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

Jung Up Park¹, Seon-Jong Kim², Chang-Su Na², Chan-hun Choi², Chang Seob Seo³, Jong-Keun Son⁴, Bok Yun Kang¹ and Young Ran Kim¹*

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-1β-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-kB and production of inflammatory mediators, such as IL-1β, 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
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 metalloproteinases (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-1β, 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-1β, 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]. Nonsteroidal 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-arthritic herbal medication [15]. In the present study, the water extracts of these 5 herbs named ChondroT was evaluated as an anti-arthritic 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-1β-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-1β, 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, 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 Herbolohy, 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 drierator (around 55 ~ 60 °C, 670 mmHg). 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-sterilized.

Table 1 Composition of ChondroT

| Compound | Purity (%) | Company | Detection wave | Source |
|----------|-----------|---------|----------------|--------|
| Chlorgenic acid | ≥ 99.0 | Aceros Organics (Pittsburgh, PA, USA) | 325 |
| Berberine chloride | ≥ 98.0 | Sigma-Aldrich Co. (St. Louis, MO, USA) | 340 |
| Nodakenin | ≥ 98.0 | NPC Technology (Yeongi, Korea) | 335 |
| Isoferulic acid | ≥ 98.0 | ChemFaces (Wuhan, China) | 325 |
| Oxypeucedanin hydrate | ≥ 98.0 | ChemFaces (Wuhan, China) | 310 |
| Decursin | ≥ 98.0 | NPC Technology (Yeongi, Korea) | 330 |
| Decursinol angelate | ≥ 98.0 | NPC Technology (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)
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 0.5–1.0 × 104/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-sulphophenyl)-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 × 105/well for 24 h. The cells were pretreated with

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

| Compound               | Linear range (μg/mL) | Regression equation | Coefficient of determination |
|------------------------|----------------------|---------------------|------------------------------|
| Chlorogenic acid       | 1.56–100.00          | \( y = 30,420.94x + 42,881.66 \) | 0.9996                       |
| Berberine Cl           | 0.78–50.00           | \( y = 81,886.13x + 16,338.31 \) | 0.9999                       |
| Nodakenin              | 0.78–50.00           | \( y = 43,427.95x + 6,116.21 \) | 0.9999                       |
| Isoferulic acid        | 0.78–50.00           | \( y = 48,822.70x + 7,991.61 \) | 1.0000                       |
| Oxypeucedanin hydrate  | 1.56–100.00          | \( y = 26,221.17x + 5,076.06 \) | 1.0000                       |
| Decursin               | 0.78–50.00           | \( y = 37,915.21x + 52,952.92 \) | 1.0000                       |
| Decursinol angelate    | 0.78–50.00           | \( y = 28,967.64x + 29,015.53 \) | 1.0000                       |

**Notes:**

- \( y \): peak area (mAU) of compounds; \( x \): concentration (μg/mL) of compounds
- \( y \) LOD = 3 × 3.3 × standard deviation of blank / slope of regression
- \( y \) LOQ = 10 × standard deviation of blank / slope of regression

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

| Compound               | Mean (mg/g) | SD × 10\(^{-3}\) | RSD (%) | Source          |
|------------------------|-------------|-----------------|---------|----------------|
| Chlorogenic acid       | 5.46        | 0.33            | 0.61    | L. japonica    |
| Berberine Cl           | 1.87        | 0.29            | 1.56    | P. amurense    |
| Nodakenin              | 1.70        | 0.18            | 1.06    | A. gigas      |
| Isoferulic acid        | 1.64        | 0.58            | 3.53    | C. chinensis   |
| Oxypeucedanin hydrate  | 2.03        | 0.33            | 1.65    | O. koreanum    |
| Decursin               | 1.09        | 0.02            | 0.16    | A. gigas      |
| Decursinol angelate    | 0.81        | 0.01            | 0.15    | A. gigas      |
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 × 10⁶/well for 4 h. The cells were pretreated with ChondroT, GHJTY, or celecoxib (20 μM) (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 μg) 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 × 10⁵/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-1β (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)-ethylenediamine 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-κB reporter assays**

