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An investigation of the effects of melatonin administration on biochemical parameters in rats with experimental cartilage damage

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ABSTRACT

Objective: This study was intended to show the effects of melatonin (MEL) in the treatment of cartilage damage in a rat model as a novel field of application.

Materials and Methods: Male Sprague Dawley rats aged 3-4 months were assigned into four groups of six rats each. Group I represented the sham group. In groups 2, 3, and 4, the right knee medial meniscus was surgically destabilized. MEL was administered to groups 3 and 4 twice a week at dosages of 0.4 µg/ml and 4 µg/ml, respectively. The application continued for four weeks. Histological examinations, imaging studies [computed tomography and magnetic resonance imaging], and biochemical tests [cartilage and bone turnover markers (COMP and CTX-I)] were performed.

Results: The application of MEL initiated regeneration in the damaged areas. However, cartilage repair was not observed in areas with experimental cartilage damage without MEL application. MEL-treated rats had higher T2 scores compared to Group 1 in the median femoral condyle at the 12th week ($p<0.05$). Serum COMP and CTX-I levels at 12 weeks were significantly higher in Group 2 compared to Group 1 ($p<0.05$). Serum COMP and CTX-I levels at 12 weeks were lower in groups 3 and 4, but were also significantly higher than in Group 1 ($p<0.05$).

Conclusion: We recommend MEL therapy for diseases related to cartilage damage. MEL seems to exert its therapeutic effect on cartilage damage through its antioxidant properties.

Keywords: Melatonin, rats, cartilage damage, COMP and CTX-I

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INTRODUCTION

Melatonin (MEL), released from the pineal area, is the hormone that regulates the human sleep–wake cycle (1, 2). It was first discovered in 1958. MEL is a prescription-only medication, but is not approved for any medical use by the US Food and Drug Administration (FDA). However, in the European Union and Australia, it may be prescribed for sleeping problems or insomnia. It was approved for medical use in 2007 (3, 4, 5). MEL is used in the treatment of insomnia in children and adolescents aged 2–18 with autism spectrum disorder (ASD), and especially in Smith–Magenis syndrome (6).

MEL is also beneficial to cartilage tissue. The protective role of MEL was found to be as significant as that of dexamethasone by Yang et al. in a study in which mouse knee joints were treated with MEL, dexamethasone, EX527, and siRNA targeted for SIRT6 (7).

According to World Health Organization (WHO) data, 25% of individuals over the age of 65 complain of joint pain and dysfunction caused by the progressive and irreversible loss of joint cartilage (osteoarthritis and calcification) (8). Despite the prevalence of cartilage damage, modalities for preventing and/or curative treatment are limited (9).

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New therapeutic options for osteoarthritis have therefore become an important research topic in recent years. MEL is a natural agent with well-known antioxidant and immunomodulatory effects.

Cartilage tissues surgically damaged through intra-articular intervention have been treated with MEL, and its effects observed, in rat models in previous studies. Such rat models can be used to evaluate the therapeutic effects of MEL for all chondral injuries. The purpose of this study was to show the effects of MEL in the treatment of cartilage damage in a rat model as a novel field of application.

MATERIAL and METHODS

This animal study was designed using a rat model of cartilage damage to show the effects of MEL on cartilage structures. The experiments commenced following approval from the Kafkas University Animal Experiments Local Ethical Committee (Turkey) (no. 2019/007 dated 21/06/2019). Twenty-four male Sprague Dawley rats aged 3-4 months and weighing 250-300 g were assigned into four groups of six animals each.

The animals were housed in a controlled room temperature (22±2°C) and in a 12-hour dark, 12-h light cycle. All experiments were performed in line with International Association for Pain Studies rules (1983). The animals were permitted ad libitum access to chow and drinking water. Eighteen-gauge soft gavage tubes have generally been used (16-20 sizes) for MEL treatment in similar studies.

Rats were randomly divided into four groups of six animals each. The right knee medial meniscus was surgically destabilized in three of these groups. MEL applications commenced after waiting four weeks for cartilage damage to develop. MEL was administered to two groups subjected to surgical destabilization through addition to drinking water twice a week at two different doses, 0.4 µg/mL and 4 µg/mL. Application continued for four weeks, at the end of which time all rats were sacrificed, and blood and tissue samples were collected.

