The Egyptian Journal of Anatomy, June 2020; 43(2): 223-239

Original Article

Influence of Omega-3 Supplementation on Adult Male Rat Adrenal Cortex Exposed to Stress

Hala Z.E. Mohamed and Rasha I. Anwar

Department of Human Anatomy and Embryology, Faculty of Medicine, Assiut University, Egypt

ABSTRACT

Background: Hypothalamus-pituitary- adrenal and sympathetic adreno-medullary axes are the systems mainly implicated in preserving body homeostasis during the stress and the adrenal gland is an important organ to both systems. Stress either subacute or chronic is usually linked with adrenal alterations. Omega-3 fatty acid (O3FAs) which is present in dietary fish oil is known to have beneficial effects on cardiovascular disease, autoimmune diseases and cancer. Also omega-3 fatty acids were mentioned to be considered as anti-apoptotic and anti-oxidant agent.

Aim of the work: Evaluation of the role of Omega-3 supplementation on adrenal cortical cells during exposure to stress.

Material and Methods: 3 groups of adult male albino rats were used (10 rats each): group 1 (control), group 2 (stressed for 6 weeks) and group 3 (stressed + Omega -3-treated; 300 mg/kg Omega-3 plus once daily 5 days/week for 6 weeks). Then the rats were sacrificed and the adrenal glands were extracted and processed for light, electron microscopic, immunohistochemical and morphometric studies.

Results: Group II revealed signs of degeneration in adrenal cortical cells; disturbed arrangement, pyknotic nuclei, vacuolated cytoplasm and destructed organelles. There was reduction in ZF thickness with weak immunoexpression of Bcl-2. Omega-3 induced relative improvement of adrenal cortex in group III. This was evidenced by light, electron microscope and confirmed by morphometric and statistical results.

Conclusion: Omega-3 FA supplementation influences sensitivity to stress and has strong anti-stress effects.

Received: 22 October 2019, **Accepted:** 6 January 2020

Key Words: Adrenal cortex, Omega-3, stress.

Corresponding Author: Rasha I. Anwar, Ph.D, Department of Human Anatomy and Embryology, Faculty of Medicine, Assiut University. **Tel.:** 01008026206, **E-mail:** ribrahem_2000@yahoo.com

The Egyptian Journal of Anatomy, ISSN: 0013-2446, Vol. 43 No. 2

INTRODUCTION

The adrenal cortex is composed of three layers: the zona glomerulosa, the zona fasciculata, and the zona reticularis, which have different structural and functional properties. Zona glomerulosa is responsible for production of aldosterone while, zona fasciculata/ reticularis synthesize cortisol in human, and corticosterone in rodents[1].

The hormones of adrenal cortex play important roles in many physiologic processes which include; electrolyte and fluid balance, cardiovascular homeostasis[2], immune and inflammatory responses, sexual development and reproductive function[3]. They also play a major role in regulating the metabolic pathways in the body, and acute response to stress[4].

Stress can be stated as physical and psychological modifications that alter the homeostasis and the balance of organisms[5]. The organism existence depends on its ability to respond and acclimate to the physical and emotional threats or stressors[6]. Various stress types have been associated with enhanced free radical generation and altered antioxidant enzyme activities[5].

Hypothalamus-pituitary - adrenal and sympathetic adreno-medullary axes are the systems mainly involved in preserving body homeostasis during the stress and the adrenal gland is an important organ to both systems[7]. Both subacute or chronic stress, as major surgery or chronic infections and chronic autoimmune diseases are usually linked with adrenal

DOI: 10.21608/EJANA.2020.18577.1044

alterations[8]. Also it can modify many factors and mechanisms that maintain the body homeostasis. Most of these processes are associated with alterations on the adrenal-pituitary-hypothalamus axis (HPA)[9]. In addition, the gland is subjected to dynamic structural changes including cellular proliferation and death. These two processes must be balanced to guarantee integrity of the adrenal gland[7].

Immobilization is one of the most common performed stresses on animals that has been used and accepted widely for studying the relation between stress and pathophysiological alterations[10].

Dietary oils and fat are known as macronutrients and are considered as a valuable source of energy necessary for the metabolic processes. Furthermore they contain fat –soluble vitamins[11]. Dietary fish oil, which contains omega-3 fatty acids (O3FAs) is known to have beneficial effects on cardiovascular disease[12], autoimmune diseases[13] and cancer[14]. Also omega-3 fatty acids were suggested to be considered as anti-apoptotic and anti-oxidant agents[15].

The benefit of the fish oil is due to the high content of omega-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Mackerel, salmon and sardines are rich sources of EPA and DHA[16]. In animal model the brain physiology can be affected by chronic stress and/or diet, however this correlation is poorly understood[17].

Thus, this study was prepared to assess the possible beneficial effects of concurrent omega-3 fatty acids administration on the adrenal cortex after immobilization induced stress in adult male albino rats.

MATERIAL AND METHODS

Animals:

30 adult male albino rats weighing 200-250 gm were used in this study. Rats were obtained from Animal House, Faculty of Medicine, Assiut University. They were kept under suitable conditions for 1 week for adaptation. They had free access to food and water and maintained under 12/12 hours light/dark cycle with suitable room temperature 23± 5 Cº in accordance with the international guidelines for the care and use of laboratory animals.

Chemicals

 Bcl-2 (B-cell lymphoma 2) was purchased from US Biological Company, United States.

 Omega-3 Plus (SEDICO, 6 October City, Egypt).

Experimental protocol

 The rats were divided randomly into three groups (10 rats each):

 Group I (Control group): The rats in this group were kept undisturbed and served as control group. They were sacrificed at the end of the experiment.

• **Group II (Stressed group):** The rats in this group were subjected to stress as following: rats were placed on a wooden plate with their trunks wrapped for 90 min 5 days/week for 6 weeks. They were able to move their limbs and heads but not their trunks[18].

• **Group III (stressed + Omega -3-treated group):** The rats in this group were exposed to stress as in group II and concurrently given Omega -3. Omega -3 plus was used as gelatinous capsules, each had 1000 mg fish oil; docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) 30%. The capsules were carefully evacuated by using syringes. The content of the capsules was given orally by the use of oro-gastric tube in a dose of 300 mg/kg once daily 5 days/week for 6 weeks[19].

• At the end of the experiment, the animals in the studied groups were sacrificed and the adrenal glands were extracted and processed for light, electron microscopic, immunohistochemical and morphometric studies.

Histological study

For light microscopic study, specimens were immersed into 10% formaldehyde solution then processed and embedded in paraffin. Sections were cut serially (5µm-thickness) to be stained with Hematoxylin and eosin (H&E) and Masson's trichrome stains[20].

For ultrastructural study, small pieces (1mm) of adrenal gland were excised and fixed in 2.5% phosphate buffered gluteraldehyde then processed for semithin sections (0.5-1µm) and stained with toluidine blue. Ultrathin sections (80-90 nm) were cut with ultramicrotome, stained with uranyl acetate and lead citrate[21], and then examined and photographed using the transmission electron microscope (Jeol-JEM-100 CXII Jeol, Tokyo, Japan) in electron microscopic unit, Assiut University.

Immunohistochemical study

Immunohistochemical staining for detection of Bcl-2, the primary antibody used was rabbit polyclonal antibody. Immunohistochemical study was conducted using the avidin–biotin peroxidase method. Paraffin sections were deparaffinized, rehydrated in descending grades of alcohol, and incubated over night with the primary antibody (diluted 1:100) at 4°C. Sections were rinsed three times with phosphate-buffered saline (PBS), then incubated for 1h with peroxidase-conjugated secondary antibody and washed three times with PBS. Visualization of the immunoreactivity was done by using 3,3′-diaminobenzidine hydrogen peroxide as a chromogen. Finally slides were counterstained with Mayer’s hematoxylin. Negative controls were performed by either replacing primary antibody with buffer or non immune serum. The positive control was tonsil. The protein product of Bcl-2 is associated with mitochondria and located on outer mitochondrial membranes, the endoplasmic reticulum, and the nuclear envelope[22]. Immunohistochemical staining was done at Faculty of Medicine, Assiut University.

Morphometric measurements and statistical analysis

Using image J software at Faculty of medicine, Assiut University, the following parameters were measured:

(1) Thickness of zona fasciculata.

(2) Area% of Bcl-2 immunoexpression.

For different subgroups, 10 values were obtained from each slide at 400 magnifications[23].

All measurements were done per area of the view field. Data in all groups were statistically analyzed using SPSS (version13) statistical package. Mann-Whitney test was used and the resulting data expressed as mean ± standard deviation. *P value* was considered significant when it is less than 0.05. P value of less than 0.001 was considered highly significant.

