Histological effects of the smokeless tobacco on the Bowman's capsule space of the kidneys of the Female Albino rats

Syna Pervaiz Singha [1], Amir Derick Isaac[2]

Abstract

Since the last decade the smokeless form of tobacco ST (gutka/snus) has gained popularity in our region. Its easy availability has made it popular among all age groups. However exposure of tobacco in the oral form also bears consequences. 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 its constituents especially nicotine which spontaneously moves into the bloodstream. Once it gains entry into the bloodstream it is circulated throughout the body systems. Many debilitating diseases such as diabetes, myocardial infarction, liver disorders and kidney diseases are the consequences of being exposed to tobacco. Hence through this study an effort is being made to evaluate the effects produced by the locally available brand of smokeless tobacco on the Bowman's capsule space of the glomeruli of the kidneys of the female Swiss albino rats. Quasi experimental study design was selected for this purpose. Study was conducted in Sindh Agricultural University (SAU), Tandojam. Further lab work including tissue processing and staining was carried out in Isra University Hyderabad. 30 adult female Swiss albino rats were randomly selected and divided into three groups (n=10). Group A were taken as control whereas Group B&C were kept as experimental groups. Rats of Group B and C were given 5 %& 10% of smokeless tobacco along with their chow diet. The feed and water were given ad libitum. Animals were sacrificed on 31st day by cervical dislocation. Their kidneys were removed and weighed. The specimens were processed routinely for light microscopy using H & E stains.A significant decrease in the weight of the kidneys was observed (P value ≤ 0.001). Kidneys of both B & C groups showed mild to marked increase in the Bowman's capsule space when compared with the kidneys of control group. This study clearly infers that the exposure of female Swiss albino rats to the smokeless form of Tobacco is associated with structural damage of the kidneys in the form of increase in the Bowman's space of the glomeruli.

Keywords: adverse effects, Bowman's capsule space, smokeless tobacco

INTRODUCTION

Previous studies have provided evidence that use of tobacco in any of its form leads to morbidity and mortality [1]. Studies have also proved statistically that globally the average age of an individual who is first time exposed to the tobacco lies between 13 – 15 years. The characteristics of the soil, drying method and packaging technique effect the composition of the smokeless tobacco (ST). Smokeless tobacco usually known as snus or gutka is an unburnt form of tobacco. It is placed into the vestibule of mouth and is either chewed or sucked. The smokeless tobacco (ST) comprises of crudely divided tobacco leaf. Sugar and molasses are mixed with this crudely divided tobacco leaves and packed in the form of a pouch [2]. "Quid" is another form of easily available smokeless tobacco. It can be used in the chewable form. The betel quid is a mixture of betel leaf, slaked lime and areca nuts. As it comes in contact with the oral mucosa, ST releases toxic chemicals. These toxic chemicals have local as well as systemic effects. As these toxins enter the blood stream they start producing deleterious effects on the organs like heart, lungs, kidneys, brain, and gonads. Smokeless tobacco contains more amount of nicotine than most cigarettes. Nicotine is the principle alkaloid found in any form of tobacco and has been known to affect many biological activities. Due to its addictive nature nicotine has been identified to be the primary reason for the use of tobacco [3,4]. Use of tobacco is known to cause many deleterious effects on different viscera. Most predominantly cardiovascular, lung diseases and cancer are occurring due to the use of ST. The two most important causes of end-stage renal disease (ESRD) are diabetes mellitus and hypertension, both of which are closely associated with exposure to tobacco in any of its form [5]. It is highly suspected that the use of smokeless tobacco could accelerate the progression of renal diseases as well [6]. Chronic kidney disease and its consequences are known to speed up the risk for cardiac insults; on the other hand, CVD increases the majority of deadly effects in patients with CKD [5,6]. While the association between the use of tobacco and cardiorenal diseases has been established for many years, the impact of ST on Bowman's space is still less recognized. Hence the aim of the present study was to test the

structural alterations in the Bowman's space of the female albino rats exposed to the smokeless tobacco [6].

