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Can intra-articular daidzein injection reduce oxidative damage and early osteoarthritis in a rabbit temporomandibular joint model?

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Abstract

Background Oxidative damage and inflammatory cytokines in osteoarthritis (OA) exacerbate the disease course. Daidzein (DZ) has antioxidant and anti-inflammatory effects. This study evaluated the early histopathological effects of intra-articular daidzein injection on experimentally induced osteoarthritis in rabbit TMJs.

Methods The predictor variable was intra-articular injection of DZ or a saline control. 50 µl of 3 mg/mL MIA solution was injected into the right TMJ of 16 New Zealand rabbits to induce experimental OA. One rabbit was sacrificed after 4 weeks to confirm the formation of the OA model and the OA model was obtained. The remaining 15 rabbits were randomly divided into 2 groups: an experimental group (9 rabbits) and a control group (6 rabbits). On days 1, 7, 14, and 21; 50 µl of saline solution was applied to the right TMJ of the control group and 50 µl daidzein solution (1.8 mg/ml) was applied to the right TMJ to the experimental group. After one week from the date of the last injection, the rabbits were sacrificed, and histopathological and biochemical evaluations were performed. The Shapiro-Wilk test was used to evaluate whether the variables in the study conformed to normal distribution. Mean ± SD (standard deviation) or median (interquartile range (IQR)) was used to show the descriptive statistics of the variables. T-test and Mann Whitney U test were used to compare the control and experimental groups for biochemical changes. The chi-square test was used to show the distribution of histopathological changes variables obtained within the scope of the study based on control and experimental groups. A P-value < 0.05 was considered significant for all evaluations.

Results There were 8 and 6 animate treated with DZ and saline, respectively. There was no statistically significant difference between groups in articular cartilage ($p = 0.3$), osteochondral junction ($p = 0.3$), subchondral bone structure ($p = 1.0$) or chondrocyte appearance ($p = 0.4$). The experimental group showed significantly lower mean values for Total Oxidant Status (TOS) ($p = 0.002$) and Oxidative Stress Index (OSI) ($p = 0.007$).

Conclusions An intra-articular DZ injection appears to show limited reduction of oxidative damage and early OA in the rabbit TMJ. DZ might represent a promising natural compound with beneficial effects in the management of TMJ-OA.

Keywords Temporomandibular joint, Osteoarthritis, Daidzein, Antioxidant

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Background

Osteoarthritis (OA) of the temporomandibular joint (TMJ) is a slowly progressive, chronic, non-inflammatory, degenerative disease characterized by progressive cartilage degeneration, subchondral bone remodeling, and chronic pain [1]. Conventional treatment of osteoarthritis involves injections of various solutions into the TMJ to eliminate pain, improve function, and modify cartilage damage [2, 3]. The most commonly applied agents to the intra-articular space for this purpose are; glucosamine, hyaluronic acid (HA), platelet-rich plasma (PRP), statins, and corticosteroids [4–10]. In cases of advanced pathology in TMD, intractable pain, and dysfunction caused by TMD-OA, surgical treatment may be considered [11]. There is currently no gold standard treatment method for TMD-OA.

Recent reports have shown that isoflavones exhibit protective effects against OA progression by interacting directly or indirectly with joint tissues and reducing inflammation, oxidative damage, and pain [12]. Among isoflavones, daidzein (DZ) is a polyphenolic isoflurane structurally similar to mammalian estrogen 17-β-estradiol. Moreover, DZ has anti-inflammatory and antioxidant activity [13–15]. It exhibits antioxidant properties by removing free radicals and/or stimulating the expression of antioxidant enzymes [15] and anti-inflammatory properties by inhibiting mediators involved in inflammation [15, 16]. DZ inhibits nitric oxide (NO) production independent of its antioxidant properties [17] and reduces the expression of proinflammatory cytokines such as TNF- α and IL-6 [18, 19]. Recent studies show that polyphenolic compounds demonstrate protective effects against OA progression by interacting directly or indirectly with joint tissues and reducing inflammation, oxidative damage, and pain. Many studies have shown the anti-inflammatory and antioxidant properties of DZ [17, 19–22]. Gündoğdu et al. [12] used an animal model to demonstrate the antioxidant and anti-inflammatory effects of DZ in treating OA in the knee joint of rats.

Therapy administered using intra-articular injection is an important approach for the management of TMJ OA. The purpose of this study is to investigate the early histopathological effects of intra-articular DZ injection on experimentally induced OA in rabbit TMJs and, specifically, evaluate the systemic and local (synovial fluid) antioxidant effect of intra-articular DZ administration. We used TAS, TOS and OSI kits, a colorimetric method, to evaluate the antioxidant activity of daidzenin locally and systemically. We hypothesize that antioxidant activity from DZ intra-articular injections will protect against early TMJ OA changes. Our specific aims are first to assess histologic changes using a TMJ OA rabbit model in joints treated with DZ versus saline controls. Second, we aim to assess the biochemical effects of DZ treatment by assaying blood TAS, TOS and OSİ.

