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Microbial chondroitin sulfate in experimental knee osteoarthritis model investigation of chondroprotective effect

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Abstract. – OBJECTIVE: Chondroitin sulfate (CS) is a glycosaminoglycan with proven anti-inflammatory, anti-apoptotic, anti-oxidant properties. CS increases type II collagen and proteoglycan synthesis in human joint chondrocytes. CS can reduce the production of pro-inflammatory mediators and proteases to improve the anabolic/catabolic balance of the extracellular cartilage matrix (ECM). Due to these characteristics, it is a natural compound that is considered to be Symptomatic Slow-Acting Drugs for Osteoarthritis (SYSADOA). Microbial chondroitin sulfate (MCS) was produced from two different bacterial sources using biotechnological methods by our team. In this study, we aimed to apply microbially produced CS and bovine-derived commercial CS forms to rabbit knees with osteoarthritis experimentally and to evaluate the results.

MATERIALS AND METHODS: In this study, a cruciate ligament cutting model was applied to 40 New Zealand rabbits to induce experimental osteoarthritis. Four weeks after the surgical procedure, rabbits were divided into 4 groups as control, animal-derived MCS, E coli-derived MCS and PaJC-derived MCS group. The standard rabbit diet was fed to the control group, and the other groups were additionally fed 17 mg/kg/day CS/MCS for 12 weeks. The rabbits were sacrificed at the 12th week after surgery and the preparations obtained were evaluated histopathologically.

RESULTS: As a result, it was observed that regeneration tissue was statistically significant in histopathological cartilage tissue compared to the control group of CS developed from different sources given to rabbits with osteoarthritis. It was determined that among the CS groups produced from different sources, the group with the highest chondroprotective effect was MCS originating from E.coli.

CONCLUSIONS: This vegan product (MCS), which we obtained as a result of our study, was produced by our team from a microbial source. According to our analysis, it has the potential to be an effective alternative therapy agent in the treatment of osteoarthritis.

Key Words:
Chondroitin sulfate, Microbial chondrotin sulfate, Osteoarthritis, Condroprotective.

Abbreviations

OA: Osteoarthritis; CS: Chondroitin sulfate; ECM: Extracellular cartilage matrix; SYSADOA: Symptomatic Slow-Acting Drugs for Osteoarthritis; MCS: Microbial chondroitin sulfate; SM: Synovial membrane; H&E: Hematoxylin Eosin; MT: Masson trichrome; SO: Safranin-O.

Introduction

Osteoarthritis (OA) is the most common form of arthritis and its prevalence increases with age. OA affects many joints and causes more disability in load-bearing joints. Knee pain seen in OA was determined to be the most common cause of physical disability in the elderly population[1,2]. There are many treatment options currently available for osteoarthritis. The ideal treatment for OA is to understand the mechanism and prevent joint destruction and disease progression[3]. However, none of the treatment methods is sufficient to completely eradicate the disease. Analgesics, NSAID, exercises, physical therapy, intraarticular agents (steroid, hyaluronic acid), orthoses, patient education, glucosamine-chondroitin sulphate (CS) preparations and topical

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capsaisin are among the main treatment methods used in the treatment of OA[4]. Cartilage is not the only tissue affected in osteoarthritis. It undergoes structural and metabolic changes in the subchondral bone and synovial membrane (SM) with the progression of the disease[5]. The management of the disease process is difficult for physicians, as the pathogenesis of OA is complex. The main approach in the treatment of osteoarthritis is symptom management with the aim of reducing pain and improving joint function. However, control of symptoms is not the only goal to be achieved in osteoarthritis. The most ideal treatment for osteoarthritis should be preserving joint structures by prioritizing increasing the quality of life of patients[6]. Nutraceutical preparations that are used and support the process are more widely used today. The contamination is a major problem in these products. Microbially produced molecules such as MCS have an important potential in this field with their high degree of purity and show a good safety profile. CS is an essential component of the extracellular matrix. It is found in the extracellular matrix of many tissues, including cartilage, bone, skin, and tendons[7,8]. It is widely used in medical procedures as cell and tissue regeneration. It has been determined that CS is absorbed by 70% when taken orally, reduces leukocyte elastarase and hyaluronidase activity, and increases the rate of hyaluronic acid[9]. Its anti-inflammatory, anti-microbial and anti-apoptotic properties make it effective in medical applications. In these uses, mostly animal based preparations are preferred. Prionic, viral and ecological risks encountered in animal products have led to the need for alternative resources in this context[10]. Microbial Chondroitin Sulfates (MCS) obtained from microbial sources are the most important alternatives with their antiallergic and biocompatible-non toxic structures that do not carry prionic and viral risks, and have high specificity[11]. In our study, unique MCSs obtained by biotechnological methods from recombinant strains created by our team were used. In the experimental osteoarthritis model created using Himalayan rabbits, the effects of microbial CS obtained from two different bacterial sources (*E. coli* and *P. aeruginosa*) on the osteoarthritis healing process were investigated for the first time and evaluated histopathologically.

CS can also reduce the activity of enzymes that damage cartilage, such as collagenase and phospholipase A2, the activity of lysosomal enzymes, and the formation of superoxide radicals[11]. MCS, on the other hand, are known for their catalytically more active, high biocompatibility and effective cell healing effects. These properties are associated with their low MA values. In our study, the positive effect of oral MCS application on cartilage regeneration was investigated.

