RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

**DOI:** 10.4274/cjms.2026.2026-24
Cyprus J Med Sci 2026;11(3):202-209

# Effects of Prenatal Gabapentin Exposure on Fetal Skeletal Development in Rats: Assessment of Bone Development and Ossification

**Muhammet Değermenci[1], İlyas Uçar[2], Seher Yılmaz[3], Esra Balcıoğlu[4], Gözde Özge Önder[4], Erdoğan Unur[2]**

[1]Department of Anatomy, Ordu University Faculty of Medicine, Ordu, Türkiye
[2]Department of Anatomy, Erciyes University Faculty of Medicine, Kayseri, Türkiye
[3]Department of Anatomy, Yozgat Bozok University Faculty of Medicine, Yozgat, Türkiye
[4]Department of Histology, Erciyes University Faculty of Medicine, Kayseri, Türkiye

## Abstract

**BACKGROUND/AIMS:** Gabapentin (GBP) is often prescribed to pregnant women to manage neuropathic pain and epilepsy. However, the impact of antiepileptic drugs on prenatal skeletal development remains controversial. The aim of this study was to investigate, using a rat model, the possible toxic effects of GBP exposure during pregnancy on fetal bone development.

**MATERIALS AND METHODS:** Pregnant Wistar albino rats were randomly assigned to five groups (n=4 each): a control group and four GBP-treated groups receiving 10, 30, 60, or 120 mg/kg/day throughout gestation. Fetuses were collected at term and evaluated using double staining. Ossification lengths and areas of forelimb and hindlimb long bones were quantitatively measured. Immunohistochemical (IHC) analyses were performed on femoral sections to assess the distribution and staining intensity of alkaline phosphatase (AP) and tartrate-resistant acid phosphatase (TRAP), key markers of osteoblastic and osteoclastic activity, respectively.

**RESULTS:** Fetuses exposed to GBP demonstrated reduced body weight and smaller morphometric measurements compared with controls. Quantitative analysis revealed a significant decrease in ossification of both forelimb and hindlimb bones across all treatment groups, with a clear dose-dependent pattern. IHC findings showed diminished AP and TRAP immunoreactivity in GBP-exposed pups, indicating impaired bone formation and resorption .

**CONCLUSION:** Continuous GBP exposure during pregnancy adversely affects fetal skeletal development in rats. Prenatal GBP administration led to lower birth weight, delayed and reduced ossification, and suppressed bone metabolic activity, particularly at higher doses. We believe these findings highlight the potential developmental risks associated with GBP use during pregnancy and underscore the need for caution.

**Keywords:** Toxicology, ossification, anatomy, immunochemistry

**To cite this article:** Değermenci M, Uçar İ, Yılmaz S, Balcıoğlu E, Önder GÖ, Unur E. Effects of prenatal gabapentin exposure on fetal skeletal development in rats: assessment of bone development and ossification. Cyprus J Med Sci. 2026;11(3):202-209

**ORCID IDs of the authors:** M.D. 0000-0002-4751-6202; İ.U. 0000-0003-3646-5320; S.Y. 0000-0003-4551-995X; E.B. 0000-0003-1474-0432; G.Ö.Ö. 0000-0002-0515-9286; E.U. 0000-0003-2033-4350.

**Corresponding author:** Muhammet Değermenci
**E-mail:** mdegermenci@yahoo.com.tr
**ORCID ID:** orcid.org/0000-0002-4751-6202

**Received:** 09.02.2026
**Accepted:** 14.05.2026
**Publication Date:** 25.06.2026

## INTRODUCTION

Epilepsy, a neurological disorder that is prevalent worldwide, affects millions of people and often requires continuous pharmacological treatment during pregnancy.[1] Gabapentin (GBP) is an anticonvulsant primarily indicated for partial-onset seizures and requently administered to pregnant women with epilepsy. In addition to its antiepileptic properties, GBP is used to treat chronic pain conditions (such as inflammatory pain) and psychiatric disorders.[2,3]

GBP is classified as a gamma-aminobutyric acid (GABA) analogue.[4] Due to its structural similarity to GABA, it readily crosses the placental barrier, thereby affecting the developing fetus. GBP is notable for its favourable safety profile, characterized by minimal drug-drug interactions and high tolerability.[5,6]

Antiepileptic drugs (AEDs) are commonly prescribed to women of reproductive age, but they are associated with a significant risk of teratogenicity.[7] Prenatal exposure to AEDs has been documented to cause both toxic and teratogenic effects, and the nature and severity of these outcomes depend on the stage of pregnancy during exposure.[8] In particular, AED use during pregnancy has been reported to adversely affect embryogenesis and osteogenesis.[9] Since the 1960s, research on skeletal toxicology has accelerated, and evidence has accumulated showing reductions in bone mineral density (BMD) and impairments in bone metabolism, particularly in paediatric and adolescent populations. Due to the increasing clinical use of GBP and the lack of comprehensive safety data during pregnancy, a significant gap exists in the literature regarding its effects on pregnancy-related outcomes.[10-12]

The exact effects of AEDs on skeletal integrity remain controversial, and the effects of GBP have been particularly understudied. Experimental studies have yielded inconsistent results regarding the developmental toxicity of GBP. However, evidence from rodent models suggests that prenatal GBP exposure may have toxic effects.[2,13] Additionally, Freyer[6] reported that oral GBP administration during organogenesis delayed ossification in many fetal bones. The relationship between GBP treatment and bone demineralisation has not been definitively established. However, other studies have highlighted negative correlations. These correlations exist among AED use, bone loss, and calcium metabolism disorders.[14,15] Recent findings indicate that in 50% of exposed individuals, AEDs contribute to reduced BMD, increased fracture risk, and skeletal malformations.[16]

Despite these findings, data specifically evaluating prenatal skeletal development following GBP exposure, particularly those obtained using double staining (DS) and immunohistochemical (IHC), remain limited. Therefore, this study evaluated the potential effects of prenatal GBP exposure on skeletal development in rats using DS and IHC.

