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The protective effect of β-cryptoxanthin against cyclophosphamide-induced lung injury in adult male albino rats

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Abstract

Background: Cyclophosphamide (CYP) is an anticancer agent widely used in chemotherapy. It has been suggested that CYP causes toxicity in many organs, including the lungs and testes. Many studies have indicated that some antioxidants have possible protective effects against CYP's side effects. β-cryptoxanthin (BCX), a major carotenoid of potential interest for health, is known for its antioxidant activities. This study aimed to investigate the protective effect of BCX on CYP-induced lung injury in rats using histologic and biochemical methods.

Methods: Forty adult male albino rats were divided into 4 groups: Group I served as the control group. Group II received BCX orally in a dose of 4 mg/kg per day for 7 days. Group III received a single dose (200 mg/kg) of CYP intraperitoneally (i.p.) on the 7th day of the study. Group IV received (CYP + BCX). On the 8th day of the experiment, lung tissues were collected for histopathological examinations. The levels of malondialdehyde (MDA), myeloperoxidase (MPO), reduced glutathione (GSH), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) were also determined in all dissected tissues.

Results: Pretreatment with BCX ameliorated lung morphological changes noticed in CYP group and the levels of MDA and MPO were significantly decreased whereas those of GSH, GSH-Px and SOD were significantly increased.

Conclusions: BCX provides a protective effect against CYP-induced lung injury by reducing oxidative stress and pulmonary fibrosis.

Keywords: Antioxidants, β-Cryptoxanthin, Lung injury, Cyclophosphamide, Oxidative stress

Background

Cyclophosphamide (CYP) is an antineoplastic agent, which is immensely used for treatment of neoplasia. It was also known as an immunosuppressant agent. CYP is employed to treat chronic and acute leukemia, myeloma, lymphoma, and rheumatoid arthritis (Meotti et al. 2013; Patra et al. 2012). It is metabolized by a microsomal cytochrome P450 enzyme in the liver, producing two active metabolites; phosphoramide mustard, and acrolein. Acrolein is said to be the cause of CYP's side effects. It inhibits the antioxidant system, causing production of reactive oxygen species within the cells, while phosphoramide mustard is attributed to CYP's therapeutic effects (MacAllister et al. 2013). It was declared that CYP-induced lung toxicity is in the form of interstitial pneumonitis and pulmonary fibrosis. These histopathologic effects are oftentimes dose-limiting and even life-threatening. The lack of the detoxifying enzymes aldehyde oxidase and aldehyde dehydrogenase in the lungs causes selective CYP toxicity in lung tissue. CYP is well known to cause various histopathological patterns of lung injury (Kim et al. 2012).

Several studies indicate that the development of oxidative stress after CYP administration leads to a decrease in

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the activities of antioxidant enzymes and an increase in lipid peroxidation (LPO) in liver and lungs of mice and rats (Premkumar et al. 2001).

Many types of antioxidant dietary supplements have been reported to have health benefits. Consumption of these products leads to a decrease in various proinflammatory and/or oxidative stress biomarkers (Vouldoukis et al. 2004). Biological compounds with antioxidant properties may contribute to the protection of cells and tissues against the deleterious effects of reactive oxygen species (ROS) and other free radicals induced by CYP (Manda and Bhatia 2003). Compounds that decrease the adverse effects and activate immunity can be highly helpful in improving cancer treatment. Recently, researchers have become interested in potential compounds of plant origin that are capable of minimizing the adverse effects of chemotherapy on normal cells without compromising its antineoplastic activity (Pratheeshkumar and Kuttan 2010).

