Original Article

Swimming Exercise Ameliorates the Chronic Immobilization Stress-Induced Alterations in Spleen and Splenic T-cell Population in Adult Male Albino Rats: Histological and Immunohistochemical Study

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ABSTRACT

Background: Stress is an integral part of modern life results in long-term alterations of the immune system. Exercise may have effects on immune functioning.

Aim of the Work: To study chronic stress-induced alterations on serum corticosterone levels, splenic antioxidants, splenic morphology, and splenic T cell population, also to investigate the influence of swimming-exercise to counteract these alterations.

Methods: Forty-rats were equally divided into four groups: control group, exercised group (EX-group), immobilization stressed group (IS-group) and exercised & immobilization stressed group (EX&IS-group). Assessment of serum corticosterone, splenic malondialdehyde (MDA), splenic total antioxidant capacity (TAC), as well as histological and immunohistochemical approaches were done.

Results: EX-group had similar results if compared to control group apart from significantly increased TAC and decreased MDA levels. IS-group had significantly increased corticosterone and MDA with significantly decreased TAC levels, significantly decreased white pulp number and size, loss of the prominent marginal zone and expansion of red pulp with decreased cellularity if compared to control and EX-groups. While, exercise in EX&IS-group significantly decreased corticosterone and splenic MDA with significantly increased TAC levels, preserved the normal architecture with significant increased number and size of the lymphatic follicles and increased cellularity compared to the IS-group. Immunohistochemicaly, IS-group had significantly decreased CD3+ and CD4+ cells with a significantly increased CD8+ and apoptotic cells. Meanwhile, exercise prevent the effects of stress in EX&IS-group which had significantly increased CD3+ and CD4+ cells and significantly decreased CD8+ and apoptotic cells compared to the IS-group.

Conclusion: Swimming exercise showed significant counteraction against the effect of chronic stress on serum corticosterone levels, splenic antioxidants, spleen microarchitecture changes and its cellular phenotypes, and also suppression of apoptosis of splenocytes. Exercise conditioned the animals to tolerate the various effects of stress, in turn, it could be hypothesized that active lifestyle is likely to be beneficial to immune function in stress exposure.

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Key Words: Caspase-3, CD, chronic stress, spleen, swimming exercise.

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INTRODUCTION

Psychological and physical disorders bi-directionally influence each other. Their comorbidity may be due to inflammatory processes[1].

Stress is an integral part of our modern life. Stressful conditions embrace a wide range of internal or environmental events, e.g. unemployment, financial worries, interpersonal conflicts, job strains, bereavement etc.[2]. Stressful life events results in long-term alterations of the immune system with decrease immune function and increase disease susceptibility[3].

Psychological and physical stress can enhance or suppress immunity depending on several factors as the severity or duration of exposure[4]. Stress exposure increases glucocorticoid release which promotes the immune response during acute stress and inhibit it during chronic stress[5]. This condition can disrupt immune cell responses particularly by altering patterns of cytokine secretion[6] resulting in suppression of T helper cells, enhancement of suppressor cells, and increases T cells apoptosis[7].

The immune system is a group of cell types that defending the body against infection and tumor cells. The adaptive arm of the system is mediated by B and T lymphocytes[1]. The lymphoid organs are the specialized tissues where lymphocytes are generated in primary organs and then transferred to the secondary organs[8]. Secondary lymphatic organs are the sites where maturation of lymphocytes

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when stimulated respond to invading pathogens occurs[9]. The spleen; one critical immunological secondary organ, harbors one-fourth of the body's lymphocytes and mediates both innate and adaptive immune responses[10]. Spleen is not only considered an important immune organ, but also plays a key role in the brain-periphery connection and can have significant effects on behavior[11]. Thus , stress and inflammation form a neuroimmune circuit[12].

To study the function of stress, several animal models have been developed. Immobilization stress considered an easy method to induce both physical (muscle work) and psychological stress (escape reaction)[13]. It was believed to be the most severe type of stress in rodent models and has a comparative effect in humans[14].

Life-style factors; diet, exercise, and sleep may have effects on immune functions. Thus, may profoundly influence a variety of preventable illnesses, as well as many physical disorders, especially those that involve an inflammatory component[1]. Swimming exercise is widely used as an appropriate model of physical exercise because swimming is a natural ability of rats. Swimming exercise under proper experimental conditions (in a moderate intensity and at body temperature) primarily involves physical exercise with little emotional arousal[15]. Despite the broad rehabilitative probable of swimming exercises, the relationship between swimming exercise and the immune system has not been fully elucidated to date[16].

It was of interest to find approaches to avoid the appearance of immune-related stress disorders especially with increased daily stress exposure. Thus, this experiment aimed to investigate chronic stress-induced alterations on corticosterone levels, splenic lipid peroxidation, cytoarchitecture of splenic tissues, splenic T lymphocyte subsets and apoptosis of splenocytes. Also, it aimed to evaluate the influence of swimming exercise to counteract stress-induced alterations in rat spleen.

MATERIAL AND METHODS

Reagents

- The enzyme-linked immunosorbent assay kit (ELISA) for measuring corticosterone levels (Sigma Aldrich, Giza, Egypt).
- Commercially available colorimetric assay kits (Bio-diagnostic, Giza, Egypt) for malondialdehyde (MDA) and total antioxidant capacity (TAC) assay.
- Antibodies:
  - Polyclonal rabbit anti-CD3 antibody (1:50 dilution, Dako. Denmark) was used for the identification of T cells.
  - Monoclonal mouse anti-CD4 antibody (1:50 dilution, Dako. Denmark) was used for identification of T helper cells.
  - Monoclonal mouse anti-CD8 antibody (ready-to-use, Dako. Denmark) was used for labeling cytotoxic T cells.
  - Polyclonal rabbit anti-caspase-3 antibody (1:200 dilution, Thermo Fisher Scientific, USA purchased from Sigma Aldrich Company, Egypt) was used for labeling apoptosis.

Animals and ethical approval

Forty adult male Wistar albino rats (8–10 weeks, 200-250 g) were purchased from the National Research Center, Cairo, Egypt. They were housed in standard control caging (five animals per cage) for 14 days for acclimatization. Cages were kept in a temperature-controlled room (22ºC±2) with a light-dark 12:12 cycle and were given free access to standard diet (commercial rat chow). This study was conducted in Histology Department, Faculty of medicine, Minia University and was approved by the Minia University ethics committee (No.553: 12/2019) in agreement with the NIH Guide for Care and Use of Laboratory Animals[17].

