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INFLUENCE OF SOME ANTIOXIDANTS IN THE TREATMENT OF ACUTE LUNG AND LIVER INJURY INDUCED BY CYCLOPHOSPHAMIDE IN MICE

Hatem A. Salem*, Nariman M. Gameil and Rania R. Abdel Aziz
Department of Pharmacology and Toxicology, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt

ABSTRACT

Cyclophosphamide (CYP), is a well known alkylating agent used as antitumor drug. Acute lung and liver injury occur in virtually all patients treated by CYP. The present study was conducted to examine the possible activity of antioxidants like 6-proanthocyanidin, melatonin and vitamin E on treatment of lung and liver injury induced by CYP. Acute lung and liver injuries were induced by a single I.P. dose (75 mg/kg) of CYP. Mice treated with CYP showed a significant increase in lung and liver lipid peroxide (MDA), decrease in reduced glutathione (GSH) in both lung and liver and a decrease in liver SOD activity. In addition CYP produced a significant reduction in blood catalase activity, lymphocyte count and marked increase in blood neutrophils. Liver injury can be manifested also through a significant increase in ALT values and AP levels. Moreover pathological examination helps to manipulate the severity of lung and liver damage. Administration of proanthocyanidin (400 mg/kg) orally normalized all the measured parameters when compared to CYP alone. Melatonin administration (14 mg/kg) orally was capable also to normalize all the measured parameters except blood catalase activity. Vitamin E (50 mg/kg) orally produced no significant increase in liver GSH, both blood catalase and AP activities when compared to CYP group. In conclusion, treatment with the previous mentioned antioxidants corrected the majority of changes in almost all biochemical markers like (MDA, SOD, GSH, ALT and AP levels) that were changed after CYP injection. Blood catalase activity, as well as blood neutrophils, lymphocytes count and histopathological changes associated with CYP returned back similar to the control group. Also we found that proanthocyanidin is the most effective antioxidant in treatment of acute lung and liver injury induced by CYP when compared with melatonin and vitamin E.

INTRODUCTION

Cyclophosphamide (CYP), an alkylating agent is a broad spectrum anticancer drug which has been shown to be inactive *in vitro* but, was found to be activated in the liver by the microsomal enzyme system in the presence of oxygen and reduced NADPH to cytotoxic metabolites[1]. Acute lung and liver injury are the most distressing side effects occuring after CYP administration[2]. The main mechanism of chemotherapy-induced lung and liver injury was the generation of free radicals ,as well as, oxidative stress which produce great modifications of the glutathione system. This system includes enzymes glutathione peroxidase and reductase, which maintain a high ratio of reduced to oxidized glutathione and a favorable redox state. Reduced glutathione is the major antioxidant in the lung and liver and its concentration in the human epithelial lining fluid is 140-folds higher than in plasma[3]. Veno-Occlusive Disease (VOD) is a liver disease which produced at high doses of cyclophosphamide and it associated with multi-organ failure, if CYP is taken for prolonged time[4].

The reduction of molecular oxygen to water is accompanied by a large free energy release that can give rise to Free Radicals (FR) and/or Reactive Oxygen Species (ROS)[5, 6]. The most important free radicals in biological systems are oxygen radical derivatives. Other highly reactive compounds are known as ROS. Reactive oxygen species, include not only oxygen FR but also non-radical oxygen derivatives involved in oxygen radical production[7]. An "anti-oxidant" is any substance that when present at low concentrations compared to those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate[8]. Organism welfare depends on the activity of efficient defense systems against oxidative damage induced by FR/ROS. On this basis, anti-oxidant defense systems *in vivo* are mainly of three kinds: preventive antioxidants (catalase, glutathione peroxidase and transferase, superoxide dismutase and carotenoid) radical scavengers (vitamin A, vitamin E, bilirubin and albumin) and finally, repair and de novo enzymes (protease, transferase and lipase).

Proanthocyanidins in grape seed refer to procyanidin mixtures. Proanthocyanidin extract demonstrates better antioxidant activity than other free radical scavenger. It is a scavenger of the superoxide radical anion, the hydroxyl radical, the lipid peroxyl radical, the peroxynitrite radical and singlet oxygen [9]. It has also been shown to protect Low-Density Lipoprotein (LDL) from oxidation[9]. Melatonin (N-acetyl-5-methoxytryptamine) is a hormone produced especially at night in the pineal gland, a gland that is located in the center of the brain. Its secretion is stimulated by the dark and inhibited by light[10]. Unlike other antioxidants, melatonin does not undergo redox cycling, the ability of a molecule to undergo reduction and oxidation repeatedly. Melatonin, once oxidized, cannot be reduced to its former state because it forms several stable end-products upon reacting with free radicals. Therefore, it has been referred to as a terminal (or suicidal) antioxidant[11]. Vitamin E is the integral part of cellular membranes whose main role is to defend the cell against oxidation. Within cells and organelles (e.g. mitochondria) vitamin E is the first line of defense against lipid peroxidation[12]. Vitamin E reduces pentane production and lipid peroxidation products from the mitochondria in vitamin E supplemented subjects[13].