RESULTS

A. Histological results:

I. Light microscopic results:

1- Hematoxylin and Eosin stain (H&E):

The stained sections of group I (control) reveal the normal arrangement of adrenal cortical layers; outer connective tissue capsule, zona glomerulosa (ZG), zona fasciculate (ZF), and zona reticularis. Zona glomerulosa cells (ZG) are arranged in groups with rounded deeply stained nucleus and pale vacuolated cytoplasm. The cells of zona fasciculata(ZF) appear as cords, polyhydral in shape with rounded nucleus and tiny cytoplasmic vacuoles. Blood sinusoids are seen between the cells (Fig. 1). In group II (stressed), the sections of adrenal cortex show that the cells of (ZG) appear with disturbed arrangement, swollen with vacuolated cytoplasm. In addition, (ZF) cells appear swollen with disorganization in arrangement and increased thickness of this layer. Pyknotic nuclei in some cells and karyolytic in others are noticed. Dilated sinusoids are clearly visible (Fig. 2). Stained sections in group III (stressed + Omega -3), the cells of (ZG) are nearly similar to control. Moreover, the cells of (ZF) show improvement in their arrangement. Although; some cells appear with normal nuclei, others still have karyolytic nucleus (Fig. 3).

2- Masson's trichrome stain:

By Masson’s trichrome stain, sections of group I show green colored collagen fibers in the capsule and in between the cords of cells (Fig. 4). While, in group II there is an increase of greenish colored collagen fibers (Fig. 5). The green colored collagen fibers are nearly similar to control in group III (Fig. 6).

3- Immunohistochemical stain:

In group I, strong positive immunoreactivity for Bcl-2 is detected in adrenal cortical cells as brown nuclear reaction (Fig. 7); while it shows weak immunoexpression in group II (Fig. 8). Meanwhile, in group III the immunoexpression is mild in the cells of adrenal cortex (Fig. 9).

4- Toluidine blue stain:

The zona fasciculata cells in group I, appear with rounded vesicular nucleus and one or more nucleoli. Clear vacuolated cytoplasm with lipid droplets and blood sinusoids are seen (Fig. 10). in group II, the cells of zona fasciculata appear destructed and disorganized. The cytoplasm shows foamy appearance and multiple vacuoles. Some cells appear with irregular nuclei while others loss their nuclei (Fig. 11). Zona fasciculata cells in group III restore their arrangement to great extent. The foamy cytoplasm and vacuoles can be still seen in some cells while other cells have dark stained nuclei. Blood sinusoids can be also seen in between the cells (Fig. 12).

II. Ultrastructural results:

Ultra structurally, zona fasciculata cells in group I appear with normal euchromatic nucleus, many rounded mitochondria, lipid droplets as well as smooth endoplasmic reticulum in the cytoplasm (Figs. 13a & b). In group II, the cells show shrunken pyknotic nuclei with irregular nuclear membrane. The cytoplasm has vacuoles, lipid droplets and destructed mitochondria (Figs. 14a & b). Meanwhile in group III there is partial restoration of the normal architecture of zona fasciculata cells. The nucleus preserves its shape to great extent, some mitochondria appear normal, while others are still damaged. Vacuoles and lipid droplets are seen in the cytoplasm (Figs. 15a & b).

B- Morphometric and statistical results:

The mean values of ZF thickness in the studied groups show significant increase in group II (179.20) as compared with group I (159.19). The thickness improves in group III (163.22), but the increase is still significant in comparison to group I (Table 1 & Histogram 1).

Meanwhile, the area% of Bcl-2 immunoexpression shows significant decrease in group II (9.40) when compared to group I (13.34). While the difference is not significant in group III (12.50) comparing to group I (Table 2 & Histogram 2).

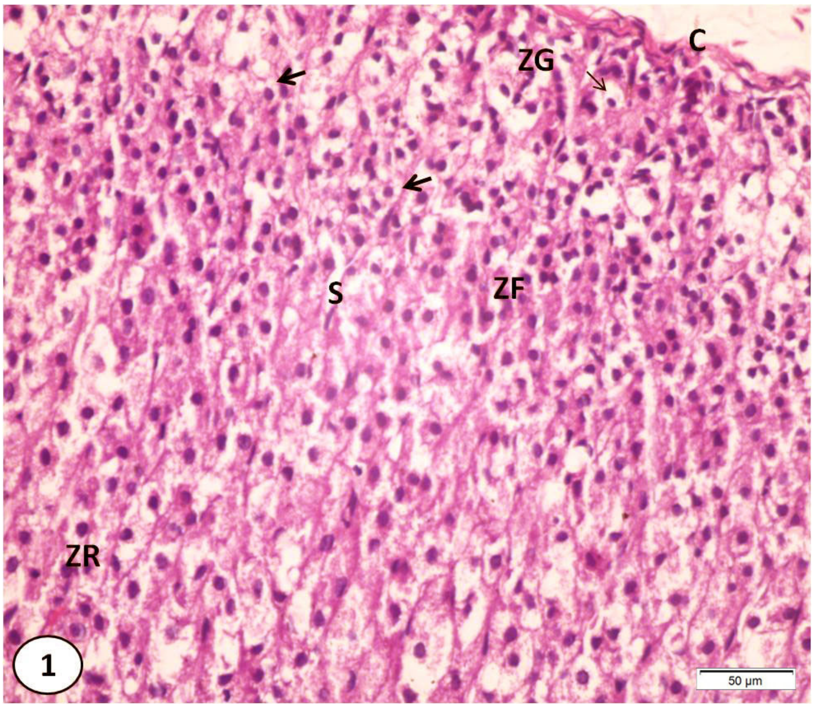


Fig. 1: A photomicrograph of adrenal cortex section of adult rat (group I, control) showing: outer connective tissue capsule (C), zona glomerulosa (ZG), zona fasciculata (ZF), and zona reticularis(ZR). The cells of (ZG) are arranged in groups and appeared large with rounded deeply stained nucleus and pale vacuolated cytoplasm (thin arrow).The cells of (ZF) arranged in cords, polyhydral in shape with rounded nucleus and tiny cytoplasmic vacuoles (thick arrows). Blood sinusoids (S) could be seen between the cells. H&E X 400

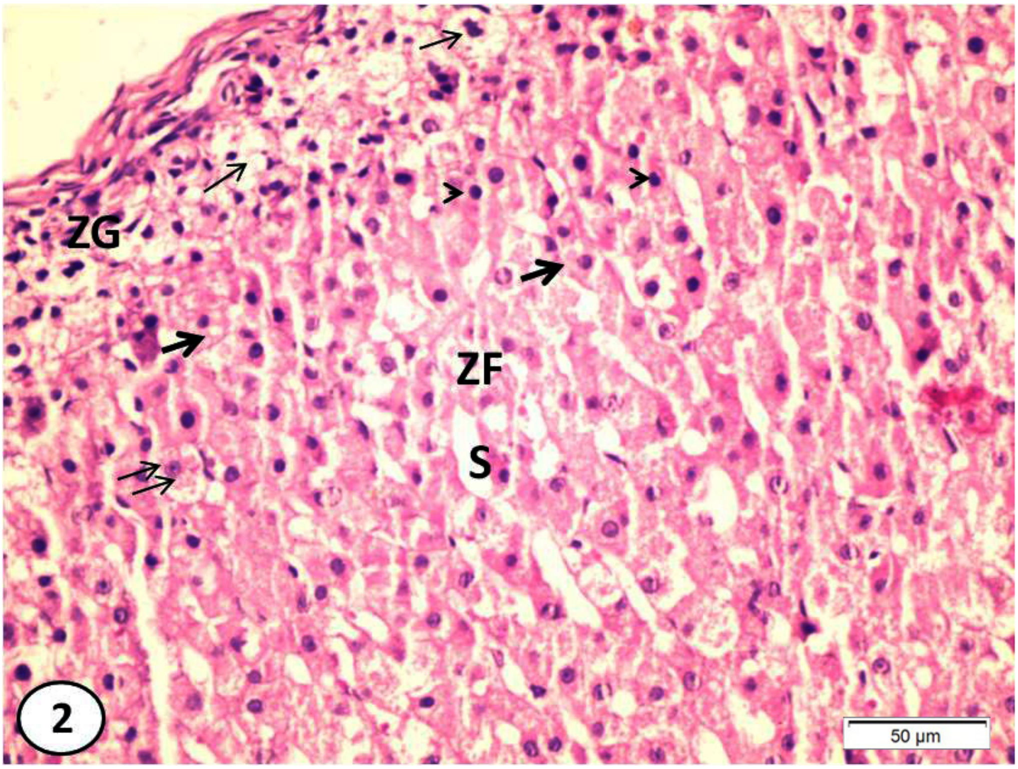


Fig. 2: A photomicrograph of adrenal cortex section of adult rat (group II, stressed) showing: the cells of (ZG) appeared disturbed in arrangement, swollen with vacuolated cytoplasm (thin arrows). ZF cells appeared swollen with disorganization in arrangement (thick arrows). Some cells showed pyknotic (arrow heads) while others karyolytic (double arrow) nuclei. Dilated sinusoids are clearly seen (S). H&E X 400

