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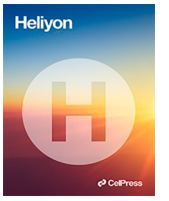

A standardized nutraceutical supplement contributes to pain relief, improves quality of life and regulates inflammation in knee osteoarthritis patients; A randomized clinical trial

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ABSTRACT

Osteoarthritis (OA) is a degenerative disease of the joints that affects greatly the elderly population and the health care systems and is on the increase due to aging and obesity. Interventions aim at palliative care and pharmaceutical therapies entail serious adverse events. Whereas polyphenols constitute a promising holistic approach in the arsenal of physicians, trials investigating biomarkers and questionnaires are scarce. As such, a randomized controlled trial (RCT) was conducted to evaluate the potency of a standardized polyphenolic supplement in the management of systemic inflammation, oxidative stress, pain and general quality of life (QoL) in patients with osteoarthritis. Sixty subjects were randomized to receive either a polyphenol supplement (curcuma phospholipid, rosemary extract, resveratrol, ascorbic acid), or an active comparator (ascorbic acid) twice, daily for 12 weeks. The group that received the polyphenols exhibited significantly lower symptoms of pain and improved physical function and QoL as it was depicted by validated questionnaires, compared to the control group. Furthermore, post intervention, inflammation was restrained in the polyphenol group. Since systemic inflammation promotes local inflammation, the decrease of pain herein might be attributed to the attenuation of systemic inflammation by the polyphenols.

1. Introduction

Osteoarthritis (OA) is a degenerative disease of the articular cartilage of the joints and the most common form of arthritis. The suffix -itis suggests inflammation, ergo inflammation of the joint, however only recently the inflammatory component of this pathophysiology has come to prominence [1]. This debilitating phenotype decreases significantly the quality of life (QoL) of the affected

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Abbreviations

OA	osteoarthritis
QoL	quality of life
YLD	years lived with disability
KOA	knee osteoarthritis
IL-6	interleukin-6
TNF-α	tumor necrosis factor alpha
IL-1β	interleukin 1β
OS	oxidative stress
oxLDL	oxidized low-density lipoprotein
NSAIDs	non-steroidal anti-inflammatory drugs
RCT	randomized controlled trial
CoA	certificate of analysis
RDA	recommended daily allowance
VAS	visual analoque scale
WOMAC	Western Ontario and McMaster Universities Osteoarthritis
PROMs	patient-reported outcome measures
PhAA	polyphenols and ascorbic acid
AA	ascorbic acid
K&L	Kellgren & Lawrence
BMI	body mass index
WHR	waist-hip ratio
SF-36	short-form-36
IPAQ	international physical activity score
METs	metabolic equivalent of task
VitD	vitamin D
HDL	high-density lipoprotein cholesterol
TG	triglycerides
CRP	C - reactive protein
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
MPO	myeloperoxidase
SD	standard deviation
ITT	intention to treat
PA	physical activity
SF	synovial fluid

population through pain, decreased functionality and reduced mental well-being. According to the Institute for Health Metrics and Evaluation (IHME), in 2019 OA was responsible for 2.2% (95% UI 1.3–4.1) of the total global years lived with disability (YLD) and specifically knee OA (KOA) contributed 60.9% to the YLDs [2].

OA may be triggered by a plethora of cues that span from physiological processes such as aging and injuries, to pathological such as low-grade systemic inflammation due to concomitant diseases [3]. Mechanistically, it takes place a disruption in the balance between anabolic and catabolic reactions in the cartilage that enhances unresolved inflammatory signals that cause neuroinflammation and chronic pain [4]. In OA, all nearby tissues participate to this vicious circle producing inflammatory and oxidative stress (OS) mediators [5]. Local and systemic inflammation are co-aggravated and the produced mediators affect the inflammatory milieu in the joint inducing pathological changes that arise during OA.

Once established, OA has no cure and therapies mostly offer short term symptomatic relief. Patients in co-operation with their doctors have to determine the optimal therapeutic regimen that may include physical, pharmacological, pharmaceutical or surgical approaches, taking into account possible medical issues, comorbidities and side effects. Some common treatments include topical and oral non-steroidal anti-inflammatory drugs (NSAIDs), intra articular steroids and hyaluronic acid [6,7], orthobiologic therapies as are the injections of platelet rich plasma and mesenchymal stem cells [8] and surgical operations that include arthroscopy and total knee arthroplasty.

In view of a) the global challenge of pandemics, b) the demographic ageing [9], c) the increase in the obesity epidemic [10], and d) the shift of health and social care services towards providing the means for healthy aging [11], novel and holistic therapies devoid of side effects should be investigated and incorporated into clinical practice. A thoroughly studied nutraceutical approach in OA is phenols, plant secondary metabolites with established benefits in health, through their antioxidant and anti-inflammatory potency. Extensive research has examined the effects of individual polyphenols (i.e. resveratrol, curcumin, quercetin) *in vitro*, *in vivo*, as well as in some clinical trials, on OA settings [12,13]. Of interest is the possible synergistic action of polyphenols in restraining OS and

inflammation and eventually alleviating pain, stiffness and edema in OA patients [14].

