Original Article

Developing Chick Embryo Under the Influence of Nicotine and Camellia Sinensis

Maryam Shan[1], Kaneez Fatima[2], Anwar Soomro[3], Ali Ahmed[3], Hina Shan[4] and Ahmad Tariq Chisthti[1]

ABSTRACT

Objective: By research work to analyze the outcome of nicotine on the weight of developing embryo of chick and to estimate the safeguard effect in overcoming toxicity of nicotine by the leaves of green tea camellia sinensis.
Study Design: A randomized control study
Place and Duration of Study: This study was conducted at the Anatomy Department, HBS Medical and Dental College Islamabad, in association with Poultry Research Institute, Rawalpindi from June 2021 to November 2021.
Materials and Methods: Forty eggs of Fayoumi species fertilized in nature of chick embryo were selected at zero hour of incubation. The research work was formulated by creating four distributive groups, each consisting of ten eggs. The G1 group was labelled as control group 1 administered with dose of 0.1ml of 9% Normal saline. Experimental G2 Injected with 0.1ml of 8% Camellia sinensis. Experimental G3 Injected with 0.1ml of 0.0001% Nicotine solution. Experimental G4 Injected with 0.1ml of 0.0001% Nicotine solution and 0.1 ml of 8% Camellia sinensis. After 17 days of incubation embryo were collected for measurement. Data were entered in a database using SPSS (Statistical Package for Social Science) version 25. Data were presented as tables. Chi-square test, Anova and PosthocTukey test were used for analyzing data. P value < 0.05 was considered significant.
Results: The observations were made on the development of chick embryo by recording the weight of chick embryo and comparing with the control group and with each other. Data were entered and analysed using SPSS version 22. One-way analysis of variance (ANOVA) was used to compare weight of chick embryo among the different groups, p value of less than 0.05 was considered significant.
Conclusion: It was concluded that Nicotine oxidative stress responsible for decreasing the developmental growth of chick embryo. Whereas green tea suppresses the oxidative stress but cannot reverse the effect.

Key Words: Hatchery, Embryo, Nicotine, Weight.

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INTRODUCTION

The skeletal system is composed of bones; they are further supported by ligaments, tendons, muscles and cartilages. It is responsible for maintaining the architecture, shape to the body. Exposure to environmental insults due to many reasons, can affect the developing skeleton.

1. Department of Anatomy, Quetta institute of Medical Sciences (Qims) NUMS, Quetta.
2. Department of Anatomy, Bolan Medical College, Quetta.
3. Department of Anatomy, Jhalawan Medical College, Khuzdar.
4. Department of Community Medicine, National University of Medical Sciences (NUMS).

Correspondence: Prof. Dr. Ahmad Tariq Chisthti, Head of Anatomy Department Quetta Institute of Medical Sciences (QIMS), Quetta.
Contact No: 0303-5906452
Email: tariqchishti58@yahoo.com

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Nicotine a natural alkaloid and one of the constituent of cigarette responsible for increasing the chances of congenital malformations in humans. Nicotine is responsible for congenital defects abortions, premature delivery, and low on the size and weight in the study[2] Nicotine affects the fetus due to the stimulation of nicotinic cholinergic receptors.[3] Camellia sinensis considers as anantioxidant used in one of the type of tea known as 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[4].

Habit of smoking during gestational period (pregnancy) and during reproductive age has high public health problem. According to the data, the ratio of smoking among pregnant women was 10–23%, even up to 59.3% in some parts.[5] According to the data driven studies, it showed that smoking at the time of gestation life responsible for congenital abnormalities causing several fetal problems. Tobacco component that is nicotine responsible for passing through the placental barrier by this reason amount of tobacco consumption appear more in the blood of fetus than in the mother's blood[6]. Another research work done with a conclusion

that tobacco smoke responsible for the condition of brochiolitis during infancy. [7] It is supported by the work of Talati A et al who mentioned oxidative effect of nicotine by effecting on the reproductive and developmental of embryo[8]. Nicotine intake causes decrease size of the skeleton and body mass index in developing family of rats[9]. In some of the other studies done it showed that the bone mass accumulation affected by the intake of nicotine[10]. In a research work done on neonatal rat pups responsible for growth retardation in neonatal rat pups[11]. It is supported by another work done by Chen B et al in which it showed nicotine consumption during pregnancy caused bony problems in developing rats family.[12]

Grean tea extracts consumption provide phyto-chemical, decreasing the process of oxidation. A substance used to counteract the deterioration by nicotine.[13]

The highest case showing universality of cigarette intake during reproductive age of female is increasing worldwide. Cigarette intake suppression campaign should be launched nationally and internationally and make the large places of the world smoke free zone as it is effecting more under twelve years old youngster and low socioeconomics group people.[14]

MATERIALS AND METHODS

The study work done in the Anatomy department, HBS Medical and Dental College Islamabad with a duration of six month after the Ethical review board approval. Forty Fayoumi fertilized eggs of chick included at zero hour of incubation. The research formulated by creating four distributive groups, each consisting of ten eggs. The control group was labelled as group one administered with dose of 0.1ml of 9% Normal saline. Experimental group two had ten number of eggs injected with 8% Camellia sinensis in same quantity as of above administered dose. Another group three had ten eggs injected with 0.1ml of 0.0001% Nicotine solution. Another group four had ten number of eggs injected with 0.1ml of 0.0001% Nicotine solution and 0.1 ml of 8% Camellia sinensis. Eggs of each group were injected with their respective solutions in same quantity with the help of insulin gauge needle under laminar flow fig 1.

