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Effects of Smokeless Tobacco on the Histology and Functioning of Proximal Convoluted Tubules of the Kidneys of Female Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author SPS designed the study. Authors SPS and AFQ wrote the first draft of the manuscript. Author ADI performed the statistical analysis. Manuscript was finally reviewed by author SPS. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The consumption of oral form of smokeless tobacco has increased rapidly. Its use is associated with increased risk of chronic diseases like diabetes, myocardial infarction, liver disorders, cerebrovascular accidents and many other health issues. Use of tobacco in any form either smoked or chewed form leads to the absorption of nicotine which spontaneously moves into the bloodstream where it is circulated throughout the body system. Hence through this study an effort is being made to evaluate the effects produced by the locally available brand of smokeless tobacco on the histology and functioning of proximal convoluted tubules of the kidneys of the female Swiss albino rats.
Place and Duration of Study: The study was conducted in Sindh Agricultural University, Tandojam and further lab work was carried out in Isra University Hyderabad.
Methodology: 30 adult female Swiss albino rats were randomly selected. They were divided into three groups (n=10). Group A were taken as control. Group B&C comprised of rats which were

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given 5 %& 10% of smokeless tobacco respectively along with their chow diet. The feed and water were given ad libitum. Animals were sacrificed on 31st day and their kidneys were removed and weighed. The specimens were processed routinely for examination under light microscope. The sections were stained using H & E stains and examined under light microscope. Blood samples for analysis of creatinine and urea were collected.

Results: A significant decrease in the weight of the kidneys, an increase in the levels of creatinine and urea were observed (P value = 0.001). Kidneys of both B & C groups showed edema, congestion and presence of cast cells when compared with the kidneys of the control group.

Conclusion: From these observations, it can be inferred that the exposure of female Swiss albino rats to the smokeless form of Tobacco is associated with structural and functional damage of the kidneys.

Keywords: Kidneys; adverse effects; renal parenchyma; smokeless tobacco.

1. INTRODUCTION

Tobacco is the leading cause of morbidity and mortality all over the world [1,2]. Many studies have proved this statistically that the average age of first time tobacco user in the world is now between 13 – 15 years. The composition of the smokeless tobacco(st) delivered to the user depends on the components of tobacco, the packing technique and the characteristics of the soil in which the plant is being grown. Smokeless tobacco is an unburnt form of tobacco. It is placed into the vestibule of mouth. The smokeless tobacco (st) consists of crudely divided tobacco leaf. The leaf is mixed with sugar and molasses and packaged in a pouch [3]. A "quid" of the tobacco is either chewed or sucked. During its stay in the oral vestibule, smokeless tobacco continuously releases toxic chemicals. These toxic chemicals have local effects and also enter the blood stream to reach the organs like heart, lungs, kidneys, brain, pituitary gland, adrenal cortex and gonads. Smokeless tobacco contains large amount of nicotine than most cigarettes. Nicotine is the principle alkaloid found in any form of tobacco. It is believed to be highly responsible for the addictive aspect of tobacco. Nicotine has been identified to be the main reason for the use of tobacco since tobacco users derive satisfaction by its use [4-6]. Due to the excessive morbidity and mortality in various diseases more attention is being given to the deleterious effects of tobacco chewing on renal function. Most predominantly cardiovascular, lung diseases and cancer are occurring due to the use of st [7]. Due to known cardiovascular affects caused by the use of smokeless tobacco it is highly suspected that it could accelerate the progression of renal diseases as well [8]. A tight relationship was seen in between cardiovascular diseases (cvd) and chronic kidney disease (ckd). Chronic kidney disease and its consequences trigger and accelerate the risk for cardiac insults; on the other side, cvd increases the majority of morbidity and mortality in patients with ckd [7][8]. Although the use of tobacco is considered to be the most common risk factor for the progression of the renal diseases, studies regarding the cause effect relationship of smoking and tobacco chewing on renal function in subjects without renal disease are scarce. It is unknown whether chronic smoking and tobacco chewing affects renal function or represents a cause of renal damage in individuals without pre-existing renal diseases [8]. Hence the aim of this study was to search for the renal effects of smokeless tobacco in control and experimental groups of female albino rats.

