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Investigation of the Efficacy of Sericin in Experimental Knee Osteoarthritis Model in Rats through the TGF-Beta/Smad Pathway

Deneysel Diz Osteoartrit Modeli Geliştirilen Sıçanlarda Serisinin Etkinliğinin TGF-Beta/Smad Yolağı Üzerinden İncelenmesi

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ABSTRACT

This study was aimed to investigate the therapeutic effectiveness of sericin in rats with monosodium iodoacetate (MIA)- induced knee osteoarthritis (KOA), focusing on evaluating its effectiveness via the TGF-β/Smad pathway. The KOA model was established through the injection of MIA into the knee joint, and the rats were randomly allocated into three groups: group 1 (control), group 2 (KOA control), and group 3 (KOA+sericin). Sericin was administered intra-articularly to rats on days 1,7,14, and 21 (0.8 g/kg/mL, 50 µL). After 21 days, the rats were sacrificed, and serum samples were analyzed using the ELISA method to measure transforming growth faktör-Beta (TGF-β1), mother against decapentaplegic homolog 2 (Smad2), and connective tissue growth factor (CTGF) levels. Additionally, knee joint samples underwent histopathological evaluations with hematoxylin-eosin staining and immunohistochemical assessment using TGF-β1 and Smad2/3 antibodies. Serum TGF-β1 and CTGF levels were significantly increased in group 2 vs. group 1 ($P < .05$). A statistically significant decrease was observed in group 3 ($P < .05$). Serum Smad2 levels were not significantly different between groups. Histopathologically, group 2 showed a subchondral bone tissue, degeneration of the cartilage and deep fissures. On the other hand, group 3 showed reduced degeneration in chondrocyte cells, increased cartilage thickness, and a cartilage matrix that appeared close to normal were noted. Immunohistochemically, group 2 exhibited an increase in TGF-β1 and Smad expression, whereas group 3 decreased these expressions than group 2. Sericin demonstrates potential efficacy in the experimental KOA model in rats through the TGF-β1/Smad pathway. Consequently, sericin may emerge as a promising therapeutic agent for the treatment of KOA with further support from advanced clinical trials.

Keywords: Knee osteoarthritis, sericin, TGF-β1/smad pathway

ÖZ

Bu çalışmada; monosodyum iyodoasetat (MIA) ile diz osteoartrit (DOA) modeli oluşturulan sıçanlarda serisinin terapotik etkinliğinin incelenmesi ve bu etkinliğinin TGF-β/Smad yolağı üzerinden değerlendirilmesi amaçlanmıştır. Sıçanlarda DOA modeli oluşturmak için diz eklemine MIA enjekte edilmiş ve ardından sıçanlar rastgele 3 gruba ayrılmıştır (1. grup (kontrol), 2. grup (DOA kontrol), 3. grup (DOA+serisin)). Sıçanlara, serisin 1, 7, 14 ve 21. günlerde (50 µL, 0,8 g/kg/mL) intraartiküler olarak uygulanmıştır. Sıçanlar 21 günün sonunda sakrifiye edilerek elde edilen serum örneklerinde transforming büyüme faktör-Beta (TGF-β1), Smad2 ve bağ doku büyüme faktör (CTGF) seviyeleri ELİSA yöntemi ile belirlenmiştir. Ayrıca diz eklem örneklerinde Hematoksilen-eozin boyası ile histopatolojik, TGF-β1 ve Smad2/3 antikorları ile immünohistokimyasal değerlendirmeleri gerçekleştirilmiştir. Serum TGF-β1 ve CTGF düzeylerinde 2. grupta, 1. gruba göre anlamlı olarak artış tespit edilmiş ($P < ,05$), tedavi verilen 3. grupta ise istatiksel olarak anlamlı azalma görülmüştür ($P < ,05$). Serum Smad2 düzeylerinde gruplar arasında anlamlı fark saptanamamıştır. Histopatolojik olarak 2. grupta subkondral kemik dokusu ve kıkırdak dejenerasyonu ve derin çatlaklar görülmüştür. 3. grupta ise kondrosit hücrelerindeki dejenerasyonun azaldığı kıkırdak dokusunun kalınlığının arttığı ve kıkırdak matriksin normale yakın olduğu izlenmiştir. İmmünohistokimyasal olarak 2. grupta TGF-β1 ve Smad ekspresyonlarında artışlar görülürken, 3. grupta 2. gruba kıyasla bu ekspresyonlarda azalmalar sergilenmiştir. Serisin sıçanlarda deneysel DOA modelinde TGF-β1/Smad yolağı üzerinden potansiyel etkinlik göstermektedir. Sonuç olarak, serisin ileri klinik çalışmalarla desteklenmesiyle DOA tedavisi için umut verici bir terapötik ajan olabilir.

