HISTOLOGICAL ANALYSIS OF THE EFFECTS OF SMOKELESS TOBACCO ON THE OVARIES OF THE NON PREGNANT FEMALE SWISS ALBINO RATS

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ABSTRACT

BACKGROUND: Smokeless tobacco contains higher quantities of nicotine than most cigarettes. Low fertility rates & adverse reproductive outcomes are associated with its use. An effort is made to evaluate the effects produced by the locally available brand of smokeless tobacco on the ovaries of the female Swiss albino rats.

MATERIAL AND METHODS: 30 adult female Swiss albino rats were randomly selected. They were equally divided into three groups. Group A were taken as control. Group B&C consisted of those rats which were given 5 %& 10% of smokeless tobacco in their feed. The feed and water were given ad libitum. On 31st day, animals were sacrificed and their ovaries were removed and weighed. The specimens were processed routinely. The sections were stained using H & E and trichrome stains and examined under light microscope.

RESULTS: A significant decrease in the weight of the ovaries was observed (P value ≤ 0.001). Ovaries of both B & C groups showed significant retardation in the follicular growth, decrease in number of healthy follicles, increase in the number of cystic follicles, apoptotic cell death and necrosis in the granulosa cells (P value ≤ 0.001).

CONCLUSION: This study concluded that the smokeless form of tobacco causes adverse effects on the ovaries of the female Swiss albino rats.

Key words: adverse effects, ovaries, smokeless tobacco

INTRODUCTION

Tobacco use is the notable cause of death all over the world. These deaths have been attributed to multiple tobacco related conditions, including heart disease, cancer, chronic pulmonary disease and stroke. Statistics show that the average age of first time tobacco user in the world is now between 13 – 15 years(Backinger, c.l etal 2003, Cooke, J.P. etal 2004).

Smokeless tobacco is unburnt tobacco which is placed into the vestibule of mouth. The chewing type of smokeless tobacco consists of crudely divided tobacco leaf that is mixed with sugar and molasses and packaged in a pouch. A “quid” or “chaw” of the tobacco is either chewed or sucked. During its stay in the mouth smokeless tobacco continuously releases toxic chemicals which have local effects and also enter the blood stream to reach the organs like brain, heart, pituitary gland, adrenal cortex, ovaries & uterus(Bombard, j. M. etal 2007).

Smokeless tobacco contains higher quantities of nicotine than most cigarettes. Nicotine is the principle alkaloid found in tobacco and is believed to be the main reason for its use since many people derive satisfaction by its use(Cristine, d.d. etal 2011)

Infertility is one of the most common problems with significant medical and psychosocial concerns. There are various factors responsible for male and female infertility. (Sanders etal., 2002). Use of tobacco is associated with low fertility rates, poor reproductive outcomes and increased risk of in vitro fertilization failures. Over the past few decades the prevalence of tobacco among women of reproductive ages has increased. (Dechanet C et al., 2010)

Tobacco is harmful to the ovaries and may lead to infertility. Tobacco use impairs every stage of the reproductive process. (B. O. Iranloye et al., 2007, 2009).Studies have shown the antiovulatory and abortifacient effects of the smokeless tobacco (Kumari etal., 2010).

MATERIAL & METHODS

The study was case control. It was conducted at Animal house Sindh Agriculture University Tando Jam. 30 adult non pregnant female Swiss albino rats were used which were confirmed for adulthood by vaginal swab test (Jyoti et al., 2010**).**The animals were housed under hygienic conditions. They were provided with laboratory chow diet & distilled water ad libitum. The room temperature was maintained at $22^0C \pm 2^0C$ & 12 hrs Light / dark cycles were observed (Browman et al.,2005).The experiment was carried out for 30 days. Rats were divided into three groups comprising of ten rats each. The groups were taken as control (A), experimental group B(5%) and experimental group C(10%).

For the experimental groups B&C locally available tobacco was purchased. It was grinded and mixed with the raw diet at the rate of 5% & 10% of their diet.The weight of all the animals was checked weekly. The growth rate of the animals was calculated by using the following formula Growth rate: weight in grams at 4th week – weight in grams at zero week. The animals were sacrificed on 31^{st} day by cervical dislocation. The ovaries were removed and weighed. The organs were then placed in 10% formaldehyde solution and were prepared for light microscopy. The blocks were sectioned to a thickness of 5µm. The slides of all tissues were

stained by using Hematoxylin and eosin stain and Trichrome stains.

