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EFFECTS OF THE ANTIEPILEPTIC DRUGS CARBAMAZEPINE AND LEVETIRACETAM ON BONE HEALING IN RAT FRACTURE MODEL

Mesut Tıkman[1], Evrim Duman[2]

[1]*Republic of Turkey Ministry of Health Gölbaşı Şehit Ahmet Özsoy State Hospital, Department of Orthopaedics and Traumatology, Ankara, Türkiye*

[2]*Etlik City Hospital, Department of Orthopaedics and Traumatology, Ankara, Türkiye*

ABSTRACT

Objective: *Long-term exposure to antiepileptics causes adverse effects on bone metabolism, but the available English literature regarding fracture healing is limited. In his study,we compare the effects of carbamazepine and levetiracetam on fracture healing.*

Material and Method: *Thirty Sprague-Dawley rats were randomly assigned to three groups of ten. Carbamazepine and levetiracetam were administered to rats in the first (CAR) andsecond group (LEV), respectively; the third group (CONT) served as the control group. Drug administration was started four weeks before fracture model establishment. Then a closed fracture was created and fixed. In each group, half of the rats were sacrificed at 4 weeks and then at 6 weeks. All femurs were evaluated with plain radiography using radiographic union scale in tibial (RUST) fractures scoring, Lane & Sandhu scoring. Besides microtomography (micro-CT), and histological Huo scoring was performed.*

Results: *Radiological scores were superior in all groups in the sixth week than in the fourth week ($p<0.01$). The group with the lowest bone mineral density (BMD) was the CAR group, followed by the LEV group in the 4^{th} and 6^{th} weeks ($p<0.01$). The callus mineralization rate was lower in the drug groups in the 4^{th} week ($p=0.05$). There was no significant difference between the groups at 6 weeks in micro-CT analysis ($p=0.54$). Histologic scores were similar to micro-CT in the 4^{th} week, but the carbamazepine group had the lowest scores in the 6^{th} week ($p<0.01$).*

Conclusion: *This study demonstrated that carbamazepine and levetiracetam adversely affect the early period of fracture healing.*

Keywords: *Carbamazepine, levetiracetam, fracture healing, microtomography.*

CORRESPONDING AUTHOR: Mesut Tıkman Segmenler Mahallesi, Cumhuriyet Cad. No:134 06830 Gölbaşı, Ankara, Türkiye mesuttikman@gmail.com

MT https://orcid.org/0000-0003-1671-9661 **ED** https://orcid.org/0000-0002-3493-5125

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ANTİEPİLEPTİK İLAÇLAR KARBAMAZEPİN VE LEVETİRASETAM'IN SIÇAN KIRIK MODELİNDE KEMİK İYİLEŞMESİ ÜZERİNE ETKİLERİ

ÖZET

Amaç: Antiepileptiklere uzun süreli maruz kalmak, kemik metabolizması üzerinde olumsuz etkilere neden olur, ancak kırık iyileşmesi ile ilgili mevcut İngilizce literatür sınırlıdır. Çalışmamızda karbamazepin ve levetirasetamın kırık iyileşmesi üzerindeki etkilerini karşılaştırdık.

Materyal ve Metot: Otuz adet Sprague-Dawley sıçan rastgele onarlık üç gruba ayrıldı. Birinci gruptaki sıçanlara karbamazepin (KAR), ikinci gruptakilere levetirasetam (LEV) verildi; üçüncü grup kontrol (KONT) grubu olarak şeçildi. İlaç uygulamasına kırık modelinin kurulmasından dört hafta önce başlandı. Daha sonra kapalı kırık oluşturuldu ve tedavi edildi. Her grupta, sıçanların yarısı 4. haftada ve kalanı 6. haftada sakrifiye edildi. Tüm femurlar düz radyografi ile tibial (RUST) kırık skorlamasında radyografik kaynama ölçeği, Lane & Sandhu skorlaması kullanılarak değerlendirildi. Ayrıca mikrotomografi (mikro-BT) ve histolojik olarak Huo skorlaması yapıldı.

Bulgular: Altıncı haftada tüm gruplarda radyolojik skorlar dördüncü haftaya göre üstündü (p<0,01). Kemik mineral yoğunluğu (KMY) en düşük olan grup CAR grubu olurken, bunu 4. ve 6. haftalarda LEV grubu izledi (p<0,01). Kallus mineralizasyon oranı ilaç gruplarında 4. haftada daha düşüktü (p=0,05). Mikro-BT analizinde 6. haftada gruplar arasında anlamlı fark yoktu (p=0,54). Histolojik skorlar 4. haftada mikro-BT ile benzerdi ancak karbamazepin grubu 6. haftada en düşük skora sahipti (p<0,01).

