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Research Article

The Possible Protective Role of Ghrelin on Acute Stress Induced Thymic Atrophy in Mice. Histological and Immunohistochemical Study

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Abstract

Background: Exposure to stress down regulates the immune system. Thymus gland is sensitive to stress. Ghrelin hormone secreted by the stomach has an immune-stimulatory effect.

Aim: Aim of the Work was to study the effect of immobilization stress on mouse thymic population and the possible protective role of Ghrelin. Material and Methods: 40 animals were divided into four groups (10 mice each). Group I was considered as control group. Group II was injected with a 100 µ g/kg of ghrelin intraperitoneally. Group III was immobilized by stress restraint test. Group IV received 100 µ g/kg of ghrelin intraperitoneally prior to the stress restraint test. Thymi of mice of different groups were removed and processed for haematoxylin and eosin, immunohistochemical staining for caspase 3 and electron microscopic studies. Finally, morphometric and statistical analysis were performed.

Results: Acute stress resulted in significant decrease in thymic weight. Atrophy of thymic lobules with marked fatty and mononuclear cellular infiltration was detected. Marked decrease in cellularity of thymic cortex was noticed and confirmed by significant increase in caspase 3 positive cells. Medulla showed proliferation of epithelial reticular cells with cystic degeneration in Hassall's corpuscle. In Ghrelin protected group, the thymus regained normal histological structure with significant decrease in caspase 3 positive cells.

Conclusion: Stress resulted in loss of double positive thymocytes to the periphery. Extra thymic T cells were defective, non-functional and auto-reactive. Ghrelin allowed activation of surviving thymocytes and prevented apoptosis.

Keywords: Electron Microscope; Ghrelin; Histology; Mice; Stress; Thymus

Introduction

Our constant exposure to environmental stressors has become a major problem in our society. The increased impact of daily stressors has taken its toll on our immune system. Stress may take several forms and its effect depends on duration, intensity and nature of the effector [1]. The thymus gland is a crucial organ for homeostatic maintenance of the peripheral immune system. It is the mediastinal tissue responsible for proper T cells development and education. It is the site where thymocytes are extensively differentiated into functional types for migration to the periphery and building of an effective and self-oriented immune system [2]. Thymic atrophy is a complication that results from exposure to many factors including drugs, cancer therapies and microbial invasion. The acute stress associated thymic atrophy can have a devastating effect on the host's immune system. The loss of the natural ability to produce an adequate population of naïve T cells exposes the body to severe danger even from minor pathogens [3]. Many efforts have been made to increase our understanding of thymus biology, microenvironment and development. Provisional therapies aiming at re-establishing an adequate and effective T cell production in patients with a defective immune system is mandatory [4]. Ghrelin is a 28 amino-acid peptide hormone secreted by the stomach. It acts at the ghrelin receptor in multiple tissues throughout the body, exhibiting broad effects potentially beneficial as a treatment in human disease states [5]. Recently

Ghrelin was reported to modulate apoptotic signalling in several cell types, such as cardiomyocytes, endothelial cells, adipocytes, adrenal zona glomerulosa cells, pancreatic β-cells, osteoblasts and hypothalamic neurons [6]. The aim of our work was to study the effect of stress on thymic architecture, cellularity and function and to prove the possible protective effect of Ghrelin hormone on stressed thymus.

Materials and Methods

Animals

The experiment was conducted on forty male mice, 6 to 8 weeks old with average weight of 25 to 50 gm. The experiment was conducted in the Scientific Research Center at Ain Shams University. The animals were housed under controlled conditions of temperature and illumination, and they were allowed to feed ad libitum. Animals were kept for seven days before the beginning of the experiment for acclimatization. All animal procedures were carried out according to the recommendation of the Institutional Animal Ethics Committee at the Faculty of Medicine, Ain Shams University.

Experimental design

Restraint stress mouse model

Previously established restraint stress protocol [3] was utilized in this study. Mice were individually immobilized by placing them in plastic restraints with a ventilation pore only allowing complete restraint for 2 hours. This procedure was then repeated daily for 7 days.

Animal grouping

The animals were randomly divided to 4 groups as follows:

Group I (Control group): It included 10 mice served as control untreated group and were sacrificed on the 7th day.

Group II: It included 10 mice injected intraperitoneally with 100 µg/kg of Ghrelin [3] (Abcam UK) for 7 days.

Group III (stress group): It included 10 mice which underwent the stress restraint test. The animals were restrained for 2 hours daily for 7 days. Animals were sacrificed on the 7th day.

Group IV (stress group with exogenous Ghrelin): It included 10 mice which were injected intraperitoneally with 100 µg/kg of Ghrelin the day before the immobilization stress experiment (day-1). After 24 hours, mice were subjected to another Ghrelin treatment 30 minutes before the start of the immobilization [3]. Mice underwent the stress restraint test as Group III. This procedure was repeated daily for 7 days.

