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Efficacy and tolerability of an undenatured type II collagen supplement in modulating knee osteoarthritis symptoms: a multicenter randomized, double-blind, placebo-controlled study

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Abstract

Background: Undenatured type II collagen (UC-II) is a nutritional supplement derived from chicken sternum cartilage. The purpose of this study was to evaluate the efficacy and tolerability of UC-II for knee osteoarthritis (OA) pain and associated symptoms compared to placebo and to glucosamine hydrochloride plus chondroitin sulfate (GC).

Methods: One hundred ninety one volunteers were randomized into three groups receiving a daily dose of UC-II (40 mg), GC (1500 mg G & 1200 mg C), or placebo for a 180-day period. The primary endpoint was the change in total Western Ontario McMaster Universities Osteoarthritis Index (WOMAC) from baseline through day 180 for the UC-II group versus placebo and GC. Secondary endpoints included the Lequesne Functional Index (LFI), the Visual Analog Scale (VAS) for pain and the WOMAC subscales. Modified intent-to-treat analysis were performed for all endpoints using analysis of covariance and mixed model repeated measures, while incremental area under the curve was calculated by the intent-to-treat method.

Results: At day 180, the UC-II group demonstrated a significant reduction in overall WOMAC score compared to placebo ($p = 0.002$) and GC ($p = 0.04$). Supplementation with UC-II also resulted in significant changes for all three WOMAC subscales: pain ($p = 0.0003$ vs. placebo; $p = 0.016$ vs. GC); stiffness ($p = 0.004$ vs. placebo; $p = 0.044$ vs. GC); physical function ($p = 0.007$ vs. placebo). Safety outcomes did not differ among the groups.

Conclusion: UC-II improved knee joint symptoms in knee OA subjects and was well-tolerated. Additional studies that elucidate the mechanism for this supplement's actions are warranted.

Trial registration: CTRI/2013/05/003663; CTRI/2013/02/003348.

Keywords: Knee function, Osteoarthritis, T regulatory cell, Undenatured type II collagen

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Introduction

Osteoarthritis, which entails the destruction of joint cartilage and remodeling of the adjacent bone, is the most common form of arthritis affecting more than 25 million Americans [1]. Current therapies for OA include various over the counter analgesics, a number of nonsteroidal anti-inflammatory drugs (NSAIDs), intra-articular injections of corticosteroids or hyaluronic acid, plus tramadol and other opioid analgesics to relieve severe pain [2, 3]. While these therapies can alleviate symptoms in the near term, their ultimate impact on the pathophysiologic progression of OA is limited [4].

Previous studies reported UC-II to be efficacious for the treatment of arthritis [5, 6]. More recently, a statistically significant improvement in knee joint function over placebo was also reported in a clinical study comprising a group of healthy individuals, supplemented with UC-II, and who developed transient knee joint pain upon strenuous exercise [7]. These same individuals also took longer to experience pain after 120 days of supplementation. Based on these observations, the current study was designed to evaluate the efficacy of UC-II in knee OA subjects compared to placebo and to GC, which is a widely available supplement that is used for reducing joint pain.

Materials and methods

Investigational products

The study product UC-II® (Lot 1204004) was derived from chicken sternum. It was manufactured under current good manufacturing practice (cGMP) conditions using a patented process that preserved its native structure (Chick Cart Inc., Fort Smith, AR). Both glucosamine hydrochloride (GH) and chondroitin sulfate (CS) were purchased through Wilke Resources (Lenexa, KS). The Wellable group (Shishi City, Fujian) manufactured GH under cGMP and according to United States Pharmacopeia 26 specifications. Sioux Pharm (Sioux Center, IA) manufactured bovine-derived CS under cGMP. UC-II and GC were encapsulated in opaque, size "00" capsules with sufficient amounts of excipients (microcrystalline cellulose and silicon dioxide) such that they were sensory identical to placebo. InterHealth Nutraceuticals provided all study materials. All American Pharmaceutical (Billings, MT) verified the amount of active ingredients in the study capsules. Study materials were kept in a secure cabinet with access restricted to the site coordinator, the dispensing pharmacist, and the principal investigator.

Study design

The objective of this randomized, double-blind, placebo-controlled clinical study was to evaluate the ability of UC-II to improve knee symptoms in OA subjects, as measured by overall WOMAC score, compared to placebo and to GC. The trial was conducted at 13 centers in southern India. Because of a limitation in synovial fluid sampling procedures at multiple clinical sites, the study was conducted under two separate study protocols. Study protocols were approved by each center's Institutional Ethics Committee (IEC), and listed on the clinical trial registry of India as study protocols 003663 and 003348. Enrollment, randomization, and follow-up visits were identical for both protocols, and were carried out at days 1 (baseline), 7, 30, 60, 90, 120, 150 and 180 (Table 1). All investigators attended the same investigator meetings, used identical intake and data reporting forms, and were trained and monitored by the same group of clinical research associates.

Efficacy measurements were assessed at all visits and included WOMAC, VAS, and LFI indices. The knee flexion range of motion (ROM) test was performed at each visit. Subject diaries and study product were provided at all visits, except day 180 and were collected at all follow-up visits. Subjects were instructed to record daily the consumption of study product, use of rescue medication, as well as concomitant medications in the subject dairy for the entire duration of the study. Blood and urine were collected at screening and day 180. Pregnancy testing was done at screening and follow-up visits. Adverse events (AEs) were recorded using each subject's diary inputs plus site visit questionnaires administered by intake personnel at all study visits.

Clinical endpoints

The primary endpoint was defined as the change in total WOMAC score from baseline through day 180 for the UC-II group versus placebo and GC. Secondary clinical endpoints for both protocols were similar and included the change from baseline through day 180 versus placebo and GC for all endpoints including the following scores: (1) mean VAS; (2) mean WOMAC subscales; (3) LFI; and (4) knee flexion. Another endpoint included the change from baseline to day 180 for the serum biomarker cartilage oligomeric matrix protein (COMP). In protocol 003348, additional secondary endpoints included the change in serum biomarker, C-reactive protein (CRP) plus synovial fluid biomarkers interleukin (IL)-6, and matrix metalloproteinase (MMP)-3 from baseline to day 180.

Study subjects

A total of 234 subjects were screened and 191 randomized (Fig. 1). Study inclusion criteria were 40–75 years-old male and female subjects, a body-mass index (BMI) of 18–30 kg/m^2, moderate-to-severe OA by physical examination (crepitus, bony enlargements, joint swelling, etc.) in one or both knees, knee pain for at least

Table 1 Protocol Schedule and Activities

Procedures common to both protocols	Screening (Visit 1)	Study period		
		Day 1 (Baseline Visit 2)	Days 7, 30, 60, 90, 120, 150 (Visits 3, 4, 5, 6, 7, 8)	Day 180 (Visit 9)
Signed Informed Consent	X			
Inclusion/Exclusion Reviewed	X	X	X	
Medical/Surgical/Medication History	X			
Physical Examination	X			
Vital Signs	X	X	X	X
Height[a], Weight, BMI	X			X
Clinical Assessment for Knee Pain & Swelling	X	X	X	X
Knee Flexion Range of Motion		X	X	X
X-ray examination	X			
WOMAC Score	X	X	X	X
VAS Scale	X	X	X	X
LFI Score	X	X	X	X
Clinical Laboratory Tests (hematology, chemistry, urinalysis)	X			X
Urine Pregnancy Test (if applicable)	X		X	X
Serum biomarker analysis-COMP		X		X
Randomization Number Assigned		X		
Investigational Product Administration		X		
Dispense Subject Diary		X	X	
Collect/Review Subject Diary			X	X
Provide Directions for Concomitant Medication and Rescue Medication Use	X	X	X	
Dispense New Investigational Product		X	X	
Review Product Accountability			X	X
Assess use of Concomitant Medications		X	X	X
Adverse Events Assessed		X	X	X
Procedures Confined to Protocol 003348				
Synovial fluid biomarker—MMP-3 and IL-6		X		X
Serum biomarker analysis—CRP		X		X

[a]Height was measured only at Visit 1

3 months prior to the start of the study, an LFI score between 6 and 10 and a VAS score of 40–70 mm 7 days after withdrawal from excluded medications, plus a knee radiograph that was graded as Kellgren and Lawrence (K-L) radiograph score of either 2 or 3 [8]. All OA diagnoses were confirmed by each study site investigator and noted in the subject's case report form (CRF). In the case of bilateral knee involvement, the index knee used for the study was the one that presented with the most severe OA symptoms at baseline. Detailed inclusion–exclusion criteria are summarized in Table 2.

Ethics, consent and permissions

Subjects were recruited after they reviewed, understood the study details, and then signed the IEC-approved consent form. The study conformed to the Declaration of Helsinki (version 1996).

Randomization & blinding

Block randomization, consisting of nine individuals per block, was executed in a 1:1:1 ratio using random numbers generated by an independent statistician (SPSS version 16.0). Knowledge of the randomization code was limited to the statistician plus one QA monitor unrelated with the study. Each investigator was given opaque, sealed envelopes denoting single patient identity numbers, randomization codes, and supplementation regimen to be opened in case of an emergency. The code was broken after the clinical database was locked.

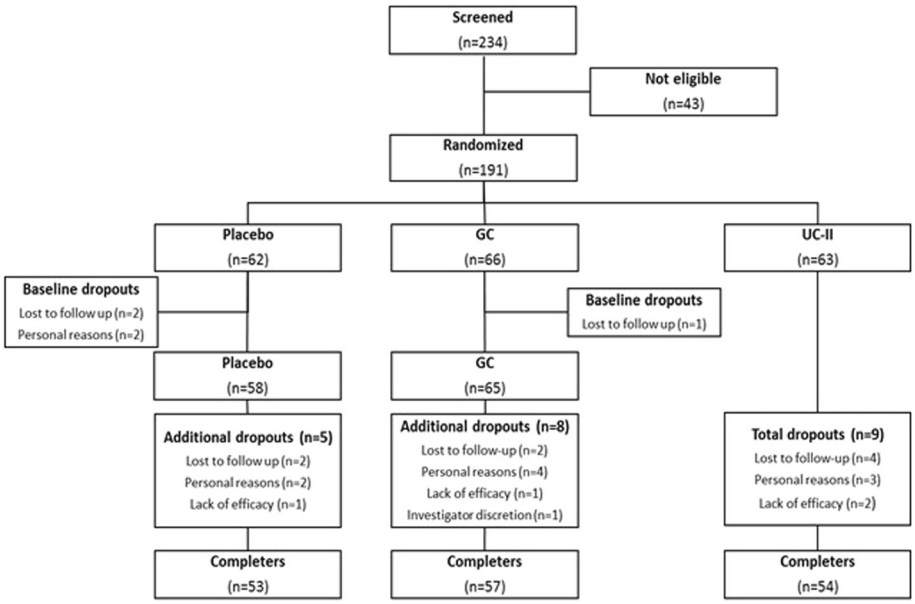

Fig. 1 Enrollment and randomization flow chart

Dosing regimen

Subjects ingested two blue pills in the morning with breakfast and two white capsules before bedtime. For the UC-II cohort, the two morning capsules were placebo, while the evening capsules contained 20 mg each of UC-II totaling 40 mg, which is identical to previously used clinical dose levels [5, 7]. This dose delivered 1.2 mg of undenatured type II collagen as determined by a newly developed and validated extraction-ELISA protocol (AIBiotech, Richmond, VA & Chondrex, Redmond, WA). For the GC group, the morning and evening doses delivered 750 mg of GH plus 600 mg of CS each totaling a daily dose of 1,500 mg of GH plus 1,200 mg of CS. The placebo group ingested identical numbers of blue and white capsules containing excipients only. Study bottles were labeled according to ICH-GCP and applicable local regulatory guidelines.

Prior and concomitant therapies

Prior medications were documented at the screening visit by the study investigator. At each visit, study personnel reviewed subject diaries and questioned each participant on the use of any concomitant medications including those on the prohibited list. Prohibited medications included ibuprofen, aspirin, other NSAIDS, or any other pain relievers (OTC or prescription), plus any dietary supplements (excluding vitamins) that could support joint health. All concomitant medications used during the study was documented in the subject's medical record by the study investigator then transcribed into their CRF by study personnel.

Rescue medications

Acetaminophen was allowed at a dose of 500 mg twice daily. Participants were instructed to not take this medication within 48 h of an evaluation visit. Usage levels and timing was entered at each visit into the subject's medical record by the study investigator. Study personnel transcribed this information into the subject's CRF.

Compliance and safety

Subjects were instructed to bring their bottles to each visit. Remaining capsules were counted and recorded in the subject's CRF and accountability log. As a secondary measure of compliance, subjects completed a diary indicating daily dosing of the study products. Safety assessments were performed at all visits by the site investigator and staff (see Table 9).

Study evaluations

WOMAC scores were determined using the WOMAC VA3.1 questionnaire containing 24 items grouped into three categories: pain, stiffness, and physical function (score range 0–2400). Each respective WOMAC subscale mean scores was determined by dividing the subscale score by the number of questions (5, pain; 2, stiffness; 17, physical function) it contained. The mean VAS score was determined using a VAS questionnaire containing 7 pain-related questions (score range 0–700), and then dividing the overall score by seven. LFI score was determined using an LFI questionnaire that assessed pain, walking distance, and activities of daily living,

Table 2 Inclusion-exclusion criteria

Inclusion

- Ambulatory, 40–75 years of age, with a BMI of 18 to 30 kg/m^2
- Females of childbearing age must agree to use a medically approved form of birth control and have a negative urine pregnancy test result throughout the study
- Female subjects of limited to no childbearing potential must be amenorrheic for at least 1 year or have had a hysterectomy, a bilateral oophorectomy, or both
- Unilateral or bilateral OA of the knee for greater than 3 months plus a Kellgren and Lawrence radiographic grade of 2 or 3
- VAS score during knee movement between 40–70 mm after 7 day withdrawal of excluded medications
- LFI score between 6–10 points after 7 day withdrawal of excluded medications
- Clinical laboratory results that are within normal range or considered not clinically significant by the Principal Investigator
- Be willing to participate in all scheduled visits, tests, and other trial procedures according to the clinical protocol
- Be willing to refrain from taking ibuprofen, aspirin or other NSAIDS, or any other pain reliever (OTC or prescription) during the entire trial other than acetaminophen (paracetamol) as rescue medication
- Provide a signed and dated informed consent indicating that the subject has been informed of all pertinent aspects and possible risks associated with participation in the trial

Exclusion

- History of hypersensitivity to the rescue medication or any of the products used in the study
- History of hypersensitivity to eggs, chicken or fowl, or shellfish
- History of inflammatory arthropathy, severe RA, OA (VAS score greater than 70), or Systemic Lupus Erythematosus
- Hyperuricemia (>440 μmol/L), past history of gout, or both
- Anticipation of surgery within the next 4 months
- Recent injury in the target knee (past 4 months)
- History of use for corticosteroid, indomethacin, glucosamine & chondroitin within 3 months of Visit 2; intra-articular treatments, including injections of corticosteroid or hyaluronic acid; consumption of Omega 3 fatty acids dietary supplements within 6 months preceding the treatment period (a 2-week washout period is allowed for subjects taking omega 3 fatty acid supplements)
- History of congestive heart failure
- Anticipated problems with product consumption
- Evidence or history of clinically significant hematological, renal, endocrine, pulmonary, gastrointestinal, cardiovascular, hepatic, neurologic diseases, or malignancies within the last 5 years
- High alcohol intake (>2 standard drinks per day) or use of recreational drugs (e.g., cocaine, methamphetamine, marijuana, etc.)
- Females who are pregnant or lactating or planning to become pregnant
- History of any mental illness that might impair the ability of subjects to provide a written informed consent
- Consumed acetaminophen (paracetamol), ibuprofen, aspirin or other NSAIDS, or any other pain reliever (OTC or prescription), or any natural health product, (excluding vitamins) within 7 days of first visit
- Participation in any clinical trials within 30 days prior to first visit

(score range 0–24). Knee flexion was measured using goniometry with the subject lying in the prone position and the leg to be tested positioned along the edge of the table [9].

Synovial fluid biomarkers

Synovial fluid (~0.5 mL) was aspirated from the knee joint using an appropriate sized needle (18–24 gauge, depending on joint size). Harvested fluid was stored frozen until tested. IL-6 and MMP-3 levels were determined using the corresponding Duoset ELISA kits (R&D Systems, Minneapolis, MN).

Serum biomarkers

COMP levels (Quantikine ELISA, R&D Systems) were determined in both study protocols. CRP levels (Latex COBAS INTEGRA, Roche Diagnostics GmbH, Mannheim) were assessed in protocol 003348. Serum was stored frozen until analyzed. Interassay and intrassay coefficients of variation for COMP and CRP were <5 %.

Statistics

We verified, using 2-way analysis of variance (ANOVA), that the results of the two protocols could be combined into a single analysis by demonstrating there was no group by study interaction and that the magnitude of the efficacy observed under the two protocols was similar.

A modified intent-to-treat (mITT) analysis was used to assess the efficacy and safety outcomes that was defined *a priori*. This included all subjects who were randomized, consumed study product, and had at least one completed post-baseline visit. An analysis of covariance (ANCOVA), that included supplementation as a fixed factor and the corresponding baseline value of the variable being tested as a covariate, was used for assessing the overall statistical significance of the primary and secondary endpoints. Following ANCOVA, the Tukey-Kramer multiple comparison test was used for determining pairwise statistical significance and calculating 95 % confidence intervals. Also, a mixed model repeated measures (MMRM) analysis of the primary endpoint was performed to account for the multiple assessments obtained during this study. In addition, the method of trapezoids was used to calculate incremental area under the curve (iAUC) for all study groups. For iAUC estimation, missing values were imputed using the expectation-maximization algorithm in SAS. Rescue medication usage between groups was compared using logistic regression followed by pairwise comparisons using the Tukey-Kramer test. In addition, a stratified analysis of the primary endpoint was performed according to baseline serum COMP levels above and below the median value for this biomarker.

Differences were considered significant if the resultant p-value was ≤0.05. An independent statistician performed the analyses and other calculations using SAS version 9.3 (Cary, NC).

Results

Demographics and baseline characteristics

Two hundred and thirty-four subjects were screened and 191 subjects who met the eligibility criteria were randomized to placebo ($n = 62$), GC ($n = 66$), or UC-II ($n = 63$) (Fig. 1). Per mITT criteria, 5 subjects were excluded from all analyses because they failed to present at any post-randomization visits resulting in an absence of clinical data. Table 3 summarizes the demographics of the remaining 186 subjects that were eligible for efficacy and safety analyses. Baseline subject characteristics, OA severity, serum CRP, COMP, IL-6 and other characteristics were similar among the three groups.

Subject dropouts

One hundred and sixty four subjects completed the study: 53, placebo; 57, GC; and 54, UC-II. The 27 dropouts, which included the five subjects mentioned previously, were allocated across the three cohorts as follows: 9, placebo; 9, GC; and 9, UC-II. The final dropout rate was 14 %. Subjects' dropout reasons are summarized in Fig. 1. No subject withdrew from the trial due to an adverse event attributable to any study product.

Study product compliance

Compliance with daily dosing of study capsules exceeded 90 % for all cohorts (data not shown).

