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The Protective Effect of Propolis Compared to Vitamins C and E Antioxidant Mixture in Cyclophosphamide-Induced Lung Toxicity In Mice

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Abstract

Background: Pulmonary toxicity could be induced by oxidative stress secondary to use of the anti-cancerous agent cyclophosphamide (CTX).

Aim of Study: This study was conducted to examine the ameliorating effects of propolis or Vitamins C +Vitamin E antioxidant mixture (Vit. E-C) on lung tissues in mice exposed to cyclophosphamide.

Material and Methods: Forty-eight mice were divided into six groups; the first three groups served as control groups and orally received saline, propolis or antioxidant mixture, respectively; group 4 was intraperitoneally (i.p.) injected with a single dose of CTX (200mg/kg); group 5 was injected with a single dose of CTX (200mg/kg) preceded by propolis (90 mg/kg) and group 6 was injected with a single dose of CTX (200mg/kg) preceded by VitE-C antioxidant mixture (50mg/kg bw each) for 14 consecutive days. One day after CTX injection, mice were sacrificed to analyze oxidative stress biomarkers and to examine the histopathological changes.

Results: CTX-treated mice developed elevated malondialdehyde and declined antioxidant enzymes. Marked pathological changes in the lung architectures were observed. Mice treated with CTX/propolis in group 5 and CTX/antioxidant mixture in group 6 showed improvements in the levels of all oxidative stress markers towards normal. This accompanied by significant restoration of normal lung histology.

Conclusions: Propolis and Vit. E-C antioxidant mixture showed a promising ameliorative effect on CTX-induced toxicity in mice.

Key Words: *Cyclophosphamide – Propolis – Lung toxicity – Vitamin C – Vitamin E– Oxidative stress.*

Introduction

THE side effects of antineoplastic drugs are limiting factor for their usage. One of the most successful and widely used antineoplastic drugs is Cyclophosphamide (CTX) that belongs to nitrogen mustard subclass of alkylating agent. Its alkylation of DNA interferes with DNA synthesis and function particularly in proliferating and non-proliferating lymphocytes. Therefore, CTX suppresses the humeral activity [1]. Such effects do not distinguish between normal and cancerous cells. In turn, systemic effects, e.g., pulmonary toxicity, nephrotoxicity and cardiotoxicity occur [2,3]. Oxidative stress and lung toxicity and are induced by uncontrolled generation of Reactive Oxygen Species (ROS) [4]. As a results, macrophages, monocytes, and neutrophils, which have a vital role in the pulmonary defense system, are activated and pulmonary fibrosis ensue [5]. Oxidative stress injures the healthy cells of bone marrow, gastrointestinal tract, kidney, urinary bladder, lung, nervous system and vascular system [1]. The source of ROS produced during CTX metabolism are the reactions catalyzed by cytochrome P450, peroxidases and lipoxygenases demonstrated in in blood cells, lung, brain and spleen [6].

Oxidative stress is counteracted by several cellular antioxidant mechanisms. One of them is repairing the damaged nucleotides and by-products of lipid peroxidation. The other method is declining the pro-oxidative state and scavenges free radicals and ROS by the enzymatic and non-enzymatic antioxidant defense systems. Vitamin E and C, urate, and melatonin are the major non-enzymatic antioxidants. Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx) and Catalase (CAT) represent the major components of the enzymatic antioxidant [7-9]. Alternative medicine, natural antioxidant agents in particular, is establishing a

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foothold in the treatment of many diseases. Drug-induced pulmonary toxicity could be alleviated by some natural agents, which have antioxidant effects [7,10]. Many studies have reported the antimicrobial, antioxidant, anti-tumor, and anti-inflammatory effects of propolis [11-14]. However, the effects of propolis on the lung toxicity, such as cyclophosphamide-induced lung toxicity, have been poorly reported. Vitamin C (L-ascorbic acid) is the major water-soluble antioxidant in intracellular and extracellular body fluids. It potently scavenges free radicals, regenerates vitamin E and protects cells against oxidative damage caused by ROS [15,16]. Vitamin E (tocopherols + tocotrienols) is the major fat-soluble antioxidant particularly against lipid oxidation and subsequent DNA damage [7,17].