The aim of the present study was to investigate the possible role of the three previous antioxidants for treatment of lung and liver injury induced by CYP. Some biochemical parameters which reflect the severity of lung and liver oxidative stress such as lipid peroxidation (MDA), Superoxide Dismutase (SOD) activity, reduced glutathione (GSH), blood catalase activity, blood neutrophils and lymphocytes counts were measured. The extent of liver injury can be manifested also by liver function tests like ALT and AP levels. Histopathological examination was also important to reflect the severity of lung and liver damage.

For correspondence; *email:* hatems57@yahoo.com

MATERIALS AND METHODS

A. Experimental animals:

Male Swiss albino mice weighing 20-30 gm were used to study the effects of drugs under investigation and evaluation of lung and liver toxicity. Animals were purchased from urology and nephrology center, Mansoura University and were kept at constant environmental and nutritional conditions throughout the experimental period and kept at room temperature 25°C ± 2 with a 12 hour on/off light schedule. Standard food and water were allowed to animals all over the experiment.

B. Drugs:

Cyclophosphamide Cycram® vial 1 gm (EIMC pharmaceuticals Co., Egypt), was dissolved in saline as 1.12% solution. 6-proanthocyanidin was obtained as a generous gift from Mr/ Qi Hong (Huzhou NBC Bio-material CO., LTD., Shanghai, China), it was prepared by dissolving in distilled water as 8% solution. Melatonin was obtained as a gift from (AMOUN pharmaceutical Co., Egypt). It was dissolved in saline as 0.28% solution. Vitamin E (± α-tochopherol acetate) is oily liquid purchased from Sigma Aldrich chemical Co. (St. Louis, MO, USA). It was prepared by dissolving in sun flower oil as 1.25 % solution.

C. Experimental protocols:

Sixty mice were grouped (10 mice in each group). CYP (75 mg/kg) was injected I.P as a single dose. CYP dose (75 mg/kg) was chosen after a preliminary study to produce acute lung and liver injury. All antioxidants were given orally. Groups were treated according to the following schedule:

Group I: Control mice received equivalent volume of normal saline (5 ml/kg).

Group II: Control mice received equivalent volume of sun flower oil (4 ml/kg).

Group III: Mice received single dose of CYP (75 mg/kg) intraperitoniallly[14].

Group IV: Mice received proanthocyanidin (400 mg/kg) orally one hour after CYP (75 mg/kg) for 3 consecutive days[15].

Group V: Mice received melatonin (14 mg/kg) orally one hour after CYP (75 mg/kg) for 8 consecutive days[16].

Group VI: Mice received vitamin E (50 mg/kg) orally one hour after CYP (75 mg/kg) for 5 consecutive days[17].

D. Sampling and biochemical parameters measured:

Twenty-four hours after the last dose of the cytotoxic drug (CYP) or antioxidants treatment, all mice were weighed and blood samples were withdrawn from the retro-orbital venous plexus under ether anesthesia. Blood was centrifuged at 3000 rpm for 15 minutes to separate serum aliquots for determination of ALT[18] and Alkaline Phosphatase (AP)[19] using colorimeter (Model CO 7500, Cambridge, England). Blood samples were withdrawn (as before) but with addition of EDTA for determination of catalase activity[20] and then mice were sacrificed by cervical dislocation. Whole lungs and livers were isolated, weighed and homogenized, the biochemical parameters like MDA as lipid peroxide[21], Superoxide Dismutase activity (SOD)[22] and reduced Glutathione (GSH)[23] were determined in lung and liver homogenates. Neutrophils and lymphocytes were counted under the microscope using a haemocytometer. Specimens of liver and lung were kept in buffered formalin for further histological manipulation.

Statistical analysis:

Data were expressed as mean ($\bar{x}$) ± Standard Error of the Mean (SEM). Statistical analysis was carried out by Student's *t*-test[24] a test of significance for comparison between two arithmetic means of the different subjects. Statistical calculations were carried out using Instat-2 computer program (GraphPad Software Inc. V2.04, San Diego, CA, USA).

RESULTS AND DISCUSSION

Cyclophosphamide is one of the most effective chemotherapeutic agents used in treatment of cancers of breast , lung , ovary , leukemia , multiple myeloma , non-hodgkin's lymphoma and sarcoma[25]. Cancer patients usually suffer from pneumonitis and hepatitis, as the most distressing side effects after CYP therapy[2]. The mechanism of such side effects is possibly through the generation of free radicals during CYP activation which cause direct injury to epithelial or endothelial cells of lung and liver. Such initial lung and liver injury may subsequently increase the influx of activated inflammatory cells into lung and liver parenchyma. The inflammatory cells, such as macrophages or polymorphonuclear cells, produce Reactive Oxygen Species (ROS), and these ROS may be important contributors to pathogenesis of CYP-induced pneumonitis and hepatitis[26]. In the last decade, the usage of antioxidant agents has been a major leap forward in the control of chemotherapy-induced pneumonitis and hepatitis due to free radical generation[1]. 6-proanthocyanidin, melatonin and vitamin E are the most commonly used antioxidants nowadays. So, the use of these antioxidants may be valuable for the prevention of some of the side effects that are produced from oxidative chemotherapeutic agents.

