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Chondroprotective and anti-inflammatory effects of ChondroT, a new complex herbal medication

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Abstract

Background: Ganghwaljetongyeum (GHJTY) is a complex herbal decoction comprising 18 plants; it is used to treat arthritis. In order to develop a new anti-arthritic herbal medication, we selected 5 out of 18 GHJTY plants by using bioinformatics analysis. The new medication, called ChondroT, comprised water extracts of *Osterici Radix*, *Lonicerae Folium*, *Angelicae Gigantis Radix*, *Clematidis Radix*, and *Phellodendri Cortex*. This study was designed to investigate its chondroprotective and anti-inflammatory effects to develop an anti-arthritic herb medicine.

Methods: ChondroT was validated using a convenient and accurate high-performance liquid chromatography–photodiode array (HPLC–PDA) detection method for simultaneous determination of its seven reference components. The concentrations of the seven marker constituents were in the range of 0.81–5.46 mg/g. The chondroprotective effects were evaluated based on SW1353 chondrocytes and matrix metalloproteinase 1 (MMP1) expression. In addition, the anti-inflammatory effects of ChondroT were studied by Western blotting of pro-inflammatory enzymes and by enzyme-linked immunosorbent assay (ELISA) of inflammatory mediators in lipopolysaccharides (LPS)-induced RAW264.7 cells.

Results: ChondroT enhanced the growth of SW1353 chondrocytes and also significantly inhibited IL-1 β -induced MMP-1 expression. However, ChondroT did not show any effects on the growth of HeLa and RAW264.7 cells. The expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) was induced by LPS in RAW264.7 cells, which was significantly decreased by pre-treatment with ChondroT. In addition, ChondroT reduced the activation of NF- κ B and production of inflammatory mediators, such as IL-1 β , IL-6, PGE₂, and nitric oxide (NO) in LPS-induced RAW264.7 cells.

Conclusions: These results show that ChondroT exerted a chondroprotective effect and demonstrated multi-target mechanisms related to inflammation and arthritis. In addition, the suppressive effect was greater than that exhibited by GHJTY, suggesting that ChondroT, a new complex herbal medication, has therapeutic potential for the treatment of arthritis.

Keywords: Anti-arthritic drug, ChondroT, Chondrocyte, MMP-1, COX-2, iNOS, Inflammatory cytokines

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Background

Arthritis is the most common inflammatory disease and a major public concern in elderly individuals. The symptoms include joint pain, tenderness, and joint inflammation. Although rheumatoid arthritis and osteoarthritis differ fundamentally in several respects, they result in cartilage degradation, which in turn leads to cartilage bone damage [1]. Matrix metalloproteases (MMPs) play a critical role in the breakdown of cartilage [2, 3]. In particular, MMP-1 is known to decompose type II collagen, which is a major component of chondrocytes [4]. Cartilage degradation in arthritis is recognized to be induced by inflammatory cytokines, such as interleukin (IL)-6, IL-1 β , and tumor necrosis factor- α (TNF- α) [5–7]. Recently, prostaglandin E₂ (PGE₂) or nitric oxide (NO) has been shown to play key roles in the induction of MMP expression in chondrocytes [8, 9]. In addition to chondrocytes, macrophages contribute to inflammation and matrix degradation in osteoarthritis tissues, and inflammatory mediators such as IL-1 β , TNF- α , IL-6, PGE₂, and NO represent potential targets for osteoarthritis disease modification [9].

Proinflammatory enzymes such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) that cause pain and inflammation, provide a measure to assess the effect of drugs for the treatment of arthritis [10]. Non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors are pharmacological treatments used for arthritis. Some oriental medicines have been used to treat arthritis [11–13]. We reported that Ganghwaljetongyeum (GHJTY), a traditional Korean herbal medicine used to treat severe joint pain, limitation of motion, fever, and swelling, inhibited inflammatory processes associated with arthritis [14]. Because GHJTY is a complex herbal decoction composed of 18 plants, we selected 5 effective herbs, i.e., *Osterici Radix*, *Lonicerae Folium*, *Angelicae Gigantis Radix*, *Clematidis Radix*, and *Phellodendri Cortex*, using bioinformatics analysis to develop a new anti-arthritis herbal medication [15]. In the present study, the water extracts of these 5 herbs named ChondroT was evaluated as an anti-arthritis herb drug. To develop a multi-functional herbal medicine for arthritis, we tested the effects of ChondroT on various arthritis-related pathomechanisms. The effects of ChondroT were evaluated on SW1353 chondrocyte protection and IL-1 β -induced MMP1 expression. In addition, the inhibitory effects of ChondroT were studied on the expression of inflammatory enzymes COX-2 and iNOS and on the production of inflammatory mediators such as IL-1 β , TNF- α , IL-6, PGE₂, and NO in RAW264.7 macrophage cells.

Methods

Plant materials

The five herbal medicines forming ChondroT – *Osterici Radix*, *Lonicerae Folium*, *Angelicae Gigantis Radix*,

Table 1 Composition of ChondroT

Latin name	Scientific name	Family	w/w %	Source
<i>Osterici Radix</i>	<i>Ostericum koreanum</i> Maximowicz	Umbelliferae	28.6	Korea
<i>Lonicerae Folium</i>	<i>Lonicera japonica</i> Thunberg	Caprifoliaceae	19.0	China
<i>Angelicae Gigantis Radix</i>	<i>Angelica gigas</i> Nakai	Umbelliferae	19.0	Korea
<i>Clematidis Radix</i>	<i>Clematis mandshurica</i> Ruprecht	Ranunculaceae	19.0	China
<i>Phellodendri Cortex</i>	<i>Phellodendron amurense</i> Ruprecht	Rutaceae	14.3	China

Clematidis Radix, and *Phellodendri Cortex* – listed in Table 1 were purchased from Omniherb (Yeongcheon, Korea). The origin of the five herbal medicines was confirmed taxonomically by Professor Jong-Kil Jeong, Dept. of Herbology, College of Oriental Medicine, Dongshin University, Republic of Korea. Voucher specimens (KYR2014-020) have been deposited at college of Pharmacy, Chonnam National University.