Experimental Design

Rats were divided randomly and equally into 4 groups (10 animals each):

1. Control group: rats were maintained without disturbances.
2. Exercised group (EX-group; 3 weeks of swimming exercise): A cylindrical tank of 50 cm diameter and 75 cm depth, containing water at 30°-32°C was used for swimming exercise (Figure Ia). Individual swimming was preferred to avoid stress-induced effect by the vigorous exercise (swimming in groups promote more vigorous exercise than rats allowed swimming alone). Additionally, to reduce stress, rats were allowed to adapt to the shallow water prior to the experiment. Swimming period was initially for 15 min/day (between 9:00 and 10:00 a.m.) and was gradually increased to perform exercise for 60 min/day over one week. Then rats were made to swim for 1 h/day, 5days/week, for 4 weeks[18].
3. Immobilization Stressed group (IS-group; 3 weeks of restraint immobilization stress): Rats were individually immobilized for 1 h /day for 3 weeks in 32 cm long and 20 cm wide wire mesh restrainers (Figure Ib). Animals were unable to turn or barrel-roll. The duration of immobilization stress has been shown to be sufficient to produce marked elevations in corticosteroids, which is consistent with a chronic physiologic stress response[19].
4. Exercised and immobilization stressed group (EX&IS-group): Those rats were permitted swimming (as in EX-group) concomitant with daily sessions of stress (as in IS-group) for 3 weeks[20].

At the end of each session, rats were returned to their home cages. The control rats were left in their home cage for the entire duration of the workout.

Fig. I: The cylindrical tank used for swimming exercise (Ia) and the wire mesh restrainers used for individual immobilization of rats in the study (Ib).

Animal sacrifice and blood and tissue collection

- At the end of the experiment, rats were then sacrificed by decapitation. Stressed rats were sacrificed after 5 min from the last stress session.
- Blood samples were collected by cardiac puncture, left to clot, and then centrifuged for obtaining sera which will be stored on − 20 °C until used.
- Spleens were obtained after dissection and segmented into parts for either tissue homogenization or histological procedures. For histological examination, parts of collected spleens were kept in 10% formalin and other parts were kept at -80 °C for tissue homogenization.

Estimation of serum levels of corticosterone

Serum levels of corticosterone were measured using ELISA-kit (Enzo Life Sciences) according to the manufacturer instructions[20].

Tissue homogenization for estimation of lipid peroxides (MDA)and total antioxidant capacities (TAC)

Splenic specimens from the studied groups were weighed and homogenized separately in potassium phosphate buffer 10 mm; pH 7.4. The ratio of splenic weight to homogenization buffer was 1:10. The homogenates were centrifuged for 10 min at 5000 rpm and 4 °C, and used for analysis of lipid peroxides; MDA and TAC[21].

Histological study

- Splenic tissues were immediately fixed in 10% formalin for 24 h and then paraffin-embedded. Five-µ paraffin sections of spleen were cut, deparaffinized, rehydrated in descending series of alcohol concentrations.
- For routine histological examination: sections were stained with hematoxylin and eosin (H&E)[22].
- For immunohistochemical staining: the anti-CD3, anti-CD4, anti-CD8, and anti-caspase-3 antibodies were used according to the manufacturer's instructions. In brief, slides were deparaffinized, rehydrated, and embedded in 3% H_2O_2 to block endogenous peroxidase (10 min.) and treated with 2% trypsin to retrieve antigens (10 min.). The non-specific protein was blocked with normal goat serum (Vectastain Universal Elite ABC kit, Vector Laboratories). Sections were then incubated with primary CD3,4 and 8 antibodies for 30 min and overnight with anti-caspase-3. After washing, sections were incubated with diluted secondary antibody and ABC reagent (Vecta-stain Universal Elite, Vector Laboratories USA) for 30 min, consecutively. The 3, 3′-diaminobenzidine tetra-hydrochloride (DAB) was added for 5- 10 min and then counterstained with hematoxylin (H)[23]. Brown staining of the cytoplasm and/or the nucleus was considered as the indicator of positive

expression for caspase-3, while for CD3,4 and 8 the expression restricted to the surface membranes of T cells. Positive control slides were the control rat splenic tissue. Negative control slides were obtained as the splenic tissues were subjected to the same previous steps but with replacing the primary antibody with BPS (images not included).

Image capture

Histological sections from all the studied groups were examined using a light microscope (Olympus, Tokyo, Japan). Images were digitally captured using a digital camera (ToupView, Zhejiang, China) mounted on the microscope and connected to a computer. The ToupView software (version ×36, 3.5.563; Hangzhou ToupTek Photonics Co., Zhejiang, China) was used to control the camera and capture photomicrographs.

Morphometric study and statistical analysis

The number of white pulps (X40, low power field; LPF), the mean surface area (μm2) of lymphatic follicles from H&E stained sections X100 and the mean number of CD3, CD4, CD8, caspase-3 immune-positive cells from their immune-stained sections (X400, high power field; HPF) were measured on equally magnified images captured randomly from 10 non-overlapping fields from 6 animals of the studied groups[9].

Numerical data were obtained firstly from the used software (ToupView software) to Microsoft Excel and were then transferred to Graph Pad Prism 5 (La Jolla, CA) for analysis. Data were represented as mean ± standard error of deviation (Mean ± SED). The significant differences among each 2 groups were done via student t test and the one-way ANOVA followed by Tukey-Kramar post hoc test for multiple comparisons. *P-values* of ≤ 0.05 considered statistically significant.

RESULTS

Serum levels of corticosterone

Serum corticosterone levels had no significant difference in the EX-group if compared to the control group (p=0.0554), while the IS-group had a significant increase if compared to the control and EX-group (both p<0.0001). Exercise in the EX&IS-group ameliorated the effect of stress with a highly significant decrease in its serum level if compared to the IS-group (p < 0.0001) but still with a significant difference compared to the control (p= 0.009) (Figure 1a).

Splenic lipid peroxides (MDA) and total antioxidant capacities (TAC)

The EX-group showed a significant decrease in the splenic tissue MDA compared to the control group (p = 0.037). The IS-group showed a significant increase if compared to the control and EX-group (both p<0.0001). Meanwhile, the EX&IS-group had a significant decrease in the splenic tissue MDA if compared to the IS-group (p < 0.0001) but with a mild significant difference compared to the control (p= 0.016) (Figure 1b).