Excessive formation of lipid peroxides are considered to be an indirect *in vivo* feasible index for contribution to free radical generation and in turn, to oxidative stress in the pathogenesis of several lung and liver injuries, particularly those caused by exposure to exogenous oxidants[27]. Lipid peroxidation within the membrane has a devastating effect on the functional state of the membrane because it alters membrane fluidity, typically decreasing it and thereby allowing ions such as Ca^{++} to leak into the cell[28].

In the present study, MDA production which is an index of lipid peroxidation was increased significantly in lung and liver homogenates (compared to control group) after CYP treatment as shown in table (1). This observation is in line with many reports that demonstrated apparent elevation in lung and liver MDA following administration of CYP[28, 29]. Treatment of mice with proanthocyanidin after CYP administration can normalize lung and liver MDA values compared with CYP-injected group. It was reported that proanthocyanidin when given in a dose of 50 mg/kg for 28 days reduces oxidative stress after bile duct ligation[30]. Also, administeration of melatonin after CYP, significantly reduced the increase in both lung and liver lipid peroxide compared to CYP group. Melatonin may act as prophylactic agent that protects against cyclophosphamide-induced oxidative stress[28].

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Concurrent treatment with vitamin E in the present study produced a significant reduction in lung and liver MDA compared to CYP-injected group as shown in tables 1 and 2. Vitamin E could diminish the adverse effect of deltamethrin on lipid peroxidation in rats [31]. In addition to that, vitamin E ameliorated liver toxicity after carbon tetrachloride but not completely[32].

Table (1): Effect of Cyclophosphamide (CYP) and its combination with Proanthocyanidin (CYP/PRO), Melatonin (CYP/MEL) or vitamin E (CYP/vitamin E) on lung MDA (nmol/gm tissue), SOD (unit/gm tissue) and GSH (µmol/gm tissue)

Treatment	MDA	SOD	GSH
Control (saline 5 ml/kg)	125.24 ± 12.3	4.78 ± 0.38	2.9 ± 0.27
Control (oil 4 ml/kg)	158.8 ± 9.2	5.7 ± 0.40	3.79 ± 0.3
CYP (75 mg/kg)	347.22±33.6 #$	5.14 ± 0.38	1.02 ± 0.12 #$
CYP / PRO (75 / 400 mg/kg)	141.9 ± 13.2*	6.86 ± 0.31 #*	2.6 ± 0.26*
CYP / MEL (75 / 14 mg/kg)	166.3 ± 12 #*	6.97 ± 0.23 #*	2.4 ± 0.17*
CYP / VIT E (75 / 50 mg/kg)	206.1 ± 7 #$*o†	6.488 ± 0.2 #*	2.3 ± 0.21 $*

Table (2): Effect of Cyclophosphamide (CYP) and its combination with Proanthocyanidin (CYP/PRO), Melatonin (CYP/MEL) or vitamin E (CYP/vitamin E) on liver MDA (nmol/gm tissue), SOD (unit/gm tissue) and GSH (µmol/gm tissue)

Treatment	MDA	SOD	GSH
Control (saline 5 ml/kg)	317.2 ± 28.2	8.4 ± 0.244	6.76 ± 0.4
Control (oil 4 ml/kg)	271.8 ± 21	8.1 ± 0.085	6.14 ± 0.21
CYP (75 mg/kg)	415.7 ± 21.2 #$	4.88 ± 0.47 #$	3.4 ± 0.39 #$
CYP / PRO (75 / 400 mg/kg)	263.1 ± 15.4*	8.3 ± 0.19*	5.9 ± 0.46*
CYP / MEL (75 / 14 mg/kg)	222.9 ± 14.8#*	8.7 ± 0.11*	7.3 ± 0.35 *o
CYP / VIT E (75 / 50 mg/kg)	352.2 ± 14 $*o†	7.84 ± 0.14 *†	3.6 ± 0.39 #$o†

Data are expressed as mean ± SEM (n=10).
CYP was injected as single ip dose.
Proanthocyanidin was administered po one hour after CYP for 3 days.
Melatonin was administered po one hour after CYP for 8 days.
Vitamin E was administered po one hour after CYP for 5 days.
#Significantly different from saline group using Student's t-test ($p < 0.05$).
$Significantly different from oil group using Student's t-test ($p < 0.05$).
*Significantly different from CYP group using Student's t-test ($p < 0.05$).
oSignificantly different from CYP/PRO group using Student's t-test ($p < 0.05$).
†Significantly different from CYP/MEL group using Student's t-test ($p < 0.05$).