Preparation of ChondroT

We combined 5 herbs containing *Osterici Radix*, *Lonicerae Folium*, *Angelicae Gigantis Radix*, *Clematidis Radix*, and *Phellodendri Cortex* in a ratio listed in Table 1. ChondroT herb material composed of above 5 herbs was extracted once using 10-fold water solvent at 100 °C for 3 h and then filtered (180 mesh). The water extract solution of ChondroT was concentrated using a continuous vacuum evaporator (around 55 ~ 60 °C, 670 mmHg) followed by lyophilization using a vacuum drier (720 mmHg) for 8 h. The water extract from GHJTY herbs was prepared as previously described [14]. Stock solutions of ChondroT and GHJTY were prepared in a concentration of 50 mg/mL using phosphate buffered saline (PBS) and filter-steriled.

Table 2 Reference compounds for quality control of ChondroT

Compound	Purity (%)	Company	Detection wave length (nm)
Chlogenetic acid	≥ 99.0	Acros Organics (Pittsburgh, PA, USA)	325
Berberine Cl	≥ 98.0	Sigma-Aldrich Co. (St. Louis, MO, USA)	340
Nodakenin	≥ 98.0	NPC BioTechnology (Yeongi, Korea)	335
Isoferulic acid	≥ 98.0	ChemFaces (Wuhan, China)	325
Oxypeucedanin hydrate	≥ 98.0	ChemFaces (Wuhan, China)	310
Decursin	≥ 98.0	NPC BioTechnology (Yeongi, Korea)	330
Decursinol angelate	≥ 98.0	NPC BioTechnology (Yeongi, Korea)	330

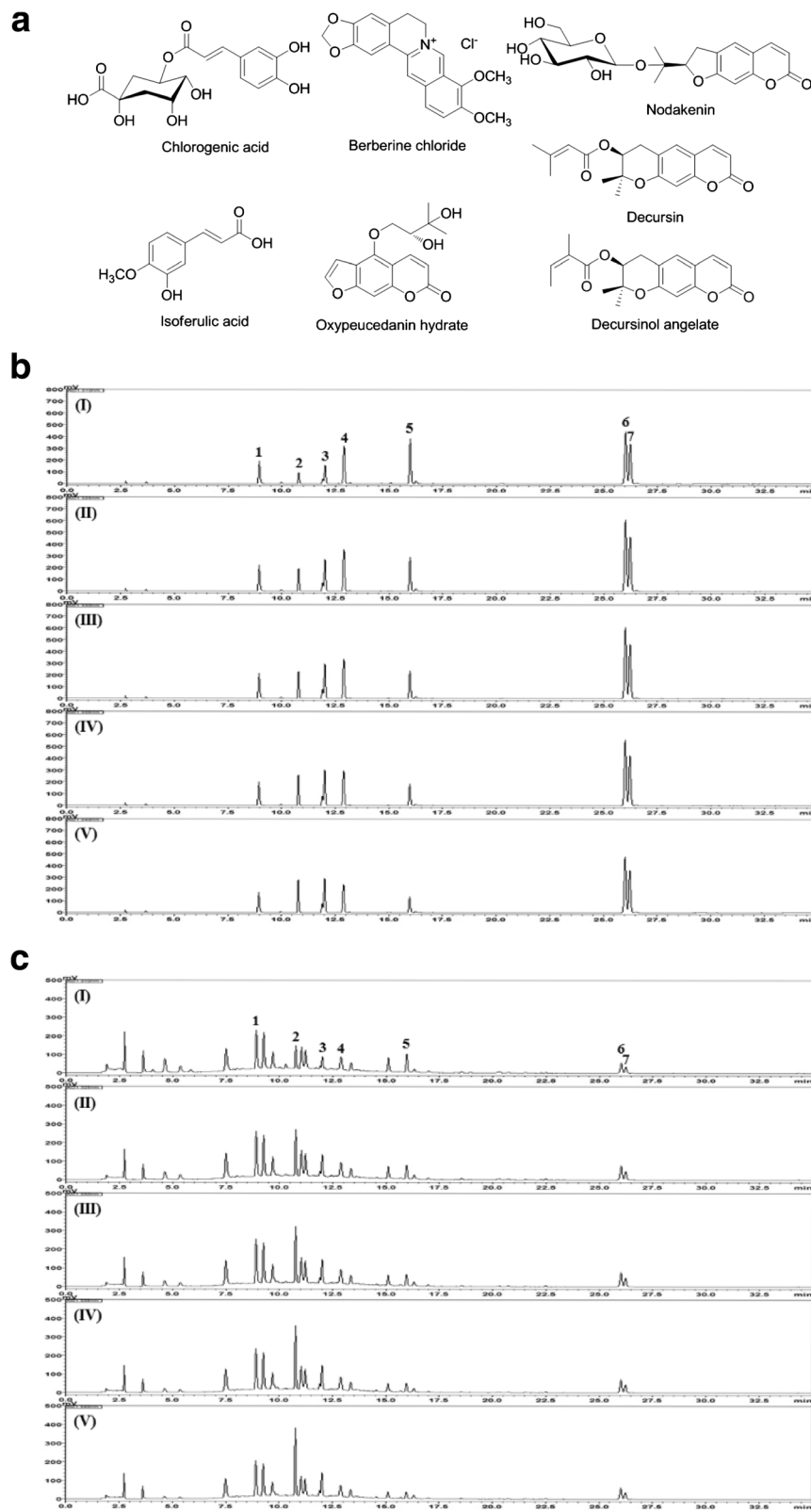


Fig. 1 Chemical structure of the seven marker compounds **(a)** and HPLC chromatogram of a standard solution **(b)** and ChondroT **(c)** with detection at 310 nm (I), 325 nm (II), 330 nm (III), 335 nm, and 340 nm (V). Chlorogenic acid (1), berberine Cl (2), nodakenin (3), isoferulic acid (4), oxypeucedanin hydrate (5), decursin (6), and decursinol angelate (7)

