Original Article

Smokeless Tobacco

Effect of Smokeless Tobacco on the Outcome of Swiss Albino Mice Pregnancy & Changes in their Offspring's Body Weight; An Experimental Study

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ABSTRACT

Objective: To study the harmful effects of smokeless chewing local tobacco on Swiss Albino mice pregnancy outcome and observe the effect on their offspring's body weight.

Study Design: An Experimental study

Place and Duration of Study: This study was conducted at Anatomy Department Al- Tibri Medical College, Isra University Karachi during June 2015 to December 2015.

Materials and Methods: Twenty pregnant Swiss albino mice and their 40 offspring male and female equal number, Selected randomly. The mice were divided into Experimental and control groups. Inclusion criteria were the healthy offspring of two weeks age. At the time of birth initial weight was taken and the final weight was taken after two weeks. Exclusion criteria was unhealthy, less or more than two week's age. Independent sample t-test was used for analysis of data through SPSS version 20.0.

Results: Total of 20 female Swiss albino mice divided equally into two groups experimental and control. Experimental group was kept on 5% smokeless tobacco. Twenty offspring from experimental and twenty offspring from control group of both sexes were taken. Initial and final weights of both groups were recorded. Significant difference was observed in initial and final weights of offspring with P-value=0.01.

Conclusion: Tobacco which is frequently used in our region without any knowledge of its harmful effects. It is proved that smokeless tobacco not only reduces the weight of offspring but during pregnancy it effects the growing fetus leading to stillbirth and neonatal deaths.

Key Words: Smokeless tobacco, Swiss albino mice, Harmful effects.

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INTRODUCTION

Tobacco the known killer of its users was a main crop of America, it is being cultivated since the prehistoric period. Americans used tobacco as medicinal and ceremonial prospect[1]. World knew it when Christopher Columbus discovered new world along with tobacco in 1492[2]. Later on tobacco was supplied to European royalty when it took place of cash crop. With the passageof time tobacco became important symbol for religious and political communities, separate rooms called withdrawing rooms now known as drawing rooms were built for smoking and Smokeless Tobacco (ST), it is used in snuff, chewing products and smoking in pipes and cigrates, in those days people used to say it is the cure for all no one was knowing about its hazards[3]. Latter on many chemical compounds were extracted including carcinogens like nicotine, arsenic, lead, chromium and nickel[4,5]. According to WHO tobacco is the only main cause of death today worldwide which can be prevented[6]. In recent years about 5.4 million people are dying every year due to tobacco use[7]. In Pakistan smokeless tobacco is being used in pan, ghutka, naswar and many other preparations[8,9]. From different reports and records it is observed that 20% males and 17% females use ST worldwide[10,11], in Pakistan about 13% females and 34% males consume tobacco in various forms[12], it is also observed that ST is as harmful as smoking, more over for females it is not only dangerous for them but also to their generation[13,14]. Swiss albino mice are small mammals, resembles humans genetically, physiologically and to some extent anatomically, due to their small size, easily maintained, polyestrous, easily handled, need small amount of water and food 4 to 8

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grams per day , and are docile, for these qualities the animals are selected in this study[15].

This study intends to see the effect of Smokeless Tobacco on the outcome of Swiss Albino Mice pregnancy and to see the changes in bodyweight of their offspring by comparing the weights of experimental and control groups. We observe only outcomes of pregnancy in 20 female Swiss Albino mice but our main objective was to observe changes in body weight of their offspring by tobacco which was given to their mothers.

MATERIALS AND METHODS

This experimental study was carried out at Anatomy Department Al- Tibri Medical College Karachi, Isra University and comprised data related to the period between June to December 2015. Simple Random sample technique was used for selection. Twenty pregnant Swiss albino mice were selected randomly then the mice were divided into Experimental and control groups equally. The sample size of offspring selection was done through the statistical software GPower 3.0.10 by taking α error probability =0.05, Power (1-β error probability)=0.95. Required sample size was calculated as 40 offspring. So we divided 40 offspring in to two groups equally into male and female randomly from both groups.

Inclusion criteria: was the healthy, adult Swiss albino mice with same age, size, breed and average weight. Offspring of two weeks age. At the time of birth initial weight was taken and the final weight was taken after two weeks.

Exclusion criteria: unhealthy, less or more than two weeks age. All female mice were kept in cages two per cage with one male to mate. Pregnancy was confirmed by vaginal plug .On confirmation of pregnancy the males were removed. 20 pregnant mice were equally divided into two groups. Group "A" Experimental group while Group "B" Control group. A group was given 5% local tobacco determined by pilot study mixed in lab cake to feed ad-libitum from day one of pregnancy to parturition, group B control group was given tobacco freecake. Cake prepared with 40% flour, 40% poultry feed and 20% dried milk. After making food 5% of total food was removed and 5% Smokeless Tobacco added. During pregnancy regular weight and any abnormal event which occurred was registered there were four stillbirths, three neonatal deaths and one offspring had left hind limb deformity all were observed in experimental group not in control group. Just before delivery all female mice were separated and kept in separate cages. After birth of baby mice 40 offsprings 20 male and 20 female were selected randomly, and tagged. The healthy offsprings were subdivided into four groups, group A1 experimental male, group A2 experimental female, B1 control male and B2 control female.Each offspring was weighed examined for any abnormality, than followed from day one up today fourteen.

Animal Protocol: All Swiss albino mice got from Al-Tibri medical college animal house were kept in well ventilated and hygienic room, animals were given water and food ad-libitum and were kept in light and dark for 12 hourly cycles and all protocols were followed as advised by animal house.

