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A study on the effect of nicotine on the pancreas of albino rat

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SUMMARY

Nicotine forms the major addictive component of the tobacco smoke. The pancreas is one of the organs where the metabolic processes of tobacco take place. This work was designed to study the effect of nicotine administration and the effect of its withdrawal on the pancreas of albino rat. Twenty-five male albino rats were separated into two groups. Group I acted as control. Rats in group II received 1.5 mg/kg body weight of nicotine by subcutaneous injection day after day divided into two subgroups, each one containing ten rats. The first one received treatment for 4 months, and then the rats were sacrificed, while the second group received treatment for 4 months, and the rats were sacrificed after one month from treatment stoppage. The pancreases were removed and processed for histological examination and electron microscopy. Histopathological and electron microscopic examination of the pancreas of nicotine-treated rats showed degenerated and distorted pancreatic acini and β cells. These changes included pyknotic nucleus, cytoplasmic swelling, vacuolization and interstitial edema in pancreatic acinar cells. Some of the islets of Langerhans showed vaculation inside their cell and others did not show apparent changes. There was also a significant decrease in lipase and glucose levels. However, after withdrawal of nicotine, the pancreas showed more degenerated pancreatic acini and β cells. There was significant increase in blood glucose level and significant decrease in lipase of treated rats.

Nicotine treatment for four months induced histopathological changes in both exocrine and endocrine pancreatic tissue that resemble the picture of chronic pancreatitis. These changes persisted long after cessation of nicotine exposure.

Key words: Tobacco – Nicotine – Smoking – Pancreas – Pancreatic acini and β cells

INTRODUCTION

Throughout the world, smoking is one of the leading causes of preventable death, yet tobacco use is still extremely common. By 2015, tobacco is thought to be responsible for 10% of all deaths worldwide (Jiang *et al.,* 2010). Nicotine is a significant constituent of tobacco and cigarettes, and potentially mediates the development of pancreatic disease (Greer et al., 2015).

When rats were given nicotine, it accumulated in their pancreas and intestine, demonstrating that cigarette toxins could become concentrated in the pancreas (Chowdhury et al., 2002).

Tobacco caused histopathological changes in the pancreas, which could result in exocrine and endocrine dysfunction. Many diseases of pancreas are related to smoking, e.g., pancreatitis, diabetes, gallstone pancreatitis. Nicotine has been proved to promote cell proliferation, angiogenesis and metastasis (Dasgupta et al., 2009). Using electron microscope, nicotine has reported to cause cytoplasmic vacuolization, interstitial cell edema, necrosis and karyolysis (Chowdhury and Rayford, 2000). Also, there is a 70% higher risk of pancreatitis and malignant changes in smokers in comparison to non-smokers (Sliwiñska-Mosson et al*.,* 2012).

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The mechanism of nicotine toxicity is not completely understood. Multiple evidence of involvement of oxidative stress, reactive oxygen species, lipid peroxidation, DNA damage, and beneficial effect of antioxidants (Kovacic and Cooksy, 2005).

MATERIALS AND METHODS

Twenty five adult male albino rats with a weight ranging from 200 to 250 gm were used in this study. They were kept in an environmentally controlled room (22±2C°, 12h light/12h dark cycle) and allowed free access to food and water. All rats received care in accordance with the rules and regulations of the Medical Research Ethics Committee of Mansoura Faculty of Medicine.

Chemicals

The treated element in this experiment was nicotine [(S)-3-(1-Methyl-2-Pyrroli-dinyl) pyridine] (Graham, 1992). Nicotine was supplied as a colourless liquid, purchased from Sigma-Aldrich-Egypt.

Experimental Design

Rats were allowed to acclimatize for two weeks, then they were divided randomly into two main groups, control and experimental as follow:

I -The control group: Formed of five rats. They were injected by 1 ml NaCl (0.9 %) subcutaneously throughout the duration of the study.

II- Nicotine treated group: It contained 20 rats. They were treated with nicotine subcutaneously in a dose of 1.5 mg/kg (Hosseini, 2011). The rats then were divided into 2 subgroups as follow:

Group IIa: It comprised ten rats that were treated every other day for four months then sacrificed immediately after the last dose.

