Med. J. Cairo Univ., Vol. 90, No. 1, March: 177-183, 2022
www.medicaljournalofcairouniversity.net

Pulmonary Toxicity Induced by Cyclophosphamide and its Modulation by Quercetin

MOHAMED M. ABDELMOATY, M.D. and WALEED A. ABDEL JALIL, M.D.

The Department of Anatomy, Faculty of Medicine, Cairo University

Abstract

Background: Cyclophosphamide (CP) is used in the treatment of many types of cancers and immune disorders.

Aim of Study: To support the role of queretin as antioxidant added to CP in the treatment of malignancies.

Material and Methods: Twenty-five rats were divided into five groups; group I were injected intraperitoneally (i.p.) with normal saline, group II were injected with a single weekly dose of CP at 500mg/kg BW for one week, group III were injected with a single dose of CP at 500mg/kg BW for one week and treated with Quercetin (QUE) dissolved in corn oil at a dose of 100mg/kg BW daily via gastric tube for 7 days, group IV were injected with a weekly dose of CP at 500mg/kg BW for 3 weeks and group V were injected with a single dose of CP) at 500mg/kg BW for 3 weeks and treated with Quercetin (QUE) dissolved in corn oil at a dose of 100mg/kg BW daily via gastric tube for 21 days. All animals were sacrificed by decapitation 4-6 hours after their last medication intake. The extracted lungs were formalin fixed in 4% buffered formaldehyde for morphological and immunohistochemical examination.

Results: CP was found to evoke histopathological alterations in the lungs via inducing oxidative stress and inflammatory reaction with improving effect by QUE.

Conclusion: QUE could modulate CP induced pulmonary toxicity by its antioxidant effect.

Key Words: *Cyclophosphamide – Quercetin – Oxidative – Inflammation.*

Introduction

CYCLOPHOSPHAMIDE (CP) is used in the treatment of many types of cancers and immune disorders but it is reported to cause lung toxicity as mentioned by Shannon and Price **[1]** and Cooper et al., **[2]** who detected pulmonary toxicity in 10% of patients with various malignancies whom their antineoplastic management included cyclophosphamide.

Correspondence to: Dr. Mohamed M. Abdelmoaty, E-Mail: mmmotey@yahoo.com)

Wilczynski et al., **[3]** described busulphan, cyclophosphamide, bleomycin and BCNU as an example of the antineoplastic drugs that may promote disturbances in lung oxidants in relation to the antioxidants, resulting in increased oxidative stress and pulmonary histological injury.

Moreover, Mahmoud et al., **[4]** reported another type of CP toxicity in the form of hepatic injury and described necrosis and inflammatory reaction as mechanisms of the injury. In addition, they used a natural antioxidant in a trial to improve these injurious effects.

MacAllister et al., **[5]** added that the metabolism of CP is made by the hepatic microsomal cytochrome P450 enzyme that transformed it into phosphoramide mustard, and acrolein and Acrolein is the part of CP which inhibits the antioxidant system, resulting in the production of reactive oxygen species.

Preiser **[6]** elucidated the oxidative stress as a mechanism mediated by most of the cytotoxic drugs to cause cellular injury resulting in different histopathological alterations in different organs.

Quercetin (QUE) is present in many fruits and vegetables as a natural flavonoid. Its antioxidants, anti-inflammatories, anti-apaptotic, anti–thrombotics, and anti-ischemics, and are anti-mutagenic, anti-cancer, anti-angiogenic, anti-proliferative, and anti-viral were documented by many trials **[7]**.

Venkatesan and Chandrakasan **[8]** explained one of the chemical actions of CP induced lung toxicity is the increase of the human cytokines, including TNF-a, that are essential mediators of inflammation promoting acute inflammatory responses.

The aim of this study was to support the role of queretin as antioxidant added to CP in the treatment of malignancies due to its antioxidative and antiinflammatory effects especially in the lungs.

Material and Methods

Animals:

Twenty five male Wistar rats (weighed from 150 to 250 grams) were put in plastic cages and fed orally at the same temperature (21-22 C) and humidity on a 12 hour light and darkness cycles at an air exchange rate of 18 changes per hour. Rats in all groups were given free access to food and water. The feeding and the drug administration were under the control of the vegetarians in the animal laboratory of the national institute of ophthalmology during the duration from 15 October 2021 to 7 November 2021.

Chemicals:

Cyclophosphamide was obtained from (Sandoz company) in the form of 500mg vials and Quercetin was obtained from Nutrissence company in the form of 500mg capsules.

