A histological study on the effect of noise on the adrenal cortex of adult male guinea pigs and the possible role of combined vitamins (A, C, and E) supplementation
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Introduction
Noise exposure is considered a stressful factor that causes changes in many organs, including the endocrine system. Timing of intervention by antioxidants obviously has a key role in the success of the therapeutic regimen.

Aim of the work
The aim of the study was to investigate the effect of noise exposure on the histological structure of the adrenal cortex in adult male guinea pigs and evaluate the role of postnoise treatment with vitamins A, C, and E

Materials and methods
Thirty adult male guinea pigs were divided equally into three groups. Group I (the control group); group II (the experimental group), which was exposed to noise 4 h/day for 30 days; and group III, which was exposed to noise in the same manner as group II and then administered vitamins A, C, and E once daily for 5 successive days, starting 1 day after noise exposure. Specimens of the adrenal cortex were processed for study by light (H&E stain) and electron microscopy. Morphometric study was also performed.

Results
The adrenal cortex of the noise-exposed group (group II) showed loss of architecture of the zona glomerulosa and fasciculata with cellular infiltration. In addition, zona fasciculata cells showed marked cytoplasmic vacuolation, whereas zona reticularis cells appeared with condensed nuclei and congested blood sinusoids. Ultrastructurally, zona glomerulosa and fasciculata cells revealed swollen mitochondria, dilated cisternae of smooth endoplasmic reticulum, and a few lipid droplets. Animals treated with combined vitamins (group III) revealed restoration of the normal adrenocortical structure, whereas zona reticularis showed increased proliferative activity. Morphometric study revealed a significant increase in the mean thickness of the cortex and surface area of both zona glomerulosa and fasciculata of the exposed animals, whereas group III revealed nonsignificant difference from the control.

Conclusion
Exposure to noise caused histological alterations in the structure of the adrenal cortex. Postnoise treatment with vitamins A, C, and E could markedly reduce these alterations.

Keywords:
adrenal cortex, guinea pig, noise, vitamins A, C, and E
effects of noise exposure on the hypothalamus–pituitary–adrenocortical axis have been performed by measuring behavioral, endocrine, and biochemical variables [5], whereas few studies have investigated the cellular effects induced by exposure to noise stress [6].

Clinically, noise exposure causes changes in the cardiovascular system, where it is responsible for increasing heart rate, peripheral vascular resistance, and blood pressure. These changes may depend on characteristics of the sound, including intensity, frequency, complexity, and duration of sound [7].

The effect of noise exposure on the DNA integrity and ultrastructure of rat cardiomyocytes was previously evaluated. The exposure to loud noise (100 dBA) for 12 h caused a significant increase in DNA damage. Genetic and ultrastructural alterations did not decrease 24 h after the cessation of the stimulus. An elevated oxyradical generation, possibly related to altered sympathetic innervation, is hypothesized as being responsible for the induction and persistence of noise-induced cellular damage [8].

In the cochlea, reactive oxygen species (ROS) levels were found to be significantly higher 1 h after exposure to 110 dB noise [9], persisting after the cessation of the exposure [10]. In this respect, it is worthy to note that DNA is a main target of ROS toxicity [11]. Oxidative damage of DNA is known to induce single-strand breaks and interstrand and intrastrand crosslinks [12]. Van campen et al. [13] reported an elevation of 8-hydroxy-2′-deoxyguanosine in the brain and liver (besides the higher cochlear involvement) of rats exposed to loud noise (120 dB). The association between noise exposure, oxidative processes, and persisting DNA damage deserves further attention because of the long-lasting consequences in term of mutagenic and carcinogenic risk [14,15].

Noise induces free radical formation; there is a nearly fourfold increase in hydroxyl radicals within 1–2 h of noise exposure [10] and a similarly significant early increase in superoxide (O₂⁻) with reaction products evident at 5 min and 2 h after noise exposure [16]. There is also a significant late formation of ROS and RNS occurring 7–10 days after noise exposure [17]. Noise induces lipid peroxidation and peroxynitrite (ONOO⁻) formation 15–30 min after noise exposure [18,19].