Group I (n=6) (sham group): The articular cavity was opened in the right knee joint, but the suturing procedure was performed without transecting the medial meniscotibial ligament.

Group II (n=6) (control group): Rats in this group were subjected to medial meniscus destabilization with transection of the ligament of the medial meniscotibial band.

Group III (n=6): Rats in this group underwent medial meniscus destabilization with transection of the ligament of the medial meniscotibial band, followed by MEL application at 0.4 µg/mL twice a week for four weeks.

Group IV (n=6): This group underwent medial meniscus destabilization with transection of the ligament of medial meniscotibial band, followed by MEL application at 4 µg/mL twice a week for four weeks.

Histological Examinations: Tissue samples collected from the knee joints for histological evaluation were fixed in 10% formaldehyde solution. Tissues were then blocked in paraffin after routine processing, and 5-micrometer (µm) thick sections were taken from the paraffin blocks. For histological examinations, the sections were stained with Hematoxylin-Eosin (H&E) and evaluated under a light microscope (Olympus Bx43). Histopathological images of the slides were then taken using a digital camera (Olympus DP21).

Imaging Studies: Animals were sacrificed at week 12. The knee joints were separated from soft tissues for histological examination. Magnetic resonance imaging (MRI) was performed 12 weeks following surgery. MRI (T1 and T2) scans were used to evaluate changes in the articular cartilage area.

Biochemical Tests: The bone and cartilage turnover markers employed were cartilage oligomeric matrix protein (COMP) and carboxy-terminal collagen I (CTX-1). These were measured initially and at 3, 6, and 12 weeks. Cartilage damage was assessed by means of biochemical measurement of the serum levels of these markers using a Biotek 50 TS plate washer and ELX808 plate reader ELISA device (BioTek Instruments Inc., Winooski, VT, USA).

RESULTS

Histological Results: Histopathological tissue examination revealed no difference between the control and sham (no damage groups) groups. However, in the experimental groups, the integrity of the knee joint cartilage was impaired in some areas due to external injury. Regeneration was observed to have commenced in the damaged areas of the knee joint as a result of MEL application in the groups with experimental cartilage damage. Partial cartilage formation was observed with fibrous connective tissue following regeneration. However, repair was negligible in rats with experimental cartilage damage without MEL application, and in some regions, no repair was observed at all (Figures 1, 2, 3, and 4). All histological examinations in this study involved cartilage tissues. The histological grading of the groups is shown in Table 1.

Imaging Studies: T1 scores at 12 weeks were higher in the lateral tibial (LT), medial tibia (MT), and lateral femoral condyle (LFC) compartments ($p<0.05$) compared to the control group. T1 scores were lower in the groups treated with MEL compared to Group 2 rats in MT, LT, LFC, and the median femoral condyle (MFC) at the 12th week ($p<0.01$). T2 scores in the cartilage were higher in Group 1 compared to Group 1. The scores were higher on the 12th week in MFC, LFC, MT, and LT ($p<0.01$) compared to the control group. T2 scores of rats treated with MEL were lower than those in Group 2 at 12 weeks in MT ($p<0.001$), MFC ($p<0.01$), and LFC ($p<0.05$). T2 scores of rats treated with MEL were higher compared to Group 1 in MFC at the 12th week ($p<0.05$) (Figures 5, 6, 7). The groups' MRI scores are shown in Table 2.

Serum Biomarkers: No change was observed in serum COMP and CTX-I levels in Group 1 compared to the other groups ($p>0.001$). Group 2 serum COMP and CTX-I levels at week 12 were higher than those in Group 1 ($p<0.05$). Serum COMP and CTX-I levels were lower in groups 3 and 4 at 12 weeks, but were higher than in Group 1 at 12 weeks ($p<0.05$). The groups' serum COMP and CTX-I levels are shown in tables 3 and 4.

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Table 1. Histological Grading of Groups.

	Group 1	Group 2	Group 3	Group 4
12th week	1.65	1.76	2.22	2.58

Table 2. Magnetic Resonance Imaging (MRI) Grading of Groups.

