EFFECT OF NICOTINE SMOKE ON LIVER AND KIDNEYS OF ADULT MALE ALBINO RATS: AN EXPERIMENTAL STUDY

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Abstract
Apart from heart and lungs, being cited as the main affected organs by nicotine smoke and hence, contributing for the morbidity and mortality, other organs like kidneys, gastrointestinal tract, liver, reproductive organs, endocrine glands, skin etc. are also affected. In the current study we planned to evaluate the effects of nicotine smoke on liver and kidneys. The study was conducted on 12 inbred adult Wistar albino rats; 6 animals acting as control and remaining 6 acting as test group. The animals of control group were given only sterile water where as animals of test group were exposed to smoke produced from the nicotine wrapped in a cotton wool in the dose of 6mg/day three times a day for each session of 5 minutes each for 5 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 sacrificed after the period of experimentation and testis were dissected out and subjected further to tissue processing for histological examination. The histological examination of tissue sections of liver of test group animals revealed distorted or normal lobular architecture of hepatic lobules with dilated and congested central vein, portal venule and hepatic sinusoids. Also, there was presence of focal inflammatory aggregates especially around bile canaliculi at portal triads. On the other hand, the tissue sections of liver of control group showed normal hepatic architecture with normal hepatocytes. The renal specimens of the control group also demonstrated normal renal architectural pattern; with glomeruli surrounded by urinary space and renal tubules present in cortex and medulla composed of collecting tubules, loop of henle and convoluted tubules. On the contrary, the renal sections of test group showed dilation of urinary space, shrunken and distorted glomeruli with some renal tubules showing solid cord like pattern and some showing eosinophilic material in lumen, focal inflammatory infiltrates and renal congestion.

Keywords: Nicotine smoke, liver, kidney

Introduction
Nicotine is the main component of tobacco smoke, and failure to quit smoking is virtually attributed to its addictive potential which is similar to that of opium and alcohol (1). Nicotine, a natural alkaloid found in the plant Nicotiana tabacum, is considered as an important component of cigarettes. Nicotine chewing gums and dermal patches are the other ways in which nicotine is consumed through non-prescription nicotine replacement therapy. However, in man it is consumed primarily through cigarettes, pipes or cigars and chewable tobacco (2). It constitutes 90-95% of the total alkaloids, which is absorbed quickly through the respiratory tract, oral mucosa and skin (3).

 Burning tobacco leads to the formation of mixture of two forms of smoke called as secondhand smoke or environmental tobacco smoke which is further composed of main stream smoke and the side stream smoke. When non-smokers are exposed to secondhand smoke it’s called involuntary smoking or passive smoking. Non-smokers who breathe in second hand smoke, take in nicotine and toxic chemicals the same way smokers do (4).

In commercial tobaccos, the major alkaloid is nicotine, accounting for 95% of the total alkaloid content (5). Tobacco use is a major preventable cause of premature death and disease worldwide. Global Adult Tobacco Survey, India (2009-2010) estimated that about 52.3% adults were exposed to second- hand smoke at home whereas 29% of adults were exposed to the same at the public places mainly in public transports and restaurants and at workplace about 29.9% of adults were exposed to the second- hand smoke (6).

Nicotine poses several health hazards. The effects of nicotine, apart from amount taken; also depends on many factors including size, weight and health of a person, along with the fact that whether the person is used to taking it or not (7). In addition to being highly addictive nicotine has diverse and adverse effect on most but not all the body organs. Nicotine produces both immediate as well as remote effects (8).
If we look at the past literature, nicotine has been mostly studied as the agent that has the carcinogenic potential. But now-a-days, the trend has been changing as scholars have started studying the side effect profile of the nicotine on other organs like gastrointestinal tract, reproductive organs, genitourinary system, respiratory system and so on and so forth.

As far as the toxicological profile of nicotine smoke on liver is concerned, these include deranged liver enzyme levels, hepatocellular carcinoma and deranged hepatocellular architecture (9). Among the pathologic effects of nicotine on renal system, increased albumin excretion, decreased glomerular filtration rates, increased incidence of renal artery stenosis and are associated with an increased mortality in patients with end-stage renal disease. Also, there is impaired response of kidneys to increased systemic blood pressure in smokers and this loss of renoprotective mechanism further deteriorates the functioning of kidneys (10).