It was assumed that pretreatment with a variety of scavengers (including vitamins A, C, and E) reduced the early formation of free radicals, which has been well characterized by Henderson et al. [19] and Ohinata et al. [20].

Unfortunately, any single protective agent will be effective only if it is given for long periods of time before noise exposure. High dose of vitamin C, as an example, did not completely prevent noise-induced hearing loss (NIHL) even with 35 days pretreatment. Its serum level has been found to be stabilized in humans after a minimum of 3 weeks of daily intake [21]. Vitamin E serum levels have also been found to stabilize after over a month of daily intake in human subjects [22]. In addition, authors recommended using Mg with other agents to improve its therapeutic efficiency [23]. Several studies showed that a combination of several agents held greater promise and was more effective than a single agent [24,25]. This was accompanied by confirmation of the safety of combined use of antioxidants in several studies in humans or experimental animals [26,27].

Considering the previous findings, the present work was designed to study the effect of noise exposure on the histological structure of the adrenal cortex in adult male guinea pigs and to evaluate the role of postnoise treatment with combined vitamins A, C, and E.

### Materials and methods

#### Experimental animals

Thirty adult male guinea pigs weighing 500–520 g were used in this study. They were obtained from the Animal House of the Veterinary Medicine, Zagazig University. They were housed in stainless-steel cages and kept under controlled laboratory conditions in a 12-h light/dark cycle and provided with a standard rodent pellet diet and water ad libitum. Animals were allowed to acclimatize to laboratory conditions for 10 days before experimental manipulation.

#### Procedure of noise generation

Noise was produced using two loudspeakers mounted 40 cm apart on opposite sides of the cage (15 W) and was driven by a white noise generator (range 0–26 kHz) installed (suspended) 30 cm above the cage. The noise level was set at an intensity of 100 dB uniformly throughout the cage and monitored by a sound level meter (RAT-M Model RE-120; Ludwigshafen, Germany).

The level of 100 dBA was chosen as it was comparable to the noise frequently detected in most of the pressure horns of trucks and buses running on the roads in cosmopolitan cities of many countries [28].

#### Experimental design

The animals were divided into three groups as follows:

1. **Group I (the control group):** Ten animals were kept under the above-mentioned controlled laboratory conditions, avoiding any influence of noise stress.
   - They were further subdivided into:
     - (a) Group Ia: this group comprised five animals that received no treatment and served as a negative control group.
     - (b) Group Ib: this group comprised five animals that received combined vitamins A, C, and E once daily for five successive days, as mentioned in the drug regimen [24].

2. **Group II (the experimental group that was exposed to noise):** This group comprised 10 animals that were exposed to noise for 4 h a day (from 16:00 to 20:00) for 30 successive days [29].

3. **Group III (the group that received combined vitamins after noise exposure):** This group comprised 10 animals that were exposed to noise in the same way as group II and then received combined vitamins.
A, C, and E once daily for 5 successive days, starting 1 day after noise exposure [24].

Drug regimen
Vitamin A was given at a dose of 2.1 mg/kg/day orally by means of an intragastric tube (β carotene forte, 15 mg capsules, equivalent natural vitamin A 25 000 IU; Medizen Pharmaceutical Industries for Arab Co. for Pharm & Medicinal Plants ‘Mepaco-Medifood’, Enshas, Sharkeya, Egypt).

Vitamin C was given at a dose of 71.4 mg/kg/day intraperitoneally (Cevalor 1000 mg ampoules; Memphis Co. for Pharmaceutical & Chemical Industries, Cairo, Egypt).

Vitamin E was given at a dose of 26 mg/kg/day orally by means of an intragastric tube (vitamin E antioxidant 400 mg capsules; Pharco Pharmaceuticals, Alexandria, Egypt).

Methods
At the end of the experiment, the animals were sacrificed after being anesthetized with thiopentone sodium (40 mg/kg intraperitoneally) [30], and the adrenals were dissected out.

