# Histopathological and immunohistochemical studies on the role of α-Lipoic acid on cyclophosphamide-induced immunosuppressive effect in mice

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## Abstract

Cyclophosphamide (CP) is an efficient anticancer drug, has been widely used for the treatment of various neoplastic diseases. α-Lipoic acid (α-LA) functions as an antioxidant in both its reduced and oxidized form. Therefore, the present work was done to investigate the possible protective effect of α-LA against CP-induced injury in the immune system of mice.

Fifty mice were divided into five groups (each of 10 mice) according to their approximately equal mean body weights as following: positive control mice were injected i.p with 0.5 ml saline solution, 3 times/week, for 6 weeks. Positive control mice were administered daily, orally by gavage with 0.5 ml sunflower oil, for 6 weeks. Mice were injected i.p. with 0.6 mg/kg bw CP, three times/week for 6 weeks. Mice were daily administered orally by gavage with 0.5 ml α- LA in a concentration of 0.1 mg/kg bw, for 6 weeks. Mice were administered with the same doses of both CP and α-LA at the same manner and for the same period as in the previous groups.

Estimation of rate of mortality, body weightchange and the lymphoid organs weights (spleen and thymus) were evaluated.Also, assessment of T- lymphocyte proliferative and T-cell function, using Con A-mitogen as well as immunohistochemical detection of CD3 as a pan T cell marker present on the mature T lymphocytes were estimated. Also, histopathological alterations in spleen and thymus tissues and evaluating the possible ameliorative effect of α- LA were investigated. The results revealed that in CP-treated mice, different symptoms of toxicity including reduced activity, general weakness were observed, and there was an increase in the rate of mice mortality and significant decrease in the body weights. Also, no significant decreases in the relative spleen weights were detected; however, the relative thymus weights were significantly decreased.The histological observations in spleen of CP-treated mice revealed severe histopathological changes which include: disorganized structure, increased size of the lymphoid follicles, marked depletion in lymphocyte population, and the increase in the number of megakaryocytes. All these pathological alterations were markedly decreased in spleen sections of CP+α-LA- treated mice. In thymus sections, CP was found to cause massive depletion in the thymocyte population, decrease in the number of the reticular cells and macrophages, and an increase in the number of Hassall's corpuscles. However, α-LA could partly reverse the changes induced by CP in thymic tissue. Theimmunohistochemical detection of CD3 in spleen sections of CP-treated mice, revealed very weak reaction. However, moderate staining reaction was found in the splenic follicles of CP+ α-LA-treated mice, indicating that α-LA. In thymus tissues, very weak staining reaction was observed in the different thymic sections of CP-treated mice, however, moderate staining reaction was observed in most areas of thymus in CP+α-LA-treated mice. Hence, the current study proved the protective effect of α-Lipoic acid on the immunosuppressive effect of CP in male mice, even if it was not a complete protection.

**Keywords: Cyclophosphamide; alpha Lipoic acid, spleen, thymus, cluster differentiation (CD), T cell proliferation, CD-ir.**

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## INTRODUCTION

Although chemotherapy is one of the most effective methods for the treatment of cancer, it is often associated with several short and long-term toxicities,and could lead to several toxic effects and clinically significant implications **(Arnon *et al.*, 2001).**

**Cyclophosphamide (CP)**is an efficient anticancer drug, has been widely used for the treatment of various neoplastic diseases **(Selvakumar*et al.*, 2006)**. It is used in the treatment of haematological malignancies, a variety of solid tumors including breast cancer **(Pritchard *et al.*, 1997)**, different types of leukemia **(Rao*et al.*, 2005)**, and cancer **(Chrystal *et al.*, 2004)**, lymphomas **(Escalon *et al.*, 2005)**, prostate cancer **(Nicolini*et al.*, 2004)**, ovarian cancer **(Stiff *et al.*, 2004)** and multiple myeloma **(Dimopoulos*et al.*, 2004)**. The acute toxicities of CP are associated primarily with its genotoxicity.

In somatic cells, CP has been shown to produce gene mutations, DNA-strand breaks, chromosomal aberrations, micronuclei and sister chromatid exchanges in a variety of cultured cells **(Monteithand Vanstone, 1995)**. Also, CP has been reported to cause several alterations in the immune system including B and T lymphocytes **(Jezernick*et al*., 2003; Shanker, 2004)**. It has been found to alter the production of cytokines such as tumor necrosis factor-alpha, transforming growth factor, beta interleukin and cytokines **(Hao*et al*., 2001)**.

**Hermeneane*t al*. (2008)** found that administration of CP is accompanied by side effects mainly affect the lymphoid organs and it can induce apoptotic cell death in variety of tissues, including thymus. **Winkelstein (2010)** studied the mechanisms of immunosuppressive effect of 20 mg/kg daily intramuscularly injection CP to guinea pigs on cellular immunity. They found that CP caused generalized depletion of lymphoid cells, including lymphopenia, and a substantial reduction in the number of macrophages found in induced peritoneal exudates.

One of the approaches for improving the therapeutic index of chemotherapy is reducing toxicity to normal cells, while leaving tumor resistance unchanged. The natural antioxidant products have gained attention on their protective effects against drug-induced toxicities especially whenever free radical generation is involved **(Freib and Higdon, 2003)**. The intake in the human diet of antioxidant compounds, or compounds that ameliorate or enhance the biological antioxidant mechanisms can prevent and in some cases help in the treatment of some oxidative-related disorders and organ toxicity events **(Havsteen, 2002)**.**Ghosh*et al*. (2002)**stated that the biological compounds with antioxidant properties may contribute to the protection of cells and tissues against deleterious effects of reactive oxygen species (ROS) and other free radicals induced by CP.

α-Lipoic acid and its reduced form referred to as a universal antioxidant **(Linnane and Eastwood, 2006).**It was shown to improve mitochondria-supported bioenergetics and also improves general antioxidant status, when given in a long-term pretreatment schedule **(Savitha and Panneerselvam, 2006). Novotny *et al*. (2008)** investigated the potential role of α-LA for use in cancer therapy. **Jariwalla*et al.* (2008)** found that α-LA may positively impact patients with HIV and acquired immune deficiency syndrome by improving functional reactivity of lymphocytes to T-cell mitogens.

The spleen is the largest secondary immune lymphoid organ in the body, where it contains large population of lymphocytes. It is composed of two functionally and morphologically distinct compartments, the red and the white pulps. The red pulp is the site of blood filtering, iron metabolism and removal of bacteria from the blood by macrophages. Also, it is the site of hematopoietic in rodents **(Mebius and Kraal, 2005; Cesta, 2006)**. The white pulpis composed of lymphocytes, macrophages, dendritic cells, plasma cells, arterioles and capillaries in a reticular framework similar to that found in the red pulp **(Saito *et al*., 1988)**.The red pulp is the site of blood filtering, iron metabolism and removal of bacteria from the blood by the macrophages. Also, it is the site of hematopoiesis in rodents The white pulp initiates the immune reactions to blood-borne antigens and for filtering the blood of foreign material and old or damaged red blood cells **(Cesta, 2006)**.

The marginal zone is an important transit area for cells leaving the blood stream and entering white pulp. Functionally, it is designed to screen systemic circulation for pathogens and contains cells involved in both innate (nonspecific) and adaptive (specific) immunity to these antigens **(Mebius and Kraal, 2005; Cesta, 2006)**. **Hermeneane*t al*. (2008)** indicated that CP lead to spleen damages both to red and white pulps, and most of lymphocyte populations were found out in different stages of apoptosis or necrobiosis.

The thymus is located in the anterior superior mediastinum, in front of the heart and behind the sternum. It is the primary lymphoid organ to be formed and grows immediately after birth. Also, it plays a central role in adaptive immune responses through its production of immunocompetent T cells **(Haynes and Hale, 1998)**.

During intrathymic maturation, thymocytes express many antigens as cluster differentiation (CD) series. This process takes place in an orderly manner mainly in the cortex of thymus and is completed by the time the cells reach the medulla **(Travers, 1990)**. CD3 is an antigen, cluster of differentiation protein, and it is a part of the T cell receptor complex on mature T lymphocytes **(Gouailliard*et al*., 2001; Kuper *et al*., 2002)**. Also, it is considered as a useful immunohistochemical marker for T cells in tissue sections **(Kuby*et al*., 2007)**. Also, it consists of five different polypeptide chains with molecular weights ranging from 16 to 28 KD. These five chains are designated gamma, delta, epsilon, zeta and eta.

**Yoon *et al.* (2003)** studied the immunohistochemical characterization of macrophage and dendritic cell subpopulations of spleen, thymus tongue and heart in CP-induced immunosuppressed rats. CD3 is initially expressed in the cytoplasm of pro-thymocytes (the stem cells from which T cells arise in the thymus), and it is at latter stage of the medullary thymocytes, CD3 antigen begins to migrate to the cell membrane of all mature T cells.

T lymphocytes can be distinguished from other lymphocyte types, such as B cells and natural killer cells by the presence of a special receptor on their cell surface called T cell receptors (TCR). The expression and relative proportion of CD3 antigens was examined using single-color staining with monoclonal antibody against CD3. The immunohistochemical reaction for CD3 antigen stain showed the presence of brown color in the nuclei of T lymphocytes of both spleen and thymus tissues, while it was negative in their nuclei. Therefore, the objective of this study was to investigate the efficacy and the possible protective activity of α-Lipoic acid against CP-induced injury to the lymphoid organs of mice. Assessment of T- lymphocyte proliferation and their functions, in addition to studying the histopathological alterations of spleen and thymus are evaluated. Also, immunohistochemical detection of CD3 as a pan T cell marker was done in both spleen and thymus tissues present in most normal mature T lymphocytes

## 2. Materials and Methods

### 2. 1. Material

#### 2. 1. 1. The chemicals used

Cyclophosphamide was purchased from the pharmacy under the international trade name, Enoxan-Asta dissolved in saline solution (0.9% NaCl). CP was used in at a dose level of 20 mg/kg/bw as it was previously described by **Ma *et al*. (2009).** The dose was calculated to be 0.6 mg/kg bw.

α-Lipoic acid (Marcyrl Pharmaceutical Industries, Egypt) and dissolved in sunflower oil as vehicle. The prepared dose of α-Lipoic acid (300 mg/kg bw) was calculated according to the equivalent daily prescribed dose for human as it was described by **Mantovani*et al*. (2002).**This equivalent calculated dose of α-LA was 0.1 mg/kg bw.

**2. 1. 2. Treatment**

Fifty adult male mice, of 3 months old and weighing nearly 30 ± 3 g each, were used as experimental animals. They were housed and adapted for acclimatization to the controlled environmental conditions for two weeks at room temperature of approximately 25C° ± 2 C°. Also, they were allowed free access of food of commercial pelleted chow (wheat, bread) and drinking tap water. All mice were divided into five groups of approximately equal body weights (each of 10 mice) as following:

**Positive control mice** were administered intraperitoneally (i.p.) with 0.5 ml saline solution,3 times/week for 6 weeks.

