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ORIGINAL ARTICLE

The effects of gabapentin and pregabalin on fracture healing: A histological, radiological, and biomechanical analysis

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Fracture healing has long been a focus in orthopedic medicine; however, despite significant advancements, challenges such as delayed union and nonunion continue to persist, with rates of bone union issues reported between 2% and 30%, leading to substantial socioeconomic impacts.[1] Factors such as aging and comorbidities such as diabetes further exacerbate these issues, increasing the burden of fracture healing complications in aging populations.[2,3]

The effects of medications on fracture healing have been a topic of growing interest.[4-7] Among these, gabapentinoids, including gabapentin and pregabalin, are commonly used as first-line

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ABSTRACT

Objectives: This study evaluated the impact of different doses of gabapentin and pregabalin on fracture healing in a rat femoral shaft model, with histological, radiological, and biomechanical assessments.

Materials and methods: Seventy male Wistar albino rats were divided into five groups: control, low-dose gabapentin (GBP-L, 300 mg/day), high-dose gabapentin (GBP-H, 3600 mg/day), low-dose pregabalin (PRG-L, 150 mg/day), and high-dose pregabalin (PRG-H, 600 mg/day), based on human equivalent doses. Bilateral femoral fractures were induced; the right femurs were prepared for radiological examination using microtomography, followed by histological analysis, whereas the left femurs were allocated for biomechanical testing. Drug administration began three weeks preoperatively and continued until sacrifice at either two or four weeks. Histological assessments included inflammation and transformation scoring and microtomography-measured callus volume. Biomechanical testing assessed maximum force and stiffness.

Results: At the fourth week, inflammation levels were significantly higher in the GBP-H, PRG-L, and PRG-H groups compared to control ($p<0.01$, $p<0.05$, and $p<0.01$), while transformation scores were significantly lower in these groups ($p<0.01$, $p<0.05$, and $p<0.001$). Low-dose pregabalin showed a borderline transformation difference ($p=0.051$). Microtomography analysis showed that the GBP-H group had significantly reduced callus volume versus control by the second week ($p<0.01$), persisting at a lower significance by week four ($p<0.05$). By the fourth week, PRG-H also had reduced callus volume ($p<0.05$). Maximum force values by the fourth week were significantly lower in the GBP-L, GBP-H, and PRG-H groups compared to control ($p<0.05$ for GBP-L; $p<0.01$ for GBP-H and PRG-H).

Conclusion: These findings suggest that these drugs, particularly with their high-dose applications, may lead to prolonged inflammation and hinder fracture healing by reducing callus volume and biomechanical integrity, potentially disrupting the transition from the inflammatory to reparative phases of healing.

Keywords: Bone, fracture, gabapentin, gabapentinoid, healing, pregabalin.

treatments for neuropathic pain, which affects 3 to 17% of the general population and is more prevalent among women aged 50 to 64 years (60.5% of patients), manual workers, and rural residents.[8,9] Gabapentinoids have also been used as adjunct therapies in perioperative pain management and low back pain, with an increasing prevalence in clinical practice.[10,11] On the overlooked side, the abuse and misuse of gabapentinoids have been increasing.[12,13] However, concerns have been raised regarding their potential negative effects on bone metabolism, such as reduced bone formation and increased bone resorption, leading to a higher risk of fractures, particularly in older adults.[14-16]

Our review of the English literature identified only two experimental rat studies that evaluated the effects of gabapentinoids on fracture healing. Sofu et al.[17] reported that gabapentin disrupted fracture healing both histologically and biomechanically on the 30th day, while Koçkara et al.[18] found that pregabalin had a negative effect on radiological healing on the 15th day, with no differences in histological or biomechanical outcomes observed between groups on the 30th day.

Due to the increasing use of gabapentinoids in fracture patients, both in emergency settings and outpatient follow-ups, the effects of these drugs on fracture healing remain unclear.[13] Therefore, this study aimed to evaluate the effects of maximum and minimum daily doses of pregabalin and gabapentin on fracture healing in an experimental rat femoral shaft model, from histological, radiological, and biomechanical perspectives, and to compare their effects.

MATERIALS AND METHODS

This experimental study was carried out at the animal research center of Balıkesir University using 70 male Wistar albino rats (14 to 16 weeks old). The rats were randomly divided into five groups, each containing the same number of animals, and each group was further subdivided into two based on the time of sacrifice: two weeks or four weeks. All of the rats underwent standard bilateral closed femoral shaft fractures. The first group served as the control group, while the others were low-dose gabapentin (GBP-L), high-dose gabapentin (GBP-H), low-dose pregabalin (PRG-L), and high-dose pregabalin (PRG-H) groups. This study was conducted with the approval of the Balıkesir University Animal Experiments Local Ethics Committee (date: 28.03.2024; no: 2024/3-6). All procedures involving animals were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and adhered to international guidelines on animal research ethics. Every effort was made to minimize animal suffering and to provide humane care throughout the study.

