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Effect of Cyclosporin - A on the Lung of Adult Male Albino Rats and The Possible Protective Role of Vitamin E

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Abstract

Background: Cyclosporine A (Cs A) is an immunosuppressive agent which used in treatment of various autoimmune diseases.

Aim of Study: Evaluation of the effect of cyclosporin-A administration on the histological structure on the lung in adult male rat and the protective role of vitamin E.

Material and Methods: Forty adult male albino rats were divided equally into four groups. Group 1 received distilled water, Group 2 received oral daily dose of cyclosporine A (10mg/kg/body weight) for 4 weeks, Group 3 (a co-treated group) received oral daily dose of vitamin E (200mg/kg/body weight) simultaneously with cyclosporine A (10mg/kg/body weight) for 4 weeks, and Group 4 (a pre-treated group) received oral daily dose of vitamin E (200mg/kg) 2 hours prior to treatment with cyclosporine A (10mg/kg body weight) for 4 weeks. Lung specimens were taken out and processed for histopathological examination and for assessment of lung tissue levels of oxidative stress markers (MDA, SOD, GSH, and CAT).

Results: Histopathological examination using light and electron microscope revealed that cyclosporine-A induced deleterious histopathological changes in the lungs of adult male albino rats. Focal destruction of alveolar walls, formation of emphysematous air spaces, distortion of the interalveolar septa, peri-bronchial inflammatory cell infiltration and congestion with fibrosis. Pneumocytes type I showed an irregular nucleus. Pneumocytes type II revealed irregular pyknotic nuclei, numerous vacuolated or empty lamellar bodies with loss of their lamellar arrangement, and distorted surface microvilli. There was significant increase in lung malondialdehyde (MDA) level, with significant decrease in lung superoxide dismutase (SOD), glutathione peroxidase (GSH), and catalase (CAT). Vitamin E either with or before receiving cyclosporin-A reduced lung affection with improved level of oxidative markers in the lung homogenates.

Conclusion: Vitamin E reduced cyclosporin-A lung affection with improved level of oxidative markers in the lung homogenates.

Key Words: *Cyclosporine A – Oxidative stress – Lung – Vitamin E.*

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Introduction

CYCLOSPORINE A is an immunosuppressive agent used in the treatment of various autoimmune diseases and during organ transplantation to prevent rejection **[1]**. However, many adverse effects, including nephrotoxicity, hepatotoxicity, cardiotoxicity, vascular toxic effects have been associated with its clinical use **[2]**.

Vitamin E (tocopherol) reduces the pulmonary damage and attenuates lipid peroxidation reactions **[3]**. It seems to be the first line of defence against peroxidation of the polyunsaturated fatty acids of the cell membranes including mitochondrial membranes **[4]**.

It can block the development of some degenerative diseases by scavenging reactive oxygen species (ROS) **[5]**. The present work aimed to investigate the effect of cyclosporine A on the lung of adult male albino rat, and the possible protective role of vitamin E.

Material and Methods

Experimental animals:

Forty adult male albino rats of a local strain weighing 130-150 g were obtained from the animal house of the Faculty of Medicine, Al-Azhar University, Cairo, Egypt. They were housed in separate clean cages (five rats/cage) under standard laboratory and environmental conditions during January 2019-December 2019. All animal procedures were performed in accordance with the guide for the care and use of laboratory animals and approved by the Animal Ethical Committee at The Faculty of Medicine for Girls, Al-Azhar University. The principles of laboratory animal care were followed, as well as the specific national laws, when applicable. Rats were kept in metallic cages (50x45x35-

5 rats per cage) for 3 weeks for acclimation before starting of the experiment.

Drugs:

Cyclosporine (CsA) presented in the form of soft gelatine capsules containing 50mg cyclosporine with trade name (Sandimmune®, Neoral®) from (Novartis Pharma AG, Basilea, Suiza). Vitamin E presented in the form of capsule, each contained 400mg vitamin E from (El Kahira Pharmaceutical Co, Egypt).

Experimental design:

Animals were divided into four equal groups:

Group 1: Control group, were left without treatment.

Group 2: (Cyclosporine A treated group) received a single daily dose of cyclosporine A (10mg/kg/body weight) by oral gavage for four weeks according to [6].

Group 3: (Co-treated group) received a single daily dose of vitamin E (200 mg/kg/ body weight) by oral gavage according to [7] simultaneously with cyclosporine (10mg/kg/body weight) daily for four weeks.