The antioxidant and health benefits of propolis and the antioxidant vitamin E (Vit. E) and Vitamin C (Vit. C) were previously extensively studied in several disease models. However, their ameliorative efficacy against CTX-induced pulmonary toxicity was not well-studied. In the current study, we investigated the possible protective impact of propolis and the antioxidant Vit. E-C mixture (Vit. E-C) on the pulmonary toxicity induced by CTX injection in mice. Biochemical investigation of oxidative stress and histopathology were employed to document and explain such effects.

Patients and Methods

Chemicals and kits:

Cyclophosphamide (Endoxan) 1g vial (Baxter Oncology GmbH, Germany) was freshly dissolved in 10mL saline for use [18]. Propolis was collected from honeybee hives at an Egyptian farm after freezing. It was ground and 10 grams of it was added to 100mL sterile distilled water. The mixture was boiled for one hour with frequent shaking, left to cool, filtered through gauze to recovered the clear working solution [12]. Tablets of Vit. C (each 500mg of L-ascorbic acid) were purchased in the form Hikma Pharma Co., Egypt. Vit. E was purchased in the form of capsules (400mg each) from Pharco Pharmaceutical Co., Egypt. For quantitative assay of Malondialdehyde (MDA), Superoxide Dismutase (SOD), Catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione-S-transferase (GST) as well as all other chemicals was obtained from Sigma (St. Louis, MO, USA). Kits were used as instructed. All investigations were normalized to the protein content of the homogenate measured by Biuret reagent.

Experimental animals and experimental design:

The current study was carried out in the Pathology Department, College of Medicine, Jouf University, Sakaka, Saudi Arabia during 2017. All animal handling and procedures were carried out according to the council directive of the European Communities of 1986 (EC 86/609) and complied with the guidelines of Bioethical Committee, Jouf University. Forty-eight, eight-weeks-old, male Swiss albino mice (22-26g) were breading in the animal house of the College of Medicine and were fed standard chow and allowed unrestricted access to water in a controlled environment maintained at 2ºC±2ºC, 54-56% relative humidity, and a 12h light/dark cycle during an acclimatization period of two weeks before experimental inception. The mice were divided into six groups (8 mice/group): 1- Negative control group (G1-C) receives only distilled water orally, 2- Propolis control group (G2-P) received 90mg/kg/day of propolis dissolved in distilled water orally by orogastric gavage once daily for 14 days [19], 3- Antioxidant mixture control group (G3-Vit. E-C) received a mixture of two antioxidants (Vit. E and Vit. C; 50mg/kg bw of each) orally by orogastric gavage once daily for 14 days [7], 4- CTX group (G4-CTX) received a single i.p. injection of CTX 200mg/kg at the end of the 14th day [18], 5- CTX-P group (G5-CTX-P) received oral propolis for 14 days as in G2 followed by a single i.p. injection of CTX 200mg/kg at the end of the 14th day, and, 6- CTX-VitE-C group (G6-CTX-Vit. E-C) received the oral antioxidant vitamin for 14 days as in G3 followed by a single i.p. injection of CTX 200mg/kg at the end of the 14th day. Body weight of each animal was measured immediately before starting of the experiment (day 0) and just before scarification to calculate the percentage change of body weight as final body weight/initial body weight X100.

Sample preparation:

At the end of the 15-days experiment, control and treated groups were fasted overnight before being sacrificed in the morning under light diethylether inhalation anesthesia. After sacrifice, lungs were removed, washed with cold phosphate buffered saline (pH 7.4 containing 0.16mg/mL heparin) to remove red blood cells and clots, and weighed. The relative final lung weight was calculated as lung weight/final body weight X100. Left lung tissue was homogenized for 3 minutes in 1:10 volumes of ice-cold potassium phosphate buffer (50mM; pH 7.5) on ice (Ultra-Turrax T25 Tissue Homogenizer, Germany). The homogenates clear supernatant was recovered by centrifugation at

4000rpm and 4ºC for 10 minutes. The supernatants were stored frozen at –80ºC until used.