MATERIAL AND METHOD

Healthy Swiss albino female rats (n=30) were obtained from the animal house of the Department of Animal Husbandry and Veterinary Sciences Sindh Agriculture University (SAU), Tandojam. All animal procedures were conducted under animal protocol approved by Sindh Agriculture University, Tandojam. Rats were placed in cages with saw dust bedding. The room temperature was kept at 23°–26°C with 55% humidity. The light/dark cycle was maintained on 12 h intervals. Cages were equipped with stainless steel feed containers and plastic drinkers with stainless steel nozzles. Rats were provided food and tap water ad libitum. They 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. 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% and 10% of grinded tobacco. On the 31st day, animals were sacrificed by cervical dislocation. Viscera were preserved in 10 % formalin. 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. 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.

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 Group A were found to be 2.18 ± 0.11gm. However the organ weights of the animals in Groups B & C was found to be 1.81 ± 0.05 and 1.36 ± 0.04 respectively. On gross examination, the rat kidneys were located alongside the vertebral column in the abdominal cavity. Kidneys of the albino rats were found to be bean shaped. Each kidney had concave and convex borders. The medial border was found to be concave indented with a hilum. The hila and borders of both kidneys were covered by adipose tissue. The suprarenal glands were situated at their upper poles. On histological examination of kidney under H & E staining, glomeruli of the control group exibited normal histological features. The kidneys of the control group exhibited delicate mesangium and small Bowman's capsule space.(photomicrograph No.01). However when the experimental groups B & C were observed marked increase in the Bowman's capsule space was observed. Mild mesangial cell proliferation was also observed in the kidneys of the animals in Group C. (Photomicrograph No. 02) (Fig. 01)

Discussion:

The gross examination of the kidney of the albino rat was found to be bean–shaped and smooth. 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 2011 who also noted that the kidneys of Wistar rat were bean shaped and smooth. The hilus and sides of the kidney of albino rat were surrounded by adipose tissue.[1,6] Anatomically rat kidneys lie 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 Theophilus et al 2012.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 [7, 8] This result is similar to the result of Theophilus et al 2012 and Tsuji H, F. H et al 2013. On histological examination of the kidneys, mild mesangial cell proliferation and increase in the Bowman's space was observed in the experimental groups. Like many debilitating conditions such as diabetes mellitus, early increase in mesangial matrix and increase in the Bowman's space was observed in this study. In experimental Group C, there was marked increase in Bowman's capsule space which was statistically significant. This increase was mainly attributable to an increase in the Bowman's space area, rather than the glomerular tuft area (which was increased by only a change that was not statistically

significant). The mechanisms responsible for the enlargement of Bowman's capsule space are uncertain but may be related to the glomerular hyperfiltration. Study conducted by Adedayo AD et al 2011 has also provided evidence that the glomerular filtration rate (GFR) increases by almost the same percentage as the Bowman's capsule space increases. [9, 10] The present study has provided the first evidence that Bowman's capsule space increases in albino rats which are exposed to the locally available form of smokeless tobacco. A single experimental study done by Jeffrey R. et al 2001 and Staplin N et al 2016 revealed an increased Bowman's space in obese dogs. In that model, glomerular hyperfiltration was also associated with increased glomerular volume; however, glomerulomegaly was mostly accounted for by an increase in Bowman's space volume and not by an increase in glomerular tuft volume [11, 12].

Nicotine, the main constituent of the smokeless tobacco suppresses an important vasodilator (nitric oxide). This phenomenon was established in this study with the help of dose dependent marked increase in bowman's space and mild mesangial cell proliferation when compared with the control group. [13, 14] Increases in bowman's space visible under light microscopy may compromise the ultra-filtrating functions of the glomeruli. This increase in the Bowman's space may also result in a decrease in glomerular filtration rate. The findings are in correlation with previous studies which have also reported that persistent exposure to nicotine alters renal function. This alteration in renal functions ultimately leads to the worsening of the renal disease [15, 16, 17].