ß-cryptoxanthin (BCX), an oxygenated carotenoid (xanthophyll) with pro-vitamin A activity, is found at high levels in citrus fruits, pumpkins, red peppers, papayas, carrots, plums, watermelon, corn, and peaches. It has shown a promise as a chemopreventive agent against lung cancer (Gallicchio et al. 2008; Yuan et al. 2003). This is supported by the results of many studies showing a reduced risk of developing lung cancer with higher intakes of β-cryptoxanthin (Mannisto et al. 2004). Studies revealed that BCX could inhibit chemically induced skin tumorigenesis (Nishino et al. 2000), and rat colon carcinogenesis with moderate BCX intake (Narisawa et al. 1999). Like other carotenoids, BCX is considered to be an important antioxidant capable of scavenging various types of ROS and nitrogen species-induced oxidative stress in experimental animals. The scavenging oxygen radicals have been considered the first line of defense against lipid peroxide (LPO) incorporation into biological membranes (Stahl and Sies 2005).

Therefore, this study aimed to evaluate the protective effects of BCX against CYP-induced lung injury in rats using histologic and biochemical methods.

Methods

Animals

The present study was carried out on 40 healthy adult male albino rats weighing from 200 to 250 g. They were purchased from the animal house of Assiut Faculty of Medicine, Assiut University, Egypt. The rats were housed in polypropylene cages under standard lightening in a temperature-controlled room (25 ± 2 °C) and had free access to laboratory food and water throughout the experiment. They were acclimatized to their environment for at least two weeks before starting the experiment. Animal experiments were performed in accordance with the national guidelines for the use and care of laboratory animals and were approved by the *local Institutional Animal Ethical Committee* of Faculty of Medicine, Sohag university, Egypt.

Experimental design

After the acclimatization period, rats were weighted, randomly divided into four groups (ten rats in each) as follows:

Group I (Control group) Received corn oil orally (vehicle) for 7 days.
Group II Received BCX dissolved in corn oil orally (Sigma-Aldrich Chemical Co. St. Louis, MO, USA) in a dose of 4 mg/kg per day for 7 days (Orhan et al. 2021).
Group III Received a single dose (200 mg/kg) of CYP intraperitoneally (i.p.) (Sigma-Aldrich Chemical Co. St. Louis, MO, USA) on the 7th day of the study. (Şengül et al. 2017).
Group IV Received BCX dissolved in corn oil orally (4 mg/kg/day) and a single injection of i.p. CYP (200 mg/kg) was administered on the 7th day.

The animals were weighted at the beginning and at the end of the experiment. The changes in body weight were recorded. Twenty-four hours after the last drug regimen, the rats were sacrificed by exsanguination via resection of the aorta. A median sternotomy was performed, and lungs were removed from the thoracic cavity.

Biochemical study

The right lungs were immediately snap frozen in liquid nitrogen and stored at − 80 °C for biochemical analysis. The lung tissues were rinsed with 10% cold phosphate buffered saline (PBS) solution (PH 7.4) to remove any residual blood clot. Tissues were homogenized in PBS and centrifuged at 8000 rpm for 15 min at 4 °C to collect supernatant fluids. These supernatant fractions were used to measure the desired biochemical markers.

Analysis of tissue malondialdehyde (MDA) level as an indicator of lipid peroxidation was performed by the spectrophotometry method (Kurokawa et al. 2006). This method was used to obtain a spectrophotometric measurement of the color produced during the reaction to thiobarbituric acid (TBA) with MDA at 535 nm. The MDA level is expressed as nmol/g tissue protein.

Myeloperoxidase (MPO) activity, an index of the degree of neutrophil accumulation, was measured in tissues with commercially available ELISA kit (Bioxytech MPO-EIA, USA). The absorbance was read at 405 nm using Multi-Detection Microplate Reader. Quantifications were

achieved by the construction of standard curve using known concentrations of MPO. Results were expressed as ng/mg tissue protein (Guneli et al. 2007).

Antioxidant activity was detected by measuring reduced glutathione (GSH), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD). Colorimetric assay for assessment of GSH concentration was used, and the level of GSH was measured at 412 nm by spectrophotometer. Results were expressed as μ0mol/g tissue protein (Vardi et al. 2008).

The GSH-Px enzyme activity was measured in tissues with commercially available Glutathione Peroxidase Assay Kit (ab102530; abcam, Cambridge, United Kingdom), and the level was measured at 340 nm by spectrophotometer. Results were expressed as units/g tissue protein (Shi et al. 2009).