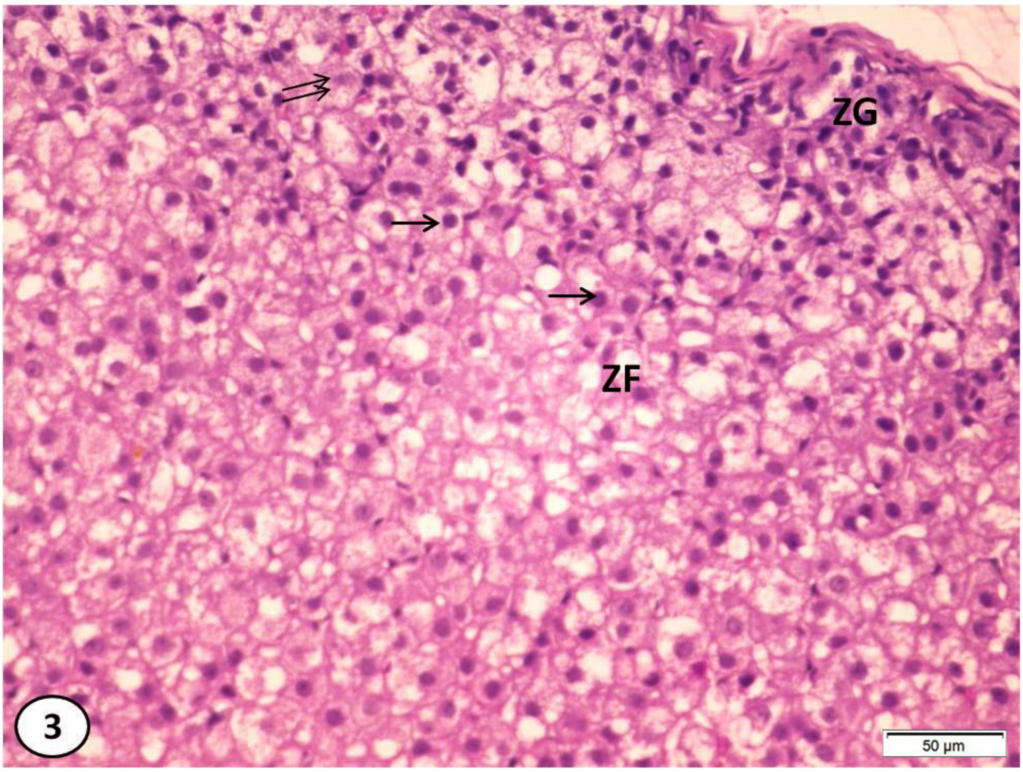


Fig. 3: A photomicrograph of adrenal cortex section of adult rat (group III, stressed + Omega -3) showing: The cells of (ZG) are nearly similar to control. In (ZF) the cells restored their normal arrangement. Some cells appeared with normal nuclei (arrows), while others with karyolytic nucleus (double arrow). H&E X 400

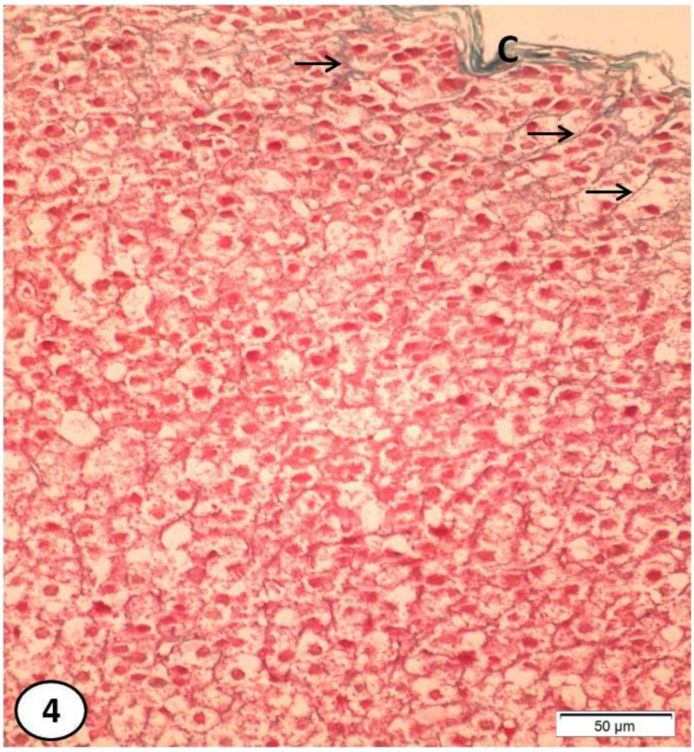


Fig. 4: A photomicrograph of adrenal cortex section of adult rat (group I, control) showing: green colored collagen fibers in the capsule (C) and in between the cords of cells (arrows). Masson's Trichrome X 400

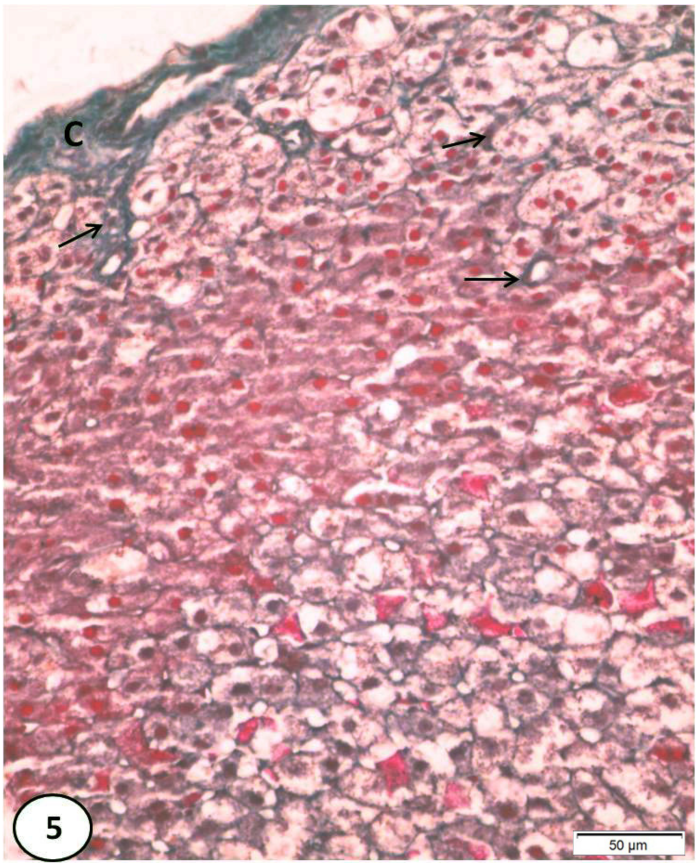


Fig. 5: A photomicrograph of adrenal cortex section of adult rat (group II, stressed) showing: an increase of greenish colored collagen fibers in the capsule (C) and between cell cords (arrows). Masson's Trichrome X 400

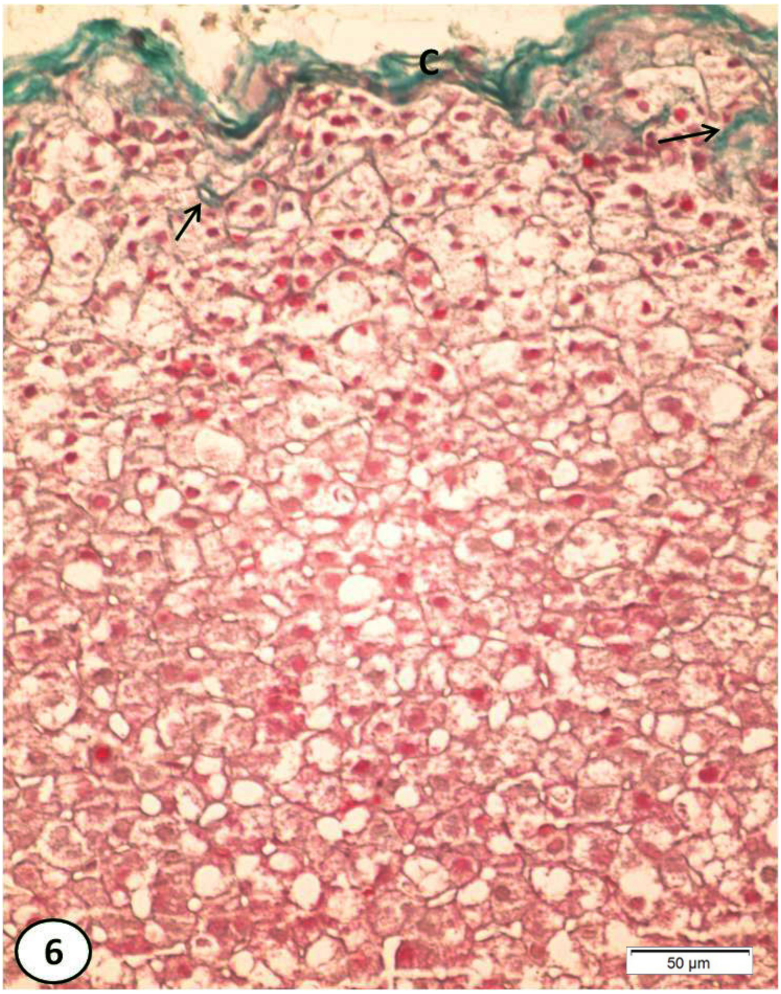


Fig. 6: A photomicrograph of adrenal cortex section of adult rat (group III, stressed + Omega -3) showing: the green colored collagen fibers is nearly similar to control in the capsule (C) and between the cells (arrows). Masson's Trichrome X 400

