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Investigation of Teratogenic Effects of Letrozole on Fetal Bone Development

Investigación de los Efectos Teratogénicos de Letrozol en el Desarrollo Óseo Fetal

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SUMMARY: Letrozole is mainly used for the treatment of unexplained infertility, breast cancer and polycystic ovarian syndrome, with secondary use in ovarian stimulation. In cases of unexpected or unknown pregnancy during the use of letrozole, letrozole may cause a teratogenic effect on the fetus. In this reason, in this study, we aimed to determine the effect of letrozole on fetal bone development. In this study, 32 pregnant Wistar albino rats were used. The rats were divided into four groups: Control (saline) and high; 0.3 mg/kg, medium; 0.03 mg/kg, low; 0.003 mg/kg letrozole. Saline and letrozole were administered in 100 mL solutions by intraperitonaly from day 11 to day 15 of pregnancy. The skeletal system development of fetuses was examined with double skeletal staining, immunohistochemical staining methods and mineral density scanning electron microscopy. A total of 100 fetuses from female rats, 25 in each group, were included in the study. As a result of that, ossification rates were observed to decrease depending on the dose of letrozole in the forelimb limb (scapula, humerus, radius, ulna) and hindlimb (femur, tibia, fibula) limb bones. As a result of the statistical analysis, a statistically significant decrease was found in the ossification rates of all bones between the control group and low, medium, high letrozole groups ($p<0.001$). Exposure to letrozole during pregnancy adversely affected ossification and bone growth. However, the teratogenic effects of letrozole are unclear. Therefore, it needs to be investigated more extensively.

KEY WORDS: Double skeleton staining method; Letrozole; Ossification.

INTRODUCTION

Aromatase is an enzyme that is a microsomal member of the hemoprotein-containing cytochrome P-450 family. This enzyme converts 19-carbon androgens to 18-carbon aromatic estrogenic steroids as a result of three sequential hydroxylation reactions. Aromatase inhibitors were first used in the late 1990s, in the early stages of the menstrual cycle. Aromatase enzyme converts androstenedione to estrone and testosterone to estradiol, showing its activity on the ovaries, brain, placenta, adipose tissue, muscle, liver, breast tissue and skeletal system (Casper & Mitwally 2006).

Calcitonin, calciotropic hormones, growth hormone, thyroid and sex hormones (testosterone and estrogen) are important regulators that play an important role in skeletal metabolism and bone growth. In particular, estrogen has an increasing effect on both the plasma calcium level and the calcitonin level (Atay *et al*., 2019).

Letrozole is a non-steroidal selective third generation aromatase inhibitor that blocks the rate-limiting step in estrogen production from androstenedione and testosterone substrates. By providing estrogenic negative feedback of the hypothalamic pituitary axis, it causes an increase in gonadotropin secretion and ovarian follicular stimulation, and exerts its effect by blocking estrogen production from all sources (Mason *et al*., 1989). The spectrum of use in the clinic is wide and it is especially used in the treatment of diseases such as breast cancer, unexplained infertility, polycystic ovary syndrome (Mauri *et al*., 2006). It is often preferred by obstetricians to provide ovarian stimulation because its inhibitory effects are much higher than other aromatase inhibitors. Letrozole has no significant active metabolites and is excreted by the liver from the systemic circulation (Casper & Mitwally, 2006).

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The use of Letrozole in unexpected or unknown pregnancy may cause teratogenic effects on the fetus. This situation was seriously discussed at the meeting of the American Society for Reproductive Medicine in 2005, and the safety of the fetus in mothers using letrozole was found to be of concern (Biljan *et al*., 2005). In addition, although letrozole is in category D in the Food and Drug Administration (FDA) classification, it has been reported in the literature that letrozole is not directly associated with general congenital anomalies in fetuses but the incidence of heart and bone anomalies increases in correlation with the use of letrozole (Forman *et al*., 2007; Bajpai *et al*., 2010; Pérez-Saborido *et al*., 2012; Pouliot *et al*., 2013; Lima *et al*., 2014). It has been reported that estrogen adversely affects osteoblastic/ osteoclastic balance, especially by having an adverse effect on bone development (Kumru *et al*., 2007; Imai *et al*., 2009). Based on this information, our aim in our study is to investigate the teratogenic effects of letrozole on fetal bone development by double skeletal staining, immunohistochemical staining (IHC) and bone mineral analysis.

MATERIAL AND METHOD

In this study, 32 adult female rats, 4-7 months old, Wistar albino breed, weighing between 200-250g and not used in any study before, were used. Rats were purchased from Afyon Kocatepe University (AKU) Experimental Animals Application and Research Center and were housed in ventilated isolator cages with ad libitum access to food and water.

The female rats used in the study were mated with male rats to get pregnant. Females with spermium in the examination of vaginal smear samples after mating were accepted as 0.5 days pregnant and the rats were randomly divided into 4 groups (n=8). The number of pregnant rats in each group was determined by considering the possible losses in scanning electron microscopy (SEM) and Energy dispersive X-Ray (EDX) analyzes, double skeletal and IHC staining, dissection procedures, and the desire to measure at least 25 fetuses in the groups.

