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Article

Therapeutic Effect of Enzymatic Hydrolyzed Cervi Cornu Collagen Np-2007 and Application in Osteoarthritis

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Abstract: Cervi cornu has been extensively used in traditional medicine as an antioxidant and for the treatment of various disorders, including osteoporosis. However, since it is not easy to separate the active ingredient, absorption is not performed well. In this study, we extracted the low molecular weight (843Da) collagen NP-2007 from cervi cornu by enzyme hydrolyzation, and evaluated the therapeutic effect in a monosodium iodoacetate (MIA)-induced rat osteoarthritis model, and identified their molecular mechanisms. NP-2007 was orally administered at 50, 100, and 200 mg/kg for 21 d. Efficacy was evaluated based on serum parameters, inflammatory mediators, and morphological and histopathological analyses of knee joints. Production of matrix metalloproteinases, including MMP-2, MMP-3, and MMP-9, in the serum was higher in MIA-treated rats; however, these levels decreased after NP-2007 treatment. Serum levels of tumor necrosis factor-α, interleukin (IL)-1β, IL-6, and prostaglandin E2 were lower in the NP-2007 group than in the MIA group. Furthermore, the administration of NP-2007 resulted in effective preservation of both the synovial membrane and knee cartilage, and significantly decreased the transformation of fibrous tissue. This study indicates that NP-2007 can prevent and alleviate symptoms of osteoarthritis and have applications as a novel arthritis treatment.

Keywords: enzyme-hydrolyzation; cervi cornu collagen; NP-2007; osteoarthritis; monosodium iodoacetate

1. Introduction

Osteoarthritis (OA) is a prevalent degenerative joint disease that primarily affects cartilage and is accompanied by inflammation in the synovial tissue [1, 2]. OA occurs when there is an imbalance between the synthesis and degradation of proteoglycans and collagen in cartilage cells [3]. Common manifestations of osteoarthritis (OA) encompass gradual deterioration of articular cartilage, mild inflammation in the intra-articular and periarticular tissues, and occasionally the development of osteophytes and subchondral cysts in the affected joints [2], eventually leading to pain and disability, reducing the quality of life [4] Therefore, the aims of osteoarthritis treatment are to reduce pain, alleviate the disease, or prevent further cartilage damage [5]. The recognition of inflammation as a crucial factor in the development and progression of arthritis has led to the emergence of anti-inflammatory agents as a promising therapeutic strategy for the treatment of this condition. Several studies have highlighted the involvement of inflammation in the pathogenesis of arthritis, thereby emphasizing the importance of targeting inflammatory processes for effective management of the disease [6]. Although

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non-steroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed for the treatment of OA, their long-term use can be associated with various side effects. These include an increased risk of gastrointestinal complications such as bleeding and stomach ulcers, particularly when used over an extended period of time [7]. Therefore, there is a need for alternative treatment approaches that provide effective pain relief and anti-inflammatory effects without the potential risks associated with long-term NSAID, and recent studies have focused on the development of safer and more effective treatment strategies [8].

Cervi cornu (Deer antler) has various pharmacological, analgesic and anti-inflammatory effects [9]. It has been reported that deer antlers prevent osteoporosis, and treat breast enlargement and mastitis [10, 11]. In addition, velvet antler, which refers to the cartilaginous antler in its precalcified stage, has a long history of use in traditional Chinese medicine and as functional foods. For thousands of years, it has been recognized for its potential in promoting overall wellness and maintaining good health [12]. Recent studies have highlighted the pharmacological potential of velvet antler [13], wound-healing [9], and anti-cancer effects [14]. In particular, anti-inflammatory peptides consisting of 68 amino acid residues (7.2 kDa) have been identified from the antlers of Cervus nippon Temminck [15]however, there is a lack of extensive information regarding the specific anti-inflammatory peptides derived from protein hydrolysates of velvet antler, including their primary structure. Recent research findings have indicated that the use of tortoise shells and antler gelatins can lead to improvements in serum insulin-like growth factor levels and stimulate the proliferation of osteoblasts [16]. Although the cervi cornu has various functional benefits, the effect of the low-molecular-weight collagen derived from deer antlers has not been reported. Actually, hydrolyzed collagens consist of amino acids and peptides, including dipeptides and tripeptides, that are resistant to intracellular hydrolysis and systemic hydrolytic enzymes, preventing their degradation by peptidases. As a result, these peptides have high bioavailability, enabling them to enter the bloodstream and accumulate in cartilage tissue. They stimulate chondrocytes and induce the synthesis of cartilage extracellular matrix (ECM), promoting cartilage health and regeneration. The bioavailability of amino acids and peptides derived from hydrolyzed collagens plays a crucial role in understanding the effects of these products on joint health. Studies have shown that peptides resistant to intracellular hydrolysis have smaller molecular weights, which facilitates their absorption in the intestines. This enhanced intestinal absorption leads to higher bioavailability of these peptides, allowing them to exert their effects at the articular level and potentially promote joint health and function[17].