## MATERIALS AND METHODS

This is an experimental animal study. This study has been approved by the Erciyes University Animal Experiments Local Ethics Committee (approval no: 16/141, date: 16.11.2016).

### Animals and Study Protocol

Twenty adult female Wistar albino rats (8 weeks old) were included. For mating purposes, female rats were housed overnight with male rats. The presence of spermatozoa in vaginal smears the following morning was considered gestational day (GD) 0. All animals were kept under standardized environmental conditions.

### Study Groups and Drug Administration

In anticipation of an average litter size of ten-twelve fetuses per dam and considering potential fetal loss due to treatment toxicity, the animals were randomly divided into five groups of four dams each. GBP was dissolved in saline solution and administered via oral gavage once daily from GD 1 to GD 20. The groups were as follows:

- Control: received saline vehicle only
- 10 mg/kg/day GBP (low dose)
- 30 mg/kg/day GBP
- 60 mg/kg/day GBP
- 120 mg/kg/day GBP (high dose)
- The administered doses were selected based on previous studies.[13,17]

### Experimental Procedure and Fetal Evaluation

On the twentieth GD, all pregnant rats were anesthetized intraperitoneally with xylazine and ketamine. After adequate anaesthesia had been achieved, a midline incision was performed, and the uterus was carefully excised with the fetuses *in situ*. Fetuses were collected and examined under a stereomicroscope for external malformations and gross skeletal deformities.

Each fetus and placenta was individually weighed. Cranio-caudal (CC) length, occipitofrontal diameter, and biparietal diameter were measured using a high-precision digital caliper. Following these assessments, fetuses were randomly allocated to either DS or IHC evaluation for further analysis of skeletal development and bone metabolism.

### Double Staining Evaluation

The fetus's skeleton was stained using the DS method (Supp1). High-resolution images of the skeletal bones of the fetal fore- and hindlimbs were captured using an Olympus SZX16 stereomicroscope (Olympus Corporation, Tokyo, Japan) equipped with a digital imaging system. Specimens were placed on millimeter graph paper for spatial calibration, and the images were transferred to a computer for quantitative analysis.

Morphometric measurements were performed using ImageJ software (version 1.51r, NIH, USA). For each specimen, the total bone length, ossified zone length, total bone surface area, and ossified zone surface area were measured. Ossification ratios were calculated using these values. This analysis enabled a precise evaluation of both longitudinal and surface-based ossification in the developing limb bones, allowing for comparative assessment across experimental groups.

### Immunohistochemical Evaluation

IHC evaluations were performed in a blinded manner. For IHC evaluation, fetuses from each experimental group were randomly selected and euthanized under appropriate anaesthesia. The femurs were carefully dissected and processed for histological and IHC analyses. Initially, a standard histological protocol was employed to assess the general tissue architecture of the bone. Subsequently, haematoxylin-eosin staining was performed to evaluate the histopathological features (Supp2).

In this study, the expression patterns of two key markers were investigated: alkaline phosphatase (AP), an established marker of osteoblastic activity and bone formation, and tartrate-resistant acid phosphatase (TRAP), a marker indicative of osteoclastic activity and bone resorption. IHC staining for AP and TRAP was conducted on femoral sections to assess the potential alterations in bone metabolism resulting from prenatal GBP exposure.

### Statistical Analysis

Statistical analyses were performed using SPSS software (version 28.0.1; IBM Corporation, Armonk, NY, USA). The normality of the distribution was assessed by the Shapiro-Wilk test. Intergroup comparisons were conducted using ANOVA, followed by Tukey's post-hoc test for multiple comparisons. A $p<0.05$ value was considered significant.

## RESULTS

No miscarriages or stillbirths were observed. Fetal and placental weights were significantly reduced in GBP-treated groups compared to the control, with a more pronounced effect at higher doses, indicating a dose-dependent pattern. Similarly, all fetal length parameters showed significant decreases across treatment groups, suggesting impaired overall fetal growth. Despite these changes, maternal body weight gain was relatively preserved, with only slight reductions observed at higher doses. These findings indicate that GBP induces fetal growth restriction independently of overt maternal toxicity.

### Morphometric Findings

The numbers, body weights, and heights of the fetuses were recorded prior to histological staining. All morphometric parameters were highest in the control group. In the GBP-administered groups, a statistically significant reduction in these parameters was observed, and this reduction correlated with dose escalation. Notably, the differences between the control group and the 120 mg/kg GBP group were statistically significant ($p<0.05$). These findings imply that GBP may adversely affect fetal growth and development in a dose-dependent manner (Figure 1).

### Double Staining Findings

In DS, ossified regions were stained red, while cartilaginous structures were stained blue. Morphometric evaluations included measurements of length and surface area of the clavicle, scapula, humerus, radius, and ulna in the forelimb and of the femur, tibia, and fibula in the hindlimb (Figure 2). Changes in overall bone dimensions among the GBP-treated groups are summarized in Table 1, whereas Figure 3 presents a comparison of ossified region sizes across groups. The control group demonstrated the highest values for both total bone length and ossification percentage, while the lowest values were recorded in the 120 mg/kg GBP group. An Analysis of ossification length percentages showed significant differences between the control group and all treatment groups for the scapula, humerus, and femur. Furthermore, significant differences for the remaining bones were detected between the control group and the 120 mg/kg GBP group ($p<0.05$).

Group comparisons of ossification area percentages indicated that proportions of both total bone area and ossified area were greater in the control group (Figure 3). Statistically significant differences in ossification area percentages were identified between the control group and the 30, 60, and 120 mg groups for the scapula and radius; between the control group and the 120 mg group for the humerus and ulna; between the control group and the 60 and 120 mg groups for the tibia; and between the control group and all treatment groups for the femur ($p<0.05$) (Table 1).

Based on the DS findings, GBP administration-particularly at higher doses (60 and 120 mg/kg/day)-was associated with a general tendency toward delayed ossification and reductions in total bone length and surface area. However, these changes did not follow a consistent dose-dependent pattern across all skeletal parameters.

### Immunohistochemical Findings

In the control group, IHC analysis revealed a well-preserved hyaline cartilage architecture within the quiescent zone. In the proliferative zone, chondrocytes exhibited mitotic activity, forming characteristic isogenic cell clusters. The hypertrophic zone was populated by enlarged, terminally differentiated chondrocytes. In the zone of calcification, degenerative changes in chondrocytes were evident, while in the zone of ossification, osteoprogenitor cells were observed differentiating into osteoblasts (Figure 4).

### Alkaline Phosphatase Density Findings

IHC samples from all groups were evaluated by light microscopy (Table 2). The highest level of AP expression ($89.94\pm3.50$) was detected in the ossified regions of the control group (Figure 4). Statistical analysis revealed significant differences between the control group and the GBP-treated groups (60 mg and 120 mg) ($p<0.05$).

### Tartrate-Resistant Acid Phosphatase Density Findings

IHC evaluation of tissue specimens from all groups demonstrated TRAP expression localized to the ossification zones. The highest intensity of TRAP expression was observed in the control group ($76.73\pm5.20$) (Table 2). Statistical analysis revealed a significant difference in TRAP expression between the control group and the high-dose GBP group ($p<0.05$). While the activities of osteoclasts and osteoblasts appeared to be in physiological balance under normal conditions, the findings suggest that increasing doses of GBP disrupt this homeostasis, thereby impairing the ossification process (Figure 4).

## DISCUSSION

Epilepsy is one of the most prevalent neurological disorders worldwide, and it particularly affects women of reproductive age. The management of epilepsy during pregnancy is critical because seizures, inherent to the chronic nature of the disease, may adversely affect maternal physiology and, via placental transfer, fetal development.[16,18]

Given that women with epilepsy often require continued AED therapy during gestation, it is essential to evaluate the potential developmental toxicity of these medications on both the mother and fetus. The increasing use of GBP underscores the need for robust evidence to inform women of childbearing potential about the risks and benefits of GBP treatment regarding pregnancy-related outcomes. While current data suggest that the risk of major congenital malformations associated with early gestational exposure to GBP is relatively low, its toxicological profile remains insufficiently elucidated.[11,12,19]

GBP is widely utilized in the treatment of neuropathic pain, spasticity, and inflammatory conditions, such as migraine.[2] Despite its therapeutic efficacy, GBP has been associated with adverse effects