For groups to be applied letrozole (Chempur; FL36495-1G), Pouliot *et al*. (2013) study referenced. Accordingly, the study groups; control saline (0.9 % NaCl) and low dose 0.003 mg/kg/day letrozole, medium dose 0.03 mg/kg/day letrozole, high dose 0.3 mg/kg/day letrozole were determined. Letrozole was dissolved in 0.1 % DMSO and a stock solution was obtained and diluted with distilled water according to the determined dose groups. The prepared solutions were injected intraperitoneally (ip) once a day at 4 pm in volumes of 1 ml from the 11th to the 15th day of pregnancy. Pregnant rats were sacrificed on the 20th day of their pregnancy. Fetuses of rats in all groups were randomly distributed for double skeletal staining, IHC and SEM+EDX analysis. Preliminary protocols for dual skeleton staining and IHC were applied in the studies specified in the literature (Atay *et al*., 2019; Suna *et al*., 2021). Photos of anterior and posterior limb bones were taken for morphometric measurements of fetuses whose bone and cartilage structures were determined by double skeleton staining method. After transferring the photos to the computer, morphometric measurements of bone and cartilage areas were made using ImageJ software program. Results were evaluated in millimeters (mm). The ratio of the ossification area to the whole bone was determined as its length and surface area. Alkaline phosphatase (AP) and tartrate resistant acid phosphatase (TRAP) expression densities were examined in 5 μm slices taken from the fetus femurs by the light microscope (Nikon E600W, Image Analysis Program NIS elements, Japan). SEM+EDX analysis were performed at AKU Technology Application and Research Center (TARC). The analysis of specimens from each experimental groups and control group were performed to determine the mineral content of the bones. SEM (LEO 1430 VP) analysis focused on the head of each femoral bone under the same voltage, current, working distance and pressure conditions. In EDX analysis, the distributions and concentrations of calcium (Ca), phosphorus (P), sodium (Na), magnesium (Mg), Carbon (C) and oxygen (O) in bone tissues were examined.

Housing conditions and experimental procedures were approved by the ethical committee of animal experimentation (AKU- 9533702/267-03.06.2020).

Statistical analysis. Statistical analysis was performed with the IBM Statistical Package for Social Sciences (SPSS) Statistics 20.0 program. The normal distribution of data was analyzed with the Shapiro Wilk test. After it was determined that the data were not homogeneously distributed, the Kruskal-Wallis test was used to determine the differences between multiple groups, and the Mann-Whitney U test was used to compare the paired groups. $p<0.05$ were considered statistically significant. Data were presented as mean±standard deviation (M±SD).

RESULTS

Growth Parameters. The height and weight of the fetuses were determined before starting the staining process in all groups. When height and weight measurements were compared between the groups, a statistically significant

decrease was found in all experimental groups depending on the dose ($p<0.05$). Weight and height measurement parameters are shown in Table I.

Double Skeleton Staining Method. In this study, measurements were made on the scapula, humerus, radius and ulna in the anterior limb, and on the femur, tibia and fibula in the posterior limb. Total bone length, length of ossification zone, total bone surface area and surface area of ossification zone of bones were measured. Ossification percentage of bones was calculated using area and length measurement data. Bilateral bone analysis was performed on each fetus. A total of 30 limbs were measured, both right and left. M±SD and p values are presented in Tables II and III.

Measurements of anterior limb bones: A proportional decrease was determined in the length and area percentages and total bone length measurements of the areas exhibiting ossification in all anterior limb bones, depending on the dose increase from the control group to the high dose group (Fig. 1). While decrease of anterior limb total bone lengths were statistically significant between the control group and all letrozole groups,

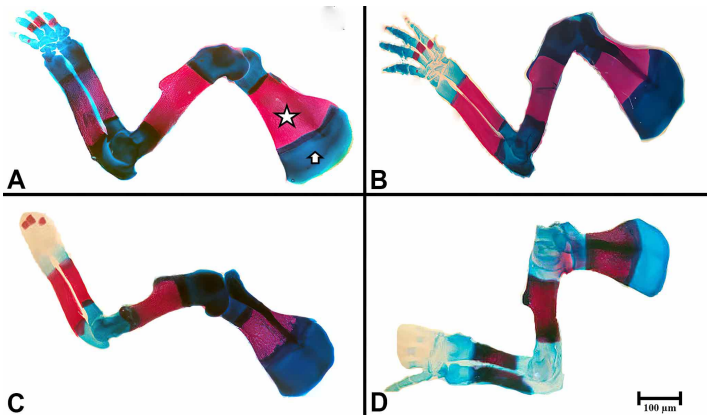

Fig. 1. Images of the anterior limb bones. A; Control, B; low dose, C; medium dose, D; High dose, Star; Ossified area, Arrow; cartilage area, (The area stained with red color (Alizarin Red-S) on the images shows the ossification area, and the area stained with blue color (Alcian Blue) indicates the cartilage area).

Table I. Weight and lengths measurements of the experimental groups.