Histopathological evaluation:

10% formalin fixed right lungs were dehydrated in ascending grades of ethyl alcohol (70, 80, 95, and 100%), cleared in xylene, and embedded in paraffin wax. Sections (5 µm) were stained with hematoxylin and eosin (H & E). Slides were examined and scored under a light microscope by a blinded pathologist to quantify the extent of lung damage evaluating the following parameters: edema, hemorrhage and/or congested alveolar capillaries, cell infiltration, and alveolar septal thickening and tissue involved [20,21]. The score was reported as: 1- Normal score 0=no lesions detected, 2- Minimal score 1=lesions involved ≤ 10%, 3- Mild score 2=lesions involved 11-40%, 4- Moderate score 3=lesions involved 41-80%, and 5- Marked score 4=lesions involved ≥81% of the lung tissue section.

Statistical analysis:

Statistical analysis was performed with SPSS Version 23.0. The data were initially tested for normal distribution using the Shapiro-Wilk test. The data were analyzed using a one-way analysis of variance (ANOVA) and Turkey's post hoc test. $p \leq 0.05$ was recognized as statistically significant in all the analyses.

Results

The findings of the current study are depicted in (Tables 1-3) and Figs. (1-3). No mortality was faced throughout the whole period of the experiment.

Table (1): Changes in the absolute and relative body and lung weights in normal (G1-C), propolis (G2-P), Vitamins E and C antioxidant mixture (G3-VitE-C) control groups, cyclophosphamide (G4-CTX), and the co-treatment mice groups.

Parameters	G1-C	G2-P	G3-VitE-C	G4-CTX	G5-CTX-P	G6-CTX-VitE-C
IBW, gm	29.0±3.25	29.37±3.16	29.62±4.03	29.63±3.96	29.0±3.46	29.37±2.14
FBW, gm	33.0±2.51	33.12±2.42	34.50±2.07	27.37±3.29^{abc}	32.75±2.76^{d}	33.12±2.53^{d}
RBW	114.26±5.81	113.36±8.63	118.01±14.91	92.72±5.39^{abc}	113.57±8.67^{d}	112.93±7.16^{d}
LW, gm	1.82±0.22	1.76±0.22	1.74±0.15	2.96±0.14^{abc}	1.84±0.141	1.85±0.14
RLW	5.57±0.92	5.35±0.85	5.05±0.54	10.92±1.0^{abc}	5.63±0.46^{d}	5.62±0.66^{d}

Results presented asthe mean ± SD. FBW: Final Body Weight. LW : Lung Weight.
IBW :Initial Body Weight. RBW: Relative Body Weight. RLW: Relative Lung Weight.
a,b,c and d significant at $p \leq 0.05$ vs. G1-C, G2-P, G3-VitE-C and G4-CTX groups, respectively.

Table (2): Changes in the lung tissue oxidative stress markers in normal (G1-C), propolis (G2-P), Vitamins E and C antioxidant mixture (G3-VitE-C) control groups, cyclophosphamide (G4-CTX), and the co-treatment mice groups.

Parameters	G1-C	G2-P	G3-VitE-C	G4-CTX	G5-CTX-P	G6-CTX-VitE-C
CAT	67.38±1.41	69.38±1.77	71.37±1.19	33.63±2.33^{abc}	60.50±1.85^{d}	68.38±1.4^{d}
SOD	64.0±1.31	65.0±1.51	66.75±1.83	36.62±2.39^{abc}	75.88±2.69^{d}	77.38±2.38^{d}
GPx	281.12±4.05	282.5±3.89	286.38±3.74	190.0±3.38^{abc}	293.62±4.03^{d}	298.75±4.46^{d}
GST	44.38±3.46	46.375±3.50	48.0±4.14	30.87±2.85^{abc}	44.25±3.73^{d}	47.50±3.33^{d}
GSH	84.25±3.37	86.375±3.46	87.75±3.73	41.62±4.44^{abc}	77.62±4.03^{d}	88.00±5.29^{d}
MDA	381.88±6.85	373.50±9.25	373.75±6.06	457.62±17.34^{abc}	381.25±8.14^{d}	361.37±8.94

Results presented as the mean ± SD.
CAT: Catalase (U/gm tissue). athione (mmol/gm tissue).
SOD: Superoxide Dismutase (U/gm t ondialdehyde (nmol/gm tissue).
a,b,c and d significant at $p \leq 0.05$ vs. G1-C, G2-P, G3-VitE-C and G4-CTX groups, respectively.

Table (3): Mean scoring of the pulmonary histopathological changes in normal (G1-C), propolis (G2-P), Vitamins E and C antioxidant mixture (G3-VitE-C) control groups, cyclophosphamide (G4-CTX), and the co-treatment mice groups.