Total WOMAC score

The UC-II supplemented group had statistically significant changes in the total WOMAC score compared to placebo (−551 vs. −414; 95 % CI −232 to −42; $p = 0.002$) and GC (−551 vs. −454; 95 % CI −190 to −3; $p = 0.04$) at day 180 (Fig. 2a, Table 4). When the total WOMAC results were analyzed, using MMRM, to account for treatment by time interactions, there remained a statistically significant difference between the UC-II and the placebo groups (−514 vs. −397; 95 % CI −210 to −24; $p = 0.0097$; Table 4). An iAUC analysis also yielded statistically significant differences between the UC-II group versus placebo (−2042 vs. -1479; 95 % CI −1012 to −113; $p = 0.0098$; Table 4). No significant changes were observed between the GC and placebo

Table 3 Demographic and baseline characteristics of the trial subjects

Characteristics	Placebo ($n = 58$)	GC ($n = 65$)	UC-II ($n = 63$)
Sex ((n) male + (n) female)	28M + 30F	28M + 37F	33M + 30F
Age (years)	53.1 ± 1.02	52.6 ± 1.02	53.5 ± 0.99
Height (cm)	162 ± 1.00	161 ± 1.12	161 ± 0.89
Body weight (kg)	64.5 ± 1.20	66.0 ± 1.13	65.5 ± 1.12
Body mass index (kg/m^2)	24.7 ± 0.40	25.5 ± 0.40	25.2 ± 0.37
Kellgren Lawrence radiographic score			
Grade 2 (n)	39	45	42
Grade 3 (n)	19	20	21
Lequesne's Functional Index	7.74 ± 0.12	8.02 ± 0.12	7.90 ± 0.13
Visual analog score (mm)	58.2 ± 0.97	59.1 ± 0.97	58.4 ± 0.99
Total WOMAC score	1382 ± 34.8	1396 ± 31.8	1398 ± 27.9
Mean WOMAC pain	56.9 ± 1.36	57.5 ± 1.33	58.1 ± 1.03
Mean WOMAC physical function	57.9 ± 1.51	58.5 ± 1.37	58.3 ± 1.24
Mean WOMAC stiffness	56.3 ± 1.63	57.3 ± 1.52	58.1 ± 1.32
Knee flexion ROM (°)	114 ± 1.62	114 ± 1.36	114 ± 1.57
Serum CRP (mg/L)[a]	5.29 ± 1.47	8.15 ± 1.79	3.35 ± 0.58
Serum COMP (ng/mL)[b]	325.2 ± 30.5	381.2 ± 44.1	334.6 ± 36.5
Synovial IL-6 (ng/mL)[c]	13.3 ± 4.73	13.9 ± 5.57	15.3 ± 6.04
Synovial MMP-3 (μg/mL)[d]	4.03 ± 1.20	2.54 ± 0.78	4.86 ± 1.74

Values presented as Mean ± SE
[a]Number of subjects used for analyses: 27, placebo; 29, GC; 29, UC-II
[b]Number of subjects used for analyses, 54, placebo; 58, GC; 55, UC-II
[c]Number of subjects used for analyses, 23, placebo; 24, GC; 21, UC-II
[d]Number of subjects used for analyses, 25, placebo; 27, GC; 23, UC-II

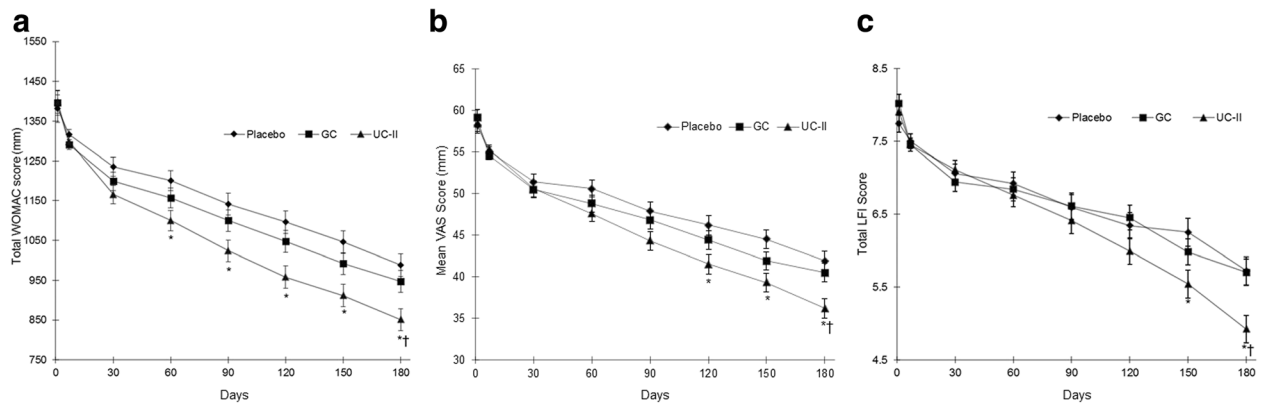

Fig. 2 Total WOMAC score (**a**), Mean VAS (**b**), Total LFI (**c**) in the UC-II, GC and placebo groups over the 180-day study period. Values are presented as mean ± SE. *Significant difference between the UC-II ($n = 54$) and the placebo ($n = 53$) group, $p < 0.05$. †Significant difference between the UC-II ($n = 54$) and the GC group ($n = 57$), $p < 0.05$

cohorts regardless of the type of analytical model used.

Total WOMAC score based on baseline COMP levels

We found that subjects supplemented with UC-II, and presented with baseline COMP levels ≥285 ng/mL (median), had a greater reduction in the total WOMAC score than both placebo and GC groups with similar COMP levels under all modeling conditions (Table 5). For study participants with baseline COMP levels <285 ng/mL, no significant differences between the study groups were noted. Interestingly, we did observe a smaller placebo effect among individuals with baseline COMP levels ≥285 ng/mL as compared to those with <285 ng/mL (28 % vs 32 %). Despite this, UC-II efficacy, as defined by a reduction in overall WOMAC score, was higher in subjects with COMP levels ≥285 ng/mL versus subjects with COMP levels < 285 ng/mL (43 % vs 36 %).

WOMAC mean subscores—pain, stiffness and physical function

At day 180, we observed significant reductions in all three WOMAC subscales for UC-II group compared to placebo (Table 6): pain (24.0 vs. 17.0; 95 % CI −11.1 to −2.8; $p = 0.0003$), stiffness (23.8 vs. 17.8; 95 % CI −10.4 to −1.6; $p = 0.004$), and physical function (22.5 vs. 17.3; 95 % CI −9.3 to −1.3; $p = 0.007$). The UC-II cohort also had significant reductions in WOMAC pain (24.0 vs. 19.2; 95 % CI −8.9 to −0.7; $p = 0.016$) and stiffness (23.8 vs. 19.4; 95 % CI −8.7 to −0.1; $p = 0.044$) at day 180 compared to GC.

Mean VAS score

The UC-II supplemented group had a significant decrease in mean VAS score at day 180 (Fig. 2b) versus both placebo (22.6 vs. 17.0; 95 % CI −9.5 to −1.8; $p = 0.002$) and GC (22.6 vs. 18.4; 95 % CI −8.0 to −0.4; $p = 0.025$). In contrast, the GC group was not significant compared to placebo at any time.

LFI score

A significant reduction was observed in the LFI score for the UC-II group at day 180 versus placebo (2.9 vs. 2.1; 95 % CI −1.4 to −0.2; $p = 0.009$; Fig. 2c). UC-II supplementation also has a greater improvement in LFI score versus GC (2.9 vs. 2.2; 95 % CI −1.4 to −0.2; $p = 0.008$). No significant change was observed between the GC and placebo cohorts. Improvement in the total LFI score for the UC-II group was attributed to a significant reduction in the LFI subscale for daily activities at day 180 ($p = 0.004$ vs. placebo; $p = 0.013$ vs. GC, data not shown).

Knee flexion

No significant differences were observed between the study groups (data not shown).

Serum biomarkers

A significant increase in the final CRP levels versus baseline occurred in all three cohorts ($p = 0.001$). However, no statistical difference between the three cohorts (Table 7; $p > 0.05$) was noted. The scientific reason behind this increase is not well understood. A significant decrease in serum COMP levels was seen in all groups versus baseline ($p = 0.04$) with no significant changes between groups.

Synovial fluid biomarkers

Similar non-significant decreases in IL-6 and MMP-3 levels were noted for all cohorts (Table 7).

Rescue medication usage

The number of subjects that used rescue medication was significantly lower in the UC-II group compared to

Table 4 Change in total WOMAC score from baseline

Analytical method	Type of analysis	Time point (Days)	Placebo ($n = 53$)	GC ($n = 57$)	UC-II ($n = 54$)	p value (95 % CI)			
						Overall[a]	GC vs PBO	UC-II vs PBO[b]	UC-II vs GC
ANCOVA	mITT	180	−414 ± 28.5	−454 ± 27.5	−551 ± 28.2	0.002	0.56 (−134 to 53)	0.002 (−232 to −42)	0.04[c] (−190 to −3)
MMRM	mITT	180	−397 ± 28.6	−452 ± 27.6	−514 ± 28.3	0.014	0.33 (−148 to 37)	0.0097 (−210 to −24)	0.25 (−153 to 30)
			($n = 58$)	($n = 65$)	($n = 63$)				
iAUC	ITT	1 to 180	−1479 ± 137	−1751 ± 130	−2042 ± 132	0.014	0.33 (−718 to 174)	0.0098 (−1012 to −113)	0.26 (−727 to 146)

Values presented as Mean ± SE
Abbreviations: *PBO* placebo
[a]Overall p value was obtained by comparing the mean changes among the three groups using ANCOVA
[b]Significant difference between the UC-II and the placebo groups using Tukey-Kramer test
[c]Significant difference between the UC-II and the GC groups using Tukey-Kramer test

Table 5 Stratified analysis for change in total WOMAC score based on baseline COMP levels

COMP (ng/mL)	Analytical method	Type of analysis	Time point (Days)	Placebo ($n = 27$)	GC ($n = 28$)	UC-II ($n = 27$)	*p* value (95 % CI)			
							Overall[a]	GC vs PBO	UC-II vs PBO	UC-II vs GC
≥285	ANCOVA	mITT	180	−368 ± 41.7	−396 ± 40.9	−574 ± 41.6	0.002	0.88 (−168 to 112)	0.002[b] (−347 to −65)	0.009[c] (−317 to −38)
	MMRM	mITT	180	−351 ± 44.1	−398 ± 41.1	−540 ± 44.2	0.006	0.71 (−188 to 94)	0.006[b] (−330 to −48)	0.048[c] (−282 to −1)
	iAUC[d]	ITT	1 to 180	−1351 ± 212	−1582 ± 204	−2384 ± 207	0.003	0.72 (−934 to 473)	0.002[b] (−1741 to −325)	0.02[c] (−1498 to −107)
				($n = 26$)	($n = 29$)	($n = 26$)				
<285	ANCOVA	mITT	180	−463 ± 38.8	−508 ± 36.6	−526 ± 38.7	0.48	0.67 (−173 to 82)	0.49 (−195 to 68)	0.94 (−145 to 109)
	MMRM	mITT	180	−442 ± 38.2	−493 ± 37.3	−521 ± 38.1	0.34	0.60 (−178 to 76)	0.32 (−208 to 50)	0.86 (−155 to 100)
	iAUC[e]	ITT	1 to 180	−1626 ± 185	−1908 ± 178	−1902 ± 185	0.49	0.52 (−896 to 333)	0.55 (−902 to 350)	0.99 (−607 to 618)

Values presented as Mean ± SE

[a]Overall p value was obtained by comparing the mean changes among the three groups using ANCOVA

[b]Significant difference between the UC-II and the placebo groups using Tukey-Kramer test

[c]Significant difference between the UC-II and the GC groups using Tukey-Kramer test

[d]Number of subjects used for analyses, 27, placebo; 29, GC; 28, UC-II

[e]Number of subjects used for analyses, 27, placebo; 29, GC; 27, UC-II

Table 6 Reduction in mean WOMAC subscores in placebo, GC and UC-II groups over 180 days

Parameter reduction	Day	Placebo ($n = 53$)	GC ($n = 57$)	UC-II ($n = 54$)	p value			
					Overall[a]	GC vs PBO	UC-II vs PBO[b]	UC-II vs GC[c]
WOMAC pain	7	3.21 ± 0.58	4.57 ± 0.54	3.88 ± 0.55	-	-	-	-
	30	6.61 ± 1.04	7.89 ± 1.00	9.18 ± 1.01	-	-	-	-
	60	8.17 ± 1.10	10.1 ± 1.07	12.7 ± 1.09	0.0149	-	0.011	-
	90	11.2 ± 1.17	12.7 ± 1.14	16.4 ± 1.16	0.0063	-	0.0059	-
	120	12.9 ± 1.28	15.6 ± 1.22	19.9 ± 1.26	0.0005	-	0.0004	0.040
	150	15.0 ± 1.21	17.5 ± 1.16	21.5 ± 1.20	0.0007	-	0.0006	0.047
	180	17.0 ± 1.25	19.2 ± 1.20	24.0 ± 1.23	0.0003	-	0.0003	0.016
WOMAC stiffness	7	3.47 ± 0.64	4.22 ± 0.61	4.24 ± 0.62	-	-	-	-
	30	6.81 ± 1.10	8.76 ± 1.05	9.28 ± 1.07	-	-	-	-
	60	9.36 ± 1.28	11.5 ± 1.25	13.1 ± 1.27	-	-	-	-
	90	11.3 ± 1.36	13.8 ± 1.32	17.0 ± 1.35	0.0158	-	0.010	-
	120	13.6 ± 1.40	15.0 ± 1.34	20.0 ± 1.39	0.0035	-	0.0039	0.029
	150	15.5 ± 1.32	17.7 ± 1.26	21.3 ± 1.31	0.0079	-	0.0058	-
	180	17.8 ± 1.31	19.4 ± 1.27	23.8 ± 1.30	0.0043	-	0.004	0.044
WOMAC physical function	7	3.17 ± 0.56	4.14 ± 0.53	3.91 ± 0.53	-	-	-	-
	30	6.30 ± 1.00	7.80 ± 0.96	9.26 ± 0.98	-	-	-	-
	60	7.75 ± 1.08	9.50 ± 1.05	11.9 ± 1.07	0.0278	-	0.020	-
	90	10.4 ± 1.17	12.1 ± 1.14	15.1 ± 1.16	0.0182	-	0.0136	-
	120	12.7 ± 1.20	14.5 ± 1.15	17.9 ± 1.19	0.0083	-	0.0064	-
	150	14.8 ± 1.19	16.9 ± 1.14	20.0 ± 1.18	0.0078	-	0.006	-
	180	17.3 ± 1.21	18.8 ± 1.16	22.5 ± 1.20	0.0068	-	0.007	-

Values presented as Mean ± SE
[a]Overall p value was obtained by comparing the mean changes among the three groups using ANCOVA
[b]Significant difference between the UC-II and the placebo groups using Tukey-Kramer test
[c]Significant difference between the UC-II and the GC groups using Tukey-Kramer test. '-'denotes a non-significant statistical outcome

placebo (Table 8; $p = 0.001$). Sixty individuals used rescue medications, at least once, during the study. Twenty-eight of these users were from the placebo group, 21 and 11 were from the GC and UC-II cohorts, respectively.

Safety assessments

No clinical or statistically significant changes were reported for any of the hematologic, blood biochemistry or vital signs results (Table 9). No significant changes were noted for the urinalyses results (data not shown).

A total of 45 AEs were reported during the 180-day study period: 9, placebo; 28, GC; and 8, UC-II (Table 10). The majority (62 %) of these occurred in the GC group. Fifteen of 45 events were classified as possibly related to supplementation, 14 of which belonged to the GC group and 1 to placebo. The 14 possible events linked to GC supplementation were primarily gastrointestinal in nature. The eight AEs noted for the UC-II cohort were deemed not related to supplementation. One individual in the GC group was removed from the study due to a respiratory tract infection (cough & fever). This infection was classified as an SAE. The event was investigated by

Table 7 Change from baseline to day 180 in serum and synovial fluid biomarkers

Matrix	Parameter reduction	Day	Placebo (n)	GC (n)	UC-II (n)
Serum	COMP (ng/mL)	180	−51.2 ± 31.3 (53)	−56.5 ± 36.0 (56)	−69.6 ± 40.8 (53)
	CRP (mg/L)	180	15.1 ± 6.33 (26)	9.09 ± 5.36 (28)	13.0 ± 4.64 (28)
Synovial	IL-6 (ng/mL)	180	−9.54 ± 4.83 (23)	−9.72 ± 5.28 (24)	−11.8 ± 5.37 (21)
	MMP-3 (μg/mL)	180	−2.24 ± 1.26 (25)	−0.93 ± 0.79 (27)	−2.67 ± 1.85 (23)

Values presented as Mean ± SE. Statistical analysis was performed on log transformed and baseline adjusted values. No significant differences were observed between the study groups ($p > 0.05$)

Table 8 Number of subjects reporting use of rescue medication

Day	Placebo	GC	UC-II
7	11/58	12/65	3/63
30	18/58	7/63	4/61
60	12/58	9/61	6/59
90	12/56	8/59	3/57
120	13/54	13/59	7/55
150	10/54	12/59	3/55
180	11/53	7/57	4/54
Entire study period	28/58	21/65	11/63[a]

The table summarizes the number of unique individuals reporting the use of rescue medication. Data presented as number of subjects using rescue medication / total number of subjects observed. [a]statistically significant versus the placebo ($p = 0.001$) based on pairwise Tukey-Kramer multiple comparison test. The overall group effect p-value was 0.002 using logistic regression

the attending physician and center staff and judged as not related to GC consumption.

Discussion

We assessed the ability of UC-II to improve joint symptoms in moderate-to-severe knee OA subjects. The results presented herein demonstrate that individuals consuming UC-II presented with better clinical outcomes versus those supplemented with placebo or GC. Analysis of the WOMAC subscales showed that reductions in all three WOMAC subscales contributed to the improvement in the overall WOMAC score observed in subjects supplemented with UC-II. In contrast, GC supplementation failed to induce a statistically significant improvement in the WOMAC, VAS or LFI scores versus placebo. These results confirm previous findings by Crowley et al. [5], which reported greater reduction in knee OA symptoms after 90 days of UC-II supplementation than what was observed with GC.

An interesting finding that emerged from this study is that stratification, according to baseline COMP levels, appears to have selected for individuals that responded better to UC-II supplementation. A greater reduction in knee OA symptom scores was observed among individuals with baseline serum COMP levels ≥285 ng/mL and supplemented with UC-II. This improvement was of sufficient magnitude that statistically significant outcomes for UC-II were observed versus both placebo and GC supplementation under all the statistical analyses we employed (ANCOVA, MMRM and iAUC). COMP, a cartilage turnover marker, is released into serum by chondrocytes and synovial cells [10–12]. Levels of this biomarker have been shown in several studies to have modest correlation with OA severity. However, serum COMP levels in groups of OA subjects overlap with levels observed in healthy populations, and this has limited the use of COMP as a prognostic marker for OA progression [12–14]. While the biologic or clinical significance to these findings remains to be understood, we find this preliminary observation an interesting one suitable for further investigation and confirmation.

The etiology behind UC-II's impact on OA symptoms is not known. However, undenatured type II collagen has been shown to protect animals against the onset of joint damage in both OA and RA experimentally induced arthritis models [15–18]. This protection is hypothesized to occur via the induction and migration of T regulatory cell (Tregs) to the area of inflammation and damage [19, 20]. The proposed role of Tregs may also have relevance to the moderation of OA symptoms, as *in vitro* studies have found that Tregs produce anti-inflammatory cytokines that stimulate chondrocytes to synthesize cartilage matrix components [21–23]. Additional studies that elucidate the precise mechanism through which UC-II mediates a reduction in knee OA symptoms are required.

The *in vivo* effects mentioned above may only be initiated by ingesting undenatured type II collagen as denatured (e.g., hydrolyzed) type II collagen fails to protect animals against the onset of arthritis [15]. This latter observation could explain why van Vijven and coworkers [24] concluded that there was insufficient evidence to support collagen for the treatment of OA as they combined data from all published clinical studies regardless whether native or denatured collagen was used in the trial.

We failed to observe any changes in knee ROM and distance walked regardless of supplementation. Improvements in these clinical outcomes are likely to be based not just on a symptomatic reduction in pain but also on physical improvements in the knee joint's overall functionality. Until we undertake trials of longer duration, it remains an open question as to whether a slow acting supplement like UC-II can impact the biomechanical status of the OA knee sufficiently to improve knee ROM.