Anahtar Kelimeler: Diz osteoartrit, serisin, TGF-β1/smad yolağı

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INTRODUCTION

Osteoarthritis (OA), the most common joint disorder, causes cartilage in the synovial membrane to degenerate, resulting in joint stiffness, pain, limited mobility, local pain, crepitation and varying degrees inflammation. OA affects not only the articular cartilage but also the subchondral bone, capsule, ligaments, synovium and surrounding muscle tissue.[1] The most common form is knee OA (KOA).[2]

Although many risk factors have been implicated in the etiology of OA, the pathophysiological processes are not fully understood. Therefore, it is not possible to talk about a single mechanism explaining the development of OA. OA is a dynamic and metabolically active process in which destruction and repair occur simultaneously due to a combination of biochemical and mechanical factors; however, over time, the balance shifts in favor of destruction.[3,4] As a result of a series of reactions in the articular chondrocytes, abnormal changes occur in the extracellular matrix (ECM) and the homeostasis of the articular cartilage is disrupted.[5] Under stress, chondrocytes release inflammatory cytokines such as interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), which induce the synthesis of metalloproteinases (MMPs), as well as other inflammatory cytokines and chemokines.[5-7] The inflammatory process in OA involves the release of pro-inflammatory cytokines, proteinases, and mediators such as reactive oxygen species (ROS) from the inflamed area and this mechanism is considered crucial in the development and progression of OA.[8,9]

Transforming growth faktör-Beta (TGF-β) is a fibrogenic factor that plays an important role throughout many processes. These include cell proliferation, migration, apoptosis, differentiation, and stimulation of ECM synthesis.[10] TGF-β binds to receptors on the cell surface, specifically the TGF-β type 1 and 2 receptors. It induces phosphorylated mother against decapentaplegic homolog 2/3 (Smad 2/3) and initiates intracellular signalling. Smads are the only known TGF-β1 receptor substrate. Therefore, Smad/ connective tissue growth factor (CTGF) signalling is essential for TGF-β-induced fibrogenesis.[7] It has been shown that TGF-β has a role to play in all stages of chondrogenesis. TGF-β's inhibitory effect on the terminal differentiation of chondrocytes relies significantly on the pivotal role played by Smad2 and Smad3 as key signaling molecules. CTGF is considered to be an important amplifier of the pro-fibrogenic effect of TGF-β1. It functions as an important down regulator of TGF-β1/Smad signalling in mesenchymal cells and fibroblasts.[7,11]

Several integrins play a significant role in the development and progression of OA by interacting with the ECM and mediating intracellular signalling pathways.[12,13] Integrin-derived growth factors like TGF-β are involved in bone formation and differentiation, with elevated expression observed in OA patients compared to those without OA.[14] Smad proteins mediate TGF-β signalling, impacting chondrocyte differentiation and the PI3K/Akt pathway, contributing to increased cholesterol synthesis in OA.[15]

Because biomaterials can mimic both the biological and mechanical functions of the natural ECM, the selection of appropriate biomaterials for treatment is important.[16] Silk proteins are biomaterials that have become the focus of research in recent years due to their natural occurrence.

The silkworm cocoon (Bombyx mori) consists of two main proteins, sericin and fibroin. The fibroin in the cocoon is a protein bound by disulphide bonds in the form of thin twin filaments and wrapped in successive layers of sticky sericin that form the silk.[17] The sericin formed by hydrolysis of silk proteins have been shown to have various biological activities: antioxidant, anti-diabetic, antitumour, antiviral, antibacterial, hypocholesterolemic, immunoregulatory.[18] Sericin has been found to have a low anti-inflammatory effect by reducing the release of inflammatory cytokines such as TNF-α, [19] while also increasing the production of anti-inflammatory cytokines including TGF-β, IL-4, and IL-10.[17] Sericin, easily accessible, natural, cost-effective, and biocompatible biomaterial. It has positive effects on tissue repair, stimulating the proliferation of fibroblasts and keratinocytes, producing regulatory cytokines essential for the wound healing process, and actively contributing to the synthesis of ECM proteins, playing a critical role in re-epithelialization and overall healing.[20] Sericin also enhances the activity of antioxidant enzymes by scavenging free radicals and ROS.[21,22] Additionally, it is known that sericin enhances the production of anti-inflammatory cytokines such as TGF-β, IL-4, and IL-10 [17], and it regulates the expression of TGF-β1-3 to prevent scar tissue formation during wound healing.[23]

There is a very limited number of studies in the literature investigating the pathogenesis and functional prognosis of experimental OA in more detail. Because of their shortcomings, there is a need to investigate alternative therapies that may shed new light on current treatments and better understand the prognosis of the disease.