**CP-treated mice** were injected i.p. with 0.6 mg/kg bw/mouse cyclophosphamide, three times/week, for 6 weeks.

**Positive control mice** were daily administered orally by gavage with 0.5 ml sunflower oil, for 6 weeks.

**α-LA-treated mice** were daily administered orally by gavage with 0.5 ml α-LA, in a dose of 0.1 mg/kg bw/mouse, for 6 weeks.

**CP + α-LA-treated mice** wereadministered with the same doses of both CP and α-LA at the same manner and for the same period as in the previous treated groups.

**2. 2. Methods**

**2. 2. 1. Signs of toxicity**

All treated animals and the control were carefully examined daily throughout the duration of experiment to depict any apparent abnormalities and/or sign of toxicity. The number of dead mice occurring among the different experimental treated mice was recorded and the percentage of mortality was calculated at the end of the experiment.

**2. 2. 2. Body weight, weight change and organs weights**

At the end of experiment, i.e. after six weeks, the final mean body weights and the body weight changes (%) of all treated mice and the control were calculated and recorded after 24 h following the last dosing.All the treated and control mice were killed by cervical decapitation. Spleen and thymus were quickly excised, weighed individually as absolute organs weight. The relative organs weights were calculated as the ratio of mean of absolute organs weight/mean of the final body weight.

**2. 2. 3.Proliferative responsiveness of T lymphocytes in spleen and thymus organs**

According to the method originally employed by **Colinge*t al.* (1994),** sufficient amounts of lymphocytes from both spleen and thymus organs were excised out and squeezed and employed for tissue culture, in Petri dishes containing phosphate buffered saline (PBS) at pH 7.2. A single cell suspension was obtained from each preparation by simple sedimentation to get rid of splenic tissue debris and cell aggregates. Then the suspensions were centrifuged at 1200 rpm for 10 min. Then, they were washed in PBS (pH 7.2) and finally re-suspended in tissue culture medium composed of RPMI-1640 supplemented with L-glutamine, 10 % heat-inactivated fetal calf serum, penicillin (100 IU/ml) and streptomycin (100 µg/ml).

Splenocytes and thymocytes were counted using heamocytometer. The viability of isolated splenocytes and thymocytes were tested by Trypan blue dye exclusion test, and the percentage of the viable cells was estimated according to the formula of **Castro-Cocha*et al*. (2005).**

Cell culture of both splenocytes and thymocytes were estimated by Con A-induced proliferative responsiveness, according to the standard method developed by **Davis (1995).** Then, *in vitro* T cell lymphoproliferative response to polyclonal T cell mitogenic stimulation by phytohaemagglutinin (PHA) was performed as previously described by **Biebacke*t al*. (2003)** with minor modifications. The tetrazolium compound MTT was added for the cultured cells for dissolving the purple Formosan dye crystals.The proliferation of lymphocytes was expressed as stimulating index (SI), where the average values from triplicate readings of PHA-stimulated versus unstimulated wells were determined to calculate the stimulating index (SI) for each sample as follows: The ratio of absorbance values for PHA-stimulated wells in testing samples/un-stimulated control lymphocytes at A 492.

**2. 2. 4. The histopathological investigation**

The histopathological examination of both splenic and thymic tissues was carried out on control and the different treated groups. At the end of the experiment, these lymphoid organs (spleen and thymus) were excised out quickly. Small pieces were fixed overnight in the freshly prepared 10% formalin, and then fixed processed for routine histological technique (**Bancroft and Gamble, 2002).** Then they were sectioned at 5 µm, stained by hematoxylin and eosin, examined and photographed under the light microscope.

**2. 2. 5. Detection of CD3-ir of T-lymphocytes in spleen and thymus**

CD3 as a primary monoclonal antibody was used, and the expression of CD3 was visualized using Streptavidin-biotin-immunoenzymatic antigen detection system **(Kupere*t al*., 1995).** The reaction product was detected using diaminobenzidinetetrahydrochloridechromogen for 10 minutes in the dark**.** Then, sections were well-rinsed in tap water, counter stained in Harris Haematoxylin, and dehydrated in ascending grades of alcohol and then cleared in xylene and cover slips added, examined and photographed by the light microscope.

The expression and relative proportion of CD3 antigens was examined using single-color staining with monoclonal antibody against CD3. The immunohistochemical reaction for CD3 antigen stain showed the presence of brown color in the nuclei of T lymphocytes of both spleen and thymus tissues, while it was negative in the cytoplasm.

**2. 2. 6. Statistical analysis**

For statistical analyses, the SPSS for Windows Software Package Version 18.0 (SPSS, Chicago, IL, USA) was used. Data were given in the form of arithmetical mean values ±SD. One-way analysis of variance (ANOVA) was performed and variant groups were determined by means of the Duncan test. *P* values were assumed to be significant at 0.05.

## RESULTS

The present results revealed that mice administered with cyclophosphamide (CP) had lost their appetite after the first time of administration with the drug,and they were progressively less active and showed general weakness. 23.3 % of these mice were died during the time of experiment. This rate was decreased markedly to 16.6 % in mice administered with α-LA+CP. Further, significant decrease in the body weight change was recorded in CP-treated mice, comparing to all treatments (Table 1& Fig. 1). In addition, most of these

mice showedmoderate to massive enlargement of spleen size (splenomegaly), however, few animals showed atrophy of it having a dark color than normal. These morphological alterations were less apparent in mice treated with α-LA alone as well as in those treated with CP + α-LA.

The thymus of most CP-treated mice showed marked decrease in their sizes, comparing to the control mice. Furthermore, the results showed no significant decreases in the relative spleen weightsof CP-treated mice, compared to mice treated with either α-LA or with CP+α-LA.The relative thymus weight showed highly significant decrease in mice treated with CP, comparing to all other treatments (Table 2 &Fig. 2).

In the proliferative responsiveness of T lymphocytes,the results revealed marked significant depletion in lymphocyte proliferation of both spleen and thymus tissues of mice treated with CP(Table 3&Fig. 3), comparing to mice either administered with α-LA alone or CP+α-LA.

**Histopathological observations of spleen**

Sections of spleen in the positive control mice that received saline solution, were appear to consist of two main compartments,the white and the red pulps. The red pulp occupies the distance between the white pulps(Fig. 4). The white pulp is so named because of its content of deeply basophilic lymphocyte aggregation, and it is composed of two main parts, the splenic follicles and the marginal zone. The splenic follicles are big, and contain eccentric arterioles (Fig. 4), and they are subdivided into an inner periarterial lymphatic sheath, where T-lymphocytes predominate, and an outer periarterial lymphatic sheath, where B lymphocytes predominate.

The red pulp is composed of a meshwork of splenic cords and the splenic sinusoids (vascular channels). The splenic cords consist of the reticular cells and associated macrophages(Fig. 5). In addition, large-sized and rough spherical bodies of megakaryocytes (MK) are observed (Fig. 6).These bodies contain large irregular multilobulated nuclei, having clumps of dispersed chromatin and they have an extensive homogenous cytoplasm.

No histopathological alterations in spleen tissues were observed in the positive control mice that received sunflower oil, and most sections appeared with normal architecture(Fig. 7).The white pulp contains the deeply basophilic lymphocyte aggregation, and the red pulp contains many reticular cells, small macrophages and large-sized megakaryocytes(Fig. 8).

In cyclophosphamide-treated mice, marked disruption of spleen structure, and many signs of pathological alterations were observed. These alterations include the marked loss in distinction between the white and red pulps (Fig. 9);the decrease in the lymphocyte populationwith marked loss in the chromatin of their nuclei (Fig. 10). Vasodilatation and congestion of splenic sinusoids were seen in the red pulp, and there was an increase number of inflammatory cell components which were polymorphous in structure, and they can be seen in layers. Most of the lymphocytes contained pyknotic nuclei (Figs. 11& 12). The reticular cells were increased in number and size, and their nuclei showed variable shapes and sizes, many being fragmented or undergoing necrosis (Fig. 12). In addiotion, the megakaryocytes were large in size and contain many nuclei (Fig. 12).

In α-Lipoic acid-treated mice, most sections of spleen showed an apparent normal structure for white and red pulps as it was observed in the normal control sections (Fig. 13). The lymphoid follicles contain predominant small dense lymphocytes, few macrophages and reticular cells(Fig. 14).Megakaryocytes were frequently seen with normal feature as in the control, and many mitotic figures was observed in this area (Fig. 15).

Spleen sections in cyclophosphamide+α-Lipoic acid-treated mice, showed a few histopathological alterations and marked improvement. There was an obvious distinction between the white and the red pulp, however, moderate cellularity was observed in the marginal zone (Fig. 16).The white pulp had normal accumulation of cellularity and the red pulp showed an increase in the number of the reticular cells and macrophages (Fig. 17).

**Histopathological observations of thymus**

Thymus sections in positive control mice showed normal structure where that it is divided into a dark high cellular outer cortex and a paler less cellular central medulla (Fig. 18).The cortical layer contains less population of B lymphocytes, plasma cells, scattered neuroendocrine cells, and dispersed reticular cells (Fig. 19). The medullary area contains many dispersed reticular cells, few macrophagesand lamellated structures known as Hassall's corpuscles (thymic corpuscles) (Fig. 20). Each one consists of concentrically arranged layers of flattened epithelial cells and their centers appear hyalinized (glassy).

In the positive control mice that received sunflower oil, no observable histological alterations were noticed in the thymic tissue, and there was marked distinction between the cortical and the medullary areas. An extensive cellular accumulation of T lymphocytes was seen in the cortical zone (Fig. 21), and the medullary area contained the usual components of the reticular cells, few macrophages and Hassall's corpuscles (Fig. 22).

In cyclophosphamide-treated mice, marked disruption of thymus organization and massive depletion of its cellularity were detected especially in the cortical region (Fig. 23). Vasodilatation and congestion of blood vessels were prominent in the medullary areas. There was marked decrease in the number and size of the reticular cells and few macrophages were seen (Fig. 24).Moreover, an observable increase in the number of Hassall's corpuscles with abnormal features was seen in most sections surfaces in the medullary region.

In α-Lipoic acid-treated mice, the division between the cortical and the medullary regions was distinct in most sections of thymus(Fig. 25),and there were no recognizable differences in structure of both the cortical and the medullary areas. There was an extensive small lymphocyte population, few reticular cells, some macrophages (Fig. 26), and small-sized Hassall's corpuscles.

