Prenatal toxicity of gabapentin on bone development in rat offsprings

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Abstract

Background

Gabapentin is a drug commonly prescribed to adult pregnant women with neuropathic pain and epilepsy. Since the effect of antiepileptic drugs used in pregnant women with epilepsy on prenatal bone development is controversial, this study was conducted to demonstrate the toxic effects of gabapentin use during pregnancy on the skeletal system.

Methods

In the study, pregnant Wistar albino rats were randomly selected and divided into 5 groups (n = 4) as control and 10 mg/kg/day, 30 mg/kg/day, 60 mg/kg/day and 120 mg/kg/day gabapentin groups. The pups were subjected to double skeletal staining (DSS) and the ossification lengths and areas of the fore and hind bones of the pups were measured. Immunohistochemistry (IHC) was used to evaluate the ossification sites and the levels of alkaline phosphatase (AP) and tartrate resistant acid phosphatase (TRAP) immunoreactivity in the pups' femurs. Results: According to the results, the weights and morphometric sizes of the pups were lower than those of the control group. It was found that ossification rates in the fore and hind bones were statistically significantly lower. It was revealed that AP and TRAP intensities which is metabolic markers for bone development were reduced in the experimental groups compared to the control group.

Conclusions

We have shown that continuous use of gabapentin during pregnancy in rats results in lower birth weight offspring, delayed ossification in the offspring and adverse effects on bone metabolism as the dose increases.

Introduction

Epilepsy is a common neurological disorder that affects millions of people and requires ongoing medication throughout pregnancy (Fan et al., 2016). GBP, which is commonly used in pregnant women with epilepsy, is an anticonvulsant for partial-onset seizures that was first introduced in 1993. It is also effective in the treatment of various chronic pain conditions, including inflammatory pain, trigeminal neuralgia, and psychiatric disorders (Rose and Kam, 2002; Afshar et a., 2008).

GBP is a third-generation gamma-aminobutyric acid (GABA) (Sills, 2006). Because it is structurally related to GABA, it readily crosses the placenta and the blood-brain barrier and has potential effects on the foetus. GBP is in pregnancy category C and is a remarkable anticonvulsant because it is well tolerated and has no drug-drug interactions (Petroff et al., 2000; Mao and Chen, 2000; Briggs et al., 2008).

AEDs are widely prescribed to women of childbearing age and have a high potential for teratogenic effects (Shihman et al., 2019). Toxic and teratogenic effects of AEDs on prenatal development have been observed, and these effects result in impaired development of organs or tissues depending on the gestational age (Calado and Dos Anjos Pires, 2018). In addition, the use of AEDs during pregnancy may have adverse effects on embryogenesis, osteogenesis, and also neurological development (Verrotti et al., 2010). Since the thalidomide disaster in the 1960s, evidence of reduced bone mineral density (BMD) and impaired bone metabolism, particularly in children and adolescents, has grown rapidly (Hant and Bolster, 2016; Lin et al., 2016; Pitetzis et al., 2017). Because of the inadequate knowledge of the safety of the use of GBP in pregnancy and its increasing use, there is a significant lack of evidence to help adult women and pregnant women understand the risks of GBP treatment for pregnancy-related outcomes (Mostacci et al., 2018; Patorno et al., 2020).

Bone is a very dynamic structure that is constantly being remodeled by bone resorption and bone formation. Under normal conditions, this balance is always maintained so that bone strength and bone mass are maintained. The imbalances that occur when this balance is disturbed can lead to abnormalities in bone formation, structure and, subsequently, function (Kanda et al., 2017; Rocha et al., 2019).

The exact effects of AEDs on bone structure are still controversial and most studies have not fully elucidated the true effects of AEDs, especially gabapentin (Rocha et al., 2009). For example, studies have shown mixed results regarding the toxicity of GBP. In particular, GBP has been shown to be developmentally toxic in animal studies during pregnancy in mice and rats (Rose and Kam, 2002; Dethloff et al., 2000; Afshar et al., 2009).

In addition, Briggs et al. have reported that oral use of this drug causes delayed ossification of several bones during the period of organogenesis (Briggs et al., 2008). Although the relationship between GBP treatment and bone loss has been inconclusive in many studies, other studies have shown a negative association between antiepileptic therapy and bone loss and calcium metabolism (Fan et al., 2016; Andress et al., 2002; Meier and Kraenzlin, 2011; Jette et al., 2011; Shen et al., 2014). Similarly, in recent years it has been reported that AEDs lead to a reduction in BMD and an increased risk of fracture, and 50% of patients have bone malformations (Güler et al., 2022). There are insufficient data to assess the prenatal skeletal development of gabapentin by both DSS and IHC. Therefore, to determine the safety of GBP, we analysed prenatal bone development in rats by DSS and IHC.

Material and Methods

Animals and Laboratory

Twenty female Wistar albino rats (160g – 180g weight, 8 weeks old) were used in this study. Female rats were kept overnight in the same cage as male rats to induce pregnancy. Female rats with sperm-positive

vaginal smears were accepted on day 0 of gestation. Optimal and appropriate room conditions (temperature, light and humidity) were provided.