Xanthine/xanthine oxidase assay was used to estimate SOD (Superoxide Dismutase Assay Kit, Item No. 706002; Cayman Chemical Company, Ann Arbor, USA) by measuring the amount of reduced nitro blue tetrazolium (NBT) with one unit of SOD, which is defined as the amount of protein that inhibits the rate of NBT reduction by 50%. SOD was expressed as units/mg tissue protein (Shi et al. 2009).

Pulmonary edema

The lower lobes of left lungs from all animals were weighted and then placed in a stove for 7 days at 37 °C. After this period, the specimens were weighted again, and the ratio of the weight before and after drying was calculated. Lung edema was represented by an increase in this ratio (Ingelse et al. 2019).

Histological examination

The upper lobes of the left lungs were maintained inflated with trapped air by ligation of the corresponding bronchus and fixed in 10% of neutral buffered formalin for 24 h. Paraffin-embedded sections (4 μm thickness) were stained with hematoxylin and eosin (H&E) (Bancroft and Layton 2013) and examined under a light microscope to detect histopathological changes. Other sections were stained with Masson's trichrome for light microscopic evaluation of the degree of fibrosis.

The degree of inflammation and destruction was scored for each group (Gokakin et al. 2013) (Table 1). A mean score for each of the variables was then calculated. A total histopathological score (maximum 12) was derived from the sum of the mean scores of the variables. All the samples were examined by the same pathologist to achieve correct score, and mean value of each group was used for statistical analysis.

Table 1 Scoring of inflammation and destruction

Pathological lesion	Score
Edema	1
Hyperemia	1
Thickness in interalveolar septum	2
Mononuclear cell infiltration	2
Loss of alveolar epithelium	3
Hemorrhage	3
Total	12

Assessment of pulmonary neutrophil sequestration

The pulmonary tissue neutrophil sequestration was determined according to the method described by Koksoy et al. (2001). A single pathologist blinded to all groups examined the pathological specimens. At least two different sections of each specimen were examined to determine the degree of injury. Lung neutrophil sequestration was quantified by counting alveolar septal wall neutrophils in the peripheral lung parenchyma. It was expressed as the mean number of neutrophils per 10 non-overlapping high-power fields (400 ×). Quantitative measurements were carried out using an image analysis system (Leica Qwin 500 C Imaging System Ltd., Cambridge, England) in Central Research Lab, Assiut Faculty of Medicine, Egypt.

Statistical analysis

All values were expressed as mean ± standard deviation (SD). The data were analyzed by unpaired Student's *t* test using the software Statistical Package for Social Sciences version 17 (SPSS Inc, Chicago, IL, USA). Differences were considered statistically significant if the probability of chance (*P* value) was < 0.05.

Results

None of the experimental rats died during the experiment period (7 days).

Evaluation of body weight

Single administration of CYP resulted in significant decrease in the body weight as compared to the control group possibly because of severe tissue damage caused by free radicals ($P < 0.05$). However, no significant difference in body weight was observed between the control, BCX and CYP + BCX groups. (Table 2).

Biochemical results

MDA level in the lung tissue of CYP group was significantly increased ($P < 0.05$) when compared with control

Table 2 Body weight of rats in the different studied groups

Parameters	Group I (Control)	Group II (BCX)	Group III (CYP)	Group IV (CYP + BCX)
Body weight (g)	225.17 ± 10.59	223.54 ± 12.42	201.21 ± 8.77[a]	218.72 ± 11.73[b]

Data are expressed as mean ± standard deviation. Results were statistically analyzed by using Student's *t* test at $P < 0.05$

[a] $P < 0.0001$ compared with the control group (group I)

[b] $P < 0.001$ compared with the CYP group (group III)

Table 3 Levels of malondialdehyde (MDA), myeloperoxidase (MPO), reduced glutathione (GSH), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) in the different studied groups