In CP + α-LA-treated mice, there wasan identifiable distinction between the cortical and the medullary zones (Fig. 27). The cortical area showed to contain an increase in cellular accumulation of T lymphocytes, however, the medullary area appeared with moderate cellularity, and the lymphocytes were larger in size and paler in color. Many reticular cells, few macrophages and Hassall's corpuscles could be seen in the medullary zone (Fig. 27).

**CD3- Immuno reactivity of T-lymphocytes**

In spleen tissue, the proliferative activity of splenic T cells was examined by the mitogenic response to anti-CD3 antibody. In the positive control mice (received saline solution), sections of spleen showed that anti-CD3 antibody provided a strong brown color to lymphocyte cytoplasm (Fig. 28). This is directly related to the expression of CD3 molecules in T cells, which varies according to the degree of lymphocyte maturation. These immunoreactive cells were abundant and populated with high density in the white pulp,

while in the red one, no staining reaction was observed in the megakaryocytes (Fig. 28).

However, very weak reaction was detected in spleen sections of CP-treated mice. Most lymphocytes were negatively-stained with light brown color indicating the degeneration in splenic tissue, and the marked loss in density of splenic lymphocytes (Fig. 29).The megakaryocytes in the red tissue were negatively-stained and appeared as pale blue structures (Fig. 30). Further, in α-LA-treated mice, there was moderate number of immunopositively-stained lymphocytes (Fig. 31). Further, the results showed positively-stained cells in the splenic follicles of CP+ α-LA-treated mice(Fig. 32), indicating that this combination of CP and α-LA could partly prevent the loss of splenocytes induced by CP.

In thymus tissue, the CD-ir detection of the positive control mice (received saline solution) exhibited a strong staining reaction in the medullary area. This was due to the presence of mature lymphocytes, while the thymic cortex was lightly stained (Fig. 33). The brownish-red staining color was distributed in the cytoplasm of most thymocytes, while it was negative in their nuclei.

Very weak staining reaction was observed in the different thymic sections of CP-treated mice, where few scattered immunopositively-stained cells were found in the cortical areas, indicating the decrease in proliferation of T lymphocytes(Fig. 34).However, massive depletion in thymocyte population was observed in the medullary areas.

In α-LA-treated mice, most of the positively-stained T cells were located in the medullary areas, while few were detected in the cortical zone (Fig. 35).

In CP + α-LA-treated mice, the thymic lymphocytes in the medullary region exhibited a moderate staining reaction, while the thymic cortex was lightly stained (Fig. 36), indicating the presence of new proliferated mature lymphocytes.

## Discussion

Use of cyclophosphamide(CP) as an effective chemotherapeutic agent is often restricted because of its widespread adverse side effects. The damage to the immune system is one of its major side effect**(DeSouza*et al.*, 2000).**

In the present study, administration of 20 mg/kg bw CP for 6 weeks caused an increase in the rate of mortality and showed significant decrease in the body weight. However, there were no significant differences in the body weights of mice administered with α-LA alone or those treated with CP + α-LA. **Lutsiak*et al*. (2005)** had indicated that CP is a well-known and powerful immunosuppressive agent, and the mechanisms of its immunosuppression may differ according to the dosage.

Further, the present results showed no significant decreases in the relative spleen weights of mice treated with CP, α-LA as well as CP+ α-LA, compared to those of the positive control (both saline and sunflower oil). However, highly significant decreases in the relative thymus weights were recorded in mice treated with CP, comparing to the all other treatments.

The decrease in these lymphoid organs weight in mice administered with CP was accompanied by the decrease in the total number and the functional ability of T lymphocytes, as it was investigated by the commonly used assay of Con A-induced T-cell proliferation. However, co-administration with α-Lipoic acid could ameliorate the toxic effect of CP. These results are in confirming with the results of **Ogiso*et al.* (1976)** who found that high dose level of CP caused a considerable decrease in thymic weight of rats along with the total number of thymic lymphocytes. **Schuurman*et al*. (1992)** explained that the thymus is a sensitive target organ following exposure to immunotoxicants, and the decrease in its size or weight is often one of the first noted measures of toxicity.

**Krzystyniak*et al.* (1995)** suggested that the marked loss in the total cellular T lymphocytes in the lymphoid organs, such as spleen and thymus affect either on the quantity of lymphocyte subpopulations, or the functional ability of them. **Youssif (1997)** found that administration of CP caused a suppressive effect on the amount of lymphocytes in both thymus and spleen.Also, **Nygaard and Lovik (2002)** explained that the observed decrease in the relative T cell number after CP treatment is probably a result of the reduced relative B cell numbers, and is most likely associated with a constant or a small reduction in absolute number of T cells.**Soleman*et al*. (2003)** reported that CP might prevent the specific proliferation of T cells and caused induction of apoptosis.

**Mochizuki *et al.* (2003)** had reported that i.p. administration of a single dose of 150 mg/kg bw CP resulted in suppression of the immune cells along with the increase in apoptotic cell count in the thymus. Also, **Smith *et al*. (2003)** investigated that 10 mg/kg CP given orally by gavage to female rats for 30 days could induce significant immunosuppressive effects.

**Murata *et al*. (2004)** explained that CP induced immune dysfunction through the intermediate reactive which caused the damage to the cells of immune system; hence, CP was effective in reducing pools of lymphocytes.Also, **Senthilkumar*et al.* (2006)** who had reported that the loss in cell populations of the lymphoid organs could be attributed to the decrease in their proliferation and the induction of cell death in them. In addition, **Hou*et al.* (2007)** investigated that oral administration of 10 mg/kg bw CP might cause marked decrease in body and lymphoid organs weights of rats.

These results had been confirmed with the current histopathological investigations of spleen of mice treated with 20 mg/kg bw CP was found to have an observable effect on splenocyte populations and their proliferative ability, indicating its defective functional ability. The decrease in splenocyte population with many degenerated and vacuolated areas in the white pulps were the most prominent features indicating the partial spleen atrophy after exposure to CP. Also, there was marked disruption of spleen organization, marked loss in distinction between the white and red pulps, vasodilatation and congestion of splenic sinusoids in the red pulp. In addition, the megakaryocytes (MKC) were large in size and contained many nuclei.

**kamel*et al.* (1992)** had reported that the term MKC for a type of multinucleated giant cells found on the lymphoid tissues in association with a variety of reactive and neoplastic disorders. These cells were decreased after treatment with α-LA, where little histopathological changes were observed in most spleen sections. **Caylak*et al*. (2008)** explained that the unique ability of α- LA made it unlike other antioxidants, where it could be easily soluble in both H2O and fat and is easily absorbed and across the cell membrane. This unique quality offers protection against free radicals both inside and outside the cell.

Furthermore, the results revealed that administration of CP caused certain atrophy of thymic cortex and medulla, decreased number of lymphocytes and epithelio-reticular cells. In addition, there was no sharp distinction between the cortical and the medullary areas. However, the results revealed that in

CP+α-LA-treated mice, slight histopathological changes were detected, comparing to mice administered with CP. Thymus did not demonstrate any recognizable differences than that of the control. However, there was slight loss in the lymphocyte populations in the medullary areas, and the division between the cortical and the medullary tissue was distinct.

Several investigators had reported that the changes in thymus histopathology and architecture are considered to be particular relevance for the determination of immunotoxicity**(Harleman, 2000; Haley *et al*., 2005).**Also, **Elmore (2006)** explained that certain chemicals that lead to direct thymus lymphocyte toxicity may result in increased numbers of apoptotic lymphocytes, and the decrease in cell density. **Prakash*et al.* (2007)** found that CP caused thymic atrophy with retardation of thymic size and a remarkable shrinkage in lobular morphology. **Bischoff *et al*. (2000) and Winkelstein (2010)** explained that the suppressive effect of CP on thymus or thymocytes might be related to excessive free radicals production and apoptosis of immune cells.

Hassall's corpuscles are unique to the thymus. They appear to act as temporary storage depots for dead nuclear material. In the normal thymus, they were in dynamic equilibrium and increased with thymocyte death. The size of these corpuscles has an important explanation in their relationship to the presence and density of thymocytes. Thus Hassall's corpuscles were increased in size and number during the acute lysis of thymocytes**(Blau, 1967).**

Furthermore, the immunohistochemical detection of CD3 showed marked loss in the proliferative T lymphocytes in both spleen and thymus tissues of mice treated with CP. The high specificity of CD3 makes it a useful immunohistochemical marker for T cells in tissue sections **(Kuby*et al*., 2007).** CD3 molecule is only found in T lymphocytes and plays a central role in formation of antigen-receptor interactions through the T cell receptor (TCR)/CD3 complex **(Gouailliard*et al*., 2001).** The results revealed very weak reaction in spleen sections of **CP-treated mice**. Most lymphocytes were stained with light brown color indicating the degeneration in splenic tissue. This indicated the damaging effect of CP on the function of spleen, and its inability to protect itself.

**Matalon*et al*. (2004)** had reported that CP requires microsomal oxidation in the liver to yield its active metabolite, 4-hydroxy-CP in target cells that spontaneously decomposes to phosphoramid mustard which exerts cytotoxic effect by the induction of DNA single strand breaks as well as crosslinks which result in different types of damage.Also, CP has been shown to induce apoptosis in the target cells through the modulation of signaling through caspases, Bcl2, Bax, NF-Kappa B, and MAP kinases **(Mirkes*et al*., 2000; Torchinsky*et al*., 2002).Bosanquet*et al*. (2002)** had reported that CP involves inhibition of cell division due to cross-linking of the drug to DNA.

**Islam (2009)** found that administration of α-LA has been shown to be effective in preventing pathology in various experimental models in which ROS have been implicated. He explained that exogenous α-LA has been shown to increase ATP production due to its role in the oxidation of pyruvate and alpha-ketoglutarate in the mitochondria. Inside cells and tissues, and during the metabolic process, lipoic acid is reduced to dihydrolipoic acid (DHLA) by glutathione reductase and lactate dehydrogenase and extensively metabolized by ß-oxidation in tissue.

**Bustamante *et al*. (1995)**found that α-LA and dihydrolipoic acid both inhibit the apoptosis of rat thymocytes after exposure to either methylprednisolone or etoposide and this inhibition was manifested at an early stage in the apoptosis as cell shrinkage, chromatin fragmentation. **Sen*et al*. (1999)**explained that the possibilities of protection by the α-LA may also include hydrogen ion donation for DNA repair and inhibition of alkylating agents when the thiol component attracts the positively charged carbonium ions.

