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Effect of a glucosamine-based combination supplement containing chondroitin sulfate and antioxidant micronutrients in subjects with symptomatic knee osteoarthritis: A pilot study

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Abstract. In the present study, we aimed to investigate the potential effect of a glucosamine (1,200 mg/day)-based dietary supplement combined with chondroitin sulfate and three antioxidant micronutrients, namely methylsulfonylmethane, guava leaf extract, and vitamin D (test supplement) on osteoarthritis (OA) of the knee. A 16-week, randomized, double-blinded, placebo-controlled trial was conducted involving 32 subjects with symptomatic knee OA. Clinical outcomes were measured using the Japanese Knee Osteoarthritis Measure (JKOM) for symptoms and a study diary-based visual analog scale (diary VAS) for pain at baseline and at weeks 4, 8, 12 and 16 during the 16-week intervention period. Furthermore, biomarkers for cartilage type II collagen degradation (C2C) and synovitis hyaluronan (HA) were measured. As compared with the baseline, the JKOM pain subscale was significantly improved at all of the four assessment time points in the test group, but was not at any time point in the placebo group. On the other hand, all of the four symptom subscales and the aggregated total symptoms were significantly improved in the two groups at one or more time points. However, all of these clinical improvements were greater in extent in the test group than in the placebo group, and there were significant differences between groups in the magnitude of changes from baseline for one subscale 'general activities' and the aggregated total symptoms at week 8 ($P<0.05$). The results of efficacy assessments with the diary VAS showed that all of the three pain subscales were significantly improved only in the test group at almost all the time points. Moreover, serum levels of C2C and HA were decreased by 10 and 25%, respectively, at week 16 in the test group, albeit not statistically significant, without any detectable changes in the placebo group. In conclusion, although the results obtained in this study were not conclusive, the tested glucosamine-based combination supplement is likely to have a beneficial effect on pain and other symptoms associated with knee OA.

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Introduction

Osteoarthritis (OA), which develops due to the progressive destruction of articular cartilage, is the most common joint disease and the leading cause of pain and physical disability in elderly people (1,2). Moreover, as the proportion of elderly individuals in the population increases, the number of OA patients is expected to further increase. In OA, particularly knee joints are affected as they are weight-bearing joints. In Japan, similar to many other developed countries, the incidence and prevalence of knee OA are currently increasing with an increase in the elderly population (3). Thus, the management of knee OA, which requires extensive utilization of health care resources, has become a major social and economic issue in the health management of the elderly.

The mainstay of pharmacotherapeutic approaches to pain management in OA involves analgesics, nonsteroidal anti-inflammatory drugs, selective cycloxygenase-2 inhibitors and an intra-articular injection of hyaluronan (HA) or corticosteroids. However, there are considerable data showing that these approaches frequently produce insufficient benefit with the associated risk of untoward side effects (4-7). It is, therefore, no wonder that patients with OA embrace complementary and alternative approaches to pain management of OA (8-12).

Orally administered glucosamine and chondroitin sulfate, used alone and in combination, both being natural components of articular cartilage, are extensively used as alternative medicines, and are suggested by several studies not only to reduce the OA-associated pain and other symptoms but also to suppress the disease progression (13,14). However, contradictory data have also been reported in a fairly large number of studies, and thus the therapeutic efficacy of glucosamine and

chondroitin sulfate alone and in combination is considered a controversial issue as described in several meta-analyses (15,16) and a manuscript reporting the results of a more recently conducted large-scale clinical trial, the Glucosamine/Chondroitin Arthritis Intervention Trial (GAIT) (17).

Besides glucosamine and chondroitin sulfate, several antioxidant micronutrients have also been reported to have the potential for relieving OA-associated pain and/or beneficially influencing osteoarthritic processes (18), probably through their analgesic, anti-inflammatory or antioxidative effects (19). The rationale is that inflammatory and oxidative damages due to over-production of nitric oxide and other reactive oxygen species (ROS) have been demonstrated in aging and osteoarthritic cartilage (20) and have been correlated with the extent of cartilage damage (21). This postulation is supported by the results of several laboratory studies demonstrating that chondrocytes are potent sources of ROS (22), and that ROS are implicated in the oxidative damage to cartilage and the development of OA (23,24).