Data Analysis: Statistical analysis was done using SPSS version 20.0. The continuous variable were presented in Mean ± Standard Deviation. To see the significant difference in the mean offspring body weight of two groups (Experimental and Control) Independent sample t-test was applied. P-value ≤ 0.05 was considered to be statistically significant.

RESULTS

A total of 20 female Swiss albino mice divided equally into two groups experimental and control. Experimental group was kept on 5% smokeless tobacco. From twenty mothers forty offspring twenty from Experimental and twenty offspring from control group of both sexes were taken. Initial and final weights of all four subgroups were recorded.

Abnormal pregnancy outcomes detected in this study were, stillbirths four 20%, neonatal deaths three 15% and malformation in one offspring 5%, all these abnormalities were observed in experimental group, while no deviation was noticed in control group.

Table No.1: Comparison of mean initial and final weights of Male offspring in grams

Male Offspring	Experimental Group (mean & SD)	Control Group (mean & SD)	p-Value
Initial weight	1.28 ± 0.15	1.85 ± 0.24	0.01
Final weight	9.15 ± 0.64	12.22± 0.51	0.01

*Data were presented in Mean± SD

*P-value < 0.05 considered to be statistically significant

Table No.2: Comparison of mean initial and final weights of Female offspring in grams

Female Offspring	Experimental Group (mean & SD)	Control Group (mean & SD)	p-Value
Initial weight	1.08 ± 0.04	1.34 ± 0.03	0.01
Final weight	8.78± 0.45	11.65± 0.73	0.01

*Data were presented in Mean± SD

*P-value < 0.05 considered to be statistically significant

The following results were observed in grams. The results were compared between initial weight (IW) of experimental group with initial weight of control group and other variables like final weight (FW) of experimental group with final weight of control group.

It was observed that Male experimental group initial Mean weight of offspring found to be 1.28±0.15 grams while in control group Mean Initial weight was 1.85±0.24 grams with P-value=0.01 as shown in Table1 and figure1.

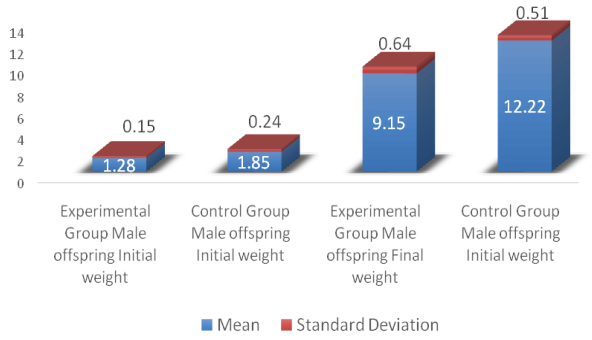

Figure No.1: Comparison of Mean & S.D of initial and final weight (gm) of Male offsprings.

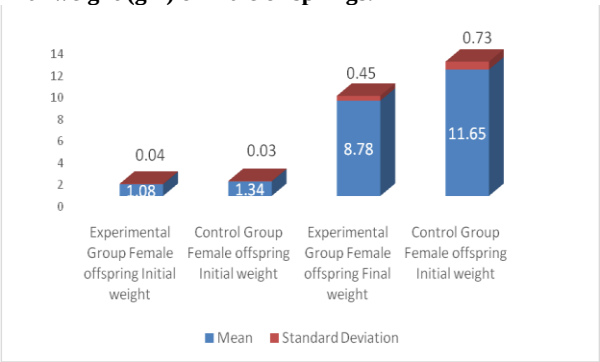

Figure No.2: Comparison of Mean & S.D of weight (gm) of Female offsprings Experimental and Control groups.

Female experimental initial Mean weight was found to be 1.08±0.04 grams while in control group it was 1.34±0.03 grams with P-value=0.01. It was observed that in male experimental group final mean weight was found to be 9.15±0.64 grams while in control group final mean weight of offspring was 12.22±0.51 grams with P-value= 0.01. Female experimental final mean weight was found to be 8.78±0.45 grams while in control group it was 11.65±0.73 grams with P-value=0.01 as shown in Table 2 and figure 2.

DISCUSSION

The use of chewing tobacco is increasing due to ban on smoking, easy to use, cheap to purchase, hide the habit, its use in communities especially in females is rising day by day. This study is designed to observe the effect of local smokeless tobacco on pregnancy outcome and on bodyweight of offspring of Swiss albino mice, to apply the results on human female population for their betterment and information.

The outcome of pregnancy in our study which showed stillbirth, neonatal deaths and deformity in the offspring of tobacco user mothers were in the line of studies of England et al[16]. The malformation or deformation of hind left limb might be due to mechanical force from fetal compression in the uterus.

Many studies were conducted on humans and on animals by using different active principles of tobacco to see their effect on offspring, all were in agreement with the our study in which we had used local chewable tobacco. In our study there was significant decrease in body weight of offspring of experimental mice in comparison to the control group, similar results were shown by Essien and Akpan in 2007[17], they used nicotine, work of EL Meligy et al in 2007[18] and Wickstrom R (2007)[19] showed the same result on Swiss albino mice offsprings by using nitrosamine a tobacco ingredient. In addition to this study of England et al 2015[20] and observation of Siddiqui et al 2015[21] supported present experimental work. Still no study is available to nullify our study.

CONCLUSION

Non smoking tobacco if used during pregnancy has harmful effects on pregnancy outcome in the form of stillbirth, neonatal deaths and decrease of bodyweight of offspring to a critical level.

Recommendations: The potential adverse effects of smokeless tobacco should be communicated to the community especially female population because females in our society are uneducated they do not know about the harmful effects of tobacco and its various preparations. There is need of conceptual work that can save them and their future generations

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

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