Sonuç: Bu çalışma, karbamazepin ve levetirasetamın kırık iyileşmesinin erken dönemini olumsuz etkilediğini göstermiştir.

Anahtar kelimeler: Karbamazapin, levetirasetam, kırık iyileşmesi, mikrotomografi

INTRODUCTION

Antiepileptic drugs (AEDs) are widely used in epilepsy and diseases such as migraine prophylaxis, essential tremor, neuropathic pain, bipolar disorder, and anxiety. However, long-term usage of AEDs, especially old-generation AEDs, causes osteopenia. Carbamazepine, valproic acid, and benzodiazepines, invented before 1990 and having similar molecular structures, are called old-generation antiepileptics. It is known that the new-generation antiepileptics (gabapentin, levetiracetam, pregabalin) have fewer side effects on the bone tissue than the old-generation ones.[1] Currently, new-generation AEDs are preferred because they are more effective in preventing seizures, and their side effects are more easily tolerated.[2,3] The choice of antiepileptic is determined by the type of epilepsy, seizure type, patient characteristics, and drug properties such as pharmacokinetics, side effects, and dose ranges.

When old-generation AEDs are used for a long time, they affect the hydroxylation stage of vitamin D in the liver and decrease the synthesis of the active form of vitamin D, reducing bone mineral density.[4] Given the combination of decreased bone mineral densityand increased risk of trauma during seizures (falls and tonic–clonic contractions), it has been reported that the fracture risk is 2-5 times higher in patients with epilepsy than in the general population.[5] There are controversial publications claiming that levetiracetam, a new-generation antiepileptic drug, does not affect vitamin D and bone metabolism. Other studies state that it increases bone fragility even if it does not change bone mineral density.[6-8] As orthopedic surgeons in daily practice, we follow up with epileptic patients' fractures as if they have no osteopenia or osteoporosis. According to Fischer *et al.*, sufficient amounts of calcium are also required for fracture callus mineralization, and compromised bone repair that is frequently observed in osteoporotic patients might be attributed to calcium and vitamin D deficiencies.[9] As a result of this knowledge, we concluded that levetiracetam should not adversely affect fracture healing.[9,10]

In the literature, there are no definite data about the effect of AEDs on fracture healing. This animal study aimed to compare the effects of the old-generation antiepileptic carbamazepine and the new-generation levetiracetam on fracture healing. It has been hypothesized that both anti-epileptic drugs negatively affect fracture healing.

MATERIAL AND METHOD

Study Plan

In this study, rats were used because of the advantages of their rapid adaptation to conditions, resistance to infection, isogeneic nature, ease of manipulation and maintenance relative to large animals, easy availability, and low cost.[11-13]

A total of 30 Sprague–Dawley male rats, aged 6-8 weeks, each weighing an average of 200 g, were randomly selected and assigned to three groups of ten rats. Exclusion criteria were defined as rejection by the veterinary surgeon, weight loss of >20% during the procedure according to daily weight track, not taking food and water regularly, and significantly reduced response to stimuli.[14] The control group (CONT) consisted of 10 drug-free rats. Rats in the second group (LEV, n:10) were administered levetiracetam 180 mg/kg/day by oral gavage (Keppra 100 mg/ml oral solution, UCB Pharma, Belgium), and rats in the third group (CAR, n:10) were administered carbamazepine 75 mg/kg/day by oral gavage (TEGRETOL® 2% syrup, Novartis, Switzerland).[7,8,15] Drugs were administered with an oral gavage tube directly to the rats' stomachs. Löscher W. examined the half-life of AEDs used in chronic epilepsy in rat models and stated that the drug half-life in rats was considerably shorter than that in humans.[15] According to previous studies, the frequency of drug administration was adjusted, paying attention to the drug dose selected for an antiepileptic effect.[8,16,17] Epilepsy patients are long-time AED users, which is why bone changes occur. The administration of the drugs was started four weeks before establishing the fracture model to mimic a patient's metabolism because it takes at least four weeks of medication usage to detect observable bone changes.[18] After four weeks of drug administration, a closed fracture was created in the right femur of all the rats. At the end of the fourth postoperative week, the first group of subjects (4 from the CAR group, 4 from the LEV group, 5 from the CONT group) were sacrificed. After two more weeks of drug administration to the remaining rats, all remaining rats were sacrificed, and the experiment was terminated. Fracture healing in rats is completed between four and six weeks.[11,19] Therefore, when examining fracture healing, it is recommended thatseparate evaluations be conducted during the 4th week (for the middle period of healing) and during the 6th week (for the late period of healing).[12] Thus, the mineralization of the maturing callus can be better evaluated, and the possible delay in fracture healing can be evaluated by comparison. Our study examined the middle and late stages of fracture healing as we thought that antiepileptic drugs might adversely affect fracture healing. After the rats were sacrificed, the right and left femurs were separated from the hip and knee joints. All the soft tissues on the femur were carefully stripped from the bone without damaging the callus tissue, and all femurs were examined radiologically and histologically.

