

Research Article

Effect of GCSB-5, a Herbal Formulation, on Monosodium Iodoacetate-Induced Osteoarthritis in Rats

Joon-Ki Kim,¹ Sang-Won Park,¹ Jung-Woo Kang,¹ Yu-Jin Kim,² Sung Youl Lee,²
Joonshik Shin,³ Sangho Lee,³ and Sun-Mee Lee¹

¹School of Pharmacy, Sungkyunkwan University, Suwon, Gyeonggi-do 440-746, Republic of Korea

²Business Development, Green Cross Corporation, Yongin, Gyeonggi-do 446-770, Republic of Korea

³Jaseng Hospital, 635 Sinsa-Dong, Gangnam-Gu, Seoul, Republic of Korea

Correspondence should be addressed to Sun-Mee Lee, sunmee@skku.edu

Received 1 April 2011; Revised 9 November 2011; Accepted 16 November 2011

Academic Editor: Andrew Scholey

Copyright © 2012 Joon-Ki Kim et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Therapeutic effects of GCSB-5 on osteoarthritis were measured by the amount of glycosaminoglycan in rabbit articular cartilage explants *in vitro*, in experimental osteoarthritis induced by intra-articular injection of monoiodoacetate in rats *in vivo*. GCSB-5 was orally administered for 28 days. *In vitro*, GCSB-5 inhibited proteoglycan degradation. GCSB-5 significantly suppressed the histological changes in monoiodoacetate-induced osteoarthritis. Matrix metalloproteinase (MMP) activity, as well as, the levels of serum tumor necrosis factor- α , cyclooxygenase-2, inducible nitric oxide synthase protein, and mRNA expressions were attenuated by GCSB-5, whereas the level of interleukin-10 was potentiated. By GCSB-5, the level of nuclear factor- κ B p65 protein expression was significantly attenuated but, on the other hand, the level of inhibitor of κ B- α protein expression was increased. These results indicate that GCSB-5 is a potential therapeutic agent for the protection of articular cartilage against progression of osteoarthritis through inhibition of MMPs activity, inflammatory mediators, and NF- κ B activation.

1. Introduction

Osteoarthritis (OA) is a degenerative joint disease characterized by joint pain and a progressive loss of articular cartilage. It has been suggested that biochemical alterations occur within the articular cartilage resulted in imbalance between synthetic and degradative pathways [1]. A key step in the pathophysiology of OA is breakdown of extracellular matrix of articular cartilage by tissue proteinases, enzymes whose expression is upregulated by inflammatory stimuli, such as primary inflammatory cytokines [2]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are effective in the management of OA inflammation. However, the adverse events secondary to NSAIDs was focused on upper gastrointestinal tolerability [3]. In recent years, gene therapy targeted at cytokines offers new hope to OA treatment, and the current focus is on the use of biological agents that block the activity of inflammatory cytokines [4]. Since there are many proinflammatory cytokines, oxidants and other factors exerting action in initiation and development of OA, it is hard to obtain

complete therapeutic effects by blocking the activity of one or two cytokines. Developing therapeutics from herbal sources may reduce the risk of toxicity or adverse effects when the drug is clinically used [5] and may exert strong, multifunctional anti-inflammatory effect like many natural products do. Therefore, efforts are being made to elucidate the role of natural products for the treatment of OA.

GCSB-5 is a purified extract from a mixture of 6 oriental herbs which are the ingredients of Chung-Pa-Juhn used in Jaseng Hospital (Seoul, Korea) and that have been used in traditional medicine to treat inflammatory diseases and bone disorders. *Ledebouriellae Radix* is reported to have anti-inflammatory effects on Freund's adjuvant-induced arthritis in rats [6]. Cimifugin, a major active component of *Ledebouriellae Radix*, exhibits inhibitory effects on the synthesis of NO induced by LPS in macrophage cell line RAW 264.7 [7]. *Achyranthis Radix* shows anti-inflammatory property and inhibits free radicals, such as ONOO⁻, HOCl, and OH radical [8]. 20-Hydroxyecdysone, which is a major active compound of *Achyranthis Radix*, has beneficial effects on

joint and bone in ovariectomized rats [9]. *Acanthopanax Cortex* is known to show antiarthritic activity [10], and *Cibotii Rhizoma* is known for its analgesic property [11] along with osteoclast formation inhibition [12]. Glycine Semen is effective in reducing swelling [13] and genistin, an active compound from Glycine Semen, shows beneficial effect on bone loss [14]. *Eucommiae Cortex* exhibits strong analgesic effect [15] and geniposide from its extract shows anti-inflammatory effect on rheumatoid arthritis rats [16] and enhances the osteoblast-like cell proliferation and inhibited osteoclast [17]. We reported strong antinociceptive and anti-inflammatory properties of GCSB-5 [11, 13]. Recently, GCSB-5 reduces the development of acute and chronic inflammation, and its anti-inflammatory property is likely due to inhibition of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 expression via downregulation of the Akt signal pathway and inhibition of nuclear factor- κ B (NF- κ B) activation [18]. In phase III clinical study, GCSB-5 was shown to exert therapeutic effects and acted to reduce OA severity and improved functional recovery without apparent hepatic or renal toxicity (unpublished data).

In this study, we examined the chondroprotective and anti-inflammatory effects of GCSB-5 on monoiodoacetate (MIA)-induced OA animal model, both *in vitro* and *in vivo*.

2. Materials and Methods

2.1. Preparation and Composition of GCSB-5. GCSB-5 was prepared by the Hanpoong Pharmaceutical Co., Ltd., Jeonju, Republic of Korea. The mixture of six crude drugs (*Ledebouriellae Radix* (4.444 g), *Achyranthis Radix* (4.444 g), *Acanthopanax Cortex* (4.444 g), *Cibotii Rhizoma* (2.778 g), Glycine Semen (2.778 g), and *Eucommiae Cortex* (1.389 g)) was powdered and boiled for 3 h in distilled water (1 L). The resulting extract was subjected to ultrafiltration, and the components with molecular weight over 10,000 were excluded. The filtrate was lyophilized as powder and kept at 4°C until use. GCSB-5 was administered orally at a dose of 300 and 600 mg/kg in saline (1 kg/10 mL), and the same volume of saline was used as a vehicle control group. The validation of GCSB-5 was performed by high-performance liquid chromatography analysis of each ingredient extract using six indicator biological components: cimifugin for *Ledebouriellae Radix*, 20-hydroxyecdysone (0.311–0.312 mg/g) for *Achyranthis Radix*, acanthoside D (0.577–0.578 mg/g) for *Acanthopanax Cortex*, onitin-4-O- β -D-glucopyranoside for *Cibotii Rhizoma*, genistin (0.0426–0.0427 mg/g) for Glycine Semen, and geniposide (0.431–0.432 mg/g) for *Eucommiae Cortex*. GCSB-5 was further standardized for quality control according to the regulations imposed by Korea Food and Drug Administration (KFDA).

2.2. Chemicals. Dulbecco's modified Eagle's medium (DMEM), penicillin/streptomycin (10,000 U/mL, 10,000 μ g/mL, resp.), and fetal bovine serum (FBS) were obtained from Gibco BRL, Life Technologies (Grand Island, NY, USA). All the other materials required for culturing of tissue were purchased from Sigma Chemical Company (St. Louis, MO, USA).

2.3. Animals. Male Sprague-Dawley rats (200–220 g) and male New Zealand white rabbits (2.0–2.2 kg) were obtained from Dae Han Biolink Ltd. (Eumseong, Korea) and housed in solid bottom cages with pellet food and water available *ad libitum*. All animal procedures were approved by the Sungkyunkwan University Animal Care Committee and were performed in accordance with the guidelines of the National Institutes of Health.