Methods

Study desing

This animal study was conducted at Karadeniz Technical University Faculty of Medicine Surgical Research Center with the approval of Karadeniz Technical University Local Ethics Committee for Animal Experiments dated 29.07.2021, file number 2021/43, and with the project support of Karadeniz Technical University Scientific Research Coordination Unit dated 30.06.2021 and coded TDH- 2021–9702. The study was designed as a randomized, controlled experimental study.

Sample

Our study used 16 six months male white New Zealand rabbits weighing 2.5–3 kg as experimental animals. The rabbits were obtained from the laboratory for animal breeding and experimentation. The inclusion criteria were that the rabbits should be male, six months old, healthy, and weigh 2.5–3 kg. The rabbits were housed separately from each other in compartmentalized cages throughout the experiment at 20–22 °C in a windowless environment in a room with both sunlight and artificial lighting by creating a living space 12 h day and 12 h night. Rabbits received standard rabbit feed without any restriction and unlimited water supply. The experiment was started after two weeks, aiming to reduce the acclimatization period and stress level.

Surgical protocol

All rabbits (16 rabbits) were weighed before anesthesia. After calculating the appropriate anesthetic dose for each rabbit, 50 mg/kg ketamine hydrochloride (Ketalar® flk., Pfizer, 50 mg/ml solution) and 5 mg/kg xylazine hydrochloride (Rompun® inject. 2% sol., Bayer, Germany) were administered intramuscularly (IM). Following anesthesia, the skin of the rabbits was exposed by shaving the hair on the TMJ region. For antisepsis, the TMJ regions of the rabbits were wiped using povidone-iodine (Betadine® sol. Kansuk, Turkey).

A mouth opener was used for the control and experimental groups to access the right TMJ cavity by moving the tip of a 29-gauge insulin syringe in the medioanterior direction behind the inferior wall of the orbit under the zygomatic process of the temporal bone and behind the condyle. 50 µl monosodium iodoacetate (MIA) injection was performed in a single dose 3 mg/mL MIA (Sigma I 2 512-25G, St.Louis, MO, United States of America) solution (according to the MIA dose recommended in the study by Güler et al.) to create experimental OA in 16 rabbit TMJs.

One rabbit was sacrificed at the 4 weeks after MIA injection and subjected to a histopathological examination of the right TMJ to confirm the formation of OA. After confirming the development of osteoarthritis in the sacrificed rabbit, the remaining 15 rabbits were randomly divided into two groups: experimental group (9 rabbits) and control group (6 rabbits). One animal in the experimental group perished on day 15 after the start of DZ injections and was subsequently excluded; the cause of death remained unknown. Therefore, the final sample comprised 14 animals (8 in the experimental group and 6 in the control). The right TMJs of the rabbits in the control group were injected with 50 µl of intraarticular saline under general anesthesia on days 1, 7, 14, and 21, the same days as the experimental group. The right TMJs of the rabbits in the experimental group received intraarticular injections under general anesthesia of 50 µl of 1.8 mg/ml DZ solution on days 1, 7, 14, and 21.

One week after the last injection, blood samples from the marginal ear vein and intra-articular synovial fluid samples were taken from all rabbits under anesthesia. Marginal ear vein was preferred for blood collection from the rabbit. The fur of the area to be sampled was shaved. The ear was held between the index finger and thumb, then a 21-gauge needle was inserted into the ear vein and blood was slowly drawn into the syringe. The surgical method involving the administration of daidzein and salin was used to access the TMJ cavity, and synovial fluid was collected using a 29-gauge insulin needle. The rabbits were euthanized with a high-dose anesthetic. For TMJ excision; an oblique incision was created superior to the zygomatic process, the tissue was elevated and retracted to access the TMJ. The exposed right TMJ was excised.

The primary outcome variables were histological characteristics assessed using light microscopy. The secondary outcome variables were biochemical changes assessed using blood total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI). There were no covariates in this study.

Histopathological examination

The dissected temporomandibular joints were placed in labeled glass jars containing 10% neutral formaldehyde solution. The fixed tissues were placed in a decalcification solution containing 10% formic acid, which was changed every 2–3 days until the bones were decalcified and softened. The bones were washed in running water for four hours, passed through an ascending series of alcohols, cleared with xylene, and blocked by paraffin embedding [23]. In this way, a paraffin block was obtained from each TMJ.