Sample collection

At the end of the experiment, the mice were sacrificed by cervical dislocation after ether inhalation anaesthesia. Midline incision was done at the chest cavity. The sternum was carefully removed and the thymi were taken and weighed by a sensitive balance. The Thymi were divided so that half of the number of the thymi of different groups were fixed in 10% buffered formalin to be processed for the paraffin technique, while the other half was fixed in cold buffered formol glutaraldehyde to be processed for transmission electron microscope.

Histological study

Preparation of paraffin sections

Thymi specimens were fixed in 10% buffered formalin, dehydrated, cleared and embedded in paraffin. Serial 5 µ m sections of the specimens were stained with Hematoxylin and Eosin (H&E), and immunohistochemically for caspase-3 antibody (dilution 1:200 and purchased from Labvision) to mark apoptotic cells [7]. Negative control sections were processed by replacing the primary antibody by phosphate buffer saline. Brown staining indicated positive reaction.

Preparation of Transmission Electron Microscope (TEM) study

Small pieces (1 mm^3) from the thymi were cut and fixed immediately in 2.5% glutaraldehyde solution, followed by 1% osmium tetroxide, dehydrated, and embedded in epoxy resin. Ultrathin sections were collected on copper grids and stained with uranyl acetate and lead citrate [8]. The ultrastructural examination was carried out with a transmission electron microscope (JEM 1200, JEOL, Tokyo, Japan) Faculty of Science, Ain Shams University.

Morphometric study

The weight of thymi of different groups was measured and the optical density of caspase-3 positive cells in immunohistochemical stained sections using 20X objective lens was also measured. Light microscope measured parameters were done in five different non-overlapping fields in thymus sections of all mice in each group. Five different readings from every captured field were taken and the mean was calculated for each specimen. Measurements were distinguished by an independent observer blinded to the specimens' details to perform an unbiased assessment. They were performed by image analyser at Histology and Cell Biology department, Faculty of Medicine, Ain Shams University using Leica Q win software installed on a Dell PC (Texas, USA). The PC was connected to a microscope (Leica microsystem, Heerburg, switzeland).

Statistical analysis

Morphometric measured parameters recorded for each group were revised and the results were expressed as mean ± SD. Statistical analysis was carried out using statistical package for the social sciences (SPSS), software program, version 20 (IBM corporation, Arm work, North castle, Westchester Country, New York, USA). Statistical difference among groups for each parameter was determined using two-way Analysis of Variance (ANOVA) followed by post hoc Least Significance Difference (LSD) for comparison between more than two groups P value ≤ 0.05 were considered statistically significant.

Histological results

Light microscopic study

Histological Examination of H&E stained sections of Group I (control) showed the thymus was formed of outer dark cortex and inner pale medulla covered by thin connective tissue capsule. The thymic cortex was heavily populated with thymocytes. The medulla appeared paler due to presence of many Thymic Epithelial Reticular Cells (TECs) with vesicular nuclei and acidophilic cytoplasm (Figure 1A). Thick capsule with outer and inner fibrous layer was noticed. The middle layer contained thymocytes (progenitor) cells (Figure 1B). Septal blood capillary was seen surrounded by row of TECs and perivascular macrophages forming blood thymic barrier (Figure 1C). The medulla showed epithelial reticular cells arranged in groups lodged in acidophilic material forming the characteristic Hassall's corpuscle. Thymocytes formed rosettes around large vesicular nuclei of mTECs (medullary) and tingible body macrophages were detected (Figure 1D).

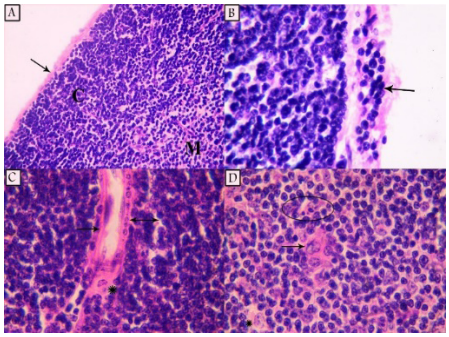

Figure 1: Photomicrographs of a mouse thymus of **Group I (control)** showing **(A)**: outer dark cortex (**C**) and inner pale medulla (**M**). Notice thin capsule (↑). **(B):** thick capsule formed of outer and inner connective tissue layers with thymocytes progenitors in between (↑). **(C):** blood capillary (↑) in connective tissue septum. Row of TECs (↕) with vesicular nuclei and acidophilic cytoplasm are present beneath the septum. Notice perivascular Macrophage (*). **(D):** group of epithelial reticular cells embedded in acidophilic material forming Hassall's corpuscle (↑). Notice large TEC (*) surrounded by thymocytes and tingible body macrophages ( ) [Hx &E (A) X 400 (B, C, D) X1000].

Moreover, examination of Group II (Ghrelin) revealed starry sky appearance of thymic cortex (Figure 2A). Higher magnification showed rounded paler areas surrounded by dark aggregation of thymocytes. The paler areas represented tingible body macrophage. They contained phagocytosed apoptotic thymocytes and cellular debris (Figure 2B). The cortex showed numerous blood vessels (Figure 2C). Two Hassall's corpuscles, blood vessels and nursing cells identified as mTEC surrounded by a group of thymocytes forming rosettes were also noticed (Figure 2D).