The rats were divided into five groups with 5 rats each:

- Group I (the control group): Were injected intraperitoneally (i.p.) with normal saline.
- Group II (the CP given group): Were injected with a single weekly dose of CP at 500mg/kg BW for one week **[9]**.
- Group III (the CP and QUE given group): Were injected with a single dose of CP at 500mg/kg BW for one week and treated with Quercetin (QUE) dissolved in corn oil at a dose of 100mg/kg BW **[10]** daily via gastric tube for 7 days.
- Group IV (the CP given group): Were injected with a weekly dose of CP at 500mg/kg BW for 3 weeks.

Group V (the CP and QUE given group): Were injected with a single dose of CP) at 500mg/kg BW for 3 weeks and treated with Quercetin (QUE) dissolved in corn oil at a dose of 100mg/kg BW daily via gastric tube for 21 days.

All animals were sacrificed by decapitation 4-6 hours after their last medication intake. Each lung was weighed separately and immediately cut in two pieces. One half was formalin fixed in 4% buffered formaldehyde for morphological and immunohistochemical examination. The other half of the lung was immediately stored at 70ºC until further analysis.

Histopathological analysis:

The right lungs were rapidly removed from the animal, sliced transversely, paraffin embedded, and prepared as 3mm thick sections stained with hematoxylin and eosin (H&E) and Masson stains for light microscopic evaluation. The tissues were examined under a microscope in a random order and by someone without the knowledge of the treatment groups **[11]**.

Preparation of lung homogenate:

The isolated left lungs were rinsed in chilled 1.15% KCl (pH 7.4) and weighed quickly. Subsequently, the lung/body weight ratio was determined. Homogenization was carried out in ice-cold KCl (1.15%, pH 7.4) to yield 10% w/v tissue homogenates and the following biochemical parameters were assessed **[12]**.

Detection of serum TNF- α by enzyme-linked immunosorbent assay:

Enzyme-linked immunosorbent assays (ELISAs) were used to detect TNF- α level in rat serum according to the manufacturer's manual (Quantikine R&D system Inc, Minneapolis, MN). All TNF- α determinations were performed in duplicate serial dilutions. Absorbance was read on a microplate reader and the concentrations were calculated from the standard curve **[13]**.

Determination of superoxide dismutase (SOD):

The enzymatic activity of SOD was assessed according to Marklund **[14]**. SOD activity was expressed as units of activity per gram wet tissue. One unit of SOD activity is defined as the amount of the enzyme causing 50% inhibition of autooxidation of pyrogallol.

Statistical analysis:

Data are expressed as mean ± SD (significance was calculated at p50.05). Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by the Tukey- Kramer multiple comparisons test, in addition to linear regression analysis for the best-fitting line of all standard points. Also, paired Student's *t*-test was used as a test of significance for comparison between two arithmetic means of the same subject before and after treatment. Chi-square test was used for the comparison of two proportions. Statistical calculations were carried out using Instat-2 computer program (GraphPad Software Inc. V2.04, San Diego, CA) **[15]**.

Imaging analyzer:

Image analysis was performed using the software Leica Qwin, Germany. The areas showing the blue Masson trichrome staining were selected and outlined by the curser. The computer system is calibrated to convert pixels into micrometer units. The computer system converted the image into a blue binary color that could be measured by the computer system and the area percent of staining was calculated. Data was expressed as mean + or - standard deviation **[11]**.

Results

I- *Histological results:*

Histopathological examination of the lungs In the histologic analyses of lung tissues, the control group sections were seen as having no lung injury or histologic changes, which were seen as normal alveolar ducts, alveolar sacs and alveoli. The broncioles were small elongated airways which opened distally into alveolar sacs. The bronchioles had numerous alveoli opening along their length. The inter-alveolar septa were formed of the epithelial lining of the alveoli and a loose connective tissue contained extensive capillary network around the alveoli, where adjacent alveoli always shared capillary bed and connective tissue (Figs. 1A,2A). Otherwise, severe lung injuries were observed in the CP-treated group for one week. There were extensive damage of the lung tissue with loss of the normal alveolar architecture. Moreover, most of the specimens showed diffuse cellular infiltration resulting in massive consolidation with alveolar collapse. Other specimens showed ruptured inter-alveolar septa with the formation of large irregular emphysematous air spaces and there were thickness in the alveolar-septa and congestion (Figs. 1B,2B). However, treatment with 100mg/kg of QUE in combination with CP treatment in group III diminished the degeneration and inflammatory changes in the previous CP-induced group (Figs. 1C, 2C). CP induced chronic lung alterations when given for 3 weeks in the rats of group IV in the form of thickening of the inter-alveolar septa, abundant fibrosis and the deposition of excessive collagen fibers as detected by Masson's trichrome stain (Figs. 1D,2D). However, addition of quercetin in group V improved these chronic changes in the form of slight increase in collagen deposition when compared to control group but, it was much less than seen in cases treated with CP alone and partial restoration of the normal thickening of the inter-alveolar septa (Figs. 1E,2E).