Superoxide dismutase is an enzyme which catalyzes the dismutation of $O^{\cdot}_2$ to a less toxic product[33] and is ubiquitously present in cells capable of aerobic metabolism[34]. Table 1 showed that cyclophosphamide administration did not alter lung SOD activity, this result was in agreement with other work[29]. On the other hand, CYP was significantly decrease SOD activity in liver as shown in table 2. It was reported that, liver SOD activity in mice was significantly reduced following single ip dose of CYP (40 mg/kg)[35]. In this current study, treatment with antioxidants like proanthocyanidin, melatonin and vitamin E significantly elevated liver SOD to reach the normal values. However, these antioxidants administration were able to increase lung SOD significantly from normal value. This effect may be due to the positive transcriptional activation of antioxidant in several antioxidant-related genes and in turn resulted in stimulation of SOD by antioxidant drugs[36, 37, 38].

Reduced glutathione is known as one of the endogenous antioxidants, being identified as a protector against the damaging effect of free radicals[39]. Administration of CYP in the current study reduced both lung and liver contents of GSH. This was in agreement with other reports which demonstrated that GSH reduction or depletion in both lung and liver following CYP challenging in animals[29, 14]. Decreased GSH levels counter the increase in lipid peroxidation in mice after single dose of CYP as a pro-oxidant[35]. In the present study, treatment with proanthocyanidin returns the values of both lung and liver content of GSH to normal values when compared to CYP group. The hepato-protective action of proanthocyanidin was investigated in other study after the hepatotoxic agent TPA (12-O-tetradecanoylphorbol-13-acetate) through increasing liver glutathione[40]. Melatonin also increased significantly both lung and liver reduced glutathione when administered after injury compared to CYP-injected group. These findings are in agreement with an experiment conducted through which melatonin (0.1 mg/kg for 15 days) is evidently effective against age-induced changes in the level of MDA, GSH, GSSG, GSH-Px and phosphatase activity[28]. Administration of vitamin E after CYP injury could increase significantly lung GSH. It was reported that, vitamin E could treat different tissue damage against the oxidative injury following the use of deltamethrin through increasing the production of GSH[31]. On the other hand, vitamin E could not produce any significant change on liver reduced GSH after oxidative stress induced by CYP as shown in tables 1 and 2. This observation could be explained on the basis that reduced liver is GSH indirectly attributed to the increased level of liver MDA[41, 42].

Serum alanine aminotransferase enzyme (ALT) and Alkaline Phosphatase (AP) are liver enzymes used to detect hepatic insufficiency. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membranes of the liver[43]. In the current study, CYP administration by a single dose increased ALT and AP significantly in mice compared to control group. It was previously reported that serum ALT and AP enzymes were increased significantly following CYP administration in mice when given in a dose (50

mg/kg ip) for 4 alternate days[44]. This was explained as CYP a hepato-toxic agent may cause cellular damage of the liver so increase the production of such enzymes and hence increase its release to serum. Proanthocyanidin administration after CYP returns ALT and AP to the normal values. Serum ALT and AP values have been decreased when proanthocyanidin was given after hepatotoxicity induced by acetaminophen in mice[45]. Administration of melatonin for 8 days after CYP reduced the elevated levels of ALT and AP to the normal values. These results are in line with other report indicated that melatonin may reduce liver damage and fibrosis caused by thioacetamide–induced liver cirrhosis in rats[46]. In the present study, vitamin E produced a significant reduction in ALT value when administered after injury by CYP, while AP level is still significantly higher compared to control untreated group as illustrated in figure 1. In another study vitamin E when injected subcutaneous 2 mg/rat for 5 alternate days, produced no significant change in AP levels following cisplatin administration[47].

Blood catalase is a common enzyme found in living organisms. Its functions include catalyzing the decomposition of hydrogen peroxide to water and oxygen[48]. Figure 1 demonstrates that administration of CYP decreased blood catalase level significantly compared to saline-treated group. This could be explained as, CYP induces immune dysfunction through reactive intermediate-induced damage to immune system. So changes of the endogenous antioxidant defenses that might be involved in the immune dysfunctions by CYP-induced immunotoxicity have occurred[49]. In this current study, treatment of lung and liver injury by proanthocyanidin produced remarkable increase of blood catalase activity compared with CYP treated group. This observation indicated that, proanthocyanidin acts as systemic antioxidant and enhances immunological antioxidant defenses through stimulation or activation of immnue function[50]. However, treatment with melatonin or vitamin E after CYP produced a non significant change in blood catalase value compared with CYP-treated group. Melatonin may not affect catalase activity when given in a dose of 1 mg/kg to rats orally[51].