Although there are several studies showing the tissue repair/regeneration efficacy of the sericin, there are no studies evaluating its efficacy in the treatment of KOA and the determination of this efficacy via the TGF-β/Smad

pathway. It is also known that the TGF-β/Smad pathway plays an important role in the mechanism of OA. The pathophysiology of OA is not fully understood. Therefore, sericin can also be used in the treatment of KOA, a common disease today. In this context, this study aimed to investigate the effectiveness of sericin in rats with monosodium iodoacetate (MIA)-induced KOA model and assess its efficacy via TGF-β/Smad pathway.

MATERIALS AND METHODS

Animal Model Induction and Experimental Treatments

Animal studies were approved by the Animal Experiments Local Ethics Committee of Pamukkale University (date 09.11.2021, number PAUHADYEK-2021/E-60758568-020-132719/08). Twenty-one female Wistar albino rats (12-14 weeks old, 200-250 g) were purchased from the Medical Experimental Research and Practice Centre of the Pamukkale University. The animals were maintained in a controlled environment at a consistent temperature of 23±2°C, with 50% humidity, and subjected to a regular light-dark cycle (lights on at 8 am, lights off at 8 pm). They were accommodated in specially designed cages and received attentive care under veterinary supervision. MIA (Sigma–Aldrich, Missouri, USA) was used to induce an experimental KOA model in rats. In the literature, MIA is defined as intra-articularly (i.a.) injection is the most commonly used method to experimentally induce KOA in rats.[24] MIA was dissolved in a 0.9% NaCl solution, and for the induction of the experimental KOA rat model, a 30 G needle was utilized to inject a solution of MIA (1.5 mg/50 µL per animal) into the right patella of the rats. A combination of ketamine (Eczacibasi, Parke-Davis, Istanbul, Turkey) and xylazine (Alfasan International BV, Woerden, Holland) general anaesthesia was used for the injection procedure.

One day after the establishment of the experimental KOA model, the rats were randomly selected and were divided into three equal groups to begin the experimental procedure. The experimental groups are shown in Table 1. The study was completed with the indicated number of rats.

Commercially purchased sericin protein (S5201, Sigma–Aldrich, Missouri, USA) was prepared by dissolving in PBS (Sigma–Aldrich, Missouri, USA) (Ph: 8.5).[25]

At the end of all treatments, the rats were fasted overnight. They were allowed free access to water. All rats were sacrificed under i.p. general anaesthesia (ketamine hydrochloride + 2% xylazine hydrochloride).

Table 1: Experimental Groups

Group Name	Experimental Treatments	n
Group 1 (Control)	Non-treatment	7
Group 2 (KOA Control)	A KOA model was induced by a single injection of MIA into the right patellar ligament of rats	7
Group 3 (KOA+Sericin)	Rats with induced KOA were exposed to sericin (0,8 g/kg/mL) through i.a. injection (50 µL per animal) on days 1, 7, 14, and 21	7

Blood samples were collected from the abdominal aorta and subsequently centrifuged at 2000 g for 15 minutes. The resulting serum samples intended for TGF-β1 (E-EL-0162, Elabscience, Texas/ABD), Smad2 (E-EL-R2582, Elabscience, Texas/ABD), and CTGF (E-EL-R0259, Elabscience, Texas/ABD) the solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) analyses were then stored at -80°C until the day of the study.

Knee joints (including patella and articular capsule) of sacrificed rats were removed and placed in 10% formalin tubes for histopathology. Samples were sent to Pamukkale University Faculty of Medicine, Histology and Embryology Laboratory for histopathological analysis using hematoxylin-eosin (H&E) and immunohistochemical analysis using TGF-β1 (sc-130348, Santa Cruz, Texas/USA), Smad2/3 (sc-133098, Santa Cruz, Texas/USA) antibodies.