Regarding splenic levels of TAC, there was a mild significant increase in the EX-group compared to the control group (p = 0.025). The IS-group showed a significant decrease if compared to the control and EX-group (both p<0.0001). Meanwhile, exercise in the EX-IS group ameliorated the effect of stress with significant increase in splenic levels of TAC if compared to the IS-group (p = 0.001) with insignificant difference compared to the control (p= 0.144) (Figure 1c).

Histological study

H&E results

The H&E stained sections of spleens from the control group (Figure 2 a-c) and EX-group (Figure 2 d-f) showed normal histological architecture with clearly identifiable two distinct compartments; the white and red pulps. The red pulp was formed of anastomosing splenic cords separated by blood sinusoids. The splenic cords were formed of lymphocytes (had large dark nuclei with little to no eosinophilic cytoplasm), macrophages (seen engulfing hemosiderin granules), plasma cells, and red blood corpuscles (RBCs). The vascular sinuses were wide channels lined with endothelial cells. The white pulp was basophilic and formed of large round or oval masses of lymphocytes (lymphatic follicles) and a periarterial lymphatic sheath (PALS). The PALS was composed of numerous lymphocytes surrounding the central arteries. The lymphoid follicles were either primary or secondary with a germinal center (lighter central zone due to proliferating B cells). The follicles showed an image of a “double halo” due to the prominence of the marginal zone separating the white pulp from the red pulp. The IS-group (Figure 2 g-i) showed marked histological disorganization. Most notable was the reduction of clearly defined splenic white pulps (including decrease in number and size of the lymphatic follicles) with loss of the prominent marginal zone. The red pulp showed expansion with marked congestion of its dilated blood sinusoids and decreased cellularity of the splenic cords. The EX&IS-group (Figure 2 j-l) preserved the normal architecture with obvious increase in the number and size of the lymphatic follicles and increased cellularity of the splenic cords compared to the IS-group. Also, the marginal zones were well defined. Morphometric study confirmed a significant decrease in the number and mean surface area of the lymphatic follicles in IS-group compared to the control and EX-group (all p≤0.05). Exercise in the EX and IS-group prevented the decline in number and surface area of lymphatic follicles seen in the IS-group with significant increase of both in EX and IS-group if compared to IS-group (all p≤0.05) (Table 1; Figure 2 m,n).

Immunohistochemical results

Expression of CD3

Spleen sections immune-stained for CD3 from the control group (Figure 3 a-c) and the EX-group (Figure 3 d-f) showed normal distribution of the CD3 immune-positive cells which were of large number in the PALS and also composed the main cells in the splenic cords with no significant difference (p= 0.313). The IS-group (Figure 3 g-i) had an obvious and significant decrease in the CD3 immune-positive cells in the white and red pulps with an obvious decrease in cellularity of spleen compared to the control group and the EX-group (both $p < 0.0001$). In the EX&IS-group (Figure 3 j-l), the CD3 immune reaction was significantly increased compared to the IS-group ($p < 0.0001$) and the cellularity of spleen was restored almost resembled those of the control group and EX-group with no significant differences (p=0.913; 0.169 respectively) (Figure 3m).

Expression of CD4

Spleen sections immune-stained for CD4 T helper cells from the control group (Figure 4 a-c) and the EX-group (Figure 4 d-f) showed normal distribution of the CD4 immune-positive cells which were the main cell of the T cells in the PALS and the splenic cords with no significant difference (p= 0.844). While there was an apparent and significant decrease in the number of CD4 immune-positive cells in IS-group (Figure 4 g-i) compared to the control group and EX-group (both $p < 0.0001$). Only a few scattered faintly stained brownish CD4 cells were observed in the PALS of the white pulp or in the cords of red pulp. Meanwhile, the EX and IS-group (Figure 4 j-l) had a significantly increased T helper population compared to the IS-group ($p < 0.0001$) resembling the control and EX-groups with no significant differences with both (p=0.458; 0.675 respectively) (Figure 4m).

Expression of CD8

Spleen sections immune-stained for CD8 cytotoxic T cells from the control group (Figure 5 a-c) and the EX-group (Figure 5 d-f) showed normal distribution where few cells were scattered either in the white or red pulps with no significant difference (p= 0.878). While there was an apparent and significant increase in the number of CD8 immune-positive cells in IS-group (Figure 5 g-i) compared to the control group and EX-group (both $p < 0.0001$). Meanwhile, the EX&IS-group (Figure 5 j-l) had a significantly decreased cytotoxic T cells compared to the IS-group ($p < 0.0001$) resembling the control group and EX-group with no significant differences with both (p=0.374; 0.436 respectively) (Figure 5m).

Apoptotic assay

Spleen sections immune-stained for caspase-3 from the control group (Figure 6 a-c) and the EX-group (Figure 6 d-f) showed few cells were scattered either in the white or red pulps with no significant difference (p=0.785). While there was an apparent and significant increase in the number of caspase-3 immune-positive cells in IS-group (Figure 6 g-i) compared to the control group and EX-group (both $p < 0.0001$). Meanwhile, the EX and IS-group (Figure 6 j-l) had a significant decrease in caspase-3 immune-positive cells compared to the IS-group ($p < 0.0001$). However, it had a significant increase compared to control group and EX-group (p=0.010; 0.015 respectively) (Figure 6m).

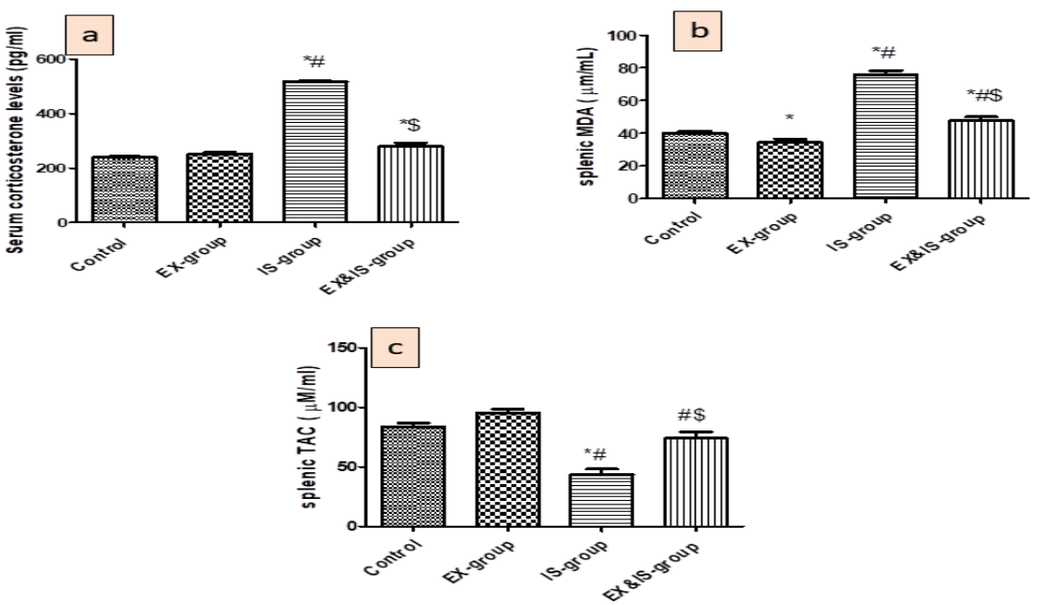

Fig. 1: The mean serum corticosterone levels (a), splenic MDA (b) and splenic TAC (c) in the studied groups (n = 6). * vs Control group, # vs EX-group, $ vs IS-group; significant at $p \leq 0.05$.