Up to date only a few clinical trials exist in regard to the evaluation of combined polyphenols in OA [15–20]. Recently, we conducted a pilot randomized controlled trial (RCT) where the effect of a standardized phenolic supplement compared to ascorbic acid (Vitamin C) on pain and function in 25 adults with symptomatic KOA after 8 weeks of administration was evaluated [21]. The visual analogue scale (VAS) for pain and the Western Ontario and McMaster Universities Osteoarthritis (WOMAC) scores decreased significantly in the group that received the phytochemical supplement compared to the group that received the ascorbic acid thus supporting the hypothesis of pain relief mediated by polyphenols. The above preliminary results reinforced us to conduct the present larger RCT to explore the efficacy of the supplement relative to ascorbic acid, as a comparator, not only on subjective patient-reported outcome measures (PROMs) that assess cardinal symptoms, functionality and QoL, but also on biomarkers of inflammation and OS. Since both local and systemic inflammation play a role in OA development, we hypothesized that the inhibition of systemic inflammation by standardized combined polyphenols, and subsequently the attenuation of local inflammation of the joint, will contribute in pain management and improvement of the QoL.

2. Materials and methods

2.1. Ethics

The protocol of the RCT was approved by Harokopio University Ethics Committee (13/21-2-2020) and Evgenidio Hospital Scientific Board (29/19-02-2019). The study followed all the principles of the Declaration of Helsinki 2013 and the Data Protection Act 2018. Patients were briefed about the protocol and voluntarily gave their informed consent to participate with the knowledge that they could withdraw consent without reprisals. The RCT was registered in the ClinicalTrials.gov database with the identifier NCT04783792.

2.2. Supplements

As previously described [21] the supplement (patent number: 20210100519/30-07-2021) contained a combination of plant-derived polyphenols and ascorbic acid, hereafter referred as PhAA with the standardized ingredients; curcuma phospholipid (148.4 mg, 52.64 %w/w), rosemary extract 40% (51.9 mg, 18.41 %w/w), resveratrol from Polygonum Cuspidatum 98% (51.9 mg, 18.41 %w/w), ascorbic acid (29.7 mg, 10.54 %w/w), VIVAPHARM® HPMC E50 - Hypromellose, and magnesium stearate. The active ingredients were purchased in the form of food grade titrated extracts from certified suppliers. Certificates of Analyses (CoA) were provided by the suppliers. Final CoA was performed by the production facility of the final capsule. Regarding the PhAA supplement its novelty lies on its unique composition of specific polyphenols with established mechanisms of action in OA and in the phytosome technology used to encapsulate curcumin in order to enhance its bioavailability, an otherwise major drawback in the use of phytochemicals in clinical practice [22,23].

Taking into account that OA is a disease with considerable psychosomatic pain it was deemed as more ethical by the physicians of the study to use an active comparator, in the present, ascorbic acid (AA, 29.7 mg each capsule). Vitamin C is an essential nutrient with a plethora of actions that include not only antioxidant capacities but also immunological and anti-inflammatory effects [24]. According to the European Food Safety Authority (EFSA) 'Vitamin C contributes to generation of collagen - An insoluble protein fiber that is the primary constituent in connective tissue (skin and tendons) and bone' [25]. To bear such a claim a supplement should have a content of more than 15% of the recommended daily allowance (RDA). The daily dose of AA studied herein covers for the 74.25% of the RDA. In OA, a depletion of antioxidant mechanisms has been observed [26] and patients with OA have increased nutritional needs for antioxidants such as AA.

2.3. Trial design & participation criteria

The design of the study was a two-arm, parallel-assignment, double-blinded, randomized controlled trial. Patients were recruited in the orthopedic outpatient clinic of Evgenidio Hospital in Athens, Greece and in the establishments of Harokopio University of Athens from 2021 to 2022. Their symptomatology and clinical manifestation of KOA was evaluated by the orthopedic doctor of the study and upon confirmation that they met the following inclusion and exclusion criteria they signed the informed consent.

In the study participated males and females above the age of 35 with monolateral or bilateral KOA as assessed by radiographic findings (Kellgren & Lawrence (K&L) score $\geq$2) [27] and by the American College of Rheumatology (ACR) clinical classification criteria for OA of the knee using history, physical examination and radiographic findings (pain in the knee and one of the following: over 50 years of age, less than 30 min of morning stiffness, crepitus on active motion and ostophytes) [28]. To ensure the presence of both structural and symptomatic OA, participants had to experience at least moderate symptoms of pain in the index knee depicted as irregular (pain during the last 7 days) or continuous pain $\geq$4 in the WOMAC pain subscale and in VAS. In bilateral KOA the index knee was concluded by the highest pain score. Participants had to be able to walk without support devices.

In the study could not participate individuals with KOA who were having physical therapy or transcutaneous electrical nerve stimulation prior the trial, who had another musculoskeletal disorder (i.e. rheumatoid arthritis), those with a scheduled surgery during the trial, whoever used corticosteroids within 2 months prior the trial or whoever changed his/her diet and food supplements 1 month prior the trial, those who used supplements that contained AA and polyphenols and finally, those judged by the researchers of the study as unable to perceive and comply with the protocol.

2.4. Blinding, randomization & compliance

Randomization was executed by an independent statistician, blind to the assigned protocols, with the use of an algorithm that allocated patients according to their gender and age to receive one of the two interventions. Investigators and participants were also blinded to the allocation as the containers and the capsules were identical. The containers for the two arms were coded as A and B and were distributed to the volunteers according to the allocation. The code breaking procedure took place after the statistical analysis. Volunteers of the two interventions were instructed to receive two capsules per day, approximately 15–30 min before breakfast and lunch and recorded their everyday compliance through diary entries. Deviations from the baseline in lifestyle, medication and possible occurrence of adverse events were monitored via telephone calls. Volunteers were allowed to use paracetamol and/or NSAIDs if they experienced symptoms of pain.