Group 4: (Pre-treated group) received a single daily dose of vitamin E (200mg/kg) 2 hours prior to treatment with cyclosporine (10mg/kg body weight) daily for four weeks. By the end of experimental period, rats were anesthetized by ether, then sacrificed by decapitation. Both lungs were dissected carefully, prepared for light, electron microscopic examination, and assessment of lung tissue levels of oxidative stress markers.

Light microscopic examination:

Lung specimens were cut into small pieces, fixed immediately in 10% formalin saline for 24 h then processed to be stained by hematoxylin and eosin (H & E) stain [8] and Masson's trichrome stain [9].

Electron microscopic examination:

Lung sections were fixed in 2% glutaraldehyde and then processed for transmission electron microscopic examination. Semithin sections (1 μm thick) were cut, stained with toluidine blue, and examined by light microscope to select the area for ultrathin cutting. The ultrathin sections were mounted on copper grids, stained with uranyl acetate and lead citrate [10], then examined and photographed using a transmission electron microscope (Joel 1010 Jem) at the Regional Centre for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo.

Assessment of oxidative stress markers:

Lung specimens were collected and homogenized in TrisHCl buffer (5mmol/L containing 2mmol/L Ethylene-Diamine-Tetra-Acetic acid (EDTA), pH 7.4) to give a 10% (w/v) lung homogenate. The homogenates were centrifuged at 10,000g for 20min at 4ºC, and the supernatants were used for the assessment of malondialdehyde (MDA) content for the determination of oxidant-antioxidant status according to [11]. Assessment of antioxidant enzyme levels such as superoxide dismutase (SOD), reduced glutathione (GSH) levels, and catalase (CAT) using colorimetric assay according to [12,13]. The biochemical measurements were carried out at Biochemistry department, Faculty of Medicine, Cairo University.

Statistical methods:

Data were coded and entered using the statistical package SPSS version 22. Data were summarized using mean and standard deviation. Comparisons between the four studied groups were done using ANOVA followed by a Tuckey post-Hoc test for multiple comparisons. p-value less than 0.05 was considered significant. Correlation between variable was done using Pearson's correlation [14].

Results

Group 1: H&E-stained sections showed the normal lung architecture with clear patent bronchial passages, alveolar sacs, clusters of alveoli, interalveolar septa and blood vessels. The alveoli were lined by alveolar epithelium. The bronchioles were lined by simple columnar epithelial cells and goblet cells, and surrounded by smooth muscle layer (Fig. 1A). Mallory's trichrome stained sections showed fine collagen fibers in the interalveolar septa and around the bronchioles. (Fig. 1B).

Ultrathin sections revealed that the alveoli were lined by two types of pneumocytes. Type I pneumocytes were flat in shape with a large flat euchromatic nuclei surrounded by thin rim of cytoplasm. Type II pneumocyte were cuboidal in shape with central rounded nuclei and cytoplasm containing mitochondria, and many electron-dense lamellar bodies that contained the pulmonary surfactant with numerous surface microvilli (Figs. 1 C,D).

Group 2: H&E-stained sections showed distorted pulmonary architecture. In the same lung section, some alveoli showed focal destruction of their alveolar walls resulting in formation of large irregular emphysematous air spaces with thickening and distortion of the interalveolar septa, whereas

other areas appeared with apparently normal pulmonary architecture. The bronchioles exhibited exfoliated epithelial cells lining, with peri-bronchial inflammatory cell infiltration, interstitial exudates, congestion and extravasated RBCs were observed (Fig. 2A). Mallory's trichrome stained sections showed increase in collagen fibers in the interalveolar septa and in perivascular and peribronchial areas (Fig. 2B).

Ultrathin sections revealed that pneumocytes type I showed an irregular nucleus surrounded by thin rim of cytoplasm. Pneumocytes type II revealed irregular pyknotic nuclei, numerous vacuolated or empty lamellar bodies with marked loss of their lamellar arrangement, and distorted surface microvilli. Marked collagen fibrils deposition were noticed (Figs. 2C,D).

Group 3: H&E-stained sections showed that the alveoli were lined by alveolar epithelium and were separated from each other by thin inter-alveolar septa with few areas of emphysematous air spaces and congestion were seen. Mallory's trichrome stained sections showed the few collagen fibers in the inter-alveolar septa and in perivascular and peribronchial areas (Fig. 3B).

Ultrathin sections revealed some of the pneumocytes type I had irregular euchromatic nuclei while the others had heterochromatic shrunken nuclei. Pneumocytes type II showed few vacuolated or empty lamellar bodies and distorted surface microvilli. Minimal collagen fibrils deposition was noticed (Figs. 3C,D).

