

## Original Article

### **Smokeless tobacco induced histological changes in the endometrial glands of the female Swiss albino rats: An experimental study**

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#### **ABSTRACT**

Tobacco use is mostly prevalent among females in the region of subcontinent. ST is used in either betel quid or gutka form .ST contains much larger quantities of nicotine than most cigarettes. Over the past few decades the use of tobacco among women of reproductive ages has increased globally. Exposure to the toxins present in the tobacco interferes with the endometrial receptivity. It disrupts endometrial angiogenesis and uterine blood flow. Quasy experimental study design was selected. 30 adult female Swiss albino rats were randomly selected and were divided into three groups (n=10 each). Group A was kept as control whereas experimental groups B&C consisted of rats which were given 5 % & 10% of smokeless tobacco in their diet respectively. Feed and water were provided ad libitum. On 31<sup>st</sup> day, animals were sacrificed by cervical dislocation. Uterus of all the animals were removed and weighed. The tissues were processed for histological examination under light microscopy using H & E. A significant decrease in the weight of the uterus was observed (P value \_ 0.001). The histological changes in the uterus of experimental groups revealed severe cystically dilated sub mucosal glands along with marked atrophy (P value < 0.001). From this study it can be concluded that the smokeless form of tobacco causes adverse effects on the endometrium of the female Swiss albino rats. Long term use of this form of tobacco causes anti – implantation, abortifacient and antifertility effects in albino rats.

**Keywords:** Endometrial glands, Swiss albino rats, Smokeless tobacco

## Introduction

The use of smokeless tobacco is a prevalent worldwide. Multiple varieties are readily available in the local markets [1]. The smokeless form of tobacco consists of crudely divided tobacco leaves. These leaves are mixed with sugar and syrup. ST is usually packaged in brightly colored packets. Gutka, zarda, paan, mainpuri ,gul, and mishri are the most commonly available preparations of smokeless tobacco in subcontinent region [2]. Multiple studies have concluded that females in the region of South Asia have easy accessibility to ST products. Due to lack of awareness regarding the tobacco products there is an increased consumption of smokeless tobacco products [2, 3]. Among other compounds, Nicotine represents 90%–95% of total alkaloids. Nicotine is a highly toxic and addictive compound [3]. Once placed in the oral vestibule it is readily absorbed through oral mucosa and circulated among the body systems. Infertility is one of the most common problem with some highly disturbing medical, psychosocial and economic implications. Nicotine which is an important alkaloid present in smokeless tobacco was also identified in the endometrial fluid [4]. Rodent models exposed to toxic compounds of

tobacco have shown abnormal endometrial maturation [5]. Exposure to the toxins present in the tobacco interferes with the endometrial receptivity. They hamper the process of endometrial angiogenesis and uterine blood flow [5, 6].

**Material and methods:** This study was carried out in the Sindh Agricultural University, TandoJam. 30 adult non-pregnant female Swiss albino rats were randomly selected. They were housed in standard cages and were provided food and water ad libitum. They were divided into three groups (n=10 each). Animals in Group A were kept as control. Experimental Groups B& C consisted of rats which were given 5 % & 10% of smokeless tobacco. The ST was grinded and mixed in their feed. The experiment was conducted for 30 days. On the 31<sup>st</sup> day, the rats were sacrificed by cervical dislocation. Uterus of all rats ere removed and weighed. Tissues were processed for examination under light microscopy using H & E stain.

**Statistical Analysis:** The statistical analysis was done by using SPSS version 21. Measures of central tendency ( mean, mode and standard deviation) were calculated. Chi- Square test and student-t test were applied to compare different groups.