From each paraffin block, 5 µm-thick serial sections were taken on the slide using a microtome (Leica RM2255, Japan). The sections were kept in the oven until the paraffin melted and then hydrated by passing through the xylene and alcohol series. The prepared sections were stained with routine hematoxylin and eosin (H&E) staining protocol. After staining, the sections were dehydrated in alcohol series and xylene, covered with Entellan, and coverslipped. Thus, two slides were made from each block.

The mandibular articular surfaces of each preparation were evaluated for histological changes in articular cartilage, osteochondral junction, subchondral bone structure and chondrocyte appearance using light microscopy (Olympus BX51, Japan). The articular cartilage (normal, abnormal (thickened-thinned)), chondrocyte appearance (normal, abnormal (hypocellular-clustered)), osteochondral junction (normal, abnormal (invaginated-poor junction)), and subchondral bone structure (normal and abnormal (trabecular bone increase)) were evaluated and scored separately and photographed with a digital camera integrated into the microscope (Olympus DP71, Japan) [24]. During the scoring, condyle cartilage in which the cellular arrangement (fibrous, proliferating, mature and hypertrophic, respectively) that the cartilage in the normal histologic condyle structure shows from the articular surface was considered normal in terms of chondrocyte appearance. Disruption of cellular arrangement, decrease in the number of chondrocyte cells and/or replacement of chondrocyte cells by fibrous tissue were considered as hypocellularity, while disruption of cellular arrangement, increase in cartilage cell proliferation and the appearance of proliferating chondrocyte clusters in all rows were considered as clustering. In terms of articular cartilage, it was considered normal if the articular surface was smooth and did not show significant irregularity-fibrillation-tearing and the cellular arrangement continued regularly. In the condyle cartilage, loss, tearing, reduction of the fibrous layer and/or loss of other layers of the cartilage layer and its replacement by fibrous tissue or bone tissue were considered as thinning of the cartilage. Thickening of the fibrous layer on the condyle surface, especially in the fibrous layer which is thinner than the other cartilage layers, cell increase and/or cell increase in all cartilage layers and increased cartilage tissue were evaluated as cartilage thickening. When the mandibular articular surface was examined in terms of osteochondral junction; separation at the cartilage-bone tissue transition or decreased and irregular cartilage-bone tissue connection at the transition was evaluated as poor juntion. The extension of bone tissue into the irregularized-thinning cartilage tissue in some areas and marked irregularity in the tidemark line were evaluated as invaginated junction. Condyles with regular tidemark and cartilage-bone tissue transition were considered normal. When the subchondral bone structure was examined, the regular formation of newly formed trabecular bone extensions

under the cartilage and the subsequent appearance of mature bone structure were considered normal; irregular and immature bone formation and increased trabecular bone structure were considered abnormal. In addition, the joints that showed normal histological structure in terms of articular cartilage, osteochondral junction and chondrocyte appearance were classified as "Normal" and the joints that exhibited signs of early osteoarthritis or pathological changes were classified as "Abnormal". The evaluations for each histological parameter were performed at x100 and x200 magnification in five randomly selected fields in each preparation by two experienced histologists blinded to the treatment groups. Random field selection was conducted to represent the beginning, middle, and end sections of the tissue, allowing evaluation of articular cartilage, osteochondral junction, subchondral bone structure, and chondrocyte appearance in each selected field.

Biochemical examination

Measurement of blood and joint fluid TAS and TOS levels

TAS and TOS levels in serum samples collected from the experimental and control groups were determined using TAS and TOS kits based on the direct colorimetric method developed by Erel [25, 26]. The results of TAS and TOS analyses were reported as mmol Trolox equivalent/L/mg protein and mmol H2O2 equivalent/L/mg protein, respectively.

Table 1 Distribution of articular cartilage, osteochondral junction, chondrocyte appearance, and subchondral bone based on the groups