ELISA Analyses

Measurement of TGF-β1, Smad2, and CTGF Levels

Serum samples were analyzed for the levels of TGF-β1 (E-EL-0162, Elabscience, Texas/ABD), Smad2 (E-EL-R2582, Elabscience, Texas/ABD), and CTGF (E-EL-R0259, Elabscience, Texas/ABD) using ready-to-use measurement kits employing the ELISA method, following the manufacturer's instructions. The obtained results were expressed in ng/mL for TGF-β1 and Smad2, and in pg/mL for CTGF.

Histological and Immunohistochemical Assessment

H&E Staining

For histopathological procedures, knee samples were taken to Pamukkale University Faculty of Medicine, Department of Histology and Embryology Laboratory in labelled bottles containing 10% buffered formaldehyde. The samples were kept in formaldehyde for 72 hours. Routine tissue tracking was performed and 5 micron thick sections were prepared from the paraffin blocks, ready for sectioning with a Leica brand microtome (RM2125RT). The sections were stained with H&E stain. Finally, each slide

was examined under a light microscope (Olympus Bx51 with DP72 camera system).

Immunohistochemical Staining

Tissue blocks were sectioned at 5 µm using a microtome. Immunohistochemistry was performed according to the manufacturer's instructions. Rat TGF-β1 (sc-515284, 1:50 dilution) and Smad 2/3 (Ab 9722, 1:50 dilution) antibodies were used for 60 minutes. A secondary antibody (Abcam HRP/DAB Detection IHC Kit, ab80436) was used according to the kit procedure. The sections were subsequently incubated with 3,3-diaminobenzidine (Dako Cytomation) and counterstained using Mayer's hematoxylin (Dako Cytomation).[26] Afterward, the sections were thoroughly washed with running water. Each of them was kept in 50%, 70%, 80%, 96%, 100% ethyl alcohol series for 2 minutes. Then the tissues were kept in xylene I and xylene II for 2 minutes each. The tissues taken from xylene were covered with entellan without waiting for the tissues to dry and examined in Olympus Bx51 high power light microscope and images were taken. TGF-β1 and Smad2/3 staining localisations were evaluated separately for each rat.

Image Analysis

An Olympus Bx51 high performance light microscope was used to examine the tissue samples. Ten randomly selected fields in each specimen were scored at 40x magnified. The scores were assessed semiquantitatively using light microscopy, analyzing specimens obtained from each rat. The scores were graded as strong staining (++++), moderate staining (+++), weak staining (+) and no staining (-).

Statistical Analysis

As a result of the power analysis, which was performed on the assumption that a large effect size (f=0.7) would be obtained in the study, it was calculated that 80% power with 95% confidence could be obtained if at least 21 rats were used (at least 7 rats for each group). It was decided to start the study with 7 rats per group, for a total of 21 rats.

The collected data were analyzed using SPSS 21.0 (IBM SPSS Statistics 21 software (IBM SPSS Corp., Armonk, NY, USA). Mean±standard deviation was used to express continuous and categorical variables. The normal distribution compatibility of the variables was assessed through the Shapiro-Wilk test. When parametric test assumptions were met, one-way analysis of variance was employed for comparing differences among independent groups. In cases where parametric assumptions were not satisfied, Kruskal-Wallis analysis of variance, with Mann-Whitney U test and Bonferroni correction as a post hoc test, was utilized to assess independent group differences. Statistical significance was considered as $P < .05$.

RESULTS

Determination of Serum TGF-β1, Smad2, and CTGF Levels

TGF-β1 levels were statistically significantly increased in group 2 (3.195 ± 0.53) compared to group 1 (1.927 ± 0.23) ($P = .001$). However, it was statistically significant lower in the group 3 (2.359 ± 0.41) compared with the group 2 ($P = .042$) (Figure 1).

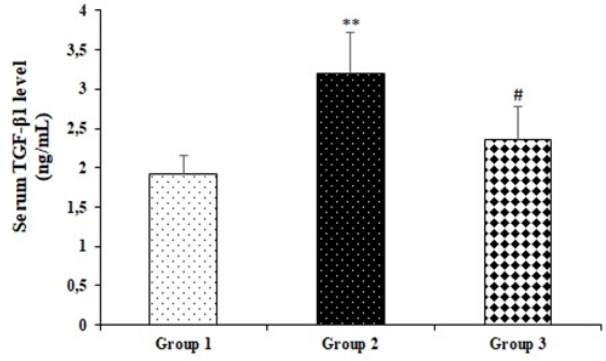

Figure 1. TGF-β1 levels in the experimental groups. (n=7; Results represent mean ± standard deviation; Mann Whitney U-Test was used; **: $P < .001$ vs. group 1; #: $P < .05$ vs. group 2)

Serum Smad2 level increased in the group 2 (0.154 ± 0.05) compared to the group 1 (0.260± 0.03) and decreased in the group 3 compared with the group 2. However, these values did not reach statistically significant level ($P > .05$) (Figure 2).