II- Image analyser:

The masson trichrome stained slides showed CP induced moderate deposition of collagen fibres in group II but significant deposition of collagen fibres in group IV with a significant improving effect when QUE added to groups III and V respectively as the area % of collagen fibres deposition measured by image analyser method (Table 1 and Histogram 1).

III- Effect of CP and/or quercetin on serum total TNF-α:

The CP-treated group produced a significant increase in the level of serum TNF-α compared to the control nontreated group (p<0.05). Animals treated with CP and quercetin showed a significant decrease in the levels of serum TNF-α compared to the CP-treated group animals (p<0.05) (Table 1 and Histogram 2).

IV- Lung SOD activity:

The SOD activity in lung homogenates from CP-treated rats was non significantly decreased when compared with the control group (p>0.05). In addition, animals treated with CP and quercetin, there was increase of SOD activities in comparison with the CP-received group especially significantly clear in groups IV and V (p<0.05) (Table 1 and Histogram 3).

Table (1): The comparison of the chemical parameters between different groups.

Chemicals Groups	Area % of collagen (mean ± SD)	TNF-a (mean ± SD)	SOD (mean ± SD)
The control group	21.8±2.1 **all**	225.3±43 **all**	206.6±14.45****
Cp received group for 1w	30.1±1*	898±30.9*,***	138.63±15.17
Cp+QUE received group for 1w	26.2±0.1*	489.7±38*,**	175.29±8.07
Cp received group for 3w	40.2±1.4*,*****	691.6±21.7*	157.64±8.93*,*****
Cp+QUE received group for 3w	35.9±1*,****	300.3±16.56*	191.42±6.65****

p-value: Significant 0.05. on-significant 0 05.
p-value: *Significant in relation to group I. ** Significant in relation to group II.
*** Significant in relation to group III. **** significant in relation to group IV
***** Significant in relation to group V.