In the current study, GC supplementation did not significantly improve the signs and symptoms associated with knee OA. The scientific literature supporting the efficacy of GC is mixed, but there are various published studies which suggest that GC may moderate OA symptoms [25–27]. The GAIT study found that GC, and each component of GC individually, failed to impact OA symptoms as measured by the WOMAC pain scale; however, the placebo effect in that study was nearly 60 % which resulted in an underpowered study to determine differences between the treatments [28]. In contrast, a significant difference in knee pain was observed in the GC subgroup with moderate-to-severe knee pain compared to the placebo treated group [28]. To confirm the observation that GC may be more efficacious in subjects with moderate-to-severe knee OA pain, Hochberg and coworkers [29] performed a study in which OA subjects with moderate-to-severe knee pain, were randomized

Table 9 Safety parameter assessment at baseline and day 180 in placebo, GC and UC-II groups

Parameter (Units)		Baseline						Day 180					
	Normal range	Placebo ($n = 58$)	GC ($n = 65$)	UC-II ($n = 63$)	p value GC vs PBO	p value UC-II vs PBO	p value UC-II vs GC	Placebo ($n = 53$)	GC ($n = 56$)	UC-II ($n = 53$)	p value GC vs PBO	p value UC-II vs PBO	p value UC-II vs GC
Hematology													
Hemoglobin (gm/dL)	12.1–17.2	12.1 ± 0.22	11.9 ± 0.21	12.1 ± 0.20	0.7613	0.9948	0.8095	12.7 ± 0.24	12.4 ± 0.20	12.7 ± 0.18	0.4454	0.9727	0.5851
ESR (mm/h)	0–29	21.1 ± 1.77	23.9 ± 2.18	17.5 ± 1.56	0.7629	0.1034	0.0144	15.1 ± 1.24	17.0 ± 1.91	13.6 ± 1.28	0.9424	0.5364	0.3387
RBC (million/mm^3)	4.7–6.1	4.29 ± 0.08	4.21 ± 0.08	4.33 ± 0.09	0.7747	0.9388	0.5498	4.32 ± 0.08	4.25 ± 0.08	4.37 ± 0.08	0.7935	0.8946	0.5129
WBC (/mm^3)	4500-10,000	7979 ± 234	8248 ± 222	7795 ± 249	0.7020	0.8483	0.3523	7984 ± 204	7981 ± 209	7769 ± 204	1.0000	0.7706	0.7639
Platelet count (x100000/mm^3)	1.5-4.5	2.77 ± 0.08	2.84 ± 0.08	2.78 ± 0.08	0.7837	0.9946	0.8319	2.77 ± 0.07	2.84 ± 0.07	2.77 ± 0.09	0.8304	0.9993	0.8113
Liver Function													
Albumin (gm/dL)	3.5–5.5	3.98 ± 0.06	4.02 ± 0.06	3.94 ± 0.06	0.8957	0.9089	0.6503	4.00 ± 0.05	4.03 ± 0.05	3.96 ± 0.04	0.8931	0.8902	0.6292
ALP (IU/L)	44–147	117 ± 5.74	118 ± 5.84	115 ± 5.57	0.9871	0.9838	0.9404	123 ± 5.72	116 ± 5.49	115 ± 5.59	0.5622	0.4847	0.9890
SGOT (U/L)	10–34	25.2 ± 0.93	24.0 ± 0.97	24.4 ± 0.60	0.5778	0.7796	0.9421	24.6 ± 0.73	23.9 ± 0.81	23.9 ± 0.65	0.7711	0.7930	0.9995
SGPT (U/L)	5–35	25.9 ± 1.23	25.0 ± 1.40	24.1 ± 0.95	0.5977	0.6004	1.0000	24.5 ± 0.94	24.3 ± 1.00	23.3 ± 0.99	0.9688	0.7119	0.8427
Total bilirubin (mg/dL)	0.3–1.9	0.78 ± 0.08	0.69 ± 0.03	0.72 ± 0.03	0.5376	0.9424	0.7343	0.72 ± 0.03	0.67 ± 0.03	0.78 ± 0.04	0.4243	0.6098	0.0718
Cardiac Function													
Systolic BP (mm Hg)	<120	125 ± 1.28	127 ± 1.35	127 ± 1.21	0.5980	0.7320	0.9752	127 ± 1.18	125 ± 1.33	128 ± 1.22	0.7263	0.8949	0.4409
Diastolic BP (mm Hg)	< 80	81.2 ± 1.19	80.2 ± 0.83	81.7 ± 1.02	0.7544	0.9283	0.5094	80.2 ± 1.03	80.5 ± 1.07	78.9 ± 0.96	0.9877	0.6233	0.5180
Pulse rate (beats/min)	60–100	80.0 ± 0.92	79.6 ± 0.98	80.3 ± 0.99	0.9149	0.9719	0.7956	80.0 ± 0.89	78.2 ± 0.82	79.2 ± 1.03	0.3201	0.8018	0.6989
Renal Function													
Creatinine (mg/dL)	0.6–1.3	1.00 ± 0.03	1.01 ± 0.04	0.96 ± 0.03	0.9995	0.5767	0.5778	0.96 ± 0.03	0.95 ± 0.02	0.96 ± 0.02	0.9904	0.9846	0.9508
BUN (mg/dL)	6–24	18.1 ± 1.08	18.0 ± 1.11	18.0 ± 1.15	0.9929	0.9878	0.9992	18.6 ± 1.11	17.8 ± 1.09	17.9 ± 1.02	0.7602	0.7953	0.9985

Results are presented as Mean ± SE. Normal ranges were obtained from Medline[a] and the Mayo Clinic[b]. Data was analyzed using ANCOVA followed by Tukey's multiple comparisons test ($p > 0.05$)

Abbreviations:

ESR erythrocyte sedimentation rate; *RBC* red blood cell; *WBC* white blood cell; *ALP* alkaline phosphatase; *SGOT* serum glutamic oxaloacetic transaminase; *SGPT* serum glutamic pyruvic transaminase; *BP* blood pressure; *BUN* blood urea nitrogen

[a]ADAM, Inc.: http://www.nlm.nih.gov/medlineplus/encyclopedia.html (accessed October 2015)

[b]Mayo Foundation for Medical Education and Research: Mayo Clinic. www.mayoclinic.org (accessed October 2015)

Table 10 Summary of analysis of adverse events in all subjects

	Study group		
	Placebo (*n* = 58)	GC (*n* = 65)	UCII (*n* = 63)
Severity			
Mild	7	21	5
Moderate	2	7	3
Severe	0	0	0
Relationship to Test Article			
Not related	8	14	8
Possible	1	13	0
Definite	0	1	0
Body System and AEs			
Gastrointestinal			
Acidity	2	3	2
Acute peptic disorder	1	0	1
Diarrhea	1	1	0
Epigastric burning	0	1	0
Febrile Enteritis	0	1	0
Heart burn	0	1	0
Vomiting	0	1	0
Nausea	0	1	0
Pain			
Arthralgia	0	1	0
Body pain	0	1	0
Low back pain	1	1	0
Neck Pain	0	1	1
Headache	2	4	0
Myalgia	0	1	0
Dermatology			
Itching	0	2	0
Xerotic skin	0	0	1
Pulmonary/Upper Respiratory			
Lower respiratory tract infection	0	0	2
Upper respiratory tract infection	0	1	0
Cough	0	2	0
Genitourinary			
Burning micturition	1	0	0
Burning sensation	0	0	1
Cardiovascular			
Palpitation	0	2	0
Constitutional Symptoms			
Fever	1	2	0
Insomnia	0	1	0
	9	28	8
Total Number of Adverse Events Experienced During Study			
Total Number of Subjects Experiencing Adverse Events: n (%)	7/58 (12 %)	20/65 (31 %)	8/63 (13 %)

to GC or celecoxib for a period of 6 months. The results showed that GC treatment reduced WOMAC measured knee pain by 50 %, comparable to the results obtained with celecoxib [28]. It is worth noting that results such as these are not consistent across a number of studies for reasons yet to be determined [25–27].

In recent years, interest has focused on developing various biomarkers for monitoring OA progression and drug development [12, 30]. We therefore assessed several biomarkers of inflammation (CRP, IL-6 and MMP-3) plus cartilage breakdown (COMP) and found no significant change for any of these biomarkers in this clinical trial. Since OA appears to impact the biology of several key components of the knee (e.g., synoviocytes, chondrocytes, etc.), the ability to achieve a significant change in any one biomarker could prove challenging for a slow acting supplement like UC-II. Also, multiple factors including ethnicity, physical activity, gender differences, and diurnal variation influence these biomarkers resulting in large variability in their levels [31–35]. Therefore, any change in these markers would have to occur as a result of a highly significant impact on the underlying pathophysiology of OA, given that the correlation between these biomarkers and OA pathophysiology are weak [12]. Such effects might be expected to occur more readily with a targeted agent [4, 36].

Conclusion

This study found that UC-II, a nutritional ingredient containing undenatured type II collagen, significantly improved knee function in OA subjects by day 180, compared to placebo and to GC, and was well-tolerated. Based on the data presented herein, we believe that additional research is warranted both to confirm and to define these findings more extensively.

Abbreviations

AEs: adverse events; ANCOVA: analysis of covariance; ANOVA: analysis of variance; cGMP: current good manufacturing practice; CI: confidence interval; COMP: cartilage oligomeric matrix protein; CRF: case report form; CRP: C-reactive protein; GC: glucosamine hydrochloride plus chondroitin sulfate; iAUC: incremental area under the curve; IEC: Institutional Ethics Committee; IL-6: interleukin-6; ITT: intent-to-treat; K-L: Kellgren and Lawrence; LFI: Lequesne functional index; mITT: modified intent-to-treat; MMP-3: matrix metalloproteinase-3; MMRM: mixed model repeated measures; NSAIDs: nonsteroidal anti-inflammatory drugs; OA: osteoarthritis; PBO: placebo; ROM: range of motion; Tregs: T regulatory cell; UC-II: undenatured type II collagen; VAS: visual analog scale; WOMAC: Western Ontario McMaster Universities Osteoarthritis Index.

Competing interests
JPL and ZMS are employees of InterHealth Nutraceuticals. NEL provided consulting services to InterHealth. This study was sponsored by InterHealth Nutraceuticals, Inc. Benicia, CA. The study was run and managed independently by Laila Pharmaceuticals Pvt. Ltd., India. Data collection was done by the clinical study staff at each respective site. Data analyses was performed by an independent statistician.

Authors' contributions
JPL and ZMS contributed in the conception and design of the study, data interpretation and manuscript preparation. NEL participated in data interpretation, manuscript drafting and revisions. All authors read and approved the final version of the manuscript.

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Safety and Efficacy of Undenatured Type II Collagen in the Treatment of Osteoarthritis of the Knee: A Clinical Trial
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Research Paper

Safety and efficacy of undenatured type II collagen in the treatment of osteoarthritis of the knee: a clinical trial

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Abstract

Previous studies have shown that undenatured type II collagen (UC-II) is effective in the treatment of rheumatoid arthritis, and preliminary human and animal trials have shown it to be effective in treating osteoarthritis (OA). The present clinical trial evaluated the safety and efficacy of UC-II as compared to a combination of glucosamine and chondroitin (G+C) in the treatment of OA of the knee. The results indicate that UC-II treatment was more efficacious resulting in a significant reduction in all assessments from the baseline at 90 days; whereas, this effect was not observed in G+C treatment group. Specifically, although both treatments reduced the Western Ontario McMaster Osteoarthritis Index (WOMAC) score, treatment with UC-II reduced the WOMAC score by 33% as compared to 14% in G+C treated group after 90 days. Similar results were obtained for visual analog scale (VAS) scores. Although both the treatments reduced the VAS score, UC-II treatment decreased VAS score by 40% after 90 days as compared to 15.4% in G+C treated group. The Lequesne's functional index was used to determine the effect of different treatments on pain during daily activities. Treatment with UC-II reduced Lequesne's functional index score by 20% as compared to 6% in G+C treated group at the end of 90-day treatment. Thus, UC-II treated subjects showed significant enhancement in daily activities suggesting an improvement in their quality of life.

Key words: undenatured type II collagen, osteoarthritis, glucosamine, chondroitin, WOMAC, visual analog scale, Lequesne's Functional Index

INTRODUCTION

Arthritis afflicts approximately 43 million Americans or approximately 16.6% of the US population. The two most common types of arthritis are osteoarthritis (OA) and rheumatoid arthritis (RA). OA of the knee and hip is a growing health concern and is the most common forms of arthritis (1-3). Pain and disease can range from very mild to very severe (3). Patients with OA have pain that typically worsens with weight bearing, including walking and standing, and improves with rest (4). Other symptoms include morning stiffness and gelling of the involved joint after periods of inactivity. Currently, OA affects

nearly 21 million people in the United States, accounting for 25% of visits to primary care physicians, and half of all Non-Steroidal Anti-Inflammatory Drugs (NSAID) prescriptions. The diverse clinical patterns of OA are observed in approximately 10% of people older than 60 years thus compromising the quality of life of millions of Americans. In addition, OA costs the North American economy approximately $60 billion per year.

Current treatment of OA includes exercise, heat/cold therapy, joint protection, weight loss, physiotherapy/occupational therapy and medications (3-5). The most common medications include acetaminophen and NSAIDs. Although these drugs are effective for reducing pain associated with OA, they do not reverse the disease. In addition, there are considerable side effects associated with the use of these drugs. As a result, OA sufferers have turned to natural nutraceuticals to ease their pain and discomfort. These products are commonly used because they are well tolerated and considered safe. Nutraceuticals are defined as functional foods, natural products, or parts of food that provide medicinal, therapeutic, or health benefits, including the prevention or treatment of disease. Currently, glucosamine and chondroitin are the two most commonly used nutraceuticals in humans as well as in animals to alleviate pain associated with arthritis (6). However, recent randomized controlled trials and meta-analysis of these supplements have shown only small-to-moderate symptomatic efficacy in human OA (7). An emerging novel nutraceutical ingredient known as UC-II has received considerable attention in the treatment of OA. UC-II is a novel undenatured type II collagen derived from chicken sternum cartilage. Previous studies have shown that undenatured type II collagen is effective in the treatment of RA (8-11), and preliminary human (12) and animal (13) trials have shown it to be effective in treating OA. Obese-arthritic dogs given 4 mg or 40 mg daily dose of UC-II for 90 days showed significant declines in overall pain, pain during limb manipulation and lameness after physical exertion (14). Greater improvement was observed with the 40 mg dose. No adverse effects or significant changes in serum chemistry were noted. Following UC-II withdrawal for a period of 30 days, all dogs experienced a relapse of overall pain, exercise-associated lameness and pain upon limb manipulation. Studies have also shown that small doses of orally administered undenatured type II chicken collagen inhibit killer T-cell attack (15). The present clinical trial evaluated the safety and efficacy of UC-II in the treatment of the knee in OA patients.

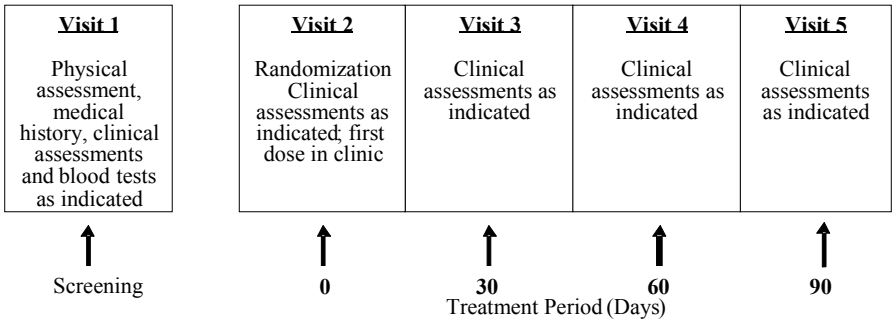


Figure 1. UC-II clinical study design. The study was a two-site, randomized, double-blind study conducted in London, Ontario and Corunna, Ontario, Canada.

Materials and Methods

Study Design

This clinical trial (Human Clinical Trial Approval #06UOHI) was managed by KGK Synergize Inc. (London, ON, Canada). The study was conducted at two sites: 1) KGK Synergize Inc., and 2) Corunna Medical Research (Corunna, ON, Canada). Figure 1 illustrates the study design while Table 1 lists the procedures and observations at each time point.

Briefly, at screening (Visit 1) the consent form was discussed, signed and a complete physical examination was performed. Activity level, diet history, medication/supplement use and medical history were recorded. The VAS score, the WOMAC Index and Lequesne scores were obtained. Urine was collected for a pregnancy test for women of childbearing potential. A blood sample was taken for determination of uric acid, CBC count and differentiation, albumin, total protein, sodium, potassium, chloride, BUN, creatinine, ALT, AST, bilirubin, erythrocyte sedimentation rate (ESR) and rheumatoid factor. Upon review of blood test results, eligible subjects were instructed to get an X-ray of the affected knees to confirm diagnosis. A total of 52 subjects were recruited using the inclusion and exclusion criteria outlined in Table 2. At the first treatment visit (Visit 2), selected subjects were randomly assigned to receive UC-II (n = 26) or glucosamine HCl plus chondroitin sulfate (n = 26, G+C). On each test day (day 0, 30, 60, 90), subjects were required to come to the clinic for clinical assessment. The clinical assessments included WOMAC, Lequesne's functional index and 100-mm VAS pain scores. A subject treatment diary was completed by each patient throughout the study period to determine side effects, medication use, and product compliance.

Table 1. Schedule of observations and procedures

Procedure	Visit 1 Screening	Visit 2 Day 0	Visit 3 Day 30	Visit 4 Day 60	Visit 5 Day 90
Informed consent	X				
Review inclusion/exclusion	X	X	X	X	X
Medical history including activity level and diet history	X				
Physical examination	X				
Biometric measurements: Weight, height*, heart rate and blood pressure.	X	X	X	X	X
Urine pregnancy test	X				
Concomitant medications	X	X	X	X	X
Blood samples: Uric acid, CBC count and differentiation, albumin, total protein, sodium, potassium, chloride, BUN, creatinine, ALT, AST, bilirubin, ESR, rheumatoid factor	X				X
WOMAC, VAS and Lequesne scores	X	X	X	X	X
X-ray	X				
Randomization		X			
Blood sample: ALT, AST, bilirubin, albumin.			X†	X†	
Knee flexion, Time to walk 50m, Swelling in the knee joint, Time for climbing 10 steps		X	X	X	X
Physician's Global Assessment		X	X	X	X
Subject's Global Assessment		X	X	X	X
Investigational Product dispensed		X	X	X	
Subject Treatment Diary dispensed		X	X	X	
Investigational Product returned Compliance calculated			X	X	X
Subject Treatment Diary returned			X	X	X
Adverse Events			X	X	X

* height was only measured at visit 1

† If acetaminophen use was greater than 2 g/day for more than 7 days

Table 2. Inclusion and exclusion criteria

Inclusion Criteria
Males and females 40-75 years old
Females of childbearing potential must agree to use a medically approved form of birth control and have a negative urine pregnancy test result
Unilateral or bilateral OA of the knee for greater than 3 months (American College of Rheumatology criteria) confirmed by radiologist's report, i.e. X-rays showing osteophytes, joint space narrowing or subchondral bone sclerosis (eburnation)
Erythrocyte sedimentation rate (ESR) < 40 mm/hr
Moderate OA as indicated by Lequesne's functional index score of 4.5-7.5 after 7 day withdrawal of usual medications
Able to walk
Availability for duration of study period (3-4 months)
Subject using other therapies for OA, such as exercise, heat/cold therapy, joint protection and physiotherapy/occupational therapy agrees to continue these therapies as normal avoiding changes in frequency or intensity and to record therapies in the study diary
Subject agrees not to start any new therapies for OA during the course of the study
Able to give informed consent
Exclusion Criteria
History of underlying inflammatory arthropathy; septic arthritis; inflammatory joint disease; gout; pseudogout; Paget's disease; joint fracture; acromegaly; fibromyalgia; Wilson's disease; ochronosis; haemochromatosis; heritable arthritic disorder or collagen gene mutations or rheumatoid arthritis
History of asthma, history of diabetes (Type I or Type II)
Hyperuricemia (urate, males > 480 umol/L, females > 450 umol/L)
Expectation of surgery in the next 4 months
Recent injury in the area affected by OA of the knee, i.e. meniscal tear (past 4 months)
Cartilage reconstruction procedure in the target knee
Severe OA as indicated by Lequesne's functional index score of 8 or greater, after 7 day withdrawal of usual medications
Intra-articular corticosteroid injections in the target knee within the last 3 months
Viscous injections in the target knee within the last 6 months
Hypersensitivity to NSAIDs
Abnormal liver or kidney function tests (ALT or AST > 2 times the upper limit of normal; elevated creatinine, males > 125 umol/L, females > 110 umol/L)

Abnormal findings on complete blood count
History of coagulopathies, history of peptic ulceration and upper GI hemorrhage
Uncontrolled hypertension
History of congestive heart failure, history of allergic reaction to chicken and/or eggs
History of allergic reaction to local anesthetic or to any ingredients in the test product including shellfish
Hyperkalemia (potassium > 6.2 mmol/L)
Anticipated problems with product consumption
History of cancer as well as gastrointestinal, renal, hepatic, cardiovascular, hematological, or neurological disorders
High alcohol intake (>2 standard drinks per day)
Pregnant, breastfeeding or planning to become pregnant during the study
History of psychiatric disorder that may impair the ability of subjects to provide written informed consent
Use of other natural health products, including glucosamine and chondroitin, one month prior to study and during the study, other than multivitamin and mineral supplements containing vitamins and minerals as the sole medicinal ingredients
Use of concomitant prohibited medication (narcotics, oral NSAIDs, topical NSAIDs) within four weeks of randomization
Use of acetaminophen or ibuprofen within 7 days of randomization
Subject is unwilling to stop taking pain medication other than the study medication (for arthritis or other types of pain) or is unwilling to stop taking other medications for the treatment of OA
Any other condition that, in the opinion of the investigator, would adversely affect the subject's ability to complete the study or its measures

Supplements

Each UC-II (InterHealth Nutraceuticals, Inc., Benicia, CA) capsule contained 20 mg UC-II standardized to 5 mg of bioactive undenatured type II collagen. Subjects in the UC-II group were instructed to take two "sugar pills" in the morning to protect blinding and two UC-II capsules in the evening accounting for a daily dose of 40 mg UC-II containing 10 mg of bioactive undenatured type II collagen.

Each G+C capsule contains 375 mg of glucosamine HCl (USP Grade) and 300 mg of chondroitin sulfate (USP Grade). The subjects were instructed to take two G+C capsules in the morning and two in the evening for a daily dose of 1500 mg glucosamine and 1200 mg chondroitin.

Removal of Patients from Therapy or Assessment

The criteria for removal of patients from the study included:

Adverse events

For any adverse event, patients were examined and appropriately managed or the patients would be referred to another medical professional for proper evaluation and treatment. If medical problems were attributed to the trial compounds, then the trial drugs were discontinued and the toxicities were reported.

Personal reasons

As stated in the Consent Form, subjects were able to withdraw from the study for any reason at any time.

Clinical judgment of physician

Subjects were withdrawn from the study (without penalty) if, in the opinion of the treating physician, it was not in the patient's best interest to continue. For instance, if during the course of the study a patient became pregnant, she would be withdrawn from the study because it was not known how the study compounds/medications might affect an unborn child.

Protocol violation

Any subject found to have entered this study in violation of the protocol or failed to follow the study protocol were discontinued from the study at the discretion of the Principal Investigator. Subjects were withdrawn for protocol non-compliance if they adhered to the dosing schedule less than 75% of the time.