Table 3 Regression equations, linearity, LOD, and LOQ of the 7 marker compounds

Compound	Linear range (µg/mL)	Regression equation ^a	Coefficient of determination	LOD ^b (µg/mL)	LOQ ^c (µg/mL)
Chlorogenic acid	1.56–100.00	$y = 30,420.94x - 42,881.66$	0.9996	0.03	0.10
Berberine Cl	0.78–50.00	$y = 81,886.13x + 16,338.31$	0.9999	0.01	0.02
Nodakenin	0.78–50.00	$y = 43,427.95x + 6,116.21$	0.9999	0.02	0.06
Isoferulic acid	0.78–50.00	$y = 44,822.70x - 7,991.61$	1.0000	0.02	0.07
Oxypeucedanin hydrate	1.56–100.00	$y = 26,221.17x + 5,076.06$	1.0000	0.04	0.12
Decursin	0.78–50.00	$y = 37,915.21x + 52,952.92$	1.0000	0.04	0.12
Decursinol angelate	0.78–50.00	$y = 28,967.64x + 29,015.53$	1.0000	0.05	0.16

^ay: peak area (mAU) of compounds; x: concentration (µg/mL) of compounds

^bLOD = $3 \times 3.3 \times$ standard deviation of blank / slope of regression

^cLOQ = $10 \times$ standard deviation of blank / slope of regression

Reagents and high-performance liquid chromatography (HPLC) analysis

Seven reference compounds used for quality control of ChondroT are shown in Table 2. The chemical structures of the seven marker compounds are shown in Fig. 1a. HPLC-grade solvents, methanol, acetonitrile, and water were obtained from J.T. Baker (Phillipsburg, NJ, USA). Analytical grade formic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). All reference compounds were dissolved in methanol at 1.0 mg/mL and stored at 4 °C. Working standard solutions were prepared by serial dilution of the stock solutions with methanol. For HPLC analysis, lyophilized ChondroT (200 mg) was dissolved in 20 mL of 70 % methanol and extracted for 60 min by sonication. All the stock solutions and ChondroT extract were passed through a 0.2-µm syringe filter (Woongki Science, Seoul, Korea) before HPLC analysis. The chromatographic analysis was conducted using a Shimadzu Prominence LC-20A series system (Shimadzu, Kyoto, Japan) consisting of a solvent delivery unit (LC-20AT), online degasser (DGU-20A₃), column oven (CTO-20A), auto sample injector (SIL-20 AC), and photodiode array (PDA) detector (SPD-M20A). Data were acquired and processed using Labsolution software (version 5.54 SP3, Shimadzu, Kyoto, Japan). The column

used was Waters SunFire C₁₈ (5 µm, 4.6 × 250 nm, Milifird, MA, USA). The flow rate was kept constant at 1.0 mL/min, while the column temperature was maintained at 40 °C, and the injection volume was 10 µL. The gradient elution of two mobile phase systems with 0.1 % (v/v) formic acid in water (solvent A) and 0.1 % (v/v) formic acid in acetonitrile (solvent B) was as follows: 10–100 % B for 0–30 min, 100 % B for 30–40 min, and 100–10 % B for 40–50 min, with a re-equilibrium time of 10 min.

Cell cultures

SW1353 chondrosarcoma cells, RAW264.7 macrophage cells, and 293T human kidney epithelial cells were purchased from American Type Culture Collection (Manassas, VA, USA) and HeLa human cervix epithelial cells were supplied from Korea Cell Line Bank (Korea). The cells were cultured in Dulbecco Modified Eagle's medium (DMEM) (Welgene, Korea) supplemented with 1 % penicillin-streptomycin and 10 % fetal bovine serum (FBS) (Gibco BRL, Rockville, MD, USA) under an atmosphere of 5 % CO₂ in a humidified 37 °C incubator.

MTS assay for cell viability test

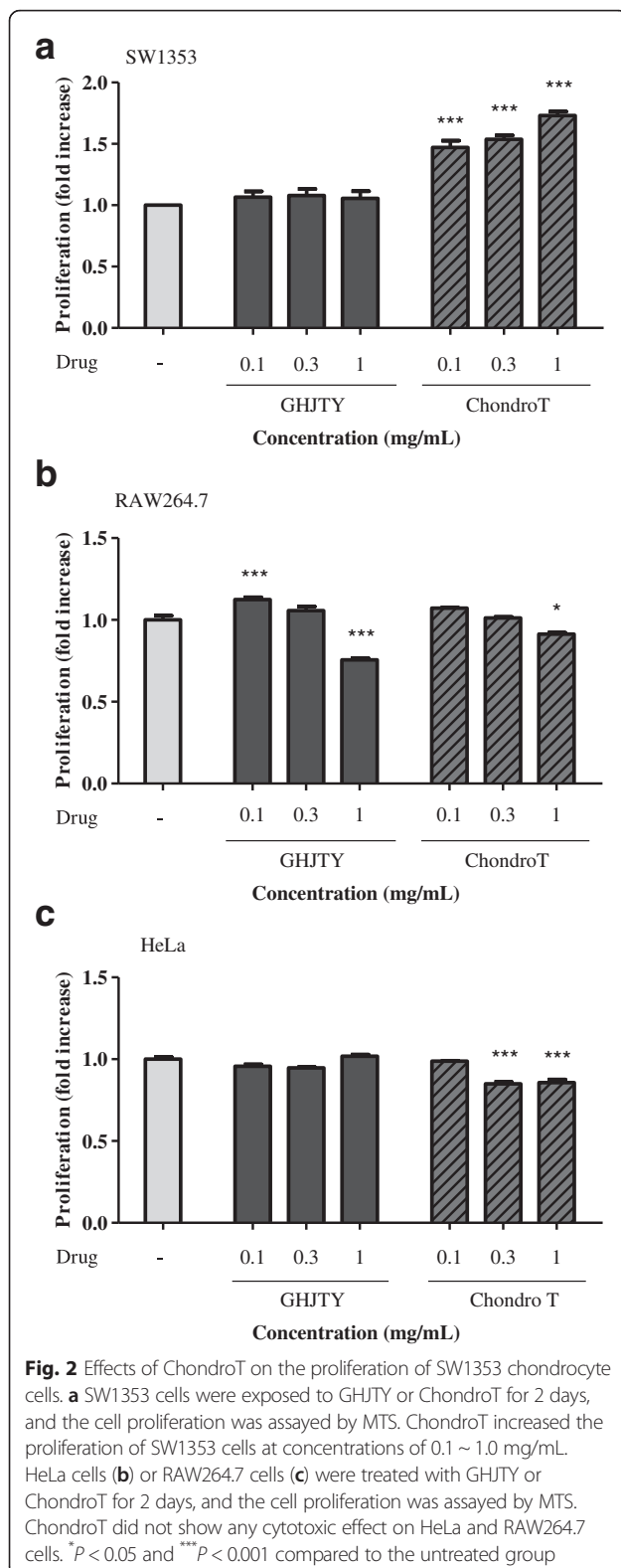
SW1353, HeLa, or RAW264.7 cells were seeded in 96 well plates (SPL life sciences Co., Pocheon, Korea) at $0.5\text{--}1.0 \times 10^4$ /well. The cells were treated with ChondroT or GHJTY for 48 h. Cell proliferation was assayed using 3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl) - 2-(4-sulfophenyl)-2H-tetrazolium (MTS), according to manufacturer's instructions (Promega, Madison, WI, USA). Absorbance was read with an ELISA microplate reader (ELx808) (BioTek Instruments, Inc., Winooski, VT, USA) at 490 nm.