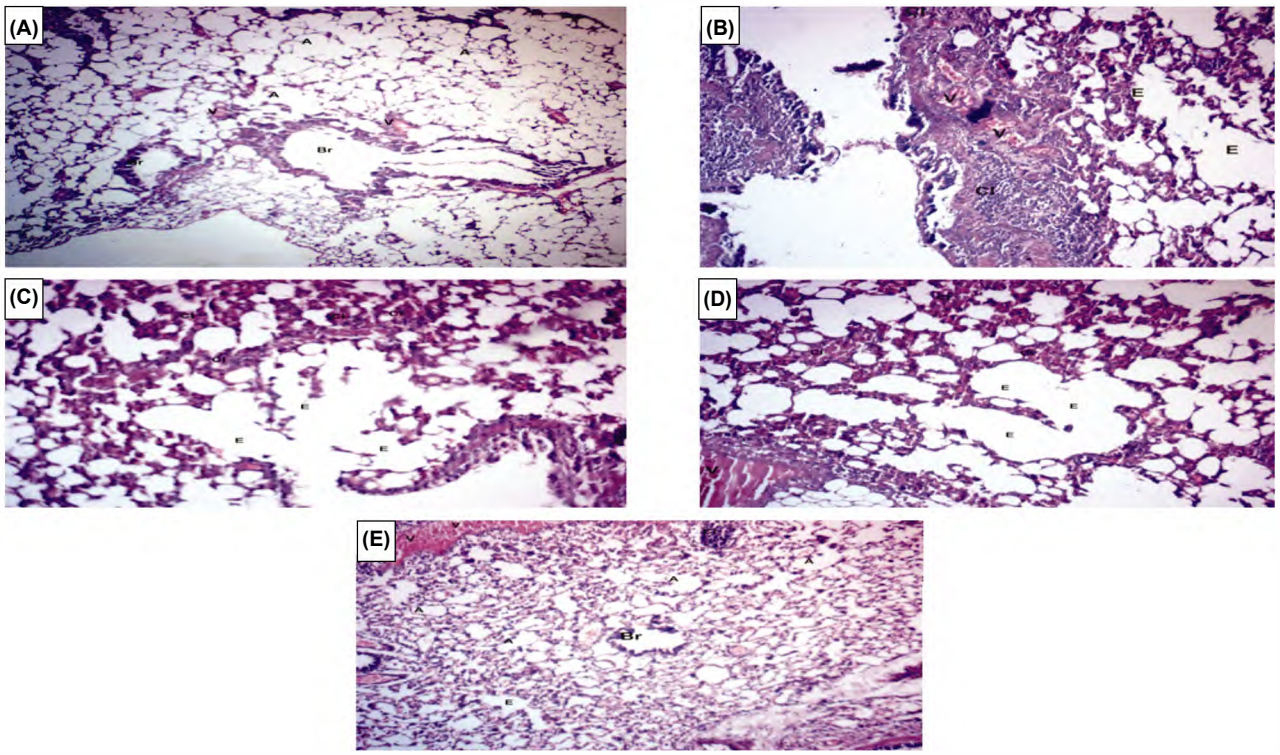


Fig. (1): A photomicrograph of the lung of the rats of (A): The control group showing normal alveoli (A), bronchioles (br) and blood vessels (V). (B): The CP received group showing massive cell infiltrates (CI), ruptured alveoli and emphysema (E) and congested blood vessels (V). (C): The CP and QUE received group showing less cell infiltrates (CI) and ruptured alveoli and emphysema (E). (D): The 3w CP received group showing thick ruptured alveoli (A), less cell infiltrates (CI) and congested blood vessels (V). (E): The 3w CP and QUE received group showing restoration of the alveoli (A), the bronchiole (br) and blood vessels (V). (Hematoxylen and eosin x100).

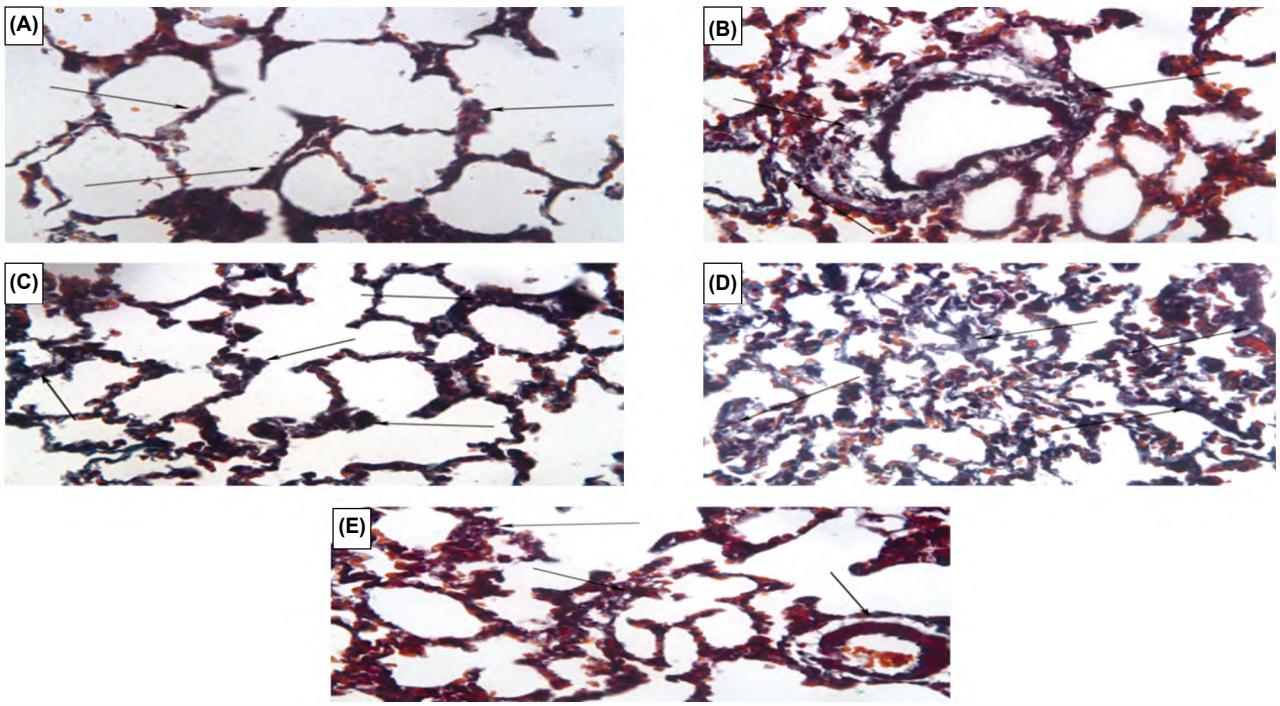
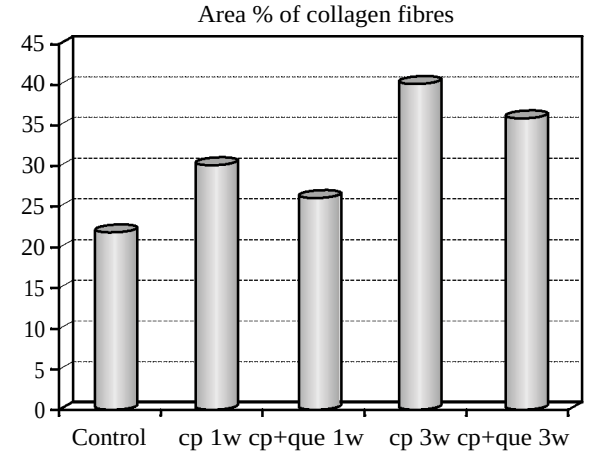
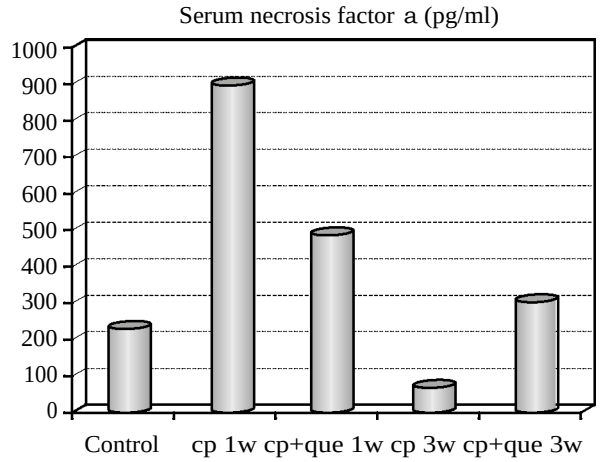