All procedures (anesthesia, surgery, sacrifice) were performed by authors within the group on the same day.

Surgical Technique

All rats were anesthetized with an intraperitoneal injection of 50 mg/kg ketamine hydrochloride (Ketalar vial, Pfizer PFE İlaçları A.Ş., Istanbul). After shaving the surgical area, the right lower extremity was disinfected entirely with povidone-iodine and sterile draped. A longitudinal skin incision with an average length of 9 mm was made from the anterior of the right knee. The patella was lateralized after medial parapatellar arthrotomy. The medulla of the femur was entered through the intercondylar notch with a 1.2 mm drill bit. Intramedullary reaming was performed over an intramedullary nitinol guidewire with a 16-gauge intravenous cannula needle. After determining the length of the intramedullary canal, a nail of the same length was prepared from an intravenous cannula needle. A closed fracture was created under sterile conditions with a device based on the three-point pressure principle while the nitinol wire was in place. After retrograde intramedullary nailing over the nitinol wire, stability was checked by manual examination and radiography. The surgery was terminated by suturing the layers and applying an antiseptic barrier spray. Sacrification with cervical dislocation was performed in the 4th and 6th weeks after fracture. Accordingly, a number representing the number of weeks was added to the end of the group name. For example, the carbamazepine-treated rats that were sacrificed in the fourth week were in a group designated CAR4.

Regarding animal experiments, it has been stated that the closed-fracture model may be more reliable than the open-fracture model in demonstrating the effectiveness of the agent or method to be examined due to the late union and nonunion seen in open-fracture models. The selection of the closed-fracture model aims to homogenize environmental factors.[12,20,21] The disadvantage of the closed-fracture model is the inability to obtain uniform fractures and the possibility of skin and soft tissue damage. Although locking was not possible in the current study, fixation with an intravenous cannula needle was preferred due to its use with a guide, ease of application, low cost, easy accessibility, and less invasive nature. The retrograde femoral nailing technique was applied because of its easy application and lower risk of hip dislocation.[12] In the current study, a closed fracture was created after advancing a flexible intramedullary guidewire.[22] Thus, the reduction process was achieved quickly and safely with the guidewire after the closed fracture was formed (Figure 1 A,B,C,D).

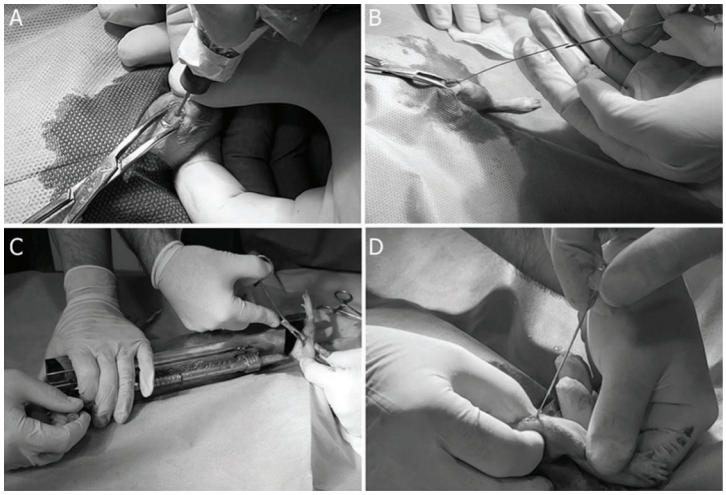

Figure 1. A,B,C,D. A longitudinal skin incision made from the anterior of the knee (A), The medulla of the femur was entered through the intercondylar notch with a drill bit. (B), A closed fracture was created under sterile conditions (C), implantation of retrograd femoral nail (D).