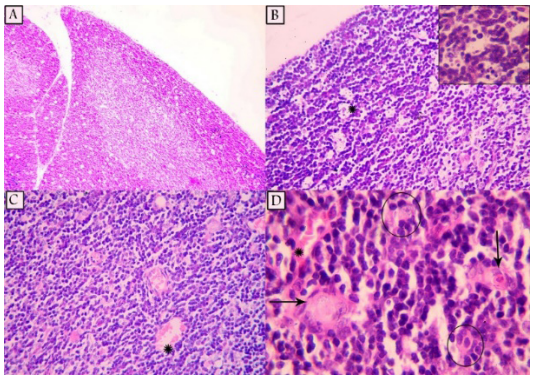

Figure 2: Photomicrographs of a mouse thymus of Group II (ghrelin) showing (**A**): starry sky appearance of the cortex in form of pale area in deep basophilic background. (**B**): central pale area (*) surrounded by deeply basophilic thymocytes nuclei. Inset: higher magnification showing tingible body macrophages containing several apoptotic Thymocytes and cellular debris. (**C**): numerous blood capillaries (*) are noticed. (**D**): Hassall's corpuscles (↑) and blood vessels (*) were seen. Notice many thymocytes forming aggregation rosettes around TECs ( ) [Hx &E (A) X 100 (B, C) x 400 (inset B, D) X1000].

However, Group III (stress) examination showed two thymic lobes with decreased size and distorted shape. One lobe was elongated, and the other lobe was shrunk and replaced by fatty tissue. Both lobes were surrounded by thick capsule with inflammatory cellular infiltration and large blood vessels (Figure 3A). Higher magnification of the capsule showed heavy infiltration of the capsule and underlying cortex with mononuclear inflammatory cells. These cells were migrating from surrounding brown adipose tissue through the capsule (Figure 3B). There was loss of cortico-medullary junction with expansion of medulla and thinning of the cortex. The medulla demonstrated hyperplasia of epithelial reticular cells. There was migration of thymocytes through blood vessels (Figure 3C). Meanwhile, Group IV (stress and ghrelin) showed normal lobulation of the thymus and thin interlobular septa. Outer dark cortex and inner pale medulla could be seen with notable cortico-medullary junction. There was normal arrangement of Hassall's corpuscles which showed relatively increased number compared to control group (Figures 3D,3E). The capsule in some specimens revealed increased thickness and noticeable thymocyte progenitor cells infiltration with prominent blood capillary (Figure 3F).

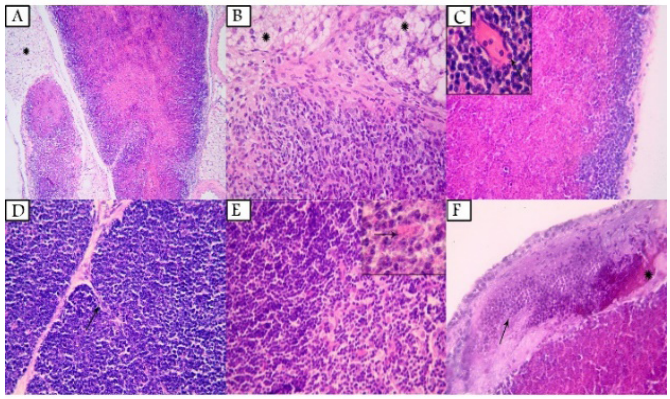

Figure 3: Photomicrographs (A, B, C) of a mouse thymus of Group III (stress) showing (**A**): One atrophied lobe is surrounded by fatty tissue (*). The other lobe appears with thin rim of basophilic cortex, thick acidophilic medulla and is surrounded by fatty tissue and blood vessels. (**B**): thymic cortex is infiltrated with heterogenous group of inflammatory cells migrating through the capsule from brown adipose tissue (*) infiltrating the other lobe. (**C**): thin cortex is noticed with thymocytes nuclei and wide acidophilic medulla. Inset: showing blood capillary containing thymocytes migrating outside the thymus (↑). Photomicrographs (D, E, F) of a mouse thymus of Group IV (stress and ghrelin) showing (**D**): normal lobulation with thin interlobular septa (↑). (**E**): demarcation of cortico-medullary junction is noticed. Inset: showing Hassall's corpuscle (↑). (**F**): thick capsule containing numerous thymocytes (↑) can be seen. Notice large blood vessel (*) [Hx &E (A) X 100 (B, C, D, E, F) × 400 (inset C, E) X1000].

Examination of caspase-3 immunohistochemical stained section of thymi of Group I (control group) showed few cells with weak positive brown immune reaction for caspase-3 more in cortex than in medulla (Figure 4A). While, some cells in Group II showed positive brown immune reaction in the cortex. These cells represent single apoptotic cells inside tingible body macrophages (Figure 4B). However, intense brown positive immune reaction was seen in the thymic cortical cells Group III (stress). Positive immune reaction significantly increased ($P \leq 0.05$) in Group III (stress) compared to Group I (Figure 4C) (Table 1). Meanwhile, groups of cellular aggregation in Group IV (stress and ghrelin) showed moderate positive caspase-3 reaction more in medulla. They probably represented site of Hassall's corpuscle formation (Figure 4D).

