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Histomorphometric Study on the Effect of Propiconazole on Bone Growth Plate in Male Rats

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Abstract

Background and Purpose: Propiconazole is a systemic fungicide from the group of triazoles that is used to control a wide range of diseases. This poison causes the cellular, genetic and metabolic damage to the animals. The bone is a hard tissue that its contents are constantly changing. The longitudinal growth of the bone is due to the growth plate, which is a cartilaginous structure at the end of the long bones of the body. During sexual maturation, the growth of the growth plate stops the longitudinal growth of the bone. The purpose of this study was to evaluate the effect of propiconazole venom on growth plate expansion (including proliferation and hypertrophy cells) in immature rats.Materials and Methods: This experimental study was performed on 12 male Wistar rats randomly divided into two groups of control and propiconazole. Treatments were performed as oral gavage and for 28 days. On the 28th day, the animals were sacrificed and the left thigh bone was removed for histomorphometric study of the width of the thoracic appendix. The studies were performed by Rasband Wayne, 40g.1 ver, ImageJ, USA, NIH, and the results were significant by ANOVA ANOVA and test s'Tukey.Findings**:** Growth plaque width in the propiconazole group was significantly (P = 0.0626) less than that of the control group, which reduced the width of the reproductive area (P <0.001) and increased the width of the hypertrophied area (P=0/016).Results: Propiconazole Leads reduction of the width of the apple peptide growth plate in the immature rat rats and can be a factor in the disruption of the longitudinal growth pattern of the bone and the premature closure of the growth plate.

Keywords: Growth plaque, bone tissue, propiconazole, Oxidative stress, Rat

Introduction

The active and dynamic tissue of the bone tissue is that its internal microscopic structure is continuously changed by bone cells (osteoblasts and osteoclasts) and the development of plate growth, a remodeling (Upledger, 2005). A highly organized cartilage between the epiphyseal bone and The diaphyses are at the end of the long bones that divide into the horizontal regions of the chondrocytes in

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different stages of differentiation (proliferative cells and hypertrophied hypertrophied cells).(Fattahi, Jorsaraei and Gardaneh, 2012) The longitudinal growth of the bone is the result of the proliferation and differentiation of the growth chondrocytes affected U Gene factors are hormones, growth factors, environments, and nutrition. Growth plaques are associated with the closed sexual maturity (bone marrow chondrocytes matrix) and the longitudinal growth of the bone ends. Growth plaques in mice for a prolonged period until after puberty and maybe Propiconazole is a systemic fungicide from the group of thriazoles that is used to control a wide range of fungal diseases in agriculture such as rice blisters, wheat rust and wheat Fusarium spp. (Battaglin et al., 2011). Propiconazole in Animals and various types of tissues have been toxic and have a wide range of Non-lethal doses of its biochemical unconscionable and can cause cell damage, genetic and environmental factors such as toxicity Grdd.az can damage the nervous system, liver cells, kidney cells, germ cells and gonads pointed out. Few studies have been conducted on the effect of propiconazole on bone and cartilage tissue in the skeletal system. (Hester et al., 2012; Allen et al., 2006) During the study of the effect of propiconazole on the chicken and queen's embryo, the teratogenic effects of this poison on cartilage and bone growth have been reported. (Taxvig et al., 2013) Also, in the study of the family who were mistakenly exposed to the toxin in the house, it was determined that propiconazole, in addition to neurotoxicity and endocrine, has had a damaging effect on the development of the skeletal system of children in this family, including delayed osteoporosis Bone growth retardation, bone growth in cysts, pathologic fractures, and lack of response to bone graft in the offspring of this family. (Barton et al., 2006) In response to the question of whether contact with propiconazole can lead to premature closure of the growth plate and subsequently delay or decrease the longitudinal growth of the bone, the present study was conducted with the aim of investigating the effect of propiconazole on apoptotic cartilage (growth plate) in rats Immature.