Study Groups

Considering that each rat would give birth to approximately 10–12 pups and possible drug-induced deaths during the experiment, experimental groups were formed with 4 rats in each group. Drug dilution was performed with normal saline (0.9% NaCl). According to dose studies in the literature (Afshar et al., 2009; Hamidi et al., 2014), pregnant rats were administered saline vehicle (control) and 10 mg/kg/day (low dose), 30/mg/kg/day, 60 mg/kg/day and 120 mg/kg/day (high dose) GBP by gavage on gestation days 1–20.

Experimental procedure

Rats were premedicated with intraperitoneal xylazine and anesthetized with ketamine on gestation day 20. The uterus was dissected together with the offspring inside. All pups were examined macroscopically for the presence of deformities and skeletal malformations. Each litter and its placenta were weighed. The cranio-caudal (height), occipitofrontal and biparietal lengths were measured using a sensitive electronic caliper. Following this procedure, the offspring were classified for DSS and IHC.

Double Skeletal Staining procedure

In order to stain the skeleton of the offspring using the DSS method, the procedure shown in Table 1 was followed.

Table 1
Procedures of the DSS method.

Steps	Procedures	Chemicals and materials	Timing
1.	Fixation	70% ethyl alcohol	4–7 days
2.	Degreasing	Pure acetone	1–3 days
3.	Skin, internal organs and eyes were removed		
4.	Staining	Alizarin Red-S (stains bone)	100 mg
		Alcian Blue (stains cartilage)	300 mg
5.	Incubation	In incubator at 38°C-40°C	7 days
6.	Decolorization	Under running water	2 hours
7.	Transparentizing	1% potassium hydroxide	Min 1 day
8.	To prevent deterioration	20% Glycerin, 50% Glycerin, 80% Glycerin, 100% Glycerin	In order

Morphometric measurements

Images of the fore and hind limbs of the offspring for morphometric measurements were taken using a stereomicroscope. These images, taken on millimeter paper, were transferred to the computer and measurements of the ossified and cartilaginous areas were made on the computer using ImageJ version 1.51r software. Measurements of total bone lengths, ossified zone lengths, total bone surface areas, and ossified zone surface areas of the bones were made. The ossification percentages of the bones (ossification measure / total bone measure x 100) were calculated from the measurement data.

Immunohistochemical procedure

For immunohistochemical analysis, offsprings in all groups were randomly selected, sacrificed and femurs dissected. First, a standard histological evaluation method was used to determine the histological structures of the bone. Then, hematoxylin and eosin staining were performed to determine the histopathological structure (Table 2). In this study, AP expression intensity, a marker of bone formation, and TRAP expression intensity, a marker of bone destruction, were examined in the femur tissues of the offsprings.

Table 2
Procedures of the IHC.

Steps	Procedures	Chemicals and materials	Timing
1.	Fixation	10% Formalin	
2.	Sectioning	5–6 µm sections on polylysine-covered slides	
3.	Deparaffinization	In an incubator at 60 C	1 night
4.		Xylene	15 mins
5.		Alcohol series	5 mins each
6.	Boiling	10% citrate buffer in a microwave oven at 600 W	5 mins
7.	Cooling	Citrate buffer at room temperature	10 mins
8.	Washing	PBS (Phosphate Buffered Saline)	10 mins
9.	to reduce non-specific staining	Endogenous peroxidase activity was blocked with 3% H_2O_2	10 mins
10.	IHC Staining	Large Volume Detection System (Thermo Scientific, TP-125-HL)	
11.	Washing Repeat	PBS (Phosphate Buffered Saline)	
12.	to cover the regions outside the antigenic areas	Ultra V block	10 mins
13.	TRAP and AP primary antibodies were applied at 4 C		1 night
14.	Incubation	At room temperature	30 mins
15.	Buffer Solution was used as negative control instead of primary antibody		
16.	IHC Staining	Avidin-biotin peroxidase method using streptavidin-biotin kit	
18.	Incubation	Biotin-secondary antibody	10 mins
19.		Streptavidin peroxidase	10 mins
20.	Immunoreactivity	Peroxidase substrate	1–5 mins
21.	Washing	Distilled water	5 mins
22.	Dehydration	with increasing series of alcohols (70%, 80%, 96%, 100%)	
		Xylene	5 mins
23.	Protection	Entellan®, Merck	

Steps	Procedures	Chemicals and materials	Timing
24.	Examine	Olympus BX51 microscope	

Statistical analysis

IBM SPSS Statistics 28.0.1 was used for statistical analysis of the data. Parametric data were presented as mean ± standard deviation (Mean ± SD). Analysis of normal distribution was performed using the Shapiro-Wilk test and measures of skewness and kurtosis. Tukey HSD test was used with 95% confidence interval with post-hoc multiple comparison command for group comparison. $p < 0.05$ was considered statistically significant.

Results

The number of offsprings collected from 4 rats in each group is shown in Table 3. According to the table, the highest number of offsprings was found in the control group. Based on these results, it was determined that there was a decrease in the number of births due to the increase in the dose of gabapentin.

Results of morphometric parameters.

The number, weight, and height of the offsprings in all groups were measured before staining. All morphometric parameters were highest in the control group (Table 3). It was found that there was a statistically significant decrease in the numerical parameters in the experimental groups as a function of dose increase, and we found that there was a statistically significant difference between the control group and the high-dose GBP group ($p < 0.05$). These results suggest that GBP appears to inhibit intrauterine growth and development.