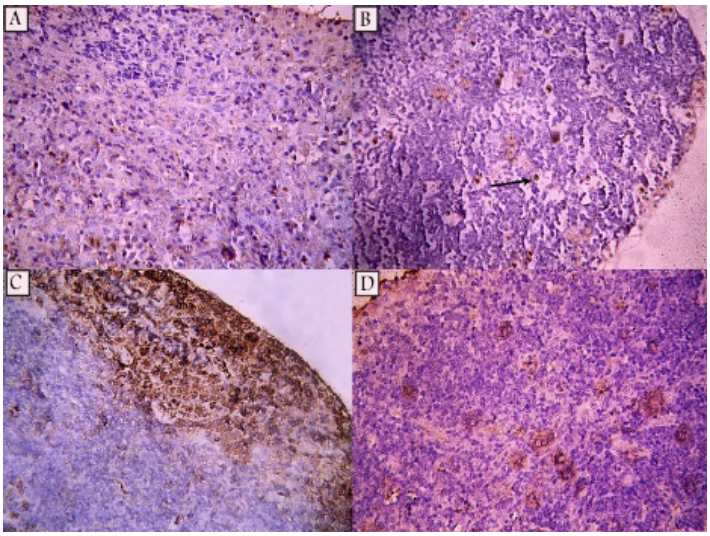

Figure 4: Photomicrographs of a mouse thymus of Group I (control) showing (**A**): weak caspase-3 immunoreactive positive cells. Group II (ghrelin) showing (**B**): weak caspase-3 immunoreactive positive cells. Notice apoptotic thymocyte inside tingible body macrophage (↑). Group III (stress) showing (**C**): strong caspase-3 immunoreactivity in many cells in the cortex. Group IV (stress and ghrelin) showing (**D**): moderate caspase-3 immunoreactivity in some cells in the medulla [Avidine biotin peroxidase technique X400].

Groups	Weight of the Thymus (mg)	Optical density of caspase-3
Control Group I	51.4 ± 1.14	61.36 ± 0.8
Group II	49.6 ± 0.54	57.17 ± 1.82
Group III	26.8 ± 0.83*	90.26 ± 2.23▲
Group IV	43.8 ± 0.84○■	69.77 ± 0.63♠♦

*-Significant decrease compared to all groups, ○Significant increase compared to Group III,■-Significant decrease compared to Groups I & II, ▲-Significant increase compared to all Groups, ♠-Significant decrease compared to Group III, ♦-Significant increase compared to Groups I & II.

Table 1: showing mean ± standard deviation of weight of thymi and optical density of caspase-3 immunohistochemical stain in different groups.

Electron-microscopic study

Ultrastructure examination of sections of Group I showed thin capsule with sub capsular population of thymocytes of different sizes. Thymocytes were rounded with heterochromatic nuclei and thin rim of cytoplasm surrounded by processes of TECs. Type I Cortical Epithelial Reticular Cells (cTECs) were seen sub capsular resting on basal lamina. They had elongated or rounded cell body and few processes with multiple vacuoles (Figure 5). TECs had dark cytoplasm and numerous processes. Tingible body macrophages containing cellular debris and apoptotic thymocytes were detected (Figure 6). Medullary TEC was distinguished by its euchromatic nucleus and remarkable number of desmosomes. Many thymocytes surrounded mTECs (Figure 7). The grouping of TECs in the medulla formed the characteristic Hassall's corpuscle. The outer most layer was formed of flat dark TECs. Inner mTECs had euchromatic nuclei. Several processes of mTECs could be detected in addition to thymocytes (Figure 8). The ghrelin injected Group II showed thin capsule and population of thymocytes of varying sizes with processes of TECs in between (Figure 9). Dark, elongated dendritic cells were found. Their dark processes formed a network that ran in between thymocytes. (Figure 10). However, Group III (stress) showed increased thickness of capsule with widely separated thymocytes. TECs showed increased vacuolation and cellular debris in their processes (Figure 11). Degenerative changes in TECs were seen resulting in widening of intercellular spaces which contained necrotic material and fat droplets (Figure 12). There was a notable increase of terminally differentiated TECs with clumps of heterochromatin in their nuclei. Hassall's corpuscle was found in medulla with central cystic degeneration containing cellular debris (Figures 13,14). Meanwhile, Group IV (stress and ghrelin) showed a thin capsule with mitotically active TEC. Normal processes of TECs containing mitochondria were present in between thymocytes. (Figure 15). Distinctive presence of large nursing cell was observed. It was identified by its large size, euchromatic nucleus, numerous mitochondria and lysosomes and its processes harboured thymocytes (Figure 16). Medulla showed Hassall's corpuscle surrounded by flat heterochromatic TECs, euchromatic TECs and with dendritic and plasma cells (Figure 17).