Conclusion:

The present study clearly provides us with the evidence that exposure to the constituents of the smokeless form of tobacco alter the histology of the kidneys. These structural alterations lead to widening of the Bowman's capsule space which might ultimately 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 preparations in our region.

Conflict of interest: None

Acknowledgements:

I would like to acknowledge the In-charge and staff of SAU, Tandojam. I also want to acknowledge Prof. Dr. Shankar Rathi & Prof. Dr. Usha Isaac for their contribution and support in the histological analysis.

References:

1. Adekomi Damilare Adedayo, A. A. T., 1 A. A. Musa, and T. D. Adeniyi1 (2011). "Histological study of smoke extracts of Tobacco nicotiana on the heart, liver, lungs, kidney, and testes of male Sprague-Dawley rats." Nigerian Medical Journal. 52(4): 217-222.

2. Begum S., S. J. J., Nair S., Donta B (2015). "smokeless tobacco use across reproductive stages." Asian Pacific Journal of Cancer Prevention (16): 7547–7554.

3. Chen H, A.-O. I., Pollock C, Saad S (2013). "Fetal programming of renal development–influence of maternal smoking." Journal of Diabetes and Metabolism: 003.

4. Chenlin Yu, Ziteng Zhang, Yangang Liu, Ying Zong, Yongchun Chen, Xiuming Du, Jikuai Chen, Shijie Feng, Jinlian Hu, Shufang Cui, and Guocai Lu (2016). "Toxicity of Smokeless Tobacco Extract after 184-Day Repeated Oral Administration in Rats." International Journal of Environmental Research and Public Health.13(3): 281.

5. Christopher A. Drummond, L. E. C. A., Steven T. Haller, Xiaoming Fan, Jeffrey X. Xie, David J. Kennedy, Jiang Liu, Yanling Yan, Dawn-Alita Hernandez, Denzil P. Mathew, Christopher J. Cooper, Joseph I. Shapiro, and Jiang Tian (2016 Dec 1). "Cigarette smoking causes epigenetic changes

associated with cardiorenal fibrosis." Physiological Genomics. 48(12): 950–960.

6. Prabhakar V., J. G., Nair S.V., Ranganathan B (2013). "Determination of trace metals, moisture, pH and assessment of potential toxicity of selected smokeless tobacco products." Indian Journal of Pharmaceutical Sciences(75): 262–269.

7. Willis D.N., P. M. A., Gany F., Hoffman C., Blum J.L., Zelikoff J.T (2014). "Toxicity of gutkha, a smokeless tobacco product gone global: Is there more to the toxicity than nicotine?" (11): 919–933.

8. Theophilus E.H., H. J. R., Ayres P.H., Morgan W.T., Potts R.J., Garner C.D., Fallacara D.M., Hejtmancik M.R., Singer A.W (2015). "Toxicological evaluation of smokeless tobacco: 2-Year chronic toxicity and carcinogenicity feeding study in Wistar Han rats." (67): 539–550.

9. Adedayo AD, T. A., Musa AA, Adeniyi TD (2011). "Histological study of smoke extract of Tobacco nicotiana on the heart, liver, lungs, kidney, and testes of male Sprague-Dawley rats." Nigerian Medical Journal (52): 217-222.

10. Tsuji H, F. H., Matsuura D, Nishino T, Lee KM, Yoshimura H (2013). "Comparison of biological responses in rats under various cigarette smoke exposure conditions." Journal of Toxicologic Pathology (26): 159–174.

11. Jeffrey R. Henegar, S. A. B., Lisa K. Henegar, Suresh C. Tyagi, and John E. Hall (2001). "Functional and Structural Changes in the Kidney in the Early Stages of Obesity." Journal of American Society of Nephrology (12): 1211–1217.