Peripheral blood neutrophils and lymphocytes are common indicators for immunology and inflammatory response and they are calculated as a percentage of the total blood volume. Neutrophilia occur secondary to inflammation or stress or any acute insult to the body[52]. Lymphocytosis is seen in infectious mononucleosis and viral infection while lymphopenia is characteristic for acute infection and inflammation as the decline in its count is a first line of sickness[53]. In the present study, administration of a single dose of CYP produced significant increase in neutrophils counts and decrease in lymphocytes counts compared to vehicle-treated group as shown in figure 2. These results indicated that CYP produced inflammatory and immune dysfunction effect, as well as, immunosuppression properties through lowering lymphocytes counts. These results confirm other report that observed lymphocyte killing both *in vivo* and *In vitro* through CYP injection[49]. During injury, unregulated activation of neutrophils has been occurred by transmigration and this result in disruption of endothelial and epithelial junction and allow passage of neutrophils[49]. Treatment with proanthocyanidin or melatonin produces normalization of neutrophils and lymphocytes count. This observation may be due to rapid recovery of leukocytes following antioxidant. When the oxidant is the last stimulus, there is a change in leukocytes count but when the antioxidant is after the oxidant agent there is a possibility of good recovery[54]. Administration of vitamin E after injury produced more reduction and elevation of neutrophils and lymphocytes counts, respectively than control groups as illustrated in figure 2. This could be explained as vitamin E prevents membrane alteration thus diminishing neutrophils transmigration and prevent the death of lymphocytes, in addition it increases its activation[55].

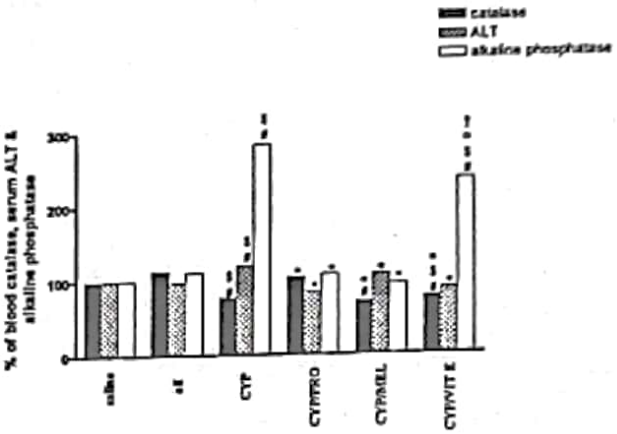

Figure (1): Percent change in blood catalase activity, serum ALT and alkaline phosphatase level of mice after cyclophosphamide (CYP, 75 mg/kg), concuurent treatment with proanthocyanidin (CYP/PRO, 75/400 mg/kg), melatonin (CYP/MEL, 75/14 mg/kg) or vitamin E (CYP/vitamin E, 75/50 mg/ kg)

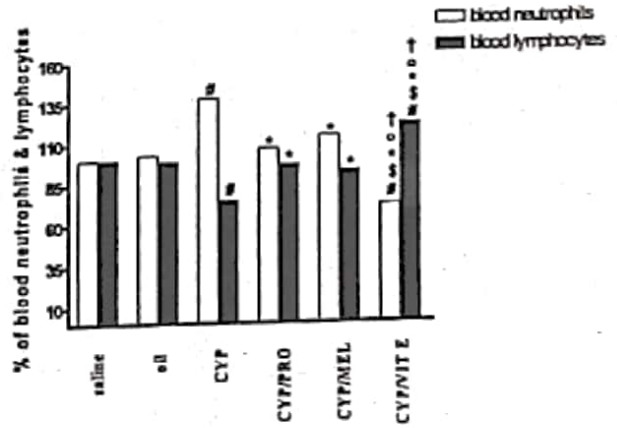

Figure (2): Percent change in blood neutrophils and lymphocytes count of mice after cyclophosphamide (CYP, 75 mg/kg), concurrent treatment with Proanthocyanidin (CYP/PRO, 75/400 mg/kg), melatonin (CYP/MEL, 75/14 mg/kg) or vitamin E (CYP/vitamin E, 75/50 mg/kg)
Values represent 10 mice/group.
CYP was injected as single ip dose.
Proanthocyanidin was administered po one hour after CYP for 3 days.
Melatonin was administered po one hour after CYP for 8 days.
Vitamin E was administered po one hour after CYP for 5 days.
#Significantly different from saline group using Student's t-test ($p < 0.05$).
$Significantly different from oil group using Student's t-test ($p < 0.05$).
*Significantly different from CYP group using Student's t-test ($p < 0.05$).
oSignificantly different from CYP/PRO group using Student's t-test ($p < 0.05$).
†Significantly different from CYP/MEL group using Student's t-test ($p < 0.05$).