Histological study
(1) For light microscopy: the adrenals of one side were fixed in 10% formol-saline and processed for paraffin blocks. Sections of 5-µ thickness were cut and stained with H&E [31].

(2) For transmission electron microscopy: specimens from the other adrenal gland cortex were immediately fixed in 2.5% glutaraldehyde buffered with 0.1 mol/l PBS at pH 7.4 for 2 h at 4°C and postfixed in 1% osmium tetroxide in the same buffer for 1 h at 4°C, dehydrated, and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate [32] to be examined and photographed using a JEOL-JEM 1010 electron microscope (Jeol Ltd, Tokyo, Japan) in the Histology Department, Faculty of Medicine, Zagazig University, and a JEOL JEM 1200 EXII Electron Microscope (Jeol Ltd) at the Research Laboratory, Faculty of Science, Ain Shams University (Egypt)

Morphometric study
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 the mean thickness of the whole adrenal cortex (µm) and the mean surface area of each zone (µm²). Ten readings from five nonoverlapping sections from each guinea pig of each group were obtained. These measurements were taken using H&E-stained sections at total magnification × 200.

Statistics
Statistical analysis
Data for all groups were expressed as mean ± SD (X ± SD). The data obtained from the image analyzer were subjected to SPSS program version 14 (http://www.spss.com, SPSS Inc., Chicago, Illinois, USA). Statistical analysis using the one-way analysis of variance test was carried out. The results were considered statistically significant, highly significant, and nonsignificant when the P value was less than 0.05, less than 0.001, and greater than 0.05, respectively.

Results
Light microscopic results
Group I (the control group)
Examination of stained sections of the control subgroups (Ia and Ib) showed that both subgroups had the same histological structures. Figures of subgroup Ia were used for comparison with other groups.

Examination of H&E-stained sections of the control group revealed a normal histological architecture for the adrenal cortex.

The cells of zona glomerulosa (ZG) were arranged in the form of rounded or arched clusters beneath the adrenal gland capsule and separated by blood sinusoids. Its cells were closely packed with spherical densely stained nuclei. The cells of zona fasciculata (ZF) were arranged in long straight parallel cords separated by blood sinusoids. These cells were large and polyhedral with pale vacuolated acidophilic cytoplasm and vesicular rounded nuclei. Zona reticularis (ZR) cells were disposed in the form of anastomosing cords. The cells were small, closely packed, and deeply stained. Blood sinusoids were observed between the cords (Fig. 1a and b, Fig. 2a and b).

Group II (the experimental group; the noise-exposed group)
Examination of group II, which was exposed to noise, showed loss of architecture of the ZG and fasciculata with cellular infiltration. Some glomerulosa cells were vacuolated. Most of the ZF cells appeared large and vacuolated, whereas ZR contained many cells with condensed nuclei and congested blood sinusoids (Fig. 3a and b, Fig. 4a and b).

Group III (the group that received combined vitamins after noise exposure)
Examination of different sections from group III revealed that most of the changes observed in group II had decreased. ZG cells revealed pale rounded nuclei with few vacuoles. ZF cells showed more or less a picture similar to that of the control group. Mitotic activity was noticed in some of the ZR cells with an apparent increase in number (Fig. 5a and b, Fig. 6a and b).

Electron microscopic results
Group I (the control group)
Examination of ultrathin sections from the control group revealed a normal ultrastructure for the cells of the three zones of the adrenal cortex. ZG cells showed large rounded or oval euchromatic nuclei with clumps of heterochromatin. The cytoplasm of cells in this area was largely occupied with mitochondria and free ribosomes. Few lipid droplets were present in cells of ZG, together with some electron-dense bodies of lysosomal nature (Fig. 7).

ZF cells contained irregular heterochromatic nuclei and a high content of lipid droplets. The droplets
were abundant, of variable size, and surrounded by mitochondria (Fig. 8).

