Original Article

Histomorphology of Fayoumi Species Embryo Effected by the Intake of Nicotine and Green Tea

Effect on Embryo by taking Nicotine and Green Tea

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ABSTRACT

Objective: The objective of this study is to consider how the nicotine consumption responsible of affecting the growth of avian species that is embryos of chick.
Study Design: Randomized control study
Place and Duration of Study: This study was conducted at the Quetta institute of Medical Sciences (NUMS), Islamabad for six month from June 2021 to November 2021.
Materials and Methods: The study was designed and conducted with the collaboration of poultry research institute of Rawapindi (PRI). It is further supervised and conducted in the department of Anatomy of Quetta Institute of Medical sciences.(NUMS).The study was a randomized control in nature. Eggs of Fayoumi species were placed at the zero hour of incubation by dividing thirty eggs in three experimental groups. Ten eggs for group G1 was considered as a control group, experimental group G2, experimental group G3 and experimental group G4 all groups were injected with 0.1ml quantity of working solution through airsac at the blunt end of the eggs by the insulin guage needle. Fixation of specimen were done by placing them in the formalin filled jars at 17th day of incubation. Data analysis was done by using SPSS (Statistical Package for Social Science) version 25.
Results: The results were calculated by calculating the CRL that is crown rump length, and comparing with the control group and with each other. Data were entered and analyzed using SPSS version 25. p value of less than 0.05 was considered significant.
Conclusion: It was concluded that oxidative stress by nicotine causes the developmental growth defects of chick embryo. Whereas green tea minimizes the oxidative stress caused by nicotine.
Key Words: Histomorphology, Fayoumi Species, Embryo, Nicotine, Green Tea

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INTRODUCTION

Cigarette smoke component that is nicotine responsible for the affect if it is consumed during gestational period causes harmful affects leading to skeleton abnormalities in early childhood and young adulthood. The present worked to show protection can be provided by intake of green tea (Camellia sinensis) in the toxic effect produced by nicotine intake problem associated with abnormalities like histomorphological changes in chick embryo. Chick embryo received 0.0001% concentration of nicotine through insulin gauge needle. The developing embryo were given 17th day of incubation. After that the nicotine-exposed newborns showed significantly low crown rump length at Day 17. According to new health research on nicotine intake has shown vast increased in its consumption. Nicotine intake control can be done at the government level and educating the community through health care providers. Many research work done in assessing how nicotine causes its affect on health in relation to cigarette smoking. However, in vitro and in vivo preclinical studies strongly indicate that nicotine exposure alone can adversely affect the developing organs especially during early trimester of gestational life. The mandible bone cephalometric analysis depicted indicated prenatal and postnatal changes in skeleton[1]. This observation is supported by rise in proliferating growth near the molars witnessed with nicotine exposure may be evidence of delayed tooth formation as proliferating cells may not be also differentiating appropriately. This delay may precipitate additional clinical dental issues including an increased need for orthodontics and an increased risk for caries.[2]

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Camellia sinensis has the potential of protecting against perinatal nicotine-induced neurobehavioral alterations, tissue injury, and oxidative stress in mice newborns[3]. This work is again supported by another research to show that the oxidative damage can be minimized by the usage of green tea that is camellia sinensis[4].

MATERIALS AND METHODS

The study was designed and conducted with the collaboration of poultry research institute of Rawalpindi (PRI). It is further supervised and conducted in the department of Anatomy of Quetta Institute of Medical sciences (NUMS).The study was a randomized control in nature. Eggs of Fayoumi species were placed at the zero hour of incubation by dividing thirty eggs in three experimental groups. Ten eggs for group G1 was considered as a control group, experimental group G2, experimental group G3 and experimental group G4 all groups were injected with 0.1ml quantity of working solution through air sac at the blunt end of the eggs by the insulin guage needle. A study was carried out on eggs keeping at the Poultry Research Institute (PRI), Rawalpindi. Fertilized oval eggs with normal shape, off-white colour and not very large size were part of the study. Eggs with not proper time of laying, not proper shell and regular shape were not taken into account of study project. The eggs were divided into four groups numbered 1, 2, 3, and 4. Then randomly groups were made. Group-1 was named as control groupG1 was given normal saline; groupG2 was given green tea extract; groupG3 was given 0.0001% nicotine solution; and group-4 was given both 0.0001% Nicotine solution and green tea extract. After fumigating and maintaining the temperature around 37.5°C and 75% relative humidity maintained with proper ventilation. Egg rotation in a duration of 4 hourly basis. First day is a zero time of placement of eggs in hatchery. In all the groups, the solutions were put by blunt ends through insulin needle and same point was taken for opening and collecting embryo of 17th days old and placing them in formalin filled jars [7].