Hence, in light of above stated facts the current study was planned to evaluate the histopathological effects of nicotine smoke on liver and kidney of adult albino rats.

Materials and Methods:

Healthy Wistar Albino rats, twelve in number of male sex 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. The animals were left for acclimatization to the laboratory conditions for a week and were provided standard rodent chow/feed and water *ad-libitum* during the period of experimentation. Later, the rats were randomly divided into two groups i.e., control group (A) and test group (B) according to block permuted randomization plan and an identification number was given to rats of each group.

The animals were housed in polypropylene cages (4 animals per cage) with dust free rice husk as a bedding material under laboratory conditions with control environment of temperature 18 to 29ºC, humidity (30% to 70%) and 12h light/dark cycle (16.00-18.00) as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India, guidelines which are in accordance with the internationally accepted principles for laboratory animal use 9and care. The animals were fasted overnight and were weighed before the initiation of the experiment; using electronic weighing scale.

Group A rats received only distilled water and served as healthy control. The animals of control group were not subjected to any kind of smoke. Group B rats served as the experimental group and were exposed to smoke produced from the nicotine wrapped in a cotton wool in the dose of 6mg/day three times a day for each session of 5 minutes each for 5 days. Each rat was exposed to the smoke produced due to nicotine separately in a closed inhalational chamber and not in groups.

All animals of test as well as control group were observed daily for physical or behavioral change throughout the experimental period. After the completion of experimental period, the animals were sacrificed 48 hrs after the administration of last dose by giving injection Thiopentone sodium. The experimental protocol was approved by the Institutional Animal Ethic Committee (IAEC).

The sacrificing of animals was followed by dissection. The liver and kidneys were dissected out from each rat. The naked eye examination was done to see any external changes. The dissected out kidneys were cut into two halves and were placed in fixative whereas liver were grossed into small pieces of 1cm x 1cm and fixed in the fixative. Later the tissues were embedded in paraffin wax, blocks were made, and sectioned using a rotatory microtome and sections of 5μm thickness were obtained. These sections were stained with Hematoxylin and Eosin and were examined under microscope.

Results:

**Behavioral Changes:**

Rats in Group A showed no behavioral alterations. Rats were active and alert throughout the study period. They consumed the standard rodent chow/feed and water *ad-libitum* regularly. All the rats completed the experimental period and did not exhibit any case of mortality.

Rats in Group B used to become lethargic after exposure to nicotine smoke, but regained activity after sometime. They consumed the standard rodent chow/feed and water *ad-libitum* regularly. All the rats completed the experimental period and did not exhibit any case of mortality.

**Microscopic Changes of Liver:**

Light microscopic examination of Hematoxylin and Eosin stained liver sections of Group A (control) rats revealed the normal basic architecture of the liver, showing the hexagonal classic hepatic lobules with central veins located in the centre of the lobule (Fig.1) and portal areas containing portal triads formed by portal venule, hepatic arteriole and bile ductule surrounded by connective tissue at corners of the lobule (Fig.2).

Within the classical hepatic lobule, the central vein had a thin connective tissue wall lined internally by endothelial cells and was present in the centre of the lobules. The cords of the hepatocytes which were one cell thick at most of the places were found to radiating from the central veins towards the periphery of the lobule which contained portal areas. The sinusoids were lined by the endothelial cells and contained few Kupffer cells (Fig.3). The portal...
areas present at the corners of the classical hepatic lobule contained connective tissue, fibroblasts and stained light pink in color. Embedded within the connective tissue were seen portal venule, hepatic arteriole and bile ductule.

Hepatocytes were seen as polygonal, eosinophilic structures with centrally placed spherical, basophilic nucleus and one nucleolus. Occasional hepatocytes containing two nuclei were also seen.

On histological examination of the liver of Group B (experimental group) rats, the lobular architecture of the liver was found to be distorted (Fig.4) whereas in some liver specimens hepatic cord pattern was preserved. The central veins were dilated and congested at certain places (Fig.5). The sinusoids were also dilated and congested in some areas with focal inflammatory infiltrates and presence of red blood cells (Fig.6). The portal areas showed periportal cuffing of lymphocytes, especially around bile duct. Dilated and congested portal venules were present in these portal areas (Fig.7).