Parameters	G1-C	G2-P	G3-VitE-C	G4-CTX	G5-CTX-P	G6-CTX-VitE-C
Edema	0	0	0	4	0	0
Hemorrhage/congested blood vessels	0	0	1	4	0	1
Cell infiltration	0	1	2	4	2	2
Alveolar septal thickening	0	1	2	4	2	2
Total score	0	2	5	16	4	5

Score 0: Normal histology.
Score 1: Minimal lesions involving ≤10% of the lung tissue section.
Score 2: Mild lesions involving 11-≤40% of the lung section.
Score 3: Moderate lesions involving 41-80% of the lung section.
Score 4: Marked lesions involving ≥81% of the lung section.

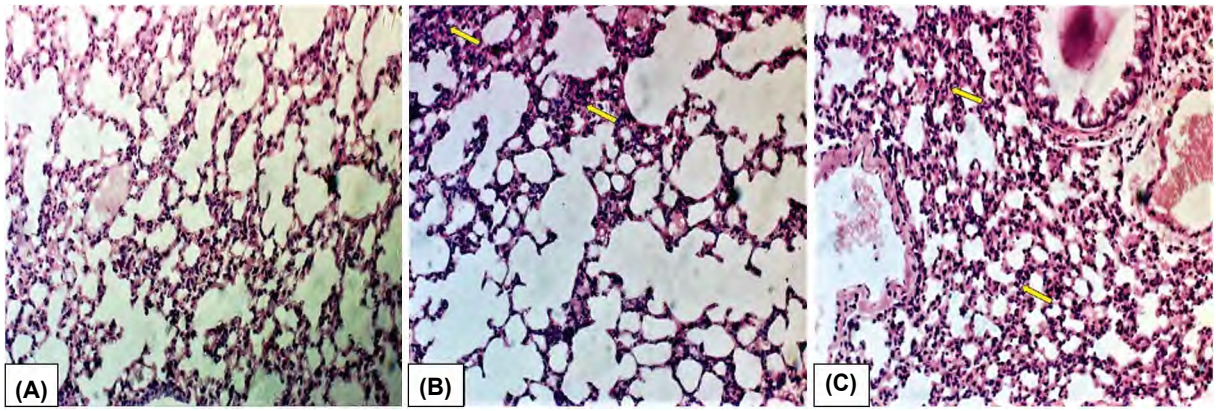

Fig. (1): Photomicrographs of different sections show the histopathological changes in the lung of a cyclophosphamide-treated mouse and amelioration by propolis and antioxidant mixture (VitE-C) administration. (A) Control group revealing normal lung structure with uniform alveoli and no abnormality detected in both alveolar architecture and epithelium. (B) Propolis group revealing mild focal thickening of alveolar septa (10% of alveoli) (yellow arrows). (C) Antioxidant mixture-treated mice lung revealing normal alveolar structures (yellow arrows) that look alike the control. Lung histology was performed on all mice (n=8 for all groups), and lungs were stained with hematoxylin & eosin with magnification of 20x.