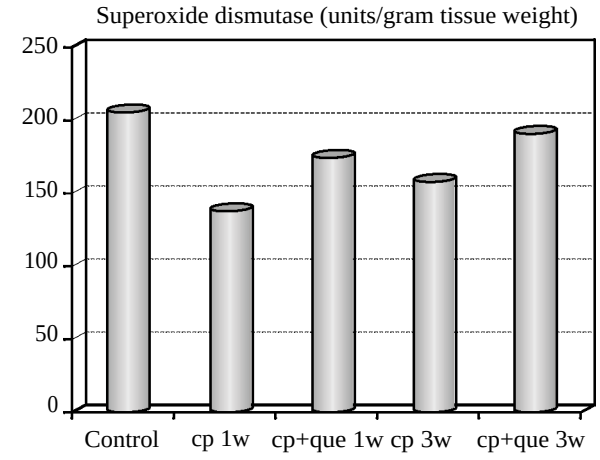
Fig. (2): A photomicrograph of the lung of the rats of (A): The control group showing normal alveolar septa and collagen fibres (arrows). (B): The CP received group showing congested blood vessels with collagen deposition around (arrows). (C): The CP and QUE received group showing mild collagen fibres deposition (arrows). (D): The 3w CP received group showing thick alveoli with massive collagen deposition (arrows). (E): The 3w CP and QUE received group showing moderate collagen fibres deposition (arrows). (Masson trichrome x400).



Histogram (1): The mean collagen area % in different groups.



Histogram (2): The mean TNF-a values in different groups.



Histogram (3): The mean SOD activity in different groups.

Discussion

Cyclophosphamide (CP) is one of the most effective chemotherapeutic agents used in the treatment of cancers of breast, lung, prostate, ovary, leukemia, lymphomas and non-Hodgkin's lymphoma **[16]**.

In our study, we described the lung toxicity induced by high dose of cyclophosphamide as two forms; one of rapid onset when CP given for one week in the form of cell infiltrates, diffuse alveolar damage, bronchiolitis obliterans organizing pneumonia and/or alveolar hemorrhage and the late onset disorder when CP given for three weeks in the form of lung fibrosis and increased deposition of collagen fibres as a prominent feature.

With agreement with our experiment, Malik et al., **[17]** reported two clinical forms of cyclophosphamide induced lung toxicities; one developed within one month of exposure in the form of cough, fever, fatigue, reticular to reticulonodular lesions on chest radiograph and the other developed within 6 months of exposure as a restrictive pattern seen on pulmonary function tests.