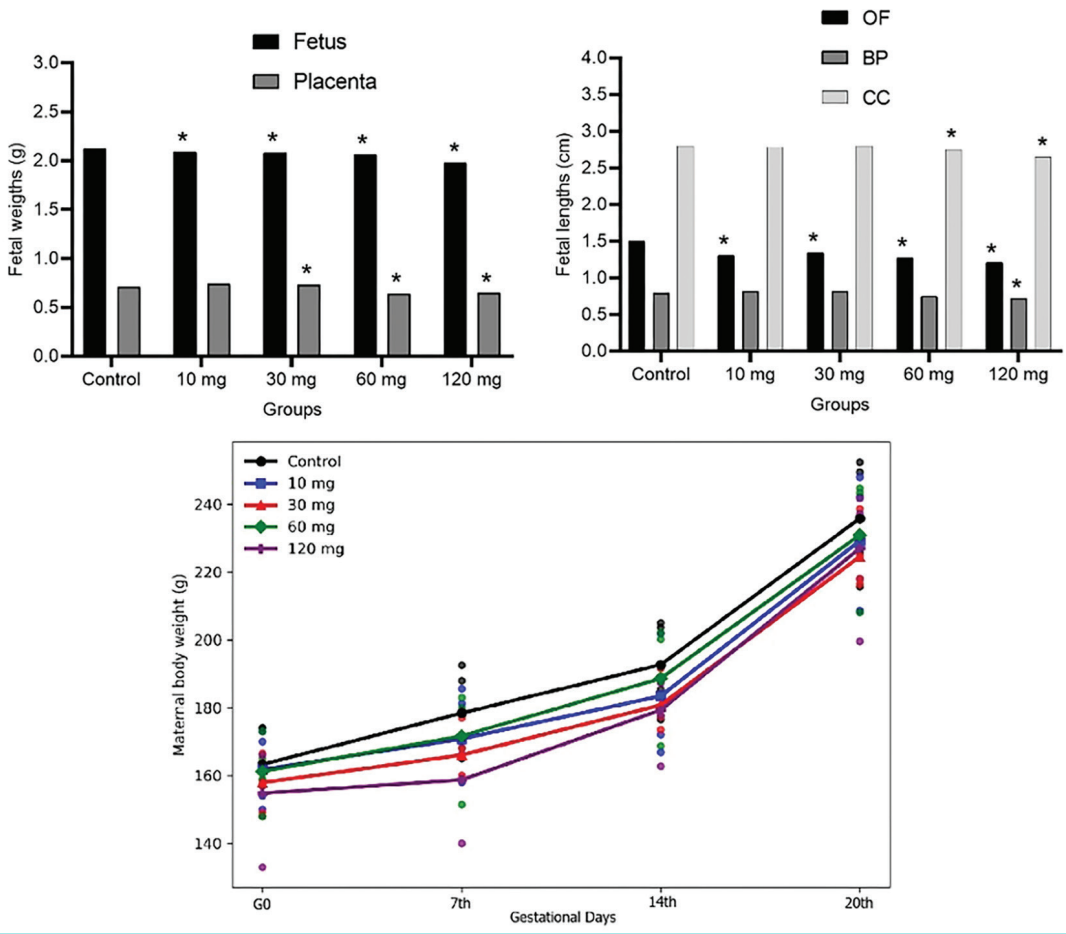


**Figure 1.** Effects of GBP exposure on fetal development parameters and maternal weight during gestation. Comparison of fetal and placental weights across experimental groups (Control, 10, 30, 60, and 120 mg/kg). Comparison of fetal lengths, including occipitofrontal (OF), biparietal (BP), and cranio-caudal (CC) measurements among groups. Changes in maternal body weight throughout gestation (G0, 7th, 14th, and 20th days) across all experimental groups. Data are presented as mean values. Asterisks (*) indicate statistically significant differences compared to the control group ($p<0.05$).

GBP: Gabapentin.

on bone development, including reductions in BMD and increased bone fracture risk. Moreover, it may cause mild side effects across multiple organ systems. Commonly reported adverse effects include weight gain and bone fragility.[20, 21] Although the precise impact of AEDs on bone metabolism remains controversial, accumulating evidence indicates that these drugs disrupt bone remodelling and increase fracture risk, potentially through indirect mechanisms, such as impaired calcium absorption, mediated by alterations in vitamin D metabolism.[10,22]

The analgesic effect of GBP is primarily exerted through a reduction in calcium influx. Nevertheless, sustained exposure to GBP may disrupt the normal physiological role of the α2β1 (alfa2/delta-1) subunit, which is critical for the proper development of musculoskeletal tissues. Consequently, long-term administration of GBP may adversely affect skeletal integrity.[23]

In the present study, we aimed to investigate the developmental and fetotoxic effects of GBP on prenatal bone development using DS and IHC. Both DS and IHC are reliable methods for evaluating skeletal alterations during the prenatal and postnatal periods in developmental toxicology research.[24] Previous studies conducted in both humans and animal models have demonstrated developmental toxicity associated with GBP exposure during pregnancy. Consistent with our findings, a study observed adverse effects on embryo-fetal development in rats following prenatal administration of pregabalin, a GBP AED whose effects are very similar to those of GBP.[25]

The effects of GBP on bone development may also manifest as alterations in morphometric parameters, such as fetal weight and CC length. In a study assessing these parameters, Prakash et al.[26] administered GBP at doses of 113, 226, and 452 mg/kg/day to rats during early, mid, and late gestation. The authors reported significant growth retardation, reduced litter size, and multiple gross malformations, particularly in the mid- and late-gestation groups. Similarly, Afshar et al.[13] administered GBP intraperitoneally at doses of 25 and 50 mg/kg/day during the first 15 days of gestation in mice and observed significant reductions in fetal body weight, together with skeletal and macroscopic malformations.

In our study, no major congenital anomalies were identified. However, we observed a dose-dependent decrease in fetal and placental weights, intrauterine growth restriction, and reduced litter size, particularly at higher GBP doses. These reductions were statistically significant compared with the control group.

Jetté et al.[20] conducted a retrospective study and reported a significantly increased risk of non-traumatic wrist, hip, and vertebral fractures among users of most AEDs, including GBP, with the exception of valproic acid, which showed no association with fracture risk. A related study involving 1,385 AED users found that postmenopausal women using AEDs had an increased risk of falls and fractures, emphasizing the importance of fall-prevention strategies in this population.[27] Additional studies have supported a strong association between AED use and fracture susceptibility.[28,29]

Although fracture risk was not directly evaluated in our study, we observed marked ossification delays, reductions in total bone length and area, and diminished ossification zones, particularly in the high-

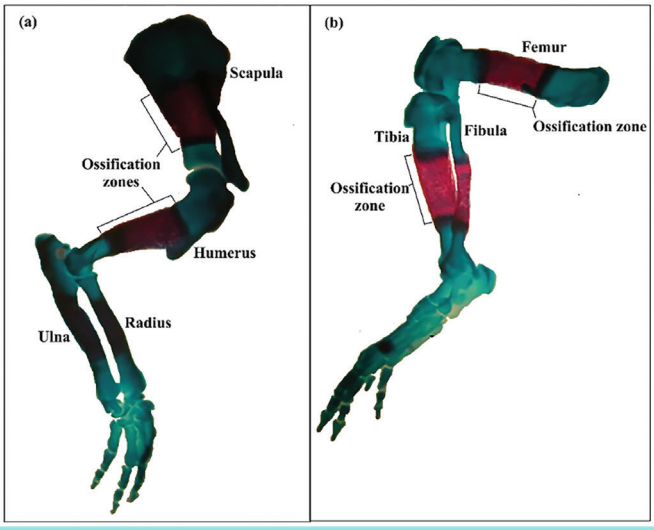


**Figure 2.** Images of forelimb (a) and hindlimb (b) in the control group stained with DS.

DS: Double staining.

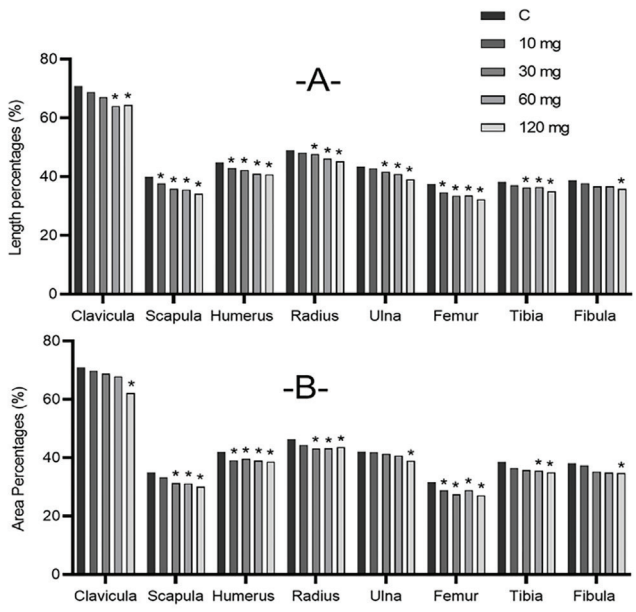


**Figure 3.** A comparison of the length (A) and area (B) of the ossified zones between the groups (* indicates that the difference is statistically significant compared to the control group).

**Table 1. Total bone length and area values of fetuses selected for groups.**

| | | GBP groups | | | | |
|---|---|---|---|---|---|---|
| | | 10 mg/kg | 30 mg/kg | 60 mg/kg | 120 mg/kg | Control |
| **Bones** | **Parameter** | **n=27** | **n=24** | **n=27** | **n=26** | **n=22** |
| Clavicula | TL | 3.04±0.19 | 3.05±0.25 | 3.15±0.17 | 3.00±0.21 | 3.16±0.22 |
| | TA | 0.99±0.13 | 1.03±0.18 | 1.08±0.11 | 0.97±0.15 | 1.12±0.20 |
| Scapula | TL | 4.26±0.24 | 4.21±0.28 | 4.30±0.28 | 4.25±0.27 | 4.37±0.20 |
| | TA | 6.16±0.68 | 6.02±0.80 | 6.33±0.69 | 6.16±0.72 | 6.47±0.87 |
| Humerus | TL | 4.42±0.20 | 4.39±0.25 | 4.50±0.18 | 4.39±0.21 | 4.57±0.25 |
| | TA | 4.16±0.39 | 4.14±0.49 | 4.33±0.38 | 4.15±0.41 | 4.37±0.53 |
| Radius | TL | 3.27±0.17 | 3.21±0.25 | 3.33±0.18 | 3.27±0.14 | 3.37±0.18 |
| | TA | 1.60±0.18 | 1.52±0.23 | 1.61±0.25 | 1.54±0.17 | 1.70±0.25 |
| Ulna | TL | 4.51±0.25 | 4.41±0.40 | 4.63±0.27 | 4.5 ±0.22 | 4.73±0.22 |
| | TA | 2.44±0.30 | 2.32±0.42 | 2.48±0.42 | 2.51±0.27 | 2.63±0.30 |
| Femur | TL | 3.79±0.18 | 3.75±0.30 | 3.84±0.21 | 3.82±0.24 | 3.86±0.24 |
| | TA | 2.97±0.33 | 2.95±0.45 | 3.12±0.27 | 3.06±0.33 | 3.19±0.37 |
| Tibia | TL | 3.70±0.24 | 3.64±0.31 | 3.78±0.24 | 3.74±0.26 | 3.81±0.24 |
| | TA | 2.36±0.28 | 2.34±0.33 | 2.54±0.30 | 2.43±0.32 | 2.55±0.30 |
| Fibula | TL | 3.46±0.20 | 3.45±0.29 | 3.58±0.20 | 3.54±0.20 | 3.60±0.16 |
| | TA | 1.04±0.15 | 1.06±0.15 | 1.15±0.12 | 1.13±0.12 | 1.18±0.14 |

GBP: Gabapentin. n: number of fetuses in the groups selected for DS.
TA: Total bone area ($mm^2$), TL: Total bone length (mm).

**Table 2. Intensities of AP and TRAP expression in all experimental groups**

| | GBP groups | | | | |
|---|---|---|---|---|---|
| | 10 mg/kg | 30 mg/kg | 60 mg/kg | 120 mg/kg | Control |
| | n=21 | n=16 | n=24 | n=15 | n=25 |
| AP | 86.63±4.60$^{ac}$ | 86.34±3.72$^{ac}$ | 85.09±6.90$^{a}$ | 80.95±6.80$^{b}$ | 89.94±3.50$^{c}$ |
| TRAP | 75.64±4.64$^{a}$ | 74.54±4.98$^{ab}$ | 73.88±5.05$^{ab}$ | 71.08±8.94$^{b}$ | 76.73±5.20$^{a}$ |

($x^{a}$, $y^{b}$): group x is statistically different from group y.
($x^{a}$, $y^{b}$, $z^{ab}$): groups x and y are statistically different, group z is similar to groups x and y.
n: number of fetuses in the groups selected for IHC analysis.
AP: Alkaline phosphatase, TRAP: Tartrate-resistant acid phosphatase.

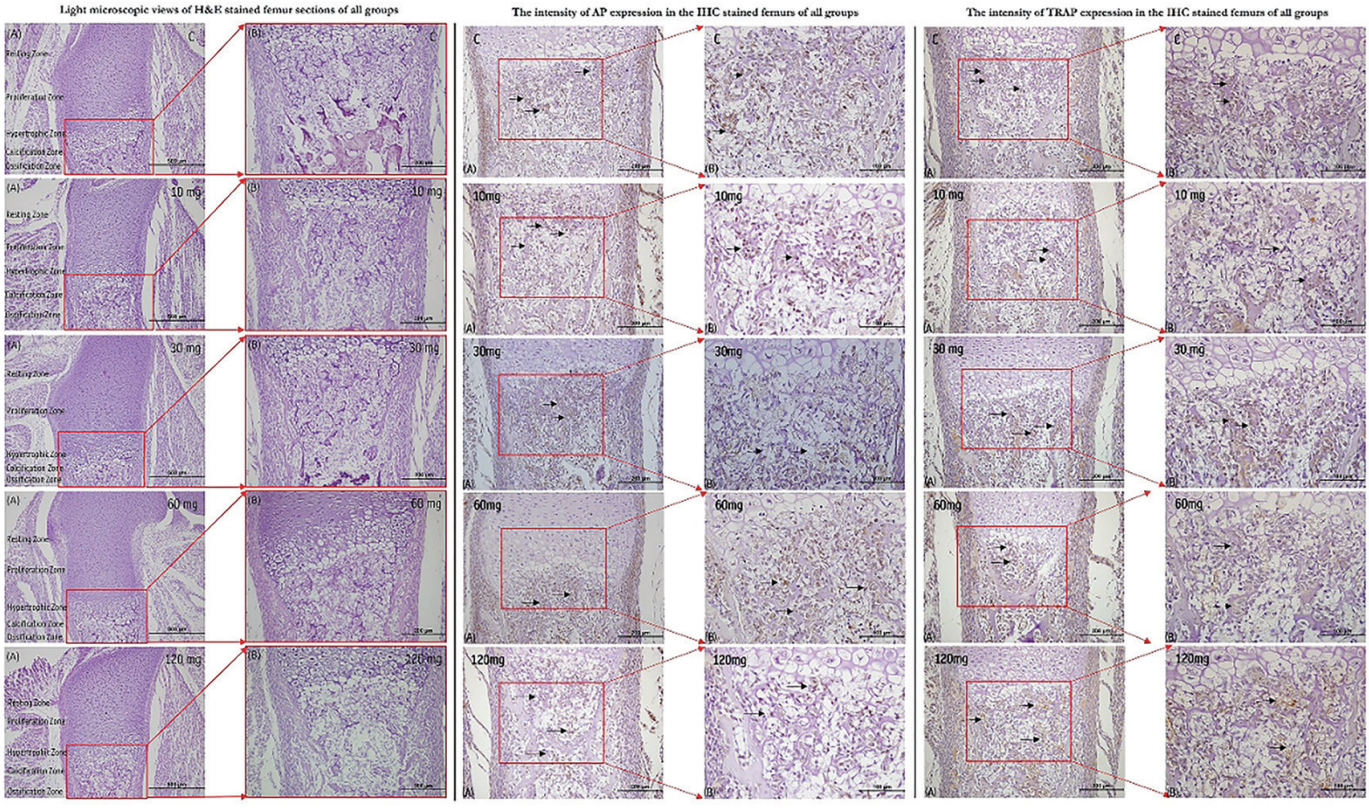


**Figure 4.** The zones of the femur in control group were identified using IHC. Arrows indicate the intensity of AP and TRAP expression in all groups (A) x20 and (B) x40, (C) Control group 10 mg, 30 mg, 60 mg and 120 mg GBP groups.

IHC: Immunohistochemical, AP: Alkaline phosphatase, TRAP: Tartrate-resistant acid phosphatase, GBP: Gabapentin.

dose GBP group. Moreover, IHC analysis revealed a disruption of bone homeostasis, evidenced by decreased AP and TRAP expression levels, suggesting a potential increase in fracture risk.

TRAP is a key enzyme involved in the development of the growth plate and metaphysis, and is widely used as a specific histochemical marker of osteoclastic and cartilaginous activity. Although TRAP deficiency in mice does not prevent overall development, it results in short, fragile bones due to dysfunction of the growth plate.[30] Simko et al.[29] examined the impact of sex hormone imbalance on susceptibility to AED-induced bone loss in orchiectomized Wistar rats and reported notable decreases in BMD, body weight, and bone strength. In addition, Blumer et al.[30] demonstrated that TRAP expression plays a crucial role in skeletal maturation, with its deficiency resulting in reduced ossification activity, particularly in the metaphyseal regions of long bones. Consistent with these observations, our findings showed that increasing doses of GBP were associated with reduced expression of bone turnover markers, including AP and TRAP, at ossification sites, suggesting impaired skeletal development and a potential increase in fracture risk.

### Study Limitations

This study has many methodological limitations. Although the number of pregnant rats in each group was limited, this sample size is comparable to that in similar experimental toxicology studies and sufficient to demonstrate consistent, dose-dependent effects. During the experiment, the loss of some animals-particularly at higher GBP doses-may have reduced group sizes; however, the remaining samples permitted reliable morphometric, skeletal, and IHC assessments. Second, the IHC assessment of AP and TRAP expression was limited to the femur,

a long bone. This approach is commonly used to evaluate endochondral ossification. Despite these limitations, the combination of DS and IHC ensured a robust evaluation of prenatal skeletal development and bone metabolism. Third, a significant limitation of this study is that fetuses were treated as independent observational units, and the absence of maternal identifiers in the dataset prevented clear consideration of the litter effect. Since fetuses from the same mother may share biological and environmental similarities, this could lead to pseudo-replication. However, similar fetal-level analytical approaches have been reported in experimental toxicology studies.

## CONCLUSION

The findings of this study demonstrate that maternal GBP exposure throughout gestation results in reductions in key morphometric parameters, including fetal and placental heights and weights, as well as delays in long bone development and ossification. Furthermore, IHC analyses revealed dose-dependent decreases and/or dysregulation in expression levels of AP and TRAP, enzymes critical for osteoblastic and osteoclastic activity, respectively. These results suggest that GBP administration during pregnancy may impair normal skeletal development by disrupting bone metabolism. The outcomes of this study provide valuable insights into the potential developmental effects of AEDs and underscore the need for further comprehensive investigations of the safety of GBP use during pregnancy.

### MAIN POINTS

- Prenatal gabapentin (GBP) exposure reduced fetal and placental growth parameters.
- Long bone development and ossification were delayed in rat fetuses.
- Expression of alkaline phosphatase and tartrate-resistant acid phosphatase showed dose-dependent dysregulation, indicating altered bone turnover.
- Gestational GBP exposure may impair normal fetal skeletal development.

### ETHICS

**Ethics Committee Approval:** This study was performed at Erciyes University Experimental Research Application Centre. Ethical approval of this study was granted by Erciyes University Animal Experiments Local Ethics Committee (approval no: 16/141, date: 16.11.2016).

**Informed Consent:** This is an experimental animal study.

### Footnotes

#### Authorship Contributions

Surgical and Medical Practices: M.D., İ.U., S.Y., E.B., G.Ö.Ö., Concept: M.D., E.B., E.U., Design: M.D., İ.U., S.Y., E.U., Data Collection and/or Processing: M.D., İ.U., E.B., G.Ö.Ö., Analysis and/or Interpretation: M.D., S.Y., E.B., G.Ö.Ö., Literature Search: M.D., Writing: M.D., E.U.

## DISCLOSURES

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** The study was funded by Erciyes University Scientific Research Projects Unit (TDK-2017-7378).

**Artificial Intelligence (AI) Use Statement:** During the preparation of this manuscript, artificial intelligence-based tools were used in a limited capacity for language refinement, clarity improvement, and overall enhancement of academic writing.

**Supplementarys Link:** https://d2v96fxpocvxx.cloudfront.net/a426c3a3-a110-40af-a6dd-1b2b563ce9ac/content-images/9209f86d-915b-42d7-a65f-9f6c1ef50311.pdf

## REFERENCES

1. Fan HC, Lee HS, Chang KP, Lee YY, Lai HC, Hung PL, et al. The impact of anti-epileptic drugs on growth and bone metabolism. Int J Mol Sci. 2016; 17(8): 1242.
2. Rose MA, Kam PC. Gabapentin: pharmacology and its use in pain management. Anaesthesia. 2002; 57(5): 451-62.
3. Afshar M, Golalipour MJ. Teratogenic effects of gabapentin on neural tube and limb development in mice. Neurosciences (Riyadh). 2008; 13(3): 321-3.
4. Sills GJ. The mechanisms of action of gabapentin and pregabalin. Curr Opin Pharmacol. 2006; 6(1): 108-13.
5. Petroff OA, Hyder F, Rothman DL, Mattson RH. Effects of gabapentin on brain GABA, homocarnosine, and pyrrolidinone in epilepsy patients. Epilepsia. 2000; 41(6): 675-80.
6. Freyer AM. Drugs in pregnancy and lactation 8th edition: a reference guide to fetal and neonatal risk. Obstet Med. 2009; 2(2): 89.
7. Shihman B, Goldstein L, Amiel N, Benninger F. Antiepileptic drug treatment during pregnancy and delivery in women with epilepsy-a retrospective single center study. Epilepsy Res. 2019; 149: 66-9.
8. Calado AM, Dos Anjos Pires M. An overview of teratology. Methods Mol Biol. 2018; 1797: 3-32.
9. Verrotti A, Coppola G, Parisi P, Mohn A, Chiarelli F. Bone and calcium metabolism and antiepileptic drugs. Clin Neurol Neurosurg. 2010; 112(1): 1-10.
10. Hant FN, Bolster MB. Drugs that may harm bone: mitigating the risk. Cleve Clin J Med. 2016; 83(4): 281-8.
11. Pitetzis DA, Spilioti MG, Yovos JG, Yavropoulou MP. The effect of VPA on bone: from clinical studies to cell cultures-the molecular mechanisms revisited. Seizure. 2017; 48: 36-43.
12. Patorno E, Hernandez-Diaz S, Huybrechts KF, Desai RJ, Cohen JM, Mogun H, et al. Gabapentin in pregnancy and the risk of adverse neonatal and maternal outcomes: a population-based cohort study nested in the US Medicaid Analytic eXtract dataset. PLoS Med. 2020; 17(9): e1003322.
13. Afshar M, Hassanzadeh-Taheri MM, Moallem SA, Tamizi A, Golalipour MJ. Teratogenic effects of gabapentin on the skeletal system of Balb/C mice fetuses. Neurosciences (Riyadh). 2009; 14(3): 239-44.
14. Meier C, Kraenzlin ME. Antiepileptics and bone health. Ther Adv Musculoskelet Dis. 2011; 3(5): 235-43.
15. Shen C, Chen F, Zhang Y, Guo Y, Ding M. Association between use of antiepileptic drugs and fracture risk: a systematic review and meta-analysis. Bone. 2014; 64: 246-53.
16. Güler H, Esen EE, Balcıoğlu E, Göktepe Ö, Yılmaz H, Yay AH, et al. Bone development in offspring of pregnant rats treated with carbamazepine: evaluation by three different methods. Epilepsia. 2022; 63(12): 3066-77.
17. Hamidi GA, Jafari-Sabet M, Abed A, Mesdaghinia A, Mahlooji M, Banafshe HR. Gabapentin enhances anti-nociceptive effects of morphine on heat, cold, and mechanical hyperalgesia in a rat model of neuropathic pain. Iran J Basic Med Sci. 2014; 17(10): 753-9.

18. Perucca P, Scheffer IE, Kiley M. The management of epilepsy in children and adults. Med J Aust. 2018; 208(5): 226-33.

19. Montouris G. Gabapentin exposure in human pregnancy: results from the gabapentin pregnancy registry. Epilepsy Behav. 2003; 4(3): 310-7.

20. Jetté N, Lix LM, Metge CJ, Prior HJ, McChesney J, Leslie WD. Association of antiepileptic drugs with nontraumatic fractures: a population-based analysis. Arch Neurol. 2011; 68(1): 107-12.

21. Woodruff TJ, Zota AR, Schwartz JM. Environmental chemicals in pregnant women in the United States: NHANES 2003-2004. Environ Health Perspect. 2011; 119(6): 878-85.

22. Tsukahara H, Kimura K, Todoroki Y, Ohshima Y, Hiraoka M, Shigematsu Y, et al. Bone mineral status in ambulatory pediatric patients on long-term antiepileptic drug therapy. Pediatr Int. 2002; 44(3): 247-53.

23. Reyes Fernandez PC, Wright CS, Warden SJ, Hum J, Farach-Carson MC, Thompson WR. Effects of gabapentin and pregabalin on calcium homeostasis: implications for physical rehabilitation of musculoskeletal tissues. Curr Osteoporos Rep. 2022; 20(6): 365-78.

24. Zin SRM, Alshawsh MA, Mohamed Z. Multiple skeletal anomalies of sprague dawley rats following prenatal exposure to anastatica hierochuntica, as delineated by a modified double-staining method. Children (Basel). 2022; 9(5): 763.

25. Morse DC. Embryo-fetal developmental toxicity studies with pregabalin in mice and rabbits. Birth Defects Res B Dev Reprod Toxicol. 2016; 107(2): 85-93.

26. Prakash, Prabhu LV, Rai R, Pai MM, Yadav SK, Madhyastha S, et al. Teratogenic effects of the anticonvulsant gabapentin in mice. Singapore Med J. 2008; 49(1): 47-53.

27. Carbone LD, Johnson KC, Robbins J, Larson JC, Curb JD, Watson K, et al. Antiepileptic drug use, falls, fractures, and BMD in postmenopausal women: findings from the women's health initiative (WHI). J Bone Miner Res. 2010; 25(4): 873-81.

28. Kanda J, Izumo N, Kobayashi Y, Onodera K, Shimakura T, Yamamoto N, et al. Effects of the antiepileptic drugs phenytoin, gabapentin, and levetiracetam on bone strength, bone mass, and bone turnover in rats. Biol Pharm Bull. 2017; 40(11): 1934-40.

29. Simko J, Karesova I, Kremlacek J, Eva Z, Horacek J, Fekete S, et al. The effect of gabapentin and pregabalin on bone turnover and bone strength: a prospective study in Wistar rats. Pharmacol Rep. 2019; 71(6): 1213-8.

30. Blumer MJ, Hausott B, Schwarzer C, Hayman AR, Stempel J, Fritsch H. Role of tartrate-resistant acid phosphatase (TRAP) in long bone development. Mech Dev. 2012; 129(5-8): 162-76.