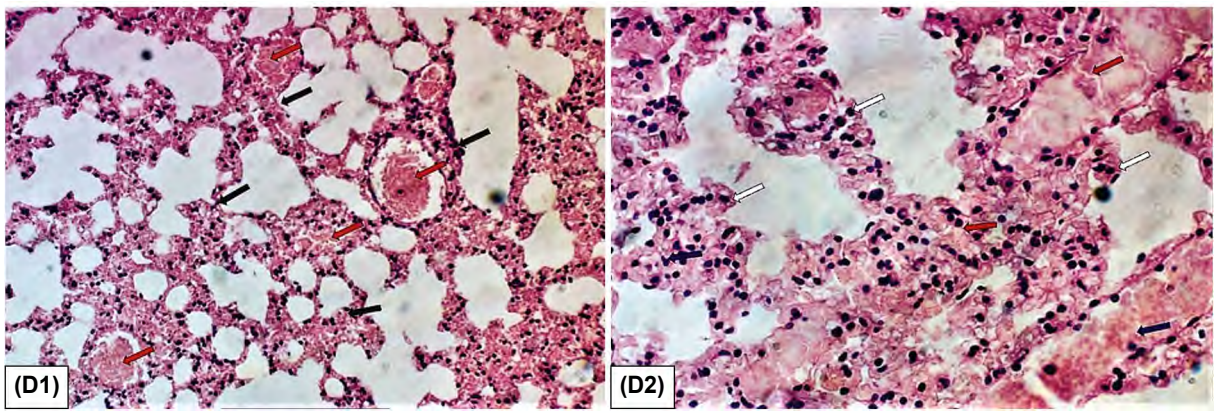

Fig. (2): Photomicrographs of different sections show the histopathological changes in the lung of a cyclophosphamide-treated mouse and amelioration by propolis and antioxidant mixture (VitE-C) administration. (D1) CTX group mice lung showing alveoli with diffuse mild thickening of alveolar septa, with chronic inflammatory cell infiltrate (black arrows) and congested alveolar capillaries (red arrows), (D2) alveolar spaces are lined with type II alveolar pneumocytes; cuboidal swollen cells (white arrows). Alveolar septa are thickened with mononuclear inflammatory infiltrate and edema (blue arrows). Congested alveolar capillaries are evident (red arrows) (H & E, 40X). Lung histology was performed on all mice (n=8 for all groups), and lungs were stained with hematoxylin & eosin with magnification of 20X.

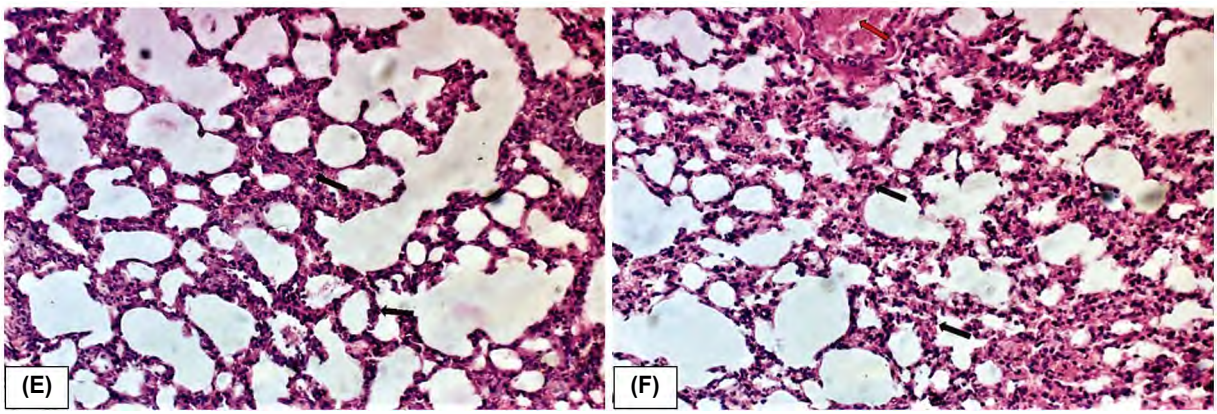

Fig. (3): Photomicrographs of different sections show the histopathological changes in the lung of a cyclophosphamide-treated mouse and amelioration by propolis and antioxidant mixture (VitE-C) administration. (E) Sections of lung in CTX + Propolis mice reveal mild focal thickening of alveolar septa (20% of alveoli) with chronic inflammatory cell infiltrate (black arrows). No edema, hemorrhage, or fibrosis could be noted. (F) Lung section of CTX + antioxidant mixture group animal showing mild alveolar capillaries congestion and few inflammatory cell infiltrate (Black arrows) with no edema, hemorrhage, or fibrosis except for mild alveolar capillaries congestion (red arrows). Lung histology was performed on all mice (n=8 for all groups), and lungs were stained with hematoxylin & eosin with magnification of 20X.

Effect of cyclophosphamide (CTX), propolis, and VitE-C antioxidant mixture on the lung and body weights:

Table (1) indicates a significant decrease ($p \leq 0.05$) in both final and Relative Body Weights (RBW) in Group 4 treated with CTX as compared with the normal, propolis and Vit. E-C treated control Groups 1-3, respectively. Propolis or Vit. E-C co-treatment with CTX in Groups 5 and 6 significantly normalized the weight changes ($p \leq 0.05$) as compared to CTX treated Group 4 mice. On the other hand, a significant increase ($p \leq 0.05$) in both absolute and relative lung weight was found in CTX Group 4 as compared with Groups 1-3. Propolis or Vit. E-C co-treatment in Groups 5 and 6 significantly normalized the absolute and relative lung weights as compared with CTX Group 4 mice ($p \leq 0.05$). Differences in these measurements were non-significant among the three control Groups 1-3.