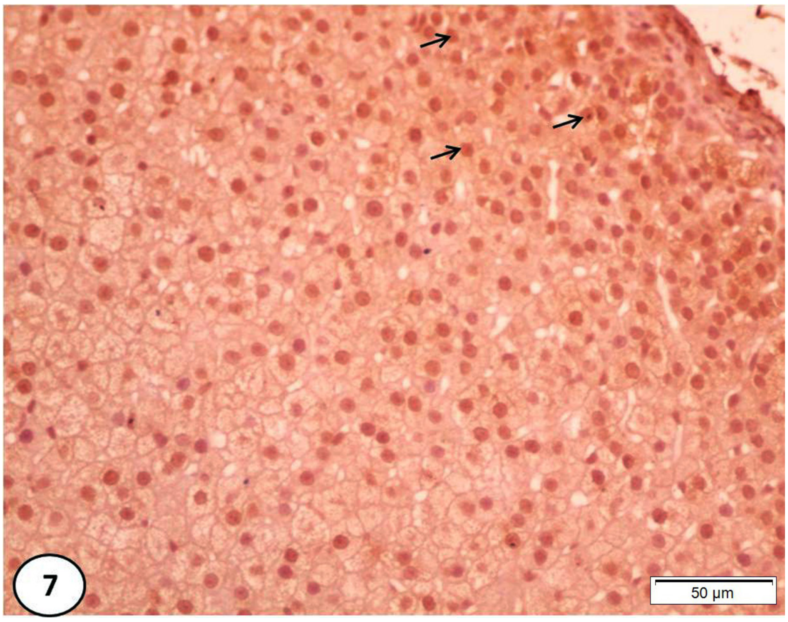


Fig. 7: A photomicrograph of adrenal gland cortex section of adult rat (group I, control) showing: strong positive immunoexpression of Bcl-2 in cortical cells (arrows). Bcl-2 X400

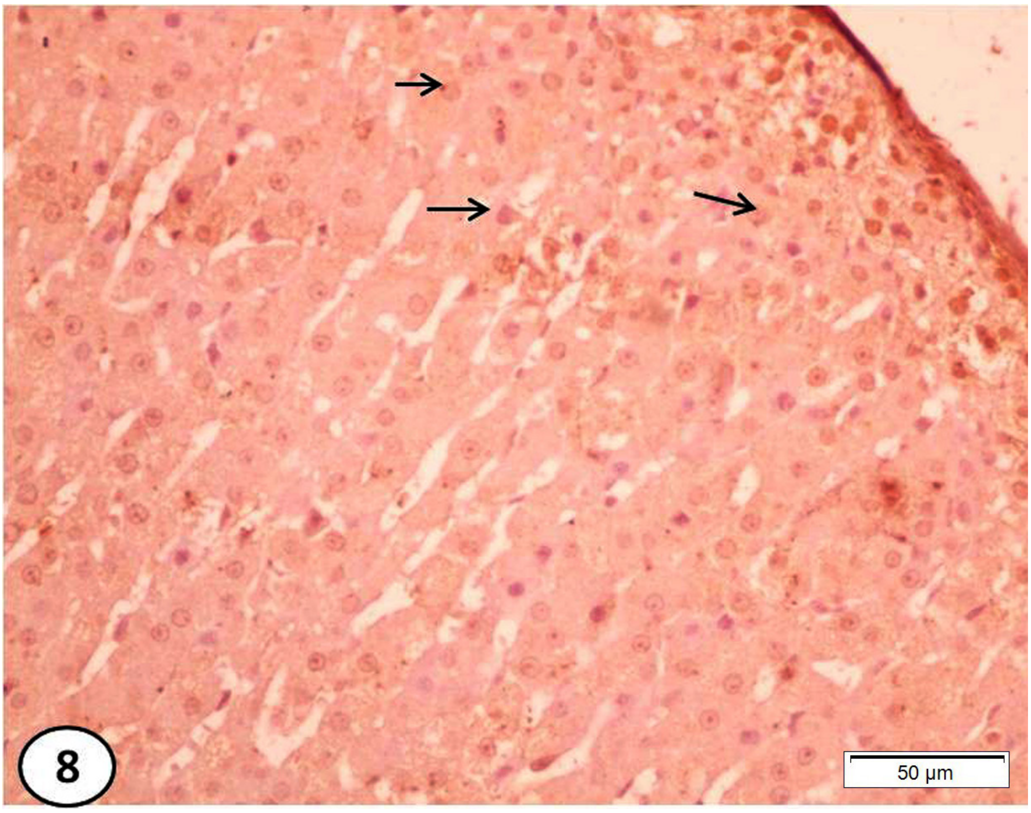


Fig. 8: A photomicrograph of adrenal cortex section of adult rat (group II, stressed) showing: weak positive immunoexpression of Bcl-2 in cortical cells (arrows). Bcl-2 X400

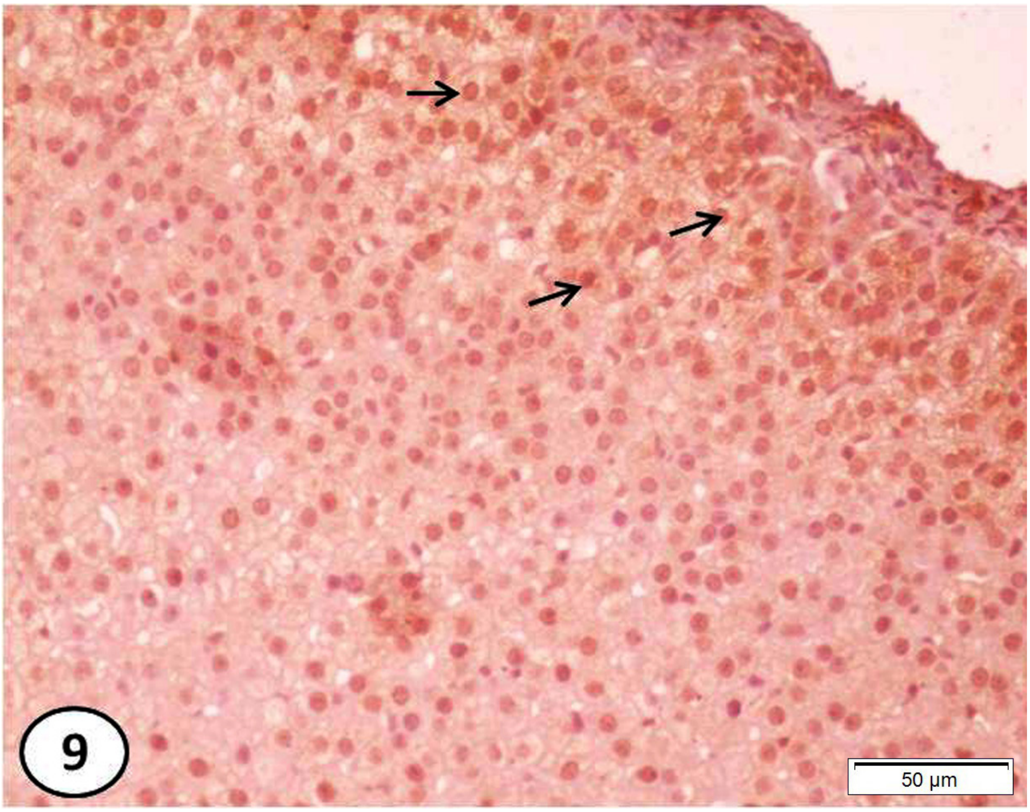


Fig. 9: A photomicrograph of adrenal cortex section of adult rat (group III, stressed + Omega -3) showing: mild positive immunoexpression of Bcl-2 in cortical cells (arrows). Bcl-2 X400

