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Effect of Green Tea on Nicotine Induced Histological Changes in Chick Femur

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Abstract

Objective: To observe histological features of developing chick skeleton undergoing the effect of nicotine and camellia sinensis.

Methodology: This randomized controlled trial study was conducted in association of Poultry Research Institute, Rawalpindi, in the department of Anatomy at Army Medical College Rawalpindi, National university of science and technology, Islamabad. The study sample was consisting of four groups each having ten eggs. Chicks were given double exposure with the same quantity of working solutions. First exposure time was at forty eight hours of incubation and second after forty eight hours of chick birth. The group1 was known as G1 and was given 0.0001ml of normal saline. Different experimental groups were injected with different solution. Group (G2) was given in 0.1ml amount of camellia sinensis (green tea)extract, Group (G3) was administered with 0.1ml in quantity of 0.0001% nicotine solution and Group (G4) was administered both 0.0001% nicotine solution and green tea extract in the amount of 0.1ml. Chicks hatched from each group were given four weeks time to grow. Chicks at the age of one month were sacrificed for the collection of specimen. Femur from each group was collected for the slide formation and histological preparation done to calculate the height of proliferative zone.

Results: Control group G1 in comparison to experimental group G2 showed p value (0.385). G1 in comparison with G3 and G4 showed statistically significant result. Experimental groups when compared with each other such as, G2 in comparison with G3 and G4 showed result with p value (0.000).

Conclusion: From our research work it was concluded that nicotine, responsible of causing toxic effects on the developing thigh bone of chick and green tea to reduce its toxicity.

Key Words: Incubation, Cigarette smoke, Nicotine, Camellia sinensis, Height of proliferative zone and Femur.

Conflict of Interest: None **Funding Source**: None

Introduction

The intake of nicotine is either in the form of first and second hand smoking considered to be the biggest worldwide human health threats and killing more than 7 million people a year.[1] Cigarette smoking is causing many health diseases and its negative effects bringing environmental hazard. The skeleton of avian species was selected for research as it is best skeleton to study the teratogenicity. The calcification of femur of chick embryo starts on the 5th day while in case of tibia and fibula it starts on the 10th day of embryonic life.[2]

Camellia sinensis is a plant species whose leaves and leaf buds are involved in production of green tea. Green tea used in different countries of Asia with different cultures. The different constituents of green tea had protective role against free radical production in body.[3]

In the present research work was done to state the consequences of nicotine and *Camellia sinensis* on growing femur and able to see how green tea reverse the detrimental effects of nicotine. Nicotine affected the metabolism of bone by remodeling bone process and suppressing osteogenesis by decreasing in alkaline phosphatase and type 1 collagen production by osteoblast.[4] Chick femur undergoes the process of endochondral calcification that initiates at its center and move towards its end.[2]

The objective of this research work was done to study and observe how the nicotine effects the developing

thigh bone and can its toxic effects be prevented by the intake of *camellia sinensis*.

Methodology

The present study was conducted in National university of science and technology (NUST) in the department of Anatomy, Army Medical College Rawalpindi, in association of Poultry Research Institute, Rawalpindi. All methods granted approval by the Ethical review Committee of Army Medical College**.** For the experimental purpose fertilized chick eggs of Fayoumi species at zero hour of incubation eggs were included. From the Poultry Research institute, Rawalpindi eggs were purchased. Study technique was simple random sampling**.**[5] Proper process of fumigation and disinfecting the hatchery was conducted. The proper time monitoring for the incubation of egg was done very regularly. The criteria for temperature regulation was controlled at 37.5^0C. Eggs rotations were monitored 4 hourly. Placement of eggs were placed in hatchery at the age that is day zero. Four experimental groups labeled as G1, G2, G3 and G4. Every group was having count of ten eggs. G1 was known as control group, provided with 0.1ml of normal saline G2 with green tea extract G3 with 0.0001% nicotine solution and G4 was given both 0.0001% nicotine solution and green tea extract in 0.1ml of quantity.[5] All the groups provided their working solutions by the piercing at the blunt end of the eggs with the help of insulin gauge needle. Injection of doses were given two times. First injection at forty eight hours of incubation and 2^{nd} at forty eight hours of post natal period.

Chicks at age of one month, were dissected at their pelvic region by removing the lumbar vertebrae at L4 from L5. Sampels were collected for histological observation. By using the decalcification technique tissue were processed (Figure 1). Paraffin wax was used with melting point range from 40-70℃ for embedding. The block was allowed to cool on cold plate. The oculometer scale was aligned with the help of stage micrometer at 10X magnification. Heights were measured by aligning the oculometer parallel to the chondrocyte columns. In the central and peripheral part of this hypertrophy zone, height was measured then the observations were calculated.[4]

All 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

For result the height of proliferative zone of control group G1 and G2 both groups showed mean value 211.111±6.054µm.Whereas G3 and G4 showed mean value 165.500±0.500 and 180.000±3.061 respectively (Table I). Control group G1 in comparison to experimental group G2 showed p value (0.385). G1 in comparison with G3 and G4 showed statistically significant result with p values of both groups were (0.000). Experimental groups when compared with each other such as, G2 in comparison with G3 and G4 showed result with p value (0.000). Comparison of G3 and G4 with each other showed statistically insignificant result with p value (0.626) (Table II).