Effect of cyclophosphamide (CTX), propolis, and VitE-C antioxidant mixture on the lung oxidative markers:

As presented in (Table 2), lung tissue SOD, CAT, GPx, GST activities and GSH level were significantly decreased in CTX Group 4 mice in comparison with normal controls ($p \leq 0.05$). Differences in these parameters were non-significant among the three control Groups 1-3. Propolis or VitE-C co-treatment with CTX in Groups 5 and 6 significantly increased ($p \leq 0.05$) the activities of SOD, CAT, GPx, GST activities and GSH content as compared with CTX Group 4 mice. There was a significantly elevated lung MDA level ($p \leq 0.05$) in CTX Group 4 as compared to the control Groups 1-3. Propolis or VitE-C co-treatment along with CTX significantly decreased lung MDA levels ($p \leq 0.05$) compared to CTX Group 4. The improvement in Group 6 CTX-VitE-C was better than Group 5 CTX-Propolis ($p \leq 0.05$).

Effect of cyclophosphamide (CTX), propolis, and Vit. E-C antioxidant mixture on the induced histopathological alterations in lung tissue:

Microscopic examination of lung from the three control Groups 1-3 revealed normal histological structure-cuboidal or simple columnar epithelium lining the respiratory bronchioles and pulmonary alveoli with regular wall structures Figs. (1A-C). In contrast, there was wide spread lung damage in mice treated with CTX with clear alveoli with diffuse thickening of alveolar septa, with mononuclear inflammatory cell infiltrate, edema, and congested alveolar capillaries as well as swollen cuboidal type II alveolar pneumocytes Fig. (1D). Co-treatment with propolis or Vit. E-C reduced CTX-induced these pathological abnormalities; particularly in Vit. E-C co-administered mice. The latter showed less cellular inflammatory infiltrates, hemorrhage, edema, and thickening of alveolar septa Figs. (1E,F). CTX mice lung showed much higher lung injury score than what observed in the control groups (Table 3). Propolis and antioxidant mixture groups also revealed lung injury with lower score than appeared in mice CTX-treated mice ($p \leq 0.05$; Figs. (1E,F) & (Table 3).

Discussion

Cyclophosphamide (CTX) is an anti-cancerous and immunosuppressive agent for autoimmune diseases [22]. Many antioxidants were previously used to protect against the toxic effects of chemotherapeutic drugs, as they modulate the generated oxidative stress [23-25]. The current study was conducted to evaluate the possible protective role of the bee glue propolis and a mixture of an antioxidant mixture of vitamins E and C in lung toxicity induced by CTX. Control mice in groups 1-3 showed no significant changes in the final and relative body weights. The final and relative body weights of CTX-treated mice showed a significant decrease in body weights. On the contrary, there was a significant improvement in body weight gain in the protected groups that received propolis or antioxidant mixture. There is a significant increase in the relative weights of lungs in CTX-treated mice due to its edema and inflammation. Lung weight was normalized in the co-administration of propolis or antioxidant mixture groups. Johnstone and his coworkers [26] reported that CTX injection in animals decreases the body weight due to its toxic effects on various systems and organs in the body. Our findings are also in agreement with Tan and his coworkers showing pulmonary edema, which caused increased ratio of lung/body weight, increased content of neutrophil and lung permeability [27]. Such pathological changes were reversed in mice co-treated with propolis or antioxidant mixture in our study, although the antioxidants were more efficient.