**Results:** The uterus of the animals in Group B showed decrease in the weight with a mean value of  $0.596 \pm 0.009$  gm. However a highly significant reduction in the uterine weights of the rats of Group C was observed with a mean value of  $0.499 \pm 0.011$  gm as compared to the controls having mean value of  $1.115 \pm 0.005$  gm. (p-value  $< 0.001$ ) (Table No: 1)

**Histological changes in the uterus:** The histological examination of the uterus of the rats of Group A showed the normal structure of the endometrial glands and surrounding stroma (Photomicrograph No.1). However a significant reduction in the size of endometrial gland was also observed in the experimental groups. The endometrial glands of the group C also showed severe cystically dilated sub mucosal glands. The endometrial glands of Group C also showed marked atrophy (P value  $< 0.001$ , Fig:01, photomicrograph No.2)

### Discussion

It is well known that, hypothalamus regulates the rhythmic release of pituitary gonadotrophins, i.e., FSH, LH and prolactin through neural stimulus to GnRH. Previous studies have revealed that the constituents of the tobacco especially nicotine being a central nervous system inhibits the release of gonadotrophins from pituitary [6].

Uterine growth depends upon the secretion of the ovarian estrogen. Estrogen primarily acts upon the surface epithelium of the gonads and the glands that are present within endometrium [6]. Progesterone acts on uterus that has been primed by estrogen. It prepares the uterine epithelium from proliferative to secretory state. In the present study, reduced thickness of endometrium and reduction in the size of endometrial glands indicates the inhibition of ovarian steroid. This ovarian steroid biosynthesis vital for growth of the uterus and reproductive cyclicity[7]. Toxins present in the tobacco interfere with the endometrial receptivity, endometrial angiogenesis and uterine blood flow[6,7].

In our study the use of smokeless tobacco produced endometrial degeneration along with edema and fibrosis.

Necrosis, atrophy and cystic dilatation in endometrial glands were also observed in the uterus of the tobacco treated rats. Similar findings were observed showing that the uterus of the animals receiving nicotine administration exhibited marked reduction in the thickness of both endometrium and myometrium with an observable reduction in the endometrial glands [9,10].

## References

[1] Backinger, C.L., Fagan, P., Matthew, E and Grana, R (2003) Adolescent and young adult tobacco prevention and cessation: current status and future directions. *Journal of Tobacco Control*, Vol.12, pp. 46-53.

[2] Srivastava, M., Parakh, P and Srivastava, M. (2010) Predictors and prevalence of nicotine use in females: a villagebased community study *Indian Journal of Psychiatry*, Vol.19, pp. 125-129.

[3] Warren, C.W., Riley, L., Asma, S., Eriksen, M.P., Green, L., Blanton, C., Loo, C., Batchelor, S and Yach, D (2000) Tobacco use in youth: a surveillance report from the Global Youth Tobacco Survey Project. *Bulletin of World Health Organization*. Vol. 78, pp. 868-876.

[4] Cooke, J.P and Bitterman, H. (2004) Nicotine and angiogenesis: a new paradigm for tobacco-related diseases. *Annals of Medicine*, Vol. 36, pp. 33-40.

[5] Zhang, W., Yang, J., Wang, J., Xia, P., XU, Y., Jia, H and Chen Y. (2007) Comparative studies on the increase of uterine weight and related mechanisms of cadmium and p-nonyl phenol, *Journal of Toxicology*, Vol.241, pp. 84-91.

[6] Xiao, D., Huang, X., Yang, S., Zhang, L (2007) Direct effects of nicotine on the contractility of the uterine artery in pregnancy. *Journal of Pharmacology and Experimental Therapeutics*, Vol.322, pp. 180-185

[7] Anastasia A. Varvarigou., Spyros G. Liatsis., Pavlos Vassilakos, George D and Nicholas G. Beratis (2009) Effect of maternal smoking on cord blood estriol, placental lactogen, chorionic gonadotropin, FSH, LH, and cortisol, *J. Perinat. Med.* 37 (2009) 364–369.

[8] El-Meligy, M.M.S., Hady, R.H.A., AbdelSamaei, A.R and SaadEldien, H.M. (2007) effect of nicotine administration and its withdrawal on the reproductive organs, fertility, and pregnancy outcome in female rats. *Journal of Forensic Medicine and Clinical Toxicology*, Vol. 15, No. 1, pp. 95-132.

