

Role of nicotine and camellia sinensis on the developing femur of chick

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Abstract

Objective: To study the harmful effects of nicotine on the developing femur of chick and to observe the role of antioxidant camellia sinensis in the prevention of these effects.

Methods: The experimental study was conducted at Army Medical College, Rawalpindi, from November 2, 2011, to November 1, 2012, and comprised fresh fertilised eggs of Fayoumi species at zero hour of incubation of Fayoumi species. The eggs were divided into four equal groups. Control group G1 was given normal saline; G2 was given green tea extract; G3 was given 0.0001% nicotine solution; and G4 was given both 0.0001% nicotine solution and green tea extract. First exposure was at 48 hours of incubation and second at 48 hours of hatching (post-natal dose). At the age of one month, the chicks were sacrificed. The length of the femurs was measured with the help of measuring scale by keeping the ruler between greater trochanter and the lateral condyle. SPSS 16 was used for statistical analysis.

Results: The four groups had 10 eggs each. G3 and G4 showed decreased growth compared to G1 and G2 ($p < 0.05$). In G4, better growth was noticed compared to G3 ($p < 0.05$), but its growth was less in comparison with G1 and G2 ($p > 0.05$).

Conclusion: Camellia sinensis antioxidant property helped to neutralise the oxidative injury by the use of nicotine, but was unable to recover it fully.

Keywords: Incubation, Femur, Nicotine, Length. (JPMA 65: 1094; 2015)

Introduction

Cigarette smoking is a global problem. Smoking stress occurs both actively and passively. Chick skeleton is one of the best avian skeletons for considering teratogenic effects. Chick embryo femur starts calcifying on the 5th day and other bones of leg such as tibia and fibula starts calcifying on the 10th day of embryonic life.¹ Green tea contains different constituents that have protective role against free radical production in the body. Smoke generated by the use of tobacco causes production of free radicals.²

The current study was planned to see the harmful effects of nicotine on the developing femur of chicks, and to observe the role of antioxidant camellia sinensis in the prevention of these effects.

Material and Method

The experimental study was conducted at the Department of Anatomy, Army Medical College, Rawalpindi. After approval by institutional ethics review committee, fertilized chick eggs of Fyousispecies were selected at zero hour of incubation. The eggs were collected from the Poultry Research Institute (PRI),

Rawalpindi. Random sampling technique was used to pick fertilized eggs with normal shape (oval), colour (off-white) and size (medium). Eggs with unknown time of laying, broken shell, abnormal shape (football shaped, pear shaped), colour (other than off-white) and size (very tiny eggs) were excluded.

After properly fumigating and clearing the hatchery, the eggs were incubated. The temperature was controlled at 37.5°C and the relative humidity was kept around 75% with proper ventilation. Rotations of eggs were done every 4 hours. Day zero was considered when eggs were placed in the hatchery (Figure-1). The eggs were divided into four equal groups, and eggs in each group were given numbers through computer-generated technique.

Control group G1 was given normal saline in 0.1ml quantity; experimental group G2 was given green tea extract in 0.1ml quantity; experimental group G3 was given 0.0001% nicotine solution in 0.1ml quantity; and experimental group G4 was given both 0.0001% nicotine solution and green tea extract in 0.1ml quantity. All the solutions were given through the blunt end of the egg with the help of insulin gauge needle (Figure-2). 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, the chicks were sacrificed and the pelvic region was dissected by separating the lumbar vertebrae at L4 from L5. From both

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Figure-1: Selection of eggs at zero day of incubation before placing them in hatchery.



Figure-2: Eggs being injected with the intervention solutions under laminar flow.

sides, head of the femur was separated from acetabular region and the lower end of femur was separated from the knee joint by carefully separating the ligaments from it. The femur's length was noted by keeping the ruler between greater trochanter and the lateral condyle (Figure-3).³

SPSS 16 was used for statistical analysis. Mean values and standard deviations were calculated for quantitative variables. One-way analysis of variance (ANOVA) was used to compare mean length of chick femur among the four groups. P<0.05 was considered significant.

