EFFECT OF NICOTINE AND PREVENTIVE ROLE OF CAMELLIA SINENSIS ON THE HISTOMORPHOLOGY OF DEVELOPING EPIPHYSEAL PLATE OF THIGH BONE OF CHICK

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ABSTRACT

Objective: To determine the effect of nicotine and camellia sinensis (green tea) on the developing epiphyseal plate of thigh bone of chick.

Design: Randomized controlled trial.

Place and Duration of Study: Army medical college, Rawalpindi, Pakistan from April 2012 to May 2012.

Material and Method: Freshly laid fertilized eggs of Fayoumi breed chick eggs were selected at zero hour of incubation. Four groups were made, group G1 was control group treated with normal saline. Experimental group G2 was treated with camellia sinensis extract (green tea), group G3 was given nicotine whereas group G4 was injected with working solution nicotine and camellia sinensis (green tea), in 0.1ml quantity. Double exposure one at 48 hour of incubation and other at 48 hours after hatching of chicks. SPSS version 15 was used to analyze the data.

Results: It was observed that the weight of chick at one month of age and weight of femur of chicks of nicotine treated groups G3 and group G4 were reduced in comparison to control group G1. Mean number of cells in hypertrophy zone of developing epiphyseal plate of thigh bone were reduced of nicotine treated groups in comparison to control group.

Conclusion: Camellia sinensis (green tea) helped to reduce the harmful effects of nicotine treated group but cannot reverse the oxidative injury.

Keywords: Incubation, Nicotine, Thigh bone, Weight.

INTRODUCTION

Avian skeleton resembles the human skeleton; it is considered to be the most important structure for studying the teratogenic effect with the induction of different chemicals. Decreased amount of oxygen and nutrient supply to the fetal tissues the effects of nicotine, can be seen from the first trimester of pregnancy in the form of spontaneous abortions[1]. Nicotine exposure is also responsible for causing premature birth, and an increase in heart rate[2]. Chick skeleton is layed down in hyaline cartilage which is subsequently ossified and represents bone[3]. Calcification of thigh bone starts on the 5th day of embryonic period[4]. Nicotine is a direct inhibitor of osteoblast differentiation and also stimulates the osteoclastic activity[5]. Green tea catechins have been found to have many unique anti microbiological activities such as antibacterial, antifungal, antiviral and antioxidant effects[6]. The rationale of current study is to observe teratogenic effects of nicotine on developing thigh bone and protective role of green tea.

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MATERIAL AND METHODS

This randomized controlled trial, was carried out at Anatomy department of Army Medical College Rawalpindi in collaboration with the Poultry Research Institute (PRI) Rawalpindi. All the procedures were approved by Ethical Review Committee of Army Medical College Rawalpindi.

Incubation

Freshly laid fertilized Fayoumi breed of chick were selected at zero hour of incubation. Eggs were placed in hatchery after properly fumigating and clearing the hatchery.

Temperature was maintained at 37.5 degree centigrade, relative humidity was kept between 75% and proper ventilation was maintained. Rotations of eggs were done 4 hourly. Placement of eggs in hatchery was taken as day zero. Four groups were made each comprising of ten eggs. Group G1 was control group injected with normal saline. Experimental group G2 was injected with camellia sinensis, group G3 was given nicotine whereas group G4 was injected with nicotine both and camellia sinensis solution.

Chicks hatched from each group were given four weeks time for their development. The alive chicks were weighed with the help of digital balance[7]. After decapitating the chick, pelvic region was dissected. The lower half, along with pelvic girdle and hind limbs was fixed in formalin filled jars for 48 hours. After fixation, muscles were removed to expose the underlying thigh bone. Femur was collected after separating it from hip and knee joints. The bone samples from each specimen were fixed in 10% formalin containing bottles which were labelled by the side of the specimen. After fixing, samples were placed into decalcifying solution that is 5% Nitric acid for 18 -24 hrs[4]. Weight of both femurs of right and left sides were obtained by using precision digital balance with 0.001 g readability after properly dissecting it. The bone tissue of right and left sides were placed in the duly labelled by tissue cassettes and processed and embedded. Tissues were cut into 7-8 micron thick sections using a rotary microtome. The sections were stained with autostainer with Hematoxylin and Eosin (H & E).

Observations were made using objective 40X. In 40X mean numbers of cells of hypertrophy zone which extends between the flat shape cells of

Table-1: Mean values of weight of chick (g), weight of femur (g), and mean number of cells in hypertrophy zone of one month old chicks of different groups.

Groups	Weight of chick (g) Mean ± SEM	Weight of femur (g) Mean ± SEM	Mean number of cells in hypertrophy zone Mean ± SEM
G1	155.38 ± 0.07	1.36 ± 0.007	26.43 ± 0.97
G2	155.38 ± 0.07	1.36 ± 0.007	26.32 ± 0.85
G3	95.45 ± 0.05	0.40 ± 0.002	18.90 ± 0.50
G4	105.56 ± 0.04	0.87 ± 0.073	24.00 ± 0.40
p value	<0.001	<0.001	<0.001

Table-2: Comparison of groups for weight of chick (g), weight of femur (g), and mean number of cells in hypertrophy zone of one month old chicks of different groups.

