Effect of Low Frequency Noise on Fundic Mucosa of Adult Male Albino Rats and the Role of Vitamin E Supplementation (Histological and Immunohistochemical Study)

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Abstract

Low frequency noise (LFN) is an environmental problem particularly to sensitive people. Stomach is one of the main targets of noise stress. Our aim was to detect histological and immunohistochemical changes that may occur in fundic mucosa of adult wistar male albino rats exposed to LFN and the role of vitamin E administration. Fifty adult wistar male albino rats were classified into three groups: control group, experimental group subdivided into two subgroups: [LFN exposed and LFN+vit.E] and recovery group. Results: fundic mucosa of LFN exposed group showed shedding of surface epithelial cells, glandular cells with pyknotic and karyolitic nuclei, parietal cells had pyknotic nuclei and vacuolated cytoplasm, Chief cells appeared with pyknotic nuclei and separated basal lamina, mononuclear cell infiltration and thick muscularis mucosa. Highly statistically significant increase in the mean area percent of collagen fibers of fundic mucosa of LFN exposed group as compared to the control group. Highly statistically significant decrease in the mean thickness of mucous film in PAS-alcian blue stained sections of the fundic mucosa was detected in the LFN exposed group as compared to the control group and LFN+vit.E exposed group. Highly statistically significant decrease in the mean optical density of chromogranin-A immunoreaction in LFN exposed group as compared to the control group. Scanning electron microscope (SEM) examination revealed dilated gastric pits and loss of the normal mucus sheet covering the surface mucus cells with some mucous patches. LFN+vit.E group revealed marked improvement. To conclude, LFN induced alteration in fundic mucosa and its mucous barrier. Marked improvement after vitamin E supplementation was detected. So, vitamin E may be beneficial for at risk people.

Keywords: Chromogranin-A, Fundic mucosa, Low frequency noise, SEM, Vitamin E

Abbreviations


Introduction

Noise is one of the main components of modern society that has become an important environmental problem. It is not only an irritating sound but also a stress factor leading to serious health problems. It could affect people both psychologically and physiologically. Exposure to noise has a significant impact on a variety of lab animals and human [1,2].

Noise consists of sounds with broad frequencies. Low-frequency noise (LFN) is continuously generated from many occupational and daily sources including transportation systems, industrial devices, air movement devices as wind turbines, compressors, ventilation and air-conditioning units and household appliances (washing machines, refrigerators and freezers). Thus, we are routinely exposed to LFN generated from different devices [3,4].

LFN is less attenuated by walls and enclosures. Moreover, because of its band width, LFN can spread across large distances with low attenuation, passing through walls and windows and making protection very difficult [5].

There are many considerations regarding noise effects on immune function, hormonal levels, mental illness, sleep rhythm, cardiovascular and respiratory systems [6].

Most of the published medical researches on noise-induced diseases are related to hearing disorders. In the last three decades, non-auditory effects of noise were related to different systems as neurologic, cardiac, vascular, respiratory and gastrointestinal disorders [7].

Gastric mucosa is continuously exposed to harmful factors. Surface epithelium forms a physical barrier between the lumen and the underlying mucosa. An increase in the epithelial cell loss or a decrease in the cell renewal may lead to mucosal damage [8,9]. LFN not only affects cellular structure and organization, but also leads to the development of tissue fibrosis and abnormal collagen fibers proliferation [10].

Several studies suggest the involvement of oxidative stress in the etiology of stress-induced gastric lesions [11]. Increasing plasma concentrations of epinephrine and cortisone suggest that noise induces stress response in rodents [12].

Vitamin E is a powerful antioxidant. It is effective in preventing oxidation of polyunsaturated fatty acids. It is a fat soluble vitamin that is divided into two subgroups; tocopherols and tocotrienols. Tocopherol is the most abundant and active form of vitamin E homologues in vivo. Vitamin E also acts as a scavenger of reactive
Oxygen species (ROS) such as superoxide radical (O$_2^·$), hydroxyl radical (OH), hydrogen peroxide (H$_2$O$_2$), hypochlorous acid and singlet oxygen. It exerts an anti-inflammatory action by inhibiting the production of O$_2^·$ in the activated neutrophils, adhesion of neutrophils to endothelial cells and transendothelial migration of neutrophils [13,14].

Although gastrointestinal complaints are common among individuals exposed to noise, limited literatures were available regarding the gastric morphological alterations of fundic mucosa induced by LFN exposure. Therefore, the goal of the present study was to investigate fundic mucosal lesions in adult male albino rats exposed to LFN. Moreover, to evaluate the possible protective effect of vitamin E supplementation.

Materials and Methods

Animals: Fifty healthy adult Wistar male albino rats (4-6 months) weighing 200-250 gm were used in this study. The animals were obtained from the Animal House, Faculty of Medicine, Zagazig University, Zagazig, Egypt. They were fed standard balanced diet and allowed water ad-libitum. They were housed in hygienic cages in 12 h light/12 h dark cycle at room temperature according to the guidelines for animal research issued by the National Institute of Health and approved by Animal Ethics Committee, Zagazig University, Zagazig, Egypt.

Chemicals: Vitamin E 400 mg soft gelatin capsules were purchased from Pharco Pharmaceuticals, Alexandria, Egypt.

Anti- chromogranin-A antibodies, (Novacastra Laboratories Ltd, UK) were purchased from Sigma office (Egyptian International Center for Import, Cairo, Egypt).

LFN Exposure: Noise generator in the Physics Department, Faculty of Science, Zagazig University, Egypt, produced an amplified and frequency filtered signal was used creating an acoustic environment rich in low frequency components. The generator was put near the position of the exposed rats (Experimental group) [7].

Experimental procedure

The rats were classified into three groups:

**Group 1 (Control group):** included 20 animals kept in a quiet place and classified equally into two subgroups:

- Subgroup 1a: received no treatment.
- Subgroup 1b: received vitamin E by gavage at an oral dose of 60 mg/kg body weight for 5 weeks.

**Group 2 (Experimental group):** comprised 20 animals and classified equally into two subgroups:

- Subgroup 2a (LFN exposed group): rats exposed to LFN 24 hours per day for 5 weeks with band width 200 HZ and amplitude 100 dB [7].
- Subgroup 2b (LFN+vit.E group): rats exposed to LFN for 5 weeks as subgroup 2a after that vitamin E supplementation at an oral dose of 60 mg/kg body weight for 5 weeks [14].

**Group 3 (Recovery group):** included 10 animals, they were exposed to LFN for 5 weeks as in group 2a and after that they kept in a quiet place for 5 weeks [14].

At the end of the experiment, the rats were fasted overnight. They were sacrificed with intraperitoneal injection of pentobarbitone sodium 60 mg/kg body weight [15] and their stomach were dissected out, rinsed and cut along the greater curvature. Specimens from the fundic region were prepared for light and SEM examination.

**Materials and Methods**

**Figure 1:** 1.A Control fundic mucosa showing the fundic glands (arrows) in the lamina propria consists of isthmus (l), neck (N) and base (B). They open into the surface by narrow gastric pits (arrow head). Muscularis mucosa (m) is also seen. 1.B LFN exposed group showing fundic glands (arrows), exfoliation of the surface epithelial cells (arrow head) creping of collagen fibers between the basal parts of the fundic glands (curved arrow) and thick muscularis mucosa (m). 1.C LFN+vit.E group showing the fundic glands are regularly arranged (arrows) and open on the surface by narrow gastric pits (arrow head). Muscularis mucosa (m) is also noticed. 1.D Recovery group showing fundic glands (arrows) open on the surface by gastric pits (arrow head), creping of collagen fibers between the basal parts of the fundic glands (curved arrow) and thick muscularis mucosa are noticed (m). (H and E X 100. Bar, 50 μm).

**Histological study:** Specimens for light microscopic examination were fixed in 10% neutral formol saline, processed for paraffin block preparation, cut into 5 μm sections, and subjected to H and E [16]. Mallory trichrome stain for detection of collagen fibers, PAS-alcian blue histochemical method was used to differentiate neutral mucin from acidic mucin [17].