Western blot analysis of MMP1

Human SW1353 chondrosarcoma cells were cultured in a 6-well plate (SPL life sciences Co., Pocheon, Korea) at 5×10^5 /well for 24 h. The cells were pretreated with

Table 4 Amounts of the seven marker components in the ChondroT by HPLC (n = 3)

Compound	Mean (mg/g)	SD × 10 ⁻¹	RSD (%)	Source
Chlorogenic acid	5.46	0.33	0.61	<i>L. japonica</i>
Berberine Cl	1.87	0.29	1.56	<i>P. amurense</i>
Nodakenin	1.70	0.18	1.06	<i>A. gigas</i>
Isoferulic acid	1.64	0.58	3.53	<i>C. chinensis</i>
Oxypeucedanin hydrate	2.03	0.33	1.65	<i>O. koreanum</i>
Decursin	1.09	0.02	0.16	<i>A. gigas</i>
Decursinol angelate	0.81	0.01	0.15	<i>A. gigas</i>



ChondroT or GHJTY (0.3 mg/mL) for 2 h and then IL-1 β or PMA (10 ng/mL) (Sigma Co., St. Louis, MO, USA) was added to the cells for 24 h. Equal amounts of cell

supernatants were concentrated by acetone followed by boiling in sample buffer (Bio-solution, Suwon, Korea) for 10 min. The samples were subjected to 12 % SDS-PAGE and electro-transferred onto a nitrocellulose membrane (Millipore, Bedford, MA, USA). The membrane was blocked with 5 % skim milk and probed with an MMP1 antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and rabbit IgG-HRP second antibody (Dako, Japan). The blots were washed three times using Tris-buffered saline with 0.1 % Tween 20 (TBST) and visualized using enhanced electrochemiluminescent (ECL) Western blotting detection kit (Advansta Corp., Menlo Park, CA, USA). The relative amount of MMP1 protein was analyzed by azure c-300 (Azure Biosystems, CA, USA).

Western blot analysis of COX-2 and iNOS

RAW264.7 cells were cultured in a 6-well plate at 1×10^6 /well for 4 h. The cells were pretreated with ChondroT, GHJTY, or celecoxib (20 μ M) (Sigma Co., MO, USA) for 2 h and then LPS (500 ng/mL) (Sigma Co., MO, USA) was added to the cells for 24 h. Equal amounts of cell lysates (25 μ g) were subjected to 10 % SDS-PAGE and electro-transferred onto polyvinylidene fluoride membranes (PVDF) (Millipore, Bedford, MA, USA). Western blot analysis was conducted using the above mentioned methods with polyclonal antibodies specific to COX-2 (Cell signaling Tech., Danvers, MA, USA), iNOS (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), or GAPDH (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA).

Enzyme-linked immunosorbent assay (ELISA) of proinflammatory cytokines

RAW264.7 cells were cultured at 1×10^5 /well in 48-well plates (SPL life sciences Co., Pocheon, Korea) for 24 h. The cells were washed with fresh medium and treated with ChondroT or GHJTY (1, 0.3, or 0.1 mg/mL) for 2 h, followed by treatment with 500 ng/mL LPS (Sigma Co., MO, USA) for 24 h. IL-6 (Biolegend, San Diego, CA, USA), TNF- α (R&D system, Minneapolis, MN, USA), IL-1 β (R&D System, Minneapolis, MN, USA), and PGE₂ (R&D system, Minneapolis, MN, USA) in the supernatants were measured using ELISA kits following the manufacturer's experimental protocols. The assay was performed at room temperature and the optical absorbance was measured at 450 nm using an ELISA microplate reader (ELx808) within 30 min.

Griess assay

The NO in the culture supernatant was measured using Griess Reagent (1 % sulfanil-amide in 2.5 % H₃PO₄, 0.1 % N-(1-naphthyl)-ethylendiamine dihydrochloride). The cell culture supernatant was blended with Griess

Reagent for 30 min, and the absorbance was read at 570 nm using an ELISA microplate reader (ELx808).

DNA transfection and NF- κ B reporter assays

Transient transfection of a reporter plasmid, pNF- κ B-SEAP (Clontech Laboratories, Inc., Palo Alto, CA, USA) was performed for 293T cells seeded at 1×10^4 /well in a 96-well plate using Lipofectamine 3000 (Invitrogen, Carlsbad, MA, USA). One day after transfection, the cell medium was replaced with fresh DMEM and treated with ChondroT or GHJTY (0.3 and 0.1 mg/mL) for 4 h. The cells were treated overnight with PMA (Sigma Co., St. Louis, MO, USA) at a concentration of 1 ng/mL. The supernatants were incubated with QUANTI-Blue (Invitrogen, Carlsbad, MA, USA) for 2–4 h, and the absorbance was read at 630 nm with an ELISA microplate reader (ELx808).