	Group 1	Group 2	Group 3	Group 4
Initation	32.37	33.65	33.41	33.45
12th week	15.65	15.87	26.78	28.88

Table 3. Serum COMP levels in groups

	Group 1	Group 2	Group 3	Group 4
Initation	2.65	2.98	2.78	2.75
3 rd week	2.27	5.75	2.64	2.60
6 th week	1.78	4.04	2.22	2.12
12 th week	1.65	3.17	1.87	1.78

Table 4. Serum CTX-I levels in groups

	Group 1	Group 2	Group 3	Group 4
Initation	32.37	33.65	33.41	33.45
3 rd week	25.27	31.75	28.64	27.60
6 th week	19.78	25.04	18.22	16.12
12 th week	15.65	23.17	15.87	14.78

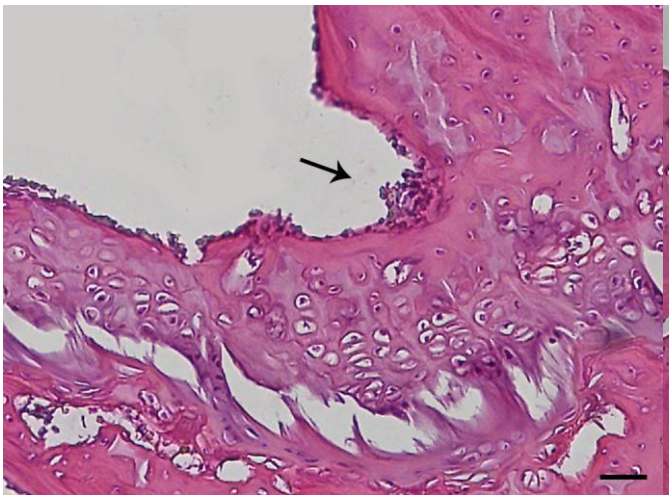

Figure 1. The histologic image of Group 1 (sham group)

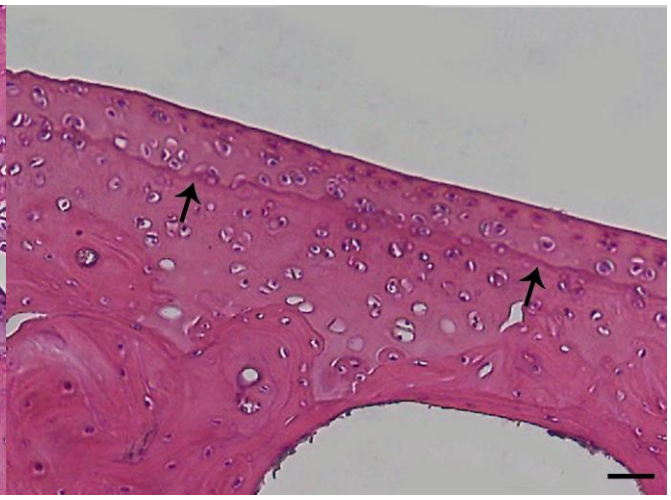

Figure 2. The histologic image of Group 2 (right knee medial meniscus surgically destabilized)

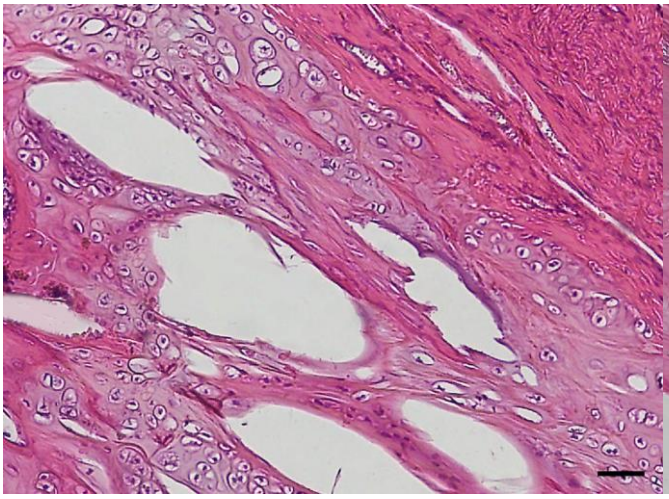

Figure 3. The histologic image of Group 3 (right knee medial meniscus surgically destabilized, MEL administration, a dose of 0.4 µg/mL, for 4 weeks)