**Immunohistochemical analysis of chromogranin-A:** Immunohistochemical reaction was carried out using the avidin-biotin complex immunoperoxidase system. Serial sections of paraffin-embedded specimens were deparaffinized on charged slides. The sections were incubated in 0.1% hydrogen peroxide for 30 min to block the endogenous peroxidase and then incubated with the primary antibody. The primary antibody used for chromogranin A was a ready-to-use rabbit polyclonal antibody (CAT-No. RB-9003-R7; Thermoscientific Laboratories, Rockford, IL, USA). The slides were then incubated with the secondary anti-rabbit antibody versusal kits (Zymed laboratories) diluted 1:200 for 30 min. Staining was completed by incubation with a substrate chromogen called diamiobenzidine (DAB). Mayer’s hematoxylin used as a counterstain. For negative
control, the primary antibody was replaced with phosphate buffer solution [18,19].

**Figure 2:** 2.A) Higher magnification of the upper part of the fundic glands of the control group showing the surface mucous cells with basal oval nuclei (double arrow) and the mucous neck cells (arrow). Parietal cells have rounded nuclei and eosinophilic cytoplasm (arrow head). Notice, the narrow gastric pits (asterisk). 2.B) LFN fundic glands are lined by glandular cells with karyolitic nuclei (arrow). Parietal cells with vacuolated cytoplasm and pyknotic nuclei (curved arrow), mononuclear cell infiltration (double arrow) are also seen. 2.B') Shedding of the surface epithelial cells and loss of its normal architecture (arrows), fundic glands are lined by cells with karyolitic nuclei (curved arrow). 2.C) LFN + vit.E group showing the fundic glands are lined by surface epithelial cells (arrow). Parietal cells are apparently normal (curved arrows). Notice, the narrow gastric pits (asterisk). 2.D) Disorganized fundic glands with wide gastric pits (asterisk), surface mucous cells have basal nuclei and foamy cytoplasm (arrow), parietal cells with cytoplasmic remnants (arrow heads) appear in the recovery group. (H and E × 400. Bar, 20 μm).

**SEM examination:** For SEM, the specimens were washed in phosphate buffer saline, fixed at room temperature in an aldehyde mixture made up of 4% formaldehyde, 1.25% glutaraldehyde and 10 nmol/L CaCl₂ in 0.05 mol/L cacodylate buffer. The samples were dehydrated in ethanol and critical point-dried in a Balzer’s apparatus using carbon dioxide as the transitional fluid. The preparations were mounted on metal stubs with conductive carbon paste. The specimens were coated with Au/Pt under vacuum and examined in a JEOL (Japan) JSM 6510 lv] SEM at Electron Microscope Unit, Faculty of Agriculture, Al Mansoura University, Egypt [20].

**Histological results:**

**Histo-morphometrical analysis:** The image analyzer computer system Leica Qwin 500 (Leica Ltd, Cambridge, UK) at the Image Analyzing Unit of the Pathology Department, Faculty of Dentistry, Cairo University, Egypt, was used to evaluate area percentage (%) of collagen fibers the thickness of mucous film and the optical density for chromogranin-A immune reaction. It was measured using the interactive measure menu. Measuring frame of a standard area equal to 118 476.6 mm² was chosen so that collagen fibers, mucous film and the brown positive immune reaction for chromogranin-A could be seen and masked by blue binary colour to be measured. Ten readings from five non-overlapping sections from each rat of all groups were examined.

**Figure 3:** 3.A) Higher magnification of the lower part of the fundic glands of the control group reveals the parietal cells (arrow heads). Chief cells appear low columnar with basal rounded nuclei and basophilic cytoplasm (arrows). Notice the muscularis mucosa (m). 3.B) LFN exposed group shows dilated fundic glands (arrow). Some glands are lined by glandular cells with pyknotic nuclei (curved arrow). 3.B') Parietal cells with pyknotic nuclei and vacuolated cytoplasm (curved arrows). Chief cells have pyknotic nuclei (arrow heads) and separated basal lamina (arrows). Thick muscularis mucosa is also noticed (m). 3.C) In LFN+vit.E group, normal mucous neck cells (double arrows), parietal cells (arrows) and chief cells (arrow heads). Notice the muscularis mucosa (m). 3.D) The recovery group shows Chief cells with pyknotic nuclei (double arrows) and separated basal lamina (arrow). Notice, the muscularis mucosa (m). (H and E × 400. Bar, 20 μm).

**Statistical analysis:** All data were expressed as mean ± SD. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software, version 13.00 (Chicago, Illinois, USA). Statistical significance was determined by one-way analysis of variance for differences between the means of different groups. Further analysis was carried out using the post-hoc test to compare the parameters between the different groups with each other. Probability of P less than 0.05 was considered statistically significant.

**Results**

**Histological results:**

**Group 1 (control group):** Histological examination of subgroups 1a and 1b showed similar histological results. So, we used the negative control subgroup 1a as the control group. H and E stained sections of control albino rats’ fundic mucosa showed the fundic mucosal layers; epithelium, lamina propria containing fundic glands and muscularis mucosa. Fundic glands were formed of isthmus, neck and base regions. They were long, straight, tubular and perpendicular to the surface occupying the whole thickness of the lamina propria and opened into the surface by narrow gastric pits (Figure 1A). LFN exposed group showed exfoliation of the surface epithelial cells, creeping of collagen
fibers between the basal parts of the fundic glands and thick muscularis mucosa. Notice, fundic glands (Figure 1B). Fundic glands of LFN+vit.E group were regularly arranged and opened on the surface by narrow gastric pits. Musculais mucosa was also noticed (Figure 1C). Fundic glands of the recovery group opened on the surface by gastric pits, creeping of collagen fibers between the basal parts of the fundic glands and thick muscularis mucosa were noticed (Figure 1D).

Figure 4: 4.A) Mallory trichrome stained sections of the control group showing very thin collagen fibers (arrow) in the lamina propria of the basal part of fundic mucosa. 4.B) Increased collagen fibers (arrow) distributed in the lamina propria of fundic mucosa in LFN exposed group as compared to control group. 4.C) LFN+vit.E group showing thin collagen fibers (arrow) distributed in the lamina propria of the fundic mucosa. 4.D) Thin collagen fibers (arrow) creeping in the lamina propria between the basal fundic glands in the recovery group. (Mallory trichrome X 100. Bar, 50 μm).

Higher magnification of the upper part of the fundic glands of the control group revealed the surface mucous columnar cells with basal oval nuclei and the mucus neck cells. Parietal cells had rounded nuclei and eosinophilic cytoplasm. Notice, the narrow gastric pits (Figure 2A). LFN fundic glands were lined by glandular cells with karyolitic nuclei. Parietal cells had pyknotic nuclei and vacuolated cytoplasm. Mononuclear cell infiltration was also seen. Other sections of LFN exposed group showed shedding of the superficial epithelial cells and loss of its normal architecture, fundic glands lined by cells with karyolitic nuclei (Figures 2B and 2B'). LFN+vit.E group, fundic glands were lined by surface mucous cells and opened into the surface by narrow gastric pits. Parietal cells had rounded nuclei and eosinophilic cytoplasm (Figure 2C). However, in the recovery group disorganized fundic glands with wide gastric pits were noticed. Surface mucus cells had basal nuclei and foamy cytoplasm. Some parietal cells with cytoplasmic remnants were noticed (Figure 2D).

Higher magnification of the lower part of the fundic glands of control group revealed the parietal cells with central rounded nuclei and eosinophilic cytoplasm. Chief cells appeared low columnar with basal rounded nuclei and basophilic cytoplasm. Musculais mucosa was also noticed (Figure 3A) however LFN-exposed group revealed dilated fundic glands. Some glands were lined by glandular cells with pyknotic nuclei. Parietal cells had pyknotic nuclei and vacuolated cytoplasm. Chief cells appeared with pyknotic nuclei and separated basal lamina. Muscularis mucosa was noticed (Figures 3B and 3B'). LFN+vit.E group showed normal mucous neck cells, parietal cells and chief cells. Musculais mucosa was also noticed (Figure 3C). The recovery group showed chief cells with pyknotic nuclei and separated basal lamina. Musculais mucosa was also noticed (Figure 3D).

PAS-alcian blue stained sections of the control group revealed positive thick mucus film over the surface epithelium extending to fill the gastric pits (arrows). Positive alcian blue reaction in mucous neck cells in the neck region of the fundic glands is also seen (arrow heads). 5.B) LFN exposed group showing negative reaction in the surface epithelium and the mucous neck cells in the neck region of the fundic glands. Notice, no mucous film (arrow). 5.C) In LFN+vit.E group, positive PAS reaction in the mucous film over the surface epithelium extending to fill the gastric pits (arrows). Positive alcian blue stained the mucous neck cells (arrow heads) is detected. 5.D) In the recovery group positive thick mucous film over the surface epithelium extending to fill the gastric pits (arrows) and negative reaction in the mucous neck cells of the neck region (arrow head) of the fundic glands. (PAS-alcian blue X 400. Bar, 20 μm).

PAS-alcian blue stained sections of the control group revealed positive PAS reaction in the thick mucus film over the surface epithelium extending to fill the gastric pits. Positive alcian blue stained the mucous neck cells in the neck region of the fundic glands was also seen (Figure 5A). LFN-exposed group revealed negative reaction in the surface epithelium and the mucous neck cells in the neck region of the fundic glands. No mucous film was noticed (Figure 5B). In LFN+vit.E group, positive PAS reaction was seen in the mucous film over the surface epithelium extending to fill the gastric pits. Positive alcian blue stained the mucous neck cells in the neck region of the fundic glands was also seen (Figure 5C). In the recovery group, positive reaction was
detected in thin mucous film over the surface epithelium extending to fill the gastric pits and negative reaction in the mucous neck cells in the neck region of the fundic glands (Figure 5D).

Immunoperoxidase reaction for chromogranin-A in control group revealed strong positive immunoreaction in chromogranin-A secreting cells cytoplasm (Figure 6A). Faint positive immunoreaction was seen in LFN exposed group (Figure 6B). Strong positive immunoreaction was detected in LFN+vit.E group (Figure 6C). The recovery group revealed positive immunoreaction in chromogranin-A secreting cells cytoplasm (Figure 6D).

SEM of control group revealed polymerized mucous sheet covering the whole surface mucous cells. Globular mucous and narrow gastric pits were also seen (Figure 7A). LFN-exposed group revealed dilated gastric pits, loss of the normal mucous sheet covering the surface mucous cells with some mucous patches and globular mucous form disappeared (Figure 7B). LFN+vit.E group showed polymerized mucous sheet covering most of the surface mucous cells. Globular mucous and narrow gastric pits were also noticed (Figure 7C). Finally, the recovery group revealed some surface mucous cells covered by mucous sheet. Mucous cells were sprouted out from the narrow gastric pits. Mucous patches were also noticed (Figure 7D).

2-Histomorphometrical and statistical results

Highly statistically significant increase in the mean area percent of collagen fibers of the fundic mucosa was detected in the LFN exposed group as compared to the control group and LFN+vit.E exposed group. Highly statistically significant increase in the mean area percent of collagen fibers of the fundic mucosa was detected in the recovery group as compared to the control group. No statistically significant difference between LFN+vit.E exposed group and the control group (Table 1).

Highly statistically significant decrease in the mean thickness of mucous film in PAS-alcian blue stained sections of the fundic mucosa was detected in the LFN exposed group as compared to the control group and LFN+vit.E exposed group. Highly statistically significant decrease in the mean thickness of mucous film in PAS-alcian blue stained sections of the fundic mucosa was detected in the LFN exposed group as compared to the recovery group. Statistically significant difference in the mean thickness of mucous film in PAS-alcian blue of the fundic mucosa was detected in the recovery group as compared to the control group. No statistically significant difference between LFN+vit.E exposed group as compared to the control group and the recovery group (Table 2).

Highly statistically significant decrease in the mean optical density of chromogranin-A immuno reaction the fundic mucosa was detected in the LFN exposed group as compared to the control group and LFN+vit.E exposed group. Statistically significant decrease in the mean optical density of chromogranin-A immuno reaction of the fundic mucosa was detected in the recovery group as compared to the control group. No statistically significant difference between LFN+vit.E exposed group and the control group (Table 3).

Discussion

Examination of fundic mucosa of LFN exposed rats in the present study showed shedding of the surface epithelial cells, glandular cells with pyknotic and karyolitic nuclei, parietal cells had pyknotic nuclei and vacuolated cytoplasm and chief cells appeared with pyknotic nuclei and separated basal lamina.

These findings were in agreement with Fonseca et al. [21] who proved that the proximal gut of LFN-exposed wistar rats suffered from diffuse gastric and duodenal erosions caused by considerable epithelial death. These epithelial cell lesions were similar to lesions previously found in other LFN-exposed tissues as rat pleura and bronchi. They were also similar to ulcerative lesions caused by whole body vibration; vibratory phenomenon comparable to noise [22].

Our findings were also in agreement with Da-Fonseca et al. [7] who detected two striking features in LFN-exposed rats; increased frequency of cell death in the glandular epithelial layer of the distal stomach, by both LM and SEM and erosions observed both macroscopically and by histological examination reflecting cellular degeneration and death.

Moslehi et al. [6] explained gastritis seen in all groups of traffic noise exposed rats via activation of the vagus nerve in the medulla. Ising et al. [23] proved that, noise exposure activates hypothalamic pituitary adrenal axis. Therefore, hypothalamus has an important role in noise pathophysiological effects. They also added that, rats exposed to repeated restraint stress were found to have a higher level of plasma noradrenalin and corticosterone compared to the non-stressed rats.
Acoustic stressors can also impact genes in two ways; by setting off chemical cascades that can lead to DNA damage and/or by altering gene expression. The neural activity required to process environmental noise leads to an increased number of free radicals, which were known to cause carcinogenic mutations [29]. ROS cause damage to DNA, as well as to proteins and lipids. ROS-induced damage was observed in the adrenal glands [30] and hearts [31] of noise-stressed rats. 

Adrenal catecholamines play a physiological role in response to stressful situations. Rats exposed to stress were found to develop gastric lesions associated with reduced brain noradrenal in content and increased plasma catecholamines and corticosterone levels [26]. The elevation in catecholamine levels generates free radicals which are cytotoxic and mediate tissue damage by injuring cellular membranes and releasing intracellular components [27].

According to Shi and Nuttall [28], exposure to noise increased the activity of inducible nitric oxide synthase with excess nitric oxide production which caused oxidative stress, generating an excess of ROS leading to DNA damage.

Acoustic stressors can also impact genes in two ways; by setting off chemical cascades that can lead to DNA damage and/or by altering gene expression. The neural activity required to process environmental noise leads to an increased number of free radicals, which were known to cause carcinogenic mutations [29]. ROS cause damage to DNA, as well as to proteins and lipids. ROS-induced damage was observed in the adrenal glands [30] and hearts [31] of noise-stressed rats.

In the current work, LFN-exposed group revealed also dilated fundic glands, creeping of collagen fibers between the basal parts of the fundic glands, mononuclear cell infiltration and thick muscularis mucosa.

These findings were in agreement with Hill et al. [32]. They stated that, the cellular components of organs particularly fibroblasts, endothelial cells and smooth muscle cells were subjected to a mechanical stress that goes beyond what happens under normal conditions. The transmission of such forces to cells in organs such as blood vessels caused production of growth factors, cytokines or hormones that led to hypertrophic, hyperproliferative or fibrotic responses. LFN increased collagen I and III in the extracellular matrix and induced ultrastructural alterations in the cardiomyocytes, interstitial collagen deposits and changes in mitochondria and intercalated discs of the cardiomyocytes in LFN-exposed animals [10].

In an experiment by Du et al. [33] rats exposed to noise, increased activation of microglial cells and macrophages in the brain and spinal cord that defend the central nervous system against immunological challenges.

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LSD (least significance difference for comparison between groups)

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*Significant (p<0.05)

**Highly Significant (p<0.001)

Table 1: Area percent of collagen fibers in different studied groups.

In the present work, Mallory trichrome stained sections of LFN-exposed rats showed increased collagen fibers distributed in the lamina propria of fundic mucosa. It was also proved statistically by highly significant increase in the mean area percent of collagen fibers of the fundic mucosa in the LFN exposed group as compared to the control group and LFN+vit.E exposed group. These results were in agreement with Inger [34,35] who stated that mechanical forces which applied to individual cells could change cell reactions to biochemical stimuli or even induce entirely different cellular responses. Mechanical forces resulting from tissue vibration may be the initial stimulus for collagen production.

Unlike the fibrotic proliferation in response to an inflammatory stimulus, the tissue exposed to LFN seems to reflect a structural reinforcement in order to assimilate the abnormal vibration stress. This structural reinforcement would be achieved by massive production of collagen [36,37]. In a study done by Fonseca et al. [21], fibrotic changes started early in the first weeks of LFN-exposure. The thickness of the submucosa of 1 week exposed rats was significantly larger than that of control rats. In their study, LFN-exposure was continuous. Consequently, the fibrotic process was constantly increased with
exposure. They found that, in rats exposed for longer periods (9 weeks or 13 weeks) fibrosis was due to collagen IV production and related to the neoangiogenesis process.

In the present study, PAS-alcian blue stained sections of the same group revealed negative reaction in the surface epithelium and the mucous neck cells in the neck region of the fundic glands. SEM revealed dilated gastric pits, loss of the normal mucous sheet covering the surface mucous cells with some mucous patches. Globular mucous form disappeared. These findings were in agreement with Mohamed [38], who attributed these changes to occurrence of damage in the gastric mucosal barriers. The first line of defense in the stomach; which is the mucous, was decreased due to suppressed prostaglandin production and damage of the surface epithelial cells and mucus neck cells.

Decreased mucus secretion allows hydrogen ions and pepsin to diffuse into the mucosa from the lumen. Back diffusion of acid and pepsin into the tissues stimulates further acid and pepsin secretion, decreases mucosal blood flow and decreases gastric motility. The acid also damages connective tissue and submucosal capillaries to cause focal mucosal hemorrhage [39].

In the current study, Faint positive immuno reaction in chromogranin-A secreting cells cytoplasm with highly statistically significant decrease in the mean optical density of chromogranin-A immuno reaction the fundic mucosa of LFN exposed group as compared to the control group and LFN+vit.E exposed group. Chromogranins are acidic glycoproteins that play an active role in hormone and neuropeptide secretion in neuroendocrine cells. Chromogranin-A is the major member of the granin family. It plays multiple roles in the secretory process [40]. Such finding was in agreement with Biswas et al. [41]. They stated that, increased microvascular injury causes ischemia which leads to gastric mucosal cells necrosis. Low oxygen tension and the subsequent depletion of ATP generation, affect sodium-potassium pump leading to influx of sodium into the cell and osmotic gain of water. At the same time, the intracellular calcium increases through influx from the extracellular fluid and its release from intracellular stores. This activates phospholipases, protease and endonucleases which result in cellular damage. Sun et al. [42] also stated that, suppressed gastric mucosal cyclo oxygenase-1 and increased gastric mucosal TNF-α, Fas and Fas ligand level, increased death signal leading to activation of caspase-3 and caspase 8.

Examination of LFN+vitamine E group rats revealed an obvious finding in the structure of fundic glands associated with strong positive PAS reaction in the mucous film over the surface epithelium extending to fill the gastric pits. Thin collagen fibers distributed in the lamina propria and strong positive immuno reaction in chromogranin-A secreting cells cytoplasm were also detected. These findings were in agreement with the study reported by Ohta et al. [13]. Prostaglandin E2, the substance that maintains the gastric mucosal integrity, gastric acidity and gastrin level were found to be decreased in the stomach of rats exposed to water immersion restraint stress (WRS). Treatment with palm vitamin E was able to reverse the detrimental effects of WRS [11]. Vitamin E plays important roles in maintaining the integrity of the gastric mucosa. It had been shown to prevent the increase in stress-induced gastric contractions [43]. This may explain the protective effect of vitamin E in reducing the formation of gastric lesions.

A decrease in gastric mucosal vitamin E level and an increase in gastric mucosal lipid peroxidation were found in ischemia-reperfusion-induced gastric mucosal injury. The severity of the injury was enhanced in vitamin E deficient rats [44]. It was also found that, stress can impair gastric blood flow and cause ischemic-like conditions leading to reperfusion-induced injury and finally development of gastric lesions [26].

In the presence of stress, vitamin E prevented the production of the enzyme xanthine oxidase in the stomach. In consequence, the free radicals formation was prevented. Xanthine oxidase promotes production of free radicals. Involvement of free radicals has been proposed as one of the mechanisms in the development of stress induced gastric ulcers. Free radicals promote lipid peroxidation and this process can be assessed by the production of its stable end product, malondialdehyde [45].