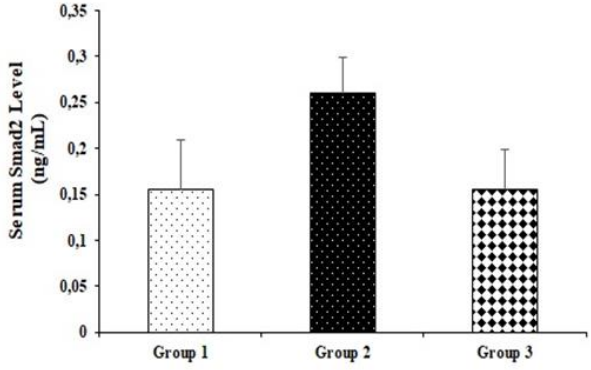

Figure 2. Smad2 levels in the experimental groups. (n=7; Results represent mean ± standard deviation; Mann-Whitney U-Test was used)

There was a statistically significant increase in serum CTGF levels in group 2 (152.42 ± 45.51) compared to group 1 (269.98 ± 41.24) ($P = .003$). However, It was statistically significant lower in the group 3 (159.26 ± 42.92) compared with group 2 ($P = .008$) (Figure 3).

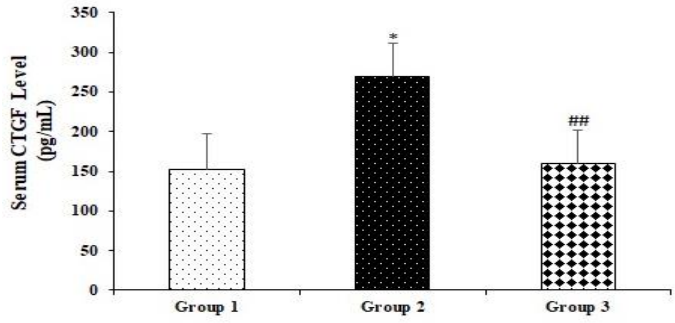

Figure 3. CTGF levels in the experimental groups. (n=7; Results represent mean ± standard deviation; Mann-Whitney U-Test was used; *: $P < .01$ vs. group 1; ##: $P < .01$ vs. group 2)

Histopathological Results

A normal morphology of the joint structure was observed in group 1. Chondrocytes had normal structure and cartilage matrix had normal density in the cartilage tissue. Group 2 had significantly thinner cartilage than group 1. Damage to the articular cartilage surface and structural fractures with reduced chondrocyte numbers were observed. In particular, marked degeneration of some chondrocytes was observed. Degeneration of subchondral bone tissue was also noted. In the treated group 3, the degeneration of chondrocyte cells decreased. The thickness of the cartilage tissue increased and a cartilage matrix close to normal was observed (Figure 4).

Immunohistochemical Results

Table 2 shows the semi-quantitative scoring results obtained from the light microscopic evaluations of the knee joint tissues obtained from the rats.