Cells of the ZR appeared with spherical or ovoid euchromatin nuclei with clumps of heterochromatin. The cytoplasm of ZR cells showed spherical mitochondria, few lipid droplets, and lysosomes (Fig. 9a and b).

**Group II (the experimental group that was exposed to noise)**

The cytoplasm of ZG cells showed swollen mitochondria with disrupted cristae and crystal-like bodies. Few lipid droplets, dilated cisternae of smooth endoplasmic reticulum (SER), and widened perinuclear space were also noticed (Fig. 10a and b). In addition, macrophages with different endocytotic vesicles were seen (Fig. 11).

Cells of ZF showed numerous swollen mitochondria with destroyed cristae, dilated profiles of SER, few electron-dense lipid droplets, and lipofuscin pigments. The nuclear changes included shrinkage with condensation of nuclear chromatin and widening of the perinuclear space (Figs 12 and 13).

Cells of ZR contained free ribosomes, lysosomes, few lipid droplets, mitochondria, multivesicular bodies, and myelin figures scattered in the cytoplasm. Macrophages and interstitial cellular infiltration could be seen (Fig. 14a and b).

**Group III (the group that received combined vitamins after noise exposure)**

Most of the cells of the three zones of the adrenal cortex appeared more or less similar to those of the control.

The surface of ZG cells appeared irregular with microvilli. The cells contained rounded nuclei, numerous lipid droplets, and mitochondria (Fig. 15). ZF cells contained mitochondria, cytoplasmic vacuoles with electron-dense cores, and lipid droplets. Their nuclei appeared rounded and euchromatic with peripheral heterochromatin. Few interstitial macrophages with cytoplasmic extensions were also seen (Fig. 16). Reticularis cells of the same group possessed euchromatic nuclei with clumps of heterochromatin. The reticularis cytoplasm had numerous rounded mitochondria, lysosomes, and lipid droplets (Fig. 17).

**Morphometric and statistical results**

The differences between the results of the studied parameters in subgroups Ia and Ib of the control group were statistically insignificant ($P > 0.05$). Thus, we chose subgroup Ia (negative control) as the control group in the statistical analysis for comparison between control group I, group II, and group III.

In this study, the mean thickness of the whole cortex showed a highly significant increase when group II was compared with group I or group III (Table 1 and Histogram 1).

The mean values of the surface area of the ZG were highly significantly increased, whereas the ZF was significantly increased when group II was compared with group I or group III, whereas the ZR of group II showed no significant difference when compared with group I or III (Table 2 and Histogram 2).
Figure 3. A section showing (a) loss of architecture of the zona glomerulosa (G) and fasciculata cells (F) with cellular infiltration (arrows). Zona fasciculata cells show swelling and vacuolation (v). (b) The zona reticularis (R) and medulla (M) possess congested blood sinusoids (s).
Group II (experimental): H&E, × 200.

Figure 4. A section showing (a) zona glomerulosa cells with small darkly stained nuclei (short arrow) and vacuolation (v) of their cytoplasm. Zona fasciculata show polyhedral cells with numerous vacuoles (long arrow). Notice the congested blood sinusoids (s).
Group II (experimental): H&E, × 400.

Figure 5. A section showing (a) normal structure of the zona glomerulosa (G) and fasciculata (F). (b) Apparent increase in the number of cells of the zona reticularis is noticed (R). The cells contain rounded nuclei (n) separated by blood sinusoids (s).
Group III: H&E, × 200.

Figure 6. A section revealing (a) zona glomerulosa cells (G) with pale rounded nuclei (n) and few vacuoles (arrows). The zona fasciculata (F) appear nearly similar to that of control. (b) Mitotic activity (white arrows) is noticed in some zona reticularis cells (R).
Group III: H&E, × 400.
**Figure 7.** An electron micrograph showing cells of the zona glomerulosa with large rounded euchromatic nuclei (N) and clumps of heterochromatin. Mitochondria (m) and free ribosomes (black long arrow) are numerous and extensively fill the cytoplasm. Few lipid droplets (L) and electron-dense bodies (white long arrow) of lysosomal nature are also seen.

