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Original Article

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Wheat Gluten Hydrolyzed Peptide Protection Joint Osteoarthritis: A Randomized, Double-Blind, Placebo-Controlled Clinical Study

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Abstract

Background: *Osteoarthritis is the most predominant joint disease, that has obsession millions of people. The purpose of this study was to evaluate the efficacy from natural plant source purifying the hydrolyzed wheat peptides (Vollapep2™) for knee osteoarthritis (OA) pain and associated symptoms compared to placebo group.*

Methods: *The assessed have the change of Visual Analogue Scale (VAS) score, Western Ontario McMaster Osteoarthritis Index (WOMAC) and Traditional Chinese Medicine (TCM) syndrome classification, from baseline through 45 days between the Vollapep2™ and placebo groups.*

Results: *At 45 days, the Vollapep2™ group demonstrated a significant reduction in VAS score compared to placebo group. Supplementation with Vollapep2™ also resulted in significant changes for all three WOMAC subscales: pain the Vollapep2™ group and placebo group were reduction rate 93% and 80%, respectively; physical function the Vollapep2™ group and placebo group were reduction rate 73% and 53%, respectively; total WOMAC score the Vollapep2™ group and placebo group were reduction rate 87% and 67%, respectively. Another TCM syndrome classification the Vollapep2™ group can reduce joint pain, swollen, tenderness compared to the placebo group. Safety outcomes did not differ among the groups.*

Conclusion: *Vollapep2™ group demonstrated can effectively reduce osteoarthritis, relieve joint swollen and tenderness, then elevate joint mobility and flexibility and improved quality of life.*

Keywords: Osteoarthritis, Wheat Gluten, Hydrolyzed Peptide, Knee Function

1. Introduction

Osteoarthritis is the most predominant joint disease, that has obsession millions of people. That often occur in knee and musculoskeletal such as age, obesity, female sex articular trauma are the main risk factors [1,2]. Osteoarthritis is a common cause of illness, functional limitations, and loss of autonomy later in life. However, the disease is widely recognized to impact multiple aspects of a person's life, including functional and social activities, body image, and psychological well- being. Osteoarthritis is a common cause of illness, functional limitations, and loss of autonomy later in life [3,4]. This has become a global problem, and novel therapeutic intervention is warranted.

Knee osteoarthritis (OA) is typically treated with non-steroidal anti-inflammatory drugs (NSAIDs) or intra-articular injection of corticosteroids. However, long-term use can lead to addiction and damage to the stomach and intestines, placing a significant burden and discomfort on the body [5-7]. Management for OA

can generally be divided into conservative and surgical measures. Conservative management broadly compromises pharmacological and non- pharmacological options and is conventionally the first line treatment to avoid or delay the need for surgical management [8].

Recent years orally supplements derived from animal chicken sternum of undenatured type II collagen (UC-II) have claimed to reduce joint inflammation, restore joint function, and alleviate joint pain [9-11]. As the progressive destruction of articular cartilage characterizes OA, undenatured type II collagen supplementation may induce cartilage matrix synthesis by stimulating the chondrocytes.

Wheat gluten (WG) is an economically important co-product in the recovery of wheat starch in wet dawdle of wheat flour. Another wheat protein is used in animal and human nutrition. Gluten is a complex mixture of main gliadin and glutenin, which are highly resistant to hydrolysis and are mediated by proteases of the human gastrointestinal tract [12]. Wheat gluten hydrolyzed proteins are primarily utilized to produce active peptides with physiological regulatory functions, including antioxidant, anti-inflammatory, immune response [13-14].

Previous studies have shown that WG can induce autoimmune responses in rats with type 1 diabetes by inducing the activation of the intestinal mucosa and regulating the immune response [15-17]. Similarly in vivo studies have shown that WG can trigger autoimmune responses in dendritic cells [18]. Furthermore, in biomedical materials, wheat protein has been found to be useful in bone reconstruction experiments. WG hydrolyzed peptides have been found in human monocytes to possess anti-inflammatory and anti-atherosclerotic properties [19-22]. Furthermore, WG supplementation appears to help improve joint pain in patients with rheumatoid arthritis [23]. Therefore, it is speculated that WG may trigger autoimmune responses in the body and regulate immune mechanisms, like the mechanism of UC-II. The innate immune system is known to play an important role in oral tolerance to dietary antigens, but the WG is unknown and not sufficiently clarified so to evaluate joint function in OA.