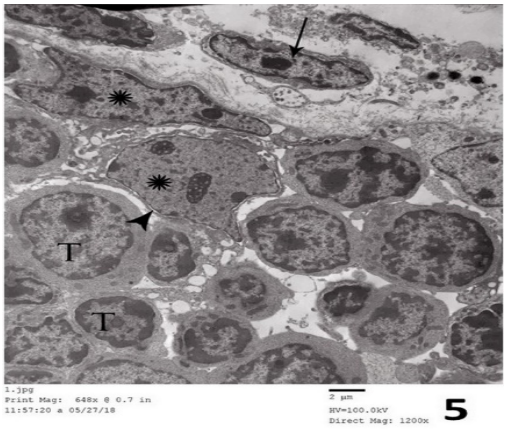

Figure 5: An electron micrograph of a section of mouse thymic cortex of Group I (control) showing connective tissue capsule containing elongated fibroblasts (↑). The cortex consists of numerous different sized thymocytes (T) surrounded by vacuolated processes of epithelial reticular cells (cTECs). Notice type I TECs (*) with flat or rounded euchromatic nucleus with two nucleoli resting on basal lamina (▲) TEM X1200.

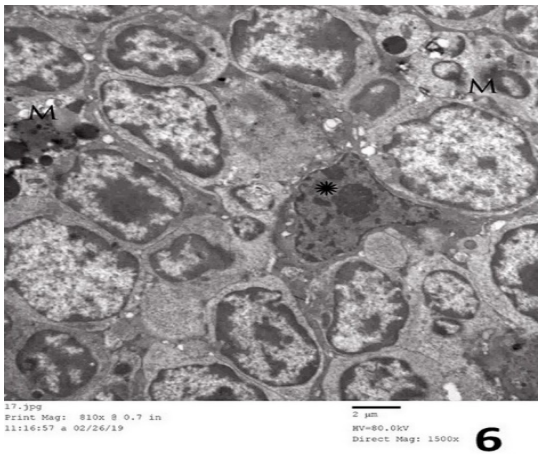

Figure 6: An electron micrograph of a section of mouse thymic medulla of Group I (control) showing medulla containing type IV TEC with euchromatic nucleus, long processes and dark cytoplasm (*). Notice macrophages (M) containing apoptotic thymocytes with cellular debris TEM X1500.

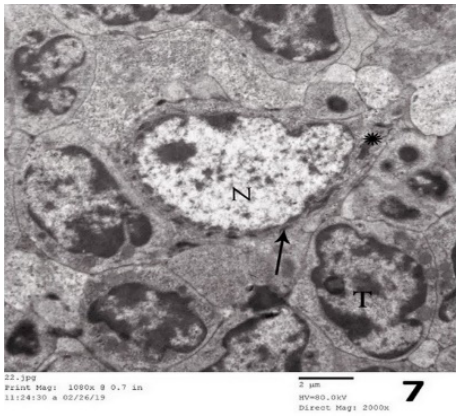

Figure 7: An electron micrograph of a section of mouse thymus of Group I (control) showing mTEC (*) having euchromatic nucleus (N) and multiple desmosomes (↑). Notice many thymocytes (T) surrounding mTEC TEM X2000.

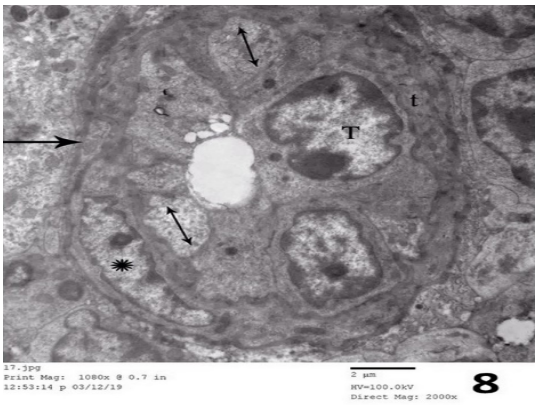

Figure 8: An electron micrograph of a section of mouse thymic medulla of Group I (control) showing Hassall's corpuscle (↑) consisting of euchromatic nucleus of mTEC (*) with cellular processes (↕), terminally differentiated TEC (t) and thymocyte (T) TEM X2000.

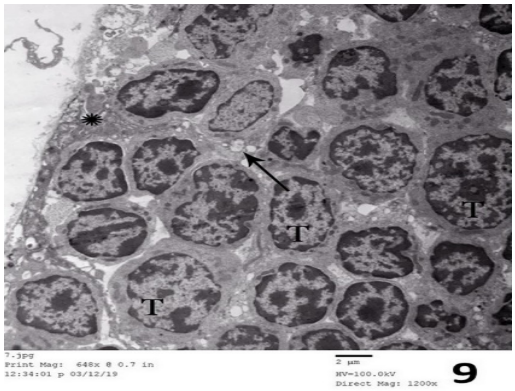

Figure 9: An electron micrograph of a section of mouse thymic cortex of Group II (ghrelin) showing thin capsule (*), cTECs with vacuolated processes (↑) and thymocytes of different sizes (T) TEM X1200.