2. MATERIALS AND METHODS

2.1 Animals

30 healthy Swiss albino female rats were obtained from the animal house of the Department of Animal Husbandry and Veterinary Sciences Sindh Agriculture University (SAU), Tandojam. Rats were housed in cages (with saw dust bedding) in temperature-controlled (23°–26°C) and humidity-controlled (55% RH) rooms. The cages were equipped with stainless steel feed containers and plastic drinkers with stainless nozzles. Rats were provided food and tap water ad libitum. Rats were provided with the diet of lab cakes having a scientifically approved composition of wheat flour, and dried milk. The dough was baked in an electric oven. Smokeless tobacco of a local popular brand was obtained from the market and used throughout the experiment. The light/dark cycle was maintained on 12 h intervals. All animal procedures were conducted under an animal protocol approved by Sindh Agriculture University, Tandojam.

2.2 Experimental Protocol

Rats were divided into three groups labeled as Group A, B & C (n=10each). Group A were kept as control. They were exposed to normal lab chow diet. Group B (n=10) were provided with lab chow diet mixed with 5% tobacco in grinded form. Group C (n=10) were given 10% tobacco mixed in grinded form in their diet. The tobacco dose was deduced from previous study done in 2015 [9]. The experiment was conducted for one month.

2.3 Blood and Tissue Sampling

At 31st day, ketamine (80 mg /kg bw) was injected to anesthetized the rats. Blood samples were collected in EDTA and plain red top bottles without anticoagulant by retro-orbital blood collection technique for the analysis of kidney function markers creatinine and urea. Later on, animals were sacrificed by cervical dislocation. The viscera were preserved in 10 % formalin. Tissue slides were prepared for histological examination under light microscopy using Haematoxylin and Eosin stains.

3. RESULTS

The collected data data (both histological and serological) was analyzed using SPSS version 21. The control group's values were compared with the tobacco treated groups using one way analysis of variance (ANOVA) test and student's t test. The p value of ≤ 0.05 was taken as significant.

A marked reduction in the weight of the animals of Group B and C was noted. 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.

A significant decrease in absolute organ weight of kidney was observed in female rats of the high-dose group compared with the control group. ($p< 0.05$). The kidneys of the animals of the control group were found to be 2.18 ± 0.11gm. However the organ weights of those in the groups B & C was found to be 1.81 ± 0.05 and 1.36 ± 0.04 respectively. On gross examination, the kidneys of the rats were found to be bean shaped having concave and convex borders. The medial border was found to be concave and was having hilum. The hila and borders of both kidneys were covered by adipose tissue. The rat kidneys were located alongside the vertebral column in the abdominal cavity. The suprarenal glands were situated at their upper poles. On histological examination of kidney under H & E staining, the proximal tubule of the control group exhibited a small, uneven lumen. The tubules were lined by a single layer of cuboidal cells with eosinophilic granular cytoplasm. A brush border lines cells (photomicrograph No.01 & 02). However when the experimental groups B & C were observed marked degeneration changes were observed in the kidneys of the rats who were placed in the group C. Severe edema was observed in the epithelial cells. Hydropic swelling along with congestion were also markedly present. A large number of casts were also seen. (Photomicrograph No. 03, 04 & 05) (Fig. 1).

The concentrations of urea and creatinine were statistically high in tobacco treated groups B & C as compared to normal group A of albino rats ($P <0.05$) (Table 1).

4. DISCUSSION

The gross examination of the kidney of the albino rat was found to be bean–shaped and smooth. They possess convex and concave borders. The lateral border of each kidney was convex while, the medial border was concave. The medial border of each kidney had indented hilus, and an upper and lower pole. This is in agreement with Adekomi et al, who also noted that the kidneys of Wistar rat were bean shape and smooth. The hilus and sides of the kidney of albino rat were surrounded by adipose tissue [1,8]. Anatomically the rat kidneys lay alongside the vertebral column in the abdominal cavity and suprarenal glands situated above their poles. The right kidney was situated more cranially than the left; this result is similar to study conducted by Theophilus et al [8] .The right kidney is located more cranial than the left kidney and was related to the liver while, the left was related to the stomach, pancreas, descending colon, spleen and small intestine [10,11].This result is similar to the result of studies conducted by Theophilus et al, and Tsuji H, F. H et al [12,13]. This research disagrees with the research work done by Shah T and P. G in 2013 who reported that the proximal convoluted tubules are lined with columnar epithelial [13] but in agreement with