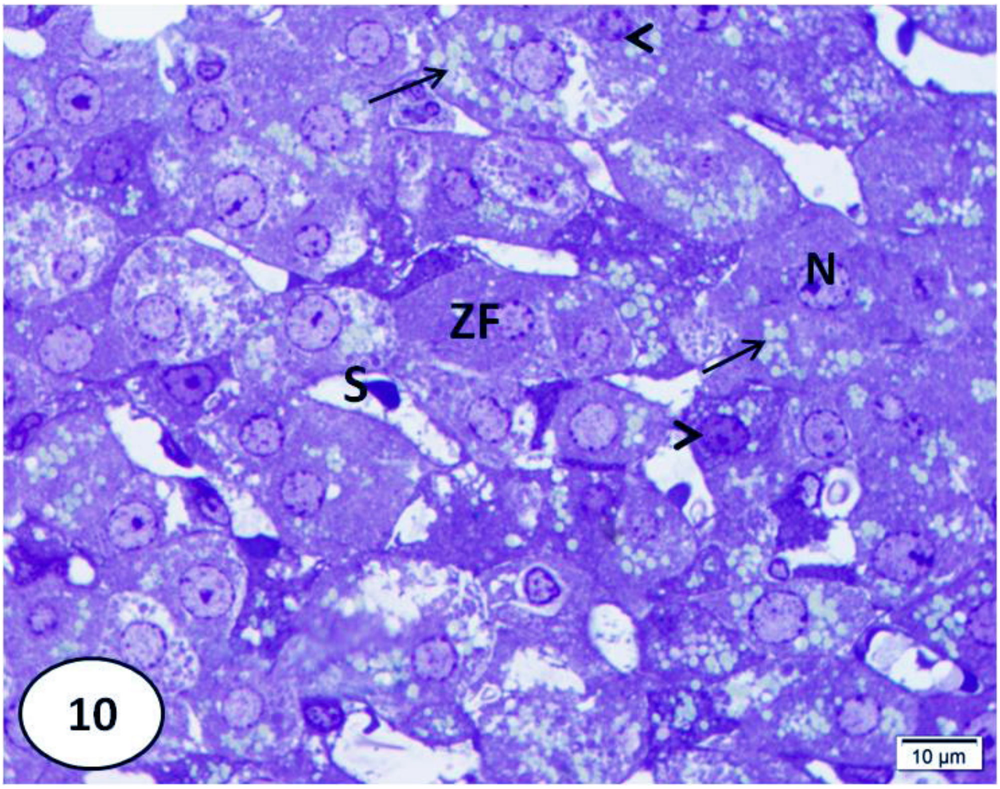


Fig. 10: A photomicrograph of adrenal cortex section of adult rat (group I, control) showing: the cells of (ZF) with rounded vesicular nucleus and one or more nucleoli (arrow heads). Note the lipid droplets in the cytoplasm (arrows) and blood sinusoids (S). Toluidine blueX1000

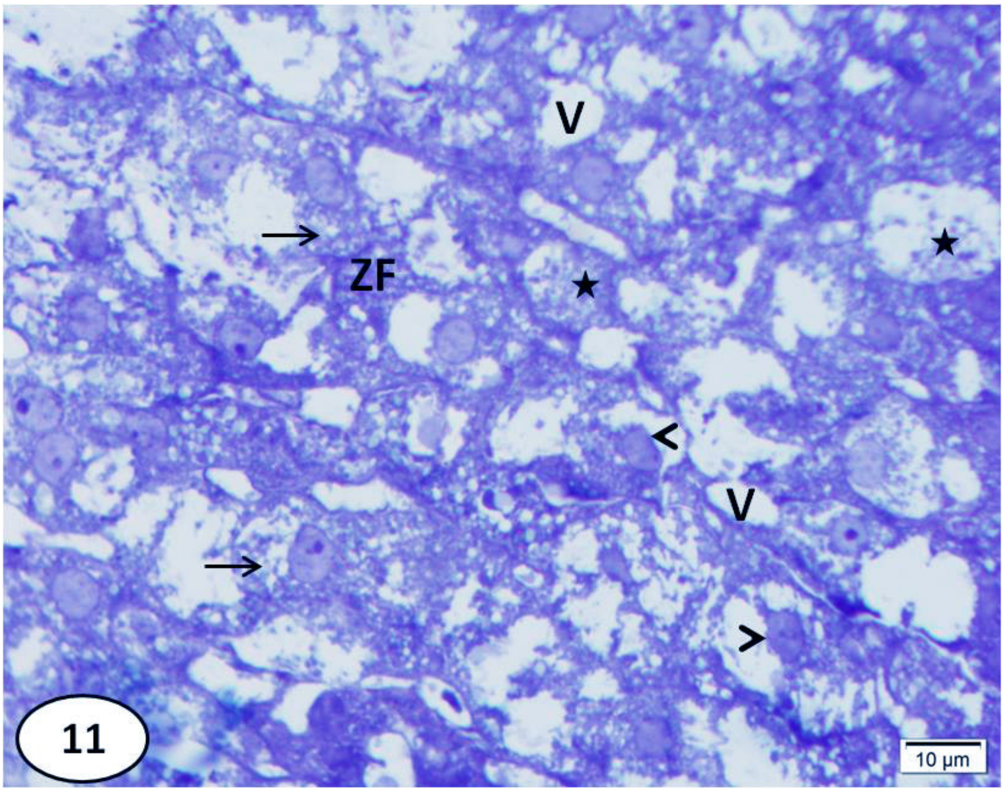


Fig. 11: A photomicrograph of adrenal cortex section of adult rat (group II, stressed) showing: destructed and disorganized cells of (ZF) with foamy cytoplasm (arrows) and vacuoles (V). Some cells appeared with irregular nuclei (arrow heads) while others lost their nuclei (stars). Toluidine blueX1000

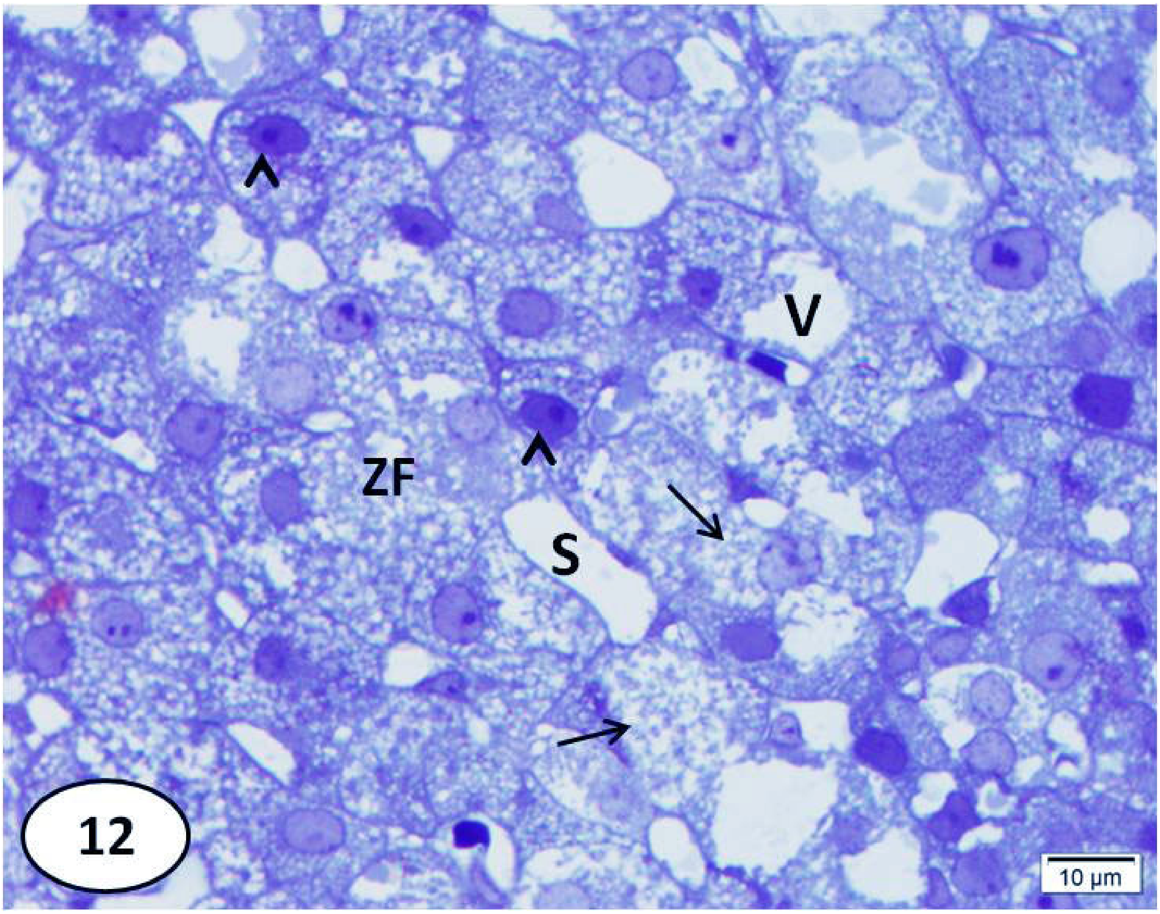
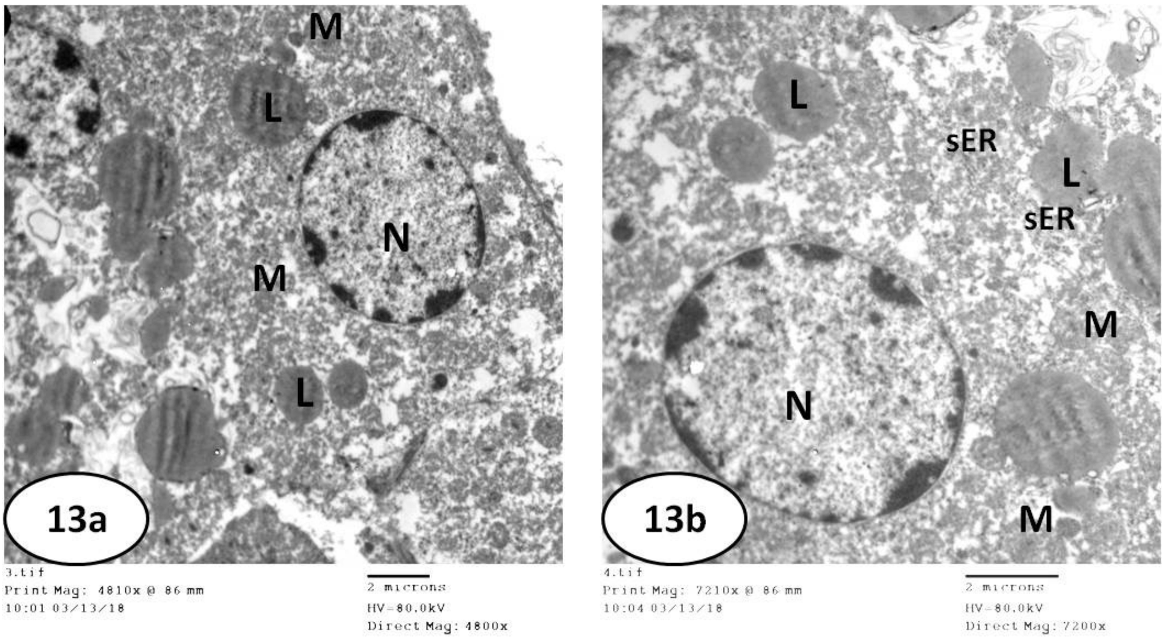
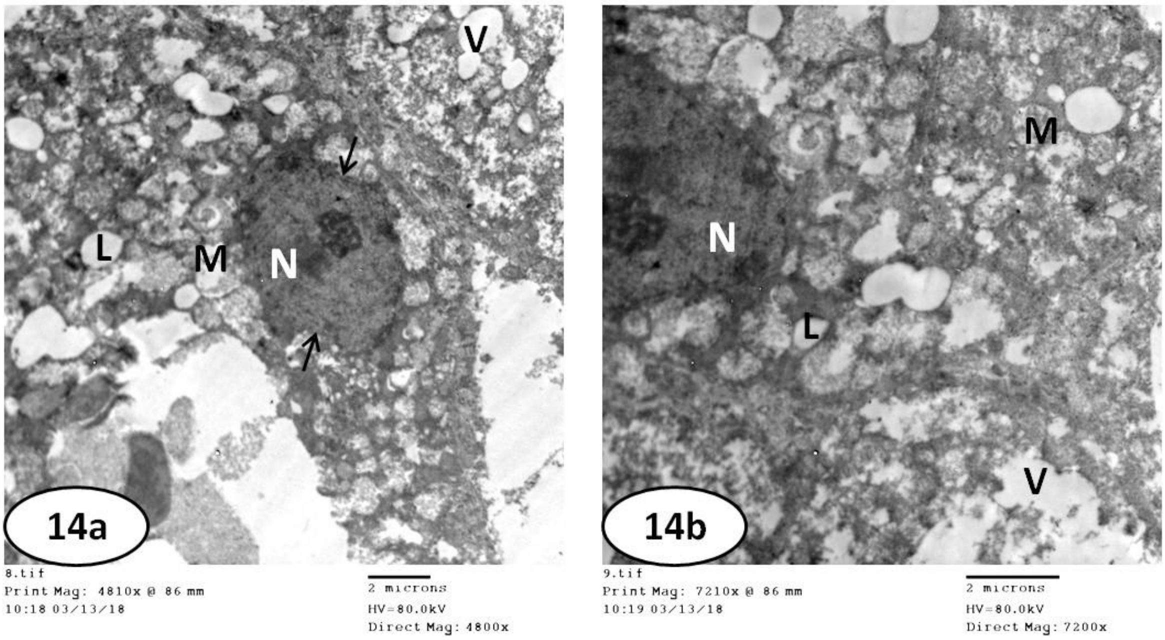