In this study is from natural plant WG source purifying the hydrolyzed wheat peptides (Vollapep2™), small molecule, high absorption, non-GMO, allergen-free (<20ppm), vegan, unlike animal-derived UC-II. In addition to different sources, the most important thing is that it is edible for whole plant sources and vegetarians. Vollapep2™ mechanism of action is to interact with lymphatic tissue in the intestine, activate immune cells, and secrete anti-inflammatory substances to explore its functional evaluation for OA. Therefore, this research aimed to evaluate the efficacy and safety Vollapep2™ in modulating knee joint function by assessing the change of Visual Analogue Scale (VAS) score, Western Ontario McMaster Osteoarthritis Index (WOMAC) and Traditional Chinese Medicine (TCM) syndrome classification, from baseline through 45 days between the Vollapep2™ and placebo groups.

2. Material & Methods

This clinical research was approved by the ethics committee of the Hefei Boe Hospital, and the study was registered with the Chinese Clinical Trial Registry (ChiCTR2500114321). All subjects recruited in this trial returned the written consent forms. It was conducted by standardized trained general practitioners and nursing staff from April to September 2025.

2.1. Study Design

The study was performed as a prospective, randomized, double-blind, placebo-controlled study in parallel design. This study was conducted following the guidelines for Good Clinical Practice set forth by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Chinese Medical Association in 2018 ,Human Use (ICH E6 [R2], Nov.2016) and following the Declaration of Helsinki (E8) for treatment of human subjects in a study [24,25].

2.2. OA Diagnosis

The diagnostic criteria for OA released by the Chinese Medical Association in 2018 were used including the following parameters:(1) Age 18-65 years old;(2) At screening, the subject's baseline study-side knee pain score on a 100-mm VAS scale (sports state: walking) should be between 40 mm and 80 mm, excluding 80 mm (all ≥40 mm and <80 mm);(3) From the screening period until the entire trial treatment period, subjects should refrain from any non-habitual physical activity.(4) According the Chinese Medical Association Osteoarthritis treatment guide (2018 edition) it was diagnosed of knee osteoarthritis [24,25].

2.3. Study Subjects

Residents were selected based on gender and age stratification, with an equal proportion of men and women, and a ratio of 1:1 for age 18-65 years old. A total of 30 subjects were selected for this study the data show in Table1. All subjects provided informed consent.

Table1: Demographic Data and Baseline Characteristics

Characteristics	Vollapep2™ Group (n=15)	Placebo Group (n=15)	p value
Age (years)	49±10	46±15	0.287
Height (cm)	165±8.9	160±10.1	0.112
Weight (kg)	66±7.8	65±12.7	0.454
Body mass index (kg/m2)	24.1±2.5	25.6±5.2	0.138

Values presented as Mean ± SE. No significant differences were observed between the placebo groups ($p > 0.05$)

2.4. Sample Treatment

The Vollapep2™ supplement was provided by Hansford Biotech Co., Ltd. (40 mg per powder). Then every day orally supplement one package in 150ml of water for 45 days.

2.5. Outcome Measures

Outcome assessments were conducted at screening, baseline and 45 days after randomization. The patient's VAS scale (0-100 mm) and pain assessment, and the physician global disease assessment were also evaluated. The each of visit, the subject responded to the 3-point Likert-type version of the WOMAC questionnaires. Primary outcomes in this study were changes in the VAS score. Secondary outcomes included the change in WOMAC, pain, psychological function and total WOMAC score. Tertiary outcomes in this study were changes in TCM syndrome classification.

2.6. Statistical Analysis

An independent two-sample Student's t-test was used to compare differences between the treatment groups in the change from baseline for continuous outcome measures. A p-value <0.05 indicating statistical significance. All analyses were performed using GraphPad Prism 6.0.

3. Results

3.1. VAS Score

The VAS Score show in Figure1, the vollapep2™ group has significantly lower compared to the baseline in 45 days of the study (p = 0.0001). The placebo group has significantly reduced compared to the baseline in 45 days of the study (p=0.0017).

