Original Article

Zoology

Study of Histological Changes in Liver, Lungs and Testes of Dermally Nicotine Treated Albino Mice

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ABSTRACT

Objective: The current study was carried out in order to assess the toxic effects enforced by nicotine on liver, lung, and testes of mice.

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the Animal House of Lahore College for Women University Lahore for a period of thirty days.

Materials and Methods: The initial body weights of mice were obtained. A control group consisting of 20 mice injected with saline solution and an experimental group containing 40 mice treated with 1mg/kg of nicotine subcutaneously were designed. The treatment lasted for 6 weeks after which final body weights were recorded. Later the mice of both groups were slaughtered and their livers, lungs, and testes were taken out and directly preserved in 10% formalin.

Results: A considerable decrease of body weight and food intake was evident in the experimental group and so was observed in the organs weight too. The decrease of body weight and food intake was from 36.89±1.31 to (35.39±1.25) and (119.41±5.76) to (115.01±5.50) respectively. All the three organs also showed a prominent decrease of the weight and resulted in the degeneration and alteration of the histology. Major histological changes in liver were widening and enlargement of sinusoids, necrosis, degeneration of hepatocytes, and fat deposition. Testes had disruptions in the seminiferous tubules and less number of Leydig cells, and experimental lungs showed proliferation of cells, damaged connective tissue network and congestion of lungs.

Conclusion: Nicotine administration to the encountered animals reduces the body weight. Decrease in body weight is considered to be due to reduction in food intake. By examining the liver, lungs, and testes affected by nicotine, it can be figured out that nicotine greatly affects the histoarchitecture of the three organs in several ways. For functional integrity of the organs, extreme and direct exposure to such drugs must be prevented.

Key Words: Liver, Lungs, Testes, Nicotine

INTRODUCTION

Nicotine is the most important component of cigarette. It is considered to be the major part of the tobacco composition [1]. Nicotine is generally considered to be the chief component in smoke of tobacco that is to be blamed for most of the deleterious effects it exerts and also that it plays a role as being developmental neurotoxicant [2].

Nicotine is readily and widely metabolized in the liver, and relatively to a lesser extent in the lungs and kidneys[3] . In the liver of humans and rabbit, the major pathway of nicotine metabolism is the formation of cotinine[4&5]. Histological modifications, and distortions such as degeneration of the hepatocytes, reduction in the population of the germ cells, enlargement of the alveoli, alveolar hemorrhage were observed in the sections of liver, lungs and testes under exposure to smoke extract[6] .It is reported that at clinical level concentration Nicotine caused pathological angiogenesis in humans[7]. In recent times it has been demonstrated that metabolism of nicotine and cotinine is considerably faster in pregnant women as compared to non-pregnant state[8]. Nicotine elevates cognitive presentation and metabolized to cotinine with a Km of 96 μM and Vmax of 56 pmol/min/mg in Zebra finches[9]. Evidences have been shown that the lungs may be affected by the direct interaction of the nicotine with nicotinic receptors that have been expressed in the developing lung[10]. Multiple studies have shown that children whose mothers smoke during the pregnancy have increased lower respiratory illness, increased bronchitis and hospital admissions, altered pulmonary functions, decreased respiratory flow rates, decreased ventilation at functional residual capacity and reduced forced expiratory flow[11].

Nicotine also affects the morphogenesis of the branching of the lungs, that is responsible for the development of the primitive tubules that later becomes the airway free, through the α7 nicotine acetylcholine receptor. These ligand gated ion channels show the most common form of nicotinic acetylcholine receptors

(nAChRs) in the body, they are most commonly found in the lungs, although their concentration is highest in the brain and muscles[12].

Nicotine is responsible for the shrinkage of male reproductive organs. It is also responsible for decrease in the level of testosterone, destruction of sperms, decrease sperm motility, relative infertility and impotence in males[13]. It was found that the administration of nicotine to mouse results in the inhibition of steroid genesis in mouse Leydig cells[14] (Patterson *et al*., 1990). It was reported that the chronic use of nicotine results in decrease in fertilization ability in male animals[15]. The release of luteinizing hormone (LH) in males is found to be inhibited by the administration of nicotine[16]. LH is the most important regulator of the function of Leydig cells without which the production of androgens is not possible[17]. The function of Leydig cells is to secrete testosterone when they are stimulated by luteinizing hormone (LH) and the concentration of testosterone raises almost in direct proportion to the quantity of LH available[18].