Moreove, Sulkowska and Sulkowski **[18]** mentioned histopathological changes of lungs extracted from CP-given rats similar with our results in the form of congestion, damage and/or edema of interalveolar septa and cell infiltrates. These alterations were also observed by Hasan et al., **[19]** who described congestion and edema evoked by CP in addition to epithelial cell damage as well as alveolocapillary destruction. In addition, Shokrzadeh et al., **[20]** reported chronic changes of lung tissues promoted by CP when given for 3 months in the form of alveolar cell injuries, fibrosis of alveolar septa and congestion.

Oxygen was postulated by Patel and Block **[21]** to enhance CP induced lung toxicity whereas the addition of the antioxidants showed lung protective effects. However, Colvin et al., **[22]** detected nonspecific increase of some antioxidants in certain clinical situations as a result in cyclophosphamide resistant tumor cells.

Administration of CP in the present study significantly decreased free radicals scavenging enzymes like SOD. Chakraborty et al., **[23]** explained the reduction of this enzyme by CP metabolism as it increased the reactive electrophiles resulting in electrophilic burden on the cells depleting SOD contents. This is in line with Choi et al., **[24]** who documented SOD reduction or depletion following CP injection in animals.

Stankiewicz et al., **[25]** assumed the oxidative stress as one of the mechanisms of CP pneumotoxicity by excessive production of reactive oxygen species (ROS) and the inability of antioxidant systems to readily scavenge the ROS or repair the resulting disturbances. However, Venkatesan and Chandrakasan **[8]** suggested another mechanism of

CP lung injury in the form of increased expression of the pro-inflammatory cytokines leading to cellular injury. Moreover, Ashry et al., **[26]** attribute these changes to excessive expression of cytokines that could be improved by the addition of anti-inflammatory.

With giving QUE to groups III,V; there was attenuation of the above-mentioned histopathological changes induced by CP in agreement with its anti-inflammation and antioxidant effects of QUE mentioned by Mlcek et al., **[27]** and Sengül et al., **[28]** with improvement of the oxidative parameters as determined in QUE groups. The antiinflammotory role of QUE was also described by Sulkowska et al., **[29]** in the pathogenesis of the reduction of CP-induced lung toxicity.

In the current study, CP administration showed a significant increase in the serum level of TNF-a compared to the control group which significantly decreased with giving QUE. This result is in line with the experimental output observed by Kumar et al., **[30]** Cruse and Bradding **[31]** also premised the anti-inflammatory role of QUE as a mechanism that inhibits the production of inflammatory mediators leading to diminishing the CO induced pneumotoxicity.

In conclusion, QUE treatment can ameliorate the lung injury provoked by CP in rats by restoring biochemical oxidative enzymes and its anti-inflammatory effects. Therefore, our experimental results recommend combination of QUE and CP during the clinical applications of CP.

Conflict of interest: There is no financial or scientific support.

References

1- SHANNON V.R and PRICE K.J.: The cancer patient. Pulmonary complications of cancer therapy. Anesth. Clin. NA, 16: 563-586, 1998.

2- COOPER J.A.D., JR., WHITE D.A. and MATFHAY R.A.: Drug induced pulmonary disease: Part I: Cytotoxic drugs. Am. Rev. Hespir. Dis., 133: 321-340, 1986.

3- WILCZYNSKI S.W., ERASMUS J.J., PETROS W.P., et al.: Delayed pulmonary toxicity syndrome following high-dose chemotherapy and bone marrow transplantation for breast cancer. Am. J. Respir. Crit. Care Med., 157: 565-573, 1998.

4- MAHMOUD A.M., HUSSEIN O.E. and RAMADAN S.A.: Amelioration of cyclophosphamide-induced hepatotoxicity by the brown seaweed turbenaria ornata. Int. J. Clin. Toxicol., 1: 9-17, 2013.

5- MACALLISTER S.L., MARTIN-BRISAC N., LAU V., YANG K. and O'BRIEN P.J.: Acrolein and chloroacetaldehyde: An examination of the cell and cell-free biomarkers of toxicity, Chem. Biol. Interact., 202 (1): 259-266, 2013.

6- PREISER J.C.: Oxidative stress, JPEN J. Parenter. Enteral Nutr., 36 (2): 147-154, 2012.

7- MENDOZA F., VALOUS N.A., ALLEN P., KENNY T.A., WARD P. and SUN D-W.: Analysis and classification of commercial ham slice images using directional fractal dimension features. Meat. Sci., 81: 313-320, 2009.

8- VENKATESAN N. and CHANDRAKASAN G.: Modulation of cyclophosphamide-induced early lung injury by curcumin, an anti-inflammatory antioxidant, Mol. Cell. Biochem., 142 (1): 79-87, 1995.