Materials and Methods

For the study, the permission was obtained from the Experimental Studies Ethics Committee of our University with the Ethics Committee No. 2015/A- 67. In the study, 40 adult New Zealand rabbits weighting between 3000 and 4000 g were used for the anterior cruciate ligament dissection model to create experimental osteoarthritis. In rabbits, 0.1 cc/kg 2% Xylazine Hydrochloride and 20 mg/kg Ketamine Hydrochloride were administered intramuscularly for anesthesia, and then, the right knee joints were reached with an anterior longitudinal incision. After medial parapatellar arthrotomy, the patella was dislocated laterally and the anterior cruciate ligament was cut, whether the cruciate ligament was completely cut was evaluated with the anterior drawer test, and the experimental animals were left to normal cage activity in the postoperative period. The rabbits were randomly divided into 4 groups 4 weeks after the surgery. The standard rabbit diet was fed daily to 10 rabbits in group 1 (control group) for 12 weeks. The rabbits in group 2 (tCS) were also given CS (17 mg/kg/day) of animal origin in addition to the standard diet. This CSs to be administered to rabbits in this group were obtained from bovine trachea and packaged in lyophilized powder. In addition, this product has been chosen from the SIGMA catalog as it is the most cost-effective and relatively easy to obtain alternative. The other 10 rabbits separated as group 3 (mECS) were given microbial KS (17 mg/kg/day) from recombinant *E.coli* produced in our project in addition to the standard diet. The other 10 rabbits separated as group 4 (mPCS) were given microbial CS (17 mg/kg/ day) originating from recombinant *P. aeruginosa* produced in our project in addition to the standard diet. The microbial CS types used in the study were produced by our team[12]. Sigma Aldrich C9819 preparation was used as commercial or animal CS. At the end of the 12th week, all rabbits were sacrificed by intramuscular high dose ketamine administration. The operated knees of the rabbits were resected, including the synovia, femur, and

tibia joint surfaces. In addition to the evaluation of macroscopic degenerative changes in femoral medial condyle cartilage tissue, various parameters were determined and evaluated for histological evaluation of synovial tissue.

Histopathological Evaluation

Arthrotomy has been performed to the right lower extremities of rabbits. Bone, cartilage and synovial tissue samples taken under appropriate conditions by osteotomy between the femur distal and tuberositas tibia were randomly numbered and pathological analyzes were performed. The materials were fixed in 10% formaldehyde for 1 week. Following fixation, the tissues were subjected to decalcification in 10% formic acid solution for 7 days. The decalcification solution was renewed every other day and, during this period, the tissues in the decalcification solution were kept on the shaker (Boeco PSU-15i) to increase the effectiveness and reduce the duration of the process. Within the scope of pathological analysis after decalcification, the tissue was divided into two sections, perpendicular to the joint space from the insertion point of the medial condyle, and cassetted in accordance with macroscopic sampling rules. The samples were washed under tap water for 3 hours to remove acid and followed-up for 14 hours in a fully automatic tissue tracking device (Shandon excelsior ES). During this procedure, the tissues underwent once 30 minutes of formaldehyde, 6 times 60 minutes of alcohol, twice 60, once 90 minutes of xylene, once for 60 minutes and twice for 90 minutes of paraffin. After the follow-up process, 3-4 micron thick sections were taken from the paraffin-embedded tissues with a microtome device (Leica RM 2255, Wetzlar, Germany). Hematoxylin & Eosin (H&E), Masson trichrome (MT) and Safranin-O (SO) staining techniques were performed to the sections taken. Staining and coverslip processes with H&E were performed with a fully automatic staining-closing device (Leica ST 5020). MT and SO staining were studied by manual method as stated in the literature. After that, the preparats were examined under a light microscope (Olympus Bx50, Olympus Optical, Tokyo, Japan), whose images were transferred to the computer environment. The changes in the structure of the joint cartilage and the findings that constitute the basis for the evaluation were examined in nine different categories. The findings were as follows: articular surface irregularity, erosion (ulceration) of the cartilage, chondrocyte necrosis, chorocyte clustering, cartilage tissue cracking, chondromalacia, reactive fibrocartilaginous hyperplasia, synovitis and synovial hyperplasia. The analysis of these parameters has been semi-quantitatively divided into four degrees. According to this, it was scored as grade 0: no change/normal, grade 1: mild, grade 2: moderate, grade 3: severe degeneration/change.

Statistical Analysis

Data are summarized as mean ± standard deviation (SD). Kruskal-Wallis Variance Analysis and Conover Paired Comparison Test were used for statistical analysis. p-value of <0.05 was considered statistically significant. IBM SPSS Statistics 22.0 program (SPSS Inc., IBM Corp., Armonk, NY, USA) was used for analysis. The differences between groups in terms of variables were evaluated by Kruskal-Wallis Variance Analysis. In variables with differences between the groups, the Conover Test determined which of the two groups had a statistically significant difference.

Results

Histopathological Analysis

When the sections prepared with H&E, MT, SO dyes of group 1 (control group) samples were observed, and we observed that joint surface irregularity was severe in focal areas for three samples, and mild to moderate in the other seven samples. Cartilage erosion, chondrocyte necrosis, cartilage cracking, synovitis and synovial hyperplasia were observed in mild/moderate levels in many samples. Chondrocyte clustering, reactive fibrocartilagenous hyperplasia and chondramalacia findings were evaluated to be mild in the samples (Figure 1).

When the group 2 (tCS) samples were compared with the control group; Joint surface irregularities, erosion of cartilaginous tissue, chondrocyte necrosis and cracking in cartilage tissue were observed to be severe across the sections. (Figure 2). Chondrocyte aggregation and synovial hyperplasia were moderate in this group where chondromalacia was observed, reactive fibrocartilagenous hyperplasia and synovitis were found to be mild to moderate. When histopathological findings were compared with the control group, it was observed that the injury findings were evident despite the insufficient repair response in the cartilage tissue and joint surface.