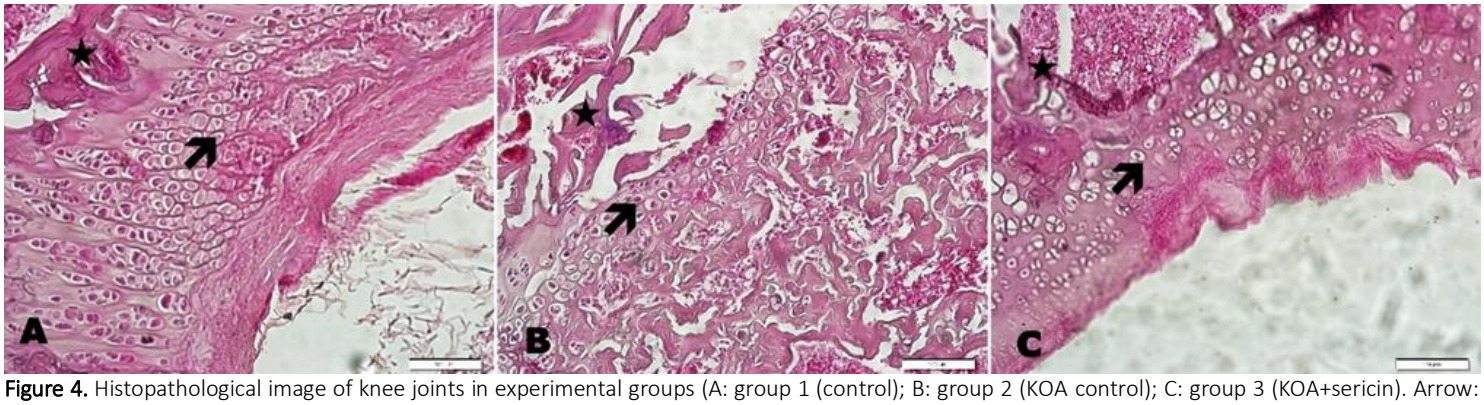

Figure 4. Histopathological image of knee joints in experimental groups (A: group 1 (control); B: group 2 (KOA control); C: group 3 (KOA+sericin). Arrow: cartilage tissue, Star: bone tissue. H&E, x100, Bar;100 µM))

The expressions of TGF-β1 and Smad2/3 in the cartilage and in the bone tissue was negative in groups 1 and 3. However, positive staining in cartilage matrix and chondrocyte membrane was found in group 2. Bone marrow showed intense positive staining in all groups (Figure 5).

Table 2. Semi-quantitative scores obtained from light microscopy of knee joint tissues from rats in the experimental groups

Groups	Group 1			Group 2			Group 3		
	Cartilage tissue	Bone tissue	Bone marrow	Cartilage tissue	Bone tissue	Bone marrow	Cartilage tissue	Bone tissue	Bone marrow
TGFβ1	-	-	+++	++	-	+++	-	-	+++
Smad2/3	-	-	+++	++	-	+++	-	-	+++

DISCUSSION

Sericin's anti-inflammatory properties in various diseases and its activity on the TGF-β1 pathway are well known. [27,28] TGF-β1 plays an important role in the development of OA. It influences chondrocyte differentiation through Smad signaling. Therefore, we hypothesized that the sericin may act through the TGF-β1/Smad pathway in the experimental KOA model. In line with our hypothesis, an experimental model of KOA induced with MIA has been established in this study. The effects of sericin on TGF-β1/Smad were evaluated by ELISA and histological analysis after sericin administration to experimental KOA rats. In present study, sericin was found to be effective on the TGF-β1/Smad pathway, which plays an important role in the pathogenesis of KOA in an experimental KOA model.