Table 3
Morphometric parameters of the offsprings.

	Gabapentin groups				Control
	10 mg/kg/day	30 mg/kg/day	60 mg/kg/day	120 mg/kg/day	
Parameters	n = 48	n = 40	n = 51	n = 41	n = 53
Weights, g	2.12 ± 0.12^{a}	2.12 ± 0.14^{a}	2.09 ± 0.16^{a}	1.98 ± 0.20^{b}	2.16 ± 0.15^{a}
Heights, cm	2.78 ± 0.12^{ac}	2.80 ± 0.11^{ac}	2.75 ± 0.15^{a}	2.65 ± 0.19^{b}	2.84 ± 0.08^{c}
Placenta, g	0.74 ± 0.12^{ac}	0.58 ± 0.13^{b}	0.64 ± 0.14^{bd}	0.70 ± 0.12^{ad}	0.80 ± 0.18^{c}
Biparietal, cm	0.82 ± 0.06^{a}	0.75 ± 0.10^{a}	0.75 ± 0.07^{a}	0.72 ± 0.08^{b}	0.82 ± 0.06^{a}
OF, cm	1.32 ± 0.06^{ad}	1.35 ± 0.07^{a}	1.27 ± 0.07^{d}	1.21 ± 0.08^{b}	1.50 ± 0.15^{c}

Same letters in groups indicate statistically significant similarity ($p > 0.05$), different letters indicate difference ($p < 0.05$). All parametric data are presented as mean ± standard deviation. One-way analysis of variance with post hoc Tukey HSD test for differences between groups.

OF: occipitofrontal; GBP: gabapentin; N: number of pups from four rats within a group.

Results of Double Skeletal Staining

In DSS, red area shows ossification, and the blue area shows cartilage (Fig. 1). The lengths and surface areas of the clavicula, scapula, humerus, radius and ulna of the forelimb and the femur, tibia, and fibula of the hindlimb were measured. The highest total bone length and ossification length percentages were found in the control group, while the lowest values were found in the high-dose GBP group. When ossification length percentages were compared between groups, a statistically significant difference was found between the control group and all other experimental groups in the scapula, humerus, and femur. A statistically significant difference was found between the control group and the high dose GBP group in all other bones ($p < 0.05$) (Fig. 2). Total bone lengths and total bone areas in the experimental groups are shown in Table 4.

The percentage of ossification area was compared between groups. When the total bone area was visualised microscopically, it was observed that the total bone area and the ossification area percentages were higher in the control group (Fig. 2). When ossification area percentages were compared between groups, a statistically significant difference was found between the control group and the 30, 60 and 120 mg groups in the scapula and radius, the 120 mg group in the humerus and ulna, the 60 mg and 120 mg groups in the tibia and all groups in the femur ($p < 0.05$).

Table 4
The total bone lengths and total bone areas.

		Gabapentin groups				Control
		10 mg/kg/day	30 mg/kg/day	60 mg/kg/day	120 mg/kg/day	
Bones	**Parameters**	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	4.57 ± 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 pups used for the DSS. TA: Total bone area, TL: Total bone length.

According to the DSS results, it was found that gabapentin, especially at doses of 60mg/kg/day and 120mg/kg/day, delayed ossification, decreased total bone area and length, and thus caused bone shortening and shrinkage. Therefore, it is predicted that prenatal bone development will be impaired, and the risk of fractures will be increased.

Results of Immunohistochemistry

IHC in the control group showed the typical hyaline cartilage structure in the quiescent zone. In the proliferation zone, chondrocytes were rapidly dividing and forming isogenic groups. Large chondrocytes were found in the hypertrophic zone. Areas of degeneration were observed in chondrocytes in the calcification zone and osteoprogenitor cells in the ossification zone were observed to transform into osteoblasts (Fig. 3a).

AP density findings

Immunohistochemical preparations from all groups were examined microscopically (Table 5). The most intense AP expression (89.94 ± 3.50) was observed in the ossification areas of the control group (Fig. 3b). A statistically significant difference was found between the control group and the 60 mg and 120 mg GBP groups ($p < 0.05$).

Table 5
Intensities of AP and TRAP expression in all experimental groups.

		Gabapentin groups				
		10 mg/kg/day	**30 mg/kg/day**	**60 mg/kg/day**	**120 mg/kg/day**	**Control**
		n = 21	n = 16	n = 24	n = 15	n = 25
AP	Min - Max	74.01–94.66	78.09–93.08	65.86–95.89	71.07–93.87	83.78–98.41
	Mean ± SD	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	Min - Max	66.90–84.23	64.80–86.16	63.99–85.66	56.94–89.96	67.64–88.89
	Mean ± SD	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}

Same letters in groups indicate statistically significant similarity ($p > 0.05$), different letters indicate difference ($p < 0.05$). All parametric data are presented as mean ± standard deviation. One-way analysis of variance with post hoc Tukey HSD test for differences between groups.