2.4. Cartilage Glycosaminoglycan Assay. Rabbit knee articular cartilage explants were obtained according to the method described by Sandy et al. [19]. Briefly, 200–220 mg articular surfaces per joint were dissected and submerged into complete medium of DMEM supplemented with heat-inactivated 5% FBS, penicillin (100 U/mL), and streptomycin (100 μ g/mL). After stabilization in incubator, the medium was replaced with basal medium made of DMEM supplemented with heat-inactivated 1% FBS, 10 mM HEPES, penicillin (100 U/mL), and streptomycin (100 μ g/mL). Cartilage pieces (50–60 mg; $2 \times 3 \times 0.35$ mm/piece) were placed in 24-well cell culture plates and treated with GCSB-5 at 1×10^{-3} , 1×10^{-2} , and 1×10^{-1} mg/mL or 30 μ M diclofenac (Sigma-Aldrich, St. Louis, MO, USA). After 1 h of GCSB-5 or diclofenac pretreatment, 5 ng/mL of rhIL-1 α (R&D Systems, Minneapolis, MN, USA) was added and further incubated at 37°C in a humidified 5% CO₂/95% air incubator. The amount of glycosaminoglycan (GAG) in the medium was determined by the 1,9-dimethyl-methylene blue method using the Blyscan Sulfated GAG Assay kit (Biocolor Ltd., County Antrim, UK) according to the manufacturer's instructions.

2.5. MIA-Induced OA. Rats were anesthetized with diethyl ether and given a single intra-articular injection of 3 mg MIA (Sigma-Aldrich, St. Louis, MO, USA) through the infrapatellar ligament of the left knee [20]. MIA was dissolved in physiological saline and administered in a 50 μ L volume. Rats were treated with saline, with 300 or 600 mg/kg of GCSB-5 or with 5 mg/kg of diclofenac by oral administration once daily, for 2, 7, and 28 days since MIA injection. These GCSB-5 doses and MIA injection volume were selected based on previous evaluations [21].

2.6. Gross Observation. After MIA injection, all experimental rats were weighed and carefully inspected every 2 days to assess knee joint swelling and gait disturbances under natural conditions in the cages, where they moved freely. Swelling and limping were classified as no change, mild, and severe on the basis of severity [22], and inspection was conducted by an inspector blinded to treatment details throughout the study.

2.7. Roentgenographic Examination and Histopathological Analysis. Seven and 28 days following MIA injection, rats were checked with roentgenography to assess chronic morphological changes of knee articular bones for narrowing, loss of joint region, cartilage erosion, and osteophyte formation [23]. For histological analysis, knee joints were removed and fixed in 10% neutral buffered formalin, decalcified with 10% formic acid, and embedded in paraffin. Five micrometer

TABLE 1: RT-PCR primers used in study.

| Gene | Accession number | Primer sequences (5' → 3') | Product length (bp) |
|---------------------------------|------------------|---|---------------------|
| <i>TNF-α</i> | X66539 | Sense: GTA GCC CAC GTC GTA GCA AA Antisense: CCC TTC TCC AGC TGG AAG AC | 346 |
| <i>iNOS</i> | D44591 | Sense: TTC TTT GCT TCT GTG CTT AAT GCG Antisense: GTT GTT GCT GAA CTT CCA ATC GT | 1061 |
| <i>COX-2</i> | U03389 | Sense: CTG CAT GTG GCT GAT GTC ATC Antisense: AGG ACC CGT CAT CTC CAG GGT AAT C | 1061 |
| <i>IL-1β</i> | M98820 | Sense: TGA TGT TCC CAT TAG ACA GC Antisense: GAG GTG CTG ATG TAC CAG TT | 378 |
| <i>IL-10</i> | X60675 | Sense: CAG TCA GCC AGA CCC ACA T Antisense: GCT CCA CTG CCT TGC TTT | 322 |
| <i>β-Actin</i> | V01217 | Sense: TTG TAA CCA ACT GGG ACG ATA TGG Antisense: GAT CTT GAT CTT CAT GGT GCT AG | 764 |

(5 μ m) sections were stained with hematoxylin and eosin (H and E) or safranin-O fast green (SOFG) and observed. Histopathological changes in each animal were quantitatively expressed by three grades for each finding [24]. Grading was done under the authority of Medplan Pathology Laboratories, Seoul, Korea.

2.8. Gelatinase Assay. Rat articular cartilage samples of MIA-induced OA were harvested 7 and 28 days after MIA injection. Gelatinase activities were measured by the gelatin zymography method described by Dumond et al. [25]. Proteins were extracted from pulverized cartilage tissues and electrophoresed on 10% zymogram precast gels. The cleared gels were captured, and the area of each band was quantified with densitometric scanning analysis program (Science Lab 98 Image Gauge, version 3.12, Fuji Photo Film Co. Ltd., Tokyo, Japan).

2.9. Serum Cytokine Levels. Commercial tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and IL-10 enzyme-linked immunosorbent assay (ELISA) kits (BD Biosciences Co., CA, USA) were used for quantification of the serum levels of TNF- α , IL-1 β , and IL-10, respectively.

2.10. Western Blot Immunoassay. 15 μ g of whole protein was used for determination of the content of COX-2 and iNOS. 20 μ g of nuclear protein was used for determination of the content of the NF- κ B/p65 subunit. 20 μ g of the cytosolic protein was used for determination of the content of the inhibitor of κ B (I κ B)- α . ImageQuantTM TL software (Amersham Biosciences/GE Healthcare, Piscataway, NJ, USA) was used for densitometric evaluation of visualized immunoreactive bands. The following primary antibodies were used: COX-2 (Abcam, Cambridge, UK; 1:1000), iNOS (Transduction Lab., CA, USA; 1:1000), phosphoryl NF- κ B/p65 (Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:1000), and I κ B- α (Santa Cruz Biotechnology; 1:5000) were used, and the signals were normalized to that of β -actin (Sigma Chemical Co.; 1:1000) or lamin B1 (Abcam; 1:2500).

2.11. Total RNA Extraction and Reverse Transcription-Polymerase Chain Reaction (RT-PCR). Articular cartilage samples collected 2 and 28 days after MIA injection were pulverized in TRI Reagent (Molecular Research Center Inc., Cincinnati, OH, USA) for RNA extraction. Equal amounts of RNA from articular cartilages were subjected to reverse transcription using iNtRON RNA PCR kit (iNtRON Biotechnology Co., Seongnam, Korea) to generate cDNA for RT-PCR analysis. RT-PCR analysis was performed with the GeneAmp PCR system 2700 (Applied Biosystems Co., Foster City, CA, USA). The primers used in the RT-PCR are listed in Table 1. All PCR reactions included an initial denaturation step at 94°C for 5 min and a final extension at 72°C for 7 min. The PCR amplification cycling conditions were as follows: 32 cycles of 94°C (30 s), 58°C (30 s), and 72°C (30 s) for *TNF- α* ; 32 cycles of 94°C (45 s), 65°C (45 s), and 72°C (60 s) for *iNOS*; 40 cycles of 94°C (45 s), 65°C (45 s), and 73°C (60 s) for *COX-2*; 36 cycles of 94°C (30 s), 60°C (30 s), and 72°C (45 s) for *IL-1 β* ; 40 cycles of 94°C (30 s), 66°C (45 s), and 72°C (45 s) for *IL-10*; 30 cycles of 94°C (30 s), 56°C (30 s), and 72°C (60 s) for *β -actin*. After RT-PCR, 10 μ L samples of the amplified products were resolved by electrophoresis on 1.5% agarose gels and stained with ethidium bromide. The intensity of each PCR product was evaluated semiquantitatively using a digital camera (DC120; Eastman Kodak, Rochester, NY, USA) and a densitometric scanning analysis program (ID Main; Advanced American Biotechnology, Fullerton, CA, USA).