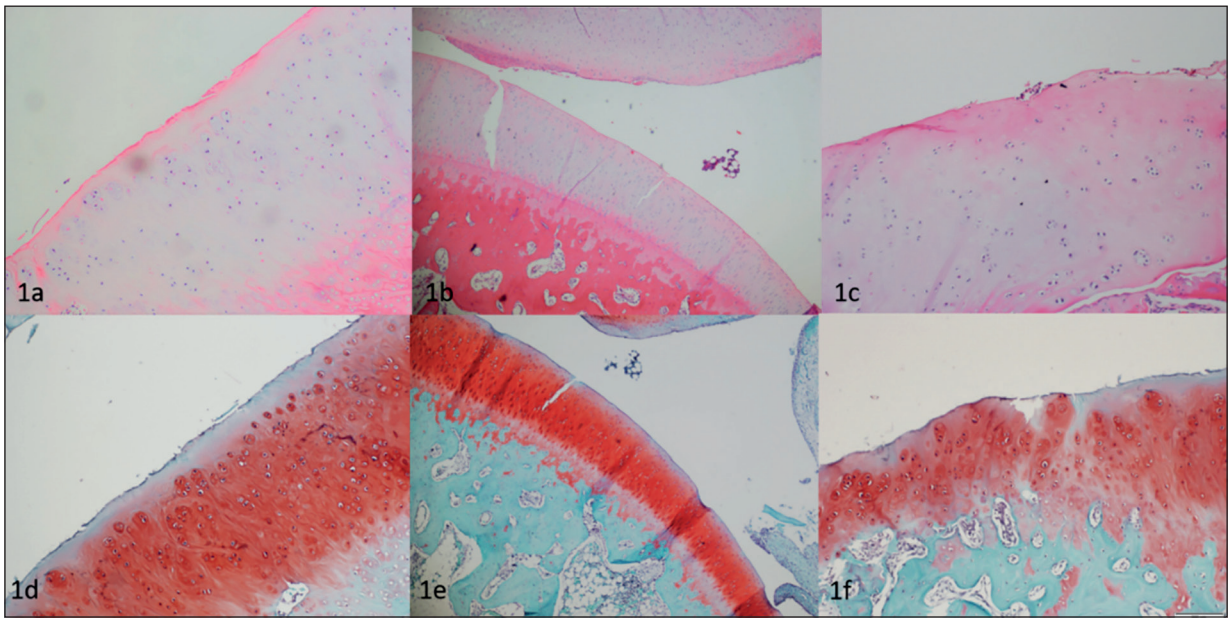

Figure 1. Joint surface irregularity **1a**, **1b**, **1c**, **1f**. Cracking in cartilage tissue, synovial hyperplasia 1b, 1e. Chondrocyte necrosis, cartilage erosion **1c**, **1f**. H&E (**1a**, **1b**, **1c**,) SO (**1d**, **1e**, **1f**) (10×).

When tissue sections belonging to group 3 (mECS) were compared with the control group and the other two groups, it was observed that all samples belonging to this group were in better condition in all parameters included in the evaluation. In addition, inflammatory and repair findings were evaluated to be mild (Figure 3).

According to nine criteria, more severe chondromalacia, joint surface irregularity, cartilage erosion, chondrocyte necrosis and synovial hy-

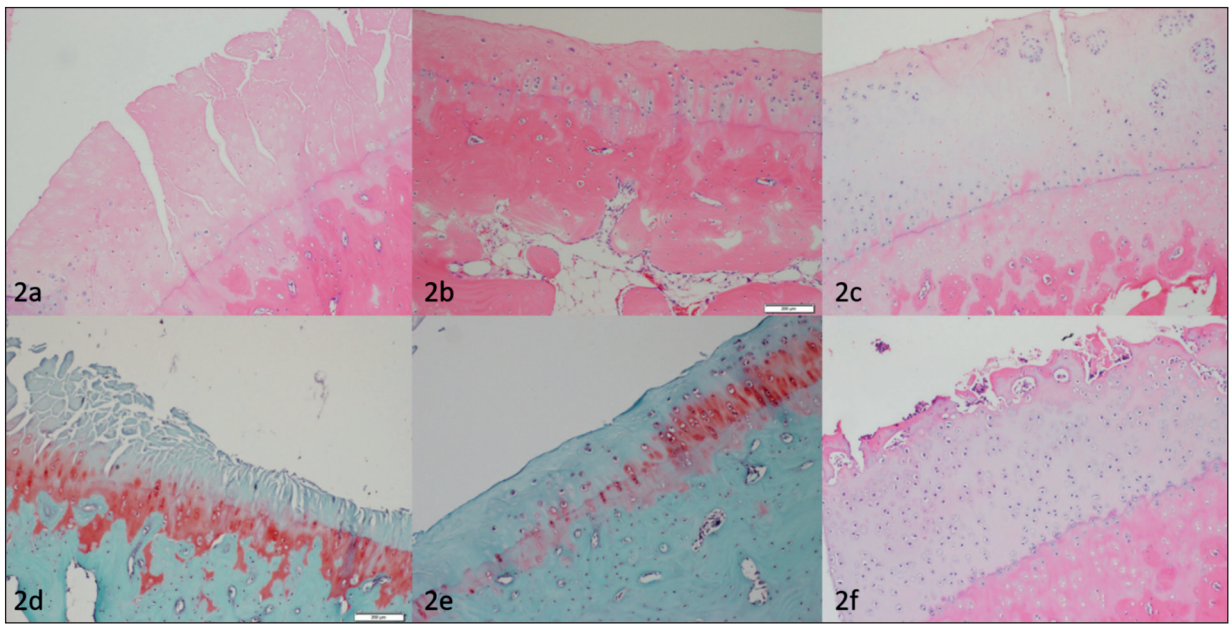

Figure 2. Cracking in cartilage tissue, Joint surface irregularity, chondrocyte necrosis **2a**, **2b**. Cartilage erosion, **2c**, **2e**. Chondrocyte cluster, synovitis **2c**, **2f**. H&E (**2a**, **2b**, **2c**, **2f**,) SO (**2d**, **2e**) (10×).