## Conclusion

The findings of the present study indicated that administration of 20 mg/kg bw/mouse CP induced lymphoid organs damage, which is illuminated by dramatic elevation in the pathological parameters.However, α-LA could produce a significant amelioration for these changes, and it may be considered as a potentially useful candidate in the combination chemotherapy with CP to combat oxidative stress mediated lymphoid organs injury. Hence, the current study proved the protective effect of α-lipoic acid on the immunosuppressive effect of CP in mice, even if it was not a complete protection. Therefore, it could be recommended to the patients who use anti-cancer or immunosuppressive drugs, such as CP, to take it in combination with a powerful antioxidant, such as α-lipoic acid, (under the doctor's advice).

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Table 1. % of morality, body weight and weight change of mice treated with cyclophosphamide, α-lipoic acid, cyclophosphamide + α-lipoic acid and of the positive control mice.

| Experimental groups | Number of mice | Number of dead mice | % of mortality | Mean of body weight | | Mean of change in body weight |
|---|---|---|---|---|---|---|
| | | | | Initial | Final | |
| Saline- treated mice (Positive control) | 30 | 0 | 0 | 29.1±1.95 | 36.8±2.98[a] | 26. 46±2.68[a] |
| Sunflower oil-treated mice (positive control) | 30 | 1 | 3.3 | 28.9±1.68 | 38.6±3.01[a] | 33.56±2.98[b] |
| Cyclophosphamide (CP)-treated mice | 30 | 7 | 23.3 | 27.4±1.82 | 22.5±2.98[b] | -17.88±1.33[c] |
| α-Lipoic acid (α-LA)-treated mice | 30 | 2 | 6.6 | 28.9±1.69 | 35.9±3.1[a] | 24.22±2.07 [a] |
| Cyclophosphamide + α-lipoic acid (CP+α-LA)-treated mice | 30 | 5 | 16.6 | 29.9±2.01 | 31.3±3.25[a] | 22.68±0.366[a] |

The data are presented as mean ± S.D,
The same small letters show no significant differences from the control,
The different small letters indicate that there were significant differences at value of $P \leq 0.05$.

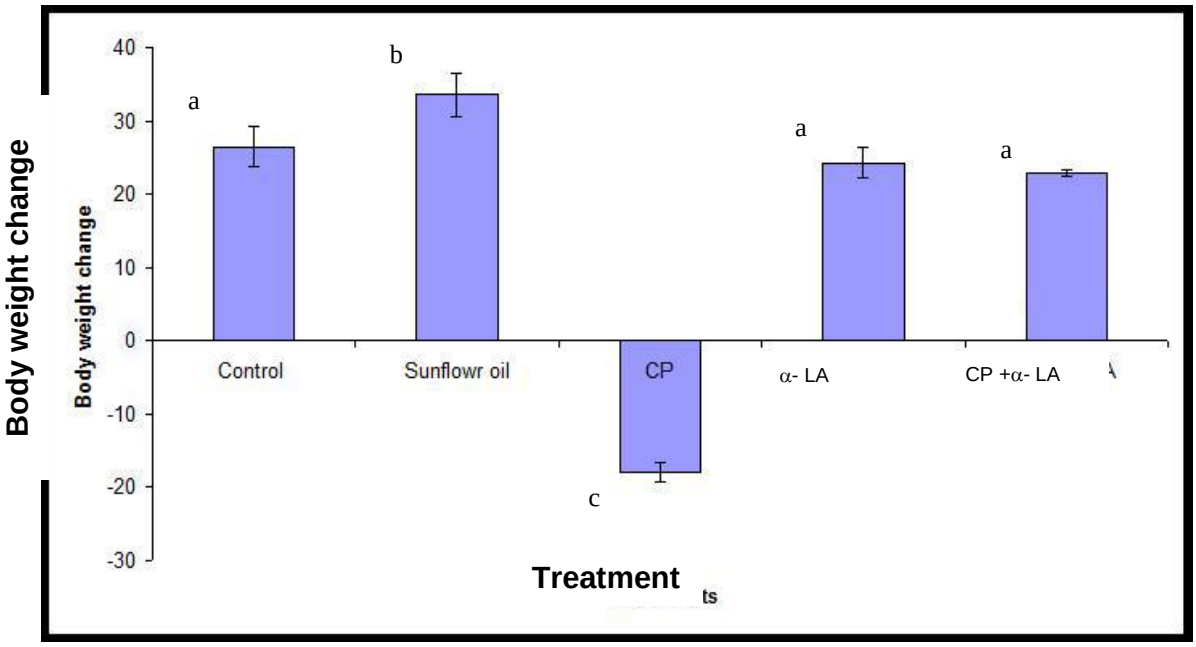


Fig. 1. Body weight change in mice administered with cyclophosphamide (CP); alpha lipoic acid (α-LA); cyclophosphamide and alpha lipoic acid (CP + α–LA) and in the positive control mice group after 6 weeks.

Table 2. Lymphoid organs weights of mice treated with cyclophosphamide, α-lipoic acid, cyclophosphamide + α-lipoic acid and of the positive control mice.

| Experiment-al groups | Spleen weight | | Thymus weight | |
|---|---|---|---|---|
| | Absolute (g) | Relative (%) | Absolute (g) | Relative (%) |
| Saline- treated mice (Positive control) | 0.416±0.106 [a] | 1.485±0.09[a] | 0.105±0.03[a] | 0.375±0.13[a] |
| Sunflower oil-treated mice (positive control) | 0.482±0.098[a] | 1.506±0.08 [a] | 0.198±0.109 [b] | 0.618±0.21[b] |
| Cyclophosphamide (CP)-treated mice | 0.173±0.082[b] | 0.678±0.12[b] | 0.018±0.002[c] | 0.071±0.08[d] |
| α-lipoic acid (α-LA)-treated mice | 0.215±0.058 [b] | 0.741±0.05 [b] | 0.041±0.003 [d] | 0.141±0.068[c] |
| Cyclophosphamide + α-lipoic acid (CP+α-LA)-treated mice | 0.198±0.065 [b] | 0.707±0.03 [b] | 0.049±0.005 [d] | 0.175±0.044[c] |

The data are presented as mean ± S.D,
The same small letters show no significant differences from the control,
The different small letters indicate that there were significant differences at value of $P \leq 0.05$.

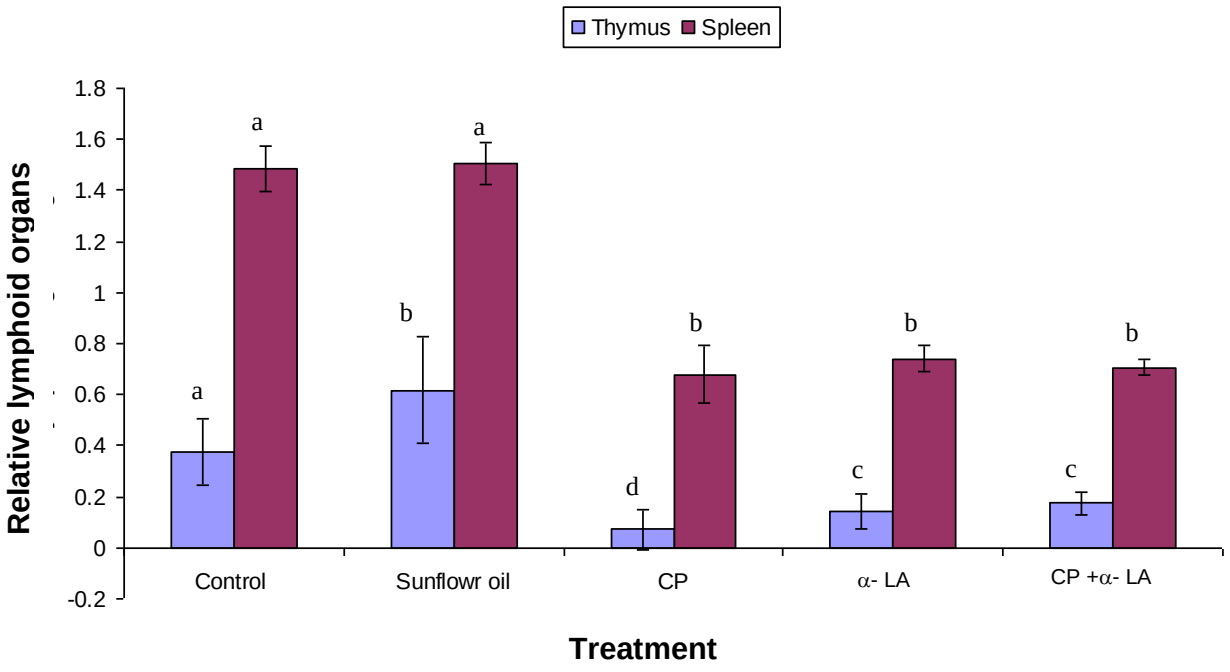


Fig. 2. The relative lymphoid organs weight in mice administered with cyclophosphamide (CP); alpha lipoic acid (α-LA); cyclophosphamide and alpha lipoic acid (CP + α–LA) and in the positive control mice group after 6 weeks.

Table 3.Thymocytes and splenocytes populations of mice treated with cyclophosphamide, α-lipoic acid, cyclophosphamide + α-lipoic acid and of the positive control mice.

| Experimental groups | Lymphocyte population | |
|---|---|---|
| | Splenocytes | Thymocytes |
| Saline- treated mice (Positive control) | 3.33 ± 0.39[a] | 3.33± 0.57[a] |
| cyclophosphamide (CP)-treated mice | 1.4± 0.16[b] | 1.03 ± 0.06[b] |
| α-lipoic acid (α-LA)-treated mice | 2.95± 0.86 [a] | 2.73± 0.17 [c] |
| Cyclophosphamide +α-lipoic acid (CP+α-LA)-treated mice | 1.93 ± 0.22[c] | 2.65 ± 0.33[c] |

The data are presented as mean ± S.D,
The same small letters show no significant differences from the control,
The different small letters indicate that there were significant differences at value of $P \leq 0.05$.