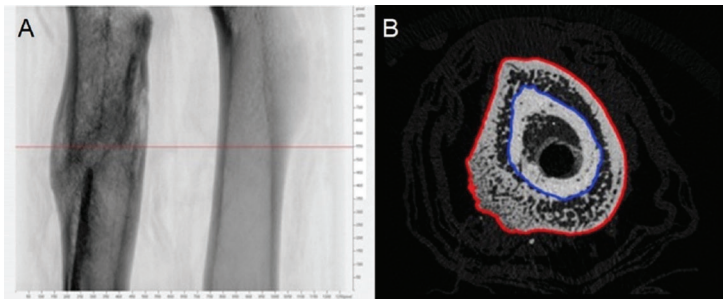

Figure 2. A,B. Microtomographic sections. Region of interest (ROI) (A), The area between the blue and red lines represents the callus area (B).

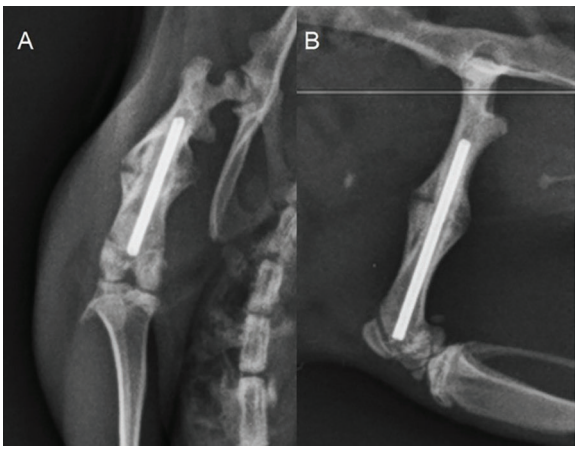

Figure 3. A,B. Anteroposterior and lateral X-ray examination for fracture lines of rat femurs for the evaluation of the RUST score.

Radiological Analysis

Anteroposterior and lateral femoral radiographs of all rats were taken immediately after surgery and at 4 and 6 weeks after fracture creation. Two different orthopedists evaluated the radiographs obtained according to the Radiographic Union Score for Tibial Fracture (RUST) and the Modified Lane-Sandhu Radiographic Score (Figure 2 A, B).[23] Microtomographic imaging of the samples was performed with X-ray microtomography (Skyscan 1272-Kontich, Belgium) within the Faculty of Dentistry of Kayseri University. While capturing the images, the source energy was selected as 80 Kv, the current was 125 µA, and a 1 mm aluminum filter was used during scanning. The pixel size was 16.1 µm. Scanning was applied in 0.4° steps through 180°. Three-dimensional models were obtained by reconstruction with the software provided by the manufacturer (Nrecon v. 1.7.1.6). The nomenclature suggested by the American Society for Bone and Mineral Research (ASBMR) was used. ImageJ software (Wayne Rasband; Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA) was used to evaluate the images.

Two hundred sections before and after the fracture line were examined for each sample using the orthogonal view (Volume of interest; VOI). First, the tissue areas to be examined were marked in each section (region of interest; ROI). Then, tissue volume (TV) and bone volume (BV) were measured as voxels. All gray values in the marked area represent TV, while BV is the total of the gray values of the bone tissue in the same area. BV measurements were made based on the gray values of the intact bone of each rat. BV/TV is the ratio of the amount of bone tissue to the entire selected volume. Callus volume (TV callus) and the amount of bone in this volume (BV callus) were measured to evaluate the mineralization of the callus tissue. These measurements were made not only for the callus but also for the whole bone tissue. BV total, TV total, BV callus, and TV callus were measured for each section. Bone mineral density (BMD) was measured with the CT-Analyzer program provided by the manufacturer using 2 phantoms at 0.25 g/cm3 and 0.75 g/cm3 (Figure 3 A, B).