Here, we found that enzyme-hydrolyzed cervi cornu collagen NP-2007 alleviated Monosodium iodoacetate (MIA)-induced OA. Therefore, we investigated the anti-inflammatory effects and molecular mechanisms of NP-2007, using a MIA-induced model and evaluated their potential for the treatment of OA. Moreover, we examine the anti-inflammatory properties of NP-2007 by evaluating its effects on RAW264.7 cells that were stimulated with LPS. The findings of this study offer valuable insights into the prevention and treatment of arthritis, presenting potential strategies that could enhance the quality of life for individuals affected by this condition. By elucidating the underlying mechanisms and exploring potential therapeutic approaches, this research holds promise for advancing arthritis care and improving patient outcomes.

2. Results

2.1. NP-2007 Mitigated Articular Cartilage in MIA-induced Osteoarthritis Rats

Following an injection of MIA to induce OA, the SD rats were orally administered with NP-2007 (50, 100, 200 mg/kg) for three-week period (Figure 1A). After sacrificed, we applied micro-CT to examine the effects of NP-2007 on the architecture of knee joints and to implement a morphological evaluation of the articular cartilage. Compared to the

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control group, the MIA treated group exhibited more deterioration of the distal femur bone architecture and decreased cartilage volume (Figure 1B). However, NP-2007 treatment efficiently preserved the architecture of the femur bone and enhanced cartilage volume.

Additionally, histopathological analysis was conducted on the knee cartilage using H&E and safranin O staining to assess the potential alleviating effect of NP-2007. The H&E staining demonstrated the presence of well-preserved cartilage in the control. However, the NC group exhibited damaged cartilage and an increased infiltration of inflammatory cells. In contrast, the NP-2007 treated groups exhibited recovered cartilage and reduced inflammatory cell infiltration group (Figure 1C). Moreover, safranin-O staining revealed decreased proteoglycan volumes in the MIA treated group, which was ameliorated by NP-2007 administration (Figure 1D).

These results indicated that NP-2007 exerts anti-inflammatory effects and protects the knee joint structure.

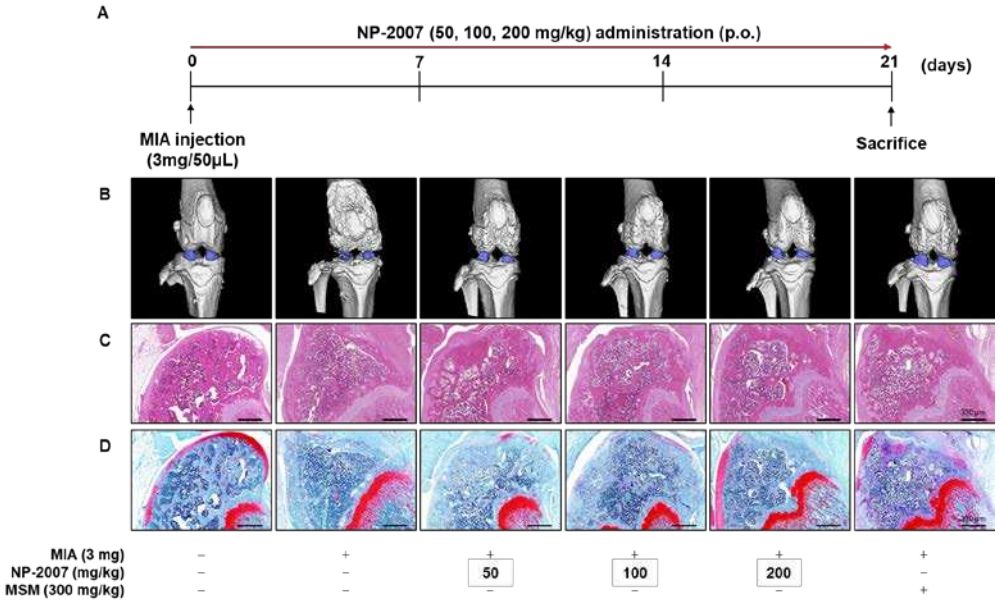

Figure 1. Effect of low molecular weight cervi cornu collagen (NP-2007) on morphological and histopathological analyze from knee joint tissue of MIA-induced osteoarthritis rats. Experimental scheme of monosodium iodoacetate (MIA)-induced osteoarthritis model in rats (A). Vehicle, NP-2007 (50, 100, 200 mg/kg) or MSM (300mg/kg) were orally administered for three weeks. Morphological and histopathological analysis showing the effect of NP-2007 on knee joint tissue of monosodium iodoacetate (MIA)-induced osteoarthritis rats. Representative (B) morphological image of the knee joint tissues measured using micro-CT. The representative histopathological images of (C) Hematoxylin & Eosin (H&E) and (D) safranin-O staining. Magnification: 40 ×.