Method of assigning patients to treatment groups

Patients were assigned to treatment groups (order of treatments) using computer-generated randomization tables. Patients were not stratified or assigned using any other specific method and were not randomized after stratification or blocking procedures.

Selection of doses in the study

The justification for the daily dose of 40 mg UC-II in capsules (providing 10 mg of undenatured collagen II) is based on efficacy demonstrated in earlier studies (8,9).

Blinding

In order to protect blinding, subjects were given bottles containing product labeled with "AM" or "PM" to distinguish the time in which treatment was to be taken. Each bottle contained descriptions of all potential products to ensure blinding was protected. Additionally, each bottle was labeled with a randomization number. In the event that an adverse effect was considered serious and related to the investigational product, the blind would be broken for

that individual subject.

Neither the patient, nor investigator, nor research staff, were aware which test compound the subject was assigned. Interim analysis was performed in order to write a preliminary report and thus preliminary unblinding occurred by an individual unrelated to the study conduct. Personnel related to analysis, statistics, and report writing remained blinded.

Prior and concomitant therapy

Uses of medications such as narcotics, oral NSAIDS, topical NSAIDS within four weeks of randomization and during the study, were not allowed.

Treatment compliance

Compliance was assessed by capsule count at visits 3, 4, and 5 and review of subject diary.

Efficacy and Safety Variables

Efficacy and safety measurements assessed

Adverse events

During the study, subjects recorded adverse effects in their subject diary. At each visit, the subjects were asked if they experienced problems or difficulties. Any adverse events were documented and recorded in the study record and was classified according to the description, duration, severity, frequency, and outcome. The investigator assessed the adverse events and decided causality. Classifications were as per the Coding Symbol Thesaurus of Adverse Reaction Terms (COSTART) U.S. Food and Drug Administration (16).

Blood tests

Blood samples were taken from all subjects during screening (visit 1) and at end of study (visit 5). Blood samples (approximately 15 ml) were taken from subjects at day 30 and day 60 (visits 3 and 4) for the determination of ALT, AST, bilirubin, and albumin if the subjects had been taking acetaminophen greater than 2 g/day for more than 7 days. All blood samples were analyzed by MDS Laboratory Services (London, Ontario, Canada).

Appropriateness of Measurements

The efficacy and safety assessments used in this study were standard for OA and are widely used and recognized as reliable, accurate, and relevant.

WOMAC scores were determined, at screening, and baseline, as well as at days 30, 60 and 90 as described in Bellamy et al (17). Other objectives also performed at days 0, 30, 60 and 90 included determination of Lequesne's functional index, VAS pain scores, knee flexion, time to walk 50 m, time to climb 10 steps, physician's and subject's global assessment. The Lequesne's functional index is described in Lequesne et al. (18).

Statistical Methods

Sample size of 25 subjects per group was based on the subject number used in Braham et al. (1). To compare UC-II with G+C group, a linear contrast was included in the analysis of variance. Data missing subsequent to 30 days were imputed using the last-observation-carried forward technique. Furthermore, comparisons between the UC-II and G+C groups were made at each visit using analysis of variance, using the baseline visit as a covariate. SAS version 9.1 has been used to perform the statistical analysis. Probability values less than 0.05 were considered statistically significant for between-group comparisons.

Results

Baseline Statistics and Compliance of Trial Subjects

Demographic and baseline characteristics of patients are summarized in Table 3. Overall, the patient profiles with respect to age, sex, height, weight, blood pressure, heart beat and target knee were similar between both treatment groups. Table 4 shows treatment compliance of the trial patients. There were no significant interaction terms or between-group differences for compliances. When compliances were compared at each visit, there were no overall between-group differences among the two treatment groups.

Table 3. Demographic and baseline characteristics of the trial subjects

	UC-II (N=26)	G + C (N=26)
Age (years)	58.9 ± 9.79	58.7 ± 10.3
Sex: male/female (%)	13/26 (50%)	17/26 (65%)
Height (cm)	167.7 ± 9.90	167.0 ± 8.73
Weight (kg)	84.3 ± 17.4	86.6 ± 21.0
Systolic Blood Pressure (mm)	128.2 ± 9.36	126.3 ± 12.5
Diastolic Blood Pressure (mm)	81.9 ± 7.43	79.7 ± 8.60
Heart Rate (bpm)	68.2 ± 7.72	67.4 ± 8.47
Target knee		
Left; n (%)	16 (61.5%)	13 (50%)
Right; n (%)	10 (38.5%)	13 (50%)
Where applicable, values are expressed as mean ± SD		

Table 4. Treatment compliance as assessed during specified visits

Visit	Treatment Group	
	UC-II	G + C
AM Capsule Compliance		
Visit 3	[25] 90.5 ± 19.2	[25] 93.6 ± 11.5
Visit 4	[24] 93.2 ± 9.66	[26] 94.5 ± 11.8
Visit 5	[23] 98.5 ± 5.15	[26] 93.3 ± 11.0
PM Capsule Compliance		
Visit 3	[25] 88.1 ± 18.7	[25] 92.5 ± 12.5
Visit 4	[24] 92.8 ± 8.97	[26] 91.6 ± 12.3
Visit 5	[22] 95.3 ± 9.92	[26] 89.7 ± 12.6

There were no significant interaction terms and between-group differences for compliances. When compliances were compared at each visit, there were no overall between-group differences among the five treatment groups. Values are expressed as [n] mean ± SD.

WOMAC Score

The interaction between visit and treatment was significant in UC-II treated group for "pain walking on flat surface" (p=0.034), "difficulty walking on flat surface" (p=0.038) and "performing heavy domestic duties" (p=0.031) as compared to G+C treated group. There was evidence that UC-II treatment has a significant effect for "ascending stairs" (p=0.013) as compared to G+C treatment. Additionally, when groups were compared at each visit, UC-II was significantly better than G+C for "ascending stairs at 30 days and 60 days" (p=0.019 & 0.040 respectively), "at night while in bed" (p=0.015) at 60 days and difficulty walking on flat surface at 90 days (p=0.035). There were no further statistically significant differences for any other individual WOMAC components or summary scores. Treatment with UC-II was most effective and reduced the WOMAC scores by 33% compared to 14% in (G+C)-treated groups after 90 days. Within-group analysis indicated that treatment with UC-II for 90 days significantly ($p<0.05$) improved WOMAC scores at all treatment time points measured. In contrast, subjects received G+C did not show any statistical significant change in WOMAC scores at Day 90 of treatment (Fig. 2).

VAS Score

The interaction between visit and treatment was non-significant for all VAS components and summary scores. However there was evidence that UC-II treatment had a significant effect for "pain during climbing up and down stairs", "night pain" and "resting pain" (p=0.035, 0.030 and 0.024 respectively). When groups were compared at each visit, UC-II was significantly better than G+C for "night pain" (p=0.040) and "resting pain" (p=0.020) at 60 days and "pain during climbing up and down stairs" (p=0.014) and "resting pain" at 90 days (p=0.034). There were no between-group differences for any of the VAS components or summary scores. Although both the treatments reduced the VAS score, UC-II was found to be more effective with a 40% decrease after 90 days of treatment compared to a 15% decrease in G+C treated groups.

Within-group analysis indicated that subjects on UC-II showed a significant reduction in total VAS scores at Day 60 and Day 90 as compared to baseline. However, subjects on G+C showed a significant reduction in total VAS scores at Day 30 and no significant difference was observed at either Day 60 or Day 90 as compared to baseline (Fig. 3).

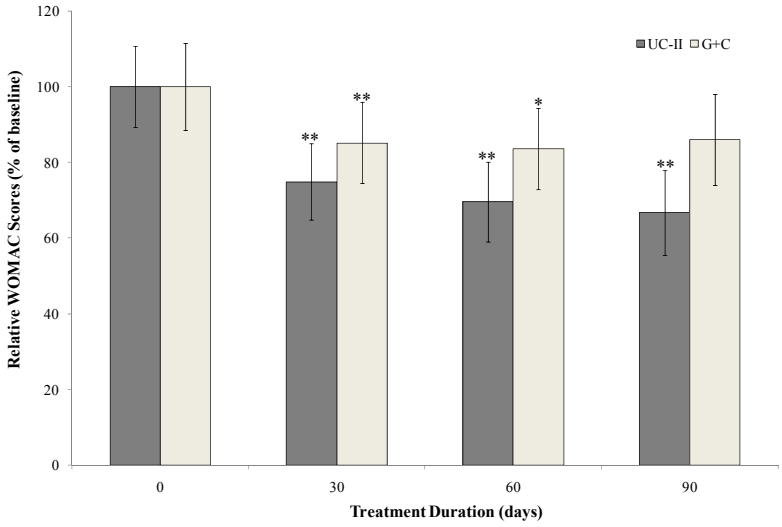


Figure 2. Changes in WOMAC scores at Day 90 from baseline. WOMAC scores from each treatment group were compared to baseline value at specified time points. Each bar presents mean ± SEM. *p<0.05, **p<0.005 indicate significantly different from baseline.

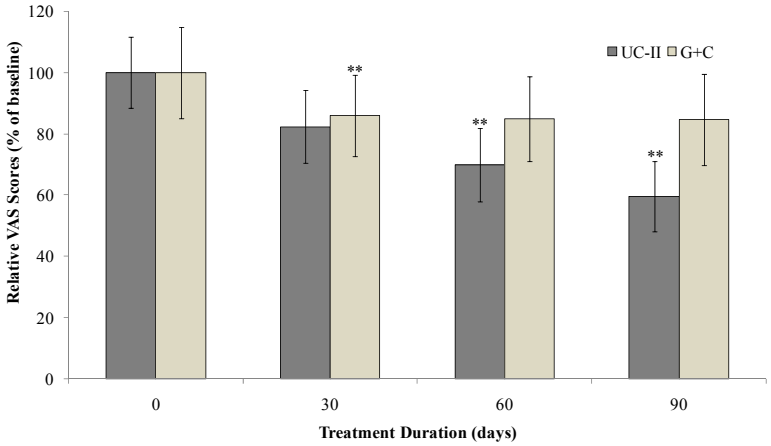


Figure 3. Changes in VAS score at Day 90 from baseline. VAS scores from each treatment group were compared to baseline value at specified time points. Each bar presents mean ± SEM. **p<0.05 indicates significantly different from baseline.

Lequesne Score

The Lequesne's functional index was used to determine the effect of different treatments on pain during daily activities. The interaction between visit and treatment was non-significant for all Lequesne's components and summary scores. Furthermore, there were no between-group differences for any of the Lequesne's components or summary scores. However there was evidence that visit has a significant effect in UC-II treated group for "pain while up from sitting" and "maximum distance walked" (p=0.036 and 0.002 respectively) as compared to G+C treated group.

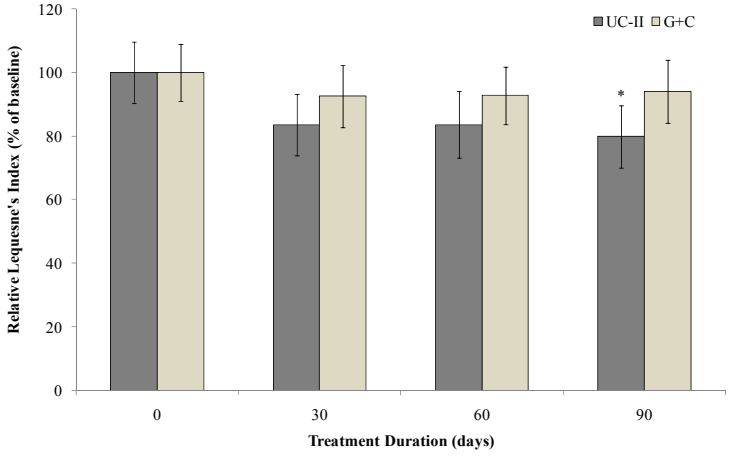
There was as a strong trend toward UC-II efficacy. UC-II treatment effectively reduced Lequesne's functional index score by 20.1% as compared to 5.9 % by G+C treatment.

Within-group analysis suggested that subjects on UC-II demonstrated a significant reduction in total Lequesne's index of severity score from baseline to Day 90, whereas no significant difference from baseline was observed for subjects on G+C at any treatment time points evaluated (Fig. 4).

Figure 4. Changes in Lequesne's functional index at Day 90 from baseline. Lequesne's functional index from each treatment group was compared to baseline value at specified time points. Each bar presents mean ± SEM. *p<0.05 indicates significantly different from baseline.

Adverse Events

Adverse effects that occurred during the 90-day trial period are summarized in Table 5. Overall, there were 58 adverse events noted in the subjects receiving G+C treatment, whereas, only 35 adverse events were observed in UC-II group. In terms of severity, 60% of mild and 38% of moderate adverse events were experienced by subjects on G+C in comparison to 43% and 54% by subjects on UC-II. In relationship to test product a higher number of subjects (23%) on G+C demonstrated adverse events possibly related to product as compared to 11.4% of subjects on UC-II. For UC-II the possible adverse events related to products were constipation and headaches (intermittently). For G+C the possible adverse events related to products were bloating, stomach pain, rash, water retention (edema around eyes and scars), hives on face and chest, and headache. However, there was no significant difference in the occurrence of adverse effects between the two treatment groups.



Rescue Medication

A greater percentage of subjects used rescue medication while on G+C as compared to UC-II at every time point assessed. From baseline to Day 30 a total of 8 subjects (33.3%) on UC-II used rescue medication as compared to 23 subjects (88.5%) on G+C. From Day 30 to Day 60, 13 subjects (54.2%) on UC-II used rescue medication as compared to 21 subjects (80.8%) on G+C. Fourteen subjects (63.6%) on UC-II used rescue medication as compared to 19 subjects (79.2%) on G+C from Day 60 to Day 90.

Table 5. Summary of analysis of adverse events in all subjects

	Treatment Group	
	UC-II (n=26)	**G + C (n=26)**
Severity (n)		
Mild	15	35
Moderate	19	22
Severe	1	1
Relationship to Test Article (n)		
Not related	17	20
Unlikely	14	30
Possible	4	8
Probable	0	0
Most Probable	0	0
Body System (n)		
Pain	10	17
Gastrointestinal	5	15
Musculoskeletal/Soft Tissue	7	5
Neurology	0	2
Pulmonary / Upper Respiratory	2	1
Hemorrhage/Bleeding	2	1
Blood/Bone Marrow	2	1
Dermatology/Skin	2	3
Allergy / Immunology	0	1
Infection	1	3
Lymphatics	0	1
Hepatobilary / Pancreatic	0	0
Renal / Genitoruinary	0	0
Constitutional Symptoms	2	3
Syndromes	1	1
Auditory/Ear	0	1
Ocular / Visual	0	1
Metabolic / Laboratory	1	2
Total Number of Adverse Events Experienced During Study (n)	35	58
Total Number of Subjects Experiencing Adverse Events: n (%)	16/26 (61.5%)	20/26 (76.9%)

Discussion

OA is the most common form of arthritis, and it is often associated with significant disability and an impaired quality of life. Clinical and radiographic surveys have found that the prevalence of OA increases with age from 1% in people <30 years to 10% in those <40 years to more than 50% in individuals >60 years of age (19). Although there are no curative therapies currently available for OA, individualized treatment programs are available to help relieve pain and stiffness, and to maintain and/or improve functional status.

In the last few years, various nutritional supplements including chondroitin, glucosamine, avocado/soybean unsaponifiables and diacerein have emerged as new treatment options for osteoarthritis (20). In this study, the efficacy of UC-II was studied in patients identified with moderate to severe OA. The objective of this study was to determine the effect of UC-II on disease specific measures and blood measures of OA of the knee compared to G+C. It was hypothesized that UC-II would reduce symptoms of OA of the knee to a greater extent than G+C.

A meta-analysis of 20 randomized control studies (2570 patients) comparing the effects of glucosamine (glucosamine sulphate, GS or glucosamine HCl, GH) vs. placebo was done. Of these only eight studies met the required controlled conditions for adequate

allocation concealment and received a quality score of 4 or higher (rated on the JADAD scale). These studies failed to show the benefit of glucosamine (GS or GH) for pain and WOMAC function. When all 20 studies were included in the meta-analysis, the results favored glucosamine with improvement in pain and functionality; however, the results were not uniformly positive and the parameters for WOMAC pain, daily function and stiffness did not reach statistical significance. Combinations of glucosamine and chondroitin have been studied in the "GAIT" study. These authors reported that glucosamine HCl and chondroitin sulphate alone or in combination did not reduce pain significantly in patients with OA of the knee. However in a subgroup of patients with moderate to severe knee pain the combination of compounds were found to be effective. Limitations to this study included a high rate of response to placebo (60.1%) and the fact that 78% of the participants were in the mild pain subgroup (21).

Previous studies have shown that UC-II is effective in the treatment of RA (8-11), and preliminary human (12) and animal (13-15) trials have shown it to be effective in treating OA. In obese-arthritic dogs given 4 mg or 40 mg per day UC-II for 90 days, significant declines in overall pain, pain during limb manipulation and lameness after physical exertion were noted (15). Greater improvement was observed with the 40 mg dose. No adverse effects or significant changes in serum chemistry (creatinine, blood urea nitrogen, alanine aminotransferase, and aspartate aminotransferase) were noted. Following UC-II withdrawal for a period of 30 days, all dogs experienced a relapse of overall pain, exercise-associated lameness and pain upon limb manipulation.

In a recent investigation, efficacy of UC-II was evaluated in arthritic horses (22). In this study, groups of horses were orally administered with a daily dose of placebo, UC-II at 320, 480 or 640 mg, or a combination of glucosamine (5.4 g) and chondroitin (1.8 g) for 150 days. Horses receiving placebo did not show any improvement in arthritic condition, while those receiving a daily dose of 320, 480 or 640 mg of UC-II exhibited significant reduction in arthritic pain. Although G+C treated group showed significant reduction in pain compared to baseline values, the efficacy was less as compared to that observed with UC-II treatment. In fact, UC-II at 480 or 640 mg/day was found to be more effective than G+C in treatment of arthritic pain in horses. Clinical conditions (body weight, body temperature, respiration rate, and pulse rate), and liver (bilirubin, GGT, and ALP) and kidney (BUN and creatinine) functions were not affected by UC-II treatment, suggesting that UC-II is well tolerated and does not cause any adverse effects (22).

In a preliminary trial of subjects with OA, taking a single oral daily dose of 40 mg UC-II on an empty stomach prior to bedtime for 42 consecutive days, an average of 26% reduction of pain was noted in four of five subjects in the study. No side effects were associated with treatment (12). The precise biochemical mechanism involved in UC-II induced pharmacological anti-arthritic effects in humans, dogs or horses is not clearly established. Type II collagen is the primary form of collagen contained in cartilage. Type II collagen extracts contain the amino acids found in the framework of human cartilage. In addition, these amino acids are required for the synthesis and repair of connective tissue throughout the body. These products reportedly aid in reducing the destruction of collagen within the body, may provide anti-inflammatory activity, and may improve joint flexibility (8-12).

The current study indicated that both treatments reduced the WOMAC scores, which measures the difficulty in physical function, stiffness and pain in the knee. However, treatment with UC-II was found to be more effective in reducing the WOMAC scores by 33% as compared to 14% in G+C treated groups after 90 days. Similar results were observed for VAS scores. Although both the treatments reduced the VAS score, UC-II was found to be more effective with 40% decrease after 90 days of treatment as compared to 15.4% in G+C treated groups. The Lequesne's functional index was used to determine the effect of different treatments on pain during daily activities. Treatment with UC-II reduced Lequesne's functional index by 20.1% as compared to 5.9 % in G+C treated groups. Thus, UC-II supplementation showed improvement in daily activities suggesting an improvement in overall quality of life in the patients receiving UC-II.

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Conflict of Interest

The authors have declared that no conflict of interest exists.

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A non-interventional, prospective, multicentric real life Indian study to assess safety and effectiveness of un-denatured type 2 collagen in management of osteoarthritis

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ABSTRACT

Background: Osteoarthritis (OA) is the most common musculoskeletal conditions affecting the quality of life. Undenatured collagen type II has emerged as one of the promising treatment options in treatment of OA. Despite being available in India, clinical safety and efficacy have not been evaluated. We performed a non-interventional, real-life study to determine its safety and efficacy in Indian population.
Methods: A non-interventional, real-life study was performed in patients with OA of knee by 18 orthopaedicians in India. Patients enrolled were followed-up at day 30 (visit 2), day 60 (visit 3) and day 90 (visit 4). Efficacy was assessed by Western Ontario McMaster Osteoarthritis Index (WOMAC) and Visual Analogue scale (VAS) on each visit. Safety was assessed by incidence of suspected adverse events (AEs), and abnormal laboratory parameters.
Results: Among 291 enrolled patients 226 patients completed the study. Mean age of the population was 56.2±8.7 years and 53.3% of them were females. In 291 patients included in safety analysis, at least one treatment emergent adverse event (TEAE) was seen in 4.47% patients. None of the AEs were serious or resulted in termination of patient from the study. Nausea (1.37%) and headache (1.03%) were the common AEs. Treatment with undenatured collagen type II was associated with significant reduction in WOMC score ($p<0.0001$) and VAS scores ($p<0.0001$) from baseline to day 90.
Conclusions: Undenatured collagen type II is safe and efficacious in Indian patients with OA. This can be considered early in the initial management of OA.