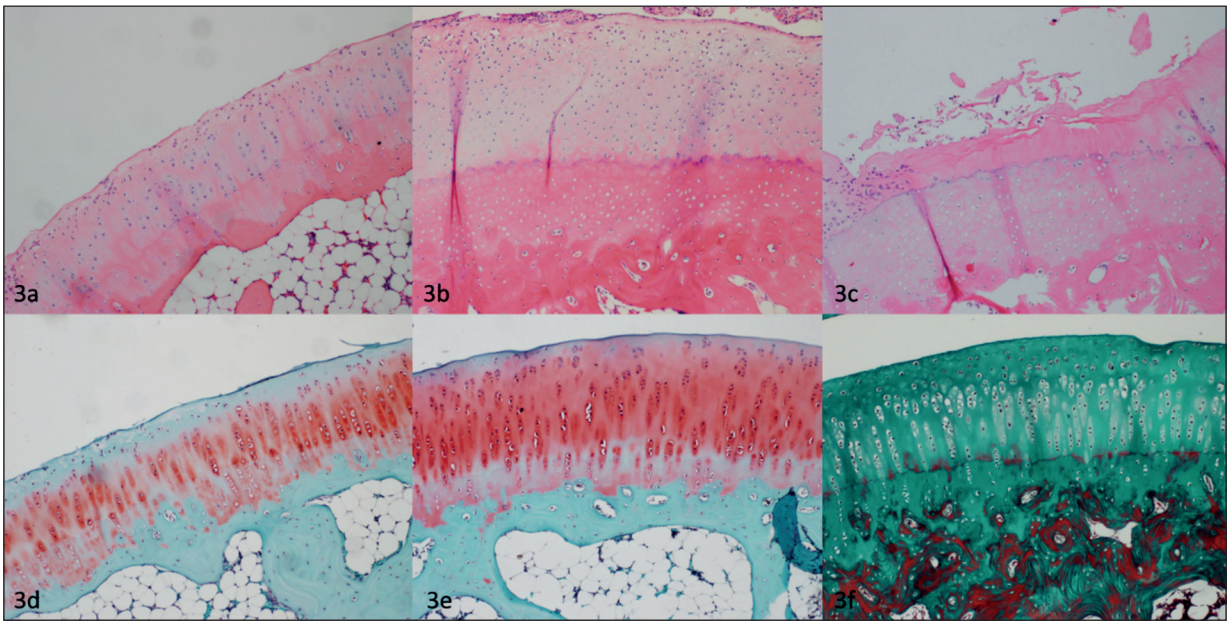

Figure 3. Articular surface irregularity **3a**, **3d**. Synovial hyperplasia **3b**. Cartilage erosion, chondrocyte necrosis **3c**. Chondrocyte cluster **3e**. H&E (**3a**, **3b**, **3c**), SO (**3d**, **3e**), MT **(3f)** (10×).

perplasia were observed in group 4 (mPCS) sections compared to the other two groups and the control group. Chondrocyte clustering and cracks in cartilage tissue were milder when compared to group two but were at a negative level compared to the control group (Figure 4).

In the general histopathological evaluation of the groups in which H&E, MT and SO stains were applied, cartilage damage, tissue damage and repair findings were found to be similar in the control group and group 2 samples. When group 4 was compared with the control group, group 2

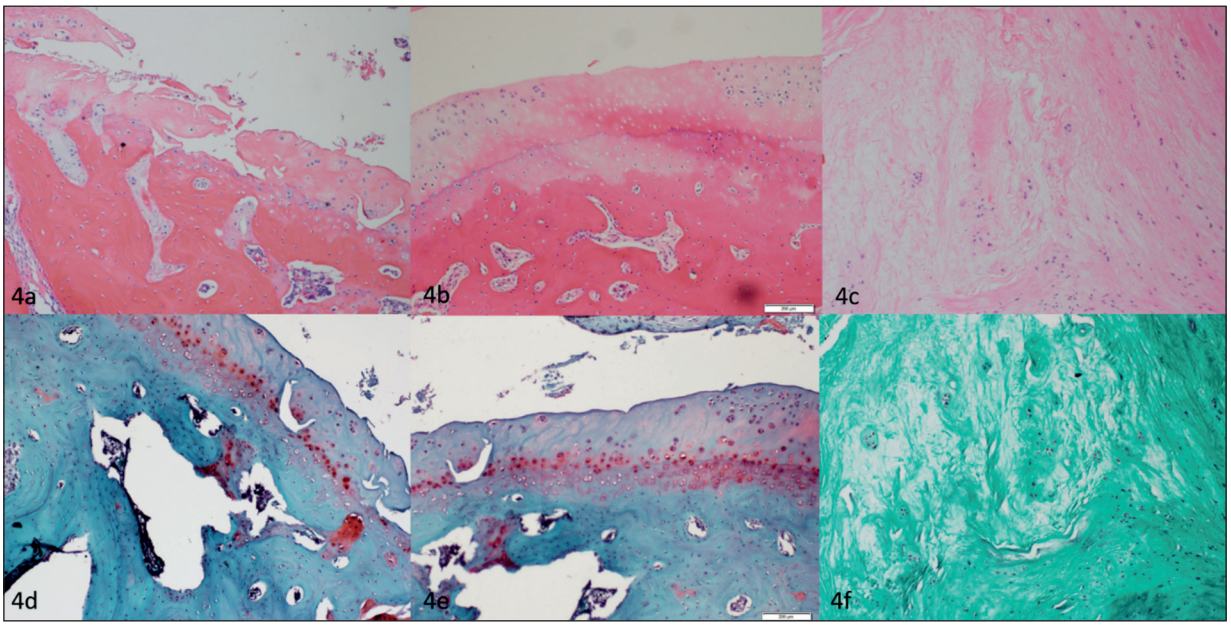

Figure 4. Joint surface irregularity, cartilage cracking **4a**, **4d**. Chondrocyte necrosis **4b**, **4e**. Chondromalacia **4c**, **4f**. H&E (**4a**, **4b**, **4c**). SO (**4d**, **4e**), MT (**4f**) (10×).

Table I. Mean and Standard Deviation Analysis Table.

Variables	Mean ± standard deviation				p
	Group 1	Group 2	Group 3	Group 4	
Joint Surface Irregularity	1.87 ± 0.99	2.62 ± 1.06	1.25 ± 0.46	2.37 ± 0.51	0.010
Cartilage Erosion	2.12 ± 1.35	2.62 ± 1.06	1.62 ± 0.51	2.12 ± 0.64	0.038
Chondrocyte Necrosis	1.62 ± 0.91	1.37 ± 1.06	1.00 ± 0.53	2.50 ± 0.75	0.015
Controcyte Clustering	1.00 ± 1.00	1.28 ± 0.95	1.00 ± 0.53	1.75 ± 0.88	0.251
Cracking in Cartilage Tissue	1.71 ± 1.11	2.12 ± 0.99	1.00 ± 0.53	1.75 ± 0.88	0.069
Chondromalacia	0.14 ± 0.37	0.57 ± 0.53	0.00 ± 0.00	1.50 ± 1.60	0.041
Reactive Fibrocartilaginous Hyperplasia	0.85 ± 1.06	1.00 ± 0.92	0.50 ± 0.53	1.37 ± 1.06	0.349
Synovitis	1.75 ± 1.03	1.37 ± 0.74	1.25 ± 0.46	1.25 ± 0.46	0.490
Synovial Hyperplasia	1.37 ± 0.91	1.62 ± 0.74	1.37 ± 0.51	2.37 ± 0.51	0.027
Total	11.71 ± 4.11	14.14 ± 4.67	9.00 ± 1.77	17.0 ± 4.44	0.006

and group 3, it was evaluated that there was severe tissue damage in all parameters. Group 3, in which microbial CS from *E. coli* was applied, was the group in which more improvement findings were observed in all parameters compared to the three groups, especially in the control group. It was found that group 3 was statistically significantly different from the other groups (group 1, 2, 4).

Biostatistical and General Evaluation of Histopathological Analysis

The changes in the structure of the joint cartilage and the findings that form the basis of the evaluation were examined in nine separate categories. The findings were as follows: joint surface irregularity, cartilage erosion (ulceration), chondrocyte necrosis, chorocyte clustering, cartilage tissue cracking, chondromalacia, reactive fibrocartilaginous hyperplasia, synovitis and synovial hyperplasia. Analysis of these parameters was made semi-quantitatively divided into four grades. According to this, it was scored as grade 0 (no change/normal), grade 1 (mild), grade 2 (moderate), grade 3 (severe degeneration/change) (Table I).

There is a statistically significant difference between the groups in terms of joint surface irregularity, cartilage erosion, chondrocyte necrosis, chondromalacia, synovial hyperplasia and total variables (Table II).

The graphical presentation of the statistically significant differences between the groups in terms of joint surface irregularity, erosion of cartilage, chondrocyte necrosis, chondromalacia, synovial hyperplasia is as follows (Figures 5-12).

Discussion

Traumas affecting the joint can cause chondral damage. Chondral damage initiates the degenerative process in the joint that results in osteoarthritis[13-15]. Today, many conservative and surgical methods are applied to prevent the osteoarthritis process or to treat the formed osteoarthritic joint[16-18]. Recently, disease modifying agents have been emphasized for the most ideal procedure. In recent years, there are studies investigating the effectiveness of the intraarticular application of CS in the literature[3,17,19]. There are few studies

Table II. Table II. Kruskal-Wallis Variance Analysis and Conover Paired Comparison Test.

Double groups	Joint surface irregularity	Cartilage erosion	Chondrocyte necrosis	Chondro-malacia	Synovial Hyperplasia	Total
Group 1-Group 2	0.100	0.298	0.471	0.107	0.530	0.32
Group 1-Group 3	0.181	0.138	0.098	0.285	0.906	0.122
Group 1-Group 4	0.264	0.463	0.052	0.094	0.028	0.018
Group 2-Group 3	0.007	0.007	0.565	0.016	0.511	0.014
Group 2-Group 4	0.097	0.045	0.037	0.455	0.040	0.149
Group 3-Group 4	0.002	0.113	0.003	0.025	0.005	< 0.001

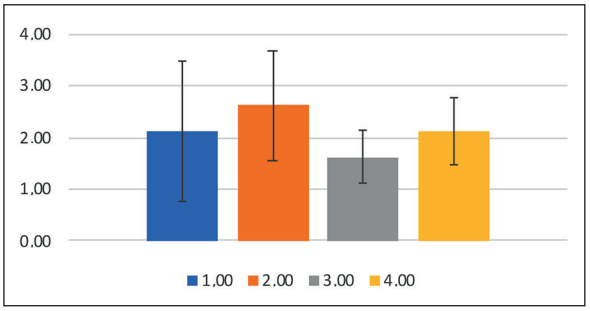

Figure 5. Cartilage erosion of group 4.