2.2. NP-2007 Alleviated Inflammation in MIA-Induced Osteoarthritis Rats

Serum levels of inflammatory factors and cytokines were evaluated in MIA-induced arthritic rats to investigate their effects on inflammation. The ELISA results demonstrated that MIA-induced OA rats had significantly elevated serum levels of TNF-α, IL-1β, IL-6 and PGE2, and that NP-2007 administration effectively reversed these alterations (Figure 2). These findings suggested that in rats with OA, NP-2007 might alleviate inflammation by suppressing inflammatory factors.

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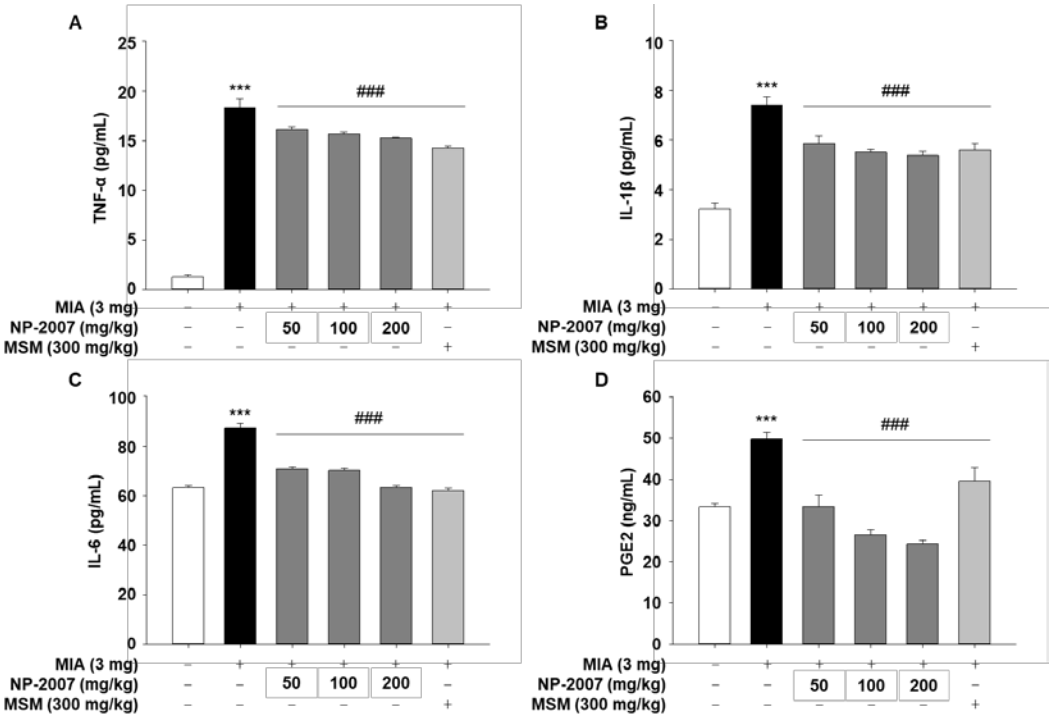

Figure 2. Effect of NP-2007 on inflammatory mediator and pro-inflammatory cytokine levels in serum of MIA-induced osteoarthritis rats. ELISA was used to quantify the amount of (A) tumor necrosis factor (TNF)-α, (B) interleukin (IL)-1β, (C) IL-6, and (D) prostaglandin E2 (PGE2) in serum.

All values represent the mean ± SD. Data were examined using Duncan's multiple comparison test. ***P <0.001 vs. control group; ###P <0.001 vs. MIA injected group.

2.3. NP-2007 Modulated the Matrix Metalloprotease levels in MIA-induced Osteoarthritis Rats

Inflammation in the joint is triggered by inflammatory factors and pro-inflammatory cytokines, which promote the excretion of cartilage-degrading enzymes such as MMPs, leading cartilage degradation to accelerate [19]. So, we analyzed the serum levels of MMP-2, MMP-3, and MMP-9 to determine whether NP-2007 directly affects MMP expression. The serum levels of MMP-2, -3, and -9 significantly increased after MIA injection, however, NP-2007 effectively suppressed MMP-2 and -3 levels in MIA-induced OA rats (Figure 3). However, for the MMP-9 level, a significant difference was observed only at 200 mg/kg. These results suggested that NP-2007 can decrease MMP production and the degradation of cartilage and bone in arthritic joints.

