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The Effect of Smokeless Tobacco on the Histopathology of Thyroid Gland in Albino Rats

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Author`s Contribution

[1,5]Substantial contributions to the conception or design of the work; or the acquisition, [3]Active participation in active methodology, [2]analysis, or interpretation of data for the work, [4,6]Drafting the work or revising it critically for important intellectual content

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ABSTRACT

Objective: To investigate the effect of smokeless tobacco on the histopathology of the thyroid gland in albino rats.

Methodology: The experimental study was conducted at the Animal House of Sindh Agriculture University over a three-month duration from June to August 2018. Thirty healthy, non-pregnant female albino rats, aged 8-10 weeks and weighing 200-230 grams, were divided into three groups. The Control group received a standard diet, Experimental Group A was exposed to 5% smokeless tobacco, and Group B to 10%. Rats were housed under hygienic conditions. After the experiment, the rats were weighed, euthanized via cervical dislocation, and their thyroid glands were removed for detailed dissection. Tissues were preserved in 10% formaldehyde, processed for microscopic examination through paraffin embedding and sectioning (2-5 µm thick), and finally stained with Hematoxylin and Eosin (H&E) and trichrome stain.

Results: Significantly reduced weight was observed in albino rats from Group 2 and 3 compared to controls (p-value < 0.0001). In the control group (Group I), histological examination revealed a normal structure of thyroid follicles. In Group 2, stroma exhibited mild infiltration of chronic inflammatory cells, whereas Group 3 showed benign lesions with mild fibrosis.

Conclusion: In summary, smokeless tobacco has adverse effects on the weight of albino rats and may alter the normal histology of the thyroid gland. Further investigation into the effects on thyroid hormone levels and a study of the dose-dependent effects of various chemicals in smokeless tobacco on the thyroid gland are recommended.

Keywords: Albino rats, histopathology, smokeless tobacco, thyroid gland.

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Introduction

Smokeless tobacco, also known as spit tobacco, dip, chew, or chewing tobacco, involves users placing tobacco leaves in their oral cavity and sucking on them as an alternative to smoking cigarettes. Users may snuff tobacco nasally or orally by placing a pinch of tobacco under the tongue or at the lower jaw between the inner cheek and gums, resulting in the sucking of tobacco juices.[1] As users consume tobacco, saliva builds up in the mouth, leading to frequent spitting. Nicotine is absorbed into the bloodstream through the gums, eliminating the need to swallow tobacco juices.

Smokeless tobacco poses an increased risk for cancers of the oral cavity, cheeks, gums, and pharynx, potentially due to various identified carcinogens, such as tobacco-specific N-nitrosamine (TSNA), N'-nitrosonornicotine (NNN), and 4(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). [2] Nicotine absorption in substantial quantities raises the risk of coronary artery disease, myocardial ischemia, and

hypertension.[3] Nicotine surges the T-mediated immune suppression of lymphocytes, altering the Th1 and Th17 pathogenic response to a protective Th2 response, which might increase the risk of Graves' ophthalmopathy in smokers. Additionally, various toxins, including thiocyanate, may further elevate the risk.[4] Salivary thiocyanate levels were found to be higher among smokers, passive smokers, and smokeless tobacco consumers in a previous study.[5] While smokers are protected against autoimmune hypothyroidism due to decreased levels of thyroid peroxidase antibodies and thyroglobulin antibodies[6], it increases the risk for Graves' hyperthyroidism.[7]

Several studies have indicated alterations in the thyroid profile in tobacco smokers. However, only one previous study showed increased T3 and T4 levels in smokeless tobacco consumers.4 Despite this, the effects of smokeless tobacco on the histology of the thyroid gland have not been discussed earlier. This study aims to fill this gap by providing information on the dose-related effects of smokeless tobacco on the histology of the thyroid gland. Future studies may be directed based on these findings. The present study was designed to evaluate the histopathological changes observed in the thyroid gland upon exposure to different concentrations of smokeless tobacco and compare them with histological changes in the thyroid gland of non-exposed albino rats.