that proximal tubule is more narrow than the distal convoluted tubule [12-14]. This study shows similar findings to the work done by Chenlin Yu et al in 2016 that the proximal convoluted epithelia of the experimental groups exhibited severe degenerative changes [7]. Several findings from scientific investigation on the implications of Tobacco use on the various organs done by several researchers Adekomi et al (2011), Christopher A. Drummond et al (2016) and Staplin et al(2016) have clearly identified varying patterns of cellular degeneration in the proximal convoluted tubules of the animals in the experimental groups [11,12] [1][14,15]. These variations may compromise the functional integrity of the proximal convoluted tubules. This intracellular edema and hydropic alterations may lead to the retention of waste products of metabolism and persistence of such abnormalities may result in loss of the sensitive homeostatic mechanisms of the kidney [13-15]. The results of the renal function tests showed that smokeless tobacco has portended threat to renal functioning by showing an increase in the levels of creatinine and urea. Smokeless tobacco contains nephrotoxic substances, including cadmium (Cd) and lead (Pb), which caused alterations in proximal tubular function, leading to increased creatinine and serum urea [16]. Another study conducted in 2021 showed the similar results of increased creatinine and urea level as current study after treatment with ST. The results of current research work accorded with Ugbor et al, who noted that consumption of ST caused the reduction in weight of right and left kidney [17].

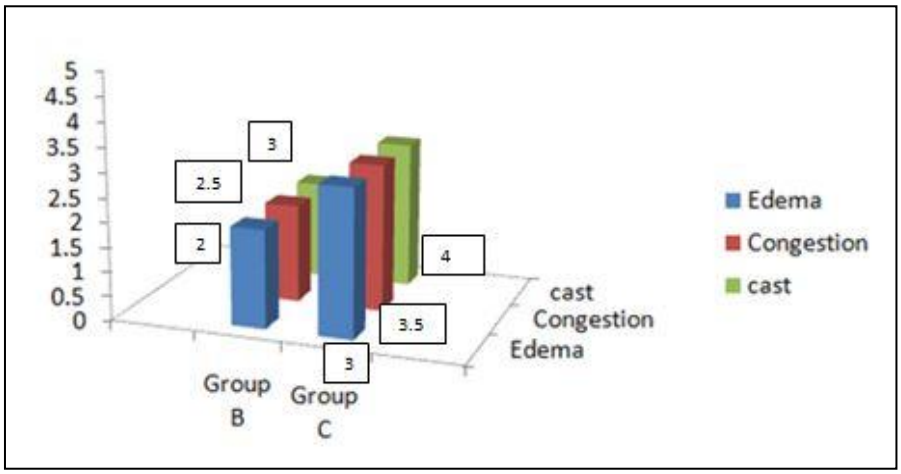
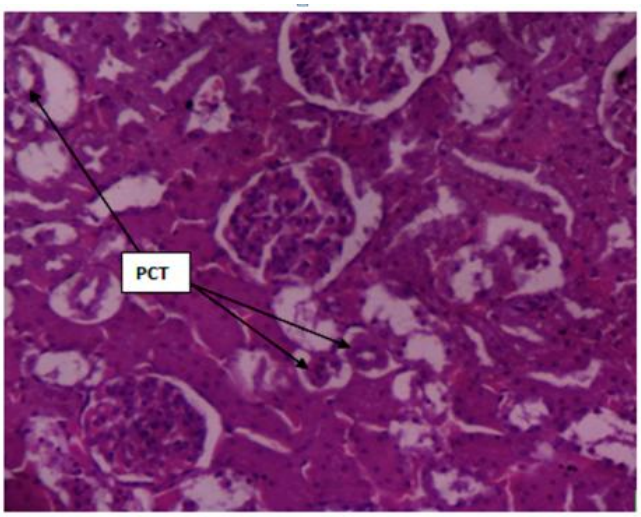
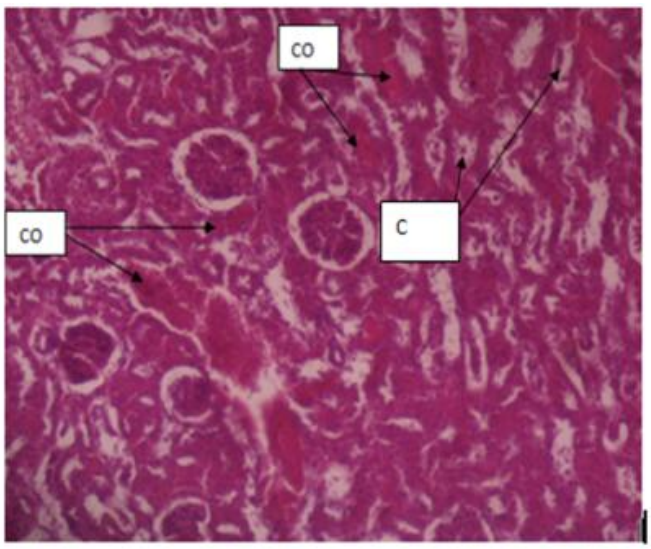