Results

The four groups had 10 eggs each. From the observation the length of femur of chick of G1 and G2 showed similar results with a mean value of 4.472±0.097cm. The mean values of G3 and G4 were 1.760±0.010cm and



Figure-3: Measurement of length of the right femur with the help of scale by keeping it between the greater trochanter and the lateral condyle.

Table-1: Mean values of length of chick femur (cm).

Groups	Mean value	p-value
G1	4.472± 0.097	< 0.05
G2	4.472± 0.097	
G3	1.760± 0.010	
G4	3.250± 0.224	

*p value ≤ 0.05 statistically significant.

Table-2: Comparison of length of femur of one-month-old chicks (cm) among different groups.

Dependable Variable	Comparison Between Groups		P value	
	Group	Group		
Length of Femur (cm)	G1	G2	1.000	
		G3	< 0.0001*	
		G4	< 0.0001*	
		G2	1.000	
	G2	G3	G1	< 0.0001*
			G2	< 0.0001*
			G4	< 0.0001*
			G1	< 0.0001*
	G3	G4	G1	< 0.0001*
			G2	< 0.0001*
			G3	< 0.0001*
			G4	< 0.0001*

*p value ≤ 0.05 statistically significant.

3.250±0.224cm respectively (Table-1). G1 compared to G2 showed insignificant result (p=1.000). The mean value was same as of G2 and G1 as green tea was not able to affect the length of femur (p>0.05). G1 compared to G3 and G4

was significantly different ($p < 0.0001$).

Experimental groups were compared with each other and G2 was significantly different from G3 and G4 ($p = 0.000$ each). G3 and G4 also showed statistically significant difference ($p = 0.000$) between themselves (Table-2).

Discussion

Nicotine has the property of natural alkaloid, and its administration decreases the production of collagen and activity of alkaline phosphate in osteoblasts like cells.⁴ It also affects the developing process of bone formation in the growing foetus by crossing the placenta and resulting in delayed growth of skeletal development.⁵ Many studies showed that nicotine used in doses that is toxic delays the process of bone ossification of vertebrae and limb bones.⁶ The length of femur showed insignificant difference when G1 was compared with G2, as green tea was not significantly affecting the growth of skeletal system of chicks compared to the control group. There was statistically significant result when all experimental groups were compared with each other ($p < 0.05$).

Nicotine affects the circulatory system by compromising the flow of blood to the tissue, which results in delaying the development. As reported earlier, it was obvious that nicotine affected the process of calcification of chondrocytes by inhibiting the formation of bone matrix.⁷ Nicotine caused adverse effect on bone, but there are reports suggesting that nicotine is not responsible for causing any adverse effects on some features of bones like its density, strength of bone, bone turnover or histomorphometry in bones of female rats.^{8,9} This difference may be due to variation in different characteristic features such as gender, amount of dose, period and procedure of nicotine intervention.¹⁰ Nicotine might delay the process of ossification in rat foetuses, decreasing the bone matrix formation.¹¹ Nicotine exposure is considered to be related with abnormal blood flow by maternal vasoconstrictive property which is responsible for foetal growth retardation caused by smoking during pregnancy.¹² Another study established that the nicotine affects the foetal growth due to the vasoconstriction of the uterine vessels.¹³ In one study, delayed development of skeleton occurred as nicotine was transferred to growing foetuses by crossing through placenta and amniotic fluid.⁵ Tobacco affects the chondrocytes of the growth plate whether environmental insult takes place by active or passive smoking, as nicotine

affects endochondral ossification.¹⁴

Conclusion

Nicotine was involved in suppressing the development of femur bone by inhibiting the process of bone matrix formation. The quantity of camellia sinensis used partially reversed or inhibited the effects of nicotine. Therefore, decreasing nicotine exposure by limiting cigarette smoking habit can be a preventive strategy.

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