Histologic Analysis

Longitudinal sections 4 µm in thickness were taken from the bone tissue samples and stained with hematoxylin & eosin (HE). With HE staining, the cell nucleus is stained purple, and the cytoplasm is colored pink. Bone tissue samples were examined at x20 magnification under a light microscope (BX43 Upright, Olympus, USA) with a digital camera (DP26 5-megapixel digital camera, Olympus, USA). Five sections from each sample were evaluated. The histological preparations were evaluated by a histologist with 20 years of histopathological experience who had researched bone healing in rats. In each section, at least three similar areas were examined and scored.[24]

Statistical Analysis

The sample size was calculated with "G Power 3" software. Accordingly, it was determined that the sample size should be at least 30 for an effect size of 0.8 (Cohen's D: 0.8), a significance level of 0.05, and a power of 0.95 with 3 study groups.[25] Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS) for Windows version 20.0. Descriptive statistics for numerical variables are presented as the mean ± standard deviation, minimum-maximum values. In the analysis of numerical data, conformity to normal distribution was examined with the Kolmogorov-Smirnov and Shapiro-Wilk tests. The mean difference between two independent groups showing normal distribution characteristics was examined with the independent-samples t-test, and the mean difference between more than two independent groups was examined with one-way ANOVA. The median difference between two independent groups not showing a normal distribution was analyzed using the Mann-Whitney U test, and the median difference between more than two independent groups was examined using the Kruskal-Wallis H-test. In the analyses of more than two groups, if the result was significant, the group from which the difference originated was determined using Bonferroni correction. The data were examined at a 95% confidence level, and a value of $p<0.05$ was considered statistically significant.

Ethical Approval

This study was carried out in the KOBAY Experimental Animals Laboratory with approval granted by the Local Ethics Committee of the KOBAY Experimental Animals Laboratory (protocol number 373).

RESULTS

One of the rats in the CONT group died on the first postoperative day, and one in the CAR group died on the third postoperative day. When examined, no abnormality was found in the fracture line except for a large hematoma. Therefore, none of the rats were rejected by the veterinary surgeon due to the exclusion criteria.

Radiological Findings

X-ray Analysis

Two independent orthopedists evaluated the images according to the RUST and Lane-Sandhu scoring criteria. According to both the RUST and Lane-Sandhu scores, in the fourth and sixth weeks of fracture healing, no significant difference was found between the radiological scores within the groups (CAR4, LEV4), (CAR6, LEV6, CONT 6). A statistically significant difference was determined between the weeks (CAR4, CAR6) (LEV4, LEV6) (CONT4, CONT6) by both observers. ($p<0.001$) (Figure 4)

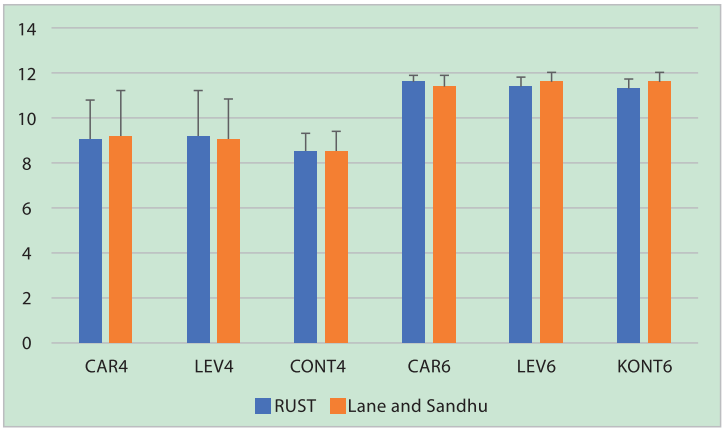

Figure 4. Statistical results of roentgenographic measurements.
CAR: Carbamazepine, **LEV:** levetiracetam

Microtomography Findings

Bone Mineral Density

When the average bone mineral density measurements were evaluated in the healthy femurs of the subjects in the 8th and 10th weeks of drug administration, a statistically significant difference was found between the groups in bone mineral density measurements, and the group with the lowest mineralization was the CAR group, followed by the LEV group ($p<0.001$).

Microtomography

In all samples, the BVcallus / TVcallus ratios in the sixth week of fracture healing were significantly higher than those in the fourth week. (CAR4, CAR6; p=0.024), (LEV4, LEV6; p=0.024), (CONT4, CONT6; $p=0.028$). BVcallus, BVcallus/TVcallus, and BV values were significantly different between the groups in the fourth week of fracture healing (CAR4, CONT4, BVcallus/TVcallus, $p=0.05$), (LEV4, CONT4,BVcallus/TVcallus, $p=0.017$). In the sixth week of fracture healing, there was no significant difference between the CAR6, LEV6, and CONT6 groups in the microtomography results ($p>0.05$) (Table).

Histological Findings

The samples were evaluated by an experienced histologist in accordance with Huo *et al*. Accordingly, in the 4th week of fracture healing in rats treated with carbamazepine, fibrous tissues were observed with

Table. Microtomography findings

	CAR4	KONT4	LEV4	CAR6	KONT6	LEV6
tv	55124483	72096961	60798174	51642547	45677528	51307713
tv callus	38844544	50015106	36065987	31532235	25315929	30046433
bv	13521427	21326132	11958387	14597204	15165392	13448879
bv callus	5832168	12004049	4794270	7862580	8182165	7372897
bv/tv	0.252143	0.299716	0.207917	0.284807	0.342484	0.265394
bvc/tvc	0.154279	0.259885	0.137697	0.253189	0.347156	0.252863
bv/tv	0.252143	0.299716	0.207917	0.284807	0.342484	0.265394
bvc/tvc	0.154279	0.259885	0.137697	0.253189	0.347156	0.252863
mean bmd	0.6837	0.79	0.732672	0.58578	0.794094	0.693358

tv: Tissue volume, **bv:** bone volume, **bmd:** bone mineral density

dense cartilage tissue, but immature bone tissue had not yet formed. In the sixth week of fracture healing, large immature bone areas and a small number of cartilage tissue areas were found in the samples treated with carbamazepine. In the evaluations of the 4th week of fracture healing in rats treated with levetiracetam, a large amount of cartilage tissue was observed, and immature bone areas were also detected. In the evaluation of the samples treated with levetiracetam, in the 6th week of fracture healing, fracture union with immature bone and patches of cartilage was observed in the two samples. In the control group's fourth week of fracture healing, cartilage tissue, and immature bone areas were equal, but the immature bone was denser in only one sample. In the evaluation of the control group in the sixth week of fracture healing, fracture union with immature bone was observed.

The CAR4 and LEV4, LEV4 and CONT4, and CAR4 and CONT4 groups were compared, and a statistically significant difference was found(p<0.001).

While there was a significant difference between CAR6 and LEV6 (p=0.47) and CAR6 and CONT6 (p=0.024), no significant difference was found between the LEV6 and CONT6 groups (p=1.0).

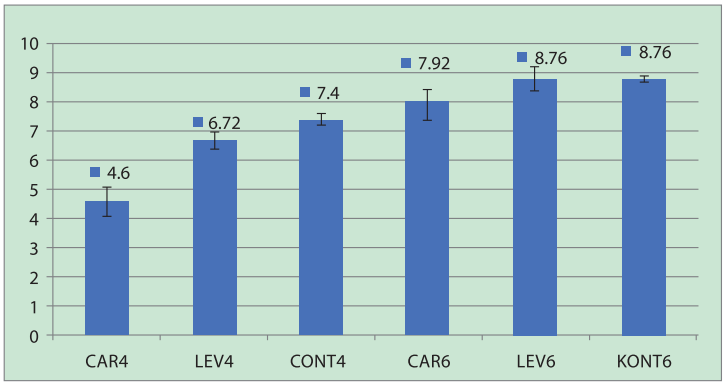

Figure 5. Statistical results of histologic scores.
CAR: Carbamazepine, **LEV:** levetiracetam

When the histological scores of all the groups in the 4th and 6th weeks of fracture healing were evaluated, a significant difference was determined: CAR4-6 (p<0.001), LEV4-6 (p<0.001), and CONT4-6 (p=0.012) (Figure 5; 6 A,B,C,D,E,F).

DISCUSSION

Upon comparing the histological evaluations of fracture healing in the fourth week, we observed that the groups receiving antiepileptic drugs exhibited significantly inferior scores compared to the control group. To clarify, our histology observations indicate that antiepileptic medicines have a detrimental impact on the initial stage of fracture repair. Furthermore, in the microtomography analysis, we determined that the BVcallus/TVcallus ratios, which indicate the level of mineralization in the callus tissue, were lower in the rats treated with carbamazepine and levetiracetam compared to the control group. This means that the mineralization of the callus was reduced in the treated rats. All results of this study indicated that both drugs have an adverse effect on fracture healing in both radiological and histological evaluations in the early period of healing.

In a study to determine the effect of phenytoin on bone metabolism in male rats, Onodera *et al*. measured bone mineral density using microtomography, trabecular bone thickness and volume in histomorphometry evaluation, serum calcium levels in the blood, pyridoline, and 25(OH)-vitamin D levels. In the same study, observable changes occurred in the bone tissue after five weeks of antiepileptic drug administration.[18] Consistent with these data, this study induced fractures in rats by administering drugs continuously for a duration of four weeks, mimicking the bone metabolism of individuals who use AEDs continuously, such as epileptic sufferers.