Group I (control), × 3000.

**Figure 8.** An electron micrograph showing cells of the zona fasciculata with irregularly outlined euchromatic nucleus (N). The cytoplasm harbors numerous lipid droplets (L) of different sizes. Mitochondria (M) are also seen.

Group I (control), × 3000.

**Figure 9.** An electron micrograph of cells of the zona reticularis showing (a) abundant mitochondria (m) and few lipid droplets (L). (b) Euchromatic nuclei (N) with patches of heterochromatin and lysosomes (white arrows) are seen.

Group I (control): (a) × 2000, (b) × 4000.

**Figure 10.** An electron micrograph of a zona glomerulosa cell showing (a) numerous swollen mitochondria (w) with disrupted cristae and crystal-like bodies (arrows). Note the nucleus (N) with widened perinuclear space (short arrow). (b) Another zona glomerulosa cell with swollen mitochondria (w), few lipid droplets (L), and dilated cisternae of SER (white arrows) is also seen.

Group II (experimental): (a) × 2000, (b) × 2500.

**Figure 11.** An electron micrograph of a zona glomerulosa cell showing a macrophage (Am) with different endocytotic vesicles (arrows).

Group II (experimental), × 3000.

**Figure 12.** An electron micrograph showing a zona fasciculata cell revealing an irregular nucleus (N) with dilated perinuclear space (arrow) and condensation of the chromatin. Swollen mitochondria (w) with destroyed cristae are also seen.

Group II (experimental), × 3000.
Figure 13. An electron micrograph showing two cells of the zona fasciculata with numerous dilated profiles of smooth endoplasmic reticulum (e). Few electron-dense lipid droplets (L), mitochondria (m), and lipofuscin pigments (l) are seen scattered in the cytoplasm. The upper cell has small irregular heterochromatic nucleus (n), whereas the lower one reveals a large euchromatic nucleus (N) with peripheral heterochromatin.

Group II (experimental), × 4000.

Figure 14. An electron micrograph showing (a) zona reticularis (ZR) containing a macrophage (Am) with different endocytotic vesicles (long arrow). Blood sinusoids (s) and lysosomes (ly) are also seen. (b) Other ZR cells are seen with numerous free ribosomes (white arrow), mitochondria (m), multivesicular bodies (short arrow), and myelin figures (star). Few lipid droplets (L) of variable size and interstitial cellular infiltration (arrow) are also seen.

Group II (experimental): (a) × 4000, (b) × 1200.

Figure 15. An electron micrograph of zona glomerulosa cells displaying rounded nuclei (N), numerous lipid droplets (L), and mitochondria (M). Irregular cell surface (long arrow) with some microvilli (vi) is noticed.

Group III, × 5000.

Figure 16. An electron micrograph showing fasciculata cells containing mitochondria (M), cytoplasmic vacuoles with electron-dense cores (v), and lipid droplets (L). Notice the rounded nucleus (N) and its nuclear envelope (short arrow). An interstitial macrophage (F) with cytoplasmic extensions (p) is also seen.

Group III, × 4000.

Figure 17. An electron micrograph showing a reticularis cell containing a euchromatic nucleus (N) with clumps of heterochromatin (Ht). Numerous rounded mitochondria (M), electron-dense lysosomes (Ly), and lipid droplets (L) are also seen.

Group III, × 1200.
The adrenal gland is an essential stress-responsive organ that is considered a part of both the hypothalamic–pituitary–adrenal axis and the sympatho-adrenomedullary system [33,34]. Acute, or short-term, and chronic stress both have similar effects, such as activation of the hypothalamic–pituitary adrenal axis and release of glucocorticoids, but the changes induced by chronic stress appear to be longer lasting [35–37].