Parameters	Group I (Control)	Group II (BCX)	Group III (CYP)	Group IV (CYP + BCX)
MDA (nmol/g tissue protein)	53.65 ± 4.22	52.41 ± 5.81	136.44 ± 8.77[a]	58.66 ± 3.53[b]
MPO (ng/mg tissue protein)	5.78 ± 0.43	5.48 ± 1.23	13.81 ± 1.08 [a]	4.89 ± 1.04[b]
GSH (µmol/g tissue protein)	6.12 ± 0.47	6.13 ± 0.76	2.82 ± 1.04[a]	5.32 ± 0.89[b]
GSH-Px (units/g tissue protein)	13.44 ± 1.12	13.75 ± 1.45	5.35 ± 2.33[a]	11.91 ± 1.57[b]
SOD (units/mg tissue protein)	4.11 ± 0.17	4.13 ± 0.12	2.55 ± 0.67[a]	3.94 ± 0.25[b]

Data are expressed as mean ± standard deviation. Results were statistically analyzed by using Student's t test at $P < 0.05$

[a] $P < 0.0001$ compared with the control group (group I)

[b] $P < 0.0001$ compared with the CYP group (group III)

group. In BCX pretreated group, a significant decrease in the MDA level was observed as compared with CYP group ($P < 0.05$). (Table 3).

MPO activity in the lung tissue of CYP group was increased significantly ($P < 0.05$) when compared with control group. However, a significant decrease in the MPO activity was observed in BCX group in comparison with CYP group ($P < 0.05$). (Table 3).

Compared with the control group, the CYP group showed a significant decrease in the level of GSH, GSH-Px, and SOD ($P < 0.05$). However, a significant increase was determined in the level of GSH, GSH-Px, and SOD activity in BCX group as compared with CYP group ($P < 0.05$). (Table 3).

Pulmonary edema

Pulmonary edema formation was assessed by wet/dry weight ratios. CYP significantly increased the lung tissue wet/dry weight ratios compared to control group ($P < 0.05$). BCX pretreatment significantly decreased the lung tissue wet/dry weight ratios compared with CYP alone ($P < 0.05$). (Fig. 1 and Table 4).

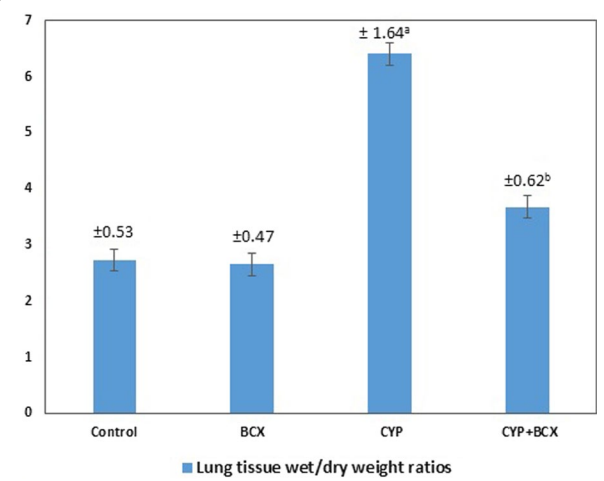

Fig. 1 Lung tissue wet/dry weight ratios in the studied groups tested by using Student's *t* test. [a]$P < 0.05$ is significant in group III versus group I and [b]$P < 0.05$ is significant in group IV versus group III

Histological results

H&E-stain

Light microscopic examination of H&E-stained sections from control group revealed normal lung architecture in which the spongy structure of the lung appeared with thin inter-alveolar septa and normal clear alveoli (Fig. 2a).

In CYP group, histological changes were variable among the animals, both in pattern and severity (Table 5). H&E-stained sections revealed a marked inflammatory cellular infiltration around bronchioles, around alveoli, in perivascular spaces, and in the inter-alveolar septa. Collapsed narrowed alveoli and thickening of the inter-alveolar septa were noticed (Fig. 2b). Extravasation of blood in the alveolar lumen, congested blood capillaries and interstitial hemorrhage were detected (Fig. 2c).