Keywords: Osteoarthritis, Undenatured collagen, Efficacy, Safety, India

INTRODUCTION

The global burden of osteoarthritis (OA) is enormous. The Centers for Disease Control and Prevention estimated that 54.4 million adults (22.7%) in the United States are affected with arthritis.[1] The two most common forms of arthritis are rheumatoid arthritis (RA) and osteoarthritis (OA). OA of large joints (e.g. knee, hip) is the most common form of arthritis. Their presence in

Indian subcontinent is also substantial.[2] In India, the reported prevalence of OA is 28.7% in >40 years age group[3]. Further, in adults aged ≥65 years, it is estimated that nearly 45% of the women have symptoms and 70% have radiological evidence of OA. The disabling pain is associated with loss of daily activities in nearly 25% of them.[4] Being overweight/obese, lack of physical activity or sedentary lifestyle, and female sex are important risk factors of OA.[3] Pain and disease activity in OA can vary from mild to severe forms. Pain is the most troubling symptom and affects the quality of life of patients with OA.[5,6] Current treatment of OA includes exercise, heat/cold therapy, joint protection, weight loss, physiotherapy/occupational therapy and medications. The most common medications used for pain relief include NSAIDs. Although these drugs are effective for reducing pain associated with OA, they do not reverse the disease. In addition, there are considerable side effects associated with the use of these drugs. As a result, physicians have turned to adjunctive therapies to ease their pain and discomfort. These products are commonly used because they are well tolerated and considered safe.[8] The understanding of pathogenesis of OA has shifted from merely a degeneration of articular cartilage to pan-joint disease involving subchondral bone and synovium.[9] Recent evidence suggest that persistent low-grade systemic inflammation is an important risk factor for OA.[10,11]

In recent years, role of undenatured collagen type II (UC II) has been explored in management of OA. Oral administration of UC II induces oral tolerance to antigens and thereby lowers the T-cell mediated attack on the joint cartilage. It is also indicated to suppress IL-17 associated Receptor activator of nuclear factor kappa B ligand (RANKL) expression of CD4+ T cells.[12,13] Multiple clinical studies including randomized trials have proved the efficacy and safety of undenatured collagen in OA of knee.[14,16] It is available and being used in India for OA patients.

Randomized controlled trials (RCTs) are the "gold standard" for evaluating treatment outcomes providing information on treatments "efficacy". The strict and controlled conditions in which they are conducted, leads to low generalizability because they are performed in conditions very different from real life usual care. Conversely, real life studies inform on the "effectiveness" of a treatment, that is, the measure of the extent to which an intervention does what is intended to do in routine circumstances. Therefore, this non-interventional, real life multi-centre study was planned with the objective to assess the safety and effectiveness of UC II in Indian patients with OA under actual practice conditions.

METHODS

The aim of the present non-interventional study was to assess the safety and effectiveness of UC II in Indian patients with OA in a 'real-life' scenario.

Study design

This was an Indian multicentric non-interventional, real life study conducted by 18 orthopaedicians across India. The study was initiated on 2nd January 2017 and was completed on 15th November 2017.

Study population

Two hundred and ninety-one patients, who were clinically &/or radiologically diagnosed to be suffering from knee OA and were prescribed UC II (as DUPACT® 40 mg capsules marketed by Wockhardt Ltd.) by investigating doctors were asked to participate in this study after provision of written informed consent for collecting their personal data.

Treatment

Enrolled patients were prescribed with undenatured collagen type II (DUPACT®, Wockhardt Ltd., Mumbai) hard-gelatin capsules of 40 mg (which yields 1.2 mg of undenatured type 2 collagen per capsule) per day. All directions regarding general care, and concomitant medications were allowed.

Study visits

All patients were assessed at baseline (visit 1) as per routine clinical practice for physical examination and baseline laboratory investigations. Activity level, diet history, medication/supplement use and medical history were recorded. OA signs and symptoms were assessed on Western Ontario McMaster Osteoarthritis Index (WOMAC) and Visual Analogue Scale (VAS) on each visit. Enrolled patients were followed-up at day 30 (visit 2), day 60 (visit 3) and day 90 (visit 4) in line with routine practice of monthly follow ups for OA knee patients.

Study endpoints

At each visit, all the patients were evaluated for safety endpoints including the incidence of suspected adverse drug reaction (AR), suspected serious adverse drug reaction (SAR), significantly abnormal clinical signs and symptoms, significantly abnormal laboratory parameters observed during treatment with UC II and effectiveness endpoints including change in total WOMAC score from baseline and change in VAS score from baseline.

Concomitant medications

All ongoing prescription & over-the counter medications consumed was recorded in the CRF.

Sample size

A total of 291 patients were evaluated of which 226 patients completed the study.

Statistical analysis

There was no formal sample size calculation. However, 291 evaluable patients were enrolled. The continuous data is presented as mean and standard deviation, whereas, categorical data is presented as frequency and percentage. The changes in WOMAC and VAS scores from baseline were compared by paired t-test. P<0.05 was considered statistically significant for all the comparisons.

RESULTS

Patient disposition

Total of 291 patients were enrolled in the study of which 64 patients were lost to follow-up and one patient underwent bilateral prosthetic transplant (Figure 1).

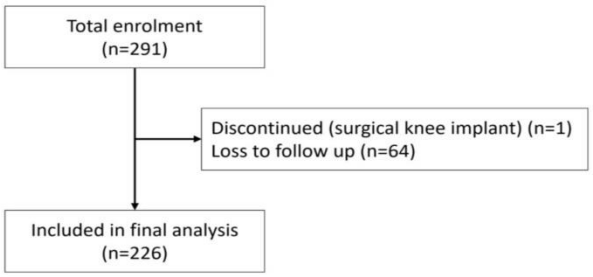


Figure 1: Patient disposition.

Baseline characteristics

The baseline characteristics of patients are shown in Table 1. Study population included equal representation of both genders with age observed to be in higher bracket of adulthood and elderly group. Mean body mass index (BMI) ranged from borderline overweight to obese in the study population.

Table 1: Baseline characteristics (n=291).

Parameters	Observation
Age (years) (range)	56.2±8.7 (28 to 79)
Gender	
Male (%)	136 (46.7)
Female (%)	155 (53.3)
Weight (kg)	69.7±9.5
BMI (kg/m^2)	26.6±3.7

Safety assessment

Thirteen (4.47%) patients reported at-least one treatment emergent adverse events (TEAE). Nausea (n=4, 1.37%) and headache (n=3, 1.03%) were the most commonly observed AEs. Seven AEs were considered of moderate intensity and eight were at least considered to be related to the study medication. There were no serious AEs or AEs leading to treatment discontinuation (Table 2).

Table 2: Safety assessment (n=291).

Parameters	N (%)
At least one TEAE	13 (4.47)
Nausea	4 (1.37)
Headache	3 (1.03)
Loose motion	2 (0.69)
Diarrhoea	2 (0.69)
Burning sensation in epigastrium	1 (0.34)
Gastritis	1 (0.34)
Terminated due to TEAE	0 (0.00)
Moderate or severe TEAE	7 (2.41)
At least one serious TEAE	0 (0.00)
At least one TEAE which is related to study medication	8 (2.75)

TEAE- treatment emergent adverse event.

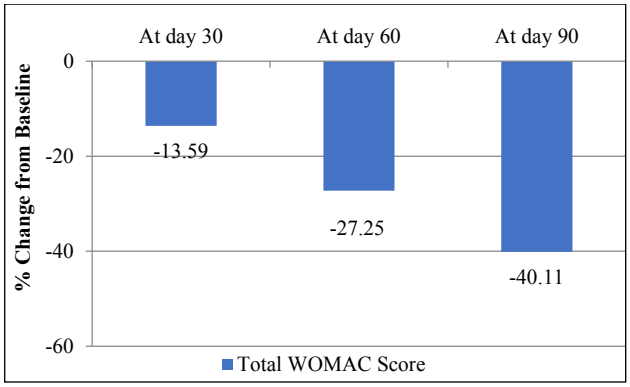


Figure 2: Percentage changes in mean total WOMAC at each visit.

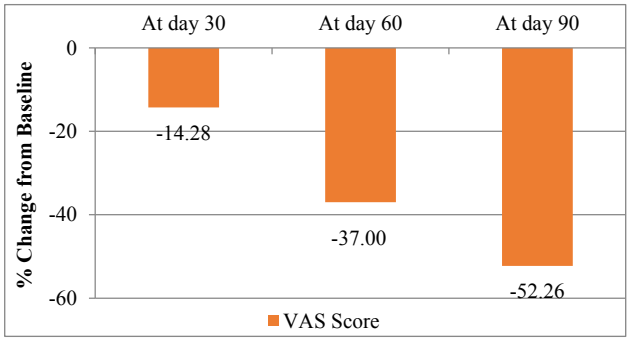


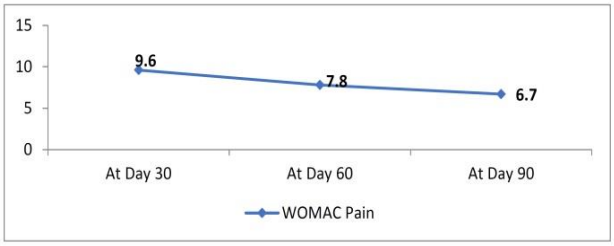
Figure 3: Percentage changes in mean VAS scores at each visit.

Efficacy assessments

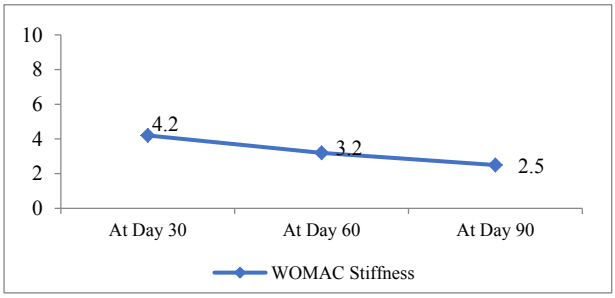
Significant reductions (Mean±SD) were observed in WOMAC and VAS scores from baseline to day 90 with mean change of -20.7±12.6, (p<0.0001) and -3.3±1.8, (p<0.0001), respectively (Table 3). Percent reduction in mean total WOMAC score and mean total VAS score also declined from day 30 to day 60 and further at day 90 as demonstrated in Figure 2 and 3. WOMAC subscales scores assessed on day 30, 60 and 90 indicated a trend of continuous decline (Figure 4a-c). Mean WOMAC-pain

score was 9.6±3.9 at day 30, which reduced to 7.8±4.1 and to 6.7±4.9 at day 60 and 90, respectively. WOMAC-stiffness score was 4.2±2.2 at day 30, which reduced to 3.2±1.9 at day 60 and to 2.5±2.1 by day 90. Physical function score reduced from 39.2±15.4 at day 30 to 36.6±16.4 at day 60 and 33.8±17.9 by day 90.

a. WOMAC - Pain



b. WOMAC - Stiffness



c. WOMAC – Physical function

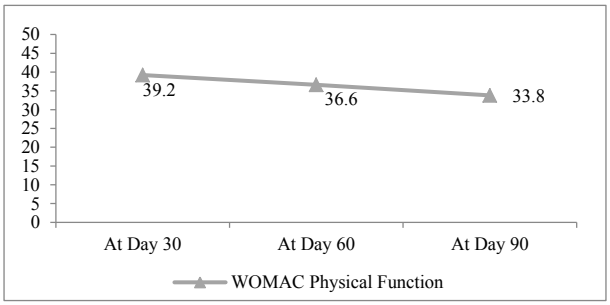


Figure 4: Changes in mean WOMAC scores (a) pain, (b) stiffness and (c) physical function at different visit.

Table 3: Efficacy assessment (n=226).

Parameter	Total WOMAC score	VAS score
Baseline	59.7±19.6	6.5±1.4
Change from baseline		
At day 30	-8.3±11.36 (p<0.0001)	-0.9±1.14 (p<0.0001)
At day 60	-14.8±12.9 (p<0.0001)	-2.2±1.6 (p<0.0001)
At day 90	-20.7±12.6 (p<0.0001)	-3.3±1.8 (p<0.0001)

DISCUSSION

OA is major musculoskeletal disease affecting adults and elderly. The pain, restriction of joint movements and limitation of physical movements affects the quality of life of patients with OA. Despite various treatments being available to manage pain and joint stiffness, none of these have any effect on OA pathogenesis. Chronic low-grade systemic inflammation has been identified as pathogenic factor in OA.[10] Thus, targeting immune modulation seems effective approach to affect the OA disease course. UC II has been found to affect the disease pathogenesis by inducing the immune tolerance. Oral use of UC II with all epitopes is presented to the gut-associated lymphoid tissues and causes antigen desensitization and therefore minimizes the T-cell induced articular damage.[12] Modulation of immune system in such way can reduce the joint damage and thereby provide symptomatic relief.

In this real life study, we observed that most of the patients were in higher bracket of adulthood and elderly group (mean 56.2±8.7 years). OA may not be only restricted to this population, but may also involve young adults. Diagnosis of OA in the young group would be more challenging considering their increased threshold of bearing pain.[17] Age group affected also is dependent on exposure to multiple factors like injuries, occupational activities, and obesity. In general, across all ages, females are more frequently affected than males in OA.[18,19] However, in our study equal distribution of gender was observed in enrolled patients. This could be due to lack of awareness about medical treatment by the female counterparts in India and opting for home-made remedies to relieve the symptoms.[20] The BMI of study patients was in range of borderline overweight to obese suggesting association of obesity with development of OA. Obesity has been long identified risk factor for OA. But, current concept pointing to presence of low-grade systemic inflammation in obese individuals suggest that obesity's association with OA is beyond the wear and tear causing joint damage.[21] Hence promotion of weight loss and modulation of inflammation should be included in treatment algorithm of OA.

Safety of UC II is well established. The incidence of at least one TEAE was seen in <5% patients. This suggests good tolerability of UC II. Out of 291 enrolled patients, only 13 patients had TEAE during the period of 3 months. None of the patient had a serious TEAE or discontinued due to TEAE. The TEAEs reported in our study included nausea, diarrhoea, gastritis, burning in epigastrium and headache indicating gastrointestinal disturbances as commonest reason. Nausea and vomiting were the only adverse events with frequency above 1%. Seven of total 13 AEs were moderate in intensity, rest being mild. Eight of them were considered possibly related to the UC II consumption. Lugo in his evaluation of the efficacy and tolerability of UC II in knee OA found only 8 subjects reporting AEs (12.7%) during the treatment period, out of which 3 (4.76%) were related to

gastrointestinal disturbances and none were considered related to UC II consumption.[15] Conversely, another study by Crowley et al. reported AEs in 11.4% patients possibly related with UC II consumption with most common being constipation and headaches (intermittently) that seem to be in line with our observations.[16]

The Western Ontario and McMaster Universities (WOMAC) index is the most widely used outcome measure in assessment of OA. It assesses pain, joint stiffness and functional capacity of patients with OA. Use of this index has been found to be a useful screening tool in patients with OA.[22] Besides this, visual analogue scale (VAS) score is used to assess pain intensity in multiple disorders. We observed that there was significant reduction in WOMAC index total scores and VAS score at each visit (days 30, 60 & 90), suggesting effectiveness of UC II. A study by Crowley et al. observed similar significant reductions in WOMAC index total (at days 30, 60 & 90) and VAS score (at days 60 & 90) in patients with knee OA.[16] A study by Lugo compared UC II with placebo in OA and found that WOMAC index total were significantly lower in UC II group at days 60, 90, 120, 150 and 180.[15] In another study, Lugo et al, found that even in absence of OA, healthy individuals who had joint discomfort after physical activity, UC II improved joint movements and increased the time for pain free strenuous exercise.[14] Thus, UC II not only diminishes pain and joint stiffness but also seems to enhance functional mobility in patients with OA. Hence indicating that its use even in patients with mild to moderate form of OA that usually is associated with no or less pain can also be helpful. This is important factor as early use of UC II can possibly stall the disease pathogenesis of OA in a safe and effective way converse to current approach of symptomatic treatment till joint condition worsens.

CONCLUSION

Evidence form this Indian real-life study suggests that UC II is safe and effective in treatment of OA in routine clinical practice. Its consumption is associated with reduction in pain, stiffness and improved functional mobility of patients with OA which can improve their quality of life. Given the disadvantages with long-term use of NSAIDs, UC II has potential to bridge the therapeutic gap in management of OA by providing safer therapeutic option that potentially stalls the disease pathogenesis through a unique mechanism.

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A Double Blind, Randomized, Placebo Controlled Clinical Study Evaluates the Early Efficacy of Aflapin® in Subjects with Osteoarthritis of Knee

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Abstract

Aflapin® is a novel synergistic composition derived from *Boswellia serrata* gum resin (Indian Patent Application No. 2229/CHE/2008). Aflapin is more efficacious as an anti-inflammatory agent compared to the existing *Boswellia* products, 5-Loxin® and traditional 65% Boswellia extract. A 30-day, double-blind, randomized, placebo-controlled study was conducted to validate the efficacy of Aflapin® in the management of clinical symptoms of osteoarthritis (OA) of the knee (Clinical trial registration number: ISRCTN69643551). Sixty eligible OA subjects selected through screening were included in the study. The subjects received either 100 mg (n=30) of Aflapin® or placebo (n=30) daily for 30 days. Each subject was evaluated for pain and physical functions by using the standard tools (visual analog scale, Lequesne's Functional Index, and Western Ontario and McMaster Universities Osteoarthritis Index) at the baseline (day 0), and at days 5, 15 and 30. A series of biochemical tests in serum, urine and hematological parameters established the safety of Aflapin. The observations suggest that Aflapin conferred clinically and statistically significant improvements in pain scores and physical function scores in OA subjects. Aflapin provided significant improvements in pain score and functional ability in as early as 5 days of treatment. In conclusion, our observations suggest that Aflapin is a safe, fast acting and effective alternative intervention in the management of OA.

Key words: Aflapin, Clinical study, *Boswellia serrata*, Osteoarthritis, Visual Analog Scale

Introduction

Osteoarthritis (OA) is a degenerative joint disorder of articular cartilage and is the most common type of arthritis in elderly persons. In OA, breakdown of cartilage and synovial proliferation result in pain and stiffness of joints. [1-3]. It has been estimated that OA affects more than 27 million people in the United States alone and is the leading cause of physical disability and impaired quality of life in elderly worldwide [4]. Unfortunately, till today there is no proper therapeutic intervention available to treat OA. Currently, acetaminophen and non-steroidal anti-inflammatory drugs (NSAIDs) including cyclo-oxygenase II inhibitors are used for relief of pain and stiffness [5,6]. Although, these pharmaceutical agents reduce both pain and improve physical functions temporarily without healing the cartilage and subchondral damage, long term usage of NSAIDs is associated with enhanced risk for renal insufficiency, gastrointestinal bleeding, hypertension and congestive heart failure [6-8]. Because of the high incidence

of adverse events associated with NSAID therapy, effective and safer alternative treatments for the management of OA pain are highly desirable.

In recent years, the gum resin extracted from the ancient herb, *Boswellia serrata* has gained considerable attention as a potent anti-inflammatory, anti-arthritic and analgesic agent [9,10]. 3-O-Acetyl-11-keto-beta-boswellic acid (AKBA) is the most active compound of *Boswellia* extract and is a potent inhibitor of 5-lipoxygenase (5-LOX), a key enzyme in the biosynthesis of leukotrienes from arachidonic acid in the cellular inflammatory cascade [11,12]. A number of independent clinical studies support the anti-inflammatory and anti-arthritic properties of *Boswellia* extracts [13-16].

Aflapin® is a novel synergistic composition derived from *Boswellia serrata* gum resin (PCT/IN2009/000505) [17-19]. Interestingly, the oral bioavailability of AKBA from Aflapin was found to be significantly higher in comparison with that of commercially available *Boswellia* extracts [17]. Aflapin exhibited enhanced 5-lipoxygenase inhibition in enzyme based *in vitro* assay and Matrix Metalloproteinase 3 (MMP3) inhibition in pro-inflammatory cytokine induced human primary chondrocytes. In a comparative analysis, various *in vitro* and *in vivo* studies have established that in comparison with regular *Boswellia* extracts Aflapin possesses more powerful anti-inflammatory efficacy and exhibits better recovery of glycosaminoglycans (GAG) in pro-inflammatory cytokine induced human chondrocytes. [17]. Furthermore, safety studies conducted according to Organization for Economic Co-operation and Development (OECD) guidelines manifested the overall safety of Aflapin in animal models [18].