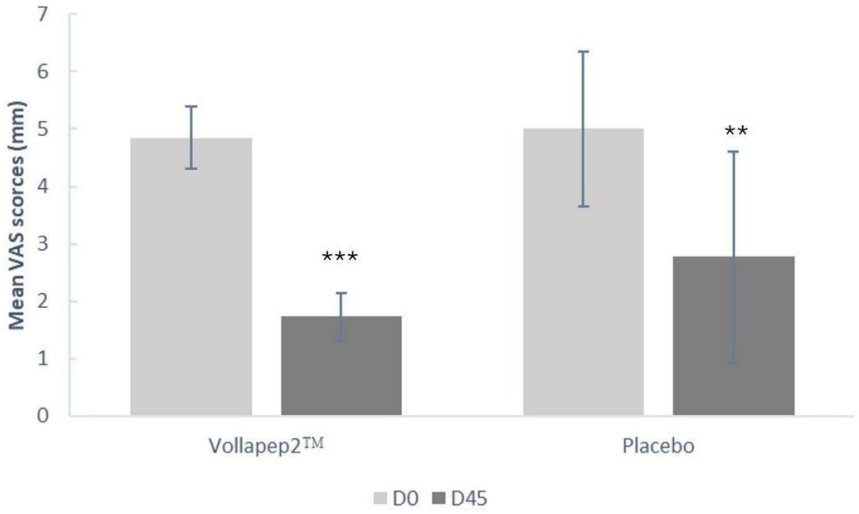

Figure 1. To evaluate of mean VAS score in Vollapep2™ groups and placebo groups over the 45days study period. Values are presented as mean ± SE. **Significant difference between 0 day and 45 days, p < 0.001. ***Significant difference between 0 day and 45 days, p < 0.0001

3.2. WOMAC Score

Whether Vollapep2™ can decrease the arthritis pain to evaluate use WOMAC index as shown in Table2. WOMAC scores were determined using the WOMAC VA3.1 questionnaire containing 24 items grouped into three categories: pain, stiffness, and physical function at 0 day and 45days. The results show that the joint pain in Vollapep2™ group were from 0 day to 45 days had a significantly lower, which got much improvement progress in comparison with placebo group. In physical function the Vollapep2™ group were from 0 day to 45 days had a significantly decrease compared to the placebo group. Since the joint stiffness in two groups the results data were not changed so did not show it. However, the total WOMAC in Vollapep2™ group from 0 day to 45 days had a significantly reduction compared to the placebo group. The joint pain reduction rate in Vollapep2™ group were 93% than the placebo group 80%. On the other hand, the physical function in Vollapep2™ reduction rate were 73% more than the placebo group 53%. Total WOMAC in Vollapep2™ reduction rate were 87% lower than the placebo group 67%. According to WOMAC Score Vollapep2™ can improve the extension of daily joint activities and reduce joint pain.

Table 2: The WOMAC-Subscale of Patients With Knee Osteoarthritis at Follow-up

Group	Vollapep2™			Placebo		
Parameters	**D45- D0(SD)**	**Reduction rate (Reduction/total)**	**p value**	**D45- D0(SD)**	**Reduction rate (Reduction/total)**	**p value**
Joint pain	-2.20±2.34	93% (14/15)	0.0018	-1.93±2.55	80% (12/15)	0.0058
Physical function	-3.07±9.71	73% (11/15)	0.1431	-3.07±7.19	53% (8/15)	0.0487
Total WOMAC	-5.27±12.94	87% (13/15)	0.0837	-5.00±10.8	67% (10/15)	0.0374

Values Presented as Mean ± se. Statistical Analysis Was Performed on 45 Days Deduct 0 day Adjusted Values.