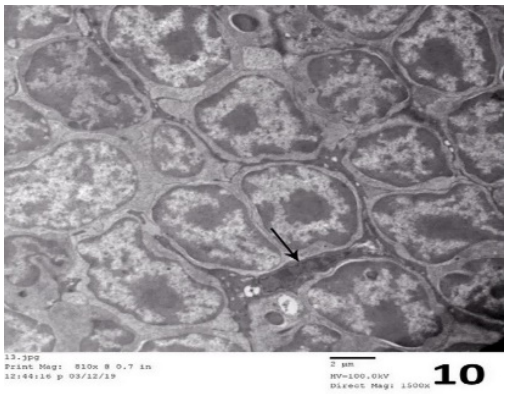

Figure 10: An electron micrograph of a section of mouse thymic medulla of Group II (ghrelin) showing dendritic cell with thin processes forming network (↑) TEM X1500.

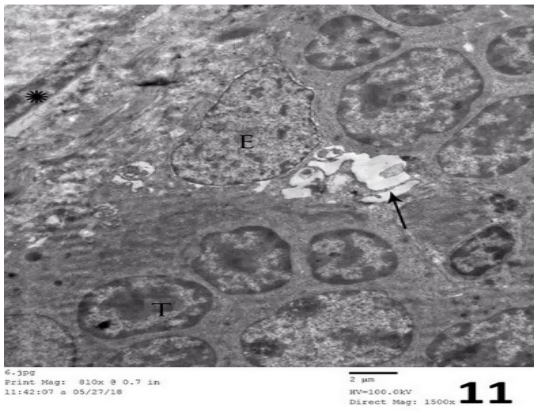

Figure 11: An electron micrograph of a section of mouse thymic cortex of Group III (stress) showing thick capsule with fibroblasts (*). Type I TEC (E) with processes containing phagocytic materials (↑). The cortex is formed of widely separated thymocytes (T) TEM X1500.

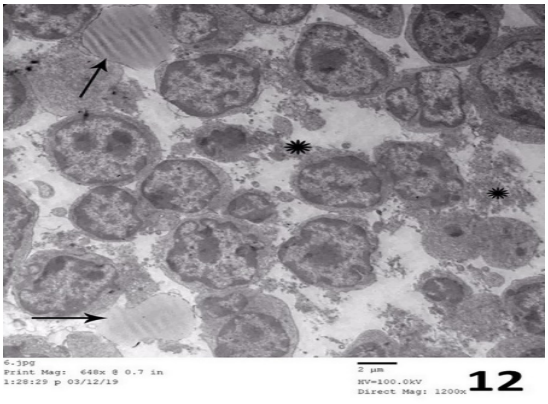

Figure 12: An electron micrograph of a section of mouse thymic cortex of Group III (stress) showing widely separated thymocytes. Notice presence of cellular debris (*) and fat droplets (↑) in intercellular space TEM X1 200.

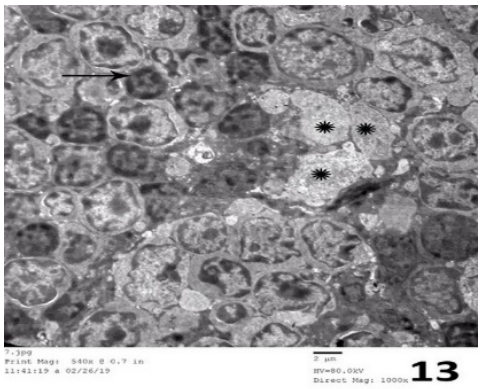

Figure 13: An electron micrograph of a section of mouse thymic medulla of Group III (stress) showing group of TECs with clumps of heterochromatin (↑). Notice wide perivascular space containing reticular fibers (*) TEM X1000.

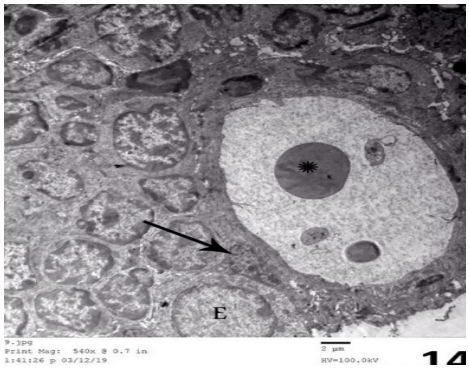

Figure 14: An electron micrograph of a section of mouse thymic medulla of Group III (stress) showing Hassall's corpuscle formed of rounded euchromatic mTECs (E) and several flat mTECs (↑) with clumps of heterochromatin in their nuclei. Notice cystic change of Hassall's corpuscle. Its lumen contains degenerative materials (*) TEM X1000.