2.5. Baseline, follow-up assessments & outcome measures

Upon completion of the screening process and randomization, a set of questionnaires, anthropometric measurements and blood were collected. Specifically, a thorough medical history along with information regarding the progress of OA i.e. age of diagnosis, relevant surgeries and treatments, demographics, and smoking were recorded. The baseline anthropometric measurements included height (cm) and body weight (kg) to calculate body mass index (BMI) as weight (kg)/height $(m)^2$ and waist circumference (cm) and hip circumference (cm) to calculate the waist-hip ratio (WHR). Blood pressure was evaluated with a digital monitor and was expressed as systolic pressure (mmHg), diastolic pressure (mmHg) and pulse.

At baseline and follow-up the following validated questionnaires [29] were filled:

VAS for pain: is a 10 cm line that spans from 0 (for no pain) to 10 (worst pain). Patients were asked to point in the scale the worst symptom of pain they experienced on the index knee during the last 7 days.

WOMAC: is a set of 24 items that are divided into 3 subscales; the subscale of pain which has 5 items: during walking, using stairs, in bed, sitting or lying, and standing upright, the subscale of stiffness with 2 items: after first waking and later in the day and the subscale of physical function with 17 items: using stairs, rising from sitting, standing, bending, walking, getting in/out of a car, shopping, putting on/taking off socks, rising from bed, lying in bed, getting in/out of bath, sitting, getting on/off toilet, heavy domestic duties, light domestic duties The 24 items are scored on a scale of 0–4, which correspond to: None (0), Mild (1), Moderate (2), Severe (3), and Extreme (4). Higher scores indicate worse pain, stiffness, and functional limitations [30].

36-Item Short Form Survey (SF-36): in this trial the freely available online version (RAND) was used. SF-36 is a health-related QoL questionnaire with 36 questions that cover 8 domains of health: Physical functioning (10 items), Role limitations due to physical health (4 items), Role limitations due to emotional problems (4 items), Energy/fatigue (4 items), Emotional well-being (5 items), Social functioning (2 items), Bodily pain (2 items) and General health (5 items). Scores were computed for each domain through an online tool [31]. Higher scores indicate a more favorable health state.

International physical activity short form (IPAQ-SF): evaluates physical activity (PA). It inquiries about walking, moderate intensity activities and vigorous intensity activities during the last week. A total score of (MET)-min/week is computed by weighing the three scores of the activities by their energy requirements defined in METs (metabolic equivalent of task) and multiplying it by the minutes performed per week [32].

Blood draws (20 mL) were also completed at baseline and follow-up after overnight fasting. For the biochemical and laboratory analysis blood was collected in vacutainers and was centrifuged for 10 min at 3000 rpm. Serum was stored in Eppendorf tubes at −80 °C. Biochemical analysis measured in serum with an automatic biochemical analyzer (Cobas 8000 analyzer, Roche Diagnostics GmbH, Mannheim, Germany) included vitamin D (VitD), high-density lipoprotein cholesterol (HDL), triglycerides (TG), glucose, urea, C-reactive protein (CRP), albumin and the hepatic enzymes serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT). Inflammation and OS markers that were quantified in duplicate in serum through sandwich enzyme-linked immunosorbent assay (ELISA) included: IL-6 (R&D Systems, Inc. Minneapolis, MN, USA), TNF-α (Thermo Fisher Scientific Inc., Waltham, MA, USA), MPO (Thermo Fisher Scientific Inc., Waltham, MA, USA) and oxidized low-density lipoprotein (oxLDL) (Mercodia, AB, Uppsala, Sweden).

Primary outcome measures of the RCT were the change in WOMAC pain subscale and VAS score for pain. Secondary outcome measures were the change in WOMAC stiffness and functionality subscales, the improvement of health as depicted in any of the domains of SF-36, the change in the levels of circulatory inflammatory and OS biomarkers.

2.6. Sample size & statistical analysis

We conducted a repeated-measures analysis of variance (ANOVA) power analysis with two levels of the between subject factor of two study groups and the within-subjects factor of time. For our design, 52 participants (26 per group) achieved a power of 0.95 for the within-subjects main effect at an effect size of 0.26, a power of 0.85 for the between-subjects main effect at an effect size of 0.38 and a power of 0.95 for the interaction effect at an effect size of 0.26. Considering possible dropouts eventually a total of 60 patients were recruited.

Continuous variables are presented with mean and standard deviation (SD). Qualitative variables are presented with absolute and relative frequencies. Concerning baseline characteristics, for the comparison of proportions chi-square and Fisher's exact tests were used, while for the comparison of means between the two groups the Student's t-test was computed. All analysis were conducted on an intention-to-treat (ITT) basis. To reduce the bias implicit in utilizing only complete cases, regression model imputation procedures in

case of missing data were implemented. Differences in changes of study variables during the follow up period between the two groups were evaluated using general linear models. Statistical significance at follow up was first assessed by looking at the interaction effect of time with study group. Log-transformations were implied in case of not normal distributed data. The study had only one repeat assessment, so no correction for multiple comparisons were made. All p values reported are two-tailed. Statistical significance was set at 0.05 and analysis were conducted using SPSS statistical software (version 26.0).

3. Results

As it is presented in the Flow diagram of the study (Fig. 1), 98 patients were assessed for eligibility. After exclusion of 38 subjects due to 34 subjects declining to participate and 4 not meeting inclusion criteria (one subject had intra-articular injections of corticosteroids recently, one had rheumatoid arthritis and two were judged as unable to comply with the protocol), 60 patients were randomized to receive either the polyphenol supplement (PhAA) (n = 31, 51.7%) or the comparator capsule with ascorbic acid (AA) (n = 29, 48.3%). The two supplements were well-tolerated and the only adverse events that were reported were 3 cases of mild rashes (n = 2 in the AA group and n = 1 in the PhAA group). Rescue medication (paracetamol and NSAIDs) was used by 36.6% of the sample. Data were analyzed in an intention to treat basis (ITT).

Demographics, anthropometrics and symptomatology by group are presented in Table 1. No significant differences were found between the two groups at baseline indicating a successful randomization.

Changes in biochemical indices in the two groups, after the intervention are pre-sented in Table 2. No significant differences were found between the two treatment groups at baseline and at follow-up. After the intervention, urea decreased signifi-cantly in both groups (p2 = 0.027 for AA and p2<0.001 for PhAA) and albumin de-creased significantly only in the PhAA group (p2 = 0.014) and the

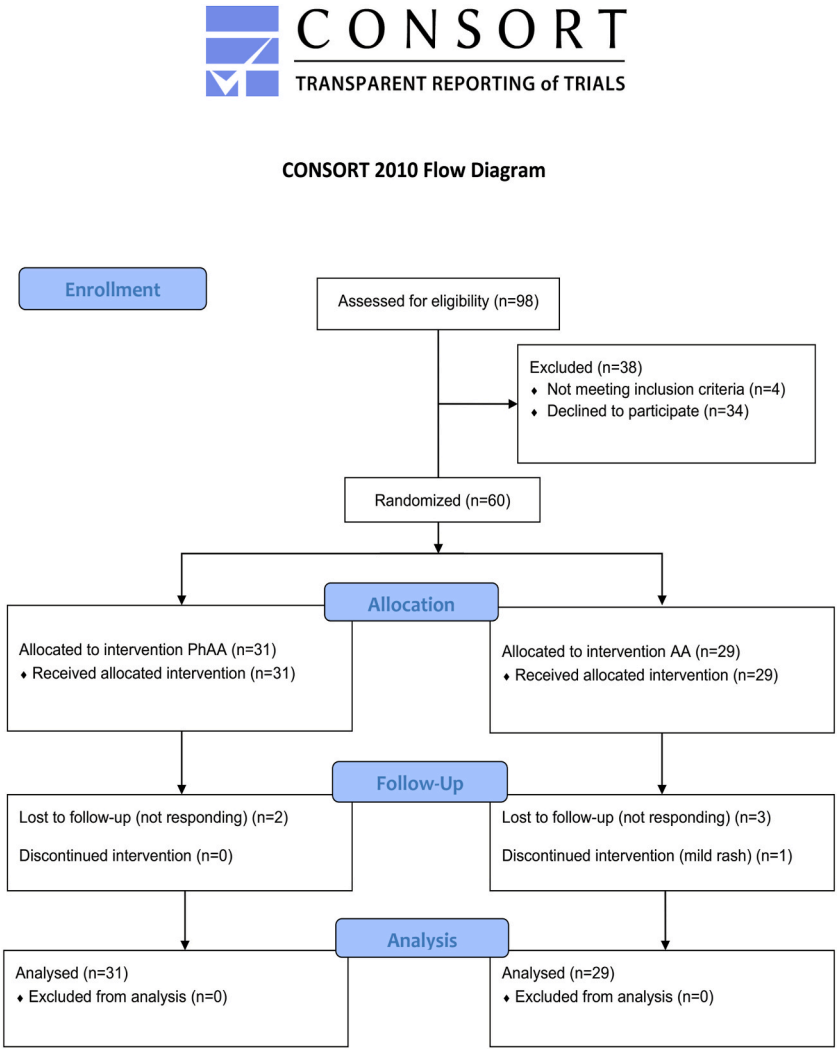

Fig. 1. Flow diagram of the study.

Table 1
Descriptive characteristics of the sample population by treatment group.

		Group		p
		AA (n = 29)	PhAA (n = 31)	
		n (%)	n (%)	
Gender	Male	9 (31)	10 (32.3)	0.919+
	Female	20 (69)	21 (67.7)	
Age (years), **mean** (SD)		62.7 (11.5)	62.5 (11.4)	0.953‡
Age of diagnosis, mean (SD)		53.5 (14.6)	52.7 (13.4)	0.831‡
Marital status	Married/divorced	24 (82.8)	26 (83.9)	>0.999++
	Unmarried	5 (17.2)	5 (16.1)	
	1–9 years	10 (34.5)	9 (29)	0.367+
Education	10–12 years	7 (24.1)	4 (12.9)	
	>12 years	12 (41.4)	18 (58.1)	
Smoking	No	21 (72.4)	26 (83.9)	0.282+
	Yes	8 (27.6)	5 (16.1)	
BMI (kg/m^2)		31.1 (5.4)	30.6 (5.6)	0.723‡
BMI, n (%)	Normal	5 (17.9)	4 (13.3)	0.817++
	Overweight	7 (25)	10 (33.3)	
	Obese	16 (57.1)	16 (53.3)	
Waist circumference (cm)		104.8 (13.4)	104.4 (14.3)	0.926‡
Hip circumference (cm)		113.6 (10.6)	112.4 (12)	0.712‡
WHR		0.9 (0.1)	0.9 (0.1)	0.847‡
Systolic blood pressure (mm Hg)		127.1 (21.1)	125.4 (16.1)	0.757‡
Diastolic blood pressure (mm Hg)		75.1 (13.9)	75.3 (11.3)	0.940‡
Pulse		71.6 (18.1)	70.1 (18.7)	0.786‡
Affected knee/s	Right	6 (20.7)	10 (32.3)	0.591+
	Left	5 (17.2)	5 (16.1)	
	Both	18 (62.1)	16 (51.6)	
Target knee	Right knee	15 (51.7)	17 (54.8)	0.809+
	Left knee	14 (48.3)	14 (45.2)	
K&L	2	3 (10.3)	7 (22.6)	0.436+
	3	17 (58.6)	15 (48.4)	
	4	9 (31)	9 (29)	
Morning stiffness on target knee	No	13 (44.8)	15 (48.4)	0.782+
	Yes	16 (55.2)	16 (51.6)	
Crepitus on target knee	No	16 (51.6)	9 (29)	0.316+
	Yes	17 (58.6)	22 (71)	

+Pearson's chi-square test, ++Fisher's exact test, ‡ Student's t-test, AA**:** ascorbic acid, PhAA: polyphenols + AA, BMI: body mass index, WHR: waist-hip ratio, K&L: Kellgren & Lawrence.

mean changes be-tween the two groups differed significantly.

Changes in VAS and in WOMAC scores, in the two groups after the intervention are presented in Table 3. No significant differences were found between the two treat-ment groups at baseline. At follow-up, the WOMAC physical function subscale was lower in the PhAA compared to the AA group (p1 = 0.026). Regarding the effect of the intervention, VAS, WOMAC pain, physical function and total score decreased signifi-cantly after the intervention only in the PhAA group (p2<0.001 for all) with the mean changes being signif-icantly different between the two groups (p3 = 0.011, p3 = 0.013, p3 = 0.047 and p3 = 0.048 respectively).

Changes in 36-Item Short Form Survey (SF-36) components and international physical activity short form (IPAQ-SF) by group are presented in Table 4. At baseline no significant differences were found between the two groups. At follow-up the PhAA group exhibited significantly higher values in the components of Emotional well-being (p1 = 0.039), Physical functioning (p1 = 0.038), Bodily pain (p1 = 0.028) and in the latter two the mean changes between the two groups for the components of Physical func-tioning and Bodily pain (p3 = 0.023 and p3 = 0.013 respectively) differed significantly. After the intervention, Physical functioning (p2<0.001), Bodily pain (p2<0.001) and Role limitations due to physical health (p2 = 0.017) increased significantly only in the PhAA group.

Changes in inflammatory and OS biomarkers are presented in Table 5. At baseline no significant differences were found between the two groups for all the biomarkers. At follow-up, MPO was lower at the PhAA group (p1 = 0.041). Also, MPO decreased only in the PhAA group (p2 = 0.011) and the mean changes between the two groups dif-fered significantly (p = 0.010). IL6 and TNF-α increased significantly after the interven-tion only in the AA group (p2 = 0.026 and p2 = 0.042 respectively), with the mean chang-es being significantly different between the two groups in the case of TNF-α (p3 = 0.034). After the intervention, CRP decreased significantly only in the PhAA group (p2 = 0.028) and the mean changes between the two groups differed significantly (p3 = 0.016).

4. Discussion

OA represents a serious problem with economic and social ramifications. Herein, a panel of serum biomarkers was explored with the corroboration of findings from validated questionnaires that capture the symptomatology and QoL in order to evaluate the response to a polyphenol intervention. Whereas biomarkers in synovial fluid (SF) are increased and theoretically reflect better the state

Table 2
Changes in biochemical indices by treatment group.

		Baseline	Follow-up	Change		
	Group	**Mean** (SD)	**Mean** (SD)	**Mean** (SD)	**p2**	**p3**
VitD (ng/ml)	AA	25.33 (11.85)	22.84 (12.52)	−2.49 (11.71)	0.199	0.714
	PhAA	26.57 (12.26)	25.06 (9.11)	−1.51 (8.86)	0.419	
	p1	0.692	0.432			
HDL (mg/dL)	AA	56.58 (11.9)	53.83 (9.76)	−2.75 (10.91)	0.141	0.254
	PhAA	51.47 (11.61)	51.67 (10.73)	0.2 (8.91)	0.910	
	p1	0.097	0.418			
TG (mg/dL)	AA	138.48 (83.42)	125.56 (50.13)	−12.93 (56.76)	0.267	0.723
	PhAA	154.71 (96.72)	147.48 (60.75)	−7.23 (66.63)	0.519	
	p1	0.491	0.134			
Glucose (mg/dL)	AA	100.71 (25.61)	101.69 (19.73)	0.99 (19.2)	0.795	0.226
	PhAA	101.09 (24.82)	95.64 (22.13)	−5.45 (21.39)	0.142	
	p1	0.953	0.269			
Urea (mg/dL)	AA	32.94 (8.78)	29.81 (8.82)	−3.13 (5.51)	**0.027**	0.261
	PhAA	37.38 (11.91)	32.06 (7.7)	−5.31 (8.86)	**<0.001**	
	p1	0.108	0.294			
Albumin (g/dL)	AA	4.4 (0.31)	4.36 (0.29)	−0.05 (0.2)	0.264	**0.012**
	PhAA	4.38 (0.24)	4.48 (0.27)	0.1 (0.23)	**0.014**	
	p1	0.716	0.101			
SGOT (U/L)	AA	18.99 (7.89)	19.1 (7.32)	0.11 (6)	0.936	0.201
	PhAA	18.18 (5.34)	20.81 (7.01)	2.63 (8.73)	0.057	
	p1	0.640	0.361			
SGPT (U/L)	AA	18.15 (10.03)	15.59 (7.76)	−2.56 (10.96)	0.192	0.332
	PhAA	19.89 (11.15)	19.97 (10.66)	0.07 (9.94)	0.968	
	p1	0.528	0.076			
Creatinine (mg/dL)	AA	0.76 (0.18)	0.72 (0.15)	−0.04 (0.1)	0.052	0.402
	PhAA	0.79 (0.2)	0.78 (0.17)	−0.02 (0.11)	0.365	
	p1	0.459	0.157			