DPPH radical scavenging activity

Radical scavenging activity was measured using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma Co., St. Louis, MO, USA) and butylated hydroxyanisole (BHA) (Sigma Co., St. Louis, MO, USA) and vitamin C (VtC) (Sigma Co., St. Louis, MO, USA) were used as positive antioxidant drugs. ChondroT and other drugs dissolved in

methanol were mixed with DPPH (0.15 mM) in a 96-well plate at room temperature for 30 min. The decrease in absorbance was measured at 470 nm using an ELISA microplate reader (ELx808).

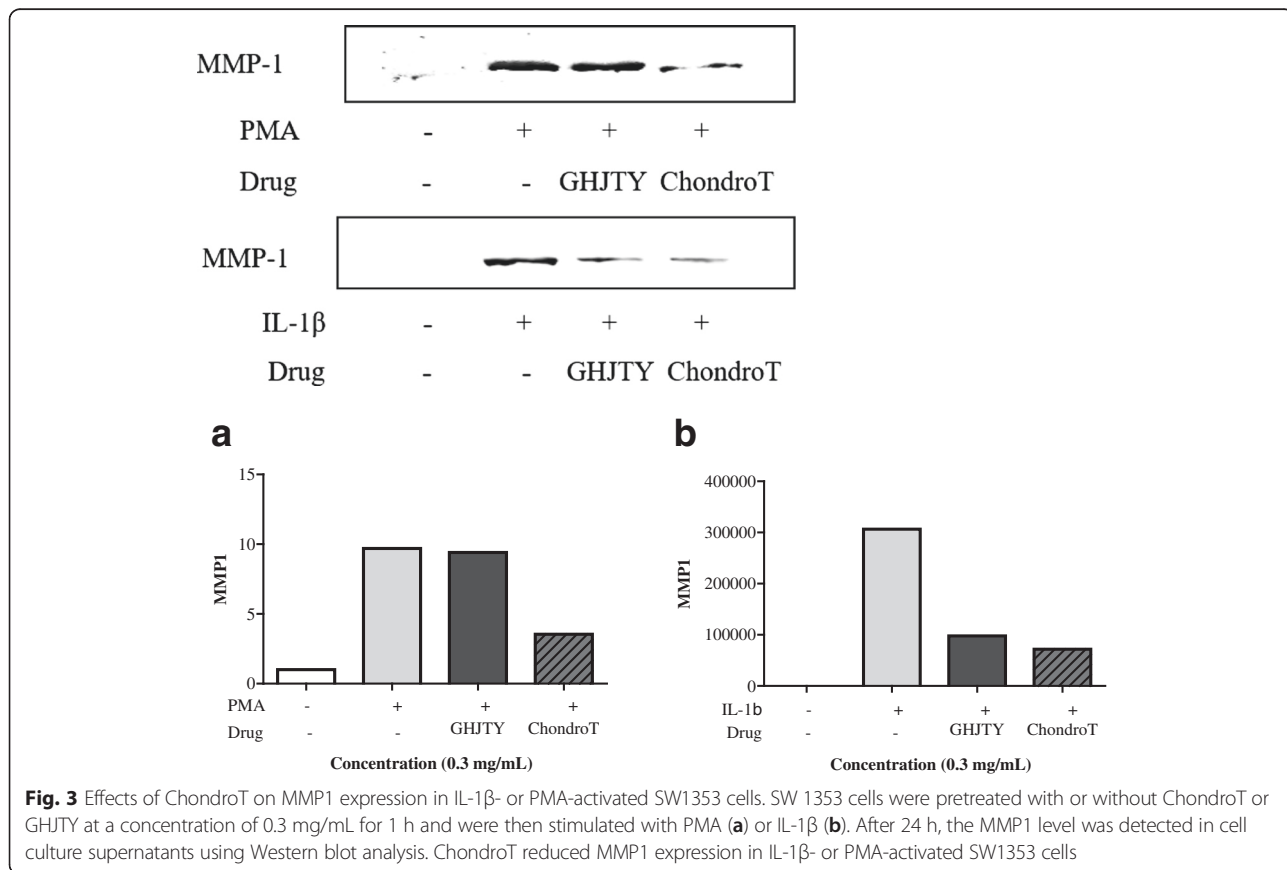
Statistical analysis

All studies were repeated at least three times. Statistical differences were evaluated using one way ANOVA. *P* value <0.05 was considered significant.

Results

The quality assessment of seven marker components in ChondroT

The HPLC-PDA method was developed for simultaneous determination of the quality assessment of seven marker components in ChondroT, which was composed of five medicinal herbs, *Ostericum koreanum* Maximowicz (Osterici Radix), *Lonicera japonica* Thunberg (Lonicerae Folium), *Angelica gigas* Nakai (Angelicae Gigantis Radix), *Clematis mandshurica* Ruprecht (Clematidis Radix), and *Phellodendron amurense* Ruprecht (Phellodendri Cortex). In this study, the seven compounds, oxy-peucedanin hydrate from Osterici Radix, chlorogenic acid from Lonicerae Folium, nodakenin, decursin, and decursinol angelate from Angelicae Gigantis Radix,



isoferulic acid from *Clematidis Radix*, and berberine Cl from *Phellodendri Cortex* were selected as marker compounds for quality control of ChondroT. The calibration curves of the seven marker components showed good linearity with a correlation coefficient (r^2) ≥ 0.9996 in the different concentration ranges, and the other parameters were shown in Table 3. Using optimized chromatography conditions, the seven marker compounds were separated within 30 min. The typical HPLC chromatogram of ChondroT is shown in Fig. 1b and 1c. The retention times of the seven components, chlorogenic acid, berberine Cl, nodakenin, isoferulic acid, oxypeucedanin hydrate, decursin, and decursinol angelate were 8.94, 10.80, 12.00, 12.86, 15.95, 26.02, and 26.24 min, respectively. The amounts of the seven compounds were 0.81–5.46 mg/g, and the results are summarized in Table 4.