Group	Control	Low	Medium	High	p value
Weight	2.47±0.15	2.18±0.7[a]	2.04±0.32[a]	1.98±0.29[a]	< 0.001
Lengths	46.14±9.48	35.02±2.15[a]	36.7±2.85[a]	36.16±4.41[a]	< 0.001

*Weight units are expressed in grams and length units in millimeters. a; There is a statistically significant difference between the control group and the control group.

Table II. Double skeletal staining measurements of anterior limb bones.

Groups	Scapula		Humerus		Radius		Ulna	
	Length Percentage	Area Percentage	Length Percentage	Area Percentage	Length Percentage	Area Percentage	Length Percentage	Area Percentage
Control	63.07±10.28	54.58±8.63	56.1±4.52	56.04±7.71	62.94±9.52	60.24±17.95	65.12±21.31	61.13±8.71
Low	50.68±6.65[a]	41.37±5.69[a]	48.03±5.98[a]	48.99±6.1[a]	54.64±5.95[a]	53.91±7.74	50±5.76[a]	50.11±6.8[a]
Medium	41.7±4.93[b]	38.54±4.73[a]	45.42±3.61[a]	44.82±4.17[a]	53.25±4.81[a]	49.14±6.81[a]	45.35±6.19[a]	44.66±8.05[a]
High	40.48±4.27[ab]	37.54±5.48[a]	41.74±5.24[ab]	40.27±4.25[a bc]	47.77±5.55[abc]	45±7.75[ab]	43.42±5.62[a b]	40.98±7.34[ab]
p value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

a; There is a statistically significant difference between the control group and the control group. b; There is a statistically significant difference between the low dose group and the low dose group. c; There is a statistically significant difference between the medium dose group.

Table III. Double skeletal staining measurements of posterior limb bones.

Groups	Femur		Tibia		Fibula	
	Length Percentage	Area Percentage	Length Percentage	Area Percentage	Length Percentage	Area Percentage
Control	50.02±5.28	47.5±6.65	60.17±5.72	59.25±7.92	59.46±8.45	54.99±10.5
Low	42.19±6.0[a]	37.93±5.48[a]	48.26±8.99[a]	45.54±9.11[a]	43.58±8.31[a]	42.09±9.1[a]
Medium	37.81±4.95[a]	33.03±4.94[a]	42.87±5.92[a]	39.46±8.77[a]	41.19±7.68[a]	37.78±10.7[a]
High	32.82±5.11[a b]	28.81±5.93[a b]	37.27±6.7[ab]	35.5±6.91[ab]	30.99±7.93[abc]	31.42±10.52[ab]
p value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

a; There is a statistically significant difference between the control group and the control group. b; There is a statistically significant difference between the low dose group and the low dose group. c; There is a statistically significant difference between the medium dose group.

and the statistical differences were also determined in some bones between the low dose group and the medium and high dose groups ($p<0.001$). In addition, when the area and length ratios of ossification areas in all anterior limb bones were evaluated the statistically significant decrease was determined in all letrozole groups compared to the control group; while at the same time a significant decrease was determined between low and the high dose letrozole groups ($p<0.001$).

Measurements of posterior limb bones: When the posterior limb bones were examined, it was determined that there was a statistical decrease in all bone measurement parameters in all letrozole groups, depending on the dose increase, as in the anterior limb bones (Fig. 2). Immunohistochemistry Staining method. In our study, AP (metabolic marker of bone formation) and TRAP (metabolic marker of bone destruction) densities in the femurs of fetuses belonging to 4 groups were evaluated by H-Score method. When the IHC slices of the control group were examined microscopically, there was intense and severe AP and TRAP expression in the ossification areas (Figs. 3 and 4). As a result of the evaluation, it was determined that AP and TRAP expression decreased as the letrozole dose increased, and there were statistically significant differences between the control group and the other groups ($p<0.05$, Table IV).

