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Clinical Trial

The efficacy and tolerability of proteoglycan F in the treatment of knee osteoarthritis: A prospective, randomized, double-blind controlled trial

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ABSTRACT

Objective: To identify the efficacy and tolerability of Proteoglycan F in patients with primary knee OA.
Design: A 24-week randomized, placebo-controlled, double-blind clinical trial with two arms: (1) Proteoglycan F (received 10 mg proteoglycan daily, for 24 weeks) and (2) control group (received placebo). Knee symptoms and joint cartilage status (evaluated by ultrasound and MRI of knee joints), quality of life, serum cytokine levels (IL-1β and TNF-α), and safety evaluation were measured before, during, and after the treatment.
Results: After 24-week treatment, pain reduction (in the KOOS pain score) of at least 20% and at least 50% (NRS scale) compared to baseline in the PGF group was significantly higher than those in the control group. The PGF group had greater reductions in the total scores of subchondral bone marrow edema, and bone cocoon under cartilage on knee MRI (classification according to WORMs), which were −2.27 (-4.0; −0.51) and −1.77 (-3.08; −0.46), respectively ($p < 0.05$). The two groups had no statistically significant difference in knee ultrasound characteristics. After 4 weeks, 12, and 24 weeks compared to baseline, there was no statistically significant difference in levels of urea, creatinine, aspartate aminotransferase, and alanine aminotransferase within the group and between the two study groups.
Conclusions: Salmon cartilage PG with 10 mg per day has potential to improve pain symptoms and subchondral bone marrow edema and bone cocoon under cartilage lesions in primary knee OA. However, the efficacy of PGF should be viewed with caution, and future studies are needed for more specific evaluation.

1. Introduction

Osteoarthritis (OA) is the most common joint condition worldwide, with the knee being the most commonly affected joint. The global prevalence of knee OA in individuals aged 40 or over was 22.9% and tended to increase in low-income and lower-middle-income countries [1, 2]. In Vietnam, the prevalence of this disorder was 34.2% [3]. This chronic illness causes a variety of consequences such as pain, chronic

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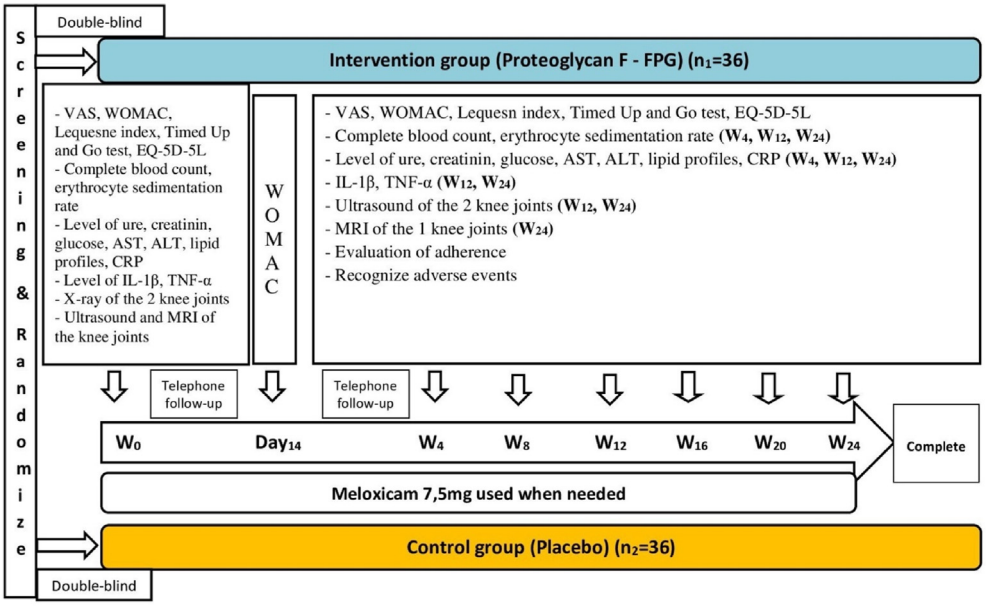

Fig. 1. Study protocol.

disability, emotional disruption, increased healthcare utilization, lower quality of life, and high financial costs to society [4].

Osteoarthritis is a degenerative articular disease with a complex pathogeny and multifactorial disorders; therefore, different approaches to OA management are critical for optimum treatment effects [5]. Therapeutic approaches for knee OA addressed symptoms, improved function, and delayed or prevented joint arthroplasty. Oral medications such as nonsteroidal anti-inflammatory drugs, acetaminophen, and opioids have the benefit of pain relief. However, long-term use of such medications brings out significant potential harm. Therefore, surgery is indicated in severe osteoarthritis patients suffering extensive pain and deformity and the conservative measures have failed but it is dependent on the OA stage, other related factors such as level of physical activity, age, and patient's comorbidities [6]. Some trials show that treatment with symptomatic slow-acting drugs for OA-modifying osteoarthritis drugs is related to pain relief and improvements in joint structure and physical function in OA patients [7,8]. However, evidence of the drug's effects are heterogeneous results between studies and still a matter of debate [9]. In recent years, beyond conventional medical and surgical interventions, there has been a surge of interest in finding alternative therapy and complementary supplements worldwide, especially in Vietnam where traditional medicine is considered mainstream. Several studies have examined the effectiveness of complementary alternative medicine use among patients with chronic diseases [10–13], including OA due to the evidence and identified analgesic, muscle relaxant properties, and anti-inflammatory for such therapies [14]. As one of the main components of the extracellular matrix, Proteoglycans have diverse functions in the cartilage [15,16]. These substances are a main ingredient in cartilage regeneration, inhibiting the enzyme elastase - an intermediate that leads to degenerative articular cartilage and decreases the formation of free oxygen radicals in cartilage tissue. Thus, the effects inhibit cartilage calcification, regenerate cartilage and reduce pain for cartilage degeneration, promoting cartilage metabolism and forming a cartilage [17].