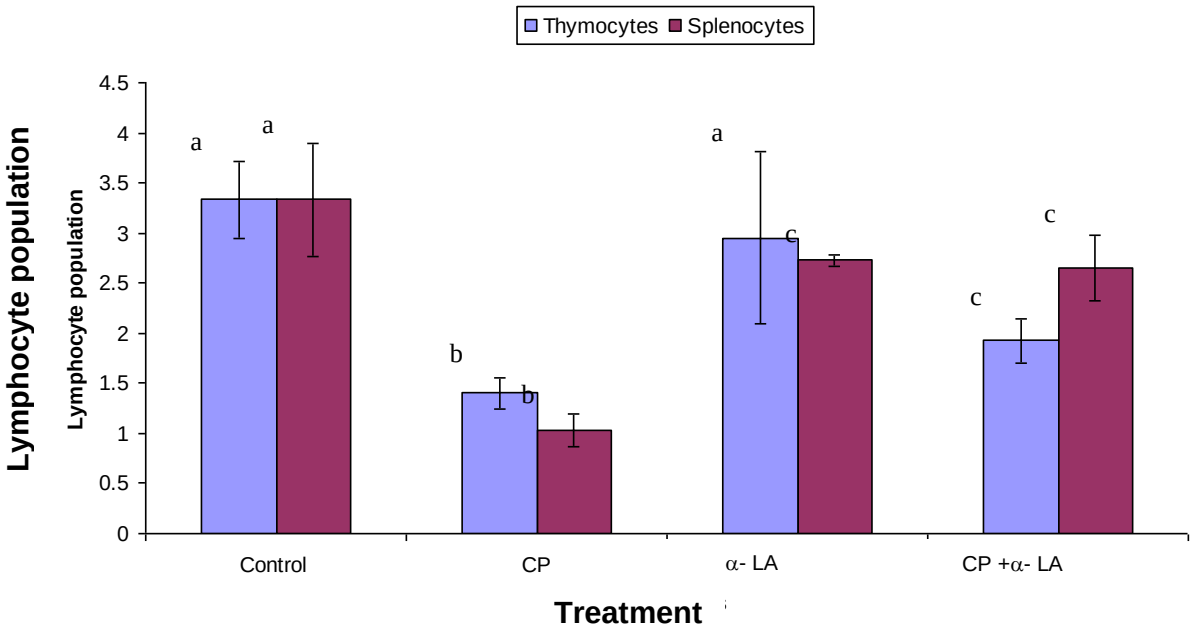


Fig. 3.Splenocyte and thymocyte population in mice administered with cyclophosphamide (CP); alpha lipoic acid (α-LA); cyclophosphamide and alpha lipoic acid (CP + α–LA) and in the positive control mice group after 6 weeks.

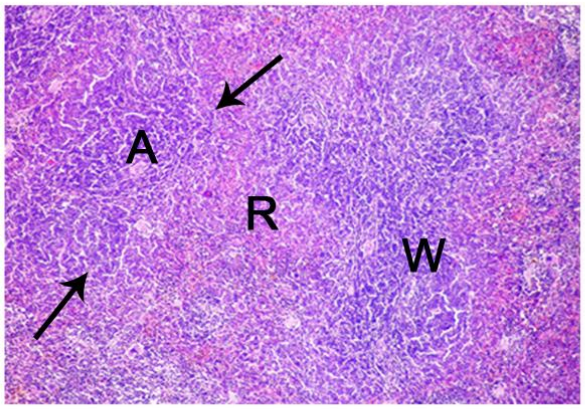


**Fig. 4: Light micrograph of spleen section of positive control mouse received saline solution showing: the white pulp (W) and the red pulp (R); the splenic follicle contains eccentric arteriole (A); arrows point at the marginal zone. H & E, X 200.**

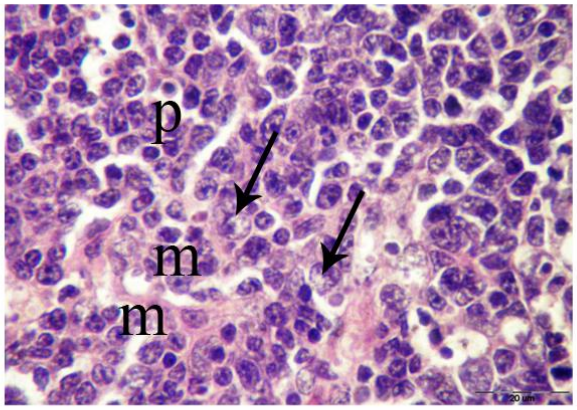


**Fig. 5. Enlarged part of the lymphoid follicle of spleen section of positive control mouse received saline solution showing: predominant lymphocytes; reticular cells (arrows); large slightly irregular-shaped macrographs (m); plasma cells (P). H & E, X 1000**

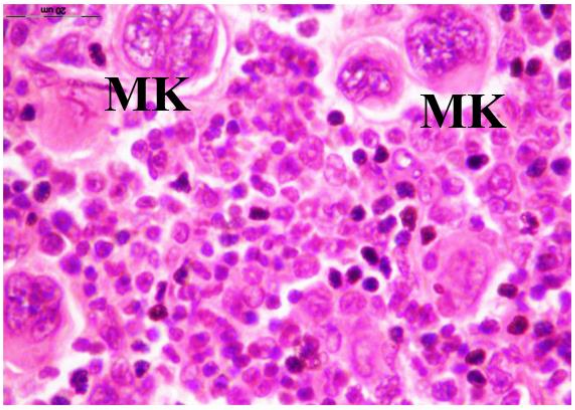


**Fig. 6. Enlarged part of the red pulp area of spleen of positive control mouse received saline solution showing: the presence of large-sized megakartocytes (MK). H & E, X 1000.**

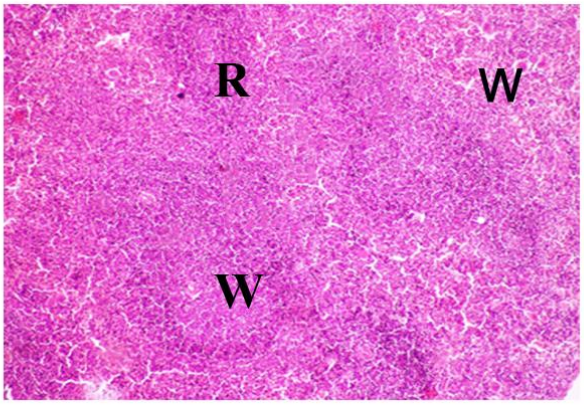


**Fig. 7. Light micrograph of spleen section of positive control mouse received sunflower oil showing: the white (W) and red (R) pulps. H & E, X 200.**

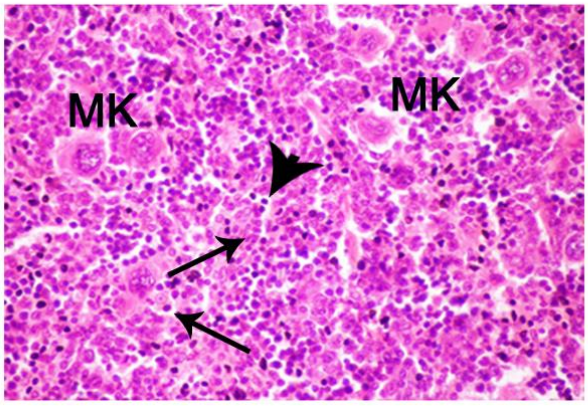


**Fig. 8. Enlarged part of the red pulp area (R) of spleen of positive control mouse received sunflower oil showing the reticular cells (arrows), macrophages (arrowheads); megakaryocytes (MK). H & E, X 630.**

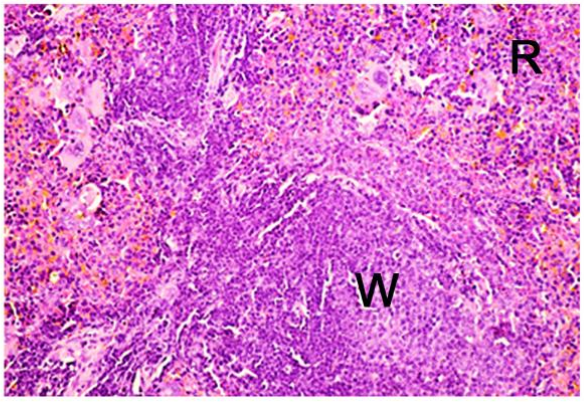


**Fig. 9: Light micrograph of spleen section of cyclophosphamide (CP)-treated mouse, showing: a general disorganization of splenic tissue and the loss in distinction between the white (W) and the red (R) pulp. H & E, X 200.**

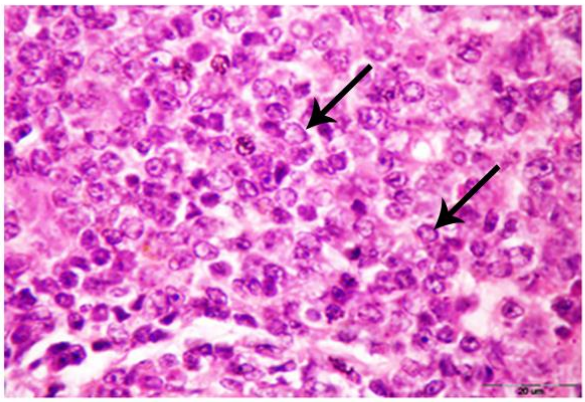

**Fig. 10: Enlarged part of the white pulp area of spleen section of CP-treated mouse showing: the marked decrease in lymphocyte population and their nuclei contain low levels of condensed chromatin (arrows). H & E, X1000.**

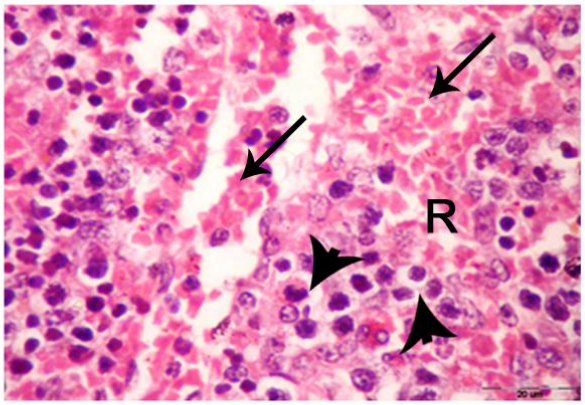


**Fig. 11: Enlarged part of the red pulp area (R) of spleen section of CP-treated mouse showing: vasodilatation and congestion of splenic sinusoid (arrows); pyknotic nuclei of lymphocytes (arrowheads); necrotic reticular cells (r). H & E, X1000.**

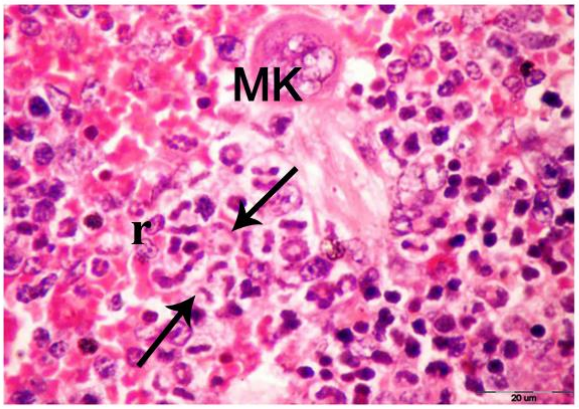


**Fig. 12: Enlarged part of the red pulp area of spleen section of CP-treated mouse showing: large-sized megakaryocyte (MK); layers of inflammatory cells (arrows) such as neutrophils and eosinophils; pyknotic nuclei of lymphocytes (arrowheads); the reticular cells (r); megakaryocytes (MK). H & E, X1000.**