The present study was aimed to determine the effects of nicotine administration on the liver, lungs and testes of experimental mice and also to test whether administration of nicotine brings about the same changes on viscera of mice as smoking of cigarette generates in humans.

MATERIALS AND METHODS

Experimental animals: In this study preserved tissue samples of adult mice were used. Sixty five adult male mice of age 30 days and weight ranging from 19-27g were included. At the Animal House of Lahore College for Women University Lahore, these mice were made acclimatized for about a month before experiment. Same maintenance and feeding conditions were provided and a 12-hr light/12-hr dark cycle was maintained throughout the experiment. An ambient temperature of about 30-35ºC and relative humidity of about 65% was given. 5 mice out of 65 were died during the period of acclimatization.

Animal treatment: Food was provided in the form of pallets. All the mice were divided at random into two groups; Group 1- Control: without treatment with nicotine (n=20), and Group 2- Experimental: treated with nicotine (1 mg/kg of body weight) (n=40). The chemical used was 1.0mg/kg nicotine which was to be administered subcutaneously to the mice.

Tissue samples: Body weights of all mice were recorded prior to experiment. All mice were dissected to examine the tissues of lungs, liver, and testes histologically. Tissues were washed in 85% saline solution and subsequently fixed in formalin so as to save them from contamination and also from decomposition.

Tissue processing: The fixed tissues were washed in water and different mixtures of ethanol solution were used to dehydrate them. Tissue specimens were cleared in xylene and were embedded in paraffin wax. At 5 micron thickness the blocks of paraffin were sectioned using rotary microtome. The resulting tissue ribbons were assembled on glass slides and Eosin and Hematoxylin stains were used to stain the slides. Slides were then observed under microscope at different magnifications.

Statistical Analysis: The results were evaluated and given as mean ± S.E.M. In control and experimental groups comparisons were made by applying independent t-test through SPSS version 14.0. The values of $p>0.05$ were found to be statistically significant.

RESULTS

The body weights (gm) of mice decreased significantly from 36.89±1.31 of control group to 35.39± 1.25 of treated group. Food intake was also observed to be decreased significantly in all mice (Fig 1 & 2). Food intake was measured to be decreased from 119.47±5.76 of control to 115.01±5.50 of experimental group

Histopathological examination:

Liver: There were clearly observed degenerated hepatocytes, widening of sinusoids, enlargement of nuclei, fat deposition and necrosis in liver of nicotine treated mice (Fig 3, A & B). These alterations in the experimental group mice reveal the fact that liver of these mice underwent a reduced cellular and functional integrity due to the high dose of nicotine. In addition to histological alterations, the liver weight also declined 5.15±0.04 upto 4.74±0.12 mg.

Lungs: The nicotine treated lungs depicted damaged cells along with large quantity of macrophages, breakage of connective tissues, alveolar wall damage, and abnormally large sized alveoli (Fig 4 C & D). The weight of lungs also reduced from 2.05±0.008 to 1.20±0.004.

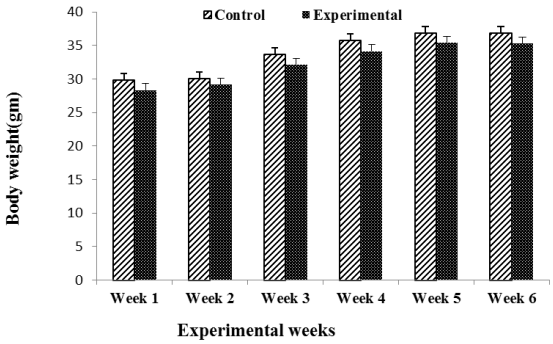

Figure No.1: Comparison of body weight (gm) between control and experimental groups of mice after 6 weeks.

Testes: The histological study of testis showed disarrangement of seminiferous tubules, lesser number

of lydig cells, loss of spermatocytes and spermatids, disruption of spermatogonia, an increase in the thickness of basal lamina in the mice of experimental group (Fig 5, E & F). Besides this a noticeable deficit in weight was observed from 1.01±0.056 to 0.83±0.07 of experimental mice (Fig 6).