Hepatocytes showed varied morphology i.e.; they had different shapes and sizes. Some hepatocytes were enlarged, swollen and edematous with irregularly clumped cytoplasm and intervening clear spaces known as Cloudy Swelling, indicative of early reversible changes. They also contained vacuoles of varying sizes, seen as small and large empty spaces within the hepatocytes known as Vacuolar or Hydropic or Dropsical Degeneration, which is indicative of severe osmotic swelling of hepatocytes. Some hepatocytes had large vacuoles pushing the nuclei to one side and contained scanty cytoplasm known as Ballooning Degeneration (Fig.8). Cytoplasm of the hepatocytes was eosinophilic with clear spaces and peripherally pushed basophilic nucleus. Hepatocytes with hyper eosinophilic cytoplasm, small shrunken condensed nuclei with increased basophilia known as Pyknotic Nuclei, smooth contour and isolated from the viable cells known as Apoptotic Cells or Counsellman’s Bodies, were seen at certain places (Fig. 9). Number of inflammatory cells was also seen to be mildly increased in the sinusoids and perivenular areas (Fig.8).

Microscopic Changes of Kidney:

The kidneys of Group A rats showed normal architecture consisting of cortex and medulla. The cortex was further composed of glomeruli, proximal convoluted tubules, distal convoluted tubules and interlobular blood vessels (Fig.10). Renal corpuscles showed glomeruli within Bowman’s capsule. Visceral and parietal layer of Bowman’s capsules were visible along with Bowman’s space. The proximal convoluted tubules were more numerous in number and were lined by a single layer of low columnar or pyramidal cells which had round nuclei and granular cytoplasm staining deeply with eosin (Fig. 11). The distal convoluted tubules were less numerous in number and they were lined by cuboidal cells and contained lightly stained cytoplasm with central euchromatic nucleus. The medullary portion contained renal tubules (Fig. 12).

The architecture of cortex and medulla of the kidneys of Group B 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 of Bowman’s space at certain places. Some of the renal corpuscles showed collapsed necrotic glomerulus with markedly dilated urinary space (Fig. 13).

The proximal convoluted tubules appeared mildly dilated with eosinophilic material in the lumina of some of the tubules. Majority of the tubules were normal appearing with empty lumina whereas some appeared as solid cord like material. The interstitium showed mild congestion and focal hemorrhage with focal chronic inflammatory infiltrate (Fig.14). The medulla appeared to be normal with normal tubules and their lining epithelium and their lumina being empty.

Figure 1: Photomicrograph of liver of showing central vein (A) & hepatic cords radiating from central vein (B) (Control group 100X).

Figure 2: Photomicrograph of liver showing portal triad (A). (Control group 100X).
**Figure 3:** Photomicrograph of liver showing central vein (A), radiating cords of hepatocytes (B), hepatic sinusoids (C) and Kupffer cells (D), hepatocytes showing double nuclei (E) and endothelial cells lining the sinusoids (F) (Control group 400X).

**Figure 4:** Photomicrograph of liver showing disrupted hepatic cord pattern (A). (Test group 100X).

**Figure 5:** Photomicrograph of liver showing preserved hepatic cord pattern (A) and dilated and congested central vein (B) (Test group 100X).

**Figure 6:** Photomicrograph of liver showing dilated and congested sinusoids with RBC’s in sinusoids (A) (Test group 400X).

**Figure 7:** Photomicrograph of liver showing dilated portal venule (A) and lymphocytic infiltration around bile duct (B) (Test group 400X).

**Figure 8:** Photomicrograph of liver showing focal lymphocytic infiltrate (A), hepatocytes with cloudy swelling (B) and hepatocytes with vacuolar degeneration (C) (Test group 400X).
Figure 9: Photomicrograph of liver showing Apoptotic cells (A) (Test group 400X).

Figure 10: Photomicrograph of renal cortex showing renal corpuscles (A), renal tubules (B) and interlobular blood vessels (C) (Control group 100X).

Figure 11: Photomicrograph of renal cortex showing glomerulus (A), parietal and visceral layer of Bowman’s capsule (B&C), Bowman’s space (D) and proximal convoluted tubules (E) (Control group 100X).

Figure 12: Photomicrograph of renal medulla showing renal tubules (A) (Control group 100X).