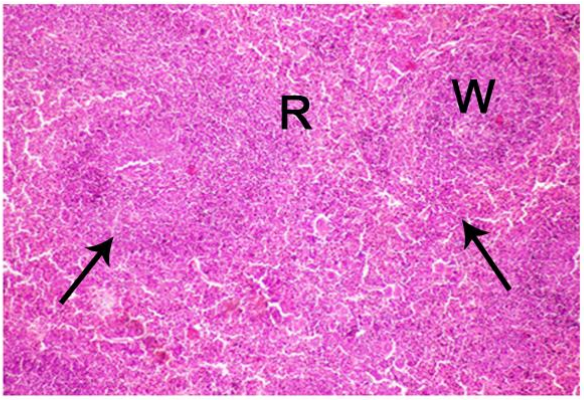


**Fig. 13: Light micrograph of spleen section of α-Lipoic acid (LA)-treated mouse showing: the normal structure of spleen tissue; white (W) and red (R) pulps; prominent lymphatic sheaths (arrows). H & E, X 200.**

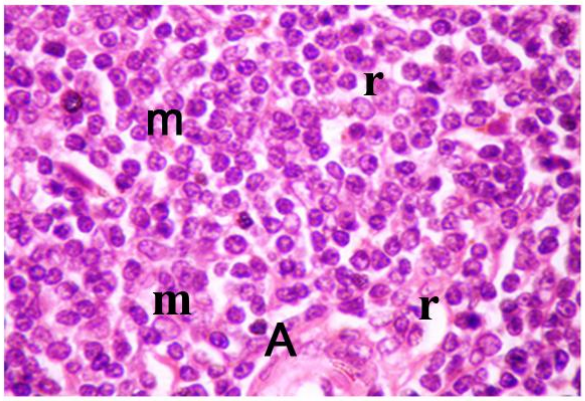


**Fig. 14: Enlarged part of the white pulp area of spleen section of α-LA-treated mouse showing: the lymphoid follicle contains lymphocyte population with eccentric arteriole (A); the reticular cells (r); macrophages (m). H & E, X1000.**

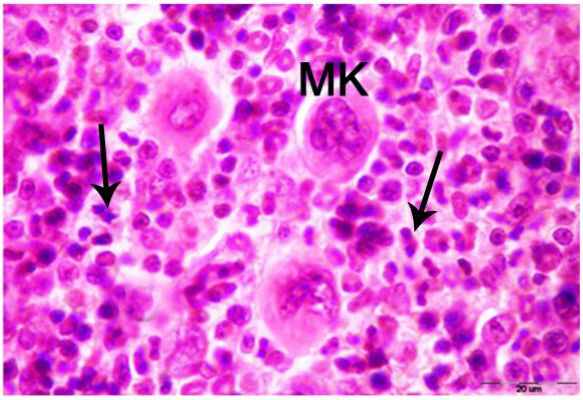


**Fig. 15: Enlarged part of the red pulp area of spleen section of α-LA-treated mouse showing: presence of many megakaryocytes (MK); mitotic figures (arrows). H & E, X 1000.**

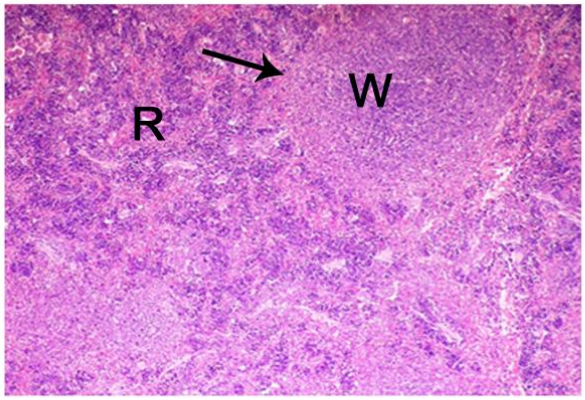


**Fig. 16: Light micrograph of spleen section of CP + α-LA-treated mouse showing: the normal organization of spleen tissue; Note: the white (W) and the red pulp (R); moderate cellularity of the marginal zone (arrow). H & E, X 200.**

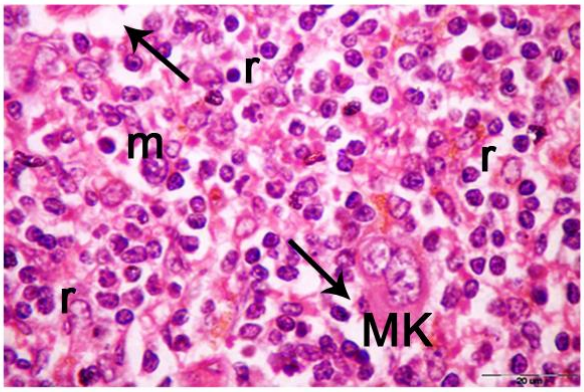


**Fig. 17: Enlarged part of the red pulp of spleen section of CP + α-LA-treated mouse showing degenerated area (arrows); congested area an increase in the number of reticular cells (r); macrophage (m); megakaryocytes (MK). H & E, X1000.**

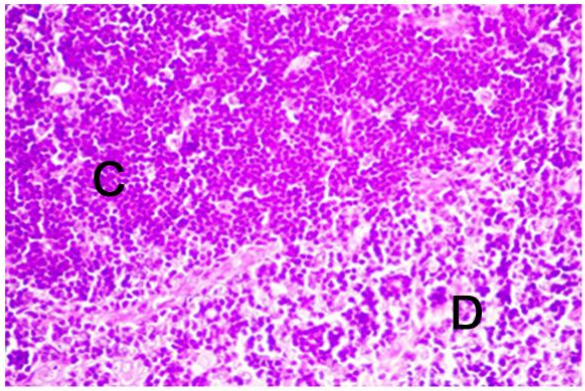


**Fig. 18: Light micrograph of thymus section of positive control mouse received saline solution, showing: the dark high cellular outer cortex (C) and the paler less cellular central medulla (D). H & E, X 400.**

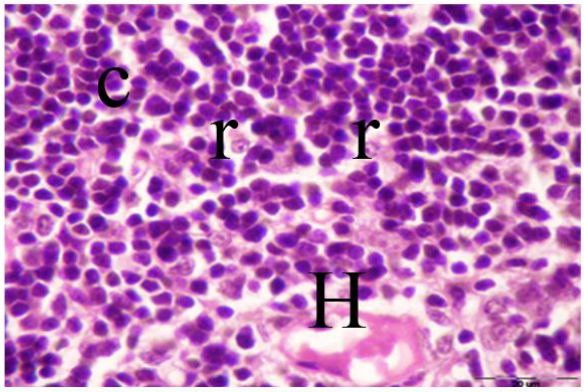


**Fig. 19: Enlarged part of the cortical area of thymus of positive control mouse received saline solution, showing: an extensive population of lymphocytes with an abundant strongly basophilic cytoplasm; dispersed reticular cells (r);Hassall's corpuscle (H). H & E, X1000.**

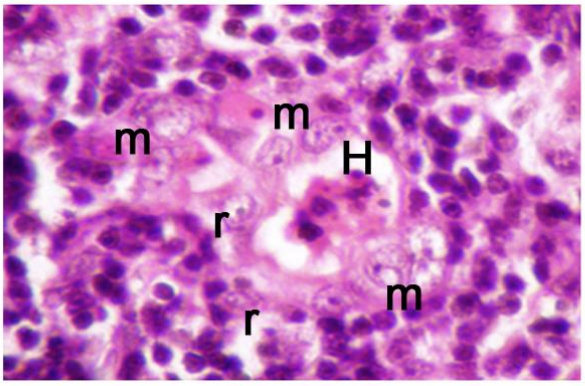


**Fig. 20: Enlarged part of the medullary area of thymus of positive control mouse received saline solution, showing: large number of the reticular cells (r); macrophages (m); Hassall's corpuscle (H). H & E, X1000.**

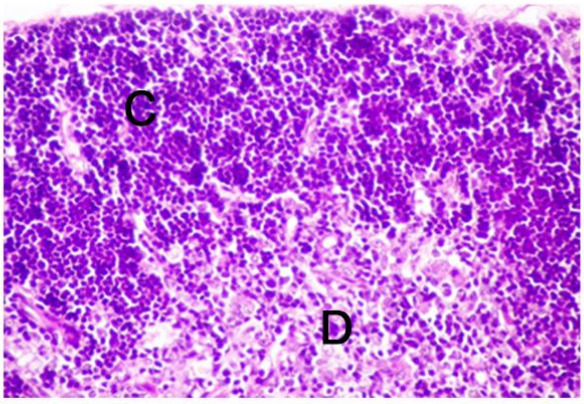


**Fig. 21: Light micrograph of thymus section of positive control mice (received sunflower oil) showing: the distinction between the cortex (C) and the medulla (D); Note: the extensive T lymphocytes in the cortical zone. H & E, X 400.**

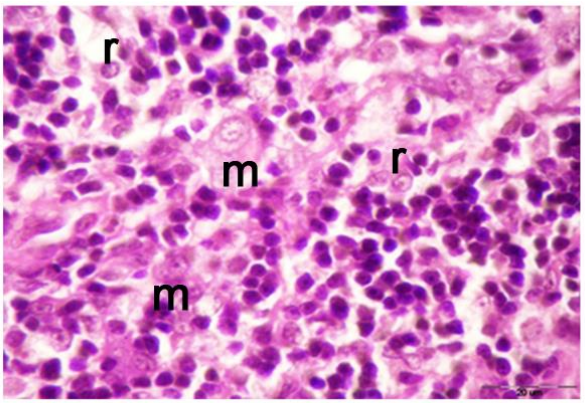


**Fig. 22: Enlarged part of the medullary area of thymus section of positive control mouse received sunflower oil showing: the reticular cells (r); few macrophages (m). H & E, X1000**

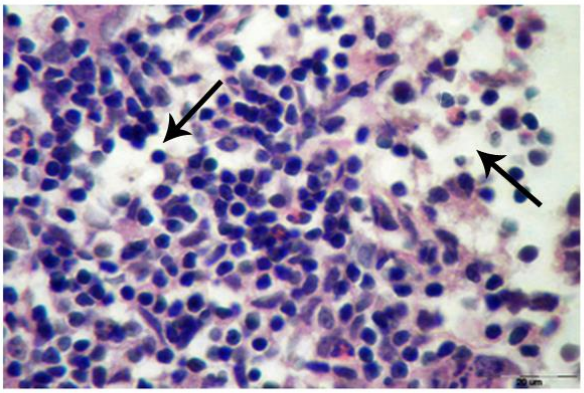

**Fig. 23: Light micrograph of thymus section of CP-treated mouse showing: the cortical area with massive depletion in lymphocytic population (arrows). H & E, X1000.**