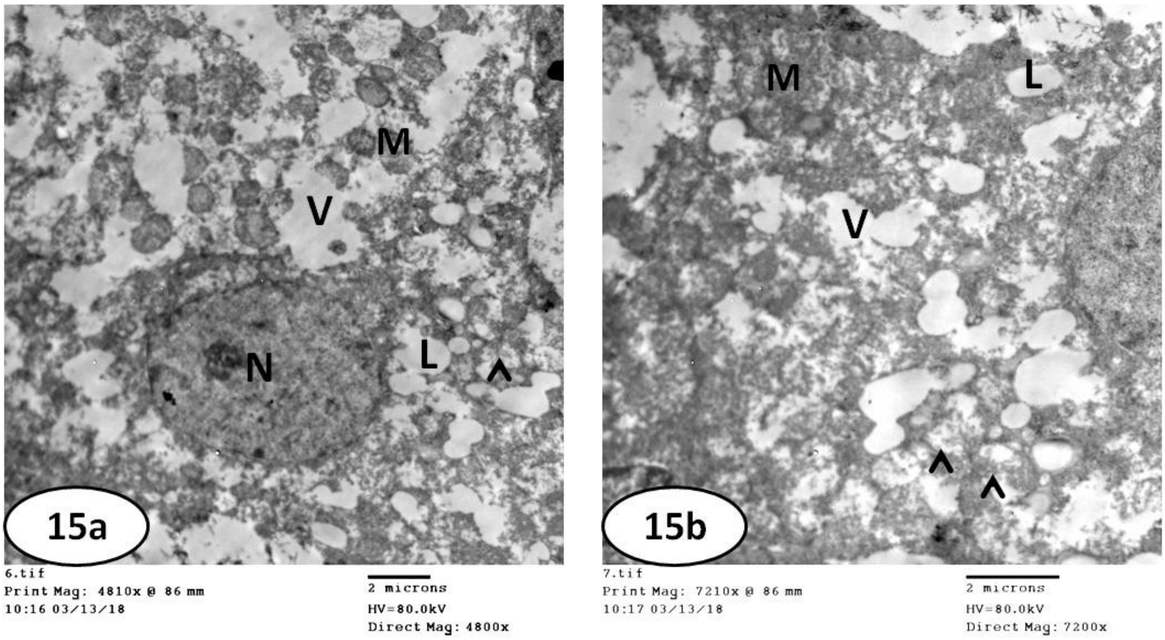
Fig. 12: A photomicrograph of adrenal cortex section of adult rat (group III, stressed + Omega -3) showing: the cells of (ZF) restored its arrangement to great extent. The foamy cytoplasm (arrows) and vacuoles (V) could be seen in some cells. Some cells had dark stained nuclei (arrow heads). Blood sinusoids could be seen (S). Toluidine blueX1000



Figs. 13 a & b: Electron micrographs of ultrathin section in adrenal cortex (ZF) of adult rat (group I, control) showing: zona fasciculata cell with euchromatic nucleus (N), many rounded mitochondria (M), lipid droplets (L) and smooth endoplasmic reticulum (sER). aX4800&bX7200



Figs. 14 a & b: Electron micrographs of ultrathin section in adrenal cortex (ZF) of adult rat (group II, stressed) showing: zona fasciculata cell with shrunken pyknotic nucleus (N) and irregular nuclear membrane (arrows). Vacuoles (V), lipid droplets (L) and destructed mitochondria (M) could be seen in the cytoplasm. aX4800&bX7200

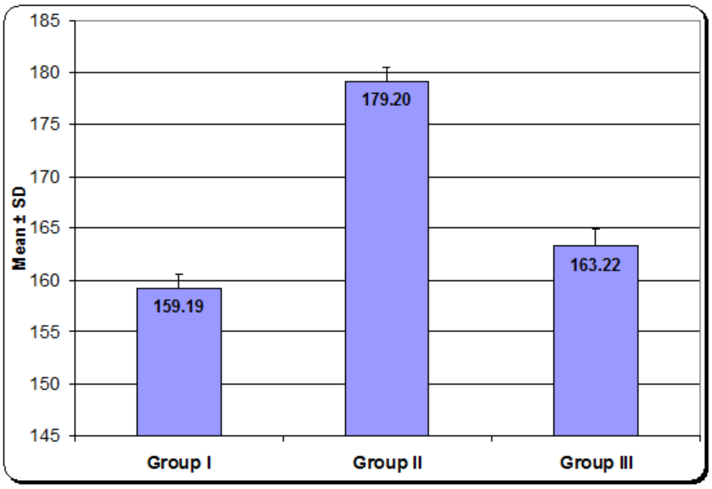


Figs. 15 a & b: Electron micrographs of ultrathin section in adrenal cortex (ZF) of adult rat (group III, stressed + Omega -3) showing: the nucleus (N) of zona fasciculata cell preserved its shape to great extent. Some mitochondria (M) appeared normal to some extent, while others still damaged (arrow heads). Vacuoles (V) and lipid droplets (L) could be seen in the cytoplasm. aX4800&bX7200

Table 1: Mean values of ZF thickness (um) in the studied groups

	Group I	Group II	Group III	*P-value[1]*	*P-value[2]*	*P-value[3]*
Mean ± SD	159.19±1.27	179.20±1.24	163.22±1.70	*<0.001**	*<0.001**	*<0.001**
Range	157.2-161.4	177.3-181.2	160.8-165.8			

SD: standard deviation
* Statistical significant difference (*P< 0.05*)
1: Comparison between groups 1&2
2: Comparison between groups 1&3
3: Comparison between group 2 &3