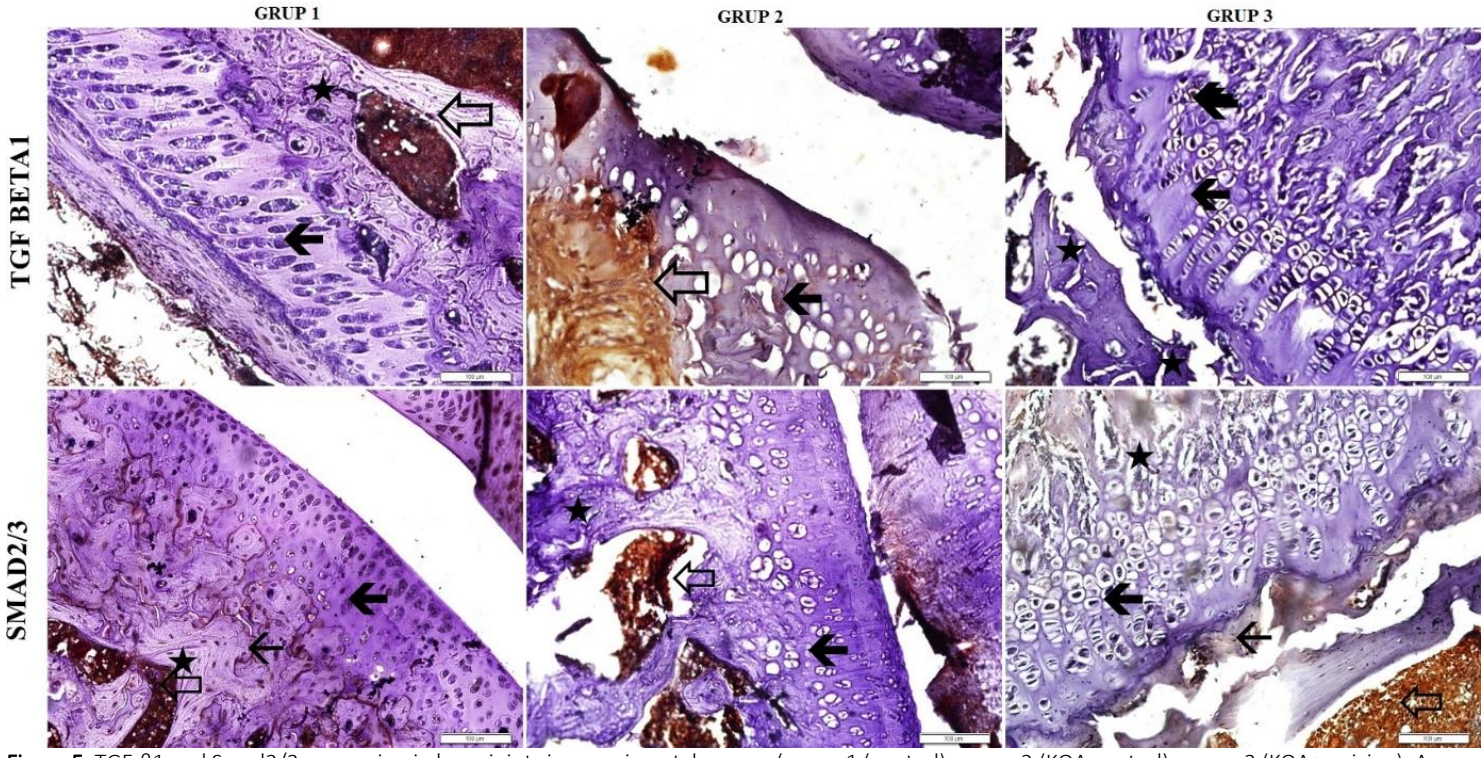

Figure 5. TGF-β1 and Smad2/3 expression in knee joints in experimental groups (group 1 (control); group 2 (KOA control); group 3 (KOA+sericine). Arrow: cartilage tissue, Star: bone tissue, Thin Arrow: Positive expression domains, Thick Arrow: bone marrow, Immunoperoxidase&Hematoxylin x40, Bar; 100µM)

Sericin has also been shown to increase reduced cartilage thickness. It has been determined that it reduces cartilage degeneration and deep cracks.

KOA is the most common joint disease and is characterized by the degeneration of the joint cartilage, which is protected by the synovial membrane.[29] Besides cartilage, OA can affect the subchondral bone, synovial membrane, tendons, capsules and surrounding muscle. OA is a slowly progressive chronic disease affecting the knee.[1] There are several risk factors associated with OA. However, the exact pathophysiological process and basic mechanisms are not fully understood. As articular chondrocytes hypertrophy in OA, the ECM is degraded and articular cartilage fragments form. This is followed by vascular invasion, subchondral bone sclerosis and the formation of osteophytes at the edge of the joint. Progressive degeneration of the articular cartilage is characteristic of OA. This leads to radiographic joint space narrowing, subchondral sclerosis and osteophyte formation.[30]

The TGF-β superfamily plays a crucial role in various biological processes such as growth control, immune response, cell differentiation, early development, and particularly skeletogenesis.[31] TGF-β is considered the main initiator of chondrogenesis, influencing all stages from condensation to terminal differentiation and mainly stimulating cartilage differentiation in early chondrogenesis. Smad signaling regulates chondrocyte terminal differentiation, believed to be significant in OA pathogenesis. However, factors beyond Smad signaling may also influence chondrocyte differentiation and contribute to OA development.[32] Recent research has focused on TGF-β's role in OA, as multiple joint cell types, including cartilage cells, synovial fibroblasts, and macrophages, can produce and release TGF-β. Alterations in the TGF-β pathway and components can disrupt cartilage homeostasis, leading to OA. Studies using mouse models have emphasized TGF-β's importance, showing that Smad3 knockout or conditional deletion causes OA-like degenerative joint disease.[33] Active TGF-β has been found in OA patients' synovial fluid, leading to OA-like changes in the knee joint upon exposure to external TGF-β, which depends on dosage and time.[34] TGF-β activation is believed to enhance cartilage proteoglycan synthesis. However, prolonged exposure or repeated intra-articular application of TGF-β can lead to adverse effects in articular cartilage, such as focal proteoglycan loss and microcracks in the deep cartilage layer. Short-term treatment of chondrocytes with TGF-β reduces MMP-13 levels, whereas prolonged stimulation upregulates MMP-13, mainly through Smad3 and Runx2 mechanisms. This dual role implies that both deficient and excessive activation of TGF-β can contribute to joint pathology in OA.[33,35] The cartilage and bone in joints experience continuous mechanical stress from daily activities, which is crucial for maintaining cartilage homeostasis. However, overuse can lead to joint damage, altering biomechanical properties seen in conditions like OA.[36] Mechanotransduction and mechanical responses have been shown to interact with TGF-β

