



## THE HISTOLOGY OF EPIPHYSEAL PLATE OF DEVELOPING THIGH BONE OF FAYOUMI UNDER THE INDUCED EFFECT OF NICOTINE AND GREEN TEA (*CAMELLIA SINENSIS*)

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### ABSTRACT

Cigarette smoking has become one of the greatest developing health hazard in young people and World Health Organization showed figures regarding million tobacco-related deaths every year. The present study was conducted during the period of November 2011 - November 2012 to assessing the induced adverse effects of nicotine. Simple Random Sampling technique was used. Freshly laid fertilized eggs of Fayoumi species were selected at zero hour of incubation. Hatchery was used for placing eggs at 37.5<sup>0</sup>C. The proper ventilation and relative humidity of 75% was maintained. Four groups were made each group was comprising of ten numbers of eggs. Control group G1 was given normal saline, experimental group G2 was given *Camellia sinensis* extract, group G3 was given 0.0001% nicotine solution, group G4 was given both 0.0001% nicotine solution and green tea extract. Double exposure of doses was given. First exposure was at 48 hours of incubation and second at 48 hours of hatching (post natal dose). At the age of one month chicks were sacrificed, thigh bones were collected dissected and duly labeled tissue cassettes and slides were made to observe the height of hypertrophy zone. Experimental groups when compared with each other such as, G2 in comparison with G3 and G4 showed significant results with p values (0.004) and (0.000), respectively. Comparison of G3 and G4 with each other showed statistically significant result with p value (0.000). Based present study, we conclude that nicotine, one of the constituent of cigarette smoke causes toxic effects on the developing thigh bone of chick.

**Keywords:** Incubation, femur, nicotine, hypertrophy zone.

### INTRODUCTION

The tobacco epidemic is one of the biggest globally human health threats and killing more than 7 million people a year (WHO, 2018). Cigarette smoking is one of the rising health problems and exposure to tobacco smoke leads to production of free radicals (Lutfi, 1970). Smoking harmful affects two ways either in active or passive smoking form. The avian skeleton is one of the best for correlating developing effects with human (Doménech *et al.*, 1999). Calcification of femur of chick embryo starts on the 5<sup>th</sup> day while in case of tibia and fibula it starts on 10<sup>th</sup> day of embryonic life (Sawad *et al.*, 2009). *Camellia sinensis* is a plant species of evergreen shrub whose leaves and leaf buds are used to produce green tea. Green tea has been related with many countries of Asia having different cultures (Tanaka *et al.*, 2005) and contains different constituents, which have protective role against free radical production in body.

The study will help in understanding the effects of nicotine and *Camellia sinensis* on developing thigh bone and concluding how green tea reverse the deleterious

effects of nicotine. Nicotine works on bone metabolism by affecting the bone remodelling process and suppressing osteogenesis by decreasing in alkaline phosphatase and type 1 collagen production by osteoblast (Grumbach, 2001). Thigh bone of chick develops with endochondral calcification which starts at its centre and extends towards its end (Sawad *et al.*, 2009).

### MATERIALS AND METHODS

The present study was arranged at the Department of Anatomy, Army Medical College Rawalpindi in collaboration with the Poultry Research Institute (PRI) Rawalpindi. Experimental procedures were granted approval by the Ethical Review Committee of Army Medical College. Eggs selected for the experiments purpose were fertilized chick eggs of Fayoumi species at zero hour of incubation. Collection of eggs were from Poultry Research institute. Simple random sampling technique was used. Corrective measures were taken after properly fumigating and clearing the hatchery, the eggs were incubated. Different parameter regarding temperature was maintained at 37.5<sup>0</sup>C, the relative humidity was kept between 75% and proper ventilation

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was maintained. Rotations of eggs were done 4 hourly. Placement of eggs in hatchery was taken as day zero. Four experimental groups were arranged, each group was comprising of ten numbers of eggs. Group G1 was Labeled as control group given normal saline in 0.1ml of quantity. Group G2 was given green tea extract in 0.1ml of quantity, group G3 was given 0.0001% nicotine solution in 0.1ml of quantity, G4 was given both 0.0001% nicotine solution and green tea extract in 0.1ml quantity. All the working solutions were administered through the blunt end of the egg with the help of insulin gauge needle. Double exposure of doses was given. First exposure was at 48 hours of incubation and second at 48 hours of hatching (post natal dose). One month old chicks, were sacrificed their pelvic region was dissected by separating the lumbar vertebrae at L4 from L5. Bone tissue was collected and secured for histology purpose. Decalcification and duly labeled Tissue cassettes were processed in Leica TP 1020 automatic tissue processor (Fig. 1). Paraffin wax was used with melting point range from 40-70°C for embedding. The block was allowed to cool on cold plate. Tissue cassettes and processed in Leica TP 1020 automatic tissue processor. Paraffin wax was used with melting point range from 40-70°C for embedding. The scale of oculometer was calibrated with the help of stage micrometer at 10X magnification. Heights were measured by aligning the oculometer parallel to the chondrocyte columns. Height of zone was measured in the central and peripheral part of this zone