	Control group *n*(%)	Experimental group *n*(%)	Total *n*(%)	*p*
Articular Cartilage				
Normal	1 (16.7)	3 (37.5)	4 (28.6)	0.3*
Thickened	1 (16.7)	3 (37.5)	4 (28.6)	
Thinned	4 (66.6)	2 (25.0)	6 (42.8)	
Normal	1 (16.7)	3 (37.5)	4 (28.6)	0.6**
Abnormal	5 (83.3)	5 (62.5)	10 (71.4)	
Osteochondral Junction				
Normal	1 (16.7)	3 (37.5)	4 (28.6)	0.3*
Invagine	2 (33.3)	4 (50.0)	6 (42.8)	
Weak junction	3 (50.0)	1 (12.5)	4 (28.6)	
Normal	1 (16.7)	3 (37.5)	4 (28.6)	0.6**
Abnormal	5 (83.3)	5 (62.5)	10 (71.4)	
Subchondral Bone				
Normal	3 (50.0)	4 (50.0)	7 (50.0)	1.000**
Increased trabecular bone	3 (50.0)	4 (50.0)	7 (50.0)	
Chondrocyte Appearance				
Normal	1 (16.7)	4 (50.0)	5 (35.7)	0.4*
Clustering	1 (16.7)	1 (12.5)	2 (14.3)	
Hypocellularity	4 (66.6)	3 (37.5)	7 (50.0)	
Normal	1 (16.7)	4 (50.0)	5 (35.7)	0.3**
Abnormal	5 (83.3)	4 (50.0)	9 (64.3)	

* Likelihood ratio results are given. ** Fisher's Exact Test results are given

Statistical analyses

Shapiro-Wilk test was used to evaluate whether the variables conformed to normal distribution. Mean ± SD (standard deviation) or median (interquartile range (IQR)) was used to represent the descriptive statistics of the variables.

The t-test and Mann Whitney U test were used to compare the control and experimental groups for blood TAS, TOS, OSI, joint TAS, TOS, and OSI values.

The distribution of articular cartilage, osteochondral junction, chondrocyte appearance, and subchondral bone variables obtained within the scope of the study based on control and experimental groups were presented as number (n) and percentage values. The chi-square test was used to compare these variables with the control and experimental groups.

IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) and MS-Excel 2007 programs were used; the statistical significance level was accepted as $p<0.05$.

Results

Study animals

Our study used 16 six months male white New Zealand rabbits. At the end of the experiment, one animal from the experimental group died and was excluded from the study. The cause of death could not be determined. All other rabbits (15 rabbits) completed the experimental procedure in good health.

Histopathological findings

Statistical analysis revealed no significant change in articular cartilage between the two groups according to the chi-square test ($p=0.3$). Histologic changes of the articular cartilage samples showed that 16.7% of the control group samples were normal, 16.7% were thickened, and 66.6% were thinned. In the experimental group, 37.5% were normal, 37.5% were thickened, and 25.0% were thinned. The samples that showed thickened or thinned articular cartilage thickness (early OA findings) were combined under the "Abnormal" category. According to this new classification, there was no statistically significant difference between the two "Normal" and "Abnormal" groups ($p=0.6$) (Table 1). In the control group, irregularities, fibrillations, and deep fissures were observed on the joint surface which are seen in the stages of osteoarthritis (Fig. 1). In the experimental group, irregularities and fibrillations on the joint surface decreased, and no fissures were observed (Fig. 2).

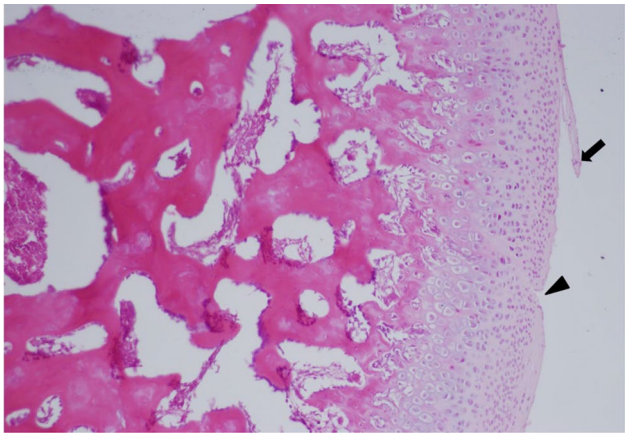

Fig. 1 Histological section of the control group. Joint surface irregularity and fibrillation (arrow) and deep fissures (arrowhead) (H&E, 100X)

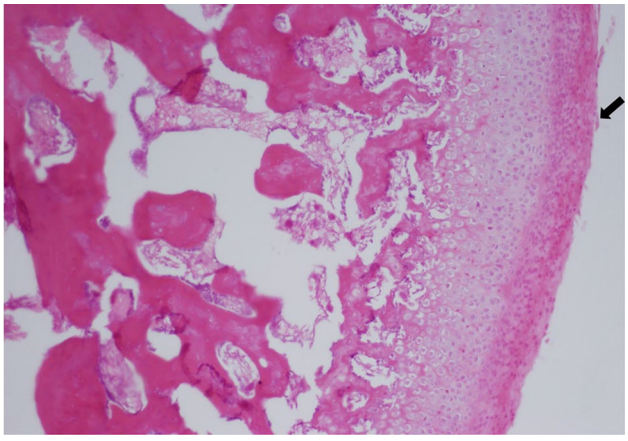

Fig. 2 Histological section of the treatment group. Joint surface irregularity and fibrillation (arrow) (H&E, 100X)

