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Biochemical and Histological Studies on the Effect of Nicotine on the Mucosa of Albino Rat Stomach.

Ali Ismail Ali Abd Alrheam[1, 2], Madiha MM Makhlouf [1], Heba F Gomaa[3]*, and Ahmed I Abd Elneam[4, 5].

[1]College of Applied Medical Sciences; Medical Laboratories Department - Shaqra University – KSA.
[2]National cancer institute – Cairo University- Egypt.
[3]Zoology Department- Faculty of science- Ain Shams University, Egypt-Biology Dept. Faculty of Arts& Sciences-Scientific Departments.- Saudi Arabia.
[4]Molecular Genetics and Enzymology Dept., Human Genetics Division, National Research Centre, 33 El Bohouth St. (former El Tahrir St.), Dokki , Cairo, Egypt.
[5]Department of Clinical Biochemistry, Faculty of Medicine Al -dawdmi, Shaqra University, Saudi Arabia.

ABSTRACT

The study of the toxicological effect of nicotine is very important to understand the mechanism of tobacco-induced human diseases and to evaluate the potential risk factors associated with the therapeutic use of nicotine as an aid in quitting smoking. There are 15 male albino rats divided into two groups; group I; the control group administered normal saline solution (0.9% NaCl) intraperitoneally; group II the experimental group received for pure nicotine dissolved in saline solution at a dose of 0.5 mg/kg body wt/day for one month and stomach mucosa of fifteen mature male albino rats was investigated after they divided into two groups, Control group, was experimental group, was administered. At the end of the experiment rats were sacrificed and blood was collected, serum was separated and parts of the stomach were homogenated; filtrated, and stored at -80 ^{0}C for biochemical investigations. The levels of prostaglandins PG; Tumor necrosis factor alpha, TNF alpha; malondialdehyde, MDA; Gasterin and Ghrelin in serum and tissue homogenate of the control and experimental group. In addition, Paraffin sections of stomach were prepared and stained with HX&E to investigate the mucosa of stomach in rats of both groups and with stained with PAS for polysaccharides. The sections are examined under the light microscope and photographed. The results showed many histological, histochemical and biochemical changes in the mucosa of the rat stomach. Decrease in the level of PG while the levels of TNF alpha, MDA, Gasterin and Ghrelin were increase in nicotine-treated group compared to the control group.

Keywords: Nicotine – Stomach –prostaglandin - malondialdehyde - Gasterin.

Corresponding author

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INTRODUCTION

The harmful effect of Smoking to the body resulted from the developed risk of many life threatening diseases like lung cancer, heart disease and many cancers and diseases of the GI tract [1]. Many studies showed that about one-fifth of all adults smoke, each year at least 434,000 American men die from diseases caused by smoking. [2]

Many diseases resulted from the use of nicotine related to GI tract like Gastro- esophageal disorders, peptic ulcers (sores on the lining of the stomach or duodenum), Crohn's disease, pancreatitis, colon polyps, and it may cause gall stones. [3]

One of the common causes of peptic ulcers is the infection with *Helicobacter pylori* and use of Non-Steroidal Anti-inflammatory Drugs. Smoking also inhibits factors that heal the lining, such as blood flow to the lining, excretion of mucus , that protects the lining from acid, Production of sodium bicarbonate a salt that neutralizes acid by the pancreas.

Nicotine is the main constituent of cigarette smoke it is known alkaloid extracted from the leaves of the tobacco plant which is a member of family Solanaceae , which includes tobacco, potato, tomato, green pepper, and egg plant. [4].

The route of administration of nicotine is very important factor as it may induce toxicity; as intravenous injection by increasing its concentration in brain and blood [7]. The resulted symptoms from nicotine poisoning include nausea, vomiting, diarrhoea, abdominal pain, headaches dizziness, sweating , confusion, weakness, convulsions, hypotension and coma[7].

Smoking was considered in the earlier studies as an associated with many factors in Peptic ulcer [9, 10]. It has been shown that early smoking resulted in development of duodenal ulcer [11].

Many studies showed more than two fold incidence in existence of peptic ulcer with smokers. In increase of more acid secretion, the high level of serum gastrin, the lower bicarbonate secretion among smokers compared to non-smoker have lower capacity of neutralization of acid secretion for the occurrence of peptic ulcer when H. pylori infection was not known as cause of the disease. Smoking caused peptic ulcer in the duodenal bulb and stomach and the risk is associated with the number of cigarettes and length of smoking.[13-14].It is reported by many scientists that long term smoking inhibits the healing of gastric ulcer[15] and duodenal ulcer of small intestine.[16-17-18] Statistical interpretation of 18 reports confirmed retardation of the healing of ulcers under treatment with anti acid and H2-receptor blocking factors. Boyd EJS et al., (1483) and Kikendall ***et al*** . (1984) found that smoking decreased the capacity of H2-receptor for induced healing of the ulcer [21- 22].

Nicotine controls the immune response by affecting the neurotransmitter serotonin in the blood.

A man who used to smoking is exposed to infections and the diseases, so, it is difficult to know, what type of nicotine response contributes to affect the immune system because the effect of a single substance on smokers cannot be isolated from the effect of other substances founded in cigarette, and their interactions. there is evidence that nicotine may reduces the production of cytokines. [23]

The absorption of nicotine may occur through the skin, oral cavity, lung, , stomach , gastrointestinal tract and urinary bladder [24] ,[25].

Gastrin is polypeptide gastric hormone produced from stomach G cells and TG cells that present in the duodenum, the hormone play central role in acid secretion. [26]. Ghrelin is peptide hormone consists of 28 amino-acid, the hormone produced by A-like cells in the parietal mucosa and involved in stimulate gastric motility and acid secretion [27]

Prostaglandin E2 is one of the main factors that involves in the mechanism of gastric mucosal defence, it regulates gastric blood flow and maintain the level of mucus in addition to bicarbonate secretion.

Prostaglandin play a role in controlling the epithelial cell proliferation and regulation of mucose immunocyte function, and acid secretion [28].

Tumor necrosis factor is a cell signaling protein (cytokine) involved in systemic inflammation and is one of the cytokines that make up the acute phase reaction. It is produced chiefly by activated macrophages, although it can be produced by many other cell types such as CD4+ lymphocytes, NK cells, neutrophils, mast cells, eosinophils, and neurons [29].

Malondialdehyde (MDA) is the organic compound and is a marker for oxidative stress. Malondialdehyde results from lipid peroxidation of polyunsaturated fatty acids [30] .

This study was done to identify the effect of nicotine on the mucosa of the stomach by using laboratory animals to determine properly the risk of nicotine and assessment the need to offer health education to population in order to avoid the problems which caused by nicotine.

MATERIALS AND METHODS

This present study was carried out in The College of Medical Applied Science – Al-Dawadmi (Shaqra University), to record the effect of the nicotine on the stomach mucosa of the albino rat. Fifteen mature male albino rats were used. They were maintained in individual wire cages, given limited food and water. Rats were reared in the animal house, College of Applied Medical Sciences – Al- Dawadmi. The animals had average weight of 150 - 200 gm (Three months old) and were divided into two groups.

Group I: served as control group, was administered normal saline (0.9% NaCl 2) solution intraperitonealy for one month.
Group II: served as the experimental group, was administered pure nicotine dissolved in saline solution at a dose of 0.5 mg/kg body wt/day for one month.

At the end of experiment the animals were sacrificed and blood was collected and serum was separated into clean tubes used for biochemical measurements.

Samples from stomach were removed and fixed in 10 % neutral formalin, processed for light microscopy to get (5µm) paraffin sections and stained with :-

1- Haematoxylin and eosin (H & E) to verify histological details.
2- PAS reaction for polysaccharides.

Biochemical Measurements

Blood samples were allowed to clot at room temperature for 0.5 h before being centrifuged at 1000 g for 20 min. Serum and tissue homogenates were separated and stored at −80 0C until measurement of PGE2 levels and TNF-alpha using enzyme-linked immunosorbent assay (R&D Systems, Inc., Minneapolis, MN, USA).

The concentrations of gastrin and ghrelin measured in serum using commercially available ELISA kits (Ray Biotech, Inc., GA, USA). Each assay was performed in duplicate according to the manufacturer's instructions.

Lipid peroxidation was evaluated through the MDA level in the rat serum and tissue according to the manufacturer's instructions using a Cayman's TBARS Assay Kit.

RESULTS

Control group: (group I)

Examination of (H & E) stained sections of control rat stomach revealed that the wall of the stomach was formed of mucosa, submucosa , muscularis externa and serosa. The mucosa was consists of surface

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epithelium which is a mucous secreting cells, gastric glands which lie perpendicular to the surface epithelium and a layer of smooth muscle (muscularis mucosa) (Figure 1). The mucous surface cells were columnar cells with pale cytoplasm. The fundic gland was formed of mucous neck cells, oxyntic cells, peptic cells and argentaffin cells (Figure 2).

Using sections stained with PAS, the surface mucous cells and mucous neck cells showed a strong positive PAS reaction in the form of deep purple granules (Figure 3).

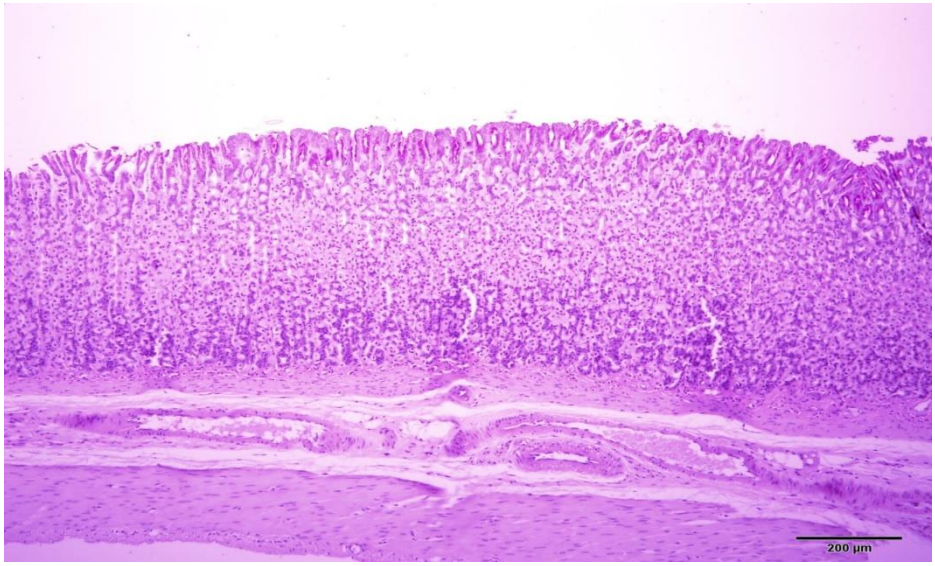

Figure 1: A section in the stomach of the control group stained with H & E showing; the normal structure of the wall of the stomach. Note: the mucosa, submucosa and the muscularis mucosa. (Hx&E X100)

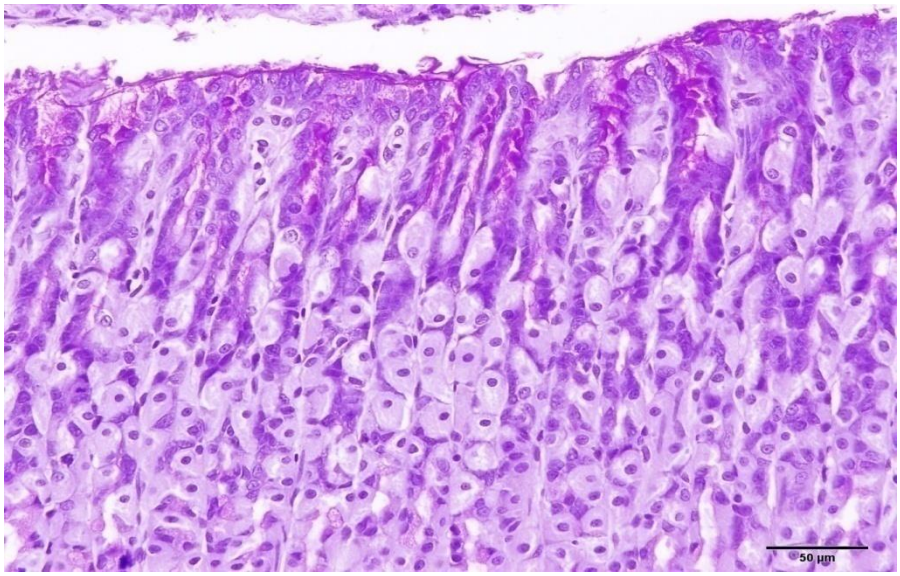

Figure 2: A section in the stomach of the Control group stained with H & E showing the surface epithelium and gastric glands. (Hx&E X400)

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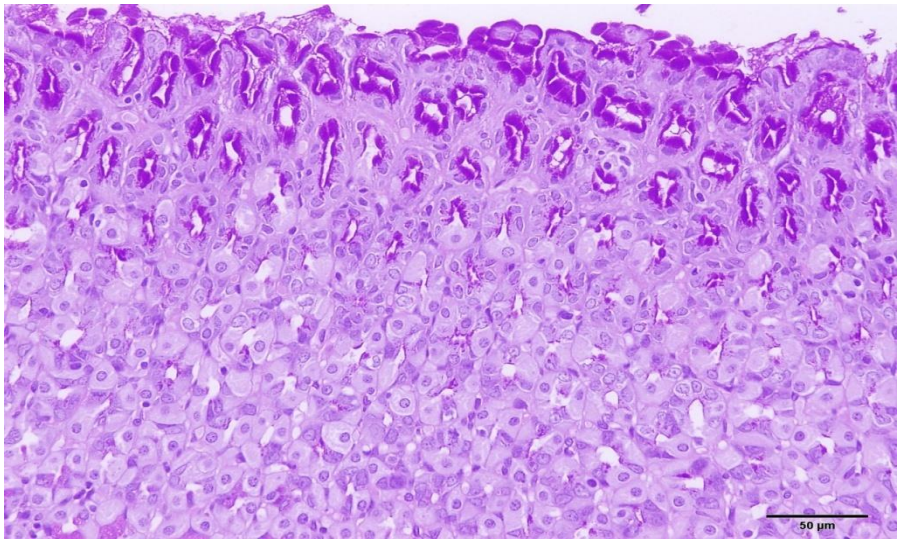

Figure 3: A section in the stomach of the Control group treated with PAS reaction showing ; positive PAS reaction in the surface epithelium and mucous neck cells in the form of deep purple granules .(X400)

Experimental group: (Group II)

Intra peritoneal administration of nicotine for one month led to histological changes in the mucosa of the stomach. Sections stained by HX & E showed sloughing of the surface epithelium in some parts. Other cells showed deeply stained pyknotic nuclei (Figure4). Regarding the PAS reaction for carbohydrates, decrease in the intensity of the positive reaction was observed compared to the control group. The sloughing parts showed negative reaction for PAS (Figure5).

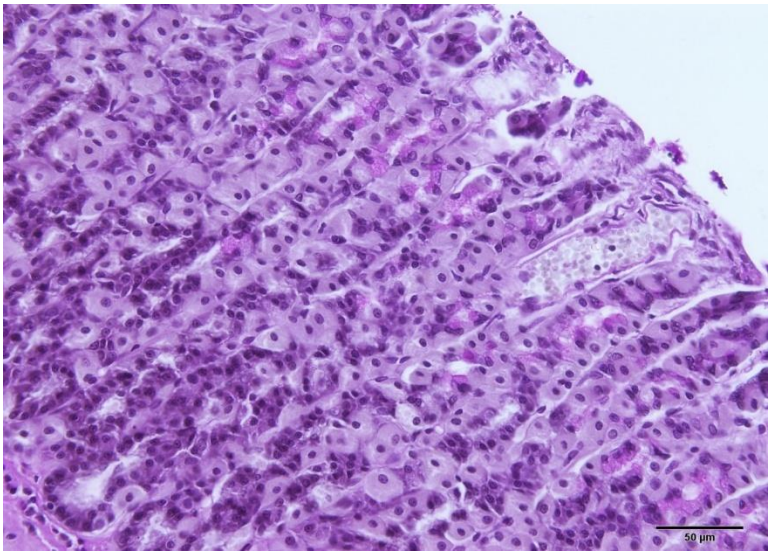

Figure 4: A section in the stomach of the Experimental group stained with Hx &E stain showing sloughing of the surface epithelium in some parts. Other cells showed deeply stained pyknotic nuclei (X400).

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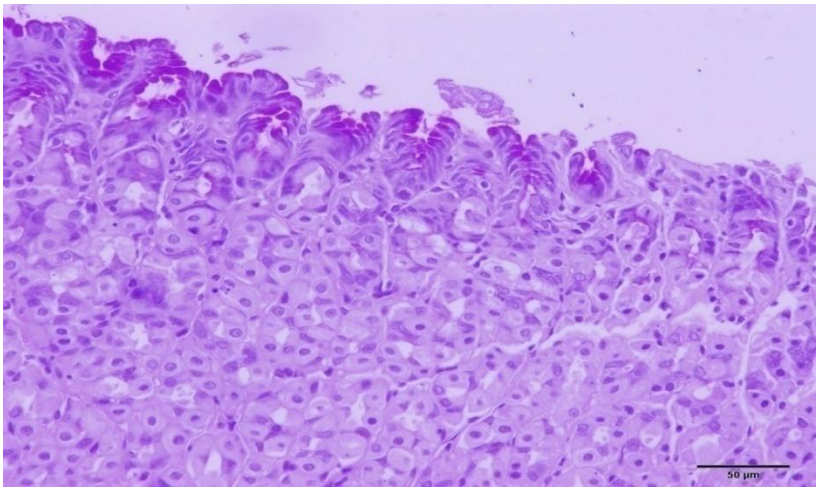

Figure 5: A section in the stomach of the Experimental group treated with PAS reaction showing a decrease in the intensity of PAS positive reaction in the surface epithelium and mucous neck cells. (X 400)

Changes in prostaglandin E2 level

Level of serum PGE2 was significantly decreased in the experimental group, as compared with the control group (279.5±1.54vs. 487.9±2.64 pg/mL, (P > 0.001). There was a marked decreased in tissue homogenate PGE2 level in the experimental group, as compared with the control group (396.3±2.47 vs. 543.6±4.62 pg/mL, P > 0.001). Mean serum and tissue PGE2 levels are shown in [Figure 6] and [Table 1].

Changes in tumor necrosis factor-alpha level

Average concentrations of serum TNF-alpha were markedly increased in the experimental group that administrated nicotine, when compared with the control group (225.3±1.74 vs. 153.8±0.85 pg/mL, P = 0.000). There was a significant decrease in gastric tissue TNF-alpha level in the nicotine group, as compared with the control group (245.3±1.25 vs. 170.6±1.33 pg/mL, P > 0.001). Mean serum TNF-alpha levels for all groups are shown in [Figure 7] and [Table 1].

Changes in malondialdehyde level

The mean level of serum and gastric MDA were significantly higher in the nicotine treated group than in the control group (13.5±0.2.6 vs. 2.89±0.23 nmol/mg protein, and (18.6±1.91 vs. 3.48±0.27 nmol/mg protein respectively, P > 0.001). Mean serum MDA levels for groups are shown in [Figure 8] and [Table 1].

Changes in Gastrin level

The mean Gastrin concentrations in serum samples and gastric homogenates were significantly higher in the nicotine treated group than in the control group (695.1±3.12 vs. 174.5±2.15 pg/mL, and (874.68±4.79 vs. 214.7±2.68 pg/mL respectively P > 0.001). Mean serum Gastrin levels for groups are shown in [Figure 9 and [Table 1].

Changes in Ghrelin level

The mean Ghrelin concentrations in serum samples was significantly higher in the nicotine treated group than in the control group (6.29±0.95 vs. 3.54±0.34 pg/mL, and but it is not much affected the gastric homogenates (8.74±0.35 vs. 7.6±0.41) pg/mL P > 0.001). Mean serum Ghrelin levels for groups are shown in [Figure 10 and [Table 1]

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Table 1: showed the (mean ±SD) serum and tissue homogenate prostaglandine PG; Tumor necrosis factor alpha, TNF alpha; malondialdehyde, MDA; Gasterin and Ghrelin levels in control and experimental group.

Group	Parameters	PGE2 (Pg/ml)	TNF-alpha (Pg/ml)	MDA (n mol/mg protein)	Gastrin (Pg/ml)	Ghrelin (Pg/ml)
Control	Serum	487.9±2.64	153.8±0.85	2.89±0.23	174.5±2.15	3.54±0.34
	Homogenate	543.6±4.62	170.6±1.33	3.48±0.27	214.7±2.68	7.6±0.41
Experimental	Serum	279.5±1.54	225.3±1.74	13.5±0.2.6	695.1±3.12	6.29±0.95
	Homogenate	396.3±2.47	245.3±1.25	18.6±1.91	874.68±4.79	10.74±0.35
P value		P > 0.001	P > 0.001	P > 0.001	P > 0.001	P > 0.001

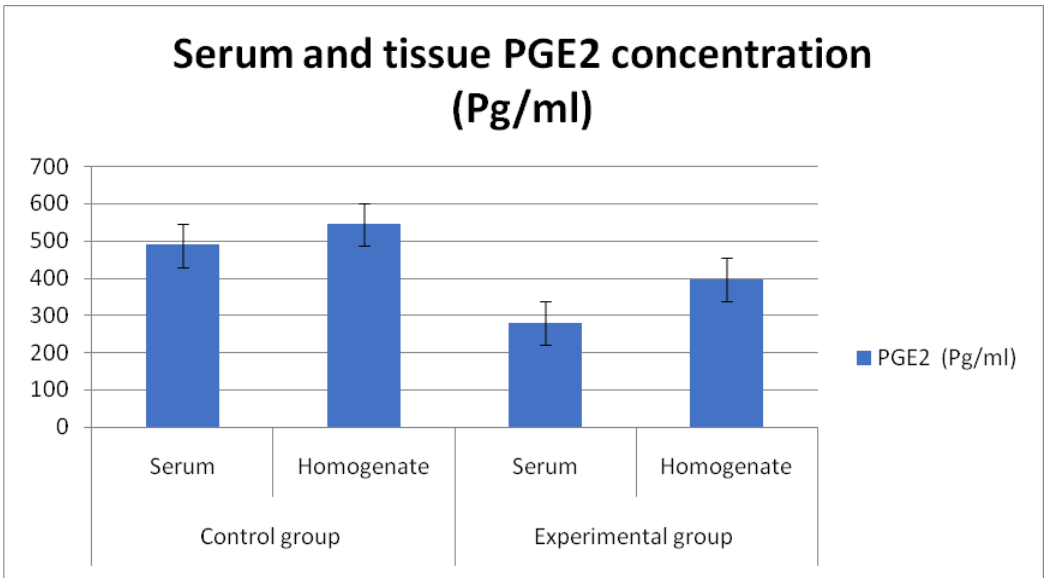

Figure 6: Showed Serum and tissue Changes in PGE2 concentration (Pg/ml) in control and experimental groups.

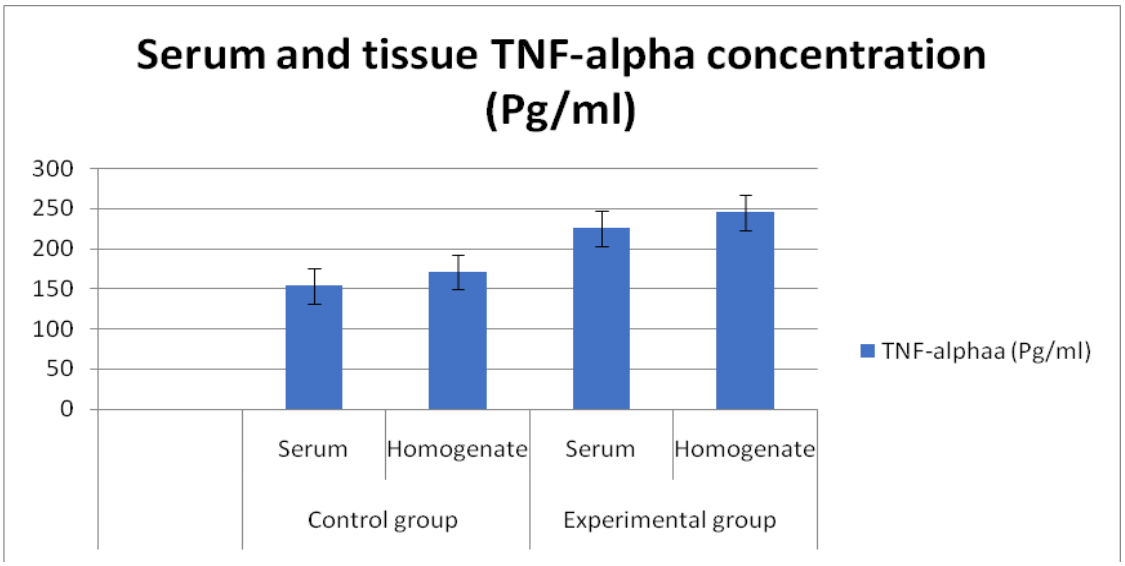

Figure 7: Showed serum and tissue changes in TNF-alpha concentration (Pg/ml) in control and experimental groups.

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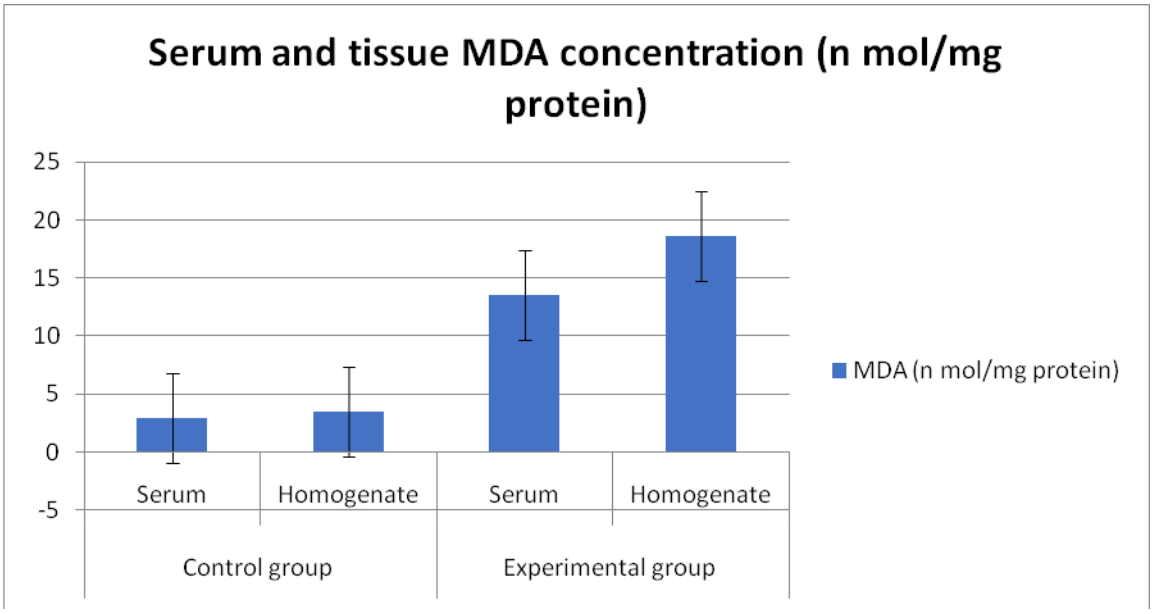

Figure 8: Showed serum and tissue changes in MDA concentration (n mol/mg protein) in control and experimental groups.

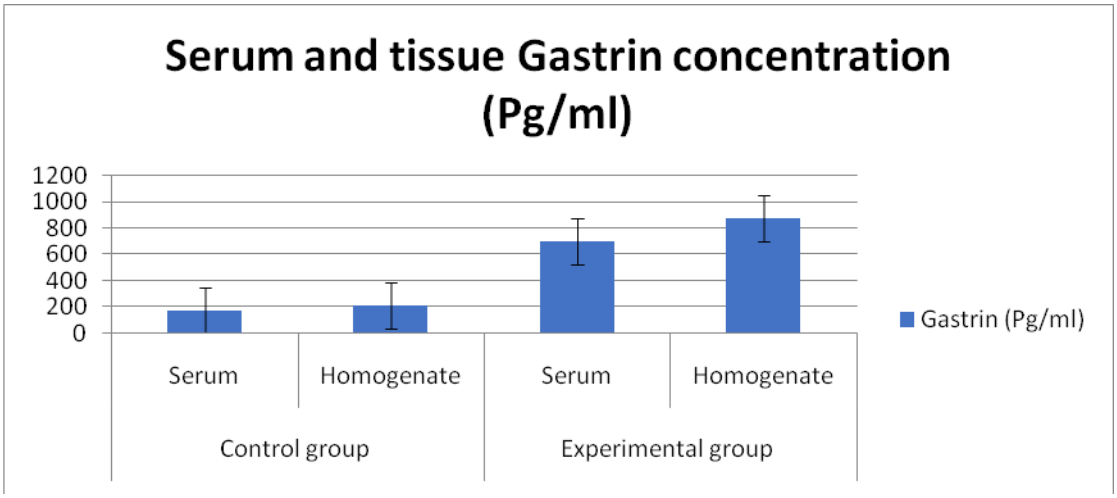

Figure 9: Showed serum and tissue changes in Gastrin concentration (Pg/ml) in control and experimental groups.

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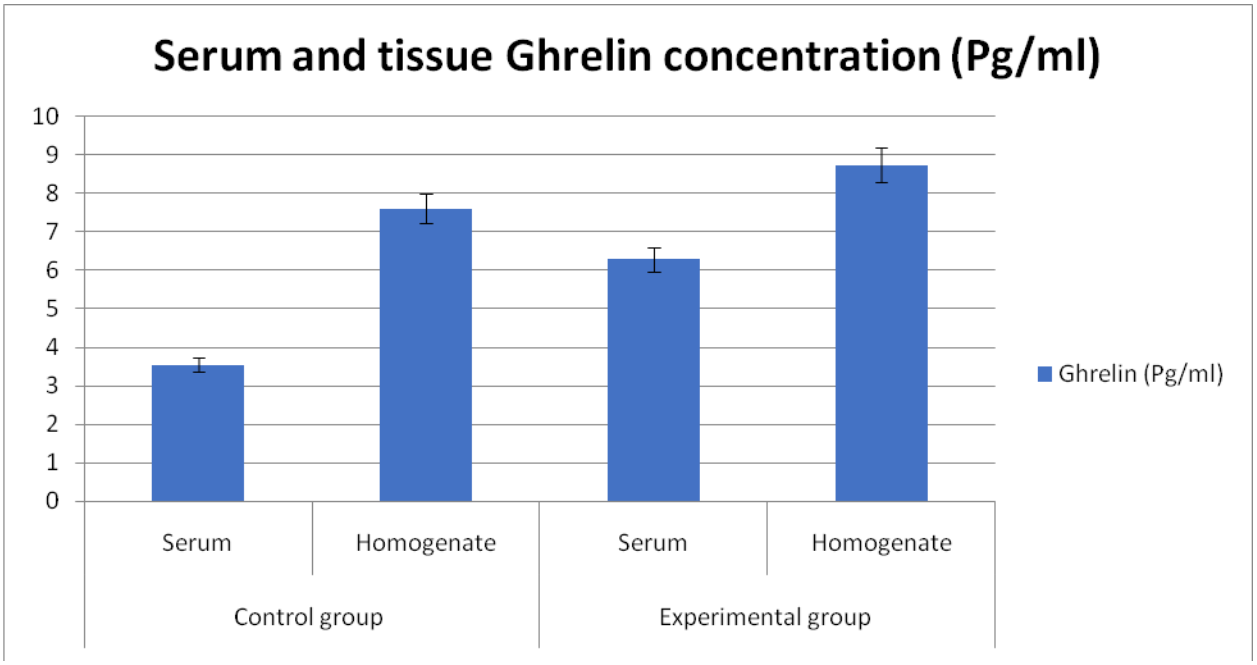

Figure 10: Showed serum and tissue changes in Ghrelin concentration (Pg/ml) in control and experimental groups.

DISCUSSION

Nicotine discovered for the first time in 1828 as it was extracted from tobacco plant and was determined to be the main component of tobacco [4].

Nicotine represents about 95% of the total alkaloids content in commercial smoking products [5] and it is the leading cause of death in the world today with 4.9 million tobacco-related deaths per year [6].

Nicotine can be absorbed and enters the blood through many paths such as the oral cavity, skin, lung, urinary bladder, and gastrointestinal tract [24] and the stomach [25].

In the current study, histological examination by light microscope showed that the administration of pure nicotine induced histological changes in the mucous surface cells (columnar cells) with pale cytoplasm and fundic gland which was formed of mucous neck cells, oxyntic cells, peptic cells and argentaffin cells.

These results are in agreements with the results of others [31] Who reported that nicotine administration via oral and inhalation paths resulted in erosion &ulcer in the gastric mucosa , and it also caused changes to the peptic cells which secrete pepsinogen; the enzyme that digested protein, and increased gastrin hormone in the stomach.

Also nicotine treatment led to depletion of secretion of gastric mucus and decrease in mucosal thickness [32].

Previous studies reported that after 24hrs from administration following nicotine resulted in that spleen, liver, lungs, and brain have high affinity for nicotine, than the adipose tissue [34].

The histological results of group II showed sloughing of the epithelial surface in some parts. Other cells showed deeply stained pyknotic nuclei. In histochemical reaction (PAS) for carbohydrates, decrease in the intensity of the positive reaction was observed compared to the control group.

The present results showed that the histological changes are in line with the biochemical changes at the levels of prostaglandin, Tumour necrosis factor alpha, malondialdehyde, Gasterin and Ghrelin levels in

nicotine-treated group compared with the control group in both serum samples and gastric homogenates [35].

The gastrin mean concentrations in serum samples as well as in gastric homogenates were significantly higher in nicotine-treated group compared with the control group .These indicated that nicotine stimulate gastrin secreted cells within stomach and duodenum. These results are in agreement with the previous studies which reported that reduced serum and gastric levels of Gastrin due to nicotine administration [36].

The results showed that nicotine administration significantly elevated the serum and gastric ghrelin concentration compared with the control group. These findings were supported by other studies as Neugebauer et al. [37]. Another study reported that Ghrelin plasma concentration decreased with smoking cessation [38]

In addition the results suggested that smoking leads to release of intracellular Ghrelin into the blood circulation. It is well known that Ghrelin has many effects on the gastrointestinal tract include regulation of secretion, gastrointestinal motility and also participation in the protective processes against ulcerogenic factors like nicotine. These changes are in line with the histological changes in the epithelial surface.

The average serum concentrations of TNF-alpha were increased in the group II that administrated nicotine compared with the control group. This can be explained by the increase in Ghrelin level that may induce many other anti-inflammatory factors like tumour necrosis factor-α in gastric mucosa that exposed to stress [39].

It was reported by many scientists that over expression of Ghrelin may induce the expression of Gastrin in gastric mucosa leading to gastric cancer and these give pointer absolutes the adverse effect of nicotine administration on gastric cells [40].

The present study also found that PEG2 concentrations in both serum and gastric homogenate were significantly decreased in animals that treated by nicotine compared with the control rats. PGE2 have various physiological mechanisms including cytoprotective effects on gastric mucosa as well as increasing epithelial mucus and bicarbonate secretion, and inhibition of free radical activities and enzymes released from neutrophils. These finding may explain the histopathological changes that nicotine induced it in the gastric mucosa [41].

The administration of nicotine increases the level of tissue MDA (a metabolite of intracellular lipid peroxidase) compared with the control group. MDA is a product of lipid peroxidation that causes cell membrane damage and is also considered as a marker of gastric ulcer due to reactive oxygen species (ROS) formation [42]. The increase of MDA observed in the present study demonstrated that administration of nicotine is able to induce lipid peroxidation and my effect the cellular integrity of the gastric mucosa [43].

CONCLUSION AND RECOMMENDATION

In many countries tobacco smoking is recognized as a serious health hazard and a major contributing factor to deaths from a number of common diseases. Results of the following study revealed that nicotine affected the stomach by changing the histological structure of the stomach. Passive smoking can consider as one of the real significant threat to public health. The first step is the promotion of effective measures protecting from indoor exposure to tobacco smoke at the workplace, in public transport, and other public places. So we recommended that the physicians must increase awareness of people against harmful effect of smoking and passive smoking not only on the cardiovascular and bronchial systems, but also about the detrimental consequences of life-long smoking on the gastrointestinal tract and the increase of its benign and malignant diseases.

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