Based on these findings, we considered a glucosamine-based dietary supplement combined with chondroitin sulfate and three antioxidant micronutrients, namely methylsulfonylmethane (MSM), guava leaf extract and vitamin D, that is commercially available in Japan as a promising candidate nutraceutical for the management of knee OA. MSM is a natural substance present in various green plants, fruits and vegetables, and has been shown to modestly reduce pain and swelling in patients with knee OA when administered alone or in combination with glucosamine (25,26). Guava leaf extract rich in quercetin and other polyphenols has anti-inflammatory and analgesic effects and is used as an antioxidant dietary supplement in Japan (27). Vitamin D has been reported to protect the development and to slow the progression of OA (28,29).

The pathophysiological hallmark of OA is the focal destruction of articular cartilage, which results from increased degradation of cartilage matrix molecules including type II collagen (CII) and proteoglycans (30,31). In this context, there is accumulating evidence suggesting that osteoarthritic processes may be beneficially modified by both glucosamine and chondroitin sulfate (32-36), and that their therapeutic effects could be evaluated by changes in the serum or urine levels of CII degradation biomarkers, such as CTX-II, C1, 2C and cartilage type II collagen degradation (C2C) (37-39). Moreover, in patients with several types of arthritis including OA, synthesis of HA was found to be enhanced in inflamed synovium or synovitis (40), eventually leading to an increase in serum levels of HA (41). Thus, although the primary objective of the present pilot study was to assess the clinical efficacy of the glucosamine-based combination supplement (test supplement) in subjects with symptomatic knee OA, its effect on biomarkers associated with CII metabolism and synovial inflammation was also investigated.

Materials and methods

Study design. A randomized, double-blinded, placebo-controlled study was designed to assess the efficacy and safety of the test supplement in adult subjects with symptomatic knee OA. The study was carried out from December 2009 to August 2010 and involved two clinical service organization centers in Yokohama, Japan. The study protocol was approved by the institutional ethics committee, and was conducted in accordance with the principles of the amended Declaration of Helsinki and 'Ethical Guidelines for Epidemiological Research (established by the Japanese Government in 2008)'. Written informed consent was obtained from all participants prior to their enrollment in the study. The overall design of the study consisted of a 16-week intervention period preceded by an approximately 4-week run-in period. The subjects accomplished full clinical and laboratory examinations at baseline and at weeks 4, 8, 12 and 16 during the intervention period.

Subjects. Male and female Japanese subjects, aged 40-83 years with clinical and radiographic evidence of mild knee OA were enrolled; 30-75 on a 100-mm visual analog scale (VAS) and radiological severity of affected knee joints mainly graded 1-2 on the Kellgren-Laurence (K/L) grade (42). Subjects with bilateral diagnosed knee OA were asked to specify the worse affected knee at baseline, and this knee was evaluated throughout the study period.

Exclusion criteria were: gout/hyperuricemia or rheumatic arthritis; prior knee surgery or its necessity; routine use of health foods containing glucosamine, chondroitin sulfate or any other constituents of the test supplement; known hypersensitivity or allergy to glucosamine or chondroitin sulfate; previous or current treatment with antiresorptive drugs such as bisphosphonates or estrogen; intra-articular injections of either corticosteroids (within the 3 previous months) or HA (within the previous 2 weeks) before the initiation of intervention; pregnant women, nursing mothers or women of child-bearing potential during the study period; participation in another clinical study; and the presence of any medical condition judged by the medical investigator to preclude the subject's inclusion in the study.

Intervention and subject assignment. The test supplement was a commercially available tablet-form preparation containing 1,200 mg of glucosamine hydrochloride, 200 mg of shark cartilage extract, of which approximately 30% (60 mg) is chondroitin sulfate, 300 mg of MSM, 105 mg of guava leaf extract and 5.6 μg of vitamin D, together with 7.35 mg of vitamin B_1 and vehicle (comprising lactose, maltitol and crystalline cellulose) at a daily dose of 7 tablets. Subjects were randomly assigned to receive 7 tablets (2,300 mg) of the test supplement (test group), or 7 tablets (2,300 mg) of dummy placebo containing only vehicle (placebo group). All subjects were instructed to take 7 tablets of the test supplement or placebo once daily at any time of the day. Intervention was continued for 16 weeks until the final visit (week 16). Adherence to the intervention was evaluated based on the consumption recorded in the study diary, and a value <80% was considered a protocol violation.