Figure 2

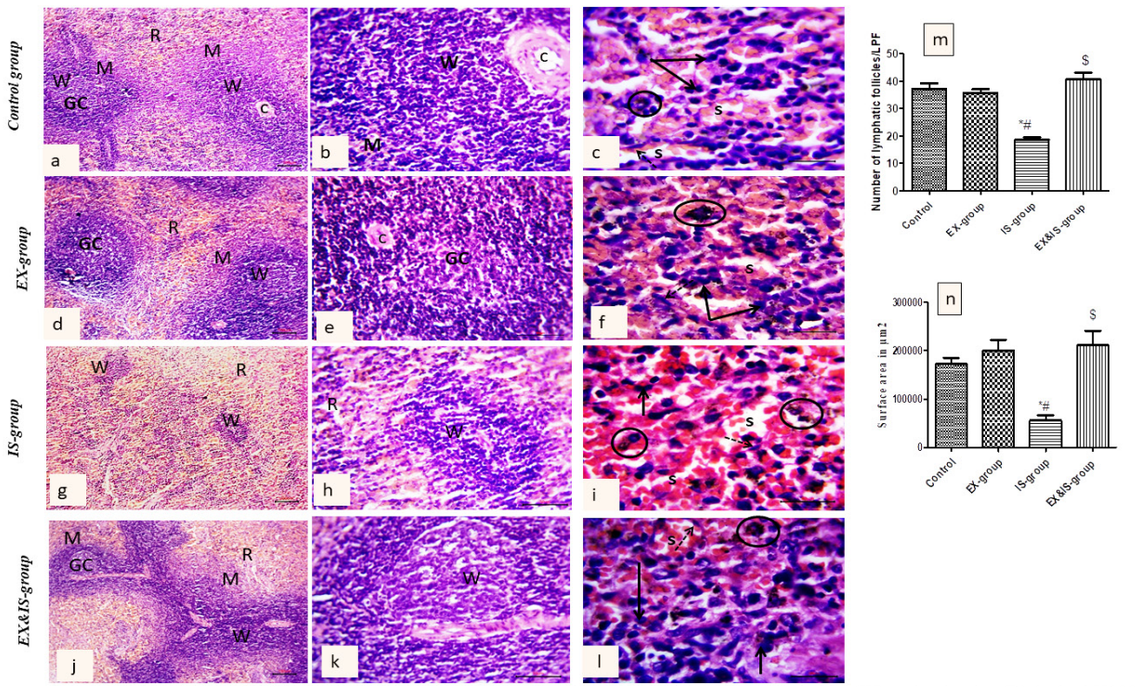

Fig. 2: Representative photomicrographs of H&E sections of the spleen tissues showing the splenic architectures; white (W) and red pulps (R) (a,d,g,j X100), the white pulp (b, e, h,k X400) and red pulp (c, f, i,lX1000). Notice the shrinkage of the white pulp (arrows) and expansion of the red pulp with decreased cord´s lymphocytes in IS-group and preservation of normal appearance in EX&IS-group. Control group (a-c), EX-group (d-f), IS-group (g-i), and EX&IS-group (j-l). W: white pulp; R: red pulp; M: marginal zone; GC: germinal center; c: central arteriole; arrow: splenic cords; s: sinusoids; dashed arrows: endothelium; circles: hemosiderin-loaded macrophages. m) The mean number of the lymphatic follicles in the studied groups (n = 6). X 40 and Scale bar =200 µm. * vs Control group, # vs EX-group, $ vs IS-group; significant at $p \leq 0.05$. n) The mean surface area of the lymphatic follicles in the studied groups (n = 6). X 100 and Scale bar=100 µm. * vs Control group, # vs EX-group, $ vs IS-group; significant at $p \leq 0.05$.