Effects of ChondroT on the proliferation of SW1353 cells

The most predominant pathological change during osteoarthritis is cartilage degradation. To evaluate the effect on cartilage protection, SW1353 cells were treated with ChondroT or GHJTY for 2 days, and the cell proliferation was evaluated by MTS. ChondroT significantly increased the proliferation of SW1353 cells at concentrations of 0.1 ~ 1.0 mg/mL (Fig. 2a). In contrast, ChondroT and GHJTY

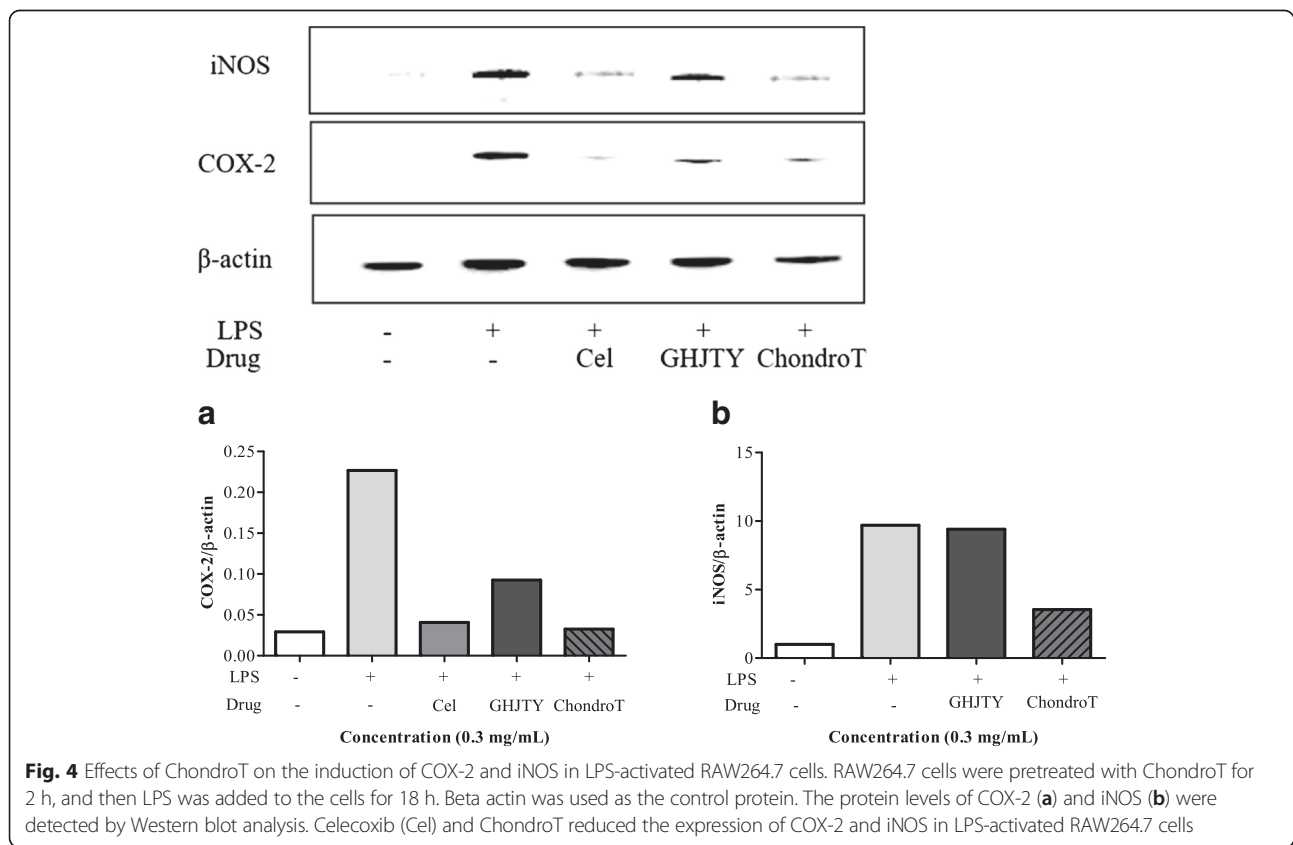
did not show any cytotoxic effect on other origin cells, RAW264.7 and HeLa cells, at concentrations of 1, 0.3, and 0.1 mg/mL (Fig. 2b and 2c). This result suggests that ChondroT has specific proliferation activity on human-born fibroblast SW1353 cells.

Effects of ChondroT on MMP1 expression in IL-1 β - or PMA-induced SW1353 cells

MMPs play a critical role in cartilage destruction in arthritic joints, and MMP-1 is known to decompose a major component of chondrocytes. The effect of ChondroT was evaluated on MMP1 expression in SW1353 cells induced by IL-1 β or PMA using Western blotting. MMP-1 expression was significantly increased by IL-1 β or PMA in SW1353 cells, which was decreased by pretreatment with ChondroT (Fig. 3). ChondroT showed inhibitory effect on MMP1 expression in SW1353 cells greater than GHJTY (Fig. 3).

Effects of ChondroT on the expression of COX-2 and iNOS in LPS-activated RAW264.7 cells

Proinflammatory enzymes, such as COX-2 and iNOS, cause pain and inflammation during arthritis. RAW264.7 cells were pretreated with GHJTY or ChondroT and LPS was added to the cells for 24 h. ChondroT significantly reduced the expression of COX-2 and iNOS in LPS-