Group 4: H&E-stained sections showed the alveoli and the bronchioles were nearly normal with few areas of thickening of the inter-alveolar septa with preservation of pulmonary architecture (Fig. 4A). Mallory's trichrome stained sections showed fine collagen fibers in the inter-alveolar septa and in perivascular and peribronchial areas (Fig. 4B).

Ultrathin sections revealed that type I pneumocytes had flat euchromatic nuclei surrounded by thin rim of cytoplasm. Type II pneumocyte had central rounded nuclei and lamellar bodies with short surface microvilli. Minimal collagen fibrils deposition was noticed (Figs. 4C,D).

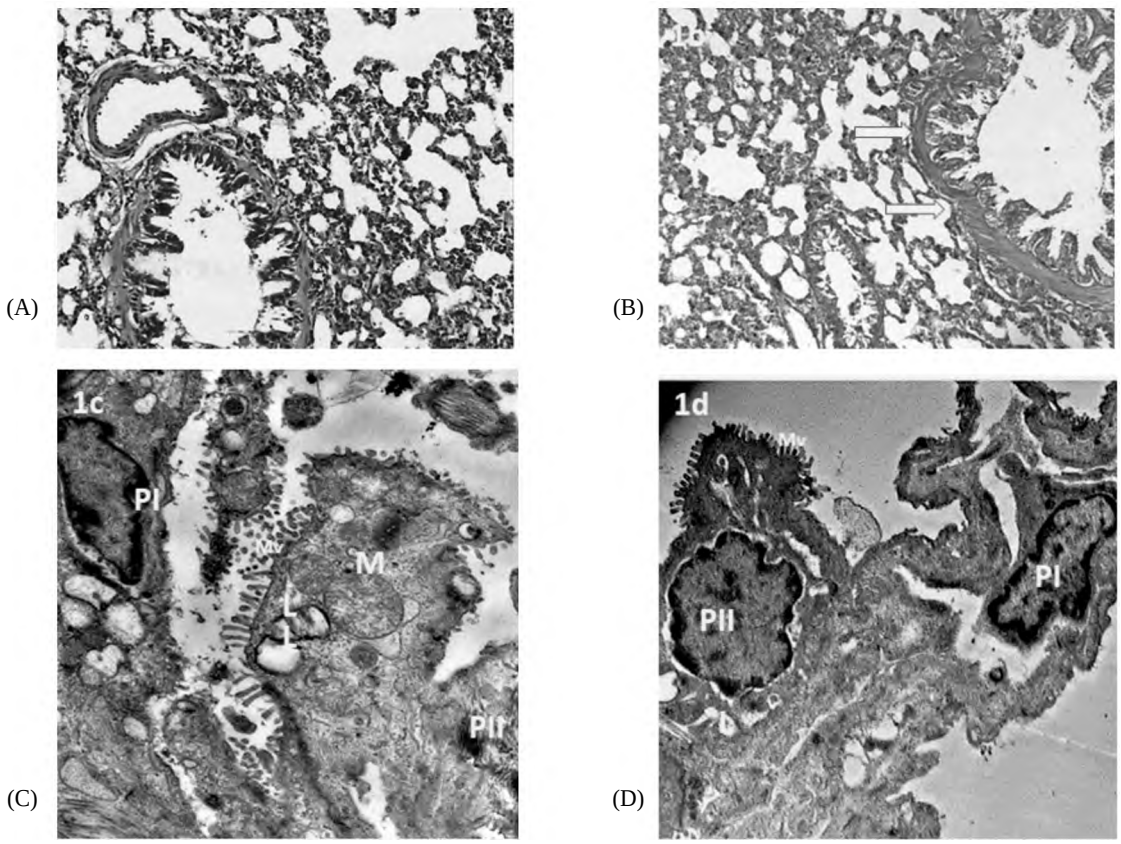

Fig. (1): Photomicrographs of sections in rat's lungs of group 1:
(A): Alveolar sacs (AS), alveoli (A), bronchiole (B) and blood vessels (v) (H &Ex 200).
(B): Fine collagen fibers around the bronchioles (arrows). (Mallory's trichrome stain x 200).
(C): Type I pneumocyte I (PI), type II pneumocyte (PII), mitochondria (M), lamellar bodies (L), and microvilli (Mv) (EMx10000).
(D): Type I pneumocyte I (PI), type II pneumocyte (PII), lamellar bodies (L), and microvilli (Mv) (EMx10000).