Figure 3

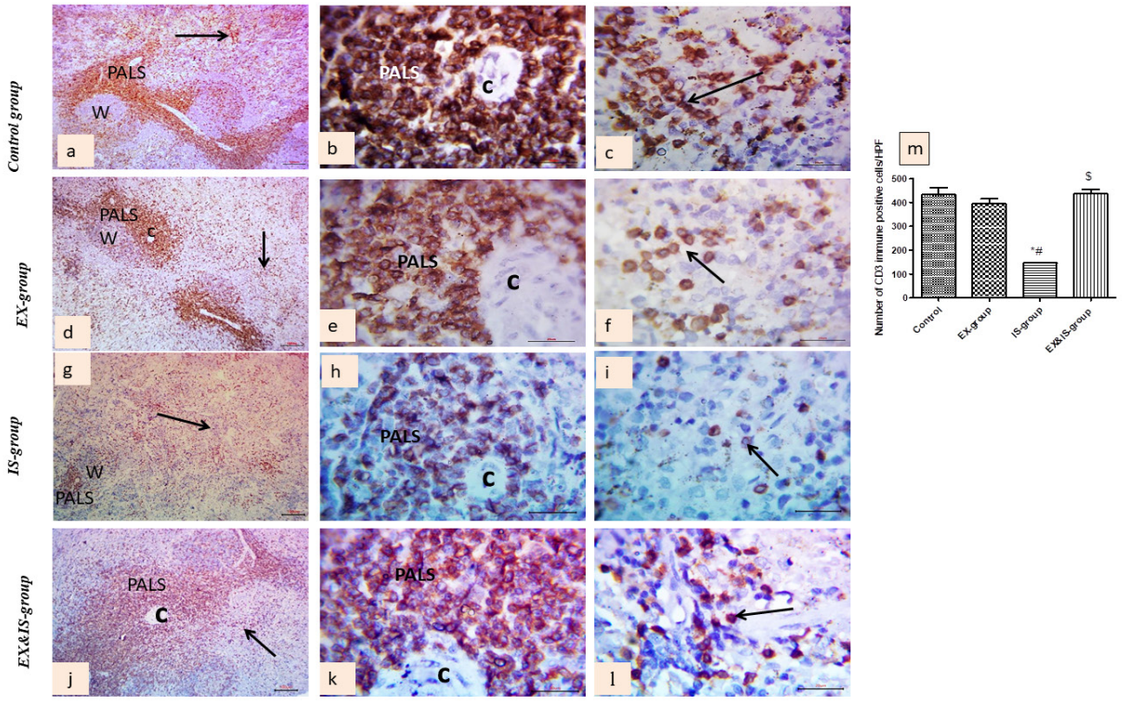

Fig. 3: Representative photomicrographs of immune-stained sections for CD3 of the spleen from the control group (a-c) and EX-group (d-f) showing a large number of CD3 immune-positive cells; T-lymphocytes (brown stained) in the PALS and red pulp cords (arrows). IS-group (g-i) showing decreased white pulps and immune-positive cells either in the PALS or in the red pulp cords (arrows). EX&IS-group (j-l) showing CD3 immune-positive cells in PALS and splenic cords resembling the control group. W: white pulp; PALS: periarterial lymphatic sheath; c: central arteriole; arrow: splenic cords. Immune-histochemistry counterstained with H (a,d,g,j X100; others X1000) m) Mean number of CD3 immune positive cells in the studied groups (n = 6)/ HPF. X 400 and Scale bar =50 µm. * vs Control group, # vs EX-group, $ vs IS-group; significant at $p \leq 0.05$.

Figure 4

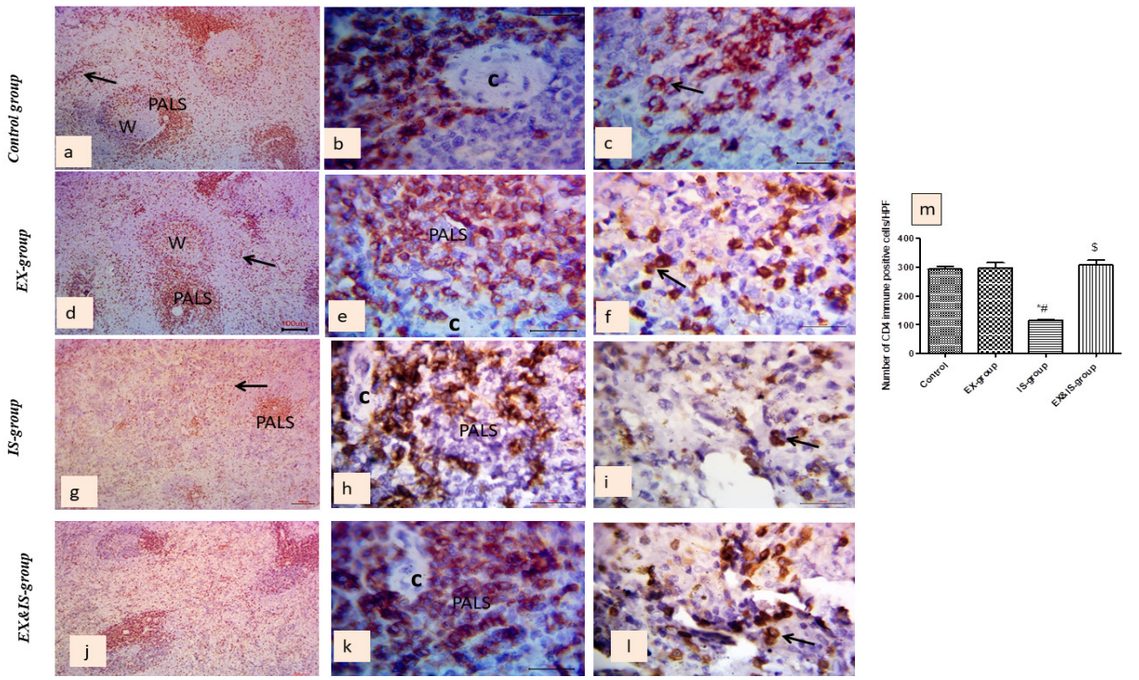

Fig. 4: Representative photomicrographs of immune-stained sections for CD4 of the spleen from the control group (a-c) and EX-group (d-f) showing most of T-lymphocytes are positive for CD4 immune-positive cells (brown stained) in the PALS and red pulp cords (arrows). IS-group (g-i) showing decreased white pulps and immune-positive cells either in the PALS or in the red pulp cords (arrows). EX&IS-group (j-l) showing CD4 immune-positive cells in PALS and splenic cords resembling the control group. W: white pulp; PALS: periarterial lymphatic sheath; c: central arteriole; arrow: splenic cords. Immune-histochemistry counterstained with H (a,d,g,j X100; others X1000) m) Mean number of CD4 immune positive cells in the studied groups (n = 6)/ HPF. X 400 and Scale bar (50 µm). * vs Control group, # vs EX-group, $ vs IS-group; significant at $p \leq 0.05$.