Crown rump length of 17th days old embryo was taken from vertex that is from the highest point between the eyeballs and tip of coccyx (Fig. 1). A thread was taken from the curve of the back of embryo to tip of the coccyx which was placed on a smooth surface with flexed hind limbs showing curved position.[5]

An ocular micrometer provided with a scale, was used to measure the size of structure under the microscope. Observations were made using objective 10X . Distal end of epiphyseal plate of thigh bone were taken into account for properly arranged structure present in between epiphysis and diaphysis of thigh bone.[6] Cells are arranged in a proper way either regular manner as the column wise or irrespective way[7] The slides were stained with haematoxylin and eosin for recording at 10X as well as 40X. At 10X alignment of cells, height of hypertrophy zone were calculated. Bone process of elongation is monitored by the rate of chondrocyte proliferation and differentiation in the growth plate region in longitudinal section. [8]

SPSS-22 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 the four groups. The p-value of ≤0.05 was considered statistically significant.

RESULTS

For 17th day old embryo, crown rump length observation was made of fayoumi species of 17th days old embryo by positioning the thread at two points one at vertex and second at tip of coccyx once it was in curved position length was measured. The distance covered by the thread was measured. Mean length of embryo of control group G1 and G2 showed similar value that is 9.780±0.013cm whereas G3 and G4 showed mean value that is 6.158 ± 0.402 cm and 7.857 ± 0.068cm respectively (Table 1).

Figure No.1: Photograph depicts crown rump length observation made of fayoumi species 17th days old embryo by positioning the thread at two points one at vertex and second at tip of coccyx once it was in curved position.

Table No.1: Mean values of crown rump length (cm) among different groups of 17th day of incubation

Dependent Variable	Groups	Mean ± SEM
CROWN RUMP LENGTH (cm)	G1	9.7700 ± 0.0133
	G2	9.7600 ± 0.0133
	G3	6.1450 ± 0.40270
	G4	7.8569 ± 0.06851

The comparison of control group G1 to experimental group G2 showed statistically insignificant result with p value that is (1.000). Comparing G1 with G3 and G4 showed p values that was (0.000) statistically significant. Experimental groups when compared with each other such as, G2 in comparison with G3 and G4

showed statistically significant result with p value (0.000). Experimental group G3 in comparison of G4 showed result with p value (0.000) which was statistically significant (Table 2).

Table No.2: Comparison of crown rump length (cm) among different groups of 17th day of incubation

Dependent Variable	Comparison Between Groups		p value
	(Group)	(Group)	
CROWN RUMP LENGTH (cm)	G1	G2	1.000
		G3	0.000
		G4	0.000
	G2	G1	1.000
		G3	0.000
		G4	0.000
	G3	G1	0.000
		G2	0.000
		G4	0.000
	G4	G1	0.000
		G2	0.000
		G3	0.000

p value ≤ 0.05 statistically significant

Table No.3: Mean values of height of hypertrophy zone (µm) among different groups of 17th day of incubation

HEIGHT OF HYPERTROPHY ZONE (µm)	G1	1042.5000 ± 6.16667
	G2	1042.5000 ± 6.16667
	G3	894.2000 ± 90.26272
	G4	1002.4714 ± 21.42857

p value ≤ 0.05 statistically significant

Table No.4: Comparison of height of hypertrophy zone among different groups of 17th day of incubation

Dependent Variable	Comparison Between Groups		p value
	Group	Group	
HEIGHT OF HYPERTROPHY ZONE (µm)	G1	G2	1.000
		G3	0.000
		G4	0.000
	G2	G1	1.000
		G3	0.000
		G4	0.000
	G3	G1	0.000
		G2	0.000
		G4	0.470
	G4	G1	0.000
		G2	0.000
		G3	0.470

p value ≤ 0.05 statistically significant

Mean value of height of hypertrophy zone of control group G1 and G2 1042.500±6.166µm whereas G3 and G4 showed mean value 894.200±90.262µm and 1002.471±21.428µm respectively (Table 3).

Comparison of control group G1 with experimental group G2 showed statistically insignificant result with p value that was (1.000). G1 in comparison with G3 and G4 showed statistically significant results with p value (0.000) and (0.000) respectively. Experimental groups when compared with each other such as, G2 in comparison with G3 and G4 showed significant result with p value (0.000) and (0.000) respectively. Comparison of G3 and G4 with each other showed statistically insignificant result with p value (0.470).