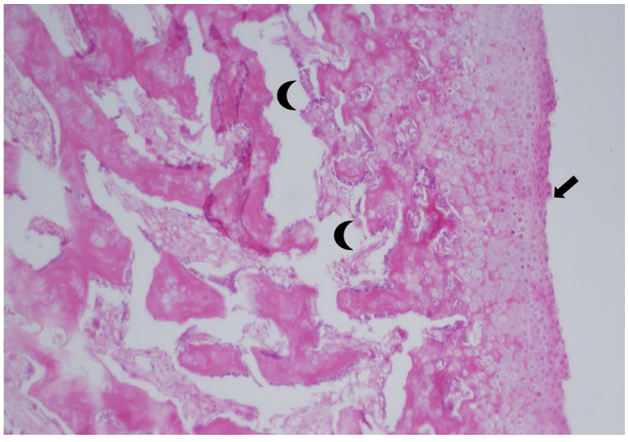

Fig. 3 Histological section of the control group. Weak osteochondral junction (moon) and irregularity and fibrillation on the articular surface (arrow) (H&E, 100X)

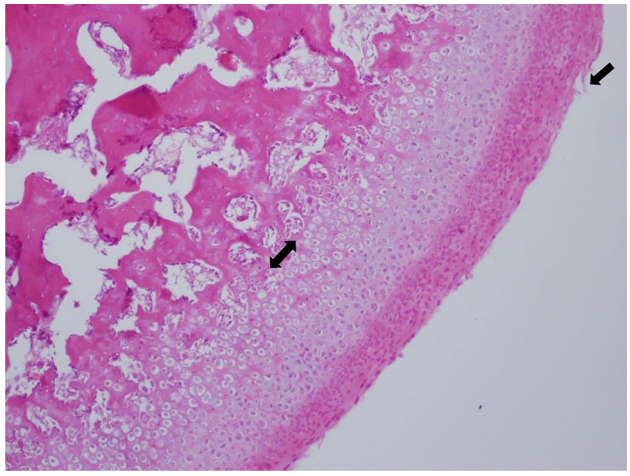

Fig. 4 Irregularity and fibrillation (arrow) on the articular surface and normal osteochondral junction (double headed arrow) in the histological section of the treatment group (H&E, 100X)

No statistically significant changes were noted at the osteochondral junction between the two groups ($p=0.3$) (Table 1). Evaluation of the osteochondral junction samples showed that 16.7% of the control group samples were normal, 33.3% were invaginated, and 50.0% ($n=3$) were weak junctions. In the experimental group, 37.5% were normal, 50.0% ($n=4$) were invaginated, and 12.5% were weak junctions. The samples that showed invaginated or weak osteochondral junction (OA findings) were combined under the "Abnormal" category. According to this new classification, there was still no difference between the "Normal" and "Abnormal" groups ($p=0.6$). Three (50%) of the joints in the control group showed poor osteochondral junction (Fig. 3), and three (37.50%) of the joints in the experimental group showed normal osteochondral junction (Fig. 4).

Analysis of the subchondral bone samples revealed that 50.0% of the control group samples were normal, 50.0% of the control group samples were increased trabecular bone and 50.0% of the experimental group samples were normal and 50.0% of the experimental group samples were increased trabecular bone. There was no significant relationship between subchondral bone and the groups ($p=1.000$) (Table 1).

Chondrocyte changes in the articular cartilage and osteochondral junction were not statistically different between the two groups ($p=0.4$) (Table 1). Evaluation of the chondrocyte appearance samples showed that 16.7%of the control group samples were normal, 16.7% had clustering, and 66.6% had hypocellularity. In the experimental group, 50% of the samples were normal, 12.5% had clustering, and 37.5% had hypocellularity. The samples that showed clustering or hypocellularity (OA findings) were combined under the "Abnormal" category, without significant difference between the "Normal" and

"Abnormal" groups ($p=0.3$). Control and experimental groups were evaluated for in terms of chondrocyte appearance. Hypocellular regions were observed in one (16.6%) of the joints in the control group (Fig. 5). In three (37.50%) of the joints in the experimental group showed, chondrocyte clustering was observed in the joint cartilage (Fig. 6).