In this study, the mean thickness of the whole cortex showed a highly significant increase when group II was compared with group I or group III. These results were in accordance with those of previous researchers [33] who additionally reported an increase in adrenal weight after chronic variable stress and attributed this increase

### Table 1. Mean thickness of the whole adrenal cortex (µm) in the different studied groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean thickness of the cortex (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>472.84 ± 56.42n</td>
</tr>
<tr>
<td>Group II</td>
<td>591.85 ± 7.13┴</td>
</tr>
<tr>
<td>Group III</td>
<td>483.9 ± 59.93d</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD.

*P<0.001, highly significant compared with group I or III.

*P<0.05, significant compared with group II.

*P>0.05, nonsignificant compared with group III.

### Table 2. Mean surface area of each zone (µm²) of adrenal cortex in the different studied groups

<table>
<thead>
<tr>
<th>Group</th>
<th>ZG</th>
<th>ZF</th>
<th>ZR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>99.88 ± 12.01n</td>
<td>150.52 ± 18.53n</td>
<td>98.57 ± 13.65n</td>
</tr>
<tr>
<td>Group II</td>
<td>163.56 ± 41.52n</td>
<td>193.27 ± 30.54n</td>
<td>119.4 ± 20.32n</td>
</tr>
<tr>
<td>Group III</td>
<td>105.62 ± 32.73n</td>
<td>159.87 ± 36.20d</td>
<td>101.60 ± 20.59n</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD.

ZF, zona fasciculate; ZG, zona glomerulosa; ZR, zona reticularis.

*P<0.001, highly significant compared with group I or III.

*P<0.05, significant compared with group I or III.

*P<0.001, highly significant compared with group II.

*P<0.05, significant compared with group II.

*P>0.05, nonsignificant compared with group II or III.
to both hyperplasia and hypertrophy that occurred in specific adrenal subregions associated with increased maximal corticosterone responses to adrenocorticotropic hormone (ACTH). The hyperplasia occurred in the outer ZF, whereas the hypertrophy occurred in the inner ZF and medulla [33].

Moreover, it had been reported that ACTH itself, or increased ACTH secretion induced by stress, exerted tropic (short-term) and trophic (long-term) effects on the adrenocortical ZF and ZR. Tropic effects have involved an immediate increase in corticosteroid hormone secretion, which appears about 10 min after the beginning of stress and reaches the maximum 15–30 min later. The trophic effect of ACTH involved an increase in adrenal mass and in the steroidogenic capacity of adrenocortical cells [38,39].

Other results suggested that the hypothalamic–pituitary adrenal axis is activated by any type of stress (acute or chronic), but the adrenal gland response varies [40].

In the present work, the ultrastructure of ZG and ZF cells after noise exposure revealed numerous swollen mitochondria and dilated vesicles of SER. These findings could be responsible for the increased vacuolation seen by LM. Similar alterations were previously noticed in the mitochondria of cardiac myocytes and were accompanied by dilution of the mitochondrial matrix and by cristolysis after exposure to loud noise (100 dBA) for 12 h [8]. In addition, at the level of the transmission electron microscopy, the nuclei of ZG and ZF of the same group showed patches of heterochromatin with widening of the perinuclear space. This may be an indication of apoptotic changes [41].

The negative effects of noise on cell structure and function were supposed to be, at least in part, mediated by the increase in ROS [9].

The involvement of ROS might play a causal role in the induction and persistence of genetic damage related to loud noise exposure in extra-auditory organs as well [12].

ROS released after noise exposure causes the peroxidation of membrane phospholipids, which can alter membrane fluidity and lead to loss of cellular integrity. Thereby, the impaired activities of mitochondrial enzymes lead to decreased energy levels [26] resulting in cell degeneration with appearance of cytoplasmic vacuolation, degeneration of the mitochondria, and nuclear changes such as indentation and pyknosis [42].

In the current study, lipid depletion was observed in the cortical zones of noise-exposed adrenals. Lipid droplets contain cholesterol esters, which are the intracellular stores of the precursors of steroid hormone in the rat adrenal. They can be rapidly utilized to meet the needs of the enhanced steroidogenic capacity. Alterations of mitochondria and dilatation of SER would enhance the steroidogenic capacity to compensate for a great demand for adrenocortical hormones, as the enzymes of steroid synthesis are located in these organelles [42].