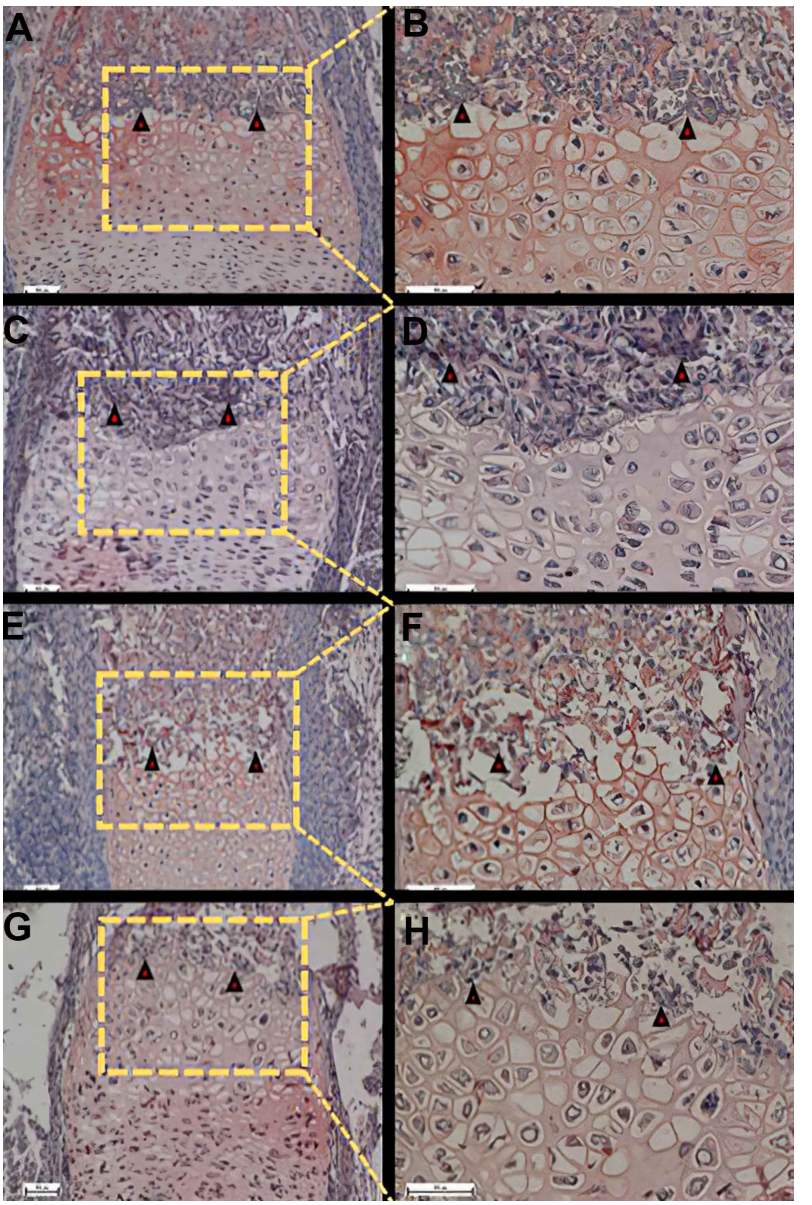

Fig. 3. Staining of AP immunoreactivity intensity according to the experimental groups by IHC method. A; Control, C; low dose, E; medium dose, G; High dose, AP; alkaline phosphatase, Arrow; AP expression.

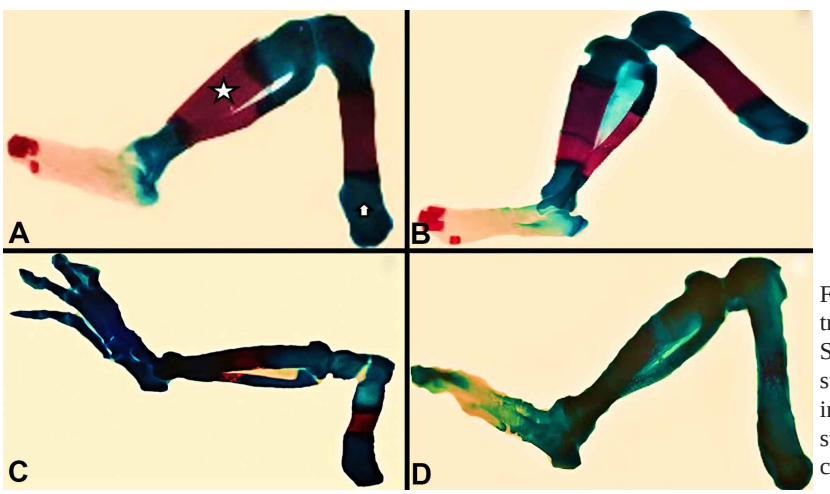

Fig. 2. Images of the posterior limb bones. A; Control, B; low dose, C; medium dose, D; High dose, Star; Ossified area, Arrow; cartilage area, (The area stained with red color (Alizarin Red-S) on the images shows the ossification area, and the area stained with blue color (Alcian Blue) indicates the cartilage area).

Table IV. H score means of AP expression.

aGroup	Control	Low	Medium	High	p value
AP	496.0±3.74	431.8±4.32[a]	431.4±7.18[a]	160.6±11.35[a]	< 0.001
TRAP	486.9±4.89	234.9±7.85[a]	234.5±9.58[a]	209.2±8.28[a]	< 0.001

*AP; alkaline phosphatase, TRAP; tartrate resistant acid phosphatase.a; There is a statistically significant difference between the control group and the control group.