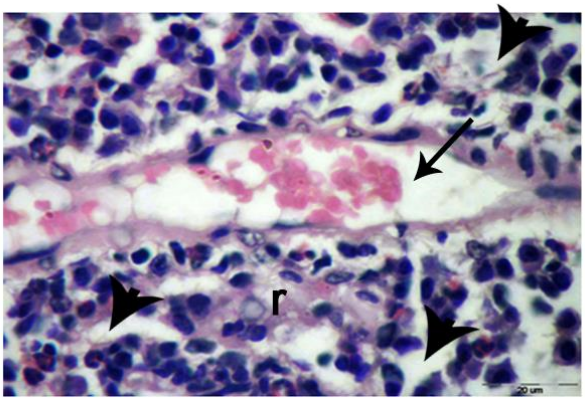


**Fig. 24: Enlarged part of the medullary area of thymus section of CP-treated mouse showing: large dilated and congested blood vessel (arrow); many degenerated areas (arrowheads); the reticular cells (r). H & E, X1000.**

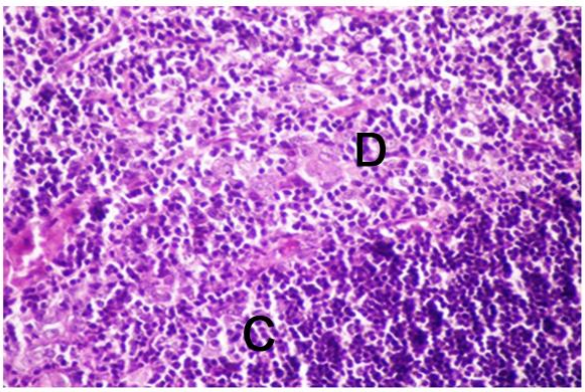


**Fig. 25: Light micrograph of thymus section of α-LA-treated mouse showing: the general normal appearance of thymus tissue with a marked distinction between the cortical (C) and the medullary area (D). H & E, X 400.**

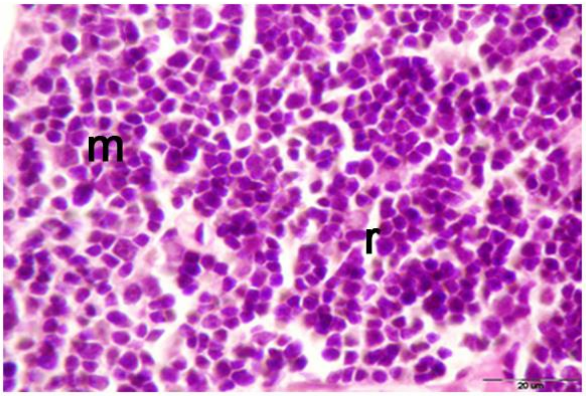


**Fig. 26: Enlarged part of the cortical region of thymus section of α-LA-treated mouse showing: an extensive small lymphocyte population; few reticular cells (r); some macrophages (m). H & E, X1000.**

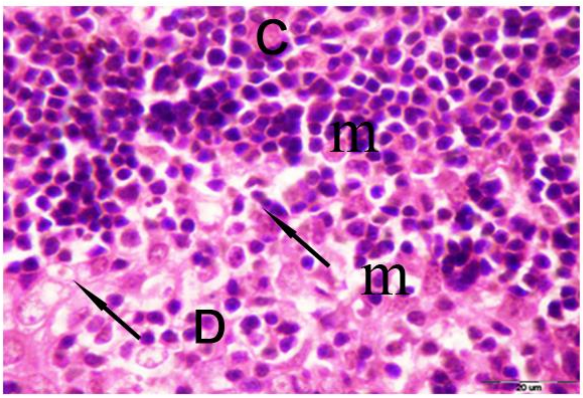


**Fig. 27: Light micrograph of CP + α–LA-treated mouse showing: the dark high cellular outer cortex (C) and the paler less cellular central medulla (D); many reticular cells (arrows); few macrophages (m). H & E, X 1000.**

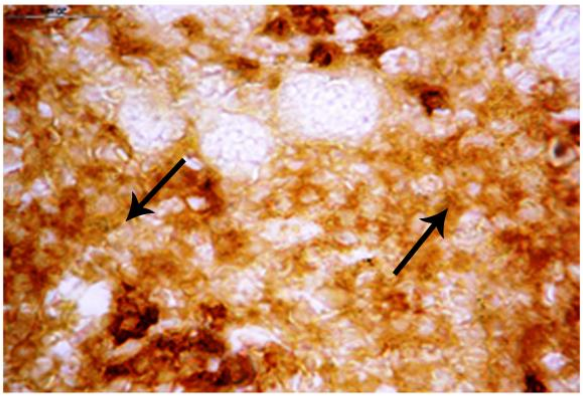

**Fig. 28: Immunohistochemical reaction of CD3 in spleen section of positive control mouse received saline solution showing: strong brown color in the lymphocyte of the white pulp (arrows). X 1000.**

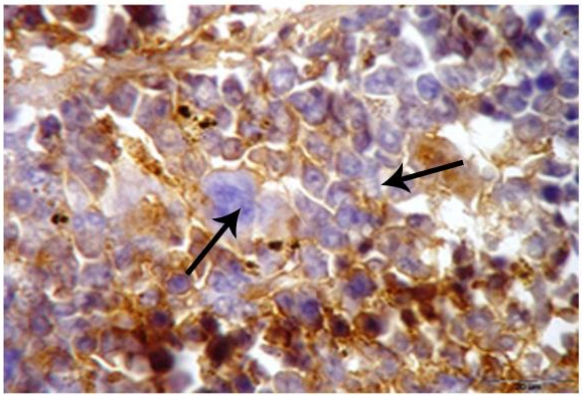

**Fig. 29: Immunohistochemical reaction of CD3 in spleen section of CP-treated mouse showing: the negatively-stained reaction of the cortical area indicated by the appearance of blue color in most splenocytes (arrows). X 1000.**

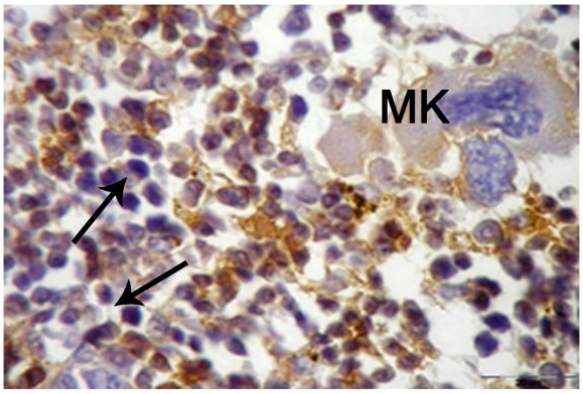


**Fig. 30: Immunohistochemical reaction of CD3 in spleen section of CP-treated mouse showing very weak reaction indicated by the appearance of blue color (arrows) in the red pulp area; Notice: the negatively-stained megakaryocytes (MK). X 1000.**

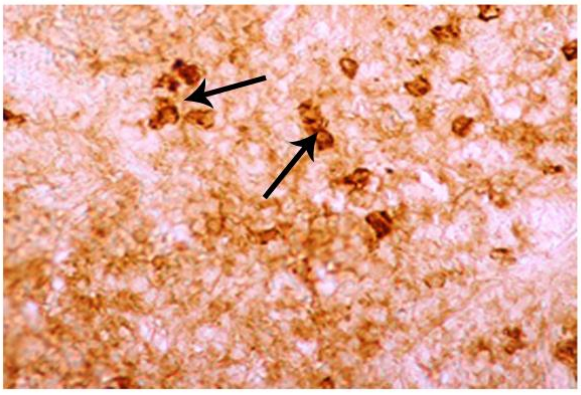

**Fig. 31: Immunohistochemical reaction of CD3 in spleen section of α-LA-treated mouse showing: moderately-stained reaction in most splenocytes (arrows). X 200.**

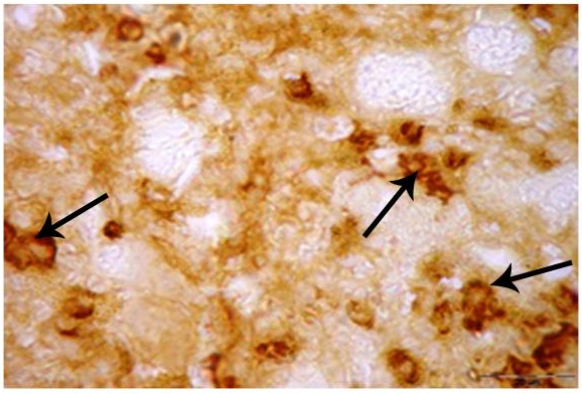

**Fig. 32: Immunohistochemical reaction of CD3 in spleen section of CP + α-LA-treated mouse showing: positively-stained reaction in most splenocytes (arrows). X 1000.**

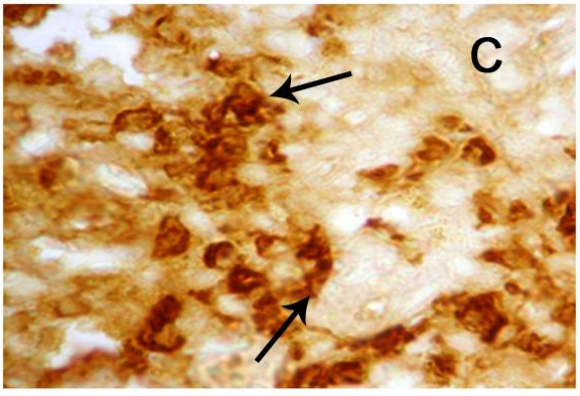


**Fig. 33: Immunohistochemical reaction of CD3 in thymus section of mouse received saline solution showing: strong brown color of the mature lymphocytes of the medullary region (arrows) and lightly-stained cortical layer (C). X 1000.**

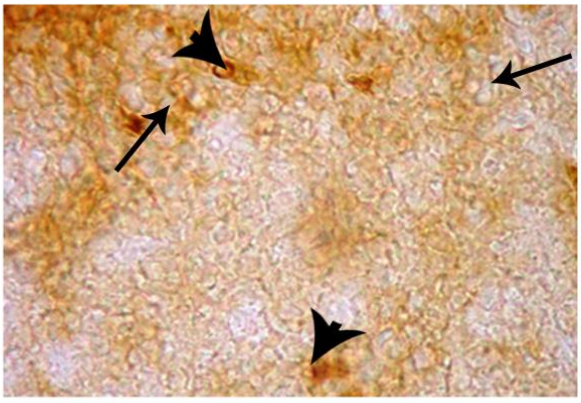

**Fig. 34: Immunohistochemical reaction of CD3 in thymus section of CP-treated mouse showing: very weak reaction in most lymphocytes (arrows); Notice: few scattered immunopositive cells in the medullary region (arrowheads). X 1000.**