In a 90-day placebo controlled clinical study the anti-arthritic efficacy of Aflapin was evaluated in OA subjects. Aflapin demonstrated a significant reduction in pain and improvement in the quality of life in OA subjects [19]. Supplementation of 100 mg Aflapin/day conferred significant improvements in pain scores and physical function. These observations led us to substantiate the anti-OA efficacy of Aflapin in a second independent clinical study. We conducted an independent double blind placebo controlled trial in a different set of subjects with OA. This study design was intended to evaluate, (i) the anti-OA efficacy of Aflapin and (ii) to assess whether Aflapin supplementation can provide fast relief from clinical symptoms of OA. The present communication describes the anti-OA efficacy of Aflapin, which substantiates the earlier observation; and demonstrates that Aflapin provides significant pain relief in subjects with OA in as early as 5 days of treatment.

Materials and Methods

Study material

Aflapin is a novel synergistic composition containing *B. serrata* extract selectively enriched with AKBA and *B. serrata* non-volatile oil. The non-volatile oil was prepared by selective removal of Boswellic acids followed by removing volatiles under high vacuum (PCT application # PCT/IN2009/000505). This composition was standardized to contain at least 20% AKBA.

Research design

This randomized, double-blind, placebo controlled trial was conducted during August 2009 to December 2009. The study protocol was approved by the Institutional Review Board (IRB) of Alluri Sitarama Raju Academy of Medical Sciences (ASRAM), Eluru, Andhra Pradesh, India (Clinical Trial Registration No. ISRCTN69643551).

Subjects

One hundred and fifty two patients of either gender were selected for screening. They were between 40 and 80 years of age, and had been suffering from unilateral or bilateral OA of the knee according to the criteria of the American College of Rheumatology [20] for more than 3 months. After the use of usual medications had ceased for 7 days, the visual analog scale (VAS) score that assessed pain during the most painful knee movement had to be more than 40, and Lequesne's functional index [21] had to be over 7 points. Participants had to be able to walk and give both verbal and written information regarding the study. Signed informed consent was obtained prior to entry. Exclusion criteria included an underlying inflammatory arthropathy, hyperuricemia, expectation of surgery in the near future, recent injury in the area affected by OA of the knee, intra-articular corticosteroid injections within the last 3 months, hypersensitivity to NSAIDs, abnormal liver or kidney function tests, major abnormal finding on complete blood count, history of coagulopathies, history of peptic ulceration and upper GI hemorrhage, uncontrolled hypertension, congestive heart failure, hyperkalemia, pregnancy, lactation and malignant tumors.

Randomization and treatment

A total of 60 subjects with symptoms of mild to moderate OA were selected and recruited into the study. Each subject was randomly assigned to the treatment group or placebo group using a randomization table generated using validated computer software CODE; IDV, Gauting, Germany. The ran-

domization codes were secured confidential by the clinical trial pharmacist and statistician. Thirty subjects were allocated each into placebo and Aflapin groups. The subjects in Aflapin group received 50 mg of encapsulated Aflapin® twice daily, whereas, the subjects in the placebo group received two capsules having similar organoleptic properties including weight, taste, color, odor and feel. Each subject filled a questionnaire, providing details regarding demographics, medical history and nutritional status, at the baseline evaluation and during each follow-up evaluation on days 5, 15 and 30.

Assessments

Functional disability was assessed at baseline and at all follow-up visits (days 5, 15 and 30) by the investigators. Pain, stiffness and physical function were assessed using Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) [22], LFI [21] and VAS [23] scores. The pain, stiffness and function subscales of the WOMAC were normalized to a scale of 0 to 100 units [24]. Analyses of these end-points were based upon the time-weighted average change from baseline over 30 days.

For assessment of safety of Aflapin®, several parameters were evaluated in serum, urine and whole blood of all subjects at each visit of the study duration (Table 1). Serum biochemical parameters and hematological parameters were measured using an automated analyzer (HumaStar 300) and a hematological counter (Humacount, Human, Wiesbaden, Germany). The urine analysis was carried out using UroColor™10 Dip Sticks and Urometer 600 (Standard Diagnostics, Kyonggi-do, Korea) and by sediment analysis using microscopy.

Rescue medication

Subjects were prescribed 400 mg ibuprofen tablets (maximum 400 mg thrice daily; total 1,200 mg) as rescue analgesia during the study based on pain intensity reported to the study physician by some subjects. Those subjects were advised not to take the rescue medicine at least 3 days before each evaluation. No other pain relieving interventions were allowed during the study period.

Statistical analysis

Detailed statistical analyses were performed using SAS software to evaluate the efficacy of Aflapin in comparison with the placebo group in terms of improvement in pain and physical function scores at baseline and on days 5, 15 and 30 of treatment. Wilcoxon's signed-rank test was used for inter group and intra-group comparisons of pain scores. Pair-wise changes were examined by carrying out least significant difference (LSD) test for all possible pairs. The significance of the effects of the treatment groups was compared by using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Results with $P<0.05$ are considered statistically significant. This is a two-arm (Aflapin and placebo), randomized, double-blind, placebo-controlled, single-centre trial conducted over 30 days. The trial's primary objective was to validate the efficacy of Aflapin on reduction of pain, joint stiffness and improvement in physical function in subjects with osteoarthritis of knee.

Table 1: Parameters tested in Serum, urine and whole blood samples

Biochemical Parameters
Blood sugar
Alkaline phosphatase
SGOT
SGPT
Total Bilirubin
Direct Bilirubin
CK Nac
Creatinine
Total Protein
Triglycerides
Cholesterol
HDL, LDL
Urea
Hematology
Total Leukocyte count
Total RBC count
Hemoglobin %
Mean Corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular hemoglobin Concentration
Platelet count
Differential count (DC)
Urine Analysis
Blood
Bilirubin, Urobilinogen
Ketone
Protein
Nitrite
Glucose
pH, Specific gravity
Leucocytes
Pus cells, Epithelial cells, Crystals

Results

Baseline characteristics

The subjects were randomly distributed into two groups and the descriptive statistics comparing demographic variables, baseline disease characteristics and baseline outcome measures (LFI, VAS, WOMAC pain, function and stiffness sub-scores) are provided in **Table 2**. The demographic variables, disease-related and baseline outcome parameters of two groups, one receiving Aflapin® 100 mg/day (n=30) and the other receiving placebo (n=29) did not differ significantly at baseline.

Table 2: Characteristics of patients in study groups.

Characteristics	Placebo (n = 29)	100 mg/day Aflapin® (n = 30)
Sex (male/female; n)	11/18	11/19
Age (years)	55.3 ± 8.8	53.2 ± 6.5
Body weight (kg)	59.7 ± 10.5	61.9 ± 10.9
Body mass index (kg/m2)	24.9 ± 2.6	25.7 ± 3.3
Visual analog score	47.6 ± 9.7	48.0 ± 6.0
Lequesne's Functional Index	12.5 ± 3.4	12.8 ± 3.7
WOMAC score		
Pain subscale	45.9 ± 10.5	47.8 ± 12.4
Stiffness subscale	37.5 ± 14.9	38.8 ± 13.3
Function subscale	40.6 ± 9.5	41.1 ± 11.8

Clinical efficacy

The data regarding the normalized pain and function scores are summarized in **Table 3**. At the end of the study, significant reductions in pain and function scores were observed in treatment group supplemented with 100 mg/day of Aflapin when compared to either baseline or placebo.

Significant ($p<0.05$) reduction in all the pain scores was observed in the Aflapin group by day 30, when compared to the placebo group. In comparison with placebo, supplementation of Aflapin for 30 days conferred 37.6, 32.0, 40.1, 41.3 and 38.8 percent reductions in VAS, LFI, WOMAC pain, WOMAC stiffness and WOMAC function scores, respectively. Interestingly, significant ($p<0.05$) reductions in VAS and LFI scores were also observed in Aflapin group over placebo by day 5. Aflapin supplementation showed 14.8 and 16.3 percent better reduction in VAS and LFI scores respectively over placebo by 5th day. Compared to the placebo group, the reductions in WOMAC scores were not significant after 5 days of treatment. Aflapin supplementation for 30 days afforded highly significant ($p<0.001$) reductions in all the pain scores exhibiting 49.1, 34.4, 49.5, 48.4 and 45.2 percent reduction, in VAS, LFI, WOMAC pain, WOMAC stiffness and WOMAC function scores, respectively, when compared to the baseline. However, significant ($p<0.05$) reductions were observed in VAS, WOMAC pain and WOMAC function scores in placebo group when compared to the base line and the magnitude of the reductions are 17.6, 12.0 and 9.24 percent respectively; which are small in comparison with those of the Aflapin group (**figure 2**).

Table 3. Normalized pain and function scores.

Parameter and treatment	Baseline mean ± SD	Day-30 mean ± SD	p value (vs. baseline)	p value (vs. placebo)
Visual analogue scale score				
Placebo (n=29)	47.6 ± 9.7	39.3 ± 9.5	<0.0001	NA
Aflapin 100 mg/day (n=30)	48.0 ± 6.0	24.5 ± 11.9	<0.0001	<0.0001
Lequesne's Functional Index				
Placebo (n=29)	12.5 ± 3.4	12.4 ± 2.6	0.7646	NA
Aflapin 100 mg/day (n=30)	12.8 ± 3.7	8.4 ± 3.8	<0.0001	<0.0001
WOMAC pain subscale				
Placebo (n=29)	45.9 ± 10.5	40.3 ± 11.4	0.001	NA
Aflapin 100 mg/day (n=30)	47.8 ± 12.4	24.2 ± 12.0	<0.0001	<0.0001
WOMAC stiffness subscale				
Placebo (n=29)	37.5 ± 14.9	34.1 ± 15.6	0.2024	NA
Aflapin 100 mg/day (n=30)	38.8 ± 13.3	20.0 ± 15.6	<0.0001	0.0014
WOMAC function subscale				
Placebo (n=29)	40.6 ± 9.5	36.8 ± 11.5	0.0029	NA
Aflapin 100 mg/day (n=30)	41.1 ± 11.8	22.5 ± 11.1	<0.0001	<0.0001

NA, not applicable; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

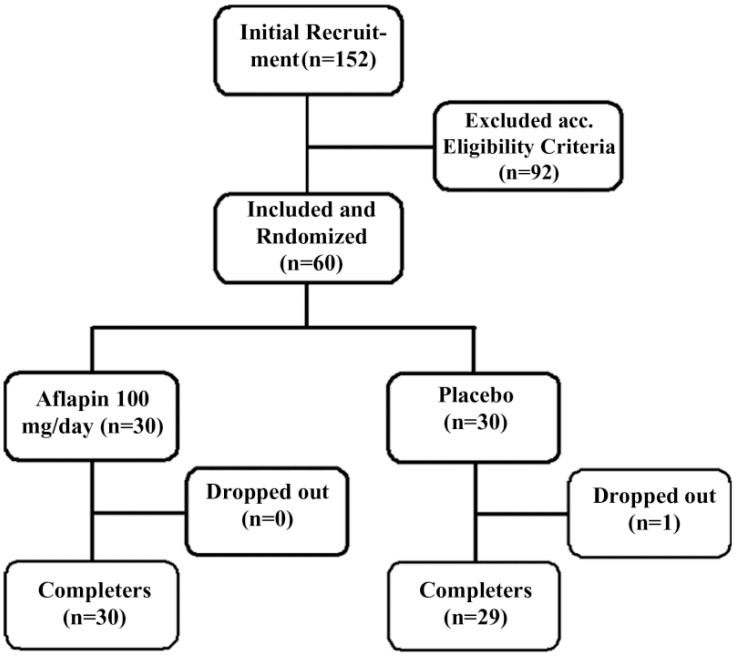

Figure 1: Flow chart of the subjects who participated in the clinical trial. Evaluations of physical activity and pain scores, serum biochemistry, hematology, urine biochemistry and pro-inflammatory biomarkers were done at baseline (day 0) and on days 5, 15 and 30 during follow up.

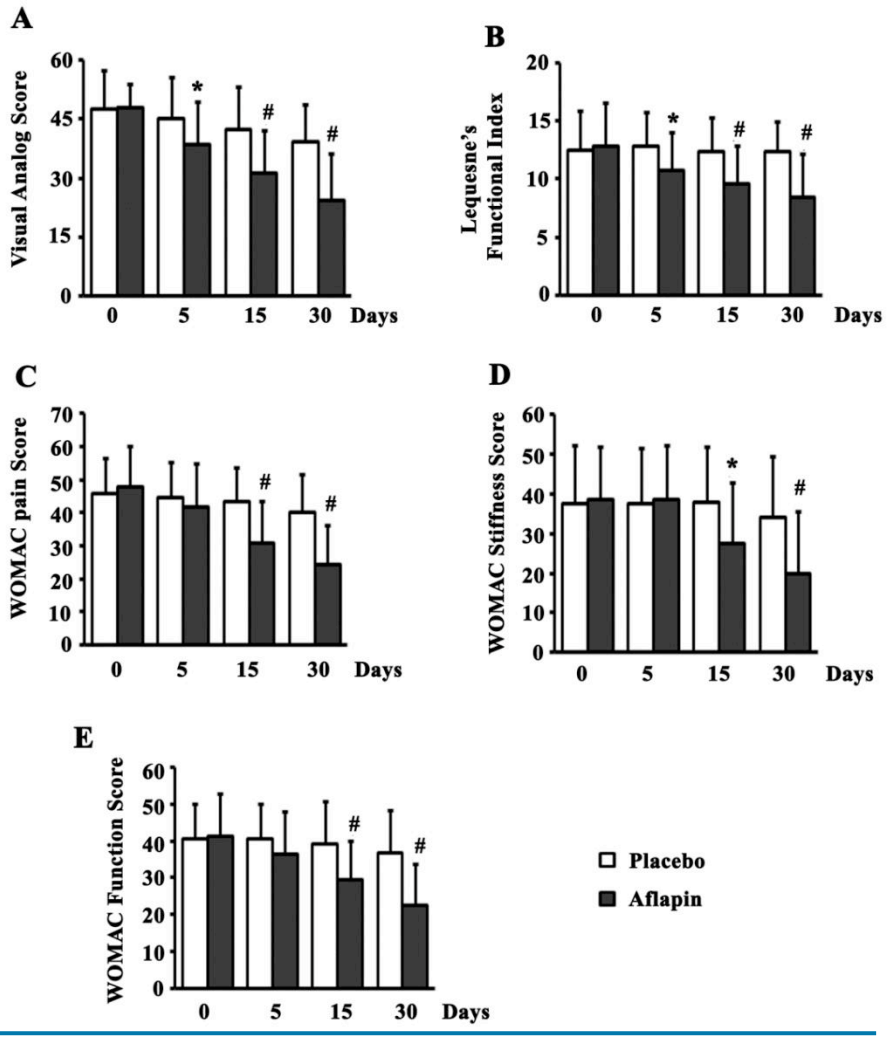

Figure 2: Pain, Function and stiffness scores. Presented are the mean scores for (a) visual analog scale, (b) Lequesne's Functional Index, (c) Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)-pain, (d) WOMAC-stiffness, and (e) WOMAC-function in placebo and 100 mg/day Aflapin® groups at different time points day 0, day 5, day 15 and day 30. Each bar represents mean ± standard deviation. In comparison with placebo the mean scores in the treatment groups were tested for significance using Wilcoxon's rank-sum test; * $p < 0.05$ and # $p < 0.01$.

Biochemical evaluations

As a part of the safety evaluation, laboratory tests were performed for assessment of different biochemical parameters (in serum and urine) and hematological parameters. The repeated measure ANOVA was used to compare the values at different evaluations over the 30 days period with those of baseline. Statistical analyses of these parameters did not indicate any significant changes. Although minor changes were observed in some of the parameters, they remained within the normal laboratory range. Similarly, no significant changes in hematological and urinary parameters were observed in the active treatment groups when compared to the placebo (data not shown).

Adverse Events and Dropouts

During the course of the 30-day study, no major adverse events were reported. However, nausea and headache were reported as minor adverse events by two subjects during the study; one each from placebo and Aflapin supplemented groups.

One subject from placebo groups was dropped out from the study due to un-availability for the follow up evaluations.

Discussion

The primary objective of conducting the present study was to substantiate the observation that Aflapin, a novel *Boswellia* extract reduces clinical symptoms of osteoarthritis, pain, physical discomfort. *Boswellia serrata* is an ancient Indian medicinal plant, and the gum resin of this plant has long been known for anti-inflammatory, anti-arthritic and analgesic properties [9,10]. Earlier studies indicate that 3-O-Acetyl-11-keto-beta-boswellic acid (AKBA) is the most active principle present in the Boswellia extracts, which mainly contributes the anti-inflammatory activities of this herbal extract by inhibiting 5-lipoxygenase activity [11,12].

To date, the anti-inflammatory and anti-arthritic efficacy of different forms of Boswellia extracts have been established in various models either *in vitro* or *in vivo* or in clinical studies [13-16,19,25-30]. However, studies indicate that upon oral administration, Boswellia extracts exhibit poor intestinal absorption of AKBA and poor bioavailability which limits its anti-inflammatory efficacy [31,32]. Aflapin is a novel synergistic composition, which contains *B. serrata* extract enriched to 20% AKBA and *B. serrata* non-volatile oil (PCT/IN2009/000505). In a recent communication Sengupta et al [17] have reported that Aflapin® provides 51.78% more bioavailable concentration of systemic AKBA after a single dose oral administration in comparison with 30% AKBA enriched Boswellia extract (5-Loxin®). In corroboration, it was observed in a recent double blind placebo controlled study that Aflapin provides significantly better improvements in clinical symptoms in OA subjects when compared with 30% AKBA enriched Boswellia extract (5-Loxin®) [19]. The present 30-day double blind, placebo controlled clinical study was designed with two approaches; (i) to reassess the anti-arthritic efficacy of Aflapin and (ii) to evaluate the early onset of action of Aflapin in pain reduction and improvement of physical function in OA subjects.

The present study demonstrates the potential of Aflapin in alleviating pain, joint stiffness and improving physical functions in OA subjects (Figure 2). Pain, stiffness of joints, reduced joint movement and physical discomfort are the major clinical manifestations of OA [24,29,30]. In comparison with the placebo, at the end of the study, the Aflapin supplemented group showed statistically significant improvements in all pain scores including VAS, LFI, WOMAC pain, WOMAC stiffness and WOMAC function scores (Figure 2). Aflapin provided significant reductions in pain scores of VAS and LFI in as early as 5 days. Whereas, in the previous study Aflapin demonstrated significant relief from joint pain and physical discomfort in OA subjects after 7 days of treatment [19]. Together, these findings clearly suggest that Aflapin confers quick and significant pain relief, improvement in physical ability and quality of life in OA subjects.

Therapeutic efficacy and fast action of Aflapine can be attributed to its role in intervening the cellular and molecular mechanisms associated with the pathologic processes of OA. Earlier we have demonstrated multiple beneficial effects of Aflapin over 5-Loxin; (1), better anti-inflammatory efficacy of Aflapin through inhibiting 5-lipoxygenase enzyme activity, and inhibiting TNFα production; (2), provides significant protection from damaging action of IL-1β by increasing chondrocytes proliferation and increasing synthesis of cartilage matrix substances such as collagen and glycosaminoglycans in human primary chondrocytes; (3), Aflapin also inhibits MMP3 production in TNFα induced human chondrocytes [17].

Overall, the data demonstrate the efficacy of Aflapin in pain management, improving physical function, quality of life and joint health. Presumably, the pleotropic beneficial effects of Aflapin might provide potential anti-osteoarthritic efficacy, which helps improving joint health in OA subjects [17,19,25].

In corroboration with the previous studies [19,25], the present investigation does not show any major changes in the hematological parameters, serum biochemical parameters and in urine analysis in

Aflapin supplemented subjects in comparison with placebo. In addition, no major adverse effect has been reported by the subjects included in Aflapin group. Taken together, these observations further demonstrate and substantiate the anti-osteoarthritic potential of Aflapin.

Conclusion

In summary, the present study validates the potential anti-OA efficacy and safety of Aflapin. In addition the present study also establishes the fast onset of therapeutic action of Aflapin® in OA subjects. Aflapin significantly improves joint function and relieves pain at as early as 5 days of treatment. This study bears potential promise in favor of Aflapin as a useful alternative therapeutic strategy for the management of OA in humans.

Abbreviations

AKBA: 3-O-acetyl-11-keto-beta-boswellic acid; ANOVA: analysis of variance; ASRAM: Alluri Sitarama Raju Academy of Medical Sciences; BMI: Body Mass Index; LFI: Lequesne's Functional Index; NSAID: nonsteroidal anti-inflammatory drug; NU: normalized units; OA: osteoarthritis; VAS: visual analog scale; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index.

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Conflict of Interest

The authors have declared that no conflict of interest exists.

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Efficacy and Tolerability of 5-Loxin and Aflapin Against Osteoarthritis of the Knee: A Double Blind, Randomized, Placebo Controlled Clinical Study
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Research Paper

Comparative Efficacy and Tolerability of 5-Loxin® and Aflapin® Against Osteoarthritis of the Knee: A Double Blind, Randomized, Placebo Controlled Clinical Study

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Abstract

Aflapin® is a novel synergistic composition derived from *Boswellia serrata* gum resin (Indian Patent Application No. 2229/CHE/2008). Aflapin is significantly better as an anti-inflammatory agent compared to the *Boswellia* extracts presently available in the market. A 90-day, double-blind, randomized, placebo-controlled study was conducted to evaluate the comparative efficacy and tolerability of 5-Loxin® and Aflapin® in the treatment of osteoarthritis (OA) of the knee (Clinical trial registration number: ISRCTN80793440). Sixty OA subjects were included in the study. The subjects received either 100 mg (n=20) of 5-Loxin® or 100 mg (n=20) of Aflapin® or a placebo (n=20) daily for 90 days. Each patient was evaluated for pain and physical functions by using the standard tools (visual analog scale, Lequesne's Functional Index, and Western Ontario and McMaster Universities Osteoarthritis Index) at the baseline (day 0), and at days 7, 30, 60 and 90. A battery of biochemical parameters in serum, urine and hematological parameters in citrated whole blood were performed to assess the safety of 5-Loxin® and Aflapin® in OA subjects. Fifty seven subjects completed the study. At the end of the study, both 5-Loxin® and Aflapin conferred clinically and statistically significant improvements in pain scores and physical function scores in OA subjects. Interestingly, significant improvements in pain score and functional ability were recorded as early as 7 days after initiation of the study in the treatment group supplemented with 100 mg Aflapin. Corroborating the improvements in pain scores in treatment groups, our *in vitro* studies provide evidences that Aflapin® is capable of inhibiting cartilage degrading enzyme MMP-3 and has the potential to regulate the inflammatory response by inhibiting ICAM-1. Aflapin® and 5-Loxin® reduce pain and improve physical functions significantly in OA subjects. Aflapin exhibited better efficacy compared to 5-Loxin®. In comparison with placebo, the safety parameters were almost unchanged in the treatment groups. Hence both 5-Loxin® and Aflapin® are safe for human consumption.

Key words: Aflapin®, 5-Loxin®, *Boswellia serrata*, anti-inflammation, osteoarthritis and clinical study.

Introduction

Osteoarthritis (OA) is the commonest form of arthritic disease, characterized by articular cartilage degradation with an accompanying peri-articular bone response [1,2]. OA affects nearly 21 million people in the USA, accounting for 25% of visits to primary care physicians. It is estimated that 80% of the population will have radiographic evidence of OA by age 65 years, although only 60% of those will be symptomatic [3]. Clinical manifestations of OA of the knee include pain in and around the joint, stiffness of the joint, crepitation on motion and limited joint motion, among others [4]. Current recommendations for managing OA focus on relieving pain and stiffness and improving physical function as important goals of therapy [5,6]. Currently available medication regimens for most cases include nonopioid analgesics such as acetaminophen and nonsteroidal anti-inflammatory drugs (NSAIDs) including cyclo-oxygenase II inhibitors. These pharmaceutical agents can reduce both pain and inflammation quite effectively, but long term use of NSAIDs has been found to associate with enhanced risk for gastrointestinal bleeding, hypertension, congestive heart failure and renal insufficiency, among other adverse effects [7-9]. Because of the high incidence of adverse events associated with both nonselective and cyclo-oxygenase II selective NSAID therapy, effective and safer alternative treatments for OA are urgently needed. In recent years, the gum resin extracted from the ancient herb, *Boswellia serrata* has gained lot of attention as a potent anti-inflammatory, anti-arthritic and analgesic agent [10,11]. 3-O-acetyl-11-keto-beta-boswellic acid (AKBA) is the most active component of *Boswellia* extract and has been demonstrated to be a potent inhibitor of 5-lipoxygenase (5-LOX), a key enzyme in the biosynthesis of leukotrienes from arachidonic acid in the cellular inflammatory cascade [12,13]. 5-Loxin® is a novel *B. serrata* extract enriched to 30% AKBA (US Patent publication no.: 2004/0073060A1). Affimatrix gene chip analysis demonstrated that 5-Loxin® can potently inhibit tumor necrosis factor α (TNFα) induced gene expression of matrix metalloproteinases (MMPs), adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1); and mediators of apoptosis in human microvascular endothelial cells [14, 15]. Cell based *in vitro* studies suggest that 5-Loxin® can inhibit pro-inflammatory cytokines such as tumor necrosis factor-α, interleukin-1β [16]. In the carrageenan-induced inflammation model, 5-Loxin® treatment yielded significant improvement in paw inflammation in albino Wistar rats. 5-Loxin® also exhibited significant Anti-arhtritic efficacy in FCA induced model of Sprague-Dawley rats [14, 15]. Extensive acute and dose dependent subchronic safety studies on rats demonstrated that 5-Loxin® is safe even at dose levels 2,000 to 3,000 times higher than the human equivalence dose [17]. In addition, 5-Loxin® was found to be non genotoxic as per the standard AMES bacterial reverse mutation assay, chromosomal aberration test in Chinese hamster cells and mouse peripheral blood micronucleus assay [18-21] The efficacy and tolerability of 5-Loxin® was assessed in a previous double blind placebo controlled clinical study. The supplementation of 5-Loxin® was well tolerated and its efficacy against osteoarthritis was found to be statistically significant. The dose dependent efficacy of 5-Loxin® was assessed against pain, joint stiffness, mobility and a cartilage degrading enzyme MMP-3 in OA subjects [22]. Aflapin® is a novel synergistic composition derived from *Boswellia serrata* gum resin (Indian Patent Application No. 2229/CHE/2008). Interestingly it was found that the oral bioavailability of AKBA from Aflapin® was better compared to that of 5-Loxin®. Aflapin exhibited better 5-lipoxygenase inhibitory activity and MMP-3 inhibition. Various *in vitro* and *In vivo* studies were performed to compare efficacy of Aflapin and 5-Loxin®. These studies proved Aflapin to be more efficacious compared to 5-Loxin® (to be presented in a separate communication). The broad spectrum safety of Aflapin was tested using a battery of safety studies conducted according to OECD guidelines and it was found to be safe [23]. Although a significant number of clinical study reports support the anti-inflammatory and anti-arthritic properties of *Boswellia* extract [24-27], no human clinical studies were done to prove the efficacy and tolerability of Aflapin in osteoarthritis. Hence in the present clinical study we sought to evaluate the comparative efficacy and tolerability of 5-Loxin® and Aflapin® in the treatment of OA of the knee.

Materials and Methods

Study materials

BE-30 (5-Loxin®) is a novel *Boswellia serrata* extract standardized to contain at least 30 percent 3-O-Acetyl-11-keto-β-boswellic acid (AKBA) using a selective enrichment process (Indian patent # 205269). The process involves selective enrichment of AKBA while simultaneously suppressing the concentration of triterpene compounds that are less active and those that antagonize the activity of AKBA. Aflapin is a

novel synergistic composition containing *B. serrata* extract selectively enriched with AKBA and *B. serrata* non-volatile oil. The non-volatile oil was prepared using a special process (PCT application # PCT/IN2009/000505) involving selective removal of Boswellic acids followed by removing volatiles under high vacuum. The composition was standardized to contain at least 20% AKBA.

Study design

This trial was performed at Alluri Sitarama Raju Academy of Medical Sciences (ASRAM), Eluru, Andhra Pradesh, India from July 2008 to December 2008 (clinical trial registration number: ISRCTN80793440). The study protocol was evaluated and approved by the ASRAM Institutional Review Board (IRB). An overview of the clinical study is provided in **Figure 1**. Briefly, 186 subjects out of 283 attending the orthopaedic outpatient department of the ASRAM hospital were selected in the first phase of the screening procedure, based on the signs, symptoms and radiological changes consistent with OA. A total of 60 subjects suffering for more than 3 months with medial tibio-femoral OA were selected using inclusion/exclusion criteria summarized in **Table 1**. All subjects signed the IRB approved consent form. Subjects, who were otherwise healthy, were aged 40 years or older and had a diagnosis of OA, fulfilling the American College of Rheumatology classification criteria [4]. After recruitment, the subjects were randomly distributed into three groups. The demographic data and baseline characteristics are summarized in **Table 2**.

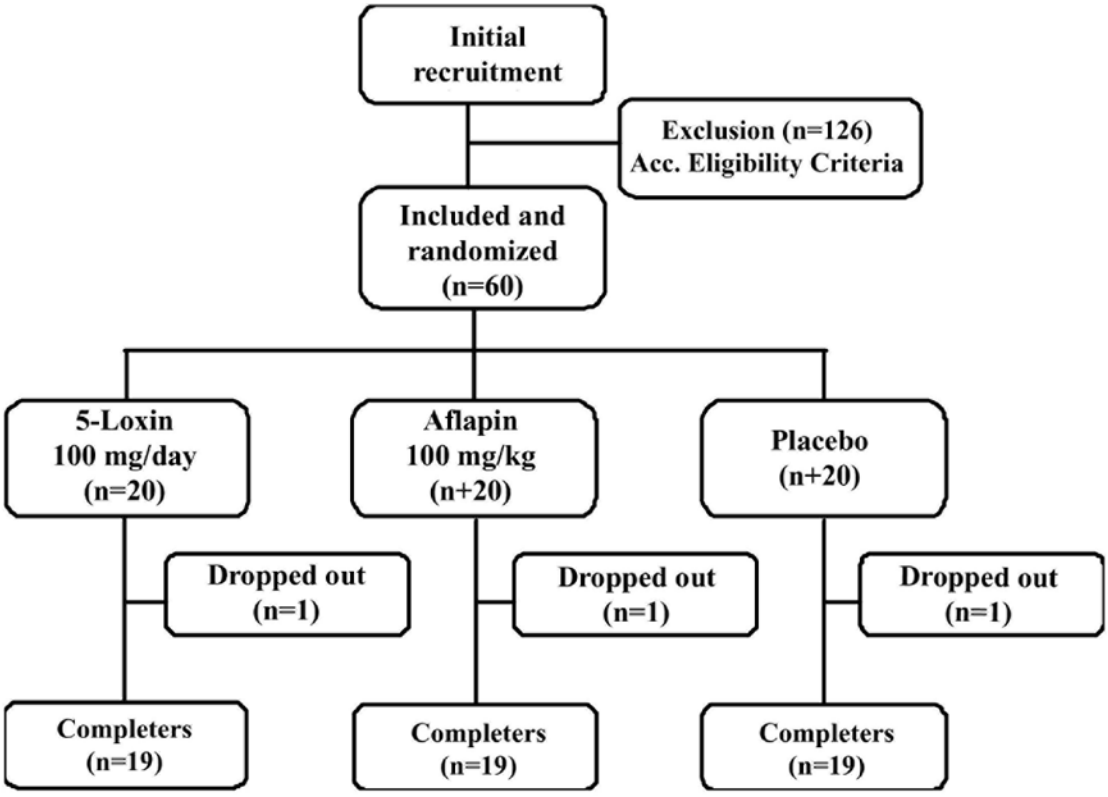

Figure 1: Flow chart of the subjects who participated in the clinical trial. Evaluations of physical activity and pain scores, serum biochemistry, hematology, and urine biochemistry were done at baseline (day 0) and on days 7, 30, 60 and 90 during follow up.

Table 1: Inclusion/exclusion criteria

Criteria	Details
Inclusion	Subjects must understand risks and benefits of the protocol and be able to give informed consent
	Male and female subjects aged 40 to 80 years
	Females of child-bearing potential must agree to use an approved form of birth control and to have a negative pregnancy test result
	Unilateral or bilateral osteoarthritis of the knee for more than 3 months
	Visual analogue scale score during the most painful knee movement between 40 and 70 mm after 7 days of withdrawal of usual medication
	Lequesne's Functional Index score greater than 7 points after 7 days of withdrawal of usual medication
	Ability to walk
	Availability for the duration of the entire study period
Exclusion	History of underlying inflammatory arthropathy or severe rheumatoid arthritis
	Hyperuricaemia (>440 µmol/l) and/or past history of gout
	Recent injury in the area affected by osteoarthritis of the knee (past 4 months) and expectation of surgery in the next 4 months
	Intra-articular corticosteroid injections within the preceding 3 months
	Hypersensitivity to nonsteroidal anti-inflammatory drugs, abnormal liver or kidney function tests, history of peptic ulceration and upper gastrointestinal hemorrhage, congestive heart failure, hypertension, cancer, hyperkalaemia
	Major abnormal findings on complete blood count, history of coagulopathies, hematological or neurological disorders
	High alcohol intake (>2 standard drinks per day)
	Pregnant, breastfeeding, or planning to become pregnant during the study
	Use of concomitant prohibited medication other than ibuprofen
	Obesity (body mass index > 30 kg/m2)

Table 2: Demographic data and baseline characteristics of the subjects

Characteristics	Placebo (n = 19)	100 mg/day 5-Loxin® (n = 19)	100 mg/day Aflapin® (n = 19)
Sex (male/female; n)	9/10	3/16	7/12
Age (years)	52.4 ± 7.5	51.6 ± 9.9	53.2 ± 7.9
Body weight (kg)	62.4 ± 14.9	57.7 ± 10.5	59.1 ± 7.4
Body mass index (kg/m2)	25.3 ± 4.4	25.1 ± 3.8	25.2 ± 3.0
Visual analog score	47.7 ± 6.5	48.2 ± 6.1	47.7 ± 7.3
Lequesne's Functional Index	12.3 ± 2.8	12.4 ± 2.6	12.0 ± 2.4
WOMAC scores			
Pain subscale	44.7 ± 11.5	46.1 ± 7.6	45.0 ± 13.3
Stiffness subscale	39.5 ± 11.2	39.5 ± 11.2	39.5 ± 13.3
Function subscale	42.0 ± 10.3	43.1 ± 7.8	42.0 ± 8.4

Before study enrollment, subjects were required to be taking an NSAID at prescription strength for at least 30 days or acetaminophen 1,200 to 4,000 mg/day on a regular basis (at least 25 of the preceding 30 days) with a history of therapeutic benefit. Eligibility requires subjects to meet specific flare criteria upon medication washout. At screening, subjects had to demonstrate a visual analog scale (VAS) score between 40 and 70 mm during the most painful knee movement, and Lequesne's Functional Index (LFI) score greater than 7 points after 7-day withdrawal of usual medication.

A total of 60 selected subjects with symptoms of moderate to mild OA were recruited into the study. Each subject was randomly assigned to a treatment group using a randomization table generated using validated computer software CODE; IDV, Gauting, Germany. The clinical trial pharmacist and statistician ensured that treatment codes remained confidential. The subjects were distributed into three groups: placebo (n=20); 5-Loxin® group, in which subjects received 50 mg encapsulated 5-Loxin® twice daily (n=20); and Aflapin group, in which subjects received 50 mg encapsulated Aflapin® twice daily (n=20). Subjects in the placebo group received two capsules of similar color, taste and appearance but filled with suitable exicipient. Each subject completed a questionnaire, providing details regarding demographics, medical history and nutritional status, at the baseline evaluation and during the follow-up evaluations on days 7, 30, 60 and 90. At the baseline evaluation, and at each visit during the 90-day follow up period, all

subjects were assessed for pain and physical function using validated pain scores. Various parameters of serum biochemistry, hematology and urine analysis were carried out on each evaluation day. Safety was monitored by clinical and laboratory assessments conducted during the study visits and subject-reported adverse experiences.

Functional disability and pain score evaluation

Functional disability was assessed by the investigators at baseline and on each follow-up visit (days 7, 30, 60 and 90). Questionnaire-based assessment of pain, stiffness and physical function were done using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) index [28], LFI [29] and VAS [30]. The WOMAC index produces scores for three subscales: pain, stiffness and physical function. The pain, stiffness and function subscales of the WOMAC were normalized to a scale of 0 to 100 units (NU) [31]. The pain subscale was the average of the first five questions of WOMAC and measured using the NU scale from 0 ('no pain') to 100 ('extreme pain') for each question. The stiffness subscale was the average of questions 6 and 7, measured using the NU scale from 0 ('no stiffness') to 100 ('extreme stiffness') for each question. The physical function subscale was the average of questions 8 through 24 of the WOMAC and measured by NU scale from 0 ('no difficulty') to 100 ('extreme difficulty') for each question. Analyses of these end-points were based upon the time-weighted average change from baseline over 90 days.

Hematological and biochemical evaluations

For assessment of safety of 5-Loxin® and Aflapin®, several parameters were evaluated in serum, urine and whole blood of all subjects at each visit of the study duration. Serum biochemical parameters and hematological parameters were measured using an automated analyzer (HumaStar 300) and a hematological counter (Humacount, Human, Wiesbaden, Germany). The urine analysis was carried out using UroColor™10 Dip Sticks and Urometer 600 (Standard Diagnostics, Kyonggi-do, Korea) and by sediment analysis using microscopy.

In vitro studies to identify mechanisms of actions of Aflapin: Effect on expression of ICAM-1 and MMP3

Adhesion molecule (ICAM-1) expression on endothelial cells: 20,000 Endothelial cell (HDMEC, Lonza Inc., USA) per well in quadruplicate wells were treated with medium, vehicle, TNFα (20ng/ml), TNFα (20ng/ml) with 5-Loxin® or Aflapin® (4μg/ml each) for 24 hour then ICAM-1 ELISA was performed on fixed cells of these wells as per our established protocol [32].

Effect on secretion of MMP3 in TNFα induced human chondrocyte: Human primary Chondrocytes (HCH) was procured from Promo Cell GmbH (Heidelberg, Germany). HCH cells were cultivated in the growth medium (Ready-to-use; Promo Cell, Catalog number C-27101) supplemented with Supplement Mix (Promo Cell, Catalog number C-39635). Equal number of HCH cells was plated in each well of 96-well cell culture plate. Cells were treated with 5 ng/ml of TNFα in presence or absence of different concentrations of 5-Loxin® or Aflapin for 24h. Vehicle control cultures received 0.01% DMSO (v/v). MMP-3 was quantitatively measured in the cell culture supernatant by human MMP-3 EIA kit (R&D Systems, USA) following manufacturer's instructions.

Rescue medication

Subjects were prescribed ibuprofen 400 mg tablets (maximum 400 mg thrice daily; total 1,200 mg) as rescue analgesia during the study based on pain intensity reported to the study physician by the patient. However, the subjects were instructed not to take medicine at least 3 days before each evaluation. No other pain relieving interventions were allowed during the study period.

Statistical analysis

Detailed statistical analyses were performed using SAS software to evaluate the efficacy of 5-Loxin® and Aflapin® in comparison with the placebo group in terms of improvement in pain and physical function scores at baseline and on days 7, 30, 60 and 90 of treatment and serum MMP-3 levels at baseline and on day 90 of treatment. Pair-wise changes were examined by carrying out a least significant difference test for all possible pairs. The significance of the effects of the treatment groups was compared by using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Results with $P<0.05$ are considered statistically significant. This is a three-arm (5-Loxin®, Aflapin® and placebo), randomized, double-blind, placebo-controlled, single-centre trial conducted over 90 days. The trial's primary objective was to determine the effects of 5-Loxin® and Aflapin® on pain, physical function and joint stiffness. For power calculations, the estimates for variability and assumed mean changes for each treatment group were based on results from previous placebo-controlled studies of celecoxib, etoricoxib and rofecoxib conducted in subjects with OA [33-36]. We believe that an intervention that gives an average improvement of mean change ± 1 standard deviation,

rather than mean change alone will provide results of greater significance [37]. Our trial is designed to have more than 80% power to detect a situation in which either active drug dosage yields an improvement to at least mean change ± 0.9 standard deviation, under a conservative assumption, and we tested differences between groups in mean improvement using ANOVA (α=0.05, two-sided). With 20 subjects per group, we would have a 93% chance of observing at least one example of any side effect occurring in 10% or more of the patient population at a specific dosage.

Results

Baseline characteristics

Descriptive statistics comparing demographic variables, baseline disease characteristics and baseline outcome measures (LFI, VAS, WOMAC pain, function and stiffness sub-scores) are provided in Table 2. Overall, the treatment groups receiving 5-Loxin® 100 mg/day, n=19, Aflapin® 100 mg/day, n=19 and placebo n=19, were similar with respect to age, Body Mass Index and pain severity (Table 2). The subjects were randomly distributed into three groups.

Clinical efficacy

We compared the scores between the treatment groups obtained at day 90. Both the treatments with 5-Loxin® and Aflapin® conferred clinically and statistically significant improvements in pain scores and physical ability scores in OA subjects between baseline and day 90 (Table 3). Tukey's multiple comparison test revealed statistically significant improvements by 31.6% (*P*=0.006), 30.3% (P=0.009) and 42.2% (p=0.006) in VAS, WOMAC pain, and WOMAC stiffness scores, respectively, in the 100 mg 5-Loxin® treated group in comparison with the placebo group (Table 3). Improvements by 18.35% (*P*=0.060) and 21.25% (*P*=0.078) in LFI and WOMAC functional ability scores, respectively were also achieved in the 5-Loxin® group (Table 3). In comparison with the placebo group, the Aflapin® 100 mg treated group also exhibited statistically significant improvements in all parameters tested (Table 3). The Aflapin group showed improvements by 47.3% (*P*<0.0001), 35.8% (*P*=0.0004), 61.7% (*P*<0.0001), 60.1% (*P*=0.0001) and 49.4% (*P*=0.0001) in VAS, LFI, WOMAC pain, WOMAC stiffness and WOMAC functional ability scores, respectively. It is worth noting that both 5-Loxin® and Aflapin® treatment groups exhibited improvement in pain scores and physical ability scores as early as 7 days after the start of treatment, and these indices continued to improve throughout the 90 days of treatment (**Figure 2**). After 7 days, the 5-Loxin® treatment group exhibited 8.09% (*P*=0.002), 8.68% (*P*=0.031) and 8.35% (*p*=0.015) reductions in VAS, WOMAC pain and WOMAC function respectively, compared with the corresponding baseline scores. After 7 days, the Aflapin treatment group exhibited 12.8% (*P*=0.0004), 9.17% (*P*=0.003), 11.78% (*P*=0.012), 18.48% (P=0.012) and 10.24% (*p*=0.005) reductions in VAS, LFI WOMAC pain, WOMAC stiffness and WOMAC function scores respectively, compared to the corresponding baseline scores (Figure 2).