Recent studies have demonstrated the safety and efficacy of proteoglycan in rats, rabbits, and individuals with knee joint discomfort but without a diagnosis of OA [15,18–20]. Previous studies found that using a proteoglycan-containing product effectively reduced knee pain and improved knee function in people with knee pain or osteoarthritis [21,22]. However, there have not been studies evaluating the effectiveness of using proteoglycan in articular cartilage. In an in vivo study, the total histological score improved significantly compared with the group without PG suggesting the efficacy of PG in cartilage repair [20]. Therefore, this randomized, double-blind controlled trial is conducted in Vietnam to demonstrate the safety and effectiveness of Proteoglycan F (PGF) in the treatment of patients with primary knee OA.

2. Materials and Methods

2.1. Study product

Proteoglycan F ingredient was manufactured by Ichimaru Pharcos, Co., Ltd. (at 318-1 Asagi, Motosu-shi, Gifu 501-0475 Japan) with GMP Certification No.原-0003.

Proteoglycan F (PGF) was manufactured in the form of a hard capsule (50 mg in a capsule) by Astrim, INC (2006, Kobora, Yamada-cho, Mizunami-Shi, Gifu, Japan - GMP Certification No.12806) and consisted of 12.5 mg salmon nasal cartilage extract (containing 10 mg proteoglycan) and 37.5 mg dextrin (as a vehicle). Salmon proteoglycan was extracted from salmon (*Oncorhynchus keta*) nasal cartilage, as in previous studies [18,19].

The placebo capsule contained only dextrin powder, which was supplied at the same dose and was similar to the PGF in shape and packaging by Matsutani Chemical Industry Co., Ltd. The PGF and the placebo have the same appearance, only the code numbers are different between the drug boxes.

2.2. Patients

Patients were recruited and followed from August 2021 to May 2022 at the National Geriatric Hospital. The treatment options and the possibility of beneficial effects and risks were explained to diagnosed patients with primary knee OA. Written informed consent was obtained from all subjects. The study protocol was approved by the research ethics board at National Geriatric Hospital, Hanoi, Vietnam (Reference number: 1337/QD-BVLKTW).

The research subjects were patients examined and treated at the Outpatient Department. Inclusion criteria were: (1) diagnosed primary knee OA according to American College of Rheumatology 1991 criteria [23] includes clinical and radiographic criteria; (2) age from 40 to 80 years old; (3) had to be symptomatic for ≥3 months before enrollment (knee pain with pain intensity according to Numeric Rating Scale ≥1); (4) had a radiologic grade II and III according to Kellgren-Lawrence