Figure 5

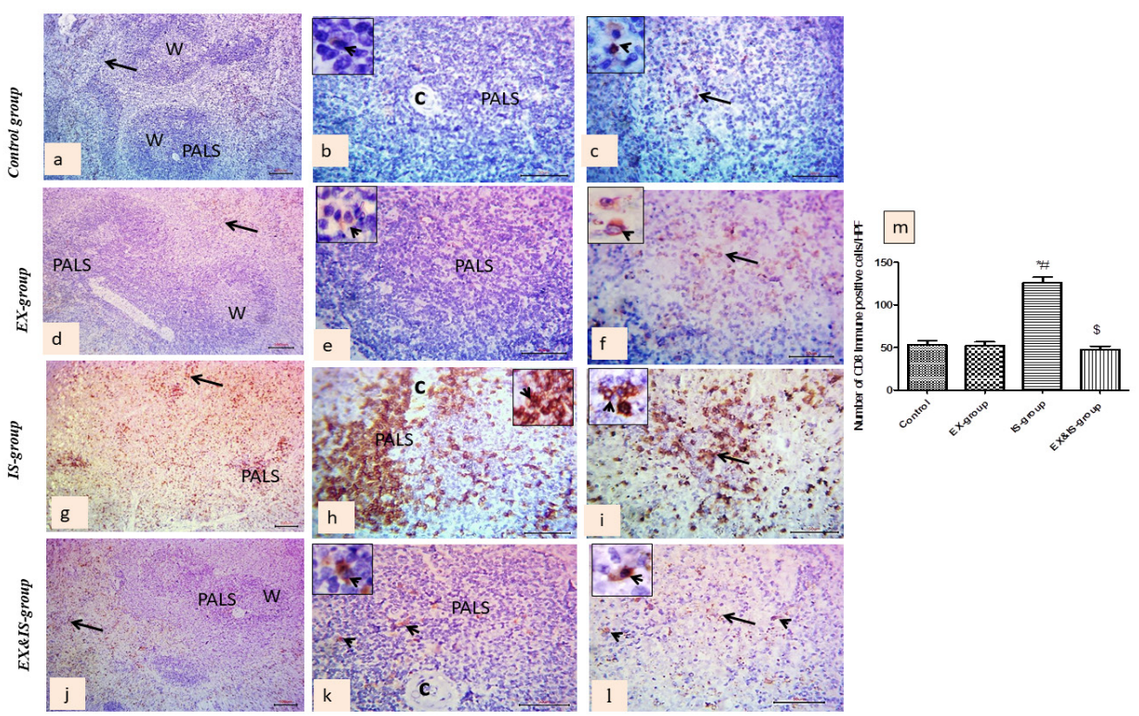

Fig. 5: Representative photomicrographs of immune-stained sections for CD8 of the spleen from the control group (a-c) and EX-group (d-f) showing few positive T-lymphocytes (brown stained) in the PALS and red pulp cords (arrows). IS-group (g-i) showing increased immune-positive cells either in the PALS or in the red pulp cords (arrows). EX&IS-group (j-l) showing CD8 immune-positive cells in PALS and splenic cords resembling the control group. W: white pulp; PALS: periarterial lymphatic sheath; c: central arteriole; arrow: splenic cords; arrowheads: immune-positive cells. Immune-histochemistry counterstained with H (a,d,g,j X100; b,c,e,f,h,iX400; insetsX1000) m) Mean number of CD8 immune positive cells in the studied groups (n = 6)/ HPF. X 400 and Scale bar (50 µm). * vs Control group, # vs EX-group, $ vs IS-group; significant at $p \leq 0.05$.

Figure 6

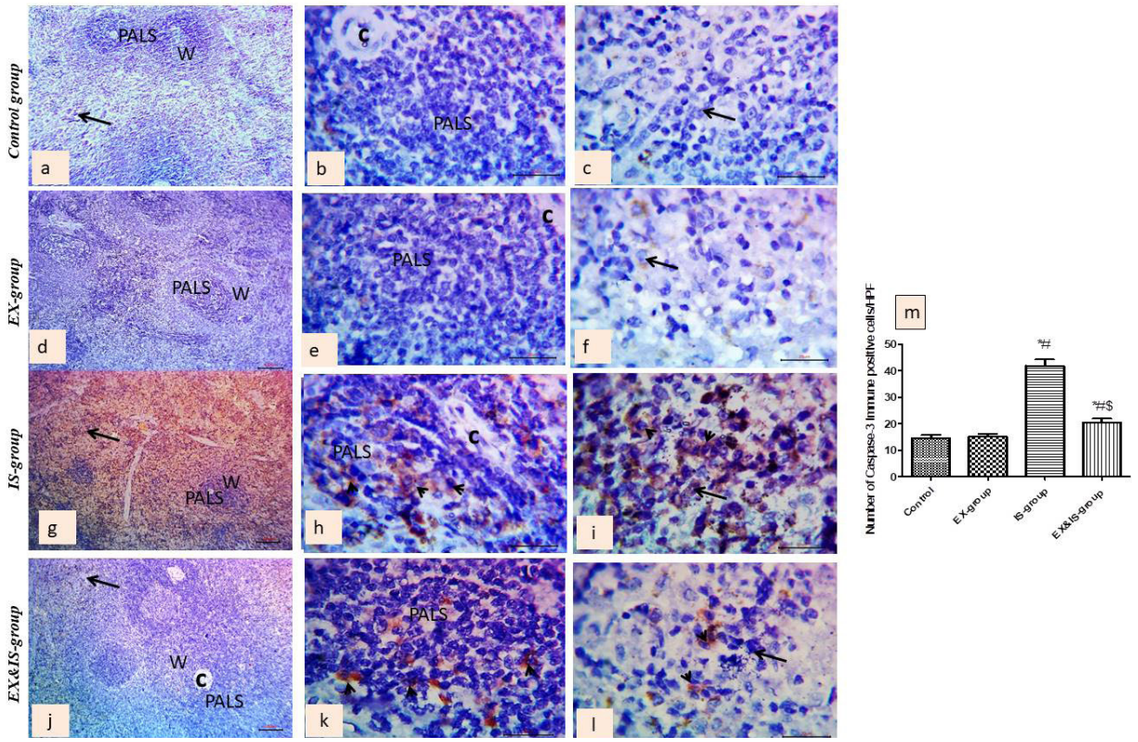

Fig. 6: Representative photomicrographs from immune-stained sections for caspase-3 of the spleen from the control group (a-c) and EX-group (d-f) showing few positive cells (brown stained) in the white and red pulps (arrows). IS-group (g-i) showing increased immune-positive cells either in the white or in the red pulps (arrows). EX&IS-group (j-l) showing decreased expression of caspase-3 immune-positive cells in white and red pulps resembling the control group. W: white pulp; PALS: periarterial lymphatic sheath; c: central arteriole; arrow: splenic cords; arrowheads: immune-positive cells. Immune-histochemistry counterstained with H (a,d,g,j X100; others X1000) m) Mean number of caspase-3 immune positive cells in the studied groups (n = 6)/ HPF. X 400 and Scale bar (50 µm). * vs Control group, # vs EX-group, $ vs IS-group; significant at $p \leq 0.05$.

Table 1: Mean follicle number and mean follicle surface area in the studied groups (n=6)

	Control group	EX-group	IS-group	EX&IS-group
Mean follicle number				
Mean ± SEM	37.17±2.09	35.67±1.31	18.67±0.84	40.67±2.33
p-value				
p^{c}		0.556	<0.0001*	0.289
p^{EX}			<0.0001$^{\#}$	0.091
p^{IS}				<0.0001$^{\$}$
Mean follicle surface area				
Mean ± SEM	172400±13020	200300±22380	55710±10930	211700±29690
p-value:				
p^{c}		0.307	<0.0001*	0.254
p^{EX}			0.0002$^{\#}$	0.766
p^{IS}				0.0006$^{\$}$

* : vs Control group, $^{\#}$: vs EX-group, $^{\$}$: vs IS-group; significant at $p \leq 0.05$.