[9] Bao H, Vepakomma M, Sarkar MA. Benzo(a)pyrene exposure induces CYP1A1

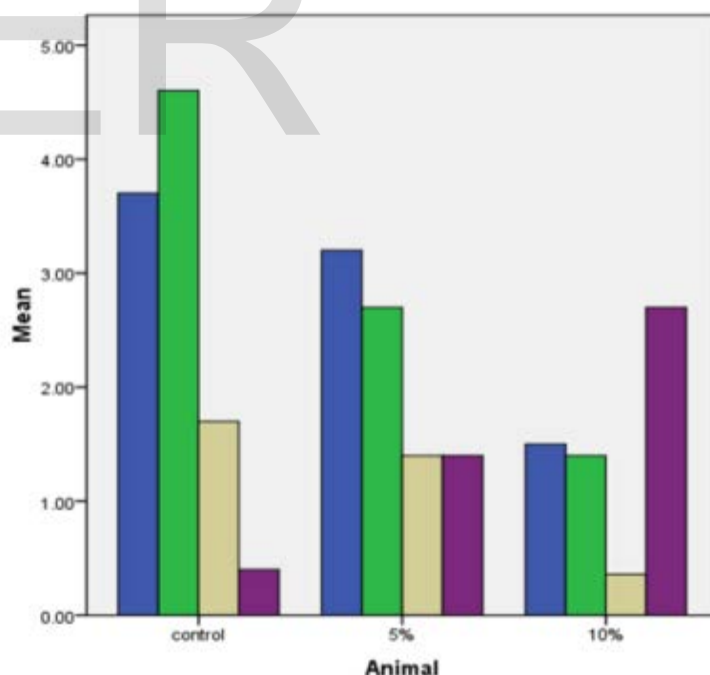
activity and expression in human endometrial cells. *J Steroid Biochem Mol Biol* 2002;81:37-45

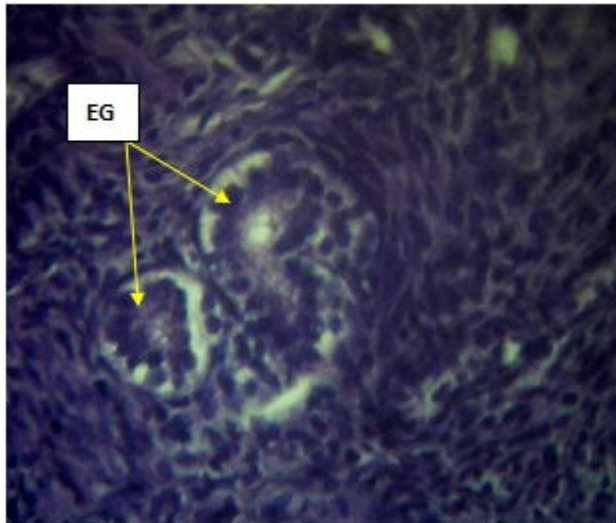
**Table No 1**

Comparison of weight of uterus between Group A and Group C using Student t-test p-value<0.05\*

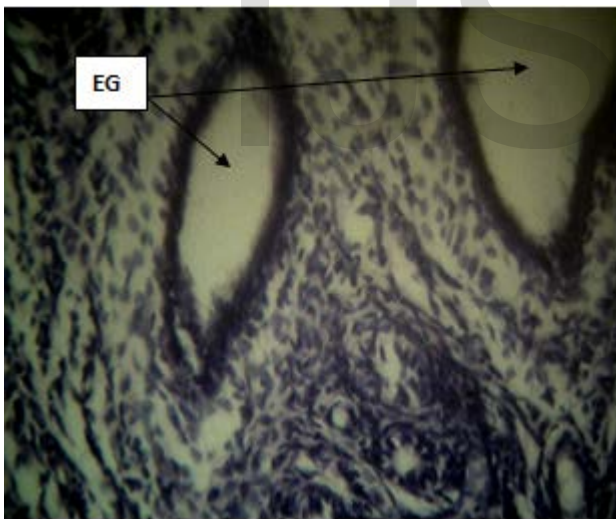
Statistics	Weight of uterus		P-value*
	Group A(n=10)	Group C (n=10)	
Mean	1.115	0.499	0.001
Std. Dev	0.005	0.011	0.001

**Fig 01:** Comparison of histological findings of uterus of rats of control, Group B & C.





Photomicrograph No01: Endometrial glands (EG) of Group A under H&E X40



Photomicrograph No02: Endometrial glands (EG) of Group C under H&E X40