Blood was obtained from subjects in a fasting state and second void of the morning urine was collected at every clinical visit. Aliquots of serum and urine samples were stored frozen until use for the blind-manner determination of OA-related biomarkers, whereas other aliquots of serum and urine samples were immediately used for the routine laboratory tests.

Efficacy assessment. Symptomatic changes over time during the intervention period were primarily assessed using the

scores of the Japanese Knee Osteoarthritis Measure (JKOM) (43) developed by the Japanese Orthopaedic Association according to the concepts of the World Health Organization's International Classification of Functioning, Disability and Health 2001 (44) and in consideration of the specific Japanese cultural lifestyle, which differs somewhat from Western countries. The JKOM consists of subject pain rating based on a 100-mm VAS and scores for a subscale of four symptoms based on a validated, disease-specific questionnaire addressing four dimensions: 'pain/stiffness', 'condition in daily life', 'general conditions' and 'health conditions', with 8, 10, 5 and 2 questions, respectively (43). Each question is rated on an ordinal scale of 0-4, with higher scores indicating a symptom or medical condition of higher severity. The four symptom subscales can be scored separately or combined to represent the aggregated total symptoms. It was previously demonstrated by a Japanese Orthopaedic Research Group that the JKOM is reliable and valid for studies of clinical outcomes for Japanese patients with knee OA (45).

Pain in the target knee was also measured using three pain subscales of the study diary-based visual analog scale for pain (diary VAS): 'pain at rest', 'pain on walking' and 'pain on ascending/descending stairs'. Each pain subscale was scored from 0 to 100, where 0 indicates no pain and 100 indicates the worst pain thus far experienced. Scores for these three pain subscales were recorded every day at home throughout the intervention period and were separately used as outcome measurements.

C2C and HA were utilized to assess the effect of the test supplement on cartilage metabolism and synovial inflammation, respectively. Serum levels of C2C and HA were measured using Collagen Type II Cleavage ELISA (IBEX Pharmaceuticals, Inc.) and LPIA-ACE HA (Mitsubishi Chemical Medience Corp.), respectively.

Data on all of the outcome measurements were collected at baseline and at weeks 4, 8, 12 and 16 during the 16-week intervention period (except for the biomarker measurements at week 4), and the within-group comparison of the mean values and the between-group comparison of the mean changes from the baseline were carried out.

Safety assessment. Safety was assessed throughout the study on the basis of the incidence and severity of intervention-related adverse events (side effects) as well as abnormal changes in blood pressure, pulse rate and laboratory tests, which included hematology, biochemical profile and urinalysis.

Statistical analysis. Values were expressed as mean ± standard deviation (SD) unless otherwise specified. Baseline data of the subjects were compared between the test and placebo groups using the unpaired Student's t-test for continuous variables and by the Mann-Whitney U test for category variables. Symptomatic scores and biomarker levels during the intervention were compared to the baseline values using the paired Student's t-test (for quantitative variables) and the Wilcoxon's signed rank test (for qualitative variables). Comparisons between the two groups were performed using the unpaired Student's t-test (for qualitative variables) and the Mann-Whitney U test (for qualitative variables). P-values <0.05 were considered statistically significant.

Results

Baseline characteristics. Data from 32 subjects who fulfilled the eligibility criteria and completed the study, were analyzed for clinical efficacy and safety. As shown in Table I, the demographic characteristics (age, male/female ratio) and physiological characteristics (height, body weight, body mass index, systolic/diastolic blood pressures, pulse rate) were not different between the test and placebo groups. Similarly, scores for the pain subscale and individual scores for all of the four symptom subscales, as well as scores for the aggregated total symptoms, of the JKOM were almost balanced between the two groups. In contrast, scores for the two diary VAS pain subscales, 'pain on walking' and 'pain on ascending/descending stairs', were significantly higher in the test group than in the placebo group (P<0.05). Moreover, another diary VAS pain subscale 'pain at rest' and the serum levels of both C2C and HA were slightly higher in the test group than in the placebo group, although the differences were not significant. Adherence to the allotted dietary supplement (test supplement or placebo) exceeded 85% in all of the 32 subjects (16 each in the two groups), and no subject discontinued the study.