Transient transfection of a reporter plasmid, pNF-κB-SEAP (Clontech Laboratories, Inc., Palo Alto, CA, USA) was performed for 293T cells seeded at 1 × 10⁴/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, oxypeucedanin hydrate from *Osterici Radix*, chlorogenic acid from *Lonicerae Folium*, nodakenin, decursin, and decursinol angelate from *Angelicae Gigantis Radix*,

![Graph](image-url)
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 ($r^2$ $\geq$ 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, oxyypeucedanin 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$\beta$- 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$\beta$ or PMA using Western blotting. MMP-1 expression was significantly increased by IL-1$\beta$ 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-
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 PGE_2 cytokines were assayed using ELISA kits. ChondroT significantly decreased the production of IL-1β, IL-6, and PGE_2 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-kB activation in 293T cells
NF-kB is a transcription factor related to inflammation and arthritis. The effect of ChondroT was tested on NF-kB activation in 293T cells. PMA increased NF-kB transcription, which was decreased by ChondroT pretreatment (Fig. 6). The inhibitory effect of ChondroT on PMA-induced NF-kB 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 synovioyte 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-kB, 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-kB 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 (Joons), 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

### Table 1: Anti-oxidant activity of ChondroT

| Drug | BHA | Vc | GHJTY | ChondroT |
|------|-----|----|-------|---------|
| 0.02 | *** | ***|       | ***     |
| 0.1  | *** | ***|       | ***     |
| 0.3  | *** | ***|       | ***     |
| 1    | *** | ***|       | ***     |

*P < 0.001 compared with the control group.
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 antioxidant 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, Ganghwajaltongyeum; HPLC–PDA, high-performance liquid chromatography–photodiode array; IL–1β, 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–KB, nuclear factor kappa B; NO, nitric oxide; NSAIDs, nonsteroidal anti-inflammatory drugs; PGE2, prostaglandin E2; PMA, phorbol 12-myristate 13-acetate; PVD, polyvinylidene fluoride membranes; TBST, tris-buffered saline and Tween 20; TNF–α, tumor necrosis factor-alpha; VtC, vitamin C

**Funding**

This study was supported by a grant (No. H11C2285) of the Korea Healthcare technology R&D Project, Ministry for Health & Welfare Affairs, Republic of Korea.

**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. KSI, NCS, and CC participated in the design of the study and performed the statistical analysis. SCS and SJK carried out all the assays. KSJ, NCS, and CC participated in the design of the study and performed the statistical analysis. KYR helped to draft the manuscript. KBY 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.

**Author details**

1. College of Pharmacy and Research Institute of Drug Development, Chonnam National University, Gwangju 500-757, Republic of Korea. 2. College of Korean Medicine, Dongshin University, Naju-si 58245, Republic of Korea. 3. Korea Institute of Oriental Medicine, Daejeon 34054, Republic of Korea. 4. College of Pharmacy, Yeungnam University, Gyeongsan-si, Republic of Korea.