Data Analysis Method

This experimental study was carried out on 12 male Wistar rats (four to five weeks old) weighted 100 gr (taken from the Faculty of Pharmacy of Tehran University and transferred to the Animal Science Laboratory of the Faculty of Science of this university). Randomly the mice were subjected to the same and standard light conditions (12 hours of light and 12 hours of darkness), ventilation Suitable and temperature (22 ± 2 ° C) and standard water and food (chaw rat Standard) The concentration of propiconazole used in this study was based on the one-time trial of 11, 17, and 18 previous studies of 30 kg / mg. The 30 mg / kg dose and even the dose of 25, 15, and 10 kg / mg resulted in The death of animals was due to the low age and maturation of the mice tested and as a result of intolerance to the dosage they consume, so a dose of 5 mg / kg, dose and non-administering propiconazole was considered in this study. Shanghai Tosco Chemical Co., Shanghai (China), 95%, was used to prepare a dose of Propiconazole. Dilution was performed using C2V2 = C1V1 formula and corn oil as solvent. Propiconazole 0.5 ml / kg / mg for propiconazole and corn oil for control group were taken as oral gavage for 28 days at 10 am. At the end of this period, animals were killed in a decoction machine and in accordance with ethical principles, and the left femur was removed immediately after removal and for histological examination (histomorphometry of the growth plate) for cellular fixation in formalin 10% for a minimum of 24 Clock was placed. To soften the bone tissue (decalcification), 7% nitric acid solution was used for 5 days and daily solution was changed. After decalcification, to remove the effect of nitric acid from sodium sulfate solution 5% and to remove the effect of sodium sulfate from running water for 24 hours. Histomorphometric studies were performed on prepared slides from the growth fractal lipophilic area of the left femur and by hematoxylin-eosin staining (E & H) to study the histological changes of the treatments. In this staining, which is a general staining, the cores are blue to violet, and the cytoplasm and the conjugated strands are pinkish (Fig. 1).

To measure the width of the apex plasticium plaque in each group, the width was different in six sections of the group and in three regions of each section, using ImageJ software, Version 1.40g (Wayne Rasband, USA, NIH) and on the photograms The microscopic sections were measured (Fig. 2) and then the mean of these values was considered as the width of the apical growth plate in each group. The amplitude area of the cells and the area of the hypertrophied cells of the growth plate were similar in each group (Fig. 1). One-way ANOVA was performed using GraphPad Prism® software version 6 Jolla, CA, USA) La Jolla, CA 92037 USA, in the statistical calculations. The means were calculated as Mean ± SEM and compared with the T test comparisons multiple Tukey test. Significance level was considered as $P < 0.05$. Charts were also plotted using Microsoft Office Excel 2007 software.

Findings

The mean growth plate width, reproductive area, and hypertrophied area in the propiconazole group were 0.4278, 0.2317, and 2383.0 mm, respectively. These values in the control group were 0 9200/0, 0/3417 and 0/1950. Table 1.

The mean width of the apple peptide plaque in the experimental groups showed a significant decrease ($P = 0.016$) in the width of the growth plate in the propiconazole group compared to the control group (Fig. 3). Also, the mean width of the reproductive area of the apple peptic region The experimental groups showed a significant decrease ($P < 0.0001$) in the width of the area in the propiconazole group compared to the control group (Fig. 3). In the study, the mean width of the hypertrophied area of the apoptotic growth plate of the experimental groups increased significantly $P < 0.0166$). The width of this region was observed in the propiconazole group as compared to the control group (Fig. 3) .

Table 1: Growth Plate Width, Growth Plate Cell Growth Region and Growth Plate Hypertrophied Cell Area in Propiconazole Group And control group

The variables studied	Propiconazole group (Deviation Mean ± Measure)	Control group (Deviation Mean ± Measure)	P
The final Plate growth (mm)	4278/0 ± 1212/0	0 3787/0 ± 9200	0126/0
The area itself Cells the door Now Multiplication Plaque Growth (mm)	2317/0 ± 03125/0	3417/0 ± 02401/0	0001/0
The area itself Plaque hypertrophied cells Growth (mm)	2383/0 ± 02927/0	0.02258 ± 0.1950	0166/0

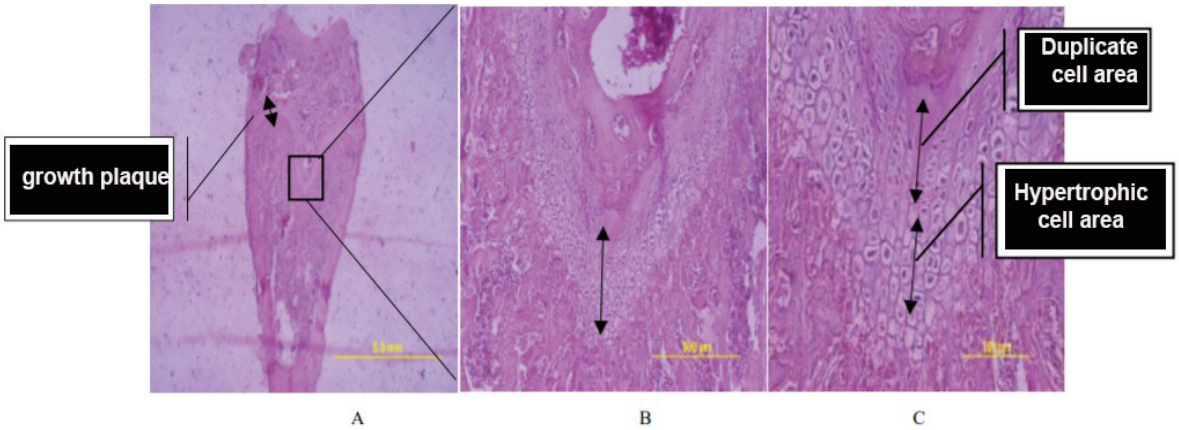

Fig. 1: Extension of the lower apex growth plate of the thighs of the rat and its different areas with E & H staining.
Figure (A) growth plate with a magnification of 10x.
Fig. B Zoom in 100X
The cell (C) of the proliferating cells and the hypertrophied cells, a magnification of 200X

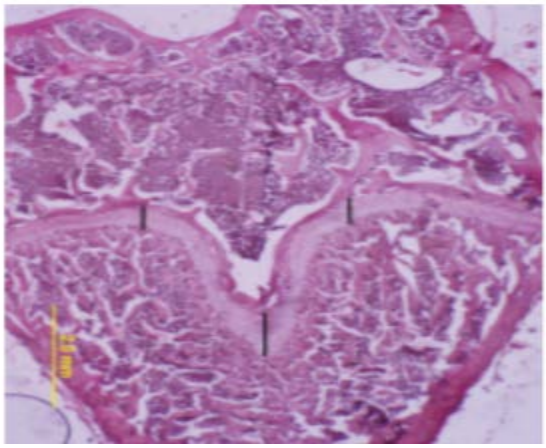

Fig. 2: Display of cartilage in the rat's apex fracture plaque with E & H staining.

Three selective regions are indicated for calculating the mean growth plate width in the test groups with dark lines.

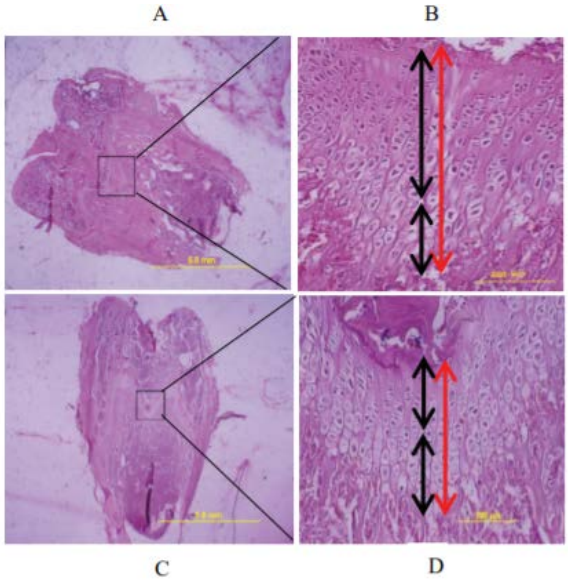

Fig. 3: DA (histological sections of the apple acne growth plate and amplified and hypertrophied regions (one sample of six selected samples from each group) by staining with hematoxylin-eosin (B, A (control group (D, C group) Propiconazole.

Discussion

Data from calculating the width of the apex peptide growth plate of the test groups showed that propiconazole significantly reduced the growth plate plaque. In the study of the effect of propiconazole on chicken and queen embryos, the teratogenic effects of this toxin on cartilage and bone growth, including the reduction of growth of the skeletal and toilets, and the reduction in the amount of clots in the foot bones, were reported by Dahlgren et al. In a study of growth retardation Bone and delayed bone calcification in contact with toxins in children exposed to this toxin. Quantitative studies on the toxic effects of external factors on the growth of the plaque have been performed with different mechanisms of propiconazole. (Dahlgren et al., 2004)

Price et al. Showed that excessive mineralization of the growth plate led to fuse and complete closure of the bone marrow growth platoon in long-term and warfarin-treated rats, and stopped longitudinal growth Height is in these animals. Warfarin, while deficient in the synthesis of coagulation factors associated with vitamin K in the liver, leads to a decrease in the amount (BGP) of an inhibitor of mineralization, which is required for the synthesis of vitamin K) leads to overproduction and closure of the growth plate. While increasing serum BGP in renal or renally impaired rats, the growth of leaves remains. (Price et al., 1982)

Propiconazole appears to have a defective effect on vitamin K and a decrease in BGP levels and, consequently, inhibition of inhibition of mineralization and, consequently, growth fusion and reduction of its plate width. This hypothesis, of course, is contrary to the effect of oxidative propiconazole on bone and cartilage cells.

Growth plaque regions have distinct morphological and biochemical characteristics controlled by growth path signaling pathways. The destructive effect of propiconazole on mitochondrial membrane transduction, vascularization and mitochondrial swelling in rat liver and heart, cytochrome P450 destruction and microsomes in the human liver have been shown to alter the hepatic enzymes and biochemical parameters of hepatocytes.

The mitochondria are the first organelles that are affected by the toxicity of propiconazole. The mitochondrial changes induced by propiconazole are an indicator of increased cell-to-energy requirement to overcome toxicity. Considering the significant presence of mitochondria in the growing region of the growth plate and the role of these organelles in the production of energy in this area, it can be argued that the decrease in energy in these cells due to propykonazole induced oxidative damage causes a disturbance in the process The proliferation and subsequent stages of cell differentiation are transitioned from reproductive to mature and hypertrophied. Also, membrane degradation of other cellular organelles, which leads to a decrease in the synthesis of intercellular matrix, can be attributed to the reduction in the width of the growing region in the present study. The reason for increasing the width of the hypertrophied area in treatment with propiconazole can be attributed to the effect of this venom on the puberty and apoptosis of the chondrocytes of this region and the disorder in the process of chondroclostogenesis and calcification of the growth plate. In fact, in this case, propiconazole also has a detrimental effect on the destruction of the membrane of organelles.

Panda et al., Using transgenic mice that have defects in vitamin D absorption, have reported that the width of the bone growth plate in this mutation has increased. In the present study, propyconazole appears to be effective in increasing the width of the hypertrophy region, along with the destruction of osteoclasts and chondrocytes and inhibition of osteo / chondroclostogenesis. (Panda et al., 2004)

Achieving more accurate results requires testing at cellular and molecular levels (including measuring serum malondialdehyde levels as the last product due to lipid peroxidation of cells in bone tissue, measuring calcium fluctuations, phosphorus, vitamin metabolites, D parathormone, sex hormones (FSH), LH, Estradiol (testosterone, biochemical markers of bone changes including osteocalcin, osteoprotegerin and bone marrow alkaline phosphatase (bone marrow markers), and bone graft markers such as collagen C (CTX) and sRANKL, osteoid measurements made by osteoblasts with R Goldner sampling, Apposition Mineral Rate Rate (MAR) measurement of apposition minerals, and estimation of the amount of sponge bone that grows along the growth plate, by 3D color maps to observe the decrease or increase in the thickness of the area, indicating that the growth plate is clysisy is.

Regarding the histopathologic effects of propiconazole treatment in rat bone tissue, we can point out the possibility of such a cell toxicity in farmers and those who are in chronic contact with this compound, and the need for care and observance of protective coatings to prevent the introduction of poison The body will lead to bone disorders and reduce or delay skeletal growth. Children, due to their natural tendency to discover the surrounding environment through the mouths of various objects and also contaminated with direct contact with surfaces, the air and foam can become more toxic.

In addition, the physiological characteristics of children, such as high water, food and air consumption per unit area of the body, can be a cause of increased risk and harmful effects. It is also necessary to protect the pregnant mothers. Based on the results of histomorphometric studies, it can be concluded that propiconazole decreases the width of the apical thrombophilic plaque in immature rats. Actually Propiconazole may interfere with long-term bone growth and cause premature closure of the plaque.

References

Allen JW, Wolf DC, George MH, Hester SD, Sun G, Thai SF, et al. Toxicity profiles in mice treated with hepatotumorigenic and nonhepatotumorigenic triazole conazole fungicides: Propiconazole, triadimefon, and myclobutanil. Toxicol Pathol 2006;34 (7): 853-62.

Barton HA, Tang J, Sey YM, Stanko JP, Murrell RN, Rockett JC, et al. Metabolism of myclobutanil and triadimefon by human and rat cytochrome P450 enzymes and liver microsomes. Xenobiotica 2006; 36: 793–806.

Battaglin W, Sandstrom M, Kuivila K, Kolpin D, Meyer M. Occurrence of azoxystrobin, propiconazole, and selected other fungicides in US streams, 2005–2006. Water Air Soil Pollut 2011; 218: 307–22.

Dahlgren JG, Takhar HS, Ruffalo CA, Zwass M. Health effects of diazinon on a family. J Toxicol Clin Toxicol 2004;42(5):579-91.

Fattahi E, Jorsaraei SG, Gardaneh M. The effects of carbaryl on the pituitary-gonad axis in male rats. Iran J Reprod Med 2012; 10(5):419-24.

Hester S, Moore T, Padgett WT, Murphy L, Wood CE, Nesnow S. The hepatocarcinogenic conazoles: Cyproconazole, epoxiconazole, and propiconazole induce a common set of toxicological and transcriptional responses. Toxicol Sci 2012; 127: 54–65.

Panda DK, Miao D, Bolivar I, Li J, Huo R, Hendy GN, et al. Inactivation of the 25-hydroxyvitamin D 1alpha-hydroxylase and vitamin D receptor demonstrates independent and interdependent effects of calcium and vitamin D on skeletal and mineral homeostasis. J Biol Chem 2004;279(16):16754-66.

Price PA, Williamson MK, Haba T, Dell RB, Jee WS. Excessive mineralization with growth plate closure in rats on chronic warfarin treatment. Proc Natl Acad Sci U S A 1982;79(24):7734-8.

Taxvig C, Hadrup N, Boberg J, Axelstad M, Bossi R, Bonefeld-Jørgensen EC, et al. In vitro-in vivo correlations for endocrine activity of a mixture of currently used pesticides. Toxicol Appl Pharmacol 2013;272(3):757-66.

Upledger J. Taming osteoporosis. Massage Today 2005;5(11):36-41.