Group IIb: It comprised ten rats that were treated every other day for four months then sacrificed one month after the last dose.

Specimens collection

At the assigned times, the rats were anaesthetized using ether inhalation, blood samples were obtained by direct left ventricle puncture and stored at -20° for serum lipase assessment. The pancreases were dissected out, preserved in 10% buffered formalin and then processed for paraffin sections.

Blood tests

Blood glucose level was determined based on Glucose Oxidase method (Barham and Trinder 1972), and results were reported as mg/dL. Lipase level was measured according to the method of Yang and Biggs (1971).

Histological processing

Pancreases were fixed in 10% buffered formalin, then embedded in paraffin, cut into 3-5 µm thick sections and stained with haematoxylin and eosin.

Transmission Electron Microscopy (TEM)

Pancreases were cut into small pieces (approx. 1 -mm^3 cubes), then fixed in 3% glutaraldehyde (in 0.1M phosphate buffer, pH 7.4) at 4° for 1 h, washed and fixed in 1% osmium tetroxide (in phosphate buffer) at room temperature for 1 h. Then tissue was washed in water, dehydrated in graded ethyl alcohol and embedded in araldite. Ultrathin sections were cut, stained with uranyl acetate and lead citrate and photographed under a Jeol transmission electron microscope (JEM-2100, Japan) at the Electron Microscopy Unit, Tanta University.

Statistical analysis

The study was done using computer software SPSS version 16. Statistical analysis was carried out for serum lipase level of the control and treated animals.

All data were expressed as the mean ± SEM. The data obtained from all the measurements taken were subjected to analysis of variance statistical test, where the significant differences between the groups were indicated. The significance level considered was $p< 0.05$.

RESULTS

I. Hematoxylin and eosin stained sections

Control group:

Sections of pancreas showed pancreatic lobules separated by connective tissue septa. The pancreatic lobules consisted of exocrine acini and showed intralobular duct system. Pancreatic islets were observed as clusters of cells that have lighter stained cytoplasm than the surrounding exocrine cells (Fig. 1a).

Group II a (Nicotine-treated rats for four months):

Sections of the pancreas showed interstitial edema between the pancreatic acini. Some immature cells could be demonstrated. The islets of Langerhans showed cytoplasmic vacuolation (Fig. 1b).

Group II b (Nicotine treated rats for four months then followed by withdrawal for one month):

There was still interstitial edema between the pancreatic acini, which showed also cytoplasmic vaculation. However, some acinar cells appeared binucleated, which might indicate newly divided cells. The islets of Langerhans showed cytoplasmic vacuolation (Fig. 1c).

II. Electron microscopic sections

Control animals:

Each pancreatic acinus was formed of pyramidal shaped cells, connected by junctional complex. The basal area contained nucleus surrounded by extensive rER and Golgi apparatus. The apical portion exhibited well preserved electron dense zymogen granules (Fig. 2a).