Examination of BCX pretreated group revealed that most of the changes which were observed in CYP group

Table 4 The mean values of lung tissue wet/dry weight ratios and mean number of neutrophils in the different studied groups

Parameters	Group I (Control)	Group II (BCX)	Group III (CYP)	Group IV (CYP + BCX)
Lung tissue wet/dry Weight ratios	2.73 ± 0.53	2.66 ± 0.47	6.41 ± 1.64[a]	3.67 ± 0.62[b]
Mean number of neutrophils	1.23 ± 0.21	1.27 ± 0.33	11.52 ± 1.93a	2.53 ± 0.37[b]

Data are expressed as mean ± standard deviation. Results were statistically analyzed by using Student's *t* test at $P < 0.05$

[a] $P < 0.0001$ compared with the control group (group I)

[b] $P < 0.0001$ compared with the CYP group (group III)

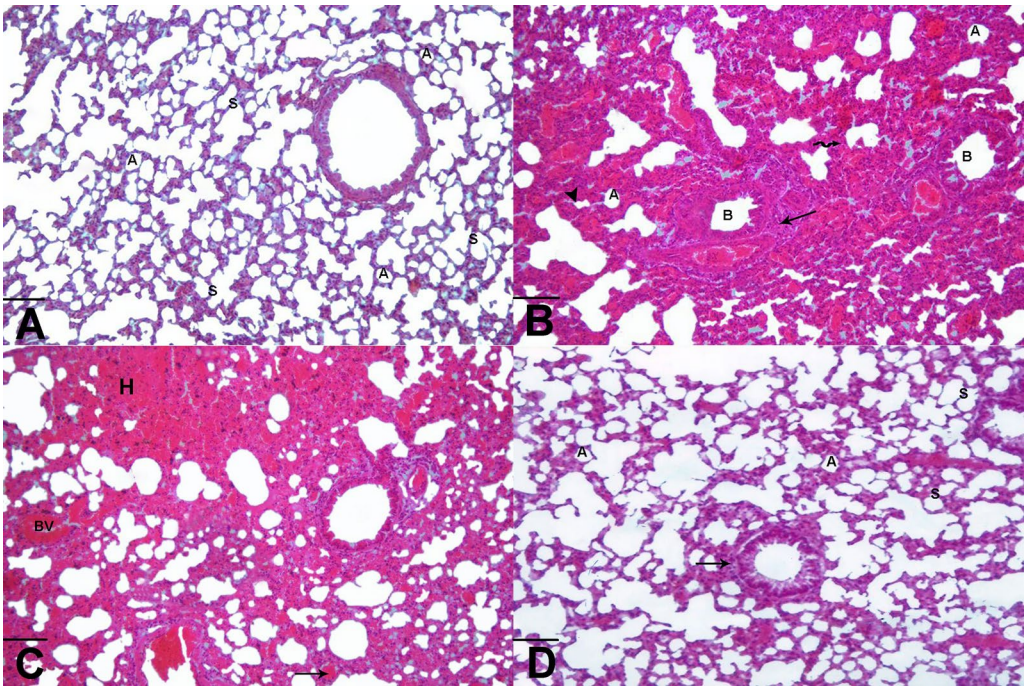

Fig. 2 **A** A Photomicrograph of normal architecture of the lung in the control group showing normal clear alveoli and thin inter-alveolar septa (H&E, scale bars = 40 µm). **B**, **C** Photomicrographs of Lung of CYP-treated rats. **B** Loss of the normal architecture of the lung with extensive infiltration by inflammatory cells (→), Marked thickening of the inter-alveolar septa (curved arrow) and narrowed alveoli (arrow head). **C** Extravasation of blood in the alveolar lumen (→), congested blood vessels (BV) and interstitial hemorrhage (H). (H&E, scale bars = 40 µm (**B**, **C**)). **D** A photomicrograph of the lung of CYP + BCX group showing normal alveolar epithelium and marked decrease in the thickening of the inter-alveolar septa. Mild mononuclear cellular infiltration was seen (→). (H&E, scale bars = 40 µm) (*A* alveoli, *S* inter-alveolar septum, *B* Bronchiole, *BV* blood vessel)

markedly decreased (Table 5). The lung alveoli were lined by a normal alveolar epithelium. The inter-alveolar septa were less thick than in the treated group. Mild mononuclear cellular infiltration was seen in the pulmonary interstitium. (Fig. 2d).

