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INTRODUCTION

Despite the presence of skeletal malformation in fetuses of rats treated with caffeine, its effects on bone formation and growth have not yet been elucidated. The objective of this study was to evaluate the effects of caffeine on the formation and endochondral bone growth in rats.

MATERIAL AND METHODS

36 Wistar rats were divided into four groups, control group and others three of which were treated with caffeine at doses of 25, 50 and 100 mg/kg daily throughout pregnancy and lactation. Both the formation and endochondral bone growth of offspring aging three and 21 days were assessed through histomorphometry.

RESULTS AND DISCUSSION

Both the formation and endochondral bone growth of offspring aging three and 21 days were assessed through histomorphometry, revealing malformations, including syndactyly and ectrodactyly, among the progeny of rats treated with higher doses of caffeine. A significant reduction in limb length and vertebral bodies as well as in the thickness of the epiphyseal plate and in the percentage of trabecular bone tissue of the primary spongiosa (Figure 2) has been found. In all groups treated with caffeine, the cartilaginous epiphyses of some long bones presented chondrocytes with pyknotic nuclei and empty lacunae of chondrocytes, characteristic of cell death (Figure 4), and glycosaminoglycans deficiency in the matrix as well. The 21-day of age offsprings of mothers treated with caffeine remained significantly smaller (Figure 1). Articular cartilage and epiphyseal plate of the vertebrae and long bones showed an impairment of differentiation of chondroblasts without distinction of growth plate zone (Figure 3). In the group treated with caffeine, there was degeneration and necrosis of chondrocytes (Figure 4, 5), mainly in offsprings of mothers treated with 100mg/kg of caffeine.



Figure 1: Photomacroscopy of the puppies from 3 and 21 days of age from the control group and group treated with 100mg/kg of caffeine. Puppies of 3 and 21 days old from treated group 100mg/kg of caffeine revealing the inferior size in comparison to the control group.

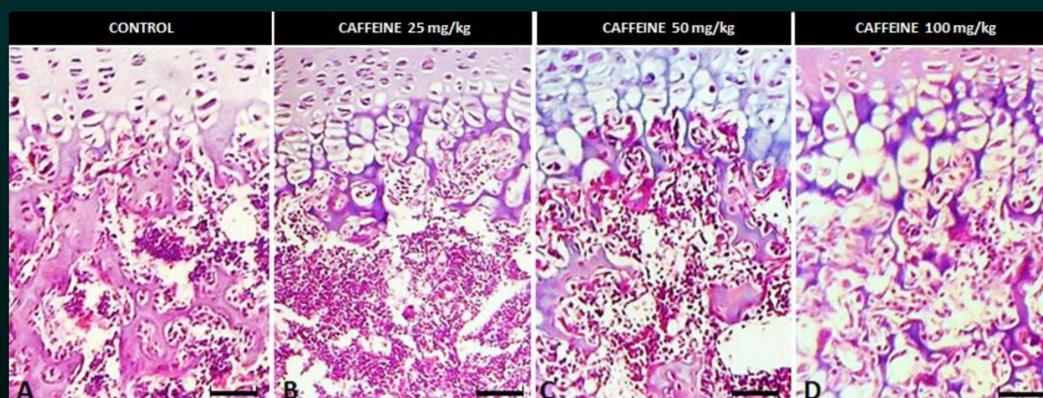


Figure 2: Primary spongiosa of the vertebrae. Newborns of 3 day old. HE. A,B,C,D- Control group and groups treated with 25,50 and 100mg/kg of caffeine respectively. Bar: 94,59 µm. The amount of trabecular bone tissue from the treated groups of caffeine in the doses 25mg (B), 50mg (C) and 100mg (D) is lower in comparison to control group (A).