2.12. Statistics. All results are presented as mean \pm S.E.M. The overall significance of the experimental results was examined by one-way analysis of variance and the two-tail Dunnet's *t*-test. Differences between groups were considered significant at $P < 0.05$ with the appropriate Bonferroni correction for multiple comparisons.

3. Results

3.1. Cartilage Glycosaminoglycan Release. In the control group, the level of GAG in the culture medium remained

TABLE 2: Quantitative summary of gross observations in MIA-induced osteoarthritic rats treated with GCSB-5.

| | | Control | Vehicle | MIA | | Diclofenac 5 mg/kg |
|-----------------|---|-----------|------------------------|--------------------------|------------------------|------------------------|
| | | | | GCSB-5 (mg/kg) 300 | 600 | |
| Swelling | | | | | | |
| No change | 0 | 10/10 | 0/10 | 1/10 | 1/10 | 0/10 |
| Mild | 1 | 0/10 | 3/10 | 8/10 | 6/10 | 8/10 |
| Severe | 2 | 0/10 | 7/10 | 1/10 | 3/10 | 2/10 |
| Average score | | 0.0 ± 0.0 | 1.7 ± 0.2 ^a | 1.0 ± 0.2 ^{a,b} | 1.2 ± 0.2 ^a | 1.2 ± 0.1 ^a |
| Limping | | | | | | |
| No change | 0 | 10/10 | 0/10 | 6/10 | 5/10 | 5/10 |
| Mild | 1 | 0/10 | 7/10 | 4/10 | 5/10 | 5/10 |
| Severe | 2 | 0/10 | 3/10 | 0/10 | 0/10 | 0/10 |
| Average score | | 0.0 ± 0.0 | 1.3 ± 0.2 ^a | 0.4 ± 0.2 ^c | 0.5 ± 0.2 ^c | 0.5 ± 0.2 ^c |

GCSB-5 or diclofenac was treated daily for 14 days after 2 weeks of OA induction by intra-articular injection of MIA.

^aDenotes significant differences ($P < 0.01$) versus the control group.

^{b,c}Denote significant differences ($P < 0.05$, $P < 0.01$) versus the vehicle-treated MIA group.

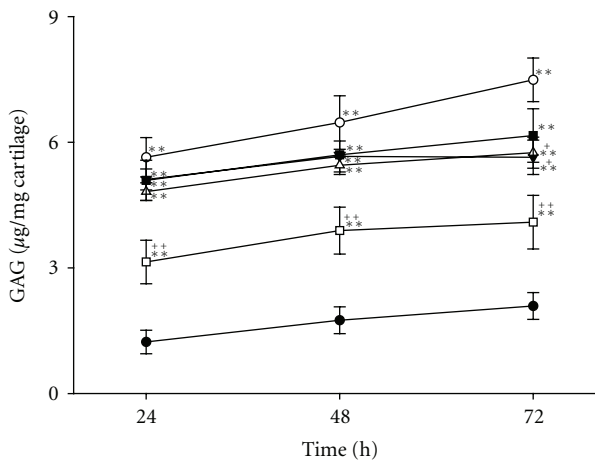


FIGURE 1: GAG release in rabbit articular cartilage explant cultures at 24, 48, and 72 h. Rabbit articular cartilage explants were stimulated with rhIL-1 α (5 ng/mL). The amount of GAG release stimulated by rhIL-1 α (\circ) increased approximately 3.6 times compared to control (\bullet) at 72 h. GCSB-5 (1.0×10^{-3} (\blacktriangledown) and 1.0×10^{-2} (\triangle) mg/mL) and diclofenac (30 μ M (\square)) efficiently inhibited the GAG release. However, a high concentration of GCSB-5 (1.0×10^{-1} (\blacksquare) mg/mL) slightly inhibited it. Each value represents the mean \pm S.E.M. from 6 articular cartilage explants cultures per group. **Significantly different ($P < 0.01$) from control. + and ++Significantly different ($P < 0.05$, $P < 0.01$) from rhIL-1 α .

constant at approximately 1.5 μ g/mg cartilage throughout the experiment. In the rhIL-1 α -treated group, on the other hand, the level of GAG in the culture medium dramatically increased to approximately 4 times the control values. GCSB-5 1×10^{-3} and 1×10^{-2} mg/mL treatments attenuated the elevation in GAG release at 72 h (Figure 1).

3.2. Gross Observation. In the MIA-injected groups, swelling and limping were first observed 7 days after MIA injection. They subsided transiently and then reappeared at 14 days.

These symptoms gradually aggravated at 21 days (data not shown) and were the most severe at 28 days. Twenty-eight days after MIA injection, swelling and limping were attenuated by both 300 and 600 mg/kg GCSB-5 treatment (Table 2).

3.3. Roentgenographic and Histopathological Analysis. Seven days after MIA injection, rats underwent the first roentgenographic examination. Their roentgenographic examinations revealed degenerative changes, such as irregularity or osteophytes on the surface of the cartilage and subchondral bone (data not shown). At 28 days, rats underwent the second roentgenographic examination (Figures 2(a)–2(d)). Morphological changes were more significant, showing rough edges of cartilage and the tendency of patellar displacement. These changes were attenuated by GCSB-5 600 mg/kg treatment. Twenty-eight days after MIA injection, H and E staining revealed irregular surface accompanied by ulceration, fibrillation, and loss of cartilage tissue (Figures 2(e)–2(h)). However, these cartilage damages were attenuated by GCSB-5 600 mg/kg treatment. SOFG staining also revealed clearly diffused PG depletion in joint cartilage tissues of MIA-injected rats (Figures 2(i)–2(l)). This loss of PG was attenuated by GCSB-5 600 mg/kg treatment. Summation of all histopathologic finding scores in vehicle-treated MIA group and in 300 and 600 mg/kg GCSB-5-treated MIA groups were 24.5 ± 1.3 , 16.1 ± 1.4 , and 12.5 ± 1.1 , respectively (Table 3).

3.4. Gelatinase Assay. Seven days after MIA injection, the activities of matrix metalloproteinase (MMP)-2 and -9 increased to 2.7 and 2.4 times that in the control group, respectively. Similarly, 28 days after MIA injection, the activities of MMP-2 and -9 increased to 2.3- and 2.8-fold higher than the control level, respectively. On day 7, GCSB-5 and diclofenac treatment showed no significant modulation on MMP activities (data not shown). However, on day 28, GCSB-5 300 mg/kg treatment exhibited significant MMP-2