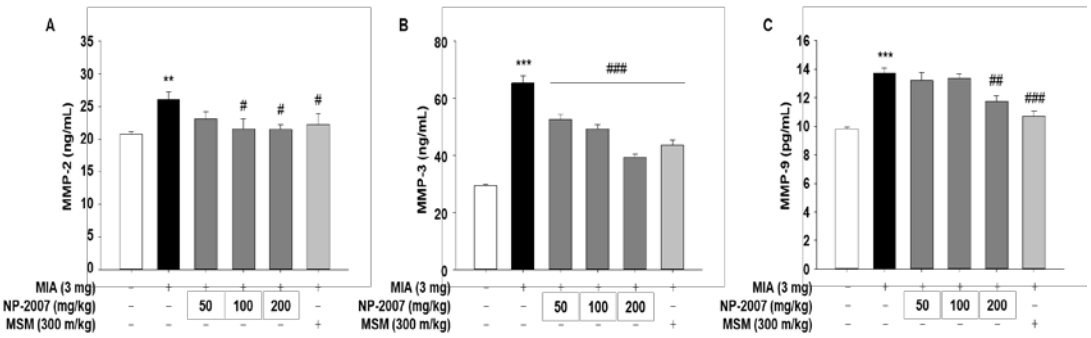

Figure 3. Effect of NP-2007 on matrix metalloproteinase (MMP) production in serum of MIA-induced osteoarthritis rats. (A) MMP-2, (B) MMP-3, and (C) MMP-9 production were measured using enzyme-linked immunosorbent assays (ELISA). All values represent the mean ± SD (n = 7). Data were examined using Duncan's multiple comparison test. **P <0.01 and ***P <0.001 vs. control group; #P <0.05, ##P <0.01 and ###P < 0.001 vs. MIA injected group.

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2.4. NP-2007 Suppresses the NF-κB Signaling in LPS-induced RAW264.7 Macrophages

To identify the molecular mechanisms related with inflammation, we used the RAW264.7 cells and confirmed whether NP-2007 could mitigate LPS-induced inflammatory effects. First, to evaluate cytotoxicity, RAW 264.7 cells were applied to various concentrations of NP-2007 and the MTS assay was implemented to evaluate cell viability. Cell viability was not significantly impacted by NP-2007 at concentrations up to 1,000 μg/mL (Figure 4A). The NP-2007 concentrations (100, 250, and 500 μg/mL) were employed in the investigations that followed.

To evaluate the inhibitory effects of NP-2007 on LPS-induced NO production, various doses of NP-2007 were applied to RAW 264.7 cells, and they were then incubated for 18 h with or without LPS (1 μg/mL). The production of NO was significantly and in dose-dependent manner reduced by NP-2007, as shown in Figure 4B. NP-2007 substantially inhibited LPS-induced protein expression of iNOS, one of the major enzymes responsible for NO production (Figure 4C, D). NP-2007 also attenuated LPS-induced COX-2 protein expression. To investigate the molecular mechanism via which NP-2007 inhibits inflammatory responses, we evaluated the protein expression of phosphorylated NF-κB, which is a critical transcription factor that regulates the production of pro-inflammatory cytokines. As shown in Figure 4C and 4D, LPS treatment stimulated the phosphorylation of NF-κB in RAW264.7 cells. However, this effect significantly decreased in a dose-dependent manner with NP-2007.

These findings suggested that NP-2007 treatment affects the inflammation reaction in RAW264.7 cells inflammatory response through altering the NF-κB signaling pathway.