then the observations were calculated (Grumbach, 2001) (Fig. 2).

#### Statistical analysis

Data was entered in a database using SPSS (Statistical Package for Social Science) version 16. Data was presented as tables. Chi-square test was used for the comparison between the groups. p value < 0.05 was considered significant.

#### RESULTS AND DISCUSSION

For results and observations the histological feature that is hypertrophy zone of one-month-old chick femur bones were taken into account (Fig. 2). Height of hypertrophy zone of control group G1 and G2 both showed mean value  $480.555 \pm 21.561 \mu\text{m}$ . Experimental groups G3 and G4 showed mean value  $437.500 \pm 2.500 \mu\text{m}$  and  $455.000 \pm 15.000 \mu\text{m}$ , respectively. Control group G1 in comparison to experimental group G2 showed statistically insignificant result with p value (1.000). G1 in comparison with G3 and G4 showed statistically significant result with p values (0.004) and (0.000), respectively. Experimental groups when compared with each other such as, G2 in comparison with G3 and G4 showed significant results with p values (0.004) and (0.000) respectively. Comparison of G3 and G4 with each other showed statistically significant result with p value (0.000) (see Table 1).

Table 1. Comparison of height of hypertrophy zone among different groups of one month old Fayoumi chicks.

| Dependable Variable                          | Comparison Between Groups |       | p value |
|--|---------------------------|-------|---------|
|  | Group                     | Group |         |
| HEIGHT OF HYPERTROPHY ZONE ( $\mu\text{m}$ ) | G1                        | G2    | 1.000   |
|  |                           | G3    | 0.004   |
|  |                           | G4    | 0.000   |
|  | G2                        | G1    | 1.000   |
|  |                           | G3    | 0.004   |
|  |                           | G4    | 0.000   |
|  | G3                        | G1    | 0.004   |
|  |                           | G2    | 0.004   |
|  |                           | G4    | 0.000   |
|  | G4                        | G1    | 0.000   |
|  |                           | G2    | 0.000   |
|  |                           | G3    | 0.000   |

p value  $\leq 0.05$  statistically significant.

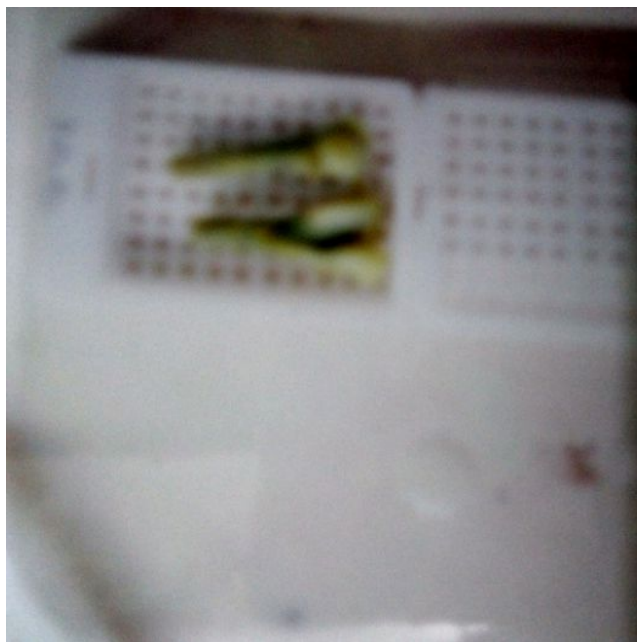


Fig. 1. Tissue placed in the cassettes for the preparation of histological slide purpose.

