Original Article

Effects of Smokeless Tobacco on Kidney

Effects of Smokeless Tobacco Alters the Histology of Kidney of Offspring's in Swiss Albino Mice

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ABSTRACT

Objective: To study the micro structural changes in the kidney of the offspring's of Swiss albino mice exposed to smokeless tobacco during pregnancy.

Study Design: Observational / descriptive study.

Place and Duration of Study: This study was conduct at the Animal House of the Department of Animal Husbandry and Veterinary Sciences (AHVS) Sindh Agriculture University, (SAU) Tandojam from July 2015 to December 2015.

Materials and Methods: Healthy adult female mice were mated. After confirmation of pregnancy, 20 pregnant mice were categorized into two categories, experimental group A and control group B. Group-A was provided Tobacco 5% mixed with standard diet along with clean water ad libitum, whereas group B, the control was provided standard diet and clean water ad-libitum throughout their pregnancy. After birth 20 offspring (10 male & 10 female) were selected randomly from each group. At 15 days after birth, the offspring were sacrificed by cervical dislocation and their kidneys were dissected out for histological analysis.

Results: The histological marked changes were seen in the kidney of offspring's of mice. In the experimental group of offspring there were very few glomeruli and also more immature glomeruli were observed. Glomerular degenerative changes, micro calcifications were noticed in both female and male offspring's of experimental group. Fatty change was observed in the renal parenchyma of the experimental group in 14 animals 9 male and 5 female offspring's showed edematous change and fatty infiltration. Glomerulus architectural distortion and displacement were also seen in kidneys of both offspring's.

Conclusion: Consumption of smokeless tobacco having significant effects on structure of kidney of offspring of mice that presented with the cellular injury to kidney parenchyma especially fatty infiltration as well asglomerular distortion and degenerative changes.

Key Words: Smokeless Tobacco, Offspring, Kidney

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INTRODUCTION

Tobacco is being used since centuries in variety of ways and forms like smoking and smokeless tobacco.[1] The smokeless tobacco (ST)usage is rising day by day because of indoor smoking bans, unproven awareness of safety, as well as reported "positive" physiological outcomes, for example relaxation, increased alertness, raised concentration, hallucination and produce anorexia.[2] The commonest types of smokeless tobacco existing and utilized in Pakistan includes: Betel/pan with tobacco, Naswar, snuff, Chaallia/supari with tobacco, Gutka and pan masala. [3]

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The utilization of ST can lead to cancer amongpeopleas well as have greater risk of gum &cheek cancer. ST utilization may be addictive, causing gingival recession, (oral mucosal lesions)oral leukoplakias, and can possibly contribute significant occurrence of peripheral vascular disorder, cardiovascular disorder, peptic ulcers, hypertension, and fetal comorbidity & mortality.[4,5] The utilization of Smokeless Tobacco imbalances the electrolytes in kidney hemodialysis patients as well as alters the renal antioxidant mechanism and renal microstructure in rodents.[6-8] Health experts have long reflected interaction to tobacco smoke injurious to reproduction, distressing features from fertility as well as pregnancy consequence to fetus and its development. The Smoke of tobacco comprises thousands of compounds, a few of them are identified to impose toxic outcomes on reproductive health, for example nicotine, carbon monoxide (CO), and metals.[9] Several surveys have reported the relationship amid maternal tobacco chewing and long-term health effects within the offspring, together with obesity, cardiovascular and respiratory disorders.[10] Maternal use of smokeless tobacco is correlated with intrauterine growth

retardation (IUGR); whereas intrauterine growth retardation in turn, is correlated with diminished quantity of nephron within the off spring.[11] Undoubtedly, human research has exhibited that either use of smoking and smokeless tobacco is strongly associated with lower fetal renal volumes in the course of 2nd&3rd trimester, and lesser the birth weight.[12,13] In human beings, nephrogenesis initiates at gestational week[12-14] and terminates at gestational week 36.[16] Majority of nephrons are created during 3rd trimester,[15] as well as the definitive quantity of nephrons in every kidney is formed at birth. Though, among rodents, nephrogenesis remains following birth for a littletime period till weaning.[17] Modification of the growth factors at any point of renal development may result in underdevelopment of kidney as well as potential kidneys dysfunction.[18,19] Although there are many studies about the hazards of usage of smokeless tobacco in the course of pregnancy and its effects in literature, but due to the structure and scope of these studies, the utero-placental mechanism of smokeless tobacco and its effects on fetal and infant organs is not clearly understood and histological experiments are also limited. The purpose of this study was to examine the modifications of renal micro structure in mice offspring due to consumption of ST during pregnancy.

