proinflammatory cytokine production and subsequent inflammatory process.[28] It activates IL-1β and IL-6 and thereby causes induction of hyperalgesia, which is mediated through downstream COX products such as prostaglandins.[28,29] 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 UP1306.

Likewise, 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,[15] inhibition of NO production, reduction of inducible NO synthase expression, inhibition of PGE2 production, and suppression of activation of NF-κB by oxyresveratrol,[16] inhibition of both NO production and iNOS, as well as reduction of pro-inflammatory mediators such as COX-2, IL-1β, IL-6 by total flavonoids from the root bark[18] and by prenylated flavonoids[17] as well as the inhibition of A disintegrin and metalloprotease with thrombospondin Type I motifs-1[30] from *M. alba* extract were reported. Hence, these collective pharmacological activities of natural actives in *M. alba* implies its wide array of application for arthritis treatments.

In comparison to the conventional NSAIDs where 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-1β, IL-6, IL-8, and TNF-α).[31] As a matter of fact, almost all of these markers have been modulated by *Acacia* and *Morus* extracts as disclosed above.

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/LOX-mediated increase in prostaglandins and leukotrienes, which leads to peripherally and centrally mediated sensitization[32] accompanied by increased tissue fluid and plasma protein exudation forming a localized edema at the site of injection.[20] In our study, considerable alleviation in pain sensitivity and reduction of paw edema were observed in rats treated with an oral dose of UP1306. These inhibitions in carrageenan-induced rat paw edema model could be due to the inhibition of proinflammatory cytokines. Substantiating our findings, previous studies have shown production of TNF-α, which initiated a cascade of cytokine release including IL-1, IL-6, and IL-8 in the carrageenan-induced mechanical hyperalgesia model.[28] Upon activation, IL-1 and IL-6, stimulate the release of hyperalgesic COX products such as PGE2 to cause inflammatory hyperalgesia. These previously documented findings suggest the possible mechanism of actions of UP1306 in alleviating pain and inflammation, in part, through the inhibitions of cytokines and hence disruption of the pro-inflammatory cascades. In fact, major enzymes COX/LOX known to be involved in inflammatory process have been moderated by UP1306 *in vitro*. Clearly interesting though, at least in this model, an unexpected synergy was observed from the combination of standardized extract from *Acacia* with *Morus* extract that the beneficial effects seen with UP1306 treatment exceeded the predicted based on simply summing the effects observed for each of its constituents.

In agreement with previously reported data, we have documented statistically significant improvement in pain resistance, and suppression of paw edema in animals orally treated with UP1306 compared to vehicle-treated diseased rats.[33-35] These marked inhibitions in pain and swelling were observed in all animals evaluated when UP1306 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.[16] 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.[33] In a similar study, when catechin was given orally at a dose level as low as 60 mg/kg to adjuvant-induced Sprague-Dawley rats, a significant suppression in secondary inflammatory paw edema, hypersensitivity, and polyarthritis index as well as the inhibition in production of IL-1, TNF-α, and PGE2 was observed.[36] While the composition UP1306 showed statistical significance inhibition in pain sensitivity and inflammation in the carrageen-induced paw edema at a dose as low as 100 mg/kg; the minimum efficacious dosage in the visceral pain was 200 mg/kg. These variations 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.

CONCLUSIONS

Collectively, the clinical application of UP1306 could be rationalized by the fact that the primary biomarkers frequently associated with patients experiencing chronic OA seemed to be modulated by individual components of the composition, extracted from *Acacia* heartwood and/or *Morus* root bark separately. It has been previously shown that the active components of UP1306 to decrease expression of pro-inflammatory cytokines TNF-α and IL-1β, NO, iNOS, and/or inhibiting the activation of transcription factor NF-κB, as well as hindering catabolic enzymes such as ADAMTS-1. In the compiled data, UP1306, a composition containing a blend of two standardized extracts from the heartwood of *A. catechu* and root bark of *M. alba*, has demonstrated its enhanced significance by improving the major cardinal signs of arthritis (Pain and inflammation) in preclinical studies. In human clinical trial, it maintained cartilage structural integrity as evidenced by significantly low level of urine CTX-II in subjects who received UP1306 compared to placebo (data not shown). Therefore, UP1306, an analgesic and anti-inflammatory agent of botanical origin, composed of two historically well-known plants with safe usage, could potentially be considered as a dietary supplement product for the management of OA.

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Conflicts of interest

There are no conflicts of interest.

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