3.3. TCM Syndrome Classification

To investigate whether the symptoms of arthritis include pain, swollen and tenderness in TCM syndrome classification scores. According to the experimental results in Table3, the symptoms of the Vollapep2™ group were significantly reduced joint pain compared to the placebo group. The joint pain reduction rate in Vollapep2™ group were 67% than the placebo group 60%. In the joint swollen were alleviated reduction compared to the placebo group. The joint swollen reduction rate in Vollapep2™ group were 33% than the placebo group 26%. The Joint swollen reduction extent ratio is not significant. Whether the joint tenderness in Vollapep2™ group were lower than the placebo group. The joint tenderness reduction rate in Vollapep2™ group and the placebo group were 67%. Nonetheless the total symptoms in Vollapep2™ group were more alleviate than the placebo group. The total symptoms reduction rate in Vollapep2™ group were 87% than the placebo group 67%. In TCM syndrome classification scores reduction percentage is not significant, maybe subjective experience is different, but the total symptoms reduction ratio compared to the WOMAC score is the same.

Tables 3: The TCM Syndrome Classification Subscale of Patients With Knee Osteoarthritis

Group	Vollapep2 ™			Placebo		
Parameters	**D45-D0(SD)**	**Reduction rate (Reduction/total)**	**p value**	**D45-D0(SD)**	**Reduction rate (Reduction/total)**	**p value**
Joint pain	-1.73±1.67	67% (10/15)	<0.0001	-1.47±1.41	60% (9/15)	0.0036
Joint swollen	-0.53±1.19	33% (5/15)	0.1233	-0.40±1.12	26% (4/15)	0.1356
Joint tenderness	-1.20±1.26	67% (10/15)	0.0043	-1.33±1.45	67% (10/15)	0.0054
Total symptoms	-3.20±3.76	87% (13/15)	0.0089	-3.60±3.79	67% (10/15)	0.0007

Values Presented as Mean ± SE. Statistical Analysis Was Performed on 45 Days Deduct 0 Day Adjusted Values.

3.4. Safety Assessments

No statistically significant changes were reported for any change to blood biochemistry, liver and renal function show in Table 4. Observe all subjects for any adverse events that occur during the clinical study, including abnormal clinical symptoms and vital signs, and abnormalities in laboratory tests.

Table 4: Safety Parameter Assessment at Baseline and 45 Days In Placebo Group And Vollapep2™ Group

Parameters	Baseline			45 days		
	Vollapep2™ group	**Placebo group**	**p value**	**Vollapep2™ group**	**Placebo group**	**p value**
Red blood cell count, RBC (× 10^{12}/L) RBC (× 10^{12}/L)	4.35±0.52	4.35±0.46	0.494	4.5±0.43	4.45±0.5	0.404
Hemoglobin (g/L)	131.15±15.22	133.77±11.13	0.311	135.18±12.62	136±14	0.441
White Blood Cell 9 count (× 10 /L)	5.2±1.13	5.85±0.89	0.058	5.41±1.22	6.56±1.39	0.052
9 Neutrophil (× 10 /L)	3.04±0.9	3.57±0.68	0.052	3.28±1.04	4.05±0.71	0.052
Lymphocyte count (× 9 10 /L)	1.75±0.42	1.83±0.4	0.314	1.7±0.3	2.24±1.09	0.065
Platelet count (× 9 10 /L)	189.39±47.4	217.54±70.09	0.121	190.73±51.76	235.23±60.41	0.034
Alanine transaminase, ALT(U/L)	15.88±6.8	14.21±6.04	0.086	12.46±4.67	13.76±3.63	0.411
Aspartate Aminotransferase, AST (U/L)	18.23±5.45	17.73±5.32	0.198	16.57±3.55	16.05±4.72	0.201
Total Bilirubin (umol/L)	11.02±3.04	12.71±4.95	0.117	13.09±5.17	10.62±5.55	0.160
Urea/ BUN (mmol/L)	5.75±1.86	5.21±1.97	0.045	4.52±1.46	5.27±1.51	0.465
Creatinine (umol/L)	64.34±13.29	60.45±16.21	0.321	63.35±13.37	56.33±16.56	0.264

Values presented as Mean ± SE. No significant differences were observed between the placebo groups ($p > 0.05$)

4. Discussion

Our research found that plant-based WG hydrolyzed peptides, assessed using VAS, WOMAC, and TCM, effectively reduced osteoarthritis, relieved joint swollen and tenderness, then improved quality of life at 45 days. Previous health supplements for reducing osteoarthritis included UCII, derived from type II collagen from chicken breast cartilage. These supplements assess VAS, WOMAC, and Lequesne's index, effectively relieved joint inflammation, swelling, and pain, promoted cartilage health, and slowed joint degeneration [9-11].