GBP: *Gabapentin*. n: *numbers of pups used for IHC.* AP: *Alkaline Phosphatase.* TRAP: *Tartrate Resistance Acid Phosphatase.*

TRAP density findings

Immunohistochemistry specimens from all groups were examined microscopically. TRAP expression was seen in zones of ossification in all groups. The highest expression intensity of the data for TRAP expression was found in the control group (76.73 ± 5.20) (Table 5). When comparing between groups, a statistically significant difference was found only between the control group and the high dose GBP group ($p < 0.05$). Although osteoclasts and osteoblasts were in equilibrium in normal bone tissue, it was concluded that with increasing GBP dose, this balance was disturbed, and ossification was adversely affected (Fig. 3c).

Discussion

Epilepsy, one of the most common neurological disorders, affects many people, especially pregnant women, and should be controlled during pregnancy. In addition, seizures may occur during pregnancy as it is a chronic disease. Therefore, seizures in pregnant women affect both the mother's body and the foetus through the placenta (Güler et al., 2022; Perucca et al., 2018).

As mothers with epilepsy have to take several medications during pregnancy, the possible side effects of the medication on the mother and the baby should be assessed. Because of the increasing use of GBP, there is a critical need for evidence to help women of childbearing age understand the risks and benefits of GBP treatment in terms of pregnancy-related outcomes. Although information on major fetal malformations caused by antiepileptic drugs such as GBP taken early in pregnancy suggests that the risk is low, the toxicological effects of GBP need to be investigated in more detail (Mostacci et al, 2018; Patorno et al., 2020; Montouris, 2003).

GBP is effective for headache, inflammatory pain and various pains associated with neuropathy, especially spasticity (Rose and Kam, 2002). GBP has adverse effects on bone development, BMD and fractures, and mild side effects in many systems, including the central nervous, gastrointestinal, and excretory systems. The most common side effects are abnormal weight gain, dizziness, fatigue, and bone weakness (Andress et al., 2002; Jette et al., 2011; Woodruff et al., 2011). The effects of AEDs on bone metabolism are currently controversial, but studies have shown that they cause disturbances in bone turnover and increased fracture risk. Indirect mechanisms such as reduced calcium absorption through stimulation of vitamin D metabolism may explain this (Pitetzis et al., 2017; Tsukahara et al., 2002).

GBP exerts its analgesic effect by selectively binding to the α2δ1 auxiliary subunit of voltage-gated calcium channels, thereby inhibiting channel function. Prolonged use of GBP may impair this function of α2δ1, which is critical for the formation of skeletal structures such as muscle, bone, and cartilage. Therefore, prolonged use of the drug is associated with adverse effects on the musculoskeletal system (Reyes Fernandez et al., 2022).

Therefore, in this study we investigated the toxic and/or teratological effects of GBP on bone development using DSS and immunohistochemistry. DSS and IHC are important methods used in teratological or toxicological investigations of the skeletal system in the prenatal and postnatal periods (Sadeghi, 2014). In studies conducted in humans and animals during pregnancy, GBP intake has been reported to be developmentally toxic, and in another study similar to ours, a negative effect on embryo-fetal development was observed in fetuses of rats given GBP during pregnancy (Morse, 2016).

The effect of GBP on bone development may have a negative impact on morphometric parameters such as litter length and weight. In a study investigating these parameters, Prakash et al. examined the effects of GBP in early, mid, and late gestation groups and administered GBP at 113, 226 or 452 mg/kg/day. They found that growth retardation was observed along with stunting of live litter size in the mid-gestation and late gestation treated groups and various gross malformations (Prakash et al., 2008). In another study, 25 mg/kg/day and 50 mg/kg/day of GBP were administered intraperitoneally to mice during the first 15 days of pregnancy. They found a decrease in fetal body weight and macroscopic and skeletal malformations. They also reported that fetal body weights were significantly lower in both treated groups compared with the control group (Afshar et al., 2009).

In our study, no significant major malformations were observed in the offsprings. In addition, we observed a decrease in litter and placenta weight (low birth weight) and growth retardation with litter size stunting as the dose of gabapentin increased. This reduction was statistically significant compared with the control group.

Jette et al. found a significant increase in fracture risk for most AEDs, including gabapentin, in their retrospective study of individuals with non-traumatic wrist, hip and vertebral fractures. However, they found that valproic acid was the only AED that was not associated with an increased fracture risk (Jette et al., 2011). Another similar study examined the association between AED use and falls, fractures and BMD and included 1,385 AED users aged 50–79 years. It reported that postmenopausal women taking AEDs were at increased risk of fractures and that attention should be paid to fall prevention in these women (Carbone et al., 2010). In addition, other studies of AED users have reported a strong association between drug use and fracture risk (Kanda et al., 2017; Simko et al., 2019).

In our study, the risk of fractures was not fully evaluated, but we found ossification delay, decrease in total bone length, and total bone area, and decrease in the ossification zones in particular in the high-dose gabapentin group. In addition, we believe that bone formation becomes unbalanced as a result of IHC, and the risk of fracture increases with decreasing AP and TRAP densities.

TRAP is critical for growth plate and metaphyseal formation. It is found in bone and cartilage and acts as a specific histochemical marker for these cells. TRAP deficiency in mice results in normal development, but offspring bones remain brittle and short due to growth plate dysfunction (Hayman et al., 2000; Blumer et al., 2012). Simko et al. investigated the influence of sex hormone balance on susceptibility to AED-induced bone loss in orchiectomized Wistar rats. They found significant reductions in BMD, weight, and biomechanical strength in orchiectomized animals (Simko et al., 2019). In addition, another study

demonstrated that TRAP density is critical for skeletal development and that TRAP deficiency leads to decreased ossification activity in adult mice, concluding that TRAP is critical for growth plate and metaphyseal development in long bones (Blumer et al., 2012). Our results show that the intensity of bone turnover markers such as AP and TRAP decreased in ossification sites depending on the increase in GBP dose.

Conclusion

In conclusion, these results showed that the use of GBP throughout pregnancy decreased litter and placenta morphological parameters such as height and weight, delayed long bone development and decreased ossification. It was also found that the density of AP and TRAP, which are responsible for osteoblast and osteoclast metabolism, decreased and/or became unbalanced with the use of GBP. We suggest that the results of this study provide an important contribution to further studies on the use of AEDs in pregnancy.

Abbreviations

GBP
Gabapentin
AP
Alkaline Phosphatase
TRAP
Tartrate Resistance Alkaline Phosphatase
DSS
Double Skeletal Staining
IHC
Immunohistochemistry
GABA
Gamma-aminobutyric acid
AEDs
Antiepileptic drugs
BMD
Bone Mineral Density
TL
Total Length
TA
Total Area

Declarations

Ethical Approval

This study was performed at Erciyes University Experimental Research Application Centre. Ethical approval of this study was granted by Erciyes University Research Centre and Animal Ethics Committee (TDK-2017-7378).

Competing Interests

The authors declare that they have no competing interests.

Authors' contributions

M.D. is the main author of the study and was involved in all phases of the study, including statistical analysis. E.B. and G.Ö.Ö. worked on the immunohistochemical analysis of the study. S.Y. and İ.U. contributed to the double skeleton staining experiment step of the study. E.U. was involved in the planning and initiation of the study.

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Availability of data and materials

The datasets obtained and analyzed during the study are available from the corresponding author upon request.

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References

1. Afshar M, Golalipour MJ (2008) Teratogenic effect of gabapentin on neural tube in limb development in mice. Neurosciences 13:320-323
2. Afshar M, Hassanzadeh-Taheri MM, Moallem SA, Tamizi A, Golalipour MJ (2009) Teratogenic effects of gabapentin on the skeletal system of Balb/C mice fetuses. Neurosciences 14:239-244
3. Andress DL, Ozuna J, Tirschwell D, Grande L, Johnson M, Jacobson AF, et al (2002) Antiepileptic drug-induced bone loss in young male patients who have seizures. Arch Neurol. https://doi.org/10.1001/archneur.59.5.781
4. Blumer MJ, Hausott B, Schwarzer C, Hayman AR, Stempel J, Fritsch H (2012) Role of tartrate-resistant acid phosphatase (TRAP) in long bone development. Mechanism of Development. https://doi.org/10.1016/j.mod.2012.04.003

5. Briggs GG, Freeman RK, Yaffi SJ (2008) Drug in pregnancy and lactation: a reference guide to fetal and neonatal risk. 8th ed, Philadelphia, Lippincott Williams&Wilkins, pp 802-803
6. Calado AM, Dos Anjos Pires M (2018) An Overview of Teratology. Methods Mol Biol. https://doi.org/10.1007/978-1-4939-7883-0_1
7. Carbone LD, Johnson KC, Robbins J, Larson JC, Curb JD, Watson K, et al (2010) Antiepileptic drug use, falls, fractures, and BMD in postmenopausal women: Findings from the women's health initiative (WHI). J Bone Miner Res. https://doi.org/10.1359/jbmr.091027
8. Dethloff L, Barr B, Bestervelt L, Bulera S, Sigler R, LaGattuta M, et al (2000) Gabapentin-induced mitogenic activity in rat pancreatic acinar cells. Toxicol Sci. https://doi.org/10.1093/toxsci/55.1.52
9. Fan HC, Lee HS, Chang KP, Lee YY, Lai HC, Hung PL, et al (2016) The Impact of Anti-Epileptic Drugs on Growth and Bone Metabolism. Int J Mol Sci. https://doi.org/10.3390/ijms17081242
10. Güler H, Esen EE, Balcıoğlu E, Göktepe Ö, Yılmaz H, Yay AH, et al (2022) Bone development in offspring of pregnant rats treated with carbamazepine: Evaluation by three different methods. Epilepsia. https://doi.org/10.1111/epi.17422
11. Hamidi GA, Jafari-Sabet M, Abed A, Mesdaghinia A, Mahlooji M, Banafshe HR (2014) 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 17:753-759
12. Hant FN, Bolster MB (2016) Drugs that may harm bone: Mitigating the risk. Cleveland Clinic Journal of Medicine. https://doi.org/10.3949/ccjm.83a.15066
13. Hayman AR, Bune AJ, Cox TM (2000) Widespread expression of tartrate-resistant acid phosphatase (Acp 5) in the mouse embryo. Journal of Anatomy. https://doi.org/10.1046/j.1469-7580.2000.19630433.x
14. Jetté N, Lix LM, Metge CJ, Prior HJ, McChesney J, Leslie WD (2011) Association of antiepileptic drugs with nontraumatic fractures: A population-based analysis. Arch Neurol. https://doi.org/10.1001/archneurol.2010.341
15. Kanda J, Izumo N, Kobayashi Y, Onodera K, Shimakura T, Yamamoto N, et al (2017) Effects of the Antiepileptic Drugs Phenytoin, Gabapentin, and Levetiracetam on Bone Strength, Bone Mass, and Bone Turnover in Rats. Biol Pharm Bull. https://doi.org/10.1248/bpb.b17-00482
16. Lin CM, Fan HC, Chao TY, Chu DM, Lai CC, Wang CC, et al (2016) Potential effects of valproate and oxcarbazepine on growth velocity and bone metabolism in epileptic children- a medical center experience. BMC Pediatrics. https://doi.org/10.1186/s12887-016-0597-7
17. Mao J, Chen LL (2000) Gabapentin in pain management. Anesth Analg. https://doi.org/10.1097/00000539-200009000-00034
18. Meier C, Kraenzlin ME (2011) Antiepileptics and bone health. Ther Adv Musculoskelet Dis. https://doi.org/10.1177/1759720X11410769
19. Montouris G (2003) Gabapentin exposure in human pregnancy: results from the Gabapentin Pregnancy Registry. Epilepsy Behav. https://doi.org/10.1016/s1525-5050(03)00110-0