9- EMIN ŞENGÜLA, VOLKAN GELENB, SEMIN GEDIKLIC, SEÇKIN ÖZKANLARD, CIHAN GÜRD, FIKRET ÇELEBIA and ALI ÇıNARA: The protective effect of quercetin on cyclophosphamide-Induced lung toxicity in rats, Biomedicine & Pharmacotherapy, 92: 303-307, 2017.

10- GUAZELLI C.F., FATTORI V., COLOMBO B.B., GEORGETTI S.R., VICENTINI F.T., CASAGRANDE R., BARACAT M.M. and VERRI W.A. (2013): Quercetin-loaded microcapsules ameliorate experimental colitis in mice by anti-inflammatory and antioxidant mechanisms, J. Nat. Prod., 76 (2): 200-208, 2013.

11- BANCROFT J.D. and GAMBLE M.: Theory and Practice of Histological Techniques. 5th Ed. Churchill Livingstone, London, New York and Philadelphia, St Louis, Sydney and Toronto, 175, 2002.

12- DABA M.H., ABDEL-AZIZ A.H., MOUSTAFA A.M., et al.: Effects of L-carnitine and Ginkgo biloba extract (EGb 761) in experimental bleomycin-induced lung fibrosis. Pharmacol. Res., 45: 461-7, 2002.

13- SULKOWSKA M. and SULKOWSKI S.: Alveolar cells in cyclophosphamideinduced lung injury: An ultrastructural analysis of type II alveolar epithelial cells in situ. Histol. Histopathol., 13: 13-20, 1998.

14- MARKLUND S.L.: Superoxide dismutase isoenzymes in tissues and plasma from New Zealand black mice, nude mice and normal BALB/c mice. Mutat. Res., 148: 129-34, 1985.

15- DANIEL WW.: Biostatistics: A Foundation for Analysis in the Health Sciences. 5 th ed. New York, Chichester, Brisbane, Toronto and Singapore: John Wiley & Sons, 1991.

16- LEVINE M.N., PRITCHARD K.I., BRAMWELL V.H., et al.: Randomized trial comparing cyclophosphamide, epirubicin, and fluorouracil with cyclophosphamide, methotrexate, and fluorouracil in premenopausal women with node-positive breast cancer: Update of national cancer institute of Canada clinical trials group trial MA5. J. Clin. Oncol., 23: 5166-70, 2005.

17- MALIK S.W., MYERS J.L., DEREMEE R.A. and SPECKS U.: Lung toxicity associated with cyclophosphamide use. Two distinct patterns. Am. J. Respir. Crit Care Med., 154: 1851-1856, 1996.

18- SULKOWSKA M. and SULKOWSKI S.: Alveolar cells in cyclophosphamideinduced lung injury: An ultrastructural analysis of type II alveolar epithelial cells in situ. Histol. Histopathol., 13: 13-20, 1998.

19- HASAN N., YUSUF N., TOOSSI Z. and ISLAM N.: Suppression of Mycobacterium tuberculosis induced

reactive oxygen species (ROS) and TNF-alpha mRNA expression in human monocytes by allicin. FEBS Lett, 580: 2517-22, 2006.

20- SHOKRZADEH M., AHMADI A., CHABRA A., NAGHSHVAR F., SALEHI F., HABIBI E. and HAGHIAMINJAN H.: An ethanol extract of Origanum vulgare attenuates cyclophosphamide-induced pulmonary injury and oxidative lung damage in mice, Pharm. Biol. 52 (10): 1229-1236, 2014.

21- PATEL J.M. and BLOCK E.R.: Cyclophosphamide-induced depression of the antioxidant defense mechanisms of the lung. Exp. Lung Res., 8: 153-165, 1985.

22- COLVIN O.M., FRIEDMAN H.S. and GAMCSIK M.P., et al.: Role of glutathione in cellular resistance to alkylating agents. Adv. Enzym Regul, 33: 19-26, 1993.

23- CHAKRABORTY P., SK U.H., MURMU N., DAS J.K., PAL S. and BHATTACHARYA S.: Modulation of cyclophosphamide-induced cellular toxicity by diphenylmethyl selenocyanate in vivo, an enzymatic study, J. Cancer Mol., 4 (6): 183-189, 2009.

24- CHOI J.-S., JOU S.-S., OH M.-H., KIM Y.-H., PARK M.-J., GIL H.-W., SONG H.-Y. and HONG S.-Y.: The dose of cyclophosphamide for treating paraquat-induced rat lung injury, Korean J. Internal Med., 28 (4): 420-427, 2013.