Table I: Mean values of height of proliferative zone (µm) among different groups of one month old chicks

Dependent Variable	Groups	Mean ± SEM
Height of proliferative Zone (µm)	G1	211.1111 ± 6.05403
	G2	211.1111 ± 6.05403
	G3	165.5000 ± 0.50000
	G4	180.0000 ± 3.06186

Table II: Comparison of height of proliferative zone among different groups of one month old chicks

Dependable Variable	**Comparison Between Groups**		**p value**
	Group	**Group**	
Height of Proliferative Zone (µm)	G1	G2	0.385
		G3	0.000
		G4	0.000
	G2	G1	0.385
		G3	0.000
		G4	0.000
	G3	G1	0.000
		G2	0.000
		G4	0.626
	G4	G1	0.000
		G2	0.000
		G3	0.626

P value ≤0.05 statistically significant

Discussion

The results showed that the chicks in the control group compared with all the experimental groups and with each other. The research work was supported by other researches done by many studies conducted that showed that nicotine's mechanism effect on weight of the developing fetus and responsible for growth defect.[6]

However the positive effect of nicotine was that the up regulation or down regulation of osteocalcin, which in turn has effects on osteogenesis.[7] The theory of a biphasic effect on the nAChR subunit showed its limitation to osteoblasts. Moreover many studies showing involving large amount of nicotine causing negative effects but, studies done at less amount doses depicted positive effects[8] In other study done has shown that the Nicotine and lipopolysaccharide stimulate the

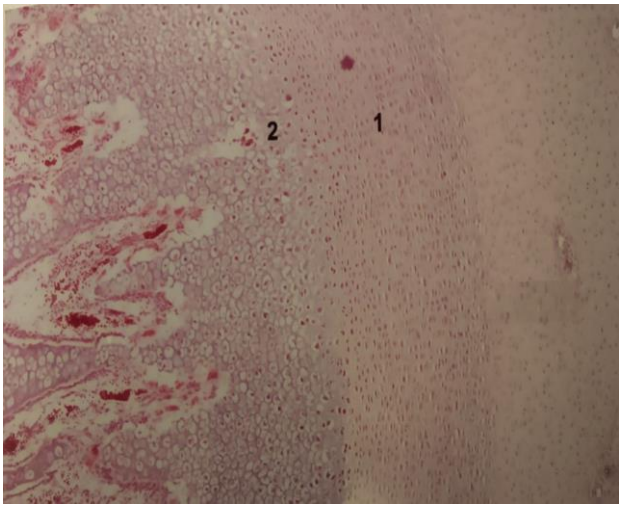

Fig.1 Photomicrograph showing one month old epiphyseal plate of contril group, '1' proliferative zone and '2' is hypertrophy zone. H&E Stain,X10.

formation of osteoclast-like cells by increasing macrophage colony-stimulating factor and prostaglandin E2 production by osteoblasts.[3] Nicotine has its influence on osteoclast as bone healing occurred by four overlapping phases; the initial inflammatory response, soft callus formation, hard callus formation, bony union and bone remodeling.[9]In many other studies done to show smoking-induced adverse biochemical changes in plasma and blood but upon green tea consumption in smokers causing protection by interacting with biomolecules at membrane and sub cellular levels by altering the pathways.[10] *Camellia sinensis* that is green tea largely used beverages in the world. Grean tea extracts consumption provide polyphenols, which act as antioxidants. An antioxidant is a molecule capable of inhibiting the oxidation of other molecules.[11]In many other researches it was observed that the plant having antioxidants properties used as the therapeutic entities.[12]

In other research work the antioxidant property of green tea also supporting this concept.[13] Nicotine one of the constituent of cigarette smoke responsible of giving negative impact on the development of neural system. [14]

Our study concluded that the nicotine responsible for causing oxidative stress in the development of skeleton of chick can be overcome by the consumption of antioxidant that is green tea extract (*camellia sinensis*). All the harm done by nicotine can not be undone but harmful effect can be decreased.

Conclusion

From this study it was elicited through the histological observations in the developing skeleton of chick under the effect of nicotine a major product of cigarette smoke and how its stressful affect can be suppressed by the use of antioxidant such as green tea. The research work revealed injection of antioxidant significantly safeguard effects of nicotine. From all the observations made we summed up that the nicotine, one of the basic part of cigarette smoke responsible of the skeleton deformity of developing chick. By injecting antioxidant counterbalance some, but not all the unpleasant effects.

Disclousre

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Authors Contribution:

[1,2]Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work & Final approval of the version to be published

[3,4,] Drafting the work or revising it critically for important intellectual content;