20. Morse DC (2016) Embryo-Fetal Developmental Toxicity Studies with Pregabalin in Mice and Rabbits. *Birth* Defects Res B Dev Reprod Toxicol. https://doi.org/10.1002/bdrb.21174
21. Mostacci B, Poluzzi E, D'Alessandro R, Cocchi G, Tinuper P (2018) Adverse pregnancy outcomes in women exposed to gabapentin and pregabalin: data from a population-based study. J Neurol Neurosurg Psychiatry. https://doi.org/10.1136/jnnp-2017-316143
22. Patorno E, Hernandez-Diaz S, Huybrechts KF, Desai RJ, Cohen JM, Mogun H, et al (2020) 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. https://doi.org/10.1371/journal.pmed.1003322
23. Perucca P, Scheffer IE, Kiley M (2018) The management of epilepsy in children and adults. Med J Aust. https://doi.org/10.5694/mja17.00951
24. Petroff OA, Hyder F, Rothman DL, Mattson RH (2000) Effects of gabapentin on brain GABA, homocarnosine and pyrrolidinone in epilepsy patients. Epilepsia. https://doi.org/10.1111/j.1528-1157.2000.tb00227.x
25. Pitetzis DA, Spilioti MG, Yovos JG. Yavropoulou MP (2017) The effect of VPA on bone: From clinical studies to cell cultures-The molecular mechanisms revisited. Seizure. https://doi.org/10.1016/j.seizure.2017.03.013
26. Prakash, Prabhu LV, Rai R, Pai MM, Yadav SK, Madhyastha S, et al (2008) Teratogenic effects of the anticonvulsant gabapentin in mice. Singapore Med J 49:47-53
27. Reyes Fernandez PC, Wright CS, Warden SJ, Hum J, Farach-Carson MC, Thompson WR (2022) Effects of Gabapentin and Pregabalin on Calcium Homeostasis: Implications for Physical Rehabilitation of Musculoskeletal Tissues. Curr Osteoporos Rep. https://doi.org/10.1007/s11914-022-00750-x
28. Rocha S, Ferraz R, Prudêncio C, Fernandes MH, Costa-Rodrigues J (2019) Differential effects of antiepileptic drugs on human bone cells. J Cell Physiol. https://doi.org/10.1002/jcp.28569
29. Rose MA, Kam PCA (2002) Gabapentin: pharmacology and its use in pain management. Anesthesia. https://doi.org/10.1046/j.0003-2409.2001.02399.x
30. Sadeghi F (2014) Two separated protocols with the most important comments for skeletal staining in embryonic and adulthood period in laboratory animals. Anatomical Sciences 11:87–92
31. Shen C, Chen F, Zhang Y, Guo Y, Ding M (2014) Association between use of antiepileptic drugs and fracture risk: A systematic review and meta-analysis. Bone. https://doi.org/10.1016/j.bone.2014.04.018
32. Shihman B, Goldstein L, Amiel N, Benninger F (2019) Antiepileptic drug treatment during pregnancy and delivery in women with epilepsy—a retrospective single center study. Epilepsy Res. https://doi.org/10.1016/j.eplepsyres.2018.11.010
33. Sills GJ (2006) The mechanism of action of gabapentin and pregabalin. Current Opinion in Pharmacology. https://doi.org/10.1016/j.coph.2005.11.003