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Figure 3 illustrates lung histopathological examination after CYP administration that revealed congestion, damage and/or edema of the interalveolar septa, neutrophilic and macrophages infiltration. This congestion and edema may be due to the changes produced by activated CYP in epithelial cell structure as well as alveolocapillary permeability[56]. It was also demonstrated that CYP induced damage to all elements forming the surfactant system, particularly to type II alveolar epithelial cells. It was reported that, activated CYP induced significant pulmonary artery endothelial cell injury[57]. Neutrophilic infilteration and macrophages were also seen at the site of inflammation[58]. Liver sections of CYP-treated group showed congestion, focal necrosis as well as microvesicular steatosis and lymphocytic inflammatory infiltrate. This congestion and focal necrosis were also observed in other study in which, cyclophosphamide-induced liver necrosis and a possible interaction with azathioprine was also conducted[59]. Microvesicular steatosis which is more severe variant, resulting primarily from deficient mitochondrial β-oxidation of fatty acids and characterized by the presence of multiple small droplets of triglycerides within the hepatocyte, which do not displace the nucleus[60]. Portal lymphocytic inflammatory infiltrate also has been found in other report[61]. Lungs and liver of animals treated by proanthocyanidin, melatonin and vitamin E showed no aberrant change from the normal animals. Adminisrtation of PRO produced regeneration of lung and liver cells but with congestion in lungs and minor inflammatory infiltrate at the site of inflammation when compared to CYP-treated group (figure 4). *In vivo* anti- inflammatory properties of proanthocyanidin may be responsible for the reduction of circulating leukocytes and plasma exudation and this anti-inflammatory effect was associated with reduction of pro-inflammatory cytokines[62]. From that observation, it was indicated that there is a parallelism between histopathological examination and the measured biochemical parameters. Treatment with melatonin showed congestion, thickness of interalveolar septa with mild inflammatory infilterate in lung. Several reports regarding to the histopathological changes following MEL administration demonestrated that MEL cannot affect haemorrhage[63]; alveolar wall thickness and inflammatory mediators in lungs[64]. On the other hand, melatonin rebuild liver cells and produced reduction in focal necrosis but not completely. Melatonin may prevent liver cirrhosis and fibrosis which are more severe variants than necrosis after thioacetamide in rats[46]. This could be explained by the capability of MEL to reduce oxidative stress through its antioxidant activity which in turn reduces cell injury[65]. Also as shown in figure 4, histopathological examination of lungs and liver of mice treated by vitamin E revealed complete recovery from injuries induced by CYP. The cells of lung and liver were completely regenerated exhibiting normal structure and size of cells with minimal inflammatory mediators. Vitamin-E was reported to be able to act directly on the endothelial or epithelial cell membranes rather than regulation of expression of cytokines[66]. This histopathological observation of vitamin E is not reflected in the measured biochemical parameters, and more time may be required for improvement of these parameters as supported by another report[67].