Figure 13: Photomicrograph of renal cortex showing shrunken glomerulus (A), collapsed necrotic glomerulus (B) and widened Bowman’s space (C) (Test group 100X).

Figure 14: Photomicrograph of kidney showing solid cord like tubules (A), pink eosinophilic material filling lumen of some tubules (B), focal inflammatory infiltrate (C) and renal congestion (D) (Test group 400X).

Discussion:
Nicotine being the active ingredient of tobacco smoke affects almost all the organs of the human body whether directly or indirectly and interferes with functions of body systems. This water soluble alkaloid can be acquired through active as well as passive smoking and is rapidly absorbed through respiratory tract, gastrointestinal tract, skin and mucus membranes. After absorption, nicotine is metabolized to cotinine in liver and thus, resulting into generation of reactive oxygen species and contributing to tissue injury.

Apart from generation of reactive oxygen species, in kidneys the process which is involved in concentration of urine also serves to concentrate metabolites of nicotine into tubular cells resulting into renal toxicity. This accumulation of metabolites further leads to precipitation of intraluminal compounds which induce renal injury.

The histological findings in the rat liver of test group showed distortion of lobular architecture which was in accordance with the findings of Dhouib et al. (2014) whereas in some specimens hepatic cord pattern was preserved which derives its support from the findings of Munir et al. (2015) (11,12).

There was significant heterogeneity in the morphology of hepatocytes and these observations are well documented in various existing literature on effect of nicotine on liver (13-16) The observation made of some of the hepatocytes showing cloudy swelling and some showing vacuolar degeneration derives its support from the observations of existing literature (12,18). Apart from these changes, there was central vein as well as sinusoidal congestion along with dilatation which was in agreement with the results of various studies of past (11,13-17).

Additionally, the liver of the test group rats also showed massive degenerative changes in form of edema, fatty degeneration and lymphocytic infiltration. This supports the findings of some existing literatures which suggest that nicotine administration is associated with massive hepatic fatty degeneration (9,11,18,19).

Furthermore, Gawish et al. (2012) observed all the above mentioned changes in a dose dependent manner along with increase in number of Kupffer cells which also thus, provided the evidence based support in favor of the observations made in the foresaid study (20).

However, Iranloye and Bolarinwa (2009) did not conclude their study with any of the above mentioned findings instead they only observed deposition of adipose tissue close to portal vein and any such kind of finding was not seen in our study. Thus, negating all the observations of the present study conducted (21).

Afore mentioned observations in the liver are attributed to direct, immunological and indirect effects produced due to nicotine exposure. Direct effects are due to metabolism of nicotine into cotinine in liver whereas immunologic and indirect effects are produced by the production of reactive oxygen species which then leads to activation of macrophage monocyte system and lipid per oxidation, thus producing changes in hepatic tissue architecture.

The present study revealed that nicotine exposure to rats caused histological changes in kidneys in 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 (22-24) but 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 and this can be attributed to long time exposure to nicotine (25). Furthermore, Odokuma and Adogbeji (2017) also observed widening of 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 (26).

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) (25,27). 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 (22).

The interstitium showed mild congestion, focal hemorrhage and chronic inflammatory infiltrate in the interstitium these findings were positively favored by the evidences in the previous indexed studies (23, 24, 28, 29).

However, Akomolafe et al. (2017) were not able to appreciate any histological changes in the nicotine treated group and found the kidney cytology to be absolutely normal which in totality is contradictory to the present study (2).

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

Conclusion:

The observations of current study reinforce that nicotine, which is one of the most active chemicals in tobacco smoke that is acquired by active or passive smoking, is highly toxic and nicotine exposure even with small duration even of 5 days, can induce severe
histopathological changes in liver and kidneys. It is also evident from the current study that nicotine not only affects the respiratory organs, as believed earlier, but other organs like liver and kidneys also. Thus, affecting the health status of a given set of population in the form of increased morbidity and mortality. Therefore, it is prudent that efforts should be increased to stop smoking and prevent exposure to nicotine smoke at public places and the persons passively exposed to nicotine smoke should be sensitized about the hazards of nicotine exposure. Furthermore, there should be standardized adoption of protective measures in order to mitigate the risks associated with nicotine exposure.

References: