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Effect of Nicotine Smoke on Genitourinary Organs of Male Albino Rats: An Experimental Study

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ABSTRACT

Background and aim: The harmful effects of nicotine have been reported on the lungs and cardiovascular system in many studies, but the most unexplored profile of the effect of nicotine has been the genitourinary organs. In the current study, we planned to evaluate the effects of nicotine smoke on one of the important genitourinary organs, i.e., the testes and kidneys.

Materials and methods: The study was conducted on 12 inbred adult Wistar albino rats; 6 animals acting as a control, and the remaining six acting as a test group. The control group animals were given only sterile water, whereas animals of the test group were exposed to smoke produced from the nicotine wrapped in cotton wool in the dose of 6mg/day three times a day for each session of 5 minutes each for five days. Each rat was exposed to the smoke produced due to nicotine separately in a closed inhalational chamber and not in groups. The rats were euthanized after experimentation, and testis and kidneys were removed and subjected further to tissue processing for histological examination.

Results: The histological examination of tissue sections of the test group revealed marked distortion and degeneration of seminiferous tubules and germ cell lineage and distorted and widened interstitial spaces with loss of Leydig cells. On the other hand, the tissue sections of the control group showed normal histological architecture of testes with normal interstitial spaces and Leydig cells. Additionally, the renal sections of the test group showed dilation of urinary space, shrunken and distorted glomeruli. On the contrary, the renal specimens of the control group also demonstrated normal renal architectural patterns.

Conclusion: the results of the present study concluded that even passive smoke exposure has drastic effects on the genitourinary organs, and hence, its use in public places should be checked.

1. Introduction

Nicotine, a natural alkaloid found in the plant Nicotianatabacum, is considered an important component of cigarettes. Nicotine chewing gums and dermal patches are the other ways nicotine is consumed through non-prescription nicotine replacement therapy. However, in man, it is consumed primarily through cigarettes, pipes, or cigars. Nicotine can also be found in electronic cigarettes (e-cig), a contemporary and widespread way of smoking.[1] Nicotine can be consumed in different forms ranging from smokeless tobacco products such as snuff and chewing tobacco but more often consumed as smoked tobacco. It constitutes 90-95% of the total alkaloids, absorbed quickly through the respiratory tract, oral mucosa, and skin.[2] Burning tobacco leads to the formation of a mixture of two forms of smoke called secondhand smoke or environmental tobacco smoke. Secondhand smoke is further composed of mainstream smoke and sidestream smoke. A smoker exhales mainstream smoke.

Sidestream smoke is the smoke from the lighted end of a cigarette, pipe, cigar, or burning tobacco in a hookah. This type of smoke has a higher concentration of cancer-causing agents (carcinogens) and is more toxic than mainstream smoke. It also has smaller particles than mainstream smoke. These smaller particles make their way into the lungs and the body's cells more efficiently. When nonsmokers are exposed to secondhand smoke, it is called involuntary smoking or passive smoking. Non- smokers who breathe in secondhand smoke take in nicotine and toxic chemicals the same way smokers do.[3]

Tobacco use is a major preventable cause of premature death and disease worldwide. Fact sheet of Global Adult Tobacco Survey, India (2009-2010) highlighted that current tobacco use in any form in India is 34.6% of adults; out of which tobacco smokers are 14% adults, 25.9% are smokeless tobacco users, and 5.3% of the adults use both smoking and smokeless tobacco. Regarding secondhand smoke exposure, Global Adult Tobacco Survey, India (2009-2010)estimated that about 52.3% of adults were exposed to secondhand smoke at home, whereas 29% of adults were exposed to the same at the public places, mainly in public transports and restaurants and at the workplace about