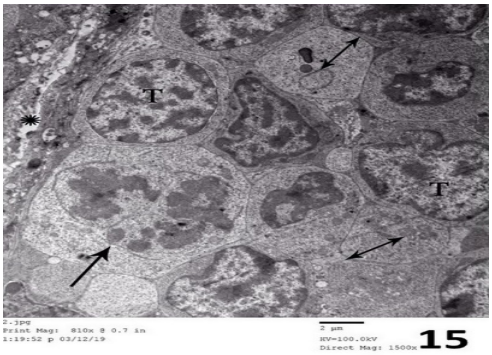

Figure15: An electron micrograph of a section of mouse thymic cortex of Group IV (stress and ghrelin) showing capsule (*), closely packed cells surrounded by normal wide processes of TECs (↕) containing mitochondria, multiple thymocytes (T) and mitotic epithelial reticular cell (↑) TEM X1500.

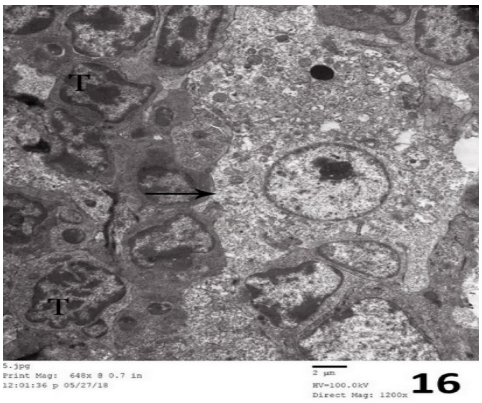

Figure 16: An electron micrograph of a section of mouse thymus of Group IV (stress and ghrelin) showing nursing cell (↑) with euchromatic nucleus and abundant cytoplasm containing mitochondria and electron dense granules surrounded by numerous thymocytes (T) TEM X1200.

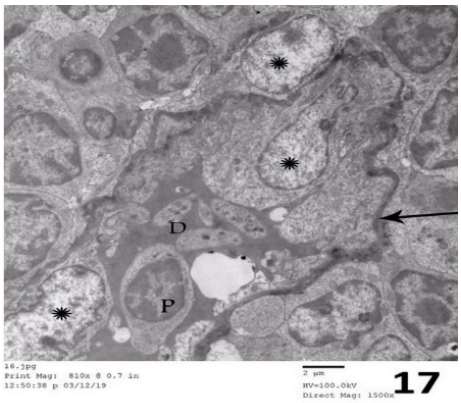

Figure 17: An electron micrograph of a section of mouse thymic medulla of Group IV (stress and ghrelin) showing Hassall's corpuscle consisting of, numerous euchromatic TECs (*), Outer flat terminally differentiated TECs (↑), a network of dendritic cell (D) and one plasma cell (P) TEM X1500.

Morphometric and statistical results

The morphometric and statistical results between the two groups (Group I) Control and (Group II) Ghrelin showed non-significant changes P>0.05.

- The mean weight of thymus in different groups showed significant decrease (P≤0.05) in Group III (stress) as compared with the other groups. Meanwhile, there was a significant increase (P≤0.05) in Group IV (stress + ghrelin) as compared with Group III (Table 1).
- The mean optical density of caspase-3 immunohistochemical stain showed significant increase (P≤0.05) in Group III (stress) as compared with the other groups. Meanwhile, there was a significant decrease (P≤0.05) in Group IV (stress + ghrelin) as compared with Group III (Table 1).

Discussion

In the present study, mouse was used as animal model because of its functional thymus and robust immune system [9]. Thymus gland is very sensitive to stress and expresses great number of glucocorticoid receptors [3]. In Group III (stress group) of the present study significant decrease in thymic weight, loss of thymic lobulation and cortico-medullary demarcation were detected. Infiltration of thymic lobules with brown adipocytes and mononuclear cellular infiltration was noticed. This was consistent with the findings of [10]. Moreover, other scientists [11] stated that trans-capsular migration of inflammatory cells could occur in stressed thymus due to decrease in cortical thymic population. Meanwhile, Others [12] declared that protein of brown adipocytes allowed thymic atrophy. Mesenchymal cells could migrate and accumulate lipid in the thymus in the presence of cellular infiltration. They also recorded that mesenchymal cells could change into adipocytes under inflammation. An increase in inflammatory cellular infiltration and elevated corticosteroid levels led to an increase in thymic permeability with loss of blood thymic barrier [13]. However, some scientists [1] stated that the increase in thymic permeability allowed recirculating T cells to enter the thymus in an attempt to compensate thymocytes depletion. Electron microscopic study of the same group revealed destruction of cellular processes of TECs with widening of intercellular spaces. Some investigators [14] stated that inflammatory cells led to destruction of TECs. This could accentuate thymic atrophy and suppressed thymic function. Moreover, other researchers [15] stated that inflammatory cytokines could activate hypothalamic-pituitary-adrenal (HPA) axis. This led to release of adrenal glucocorticoids resulting in negative feedback (decrease in cytokine release) and modulation of immune response.

Marked decrease in the cellularity of thymic cortex was recorded in Group III. This was confirmed by significant increase of capase-3 immunohistochemical cellular optical density in this study. Some researchers [16] postulated that the extensive apoptosis led to an increase of antigen presentation and development of autoimmune disease. While, others [17] stated that modification of TECs during stress led to change in their pattern of MHC expression which interfered with thymocytes education.