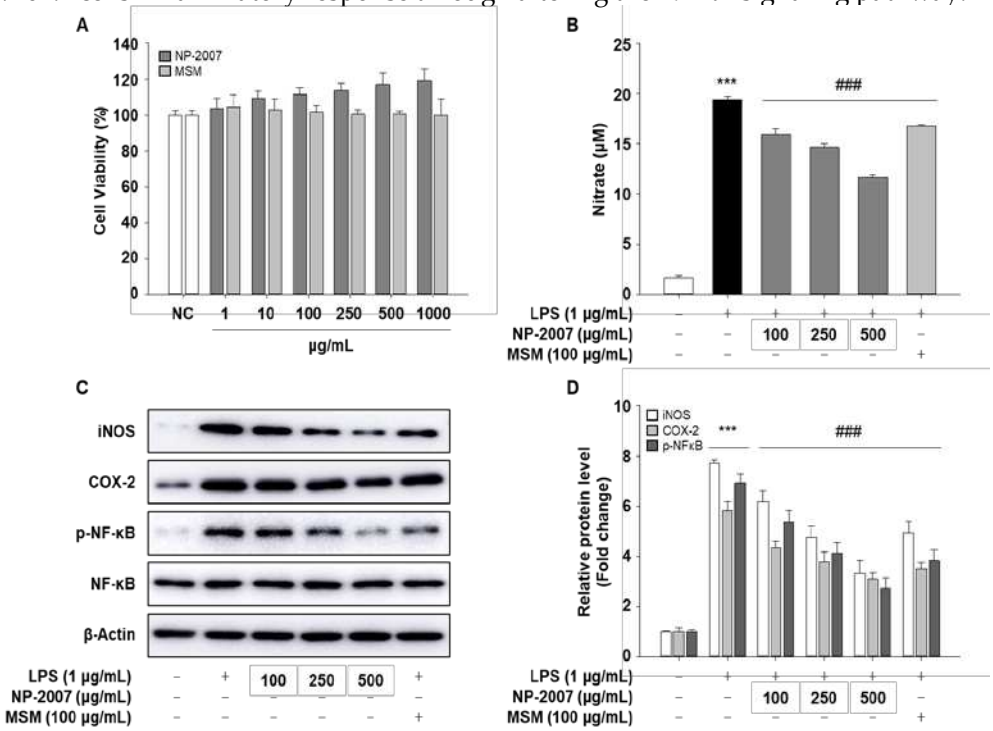

Figure 4. Inhibitory effect of NP-2007 on LPS-induced nitric oxide (NO) production and the NF-kB pathway in RAW 264.7 cells. (A) Effects of NP-2007 on RAW 264.7 cell viability. (B) NO production was determined in supernatants from RAW 264.7 cells. (C) Protein expression of iNOS, COX-2, and NF-kB signaling pathways were determined via western blotting. SDS-PAGE effectively processed equivalent amounts of total proteins. (D) Quantitative analysis of blots. The densitometry results are represented as the relative protein bands densities normalized to the β-actin level. All values represent the mean ± SD. Data were examined using Duncan's multiple comparison test. ***P <0.001 vs. control group; ###P <0.001 vs. LPS group.

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2.5. NP-2007 Suppresses the Pro-Inflammatory Cytokine Production and MAPK Signaling in LPS-induced RAW264.7 Macrophages

To investigate whether NP-2007 could mitigate LPS-induced pro-inflammatory cytokine levels, RAW 264.7 cells were pretreated with NP-2007 (100, 250, or 500 μg/mL) for 1 h before being stimulated with LPS (1 μg/mL) for 18 h. In contrast to untreated cells, LPS-treated RAW 264.7 macrophages created pro-inflammatory cytokines (TNF-a, IL-1β, and IL-6), as demonstrated by ELISA analyzes (Figure 5A, B, C). However, NP-2007 application significantly and independently lowered the levels of cytokines IL-6 and IL-1β released by LPS-treated macrophages.

In comparison to the LPS-only control, NP-2007 significantly enhanced the amount of the anti-inflammatory cytokine IL-10 that is produced when LPS is present (Figure 5D). These findings suggested that NP-2007 improved IL-10 production and attenuated LPS-induced pro-inflammatory cytokine production in RAW 264.7 macrophages.

TNF-α, IL-1b, and IL-6 cytokines cause inflammation by activating critical proteins in signaling pathways like NF-kB and MAPK. We further investigated whether NP-2007 inhibited LPS-induced MAPKs signaling. As MAPK is stimulated with phosphorylation, we performed western blotting to evaluate the levels of phosphorylation of JNK, ERK, and p38 MAPK in RAW 264.7 macrophages using western blotting. In cells treated with LPS, increased levels of phosphorylation of all MAPK signals were observed compared to the N group. NP-2007 markedly inhibited the LPS-induced phosphorylation of ERK, JNK, and p38.

These results suggested that NP-2007 could exert to its protective effects against LPS-induced RAW264.7 macrophages through modulating MAPK and NF-κB signaling.