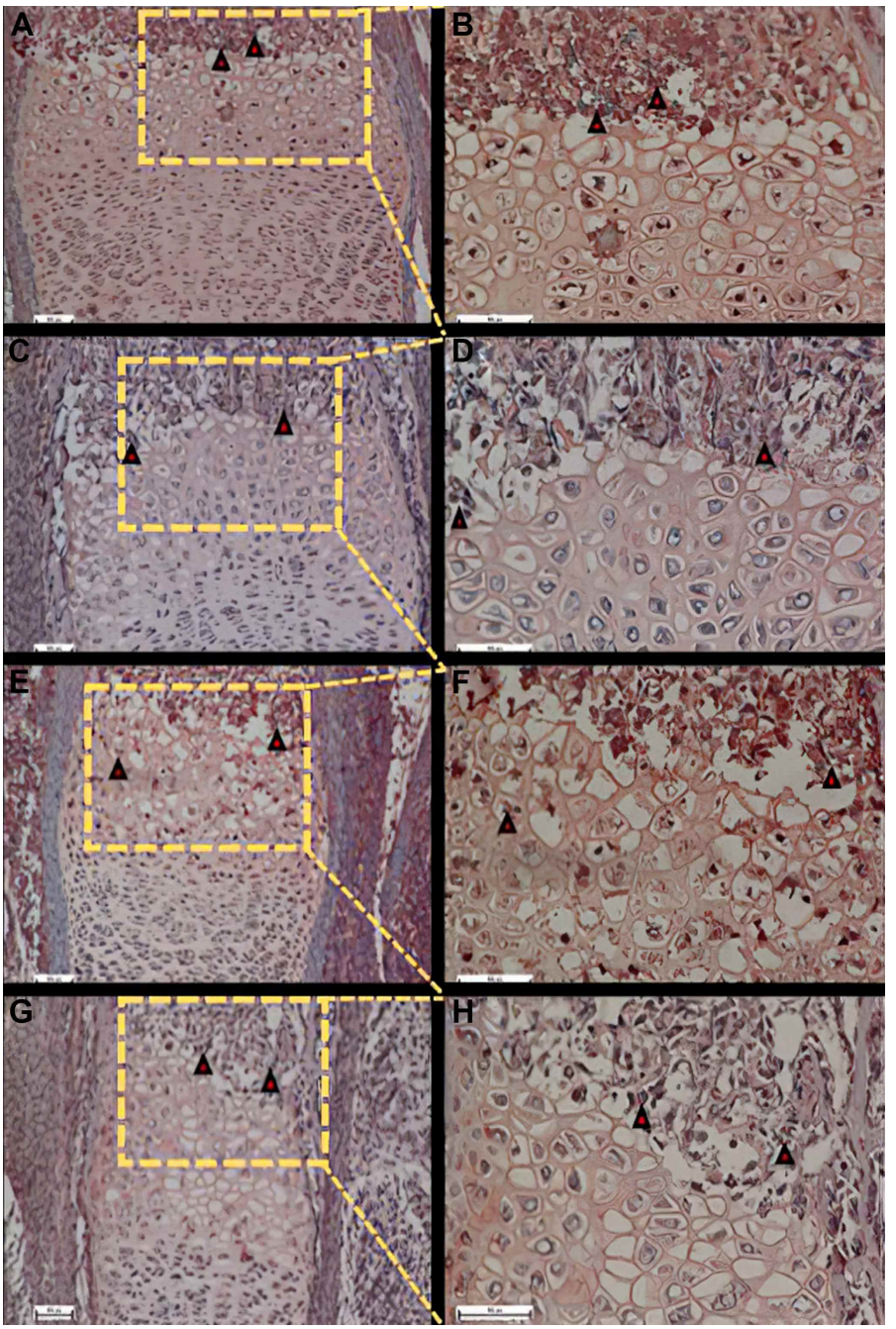

Fig. 4. Staining of TRAP immunoreactivity intensity according to the experimental groups by IHC method. A; Control, C; low dose, E; medium dose, G; High dose, Arrow; TRAP expression, TRAP; alkaline phosphatase, IHC; Immunohistochemistry.

Scanning Electron Microscopy and Energy dispersive X-Ray Method. In SEM examinations, 8 head of femoral bones from each group were morphologically examined. When the bone structures in the experimental groups were examined, a thin layer of compact bone was observed in the outer part of the femoral head, while in the middle part there was spongy bone consisting of trabeculae When the structural differences between compact and spongy bone structures were evaluated according to the applied doses, it was observed that the appearance of the compact bone in all experimental groups was the same. However, it was determined that the trabecular spicule surfaces in the spongy bone decreased from the control group to the high dose group, and the spaces between the spicules increased. Especially in the high dose group, it was observed that the bone spicules regressed, decreased in number and the spaces between them merged to form large spaces (Fig. 5).

When the mineral density in the femoral heads was analyzed, it was determined that Ca, Na, P minerals were statistically significantly decreased in the letrozole groups compared to the control group, inversely proportional to the dose increase ($p<0.05$). However, the same statistical results were not determined for C, O, Mg minerals ($p>0.05$). Statistically significant differences between the groups were found between the control group and all letrozole groups (Table V).

Table V. Mineral density measurements.

Groups	Ca	C	Na	Mg	P	O_2
Control	13.16±2.64	31.94±4.33	1.02±0.92	0.46±0.18	6.33±1.15	39.44±14.73
Low	3.62±1.19[a]	32.58±7.78	0.31±0.27[a]	0.24±0.23	1.76±1.02[a]	41.12±3.43
Medium	2.18±0.63[a]	36.98±5.53	0.27±0.18[a]	0.56±0.65	1.12±0.30[a]	45.15±3.76
High	1.41±0.35[a]	34.20±5.44	0.18±0.15[a]	0.59±0.34	0.84±0.22[a]	47.42±3.62
p value	<0.001	0.461	0.016	0.026	<0.001	0.24

Ca; Calcium, C; Carbon, Na; Sodium, Mg; Magnesium, P; Phosphorus, O2; Oxygen. a; There is a statistically significant difference between the control group and the control group.