Groups comparisons	*p* values for		
	Weight of chick (g)	Weight of femur (g)	Mean number of cells in hypertrophy zone
G1 versus G2	1.000	1.000	0.86
G1 versus G3	<0.001*	<0.001*	0.01*
G1 versus G4	<0.001*	<0.001*	0.003*
G2 versus G3	<0.001*	<0.001*	0.009*
G2 versus G4	<0.001*	<0.001*	0.002*
G3 versus G4	<0.001*	<0.001*	0.69

*p value < 0.05 significant

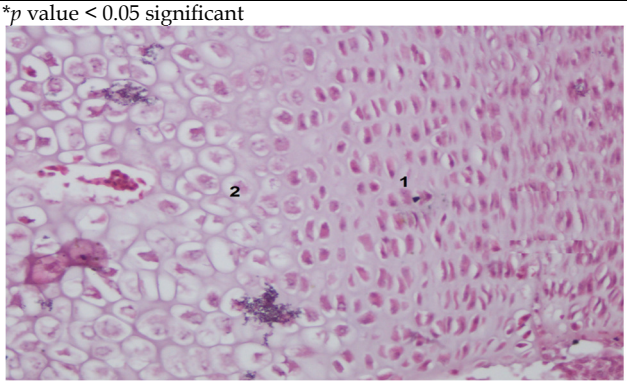

Figure-1: Hypertrophy zone cells of one month old chick labelled as 2 placed between flat shape proliferating cells labelled as 1 and metaphyseal blood vessels.

proliferative zone and invading metaphyseal blood vessels.

The numbers of hypertrophic cells were counted using ocular micrometer in approximately 15 intact columns per growth plate. The observations were done at X40[8]. (Fig-1)

Statistical Analysis

Data had been analyzed with the help of statistical program SPSS version 15. Descriptive statistics i.e mean and standard error of mean (SEM) were calculated for weight of chick, femur and mean number of cells. The *p* value <0.05 was considered statistically significant.

RESULTS

For results and observations both the gross and microscopic features were recorded. Mean weight one month old chicks was higher in control group G1 and experimental group G2. Whereas mean values of weight of experimental groups G3 and G4 were markedly reduced in comparison to control group (table-1). When comparing control group G1 with experimental groups G3 and G4 the results were statistically significant with *p* value <0.05 (table-2).

Mean value of weight of femur was higher in control group G1 as compared to the experimental group G2. Whereas mean values of experimental groups G3 and G4 were markedly reduced in comparison to control group (table-1). Weight of femur bone of chick at one month of age in control group G1 comparison with experimental group G2 showed statistically insignificant result with *p* value 1.000. When comparing control group G1 with experimental groups G3 and G4 the results were statistically significant with *p* value < 0.05. Inter experimental group comparison showed G2, significant difference between G3 and G4. Comparison of experimental groups G3 and G4 showed significance difference between the groups (table-2).

Microscopic study of thigh bone of one month old chick

Mean number of cells of hypertrophy zone were higher in control group G1 and experimental group G2. Whereas mean values of experimental groups G3 and G4 were towards lower side in comparison of control group G1 (table-1). There is significant difference between groups G1, G3 and G4. Similarly group G2 showed significantly difference from G3 and G4 (table-2).

DISCUSSION

Nicotine once enters into the body is responsible for oxidative stress[9]. It is responsible forgiving exogenous stress to human population, therefore, imposing great risks towards the health problem. Cigarette smoke constituents have high concentration of oxidants and free oxygen radicals[10]. Nicotine stimulates angiogenesis, atherosclerosis and promotes tumor growth[11] and osteoporosis[12]. Weight of chick as well as the weight of femur bone showed statistically significant result when control group G1 and experimental group G2 was compared with experimental groups G3 and G4 with *p* value 0.000. When experimental groups G3 and G4 were compared the showed results statistical significant difference.

Mean number of cells in hypertrophy zone in comparison to control group G1 and experimental group G2 were not statistically significant, but when control group was compared with experimental groups G3 and G4 showed statistically significant results. In many studies it was found that nicotine causes adverse effects on bone. The changes in trabecular bone structure and cellular bone content in the nicotine treated groups may be due to imbalance in normal remodelling process. Nicotine exposure is responsible for inhibiting collagen synthesis and alkaline phosphate activity in osteoblast cells[12]. When rats were exposed to nicotine, oxidative stress occurred, which resulted in increased free radicals, osteoclast gets activated by free radicals[13]. In this study the results of gross and

histological observations, led to the conclusion that camellia sinensis has antioxidant properties that reduced the harmful effects of nicotine.

CONCLUSION

The research work revealed that administration of antioxidant significantly prevented some of the harmful effects of nicotine in gross study and histological observations but did not completely reverse it.

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