The fractures in this study were created with a blunt-ended guillotine consisting of two fixed supports and a spiral spring slide rod. This device has many advantages, including being small, portable, easy to use, reproducible, and easily sterilizable with glutaraldehyde.

The modified radiographic score of Lane and Sandhu and the histological evaluation score of Huo *et al*. were used to evaluate fracture healing. Both scores are the most used. In addition, radiographic evaluation was performed with the RUST scoring system, which is superior in evaluating the healing of diaphyseal fractures treated with intramedullary fixation.[23,24,26] However, microtomography is the most sensitive

radiological evaluation method that shows bone geometry, changes in bone tissue, bone loss, or gain without damaging the tissue. Many publications suggest that microtomography results give similar results to conventional mechanical tests when assessing fracture healing. Therefore, in orderto avoid doubling the number of subjects to sixty, mechanical testing was not performed in the current study.[21,27]

According to the results of microtomography, the BV callus/TV callus ratio, which represents the mineralization of the callus tissue, was significantly lower in the antiepileptic drug group in the 4th week of fracture healing than in the control group. Since callus mineralization is directly proportional to the strength of the callus, we found that AEDs negatively affected fracture healing at the end of this experiment. In the 6th week results, there was no significant difference between the groups (CAR, LEV, CONT) in the BV callus/TV callus ratios. However, when the 4th and 6th week results were compared within the groups, the BV callus/TV callus ratio was significantly different in each group. Based on all these results, it can be considered that sampling earlier and at shorter intervals will yield more detailed results since maximum bone mineralization was seen to have occurred in 6 weeks in all the groups.

Contrary to the microtomography results, a result against CAR6 was obtained among the histological scores at the 6th week. Histologically, the difference between the CAR and CONT groups at 4 and 6 weeks indicated that carbamazepine had a negative effect on fracture healing and delayed healing. At 4 weeks, the drug-administered groups (LEV, CAR) had significantly different scores than the control group, but at 6 weeks, a significant difference was found only between the CAR and CONT groups. Based on this finding, it can be thought that levetiracetam, a new-generation antiepileptic drug, affects fracture healing less than carbamazepine. Except for the 6-week CAR results, all these histological results were supported by the microtomography results. From a review of the literature, it can be seen that microtomographicand histological evaluation have been widely used in studies of fracture healing. Studies have found that both provide valuable information.[12] In our opinion, the two evaluations did not reach the same result in our study because the microtomographic evaluation is based on more subjective criteria. The fractures of the CAR group were just as healed or mineralized as the unbroken side. Furthermore, we found that the CAR group had the lowest BMD values at the sixth week of fracture healing.

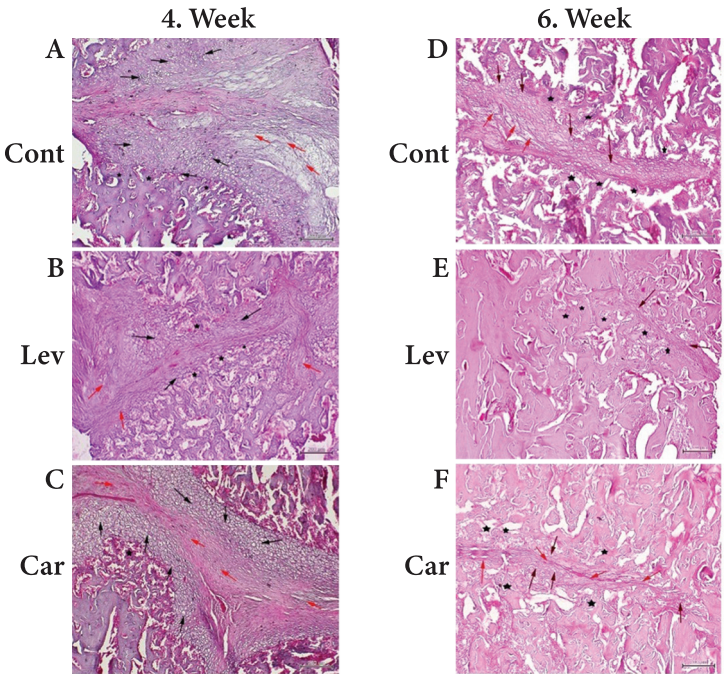

Figure 6. A,B,C,D,E,F. Red arrows indicate fibrous tissue, black arrows indicate cartilage tissue, asterisks indicate immature bone tissue. On the left, (A) in the control group, more cartilage tissue is observed compared to levetiracetam, but immature bone areas can be seen (B) In the levetiracetam group, fibrous and cartilage tissue and immature bone areas are observed together. (C) Cartilaginous areas with extensive fibrous tissues are observed in the fracture line in the carbamazepine group at 4 weeks. On the right, union in the fracture line with cartilage tissue and immature bone can be seen at 6 weeks in the control group (D). In the levetiracetam group, the fracture line healed with immature bone (E). Although fibrous tissue is seen in the fracture line of the carbamazepine group, cartilage tissue and immature bone areas can also be seen (F).