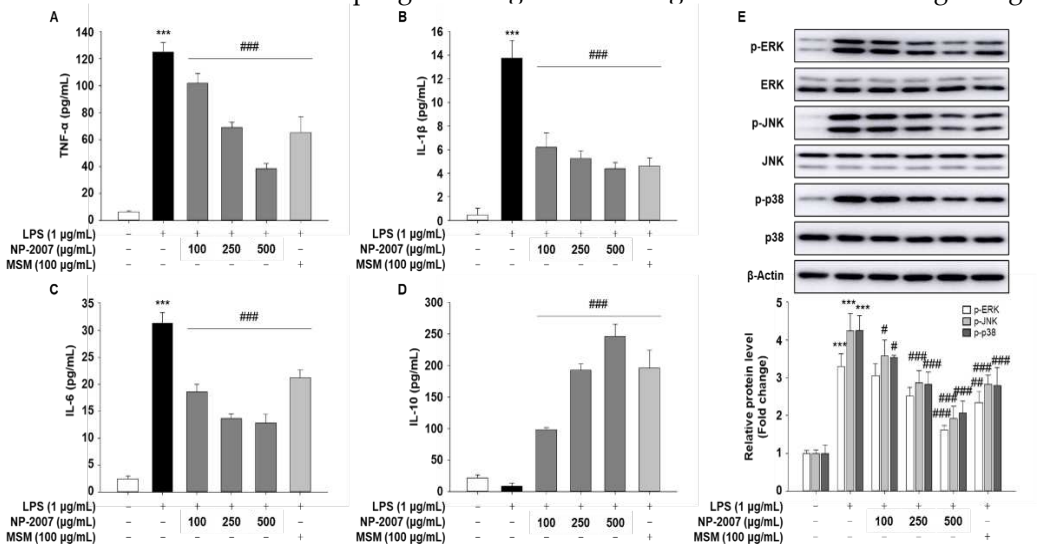

Figure 5. Inhibitory effect of NP-2007 on LPS-induced inflammatory cytokine production and the MAPK pathway in RAW 264.7 cells. Concentrations of (A) tumor necrosis factor (TNF)-α, (B) interleukin (IL)-1β, (C) IL-6, (D) prostaglandin E2 (PGE2) in RAW 264.7 macrophages. Protein expression of (E) MAPK signaling pathways were determined via western blotting. SDS-PAGE effectively processed equivalent amounts of total proteins. Quantitative analysis of blots. The densitometry results are represented as the relative protein bands densities normalized to the β-actin level. The total ERK, JNK, or p38 is shown as a loading control. All values represent the mean ± SD. Data were examined using Duncan's multiple comparison test. ***P <0.001 vs. control group; #P < 0.05, ##P < 0.01 and ###P < 0.001 vs. LPS group.

3. Discussion

In this study, we demonstrated, for the first time, that a small molecular weight collagen from deer horn, NP-2007, is effective against MIA-induced osteoarthritis. Although traditionally considered a non-inflammatory joint disease due to its primary association with cartilage degeneration, it is now well established that inflammation plays a

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critical role in the etiology of OA. Inflammation contributes to various pathological changes observed in OA, highlighting the importance of understanding and targeting the inflammatory processes for effective management and treatment of the disease [20]. Hence, the identification of inflammation and pain as potential therapeutic targets opens up new avenues for the development of novel drugs aimed at addressing these specific aspects of OA. By targeting inflammation and pain mechanisms, researchers and clinicians can work towards the development of more effective and targeted treatment strategies to alleviate symptoms, slow down disease progression, and improve the quality of life for individuals with OA.

The primary objectives of OA treatment include managing pain, minimizing cartilage damage, slowing down the progression of the disease, relieving symptoms, and improving or maintaining functional abilities. By addressing these goals, healthcare professionals aim to enhance the overall well-being and quality of life for individuals affected by osteoarthritis [21]. Despite the known limitations in the therapeutic efficacy and the potential for side effects such as cardiovascular risk, gastrointestinal upset, diarrhea, vomiting, nausea, and renal toxicity, common treatments for OA such as acetaminophen, NSAIDs, and opioids have remained largely unchanged in recent decades [21, 22]. Hence, there is a critical need to explore and develop natural products as potential treatments for OA. In recent research, various traditional medicinal herbs have been extensively studied as potential sources for alternative and complementary approaches in managing OA. These studies aim to explore the use of dietary supplements, functional foods, and nutraceuticals derived from these herbs, with the goal of reducing pain and slowing down the progression of the disease [23, 24]. In this study, we used a small molecular weight collagen, NP-2007, from deer horns. We identified its anti-inflammatory properties.