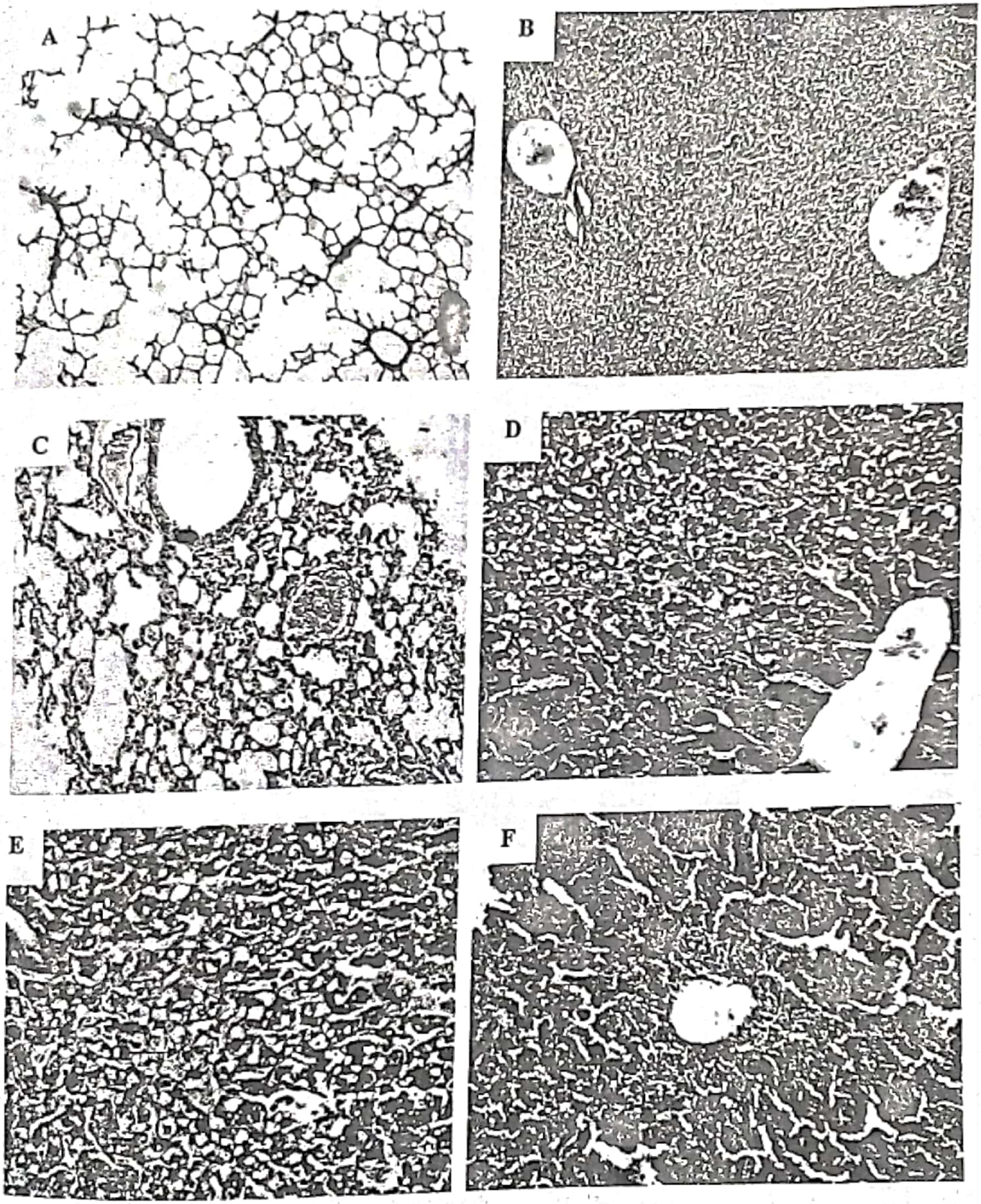

Figure (3): Photomicrographs showing sections of:

A, B) Lung and liver isolated from normal mouse, respectively, (H.& E.×10).

C) Lung isolated from cyclophosphamide-injected mouse with congestion, thickening of alveolar septa, neutrophilic and macrophages infiltration, (H&E × 10).

D, E, F) Liver isolated from cyclophosphamide-injected mouse with Focal necrosis in (D), Micro vesicular steatosis in (E) and Portal lymphocytic infiltrate in (F) (H.& E.×10).

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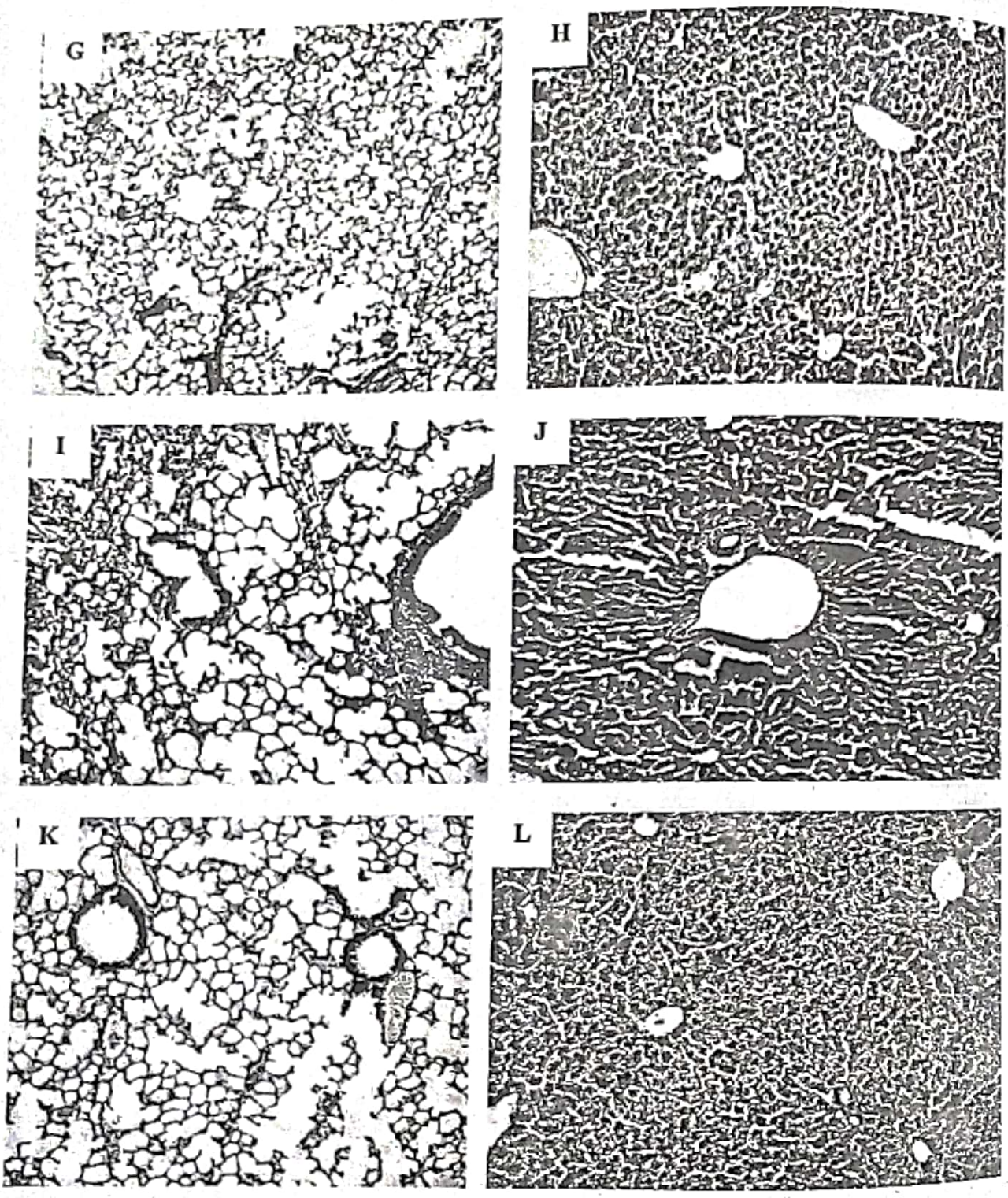

Figure (4): Photomicrographs showing sections of:
G, H) Lung and liver isolated from proanthocyanidin-treated group, respectively (H&E × 10).
I, J) Lung and liver isolated from melatonin-treated mouse, respectively (H&E × 10).
K, L) Lung and liver isolated from vitamin E-treated mouse, respectively (H&E × 10).