signalling, although the mechanisms remain incompletely understood. TGF-β / Smad2/3 signalling affected by mechanical forces.[37] TGF-β plays a role not only in regulating chondrocyte behavior and cartilage degradation but also in osteophyte formation, a hallmark of OA. Osteophytes in experimental OA show strong expression of TGF-β1 and Smad2/3. TGF-β is a multifunctional cytokine involved in inflammation and immunity. During OA development or associated inflammation, damaged joint tissue releases TGF-β. TGF-β1 expression increases in subchondral bone in OA, both in human and mouse models. In summary, it is evident that TGF-β plays a role not only in cartilage degradation but also in new cartilage and bone formation, such as in osteophytes.[32]

There are two main proteins in the silk cocoon, sericin and fibroin. Fibroin is found in the cocoon. It is a protein wrapped in layers of sericin.[17] Sericin, formed by the hydrolysis of silk proteins, has several biological activities such as anti-diabetic, hypocholesterolemic, anti-oxidant, immunoregulatory, anti-tumor, anti-viral, anti-bacterial.[18] Panilaitis and colleagues investigated the inflammatory potential of silk fibers and extracts in vitro and found an increase in the release of TNF-α.[19] Another study showed that sericin increases anti-inflammatory cytokines such as IL-4 and IL-10 production.[17] According to the study conducted by Qi et al., sericin was reported to exhibit anti-inflammatory effects in the wound healing process. It was observed to promote angiogenesis and prevent the formation of scar tissue by regulating the expression of TGF-β1-3.[23] Sericin was shown to attenuate glomerulosclerosis and renal interstitial fibrosis by blocking activation of the TGF-β1/Smad3 pathway in rats with diabetic nephropathy, and to protect and prevent renal damage in rats with diabetic nephropathy by Song et al.[38] Our previous study demonstrated the efficacy of sericin in the treatment of experimental Achilles tendonopathy in rats via the TGF-β1/Smad signalling pathway.[39]

In this study, we evaluated the effects of sericin on the KOA model through the TGF-β1/Smad signaling pathway. TGF-β1, Smad2 and CTGF levels, which are important in the pathogenesis of OA, were determined by ELISA in serum samples from rats (Figure 1-3). It was found that TGF-β1, Smad2 and CTGF levels increased in the group 2 compared to the group 1. These levels decreased with sericin treatment (group 3). However, only Smad2 did not achieve statistically significant levels. In addition, TGF-β1 and Smad2/3 expressions were evaluated by IHC staining (Figure 5). While group 2 showed increased immunostaining, the group 3 showed immunostaining similar to the group 1.

In addition, in this study, knee joint samples from all groups were histopathologically examined using H&E staining to demonstrate the efficacy of sericin in the treatment of KOA. In group 2, thinning of the cartilage tissue, damage to the articular cartilage surface and structural fractures were observed, as well as a decrease in the number of chondrocytes and degeneration. Sericin treatment was shown to decrease chondrocyte degeneration, increase chondral thickness, and restore near-normal chondral matrix (Figure 4).

Sericin is known to have a positive effect on tissue damage repair, keratinocyte and fibroblast growth and wound healing.[20] It has also been suggested that TGF-β plays a central role in cartilage destruction, osteophytosis and synovial fibrosis in the development of OA.[20] This means that sericin can also be used to treat KOA, which is now a common disease.

In this context, this study, which focused on the effects of sericin treatment on KOA through the TGF-β1/Smad signalling pathway, demonstrated its efficacy. Biochemical and immunohistochemical analysis showed that sericin effectively reduced TGF-β1/Smad signaling, a key pathway for tissue repair in KOA. In addition, histopathological evaluations of sericin showed that it increased cartilage tissue and reduced degeneration. These findings suggest that because of its beneficial effects on inflammation and tissue repair, sericin may be a promising therapeutic agent for the treatment of KOA.

Ethics Committee Approval: This study was approved by the Animal Experiments Local Ethics Committee of Pamukkale University Medical Faculty (Ethics Committee approval date 09.11.2021, decision no: PAUHADYEK-2021/E-60758568-020-132719/08).
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