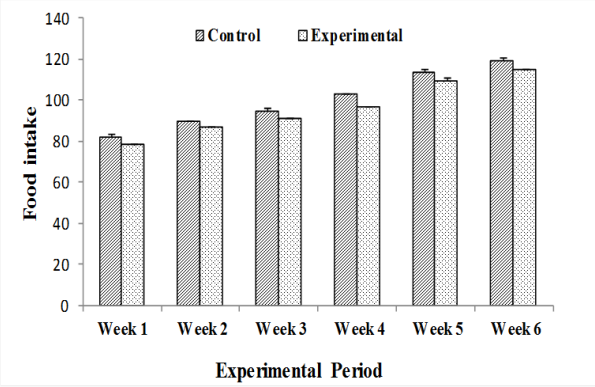

Figure No. 2: Comparison of food intake between control and experimental groups of mice.

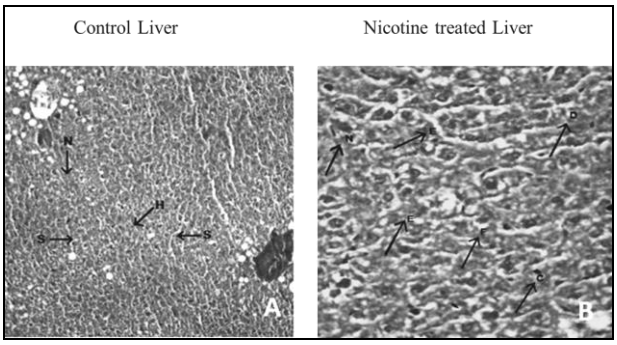

Figure No.3-*A& B*: Microphotograph (A) of Control group liver; H&E (400X). This slide shows regular hepatocytes (H), normal nuclei (N), and sinusoids (S) can be observed in control tissue whereas in Microphotograph (B) of Experimental liver; H&E (400X). Necrosis (N), enlarged nuclei (C), fat deposition (F), and sinusoidal widening (E) can be seen.

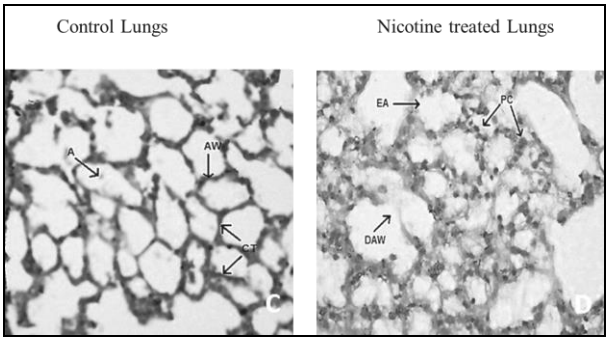

Figure No. 4-*C &D*: Microphotograph (C) of lungs of Control group; H&E (400X). Pulmonary cells with normal alveoli (A), alveolar wall (AW), connective tissue (CT), lumen (L), bronchiolar wall (BW), and peribronchiolar epithelium (PBE) whereas Microphotograph (D) of Experimental lungs; H&E (400X). Treated lungs appearing with accumulation of macrophages (M), damaged alveolar walls (DAW), enlarged alveoli (EA), and proliferated cell (PC).

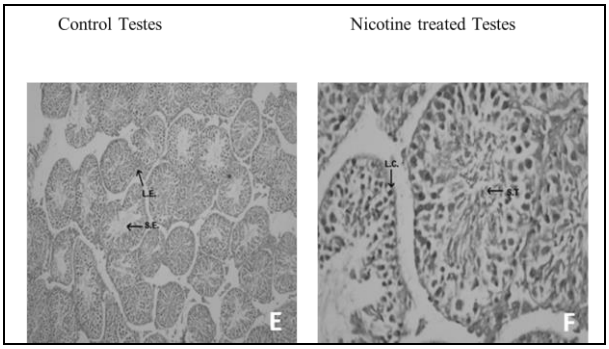

Figure No.5-E & F: Microphotograph (E) of testes of Control group; H&E (400X). Control group testis appears with fine arrangement of seminiferous tubules (S.E), and leydig cells (L.E) whereas Microphotograph (F) of experimental testes; H&E (400X): shows disruption of seminiferous tubules (S.T), loss of Ledyg cells (L.E), and spermatogenesis, and thickening of basal lamina.