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29.9% of adults were exposed to the secondhand smoke.[4] Nicotine poses several health hazards. Apart from the amount taken, the effects of nicotine also depend on many factors, including size, weight, and health of a person, along with whether the person is used to taking it or not.[5] In addition to being highly addictive, nicotine has a diverse and adverse effect on most body organs. Nicotine produces both immediate as well as remote effects.[6] Among the organs that nicotine effects, the urogenital organ system is the least explored and evaluated system. In males, the effects of nicotine on the reproductive system are erectile dysfunction, degeneration of seminiferous tubules, disruption of spermatogenesis, and affects germ cell structure and function at the cellular level.[7, 8] Among the pathologic effects of nicotine on the renal system, increased albumin excretion, decreased glomerular filtration rates, increased incidence of renal artery stenosis, and increased mortality in patients with end-stage renal disease. Also, there is the impaired response of kidneys to increased systemic blood pressure in smokers, and this loss of renoprotective mechanism further deteriorates the functioning of kidneys.[9] Further, there is huge clinical relevance in evaluating the effect of nicotine on these organs. Hence, the current study was planned to evaluate the effect of nicotine on the testes and kidneys of adult Albino rats.

2. Materials and methods

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Grouping of animals: Healthy Wistar Albino rats, twelve in several male sexes only weighing between 125- 160 gm, were taken for the study. The rats were procured from the Central Animal House of Government Medical College, Jammu. According to the block permuted randomization plan, after acclimatizing to the laboratory conditions for a week, the rats were randomly divided into two groups, i.e., test group (T) and control group (C). An identification number was given to rats of each group. The animals were housed under laboratory conditions with a controlled environment and were given food and water ad libitum as per the Committee for Control and Supervision of Experiments on Animals (CPCSEA), India, guidelines. The animals were fasted overnight and were weighed before the initiation of the experiment.

Experimental procedure

Group C rats received only distilled water and served as healthy control. The animals of the control group were not subjected to any smoke. Group T rats served as the experimental group and were exposed to smoke produced from the burning of nicotine wrapped in cotton wool in the dose of 6mg/day three times a day for each session of 5 minutes each for five days with a maximum time interval of 5 hours in between two successive sessions of smoke exposure. Each rat was exposed to the smoke produced due to nicotine separately in a closed inhalational chamber and not in groups. All animals of the test and the control group were observed daily for physical or behavioral change throughout the experimental period. After completing the experimental period, the animals have euthanized 48 hrs after administering the last dose. The animals were euthanized, and it was followed by the removal of testes and kidneys from each rat.

Analysis

The naked eye examination was done to see any external changes. Later the tissues were subjected to histological processing to make tissue sections of 5-6μ thick which were fixed and then stained with Hematoxylin and Eosin and then analyzed. The observational analysis was done using a light microscope under 100X and 400X powers.

3. Results

Microscopic changes of kidney

The kidneys of Group C rats showed normal architecture consisting of cortex and medulla. The cortex was composed of glomeruli, proximal convoluted tubules, distal convoluted tubules, and interlobular blood vessels. Renal corpuscles showed glomeruli within Bowman's capsule. The visceral and parietal layers of Bowman's capsules were visible along with Bowman's space. The proximal convoluted tubules were more numerous, whereas distal convoluted tubules were less numerous in numbers (Fig. 1.1). The architecture of the cortex and medulla of the kidneys of Group T rats showed focal disruption at places. In the cortex, the renal corpuscles showed moderate congestion of glomerular tuft of capillaries with shrinkage of glomerulus and widening Bowman's space at certain places. Some of the renal corpuscles showed collapsed necrotic glomerulus with markedly dilated urinary space (Fig. 1.2). The majority of the tubules were normal, appearing with empty lumina, whereas some appeared solid cord-like material. The interstitium showed mild congestion, and focal hemorrhage with focal chronic inflammatory infiltrate (Fig.1.3). The medulla appeared normal, with normal tubules and their lining epithelium and their lumina being empty.

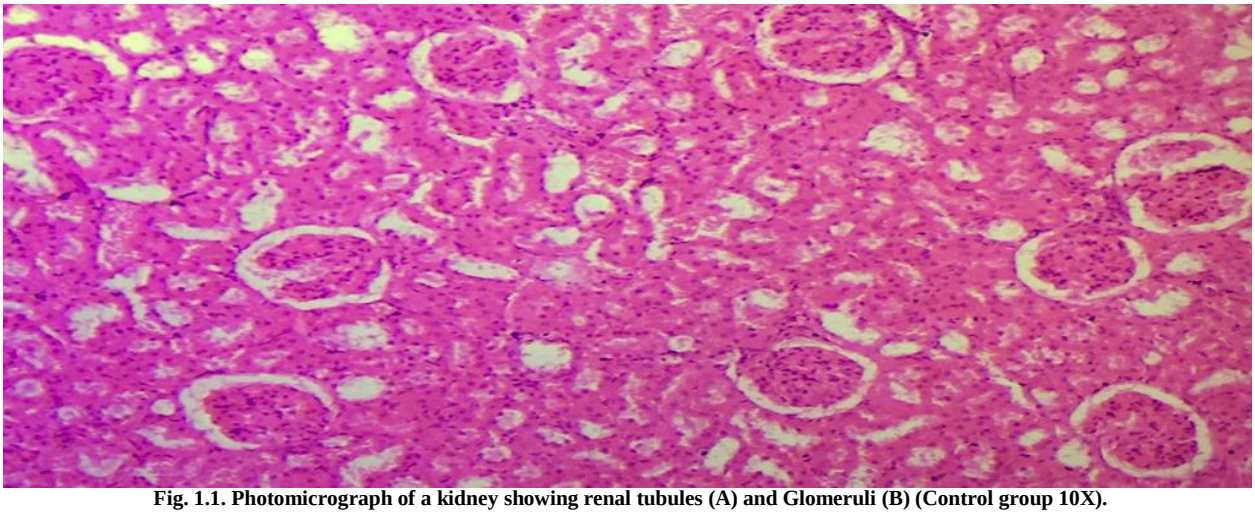

Fig. 1.1. Photomicrograph of a kidney showing renal tubules (A) and Glomeruli (B) (Control group 10X).

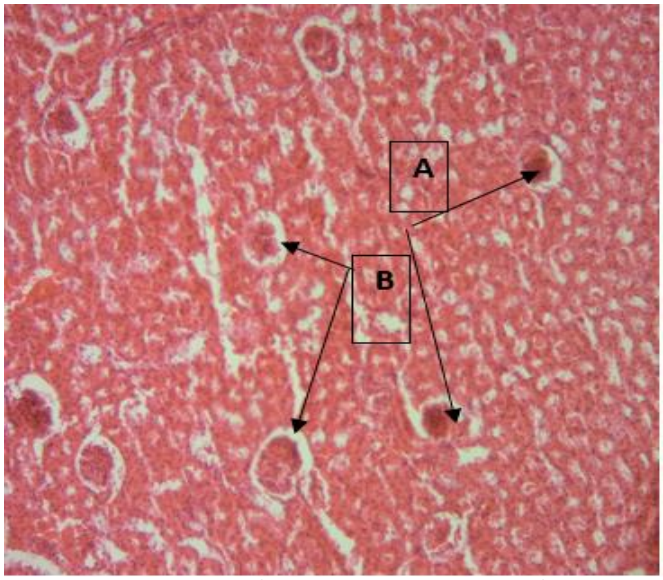

Fig. 1.2. Photomicrograph of a kidney showing shrinkage of the glomerulus (A) and dilatation of bowman's space (B) (Test group 10X).

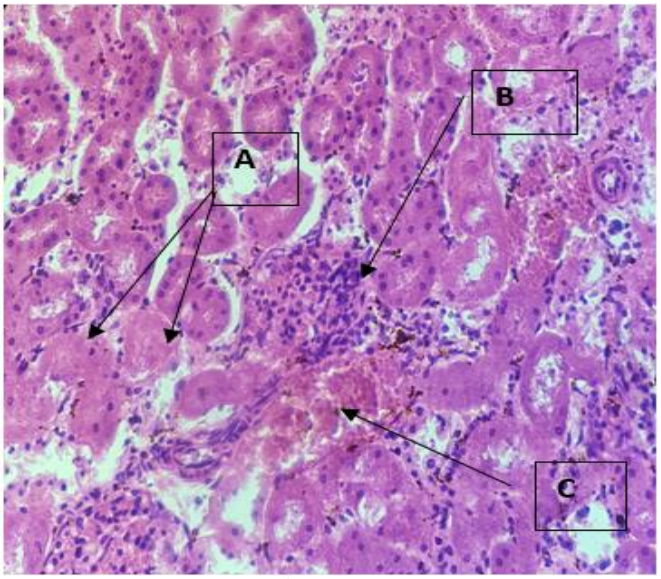

Fig. 1.3. Photomicrograph of a kidney showing solid cord-like tubules (A), inflammatory infiltrate (B) and focal hemorrhage (C) (Test group 100X).

Microscopic changes in testes

The cut section of testis of group C rats showed seminiferous tubules cut in various planes, and an outer layer of connective tissue surrounded each seminiferous tubule with fibroblasts known as Tunica Albuginea and an inner basement membrane. On the basement membrane rest, several layers of spermatogenic cells called germinal epithelium (Fig. 1.4). These spermatogenic cells were seen in different stages of development. Interstices of the testes consist of loose connective tissue lying in between the adjacent seminiferous tubules. The loose connective tissue was seen to consist of clusters of Leydig cells, blood vessels, nerves, and lymphatics. The interstitial cells of Leydig were observed as large or polyhedral-shaped large cells with lightly stained foamy cytoplasm and eccentric nucleus (Fig. 1.5). The tunica albuginea and tunica vasculosa of Group T rats were seen to be thickened (Fig. 1.6) with shrunken seminiferous tubules and wide interstitial spaces. Some seminiferous tubules appeared small in diameter, distorted, and degenerated with thickened basement membrane (Fig. 1.7). All the seminiferous tubules were widely separated with distorted interstitial tissue containing dilated blood vessels and few Leydig cells (Fig.1.7). Some of the seminiferous tubules showed vacuolations, and some accumulated cellular masses in their lumen (Fig. 1.7). The germinal epithelium showed a variable degree of degeneration. The degenerated seminiferous tubules were found to have fewer layers of spermatogonia, few germinal cells, and an empty lumen, indicating incomplete spermatogenesis. However, some seminiferous tubules appeared normal with normal stratification of germinal epithelium depicting complete spermatogenesis.

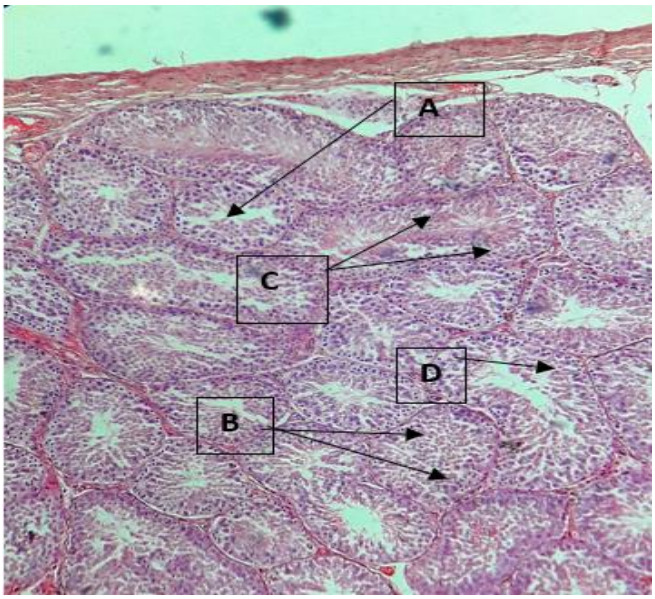

Fig. 1.4. Photomicrograph of testes showing tunica albuginea (A), seminiferous tubules (B) and interstitial tissue (C)(Control group 100X.

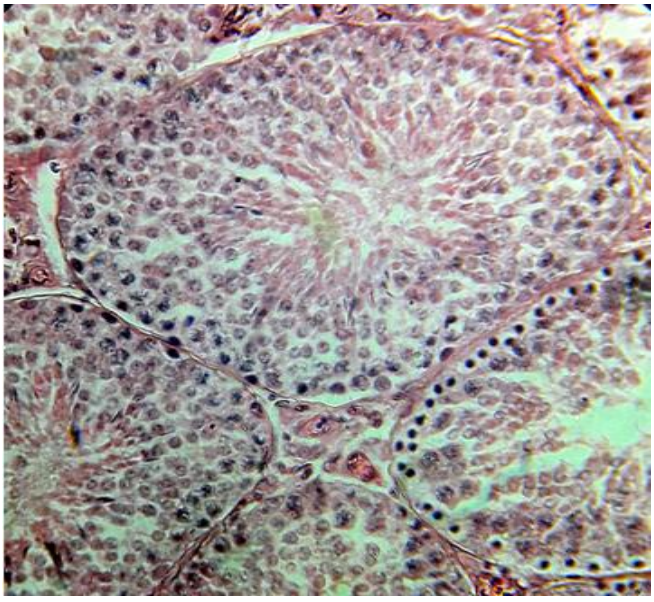

Fig. 1 .5. Photomicrograph showing spermatogenic cells in stratifie layers (A), Leydig cells (B),spermatids (C) and spermatogonia (D)(Controlgroup 400 X).

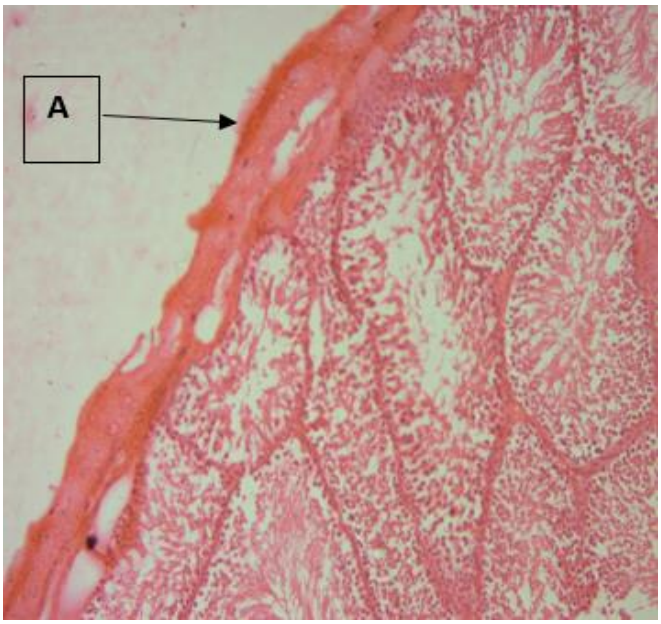

Fig. 1.6. Photomicrograph of testes showing thickened tunica albuginea (A) (Test group 400 X).

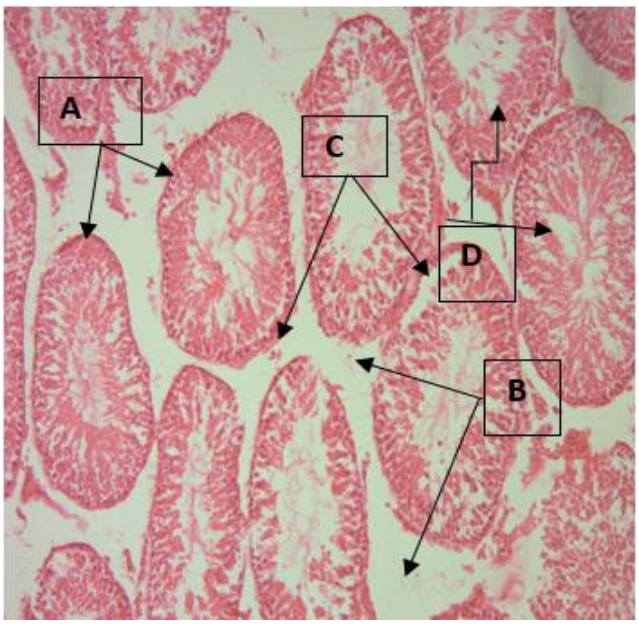

Fig. 1.7. Photomicrograph of testis showing shrunken seminiferous tubules (A), wide and empty interstitial spaces (B), thickened basement membrane (C) and vacuolations (D) (Test group 100X).