Surgical procedure and experimental designSince the long-term use of the drugs was simulated, the administration of these medications to the rats was initiated via oral gavage starting three weeks prior to surgery and continued until sacrifice.[19] During the first week of this three-week period, the GBP-H and PRG-H groups, similar to the GBP-L and PRG-L groups, were started on low doses as recommended for the clinical treatment of neuropathic pain.[20] The daily doses of gabapentin and pregabalin were respectively 300 or 3600 mg/day and 150 or 600 mg/day, as reported in the literature, calculated according to the body surface area conversion to the human equivalent dosing regimen.[20,21] These daily doses were administered to the rats via oral gavage, prepared by the same person and given as 1 mL each in the morning and evening to all groups. To standardize the stress factor, 1 mL of 1% methylcellulose was given to the control group via oral gavage twice daily.[17,18]

Under anesthesia, which involved the intraperitoneal administration of 80 mg/kg ketamine hydrochloride and 8 mg/kg xylazine, the right knee joint of the rats was initially shaved and subsequently covered with a sterile drape. An arthrotomy was then performed using a medial parapatellar approach to the right knee. Utilizing a drill motor, a 1.0-mm Kirschner wire was retrogradely advanced from the intercondylar area of the femur to the trochanteric region. The wire was retracted, and a closed fracture was established at the midshaft of the femur using the three-point bending technique as described by Bonnarens and Einhorn.[22] Afterward, the retracted Kirschner wire was reinserted retrogradely and secured at the greater trochanter (Figure 1). The wire was trimmed at the condyle in the knee region to prevent restriction of joint movement. Finally, the patella was reduced, and the medial parapatellar approach was closed.

After the surgery, the rats were allowed to move freely, and their lower limbs were not immobilized. They were maintained under laboratory conditions with free access to standard pellet feed and water. The rats were housed in an environment with a natural light/dark cycle, and room temperature and humidity were closely monitored until sacrifice, which was performed through cervical dislocation