Biochemical findings

Distribution of blood and joint fluid measurements are given according to the groups (Table 2). Systemic TAS, TOS, and OSI were tested using blood samples. The mean blood TAS value for the control group was 1.09±0.12, and the mean blood TAS value for the experimental group was 1.08±0.08. There was no significant difference between the control and experimental groups for mean blood TAS values (t=0.343, $p=0.7$). The mean blood TOS value for the control group was 14.77±2.87, and the mean blood TOS value for the experimental group was 8.66±2.75. Here, there was a significant difference between the control and experimental groups for mean blood TOS values (t=4.038, $p=0.002$). The mean blood OSI value for the control group was 1.37±0.37, and the mean blood OSI value for the experimental group was 0.81±0.28. Again, there was a statistically significant difference between the control and experimental groups for mean blood OSI values (t=3.225, $p=0.007$).

Local TAS, TOS, and OSI were tested using joint fluid. The median of the joint TAS value for the control group was 0.70 (IQR=0.22), and the median of the joint TAS value for the experimental group was 0.76 (IQR=0.19). This was not statistically significant (Z=0.289, $p=0.8$). The mean joint TOS value for the control group was 7.59±4.04, and the mean joint TOS value for the experimental group was 5.51±2.03. Again, there was no statistically significant difference between the mean joint TOS values for the control and experimental groups (t=0.923, $p=0.4$). The mean joint OSI value for the control group was 1.01±0.61, and the mean joint OSI value for the experimental group was 0.78±0.55. There was no statistically significant was noted (t=0.703, $p=0.5$) (Table 2).

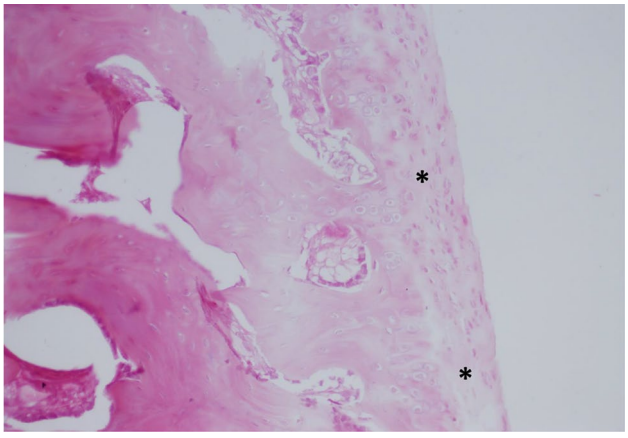

Fig. 5 Hypocellular regions (asterisk) in the thinned articular cartilage in the histological section of the control group (H&E, 200X)

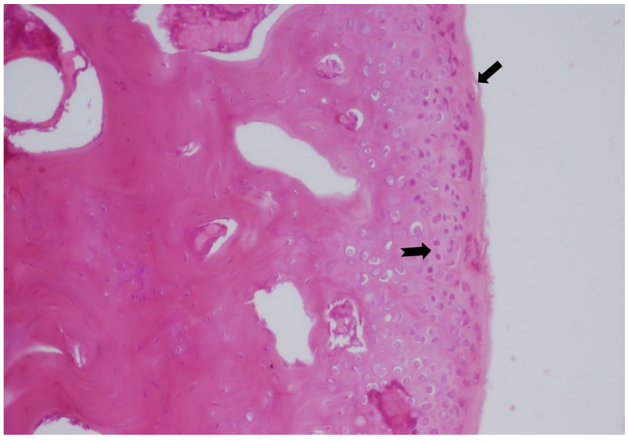

Fig. 6 Chondrocyte clustering in the articular cartilage in the histological section of the treatment group (notched arrow) (H&E, 200X)

Table 2 Distribution of blood and joint fluid measurements according to groups

	Control group	Experimental group	t, Z	*p*
	Mean ± SD IQR	Mean ± SD IQR		
Blood TAS	1.09 ± 0.12	1.08 ± 0.08	0.343	0.7*
Blood TOS	14.77 ± 2.87	8.66 ± 2.75	4.038	**0.002***
Blood OSI	1.37 ± 0.37	0.81 ± 0.28	3.225	**0.007***
Joint fluid TAS	0.70 (0.22)	0.76 (0.19)	0.289	0.8**
Joint fluid TOS	7.59 ± 4.04	5.51 ± 2.03	0.923	0.4*
Joint fluid OSI	1.01 ± 0.61	0.78 ± 0.55	0.703	0.5*

* t test results are given. ** Mann Whitney test results are given

Discussion

Antioxidant-based therapy shows promise in limiting the progression of OA. The purpose of this study was to test if the antioxidant DZ can alter histologic and biochemical changes associated with OA in a rabbit model. We hypothesized that DZ treated joints will exhibit fewer OA histologic changes than joints treated with saline alone. We also hypothesized that animals treated with DZ would show biochemical changes consistent with the antioxidant action of DZ. Our specific aims were to document differences in OA progression between the DZ and saline groups using light microscopy. In addition, we documented blood and joint biochemical changes using assays for TAS, TOS, and OSI. Our findings were suggestive that DZ treatment limited the progression of OA and demonstrated certain antioxidant responses that might impede OA.