12. Staplin N, H. R., Herrington WG, Reith C, Cass A, Fellström B, Jiang L, Kasiske BL, Krane V, Levin A, Walker R, Wanner C, Wheeler DC, Landray MJ, Baigent C, Emberson J (2016). "SHARP Collaborative Group. Smoking and adverse outcomes in patients with CKD: the study of heart and renal protection (SHARP)." American Journal of Kidney Diseases 68: 371–380.

13. Odokuma EI, A. J. (2017). "Histomorphological effects of nicotine on the kidney." Annals of Bioanthropology (5): 9-13.

14. Budisavljevic MN, P. D. (2012). "Nicotine and the kidney: Mr. Hyde, and perhaps some Dr. Jekyll." American Journal of Physiology and Renal Physiology 302: F1082-1083.

15. Avila-Tang E, A.-D. W., Ashley DL,Benowitz N, BernertJT, KimS, Samet JM and, Hecht SS. (2012). "Assessing secondhand smoke using biological markers. ." Tobacco Control (1): 1-8.

16. Shah T, P. G. (2013). "Microproteinuria in chronic cigarette smokers as a predictor of renal diseases: A case control study." Biomedical Research (24): 55-58.

17. Tsuboi N, U. Y., Kanzaki G, Koike K, Ikegami M (2012). "Low glomerular density with glomerulomegaly in obesity-related glomerulopathy." Clinical Journal of American Society of Nephrology (7): 735-741. doi:https://doi.org/10.2215/CJN.07270711