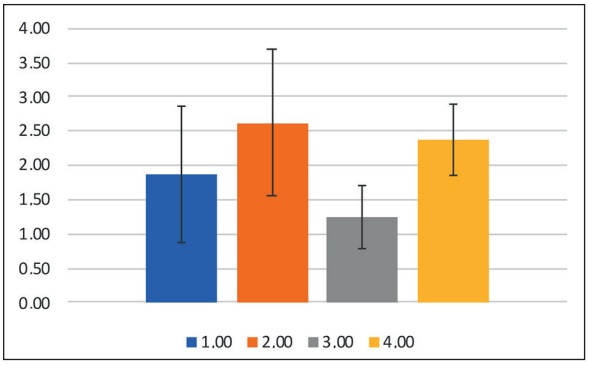

Figure 6. Joint surface irregularity of group 4.

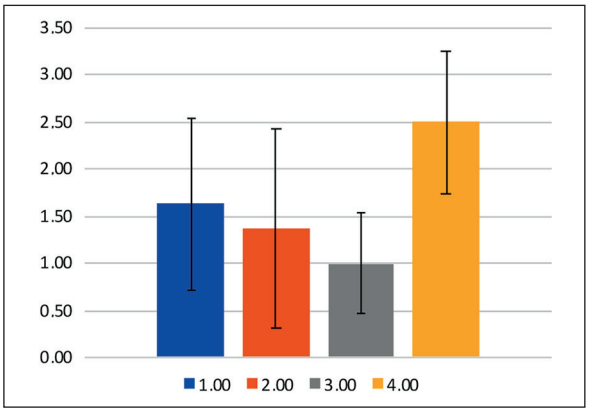

Figure 7. Condrocyte necrosis of group 4.

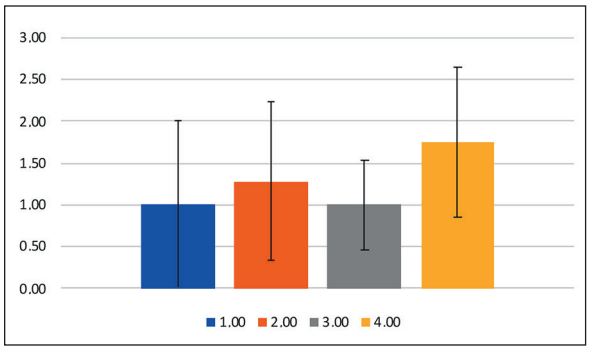

Figure 8. Condrocyte cluster of group 4.

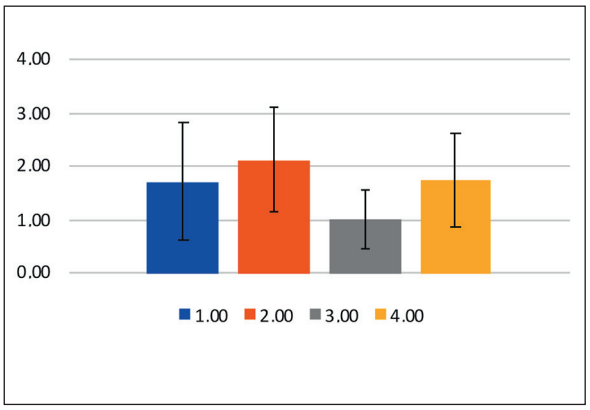

Figure 9. Cartilage tissue cracking of group 4.

in the literature comparing the oral effects of CS. In our study, it was found that microbial CS had more positive effects in the experimental osteoarthritis model in rabbits in terms of chondroprotective histopathologically compared to the control group and commercially produced animal origin CS.

Positive results were obtained in every parameter in the histopathological evaluation of *E coli*-derived MCS. In addition, it has been concluded that *E coli*-derived MCS is highly effective in healing cartilage deformity in the osteoarthritis model created by an experimental animal study. In the healing parameters, the expected effect was limited in our PAJC-derived product, which was created by obtaining the same MA value but from different bacteria (Pseudomonas aeruginosa pAJC strain) and which we encoded as pCS throughout the experiments. This requires further investigation into bacterial pathogenicity or the portability of bacterial characteristics as a marker in carbohydrate units. In this sense, it is

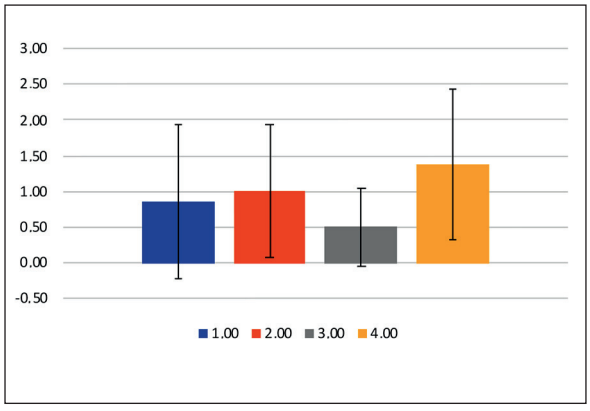

Figure 10. Reactive fibrocartilaginous hyperplasia of group 4.