Actually, collagen include insoluble undenatured native collagens, soluble native collagens, denatured collagens (gelatins), collagen hydrolysates, and collagen peptides. Each product is obtained through specific manufacturing processes, resulting in variations in structure, composition, and properties[25]. Collagen hydrolysates, formed through chemical or enzymatic hydrolysis, lack the triple helix structure and consist of amino acids and peptides. Their composition varies based on collagen source and hydrolysis method such as pepsin, alcalase, papain, with molecular weights typically ranging from 1 kDa to 10 kDa[26]. We used the cervi cornu-derived collagen hydrolysate with a molecular weight of 843 Da. Similar to other approved joint health functional materials, we studied joint health in an MIA-induced OA model. MIA is a compound that is commonly used in experimental models to induce OA-like pathology in rodents. By inhibiting the enzyme glyceraldehyde-3-phosphatase, MIA causes chondrocyte apoptosis and leads to cartilage degeneration [27]. This MIA-induced OA model is valuable for studying the mechanisms underlying OA development and evaluating potential therapeutic interventions for the disease. This model triggers inflammatory pain and therefore, is the most commonly used model, especially for pain treatment [28, 29]. Moreover, when inflammation is activated, NF-κB activated in macrophages induces the release of interleukins, which causes caspase-mediated chondrocyte damage and arthritis [30]. Through MIA induction, the joint was degraded, and MMPs were upregulated. We confirmed that the joint was degraded via MIA, and these effects were reversed upon NP-2007 treatment. Histopathological studies also revealed that the pathogenesis was reversed upon NP-2007 treatment.

Numerous research studies have consistently shown elevated levels of inflammatory cytokines, including IL-6, IL-1β, and TNF-α, in the knee joints of individuals with OA. The stimulation of cartilage tissue by pro-inflammatory cytokines has been associated with various structural changes that are characteristic of OA. These findings highlight the crucial role of inflammation in the pathogenesis and progression of OA, providing insights into potential therapeutic targets for managing the disease [31-33]. Additionally, individuals with OA often experience joint cartilage loss and disruption of

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bone structure, leading to the manifestation of joint pain [34]. So, the objective of this study was to demonstrate and examine these biomarkers in order to gain a better understanding the role of NP-2007 in the development and progression of OA. NP-2007 demonstrated inhibitory effects on the inflammatory response by reducing the expression of inflammatory mediators such as COX-2 and iNOS, along with cytokines including TNF-α, IL-1β and IL-6. In addition, NP-2007 decreased joint cartilage loss and disruption of bone structure by downregulating MMPs. Through our mechanistic investigation, we observed that NP-2007 exerts its effects by inhibiting the NF-kB and MAPK signaling pathways. Our findings highlight the potential of NP-2007 as a therapeutic agent for OA by targeting key pathways involved in the regulation of inflammation.

Collectively, our findings suggested that NP-2007 has anti-inflammatory effects and may be useful for the prevention and treatment of OA and other inflammation-related diseases.

4. Materials and Methods

4.1. Low Molecular Weight Cervi Cornu Collagen (NP-2007) Preparation

NP-2007 was prepared by enzyme hydrolyzation of cervi cornu and commercialized as a food ingredient (NP-2007) by Hanpoong Nature Pharm. Briefly, cervi cornu was extracted with purified water at 95~99 °C for 3 h for 9 times. Thereafter, extract was filtered and concentrated. The first hydrolysis was performed with neutralase and then the second hydrolysis was performed with flavorzyme. After hydrolysis, it was vacuum dried and used as a food raw material.

4.2. Cell Culture

RAW 264.7 macrophages were acquired from the American Type Culture Collection (Manassas, VA, USA) and cultivated at 37 °C with 5% CO2 environment in Dulbecco's modified Eagle's medium (DMEM) with penicillin-streptomycin sulfate (100 units/mL and 100 g/mL) and 10% fetal bovine serum.

4.3. Cell Viability Assay

Cell viability was determined utilizing the MTS assay. In 96-well culture plates, cells (1×10^4 cells/well) were seeded and treated for 24 h with small molecular weight

cervical cornu collagen (NP-2007) dissolved in DMSO. The cells were then given 10 µL of MTS solution in each well, and the cells were incubated for a further 4 h. At 490 nm, absorbance was determined utilizing a microplate reader (Multiskan Go, Thermo Scientific, Waltham, MA, USA). The values of the control were considered 100% viable.

4.4. Nitric Oxide (NO) Assay

Cells (5×10^5 cells/well) were placed in 6-well plates and cultured for 18 h with NP-2007 (100, 250, and 500 µg/mL) and LPS (1 µg/mL) for 18 h. The culture medium was analyzed using colorimetric assay kits (iNtRON Biotechnology, Sungnam, Korea) following the manufacturer's requirements. The established standard curve for sodium nitrite ($NaNO_2$) was used to calculate the nitrite concentration.

4.5. Animals

Male Sprague-Dawley rats (7-weeks-old) were purchased from Damul Science (Daejeon, Korea). The typical housing settings for rats were 22 ± 2 °C, 55 ± 5% humidity (12 h light/dark cycles). Rats were randomly divided into six groups. Before the experiments began, rats were given a week to get used to the conditions in the laboratory and were allowed free access to food and drink. The Jeonju AgroBio-Materials Institute's Animal Care Committee evaluated and approved the experimental procedure for this study, and strict compliance to the committee's guidelines (JAMI IACUC 2022001).