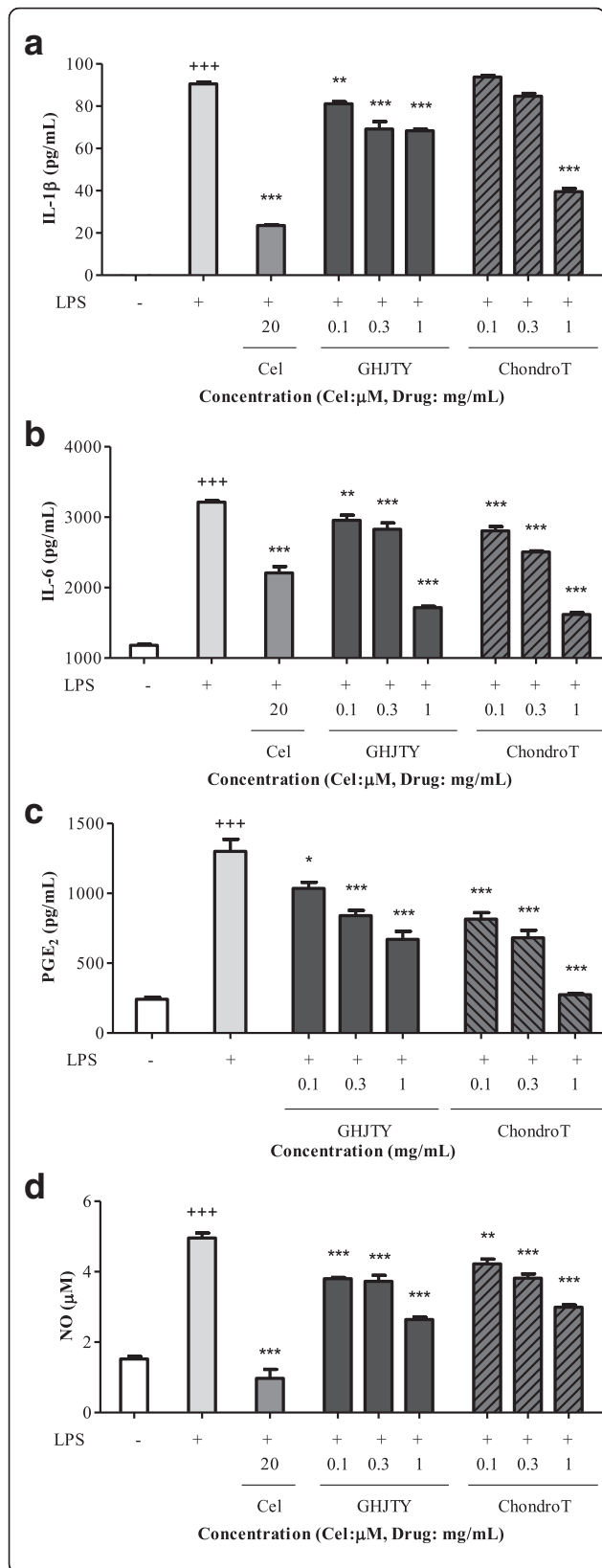


Fig. 5 Effects of ChondroT on the production of pro-inflammatory cytokines in LPS-activated RAW264.7 cells. RAW264.7 cells were pretreated with ChondroT for 2 h and then incubated with LPS for 24 h. Inflammatory mediators were measured in the cell supernatants by ELISA. ChondroT decreased the LPS-induced production of IL-1β (a), IL-6 (b), and PGE₂ (c). NO in the supernatant was detected by Griess reagent (d). LPS-induced NO production in RAW264.7 cells was decreased by ChondroT treatment. **P* < 0.01 and +++*P* < 0.001 compared with the untreated group. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 compared with the LPS-treated group

activated RAW264.7 cells similar to celecoxib (Cel), a COX-2 inhibitor (Fig. 4). The inhibitory effect of ChondroT on COX-2 and iNOS expression was greater than that exhibited by GHJTY (Fig. 4).

Effects of ChondroT on the production of inflammatory mediators in LPS-activated RAW264.7 cells

RAW264.7 cells were pretreated with ChondroT or GHJTY for 2 h prior to addition of LPS (500 ng/mL). IL-1β, TNF-α, IL-6, and PGE₂ cytokines were assayed using ELISA kits. ChondroT significantly decreased the production of IL-1β, IL-6, and PGE₂ in RAW264.7 cells activated with LPS (Fig. 5a, b, and c). NO production in LPS-activated RAW264.7 cells was also decreased by the pretreatment of ChondroT (Fig. 5d). The inhibitory effect of ChondroT on inflammatory mediator production was greater than that exhibited by GHJTY.

Effects of ChondroT on PMA-induced NF-κB activation in 293T cells

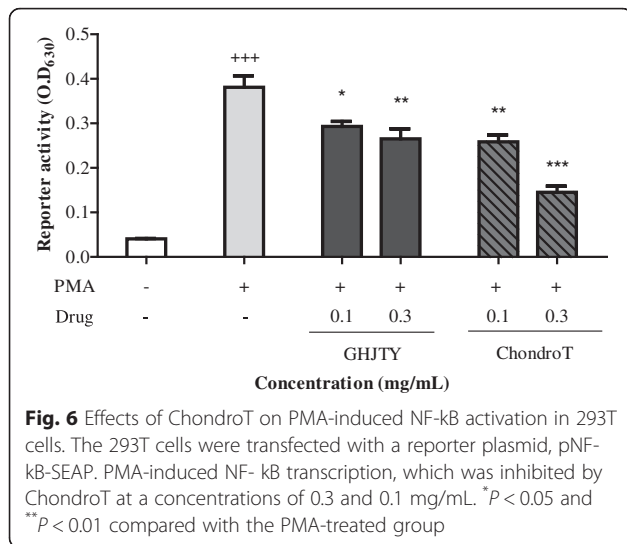
NF-κB is a transcription factor related to inflammation and arthritis. The effect of ChondroT was tested on NF-κB activation in 293T cells. PMA increased NF-κB transcription, which was decreased by ChondroT pretreatment (Fig. 6). The inhibitory effect of ChondroT on PMA-induced NF-κB activation was greater than that exhibited by GHJTY.

Anti-oxidant activity of ChondroT

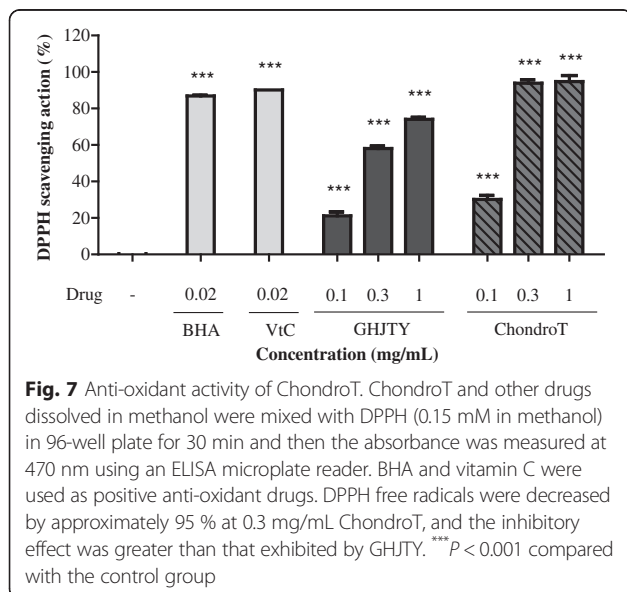
To study the anti-oxidant activity of the ChondroT, the DPPH scavenging potential was determined. Vitamin C and BHA were used as the positive controls. DPPH free radicals were decreased by approximately 95 % at 0.3 mg/mL ChondroT and the inhibitory effect was greater than that exhibited by GHJTY (Fig. 7).