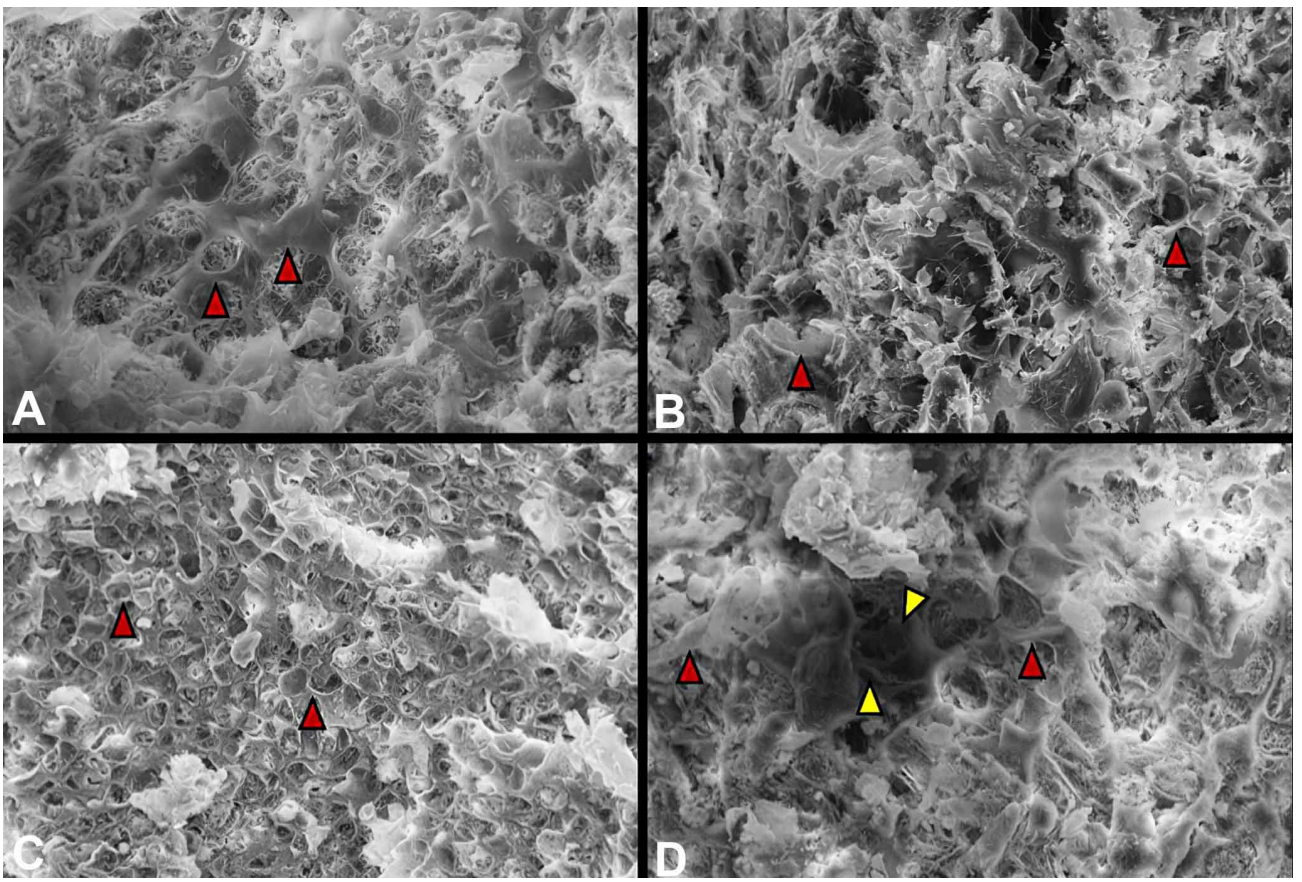

Fig. 5. Images of the head of the femoral bone taken with SEM according to the experimental groups. A; Control, B; low dose, C; medium dose, D; High dose, Red arrow; trabeculae spicules, Yellow arrow; wide spaces between trabeculae, SEM; Scanning electron microscope.

DISCUSSION

Aromatase inhibitors have also been used in pediatric practice for the treatment of peripheral precocious puberty, congenital adrenal hyperplasia, and short stature (Hero *et al*., 2010; Zhao & Zhang, 2014). However, it is mostly used in the treatment of breast cancer, unexplained infertility, polycystic ovary syndrome and ovarian simulation (Mauri *et al*., 2006). Letrozole is a competitive non-steroidal aromatase inhibitor that reduces circulating estrogen by inhibiting the conversion of androgens to estrogens in peripheral tissues (Cohen *et al*., 2002). Although it is not an important active metabolite and is excreted from the systemic circulation by the liver, exposure to letrozole at doses equal to or lower than the recommended daily dose in humans may have teratogenic effects on gestational development (Tiboni *et al*., 2008). Therefore, in our study, the effects of letrozole on intrauterine skeletal development at lower and higher exposure levels, together with the recommended therapeutic dose for humans, were investigated.

In our study, Pouliot *et al*. (2013) study examining the development of postpartum rats as a result of the use of letrozole in pregnant rats was taken as a reference in the adjustment of letrozole doses. In this study, it was determined that there was a decrease in body weight and crown-rump length of the juvenile rats, as well as a decrease in growth at all dose levels during the dosing period. In addition, in the study by Bajpai *et al*. (2010) in which they applied 1 mg/kg letrozole daily to peripubertal male rats and examined the long and short-term effects of letrozole, developmental retardation was found in the weight and growth of letrozole administered rats. However, in the study of Hero *et al*. (2010) in which they evaluated the effects of 2.5mg/day letrozole on growth and bone development in prepuberty children, they reported that letrozole did not cause any change in body composition. Contrary to this situation, in the study of Mohamed & Yeh (2009) in which they administered 2.5mg/kg letrozole to young adult female rats, it was stated that the rats increased in body weight and length. In our study, the effects of different doses of letrozole on pregnancy rats were investigated, and the effects on the fetuses in the organogenesis period were evaluated, and the lengths and weights of the fetuses were evaluated. As a result of our study, it was determined that fetal growth decreased significantly with the increase in letrozole dose in accordance with the literature. This contradiction between our results and some literature study results can be explained by the differences in the selected sample, the doses administered, and the methodology determined.

Double skeletal staining is an important method used in teratogenic research in the embryonic and postnatal period. With this method, the development levels of the skeletal system in fetuses are determined. In addition, the effects of various chemicals and drugs on bone development have been investigated by this method in the literature (Atay *et al*., 2019; Suna *et al*., 2021). In this staining method, Alizarin Red-S and Alcian Blue are used to distinguish between mineralized bone tissue and non-mineralized cartilage tissue in the skeleton of fetuses (Menegola *et al*., 2001). In our study, the effects of letrozole on fetal bone development were evaluated using the double skeleton staining method, and the area and length ratios of the regions exhibiting ossification were evaluated. It was determined that there was a statistically significant decrease in ossification areas depending on the letrozole dose in the experimental groups. In addition, the same statistical results were determined in all bone lengths of the fetuses. According to these results, our study showed that letrozole delays ossification and negatively affects bone growth. As far as we have researched in the literature, while there is no study examining the effect of letrozole on fetal bone development; it has been determined that the scope of the studies examined in the literature review is concentrated in the direction of bone length and ossification in prepubertal and pubertal subjects. Pouliot *et al*. (2013) and Mohamed & Yeh (2009) study on young rats, an increase in bone length and ossification area of female rats was observed in both studies. In addition, in the study of Pouliot *et al*. (2013), it was determined that, unlike females, male rats had a decrease in bone length and ossification area. In the study of Bajpai *et al*. (2010) on peripubertal male rats, a decrease in bone length and ossification rate was found in male rats depending on the letrozole dose, consistent with the study of Pouliot *et al*. (2013). In the studies of Li *et al*. (2019), 0.5 mg/day of letrozole was given orally to laying hens for 9 weeks, but no statistical difference was found in bone morphometry. Apart from experimental animal studies, Hero *et al*. (2010) and Zhao & Zhang (2014) examined the effects of long and short-term use of letrozole in short children under 13 years of age and stated that the bone height increased as a result of the studies. When the above-mentioned literature studies on the use of letrozole are examined, the results about letrozole are contradictory, and it has been stated in some studies that it has a beneficial effect on bone development and some adverse effects. The variability of the dose and age ranges determined in the studies and the application of letrozole on different living species can be shown as the source of the conflicts between the results of the above-mentioned studies.

In the literature, it has been stated that human and rodent bone osteoblast activity expresses the aromatase gene and estrogen induces osteoclast apoptosis in trabecular bones (Saki *et al*., 2019). Therefore, in our study, the intensity of AP and TRAP expression was analyzed as determinants of osteoblastic and osteoclastic activity, which are responsible for the architecture of bone geometry. Depending on the results of the analysis, it was determined that AP expression and TRAP density decreased as the letrozole dose increased. In the literature, in the study of Li *et al*. (2019), the TRAP density in the laying hens' bones was analyzed biochemically and it was found that the TRAP density was decreased in the letrozole-treated group. In their study on female rats, Kumru *et al*. (2007) showed that serum estrogen level decreased bone mass through estrogen receptors on both osteoclasts and osteoblasts. In addition, in Batra *et al*. (2003)'s study examining the expression of ER-a and ER-b on serial sections of human fracture callus, it was stated that estrogen expresses both osteoblasts and osteoclasts at the mRNA level. These changes in bone geometry are due to the suppression of osteoblastic and osteoclastic activities. Because changes in estrogen level cause deterioration of osteoblastic and osteoclastic balance and negatively affect bone development (Imai *et al*., 2009; Bajpai *et al*., 2010; Saki *et al*., 2019). As a matter of fact, the decrease in AP and TRAP density determined in our study and the decrease in

ossification percentage rates is one of the most important indicators of this situation.

