EFFECTS OF SMOKELESS TOBACCO ON THE UTERINE WALL OF ADULT NON PREGNANT FEMALE SWISS ALBINO RATS

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ABSTRACT

This study was aimed to evaluated effects of smokeless tobacco on the uterine wall of female rats of Swiss albino species. This was a controlled experimental study with three groups of adult female Swiss albino rats. The study was conducted over a period of 31 days, at Animal House, Husbandry, and Veterinary Sciences at Sindh Agricultural University, Tandojam, and the Department of Anatomy, Isra University, Faculty of Medicine & Allied Medical Sciences, Hyderabad, Sindh, Pakistan. Thirty adult female Swiss albino rats were randomly assigned to three groups: Group A (control, no treatment), Group B (treated with 5% smokeless tobacco), and Group C (treated with 10% smokeless tobacco). Blood samples were collected on the 31st day to analyze Follicle-stimulating hormone (FSH) and Luteinizing hormone (LH) levels in the serum. After euthanization, uteruses were removed and weighed, followed by histological examination using Hematoxylin & Eosin and Mason’s trichrome stains. The experimental groups (B and C) exhibited a significant reduction in uterine weight and decreased serum levels of FSH & LH. Histological examination showed cystically dilated submucosal glands and pronounced atrophy in the uteri of the experimental groups. Additionally, the myometrial wall thickness was also decreased. The use of smokeless tobacco negatively affects the uterine wall of female Swiss albino rats, resulting in histological changes and decreased serum levels of FSH and LH.

Key Words: Serum FSH, Serum LH, Smokeless Tobacco, Swiss Albino Rats, Urogenital Diseases, Uterine wall

INTRODUCTION

Smokeless tobacco is a popular form of tobacco consumed in many countries, particularly in South Asia. Tobacco is known for its harmful impact on human health, causing various diseases such as cancer, cardiovascular diseases, and respiratory disorders. Despite the well-established negative impact on health, the use of smokeless tobacco is still prevalent in many countries of the world(1). Recently, researchers have expressed concern regarding the effects of smokeless tobacco on female reproductive health. Smokeless tobacco contains several toxic substances, such as nicotine, polycyclic aromatic hydrocarbons, and nitrosamines. These substances have potentially detrimental effects on the female reproductive system. Earlier studies have shown that smokeless tobacco use during pregnancy can lead to adverse outcomes, including preterm delivery, stillbirths, and low birth weight (2).

Nevertheless, the impact of smokeless tobacco on the uterine wall of adult non-pregnant female rats has not been extensively studied (3). The uterine wall is a vital component of the female reproductive system, and any disruption to its function can lead to significant reproductive problems(4). Therefore, this study aimed to investigate the impact of tobacco (smokeless) on the uterine wall of adult non-pregnant Swiss female albino rats. Gaining insights into the reproductive health implications of smokeless tobacco use in females can be
significantly enhanced by comprehending the effects of smokeless tobacco on the uterine wall of non-pregnant female rats (5). The results of this study may add to the development of interventions and formulate strategies to reduce the use of smokeless tobacco and its harmful impact on female reproductive health.

**METHODS**

The research was jointly conducted at two locations: The Animal House, Husbandry, and Veterinary Sciences at Sindh Agricultural University, Tandojam, Pakistan and the Department of Anatomy, Isra University, Faculty of Medicine & Allied Medical Sciences, Hyderabad, Sindh, Pakistan. The study involved a total of thirty female adult Swiss albino rats, randomly selected and divided into three groups, each consisting of 10 rats. Group A served as the control group, while groups B and C were administered 5% and 10% smokeless tobacco, respectively, in their feed.

Throughout the study, the rats had unrestricted access to both feed and water for a duration of one month. On the 31st day, blood samples were collected from the animals' tails in each group to assess the levels of serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Subsequently, the rats were humanely euthanized by cervical dislocation, and their uteri were removed and weighed. The uterine tissues were then processed and subjected to histological examination by making Paraffin blocks after dehydration, cleaning, and infiltration of the samples. Tissues were sectioned to a thickness of 5 micron using a microtome and placed under water bath. Then slides were prepared and stained using Hematoxylin and Eosin stain and studied under light microscope. Masson's Trichrome stain was used to differentiate collagen and muscle fibers on tissue sections.