Masson's trichrome stain

Light microscopic examination of sections from control group revealed presence of collagen fibers in the interalveolar septa and the wall of the bronchiole (Fig. 3a).

The CYP group revealed the presence of an excessive increase in collagen deposition in the interalveolar septa, in the walls of the blood vessels and the walls of the bronchioles. Large fibrotic areas are also seen (Data are expressed as mean ± standard deviation. Results were statistically analyzed by using Student's *t* test at $P < 0.05$. Histopathological severity of lung injury was significantly reduced (*$P < 0.0001$) in BCX + CYP group (group IV) versus CYP group (group III).

However, examination of BCX pretreated group showed marked decrease in collagen deposition as compared with CYP group (Fig. 3c).

Pulmonary neutrophil sequestration

The neutrophil sequestration in the lung tissue was significantly higher ($P = 0.0001$) in CYP group than that

Table 5 Total scores of histopathological lesions in rat lungs in the different studied groups

Rats	Group III scores	Group IV scores
1	9	2
2	7	3
3	8	2
4	8	4
5	6	3
6	9	2
7	7	2
8	7	3
9	8	4
10	6	2
Mean ± SD	7.5 ± 0.97	2.7 ± 0.82*

Data are expressed as mean ± standard deviation. Results were statistically analyzed by using Student's *t* test at $P < 0.05$. Histopathological severity of lung injury was significantly reduced (*$P < 0.0001$) in BCX + CYP group (group IV) versus CYP group (group III)

in control group. However, BCX pretreatment significantly reduced the sequestration of neutrophils in lungs ($P = 0.0001$). (Fig. 4 and Table 4).

Discussion

CYP is an antineoplastic and immunosuppressant that is widely used. It can cause multi-organ injury in humans and experimental animals. Histological and biochemical changes caused by CYP in experimental animals are more or less similar to those detected in humans. Oxidative stress-mediated cellular damages prevent free radical scavenging enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) (Chakraborty et al. 2009; Choi et al. 2013).

Like other carotenoids, BCX was speculated to be an important antioxidant due to the presence of a hydroxyl group in its structure (Liu et al. 2016).

The present study investigated the influence of BCX on CYP-induced lung damage and evaluated its antioxidant and anti-inflammatory effects.

The administration of CYP resulted in significant decrease in the body weight possibly because of severe tissue damage caused by free radicals. This result was supported by Suddek et al. (2013) who denoted that administration of CYP alone induced a significant decrease in the average body weight (9.5% reduction). However, in combined CYP and BCX group, body weight was near to that in control group. This result agreed with Iskandar et al. (2016) who found that the mean body weights of the BCX + NNK-supplemented groups were not significantly different from the control.

In the present study, myeloperoxidase (MPO) activity was used to measure the extent of inflammation and as an indicator of neutrophils accumulation. The lung tissue content of malondialdehyde (MDA), an index of lipid peroxidation, was also investigated in this study. We found a significant increase in the MDA and MPO levels in CYP-treated group as compared with normal group.