Throughout adult life, estrogen concentration plays an important role in bone remodeling to maintain mineral content and strength in both men and women. As clinically in postmenopausal women, bone mineral loss is inevitable due to estrogen deficiency (Mohamed & Yeh, 2009). When the femur bones in our study were examined by SEM, it was observed that the trabecular volumes in the bones decreased from the control group to the high dose group, and the spaces between the trabeculae increased.

When the mineral density was analyzed according to the experimental groups, it was determined that Ca, Na and P minerals decreased in the letrozole groups depending on the dose increase. In the literature, in the dual X-ray absorptiometry studies of Goss *et al*. (2004) on ovariectomized rats and Mohammed & Yeh (2009) on female rats, a decrease in bone mineral density was observed in the groups to which letrozole was administered. The decrease in bone mineral density can be explained by the decrease in estrogen levels in fetuses due to letrozole exposure. Because estrogen has three main effects on bone metabolism. First, the decrease in estrogen causes an increase in the apoptosis of osteocytes and therefore a decrease in bone turnover. Second, estrogen inhibits differentiation and decreases bone resorption by increasing apoptosis of osteoclasts. Third, estrogen inhibits osteoblast apoptosis and provides bone formation at the cellular level (Juul, 2001). Considering the effects of estrogen mentioned above, we think that letrozole may have negative effects on bone mineral density and bone morphometry due to its suppressive effect on estrogen level. Contrary to these studies, in the dual X-ray absorptiometry study of Wickman *et al*. (2003) on adolescent children, an increase in bone mineral density was determined as a result of letrozole combined with testosterone. In addition, in the dual X-ray absorptiometry studies of Bajpai *et al*. (2010) on peripubertal male rats and Kumru *et al*. (2007) on female rats, it was stated that bone mineral density was preserved. Considering all these study results, there is no consensus on the effect of letrozole application on bone density. Although the same analysis method was used in several of the literature studies in the analysis of the mineral density of bones, different results were obtained. Although these results are of course affected by the variables in the selected samples, they do not compare the mineral contents in detail. In this context, our study has the most reliable quantitative parameter as a result of detailed calculation of bone mineral contents. For this reason, we think that the result of our study provides the most effective view on the effect of letrozole on mineral density by eliminating the existing disagreements in the literature.

As a result of our study, in the fetuses of pregnant rats applied letrozole, in proportion to the dose; decrease in birth weight, shortening in stature, delay in bone development and accordingly decrease in ossification protein expressions and bone mineral density were detected. These results were presented separately by double skeleton staining, IHC and SEM+EDX analysis methods. It is a valuable research article that provides comprehensive information to the literature on these aspects of our study. The multiplicity of evaluation parameters and the results obtained supporting each other reveal the strengths of our study. Based on this information, we can definitively say that letrozole disrupts bone metabolism and disrupts the stages of bone formation and destruction. In addition, we think that our study data will be an important source and pioneering information in terms of examining the teratogenic effects of letrozole on the development of the skeletal system of fetuses due to the use of letrozole during pregnancy.

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RESUMEN: Letrozol se usa principalmente para el tratamiento de la infertilidad inexplicable, el cáncer de mama y el síndrome de ovario poliquístico, con estimulación ovárica de uso secundario. En casos de embarazo inesperado o desconocido durante el uso de letrozol, puede causar un efecto teratogénico en el feto. Por esta razón, en este estudio, nuestro objetivo fue determinar el efecto de letrozol en el desarrollo óseo fetal. Se utilizaron 32 ratas albinas Wistar preñadas las cuales se distribuyeron en cuatro grupos: Control (solución salina) y alta; 0,3 mg/kg, medio; 0,03 mg/kg, bajo; 0,003 mg/kg de letrozol. Se administró solución salina y letrozol en soluciones de 100 mL por vía intraperitoneal desde el día 11 hasta el día 15 de la preñez. El desarrollo del sistema esquelético de los fetos se examinó con tinción esquelética doble, métodos de tinción inmunohistoquímica y microscopía electrónica de barrido de densidad mineral. Se incluyeron en el estudio un total de 100 fetos de ratas hembra, 25 en cada grupo. Como resultado, se observó que las tasas de osificación disminuían dependiendo de la dosis de letrozol en los huesos de los miembros torácicos (escápula, húmero, radio, ulna) y de las miembros pélvicos (fémur, tibia, fíbula). Se encontró una disminución estadísticamente significativa en las tasas de osificación de todos los huesos entre el grupo control y los grupos de letrozol bajo, medio y alto ($p<0,001$). La exposición a letrozol durante la preñez afectó negativamente la osificación y el crecimiento óseo. Sin embargo, los efectos teratogénicos del letrozol no están claros por lo que debe ser investigado más extensamente.

PALABRAS CLAVE: Método de tinción de doble esqueleto; Letrozol; Osificación.

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