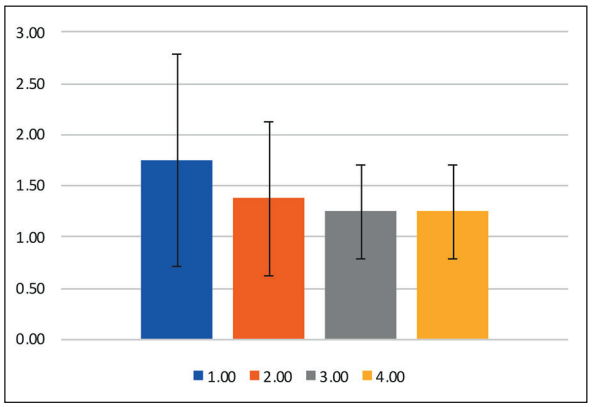

Figure 11. Synovitis of group 4.

predicted that it will have a significant quality in the literature as an important preliminary data.

In joint traumas, all intra-articular and extra-articular structures are affected[20,21]. Injury to intra-articular structures, especially cartilage tissue, initiates a pathological process in the joint and causes a painful dysfunctional joint. Cartilage diseases are an important socioeconomic problem that causes job loss and negatively affects patient comfort worldwide[22,23]. In patients with osteoarthritis, chondroprotective agents, such as HA, glycosaminoglycan and CS are used in addition to surgical approaches, such as arthroscopic debridement and arthroplasty[24-27]. The main purpose of all methods is to prevent the progression of cartilage tissue to post-operative degenerative arthritis. In addition, creating hyaline-like regeneration in damaged cartilage areas to save the joint from arthrosis is one of the aims of the treatments[28,29]. For this reason, there has been an increase in the number of studies on molecules that have or are thought to have a positive effect on cartilage tissue repair exposed to trauma[30-32].

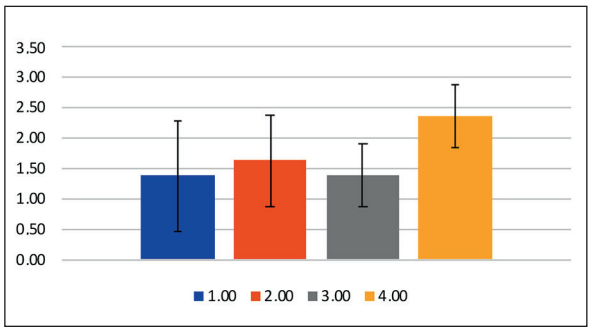

Figure 12. Synovial hiperplasia of group 4.

In two separate studies in the literature investigating the effectiveness of CS in knee osteoarthritis, 800 mg/day[33] or 1200 mg/day[34] doses of CS were reported to be effective and safe on symptoms. A significant reduction in joint swelling and effusion was also reported in these studies. The dose we used in our study is 17 mg/kg/day. The observation of chondroprotective effect at this level without the need for higher doses can be accepted as evidence that the produced MCS can be effective even at lower doses than its animal counterparts with low MA value.

We think that this study will lead to the creation of new cartilage repair preparations. We expect these new products to contribute effectively to the prevention and repair of joint cartilage damage. In this way, we aim to avoid early surgical procedures and to apply more conservative methods.

Conclusions

In our study, it was found that MCS had positive chondroprotective effects in the experimental osteoarthritis model in rabbits compared to the control group and animal CS. The beneficial effects of CS described above are likely due to chondroprotective properties associated with reducing chondrocyte apoptosis, decreased synthesis or activity of ECM metalloproteases, and enhancing the synthesis of articular cartilage PGs.

However, mCS is an important alternative for the treatment of OA because it is more reliable, the vegan version is purer, and provides a fast action mechanism with its low MA (269 daltons).

In our future research, we will focus on determining the appropriate dose of MCS. According to the results of this research, it seems possible to use mCS effectively in the treatment of osteoarthritis as a vegan agent obtained by biotechnological methods.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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References