**Received:** 12 March 2016 Accepted: 24 June 2016

**Published online:** 13 July 2016

**References**

1. Kim JH, Ryu KH, Jung KW, Han CK, Kwak WJ, Cho YB. Effects of SK306X on arachidonate metabolism and other inflammatory mediators. Biol Pharm Bull. 2005;28(9):1615–20.
2. Sondergaard BC, Henriksen K, Wulf H, Oestergaard S, Schurigt U, Brauer R, Danielsen I, Christansen C, Qvist P, Karidal MA. Relative contribution of matrix metalloproteinase and cytokine protease activities to cytokine-stimulated articular cartilage degradation. Osteoarthritis Cartilage. 2006;14(8):738–48.
3. Shin SS, Jin M, Jung HJ, Kim B, Jeon H, Choi JJ, Kim JM, Cho BW, Chung SH, Lee YW, et al. Suppressive effects of PGZ201, an ethanol extract from herbs, on collagen-induced arthritis in mice. Rheumatol (Oxford). 2003;42(5):665–72.
4. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circulation research. 2003;92(8):827–39.
5. Zeng L, Wang W, Rong XF, Zhong Y, Jia P, Zhou GQ, Li RH. Chondroprotective effects and multi-target mechanisms of Icarin in IL-1beta-induced human SW 1353 chondroasoma cells and a rat osteoarthritis model. Int Immunopharmacol. 2014;18(1):715–81.
6. Kobayashi M, Squires GR, Moua A, Tanzer M, Zokor DJ, Antoniou J, Feige U, Poole AR. Role of interleukin-1 and tumor necrosis factor alpha in matrix degradation of human osteoarthritic cartilage. Arthritis Rheum. 2005;52(1):128–35.
7. Martel-Pelletier J, Pathophysiology of osteoarthritis. Osteoarthritis Cartilage. 2004;12 Suppl A:313–31.
8. Gosset M, Pigenet A, Salvat C, Berenbaum F, Jacques C. Inhibition of matrix metalloproteinase-3 and -13 synthesis induced by IL-1beta in chondrocytes from mice lacking microsomal prostaglandin E synthase-1. J Immunol. 2010;185(10):6244–52.
9. Wu SQ, Otero M, Unger FM, Goldring MB, Phutivorapongkul A, Chiai C, Kolb A, Vennstein H, Taegsel S. Anti-inflammatory activity of an Ethanolic Caesalpinia sappan extract in human chondrocytes and macrophages. Int J Ethnopharmacol. 2011;138(2):364–72.
10. Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev. 1991;43(2):109–42.
11. Keisuke I, Iman Ei, Li XD, Takashi S, Akira I. Action mechanisms of complementary and alternative medicine therapies for rheumatoid arthritis. Chin J Integr Med. 2011;17(10):715–30.
12. Liu J, Liu RL. The potential role of Chinese medicine in ameliorating extra-articular manifestations of rheumatoid arthritis. Chin J Integr Med. 2011; 17(10):735–7.
13. Funk JL, Oyarzo JN, Frye JB, Chen G, Lantz RC, Jolad SD, Solyom AM, Timmermann BN. Turmeric extracts containing curcuminoids prevent experimental rheumatoid arthritis. J Nutr. 2006;136(3):551–5.
14. Jeoung BR, Lee KD, Na CS, Kim YE, Kim B, Kim YR. Ganghwajaltongyeum, an anti-arthritis remedy, attenuates synoviocyte proliferation and reduces the production of proinflammatory mediators in macrophages: the therapeutic effect of GHJTY on rheumatoid arthritis. BMC Complement Altern Med. 2013;13:47.
15. Choi W, Choi CH, Kim YR, Kim SJ, Na CS, Lee H. HerDing: herb recommendation system to treat diseases using genes and chemicals. Database (Oxford); 2016. 2016; doi:10.1093/database/baw011.
16. Lim HD, Kim YS, Ko SH, Yoon U, Cho SG, Chun YH, Choi BI, Kim EC. Cytoprotective and anti-inflammatory effects of melatonin in hydrogen peroxide-stimulated CHON-001 human chondrocyte cell line and rabbit model of osteoarthritis via the SIRT1 pathway. J Pineal Res. 2012;53(3):225–37.