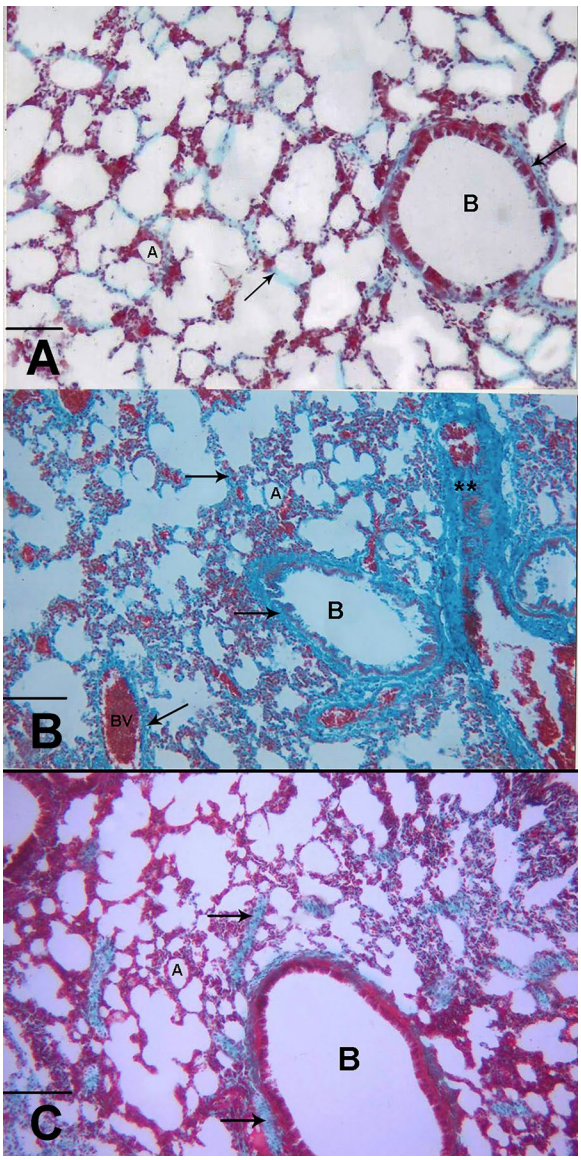

Fig. 3 **A** A Photomicrograph of lung in the control group showing normal distribution of thin layer of collagen fibers in the interalveolar septa and the wall of the bronchiole (→). (Masson's trichrome, scale bars = 40 µm). **B** A Photomicrograph of lung in the CYP group showing excessive increase in collagen deposition in the interalveolar septa, in the walls of the blood vessels and the walls of the bronchioles (→). Large fibrotic areas are seen in the parenchyma (**). (Masson's trichrome, scale bars = 40 µm). **C** A photomicrograph of the lung of CYP + BCX group showing marked decrease in collagen deposition (→). (Masson's trichrome, scale bars = 40 µm) (*A* alveoli, *B* Bronchiole, *BV* blood vessel)

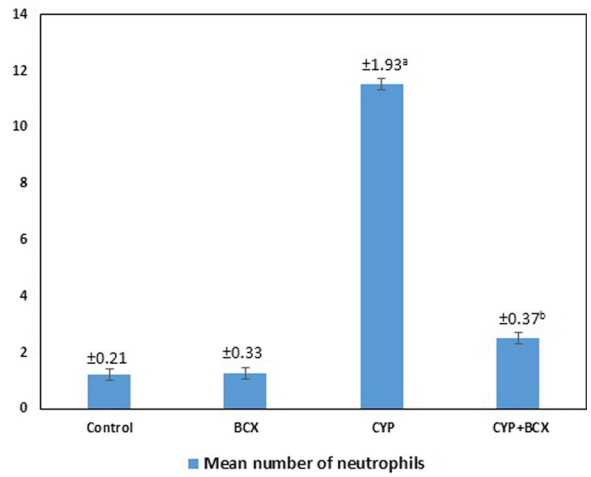

Fig. 4 Mean number of neutrophils per 10 high-power fields in the studied groups tested by using Student's t test. $^{a}P < 0.05$ is significant in group III versus group I and $^{b}P < 0.05$ is significant in group IV versus group III

On the other hand, the levels of antioxidant enzymes namely GSH, GSH-Px, and SOD were used to measure oxidative stress and the production of reactive oxygen species (ROS). We found that the antioxidant enzymes were significantly decreased. This result was supported by Şengül et al. (2017) who observed that there was a decrease in GSH levels and SOD activity and an increase in MDA levels in the lung following CYP intoxication. Another study revealed that intraperitoneal administration of CYP significantly elevated the MDA level in lung while significantly decreased the levels of GSH, SOD, CAT and GSH-Px as compared with the control group (Ghosh et al. 2015).