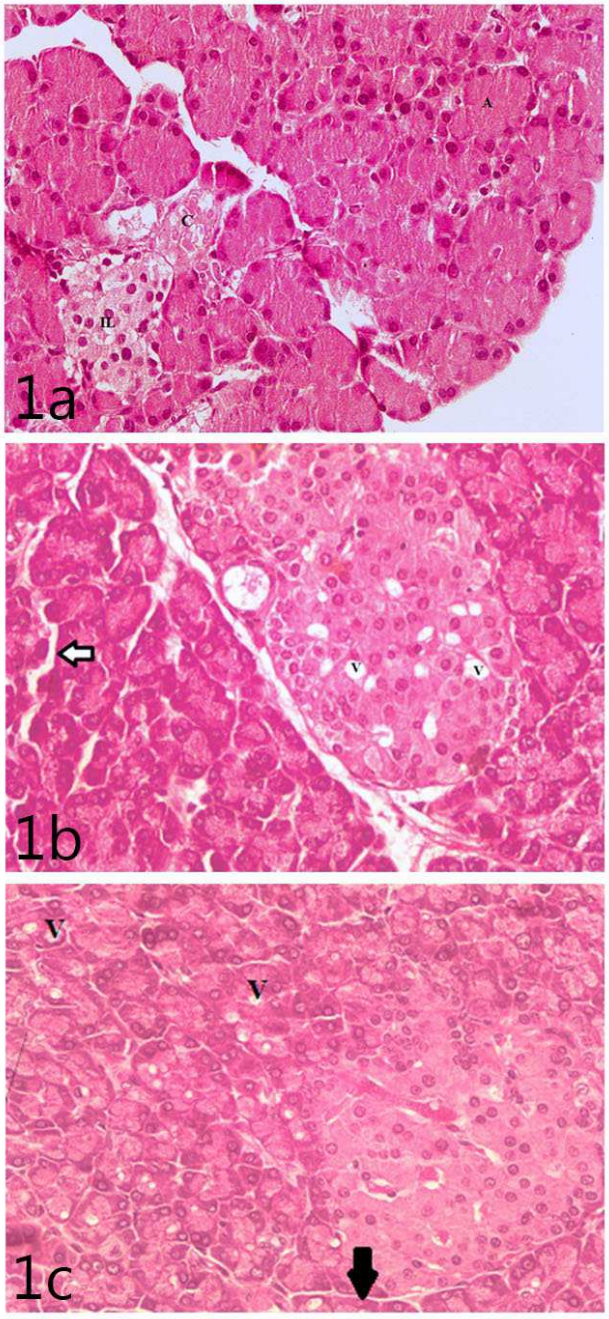

Fig 1. 1a. A photomicrograph of pancreatic section of control rat, showing acinar cells (A) with darkly stained basal nuclei and pink cytoplasm. An islet of Langerhans (IL) with pale cells appears near a capillary bud (C). **1b.** A photomicrograph of a section of the pancreas of nicotine treated rat for 4 months showing, cytoplasmic vacuolation (v) inside islets of Langerhans and interstitial edema (white arrow) in between pancreatic acini. **1c.** A photomicrograph of a section of the pancreas of nicotine treated rats for four months then followed by one month withdrawal, showing interstitial edema (black arrow) and cytoplasmic vacuolation in acinar cells (V). (Haematoxylin & Eosin, x400).