In previous animal experiments investigating the effect of AEDs on bone metabolism, it has been found that the old-generation antiepileptics phenytoin, carbamazepine, and phenobarbital decrease bone mineral density and bone strength and increase bone resorption.[6-8] Parveen *et al*. conducted a study where they administered clinically significant doses of carbamazepine and levetiracetam to female rats for a duration of 10 weeks. The results showed that this treatment had negative effects on the rats' bones, as evidenced by alterations in bone mineral density, bone microarchitecture, and biomarkers.[8] The data presented here, which are consistent with our own findings, provide more evidence that antiepileptic medications have a detrimental impact on bone structure. Thus, the fact that the bone mineral density of the carbamazepine group in the current study was significantly lower than that of the control group is consistent with the literature.

Kanda *et al*. found that phenytoin caused a significant decrease in density and strength in rat bone, while levetiracetam did not change bone strength and mass.[7] In contrast to the findings of Kanda *et al*., our study revealed a statistically significant reduction in bone mineral densitometry as a result of levetiracetam administration as compared to the control group.

Nissen-Meyer *et al*. reported that low-dose levetiracetam (50 mg/kg) does not cause any change in bone mineral density but weakens the femoral neck.[6] In the current study, a significant decrease was determined in the bone mineral density in the levetiracetam group compared to the control group ($p<0.05$). An inherent constraint of our investigation is the absence of bone strength testing. In addition, the fact that the histological scores of the levetiracetam group in the 4th week of fracture healing were found to be significantly different compared to the control group suggested that levetiracetam had adverse effects on bone metabolism.

Enzyme-inducing AEDs may cause a decrease in the active form of vitamin D in the blood and secondary osteomalacia due to suppression of the hydroxylation of vitamin D in the liver.[5,9] Therefore, to determine the effect of carbamazepine on vitamin D and calcium metabolism, the levels of vitamin D, calcium, and phosphorus should be measured. In this study, not checking the levels of vitamin D, calcium, and phosphorus in the blood was the most critical limitation; other limitations arose from not using minipump infusion to ensure optimal levels of drugs, not using locked nails for fixation, and not evaluating fracture healing in the second week.

Diemar and colleagues conducted a study to assess the impact of carbamazepine (CBZ), eslicarbazepine (ESL), valproic acid (VPA), and levetiracetam (LEV) medications on bone metabolism in rats using microtomography. According to their research, VPA has an impact on the microarchitectural characteristics of the bone. The antiepileptic drugs (AEDs) CBZ, ESL, and LEV seem to have a reduced impact on bone physiology. Our investigation revealed a detrimental impact of antiepileptic medications on bone mineral densitometry, with carbamazepine exhibiting a greater effect.[28]

Phabphal *et al*. showed a substantial increase in bone mineral density (BMD) among patients who switched from phenytoin to levetiracetam treatment. Hence, they suggested altering the prescription when the bone mineral density (BMD) levels of patients, who had been on medication for an extended duration, declined. Nevertheless, our study focused on examining fracture healing and revealed that levetiracetam had an adverse impact on the initial phase of fracture healing, as observed through histological investigations and radiographic evaluations. Modifying drugs may be considered for individuals with poor bone mineral densitometry, although we do not anticipate that such modifications will enhance the process of fracture healing.[29]

To optimize the study, infusion of drugs with a minipump can ensure that the drug remains at an optimal level in the blood, vitamin D, calcium, and phosphorus levels can be measured in the blood, and a locked nail may be used for fixation. The strengths of our study are the comprehensive analysis of fracture healing tissue using microtomography as well as the independent evaluation of radiological and histological evaluations by unbiased observers.

Further clinical studies are needed to better determine the clinical significance of this study. However, considering that fracture healing is a complex and multiparameter process, the effects of AEDs on fracture healing will be better understood with further epidemiological studies evaluating the fracture characteristics and the systemic disease metabolic in patients.

*The authors declare that there are no conflicts of interest.

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