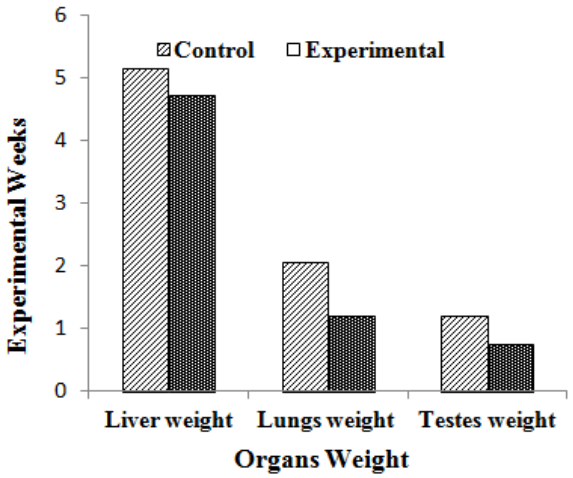

Figure No. 6: Comparison of organs weight between the control and experimental group of mice. The experimental mice were administered nicotine for 6 weeks and liver, lungs and testis were obtained on dissection and weighed. The control animals were dissected without any treatment

DISCUSSION

In the present study the nicotine decreases the body weight in experimental mice that was 35.39 ± 1.25 as compared to the control group animals 36.83 ± 1.31. Decrease in weight was observed to be evident in the experimental animals when they were exposed to nicotine treatment. It was evidenced that the nicotine is the major reason in the weight loss and our study is in agreement to these results[19].

Our results indicate that the alveolar diameter has been increased in the experimental group when exposed to the nicotine dose. In addition to this number of total alveoli per unit volume has been reduced in the experimental group as compared to the control group. Our study findings are similar to the results which

showed that nicotine causes the proliferation of the cells in the bronchial, bronchiolar, and alveolar ducts and the vessels that are associated with this system[20,21].

The stimulation of nicotine causes the production of the lung hypoplasia. The present study results shows that there was a normal size of the alveolar cells along with the normal process of respiration in the control group as compared to the experimental group in which the alveolar size differentiate from one another along with the decrease in the pulmonary capacity for the exchange of gases because of the loss of normal size of alveoli. Our study shows agreement to the work that nicotine decreases the stored pulmonary capacity and increases the respiratory illness in the experimental group as compared to control group mice [22,23,24].

The ability of the animals exposed to the nicotine also undergoes the decrease of the ability of the feeding. The experimental animals that were provided with nicotine dose have the decreased amount of food intake as compared to the control group animals. Present study results speak that the nicotine suppresses the food intake ability of the animals as compared to the other control group animals [25]. Our study is also consistent with the results that the nicotine dose administration decreases the food intake upto 30% [27]. Present study disagrees with the findings that nicotine increases the food intake.

The histopathological alterations in the liver of experimental mice observed in the present study are supported by the findings which reflect a noticed striking tissue disruptive architecture of liver of nicotine treated mice in his experiment in which presence of degenerative vacuoles in the hepatocytes, activation of Kupffer cells, enlargement of nuclei, and variation in the sinuosoid appearance was markedly evident[28].

The results also portrayed a clear picture of the liver of male Wistar rats of control and experimental group showing identical results as ours, in which nicotine was introduced orally for 32 days to the rats and at the end of the experiment the control group represented preserved histoarchitecture of the liver with normal capillaries, undisturbed and composed hepatocytes with no inflammation of the cells. Whereas, loss of hepatoarchitecure profile was most evident in the experimental group representing alteration and degeneration in the hepatocyte structure and appearance of fat globules were also notable [29].

Our results showed the disorganization of seminiferous tubules with the widening of lumen. The present study showed that nicotine treatment reduced the testicular weight with the removal of germ cells from testis. This result was varied to the reported results[30]. The histological rise in the testicular section was seen with the dis-arrangement of spermatogenesis. Our study depicted the same results as reported by others [31, 32.]

It is found that spermatogenesis might be affected by nicotine, smoking in humans affect the testicular spermatogenesis as a result of induced stress. The current study also showed the same results by the disarrangement of seminiferous tubules [33].

In the present study, nicotine treatment reduced the testicular weight with the removal of germ cells from testis. This result was varied to the reported results[30]. The histological rise in the testicular section was seen with the disarrangement of spermatogenesis and are in agreement with the results of others[31,32].

CONCLUSION

Nicotine administration to the encountered animals reduces the body weight. Decrease in body weight is considered to be due to reduction in food intake. By examining the liver, lungs, and testes affected by nicotine, it can be figured out that nicotine greatly affects the histoarchitecture of the three organs in several ways. For functional integrity of the organs, extreme and direct exposure to such drugs must be prevented.

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