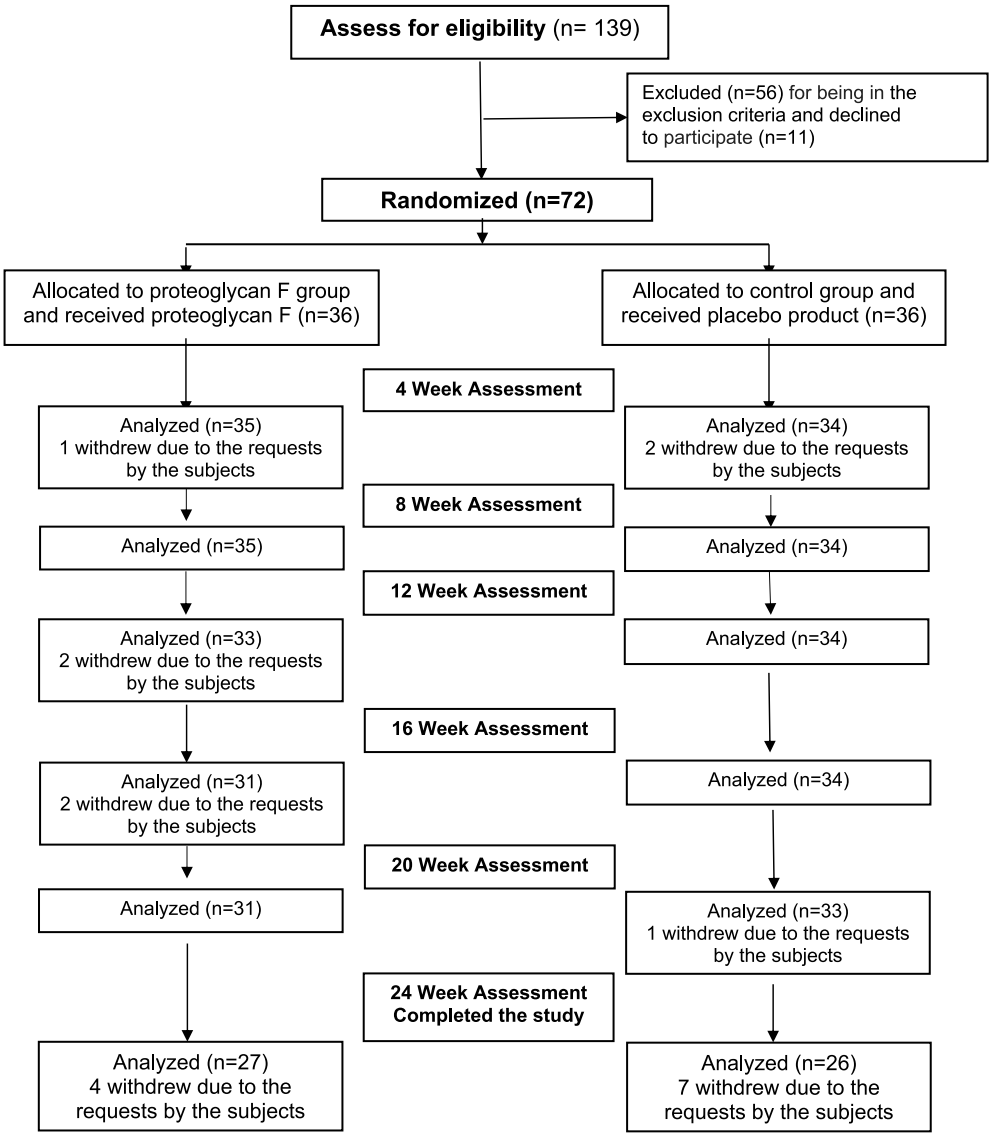

Fig. 2. Participant flow diagram (CONSORT flow diagram).

criteria [24]. Exclusion criteria confirmed clinically and, if necessary, by laboratory and instrumental findings, including (1) secondary knee OA; (2) grade-I and IV of knee OA according to Kellgren-Lawrence; (3) had joint lavage, arthroscopy, or treatment with hyaluronic acid or other disease-modifying agents during the previous 6 months, or who had been treated with intra-articular corticosteroids during the past 3 months; (4) contraindications to NSAIDs; (5) had hematologic disorders, liver disease, acute illness, renal disease, other rheumatic diseases, diabetes mellitus and disabling comorbid conditions that would make it impossible for the patient to visit the research center; (6) pregnancy.

2.3. Study designs

This study was a 24-week randomized, placebo-controlled, double-blind clinical trial (ClinicalTrials.gov ID: NCT04998825) with two arms: (1) Proteoglycan F and (2) control. The protocol was presented in Fig. 1.

Sample size estimates: Assuming a standardized effect size (ES) of 0.2 [25] for a pilot randomized trial, with 90% power and two-sided 5% significance, and allowing for a 20% dropout rate, the final sample size to be recruited is 72 (36 per study arm).

Randomization: After the screening, all selected patients were randomized into two groups: the PGF group (received PGF) and the control group (placebo group), using block method: block 4, with a ratio of 1:1 (PGF drug – placebo). Implementer: a registered nurse was trained in randomization methods and worked independently of researchers. Prepare tools: 1 block of 4 envelopes with predetermined codes (three-digit identification number) sealed and secured inside. Implementation steps: (1) After agreeing to participate in the study and randomization, patients will be sent to the registered nurse; (2) Each patient picks 01 envelope from the box, the envelope has a predetermined code corresponding to the code on the medicine box that the patient will receive; (3) Continue as above until all 4 envelopes (1 block) are gone; (4) Prepare the next block to put in the box; (5) Continue to repeat the process until all patients participate in the study. Group assignment of patients was blinded for all patients, investigations, and statisticians. The independent allocator kept the codes and revealed them only after completing treatment periods and analyses.

A nurse provided the patient with the study product with a code corresponding to the one the patient received and instructions for use at baseline and weeks 4, 8, 12, 16, and 20. The PGF group received Proteoglycan F at a dose of 50 mg per day for 24 weeks, the control group received placebo capsules. During the research period, all patients of the 2 groups will be prescribed a background regimen of meloxicam 7.5 mg, taking a maximum of 2 tablets/day divided into 2 times after eating. In

the prescription, patients only use it when they have uncontrollable pain. The amount of meloxicam used will be reported during each follow-up visit. All patients received knee osteoarthritis education, which included lifestyle and physical activities. Patients did not modify their therapeutic program (for both drug treatments and physical therapy) during the duration of the study unless an adverse event (AE) occurred and required management. They were instructed to avoid corticosteroids and hyaluronic acid infiltrations, joint lavage, and arthroscopic surgery, and to avoid treatment with disease-modifying OA drugs.

Both groups were assessed at baseline and weeks 4, 8, 12, 16, 20, and 24. Recruitment, randomization, and flow of participants are shown in Fig. 2.

3. Outcome measures

3.1. Primary outcomes

The primary outcomes were (1) symptom relief and (2) improvement in joint cartilage status before and after intervention in each group and a comparison between the two groups.

(1) Symptoms relief: pain relief and changes in knee function were evaluated using the Numeric Rating Scale, Knee Injury and Osteoarthritis Outcome Score, Lequesne index, and Timed Up and Go test.

Pain intensity was assessed using the Numeric Rating Scale (NRS). Scores range from 0 to 10. The higher the score, the more severe the pain [26].

The Knee injury and Osteoarthritis Outcome Score (KOOS) was used to assess the patients' opinions about the short-term and long-term consequences of knee OA. The KOOS consists of 42 items in 5 separately scored subscales: pain (9 items), other symptoms (7 items), function in daily living (ADL, 17 items), function in sport and recreation (5 items), and knee-related quality of life (QOL, 4 items) [27]. Each question has five possible answers scored from 1 (No problem) to 5 (Extreme problems). Then calculate the total score for each subscale and the total KOOS score. Accordingly, the higher the total score, the worse the knee joint function.

The Lequesne index has an interview format questionnaire, including 11 questions divided into three sections: pain or discomfort (0–8 scores), maximum distance walked (0–8 scores), and activities of daily living (0–8 scores). The total score ranges from 0 (no pain, no disability) to 24 (maximum pain and disability). The higher the total score, the worse the knee joint symptoms and function [28].

The Timed Up and Go test (TUG) was used to assess balance, walking ability, and functional mobility changes in study populations. A faster time indicates a better functional performance [29,30].

(2) Changes in knee joint characteristics (evaluated by ultrasound and magnetic resonance imaging of knee joints):

Knee ultrasound assessments were performed at baseline, 12, and 24 weeks using the same machine (RS80A, Samsung, Korea, 2016) by two radiologists at the National Geriatric Hospital. The knee ultrasound was based on a protocol of EULAR guidelines [31]. The cartilage thickness was measured in the medial, lateral condyles, and notch, with the knee in maximum flexion. The technique was described elsewhere [32,33]. The two radiologists with over 10 years of ultrasound experience and were trained in knee ultrasound techniques and procedures before starting the study. Each patient had a knee ultrasound performed by two radiologists separately. The final result is the average of these two assessments and is agreed upon by both doctors.

Magnetic resonance imaging (MRI) of the knee: All patients underwent MRI scans on a 1.5 T scanner (Magnetom Essenza 1.5 T, SIEMENS) using Syngo Siemens software. The biochemical composition of the medial and lateral tibiofemoral cartilage was estimated using single-slice transverse relaxation time (T2) mapping (milliseconds, ms). Sagittal T2 mapping images for the most severely damaged area of articular cartilage and the least damaged or normal articular cartilage region were evaluated pre and post-intervention. The ROY software was to assess T2 mapping. The transverse relaxation time (T2) of the cartilage has been shown to evaluate the early degeneration of articular cartilage, especially the changes in water and collagen content and tissue anisotropy [34,35]. Joint cartilage lesions, subchondral bone marrow edema, and bone cocoon under cartilage on knee MRI were classified according to the Whole-Organ Magnetic Resonance Imaging Score (WORMs) [36]. MRI of the knee was measured by a radiologist with over 10 years of experience in knee MRI at the National Geriatric Hospital, who was trained on the procedure for scanning and reading knee MRI results before starting the study.

3.2. Secondary outcome

Secondary measures included changes in quality of life, serum cytokine levels (IL-1β and TNF-α), and safety evaluation.

Quality of life was assessed by the EuroQol EQ-5D-5L index, which was validated in Vietnam [37]. The EQ-5D-5L comprises 5 dimensions ('5D'): (1) mobility; (2) self-care; (3) usual activities; (4) pain/discomfort and (5) anxiety/depression. Those are rated by a verbal 5-point rating scale allowing for the distinction of five levels ('5L') of severity: Level 1: no problems; Level 2: slight problems; Level 3: moderate problems; Level 4: severe problems; Level 5: extreme problems per dimension and providing a 1-digit number for each dimension.

Serum cytokine levels: The samples were centrifuged at 1000 g for 8 min, and then the serum was collected and stored at −20 °C until use.

Table 1
Patient demographics and baseline characteristics by treatment groups.

Variable		PGF group ($n_1 = 36$)	Control group ($n_2 = 36$)	p-value
		n (%)	n (%)	
Gender	Male	2 (5.6)	5 (13.9)	0.233
	Female	34 (94.4)	31 (86.1)	
Kellgren–Lawrence grade on radiography	II	26 (72.2)	24 (66.7)	0.798
	III	10 (27.8)	12 (33.3)	
BMI (kg/m^2)	18.5–22.9	16 (44.4)	18 (50.0)	0.637
	23–24.9	10 (27.8)	10 (27.8)	
	≥25.0	10 (27.8)	8 (22.2)	
		Mean ± SD	**Mean ± SD**	
Age (year)		63.7 ± 8.5	65.6 ± 7.8	0.310
BMI (kg/m^2)		23.9 ± 2.9	23.0 ± 2.0	0.141
Duration of knee OA (month)		66.7 ± 64.8	68.9 ± 54.9	0.873
NRS score		6.7 ± 1.8	6.3 ± 1.7	0.386
KOOS (Total score)		141.2 ± 26.5	139.3 ± 21.4	0.733
Lequesne Index (total score)		14.2 ± 4.2	13.9 ± 3.3	0.734
TUG test (second)		17.1 ± 5.0	17.5 ± 6.1	0.761
EQ-5D-5L index		0.45 ± 0.2	0.46 ± 0.2	0.892

The levels of IL-1β and TNF-α were detected using enzyme-linked immunosorbent assay kits from Abcam (USA) and Arigo Biolaboratories (Taiwan), respectively. The results then were measured on a Synergy HT reader (Biotek, USA). Tests were made according to the standard procedures and the experiment was repeated three times.

Safety evaluation: Safety and tolerability were assessed during every follow-up visit. The incidence and severity of any adverse events (AEs) were recorded and abnormal changes in physical parameters, including pulse rate and blood pressure. Laboratory tests including renal function (urea and creatinine levels), and liver function (levels of aspartate aminotransferase, and alanine aminotransferase) were also evaluated at baseline, 4, 12, and 24 weeks.

3.3. Data analysis

Data were entered and managed using REDCap electronic data capture tools hosted at Hanoi Medical University. SPSS 22.0 was used to analyze the data. Results are expressed as mean ± SD. Paired sample *t*-test was used to analyze data in the same group before and after treatment. In order to account for the within-subject correlation among the repeated measurements and to capture the changes in the outcome variable over time, we analyzed the data using a longitudinal model. To account for repeated data measurements, in which interested variables were measured three times, we fitted the Generalized Estimating Equations (GEEs) model. Survival analysis was used to compare differences between the intervention group and the placebo group. Covariates that could influence the outcome variable over time or confound the treatment effect were adjusted in the longitudinal model. The covariates include age, gender, body mass index, and knee OA stage. The selection of these covariates was predicated on biological plausibility and previous literature. P values lower than 0.05 were considered to be significant.

4. Results

The 72 patients who met the inclusion criteria and participated in this study, were randomized into the PGF group (n = 36) and placebo group (n = 36). Fifty-three subjects (27 in the PGF group and 26 in the placebo group) completed the 24-week study duration. Drop-outs were due to a loss of follow-up (Fig. 2). At baseline, there were no statistically significant differences between the PGF group and the placebo group on age, gender, BMI, and Kellgren–Lawrence grade on radiography and any of these baseline measures of NRS score, KOOS scores, the Lequesne index, the TUG test, and the EQ-5D-5L index value (Table 1).

Results of KOOS, Lequesne index, TUG test, and EQ-5D-5L index are presented in Table 2. After adjusting factors that can affect the effectiveness of treatment (age, gender, BMI, and stage of knee osteoarthritis), the degree of symptom improvement between baseline and subsequent follow-ups tended to be greater in the PGF group for these dimensions, but these differences were not significant ($p > 0.05$). The results showed that the PGF group had an improvement of 3.12 points greater than the control group, after adjusting for these factors. However, this difference was not statistically significant.

Fig. 3 shows comparisons of at least 20% improvement in symptoms and quality of life between the PGF group and the control group. Pain was reduced by at least 20% compared to baseline according to the KOOS pain score and pain level reduced by at least 50% compared to baseline according to the NRS scale in the PGF group was significantly higher than those in the control group (Fig. 3B and G). Other symptoms (in KOOS total score, Lequesne total index, Lequesne distance index, TUG test, and EQ-5D-5L index) improvement of at least 20% from baseline tended to be greater in the PGF group, but these differences were not significant ($p > 0.05$).

Comparison of the change in knee ultrasound characteristics between the PGF group and the control group are presented in Table 3. After adjusting factors that can affect the effectiveness of treatment (age, gender, BMI, and stage of knee osteoarthritis), there were no significant differences between the two groups ($p > 0.05$).

Table 2
Comparison of the change in some clinical outcomes between the PGF group and control group.