The study was done on those eggs which at the age of zero hour of incubation. Those eggs with unknown time of laying, broken shell, abnormal shape, colour, texture or eggs stored in refrigerator were not considered for the research project. Total of forty eggs were placed in hatchery. Solutions used for the study were injected to the eggs of four groups (experimental) after two days that is forty eight hours of incubation. After incubating eggs for a period of 17 days, ten eggs from each group were selected for collection of 17th day old embryos. The embryos were collected by opening the blunt end by using the forceps. By removing yolk sac embryos placed in the formalin filled jars for 48 hours. The embryos after taking out from jars kept on a piece of blotting paper. Precision digital balance with 0.001g readability were used for recording weight.[15]

RESULTS

The developing embryos of 17th days old were removed from fixative, placed on a piece of blotting paper. Each embryo weighed.[15] Mean weight of control group G1 and experimental group 2 were 8.539 ± 0.099g high in comparing of group 3and group 4 where they were 5.318±1.065g and 6.755±0.036g (Table 1). The p value in comparison of control group G1 with G2 was (1.000). The p value in comparison of control group G1 with G3 and G4 were significant that was (0.000). 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). In contrast of group 3 and group 4 depicted statistically significant result with p value (0.011) (Table 1).The recorded weight of all the surviving embryo and their recorded weight showed in Table 2.

Table No.1: In contrast to weight (g) of developing chick embryo among different groups

Dependent Variable	Comparison Between Groups		p value
	(Group)	(Group)	
Weight of Embryo (g)	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	0.011
	G4	G1	0.000
		G2	0.000
		G3	0.011

p value $\leq$ 0.05 statistically significant

Figure No.1: Injection of different working solutions by insulin gauge needle into the incubated eggs.

Table No.2: Gross Study at 17th Day of Incubation

Control Group 1	Weight (g) Group 1	Exp Group 2	Weight (g) Group 2	Exp Group 3	Weight (g) Group 3	Exp Group 4	Weight (g) Group 4
1	8.55	1	8.53	1	6.75	1	6.75
2	8.54	2	8.54	2	5.99	2	6.95
3	8.50	3	8.54	3	6.75	3	6.66
4	8.50	4	8.55	4	5.99	4	6.75
5	8.44	5	8.45	**5**	1.11	5	6.66
6	8.52	6	8.55	-	-	6	6.76
7	8.55	7	8.53	-	-	**7**	6.76
8	8.47	8	8.55	-	-	-	-
9	8.55	9	8.55	-	-	-	-
10	8.55	**10**	8.55	-	-	-	-
Mean Weight	8.517	**Mean Weight**	8.54	**Mean Weight**	5.31	**Mean Weight**	6.75

DISCUSSION

The research work done by looking at how the nicotine effects the developing stages of embryo and preventive role of camellia sinensis (green tea) on it. The control group G1 was brought in comparison with other experimental groups and with each other. Nicotine exposure comes into the degree of environment hazard during the period of pregnancy, it can be first hand smoking or second hand smoking. Nicotine responsible to affect the offspring by influencing their epigenetic regulation. Nicotine is considered to be a reason for bringing against the normal functioning of multiorgan functional genes. Many studies showed that nicotine decreases the fetal adrenosteroid synthase expression and adrenal function system as well as osteogenic process got inhibition that is responsible for lowering the mass of the developing offspring due to nicotine exposure during gestational life come across as prenatal nicotine exposure.[15]

From the previous studies it was estimated that 400,000 developing stage of human embryo got exposure to harmful ingredients of tobacco smoke that is nicotine leading to the ultimate exposure in the way of carbonmonoxide. The tobacco extract intake causing developmental defect to the growing skeleton of fetus whose mother are in their gestational period.[1]

Other research work done to brought this evidence under consideration that epigenetic transformation played vital roles in the long-term developmental toxicity due to the exposure of environmental hazards. [16] As it is supported by another work which clearly concluded that long duration of exposure of nicotine responsible for bone developmental defects in neonates as well as uterine transformation. [17]

To support our research works, it has proven that green tea antioxidant quality can be effective as a therapy of different problems.[18] It was observed that resveratrol undo pathological injury of renal tissue by decreasing the fatty acid break down. [19]In other study done that revealed green tea, prior administration to nicotine, showed better reason of nephroprotection, shown by immense reduction in oxidative damage.[20]

Camellia sinensis that is green tea beverage taken in by large population of the world especially asian subcontinent. Green tea composition had polyphenols, which act as antioxidants. An antioxidant molecules are considered as a inhibitors for oxidative stressi.[12] Green tea has got catechins that will serve as a anticanacer agent in the area of tumor growth.[21] Green tea proved itself in a case of immune dysfunction as a immune modulators reason of any oncology treatment.[22]

CONCLUSION

It was concluded by the study that green tea (camellia sinensis) polyphenol molecules reduced the oxidative stress caused by nicotine but cannot reverse the toxicity completely.

Author's Contribution:

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

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

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