DISCUSSION

Stress has been shown to either increase or decrease immune function depending on duration, severity, psychological tension and physical stress in both humans and animals[6,24]. Acute stress has enhancement effect, while chronic stress has a suppressive effect on the immune system[25]. Altered adaptive immunity involving T lymphocytes has been found in depressed patients and in stress-induced depression-like behavior in animal models[26].

Exercise is an activity that affects all body organs and can result in many health benefits[27]. Hence, the aim of this study was to investigate the effect of swimming exercise training to counteract the effect of chronic stress on spleen.

Coping with chronic stress, activation of the hypothalamic–pituitary–adrenocortical axis is a familiar response of vertebrates with significant increase in the glucocorticoid levels (cortisol in humans; corticosterone in rodents)[24,28]. The immunoregulation of corticosteroids is mediated by specific binding of glucocorticoids

to glucocorticoid receptors that are expressed in all leukocytes[29]. Elevated glucocorticoids are characteristic mediators during chronic stress inducing a negative immune response[30]. In this study, a significant increase in serum corticosterone levels confirmed stress exposure of rats in IS-group. Hence, the regressive changes in histological morphology observed in this group were a stress response. This was in agreement with other study which reported that chronic stress exposure causes immune suppression as shown by the involution of different lymphoid organs including the spleen[9].

Measurement of MDA is widely used as an indicator of lipid peroxidation and occurrence of oxidative stress. To verify whether the IS-group had oxidative damage on rat spleen and weather swimming exercise reduces it or not, MDA levels were estimated. Chronic immobilization stress in the IS-group significantly increased splenic concentrations of MDA compared with control animals. This finding was in line with Gavrilovic and his coworkers even with their different model of stress[21]. Increased MDA levels indicating possible alterations in the lipid matrix and cell membranes resulting from the indirect effect of reactive oxygen species (ROS) production[31]. Meanwhile, chronically stressed rats exposed to swimming exercise training had significantly decreased MDA levels compared to IS-group. This was founded in other studies concerned effect of exercise on red blood cells[32] and ilium[33]. This was in line with the reports of Belviranli *et al*.[34], who observed that exercise has protective role because the decreased oxidative damage is associated with improved aerobic metabolism induced by physical training. This might also explain the significant decrease in the splenic tissue MDA in the EX-group compared to the control group. However, this was in contrast with the study of da Cunha Araujo and his team[33] who showed increased MDA levels after 4 weeks and decreased levels only after 8 weeks of swimming exercise training in the ilium. This might related to their use of overloads tied to the thorax of rats during swimming.

On the other hand, the splenic TAC levels were significantly reduced in stressed rats while swimming exercise training significantly increased its levels toward the control levels in the EX&IS-group. Other studies[16,35] examined the effect of training exercise on chronic stress or other disorders and proved its role in elevating the antioxidant system. Also, swimming training induced liver mitochondrial adaptations to oxidative stress in rats improve the antioxidant status in the liver[15]. Thus, the results presented in this study confirmed that swimming exercise induced high splenic antioxidant enzyme levels in chronically stressed rats suggesting high readiness of spleen to repair or prevent damage by ROS in chronically stressed animals exposed to exercise. Again, this might explain the significant increase in the splenic levels of TAC in the EX-group compared to the control group.

Spleen is a complex, but highly organized, structure composed of white pulp, red pulp, and the marginal zone[36]. This was observed in the control group and with no disturbance in exercised group. Several studies demonstrated the effects of chronic stress on spleen composition[10,21,25]. In line with this concept, chronic stress in the present work altered both the histological structure and splenocyte subpopulations phenotypes with decreased splenic cellularity. It showed a severely disrupted microarchitecture where white pulps were dramatically attenuated either in number or sizes with no discernable T-cell zone; PALS compared to control rats.

Similar to the earlier reports[9,37], there was a relative decrease in cellularity of spleen in stressed rats compared to controls. Also, total T cells (CD3 positive cells) were significantly decreased in the stressed rats. This was in agreement with Domínguez-Gerpe and his co-worker[38] who proved that in general, T cells are more affected than B cells when exposed to stress. However, the present study revealed a cause of these changes, i.e. a significant increase in apoptosis of splenocytes in stressed rats. In a previous study[9], chronic restraint stress resulted in decreased splenic cellularity by a mechanism associated with CD95-mediated apoptosis. Zhang and his coworkers[25] added that chronic stress induced over expression of apoptosis-related proteins.

It is known that high levels of corticosterone causes apoptosis of immune cells[39]. Increased corticosterone levels in the IS-group might be the major cause of increased apoptosis. This might be enforced by significantly decreased corticosterone levels associated with significantly decreased apoptosis observed in the EX&IS-group.

In addition to the disruption in splenic histology and increased apoptosis induced by chronic stress, splenic lymphocyte populations showed immunophenotyping alteration with increased differentiation of T cytotoxic cells in spleen. Chronic stress can disrupt immune cells particularly by altering cytokine secretion[6]. This process results in the suppression of Type 1 helper T cells, increases the apoptosis of T cell and dendritic cell, and enhances the differentiation of suppressor cells affecting the defense system[25].

All T cells express the usual CD3 T-lymphocyte antigen[40]. The CD4 helper/inducer cells and CD8 cytotoxic/suppressor cells are 2 phenotypes of T lymphocytes, characterized by distinct surface markers and functions[41]. The splenic white pulp consists of arterioles surrounded by lymphoid cells, so it called the PALS and adjacent outpouchings of follicular lymphoid tissue[40]. The PALS is composed of predominantly CD3 T-lymphocyte and flattened reticular cells. T-lymphocytes are mostly CD4 positive with few CD8 positive cells in PALS[42]. The lymphoid follicles are composed mostly of B cells along with fewer scattered CD4 positive T lymphocytes. The red pulp contained CD4 and CD8 positive T cells[40]. This immune-distribution was exactly what was observed in the control and exercise groups in this study. Meanwhile beside the

decreased total CD3 positive T cells, immunophenotyping of lymphocytes revealed that CD4 helper T cells were significantly reduced with a relative increase in CD8 cytotoxic T cells which was also significant. However, this shift of lymphocyte immunophenotypic profiling was in stark contrast to those reported earlier by others[43,44] who mentioned decreased percentages of all T cells including the CD8 cytotoxic T cells.