This led to the loss of cortical thymocytes. Researchers [18] also stated that as thymic cortex contains small immature cells, these cells are sensitive to corticosteroids than mature cells. The present study revealed many thymocytes traversing the wall of the capillaries migrating to the outside. Some authors [19] noted that during stress, there was premature release of double positive thymocytes which represent 80% of the cells into the periphery. The migration of DP cells was aided by increased perivascular space that contained extracellular matrix like fibronectin that increased cellular migration. Recently scientists [1] confirmed early release of DP cells to the periphery in case of thymic atrophy by abnormal increase in DP cells in lymph nodes. Moreover, others [20] described early release of DP cells was an adaptive mechanism to protect DP cells of stress induced cell death. Other scientists [15] stated that glucocorticoid caused decrease in TCR signalling capacity of thymocytes. Decrease of TCR signalling led to death of thymocytes. The normal development of thymocytes needs energy expenditure which is down regulated in stress. On the contrary cellularity of medulla was not affected in Group III of the present study. This was confirmed by absence of caspase-3 positive cells noted in the current study. The mTECs formed groups of terminally differentiated cells with no formation of Hassall's corpuscle This was due to premature differentiation of mTECs to escape apoptosis. Some investigators [14] declared that medullary thymocytes were more mature and less affected by glucocorticoid. Moreover, other authors [21] stated that early release of double positive cells during stress allowed few cells to complete their developmental pathway through medulla. Negative selection was stopped and there was no need for cell death by apoptosis. This was confirmed by regression of Hassall's corpuscle of present study. Injection of ghrelin in normal thymus of the present study (Group II) resulted in starry sky appearance of thymic cortex. The starry sky was due to prominent increase in the phagocytic activity of macrophages in ghrelin injected group. Ghrelin increased autophagy and activated the process of removing damaged organelles and misfolded proteins [22].

In the Ghrelin protected group (Group IV) of the present study: The thymus had more or less normal histological structure. There was significant increase in thymic weight. This was in accordance with the study of [1]. Investigators [23] stated that ghrelin reorganize thymic architecture by acting on TECs and progenitor cells receptors that are markedly upregulated during stress. Ghrelin activated signalling molecules for T cell activation and proliferation. However, in some specimens, there was hyperplastic progenitor cells in thick capsule adjacent to the blood vessels. This was also recorded by researchers [5] who stated that ghrelin increased proliferation of progenitor cells. Premature delivery of thymocyte to circulation occurred in stress. As a result, progenitor cells were constantly recruited from bone marrow and penetrated vascularized department between cortex and medulla. Moreover, the same group in this study showed that the thymic cortex preserved its cellularity. This was confirmed by significant decrease in caspase-3 immunohistochemical cellular optical density in the protected group. Also, E/M revealed normal processes of TECs which contained mitochondria. Meanwhile, recently authors [3] declared that ghrelin suppressed apoptosis of cortical cells by reduction of BAX and improved BCl2/BAX ratio. Thus, promoted cell survival. Also, scientists [5] stated that ghrelin inhibits apoptosis of thymic endothelial cells and thymocytes by expression of uncoupling protein 4CP2 that buffered the production

of ROS. In addition, ghrelin has anti-inflammatory action through suppression of pro-inflammatory cytokines. Furthermore, in ghrelin protected group the presence of numerous Hassall's corpuscle was detected by light microscope. By E/M the Hassall's corpuscle was composed of euchromatic mTEC, terminally differentiated mTEC, network of dendritic cells and plasma cells. The presence of Hassall's corpuscle denoted active thymopoeisis of surviving thymocytes. Researchers [24] stated that Hassall's corpuscles appeared when lymphopoeisis was already established and cortex, medulla and cortico-medullary junction could conduct positive and negative selection of thymocytes. Moreover, there was increased appearance of dark migrating dendritic cells which were involved in formation of Hassall's corpuscles. These cells were suppressed in stress group due to lack of Hassall's corpuscle formation [25]. The protective effect of Ghrelin targeted TECs of thymus. The dark mTECs showed proliferation. TECs are responsible for production of thymus hormones including thymulin which has an important role in both intra and extra thymic T cell differentiation [26]. Regarding the mechanism of action of ghrelin: Cells of thymus (T cells and TECs) had a receptor for ghrelin. Acetylated form of ghrelin is a natural ligand for growth hormone receptor which resulted in growth hormone release. As TECs had receptor for growth hormone, so TECs were the main target for ghrelin hormone [27]. Ghrelin reduced corticosteroid level. It attenuated hypothalamic pituitary adrenal axis in response to acute stress. It acted on anterior pituitary to stimulate ACTH negative feedback on corticosteroid release. In addition, thymosin release under the effect of Ghrelin block binding of steroids to its receptor [28].

Conclusion

Thymus is important for production of mature functioning self-tolerant T lymphocytes. During stress, thymus delivered immature DP cells to the periphery. DP cells were non-functioning and auto-reactive. Ghrelin hormone had stimulatory effect on both TECs and progenitor thymocytes. In addition to its role in decrease corticosteroid level.

Conflicts of Interest

The authors declare no conflicts of interest.

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