17. Li J, Zhou XD, Yang KH, Fan TD, Chen WP, Liang LF, Bao JP, Wu LD, Xiong Y. Hinokitiol reduces matrix metalloproteinase expression by inhibiting Wnt/beta-Catenin signalling in vitro and in vivo. Int Immunopharmacol. 2014; 23(1):85–91.
18. Zhang C, Xu NW, Gao GM, Ni S, Miao KS, Li CK, Wang LM, Xie HG. Polysaccharide from Angelica sinensis protects chondrocytes from H2O2-induced apoptosis through its antioxidant effects in vitro. Int J Biol Macromol. 2016;67:322–8.
19. Roh GS, Yi CO, Cho YJ, Jeon BT, Nizamudinova IT, Kim HJ, Kim JM, Oh YM, Huh JW, Lee YH, et al. Anti-inflammatory effects of celecoxib in rat lungs with smoke-induced emphysema. Am J Physiol-Lung C. 2010;299(2):L184–91.
20. Xu XY, Jiang M, Zhang Y, Bi YL, Han MY. Celecoxib attenuates cachectic events in mice by modulating the expression of vascular endothelial growth factor. Mol Med Rep. 2015;11(1):289–94.
21. Solomon DH, Avorn J, Sturmer T, Glynn RJ, Mogun H, Schneeweiss S. Cardiovascular outcomes in new users of coxibs and nonsteroidal Antiinflammatory drugs - High-risk subgroups and time course of risk. Arthritis Rheum-U. 2006;54(5):1378–89.
22. Kisley LR, Barrett BS, Dwyer-Nield LD, Bauer AK, Thompson DC, Malkinson AM. Celecoxib reduces pulmonary inflammation but not lung tumorigenesis in mice. Carcinogenesis. 2002;23(10):1653–60.
23. Hartog A, Hougee S, Faber J, Sanders A, Zuurman C, Smit HF, van der Kraan PM, Hoijer MA, Garsen J. The multicomponent phytopharmaceutical SK306X inhibits in vitro cartilage degradation and the production of inflammatory mediators. Phytomedicine. 2008;15(5):313–20.
24. Kim JH, Ryu KH, Jung KW, Han CK, Kwak WJ, Cho YB. SK306X suppresses cartilage destruction and inhibits the production of matrix metalloproteinase in rabbit joint cartilage explant culture. J Pharmacol Sci. 2009;98(3):298–306.
25. Lee CW, Park SM, Kim YS, Jegal KH, Lee JR, Cho IJ, Park YK, et al. A four-week, randomized, double-blind trial of the efficacy and safety of SK306X: a herbal anti-arthritis agent versus diclofenac in osteoarthritis of the knee. Arq Bras Med. 2004;32(2):291–301.
26. Kim JH, Huh JE, Baek YH, Lee JD, Choi DY, Park DS. Effect of Phellodendron amurense in protecting human osteoarthritic cartilage and chondrocytes. J Ethnopharmacol. 2011;134(2):234–42.
27. Choi Y, Ahn H, Ryu JH. Polycytcleyenes from angelica gigas and their inhibitory activity on nitric oxide synthesis in activated macrophages. Biol Pharm Bull. 2000;23(7):884–6.
28. Lee CW, Lee SY, Koo SY, Park YH, Park KM, Park YK, et al. Bisabolangelone isolated from Ostericum koreanum inhibits the production of inflammatory mediators by down-regulation of NF-kappaB and ERK MAP kinase activity in LPS-stimulated RAW264.7 cells. J Ethnopharmacol. 2010;130(2):155–62.
29. Lee CW, Park SM, Kim YS, Jegal KH, Lee JH, Cho IJ, Ku SK, Lee JY, Ahn YT, et al. Biocompatibility and anti-inflammatory effects of Clematis mandshuica Ruprecht root extract in rodent cells. J Ethnopharmacol. 2014;155(2):141–55.
30. Lee JH, Ko WS, Kim YH, Kang HS, Kim HD, Choi BT. Anti-inflammatory effect of the aqueous extract from Loniceria japonica flower is related to inhibition of NF-kappaB activation through reducing I kappaB alpha degradation in rat liver. Int J Mol Med. 2001;7(1):79–83.
31. Bae S, Jung Y, Choi YM, Li S. Effects of er-miao-san extracts on TNF-alpha-induced MMP-1 expression in human dermal fibroblasts. Biol Res. 2015;488.