MATERIALS AND METHODS

Female & male Swiss albino rates were acquiredvia the animal house of the Department of Animal Husbandry and Veterinary Sciences (AHVS) Sindh Agriculture University, (SAU) Tandojam. Healthy female Swiss albino mice were mated. The animals were reared in a hygienic as well as well-aired setting. Mice were given diet (lab chow) as well as tap water ad-lib. The dark/light cycle was looked after at 12 hours intervals. 20 non-pregnant Swiss albino mice aged from 10 to 12 weeks with 28gm of average weight were selected randomly, as well as were categorized into two categories; each category with ten mice. Each female mated with male adult mice reserved for sex for four to ten days to increase their urge of sex for female mice. One male mouse was mated with two females. Pregnancy was confirmed by presence of mucus vaginal plug between 1-10 days of pairing. On pregnancy confirmation the male mice were isolated.

After confirmation of pregnancy 20 pregnant female mice were categorized into two categories, Group A was given Tobacco 5% mixed with usual food along with clean water ad-libitum, whereas group B, the control was provided regular diet and clean water ad libitum throughout their pregnancy. After birth 20 offspring (10 male & 10 female) were selected randomly from each group.

Offspring of both A and B categories were allocated sub categories as follows:

Group A-1 (Experimental group)	10	male offspring
Group A-2(Experimental group)	10	female offspring
Group B-1 (Control group)	10	male offspring
Group B-2 (Control group)	10	female offspring

15 days after birth, the offspring were sacrificed by cervical dislocation and their kidneys were dissected out for histological analysis.

The kidneys were removed and set in 10% formaldehyde later dehydration was performed in leading qualities of alcohol. The tissues then were freed from xylene quickly to eliminate the alcohol. Impregnation/infiltration was performed for two alterations of soft molten paraffin wax; each at the temperature of 58°c upto 30 minutes. Implanting & dipping in paraffin wax with two L- formed pieces of metal was performed as well as sectioning was done with a microtome. Four micron (u) thick sections were done on rotary microtome then dipped in hot water container.

The sections were fixed on slides bya thin layer of egg albumen coated on every slide. De-waxing was performed by hot plate at 37°c afterwards clearing in two alterations of xylene.

Isolation of Xylene was done through absolute alcohol and at last prior to staining, hydration was carried out. The sections were stained with hematoxylin & Eosin and fixed in Canada balsam. The slides were assessed for histopathological variations under light microscope. All data was recorded in the proforma.

RESULTS

The Histological Profile of the Kidney Section: The marked histological changes in the kidney were seen in smokeless tobacco exposed offspring.

Figure No.1: A section of kidney of offspring of mice showing normal architecture of kidney

In the experimental group of offspring there were lessglomeruli & further immature glomeruli were observed as contrasted to Control offspring. Glomerular size was also significantly decreased that there was

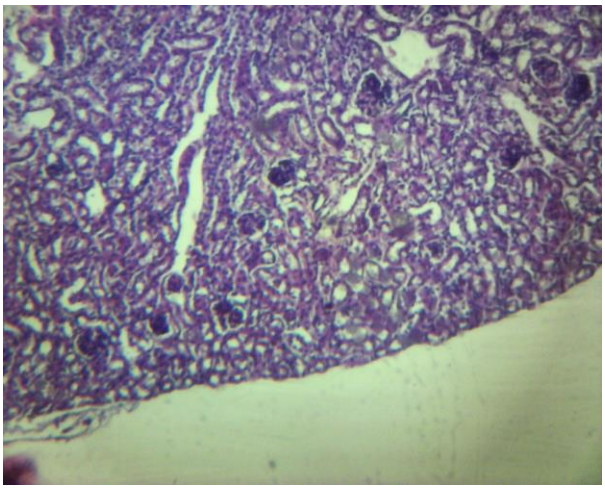

Figure No.2: A section of kidney of male offspring of mice showing shrinkage of glomeruli, necrosis and disruption of renal tubules