Assessment using the VAS scale revealed that, compared to the placebo group, Vollapep2™ significantly reduced osteoarthritis pain index from 0 day to 45 days. The VAS scale accurately quantifies pain levels to track whether joint pain intensity has decreased or increased. The WOMAC assessment measures limitations and inconvenience in daily activities, differing from the VAS. The WOMAC assessment includes joint pain, stiffness, and physical function. Regarding joint pain, Vollapep2™ significantly reduced joint pain from 0 day to 45 days compared with the placebo group. However, there was no significant difference in joint stiffness between the two groups. This may be because Vollapep2™ is a plant-derived hydrolyzed peptide protein and does not significantly affect stiffness like UCII, which is derived from animal-derived type II collagen. However, regarding physical function, Vollapep2™ significantly improved physical function on 45 days compared with the placebo group. Regarding the total WOMAC score, Vollapep2™ significantly improved the total WOMAC score on 45 days compared with the placebo group. This study found that Vollapep2™, assessed using the WOMAC scale, can reduce osteoarthritis, improve quality of life, and does not cause inconvenience in daily activities.

Since the diagnostic criteria for human trials were based on the Chinese Medical Association in 2018 Manual, the four items of joint pain, swelling, tenderness, and stiffness were assessed using the TCM symptom grading and quantification standards. Firstly, regarding joint pain, compared to the placebo group, Vollapep2™ significantly reduced joint pain on 45 days. Regarding joint swelling, compared to the placebo group, Vollapep2™ reduced joint swelling on 45 days, although the reduction was not significant, and the difference was statistically significant. However, the joint tenderness, compared to the placebo group, Vollapep2™ reduced joint tenderness on 45 days, and the difference was statistically significant. Furthermore, in the stiffness assessment and the WOMAC assessment scale, there was no significant difference between the two groups, suggesting that the plant-based WG hydrolyzed peptides protein, unlike animal-based proteins, significantly alleviates stiffness. Finally, Vollapep2™ significantly reduced overall scores on 45 days and increased joint mobility. This combination of assessments suggests that Vollapep2™ may reduce joint pain, swelling, and tenderness through immune regulation, thereby improving daily joint mobility and flexibility.

WG protein has been shown in previous studies to have the ability to activate the immune system, and previous research has confirmed that the gut immune system is related to type 1 diabetes. Dendritic cells (DC) can be able to activate both the innate and adaptive immune system and are responsible for regulating oral tolerance and immunity in the intestine. Further gliadin is known to stimulate several innate parameters in vitro using cells from non-diabetic BALB/c mice [15-18].

WG protein has been used extensively in previous studies, such as in the repair of bone and joint scaffolds and the reconstruction of cartilage tissue [20]. Due to its highly cross-linked structure, wheat protein can be used to design ultrafine fiber scaffolds using 3D technology, mimicking the natural extracellular matrix of soft tissues (such as adipose tissue). This allows it to support the attachment, proliferation, and adipogenicity differentiation of adipose-derived mesenchymal cells [21]. Furthermore, a composite scaffold formed by combining nano-magnesium phosphate and wheat protein has been applied to bone tissue repair.

Cell culture experiments showed that the composite scaffold significantly promoted the proliferation, differentiation, and infiltration of MC3T3-E1 cells, resulting in a sustained increase in newly formed bone tissue. Immunohistochemical analysis further confirmed the stimulatory effect of the composite scaffold on osteogenic cell differentiation and new bone regeneration, demonstrating its potential for bone tissue repair [22,23]. The blood analysis results for blood, liver, and kidney function showed no significant changes or differences, indicating that the plant-based hydrolyzed peptide protein is a safe health supplement that can relieve joint pain, swelling, and tenderness through immune regulation, thereby improving daily joint mobility and flexibility.

5. Conclusion

This study found that Vollapep2™, a nutritional ingredient come from plant hydrolyzed wheat peptides, that significantly improved knee function in OA subjects by 45 days, compared to placebo group. Based on these data can elevate joint mobility and flexibility and improve quality of life.

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Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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