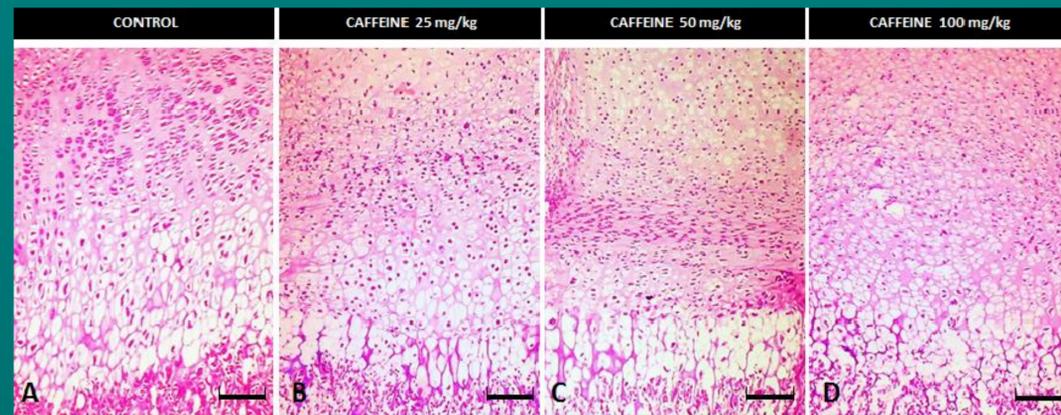


Figure 3: Newborns of 3 days old. HE. Bar = 47,29 µm. A, B, C, D: Epiphysis plaque of the tibia of groups control and treated with caffeine 25, 50 and 100mg/kg, respectively. A) Epiphysis plaque from the control group with zones (proliferation, differentiation and hypertrophy) quite distinguished. B, C) Differentiation zone narrow and poorly organized in columns. D) Undifferentiated zones of proliferation, differentiation and hypertrophy.

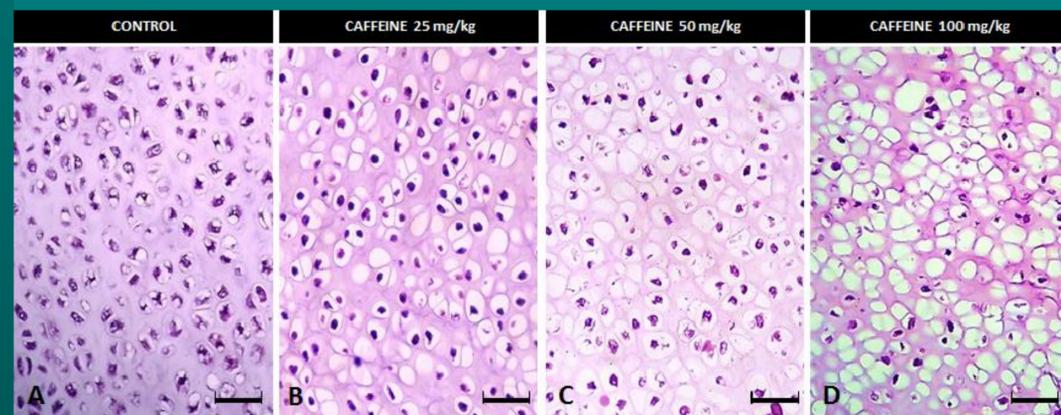


Figure 4: Cartilaginous epiphysis of the tibia. Newborns of 3 days old. HE. Bar = 23,64 µm. A,B,C and D: Control group and treated groups with 25, 50 and 100mg/kg of caffeine, respectively. Empty lacunae of chondrocytes in all groups treated with caffeine; groups treated with 50 and 100mg/kg of caffeine presented higher amounts of picnotic nucleus and empty lacunae of chondrocytes in comparison to control group.

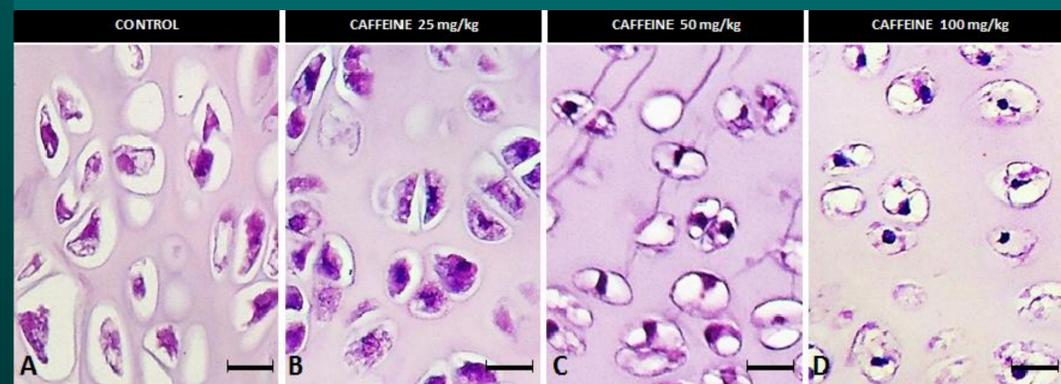


Figure 5: Proliferation zone of the epiphysis plaque from the femur of 21 days old puppies. HE, Bar= 9,45 µm. A, B, C e D) Groups control and treated with caffeine on doses 25, 50 and 100 mg/kg, respectively. A) Numerous chondrocytes with volumous nucleus and loose chromatin. B) Numerous chondrocytes with volumous nucleus and chromatin discretely condensed. C) Chondrocytes with picnotic nucleus and some empty lacunae. D) Chondrocytes with nucleus intensely picnotic and some empty lacunae.

CONCLUSION

It was concluded that offsprings of mothers treated with caffeine have reduced bone formation and endochondral bone growth at all doses studied.

REFERENCES

BARONE, L.M.; TASSINARI, M.S.; BORTELLI, R. et al. Inhibition of induced endochondral bone development in caffeine treated rats. J. Cell. Biochem., v.52, p.171-182, 1993.