34. Simko J, Karesova I, Kremlacek J, Eva Z, Horacek J, Fekete S, et al (2019) The effect of gabapentin and pregabalin on bone turnover and bone strength: A prospective study in Wistar rats. Pharmacol Rep. https://doi.org/10.1016/j.pharep.2019.08.004
35. Tsukahara H, Kimura K, Todoroki Y, Ohshima Y, Hiraoka M, Shigematsu Y, et al (2002) Bone mineral status in ambulatory pediatric patients on long-term anti-epileptic drug therapy. Pediatr Int. https://doi.org/10.1046/j.1442-200x.2002.01561.x
36. Verrotti A, Coppola G, Parisi P, Mohn A, Chiarelli F (2010) Bone and calcium metabolism and antiepileptic drugs. Clin Neurol Neurosurg. https://doi.org/10.1016/j.clineuro.2009.10.011
37. Woodruff TJ, Zota A, Schwartz JM (2011) Environmental chemicals in pregnant women in the United States: NHANES 2003–2004. Environmental Health Perspectives. https://doi.org/10.1289/ehp.1002727

Figures

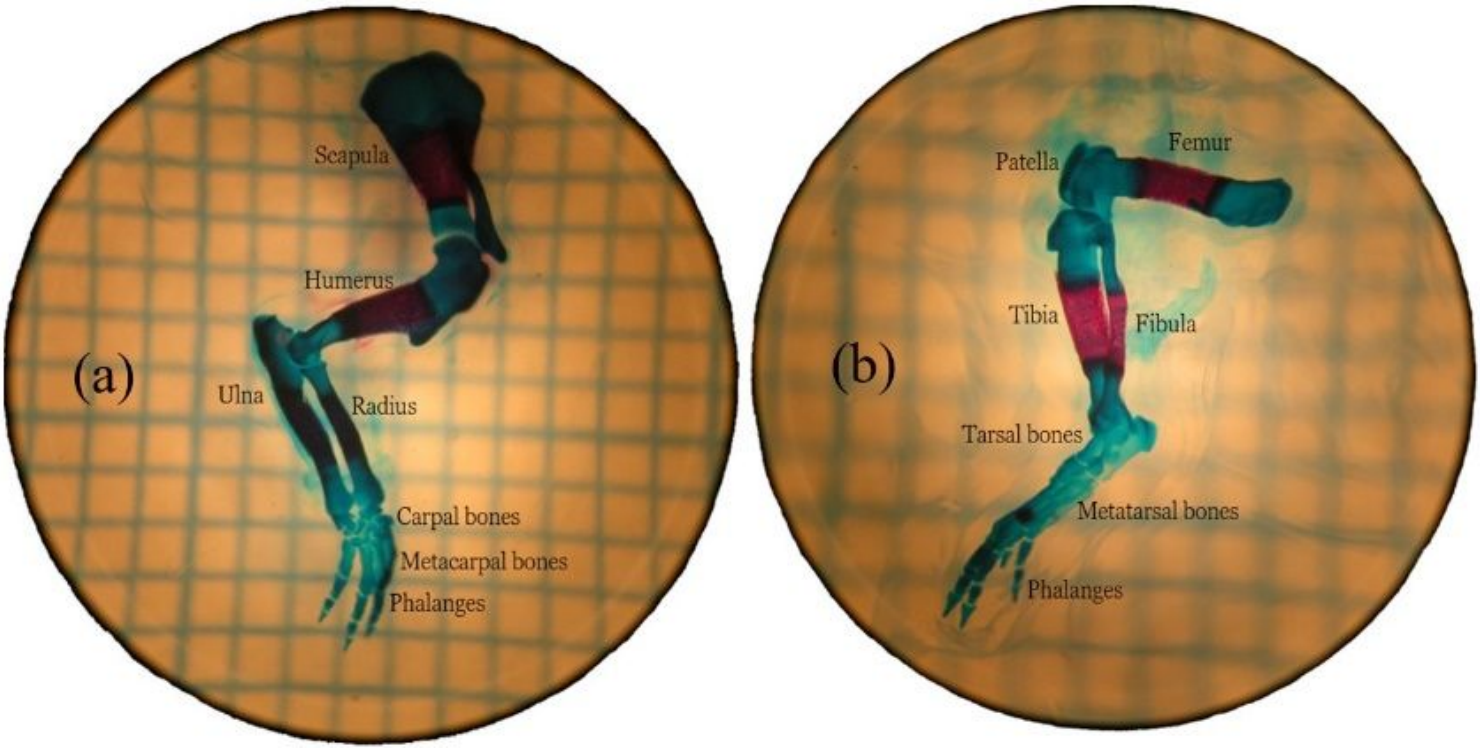

Figure 1

Images of forelimb (a) and hindlimb (b) in the control group on squared paper.