**STATISTICAL METHODS**

Data analysis was performed using Statistical Package for Social Sciences (SPSS version 22.0), with measures of central tendency employed, and Student's t-test and ANOVA were used to compare the study groups. A p-value <0.05 was considered significant.

**RESULTS**

A total of 30 rats were used; 10 in each group (A, B and C). The mean weight of the uterus in Group A was $1.115 \pm 0.005$, while in Group B, it was $0.596 \pm 0.009$ (p-value <0.001). There was a significant difference in Group A and C (p-value <0.001). Group A exhibited a mean uterus weight of $1.115 \pm 0.005$, significantly higher than Group C with a mean weight of $0.459 \pm 0.011$. The FSH and LH levels of all three groups was compared and there was significantly higher concentration of hormones found in Group A while lowest was observed in Group C (p-value <0.001). The histological presentation of the endometrial glands of uterus removed from Group A is presented in Figure 1, the endometrium and the stroma appears normal while Figure 2 presents uterus removed from Group C, which illustrated severe cystically dilated submucosal glands with marked atrophy in this group. Furthermore, there was a reduced thickness of the myometrial wall, with atrophic changes observed under H&E staining. Additionally, trichrome staining indicated pronounced fibrosis in both the endometrium and myometrium (Figure 3).

**Table 1: Comparison of weight of uterus of Swiss albino rats: Control Group (A) versus 5% smokeless tobacco Group (B) and 10% smokeless tobacco Group (C)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A versus B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1.115±0.005</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B</td>
<td>0.596±0.009</td>
<td></td>
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<tr>
<td>A versus C</td>
<td></td>
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</tr>
<tr>
<td>A</td>
<td>1.115±0.005</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.459±0.011</td>
<td></td>
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</tbody>
</table>
Table 2. Comparison of follicle stimulating hormone and Luteinizing Hormone hormonal levels between Group A, B and Group causing ANOVA test where n=10/group

<table>
<thead>
<tr>
<th>Group</th>
<th>Follicle Stimulating Hormone Mean ± SD</th>
<th>Luteinizing Hormone Mean ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>21.92 ± 2.95</td>
<td>6.12 ± 1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B</td>
<td>16.01 ± 1.22</td>
<td>3.32 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>14.03 ± 1.18</td>
<td>2.80 ±0.15</td>
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</tbody>
</table>

Figure 1. Uterus of group A where the arrowheads indicate healthy endometrial glands x 10

Figure 2. Uterus of group C where the arrowheads indicate cystically dilated endometrial glands x 40

Figure 3. Uterus of group C where E, M & P indicate endometrium, myometrium & perimetrium respectively under Mason’s Trichrome staining x 40
DISCUSSION

The periodic secretion of pituitary gonadotrophins, such as FSH and LH, is regulated by the hypothalamus and prolactin, through neural stimulus to Gonadotropin-releasing hormone (GnRH). Nicotine, a central nervous system influencing agent found in tobacco, has been shown to inhibit the release of gonadotrophins from the pituitary gland, leading to dysregulation of the reproductive hormonal system in both healthy women and animals (6). In the context of this study, estrogen plays a crucial role in uterine growth, primarily affecting the surface epithelium and endometrial glands. Subsequently, progesterone prepares the uterine epithelium, transitioning it from a proliferative to a secretory state. Moreover, the study revealed a decrease in serum concentrations of FSH and LH hormones, indicating inhibition of ovarian steroid biosynthesis, which is indispensable for uterine development and reproductive cyclicity(7).