p1: p-value for group effect, p2: p-value for time effect, p3: p-value for interaction effect (repeated measures ANOVA), AA: ascorbic acid, PhAA: polyphenols + AA, HDL: high-density lipoprotein cholesterol, TG: triglycerides, SGOT: serum glutamic oxaloacetic transaminase, SGPT: serum glutamic pyruvic transaminase.

Table 3
Changes in VAS and in WOMAC scores by treatment group.

		Baseline	Follow-up	Change		
	Group	**Mean** (SD)	**Mean** (SD)	**Mean** (SD)	**p2**	**p3**
VAS	AA	5.81 (1.96)	5.72 (2.67)	−0.09 (2.99)	0.867	**0.011**
	PhAA	7.08 (1.79)	5.03 (3.04)	−2.05 (2.78)	**<0.001**	
	p1	0.111	0.354			
WOMAC pain	AA	7.07 (3.54)	6 (3.24)	−1.07 (3.42)	0.087	**0.013**
	PhAA	8 (3.56)	4.73 (4.15)	−3.27 (3.19)	**<0.001**	
	p1	0.319	0.198			
WOMAC physical function	AA	24.03 (15.21)	20.94 (12.12)	−3.1 (13.23)	0.157	**0.047**
	PhAA	22.82 (13.92)	13.61 (12.7)	−9.21 (9.93)	**<0.001**	
	p1	0.748	**0.026**			
WOMAC stiffness	AA	2.24 (1.88)	1.97 (2.04)	−0.28 (1.94)	0.409	0.512
	PhAA	2.29 (1.95)	1.71 (1.55)	−0.58 (1.63)	0.076	
	p1	0.922	0.586			
WOMAC total score (%)	AA	34.61 (19.71)	29.1 (16.17)	−5.5 (17.2)	0.069	**0.048**
	PhAA	34.92 (17.68)	21.09 (17.12)	−13.83 (14.74)	**<0.001**	
	p1	0.948	0.068			

p1: p-value for group effect, p2: p-value for time effect, p3: p-value for interaction effect (repeated measures ANOVA), AA: ascorbic acid, PhAA: polyphenols + AA, VAS: visual analogue scale, WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index.

of the disease, the intrusive nature of the arthrocentesis procedure, the heterogeneity of the proposed markers and their controversial results render it a difficult candidate for the evaluation of the efficacy of an intervention [33]. As such, we hypothesized that attenuation of systemic inflammation induced by polyphenols can be reflected in the reduction of pain and we evaluated this hypothesis through a combination of subjective and objective tools. The two supplements were well tolerated as markers of general health, serum urea and hepatic enzymes remained within normal range in both arms, indicative of the negligible toxicity induced by the interventions.

Our primary outcome measures, VAS and WOMAC pain, decreased significantly only in the group that received the polyphenols, with the mean changes between the two interventions being significantly different as well. A similar pattern was observed in all

Table 4
Changes in SF-36 components and in IPAQ-SF by treatment group.

		Baseline	Follow-up	Change		
	Group	**Mean** (SD)	**Mean** (SD)	**Mean** (SD)	**p2**	**p3**
SF-36_Physical functioning	AA	49 (22.3)	52.9 (20.5)	4 (19.3)	0.219	**0.023**
	PhAA	50.6 (23.3)	65 (23.4)	14.4 (14.9)	**<0.001**	
	p1	0.777	**0.038**			
SF-36_Bodily Pain	AA	49.7 (26.4)	54.6 (21.1)	4.9 (20.8)	0.292	**0.013**
	PhAA	46.9 (27.2)	68.3 (25.4)	21.4 (28.1)	**<0.001**	
	p1	0.685	**0.028**			
SF-36_Role limitations due to emotional problems	AA	60.2 (45.5)	68.8 (36.1)	8.6 (45.5)	0.419	0.795
	PhAA	67.6 (38.3)	78.4 (45.2)	10.9 (54.9)	0.667	
	p1	0.238	0.394			
SF-36_Energy/fatigue	AA	52.9 (21.2)	61.4 (20.3)	8.5 (31.2)	0.145	0.612
	PhAA	55.2 (25.8)	64.5 (19.6)	9.3 (21.8)	0.464	
	p1	0.274	0.576			
SF-36_Emotional well-being	AA	59 (25.2)	58.5 (19.8)	−0.5 (20.8)	0.936	0.373
	PhAA	60.6 (19.6)	69.2 (16.7)	8.5 (14.3)	0.187	
	p1	0.317	**0.039**			
SF-36_Role limitations due to physical health	AA	52.6 (40.8)	61.2 (35.7)	8.6 (39.7)	0.251	0.382
	PhAA	52.4 (42)	70.2 (31.9)	17.7 (40.4)	**0.017**	
	p1	0.988	0.309			
SF-36_Social functioning	AA	65.1 (31.9)	62.9 (28)	−2.2 (36.1)	0.728	0.114
	PhAA	60.9 (30)	72.8 (22.7)	11.9 (32)	0.057	
	p1	0.604	0.136			
SF-36_General health	AA	62.6 (17.7)	61.3 (16.3)	−1.2 (14.6)	0.625	0.174
	PhAA	65.2 (16.7)	68.7 (21.2)	3.5 (12)	0.146	
	p1	0.551	0.137			
IPAQ-SF (MET-min/week)	AA	1491.17 (1726.48)	1861.7 (1757.26)	370.53 (1785.61)	0.680	0.327
	PhAA	1837.15 (2084.2)	1300.9 (1712.78)	−536.25 (1834.31)	0.325	
	p1	0.932	0.243			