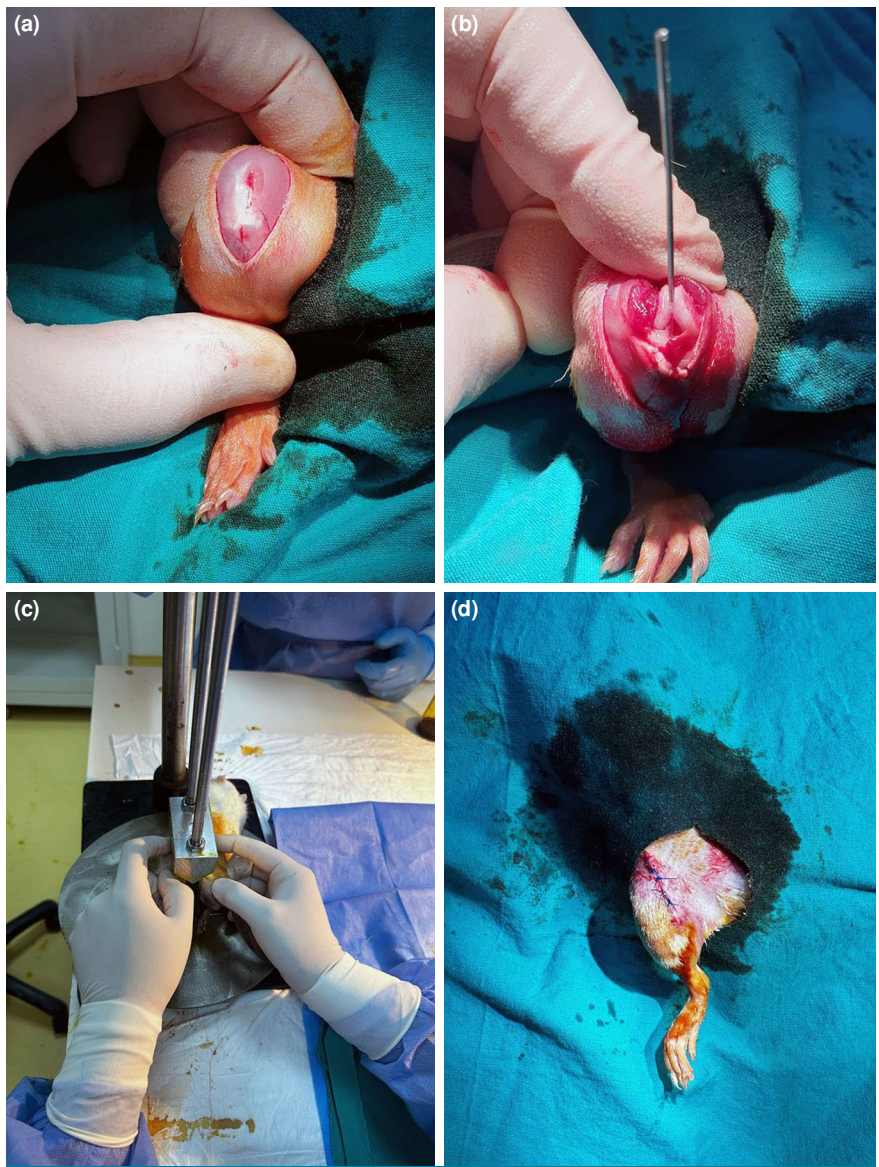


FIGURE 1. (a) Arthrotomy performed using a medial parapatellar approach. **(b)** A Kirschner wire 1.0 mm in diameter was advanced retrogradely from the intercondylar area of the femur to the trochanteric region. **(c)** The wire was then withdrawn, and a closed fracture was created at the midshaft of the femur using the three-point bending technique described by Bonnarens and Einhorn (29). **(d)** The withdrawn Kirschner wire was reinserted retrogradely and trimmed at the condyle. Finally, the patella was reduced, and the layers were sutured.

under high-dose anesthesia. For each group, the first subgroup was sacrificed at two weeks, while the second subgroup was sacrificed at four weeks. The entire femur, along with the fracture site and callus tissue, was carefully extracted from the hip and knee joints. The Kirschner wire was then removed from the trochlear entry point. The right femurs were prepared for radiological examination using microtomography (micro-CT), followed by histological analysis, while the left femurs were used for biomechanical testing.

Radiological imaging

The distal femurs of the rats were preserved in a 10% formaldehyde solution until they were transported for micro-CT analysis. The micro-CT scans were conducted by positioning the femurs in a plastic holder and scanning them at a dose

of 50 kVp and 45 mA, with a step angle of 0.75° and a length of 40 msec for each projection using the U-CT system (MILabs MicroCT-OI; U-CT OI, MILabs B.V., Utrecht, the Netherlands). The scanned sections were reconstructed with voxel sizes of 60 µm. Following reconstruction in the coronal, sagittal, and three-dimensional (3D) formats, the callus volume, bone volume, and the callus volume/bone volume, were measured using 3D Slicer version 5.7.0 (https://www.slicer.org/), an open-source software (Figure 2).

Biomechanical testing

Rat femur bones were thawed overnight at 4°C and kept at room temperature, wrapped in saline, prior to testing. All tests were conducted under bending load using an electromechanical actuator (5 kN AG-X; Shimadzu, Kyoto, Japan). The load (N) and displacement (mm) values recorded during the tests were captured simultaneously by the software associated with the tester. A three-point bending model was utilized to evaluate the load distribution experienced by the rat femur in the sagittal plane and to assess extensional stability. The upper loading device was positioned at the center of the femoral shaft, while the two sides of the lower device rested on the support fixture, with a span distance of 20 mm between the supports. Each test was conducted at a speed of 5 mm/min until failure, with displacement and load values being logged.

Histological evaluation

Following micro-CT scanning and a two-day fixation in 10% formalin, each sample was immersed in a 10% EDTA solution, which was replaced every two days throughout the four-week decalcification process. The decalcified samples were then embedded in paraffin and cut into 5-µm-thick sections. After deparaffinization, the tissue sections were stained using hematoxylin and eosin (Figure 3). A blinded pathologist subsequently evaluated the histological healing at the fracture site, using the healing scale developed by Huo et al.,[23] which ranges from 0 (indicating poor healing) to 10 (indicating optimal healing; Table I).

Statistical analyses

The minimum number of rats required for the study was calculated using G*Power version 3.1.2 software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany), with an effect size of 0.40, a confidence level of 0.90, and a power of 0.80, resulting in a total of 65 rats. To ensure equal group distribution and account for potential dropouts, the study was conducted with 70 rats.

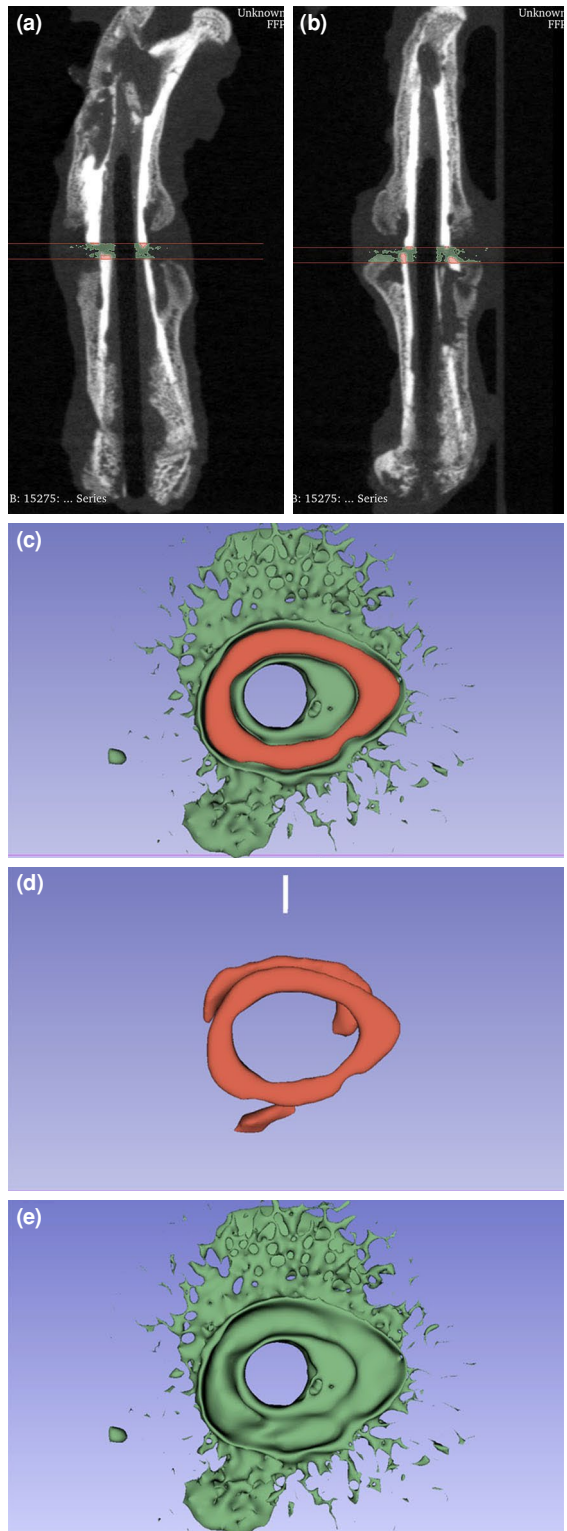


FIGURE 2. (a) Coronal views of the micro-CT images of the rat femur. **(b)** Sagittal views of the micro-CT images of the rat femur. **(c)** 3D reconstruction of the fracture site by using 3D Slicer illustrating bone (orange) and callus (green) tissue. **(d)** Illustrates only bone (orange). **(e)** Illustrates only callus (green)
CT: Computed tomography; 3D: Three-dimensional.

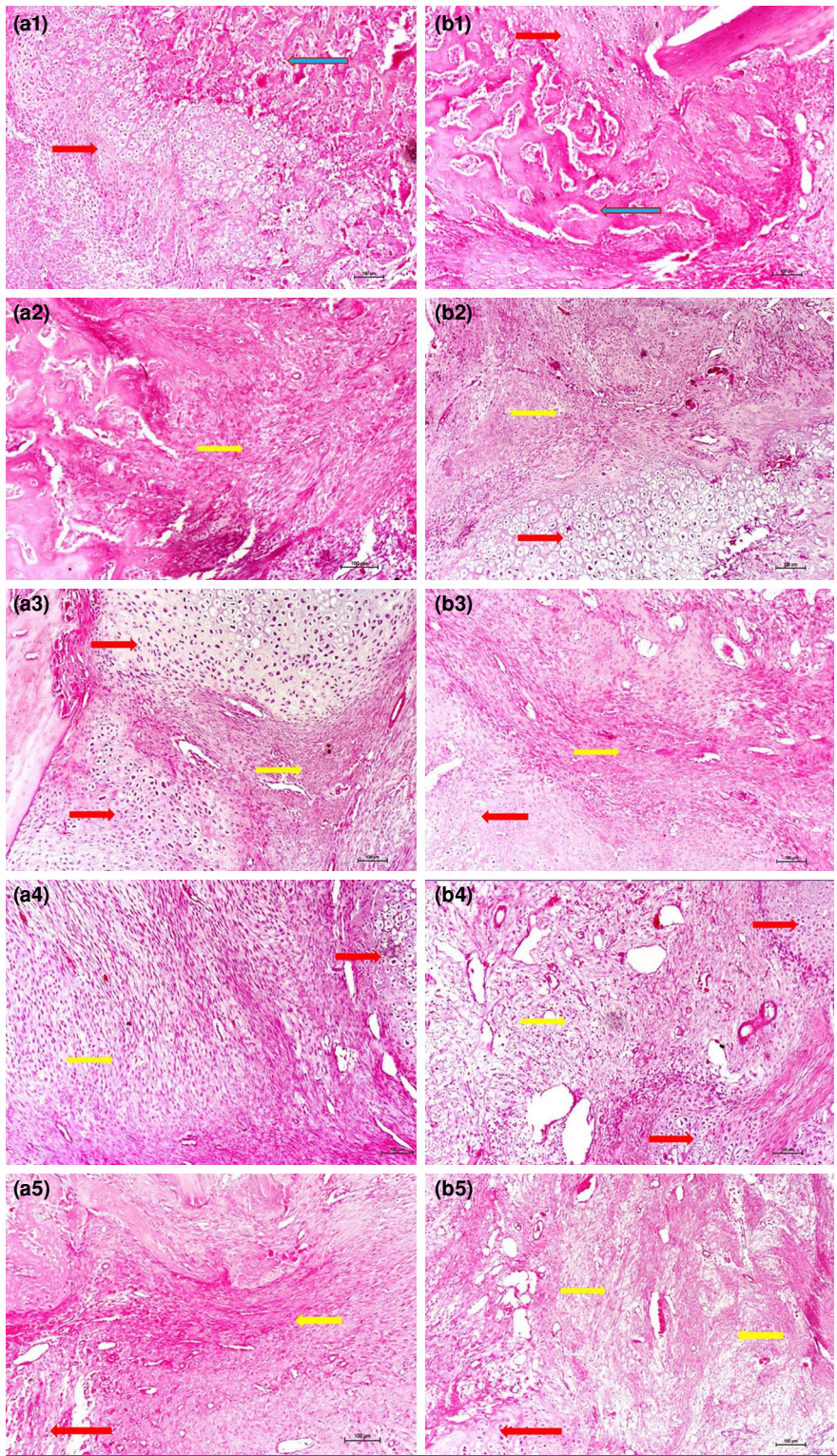


FIGURE 3. (a) (1-5): The histologic images (H-E, ×200) correspond, from left to right, to the control, GBP-L, GBP-H, PRG-L and PRG-H groups at two weeks, respectively. In the figure, the red, blue, and yellow arrows indicate callus tissue, bone tissue, and fibrosis, respectively. **(b)** (1-5): The histologic images (H-E, ×200) correspond, from left to right, to the control, GBP-L, GBP-H, PRG-L and PRG-H groups at two weeks, respectively. In the figure, the red, blue, and yellow arrows indicate callus tissue, bone tissue, and fibrosis, respectively.

TABLE I
The scoring table for the histologic evaluation of fracture healing

Score	Associated finding at fracture site
1	Fibrous tissue
2	Predominantly fibrous tissue with small amount of cartilage
3	Equal mixture of fibrous and cartilaginous tissue
4	Predominantly cartilage with small amount of fibrous tissue
5	Cartilage
6	Predominantly cartilage with small amount of immature bone
7	Equal mixture of cartilage and immature bone
8	Predominantly immature bone with small amount of cartilage
9	Union of fracture by immature bone
10	Union of fracture fragments by mature bone

Data were analyzed using IBM SPSS version 25.0 software (IBM Corp., Armonk, NY, USA). The mean values were compared using Kruskal-Wallis and pairvise comparison tests. As the descriptive statistics, mean ± standard deviation (SD) of each group were presented. A p-value <0.05 was considered statistically significant.

RESULTS

On the day the experimental fractures were induced, three rats (two from the GBP-H subgroup scheduled for the fourth week, and one from the control subgroup scheduled for the second week) were excluded due to comminution. Additionally, two rats (one from the control subgroup scheduled for the fourth week, and another from the GBP-H subgroup scheduled for the fourth week) died the day after surgery, resulting in a total of five exclusions. To maintain group sizes as determined by power analysis, the surgical procedure was repeated for the control group the following day with new rats, while a new three-week drug treatment regimen was initiated for the three replacement rats in the GBP-H group.

In the second week, the maximum force measurement means were 20.25±7.9 N in the control group, 16.74±4.5 N in the GBP-L group, 10.63±1.2 N in the GBP-H group, 17.99±5.5 N in the PRG-L group, and 13.42±4.1 N in the PRG-H group. By the fourth week, these values increased to 52.05±8.9 N in the control group, 27.52±7.4 N in the GBP-L group, 24.24±5.1 N in the GBP-H group, 29.06±9.0 N in the PRG-L group, and 22.99±5.6 N in the PRG-H group. In the second week, the mean maximum force in the GBP-H group was significantly lower compared to the control and PRG-L groups ($p<0.01$ and $p<0.05$, respectively). By the fourth week, the mean maximum force levels in the GBP-L, GBP-H, and PRG-H groups were significantly lower than those in the control group ($p<0.05$, $p<0.01$, and $p<0.01$, respectively; Figure 4).

In the second week, stiffness measurement means were 8.62±3 N/mm in the control group, 6.64±2.5 N/mm in the GBP-L group, 6.64±5.2 N/mm in the GBP-H group, 11.75±6.6 N/mm in the PRG-L group, and 9.47±5.8 N/mm in the PRG-H group. By the fourth week, these values increased to 62.33±50.8 N/mm in the control group, 19.95±7.2 N/mm in the GBP-L group, 24.6±17 N/mm in the GBP-H group, 25.04±19.9 N/mm in the PRG-L

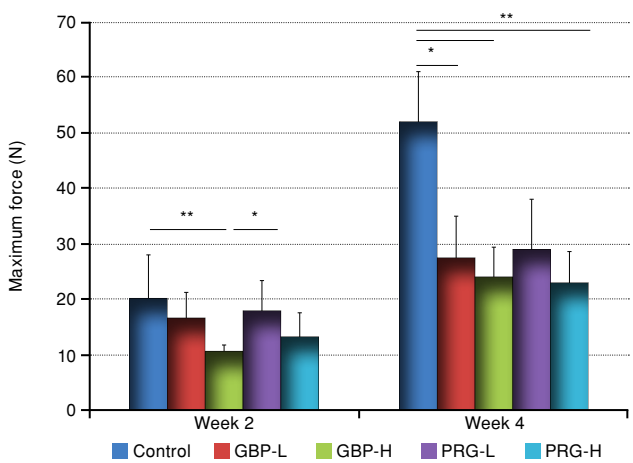


FIGURE 4. The graph illustrates the mean maximum force measurements in newtons at two and four weeks.
GBP-L: Gabapentin low-dose; GBP-H: Gabapentin high-dose; PRG-L: Pregabalin low-dose; PRG-H: Pregabalin high-dose; * p<0.05; ** p<0.01.

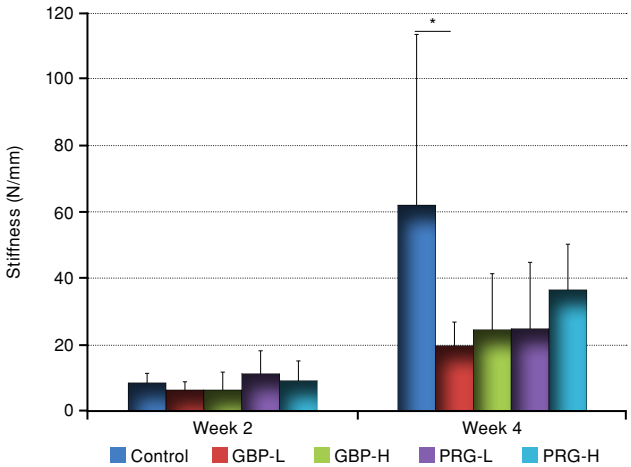


FIGURE 5. The graph illustrates the mean stiffness measurements at two and four weeks.
GBP-L: Gabapentin low-dose; GBP-H: Gabapentin high-dose; PRG-L: Pregabalin low-dose; PRG-H: Pregabalin high-dose; * p<0.05.

group, and 36.7±13.8 N/mm in the PRG-H group. In the second week, there were no statistically significant differences in stiffness among the groups. However, by the fourth week, the GBP-L group had significantly lower stiffness compared to the control group (p<0.05; Figure 5).

At the second week, there were no statistically significant differences in inflammation levels between the groups. However, by the fourth week, the inflammation levels in the GBP-H, PRG-L, and PRG-H groups were significantly higher than those of the control group (p<0.01, p<0.05, and p<0.01, respectively; Table II).

There were no significant differences in transformation levels between the groups at the second week (p>0.05). However, by the fourth week, the GBP-H, PRG-L, and PRG-H groups had significantly lower transformation levels compared to the control group (p<0.01, p<0.05, and p<0.001, respectively; Table II). The statistical difference between the control and GBP-L groups was on the borderline of significance (p=0.051).

In the second week, the mean callus volumes were 35.38±5.3 mm^3 in the control group, 26.16±5.1 mm^3 in the GBP-L group, 24.55±4.2 mm^3 in the GBP-H group, 33.22±6.4 mm^3 in the PRG-L group, and 30.86±4.2 mm^3 in the PRG-H group. By the fourth week, these values change to 37.57±5.1 mm^3 in the control group, 32.6±4.6 mm^3 in the GBP-L group, 28.86±2.6 mm^3 in the GBP-H group, 33.27±4.4 mm^3 in the PRG-L group, and 29.25±2.6 mm^3 in the PRG-H group. In the second week, the GBP-H group had a significantly lower callus volume than the control group (p<0.01). Although the callus volume in the GBP-H group remained lower than that of the control group by the fourth week, the significance level decreased to p<0.05. Additionally, the PRG-H group also showed a significantly lower callus volume compared to the control group at the fourth week (p<0.05; Figure 6).

In the second week, the mean bone volume values were 10.48±0.9 mm in the control group, 9.7±1.7 mm in the GBP-L group, 8.92±1.1 mm in the GBP-H group, 10.36±1.2 mm in the PRG-L group, and 8.79±0.8 mm in the PRG-H group. None of the comparisons showed statistically significant differences between groups. By the fourth week, the mean bone volumes were 11.59±1.3 mm in the control group, 9.84±0.5 mm in the GBP-L group, 8.93±0.7 mm in the GBP-H group, 10.05±1.1 mm in the PRG-L group, and 9.09±0.9 mm in the PRG-H group. The mean bone volumes in the GBP-H and PRG-H groups were significantly lower

TABLE II
Comparison of histological scores

	Inflammation		Transformation	
	2nd week	4th week	2nd week	4th week
Groups	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Control	1.29±0.5	0.71±0.8	3.43±0.5	7.14±0.7
GBP-L	1.86±0.7	2.29±0.5	2.43±0.5	2.29±0.5
GBP-H	2.29±0.5	2.57±0.5**	2.43±0.5	1.86±0.7**
PRG-L	2.14±0.7	2.43±0.5*	2.43±0.5	2.14±0.4*
PRG-H	2.14±0.7	2.71±0.5**	2.43±0.5	1.71±0.8#

SD: Standard deviation; GBP-L: Gabapentin low-dose; GBP-H: Gabapentin high-dose; PRG-L: Pregabalin low-dose; PRG-H: Pregabalin high-dose; * p<0.05; ** p<0.01; # p<0.001 (compared to control).

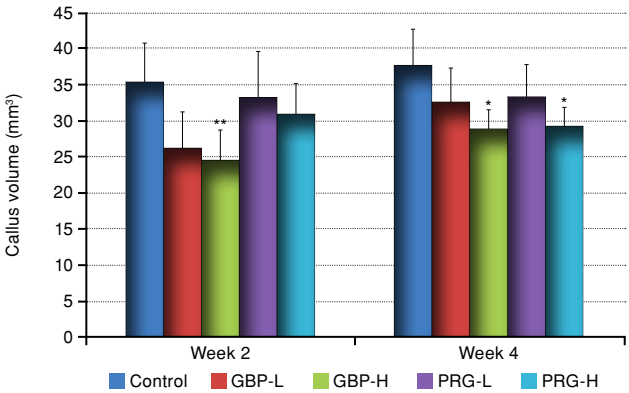


FIGURE 6. The graph illustrates the mean callus volumes at two and four weeks.

GBP-L: Gabapentin low-dose; GBP-H: Gabapentin high-dose; PRG-L: Pregabalin low-dose; PRG-H: Pregabalin high-dose; * p<0.05; ** p<0.01 (compared to control).

than those in the control group in the fourth week (p<0.01 for both).

For the mean callus/bone volume ratios, the second week values were 3.37±0.3 in the control group, 2.76±0.7 in the GBP-L group, 2.81±0.7 in the GBP-H group, 3.25±0.7 in the PRG-L group, and 3.51±0.3 in the PRG-H group. By the fourth week, these values were 3.25±0.3 in the control group, 3.31±0.4 in the GBP-L group, 3.23±0.2 in the GBP-H group, 3.31±0.2 in the PRG-L group, and 3.23±0.2 in the PRG-H group. There were no statistically significant differences between groups at either the second or fourth week.

A statistically significant difference was identified between the GBP-H and PRG-L groups in maximum force values at the second week. However, no other statistically significant differences were observed among the GBP-L, GBP-H, PRG-L, and PRG-H groups with respect to maximum force, rigidity, inflammation, transformation, callus volume, bone volume, or callus-to-bone volume ratio.