Spleen is the largest secondary lymphoid organ which defends the body against blood borne pathogens[9]. Hence, such changes in lymphocyte proportions observed in the IS-group possibly have a profound effect on immune responses. The CD4+/CD8+ ratio is the ratio of T helper cells (with the surface marker CD4) to cytotoxic T cells (with the surface marker CD8). This ratio has been used as a clinically index to evaluate patients› immunity[45]. Normal ratios can invert through isolated apoptotic or targeted cell death of CD4 cells, expansion of CD8 cells, or a combination of both phenomena[41]. An altered ratio is an immune risk phenotype and can indicate diseases relating to immunodeficiency or autoimmunity[46] and a reduced ratio is associated with reduced resistance to infection[45], and is an indicator of immunosenescence[47,48] Antu</author><author>Lian, Zeqin</author><author>Wu, Huaizhu</author></authors></contributors><titles><title>T cells in adipose tissue in aging</title><secondary-title>Frontiers in immunology</secondary-title></titles><periodical><full-title>Frontiers in immunology</full-title></periodical><pages>2945</pages><volume>9</volume><dates><year>2018</year></dates><isbn>1664-3224</isbn><urls></urls></record></Cite></EndNote>.

Exercise is an activity that affects all organs and tissues and can result in many health benefits[27]. Here, the available evidence showed that exercise in the EX&IS-group had an immune-phenotypic proportion of T cells resembling those of the control with an important modulatory effect on T immunocytes and possibly on immune function.

In the last decade, there has been a remarkable increase in the number of descriptive studies on exercise and the immune system[47]. Epidemiological evidence indicates that regular physical activity and/or frequent structured exercise reducing the risk of non-communicable and diminishes the risk of a range of communicable diseases[47,49]. A common belief is that regular exercise improves one's resistance to disease by enhancing immunological function[49]. Not surprisingly then, the observations obtained in the current study where swimming exercise resulted in improvement of the tissue lipid peroxidation, morphological changes and T cells phenotypic alteration induced in the spleen by chronic stress exposure thereby leading to high rates of infectious complications in whom chronically exposed to stress providing further evidence that exercise is beneficial for immunological health.

CONCLUSION

Findings of the present study revealed that swimming exercise showed a significant counteraction against the effect of chronic stress on serum corticosterone levels, splenic antioxidants, changes of the spleen microarchitecture and its cellular phenotypes, and also the suppression of apoptosis of splenocytes. Exercise conditioned the animals to tolerate the various effects of immobilization stress, in turn, it could be hypothesized that active lifestyle is likely to be beneficial to immune function in stress exposure.

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CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

ممارسة السباحة يخفف من التغييرات التي يحدثها الإجهاد المزمن في الطحال وخلايا الطحال التائية في ذكور الجرذان المهق البالغة: دراسه هستولوجيه وهستوكيميائية مناعية

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الخلفية العلمية: الإجهاد هو جزء لا يتجزأ من الحياة العصرية يؤدي إلى تغييرات طويلة الأجل في الجهاز المناعي. التمرينات قد يكون لها تأثيرات على الأداء المناعي.

الهدف من الدراسه: دراسة التغيرات التي يسببها الإجهاد المزمن على مستويات الكورتيكوستيرون في الدم، مضادات الاكسده بالطحال، تركيب الطحال، والخلايا الطحالية ، للتحقيق في تأثير ممارسة السباحة لمقاومة هذه التغييرات.

الطريقة: تم تقسيم الجرذان الأربعين بالتساوي إلى: المجموعة الضابطة، المجموعة التي تمارس التمارين (مجموعة التمارين)، المجموعة المتعرضه للتوتر المزمن (مجموعة التوتر المزمن) والمجموعة المتعرضه للتوتر المزمن وتمارس التمارين (مجموعة التمارين والتوتر المزمن). تم قياس مستوي الكورتيكوستيرون في الدم، مالوندهايد في الطحال، والقدرة الكلية المضادة للأكسدة في الطحال، كماأجريت الدراسات الهستولوجية والهستوكيميائية المناعية.

النتائج: مجموعة التمارين كانت لها نتائج مماثلة إذا ما قورنت بالمجموعة الضابطه فيما عدا إرتفاع مستوي القدرة الكلية المضادة للأكسدة وانخفاض مستوي مالوندهايد في الطحال بدلالة احصائيه هامة. بينما اظهرت مجموعة التوتر المزمن زياده بدلالة احصائيه هامة في مستويات الكورتيكوستيرون والمالوندهايد مع إنخفاض في مستوي القدرة الكلية المضادة للأكسدة، وانخفض بشكل كبير عدد اللب الأبيض وحجمه، وفقدت المنطقة الهامشية البارزة وتوسع للب الأحمر مع انخفاض في الخلايا إذا ما قورنت بالمجموعات الضابطة و مجموعةالتمارين. في حين أن التمارين في مجموعة التمارين والتوتر المزمن قللت بدلالة احصائيه هامة مستويات الكورتيكوستيرون ومالوندهايد مع زيادة مستوي القدرة الكلية المضادة للأكسدة، وحافظت على البنية الطبيعية للطحال مع زيادة عدد وحجم البصيلات اللمفاوية وزيادة الخلايا مقارنةً بمجموعة التوتر المزمن. واظهرت الدراسه المناعية الهستوكيميائية ان مجموعة التوتر المزمن انخفضت بها خلايا +CD3 و+CD4 مع زيادة كبيرة في خلايا +CD8 وخلايا الموت المبرمج بدلالة احصائيه هامة. وفي الوقت نفسه، ممارسة الرياضه منعت تأثيرات الإجهاد المزمن في مجموعة التمارين والتوتر المزمن التي زاد فيها خلايا +CD3و +CD4 وانخفضت خلايا +CD8 وخلايا الموت المبرمج بدلالة احصائيه هامة مقارنة مع مجموعة التوتر المزمن.

الخلاصة: أظهرت الدراسه أن ممارسة السباحة كان لها رد فعل كبير ضد تأثير الإجهاد المزمن على مستويات الكورتيكوستيرون في الدم ومضادات الأكسده في الطحال، والتغيرات في بنية الطحال الدقيقة وأنماطها الخلوية، وكذلك قمع موت الخلايا المبرمج في الطحال. وعليه فإن ممارسة الرياضة جهزت الحيوانات لتحمل الآثار المختلفة للإجهاد وبدوره يمكن إفتراض أن نمط الحياة النشط من المرجح أن يكون مفيداً لوظيفة المناعة عند التعرض للإجهاد