p1: p-value for group effect, p2: p-value for time effect, p3: p-value for interaction effect (repeated measures ANOVA), note: for IPAQ-SF analyses were based on logarithmic transformations, AA: ascorbic acid, PhAA: polyphenols + AA, IPAQ- SF: international physical activity questionnaire short form, SF-36: 36-item short form survey.

Table 5
Changes in biomarkers of inflammation and OS by treatment group.

		Baseline	Follow-up	Change		
	Group	**Mean** (SD)	**Mean** (SD)	**Mean** (SD)	**p2**	**p3**
IL-6 (pg/ml)	AA	2.7 (1.66)	3.47 (1.98)	0.77 (1.31)	**0.026**	0.065
	PhAA	3.05 (1.93)	2.91 (1.57)	−0.14 (1.09)	0.737	
	p1	0.456	0.495			
TNF-α (pg/ml)	AA	0.95 (0.42)	1.21 (0.58)	0.27 (0.62)	**0.042**	**0.034**
	PhAA	1.32 (1.37)	1.05 (0.42)	−0.27 (1.41)	0.334	
	p1	0.104	0.327			
CRP (mg/L)	AA	2.95 (2.31)	4.02 (4.57)	1.07 (4.61)	0.808	**0.016**
	PhAA	3.69 (4.75)	2.64 (3.59)	−1.05 (2.87)	**0.028**	
	p1	0.922	0.124			
oxLDL (U/L)	AA	68.47 (23.25)	65.84 (21.69)	−2.63 (17.32)	0.454	0.618
	PhAA	71.31 (27.57)	65.12 (20.93)	−6.2 (16.78)	0.139	
	p1	0.809	0.893			
MPO (ng/ml)	AA	95.17 (78.4)	103.64 (77.79)	8.47 (77.04)	0.245	**0.010**
	PhAA	109.53 (121.81)	76.06 (77.88)	−33.46 (83.28)	**0.011**	
	p1	0.799	**0.041**			

p1: p-value for group effect, p2: p-value for time effect, p3: p-value for interaction effect (repeated measures ANOVA), note: for IPAQ-SF analyses were based on logarithmic transformations, OS: oxidative stress, AA**:** ascorbic acid, PhAA: polyphenols + AA, IL-6: interleukin-6, TNF-α: tumor necrosis factor alpha, CRP: C - reactive protein, oxLDL: oxidized low-density lipoprotein, MPO: myeloperoxidase.

WOMAC subscales (except for stiffness). Improvements in the symptoms were corroborated by the same findings in SF-36, a tool with good correspondence in KOA trials evaluating nutraceutical interventions [34]. Specifically, *Andrographis paniculata* (ParActin®) improved significantly the total score of SF-36 compared to placebo in KOA patients [35]. Another study on the effects of methylsulfonylmethane in KOA patients showed a non-significant improvement in SF-36 total score, compared to a deterioration of symptoms in the control group [36].

Despite improvement in measures of QoL, the PA level, assessed by the IPAQ score, showed that participants of the two groups had stable PA before and after the intervention. The measurement of PA is a challenge in OA and the instruments for its evaluation have

limited reliability [37] with over- or under-reporting of activities being common [38]. Moreover, herein this lack of increase in PA might be also due to the confinements of COVID-19 pandemic.

The polyphenol-rich supplement managed to attenuate systemic inflammation. Specifically, it decreased significantly CRP compared to the active control. CRP is an acute-phase protein elevated in inflammation. In OA, findings regarding its sensitivity are conflicting due to the heterogeneity of the protocols. Nevertheless, a meta-analysis of 32 studies found that serum hs-CRP levels were significantly higher in patients with OA compared with controls and were associated with pain and decreased function [39]. Additionally, IL-6 and TNF-α increased significantly only in the comparator group and MPO decreased significantly in the polyphenol group. Although MPO has been found elevated in OA [40], only one study evaluated the effect of phytochemicals on its levels, where a ginger-curcumin use decreased serum MPO levels in an OA rat model [41]. Given that BMI did not change after the intervention in neither arm (data not shown), our results agree with current knowledge on the modulation of inflammatory molecules by dietary polyphenols, mainly by inhibiting apoptosis of cells and by controlling articular cartilage damage [13].

Regarding the biomarker of OS, no changes in oxLDL levels were observed. Although in one study this biomarker has been found increased in OA patients compared to healthy controls and it was correlated with radiographic severity and WOMAC score [42], data on OS biomarkers in OA are scarce. However since OS is implicated in OA pathophysiology they merit more investigation.