4. Discussion

The study focused on depicting the histological changes in the rat testes and kidneys produced due to nicotine smoke. The nicotine smoke exposure at the given dose for such a short period of 5 days produced thickening of tunica albuginea and tunica vasculosaofGroup T rats with shrunken seminiferous tubules and wide interstitial spaces. Some seminiferous tubules appeared small in diameter, distorted, and degenerated with the thickened basement membrane. The seminiferous tubules were widely separated with distorted interstitial tissue containing dilated blood vessels and few Leydig cells. These observations are well documented in the various existing literature on the effect of nicotine on testes.[11-13, 15, 17] Additionally, it was observed that there was reduced spermatid volume, tubular depletion in the form of small diameter, and germinal epithelium dissociation. The supports the findings of some existing literature, which suggests that nicotine administration is associated with reduced spermatid volume.[2, 12, 15, 16] The stratified epithelium lining seminiferous tubules was found to be formed of two or three rows of spermatogenic cells, including Sertoli cells denoting the arrest of spermatogenesis. Some seminiferous tubules appeared small in diameter and distorted with incomplete spermatogenesis and empty lumen, whereas some of the seminiferous tubules appeared normal with intact germ cells and complete spermatogenesis. These findings were supported positively by the evidence derived from the observations of the previously indexed studies.[7, 11, 13, 14, 16, 17]

However, few studies concluded with the findings of disruption of normal orderly progression of the spermatogonia with tubules showing only one layer of spermatogonia with no changes in the interstitial tissue and Leydig cells.[18] Furthermore, few authors also documented a gradual increase in the number of spermatogonia with decreased number of spermatocytes and spermatids.[19] These findings were not in accordance with the current study's observations, which showed a decrease in all types of spermatogenic cells with changes in the interstitial tissue. Some of the seminiferous tubules showed vacuolar spaces as vacuoles have replaced the depleted cells with distorted germinal epithelium and degenerated germinal epithelial cells sloughed into the lumen of tubules. These findings concordance with some existing studies' observations.[2, 11, 14, 16, 20] The testicular histological changes were seen in the present study, thus produced, can be attributed to nicotine-induced lipid peroxidation and the reduction in testosterone hormone level either directly through the decrease of Leydig cells or indirectly by affecting the central nervous system and release of gonadotropin-releasing hormones (GnRH) from the pituitary.

The present study revealed that nicotine exposure to rats caused histological changes in kidneys in the form of the renal corpuscles showing moderate congestion of glomerular tuft of capillaries with shrinkage of glomerulus and widening of Bowman's space at certain places. These findings were supported positively by the evidence derived from the observations of the previously indexed studies.[21-23] However, Hassan et al. (2016)contradicted these findings as the results of their study revealed swelling of glomerulus due to congestion and edema with normal Bowman's space, which can be attributed to long time exposure to nicotine.[24] Furthermore, few scholars also observed widening Bowman's space on acute and subacute exposure, whereas, on chronic exposure, the Bowman's space appeared to be normal, which again gave positive supporting evidence in favor of the observations made in our study.[25] The proximal convoluted tubules were lined by low columnar cells, intensely stained with eosin which showed cloudy swelling at places and loss of brush border, and these observations were in accordance with the findings of Hassan et al. (2016) and Mahmoud and Amer (2014)[24, 26] However, some tubules appeared solid cord-like as pink eosinophilic hyaline material, and this finding was consistent with the changes observed by Metwally et al. (2015)in rats exposed to nicotine.[21] The interstitium showed mild congestion, focal hemorrhage, and chronic inflammatory infiltrate in the interstitium. The evidence positively favored these findings in the previous indexed studies.[22, 23, 27, 28]

However, Akomolafe et al. (2017) could not appreciate any histological changes in the nicotine-treated group and found the kidney cytology to be normal, which in totality is contradictory to the present study.[1] All the

changes mentioned above were due to the production of reactive oxygen species, which damage the tubular epithelium and the concentrating and filtering of toxins by the kidney leading to precipitation of toxic substances in the glomerulus and the tubules, thus affecting them.

5. Conclusion

The observations of the current study reinforce that nicotine, one of the most active chemicals in tobacco smoke acquired by active or passive smoking, is highly toxic, and nicotine exposure even with a small duration of 5 days can induce severe histopathological changes in testes and kidneys. It is also evident from the current study that nicotine affects the respiratory organs, as believed earlier, but other organs like the testes and kidneys.

Conflict of Interest

The authors declared that there is no conflict of interest.

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