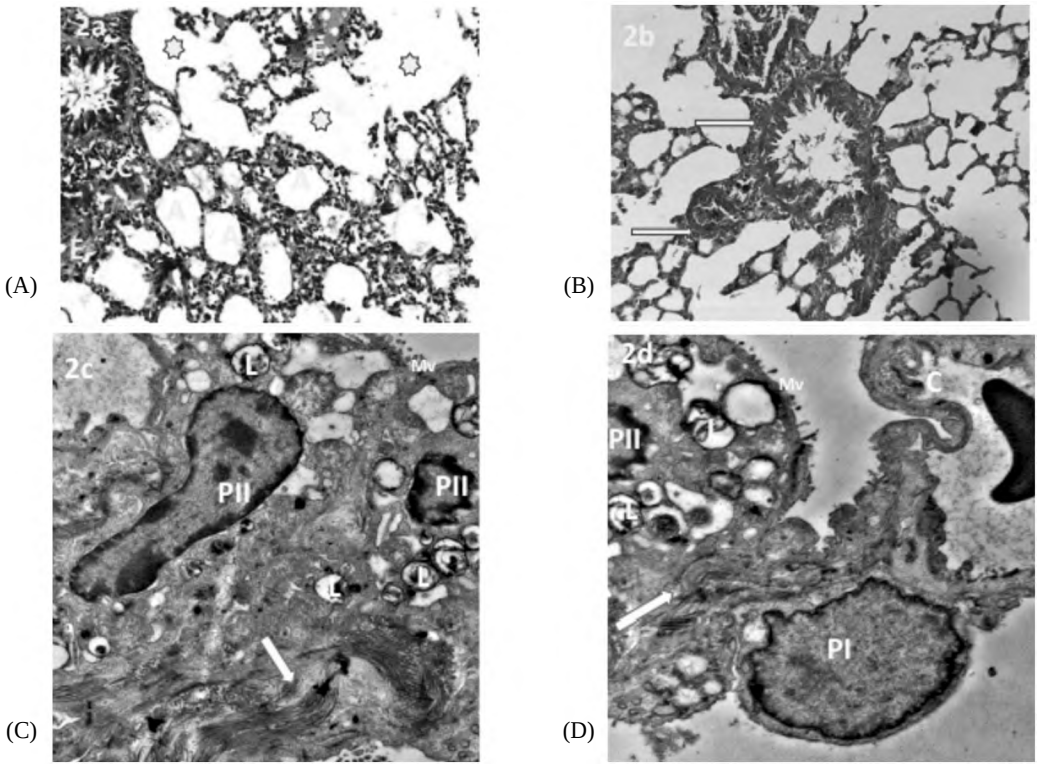

Fig. (2): Photomicrographs of a sections in rat's lungs of group 2:
(A): Destructed alveoli (A), large irregular emphysematous air spaces (stars), congestion (C) and exudate (E) surrounding bronchiole (B) (H &Ex 200).
(B): Increase collagen fibers in perivascular and peribronchial areas (arrows) (Mallory's trichrome stain x 200).
(C): Type II pneumocyte (PII), lamellar bodies (L), microvilli (Mv) interstitial collagen fibrils (arrow) (EMx10000).
(D): Type I pneumocyte I(PI), type II pneumocyte (PII), lamellar bodies (L), microvilli (Mv) and interstitial collagen fibrils (arrow) (EMx10000).