Table 3: Student's *t*-test (paired) analyses for comparison of the scores obtained from the Aflapin and 5-Loxin groups at day 90

	n	Baseline		Day 90		95% CI (versus placebo)	p
		Mean	SD	Mean	SD		
Visual analogue scale score							
Placebo	19	47.7	6.5	38.3	9.0	34.0, 42.7	0.0013
5-Loxin 100 mg/day	19	48.2	6.1	26.2	16.5	18.2, 34.1	<0.0001
Aflapin 100 mg/day	19	47.7	7.3	20.2	12.3	14.2, 26.1	<0.0001
Lequesne's Functional Index							
Placebo	19	12.3	2.8	10.9	3.0	9.4, 12.3	0.0496
5-Loxin 100 mg/day	19	12.4	2.6	8.9	3.7	7.1, 10.7	<0.0001
Aflapin 100 mg/day	19	12.0	2.4	7.0	2.6	7.1, 9.6	<0.0001
WOMAC pain subscale							
Placebo	19	44.7	11.5	36.3	10.5	31.2, 41.4	0.0021
5-Loxin 100 mg/day	19	46.1	7.6	25.3	17.2	17.0, 33.6	<0.0001
Aflapin 100 mg/day	19	45.0	13.3	13.9	8.3	10.0, 17.9	<0.0001
WOMAC stiffness subscale							
Placebo	19	39.5	11.2	29.6	9.5	25.0, 34.2 p	0.0059
5-Loxin 100 mg/day	19	39.5	11.2	17.1	16.8	9.0, 25.2	0.0001
Aflapin 100 mg/day	19	39.5	13.3	11.8	12.8	5.7, 18.0	<0.0001

	n	Baseline		Day 90		95% CI (versus placebo)	p
		Mean	SD	Mean	SD		
WOMAC function subscale							
Placebo	19	42.0	10.3	32.0	10.8	26.8, 37.2	0.0025
5-Loxin 100 mg/day	19	43.1	7.8	25.2	15.0	17.9, 32.4	<0.0001
Aflapin 100 mg/day	19	42.0	8.4	16.2	8.1	12.3, 20,1	<0.0001

CI, confidence interval; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

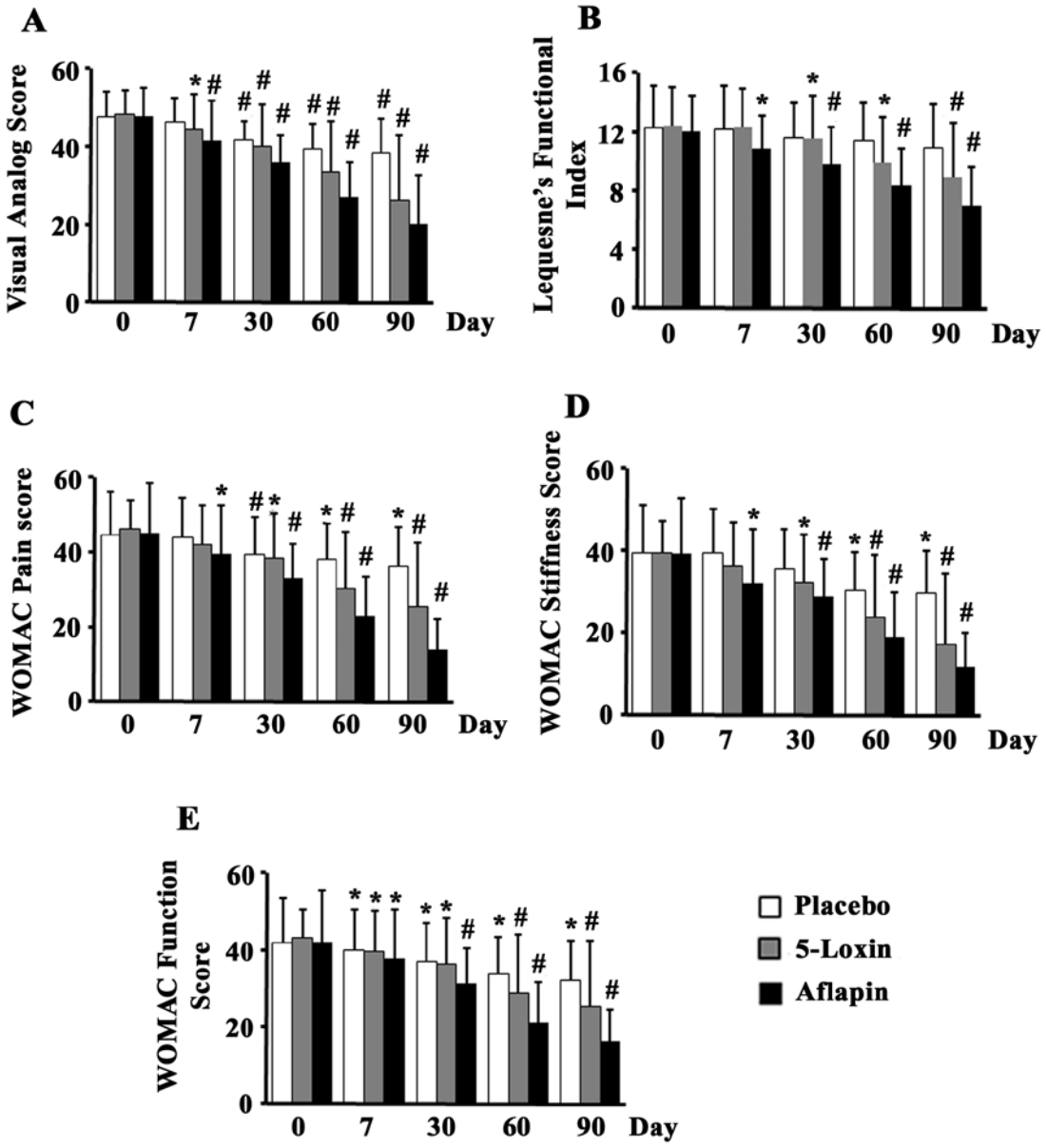

Figure 2: Bar diagrams represent the mean scores of (a) visual analog scale (VAS) (**a**); Lequesne's Functional Index (LFI) (**b**); Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)-pain (**c**); WOMAC-stiffness (**d**); and WOMAC-function (**e**) in placebo, 100 mg/ day 5-Loxin® and 100 mg/day Aflapin® groups, respectively. 1 to 5, represent days of evaluations such as day 0, day 7, day 30, day 60 and day 90, respectively. Each bar represents mean ± standard deviation. In comparison with corresponding baseline data, the change in scores in the treatment groups was tested for significance using Tukey's multiple comparison test; * $p<0.05$; ** $p<0.005$.

Aflapin inhibits secretion of MMP-3 in TNFα-induced human primary chondrocytes

In OA, the loss of collagen from articular cartilage is proportional to the disease severity (38). Under the influence of pro-inflammatory cytokines, increased production and secretion of collagenases such as MMP-3, MMP-13 is the crucial event for enhanced collagen degradation in OA [39]. Therefore, we sought to evaluate whether 5-Loxin and Aflapin can modulate MMP-3 secretion in TNFα, a potent pro-inflammatory cytokine induced human primary chondrocytes. **Figure 3** shows a steep increase in MMP-3 secretion in TNFα-induced chondrocytes and dose-dependent inhibition of MMP-3 secretion in 5-Loxin and Aflapin treated cultures. Interestingly, we observed, Aflapin (IC_{50} at 18.5 μg/ml) provided (41.36%) better efficacy than 5-Loxin (IC_{50} at 31.71 μg/ml) in inhibiting MMP-3 secretion from TNFα-induced human chondrocytes.

Aflapin inhibits ICAM-1 expression in activated endothelial cells

OA is a degenerative joint disorder. However, there are migrations of inflammatory cells in the synovial fluid. Adhesion molecule expression on endothelial cells helps in the diapedesis of these cells. Therefore, in order to determine whether 5-Loxin® and Aflapin® treatments can ameliorate the ICAM-1 expression, we evaluated the ICAM levels on HDMEC. **Figure 4** depicts that 5-Loxin® and Aflapin® significantly reduce TNFα induced ICAM-1 expression ($p<0.01$, student t-test). Interestingly, Aflapin® shows more capability to reduce ICAM-1 secretion than that of 5-Loxin®.

Biochemical evaluations

As a part of the safety evaluation, laboratory tests were performed to evaluate different biochemical parameters (serum and urine) and hematological parameters. The significance of the differences between baseline and 90 days was tested by using repeated measures ANOVA. The f ratio is considered significant if $P<0.05$. Although minor changes were observed in some of the parameters, they remained within the normal laboratory range. Statistical analyses of these parameters did not indicate any significant changes. Similarly, no significant changes in hematological and urinary parameters were observed in the active treatment groups when compared to the placebo (data not shown). These findings further demonstrate the safety of 5-Loxin® and Aflapin® in humans.

Adverse Events and Dropouts

During the course of the 90-day study, no major adverse events were reported. However, acidity was reported as a minor adverse event by two subjects during the study; one each from placebo and Aflapin supplemented groups, respectively.

Three subjects one from each placebo, 5-Loxin® and Aflapin® supplemented groups were dropped out from the study due to their un-availability during the entire study period.

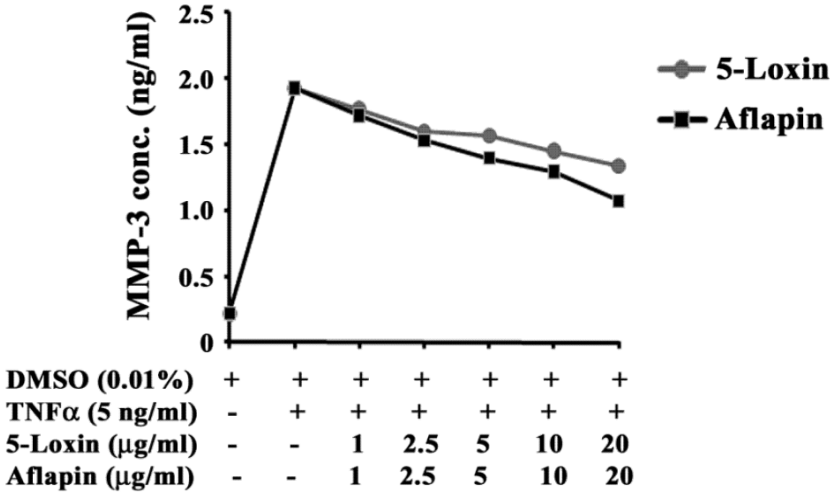

Figure 3: Aflapin and 5-Loxin inhibit matrix metalloproteinase-3 secretion from TNFα-induced human primary chondrocytes. Line diagram represents MMP-3 concentrations in the culture supernatants of chondrocytes treated with 5 ng/ml of human recombinant TNFα in presence or absence of different doses of either 5-Loxin or Aflapin as indicated. Vehicle control cultures received 0.01% DMSO. Each data point represents the mean of quadruplicate wells.

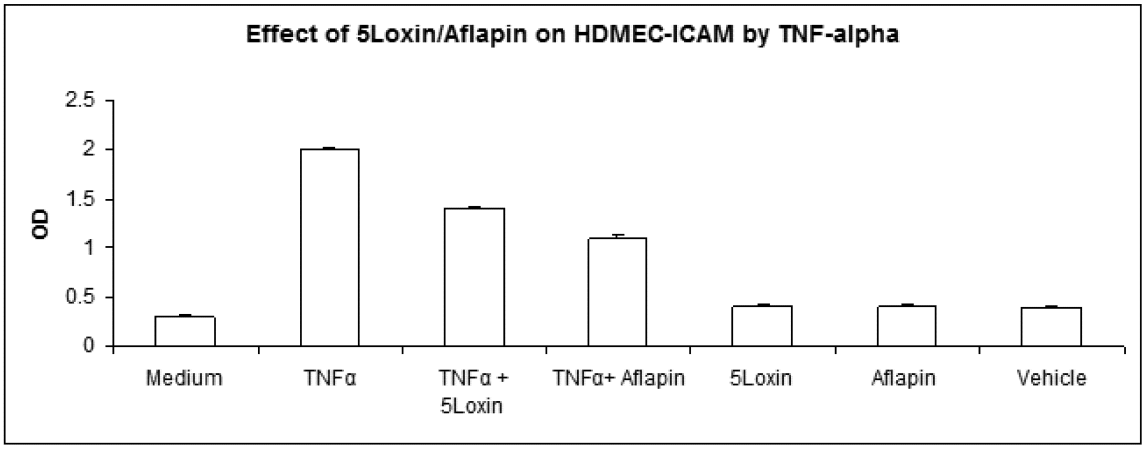

Figure 4: Aflapin and 5-Loxin inhibit TNFα-induced ICAM-1 expression on human dermal microvascular endothelial cells (HDMEC). Bar diagrams represent the ICAM-1 expression on HDMEC treated with 20ng/ml of human recombinant TNF-α in presence or absence of either 5-Loxin (4µg/ml) or Aflapin (4µg/ml) as indicated. Vehicle control cultures received 0.01% DMSO. Each experiment is done in quadruplicate wells. The results are expressed as the mean±SD of five experiments in quadruplicate wells. 5-Loxin and Aflapin significantly inhibits ICAM-1 expression induced by TNF-α ($p<.01$, student t-test).

Discussion

This is the first clinical study to evaluate the efficacy of Aflapin® in OA subjects. Aflapin is a novel synergistic composition comprising AKBA enriched *B. serrata* extract and non acidic gum extract of *B. serrata*. In a battery of preclinical studies designed in *in vitro* cellular models and *in vivo* animal models, Aflapin exhibited significantly better anti-inflammatory activities in comparison with 5-Loxin® (Data to be presented in a separate communication). 5-Loxin® is a *Boswellia serrata* extract standardized to 30% AKBA. Its multidirectional activities related to anti-inflammatory efficacies obtained in appropriate cellular, animal models and in human subjects have established that 5-Loxin® is a potent dietary supplement for the management of inflammatory diseases such as osteoarthritis [14-22]. In a series of experiments designed in *in vitro* cellular and *in vivo* animal models, Aflapin showed significantly better efficacy in comparison with 5-Loxin®. In addition, Aflapin exhibited better AKBA bioavailability than 5-Loxin® in Wistar rat model. Broad spectrum safety of Aflapin was also established in a battery of acute and sub-acute toxicity studies in rat and rabbits. These findings altogether motivated us to evaluate efficacy of Aflapin in comparison with 5-Loxin® against osteoarthritis in human subjects. In the present 90-day clinical study, we assessed the efficacy and tolerability of Aflapin in comparison with 5-Loxin® in OA subjects. Pain, stiffness of joints, reduced joint movement and physical disability are the major clinical manifestations of OA [1,40,41]. Our study demonstrates that Aflapin potentially improves pain, joint stiffness and physical function in OA subjects (**Figure 2**). In order to check improvements in the treatment groups, we compared the data for all parameters between the baseline and day 90. Paired *t*-test revealed that both treatment groups showed statistically significant improvements in all parameters.

Compared to the placebo, 5-Loxin® supplementation for 90 days, significantly reduced VAS, WOMAC-pain, WOMAC-stiffness (**Table 3**), which are consistent with our previous observations [22]. Whereas, Aflapin supplementation for 90 days, resulted in significant reduction in all pain scores tested in comparison with placebo. These findings suggest that Aflapin has better therapeutic efficacy against OA compared to 5-Loxin®. We observed that, in comparison with baseline, there were downward trends in VAS score and WOMAC scores in the placebo group. We believe that this might be partly attributable to the placebo effect [42,43] manifested while administering the questionnaires to placebo subjects and partly due to the consumption of ibuprofen as rescue medication by more subjects in the placebo group during the study. It is noteworthy that 5-Loxin® possesses significant efficacy in lowering VAS score by 8.09% (P=0.022), WOMAC pain score by 8.68% (0.031) and WOMAC function score by 8.35% (P<0.015) in OA subjects as early as 7 days after the initiation of treatment. In comparison, Aflapin showed significant reduction in all the pain scores assessed including VAS score by 12.8% (P=0.0004), LFI score by 9.17% (P=0.003), WOMAC pain score by 11.78% (P=0.012), WOMAC stiffness score by 18.48% (P=0.012) and

WOMAC function score by 10.24% (P=0.005) (**Figure 2**). These findings therefore indicate that 5-Loxin® and Aflapin® confers prompt and significant pain relief, improvement in physical ability and quality of life in OA subjects. However, Aflapin showed better reduction in all the tested pain scores and hence can be considered superior to 5-Loxin®.

Pathogenesis of osteoarthritis is a complex process. These include mechano-transduction, the interplay between metalloproteases (MMP3, MMP13), protease inhibitors and cytokines on cartilage degradation and mechanisms of cartilage repair [40,44,45]. MMP-3 is over-expressed in OA and cause degeneration of cartilage tissue [44,45]. Cytokines act via autocrine and endocrine functions to alter cartilage homeostasis. Interleukin-1 (IL-1) and TNF-α are perhaps the best characterized cytokines for cartilage degradation (46,47). They are synthesized by chondrocytes and FLS. These cytokines act in various ways in the pathogenesis of OA such as inhibition of synthesis of type 2 (articular) cartilage and activation of catabolic metalloproteases including MMP-3 which plays a critical role in cartilage degradation [44,45]. A role of synovitis in OA can't be disregarded either. It is a well established clinical observation that pain and swelling in OA improves for months following intra-articular corticosteroid injection. In addition, histologic studies suggest that localized inflammatory changes characterized by foci of inflammatory cells occur in up to 50% of OA patients [48]. In this study to find out possible mechanism of actions of Aflapin we carried out *in vitro* studies to evaluate whether Aflapin can inhibit metalloprotease secretion or influence the inflammatory component of osteoarthritis. We observed: (1) Aflapin inhibits TNFα induced MMP-3 secretion in chondrocytes; (2) Aflapin inhibits TNFα induced expression of ICAM-1 in endothelial cells.

Overall, the foregoing data together demonstrates the better ability of Aflapin compared with 5-Loxin® in terms of reducing the pain, improving physical function, quality of life and joint health. Presumably these improvements might occur through down regulation of cartilage degrading enzymes such as MMP-3 in OA subjects. The present study also demonstrates no major changes in the hematological parameters, serum biochemical parameters and in urine analysis in the treatment groups compared to placebo. In addition, no major adverse effect was reported by the subjects in the treatment groups. Taken together, these observations further demonstrate that 5-Loxin® and Aflapin® are potentially safe in the treatment of OA in humans and more specifically Aflapin® is more efficacious in the management of osteoarthritis than 5-Loxin®.

Conclusion

In summary, the present study provides the evidence in support of the potential efficacy and tolerability of 5-Loxin® and Aflapin® in subjects with OA; 5-Loxin® and Aflapin significantly improved joint function. Aflapin exhibited better therapeutic efficacy over 5-Loxin® at 100 mg/day; it reduces pain rapidly, as early as after 1 week of treatment. Furthermore, *in vitro* studies also provide evidences that compared to 5-Loxin, Aflapin is capable of inhibiting cartilage degrading enzyme MMP-3 and has the potential to regulate the inflammatory component in by inhibiting ICAM-1. Most importantly, we have observed that 5-Loxin® and Aflapin® are safe for human consumption, even for long term supplementation. 5-Loxin® and Aflapin® are promising alternative therapeutic options, that may be used as nutritional supplements for management of OA.

Authors' contributions

KS contributed to the design of the project and data analysis, and was primarily responsible for writing the manuscript. KVA contributed to the design of the project, patient recruitment and management, and data collection. ARS and AM worked with subjects to obtain informed consent, conducted clinical evaluations, took samples and evaluated therapeutic response of 5-Loxin® and Aflapin®. TG contributed as the study coordinator and helped to review the manuscript. KVSS and DD helped in clinical data analysis. SMR helped in designing and executing the mechanisms of action studies. SPR helped in designing the study, conducting data analysis and writing the manuscript.

Abbreviations

AKBA: 3-O-acetyl-11-keto-beta-boswellic acid; ANOVA: analysis of variance; ASRAM: Alluri Sitarama Raju Academy of Medical Sciences; BMI: Body Mass Index; ELISA: enzyme-linked immunosorbent assay; LFI: Lequesne's Functional Index; MMP: matrix metalloproteinase; NSAID: nonsteroidal anti-inflammatory drug; NU: normalized units; OA: osteoarthritis; VAS: visual analog scale; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index.

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Conflict of Interest

The authors have declared that no conflict of interest exists.

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