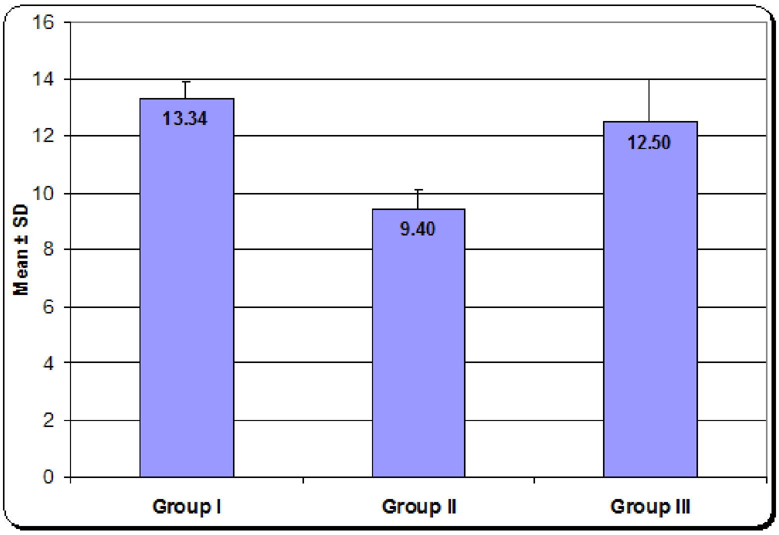


Histogram 1

Table 2: Mean values of area % of BCL-2 in the studied groups

	Group I	Group II	Group III	*P-value[1]*	*P-value[2]*	*P-value[3]*
Mean ± SD	13.34 ± 0.57	9.40 ± 0.71	12.50 ± 1.46	*<0.001**	*0.109*	*<0.001**
Range	12.6-14.3	8.0-10.3	10.6 - 14.9			

SD: standard deviation
* Statistical significant difference (*P< 0.05*)
1: Comparison between groups 1&2
2: Comparison between groups 1&3
3: Comparison between group 2&3



Histogram 2

DISCUSSION

The adrenal gland reacts constantly to recurrent and/or transient stimuli, such as stress[9]. Stress can be described to alter the body balance and homeostasis[5]. As a result of Immobilization stress, Y neuropeptide (NPY) concentration increases in adrenal gland with higher proportions in fascicular and reticular zones[7].

The present study showed changes in the cells of adrenal gland cortex after exposure to stress such as; disorganization of cells, vacuolated cytoplasm, pyknotic nuclei and dilated sinusoids. Moreover, there was an increase of the greenish colored collagen fibers in the capsule and between cell cords seen by Masson's trichrome stain. Ultrastructurally, cells of zona fasciculata showed many signs of degeneration as shrunken irregular nuclei, many lipid droplets and vacuoles in the cytoplasm and destructed mitochondria. These results were in agreement with Koko *et al*.[24] who reported that ACTH secretion increased with stress and this will exert tropic (short-term) and trophic (long-term) effects on the adrenocortical zona fasciculata (ZF) and zona reticularis (ZR). At the same time, Kamal[5] declared that, the various types of stress have been associated with intensified free radical generation and disrupted antioxidant enzyme activities. Meanwhile, they found that immobilization stress may promote the generation of reactive oxygen species (ROS) which are engaged in the pathogenesis of many diseases and pathologic processes and play a chief role in the complex physiological processes such apoptosis. In addition, cell growth arrest is supposed to be mediated by ROS which also activate proteins that dampen the cell cycle[25].

Pérez *et al*.[17] stated that the release of corticotrophin releasing factor (CRF) from the hypothalamus is augmented by stressors, which in turn will promote the release of adrenocorticotropic hormone from the anterior pituitary, and induces the secretion of adrenal corticosterone.

The increased greenish color of collagen fibers observed in this study under stress supported by the findings of Koko *et al*.[24] who stated that, there is a collagen-binding glucoprotein (47•kDa heat shock protein, HSP 47) involved in collagen formation under normal conditions. HSP 47 synthesis is increased under stress conditions, which correlates with collagen synthesis in several cell lines. However, some authors did not detect signs of fibrosis, this could favor the idea that adrenal gland react by different ways to the various stress factors, regardless of the same reaction in the hypothalamic–pituitary axis[26].

The morphometric results of this study revealed significant increase in ZF thickness in group II when compared with control. These results are in concurrent with Kocaman *et al*.[27] who found that there was increase in adrenal gland volume and in cortical thickness observed in the Electromagnetic fields (EMF) exposed groups; principally, ZF and medulla. The possible explanations for the volume increase; may be hyperplasia and hypertrophy that occurred in this zone.

In addition, the immunohistochemical results in this study revealed strong positive immunoreactivity for Bcl-2 in control adrenal cortical cells. These results were in agreement with Attia *et al*.[1] who noticed prominent Bcl-2 staining in the adrenal cortex. However, weak immunoreactivity for Bcl-2 was detected in adrenal gland cells exposed to stress. These results were evidenced by the morphometric and statistical results which showed significant decrease in area% of Bcl-2 immunoexpression in group II as compared to group I which was improved in group III.

The apoptotic process is regulated at several checkpoints. One of them is mainly controlled by members of the Bcl-2 family[28]. The Bcl-2 protein is a regulator of the apoptotic process (programmed cell death), and elevated levels can assist cell resistance to death so it supports cell survival[29,30]. Their relative abundance may impact the cellular vulnerability to undergo apoptosis as result to stimulus exposure. In young aged buffalo's adrenal cortical cells, the expression of Bcl-2 was more prominent (incidence of apoptosis was lower) than in the adrenal cortical cells of the old ages (incidence of apoptosis was higher). This may point to that stress may promote aging changes[1].

Dietary fish oil, which contains omega-3 fatty acids (O3FAs) is known to have valuable effects on cardiovascular disease[12], autoimmune diseases[13] and cancer[14].

As Omga-3 is an important component of cell membrane structure, therefore it plays a crucial role in cell movement, receptor placement and preserving cell membrane cohesion and fluidity[31].

The results of this study showed that the adrenal gland cells after receiving Omega-3 restored their arrangement. In addition there was amelioration in cellular structure, collagen fibers content and Bcl-2 expression as shown by light and electron microscope. Also these results were confirmed by morphometric results which revealed improvement in area% of Bcl-2 immunoexpression in group III.

These findings agreed with El Kalawy *et al*.[32] who stated that these effects may be due to that Omega-3 FAs have been pointed to act as anti-oxidant and anti-apoptotic agents. It enhances antioxidant enzymes actions through regulating expression of genes and has a significant role in stabilizing oxidative stress in the cell; it also affect the molecular mechanism of cells exposed to stress and thus diminish oxidative stress[27].

Liu *et al*.[33] reported that, activation of the HPA axis during stress is due to release of pro inflammatory cytokines which participate as mediators. So, dietary fish oil (FO) supplementation may have beneficial effects in decreasing this pro inflammatory cytokines expression. The association of stress and a lack of omega-3 FA could be a powerful stimulus disturbing cognition and mood[34]. Moreover, Larrieu *et al*.[35] revealed that dietary n-3PUFA (polyunsaturated fatty acid) supplementation for long periods inhibit chronic stress-induced emotional and neuronal deterioration by disrupting HPA axis hyperactivity.

CONCLUSIONS

In conclusion, it was found that a diet enriched in omega-3FA attenuates some of the deleterious effects of chronic stress. This suggests that omega-3 FA supplementation influences sensitivity to stress and has strong anti-stress effects.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Attia HF, Abdel-Rahman GH, Nassan MA, and Baiomy AA. Immunohistochemical Evaluation of Bcl-2 Oncoprotein in Buffalo's Adrenal. Journal of Cytology and Histology. 2013; 4: 167.

2. Sadek HL and Mohamed DS. Histological and morphometric changes in adrenal cortex of aged male albino rats. Journal of American Science. 2015; 11(3): 162-173.

3. Hart KA and Barton MH. Adrenocortical insufficiency in horses and foals. Journal of Veterinary Clinics of North America: Equine Practice. 2011; 27(1):19-34.

4. El-Feky H, Salah A, Hussein Y and Sabry M. Histological study of the effect of induced diabetes on the zona glomerulosa of the adrenal cortex of adult male albino rat. Egyptian Journal of Histology. 2010; 33(1): 17-22.

5. Kamal HM. The effect of immobilization induced stress on the lung of the adult male albino rat and the possible protective role of melatonin: A light, electron microscopic and immunohistochemical study. Egyptian Journal of Anatomy. 2017; 40(2): 358-380.

6. Ahmed AM. Light and electron microscopic study of the effect of experimentally- induced colitis on the adrenal gland of albino rats. The Egyptian Journal of Histology. 2005; 28(2): 263-272.

7. Bozzo AA, Soñez CA, Cobeta IM, Rodolfo A R, Rolando AN, Romanini MC, *et al*. Chronic Stress Effects on Adrenal Cortex CellularProliferation in Pregnant Rats. International Journal of Morphology. 2011; 29(4):1148-1157.