STATISTICAL ANALYSIS:

The statistical analysis was done by using SPSS version 16.0. Chi- Square test and student-t test were applied to compare different groups.

RESULTS

A marked reduction in the weight of the animals of Group B and C was noted(Table No.01). The mean of the Group A was found to be 2.13±1.27gm. However the body weight of Group B and C was found to be 1.98 ±8.97 and 1.55 ±1.89 respectively. These findings were found to be highly significant when analyzed for comparison between Group A and C using chi- square and student t- test (p-value < 0.05) but were found to be significant when compared between Group A and C.

Table No. 01 Comparison of body weight between Group A and C

using Student t- test p-value <0.05*

Statistics	Body Weight		p-Value*
	GROUP A(n=10)	GROUP C (n=10)	
Mean	2.1380	1.5540	0.001
Std. Dev	12.761	18.963	0.001

A highly significant reduction in the weight of the ovaries was observed in the experimental groups C with a mean value of 0.025 ± 0.005 gm. as compared to the controls having mean of0.128 ± 0.013gm(Table No 02)

Table No. 02. **Comparison of ovarian weight between Group A and C using Student t-test p-value<0.05 ***

Statistics			p-Value*
	Group A (n=10)	Group C (n=10)	
Mean	0.128	0.025	0.001
Std. Dev	0.013	0.005	0.001

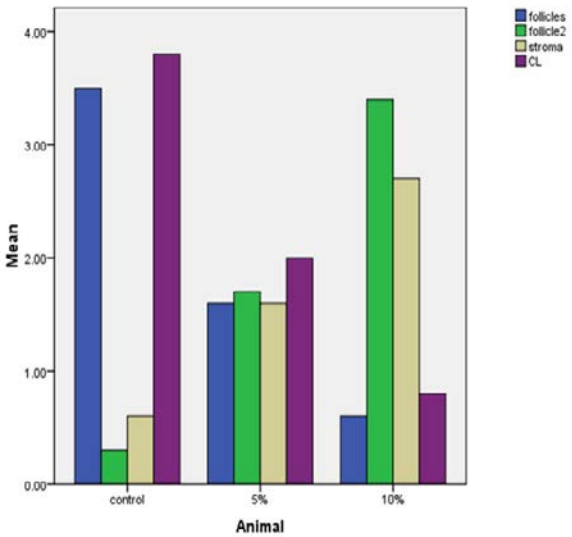

Chart No. 01 Comparison of histological findings of ovaries of rat of control , Group B (5%) and Group C (10%). p-value < 0.05

:Healthy follicles :atretic follicles

:stromal change :corpora lutea

Under H&E staining, the ovaries of the control rats showed multiple follicles in various stages of development occupying the cortex. Multiple corpora lutea are also visible (Photomicrograph a). The ovaries of the rats of Group B showed mildly decreased number of healthy follicles. Reduction in the number of corpora lutea was also observed .(photomicrograph b). The ovaries of experimental group C showed multiple cystic and degenerated follicles in various stages of development (photomicrograph c).Increased number of atretic follicles were also seen. Hemorrhage within the ovarian stroma was noted(photomicrograph d) The no. of corpora lutea was markedly reduced. Apoptotic changes in the granulosa cells were visible. Multiple degenerated granulosa cells characterized by vacuolated cytoplasm and dense nuclei were also observed (photomicrograph d& e)

Ovaries of the rats of Group C when viewed under trichrome stain showed increase in fibrous tissue in stroma especially around the maturing follicles. Marked fibrosis around the graaffian follicle accompanied by necrosis in the granulosa cells of the follicles was also observed. (photomicrograph f&g & Bar Chart No 01)

Discussion:

In this study the observed reduction in the ovarian weight clearly states that there is inhibition of ovulation due to the administration of smokeless tobacco in the albino rats. Few mature graafian follicles and reduced number of corpus luteum are also indicative of the same findings. These findings are in agreement with the work done by Tuttle et al., 2009, Neal et al., 2007 .The primordial follicles and follicular growth appears to be sensitive to the components of tobacco. Degenerative and atrophic changes were observed in the granulosa cells. There were marked vacuolations appearing in the ovarian stroma. The apoptotic changes were also visible in the granulosa cells. Research done by Jurisicova et al., 2007 also suggests that ovarian tissue retains tobacco compounds leading to the consequences of follicular loss, follicular atresia and necrotic changes in the granulosa cells of the

rodents when treated with different form of tobacco.

CONCLUSION:

Based on the histological findings in this study it can be easily stated that the exposure to the oral smokeless tobacco alters stages of the ovulation in the Swiss albino rat and may lead to the pathology of this tissue as well if given for a longer duration.