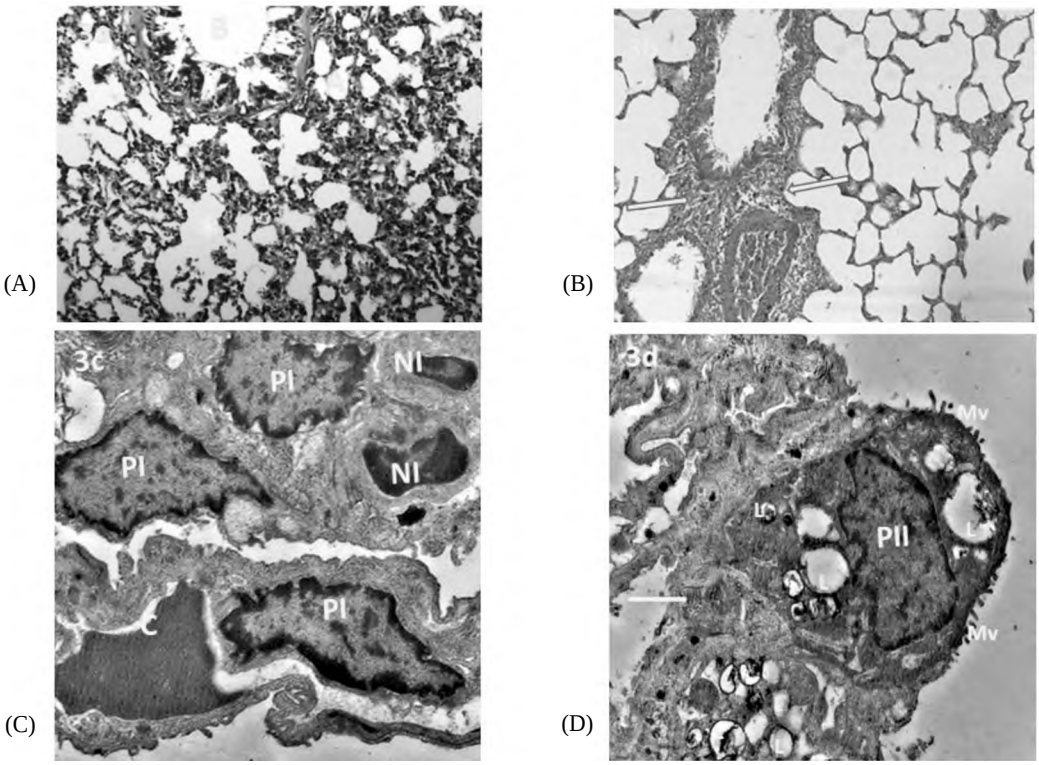

Fig. (3): Photomicrographs of sections in rat's lungs of group 3:
(A): Alveoli (A), bronchiole (B) emphysematous air spaces (star) (H &Ex 200).
(B): Increase collagen fibers in perivascular and peribronchial areas (arrows) (Mallory's trichrome stain x 200).
(C): Pneumocytes type I had irregular euchromatic nuclei (PI), while the others had heterochromatic shrunken nuclei (NI), and blood capillary (C) (EMx10000).
(D): Type II pneumocyte (PII), lamellar bodies (L), microvilli (Mv), and interstitial collagen fibrils (arrow) (EMx10000).