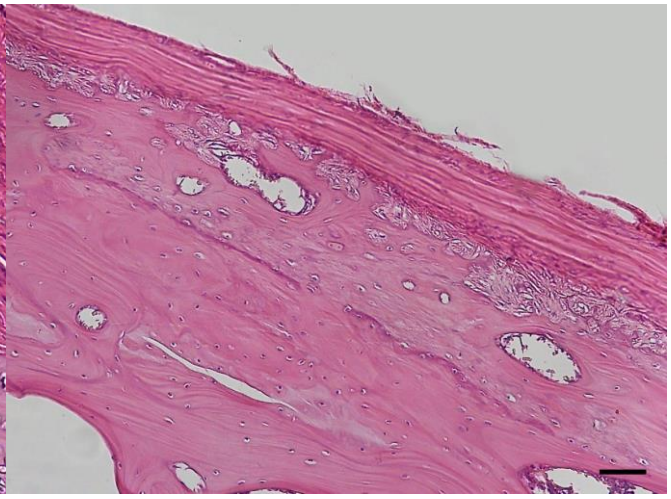

Figure 4. The histologic image of Group 4 (right knee medial meniscus surgically destabilized, MEL administration, a dose of 4 µg/mL, for 4 weeks)

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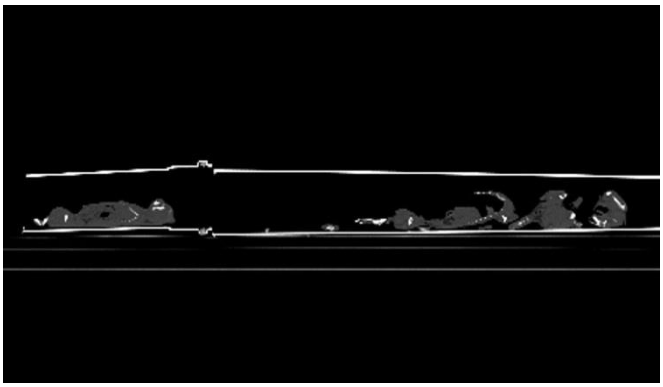