RECOMMENDATION:

In the light of present study it is recommended that measures should be taken to encourage females in the reproductive age to stop use of smokeless tobacco. Education about the effects of tobacco at adolescent level should be provided.The proper implementation of already existing tobacco laws should be sorted out. For further studies the effects of smokeless tobacco on the fertility rate and its effects on the fetal outcomes are also necessary to be carried out.

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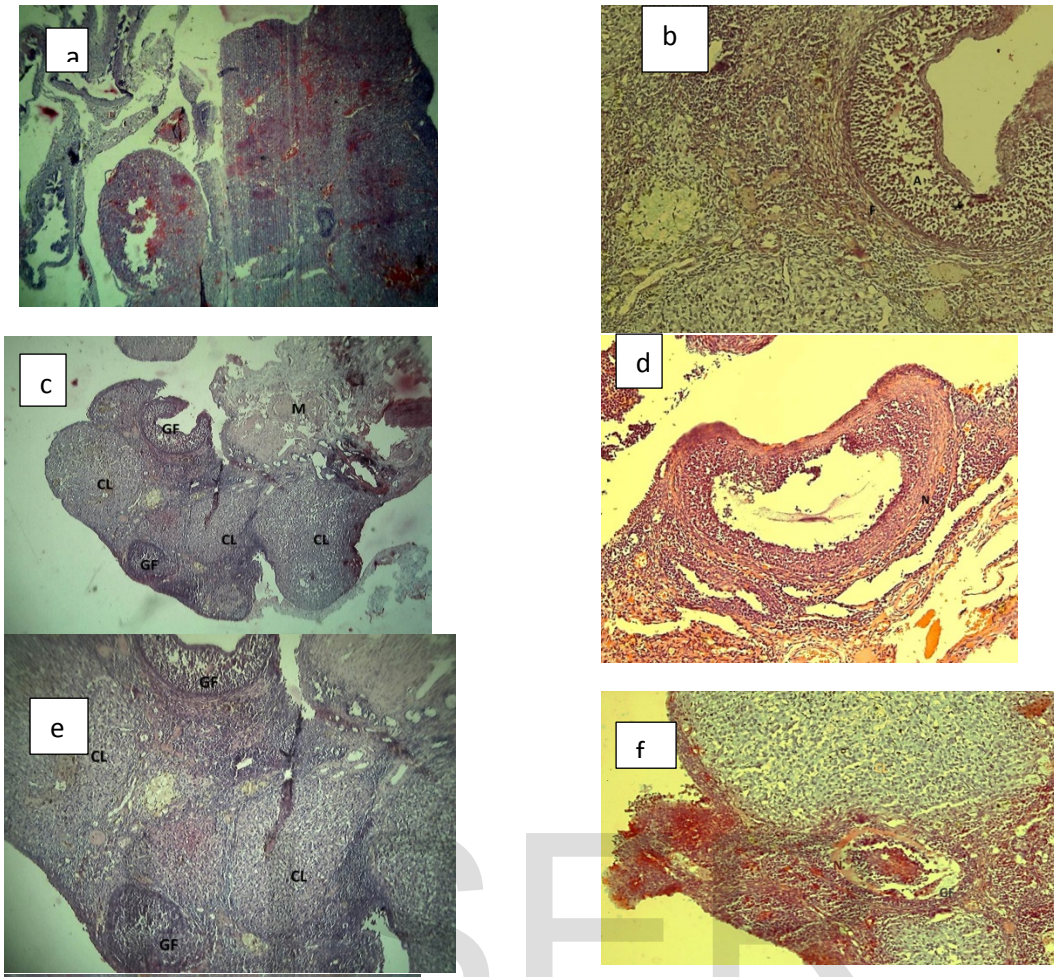

(a) showing ovary (O) and oviduct (OD) under H & E X100.(b) from Group B showing multiple corpora lutea (CL), Graafian follicle (GF) and medulla (M) under H&E X 100.(c) from Group B showing multiple corpora lutea (CL), Graafian follicle (GF) and medulla (M) under H&E X 400.(d) from Group C showing atrophied corpus luteum (CL), Graafian follicle (GF) and Hemorrhage (H) under H&E X 100.(e)from Group C showing atretic Graafian follicle (GF) under H&E X 400.(f & g) from Group C showing apoptosis(A) within granulosa cells and fibrosis (F) around the graafian follicle under Trichrome stain X 400