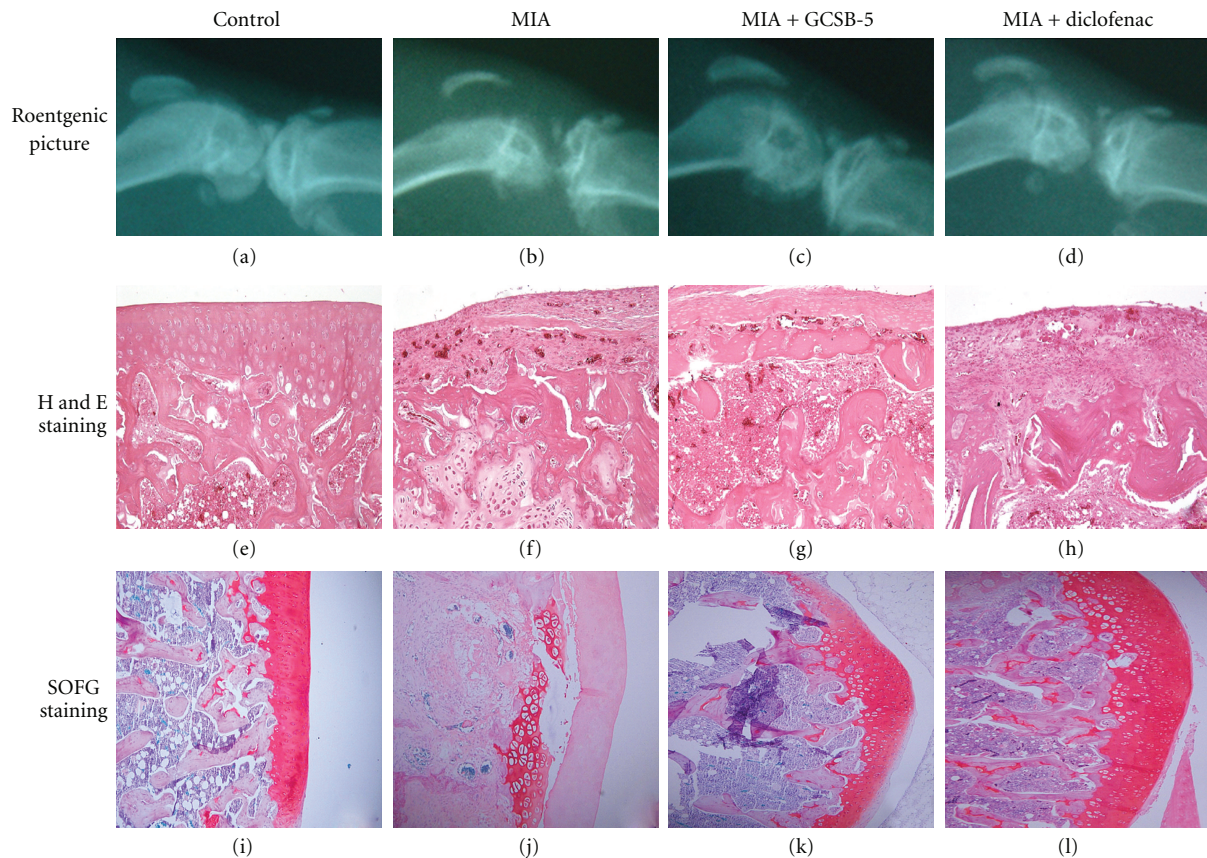


FIGURE 2: Roentgenography and histopathological features of osteoarthritic lesion in the knee joint of rats 28 days after intra-articular injection of MIA (H and E staining, $\times 100$; SOFG staining, $\times 100$). Control (a) represents intact normal joint feature. Vehicle-treated MIA (b) shows a severely damaged joint with rough edges around the tibia and femur, indicative of bone lysis, swelling, and tendency of patellar displacement. However, these damages were reduced significantly by treatment with 600 mg/kg GCSB-5 (c) and 5 mg/kg diclofenac (d). SOFG-stained control (e) represents normal cartilage PG staining, whereas vehicle-treated MIA (f) represents severely damaged cartilage showing marked fibrillation and the depletion of SOFG staining with separation of cartilage from subchondral bone. 600 mg/kg GCSB-5 (g) and 5 mg/kg diclofenac (h) treatments significantly reduced cartilage damage. H and E stained control (i) represents the normal status of joint cartilage, whereas vehicle-treated MIA (j) represents severely damaged cartilage showing widespread cell necrosis and inflammation. However, treatment with 600 mg/kg GCSB-5 (k) and 5 mg/kg diclofenac (l) treatments significantly reduced joint cartilage damage.

and -9 activities attenuation (79.6%, $P < 0.01$ and 81.2%, $P < 0.01$, resp.), while GCSB-5 600 mg/kg treatment did not affect the MMP-2 and -9 activities (91.0% and 91.6%, resp.) (Figure 3).

3.5. Inflammatory Mediators. The serum levels of TNF- α , IL-1 β , and IL-10 were 30.0 ± 4.5 pg/mL, 29.1 ± 3.7 pg/mL, and 27.8 ± 0.6 pg/mL in the control. 2 days after MIA injection, the serum levels of TNF- α , IL-1 β , and IL-10 increased to 2.8-, 3.4- and 2.2-fold higher than the control level, respectively. Increase in TNF- α level was significantly suppressed by treatment with GCSB-5, while increase in IL-10 level was significantly potentiated by GCSB-5. However, GCSB-5 did not affect the serum level of IL-1 β (Table 4). The levels of COX-2 and iNOS protein expression increased 3.3 and 12 times in the vehicle-treated MIA groups, compared to those in the control group 2 days after MIA injection, respectively (Figure 5). Increase in COX-2 and iNOS protein expression was significantly suppressed by treatment with GCSB-5.

The levels of TNF- α , IL-1 β , IL-10, COX-2, and iNOS mRNA expression increased 5.3, 2.1, 1.3, 7.8 and 8.8 times in the vehicle-treated MIA groups, compared to those in the control group 2 days after MIA injection, respectively (Figures 4 and 6). Increase in TNF- α , COX-2, and iNOS mRNA expression was significantly suppressed by treatment with GCSB-5. However, GCSB-5 did not affect the level of IL-1 β mRNA expression. Interestingly, increase of IL-10 mRNA expression was significantly potentiated by GCSB-5. At 7 and 28 days, there were no significant differences in the level of inflammatory mediators mRNA expression among any of the experimental groups (data not shown).

3.6. Nuclear NF- κ B and Cytosolic I κ B- α Immunoblot Assay. The nuclear localization of NF- κ B was measured by the protein level of NF- κ B p65 subunit in the nucleus. Cytosolic I κ B- α was also examined from cytosol fraction as an endogenous NF- κ B inhibitor. The level of nuclear NF- κ B p65 protein expression increased 2.5 times, whereas the level of cytosolic

TABLE 3: Summary of microscopic findings.

| | | Vehicle | MIA | | Diclofenac 5 mg/kg |
|--|-----|----------------|-----------------------------|-----------------------------|-----------------------------|
| | | | GCSB-5 (mg/kg) 300 | 600 | |
| Structural changes in the joint | | | | | |
| Surface irregularities | + | 0/4 | 2/4 | 1/4 | 2/4 |
| | ++ | 0/4 | 1/4 | 2/4 | 2/4 |
| | +++ | 4/4 | 1/4 | 1/4 | 0/4 |
| Average pathology score | | 3 | 1.8 | 2 | 1.5 |
| Ulceration | | | | | |
| Ulceration | + | 0/4 | 1/4 | 3/4 | 1/4 |
| | ++ | 1/4 | 1/4 | 1/4 | 2/4 |
| | +++ | 3/4 | 2/4 | 0/4 | 1/4 |
| Average pathology score | | 2.8 | 2.3 | 1.3 | 2 |
| Fibrillation of cartilage surface | | | | | |
| Fibrillation of cartilage surface | + | 0/4 | 1/4 | 2/4 | 3/4 |
| | ++ | 3/4 | 3/4 | 1/4 | 1/4 |
| | +++ | 1/4 | 0/4 | 1/4 | 0/4 |
| Average pathology score | | 2.3 | 1.8 | 1.8 | 1.3 |
| Disorganization of chondrocytes | | | | | |
| Disorganization of chondrocytes | + | 0/4 | 1/4 | 3/4 | 3/4 |
| | ++ | 2/4 | 3/4 | 1/4 | 0/4 |
| | +++ | 2/4 | 0/4 | 0/4 | 1/4 |
| Average pathology score | | 2.5 | 1.8 | 1.3 | 1.5 |
| Exposure of subchondral bone | | | | | |
| Exposure of subchondral bone | + | 2/4 | 1/4 | 0/4 | 0/4 |
| | ++ | 1/4 | 0/4 | 1/4 | 0/4 |
| | +++ | 1/4 | 0/4 | 0/4 | 0/4 |
| Average pathology score | | 1.8 | 0.3 | 0.5 | 0 |
| Cellular changes of chondrocyte hypertrophy | | | | | |
| Cellular changes of chondrocyte hypertrophy | + | 1/4 | 3/4 | 1/4 | 0/4 |
| | ++ | 2/4 | 1/4 | 3/4 | 4/4 |
| | +++ | 1/4 | 0/4 | 0/4 | 0/4 |
| Average pathology score | | 2 | 1.3 | 1.8 | 2 |
| Degeneration/necrosis | | | | | |
| Degeneration/necrosis | + | 0/4 | 1/4 | 4/4 | 2/4 |
| | ++ | 1/4 | 0/4 | 0/4 | 2/4 |
| | +++ | 3/4 | 3/4 | 0/4 | 0/4 |
| Average pathology score | | 2.8 | 2.5 | 1 | 1.5 |
| Inflammatory cell infiltration in synovial tissue | | | | | |
| Inflammatory cell infiltration in synovial tissue | + | 1/4 | 3/4 | 2/4 | 3/4 |
| | ++ | 1/4 | 1/4 | 1/4 | 0/4 |
| | +++ | 2/4 | 0/4 | 0/4 | 1/4 |
| Average pathology score | | 2.3 | 1.3 | 1 | 1.5 |
| Synovial cell proliferation | | | | | |
| Synovial cell proliferation | + | 1/4 | 3/4 | 3/4 | 3/4 |
| | ++ | 2/4 | 0/4 | 0/4 | 1/4 |
| | +++ | 1/4 | 1/4 | 1/4 | 0/4 |
| Average pathology score | | 2 | 1.5 | 1.5 | 1.3 |
| Safranin-O staining | | | | | |
| Reduction of staining in cartilage | + | 0/4 | 3/4 | 1/4 | 1/4 |
| | ++ | 0/4 | 0/4 | 0/4 | 1/4 |
| | +++ | 4/4 | 1/4 | 0/4 | 0/4 |
| Average pathology score | | 3 | 1.5 | 0.3 | 0.8 |
| Total pathology score (average \pm S.E.M) | | 24.5 \pm 1.3 | 16.1 \pm 1.4 ^a | 12.5 \pm 1.1 ^b | 13.4 \pm 2.2 ^b |

+: mild, ++: moderate, and +++: severe.

^{a,b}Denote significant differences ($P < 0.05$, $P < 0.01$) versus the vehicle-treated MIA group. $N = 4$.

TABLE 4: Effect of GCSB-5 on serum TNF- α , IL-1 β , and IL-10 levels in MIA-induced osteoarthritic rats.

| Group | TNF- α (pg/mL) | IL-1 β (pg/mL) | IL-10 (pg/mL) |
|--------------------|-------------------------------|-------------------------------|-------------------------------|
| Control | 30.0 \pm 4.5 | 29.1 \pm 3.7 | 27.8 \pm 0.6 |
| MIA | | | |
| Vehicle | 85.4 \pm 6.6 ^b | 99.5 \pm 11.2 ^b | 60.4 \pm 7.2 ^b |
| GCSB-5 | | | |
| 300 mg/kg | 60.0 \pm 4.6 ^{a,c} | 102.6 \pm 10.4 ^b | 91.4 \pm 8.8 ^{b,d} |
| 600 mg/kg | 55.3 \pm 6.2 ^c | 93.9 \pm 9.0 ^b | 84.5 \pm 8.5 ^{b,c} |
| Diclofenac 5 mg/kg | 50.1 \pm 4.0 ^d | 49.5 \pm 7.5 ^d | 86.1 \pm 9.2 ^{b,c} |

The serum concentration of TNF- α , IL-1 β , and IL-10 was determined using enzyme-linked immunosorbent assay. The results are presented as mean \pm S.E.M. of 6 rats per group.

^{a,b}Denote significant differences ($P < 0.05$, $P < 0.01$) compared with control group.

^{c,d}Denote significant differences ($P < 0.05$, $P < 0.01$) compared with vehicle-treated MIA group.

I κ B- α protein expression decreased 2.0-fold in the vehicle-treated MIA group, compared to that in the control group 2 days after MIA injection. These changes were significantly inhibited by GCSB-5 treatment (Figure 7).

4. Discussion

Today, cure for OA remains elusive. The management of OA is largely palliative focusing on the alleviation of symptoms. Current recommendations for the management of OA include a combination of nonpharmacological (weight loss, education programs, and exercise) and pharmacological interventions (paracetamol, NSAIDs, etc.) [26]. Of the pharmacological intervention available, analgesics and NSAIDs have been proven to be highly effective in controlling the symptoms and signs of OA. However, they have potential gastrointestinal (GI) adverse effects. Herbal medicinal products (HMPs) are not yet among the recommended treatment options, although they are used in a variety of oral and topical forms in the treatment of OA. The mechanism of action of HMPs is broader than that of NSAIDs and/or analgesics in current use for symptomatic OA. Although the exact mechanisms of action have not yet been elucidated, there is no doubt that all herbal medicines act via several pathways, including inhibition of COX and/or lipoxygenase, inhibition of cytokine release, inhibition of elastase or hyaluronidase, and induce antioxidative activity [27]. On the basis of this knowledge, our experimental herbal extract, GCSB-5, consisted of various herbs known to exhibit antiarthritic, anti-inflammatory and analgesic effects, is expected to show therapeutic activity against OA.

Articular cartilage destruction is a key pathological characteristic of OA. MIA is an inhibitor of glyceraldehyde-3-phosphate dehydrogenase activity, and therefore an inhibitor of glycolysis shown to induce chondrocyte death *in vitro* [28]. Intra-articular injection of MIA induces chondrocyte death in the articular cartilage of rodent and nonrodent species [29]. Injection of MIA into the knees of rats provides a

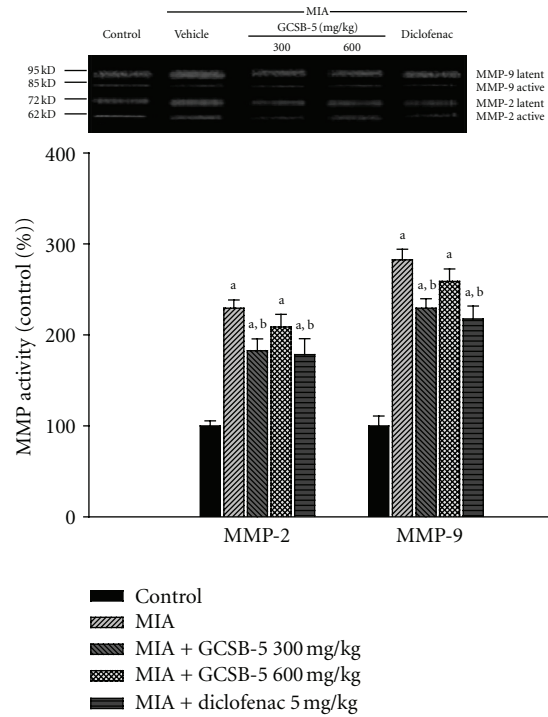


FIGURE 3: Activities of MMP-2 (Gelatinase A) and MMP-9 (Gelatinase B) assessed by zymography in knee joint cartilages obtained 28 days after MIA injection. The latent and active amounts of gelatinase were combined to give a total value for each gelatinase. Each value represents the mean \pm S.E.M. from 6 rats per group. ^aSignificantly different ($P < 0.01$) from control. ^bSignificantly different ($P < 0.05$) from vehicle-treated MIA.

model in which lesions resembling some aspects of human OA produced quickly and has been suggested as a model for the study of chondroprotective drugs [30]. In the present study, we investigated GCSB-5 on the clinical and behavioral changes associated with MIA-induced OA. Swelling and limping were apparent as early as 7 days after MIA injection, after which they became transiently subsided. At 14 days, there was a second period of knee joint swelling and limping that was progressively aggravated until day 28. Administration of GCSB-5 once daily for 28 days significantly reduced the severity of swelling and limping. These results suggest that GCSB-5 may have potential as a treatment for OA. Roentgenographic and histological observations strongly supported the behavioral changes following MIA injection as well as the protective effect of GCSB-5.

Cartilage comprises an extracellular matrix consisting of PGs, collagens (types II, IX, XI, and others), and water. Cartilage PGs consist of a protein core with GAG side chains [31]. When cartilage is damaged by inflammatory mediators such as rhIL-1 α , PGs degrade and consequently release GAG, which is a typical clinical symptom of OA. GCSB-5 did not inhibit GAG release at low concentrations, but showed an inhibitory effect at moderate-to-high concentrations. This analysis reflected the histochemical appearance of cartilage. SOFG staining showed significant PG loss and lesion development in subchondral bone which were induced by MIA

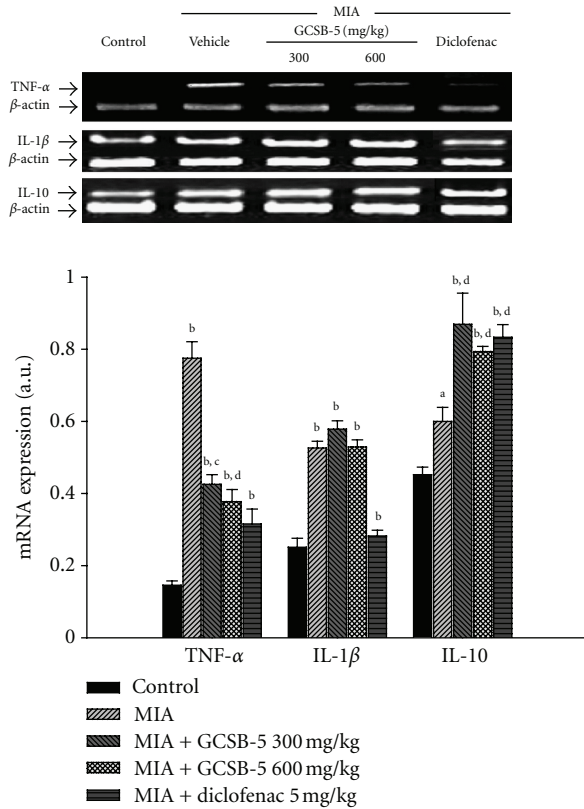


FIGURE 4: *TNF-α*, *IL-1β*, and *IL-10* mRNA expressions in cartilage from knee joints of rats at day 2 after MIA injection. Each value represents the mean \pm S.E.M. from 6 rats per group. ^{a,b}Significantly different ($P < 0.01$, $P < 0.05$) from control. ^{c,d}Significantly different ($P < 0.01$, $P < 0.05$) from vehicle-treated MIA.

injection. These cartilage damages were attenuated by GCSB-5 treatment. Our data suggest that GCSB-5 may protect articular cartilage from degradation.

MMPs are a family of proteinases that together can degrade all extracellular matrix components. Type IV collagenases (gelatinases) are members of the MMP family and are thought to play an important role in the degradation of extracellular components. The gelatinase subclass can be divided into gelatinase-A (MMP-2) and gelatinase-B (MMP-9), which is capable of degrading types IV and V collagens, elastin, and gelatin [32]. MMP-2 is known to be produced by osteoblasts and tissue structural cells, including fibroblasts and endothelial cells, whereas MMP-9 is produced by inflammatory cells such as macrophages, neutrophils, and eosinophils [33, 34]. These MMPs are secreted as latent precursors and can be activated by limited proteolysis. The increased expression of MMP-2 and -9 in the synovium of patients with arthritic effusions superiorly reflects the inflammatory condition of the joints, and a positive correlation between MMP-9 production and rapid destruction of the hip joint has been described in OA [35]. Although GCSB-5 did not affect MMP-2 and -9 activities 7 days after MIA injection (data not shown), GCSB-5 especially at a dose of 300 mg/kg suppressed MMP-2 and -9 activities 28 days after

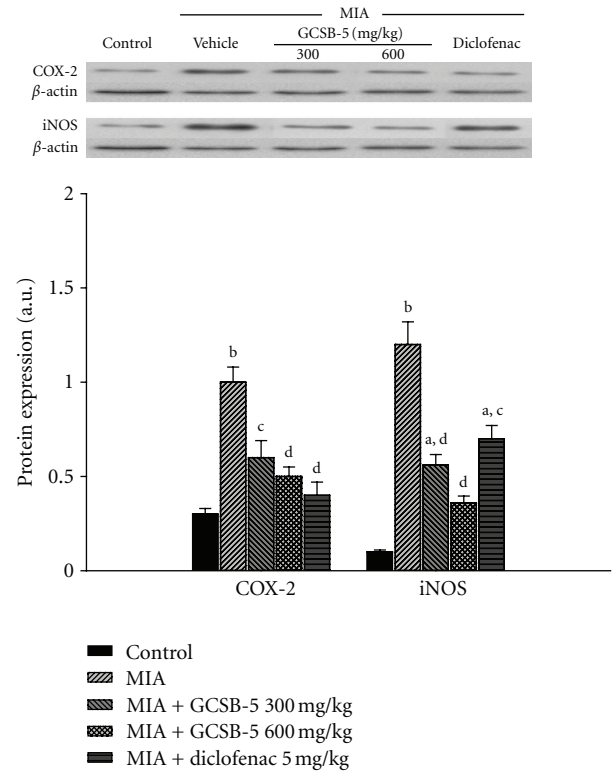


FIGURE 5: COX-2 and iNOS protein expressions in cartilage from knee joints of rats at day 2 after MIA injection. Each value represents the mean \pm S.E.M. from 6 rats per group. ^{a,b}Significantly different ($P < 0.01$, $P < 0.05$) from control. ^{c,d}Significantly different ($P < 0.01$, $P < 0.05$) from vehicle-treated MIA.

MIA injection. Our results indicate that GCSB-5 inhibits collagen degradation through inhibition of MMP-2 and -9 activities in late stage of OA.

Matrix turnover is solely dependent on chondrocytes, which are believed to be the main site of inflammatory mediators production in human OA [36]. Overexpression of MMPs is induced by several cytokines, such as *TNF-α*, *IL-1*, *IL-17*, and *IL-10*. *TNF-α* and *IL-1β* drive the catabolic processes in OA, leading to cartilage degradation. In this study, the levels of *TNF-α*, *COX-2*, and *iNOS* mRNA expression were significantly increased 2 days after MIA injection and returned to control level at 28 days (data not shown). These increases were attenuated by GCSB-5 treatment. On the other hand, a critical function of *IL-10* is to limit inflammatory responses [37]. This cytokine inhibits *IL-1β* and *TNF-α* expression and is present in OA chondrocytes, where it may counteract their catabolic effects [38]. Interestingly, GCSB-5 treatment significantly potentiated this increase. Our results indicate that GCSB-5 shows a significant anti-inflammatory action in the early stage of OA.

Inappropriate regulation of NF- κ B activity has been implicated in the pathogenesis of inflammatory diseases, such as rheumatoid arthritis and OA [39]. NF- κ B signaling pathways mediate critical events in the inflammatory

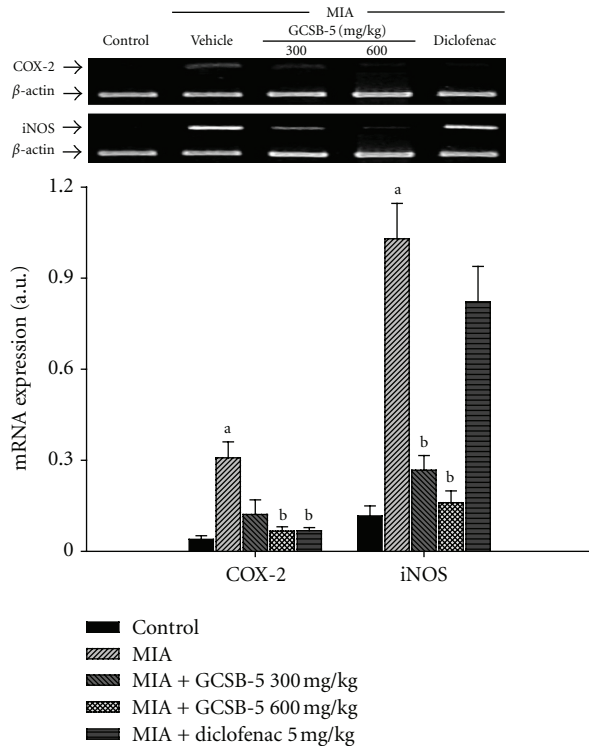


FIGURE 6: COX-2 and iNOS mRNA expressions in cartilage from knee joints of rats at day 2 after MIA injection. Each value represents the mean \pm S.E.M. from 6 rats per group. ^aSignificantly different ($P < 0.05$) from control. ^bSignificantly different ($P < 0.05$) from vehicle-treated MIA.

response by chondrocytes, leading to progressive extracellular matrix damage and cartilage destruction. NF- κ B mediates fibronectin fragment-induced chondrocyte activation and increased expression of proinflammatory cytokines, chemokines as well as MMPs such as IL-6, IL-8, MCP-1, growth-related oncogene- α , - β , - γ , and MMP-13 by human articular chondrocytes [40, 41]. In this study, we showed that GCSB-5 inhibits nuclear translocation of NF- κ B/p65 subunit and degradation of I κ B- α .

Although the results of present study provided clues for further studies on the pharmacological mechanisms of GCSB-5, the relationship between the effects and its active components remains to be clarified. Therefore, the detailed molecular mechanisms of GCSB-5 and further studies of anti-inflammatory properties of the active ingredients should be elucidated.

5. Conclusions

These results indicate that GCSB-5 improves OA-induced cartilage damage, which inhibits MMP activities, down-regulates the expression of inflammatory mediators, and suppresses NF- κ B activity, suggesting that GCSB-5 may be a potential therapeutic agent for OA.

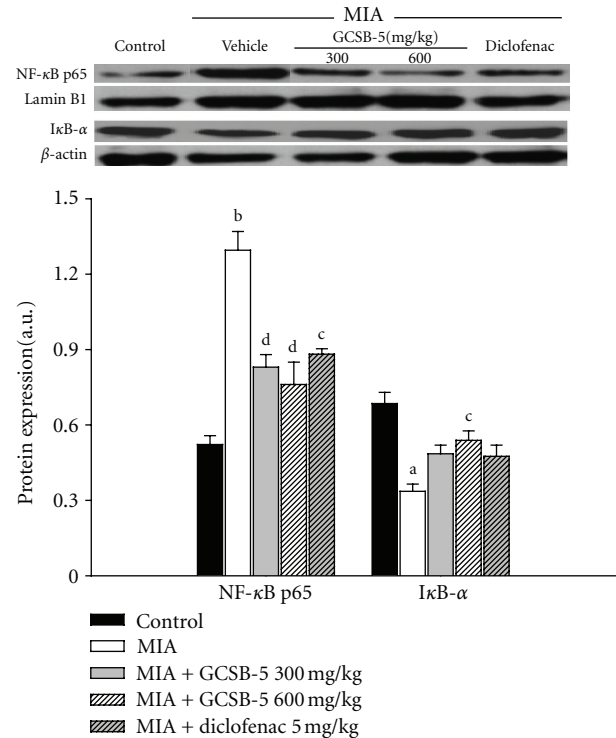


FIGURE 7: Nuclear NF- κ B p65 and cytosolic I κ B- α protein expressions in cartilage from knee joints of rats at day 2 after MIA injection. Each value represents the mean \pm S.E.M. from 6 rats per group. ^{a,b}Significantly different ($P < 0.05$, $P < 0.01$) from control. ^{c,d}Significantly different ($P < 0.05$, $P < 0.01$) from vehicle-treated MIA.

Acknowledgments

This paper was supported by Green Cross Corporation, Korea, and Jaseng Hospital, Korea.

References

- [1] M. B. Goldring and S. R. Goldring, "Osteoarthritis," *Journal of Cellular Physiology*, vol. 213, no. 3, pp. 626–634, 2007.
- [2] J. Saklatvala, "Inflammatory signaling in cartilage: MAPK and NF- κ B pathways in chondrocytes and the use of inhibitors for research into pathogenesis and therapy of osteoarthritis," *Current Drug Targets*, vol. 8, no. 2, pp. 305–313, 2007.
- [3] F. E. Silverstein, G. Faich, J. L. Goldstein et al., "Gastrointestinal toxicity with Celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: a randomized controlled trial," *Journal of the American Medical Association*, vol. 284, no. 10, pp. 1247–1255, 2000.
- [4] L. X. Chen, L. Lin, H. J. Wang et al., "Suppression of early experimental osteoarthritis by in vivo delivery of the adenoviral vector-mediated NF- κ Bp65-specific siRNA," *Osteoarthritis and Cartilage*, vol. 16, no. 2, pp. 174–184, 2008.
- [5] S. W. Park, C. H. Lee, S. H. Kim et al., "General pharmacological study of GCSB-5, a herbal formulation," *The Journal of Applied Pharmacology*, vol. 14, pp. 194–201, 2006.
- [6] H. W. Kim, Y. B. Kwon, T. W. Ham et al., "The antinociceptive and anti-inflammatory effect of ethylacetate extracts from