	KOOS total (42–210 scores)	KOOS pain (9–45 scores)	Lequesne index (0–24 scores)	Lequesne distance (0–8 scores)	TUG (seconds)	EQ-5D-5L index
PGF group	−3.12 (−12.2; 5.96)	−0.53 (−2.64; 1.57)	−0.61 (−1.99; 0.78)	−0.007 (−0.1; 0.09)	−0.26 (−0.93; 0.4)	0.10 (−1.92; 2.12)
Time to follow up						
30 days	−3.23 (−8.43; 1.97)	0.39 (−0.88; 1.65)	−0.04 (−0.85; 0.77)	0.57 (0.1; 1.05)*	0.94 (−0.52; 2.4)	−0.11 (−0.17; −0.04)**
60 days	−6.53 (−12.08; −0.99)*	−0.9 (−2.4; 0.61)	0.08 (−0.74; 0.89)	0.59 (0.12; 1.07)*	0.22 (−0.86; 1.3)	−0.25 (−0.32; −0.17)***
90 days	−11.31 (−16.19; −6.43)***	−1.44 (−2.74;−0.14)*	−0.34 (−1.09; 0.42)	0.44 (0.002; 0.88)*	−0.83 (−1.92; 0.25)	−0.31 (−0.39; −0.23)***
120 days	−16.08 (−21.05; −11.11)***	−2.04 (−3.26;−0.81)**	−0.54 (−1.29; 0.22)	0.45 (0.02; 0.87)*	−1.02 (−1.96; −0.08)*	−0.40 (−0.48; −0.31)***
150 days	−20.55 (−26.22; −14.87)***	−3.53 (−4.87;−2.19)***	−0.94 (−1.79; −0.09)*	0.34 (−0.14; 0.82)	−1.41 (−2.36; −0.46)**	−0.47 (−0.55; −0.39)***
180 days	−19.82 (−26.22; −13.42)***	−4.22 (−6.14; −2.31)***	−1.2 (−2.21; −0.19)*	−0.06 (−0.56; 0.43)	−1.86 (−3.08; −0.64)**	−0.52 (−0.61; −0.43)***
Male	25.83 (15.91; 35.74)***	7.19 (5.23; 9.14)***	4.78 (2.38; 7.18)***	0.30 (0.17; 0.42)***	1.41 (0.43; 2.39)**	2.29 (0.08; 4.5)*
Age	0.56 (−0.06; 1.17)	0.14 (0.001; 0.27)*	0.12 (0.02; 0.21)*	0.005 (−0.001; 0.01)	0.03 (−0.01; 0.07)	0.23 (0.12; 0.33)***
BMI	0.82 (−1.0; 2.64)	0.19 (−0.27; 0.65)	0.17 (−0.1; 0.43)	0.01 (−0.01; 0.03)	0.07 (−0.04; 0.19)	0.09 (−0.32; 0.49)
Stage of knee OA	7.64 (−0.80; 16.09)	2.25 (0.11; 4.4)*	1.32 (0.05; 2.59)*	0.11 (0.02; 0.19)*	0.93 (0.34; 1.52)**	−0.64 (−2.34; 1.06)

*p < 0.05; **p < 0.01; ***p < 0.001.

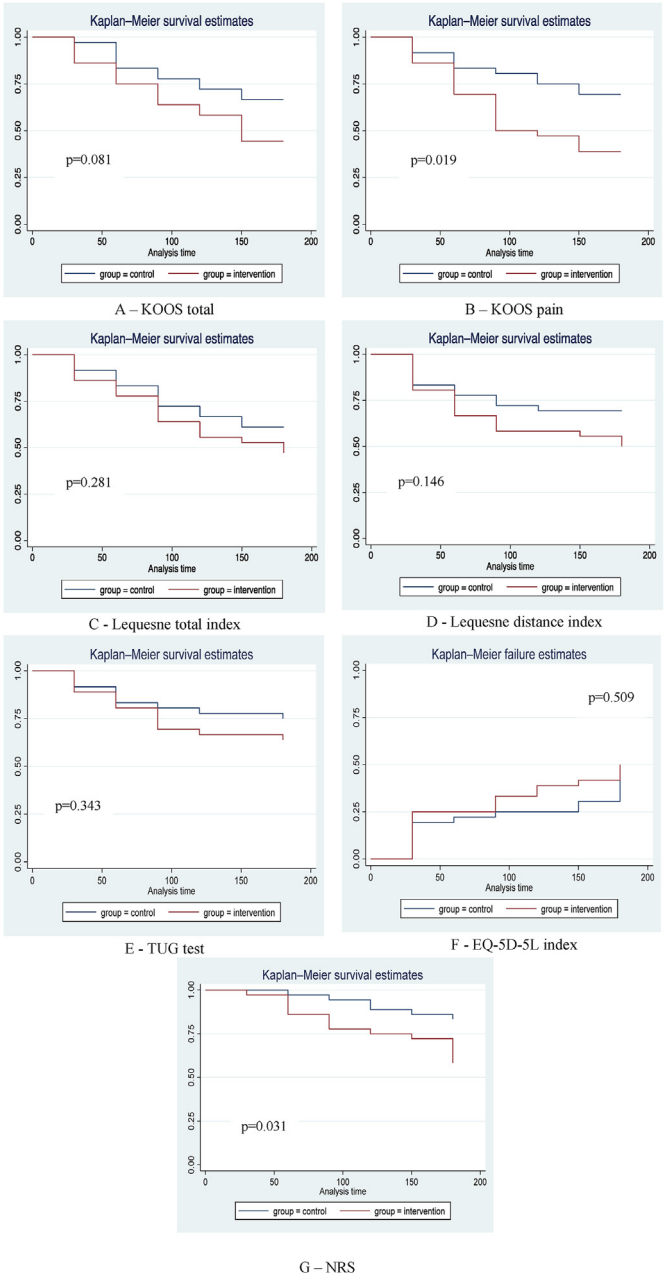

Fig. 3. Outcome measures were defined as a 20% improvement from baseline and pain intensity reduced by at least 50% compared to baseline (NRS).

Table 4 compares the change in knee MRI characteristics between the PGF group and the control group. When adjusted for time, gender, age, BMI, and stage of knee OA, the total scores of subchondral bone marrow edema and bone cocoon under the cartilage of the PGF group decreased more than those in the control group by 2.27; 1.77 points, respectively. These differences were significant ($p < 0.05$).

The study results showed that there was no statistically significant difference between the number of rescue drugs (Meloxicam) used between the PGF group and the control group (Table S6 - Supplements). The change in the level of TNF-alpha and IL1-beta between the two study groups at the follow-up time points did not have a statistically significant difference (Tables S2 and 3 - Supplements).

Tolerance: no death occurred and no significant change in vital signs was found during 24 weeks of follow-up. Results of renal function (urea and creatinine levels) and liver function (levels of aspartate aminotransferase - AST, alanine aminotransferase - ALT) were shown in Table 5. At the time of evaluation after 4 weeks, 12, and 24 weeks compared to baseline, there was no statistically significant difference in the above indicators within the group and between the two study groups ($p > 0.05$).

5. Discussion

This prospective, randomized, double-blind controlled trial demonstrated improved knee OA symptoms and degenerative joint injury characteristics on knee MRI in patients treated with oral administration of proteoglycan (10 mg/day), compared to patients who received a placebo after a 24-week intervention. The results favor the efficacy of Proteoglycan F in alleviating the pain symptom of knee OA, as assessed by improvement in KOOS pain score. The comparable efficacy was also shown by the change in the total scores of subchondral bone marrow edema and bone cocoon under cartilage on MRI of the knee joint. The comparable safety was shown by the result that there was no statistically significant difference in the incidence of AEs between the two groups.

Our finding showed that the percentage of patients with a 20% reduction in KOOS pain score and pain level reduced by at least 50% compared to baseline according to the NRS scale in the group of patients using salmon nasal proteoglycan (10 mg/day for 24 weeks) was significantly higher than that in the group receiving placebo. Besides, there was no difference in the number of rescue anti-inflammatory analgesics used between the two groups. This result was similar to the previous study suggested that administration of salmon nasal cartilage proteoglycan (10 mg/day for 12 weeks) reduces symptoms of OA, including the subscale scores of Japan Knee Osteoarthritis Measure (JKOM) and visual analog scale (VAS), in patients with knee OA [22]. On the contrary, using salmon nasal proteoglycan (10 mg/day for 16 weeks) did not relieve the symptoms of knee discomfort in people without knee OA [18]. This suggests that the salmon nasal cartilage proteoglycan might be effective in improving symptoms in subjects with arthritis and experiencing greater pain and dysfunction. However, our results indicated that the degree of other symptom improvements (functional performance) between baseline and subsequent follow-ups tended to be greater in the Proteoglycan F group, but these differences were not significant.

The results of knee MRI before and after the intervention in the two groups showed an improvement in subchondral bone marrow edema and bone cocoon under cartilage lesions in the group that received salmon nasal

Table 3
Comparison of the change in knee ultrasound characteristics between the PGF group and the control group.

	Joint cartilage thickness – *intercondylar notch (mm)*	Joint cartilage thickness – *lateral condyle (mm)*	Joint cartilage thickness – *medial condyle (mm)*	Joint fluid thickness (mm)	Synovial membrane thickness (mm)
PGF group	−0.15 (–0.34; 0.04)	−0.005 (−0.18; 0.17)	0.03 (−0.31; 0.38)	−0.54 (–1.9; 0.83)	0.005 (–0.63; 0.64)
Time to follow up					
90 days	0.03 (–0.13; 0.18)	0.01 (−0.15; 0.17)	0.06 (−0.1; 0.22)	−0.92 (–1.84; 0.002)	−0.61 (–1.1;–0.11)[a]
180 days	0.0004 (–0.16; 0.48)	0.1 (−0.1; 0.31)	0.29 (−0.15; 0.73)	−0.5 (–1.73; 0.75)	−0.55 (–1.1;–0.002)[a]
Male	0.16 (–0.16; 0.48)	0.13 (–0.2; 0.47)	0.19 (−0.27; 0.64)	0.7 (–1.81; 3.21)	−0.16 (–1.65:1.32)
Age	−0.003 (−0.01; 0.01)	0.004 (–0.01; 0.02)	−0.01 (−0.02; 0.01)	0.04 (–0.05; 0.12)	0.03 (–0.01; 0.07)
BMI	0.01 (–0.02; 0.04)	0.01 (−0.01; 0.04)	−0.02 (−0.1; 0.06)	−0.11 (–0.40; 0.18)	−0.02 (–0.17; 0.12)
Stage of knee OA	0.11 (−0.12; 0.35)	−0.01 (–0.22; 0.19)	0.24 (−0.29; 0.78)	1.96 (0.38; 3.54)[a]	0.99 (0.13; 1.84)[a]

[a] $p < 0.05$.

Table 4
Comparison of the change in knee MRI characteristics between the PGF group and the control group.

	Joint cartilage lesions	*Subchondral bone marrow edema*	*Bone cocoon under cartilage*	*Relaxation time T2 mapping (milisecond) The most severely damaged area*	*Relaxation time T2 mapping (milisecond) Normal/least damaged*
***PGF g*roup**	−5.78 (−13.07; 1.52)	*−2.27(–4.0; –0.51)**	*−1.77(–3.08; –0.46)**	0.9 (–40.18; 41.98)	0.62 (–13.21; 14.45)
Time to follow up					
180 days	1.02 (−0.89; 2.93)	−0.64 (–1.63; 0.35)	0.3 (−0.02; 0.62)	−22.24 (−42.38; −2.09)*	−8.79 (–18.84; 1.26)
Male	14.55 (6.31; 22.78)**	1.05 (–1.15; 3.26)	1.59 (0.13; 3.05)*	16.21 (–55.72; 88.14)	−29.57 (–62.69; 3.55)
Age	0.96 (0.54; 1.37)***	0.13 (0.01; 0.25)*	0.03 (−0.05; 0.11)	1.81 (–0.8; 4.42)	−0.52 (−1.47; 0.43)
BMI	0.07 (−1.37; 1.51)	−0.04 (–0.35; 0.26)	−0.11 (–0.33; 0.10)	6.75 (–1.54; 15.05)	−2.43 (−5.55; 0.67)
Stage of knee OA	10.61 (2.56; 18.67)*	1.09 (−0.81; 3.0)	−0.26 (–1.64; 1.11)	64.91 (15.13; 114.7)*	10.80 (–2.66; 24.26)

*p < 0.05; **p < 0.01; ***p < 0.001.

Table 5
Results of urea and creatinine, AST, and ALT levels.

		Urea (mmol/L)	Creatinine (μmol/L)	AST (U/L)	ALT (U/L)
Time					
Baseline	PGF group	4.9 ± 1.0	62.4 ± 8.4	23.0 ± 7.4	23.9 ± 11.3
	Control group	5.5 ± 1.5	65.9 ± 9.5	23.8 ± 6.6	24.1 ± 10.2
4 weeks	PGF group	4.8 ± 1.1	61.6 ± 8.4	23.6 ± 8.0	22.3 ± 10.7
	Control group	5.3 ± 1.4	65.9 ± 10.1	24.3 ± 7.1	25.0 ± 11.5
12 weeks	PGF group	5.0 ± 1.2	60.3 ± 12.5	22.2 ± 7.2	21.2 ± 11.7
	Control group	5.2 ± 1.0	64.4 ± 10.7	23.9 ± 8.3	23.9 ± 10.3
24 weeks	PGF group	5.1 ± 1.2	62.9 ± 9.4	24.4 ± 10.4	27.7 ± 18.2
	Control group	6.0 ± 1.4	68.2 ± 15.6	28.7 ± 11.6	32.1 ± 16.6

p > 0.05.

proteoglycan (10 mg/day for 24 weeks) compared to the control group. Although there was no difference in the lesion characteristics on ultrasound of the knee joint. This shows the advantage of the MRI method when it is possible to detect very small structural changes that ultrasound cannot detect. Indeed, although the exact mechanism by which salmon nasal cartilage exerts its protective effects on cartilage structure is still to be elucidated, proteoglycan has been shown to possibly have anti-inflammatory effects through inhibition of inflammatory cytokine production IL-1, IL-6, TNF-α, and immunomodulation in vitro and in vivo [19,20,38,39]. Furthermore, salmon nasal proteoglycan was shown to promote chondrocyte proliferation and innate PG production, thereby providing an anti-inflammatory and chondroprotective effect in the joint tissue [20].

Proteoglycan F was generally well-tolerated. Furthermore, no adverse effects were documented in the treatment with salmon nasal proteoglycan and placebo extract. This result was similar to a previous study showing that changes in body weight, systolic and diastolic blood pressures, and pulse rate were minimal and within the reference range during the intervention in both groups [18]. In addition, in the study, neither hepatotoxicity nor nephrotoxicity developed during the use of the product, in support of the biosafety of salmon nasal proteoglycan used as a supplement in the treatment of knee OA. Indeed, the study had good compliance (>95%).

This is the first randomized double-blind placebo-controlled clinical trial to show the efficacy and tolerability of oral administration of salmon nasal proteoglycan on knee OA patients. The relatively modest sample size may be a limitation of the study. In addition, the patients were recruited and followed during the period when Vietnam was seriously affected by the COVID-19 epidemic, with some periods requiring quarantine, which also increased the number of drop-out patients. Therefore, appropriate analysis of results in the context of epidemics also needs to be noted. Since this is one of the first studies to evaluate the efficacy of Proteoglycan F in primary knee osteoarthritis, we used multiple outcomes and multiple comparisons. This may also be a limitation of the study with the results should be viewed cautiously. On the other hand, in this study, the effectiveness was evaluated not only by clinical symptoms but also by magnetic resonance imaging of the knee to further clarify even little improvement. Future clinical trials with larger sample sizes and more rigorous designs should be performed to overcome the above points.

6. Conclusions

This study demonstrated that salmon cartilage PG at the doses of 10 mg/day improved pain symptoms and subchondral bone marrow edema and bone cocoon under cartilage lesions and this improvement was sustained over 24 weeks. The biosafety of the product was suggested clinically. There were no acute hepatotoxic and nephrotoxic or other adverse effects were observed in the trial. Our results could offer a complementary oral solution for the treatment of knee OA using a dietary supplement containing salmon nasal PG. However, the efficacy of PGF should be viewed with caution, and future studies are needed for more specific evaluation.

Contribution

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work.

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Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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