Clinical efficacy. Table II shows the changes over time in scores for the pain subscale and individual scores for all of the four symptom subscales, together with scores for the aggregated total symptoms, of the JKOM during the 16-week intervention period. The pain subscale scores decreased time-dependently and reached a significant level at weeks 8, 12 and 16 compared with the baseline (P<0.05) in the test group, whereas no such significant changes were noted at any time points in the placebo group. In respect to the JKOM symptom subscales, individual scores for three of the four subscales, namely 'pain/stiffness', 'condition in daily life', and 'general activities', as well as scores for the aggregated total symptoms, were all significantly improved at weeks 8, 12 and 16 compared with baseline in the test group (P<0.01 each), although improvements in scores for the subscale 'health conditions' were lower in extent, reaching a significant level only at week 16 (P<0.05). Significant decreases in individual scores for the three subscales, 'pain/stiffness', 'condition in daily life', and 'general activities', and scores for the aggregated total symptoms were also noted in the placebo group (P<0.05 each). However, the magnitude of changes from baseline in scores for these symptom subscales and the aggregated total symptoms were all greater in the test group than in the placebo group. In particular at week 8, significant differences between the test and placebo groups were noted in scores for the 'general activities' (-2.7±2.3 vs. -0.9±1.9; P<0.05) and in scores for the aggregated total symptoms (-13.0±8.9 vs. -5.0±10; P<0.05).

Table III shows the changes in scores for the three diary VAS pain subscales, 'pain at rest', 'pain on walking' and 'pain on ascending/descending stairs', in the two groups during the 16-week intervention. Scores for all of the three subscales significantly decreased from baseline at almost all of the four assessment time points (weeks 4, 8, 12 and 16) in the test group (P<0.05 each), whereas none of the subscale scores showed a significant decrease at any time point in the placebo group. Moreover, the magnitude of score reductions from baseline appeared greater at all of the time points in the test group than

Table I. Baseline data of subjects in the test and placebo groups who completed the study[a].

Baseline variables	Test group (n=16)	Placebo group (n=16)
Age (years)	56.4±7.7	54.5±9.1
Male/female (no. of subjects)	2/14	2/14
Height (cm)	156.9±6.8	157.1±7.7
Weight (kg)	59.8±6.1	55.8±10.2
Body mass index (kg/m^2)	24.4±2.9	22.6±3.9
Systolic blood pressure (mmHg)	128.1±18.1	125.6±14.5
Diastolic blood pressure (mmHg)	80.4±9.8	76.9±8.9
Pulse rate (beats/min)	62.6±6.9	63.7±6.1
JKOM, VAS pain subscale scores (mm)	53.3±14.4	47.2±15.2
JKOM, symptom subscale scores		
Pain/stiffness	20.9±4.2	18.7±4.9
Condition in daily life	19.4±4.8	17.2±5.6
General activities	11.0±3.5	9.3±2.6
Health conditions	4.6±1.3	4.1±1.2
Aggregated total symptoms	53.3±14.4	47.2±15.2
Diary VAS pain subscale scores (mm)		
Pain at rest	31.1±27.2	16.3±21.2
Pain on walking	59.0±14.9[b]	43.3±20.7
Pain on ascending/descending stairs	66.2±13.3[b]	51.3±22.5
Kellgren-Laurence grades, 1-2 (%)	94	88
Serum C2C (ng/ml)	212.0±39.6	188.8±33.7
Serum HA (ng/ml)	46.5±42.1	26.6±12.3

[a]All values are expressed as the mean ± SD except for 'male/female' and 'Kellgren-Laurence grades'. [b]P<0.05 according to the unpaired Student's t-test (comparison between the two groups).

Table II. Changes in mean JKOM scores for VAS pain, the individual four symptom subscales and the aggregated total symptoms during the 16-week intervention period in the test and placebo groups (n=16 each).