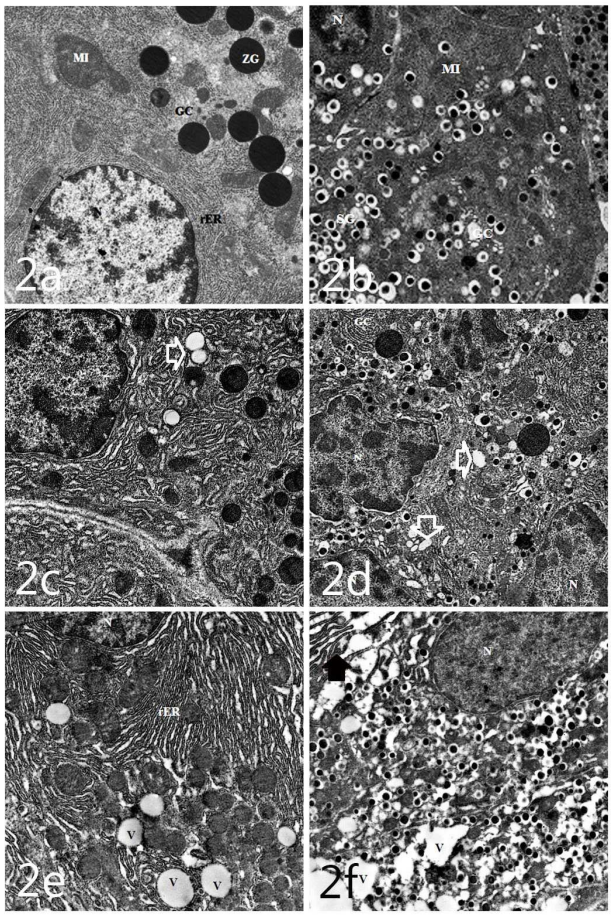

Fig. 2.- Electron micrographs of sections of the pancreas of different groups. **2a.** Pancreatic acinar cell of a control rat showing the nucleus (N) present at the bottom, the apical cytoplasm contains extensive rough endoplasmic reticulum (rER), mitochondria (MI), zymogen-containing secretory granules (ZG), and Golgi complex (GC). **2b.** A β-cell in Langerhans islet of a control rat containing nucleus (N), mitochondria (MI), Golgi complex (GC) and secretory granules (SG). **2c.** a portion of pancreatic acinar cell of a nicotine treated rat for four months having a nucleus (N) with increased heterochromatin and perinuclear space (white arrow). The cytoplasm contains extensive dilated rough endoplasmic reticulum (rER) and a decrease in the electron density of zymogen-containing secretory granules (ZG). **2d.** β-cells of Langerhans islet of a pancreas of nicotine treated rat for four months, their nuclei (N) showing increased heterochromatin and surrounded by perinuclear space. Many secretory vesicles in the cytoplasm appear empty with loss of the electron dense core (arrows). There is dilatation of Golgi complex (GC). **2e.** An acinar cell of the pancreas of a nicotine treated rat for four months then withdrawal for one month showing dilated rER and cytoplasmic vacuolation (v). **2f.** A β-cell of a nicotine treated rat for four months then withdrawal for one month showing intact nucleus (N). The cytoplasm shows increase in empty granules (V) and dilated rER (red arrow). x 3000.

The islet cells are polyhedral in shape and tightly packed, close to a fenestrated capillary. β cell has a vesicular nucleus, secretory vesicles, well developed Golgi complexes and mitochondria, scattered throughout the cytoplasm (Fig. 2b).

Group II a (Nicotine treated rats for four months):

Some pancreatic acinar cells showed pyknotic nuclei with increased heterochromatin and electron -lucent perinuclear space. An extensively dilated rER and a dilated Golgi apparatus were seen around the nucleus. Zymogen granules were distributed all over the cell. Cytoplasmic vacuolations were scattered in the cytoplasm (Fig. 2c).

β cells decreased in size as assumed from their increased number. Some cells showed pyknotic

nuclei with increased heterochromatin and surrounded by electron lucent space. There were a number of empty β cell secretory vesicles with loss of electron-dense part of the granules, so they appeared as cytoplasmic vaculation. Dilated Golgi complexes were also detected (Fig. 2d).

Group II b (Nicotine treated rats for four months followed by withdrawal for one month):

The cells of some pancreatic acini showed pyknotic nuclei with increased heterochromatin and electron-lucent perinuclear space. The rER appeared extensively dilated. The zymogen granules inside the cell showed apparent increase. Cytoplasmic vaculations were observed in some cells (Fig. 2e).

Some of β cells showed normal nuclei, while others had pyknotic nuclei with increased heterochromatin and electron-lucent space around them. There was more increase in the number of empty β cell secretory vesicles with loss of their electron dense part. More dilated rER were noticed (Fig. 2f).

Lipase enzyme level measurement

By using Post Hoc multiple comparisons for observed means, there was a highly significant decrease in lipase enzyme in group II a (nicotine treated for 4 months), and group II b (Nicotine treated for four months then withdrawal for one month) as compared to control group ($P < 0.005$). There was also a significant decrease in Lipase enzyme in group II b, as compared to group II a ($P < 0.05$) (Graph 1).

Serum glucose level measurement

By using Post Hoc multiple comparisons for observed means, there was significant increase in glucose level obtained from group II b (Nicotine treated for four months then withdrawal for one month) as compared to control. However, there was no significant difference in glucose level in group II a (nicotine treated for 4 months) as compared to control one ($P > 0.05$) (Graph 2).

DISCUSSION

The role of nicotine in the induction of pancreatic inflammation and pancreatic cancer as a component of cigarette smoking is now well recognized. The exact mechanism by which nicotine induces such pathologies is not known yet, but it is suggested that they are caused by alterations in cellular, subcellular and/or genetic mechanisms (Chowdhury, 2011).