Oxidative stress ensues when the antioxidant molecules and systems of the body are overwhelmed by oxidants and free radicals and the body cannot reverse the induced damage [28]. For evaluation of the oxidative stress and redox balance in mice lungs after CTX exposure, it is common to monitor changes in cellular antioxidant components such as SOD and the resulted lipid peroxidation end-product MDA [29,30]. We observed increased MDA level while CAT, GPx, GST, and

SOD activities and GSH content were reduced in lung tissue of CTX-exposed groups. This reflects an oxidative stress state. These findings are comparable to several reports dissecting CTX toxicity [31,32]. The liver and lung metabolites (hydroxycyclophosphamide, acrolein and phosphoramide mustard) rather than the parent compound are the toxic moieties of CTX [6]. Acrolein disturbs the defense antioxidant systems of the tissues and generates ROS, which damages cells, leading to multi-organ toxicity [33]. Genetic differences may determine the ability of the lung to metabolize cyclophosphamide and hence its susceptibility to its toxic effects including fibrosis. This is evident by the lack of obvious dose-response relationship for pulmonary toxicity development in humans [34]. Moreover, the pulmonary tissue is exceptionally vulnerable to the toxicity of CTX due to the absence of detoxifying enzymes [35].

CTX-exposed mice had decreased GPx activity in their lungs. This could be the sequela of the observed reduction in lung tissue content of reduced glutathione. Oxidants consume GSH as the major cellular antioxidant. This leaves peroxides (H_2O_2 and organic) undetoxified GPx and a vicious cycle ensues [36]. These CTX-mediated effects were prevented by simultaneous administration of VitE-C or propolis. GSH and Vitamin C act to recycle vitamin E from its innocent free radical form to enable its return to cellular membranes to keep protecting them from oxidant attacks. Moreover, GSH reduces the resulted ascorbate radical to reform ascorbate. This utmost important role of GSH highlights the importance of its regeneration through NADPH glutathione reductase system [37]. Several types of antioxidant agents that scavenge free radicals or promote internal antioxidant defenses are able to alleviate the anticancer chemotherapy-induced lung toxicity [38]. Along with its direct antioxidant activity, GSH has an important role in GST and GPx catalytic cycles [39]. In our experiment, the lung antioxidant enzyme activities were affected by propolis, antioxidant mixture and CTX. Detoxification through GST is one of the antioxidant mechanisms that avoid generation of oxidants [40]. Our results showed significant enhanced of lung tissue antioxidant enzyme activities upon treatment with each of propolis and antioxidant vitamin mixture.

Reportedly, propolis, acting through its antioxidant and anti-inflammatory activities alleviates lead-induced neurotoxicity [13], aluminum chloride-induced reproductive toxicity [14], carbon tetrachloride-induced hepatic toxicity [41], pulmonary inflammation induced by short-term cigarette smoke inhalation [42], and, CTX-induced lung toxicity [43]. Propolis antioxidant properties are reasoned to its content of coumaric acid, artepillin C and drupanin active against superoxide and alkoxyl radicals [44]. This why propolis, in our experiment, protected against oxidants CTX-induced oxidative damage to lung tissue and prevented the initiation of the inflammatory process. This is confirmed in the CCL4-induced liver injury and other models [41]. Propolis has an SOD-like characteristic since it is able to scavenge superoxide anions [45]. In this study, the SOD-like action of propolis may have circumvented the necessity of increasing SOD and CAT activities in CTX-P co-treatment mice. In addition, Capucho et al., showed that propolis increased sulfhydryl groups bioavailability in epididymis and sperm and liver injury models through saving them the attack of free radicals [46]. In this study, we observed an increase in lung tissue contents of GSH mice co-treated with CTX-P.

Antioxidant vitamins prevent procarcinogen activation and DNA damage, induce immune stimulation and control cell fate and activities [47]. The protective effect of Vitamin E as the major lipid-soluble antioxidant has been described in several previous reports [17,48,49] in various experimental models. Vitamin E suppresses the formation of free radicals and safeguards against the peroxidation-induced cellular membrane and lipoprotein damage [50]. Vitamin C along with GSH are the major water phase antioxidants against water-soluble oxygen and nitrogen radicals [51,52]. However, the main duty of ascorbic acid is to recycle the tocopheroxyl radical [43,54]. Vitamins E and C combination decline oxidative damage induced in human and animal models [7]. In the current study VitE-C co-treatment ameliorated lung effects induced by CTX. This is likely secondary to suppression of lipid peroxidation, deterioration of cell wall, cell lysis, and necrosis [54]. Furthermore, vitamin E antagonizes tissue ischemia via elevated synthesis of endothelial nitric oxide, which promotes vasodilatation, raise blood flow to ischemic tissue and inhibit necrosis [55,56].