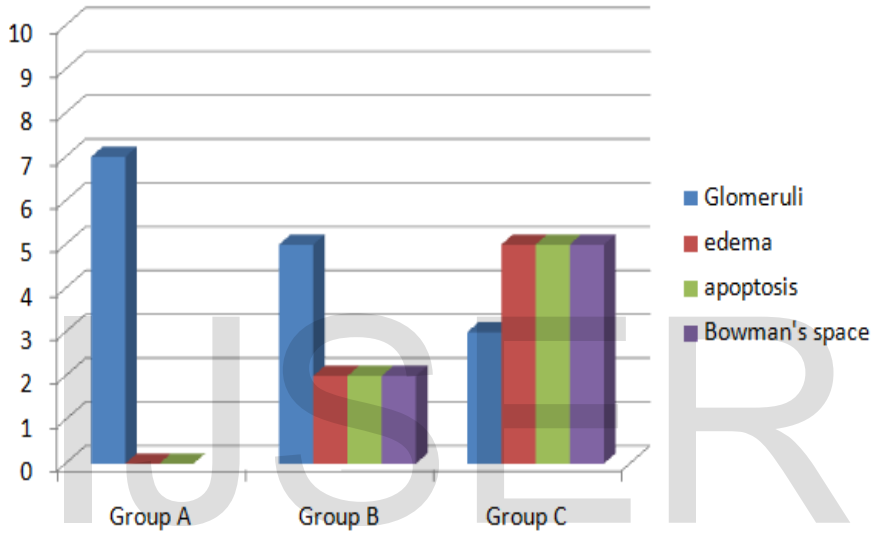
Table.1, Weight of organs

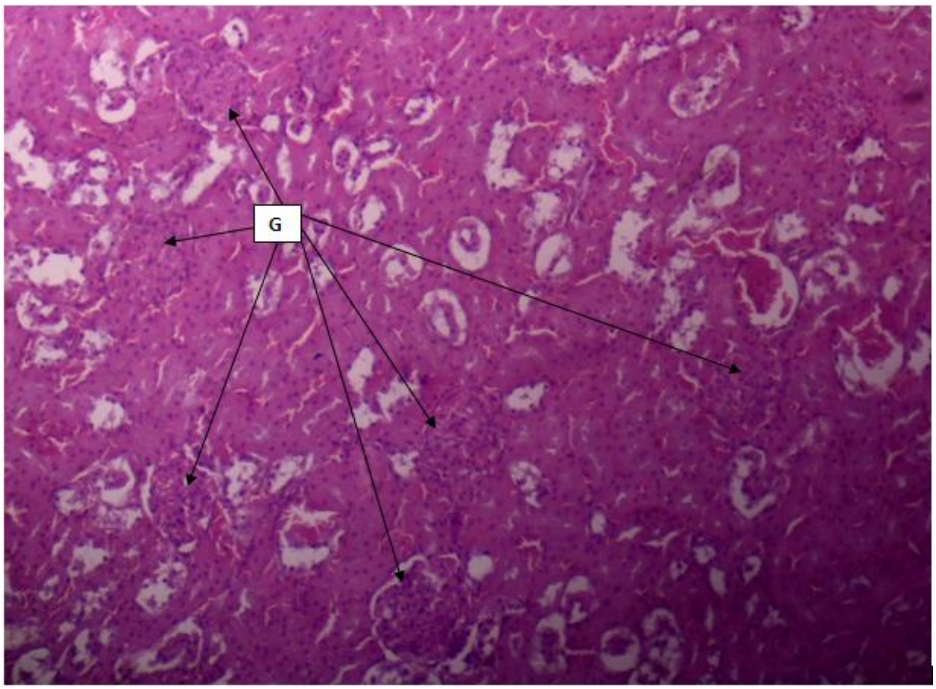
Parameter	Group(n=10 each)	Mean	Std. Deviation	p-value*

Weight of the kidney in mg	A	2.1880	.11987	0.001
	B	1.8120	.05263	
	C	1.3680	.04382	

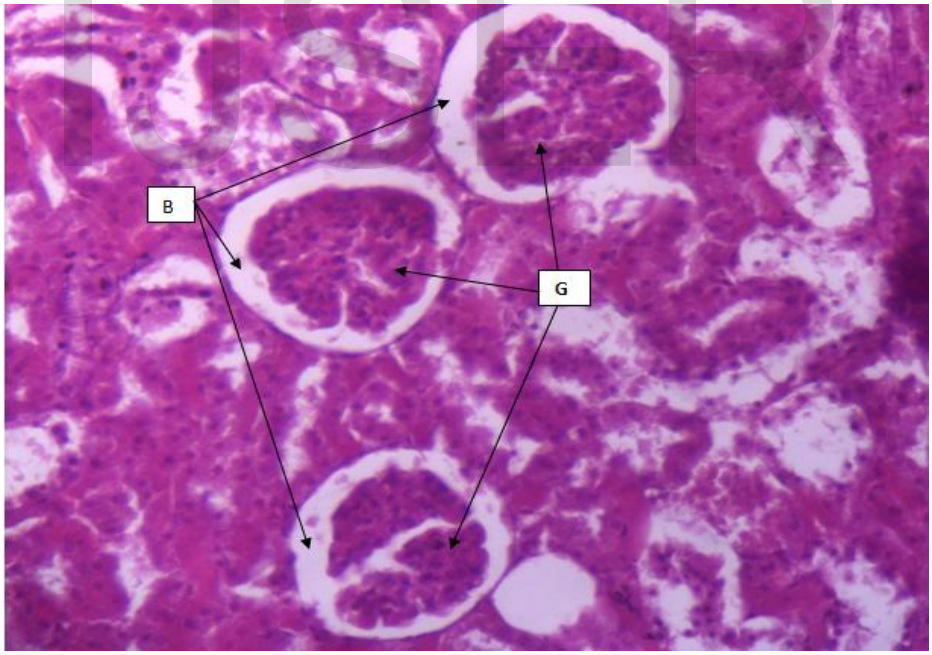
*p-value <0.05 taken as significant

Fig 01: showing histological changes between the groups





Photomicrograph 01 showing multiple glomeruli (G) with no Bowman's space visible under H & E X20



Photomicrograph 02 showing Glomeruli (G) of Group C having marked bowman's space under H& E X40