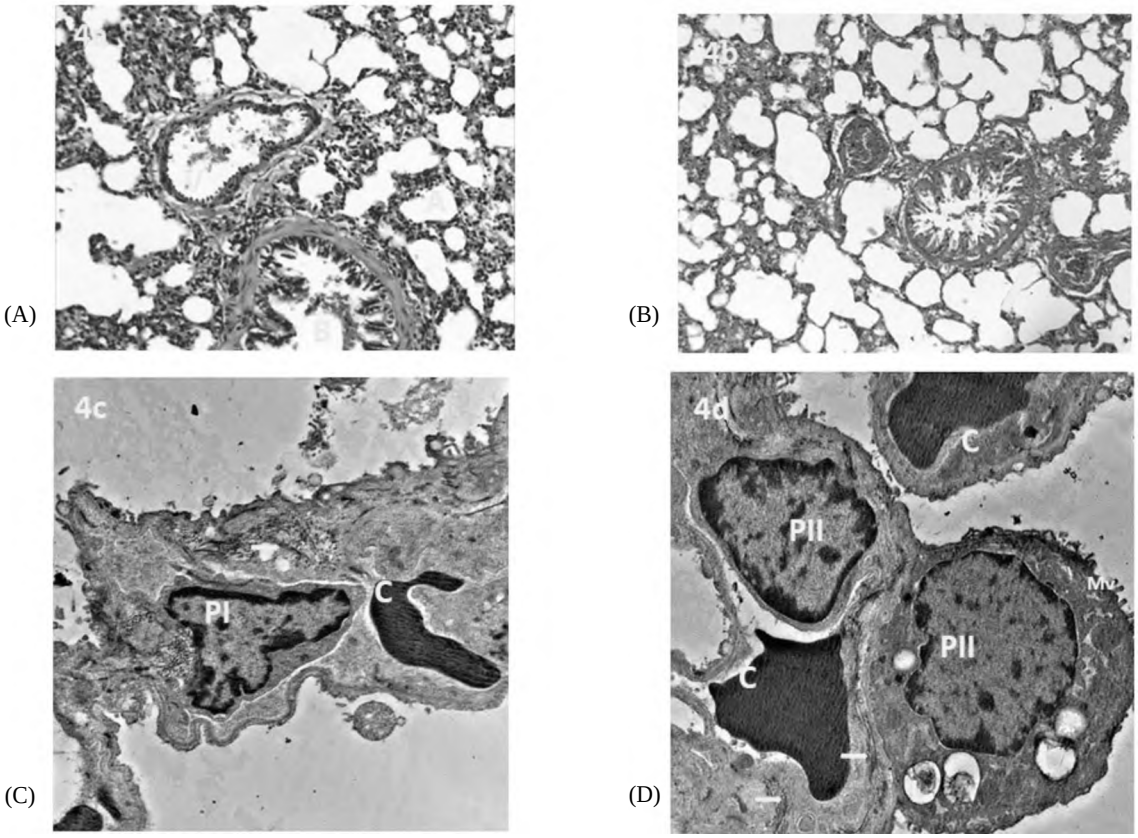

Fig. (4): Photomicrographs of sections in rat's lungs of group 4 :
(A): Alveoli (A), bronchiole (B) and blood vessels (v) (H &Ex 200).
(B): Fine collagen fibers in perivascular and peribronchial areas (arrows). (Mallory's trichrome stain x 200).
(C): Type I pneumocyte (PI), blood capillary (C), and collagen fibrils (arrow) (EMx10000).
(D): Type II pneumocyte (PII), lamellar bodies (L), microvilli (Mv), and interstitial collagen fibrils (arrow) (EMx10000).

Assessment of oxidative stress markers:

The lung MDA levels showed significant increase in groups (2,3, and 4) compared to group 1 (p-value <0.001). Significant decrease in MDA level in groups (3, and 4) compared to group 2 (p-value <0.001). Significant decrease in MDA level in group 4 compared to group 3 (p-value <0.001) (Fig. 5).

The lung SOD levels showed significant decrease in groups (2, 3, and 4) compared to group 1 (p-value <0.001, 0.012, 0.023) respectively. No significant difference between groups (2,3, and 4) (p-value <0.05) (Fig. 6).

The lung GSH levels showed significant decrease in groups (2,3, and 4) compared to group 1 (p-value <0.001). Significant increase in GSH level in group 3, and 4 compared to group 2 (p-value <0.001). No significant differences between groups 3, and 4 (p-value 0.15) (Fig. 7).

The lung CAT levels showed significant decrease in groups (2,3, and 4) compared to group 1 (p-value <0.001). Significant increase in CAT level in groups 3, and 4 compared to group 2 (p-value <0.001). Significant decrease in CAT level in group 4 compared to group 3 (p-value <0.001) (Fig. 8).

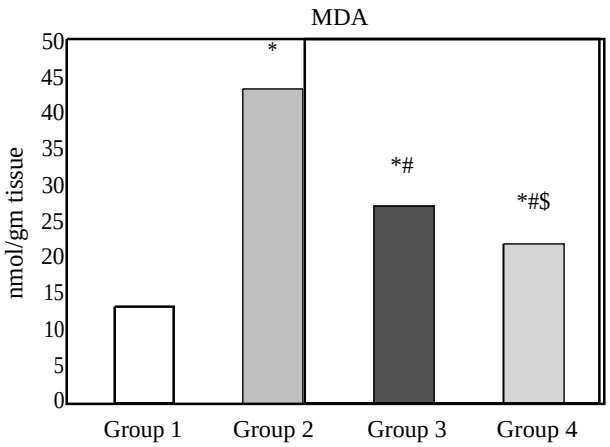

Fig. (5): MDA level in all experimental groups.

Data were expressed as mean ± SD.
(*): Significant difference versus group 1.
(#): Significant difference versus group 2.
($): Significant difference versus group 3.

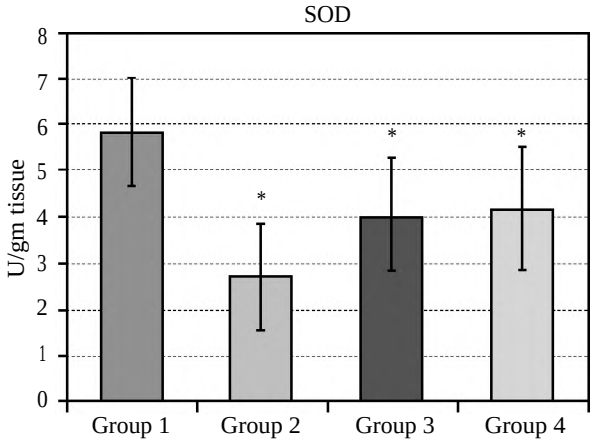

Fig. (6): SOD level in all experimental groups.

Data were expressed as mean ± SD.
(*): Significant difference versus group 1.
(#): Significant difference versus group 2.
($): Significant difference versus group 3.