Methodology

This experimental study was carried out collaboratively at Isra University and the Animal House of Sindh Agriculture University, Tando Jam, after obtaining approval from the ethics review committee. The study involved the selection of 30 healthy, non-pregnant female albino rats, aged 8-10 weeks, and weighing between 200-230 grams. These rats were evenly distributed into three groups: Group 1 (Control), Group 2 (Experimental A), and Group 3 (Experimental B).

The Control group received a standard diet without any exposure to smokeless tobacco, while Experimental Group A was subjected to a 5% concentration of smokeless tobacco, and Group B to a 10% concentration. The rats were housed in standard hygienic conditions within steel cages, accommodating three rats per cage. Before the initiation of the experiment, a three-day acclimatization period was provided, during which the rats had unrestricted access to laboratory chow and distilled water.

The animals were kept in a well-ventilated facility, maintaining a 12-hour light/dark cycle, a temperature of 22±2°C, and a relative humidity of 55±10%. Throughout the 40-day duration of the experiment, the rats were provided with different diets based on their assigned groups, and their well-being was consistently monitored. One kilogram of diet was prepared according to the following ratios:

Group 1 (Control) was provided with a diet consisting of standard laboratory raw feed (comprising Wheat, Oats, Canola, Sunflower, Barley, Rye, Soybean, Cottonseed), dried milk, and wheat flour in a ratio of 4:4:2.

Group 2 (Experimental Group A) was fed a diet consisting of raw feed, dried milk, wheat flour, and smokeless tobacco in a ratio of 3.8:1.9:3.8:0.5. The smokeless tobacco, which was locally sourced, contained ingredients such as tobacco, menthol, aromatic compounds, spices, and synthetic flavors.

Group 3 (Experimental Group B) received a diet composed of raw feed, dried milk, wheat flour, and smokeless tobacco in a ratio of 3.6:1.8:3.6:1

All animals were weighed (measured on electronic weight machine in grams) weekly, till the 40th day of completion of study. Afterwards, the animals were sacrificed by a procedure of cervical dislocation euthanasia. Thyroid glands removed and fine dissection performed. For tissues fixation 10% formaldehyde solution was used. Next, the tissues were prepared for microscopic examination. For light microscopy, paraffin embedding was performed, paraffin blocks sectioned (2-5 µm thick) and glass slides prepared. The slides were stained by Hematoxylin and eosin (H&E) stain and trichrome stain.

Results

The mean weight of the Control Group (Group 1) was 202.14 ± 12.76 grams. In contrast, the body weight of Group 2 was 166.90 ± 8.98 grams, and that of Group 3 was 153.42 ± 18.96 grams. There were statistically significant differences in mean weight between Group 1 and Group 2 (p-value < 0.0001) and between Group 1 and Group 3 (p-value < 0.0001). However, the mean weight between Group 2 and Group 3 did not show a significant difference (p = 0.057). The histological findings of Group 1 (Control), Group 2 (Experimental A), and Group 3 (Experimental B) are depicted in Figure 1. In Group 1 (Control), the histological examination revealed a normal thyroid follicle structure closely packed with thin connective tissue septa, containing a minimal amount of collagen. The thyroid follicles displayed variable sizes and shapes, lined by cuboidal epithelium with rounded nuclei,