1) Altman RD, Lozada CJ. Clinical features of osteoarthritis In: Hochberg MC, Silman AJ, Smolen JS, Weinblatt ME, Weisman MH, editors. Rheumatology. 4th ed. Spain: Mosby Elsevier 2008; pp. 1703-1710.
2) Peat G, McCarney R, Croft P. Knee pain and osteoarthritis in older adults: a review of community burden and current use of primary health care. Ann Rheum Dis 2001; 60: 91-97.
3) Ozkan FU, Ozkan K, Ramadan S, Guven Z. Chondroprotective effect of N-acetylglucosamine and hyaluronate in early stages of osteoarthritis--an experimental study in rabbits. Bull NYU Hosp Jt Dis 2009; 67: 352-357.
4) Dıracoglu D. Intra-articular hyaluronic acid in the management of osteoarthritis. Turk J Phys Med Rehabil 2007; 53: 154-159.
5) Samuels J, Krasnokutsky S, Abramson SB. Osteoarthritis: a tale of three tissues. Bull NYU Hosp Jt Dis 2008; 66: 244-250.
6) Hochberg M, Chevalier X, Henrotin Y, Hunter DJ, Uebelhart D. Symptom and structure modification in osteoarthritis with pharmaceutical-grade chondroitin sulfate: what's the evidence? Curr Diabetes Rev 2013; 29: 259-267.
7) Doral MN, Donmez G, Atay OA, Bozkurt M, Leblebicioglu G, Uzumcugil G, Uzumcugil A, Aydog T. Degenerative Joint Diseases. Journal of the Turkish Orthopedics and Traumatology Association 2007; 6: 56-65.
8) Sen C, Güneş T, Saygi B, Erdem M, Köseoğlu RD, Kiliç N. Eklem içine uygulanan hiyalüronik asidin erken evreli osteoartritte kikirdak koruyucu etkisi: Tavşanda deneysel çalişma [The chondroprotective effect of intra-articular hyaluronic acid at early stages of osteoarthritis: an experimental study in rabbits]. Acta Orthop Traumatol Turc 2004; 38: 348-352.
9) Horton D, Wander JD. The carbohydrates. New York: Academic Press. 1980; 1(B).
10) Martin CW. Glucosamine: review of its effectiveness in treating knee osteoarthritis. Workspace BC; January 2004.
11) Glucosamine Sulfate. Alternative Medicine Review. Monograph 1999; 4: 193-195.
12) Erenler AS, Geckil H, Karabulut AB, Akpolat N, Sevimli R, Ulke E, Aliyeva A. Cloning and expression vgb-kfo Genes in E. coli and Microbial Chondroitin Sulfate Production. Science of Advanced Materials 2019; 11: 1745-1754.
13) Creamer P, Hochberg MC. Osteoarthritis. Lancet 1997; 350: 503-508.
14) Arasıl T. Osteoarthritis, history, definition and classification. Istanbul: Nobel Medicine Bookstores 2007; 1: 1-7.
15) Sarzi-Puttini P, Cimmino MA, Scarpa R, Caporali R, Parazzini F, Zaninelli A, Atzeni F, Canesi B. Osteoarthritis: an overview of the disease and its treatment strategies. Semin Arthritis Rheum 2005; 35: 1-10.
16) Brief AA, Maurer SG, Di Cesare PE. Use of glucosamine and chondroitin sulfate in the management of osteoarthritis. J Am Acad Orthop Surg 2001; 9: 71-78.
17) Kazakçıoğlu M. Osteoartrit ve glukozamin. Turkish Journal of Rheumatology 2009; 24: 94-97.
18) Beaumont GH, Rovari LC. Use of cyrstalline glucosamine sulfate in osteoarthritis. Future Rheumatology 2006; 1: 397-414.
19) Wang CT, Lin J, Chang CJ, Lin YT, Hou SM. Therapeutic effects of hyaluronic acid on osteoarthritis of the knee. A meta-analysis of randomized controlled trials. J Bone Joint Surg Am 2004; 86: 538-545.
20) Karaduman ZO, Yucel I, Solak K. Condroprotective Effect of Intraarticular Glucosamine Sulfate Application on The Model of Experimental Knee Osteoarthritis Created on Rats. Duzce University Journal of Health Sciences Institute. 2013; 3: 3-18
21) Mankin HJ, Dorfman H, Lippiello L, Zarins A. Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. II. Correlation of morphology with biochemical and metabolic data. J Bone Joint Surg Br 1971; 53: 523-537.
22) Murat N, Karadam B, Ozkal S, Karatosun V, Gidener S. Quantification of papain-induced rat osteoarthritis in relation to time with the Mankin score. Acta Orthop Traumatol Turc 2007; 41: 233-237.
23) Tiraloche G, Girard C, Chouinard L, Sampalis J, Moquin L, Ionescu M, Reiner A, Poole AR, Laverty S. Effect of oral glucosamine on cartilage degradation in a rabbit model of osteoarthritis. Arthritis Rheum 2005; 52: 1118-1128.
24) Ameye LG, Chee WS. Osteoarthritis and nutrition. From nutraceuticals to functional foods: a systematic review of the scientific evidence. Arthritis Res Ther 2006; 8: 125-127
25) Kim LS, Axelrod LJ, Howard P, Buratovich N, Waters RF. Efficacy of methylsulfonylmethane (MSM) in osteoarthritis pain of the knee: a pilot clinical trial. Osteoarthritis Cartilage 2006; 14: 286-294.
26) Usha PR, Naidu MU. Randomised, double-blind, parallel, placebo-controlled study of oral glucosamine, methylsulfonylmethane and their combination in osteoarthritis. Clin Drug Investig 2004; 24: 353-363.
27) Michel BA, Stucki G, Frey D, De Vathaire F, Vignon E, Bruehlmann P, Uebelhart D. Chondroitins 4 and 6 sulfate in osteoarthritis of the knee: a randomized, controlled trial. Arthritis Rheum 2005; 52: 779-786.
28) Reginster JY, Bruyere O, Neuprez A. Current role of glucosamine in the treatment of osteoarthritis. Rheumatology 2007; 46: 731-735.
29) Tiku ML, Narla H, Jain M, Yalamanchili P. Glucosamine prevents in vitro collagen degradation in chondrocytes by inhibiting advanced lipoxidation reactions and protein oxidation. Arthritis Res Ther 2007; 9: 75-76.

30) Vlad SC, LaValley MP, McAlindon TE, Felson DT. Glucosamine for pain in osteoarthritis: why do trial results differ? Arthritis Rheum 2007; 56: 267-277.

31) Krasnokutsky S, Samuels J, Abramson SB. Osteoarthritis in 2007. Bull NYU Hosp Jt Dis 2007; 65: 222-228

32) Richy F, Bruyere O, Ethgen O, Cucherat M, Henrotin Y, Reginster JY. Structural and symptomatic efficacy of glucosamine and chondroitin in knee osteoarthritis: a comprehensive meta-analysis. AMA Arch Intern Med 2003; 163: 1514-1522.

33) Gabay C, Medinger-Sadowski C, Gascon D, Kolo F, Finckh A. Symptomatic effects of chondroitin 4 and chondroitin 6 sulfate on hand osteoarthritis: a randomized, double-blind, placebo-controlled clinical trial at a single center. Arthritis Rheum 2011; 63: 3383-3391.

34) Zegels B, Crozes P, Uebelhart D, Bruyère O, Reginster JY. Equivalence of a single dose (1200 mg) compared to a three-time a day dose (400 mg) of chondroitin 4&6 sulfate in patients with knee osteoarthritis. Results of a randomized double blind placebo controlled study. Osteoarthritis Cartilage 2013; 21: 22-27.