DISCUSSION

This study is the first to evaluate the effects of long-term pregabalin and gabapentin use on fracture healing by assessing histological, radiological, and biomechanical outcomes, comparing these agents both with each other and a control group. Previous studies by Sofu et al.[17] and Koçkara et al.[18] have explored the acute effects of one of the gabapentinoids on bone metabolism; however, none have investigated their long-term use in the context of fracture healing. In those studies, drug administration began via oral gavage starting 4 h postoperatively.[17,18] In contrast, in our study, the medications were initiated three weeks before surgery, although the literature generally suggests a two-week duration for long-term use.[19] With regular dosing, pregabalin reaches a stable plasma concentration within 24 to 48 h, whereas for gabapentin, this process takes one to two weeks.[24] This may be an important difference between acute and long-term use in terms of fracture healing, particularly affecting the inflammatory phase, which is reported to occur within the first one to three days in rats.[25] Our findings provide important insights into the potential impact of long-term gabapentinoid use on fracture healing, particularly in clinical practice, where their use is becoming increasingly common.

The most interesting finding of this study was the persistence of inflammation and regression in histological transformation at the fourth week in all medication groups, suggesting that ongoing inflammation may have a detrimental effect on callus transformation (Table II). This is supported by existing literature, where prolonged inflammation has been associated with delayed bone healing and impaired callus formation.[26] Chronic inflammation may interfere with the transition from the inflammatory phase to the reparative phase, hindering the proper development and transformation of the callus, as evidenced by our histological evaluations. Sofu et al.[17] reported significant histological differences at 30 days between the gabapentin and control groups, wheras Koçkara et al.[18] reported no difference between the pregabalin and control groups. However, both of these studies reported histological progression from the second to the fourth week in the treatment group. Regression of the histological transformation has not been reported in the literature. The stable plasma concentration levels reached during the inflammatory phase in our study may be the primary reason for these results.[26]

Furthermore, the reduction in callus volume observed in the GBP-H group at the second week, as well as both the GBP-H and PRG-H groups by the fourth week, indicates that gabapentinoids may not only disrupt callus transformation but also reduce the overall volume of callus formation. As callus volume is a critical marker of early fracture healing, reductions in this parameter suggest that gabapentinoids may interfere with the bone's ability to initiate proper reparative processes.

Sofu et al.[17] used a human equivalent dose of 1200 mg/day of gabapentin, which does

not correspond to the doses used in our study (600 mg/day in the GBP-L group and 3600 mg/day in the GBP-H group). In their study, they reported no statistically significant radiological differences between the control and gabapentin-treated groups at 15 and 30 days, while significant histological difference was observed at 30 days.[17] Although we cannot directly compare the dose used in their study with ours, it can be inferred that, similar to the 600 mg/day dose in the GBP-L group in our study, increasing the dose to 1200 mg/day may not significantly affect radiologic score at either the second or fourth week. However, the GBP-H group in our study demonstrated that increasing the dose to the maximal level caused a reduction in callus volume at both two and four weeks, suggesting a dose-dependent effect of gabapentin on callus volume. In addition, the presence of a statistical difference in histological transformation between the control and GBP-H groups but not between the control and GBP-L groups at the fourth week also suggests a dose-dependent effect. However, our findings on pregabalin do not align with those of Koçkara et al.,[18] who used a human equivalent dose of 150 mg/day, similar to our PRG-L group. They reported that pregabalin negatively impacted radiological healing at the second week but not significantly at the fourth week. In contrast, we found no statistical difference in callus volume at the second or fourth week. Additionally, they observed no significant effect on healing histologically. However, we observed significantly lower transformation level in the PRG-L group compared to the control group at the fourth week ($p<0.05$; Table II). Additionally, the more significant histological difference between the PRG-H and control groups compared to the PRG-L and control groups ($p<0.001$ and $p<0.05$, respectively) likely suggests the presence of a dose-dependent effect.

Moreover, the biomechanical data from this study show that at the second week, only the GBP-H group exhibited a reduction in maximum force compared to the control group, while by the fourth week, a statistically significant reduction was observed not only in the GBP-H group but also in the GBP-L and PRG-H groups. These findings are consistent with the study by Sofu et al.[17] using a human equivalent dose of 1200 mg/day gabapentin but not with the study by Koçkara et al.[18] using a human equivalent dose of 150 mg/day.

The major limitation of this study was the limited number of rats included in each group. Additionally, an intermediate dose group could have been included alongside the maximum and minimum doses. Moreover, if blood samples to evaluate bone markers and cytokines had been collected at the time of sacrification and pathological examinations had included immunohistochemical analysis, the pathophysiological mechanisms could have been investigated more thoroughly.

In conclusion, this study's findings reveal that the long-term use of these drugs, particularly at high doses, may lead to prolonged inflammation, regression in histological transformation, and a reduction in callus volume. These results highlight the need for further research into the long-term implications of gabapentinoid use on fracture healing, given their expanding role in managing neuropathic pain and perioperative care. Future studies incorporating intermediate dosing and biomarker analyses will be essential to deepen our understanding of these pathophysiological mechanisms, ultimately contributing to a clearer perspective on the role of gabapentinoids in fracture healing.

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