		Scores at[a]				
Subscale	Group	Baseline	Week 4	Week 8	Week 12	Week 16
VAS pain	Test	53.3±14.4	44.9±16.8	32.2±19.1[c]	29.8±21.2[c]	22.6±22.9[b]
	Placebo	47.2±15.2	43.4±15.9	36.9±20.9	25.2±22.3	20.0±25.0
Pain/stiffness	Test	20.9±4.2	17.3±2.8[c]	15.8±3.5[c]	16.0±4.3[c]	13.4±4.0[c]
	Placebo	18.7±4.9	16.8±5.4	16.8±5.9	15.2±5.9[c]	14.3±5.6[c]
Condition in daily life	Test	19.4±4.8	16.1±3.1[c]	14.4±2.4[c]	14.8±5.6[c]	13.5±3.9[c]
	Placebo	17.2±5.6	15.6±5.6	15.1±5.6[b]	13.7±5.3[c]	13.3±4.9[c]
General activities	Test	11.0±3.5	8.3±2.1[c]	8.3±2.1[c]	8.8±3.7[c]	8.2±2.8[c]
	Placebo	9.3±2.6	8.1±2.1[b]	8.4±3.2	7.9±2.4[b]	7.8±2.8[b]
Health conditions	Test	4.6±1.3	4.2±0.8	4.4±1.1	4.1±1.2	3.7±1.2[b]
	Placebo	4.1±1.2	4.3±1.4	4.0±1.0	3.6±1.0	3.7±1.6
Aggregated total symptoms	Test	55.9±10.9	45.9±5.3[c]	42.9±5.8[c]	43.6±12.7[c]	38.8±13.2[c]
	Placebo	49.3±12.4	44.8±11.8	44.3±14.0[b]	40.4±13.0[c]	39.0±13.2[c]

[a]Values are expressed as the mean ± SD. [b]P<0.05, [c]P<0.01 against baseline (paired Student's t-test).

Table III. Changes in mean scores for the three diary VAS pain subscales during the 16-week intervention period in the test and placebo groups (n=16 each).

Subscale	Group	Scores at[a]				
		Baseline	Week 4	Week 8	Week 12	Week 16
Pain at rest	Test	31.1±27.1	14.9±15.6[a]	15.6±19.0	15.7±19.3[a]	12.3±22.1[a]
	Placebo	16.3±21.2	12.4±16.6	12.2±14.5	10.6±16.8	13.1±25.2
Pain on walking	Test	59.0±14.9	34.1±19.3[b]	27.4±19.6[b]	26.6±21.7[b]	17.7±19.8[b]
	Placebo	43.3±20.7	34.9±24.6	29.9±21.8	22.1±28.6	21.9±32.5
Pain on ascending/ descending stairs	Test	66.2±13.3	44.2±16.8[b]	30.7±20.8[b]	31.5±24.1[b]	24.7±25.1[b]
	Placebo	51.3±22.5	45.1±26.9	34.6±25.9	26.5±29.9	24.4±32.4

[a]Values are expressed as the mean ± SD. [b]P<0.05, [c]P<0.01 against baseline (paired Student's t-test).

Table IV. Changes in mean serum levels of a CII degradation biomarker C2C and a synovitis biomarker HA during the 16-week intervention period in the test and placebo groups (n=16 each).

Biomarker	Group	Mean values (ng/ml) at[a]			
		Baseline	Week 8	Week 12	Week 16
C2C	Test	212.0±39.6	196.8±21.0 (-7)	198.2±27.7 (-7)	190.3±35.8 (10)
	Placebo	188.8±33.7	193.3±25.2 (2)	200.4±29.3 (6)	198.1±31.0 (5)
HA	Test	46.5±42.1	36.4±24.8 (-12)	35.6±19.7 (-23)	35.1±26.2 (-25)
	Placebo	26.6±12.3	29.0±12.5 (9)	28.3±10.2 (6)	28.0±13.9 (5)

[a]Values are expressed as the mean ± SD and percent changes from baseline in parentheses.

in the placebo group; differences in scores for 'pain on walking' between the test and the placebo group at week 8 (-31.6±22.4 vs. -13.4±30.5) and those at week 16 (-41.3±20.5 vs. -21.4±30.5) achieved a statistical significance (P<0.05 each).

Effect on biomarkers. As shown in Table IV, the serum levels of C2C and HA were substantially reduced during the intervention period at weeks 8, 12 and 16 in the test group, although the extent of changes were not statistically significant. In the placebo group, in contrast, C2C levels rather increased and HA levels were virtually unchanged throughout the intervention periodin the placebo group. Notably, the differences in the magnitude of reduction from baseline between the test and placebo groups were significantly larger in the former group than in the latter for C2C levels at week 12 (-13.8±30.6 vs. 11.6±35.4 ng/ml) and those for HA at week 8 (-10.1±20.6 vs. 2.4±11.7 ng/ml), both achieving a statistical significance (P<0.05 each).