We used the New Zealand rabbit model for OA, which shows structural similarities to human TMJ, low phylogenetic class, and favorable anatomy [27–29]. To minimize

disruption to the rabbits' dietary patterns, unilateral injection was administered randomly into the right joints. Like many studies, we chose male subjects to minimize hormonal effects [30–33]. The induction methods for OA models are mechanical, surgical, or chemical [34–37]. Pharmacologic agents administered via the intra-articular (IA) route have advantages such as easy adjustment of concentration, controllability of OA severity, rapid onset of action, and easy administration [33, 34]. Guler et al. [23] histologically proved OA formation in the 4th week after IA injection of 50 µl of MIA solution at a concentration of 3 mg/ml in their study on rabbit TMJ. This study used MIA to create an OA model. In our study, a mixture of xylazine and ketamine hydrochloride was injected IM to provide general anesthesia before performing all minimally invasive procedures on rabbits [38]. No complications related to general anesthesia were encountered.

Isoflovanes are an important therapeutic modality in OA management [13–15]. In this study, we investigated DZ, a flavonoid untested in the TMJ. Shahi et al. [18] reported that DZ decreased the serum concentrations of TNF-α, IL-6, adiponectin, and leptin in a rat model of rheumatoid arthritis and significantly reduced RA symptoms. They predicted that DZ may be effective in treating inflammatory diseases. Sakamato et al. [19] showed that DZ controls the expression of proinflammatory cytokines such as IL-6 via PPAR-alpha-gamma (peroxisome proliferator-activated receptor-alpha-gamma) and JNK (Jun-N-terminal kinase) pathways in adipocyte and macrophage cultures. In our study, we used a single concentration of DZ. Due to the paucity of previous studies on this subject, we used the dose and the timepoint of examination amount recommended by Gündoğdu et al. [12] which was calculated and adapted to the rabbit.

We tested if DZ injection could limit OA histopathologic changes. Inflammatory cytokines or ROS can lead to chondrocyte death [39]. Degradation of various joint components, such as collagen, proteoglycans, and hyaluronic acid, increases ROS. Oxidative stress caused by ROS exacerbates the inflammatory process in OA and accelerates ECM degradation [40–42]. The viscoelastic property of synovial fluid decreases due to hyaluronic acid (HA) degradation [43]. Reports show that the source of inflammation in the TMJ causing pain and dysfunction may be related to free radical accumulation [44]. Oxidative stress, the accumulation of free radicals deactivated by molecules known as antioxidants, may significantly affect osteoarthritis, disc displacement, and internal derangement [45, 46]. Many studies have shown that an oxidant/antioxidant imbalance may be involved in the pathogenesis of TMJ and related diseases [25, 44, 47, 48]. Free radicals can promote chronic inflammation, causing further tissue damage and pain [44].

This is the first study to evaluate the effect of DZ injection into the TMJ. In this study, we aimed to evaluate early osteoarthritis outcomes through histopathological analysis. The earliest signs of joint cartilage degeneration include excessive production of proteoglycans and other extracellular matrix molecules, along with the emergence of chondrocyte clusters [23]. Hypocellularity associated with apoptotic cell death and cartilage erosion due to subchondral bone exposure are reported to occur in later stages [33]. Our study observed these two findings, with hypocellularity rates, a recognized indicator of late-stage OA, being higher in both groups. Our findings indicate that articular cartilage treated with DZ exhibited a greater presence of normal tissue compared to the control group. However, these differences did not reach statistical significance. Analysis of the rates of thickening and thinning, particularly in the fibrous layer of the articular cartilage—considered an early indicator of OA—revealed that the beneficial histopathological effects of DZ on the articular cartilage were not substantial enough. Despite that normal osteochondral junction had a higher percentage in the study groups treated with DZ, there was no significant difference between the groups. Although there were no significant results in the histological results obtained, fewer OA-related alterations were noted in the osteochondral junction and chondrocytes of the experimental group compared to the control group. Mahmoud et al. also found that daidzein applications in cultured human chondrocyte cells resulted in bioactivity regulation and ECM enhancement in chondrocytes [49]. This result can be attributed to the regeneration ability of DZ on chondrocytes. There was no superiority in subchondral bone appearance. Due to ethical constraints, the limited number of animals and the absence of a control group have led to a restricted sample size in certain parameters. The short duration of the observation period may also have influenced the results. In addition, the significant results previously found by Gundogdu et al. [12] in the rat knee joint suggest that the effect of DZ may be joint-dependent. For these reasons, significant histologic results may not have been obtained.