In the present investigation, the adrenal cortex of experimental animals showed marked sinusoidal congestion, especially in the ZR. This congestion was attributed to an increase in ACTH, which stimulated the formation of prostaglandins leading to congestion [43].

The same result was noticed by other investigators [44] who reported that the intracortical capillaries of the unstimulated adrenal gland were constricted, and after operative stress, or following a 1 h period of ACTH perfusion, they become massively expanded.

In contrast, in rats exposed to acute heat stress, the mean diameter and length of blood vessels in the ZF and ZR regions did not significantly change despite the fact that the ACTH level in heat-stressed rats was very high [45].

In addition, ultrastructural examination of the adrenal cortices of noise-exposed animals in this study revealed macrophages with different endocytotic vesicles in both the ZG and the reticularis. This result was in agreement with those of previous researchers [46] who clarified that some cytokines released by macrophages have been stated to induce adrenocortical steroidogenesis independent from adrenocorticotropic hormones through medullary catecholamines. Thus, macrophages may have a significant role in the immune-adrenocortical communications within the adrenal [46].

The lipofuscin pigment noticed in the present work may be the result of increased cellular oxidative stress. It may also impair both proteosomal function and lysosomal degradation of polyfunctional organelles [47].

Oxidative stress is a state in which significant imbalance between oxidants and antioxidants occurs, leading to damage, dysfunction, or cellular death [48].

An examination of animals of group III, which received combined vitamins after noise exposure, showed that most of the cells of the three zones of the adrenal cortex were more or less similar to those of the control. They showed numerous lipid droplets and many mitochondria.

In contrast, the ZR of the same group showed apparently increased proliferative activity. In agreement with the present work, previous clinical studies in humans and animals have proven that circulating levels of ACTH are positively correlated to adrenal size and that the ACTH chronic hypersecretion induces diffuse bilateral hyperplasia of the adrenal gland [49].

ACTH is known to have a proliferative effect on the adrenal gland. It was demonstrated that its chronic liberation induces extracellular signal-regulated kinase activation, which plays a crucial role in cellular proliferation induction [50].

In addition, the current study showed the presence of interdigitating microvilli between adjacent cells of ZG, which may also be associated with an increased hormonal output supporting the idea of increased cellular synthetic activity [51].
Most of the recent studies have focused on antioxidants in the treatment of NIHL. The antioxidant properties of vitamins A, E, and C have been well known and documented by many studies. β-Carotene, which is metabolized into vitamin A in vivo, has been found to scavenge singlet oxygen radicals. These free oxygen radicals are known to react with lipid to form lipid hydroperoxides; thus, scavenging them with vitamin A prevents lipid peroxidation [24]. Vitamin E is also a well-known fat-soluble antioxidant that reacts with and decreases peroxyl radicals within the cell membrane, inhibiting the propagation of the lipid peroxidation cycle [52,53]. In contrast to vitamins A and E, vitamin C is a water-soluble antioxidant detoxifying free radicals by reducing and scavenging them in the aqueous phase [21].

The present study showed that these vitamins acted in synergy as they significantly inhibited most of the noise-induced histological alterations occurring in the adrenal cortex.

Together, the antioxidant vitamin A used here has scavenged singlet oxygen, whereas vitamin E has reacted with and reduced peroxyl radicals in cell membranes. Vitamin C has detoxified free radicals by reducing them in the aqueous phase. Although there are well-characterized differences in the primary mechanism and site of action of these agents, there is also potential for overlap in their effects [54]. Specifically, antioxidant scavenging of ROS may reduce the vasoconstriction that occurs with ROS production. A combination of these agents clearly attenuated NIHL even when treatment is initiated close to the time of noise exposure (i.e. 1 h before noise) [23].

Timing of intervention by antioxidants obviously has a key role in the success of either protection or therapeutic regimen. A previous study reported that initiation of combined treatment as shortly as 1 h before noise exposure failed to prevent hair cell death [24]. On the other hand, delayed treatment initiated 5 days after noise exposure has not met any mentioned success [17].

