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HISTOLOGICAL EFFECTS OF THE SMOKELESS TOBACCO ON THE GLOMERULI OF THE KIDNEYS OF THE FEMALE ALBINO RATS

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Abstract

The use of smokeless form of tobacco has increased worldwide. Its consumption is associated with increased frequencies of chronic diseases like diabetes, Ischemic heart diseases, hepatic disorders, cerebrovascular accidents and many other morbid conditions. Use of tobacco in any form either inhaled/smoked or chewed/pouch/snus form leads to the absorption of nicotine which spontaneously moves into the bloodstream. Once it enters the bloodstream it is circulated throughout the body systems. Hence through this study an effort is being made to evaluate the effects produced by the locally available brand of smokeless tobacco on the glomeruli of the kidneys of the female Swiss albino rats. Quasi experimental study design was selected. The study was conducted in Sindh Agricultural University, Tandojam. The lab work was carried out in Isra University Hyderabad. 30 adult female Swiss albino rats were randomly selected and were placed into three groups (n=10). Group A were kept as control. Experimental Groups B&C consisted of rats which were given 5 %& 10% of smokeless tobacco along with the lab chow diet. The feed and water were provided ad libitum. The animals were sacrificed on 31st day by cervical dislocation. The kidneys were removed and weighed. The specimens were processed routinely for examination under light microscope using H & E stains.A significant decrease in the weight of the kidneys was observed (P value ≤ 0.001).The glomeruli of both B & C groups showed interstitial edema, increased cellularity, apoptosis and enlargement of the Bowman' space when compared with the glomeruli of the control group.It can be inferred that the exposure of female Swiss albino rats to the smokeless form of tobacco is associated with structural damage of the glomeruli.

Keywords: *adverse effects, kidneys, renal parenchyma, smokeless tobacco*

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INTRODUCTION:

Tobacco use is one of the preventable cause of morbidity and mortality all over the world [1]. Many researchers have proved this statistically that the average age of first time tobacco user in the world is now lying between 13 – 15 years. The composition of the smokeless tobacco (ST) delivered to the consumer usually depends upon the components of tobacco, the packaging technique and the characteristics of the soil in which the plant is being sowed. Smokeless tobacco/snus is an unburnt form of tobacco. It is placed in the vestibule of mouth. The smokeless tobacco (ST) consists of crudely divided tobacco leaf which is mixed with sugar and molasses. After mixing it is packaged in a pouch and sold in local markets [2]. A "quid" of the tobacco is placed in the vestibule of the mouth and is either chewed or sucked. Once it comes in contact with the oral mucosa, ST continuously releases toxic chemicals. These toxic chemicals lead to local insults plus these chemicals also enter the blood stream to reach the organs like heart, lungs, kidneys, brain, pituitary gland, adrenal cortex and gonads. Nicotine is the principle alkaloid found in ST. The amount of nicotine present in the ST is far greater than that present in the cigarettes. It is also known to be highly addictive. It addictive aspect becomes the sole reason for the use of tobacco since its users derive satisfaction by it [3,4]. Due to the deleterious effects of ST more attention is being given to the effects of tobacco chewing on renal function. Due to known cardiovascular insults caused by the use of smokeless tobacco it is highly suspected that it could accelerate the progression of renal diseases as well [5, 6]. Multiple studies have proved that nicotine worsens renal injury in rats and has also shown to promote mesangial cell proliferation and hypertrophy. 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 [5,6]. 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. Hence the aim of this study was to search for the histological effects of the components of ST on the glomeruli of the animals exposed to ST. [6]

MATERIAL AND METHOD:

30 healthy Swiss albino female rats were used in this experiment. Rats were placed in the animal house Tandojam. They 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 along with plastic drinkers with stainless nozzles. Rats were provided standard lab. Chow diet and tap water ad libitum. 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. 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 & C (n=10 each) were provided with lab chow diet mixed with 5% & 10 % tobacco in grinded form respectively. The experiment was conducted for one month. On 31st day, the animals were sacrificed by cervical dislocation. The viscera were preserved in 10 % formalin solution. Tissue slides were prepared for histological examination under light microscopy using Haematoxylin and Eosin stains.

Statistical analysis:

The collected data was analyzed using SPSS version 21. The values of central tendencies (mean, mode, median & Std. Deviation) were analyzed applying one way Anova test. The p value of <0.05 was taken as significant.

RESULTS:

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.