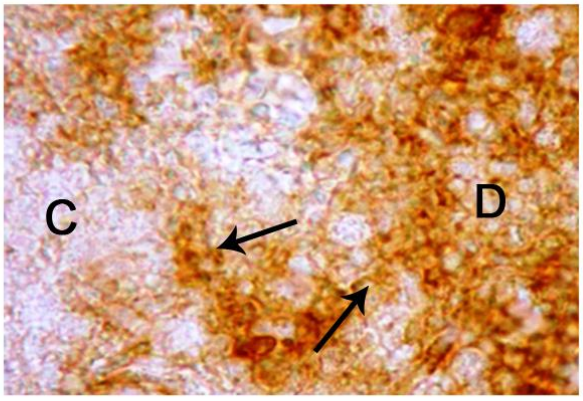


**Fig. 35: Immunohistochemical reaction of CD3 in thymus section of α-LA-treated mouse showing: most of the positively-stained T cells are located in the medullary area (D); few are detected in the cortical region (C). X 400**

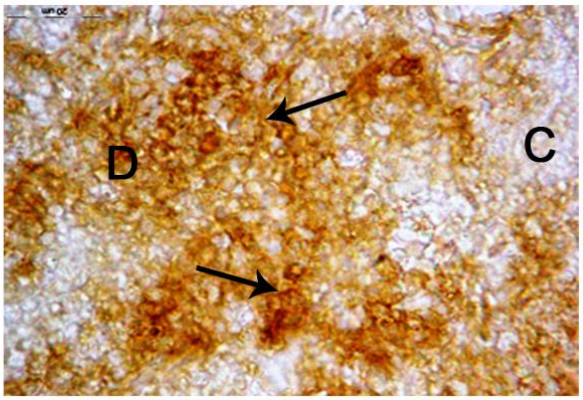


**Fig. 36: Immunohistochemical reaction of CD3 in thymus section of CP + α-LA-treated mice showing: moderate staining reaction of thymocytes (arrows) in the medullary area (D), and more lightly staining cortex (C). X 1000.**

# الملخص العربى

## دراسات هستولوجية ونسيجية مناعية عن دور حامض ألفا الليبويك على السيكلوفوسفاميد-المحث للتثبيط فى المناعة عند الفئران.

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يستخدم عقار السيكلوفوسفاميدلفترات طويلة فى علاج الكثير من أنواع السرطانات المختلفة، وكذلك سرطان الدم (اللوكيميا)، والعديد من أنواع الأورام السرطانية الصلبة. من جهة أخرى، تستطيع بعض المركبات البيولوجية والتى لها خواص مضادة للأكسدة، حماية الخلايا والأنسجة من نواتج الأكسدة النشطة الناتجة عن استخدام عقارالسيكلوفوسفاميد. من أهم هذه المركبات هو حمض ألفا الليبويك.

الهدف من هذه الدراسة هو التحقق من دورحمض ألفا الليبويك كعامل مضاد للأكسدة ضد الضرر الناتج عند إستخدام عقار السيكلوفوسفاميد على بعض الأعضاء الليمفاوية لذكور الفئران. لتحقيق هذا الهدف، تم شراء عدد من الفئران، وتركت لتتأقلم طبقا" للظروف البيئية والمعملية المناسبة لمدة أسبوعين قبل بداية التجربة. قسمت هذه الحيوانات إلى خمسة مجموعات تجريبية مختلفة، و احتوت كل واحدة منها على عشرة فئران حسب متوسط أوزان أجسامها، كما يلى:

أعطيت مجموعة فئران داخل التجويف البريتونى ½ مل من المحلول الملحى ، ثلاث مرات أسبوعيا"، لمدة ستة أسابيع متتالية، واعتبرت هذه الفئران ضابطة موجبة. المحلول الملحى هو المادة المستخدمة اللازمة لذوبان عقارالسيكلوفوسفاميد فيها. أعطيت مجموعة اخرى من الفئران يوميا"عن طريق الفم ½ مل من زيت عباد الشمس، لمدة ستة أسابيع متتالية، واعتبرت هذه الحيوانات ضابطة موجبة. زيت عباد الشمس هو المادة المستخدمة اللازمة لذوبان حمض ألفا الليبويك، وأعطيت مجموعة فئران داخل التجويف البريتونى ½ مل من مادة السيكلوفوسفاميد بتركيز 0.6 مجم/كجم وزن جسم، ثلاث مرات أسبوعياً ولمدة ستة أسابيـــــع. أما مجموعة فئران اخرى فقد أعطيت يومياً وعن طريق الفم ½ مل من حمض ألفا الليبويك بتركيز 0.1 مجم/كجم وزن جسم، لمدة ستة أسابيع متتاليـــــة. كما أعطيت مجموعة فئران نفس الجرعات السابقة من السيكلوفوسفاميد وكذلك حمض ألفا الليبويك بنفس الجرعات ولنفس المدة كما فى المجموعات السابقة.

فى نهاية مدة التجربة، أى بعد ستة أسابيع، تم وزن جميع حيوانات التجربة وكذلك الطبيعية منها. ثم ذبح الفئران وأخذ عينات الدم من القلب، وأخذت الأعضاء الليمفاوية مثل الطحال والغدة التموسية لفحصها ووزنهـا، وكذلك لدراسة التركيب النسيجى لها. كما تم أيضا قياس التكاثر الخلوى للخلايا Tالليمفاوية (التائية) فى كلا من نسيج الطحال والغدة التوتية، للتحقق من حيوية هذه الخلايا وقدرتها الوظيفية، وذلك باستخدام Con A mitogen. وكذلك تمت دراسة التركيبات النسيجية المناعية باستخدام (CD3) كمستقبل موجود على عموم أسطح الخلايا التائية للأعضاء الليمفاوية مثل الطحال والغدة التوتية.

أظهرت النتائـــــج أن الفئران التي تم إعطاؤها مادة السيكلوفوسفامايد انخفاضا" واضحا" فى نشاطها العام وأوضحت ضعفا" عاما" فى حيويتها، تم تسجيل انخفاضا" فى وزن الجسم، كما وجد ارتفاعا" ملحوظا" في معدلات الوفيات. من ناحية أخرى، فقد لوحظت زيادة معنوية فى أوزان الجسملدى الفئران التي تم إعطاؤها حمض ألفا الليبويك مع عقار السيكلوفوسفاميد.

سجلت النتائج المناعية عند إستعمال Con A mitogenانخفاضاً ملحوظاً في وظيفة الخلايا التائية وكذلك فى نسبة تكاثرها فى خلايا الطحال والغدة التوتية المنزوعة من مجموعة الفئران المعاملة بالسيكولوفوسفاميد. تمت معالجة هذا الإنخفاض بشكل طفيف فيالطحال وبشكل كبير في الغدة التوتية في مجموعة الفئران المعالجة بالمادتين معا".

أظهرت دراسة التركيب النسيجي لطحال الفئران التى أعطيت عقار السيكولوفوسفاميد تغيرات نسيجية واضحة مشتملة على:نقصاً كبيراً فى تجمعات الخلايا الليمفاوية وتحطماً فى النسيج الأحمر حيث ظهرت تجمعات دموية فى معظم أجزاء هذا النسيج. كما لوحظت أيضاً زيادة كبيرة فى عدد الخلايا الضخمة (megakaryocytes) فى معظم أجزاء نسيج الطحال، وأظهرت هذه الخلايا تعددا" ملحوظا" في الشكل وفقدانا" للتنظيم السيتوبلازميومحتوىالكروماتين. أما مجموعة الفئران التىأعطيت حمض ألفا الليبويك، فقد أظهرت تركيبياً طبيعياً

واضحاً لنسيج الطحال، حيث ظهرت الجريبات الطحالية كبيرة ويتوسطها شريان صغير كما فى الحالة الطبيعية. كما لم تظهرأنسجة الطحال للفئران التى أعطيتعقارالسيكلوفوسفاميد مع حمض ألفاليبويك تغيرات نسيجية كبيرة عند مقارنتها بتلك التى أعطيت عقار السيكلوفوسفامايد فقط.

أوضحت نتائج التركيب النسيجي للغدة التيموسية نقصاً ملحوظاً فى تجمع خلاياها، وكذلك فى الخلايا الشبكية والبلعمية ، أما فى منطقة النخاع فقد ظهر احتقان ملحوظ للأوعية الدموية، كما نقصت أيضاً أعداد وأحجام الخلايا الشبكية والبلعمية وكان هذا مصحوباً بزيادة في الخلايا الإلتهابية.إضافة إلىذلك، لوحظت زيادة في أعداد جسيمات هاسل مع التغير الواضح في تركيبها. أظهرت النتائج أن معظم التغييرات النسيجية السابقة فى نسيج الغدة التوتية قد تم انعكاسها في الفئران التى عولجت بحمض ألفا ليبويك بالاتحاد مع عقار السيكلوفوسفاميد، حيث ظهر نسيج هذه الغدة طبيعياً إلى حد كبير.

أظهرت نتيجة الفحص النسيجي المناعي بإستخدام CD3، تفاعلاً ضعيفاً جداً في مجموعة الفئران المعالجة بعقارالسيكلوفوسفامايد حيث أبدى معظم أجزاء النسيج الطحالي وكذلك نسيج الغدة عدم اصطباغه باللون البنى كما فى الأنسجة الطبيعية، مما يدل على النقص الواضح فى قدرة تكاثر الخلايا التائية الليمفاوية وعدم قدرتها على استكمال نضوجها.

على العكس من ذلك، أظهرت مجموعة الفئران التى عوملت إما بحمض ألفا ليبويك فقط أوعند إعطائه مع عقارالسيكلوفوسفاميد وجود الكثير من الخلايا الليمفاوية التى اصطبغت باللون البني مما يدل على وجود خلايا ليمفاوية ناضجة فى معظم أنسجة الطحال وكذلك أجزاء من الغدة، وهذا يدل على قدرة حمض ألفا الليبويك على استعادة إنتاج الخلايا الليمفاوية وزيادة تكاثرها وكذلك القيام بوظائفها فى كثير من مناطق النسيج الطحالي أوالنسيج الغدي، مما يثبت قدرة حمض ألفا الليبويك على علاج معظم التغيرات التى أحدثها عقار السيكلوفوسفاميد. واستنتاجا" أثبتت الدراسة الحالية الدور الوقائي لحمض ألفاليبويك ضد التأثير المثبط للمناعة والذي تسببه مادة السيكلوفوسفاميد في ذكور الفئران حتى وإن كانت هذه الحماية جزئية.