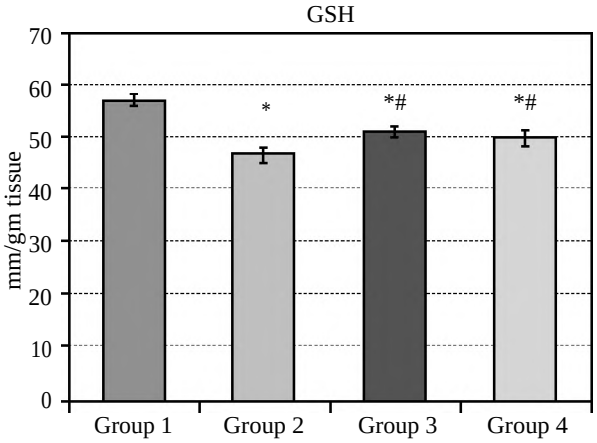

Fig. (7): GSH level in all experimental groups.

Data were expressed as mean ± SD.
(*): Significant difference versus group 1.
(#): Significant difference versus group 2.
($): Significant difference versus group 3.

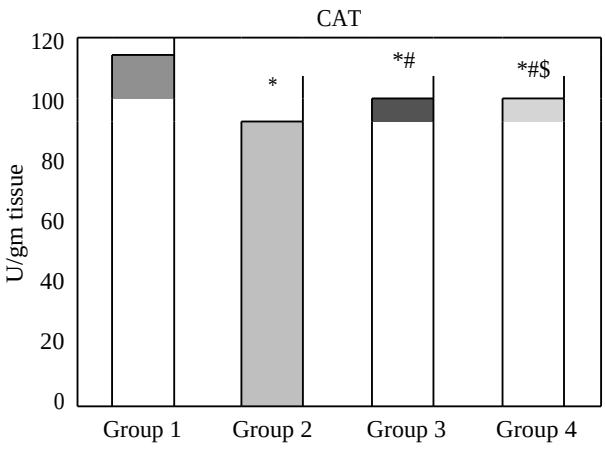

Fig. (8): CAT level in all experimental groups.

Data were expressed as mean ± SD.
(*): Significant difference versus group 1.
(#): Significant difference versus group 2.
($): Significant difference versus group 3.

Discussion

The present study showed that cyclosporin-A exerted deleterious histopathological changes in the lungs of adult male albino rats. Focal destruction of alveolar walls, formation of emphysematous air spaces, distortion of the interalveolar septa, peribronchial inflammatory cell infiltration and congestion with fibrosis. Pneumocytes type I showed an irregular nucleus. Pneumocytes type II revealed irregular pyknotic nuclei, numerous vacuolated or empty lamellar bodies with loss of their lamellar arrangement, and distorted surface microvilli.

These findings were matched with **[15]** who found that cyclosporine induced pulmonary changes in mice in the form of thickened interalveolar septa, pneumocytes vacuolation, congestion and haemorrhage of most blood capillaries. Similar results were reported by **[16]** who found degeneration of alveoli, pneumocyte type II apoptosis, pulmonary vessels congestion and emphysema with cyclosporine in rats. Our results revealed that cyclosporine induced lung fibrosis with collagen fibers deposition which were detected by Mallory's trichrome stain and confirmed by electron microscopic examination. This result was clarified by previous studies of **[17]** who found that cyclosporine can initiate fibroblast proliferation through stimulation of mediators of the epithelial cells of lung airway passages.

Our results revealed that cyclosporin administration resulted in significant elevation in lung tissue MDA level along with significant decrease in antioxidant enzymes (SOD, GSH, and CAT) levels. Many published articles explained the role of oxidative stress in the development of cyclosporine pulmonary toxicity **[18]** found that cyclosporin increases the production of reactive oxygen species (ROS) and lipid peroxidation products. Similarly **[19]** suggested that cyclosporine induced oxidative stress thus leading to molecular and cellular damage in different body organs.

Co-administration of vitamin E with cyclosporine-A resulted in reduced lung tissue damage with few areas of emphysematous air spaces and congestion. In addition, marked reduction in the collagen deposition and fibrosis in lung was detected by Mallory's trichrome and confirmed by electron microscopic examination. Ultrastructurally minimal affection and reduced apoptosis of both type I pneumocytes and type II pneumocytes. Moreover, significant decrease in lung MDA level with significant increase in antioxidant enzymes levels (SOD, GSH, and CAT) in this group. Similar

results were reported by **[20]** who found that Vit E decrease the inflammation appeared in the lung tissues together with the decrease in the levels of malondialdehyde (MDA).

Pre-administration of vitamin E 2 hours prior to treatment with cyclosporine-A resulted in preservation of pulmonary architecture with amelioration of fibrosis as detected by Mallory's trichrome and confirmed by electron microscopic examination. Ultrastructurally type I pneumocytes and type II pneumocytes appeared nearly normal. Significant decrease in lung MDA level with significant increase in antioxidant enzymes levels (SOD, GSH, and CAT). This was in harmony with the results of **[21]** who found pre-administration of vitamin E 2 hours prior to treatment with amiodarone revealed noticeable improvement of the histological and ultrastructural architecture of the lung with decrease in fibrosis of lung which might be due to down regulation of pro-fibrotic and pro-inflammatory genes by vitamin E.

In this study the vitamin E was found to be effective in reducing lung damage produced by cyclosporine-A and this improvement was more marked with pre- administration **[22]** suggested that alveolar type II cells play a central role in the biosynthesis and assembly of surfactant lipids. Vitamin E is important lipophilic antioxidant which acts by protecting surfactant lipids against oxidation and shield alveolar type II cells.

These results coincided with **[23]** who stated that vitamin E supplementation has reduced the alveolar damage and collagen deposition caused by intratracheal amiodarone. The mechanism by which vitamin E protected the lungs was thought to be due to reduction in oxidative stress biomarkers and hence decreased inflammation through inhibition of release of cytokines **[24]**. Vitamin E is effective on the prevention of bleomycin-induced pulmonary fibrosis and increased the GSH level and CAT activity and decreased the MDA level and reduced the histopathological fibrotic lesions **[25]**.

Conclusion:

Our study showed that administration of vitamin E is effective against the cyclosporine-A induced damaging effect on the histological structure of the lung. This improvement was more marked with pre- administration than co-administration.

Declaration of conflicts of interest:

No conflicts of interest.

Funding details:

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تأثير السيكلوسبورين أ على الرئة فى ذكور الجرذان البالغة البيضاء والدور الوقائى المحتمل لفيتامين (هـ)

خلفية البحث: السيكلوسبورين A (CsA) هو عامل مثبط للمناعة يستخدم فى علاج أمراض المناعة الذاتية المختلفة.

الهدف من البحث: تقييم تأثير إعطاء السيكلوسبورين أ على التركيب النسيجى للرئة فى ذكور الجرذان البالغة والدور الوقائى لفيتامين هـ.

مواد وطرق البحث: قسمت أربعون فأراً بالغاً من ذكور الجرذان البيضاء بالتساوى إلى أربع مجموعات:

- المجموعة ١: تلقت الماء المقطر.
- المجموعة ٢: تلقت جرعة يومية فموية من السيكلوسبورين أ (١٠ مجم/كجم/وزن الجسم) لمدة ٤ أسابيع.
- المجموعة ٣: مجموعة معالجة مشتركة تلقت جرعة يومية فموية من فيتامين (هـ) (٢٠٠ مجم/كجم) بالتزامن مع السيكلوسبورين (١٠ مجم /كجم/وزن الجسم) لمدة ٤ أسابيع.
- المجموعة ٤: (مجموعة مُعالجة مسبقاً) تلقى كل فأر جرعة يومية فموية من فيتامين (هـ) (٢٠٠ مجم/كجم) ساعتين قبل العلاج بالسيكلوسبورين A (١٠ مجم/كجم من وزن الجسم) لمدة ٤ أسابيع.

تم أخذ عينات الرئة معالجتها لفحص الأنسجة ولتقييم مستويات أنسجة الرئة لعلامات الإجهاد التأكسدى (MDA و SOD و GSH وCAT).

النتائج: أظهر الفحص التشريحى المرضى بإستخدام المجهر الضوئى والالكترونى أن السيكلوسبورين-A تسبب فى تغيرات نسيجية ضارة فى رئتى الجرذان البيضاء البالغة. التدمير البؤرى للجدران السنخية، وتشكيل مساحات هوائية انتفاخية، وتشويه الحاجز بين السنخ، وتسلل الخلايا الالتهابية حول القصبات واحتقان التليف. الخلايا الرئوية من النوع الأول أظهرت نواة غير منتظمة. وكشفت الخلايا الرئوية من النوع الثانى عن نوى متحدرة غير منتظمة، والعديد من الأجسام الصفائحية الفارغة أو الفارغة مع فقدان ترتيبها الرقائقى، وتشوه لزغابات السطحية.

أنت هناك زيادة معنوية فى مستوى مالو نديالديهيد الرئة (MDA) مع انخفاض كبير فى ديسموتاز الرئة الفائق (SOD)، والجلوتاثيون بيروكسيديز (GSH)، والكتلاز (CAT).

استخدام فيتامين (هـ) مع أو قبل تناول السيكلوسبورين أ – يقلل من تأثر الرئة مع تحسن مستوى علامات الأكسدة فى متجانسات الرئة.