Fig. 1. Comparison of histological changes between group B & C



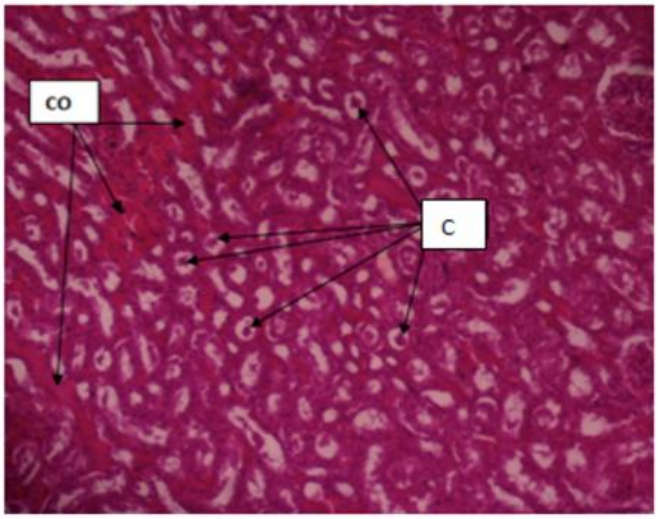
Photomicrograph 02 showing proximal convoluted tubules (PCT) of the control group under H& E X40

Fig. 2



Photomicrograph 03 showing proximal convoluted tubules (PCT) of the Group B under H& E X20 (CO=congestion, C=cast)

Fig. 3



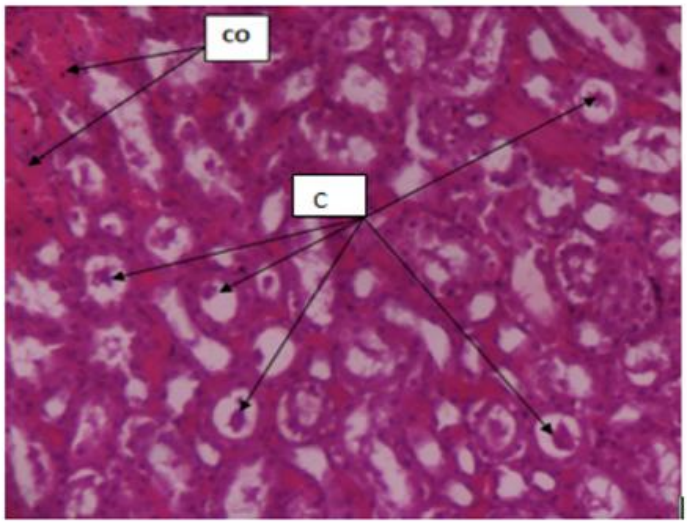
Photomicrograph 04 showing proximal convoluted tubules (PCT) of the Group C under H& E X 20(co=congestion, c=cast)

Fig. 4

Table 1. Weight of organs and serum levels of kidney function markers (creatinine & Urea)

Group (n=10)	Weight of Kidneys (mg)	Creatinine (mg/dl)	Urea (mg/dl)	p-value*
A	2.18 ± 0.11	0.60 ± 0.06	18.60 ± 2.91	0.001
B	1.81 ± 0.05	1.79 ± 0.05	21.60 ± 1.26	
C	1.36 ± 0.04	2.13 ± 0.15	27.20 ± 3.70	

*Date were expressed as mean ± SD. *p-value <0.05 taken as significant.*



Photomicrograph 05 showing proximal convoluted tubules (PCT) of the Group C under H& E X 40(co=congestion, c=cast)

Fig. 5

5. CONCLUSION

The present study clearly provides us with the evidence that exposure to the constituents of the smokeless form of tobacco alter the histology and functioning the proximal convoluted tubules of kidney. These histological alterations then lead to the functional compromise of the kidneys.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Study was approved by Ethical board of Isra University.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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