INTRODUCTION

The immense consumption of caffeine, a methylxanthine alkaloid in eatables, drinks and various medicines, has been estimated at 120,000 tons per year, and has created interest in clarifying mechanisms of response and the multiple influences of this drug in daily use.\(^1\) 

The influence of caffeine on metabolism of bone is somewhat contentious, although linked with a considerable rise in danger of fracture, periodontal disease and osteoporosis.\(^2\) Empirical studies have revealed that caffeine impedes development of bone and reduces mineral-content of the maxilla, femur and mandible in fetal, infant and juvenile rats.\(^3\) In-vitro researches have indicated that caffeine slows down mineralization of bone, configuration of extracellular matrix and osteoblast differentiation.\(^3\) In neonates of lactating mothers, intake of caffeine can cause less osteocytes in an area of femur cross-section, delayed structural re-modeling of lateral tibial metaphysis, unusual osteocytes and osteoblasts with enlarged mitochondria.\(^4\) Zn contents in the bone are also consistently lowered by the caffeine.\(^5\)

Epidemiological studies related to caffeine and development of bone have usually concentrated on females to observe decreased bone mineral density (BMD).\(^5\)

Moreover, caffeine possesses the potential to disturb the mechanism involved in cell proliferation. Caffeine quickly passes through the placenta and reaches the fetus. Embryo from mothers, treated with caffeine, exhibited a remarkable reduction in crown-rump length; and maternal treatment with caffeine altered utero-placental circulation to such an extent that normal embryonic development was diminished.\(^4\) Caffeine had a detrimental effect on balance of calcium which got decreased through intestinal calcium absorption or increased urinary excretion.\(^6\)

Vitamin D, a fat soluble vitamin, had its chemical structure determined during 1930s. In the beginning of the 20th century, therapeutic use of cod liver oil, having vitamin D as an ingredient, led to a sharp decrease in the incidence of rickets. Vitamin D is obtainable from diet, supplements or from endogenous production in the skin. The cutaneous production is very effective and within few minutes of exposure to the sun, increases circulating vitamin D concentration.\(^7\) There are two forms of vitamin D namely, ergocalciferol (vitamin D\(_2\)) and cholecalciferol (vitamin D\(_3\)). Vitamin D, and in particular 1,25(OH)\(_2\)D\(_3\), is of critical importance to bone formation.\(^8\)

ABSTRACT

Objective: To determine the effects of caffeine ingestion on the development of femur and role of vitamin D\(_3\) in preventing these effects in BALB/c mice.

Study Design: Experimental study.

Place and Duration of Study: Department of Anatomy, Army Medical College, Rawalpindi, in cooperation with NIH (National Institute of Health), Islamabad, from October 2014 to October 2015.

Methodology: Thirty (100%) BALB/c mice, 50% male and female each, three weeks old, weighing 12-14 grams were taken and divided equally and randomly into three groups, each having 10 (33.3%) mice; 5 (16.6%) male and female. G\(_1\) (control group) was given normal diet with water ad libitum. G\(_2\) and G\(_3\) (experimental groups) were given 10 mg of caffeine per 100g body weight, three days a week, through oral gavage for 60 days on alternate days. However, experimental group G\(_3\) was additionally provided 0.1µg vitamin D\(_3\) daily, through oral gavage for 60 days. Experimental groups were compared with control group and data was analyzed statistically.

Results: The mean weight of mice femur of G\(_1\) (control group) was 0.387 ±0.019 g; while mean weights of right femur of G\(_2\) and G\(_3\) (experimental groups) were 0.316 ±0.020 g and 0.345 ±0.020 g, respectively. Similarly, mean right femur length of group G\(_1\) was 20.70 ±0.609 mm; while for groups G\(_2\) and G\(_3\), it was 24.382 ±1.087 mm and 22.966 ±0.822 mm, respectively. In comparison with group G\(_1\) for groups G\(_2\) and G\(_3\), femur weight decreased, however femur length increased.

Conclusion: Caffeine intake caused femur length to increase and weight to decrease, but treatment with vitamin D\(_3\) ameliorated these effects of caffeine.

Key Words: Caffeine.  Femur.  Vitamin D\(_3\).  Mice.
Caffeine is consumed in Pakistan in different forms through foods and beverages. However, society is generally unaware due to non-availability of data on its various harmful effects. The present study is an effort towards generating this understanding by gathering information and demonstrating the effect of high caffeine consumption on the development of bony tissues, and to demonstrate the role of vitamin D$_3$ in preventing these adverse effects of caffeine ingestion.

The objective of this study was to determine the effects of caffeine ingestion on the development of femur and role of vitamin D$_3$ in preventing these effects in BALB/c mice.

**METHODOLOGY**

This research was a laboratory-based experimental study performed in the Anatomy Department, Army Medical College Rawalpindi, in cooperation with National Institute of Health (NIH), Islamabad, from October 2014 to October 2015 after requisite approval from Army Medical College ethical committee on animal experiments.