25- STANKIEWICZ A., SKRZYDLEWSKA E. and MAKIEŁA M.: Effects of amifostine on liver oxidative stress caused by cyclophosphamide administration to rats. Drug Metabol. Drug Interact, 19: 67-82, 2002.

26- ASHRY N.A., GAMEIL N.M. and SUDDEK G.M.: Modulation of cyclophosphamide-induced early lung injury by allicin. Pharm. Biol., 51: 806-811, 2013.

27- MLCEK J., JURIKOVA T., SKROVANKOVA S. and SOCHOR J.: Quercetin and its anti-Allergic immune response, Molecules, 21 (5): 623, 2016.

28- SENGÜL E., GELEN V., GEDIKLI S., ÖZKANLAR S., GÜR C., ÇELEBI F. and ÇıNAR A.: The protective effect of quercetin on cyclophosphamide-induced lung toxicity in rats. Biomed. Pharmcother., 92: 303-307, 2017.

29- SULKOWSKA M., SKRZYDLEWSKA E., SOBANIEC-LOTOWSKA M., FAMULSKI W., TERLIKOWSKI S., KANCZUGA-KODA L., RESZEC J. and DANISZEWSKA I.: Effect of cyclophosphamide-induced generation reactive oxygen forms on ultrastructure of the liver and lung, Bull.-Vet. Inst. Pulawy, 46 (2): 239-246, 2002.

30- KUMAR S., DHANKHAR N. and KAR V., et al.: Myocardial injury provoked by cyclophosphamide, protective aspect of hesperidin in rats. Int. J. Res. Pharm. Biomed. Sci., 2: 1288-96, 2011.

31- CRUSE G. and BRADDING P.: Mast cells in airway diseases and interstitial lung disease, Eur. J. Pharmacol., 778: 125-138, 2016.

التسمم الرئوى الناتج عن عقار السيكلفوسفاميد فى الفأر الأبيض البالغ و إمكانية تعديله بإستخدام الكريستين

إن عقار السيكلفوسفاميد يستخدم فى علاج العديد من الأورام الخبيثة و لهذا السبب تم دراسة الدور الو قائى المحتمل لمضادالء كسدة النباتى (كريستين) للتخفيف من آثاره الجانبية على الرئة وَهذا الغرض تم الإستعانة بخمسة وعشرون فأراً أبيض بالغ حيث تم تقسيمهم إلى خمسة مجموعات وتحتوى كل مجموعة على خمسة فئران المجموعة تم إعطاؤها ماء ملح عن طريق الحقن فى الغشاء البرويتونى وتمثل الكنترول والمجموعة الثانية وتم إعطاؤها جرعة وحيدة من عقار السيكلفوسفاميد بجرعة ٥٠٠ ملليجرام/كجم وتم ذبحها بعد أسبوع والمجموعة الثالثة وتم إعطاؤها جرعة وحيدة من عقار السيكلفوسفاميد بجرعة ٥٠٠ ملليجرام/كجم مذاباً فى ماء الملح عن طريق الحقن البريتونى وكريستين بجرعة ١٠٠ ملليجرام/كجم مذاباً فى زيت الذرة عن طريق الأنبوبة المعدية وتم ذبحها بعد أسبوع والمجموعة الرابعة وتم إعطاؤها جرعة أسبوعية من عقار السيكلفوسفاميد بجرعة ٥٠٠ ملليجرام/كجم مذاباً فى ماء ملح عن طريق الحقن البريتونى والمجموعة الخامسة وتم إعطاؤها جرعة أسبوعية من عقار السيكلفوسفاميد بجرعة ٥٠٠ ملليجرام/كجم مذاباً فى ماء الملح عن طريق الحقن البريتونى و كريستين بجرعة ١٠٠ ملليجرام/كجم مذاباً فى زيت الذرة عن طريق الأنبوبة المعدية وتم ذبحها بعد ثلاثة أسابيع وقد أوضحت الدراسة التأثير الضار لعقار السيكلفوسفاميد على الرئة حيث تسبب فى تغيرات هستولوجية حادة فى رئات فئران المجموعة الثانية وزيادة التليف فى رئات فئران المجموعة الرابعة وقد لوحظ تحسن هذه التغيرات عندما تم إعطاء الكريستين فى المجموعة الثالثة والخامسة ولذلك ننصح بإضافة الكريستين إلى بروتوكيلات العلاج التى تستخدم السيكلفوسفاميد.