- Bang-Poong (*Radix ledebouriellae*) on the Freund's adjuvant-induced arthritis in rats," *Journal of Veterinary Science*, vol. 3, no. 4, pp. 343–349, 2002.
- [7] B. Zhao, X.-B. Yang, X.-W. Yang et al., "Intestinal permeability of the constituents from the roots of *saposhnikovia divaricata* in the human caco-2 cell monolayer model," *Planta Medica*, vol. 77, no. 13, pp. 1531–1535, 2011.
- [8] Y. Ida, Y. Satoh, M. Katsumata et al., "Two novel oleanolic acid saponins having a sialyl Lewis X mimetic structure from *Achyranthesfauriei* root," *Bioorganic and Medicinal Chemistry Letters*, vol. 8, no. 18, pp. 2555–2558, 1998.
- [9] D. Seidlova-Wuttke, D. Christel, P. Kapur, B. T. Nguyen, H. Jarry, and W. Wuttke, " β -Ecdysone has bone protective but no estrogenic effects in ovariectomized rats," *Phytomedicine*, vol. 17, no. 11, pp. 884–889, 2010.
- [10] H. C. Kim, S. I. Lee, and D. K. Ahn, "Effect of *acanthopanax cortex* on the IL-8 production in human monocyte as a rheumatoid arthritis remedy," *Journal of Herbalogy*, vol. 10, no. 1, pp. 49–59, 1995.
- [11] C. H. Lee, S. H. Kim, J. S. Lee et al., "Evaluation of the antinociceptive properties of GCSB-5, a herbal formulation," *Korean Journal of Pharmacognosy*, vol. 36, no. 4, pp. 299–304, 2005.
- [12] X. C. Nguyen, V. M. Chau, V. K. Phan et al., "Inhibitors of osteoclast formation from rhizomes of *Cibotium barometz*," *Journal of Natural Products*, vol. 72, no. 9, pp. 1673–1677, 2009.
- [13] S. H. Kim, C. H. Lee, J. S. Lee et al., "Anti-inflammatory activities of a herbal preparation GCSB-5 on acute and chronic inflammation," *Korean Journal of Pharmacognosy*, vol. 36, no. 4, pp. 311–317, 2005.
- [14] R. W. Wong and A. B. Rabie, "Effect of genistin on bone formation," *Frontiers in Bioscience*, vol. 2, pp. 764–770, 2010.
- [15] N. D. Hong, Y. S. Rho, J. W. Kim, D. H. Won, N. J. Kim, and B. S. Cho, "Studies on the general pharmacological activities of *Eucommia ulmoides* Oliver," *Korean Journal of Pharmacognosy*, vol. 19, pp. 102–110, 1998.
- [16] J. Zhu, X. Gao, W. L. Xie, Y. Z. Jin, and W. J. Sun, "Effect of geniposide on serum IL-1 β and TNF- α of rheumatoid arthritis rats," *Zhongguo Zhongyao Zazhi*, vol. 30, no. 9, pp. 708–711, 2005.
- [17] H. Ha, J. Ho, S. Shin et al., "Effects of *Eucommiae Cortex* on osteoblast-like cell proliferation and osteoclast inhibition," *Archives of Pharmacol Research*, vol. 26, no. 11, pp. 929–936, 2003.
- [18] H. J. Chung, H. S. Lee, J. S. Shin et al., "Modulation of acute and chronic inflammatory processes by a traditional medicine preparation GCSB-5 both in vitro and in vivo animal models," *Journal of Ethnopharmacology*, vol. 130, no. 3, pp. 450–459, 2010.
- [19] J. D. Sandy, H. L. G. Brown, and D. A. Lowther, "Degradation of proteoglycan in articular cartilage," *Biochimica et Biophysica Acta*, vol. 543, no. 4, pp. 536–544, 1978.
- [20] S. E. Bove, S. L. Calcaterra, R. M. Brooker et al., "Weight bearing as a measure of disease progression and efficacy of anti-inflammatory compounds in a model of monosodium iodoacetate-induced osteoarthritis," *Osteoarthritis and Cartilage*, vol. 11, no. 11, pp. 821–830, 2003.
- [21] M. J. Yeom, H. C. Lee, G. H. Kim, I. Shim, H. J. Lee, and D. H. Hahm, "Therapeutic effects of *Hominis placenta* injection into an acupuncture point on the inflammatory responses in subchondral bone region of adjuvant-induced polyarthritic rat," *Biological and Pharmaceutical Bulletin*, vol. 26, no. 10, pp. 1472–1477, 2003.
- [22] J. H. Choi, J. H. Choi, D. Y. Kim et al., "Effects of SKI 306X, a new herbal agent, on proteoglycan degradation in cartilage explant culture and collagenase-induced rabbit osteoarthritis model," *Osteoarthritis and Cartilage*, vol. 10, no. 6, pp. 471–478, 2002.
- [23] Y. Sakano, N. Terada, H. Ueda et al., "Histological study of articular cartilage in experimental rat knee arthritis induced by intracapsular injection of cationic polyethyleneimine," *Medical Electron Microscopy*, vol. 33, no. 4, pp. 246–257, 2000.
- [24] K. Kobayashi, R. Imaizumi, H. Sumichika et al., "Sodium iodoacetate-induced experimental osteoarthritis and associated pain model in rats," *Journal of Veterinary Medical Science*, vol. 65, no. 11, pp. 1195–1199, 2003.
- [25] H. Dumond, N. Presle, P. Pottier et al., "Site specific changes in gene expression and cartilage metabolism during early experimental osteoarthritis," *Osteoarthritis and Cartilage*, vol. 12, no. 4, pp. 284–295, 2004.
- [26] L. G. Ameye and W. S. S. Chee, "Osteoarthritis and nutrition. From nutraceuticals to functional foods: a systematic review of the scientific evidence," *Arthritis Research and Therapy*, vol. 8, no. 4, article R127, 2006.
- [27] M. Cameron, J. J. Gagnier, C. V. Little, T. J. Parsons, A. Blümle, and S. Chrusasik, "Evidence of effectiveness of herbal medicinal products in the treatment of arthritis—Part 1: osteoarthritis," *Phytotherapy Research*, vol. 23, no. 11, pp. 1497–1515, 2009.
- [28] C. Cournil, B. Liagre, L. Grossin et al., "Overexpression and induction of heat shock protein (Hsp) 70 protects in vitro and in vivo from mono-iodoacetate (MIA)-induced chondrocytes death," *Arthritis Research and Therapy*, vol. 3, supplement 1, p. 41, 2001.
- [29] J. Dunham, S. Hoedt-Schmidt, and D. A. Kalbhen, "Prolonged effect of iodoacetate on articular cartilage and its modification by an anti-rheumatic drug," *International Journal of Experimental Pathology*, vol. 74, no. 3, pp. 283–289, 1993.
- [30] R. A. Barve, J. C. Minnerly, D. J. Weiss et al., "Transcriptional profiling and pathway analysis of monosodium iodoacetate-induced experimental osteoarthritis in rats: relevance to human disease," *Osteoarthritis and Cartilage*, vol. 15, no. 10, pp. 1190–1198, 2007.
- [31] A. L. Stevens, C. A. Wheeler, S. R. Tannenbaum, and A. J. Grodzinsky, "Nitric oxide enhances aggrecan degradation by aggrecanase in response to TNF- α but not IL-1 β treatment at a post-transcriptional level in bovine cartilage explants," *Osteoarthritis and Cartilage*, vol. 16, no. 4, pp. 489–497, 2008.
- [32] R. Trelstad and P. Kemp, *Matrix Glycoproteins and Proteoglycans*, WB Saunders, Philadelphia, Pa, USA, 1993.
- [33] K. Kusano, C. Miyaura, M. Inada et al., "Regulation of matrix metalloproteinases (MMP-2,-3,-9, and-13) by interleukin-1 and interleukin-6 in mouse calvaria: association of MMP induction with bone resorption," *Endocrinology*, vol. 139, no. 3, pp. 1338–1345, 1998.
- [34] G. Murphy and A. J. Docherty, "The matrix metalloproteinases and their inhibitors," *American Journal of Respiratory Cell and Molecular Biology*, vol. 7, no. 2, pp. 120–125, 1992.
- [35] S. C. Chu, S. F. Yang, K. H. Lue, Y. S. Hsieh, T. Y. Hsiao, and K. H. Lu, "The clinical significance of gelatinase B in gouty arthritis of the knee," *Clinica Chimica Acta*, vol. 339, no. 1-2, pp. 77–83, 2004.
- [36] C. Melchiorri, R. Meliconi, L. Frizziero et al., "Enhanced and coordinated in vivo expression of inflammatory cytokines and nitric oxide synthase by chondrocytes from patients with osteoarthritis," *Arthritis and Rheumatism*, vol. 41, no. 12, pp. 2165–2174, 1998.

- [37] K. W. Moore, R. De Waal Malefyt, R. L. Coffman, and A. O'Garra, "Interleukin-10 and the interleukin-10 receptor," *Annual Review of Immunology*, vol. 19, pp. 683–765, 2001.
- [38] F. Iannone, C. De Bari, F. Dell' Accio et al., "Interleukin-10 and interleukin-10 receptor in human osteoarthritic and healthy chondrocytes," *Clinical and Experimental Rheumatology*, vol. 19, no. 2, pp. 139–145, 2001.
- [39] A. S. Baldwin, "The transcription factor NF- κ B and human disease," *Journal of Clinical Investigation*, vol. 107, no. 1, pp. 3–6, 2001.
- [40] C. B. Forsyth, A. Cole, G. Murphy, J. L. Bienias, H. J. Im, and R. F. Loeser Jr., "Increased matrix metalloproteinase-13 production with aging by human articular chondrocytes in response to catabolic stimuli," *Journals of Gerontology A*, vol. 60, no. 9, pp. 1118–1124, 2005.
- [41] J. I. Pulai, H. Chen, H. J. Im et al., "NF- κ B mediates the stimulation of cytokine and chemokine expression by human articular chondrocytes in response to fibronectin fragments," *Journal of Immunology*, vol. 174, no. 9, pp. 5781–5788, 2005.