Nur-Azlina et al. [26,46] showed that gastric PGE2 content after 3.5 hours exposure to WRS was significantly suppressed compared to that of the control group. They recorded that, the increased gastric PGE2 content in their study after tocotrienol (TT) administration as a source of vitamin E, could possibly be due to the effect of vitamin E which was reported to stimulate prostaglandin synthesis by activating the calcium-dependent phospholipase enzyme A2 and inhibiting the lipoxynase enzyme. They also stated that, gastroprotective effect of vitamin E was not only due to its antioxidant action but also its inhibitory action on neutrophil infiltration into the gastric mucosa.

Examination of recovery group showed partial improvement in fundic glands structure with positive PAS reaction in the mucous film over the surface epithelium extending to fill the gastric pits and negative reaction in the mucous neck cells in the neck region of the glands. Positive immuno reaction in chromogranin-A secreting cells cytoplasm with significant decrease in the mean optical density of chromogranin-A immuno reaction of the fundic mucosa was detected in the recovery group as compared to the control group. Some authors [46,47] found that the exposure to stress led to ischemia reperfusion, which produced a significant fall in PGE2 generation in the gastric mucosa, but it was gradually restored during mucosal recovery from gastric lesions, suggesting that endogenous prostaglandin is involved in the spontaneous healing of these lesions. This is supported by the

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<tr>
<td>G2b</td>
<td>55.2 ± 11.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>45.5 ± 11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Significant (p<0.05)**

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G2 a</td>
<td>&lt;0.001&quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G2 b</td>
<td>&gt;0.05</td>
<td>&lt;0.001&quot;</td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>&lt;0.01&quot;</td>
<td>&lt;0.001&quot;</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

**Highly Significant (p<0.001)**

**Table 2: Thickness of mucous film in different studied groups.**
fact that PGE2 generation reached higher values during the course of healing of ulcerated gastric mucosa than it did in nonulcerated mucosa.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>45.2 ± 14.2</td>
<td>17.23</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>G2a</td>
<td>15.07 ± 6.1</td>
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</tr>
<tr>
<td>G2b</td>
<td>37.3 ± 5.6</td>
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<td></td>
</tr>
<tr>
<td>G3</td>
<td>20.5 ± 10.8</td>
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<td></td>
</tr>
</tbody>
</table>

LSD for comparison between groups

<table>
<thead>
<tr>
<th></th>
<th>G1</th>
<th>G2 a</th>
<th>G2 b</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2 a</td>
<td>&lt;0.001**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G2 b</td>
<td>0.227</td>
<td>&lt;0.001**</td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>0.029*</td>
<td>0.137</td>
<td>0.037*</td>
</tr>
</tbody>
</table>

*Significant (p<0.05)
**Highly Significant (p<0.001)

Table 3: Optical density of chromogranin-A immunoreaction in different studied groups.

Konturek et al. [48] also showed that, the healing of stress lesions resulted in the restoration of mucosal prostaglandin generation, and this effect was accompanied by overexpression of epithelial growth factor (EGF) and tumor necrosis factor alpha (TNFα) as well as cyclooxygenases COX-1 and COX-2 mRNA and by the increased biosynthesis of gastroprotective prostaglandins.

Collagen fibers in the recovery group were creeping in the lamina propria between the basal fundic glands with highly significant increase in the mean area percent of collagen fibers as compared to the control group. This was explained by Verrecchia and Mauivel [49] who reported that, in fibrosis caused by chronic stimulation, the tissue injury and the attempt of regeneration process were implicated. Also, it was reported that despite the fibrogenesis is a repair mechanism; it tends to imbalance with extensive deposition of the matrix proteins and fibrosis with the chronicity of the stimulus on the tissue [50]. According to Kight and Swaddle [51], animals may habituate to stressors over time. Many neuroendocrine responses to noise are highly plastic; thus, ecological control of noise pollution could allow animals to achieve both structural and functional recovery.

Conclusion

Exposure to LFN has marked effects on fundic mucosa and its mucus barrier. So, it is recommended to meticulously follow up people exposed to LFN to avoid any possible complications. Moreover, marked improvement with vitamin E administration was approved. So, vitamin E is recommended to protect the fundic mucosa against these effects.

References