In this study, histopathological changes were observed in the pancreatic acinar cells of nicotine-treated animals, which are in accordance with those mentioned by **Chowdhury (2003), and** which resemble the histological picture of pancreatitis. Electron microscopic examination used in the present study clarified the changes in the acinar cells, which included pyknotic nuclei with increased heterochromatin and perinuclear space, dilated rER, dilated Golgi apparatus and retention of zymogen granules. Cytoplasmic vacuolations were also present in some cells. Similarly, multiple studies have identified cigarette smoking as an independent and dose-dependent cause for development of both acute pancreatitis (Sadr-Azodi et al., 2012; Yuhara et al., 2014) and chronic pancreatitis (Law et al., 2010; Alsamarrai et al., 2014). Nicotine can induce pancreatic injury partly through oxidative stress (Chowdhury and Walker, 2008).

The acinar cells of the pancreas are the main source of Pancreatitis-associated protein (PAP) in pathological situations. It is a secretory protein that modulates inflammatory response particularly in acute pancreatitis. PAP1 is aberrantly expressed by acinar cells upon cellular stress (Closa et al., 2007). PAP1 is believed to form protein plugs when interacting with active digestive enzymes that seal leaks of small pancreatic ducts in acute pancreatitis. In cigarette smoke-induced pancreatic damage, PAP1 secreted by damaged acinar cells and subsequently protein precipitates form in small pancreatic ducts, interrupting the downstream drainage of the pancreatic juice. This leads to formation of a vicious cycle of reduced drainage that aggravates the acinar cell damage of the affected pancreatic segment (Wittel et al., 2008).

Serum level of lipase enzyme demonstrated a significant decrease in both nicotine treated and withdrawal after four months of nicotine treatment. Multiple experiments have supported the results of the present study as they stated that nicotine could alter the pancreatic secretion manner. Nicotine caused reduction in pancreatic amylase secretion in rats, accompanied by retention of pancreatic zymogens (**Chowdhury et al., 2007**; **Chowdhury and Udupa, 2006**). Azab and Dawood (2012) found that serum lipase level was significantly lower in smokers compared to non-smokers.

In this study, it has been found that some islets of Langerhans showed cytoplasmic vacuolization, while other islets did not show any apparent changes. Electron microscopic examination showed that β cells had decreased in size, had pyknotic nuclei with increased heterochromatin and perinuclear space, increased empty β cell granules, cytoplasmic vacuolization, dilated Golgi complex and dark stained electron dense, mitochondria. The effects of nicotine on the islets have been studied following fetomaternal exposure. The results clearly indicated that nicotine does adversely affect pancreatic mitochondrial and beta cell function by reduction of pancreatic respiratory chain enzyme activity, degranulation of beta cells, elevated islet oxidative stress and impaired glucose-stimulated insulin secretion (Bruin et al., 2008).

Statistical results showed normal level of glucose

in nicotine-treated animals for four months in spite of the presence of pathological changes in some βcells. This can be attributed to increased insulin sensitivity in normal rats as indicated by Xu et al. (2012) through activating a7-nAChR-STAT3 signaling pathway. Vu et al. (2014) determined that nicotine initially provokes hyperglycemia through glycogen breakdown in a catecholamine/NO-dependent manner. Hyperglycemia is then reduced with chronic exposure, due to decreased glucose production. Liu et al. (2003) also showed that long-term oral nicotine administration reduces insulin resistance in obese rats. However, Chowdhury (2003) found that plasma glucose and insulin levels were significantly reduced in nicotine-treated rats for various lengths of time when compared to the control, and attributed that to increased energy utilization.

After withdrawal of nicotine, the glucose level increased above its normal values. In this case, the effect of nicotine on insulin sensitivity was abolished, but its destructive effect on some islet cells persisted in the form of increased numbers of empty β cell granules and pyknotic cells.

It could be concluded from the present study that chronic nicotine intake resulted in pathological changes in the pancreatic acini and islet cells that persisted long after cessation nicotine administration. So, it is advisable to stop smoking as early as possible to avoid long lasting damage of β cells and exocrine pancreatic tissues.

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