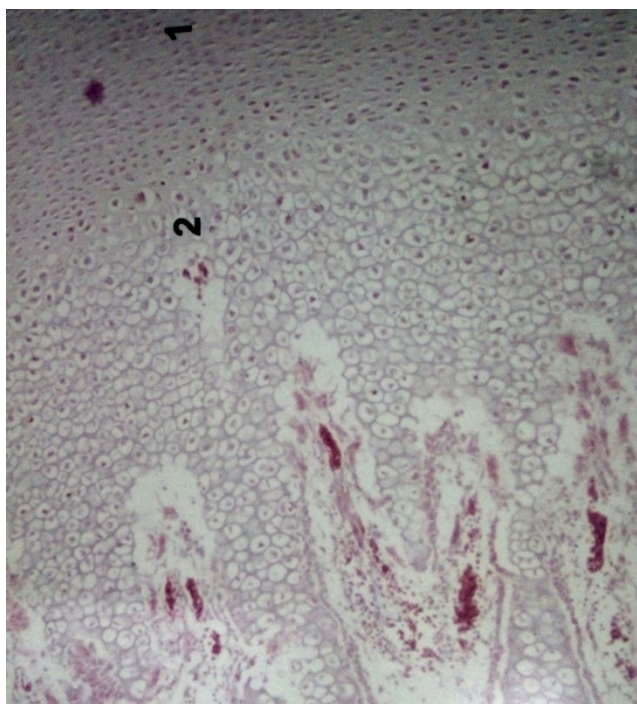


Fig 2. The slide is marked with 1 for the proliferating zone and “2” for the hypertrophy zone.

One of the most harmful constituent of cigarettes smoke is nicotine. Nicotine, the chief alkaloid in tobacco, is known to have effects on development. The avian skeleton gives resemblance with the human skeleton, it is considered to be the most suitable for the teratogenic

effect induced by different chemicals. Green tea extract used to detoxify the free radical effect of nicotine. Tobacco usage and increased requirement is the leading cause of death in the world today. With 4.9 million tobacco-related deaths per year, has been notice no other consumer product is as dangerous or kills as many people as tobacco (WHO, 2003). The study done to know whether green tea reverse the deleterious effects of nicotine. Green tea intake related with a decrease in blood cholesterol levels (Maron *et al.*, 2003). Over past years the epidemiological and clinical studies have shown various physiological responses to green tea for the promotion of health and the prevention or treatment of some chronic diseases. However, the results from epidemiological and clinical studies of the relationship between green tea consumption and human health are mixed (Green teas Brief history, 2010). Green tea leaves extracts to the old age as well as young age male subjects may be beneficial, especially for the nicotine subjects to improve the health status and life span. Nicotine administration had also been reported to inhibit collagen synthesis and alkaline phosphate activity in osteoblast like cells (Lutfi, 1970).

In the present study, height of hypertrophy zone of G1 and G2 in comparison to G3 and G4 results showed that height of hypertrophy zone of epiphyseal plate in comparison to those of G1 and G2, but in comparison of G3 and G4 showed that *Camellia sinensis* helped to bring the oxidative injury back to normal level. This study was supported by another work which concludes that Nicotine has a significant qualitative and quantitative effect on bone healing at later stages of the healing mainly owing to its inhibitory effects on neovascularization and osteoblast differentiation (Tanaka *et al.*, 2005). Although many studies showed that nicotine caused adverse effect on bone, but other studies reported that nicotine did not cause any adverse effects on bone density, bone strength, bone turnover or histomorphometry of bone seen in female rats (Syversen *et al.*, 1999) this work is reported by Fang *et al.* (1991). This difference may be due to variation in species gender, dosage, treatment period and method of nicotine administration (Hermizi *et al.*, 2007). Another research reported that nicotine has a significant qualitative and quantitative effect on bone healing at later stages of the healing cascade mainly owing to its inhibitory effects on neovascularization and osteoblast differentiation (Dilip *et al.*, 2014). The study done on kidney tissues of rats showed *Camellia sinensis* leaves extract play a prominent role in preventing nicotine induced oxidative stress by promoting the GSH-Px activity in the kidney tissue in young and as well as old age rats (Katika *et al.*, 2015).

The present work was designed to study the histological changes in the developing thigh bone of chick exposed to nicotine one of the main constituent of tobacco smoke and to observe the preventive effects of antioxidant such as

*Camellia sinensis*. The study proved that injection of antioxidant significantly prevented some of the harmful effects of nicotine.

## CONCLUSION

Based present study, we conclude that nicotine, one of the constituent of cigarette smoke causes toxic effects on the developing thigh bone of chick. Administration of antioxidant neutralizes some, but not all of the harmful effects of tobaccos smoke. Therefore the only possible way to prevent the damage is to avoid it.

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