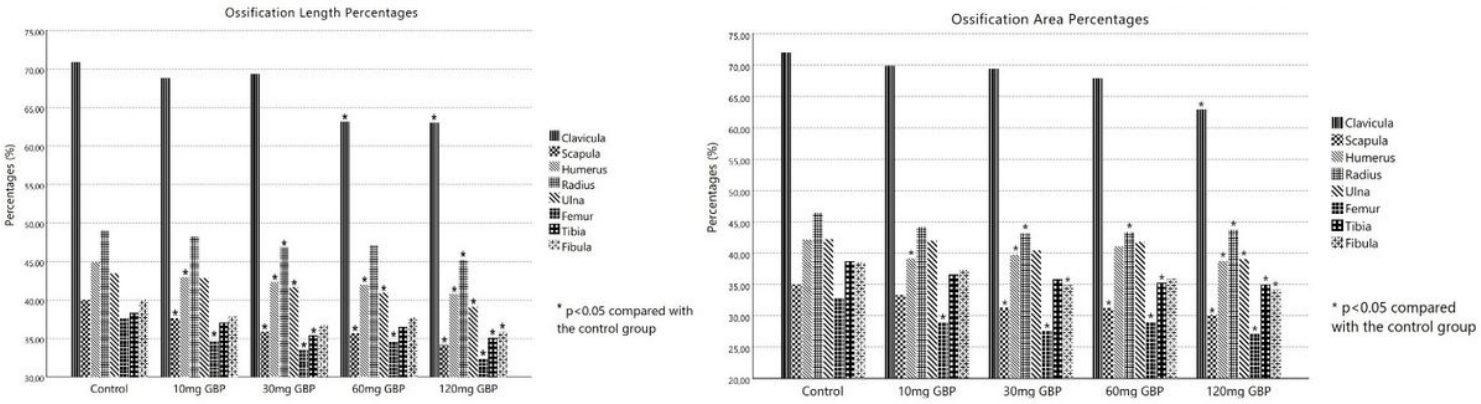

Figure 2

Comparison of the length and area of ossification zones between groups.

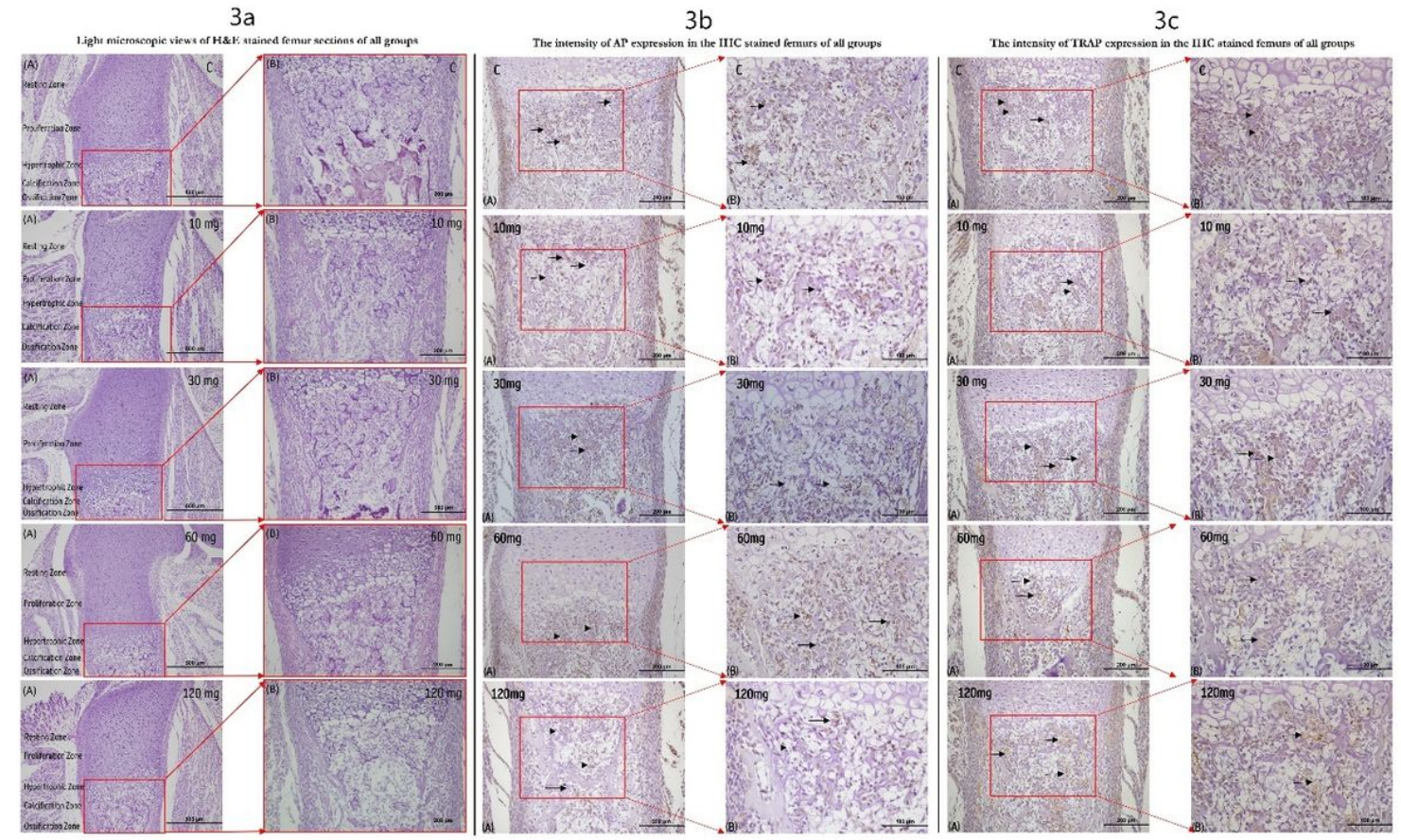

Figure 3

(3a): Histological zones of a control femur identified by IHC, (3b): Arrows indicate the intensity of AP expression in all groups, (3c): Arrows indicate the intensity of TRAP expression in all groups [(A): x20, (B): x40].

Supplementary Files

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- GraphicalAbstract.jpg