The biochemical disturbances we observed in lungs of CTX-treated mice were supported by the histological findings in the current study, where, diffuse thickening of alveolar septa, inflammatory cell infiltrate, congested alveolar capillaries and swollen cuboidal pneumocytes were observed. In agreement, Ashry et al., [57] showed that CTX induces dose-dependent pulmonary histopathological abnormalities that included lung fibrosis and interstitial pneumonia. Tan et al., [27] showed

similar findings of cellular inflammatory infiltrates, thickening of inter-alveolar septa and hemorrhage. As in CTX-exposed animals, activated macrophages and neutrophils during inflammation generate ROS and release lysosomal enzymes which disturb the normal cellular components **[58]**. This injure the endothelial cells of pulmonary vasculature and disturbances of the membrane of alveolar capillary with proteinaceous fluid leakage into the pulmonary parenchyma **[59]**, eventually, lung fibrosis ensues **[60]**. In the current study, histopathologically, the protective effect of propolis and antioxidant mixture against CTX-induced lung toxicity was documented.

Conclusion:

Pulmonary toxicity was induced by CTX functioning mainly through induction of oxidative stress and inflammatory damage. Propolis and antioxidant mixture of Vitamins E and C prevented oxidative stress and inflammatory response. This highlights their utility in the management of CTX-induced pulmonary damage. Further studies in the future concentrating on molecular genetics and epigenetic mechanisms devoted to explain the protective effect of propolis and antioxidant mixture on CTX pulmonary toxicity are desired.

Conflict of Interest:

The authors declare that there are no conflicts of interest.

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التأثير الوقائى للبروبوليز مقارنة مع فيتامين ج، هـ كمضادات الأكسدة فى سمية الرئة المستحثة بواسطة سيكلوفوسفاميد فى الفئران

الخلفية: يمكن حدوث التسمم الرئوى عن طريق الإجهاد التأكسدى الثانوى لإستخدام سيكلوفوسفاميد كعامل مضاد للسرطان. ولقد أجريت هذه الدراسة لفحص التأثيرات المحسنة للبروبوليز مقارنة لمزيج من مضادات الأكسدة (فيتامين ج، هـ) على أنسجة الرئة فى الفئران المعرضة لسيكلوفوسفاميد.

المواد والطرق: تم تقسيم ثمانية وأربعين من الفئران إلى ست مجموعات الثلاث الأولى خدمت كمجموعات ضابطة وتم أعطاؤها محلول ملحى أو البروبوليز أو مضاد للأكسدة (فيتامين ج، هـ)، على التوالى، المجموعة الرابعة فقد تم حقنها داخل البريتون بجرعة واحدة من سيكلوفوسفاميد (٢٠٠مجم/كجم). وتم حقن المجموعة الخامسة بجرعة واحدة من سيكلوفوسفاميد (٢٠٠مجم/كجم) مسبوقة بالبروبوليز (٩٠ مجم/كجم) وتم الحقن المجموعة السادسة بجرعة واحدة من سيكلوفوسفاميد (٢٠٠مجم/كجم) مسبوقة بمزيج مضادات الأكسدة (فيتامين ج، هـ) ٥٠مجم/كجم لكل منهما ١٤يوماً متتالية. بعد يوم واحد من حقن سيكلوفوسفاميد، تمت التضحية بالفئران لتحليل المؤشرات الحيوية للأكسدة وفحص التغيرات النسيجية.

النتائج: أظهرت الفئران التى عولجت بسيكلوفوسفاميد زيادة مادة المالونداياالدهيد وأنخفضت أنزيمات مضادات الأكسدة. وأظهرت تغيرات مرضية ملحوظة فى نسيج الرئة. وأظهرت الفئران التى عولجت بالبروبوليز مع سيكلوفوسفاميد فى المجموعة الخامسة ومزيج مضادات الأكسدة (فيتامين ج، هـ) فى المجموعة السادسة تحسينات فى مستويات جميع علامات الإجهاد التأكسدى نحو وضعها الطبيعى. وقد رافق ذلك أستعادة كبيرة من أنسجة الرئة الطبيعية.

الأستنتاجات: أظهرت مادة البروبوليز ومزيج مضادات الأكسدة (فيتامين ج، هـ) تأثير تخفيفى واعد على السمية التى يسببها سيكلوفوسفاميد فى الفئران.