Discussion

In this study, we evaluated the multifunctional effects of ChondroT, a new complex herbal medication, for arthritis pharmacologic treatment. ChondroT is a water extract of 5 herbs, *Osterici Radix*, *Lonicerae Folium*, *Angelicae Gigantis Radix*, *Clematidis Radix*, and *Phellodendri Cortex*. We previously reported that Gangwhaljetongyeum



(GHJTY), a traditional Korean herbal medicine comprised with 18 herbs, attenuates synoviocyte proliferation and reduces the production of proinflammatory mediators in macrophages [14]. GHJTY as an anti-arthritis drug may be limited because it is composed of 18 plants. To reduce the numbers of plants and to increase the potency as multifunctional anti-arthritis drugs, we conducted bioinformatics analysis [15]. In addition, oriental clinical doctors also suggested 4 complex herb medication candidates according to their clinical experiences. We compared the inhibitory effects of GHJTY and 4 complex herb medications on COX-2 and iNOS expression. Among them, an herb material ChondroT showed the most potent activity in chondrocyte protective effects and anti-inflammatory



effects. ChondroT enhanced the proliferation chondrocytes (Fig. 2a) and also significantly inhibited IL-1β- or PMA-induced MMP-1 expression in the chondrocytes (Fig. 3). In addition, ChondroT decreased the expression of inflammatory enzymes COX-2 and iNOS (Fig. 4) and reduced the production of inflammatory mediators, such as IL-1β, IL-6, PGE₂, and NO, thereby playing important roles in arthritis (Fig. 5). ChondroT also decreased the PMA-induced activation of NF-κB, a transcription factor related to inflammation and arthritis (Fig. 6). These results show that ChondroT exerted a chondroprotective effect and demonstrated a multi-target mechanism on inflammation and arthritis.

Oxidative stress was reported to induce apoptic cell death of chondrocytes and excessive production of various inflammatory cytokines in osteoarthritis, which further promote the expression of MMPs [16, 17]. Polysaccharide from *Angelica sinensis* was reported to protect chondrocytes from H₂O₂-induced apoptosis through its antioxidant effects in vitro [18]. The anti-oxidant effects of ChondroT (Fig. 7) can help patients suffering arthritis. These results suggest that ChondroT composed of 5 herbs has therapeutic potential for the treatment of arthritis.

Arthritis has become a significant clinical problem worldwide with an increase in the aging populations. NSAIDs and selective COX-2 inhibitors are used for pharmacologic treatment of arthritis. Recent study showed that celecoxib, a COX-2 inhibitor decreased NO production in chondrocytes from osteoarthritic rat joints and reduced inflammation by blocking NF-κB activation in a murine model [19–22]. However, some patients treated with these drugs complained of side effects such as gastrointestinal, cardiovascular, and other complications. Our results show that the suppressive effect of ChondroT on COX-2 and iNOS expression was similar that exhibited by celecoxib, a COX-2 inhibitor used for the treatment of arthritis (Fig. 4). In addition, ChondroT did not show any cytotoxicity to various origin cells (Fig. 2a, b, and c). Traditional herb medications have been concerned for the treatment of arthritis. Recently, SKI306X (Joins), an oriental herbal mixture were developed for osteoarthritis patients [1, 23–25].

ChondroT is a water extract of 5 herbs, *Osterici Radix*, *Lonicerae Folium*, *Angelicae Gigantis Radix*, *Clematidis Radix*, and *Phellodendri Cortex*. Effect of *Phellodendri Cortex* was reported in protecting human osteoarthritic and cartilage and chondrocytes [26]. Anti-inflammatory effects of ChondroT can be attributed to the action of all 5 herbs [27–31]. We evaluated the remedial value of ChondroT compared with GHJTY. The suppressive effect of ChondroT was greater than that exhibited by GHJTY, and it showed multifunctional therapeutic effects on inflammation and arthritis. For standard validation of ChondroT, a

convenient and accurate HPLC–PDA detection method was used for simultaneous determination of seven reference components. In conclusion, ChondroT treatment increased chondrocyte proliferation, in part through a reduction in oxidative damage. In addition, ChondroT attenuated the severity of cartilage degradation factor. With its anti-inflammatory and anti-oxidative properties, ChondroT may constitute a promising therapeutic option for the management of arthritis.

Conclusion

ChondroT exerted a chondroprotective effect and demonstrated a multi-target mechanism involving effects on inflammation and arthritis. In addition, the suppressive effect was greater than that exhibited by GHJTY, suggesting that ChondroT, a new complex herbal medication, has therapeutic potential for the treatment of arthritis.

Abbreviations

BHA, butylated hydroxyanisole; Cel, celecoxib; COX-2, cyclooxygenase-2; DMEM, Dulbecco Modified Eagle's medium; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; GHJTY, Ganghwaljetongyeum; HPLC–PDA, high-performance liquid chromatography–photodiode array; IL-1 β , interleukin 1 beta; IL-6, interleukin 6; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharides; MMP1, matrix metalloproteinase 1; MTS, 3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; NF- κ B, nuclear factor kappa B; NO, nitric oxide; NSAIDs, nonsteroidal anti-inflammatory drugs; PGE₂, prostaglandin E₂; PMA, phorbol 12-myristate 13-acetate; PVDF, polyvinylidene fluoride membranes; TBST, tris-buffered saline and Tween 20; TNF- α , tumor necrosis factor-alpha; VtC, vitamin C

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Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

Authors' contributions

PJU carried out all the assays. KSJ, NCS, and CC participated in the design of the study and performed the statistical analysis. SCS and SJK carried out HPLC analysis of ChondroT. KBY helped to draft the manuscript. KYR participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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