8. Marx C, Wolkersdorfer G and Bornstein SR. A new view on immune-adrenal interaction: role for Fas andFas ligand. Neuroimmunornodulation. 1998; 5:5-8.

9. Bertorello C M, Bozzo A, Cots D, Rolando A, Diaz T and Romanini MC. Determination of the neuropeptide Y in the adrenal cortex

of stressed pregnant rats. Investigacion Veterinaria 2015; 17 (2): 187-194.

10. Jaggi AS, Bhatia N, Kumar N, Singh N, Anand P and Dhawan R. A review on animal models for screening potential anti-stress agents. Neurological Sciences. 2011; 32(6): 993-1005.

11. Al-Attar AM and Al-Rethea HA. Chemoprotective effect of omega-3 fatty acids on thioacetamide induced hepatic fibrosis in male rats. Saudi Journal of Biological Science. 2017; 24:956-965.

12. Xin W, Wei W and Li X. Effects of fish oil supplementation on cardiac function in chronic heart failure: a meta-analysis of randomized controlled trials. Heart. 2012; 98: 1620-1625.

13. Chapkin RS, Kim W, Lupton JR and McMurray DN. Dietary docosahexaenoic and eicosapentaenoic acid: emerging mediators of inflammation. Prostaglandins, Leukotrienes and Essential Fatty Acids Journal. 2009; 81: 187-191.

14. Cockbain J, Toogood GJ and Hull MA. Omega-3 polyunsaturated fatty acids for the treatment and prevention of colorectal cancer. Gut. 2012; 61: 135-149.

15. Fahmy HA, Abd El Azim AS and Gharib OA. Protective Effects of omega-3 fatty acids and/ or nanoselenium on cisplatin and ionizing radiation induced liver toxicity in rats. Indian Journal of Pharmaceutical Education and Research. 2016; 50(4):649-656.

16. Bolles KL and Begg GA. Distinction between silver hake (Merluccius bilinearis) stocks in US waters of the northwest Atlantic based on whole otolith morphometrics Fishery Bulletin 2000; 98: 451-462.

17. Pérez MÁ, Terreros G and Dagnino-Subiabre A. Long-term ω-3 fatty acid supplementation induces anti-stress effects and improves learning in rats. Behavioral and Brain Functions. 2013; 9:25.

18. Bertsch K, Schmidinger I, Neumann ID and Herpertz SC. Reduced plasma oxytocin levels in female patients with borderline personality disorder. Hormones and Behavior. 2013; 63:424-429.

19. Meganathan M, Gopal KM, Sasikala P, Mohan J, Gowdhaman N, Balamurugan K, *et al*. Evaluation of hepatoprotective effect of omega 3-fatty acid against paracetamol induced liver injury in albino rats. Global J of Pharmacol. 2011; 5 (1): 50-53.

20. Bancroft JD and Gamble M. Theory and practice of histological techniques. 2008; 6th ed. Churchill Livingstone Elsevier, Philadelphia.

21. Graham L and Orenstein JM. Processing tissue and cells for transmission electron microscopy in diagnostic pathology and research. Nature Protocols. 2007; 2 (10): 2439-2450.

22. Desouky MK, Anwar RI and Algaidi SA. Immunohistochemical Expression of Bcl-2 and Microvessel Density in Uterine Fibroids in Saudi Patients. West Indian Medical Journal. 2015; 2(3):121-126.

23. El-Helbawy NF,Radwan DA,Salem MF,El-Sawaf ME. Effect of monosodium glutamate on body weight and the histological structure of the zona fasciculata of the adrenal cortex in young male albino rats. Tanta Medical Journal. 2018; 45:104–113.

24. Koko V, Djordjeviæ J, Cvijiæ G and Davidoviæ V. Effect of acute heat stress on rat adrenal glands: a morphological and stereological study. The Journal of Experimental Biology. 2004; 207: 4225-4230.

25. Ozgur F, Ozer M, Levent Y, Goksel G, Cigdem Y, Ahmet C. Omega-3 fatty acids inhibit oxidative stress in a rat modelof liver regeneration. Annals of Surgical Treatment and Research. 2017; 93(1):110.

26. Pacak K and Palkovits M. Stressor specificity of central neuroendocrine responses:

implications for stress-related disorders. Endocrine Reviews. 2001; 22: 502-548.

27. Kocaman A, Gül M, Yurt KK, Altun G, Zayman E and Kıvrak EG. Does omega-3 have a protective effect on the rat adrenal gland exposed to 900 MHz electromagnetic fields? Journal of Microscopy and Ultrastructure. 2017; 5:185–190.

28. Gross A, McDonnell JM and Korsmeyer SJ. Bcl-2 family members and the mitochondria in apoptosis. Genes and Development. 1999; 13: 1899-1911.

29. Digiuseppe JA, LeBeau P, Augenbraun J and Borowitz MJ. Multiparameter flow-cytometric analysis of bcl-2 and Fas expression in normal and neoplastic hematopoiesis. American Journal of Clinical Pathology. 1996; 106: 345-351.

30. Reed JC. Double identity for proteins of the Bcl-2 family. Nature. 1997; 387: 773-776.

31. Valentine RC and Valentine DL. Omega-3 fatty acids in cellular membranes: a unified concept. Progress in Lipid Research. 2004; 43: 383-402.

32. El Kalawy SA, Mostafa MHA, Abd El Fattah LI and Kamar SSI. Histological and immunohistochemical study of the effect of omega-3 fatty acids on ifosfamide-induced liver injury in adult male albino rat. Journal of Medical Histology. 2017; 1 (2): 134-145.

33. Liu Y, Chen F, Li Q, Odle J, Lin X, Zhu H, *et al*. Fish Oil Alleviates Activation of the Hypothalamic- Pituitary-Adrenal Axis Associated with Inhibition of TLR4 and NOD Signaling Pathways in Weaned Piglets after a Lipopolysaccharide Challenge. The Journal of Nutrition. 2013; 4: 1799- 1807.

34. Hennebelle M, Balasse L, Latour A, Champeil-Potokar G, Denis S, Lavialle M, *et al*. Influence of Omega-3 Fatty Acid Status on the Way Rats Adapt to Chronic Restraint Stress. PLoS ONE. 2012; 7(7): e42142.

35. Larrieu T, Hilal LM, Fourrier C, Smedt-Peyrusse V, Sans N, Capuron L, *et al*. Nutritional omega-3 modulates neuronal morphology in the prefrontal cortex along with depression-related behavior through corticosterone secretion. Translational.

تأثير مكملات اوميجا – 3 علي قشرة الغدة الكظرية في الفئران البالغة المعرضة للتوت

رشا ابراهيم أنور , هالة زين العابدين محمد

قسم التشريح الأدمي و علم الأجنة- كلية الطب-جامعة أسيوط

ملخص البحث

الخلفية: الغدة النخامية, الغدة الكظرية و الجهاز السمبتاوي هي المحاور و الاجهزة الاساسية التي تساهم في الحفاظ علي توازن الجسم أثناء التوتر. التوتر تحت الحاد و المزمن يصاحبه عادة تغييرات في الغدة الكظرية. من المعروف أن الحمض الدهني أوميغا- ٣ الذي يوجد في زيت الأسماك الغذائي له تأثير مفيد علي أمراض الجهاز الدوري, الأمراض المناعية و السرطان. و يعتبر اوميغا-٣ أيضا من عوامل مكافحة موت الخلايا المبرمج و الأكسدة.

الهدف من البحث: تقييم دورمكملات أوميغا-٣ علي خلايا قشرة الغدة الكظرية أثناء التعرض للتوتر.

المواد و الطرق المستخدمة: تم استخدام ٣ مجموعات من ذكور الجرذان البالغة (١٠ في كل مجموعة): مجموعة ١ (مجموعة ضابطة) ,مجموعة٢ (تعرضت للتوتر لمدة ٦ أسابيع) و مجموعة ٣ (تعرضت للتوتر و في نفس الوقت تم أعطاؤ ها أوميغا-٣} ٣٠٠كجم/كجم{يوميا/٥ ايام في الاسبوع لمدة ٦ أسابيع. ثم تمت التضحية بالفئران و استخراج الغدة الكظرية و تمريرها للدراسة الهستولوجية, والهيستوكيميائية مناعية و القياسات المورفومترية.

النتائج: ظهر في المجموعة الثانية علامات التحلل في خلايا قشرة الغدة الكظرية و التي تشمل إختلال في ترتيب الخلايا, تغلظ الأنوية, السيتوبلازم المفرغ و تكسر في عضيات الخلية. كما انه يوجد نقص في سمك طبقة Zona Fasciculata مع ضعف التعبير المناعي ل Bcl-2. و قد حث أوميغا-٣ علي التحسن النسبي في خلايا قشرة الغدة الكظرية في المجموعة الثالثة. و هذا تم إثباته بالميكروسكوب الضوئي و الإلكتروني وتأكيده بالنتائج المورفومترية و الإحصائية.

الخلاصة: إستخدام مكملات أوميغا-٣ يؤثر علي الحساسية للتوتر و له تأثير قوي ضد أثار التوتر.