DISCUSSION

Our study depicted that toxic effects by nicotine was to some extent reverse by the intake of camellia sinensis. This work is supported by the study which suggested that the intake of cigarettes has become a increased health hazard for community with harmful mental diseases and lung problems. Cigarette intake is a causative factor of harmful effects on human body embryo. Individuals aged more than 60 and have a habit of cigarette smoking have two times more chances in mortality as compared with those who are nonsmoker.[8,9] Stop inhaling cigarette smoke as a first and secondhand smoker improved health benefits whereas some or all the reduced life expectancy can be improved depending on the age a person quits. As it is responsible for many effects like mental problems.[10] Another research work supported that the emphysema caused by many intake conitine and thyroxine. [11,12]

In many recent research works has shown that the antioxidant property of some plant used as therapeutic entities. Green tea (Camellia sinensis) largely consumed tea in the world. Green tea extracts consumption provides polyphenols, which act as antioxidants[13]. An antioxidant is a molecule capable of inhibiting the oxidation of other molecules.[14]In other research work done animals which shown treated with green tea, cigarette smoking responsible for many harmful biochemical changes in plasma and blood leading to ill health effects for which therapeutic approaches are seen by the intake of green tea.[15]As a large amount of high natural polyphenols, constituent of green tea healthiest nonalcoholic drink intake worldwide. Epigallocatechin-3-gallate (EGCG) is the predominant catechin found in green tea, which has largely increase the benefits of health.

In our research work, the threatening effect by induction of nicotine on developing fayoumi species and crown rump length of chick embryo were reduced by the intake of green tea though it was not totally reversed but some of the oxidative stress can be neutralized.

CONCLUSION

It was shown through the research work that Camellia sinensis (green tea) has antioxidant nature responsible of decreasing the oxidative stress caused by the nicotine, however, everything cannot be reverse back.

Author's Contribution:

Concept & Design of Study:	Maryam Shan, Kaneez Fatima
Drafting:	Ali Ahmed, Hina Shan
Data Analysis:	Ahmad Tariq Chisthti, Anwar Soomro
Revisiting Critically:	Maryam Shan, Kaneez Fatima
Final Approval of version:	Maryam Shan, Kaneez Fatima

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Durham EL, Balog C, Howie RN, Boyce MA, Arand JR, Warren G, et al. Effects of nicotine exposure on murine mandibular development. PLoS One 2019;14(6):e0218376.
2. Zhou S, Rosenthal DG, Sherman S, Zelikoff J, Gordon T, Weitzman M. Physical, Behavioral, and Cognitive Effects of Prenatal Tobacco and Postnatal Secondhand Smoke Exposure. Current Problems in Pediatr Adolescent Health Care 2014; 44(8):219–41.
3. Ajarem JS, Al-Basher G, Allam AA, Mahmoud AM. Camellia sinensis Prevents Perinatal Nicotine-Induced Neurobehavioral Alterations, Tissue Injury, and Oxidative Stress in Male and Female Mice Newborns. Oxid Med Cell Longev 2017;2017:5985219.
4. Martins A, Schimidt HL, Garcia A, Colletta Altermann CD, Santos FW, Carpes FP, et al. Supplementation with different teas from Camellia sinensis prevents memory deficits and hippocampus oxidative stress in ischemia-reperfusion. Neurochem Int 2017;108:287-295.
5. Kamran K. The effect of ethanol vapour exposure on the development of heart and great vessels of chick embryo. FCPS thesis, CPSP 2007.
6. Blumer MJF, Longato S, Ritcher E, Perez MT, Konakci KZ, Fritsch H. The role of cartilage canals in endochondral and perichondral bone formation is their similarities between these two processes. J Anat 2005;206(4): 359–372.
7. Shan M, Qamar K, Iqbal I. Role of nicotine and camellia sinensis on the developing femur of chick. JPMA. J Pak Med Assoc 2015;65(10):1094-6
8. Schwartz AG, Cote ML. Epidemiology of lung cancer. Lung Cancer and Personalized Med 2016: 21-41.
9. Glasheen C, Hedden SL, Forman-Hoffman VL, Colpe LJ. Cigarette smoking behaviors among adults with serious mental illness in a nationally representative sample. Annals Epidemiol 2014; 24(10):776-80.
10. Xu X, Su Y, Fan ZH. Cotinine concentration in serum correlates with tobacco smoke-induced emphysema in mice. Scientific reports 2014;4: 3864.
11. Howie RN, Durham EL, Black L, Bennfors G, Parsons TE, Elsalanty ME, et al. Effects of In Utero Thyroxine Exposure on Murine Cranial Suture Growth. PLoS One 2016;11(12):e0167805.
12. Kasote DM, Katyare SS, Hegde MV, Bae H. Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int J Biological Sciences 2015;11(8):982.
13. Vishnoi H, Bodla RB, Kant R. Green Tea (Camellia sinensis) and its antioxidant property: A review. Int J Pharm Sci Res 2018;9(5):1723-36.
14. Begum MS, Saradamma B, Reddy VD, Padmavathi P, Maturu P, babu Ellutla N, et al. Influence of green tea consumption on cigarette smoking-induced biochemical changes in plasma and blood. Clin Nutr Experimental 2017;16:1-2.
15. Hung SW, Li Y, Chen X, Chu KO, Zhao Y, Liu Y, et al. Green Tea Epigallocatechin-3-Gallate Regulates Autophagy in Male and Female Reproductive Cancer. Front Pharmacol 2022; 13:906746.