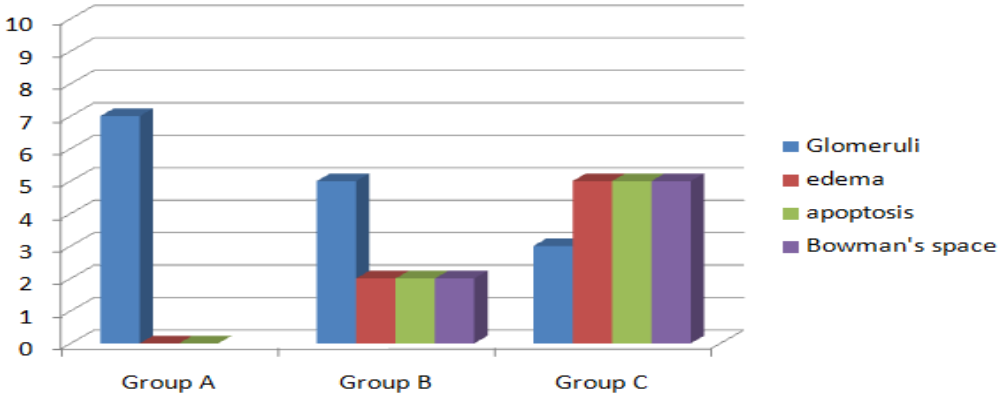
Table.1, Weight of organs

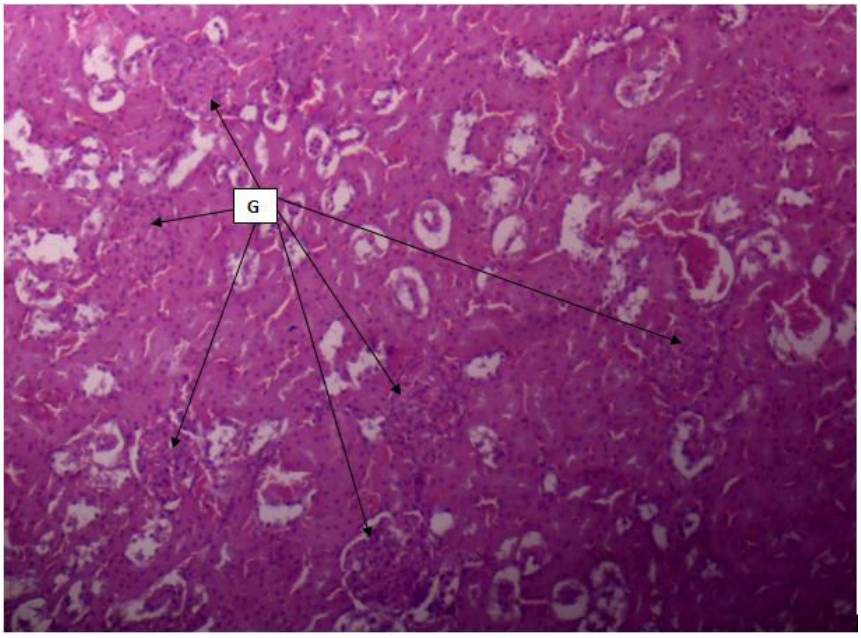
Parameter	Group(n=10 each)	Mean	Std. Deviation	p-value*
Weight of the kidney in mg	A	2.1880	.11987	0.001
	B	1.8120	.05263	
	C	1.3680	.04382	

*p-value <0.05 taken as significant

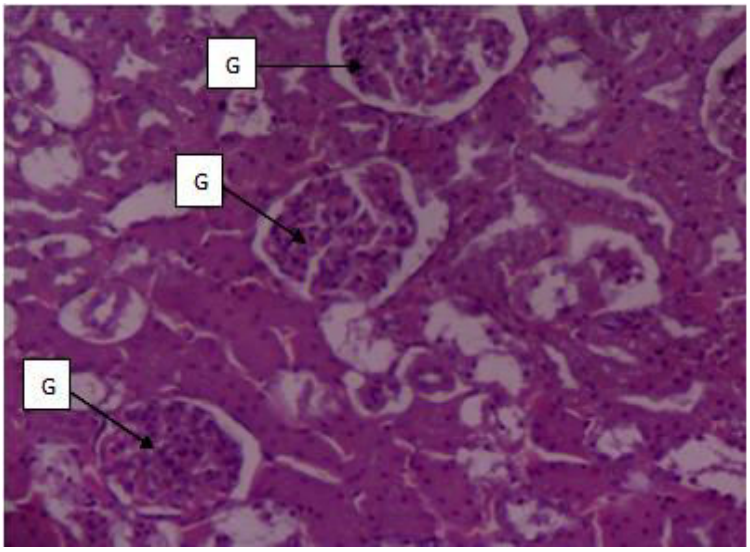
A significant decrease in absolute organ weight of kidney was observed in female rats of the high-dose group compared with the control group. ($p < 0.05$). The kidneys of the animals of the control group were found to be 2.18 ± 0.11gm. However the organ weights of those in the groups B & C was found 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 glomeruli of the control group exibited no evidence of glomerular, tubular or interstitial injury and there were no protein casts in the medulla.(photomicrograph No.01). However when the experimental groups B & C were observed sections showed glomeruli having diffused edema and congestion in the interstitium. Severe edema in the interstitium of the Group C animals was observed. Few glomeruli also exhibited increased bowman's space along with the atrophic changes. (Photomicrograph No. 02 & 03) (Fig. 01)