A total of 30 healthy (100%), 15 (50%) male and 15 (50%) female BALB/c mice having age of three weeks and weighing 12-14 g were taken for study. Unhealthy and under-three weeks mice were excluded. They were housed in a well-ventilated environment with temperature control between 20-26°C. Random division of mice was done into three groups. Each group contained 5 (16.6%) male and 5 (16.6%) female mice; making a total of 10 (33.3%) animals in a group. Separate cages were used for male and female mice to avoid pregnancy. The mice in control group G$_1$ were provided a standard defined lab diet for 60 days. Experimental group G$_2$ mice were administered with caffeine dosage of 10 mg/100 g body-weight, on alternate days, 3 days a week for 60 days. All mice of G$_3$ were given caffeine 10 mg/100 g body weight on alternate day, three days in a week and vitamin D$_3$ 0.1 μg per day by oral gavage for 60 days.

At the conclusion of study, the mice were sacrificed with anesthesia of ether. After dissection, the right femur was eliminated by detaching from knee and hip joints. Weighing of right femur was conducted by electronic balance, while its length was gauged using vernier calliper (digital) from lateral condyle to greater trochanter.

Data was analyzed using IBM-SPSS version 20. ANOVA test was performed and then for comparison of quantitative variables between groups, Post Hoc Tukey’s Test was applied. Significance criterion was taken as p-value of ≤ 0.05.

**RESULTS**

Significant reduction in mean weight of mice femur in experimental group G$_2$ was observed in comparison with control group G$_1$ and experimental group G$_3$ (Table I). However, increase in the mean femur length of experimental group G$_2$ was found in comparison with control group G$_1$ and experimental group G$_3$ (Table I). The length and weight of femur of group G$_2$ when compared with groups G$_1$ and G$_3$ turned out to be significantly different (Table II).

**DISCUSSION**

Caffeine is consumed in Pakistan in different forms through foods and beverages. However, society is generally unaware due to non-availability of data on its various harmful effects. The present study is an effort towards generating this understanding by gathering information and demonstrating the effect of high caffeine consumption on the development of bony tissues, and to demonstrate the role of vitamin D$_3$ in preventing these adverse effects of caffeine ingestion.

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development stage. For mice, in distal femoral epiphysis, bone development takes place rapidly and firstly various small nuclei of ossification appear, which unite to form enormous secondary ossification centre.\textsuperscript{15}

The growth of long bone is due to the secretion of growth hormone. The findings pertaining to femur length were found statistically significant among the G\textsubscript{1}, G\textsubscript{2} and G\textsubscript{3} groups. The weight of femur was lower in experimental group G\textsubscript{3} than control group G\textsubscript{1} and experimental group G\textsubscript{2}. The results of weight of femur were also found statistically significant among all the groups G\textsubscript{1}, G\textsubscript{2} and G\textsubscript{3}. Increased caffeine consumption altered bone mineral mass and density by increasing calcium loss.\textsuperscript{9}

Caffeine reduced hydroxyproline as well as mineral contents in bones; amount of hydroxyproline was indicative of collagens in the bones.\textsuperscript{16}

Although caffeine has been a subject of interest for the medical researchers globally; however, limited local literature is available on harmful effects of caffeine on the animals. The results of the current study are comparable with an earlier international study done on male Wistar rats where, after exposure to caffeine, femur length and weight reduced significantly.\textsuperscript{17} Yet another study illustrates that caffeine impairs the growth and composition of osseous tissues due to reduction of the mineral content and mineral density.\textsuperscript{18}

Caffeine provoked various detrimental influences on the bone metabolic process, including reduced bone weight, lesser BMD, and decreased calcium contents. The reduced calcium content was also linked with caffeine instigated impairment of bone during growth. The copious amount of caffeine reduced the weight of femur as well as calcium content in the femur.\textsuperscript{19} Caffeine caused harmful effects on normal development and growth of the bone. Caffeine also resulted in decreased zinc levels in bones as well as various tissues.\textsuperscript{20} The insufficiency in zinc level in caffeine-administered-rats has modified bone’s metabolism and forever changed bone framework.\textsuperscript{7}

In experimental group G\textsubscript{3}, there was considerable protection of the cytoarchitecture of femur due to vitamin D\textsubscript{3}. There was 5 to 7\% increase in the mean degree of mineralization of bone tissue by vitamin D.\textsuperscript{21} Vitamin D deficiency decreased bone mineral density and resulted into increased risk of fractures. Vitamin D stimulated the absorption of calcium from gut and influenced the overall mineralization of the skeleton.\textsuperscript{22} Vitamin D has been extensively used for the prevention and treatment of osteoporosis and known as a bone resorbing hormone, mainly in in-vitro studies.\textsuperscript{23}

CONCLUSION

It was observed in the current study that caffeine caused harmful effect on the developing femur of BALB/c mice. Caffeine instigated increase in femur length, may be due to stimulation of growth hormone; and the decrease in femur weight may be due to demineralization of femur. However, the treatment with vitamin D\textsubscript{3} ameliorated these adverse effects of caffeine.

REFERENCES