The polyphenols curcumin and resveratrol used in the present supplement are supported by extensive bibliography that is related to biomarkers of inflammation and PROMs in OA. Namely, regarding curcumin, a meta-analysis of 15 RCTs evaluating the efficacy and safety of such supplements, concluded that curcumin can improve pain, function and stiffness compared to placebo [43]. Importantly, curcumin was found safer than NSAIDs with its positive effects being greater when administered adjunct to NSAIDs. Concerning, resveratrol, although many experimental models have highlighted its anti-inflammatory capacities [44] not many trials have investigated them in KOA. Resveratrol adjunct to meloxicam was found to relieve pain in patients with KOA as it was shown through WOMAC in one study [45] and through WOMAC, knee injury and osteoarthritis outcome score (KOOS), and VAS in another [46]. In the latter, markers such as TNF-α, IL-6 and IL-1β were remarkably reduced but correlations with the clinical outcomes were non-significant. Additionally, two studies reported a reduction in chronic pain in age-related OA in post-menopausal women after resveratrol supplementation [47,48]. Rosmarinic acid has been evaluated only in one clinical trial, where administration of a spearmint tea high in rosmarinic acid managed to reduce significantly Bodily pain in SF-36 and in WOMAC [49].

On the other hand, the number of trials that have investigated mixtures of specific phenols in OA is limited and none of them has explored the responsiveness of serum biomarkers together with questionnaires. Briefly, a formulation of curcumin together with gingerols and piperine that was administered in patients with KOA for one month achieved a similar significant decrease in the inflammatory marker PGE2 to Naproxen [16]. Flavocoxid, a mixture of flavonoids, was also found as effective as Naproxen in decreasing VAS and WOMAC after one month of administration in patients with moderate KOA [15]. Another novel propriety of extracts of *Terminalia chebula*, *Curcuma longa*, and *Boswellia serrata* demonstrated alleviation of symptoms in KOA patients compared to placebo even from day 14 as it was depicted in VAS, WOMAC and Lequesne's functional index (LFI) [19]. Interestingly, Mulek and colleagues found that the polyphenols of the extensively analyzed standardized maritime pine bark extract, Pycnogenol were present not only in serum and blood cells, but also in the SF, verifying that these constituents can reach the tissues that are most in need [20].

Albeit there are confounding results regarding the effect of the dietary intake of AA on the incidence of OA that are possibly attributed to the antioxidant capacities of the vitamin, the positive effects that have been found in animal models and *in vitro* studies could be ascribed to other mechanisms [50–54]. For instance, AA can stimulate anabolic processes as shown *in vitro* [55,56]. Furthermore, in a most recent *in vitro* study, the potency of AA to exert synergistic effects together with β-caryophyllene and D-glucosamine was reported by decreasing the expression of inflammatory molecules and increasing the expression of collagen type II and aggrecan [57]. In our study we used AA as the active comparator since (a) AA is a common vitamin used in OA supplements and (b) patients enrolled in the study could not be treated only with placebo (ethical issues could be risen).

From the aforementioned, it is evident that phenolic compounds; a) have the potency to halt inflammation and progress of OS, b) can manage the symptoms of OA, c) can achieve quick results with no adverse events, and d) can be distributed to the tissues of interest. Nutraceutical supplements have risen to be a great option for the management of chronic illnesses such as OA and adjunct to standard therapies can maximize the benefits that the patients can receive [58,59]. Nevertheless, there is a lack of studies that analyze PROMS together with biomarkers of inflammation and OS after interventions with standardized phytochemicals and adequate duration in KOA. Only a recent clinical trial evaluated the 12-month effect of a *Curcuma longa* extract compared to placebo and found that although VAS was significantly improved, effusion-synovitis volume did not change [60]. Furthermore, on a subgroup from the trial researchers found no significant changes in hsCRP, IL-6, and TNF-α levels compared to placebo [61].

To the best of our knowledge this study is the first one to explore the efficacy of a combination of polyphenols on systemic inflammation using serum biomarkers and PROMs outcomes on KOA patients. However, there are some limitations. These include the heterogeneity of OA subjects in regard to their disease status as they were not recruited nor divided through the evaluation of phenotypes or endotypes. This had to be made to ensure the sample size during Covid-19 pandemic. Finally, the self-reported compliance may have skewed the diary entries.

5. Conclusions

Herein, we demonstrate the positive effects of a novel and standardized polyphenol-rich supplement on the regulation of systemic inflammation and relief of KOA symptoms. These promising results in moderate to severe KOA indicate that this supplement can alleviate pain through the attenuation of systemic inflammation even in advanced stages of the disease with no adverse events. Future studies in larger cohorts should be conducted to confirm our findings. Overall, the food supplement was found to be safe and effective

in managing pain in KOA patients.

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Author contribution statement

Andriana C. Kaliora: Conceived and designed the experiments; Analyzed and interpreted the data. Evdokia Valsamidou: Performed the experiments; Analyzed and interpreted the data; Wrote the paper. Charalampia Amerikanou: Performed the experiments; Analyzed and interpreted the data. Chara Tzavara: Analyzed and interpreted the data. Panagiotis Zoumpoulakis, George Skarpas, Theodoros D. Mariolis-Sapsakos: Contributed reagents, materials, analysis tools or data.

Data availability statement

Data will be made available on request.

Ethics declaration

This study was reviewed and approved by Harokopio University Ethics Committee with the approval number 13/21-2-2020 and Evgenidio Hospital Scientific Board with the approval number 29/19-02-2019). All participants/patients (or their proxies/legal guardians) provided informed consent to participate in the study.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Author E.V. was an employee of Qualia Pharma during the trial but owned no shares in the company. The funding source provided the supplements and did not have any role in study design, collection, and analysis, interpretation of the data or decision to submit this manuscript.

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Appendix A. Supplementary data

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