Safety. Eight subjects (50%) in the test group and 6 subjects (38%) in the placebo group reported at least one intervention-associated adverse event. None of subjects in the two groups discontinued the intervention for the reasons of adverse events. Adverse events most frequently reported from both the test and placebo subjects were respiratory symptoms (asthma, sore throat, cough, rhinorrhea, malaise and/or fever) and pain (hip, tooth or head). All adverse events were of mild intensity and were judged by the medical investigator as unrelated to the intervention. In both groups, routine laboratory tests and measurements of physiological parameters (body weight, body mass index, blood pressures and pulse rate) did not show any significant abnormalities throughout the intervention.

Discussion

In this pilot randomized, double-blinded, placebo-controlled trial, the tested glucosamine-based combination supplement containing chondroitin sulfate and three antioxidant micronutrients was primarily evaluated for its clinical efficacy in subjects with mild knee OA (K/L grades mainly 1-2). Efficacy of the test supplement for pain relief in knee OA subjects was basically supported by data indicating significant reduction from baseline in scores for the JKOM pain subscale and scales for the diary VAS pain subscales at almost all of the four assessment time points (weeks 4, 8, 12 and 16) in the test group compared to the placebo group. Moreover, individual scores for the four JKOM symptom subscales, as well as scores for the aggregated total symptoms, which are primarily related to knee OA-associated general symptoms, physical functions and health-related quality of life, were also significantly improved from baseline in the test group. However, when comparing the magnitude of changes in scores from baseline between the two groups, significant improvements were observed only

in scores for the 'general activities' subscale and the aggregated total symptoms of the JKOM at week 8 and scores for the 'pain on walking' subscale of the diary VAS at weeks 8 and 16. The discrepancy in the significance in the within- and between-group differences may be partly explained by previous findings that a strong placebo effect is observed in many OA clinical studies conducted for relatively short intervention periods (46,47). Thus, although there were substantial limitations to clearly assess the therapeutic efficacy of the test supplement in the present study, the results of the JKOM- and dairy VAS-based efficacy assessments strongly suggest that the test supplement is potentially effective in improving pain and other symptoms associated with knee OA.

In connection with the possible symptomatic efficacy, glucosamine and chondroitin sulfate have been reported to reduce the osteoarthritic joint damage and to slow the progression of OA (13,14,48). Recently, various biomarkers have been developed to detect the articular alterations in OA (49,50), and there are accumulating data supporting the association of the changes in various CII degradation biomarkers, such as CTX-II, C1, 2C and C2C (51-53), and a synovial inflammation biomarker HA (54,55) with the disease progression in knee OA. In the present study, we utilized C2C and HA for evaluating the effects of the test supplement on the pathophysiological changes in the cartilage and synovium, respectively, as described previously (56). The results demonstrated that the serum levels of both C2C and HA time-dependently decreased only in the test group. This observation suggests that the test supplement may suppress the CII degradation and synovial inflammation in osteoarthritic joints, thereby possibly reducing cartilage loss and inflammatory responses. Along with the CII degradation in the articular cartilage by collagenases, persistent synovitis, as reflected by the increased serum HA levels, has been shown to predict the disease process of OA (54,57). These findings lead us to the speculation that substantial antioxidant activities of the micronutrients which were contained in the test supplement may be effective in not only preventing the activation of collagenases by ROS (58) but also protecting HA molecules in the synovial fluid from degradation by ROS (59). This could partly explain the reduction in the serum levels of C2C and HA noted in the OA subjects treated with the test supplement. It appears, therefore, likely that such pathophysiologically favorable effects of the test supplement on the osteoarthritic joint tissues may be involved in its therapeutic efficacy in subjects with knee OA.

The present pilot clinical study has some limitations. First, the number of subjects enrolled in both the test and placebo groups was small, which may have decreased the power to detect significant difference between the two groups. Second, as we did not use antioxidant micronutrient-free control dietary supplements, we were unable to learn the role of specific antioxidant components of the test supplement in its therapeutic efficacy. Extended larger studies are necessary to assess the potential of this combination supplement for improving clinical symptoms, as well as for preventing cartilage loss and/or suppressing synovial inflammation, in subjects with knee OA.

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