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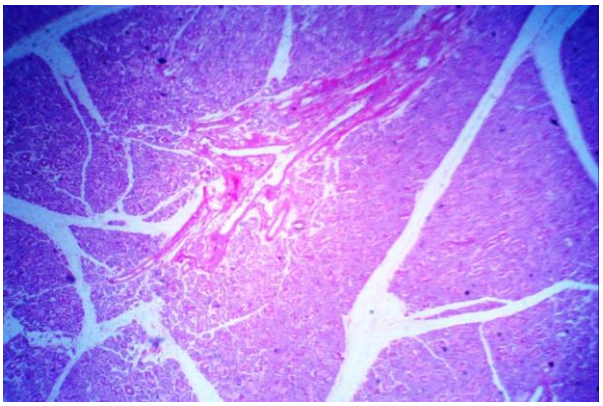
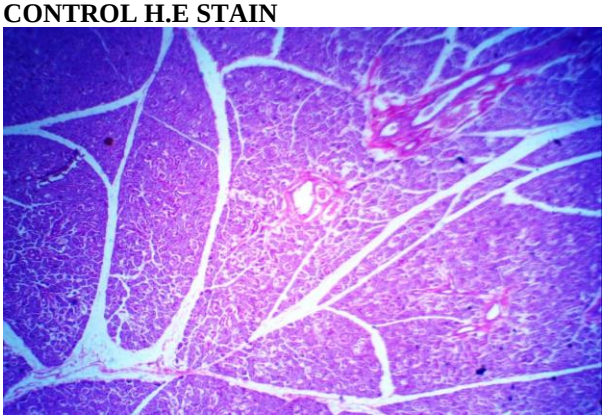
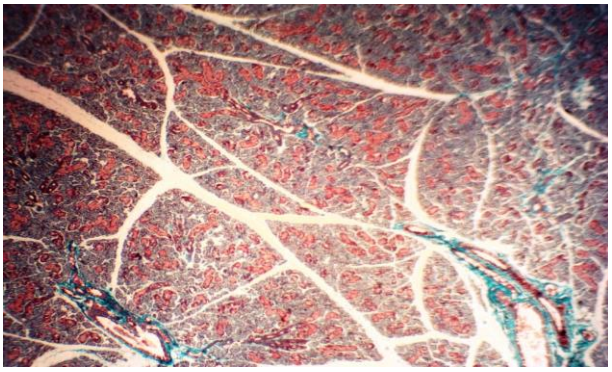
and the lumen contained a uniform, acidophilic colloid with peripheral vacuoles. Conversely, in Group 2 (Experimental A) and Group 3 (Experimental B), the thyroid follicles were organized in nodules, encased by a thin fibrous capsule, and surrounded by congested and dilated blood vessels. Furthermore, in Group 2 (Experimental A), there was evidence of stroma with a mild infiltration of chronic inflammatory cells, while Group 3 (Experimental B) displayed benign lesions with mild fibrosis.