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4.6. Induction of MIA-induced arthritis in rats

Rats with MIA-induced arthritis were generated as previously described [18]. First, the rats were anesthetized with avertin (250 mg/kg intraperitoneally). Using an insulin syringe, MIA solution consisting of 3 mg of MIA dissolved in 50 μL of 0.9% saline was injected precisely into the intra-articular space of the right knees of rats using an insulin syringe. After MIA injection, the rats with MIA-induced OA were treated with NP-2007 at various doses (50, 100, and 200 mg/kg) of NP-2007 dissolved in water for 21 d. The positive control group was treated with 300 mg/kg methyl sulfonyl methane (MSM). Distilled water was orally administered to the N and NC groups.

4.7. Cytokine Assay

Cell supernatants and rat serum were prepared to measure cytokine concentrations. The enzyme-linked immunosorbent assay (ELISA) kits provided by R&D Systems were used for assessing the levels of matrix metalloproteinase (MMP)-9, interleukin (IL)-1β, IL-6, prostaglandin E2 (PGE2) and tumor necrosis factor-α (TNF)-α. The MMP-2 and MMP-3 concentrations were measured using Quantikine ELISA kits (Abcam, Cambridge, UK). Instructions provided by the manufacturer were followed for all measurements.

4.8. Immunoblotting

SDS-PAGE (8% or 10%) gels with polyvinylidene difluoride membranes (GE Healthcare, Little Chalfont, Buckinghamshire, UK) were utilized to resolve proteins (20 μg per lane). Primary antibodies were incubated with the blots overnight at 4 °C. After blocking buffer washes, secondary antibodies combined with horseradish peroxidase were incubated with the blots for 1 hour at room temperature. An enhanced chemiluminescence system (Bio-Rad, Munich, Germany) was used to detect antibody binding. Densitometric scanning (Amersham Imager 600; GE Healthcare) was utilized to evaluate all immunoreactive signals.

4.9. Histological Analysis

The microarchitecture of the knee joints was measured by a microcomputed tomography (micro-CT) system (Sky-Scan 1076; SkyScan, Belgium). The tissues of knee joint were fixed for 24 h in 4% paraformaldehyde. Sections were embedded in paraffin after being fixated.

The serial sections, measuring 4 μm in thickness, were mounted onto slides coated with silane and subsequently stained for the detection of proteoglycans with Safranin O or for general histopathology with hematoxylin and eosin (H&E). A microscope using an optical system was employed to examine and photograph images of all stained specimens (Olympus, Tokyo, Japan).

4.10. Statistical Analyses

The data represent the means ± standard deviation (SD) derived from a minimum of three separate experiments. Student's t-test was applied to compare parameters between two groups, whereas while analysis of variance (ANOVA) followed by Duncan's post hoc test was employed to compare parameters among the three groups. Statistics were judged significant at $P < 0.05$.

5. Conclusions

In our study, we presented novel findings indicating that NP-2007 exhibited inhibitory effects on MIA-induced OA, and this is the first study to demonstrate the anti-inflammatory properties of NP-2007, which was shown to modulate the MAPK and NF-κB signaling pathways. These results provide valuable insights into the potential therapeutic applications of small molecular collagen in mitigating inflammation-associated conditions. Building upon the promising outcomes of our preclinical

study, we are currently in the process of designing a clinical study to investigate the effects of small molecular weight collagen on patients diagnosed with OA.

Supplementary Materials:

Author Contributions: Conceptualization, H.-R.K., M.H.P., S.-Y.K., and B.S.C.; methodology, H.-R.K.; software, H.-R.K. and S.-H.L.; validation, H.-R.K. and S.-H.L.; formal analysis, H.-R.K. and S.-H.L.; investigation, E.-M.N.; resources, B.S.C.; data curation, H.-R.K., S.-H. L., and M.H.P. ; writing—original draft preparation, H.-R. K, M.-H.P, and S.-Y.K.; writing, review, and editing, M.-H. P., S.-Y.K; visualization, M.-H.P. and S.-Y.K.; supervision, S.-Y. K; project administration, H. Y. S., H.S.J., and S.-Y. K.; funding acquisition, H. Y. S., H.S.J., and S.-Y.K. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The animal study protocol was approved by the Institutional Animal Care and Use Committee of Jeonju Agrobio-Materials Institute (approval number: JAMI IACUC 20220101).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available upon request.

Acknowledgments:

Conflicts of Interest: The authors declare no conflicts of interest.

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