CONCLUSION

CYP in a single dose (75 mg/kg) was able to induce lung and liver injury through increasing lung and liver MDA, ALT and AP levels and blood neutrophils counts. It also decreased liver SOD, lung and liver GSH, blood catalase activity and lymphocytes counts. Significant histopathological changes in both lung and liver have been observed following CYP administration. Treatment with proanthocyanidin ameliorated the changes of all biochemical parameters, as well as, histopathological changes associated with CYP-induced lung and liver injury. Melatonin administration corrected the changes of liver SOD, GSH, AP, blood neutrophils, lymphocyte counts and liver histopathological examination. Vitamin E administration after injury induced by CYP normalized only lung GSH, ALT and lung and liver histopathology. Also it was found that proanthocyanidin is the most effective antioxidant for treatment of acute lung and liver injury induced by CYP when compared with melatonin or vitamin E.

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تأثير بعض مضادات الأكسدة فى علاج الإصابة الحادة للرئة والكبد المحدثة بالسيكلوفوسفاميد فى الفئران البيضاء الصغيرة

حاتم عبدالرحمن سالم – ناريمان محمد جميل – راتيا رمضان عبدالعزيز

قسم الفارماكولوجي – كلية الصيدلة – جامعة المنصورة – المنصورة 35516– مصر

عقار السيكلوفوسفاميد أحد أهم العقاقير المستخدمة في علاج مرض السرطان ، والعديد من الأورام السرطانية ، ولكن نظرا للآثار الجانبية والتي من أهمها الإصابة الحادة لكل من الرئة والكبد ، فقد قامت هذه الدراسة لمحاولة التخفيف من هذه الأعراض.

تضمنت هذه الدراسة إستخدام بعض مضادات الأكسدة مثل البروانثوسيانيدين ، ميلاتونين وفيتامين هـ لدراسة فعاليتها في علاج الإصابة الحادة للرئة والكبد المحدثة تجريبيا في الفئران الصغيرة بإستخدام عقار السيكلوفوسفاميد بجرعة (75 مجم/كجم) كجرعة واحدة فقط.

وقد أشارت نتائج هذه الدراسة إلى أن السيكلوفوسفاميد أدى إلى زيادة ذات دلالة إحصائية في نسبة الدهون المؤكسدة في الرئة والكبد ، إنزيمي إ.س.ج.ب.ت ، الألكالين فوسفاتيز ، عد خلايا النيتروفيل في الدم.

وقد أدى السيكلوفوسفاميد إلى نقص ذو دلالة إحصائية في نشاط الجلوتاثيون المختزل في الرئة والكبد ، نشاط إنزيم السوبر أكسيد ديسميوتيز في الكبد ، نشاط إنزيم الكتاليز وعد الخلايا الليمفاوية بالدم.

كما ظهر في الفحص الباثولوجي أن السيكلوفوسفاميد ادي إلى إحتقان ونزيف في خلايا الرئة والكبد وترسيبات دهنية في الخلايا الكبدية ، مما يعكس وجود تفاعلات مؤكسدة داخل هذه الخلايا.

ومن نتائج هذه الدراسة يتضح أن العلاج بعقار البروانثوسيانيدين يلاشي كل التغيرات البيوكيميائية وتغيرات الفحص الباثولوجي الناتجة من السيكلوفوسفاميد.

بينما أدى الميلاتونين إلى ضبط كل من إنزيم السوبر أكسيد ديسميوتيز في الكبد ، الجلوتاثيون، الألكالين فوسفاتيز ، عدد خلايا النيتروفيل ، الخلايا الليمفاوية بالدم ، وضبط الفحص الباثولوجي في الكبد فقط.

كذلك كان لفيتامين هـ القدرة على إعادة مستويات الجلوتاثيون المختزل في الرئة ، نشاط إنزيم إ.س.ج.ب.ت ، والفحص الباثولوجي لكل من الرئة والكبد إلى المستوى الطبيعي.

مما سبق نستنتج أن إستخدام أي من مضادات الأكسدة الثلاثية السابق ذكرهم في العلاج يقلل من التأثيرات المؤكسدة السامة للسيكلوفوسفاميد على كل من الرئة والكبد ، ولكن أظهرت الدراسة أن البروانثوسيانيدين فعاليته كبيرة في العلاج مقارنة بالميلاتونين والفيتامين هـ.