The present research demonstrated that consumption of smokeless tobacco resulted in endometrial degeneration, fibrosis, and edema. The uterus of the tobacco-treated rats exhibited necrosis, cystic dilatation, and atrophy in the endometrial glands. These results were in agreement with the preceding study, which also found a noticeable decrease in the thickness of both the endometrium and the endometrial glands in rats receiving nicotine administration(6). Moreover, toxins constituting tobacco can also impede the receptivity of the endometrial, uterine blood flow, and endometrial angiogenesis. The findings from the current study, which include a diminished endometrial thickness and reduced size of endometrial glands, suggest the inhibition of ovarian steroid biosynthesis. These hormones are crucial for uterine growth and reproductive activity(7,8). The present study provides evidence that the consumption of smokeless tobacco causes dysregulation of the reproductive hormonal system and impairs uterine growth through endometrial degeneration, edema, and fibrosis. These findings are consistent with the above studies and highlight the harmful effects of tobacco use on the health of the female reproductive system as previously reported(7).

One study that investigated the weight of the uterus in Pakistani women measured the weight of the uterus in 126 women who underwent hysterectomy for benign conditions(9). The mean weight of the uterus was 82.7 ± 45.1 grams. The study found no substantial changes in the weight of the uterus between women of different age groups or parity(9).

Another study conducted by Octaviana et al. examined hormonal levels in 100 women with irregular menstrual cycles. They measured estrogen, progesterone, and follicle-stimulating hormone levels and identified notable distinctions in these hormone levels among women with varying menstrual patterns(10). The present study validated that the consumption of smokeless tobacco in rats led to myometrial degeneration along with fibrosis and edema. This is in agreement with previous studies, such as the study by Khorram et al. (2010), which showed a significant reduction in the thickness of the uterine wall, particularly in the myometrium, in response to smokeless tobacco exposure(11). Another study also demonstrated that smokeless tobacco compounds were capable of altering the endometrial strata and myometrium. These findings suggest that smokeless tobacco use can have significant impacts on the structure and function of the uterus.

Moreover, smokeless tobacco has been known to lead to stromal inflammation and cellular edema, which can further exacerbate the damage to the uterus(12). The findings of the current study align with this observation, as it also demonstrated that the administration of smokeless tobacco to rats resulted in compromised endometrial receptivity and angiogenesis. These results highlight the potential hazards of smokeless tobacco use on reproductive health and suggest that women who use smokeless tobacco may be at increased risk for infertility and other reproductive disorders(13).
Previous studies have shown that the effects of tobacco use on the reproductive system are similar in animals (rats) and humans. Therefore, it is essential to further investigate the potential impacts of smokeless tobacco use on reproductive health in human population.

This study provides evidence that the use of smokeless tobacco can have significant impact on the structure and function of the uterus, leading to myometrial degeneration, fibrosis, and edema. These findings underscore the need for increased public health efforts to educate women on the potential dangers of smokeless tobacco use and to encourage cessation of tobacco use to protect reproductive health(14). Moreover, smokeless tobacco has been known to lead to stromal inflammation and cellular edema, which can further exacerbate the damage to the uterus. This is consistent with this study, as results of the current study showed that the use of smokeless tobacco in rats led to impairment in the endometrial receptivity & angiogenesis.

It is significant to note that although current study was conducted in rats, the findings are likely to have implications for human health as well. Previous studies have shown that the effects of tobacco use on the reproductive system are similar in rats and humans. Therefore, it is essential to further investigate the potential impacts of smokeless tobacco use on reproductive health in human populations. The present study provides evidence that the use of smokeless tobacco can have significant impacts on the structure and function of the uterus, leading to myometrial degeneration, fibrosis, and edema. These findings underscore the need for increased public health efforts to educate women on the potential dangers of smokeless tobacco use and to motivate cessation of tobacco use to protect their reproductive health.

CONCLUSION
The study has shown substantial differences in the weight of the uterus and hormonal levels between different groups of rats based on the smokeless consumption. The use of smokeless tobacco was associated with reduction in the weight and hormonal levels in rats. Further research is needed to confirm and expand these findings in human subjects.

CONFLICT OF INTEREST:
Authors declare no conflict of interest

FUNDING SOURCE:
The study did not receive any external funding

ETHICAL APPROVAL:
This study was approved by Local Ethics Committee.

REFERENCES