Figure 5. Computed Tomography images of rats

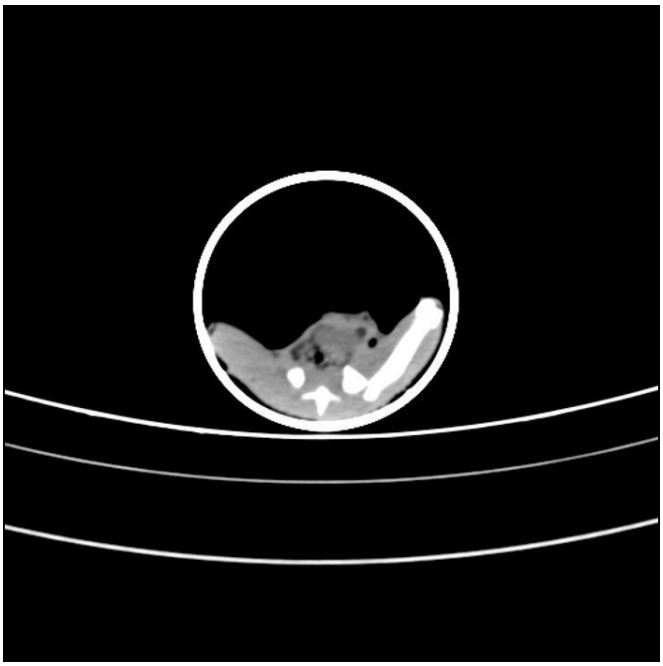

Figure 6. Computed Tomography axial images of rats

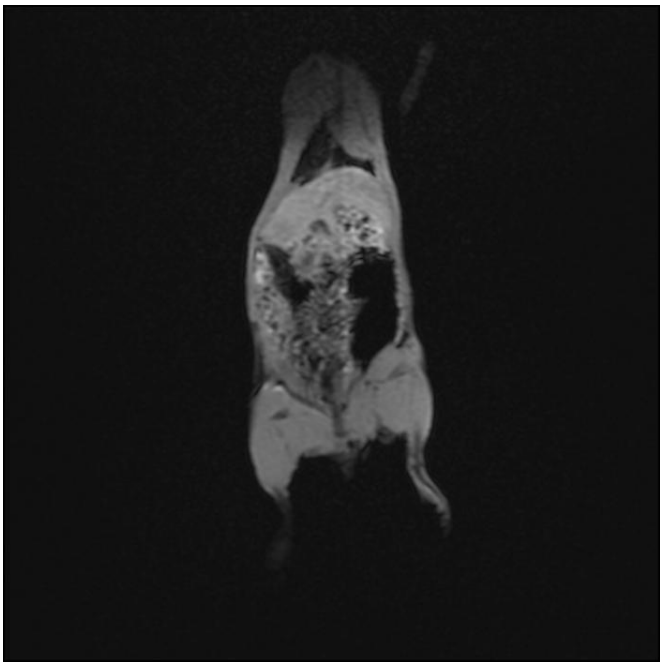

Figure 7. Magnetic Resonance Imaging scans of rats

DISCUSSION

No histological difference was observed between the rats in the control and sham (no damage) groups. In the experimental groups, the integrity of the joint cartilage was impaired in some areas due to external damage to the joint. In the groups with experimental cartilage damage, the application of MEL initiated regeneration in the damaged areas. Following regeneration, partial cartilage formation with fibrous connective tissue was observed. However, no cartilage repair was found in damaged areas with experimental cartilage damage without MEL application. T1 scores at 12 weeks were higher in MT, LT, and LFC compared to the control group ($p<0.05$). MEL treatment resulted in lower T1 scores at 12 weeks compared to Group 2 in MT, LT, LFC, and MFC ($p<0.01$). T2 score elevation was observed in cartilage in groups 3 and 4. At week 12, the scores were higher in MT, LT, MFC, and LFC compared to the control group ($p<0.01$). MEL treatment resulted in lower T2 scores compared to Group 2 in MFC ($p<0.01$), LFC ($p<0.05$), and MT ($p<0.001$) at 12 weeks. MEL-treated rats exhibited higher T2 scores than those in Group 1 in the MFC at 12 weeks ($p<0.05$). No significant difference was determined in serum COMP and CTX-I levels in Group 1 compared to the other groups ($p>0.001$). Serum COMP and CTX-I levels were significantly higher at 12 weeks in Group 2 compared to Group 1 ($p<0.05$). Serum COMP and CTX-I levels were lower in groups 3 and 4 at 12 weeks, but were higher than in Group 1 ($p<0.05$).

A previous study reported that MEL reversed the adverse effects of dexamethasone. Inhibition of SIRT1 was also observed to block the protection provided by MEL. The most important results of that study were that the chondroprotective effects of MEL increase with NAD-dependent SIRT1 activity, and that MEL reduces dexamethasone-induced cartilage damage (7). The removal of the pineal gland accelerates intervertebral disc degeneration (IDD). Li et al. demonstrated the presence of MT1 and MT2 (melatonin membrane receptors) in the intervertebral disk tissues and the cells of the nucleus pulposus (NP). MEL inhibits the cellular proliferation of NP in a dose-dependent manner and regulates its cell function. It is also an important factor in the prevention of IDD (**10**). MEL exhibits cytoprotective effects through a pathway dependent on numerous molecules and receptors, including NF-κB. The disturbance of circadian timekeeping has been linked to inflammatory arthritis. MEL stimulates the cartilage destruction/regeneration process through direct/indirect modulation of the main circadian clock genes. These genes are BMAL, CRY, and/or DEC2. BMAL, CRY, and/or DEC2. The effects of MEL on major arthritis disease as rheumatoid arthritis and osteoarthritis demonstrated by Jahanban-Esfahlan R et al. (**11**).

MEL is a powerful antioxidant capable of scavenging a variety of reactive oxygen species. MEL levels in vivo are relatively low, and it is difficult to explain this antioxidant activity in terms of scavenging activity. It is probably capable of altering the antioxidant activity of the cell in an indirect manner. High local concentrations of MEL in the brain produce significant protective effects against head trauma in rats (**12**).

CONCLUSION

MEL application provided regeneration in the damaged areas of the knee joint following experimental cartilage damage. The fibrous connective tissue was maintained following this regeneration process. Cartilage repair was almost non-existent in rats without MEL application. We recommend the use of MEL therapy for cartilage diseases.

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Ethical issues: All authors declare originality of research.

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