Previous investigations documented the presence of late-forming free radicals in NIHL. Several studies reported that noise-induced oxidative stress begins early and becomes substantial with time [10]. This would potentially explain the observations of hair cell death that accelerated with time up to 14 days after noise exposure. It was confirmed that the peak in ROS production in cells of the organ of Corti occurred 7–10 days after noise exposure, and that noise-induced hair cell death is similarly delayed [17].

It could be assumed that pretreatment with a variety of scavengers reduced the early formation of free radicals; this has been well characterized by previous investigators [10,19]. However, the present work could justify that postnoise treatment with antioxidants for 5 more days was more effective than prenoise treatment administered in previous studies [10,19] as daily treatment that continued after noise exposure presumably reduced the late-forming radicals [17].

**Conclusion**

Exposure to noise caused histological alterations in the structure of the adrenal cortex of male guinea pigs. The present work provides morphological evidence that postnoise treatment with combined vitamins could markedly reduce these alterations.

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**Conflicts of interest**

There is no conflict of interest to declare.

**References**

الملخص العربي

دراسة هستولوجية عن تأثير الضوضاء على القشرة الكظرية لذكور الخنازير الغينية البالغة والدور المحتمل للإمداد بفيتامينات (أ و ج و ه) مجتمعة

رانيا أحمد زيدان و هبة محمد النجرس
قسم الهستولوجيا و بيولوجيا الخلوية - كلية الطب البشري - جامعة الزقازيق

المقدمة: يسبب التعرض للضوضاء تغييرات في العديد من الأجهزة بما في ذلك الغدد الصماء، ويشكل توقيت التدخل بالعوامل المضادة للاصابة دورا رئيسيا في نجاح أي نظام علاجي.

الفكرة من العمل: التعرض على التخرج النسيجي للقشرة الكظرية في ذكور الخنازير الغينية البالغة عند التعرض للضوضاء والدور المحتمل لمجموعة من الفيتامينات (أ و ج و ه) عند اخذها بعد التعرض للضوضاء.

المواد والطرق المستخدمة: تم تقسيم ثلاثون من ذكور الخنازير الغينية البالغة إلى ثلاث مجموعات، المجموعة الأولى بوتلي زويا. المجموعة الثانية (المجموعة التجريبية): تعرضت للضوضاء المستمرة لمدة 30 يوما، المجموعة الثالثة: مرتين فيتامينات أ، ج، ج، ه مرة واحدة يوميا، بدأت بعد يوم من التعرض للضوضاء لمدة 5 أيام. تم تجميع عينات من قشرة الغدد الكظرية لعمل دراسة نسيجية بواسطة المجهر الضوئي (الهيماتوكسيلين والأيزين) والمساحة السطحية بمساحة الدهون والمساحة النسبية لكل طبقة.

النتائج: عند فحص المجموعة التجريبية بالمجهر الضوئي ظهرت خلايا المنطقة الكبيبية والمزمنة غير منظمة وظهرت خلايا المنطقة الحزية ملنبة بالغذاء من مختلف الأحماض، مما أدى إلى منطقة الكبيبية على النحوية دموية متغيرة. وظهر نقص واضح في الإزهار الشبقي للمنطقة الكبيبية والمزمنة. وكان من الناحية نفسية وظهر واضح للشبكة الأندية الشبكية ذات الخلايا النسبية ووجود القليل من الحبيبات الدهنية. وظهر نقص المجموعة الثالثة أنها استرجعت تقريبا تركيبها الطبيعي مع زيادة في تجدد خلايا المنطقة الشبكية.

الخلاصة: يسبب التعرض للضوضاء تغييرات هستولوجية في التركيب النسيجي لخلايا القشرة الكظرية و يمكن أن تقل هذه التغيرات بشكل ملحوظ باستخدام مجموعة من الفيتامينات (أ و ج و ه) بعد التعرض للضوضاء.