CONTROL H.E STAIN



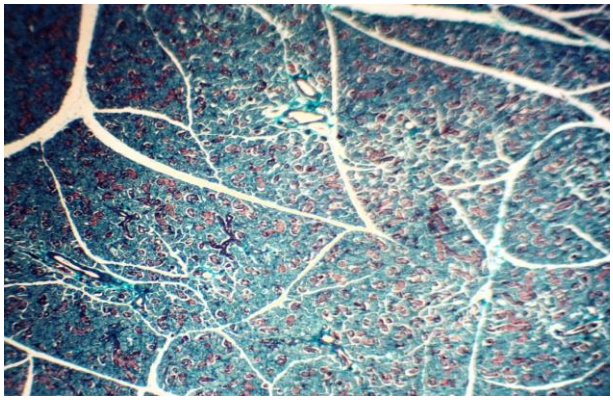
GROUP B 5% H.E STAIN



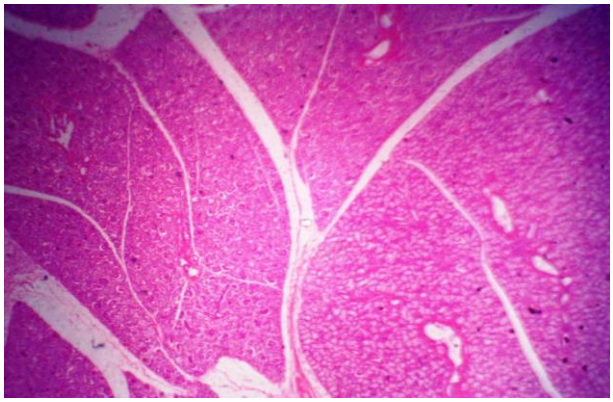
Control Trichome Stain

Discussion

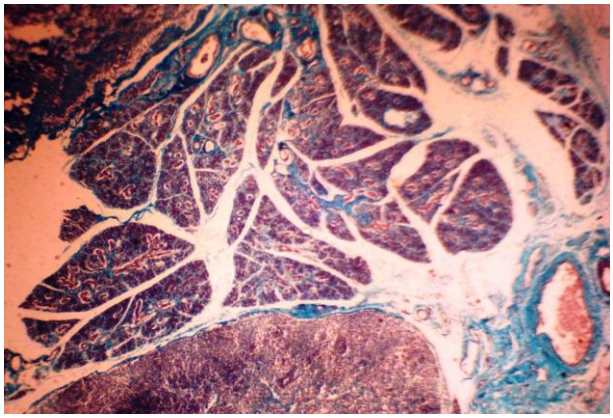
Over the past few years, research has identified various chemicals in cigarette smoke and their effects on different tissues and organs. One such chemical, Thiocyanate, which is a derivative of hydrogen cyanide, is believed to be the primary factor responsible for the thyroid's response to cigarette smoke. Thiocyanate affects thyroid function in at least three distinct ways. Firstly, it hinders the absorption of iodine, leading to iodine deficiency. Secondly, during the organification process, thiocyanate competes with iodide, thereby inhibiting the production of thyroid hormones.



GROUP B 5% TRICHROME STAIN



GROUP C 10% H.E STAIN



GROUP C 10% TRICHROME STAIN

Finally, it causes an increase in iodine excretion in the kidneys. Thiocyanate inhibits iodide transport regardless

of thyroid stimulating hormone (TSH) levels, although it competes with iodine concentration.[8,9] Iodine insufficiency amplifies the antithyroid activity of thiocyanate due to this competitive inhibition, whereas iodide excess reduces its detrimental impact. As a result, thiocyanate might be to blame for the goitrogenic impact of cigarette smoking, which has been observed in iodine-deficient locations.[10] Smoking has been linked to fatal hyperthyroidism for a long time, and the evidence is mounting.[11,12] Sajid F & Bano S studied the effects of Naswar, a type of smokeless dipping tobacco on thyroid hormones and interleukin levels, and found the significant higher FT3 and FT4 levels in Naswar users as compared to the control group; however, no difference of TSH levels was observed between both groups.[4] Additionally, the immune suppressive state was observed due to the low interleukin 1β levels. Theophilus EH et al. conducted a 90-day toxicology study to investigate the subchronic effects of smokeless tobacco. They evaluated plasma nicotine and cotinine levels in conjunction with body weights, clinical observations, and histopathological examinations of various organs. The study revealed significant changes, including reduced body and organ weights, as well as notable histopathological alterations, primarily observed at higher doses.[13] Khaitan T et al[14] also partially supported our findings as they observed, significant alteration alterations noted in the liver enzyme levels and thyroid profile of individuals using smokeless tobacco in comparison to their control healthy population. Furthermore, they observed negative correlation with thyroid profil.[14] However, no study previously has reported the histological changes due to the effects of smokeless tobacco on the thyroid gland. In our study, we found that in comparing the histological characteristics of the thyroid gland in the control group to those exposed to a 5% concentration of smokeless tobacco, we observed a distinct pattern of alterations. Notably, there was a noticeable infiltration of chronic inflammatory cells, suggesting an immune response to the presence of smokeless tobacco constituents. Moreover, we documented the presence of congested and dilated blood vessels, which could be indicative of increased vascular permeability and a potential response to the pro-inflammatory properties of the tobacco components.

As the dose of smokeless tobacco increased, the observed changes became more pronounced and severe. These changes included the emergence of benign findings, suggesting the development of tissue abnormalities consistent with early pathological changes. The presence of mild fibrosis further emphasizes the potential harm imposed by smokeless tobacco, as fibrosis can disrupt normal tissue structure and function.

However, our preliminary study has some limitations. We did not measure the weight of the thyroid gland, and there is an absence of correlation between histological changes and serum FT3, FT4, and TSH levels. Despite these limitations, our study is the first to report and identify histopathological changes associated with smokeless tobacco use, which is a widespread addiction in many Asian countries.4

Conclusion

In conclusion, smokeless tobacco demonstrates detrimental effects on the overall weight of albino rats and the histology of the thyroid gland.

Recommendations: Further large scale experimental studies are recommended to assess thyroid hormone levels in tobacco smokers, smokeless tobacco users, and passive smokers, while considering their association with histopathological changes. Additionally, investigating dose-dependent effects of the various chemicals present in smokeless tobacco on the thyroid gland may yield further insights.

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