Fig 01: showing histological changes between the groups

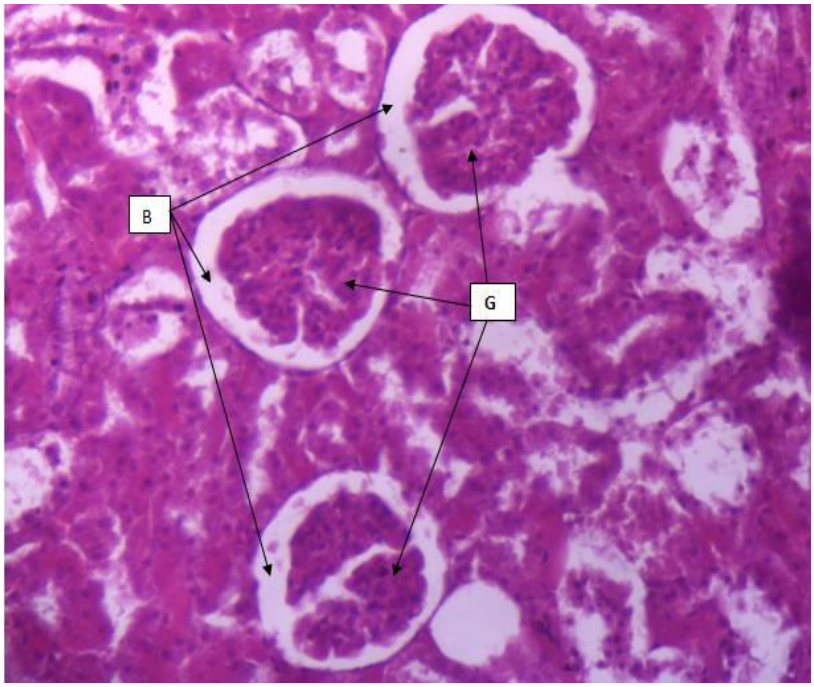




Photomicrograph 01 showing glomerulus (G) of the control group under H& E X 20



Photomicrograph 02 showing glomerulus (G) of the Group B under H& E X 20



Photomicrograph 03 showing glomeruli (G) of the Group C under H& E X 40(B= Bowman's space)

DISCUSSION:

The gross examination of the kidney of the albino rat was found to be bean–shaped and smooth. The medial and lateral borders of each kidney were concave and convex respectively. Each kidney had an upper and a lower pole with the hilum present at the medial border. This is in agreement with Adekomi et al (2011) who also noted similar findings on gross examination of the kidneys of Wistar rat. [1, 6] The rat kidneys were located 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. The right kidney is located more cranially than the left kidney and was related to the liver. The left kidney was related to the stomach, pancreas, descending colon, spleen and small intestine. These results are similar to the results of Theophilus et al (2012) and Tsuji H, F. H et al (2013). [7, 8] This study demonstrates that oral use of the smokeless form of tobacco causes

significant histological changes in the kidney of the female albino rats. These changes included diffused edema and congestion in the interstitium. The glomeruli also exhibited increased bowman's space along with the atrophic changes. These findings were consistent with other epidemiologic study conducted by Rezonzew G et al 2012 which have also suggested that use of tobacco accelerates the rate of progression of CKD of diverse etiologies including diabetes mellitus type-1, hypertension, lupus nephritis, polycystic kidney disease, IgA nephropathy, and postkidney transplantation.[9] In the present study , an increase in the Bowman's space was observed in the animals that were given 5 & 10% of the smokeless form of tobacco. The mechanism responsible for this increased glomerular space maybe due to the marked glomerular hyperfilteration. This experimental finding is similar to the work done by Zhu Y et al 2013 which also demonstrated that the exposure to smokeless tobacco worsens renal injury in rat models. [11] Nicotine is one of the biologically active and stable compounds present in large concentrations in tobacco that can be acquired through active and passive use of tobacco. In addition to its addictive properties, nicotine promotes atherosclerosis and angiogenesis. The present study has also demonstrated that nicotine worsens glomerular injury in the albino rat model. The structural changes such as increased cellularity of the glomerular capillaries and interstitial edema are confirming the deleterious effects due to the exposure of ST. [10-12] Nicotine has been known to increase oxidative stress, which causes the progression of multiple nephropathies and chronic kidney disease in in vivo experimental models. Consistent with these observations our experiment demonstrated that the glomerular and tubular apoptosis may be as a result of increase in oxidative stress caused by nicotine exposure.

CONCLUSION:

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

Recommendations:

Further in-depth mechanistic studies should be conducted in order to determine other epigenetic and biomolecular factors that lead to accelerated renal insults and functional compromise due to the exposure of the constituents of readily available tobacco compounds in our region.

Conflict of interest: None

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