Our biochemical analysis showed DZ protection in the blood samples but not joint fluid. Studies suggest that oxidative stress caused by free radicals may initiate degenerative changes in TMJ. Free radicals in healthy TMJs cannot induce disease if increased oxidative stress is regulated by antioxidant enzymes [44]. Therefore, possible tissue damage within the TMJ depends on the local antioxidant capacity of the joint tissues against free radicals [50]. Increased oxidative stress caused by free radicals in the affected TMJ can impair local antioxidant defense [46]. We performed measurements in joint fluid and blood samples using TAS, TOS, and OSI kits to evaluate the efficacy of daidzein. Hämäläinen et al. [16] reported that daidzein inhibited NO

(nitrous oxide) and INOS (inducible nitric oxide synthase) expression. They investigated the anti-inflammatory properties of flavonoids and showed that they inhibited STAT-1 (signal transducer and transcription-1) and NF- κB (nuclear factor kappa B) activation, which are important transcription methods for INOS.Gundogdu et al. [12] examined serum samples to evaluate TAS-TOS levels in the group with IA DZ application for treating experimental OA in rat knee joints. Results showed higher TAS levels in the IA DZ group and significantly lower TOS levels than in the control group. In our study, blood and joint fluid samples were taken from the subjects. Synovial fluid samples were evaluated for local examination of TAS, TOS and OSI values and blood samples were evaluated for systemic examination of TAS, TOS and OSI values. Blood fluid samples showed no difference in the mean blood-TAS level for the experimental group compared to the control group, with significantly decreased mean blood-TOS and mean blood-OSI levels. Similarly, mean joint fluid-TAS level increased in the experimental group, mean joint fluid- TOS level decreased in the experimental group, and mean joint fluid-OSI values decreased in the experimental group, with no significant results in joint fluid evaluations (Table 2). These results have demonstrated the systemic effects of DZ while failing to fully elucidate its local effects. Obtaining intra-articular synovial fluid for evaluating the local effect of DZ is challenging. The researchers had difficulty removing synovial fluid from the joint capsule for two reasons. The first reason was the difficulty of accessing the small amount of synovial fluid in the already narrow joint capsule. The second reason was that the region is a risky area in terms of bleeding and our calculation kits work colorimetrically. For these reasons, synovial fluids with hemorrhage were excluded from the evaluation. This situation prevented statistically significant results from being obtained. Due to this difficulty, it is recommended to use a calculation method other than the colorimetric method for future research.

Our study has several limitations. Any findings based on an animal study have to be viewed with caution until confirmed by clinical studies. Our study is based on a single dose and single time point; therefore, the opportunity to capture a more comprehensive appreciation of DZ treatment were likely missed. In addition, our findings were not compared against a gold standard to allow us to assess the relative importance of DZ. Our observations are descriptive in nature. More targeted functional studies will need to be performed to understand the mechanisms of DZ's potential role in TMJ OA management.

Conclusion

In conclusion, an intra-articular DZ injection appears to show limited reduction of oxidative damage and early OA in the rabbit TMJ. DZ might represent a promising natural compound with beneficial effects in the management of TMJ-OA. Future studies that compare DZ with current antioxidant in use, as well as explore dose dependency, expanded timepoints during TMJ OA progression and underlying mechanisms of DZ action, need to be performed. Using the New Zealand rabbit TMJ model, we believe we can refine our understanding of the therapeutic benefits of DZ in order to improve future management of TMJ OA.

Abbreviations

OA	Osteoarthritis
DZ	Daidzein
TAS	Total antioxidant status
TOS	Total oxidant status
OSI	Oxidative stress index
TMJ	Temporomandibular joint
HA	Hyaluronic acid
PRP	Platelet-rich plasma
NO	Nitric oxide
IM	Intramuscularly
MIA	Monosodium iodoacetate
IQR	Interquartile range
IA	Intra-articular
STAT-1	Signal transducer and transcription-1
NF- κB	Nuclear factor kappa B
NO	Nitrous oxide
INOS	Inducible nitric oxide synthase

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Author contributions

G.E.E Creation of the methodology, performing experiments, writing the tables and figures, writing the manuscript. O.Y. Performing experiments, writing the manuscript. G.K. Histopathologic evaluations, writing the manuscript. E.S. Biochemical assessments. S.Y.D. Histopathologic evaluations. All authors reviewed the manuscript.

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Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Ethics approval and consent to participate

This study performed on rabbits at the Karadeniz Technical University Faculty of Medicine Surgical Research Center with the approval of Local Ethics Committee (2021/43).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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