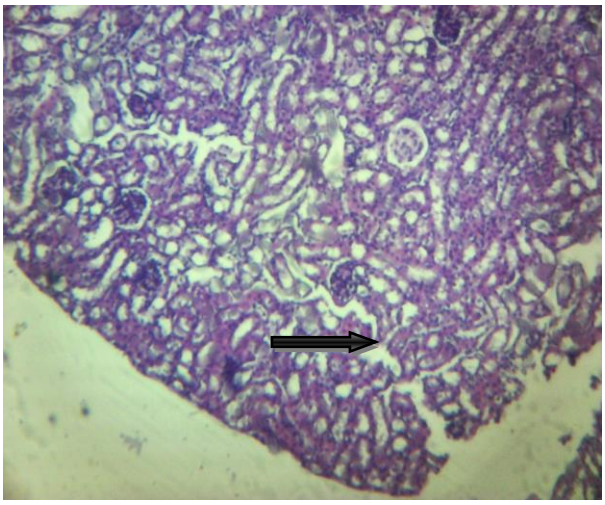

Figure No.4: A section of kidney of offspring of mice showing micro calcifications

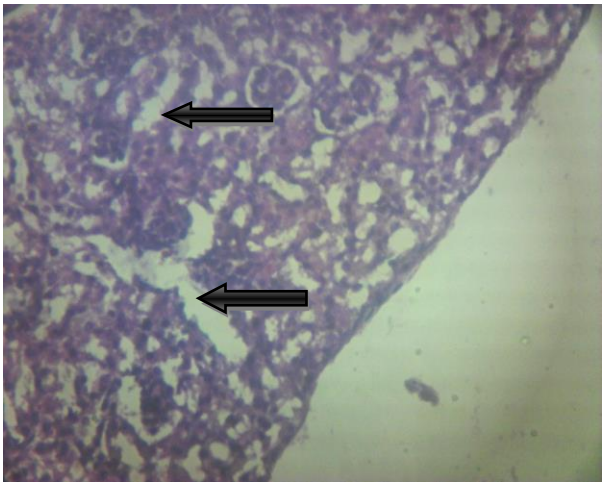

Figure No.3: A section of kidney of offspring of mice showing Distortionand Edematouschange of Glomerulus and Tubular Necrosis

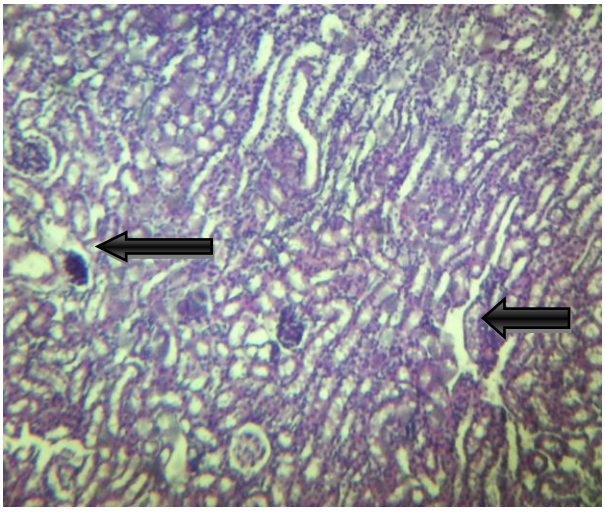

Figure No.5: A section of kidney of offspring of mice showing glomerulusdegenerativechanges, loss of architecture & tubular inflammation

Table No.1: Microscopic findings in Kidney from male & femaleoffsprings of experimental and control groups.