Treatment with BCX in combination with CYP significantly increased levels of SOD, GSH, and GSH-Px and significantly decreased MDA and MPO levels when compared with CYP-treated group. These findings are in agreement with Liu et al. (2016) who found that the administration of BCX significantly prevented the increase in the LPO and MDA levels and induced a significant increase in the activities of SOD, GSH and CAT in Cd-induced testicular damage in experimental rats.

The present study showed that CYP-treated rats induced pulmonary edema as indicated by significant increase in the lung tissue wet/dry weight ratios compared with control group. This finding was consistent with another study showing that CYP administration induced a significant increase in lung/body weight ratio (indicating the occurrence of pulmonary edema) as compared to control group (Ashry et al. 2013). On the other hand, BCX pretreatment prevented the occurrence of pulmonary edema as indicated by significantly decreased lung tissue wet/dry weight ratios compared with CYP-treated group.

Histological examination of H&E-stained sections of CYP-treated rats revealed a marked inflammatory cellular infiltration around bronchioles, around alveoli, in perivascular spaces, and in the inter-alveolar septa. Collapsed narrowed alveoli and thickening of the inter-alveolar septa were noticed. Extravasation of blood in the alveolar lumen, congested blood capillaries and interstitial hemorrhage were also detected. The findings of the present study were consistent with Şengül et al. (2017) who observed severe lung injuries in the CYP-treated group in the form of obvious degenerated alveolar cells, thickness in the alveolar septa, polymorphonuclear cells, and erythrocytes in the alveolar lumen. Another study revealed foci of edema and congestion, alveolar septal thickening, lymphocytes and macrophage infiltration, and prominent alveolar lining in the lungs of CYP-treated animals (Suddek et al. 2013).

Marked histological amelioration was observed in the lung tissue of rats treated with a combination of CYP and BCX. The lung alveoli were lined by a normal alveolar epithelium, and the inter-alveolar septa were less thick than in the CYP-treated group. Mild cellular infiltration was seen in the pulmonary interstitium. These findings were supported by a previous study showing that BCX significantly lowered smoke-induced lung inflammation in ferrets (Liu et al. 2011). Another study showed that BCX inhibited the cadmium-induced testicular tissue injury. The study denoted that the protective effects associated with BCX treatment are due to the presence of a hydroxyl residue leading to antioxidant properties (Liu et al. 2016).

Histological examination of Masson's trichrome-stained sections of CYP-treated rats revealed the presence of an excessive increase in collagen deposition in the interalveolar septa, in the walls of the blood vessels and the walls of the bronchioles. Large fibrotic areas are also seen within the lung parenchyma. However, examination of BCX pretreated group showed marked decrease in collagen deposition as compared with CYP group.

The present study showed that CYP-treated rats induced significant neutrophil sequestration in the lung tissue compared with the control group. However, BCX pretreatment significantly reduced the sequestration of neutrophils in lungs. The presence of increased numbers of activated neutrophils may be the cause of induced pulmonary injury and pulmonary edema via the excessive elaboration of inflammatory cytokines, proteolytic enzymes, and oxygen radicals. This finding was in agreement with a previous study denoting that CYP significantly stimulated the release of neutrophil chemoattractant by the lung fibroblasts, which cause neutrophil

migration from the vascular compartment to the interstitium (Koyama et al. 2001).

Conclusions

In conclusion, usage of BCX showed promising results and exerted a protective effect against lung damage induced by CYP treatment in rats by reducing oxidative stress and histopathological changes. Therefore, BCX may be a useful therapeutic agent during treatment with CYP after validation of the study results in human studies.

Abbreviations

CYP: Cyclophosphamide; BCX: β Cryptoxanthin.

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Author contributions

I am the only author.

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Availability of data and materials

All data needed are discussed in details in the manuscript.

Declarations

Ethics approval and consent to participate

The manuscript does not involve human participants, human data or human tissue.

Consent for publication

The manuscript does not contain any individual person's data in any form.

Competing interests

The author declares that there are no conflicts of interest related to the subject matter or materials discussed in this article.

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