Histological findings	Male offsprings n=10		Female offsprings n=10	
	Experimental	Control	Experimental	Control
Glomerulus immaturity and decrease in number	4	0	3	0
Tubular inflammation & destruction	6	0	4	0
Edematous and fatty infiltration of parynchyma	9	0	5	0
Necrotic changes	2	0	2	0
Glomerulus shrinkage	7	0	4	0
Glomerulus distorsion	7	0	4	0
Micro calcification	5	0	5	0

shrinkage and distortions of glomerular architecture seen in the experimental male and female offspring. The animals of treated group subjected to smokeless tobacco exhibited significant degenerative variations, fatty infiltration and edematous changes in kidney parenchyma. The more potent destructive structural changes of glomerulus as well as tubules were seen in male offspring as compared to female offspring. In which 14 male offspring and 9 female offspring showed edematous change and fatty infiltration. Glomerulus shrinkage and distortion were seen in both offsprings in which 7 male and 4 female offspring showed these

findings in their kidney architecture. While no structural change was observed in kidneys of control male and female offsprings.

The comparison of results in control and experimental groups in male and female offsprings shown in Table 1 and Figures 1 (control) and 2,3,4,5(experimental)

DISCUSSION

This study showed that smokeless tobacco having significant effects on the microstructure of kidney of offspring mice. The most important finding of this study exhibits that maternal smokeless tobacco exposure in gestational period is evidently correlated with loss of kidney architecture causing kidney dysfunction as well as raised inflammatory indicators. Structurally, subtle variations were noticed in glomeruli and tubules within the kidneys of ST exposure progenies. Inflammation associates with kidney impairment and plays a vital role in the development of chronic renal disorder, which was well exhibited in current study. Consequently, the progenies of SE dams can possibly be inclined to more kidney impairment with the progression of adulthood. Varying forms of cellular degeneration were noticed in the proximal convoluted tubules that can possibly settle the functional reliability of proximal convoluted tubules. This attribute can possibly cause the retention of metabolic waste products as well as endurance of such abnormalities can possibly lead to loss of delicate homeostatic systems of the kidney.[20,21] as well as histological cardiac, hepatic lungs and renal surveys, as well as testes were conducted in terms of procedures defined by Disbrey & Rack[22] and Drury & Wellington.[23]

Usage of smokeless tobacco is relatively prevalent in the Middle East, Far East as well as European nations[5]. Chewing tobacco of different brands is available in most part of our country. The commonest types of smokeless tobacco present and utilized in Pakistan comprise: catechu (Acacia catechu), tobacco, Betel/pan with tobacco a chewed areca nut mixture (Areca catechu), slaked lime [calcium hydroxide $(CaOH)_2$ and calcium oxide (CaO)], wrapped in a (Piper betel)betel leaf with sweetening agents.[28]

Some studies[24,25] have been conducted to determine the outcome of nicotine on fetal growth as well as whether this could be correlated with the activities of this drug over the metabolism of maternal adipose tissue. It has though been speculated that nicotine existing in tobacco smoke can possibly result in reduce maternal appetite, uterine vasoconstriction, or somehow produce metabolic variations within the mother and/or fetus reported by Nakamoto T, et al[26].

The placenta contributes significantly in prenatal development through carrying nutrients as well as waste products amid fetal & maternal circulation and by offering hormones required for typical development. Human placental explants can be developed as well as examined experimentally in vitro reported by Jauniaux E, et al[24]. In one such study, it was shown that nicotine unaccompanied was capable of inhibiting variation and thus inhibitcy to trophoblast invasion within an in vitro test. These authors more over exhibited that nicotine retarded synthesis as well as activation of type-IV collagenase, which is essential for cytotrophoblast invasion. A few placental surveys have included an fascinating association of in-vitro and in-vivo experimentation[24,25]. This study showed in the tubular structure of the cortex of kidney of animal's nicotine exhibited disturbance in the histological structure of kidney.Nicotine is a fundamental component of tobacco that retards the development and variation of cytotrophoblasts within human placenta. Nicotine can possibly caused decreased blood flow and vasoconstriction reported by Kazi AS et al.[28] Nicotine can as well escalate maternal blood pressure (BP) and cardiac rate, dropping uterine blood flow[26]. In our study the necrotic and inflammatory changes were noticed in the ST dams, which are in relation to the study of Agarwal R, et al.[27] similarly in the study of Jagadapillai R, et al[29] reported that renal proteins expression was affected by CSE belonged to inflammatory diseases, as well as indicated that CSE altered kidney proteome.

CONCLUSION

From these interpretations, it can be concluded that exposure of the smokeless extract may be associated with structural damage of kidney in the offspring of mice.

Conflict of Interest: The study has no conflict of interest to declare by any author.

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