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Lutein's Protection Against Cisplatin's Pulmonary Toxicity

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Abstract: One of the biggest problems of cancer treatment is the harmful effects of these drugs on the healthy tissues and organs of the organism. The aim is to determine the possible protective effects of Lutein (L) against Cisplatin (CS) toxicity in rat lungs by biochemical tests. In our study, lutein (L) (orally, 100 mg/kg) was administered for CS-induced lung toxicity (intraperitoneally (i.p.), 10 mg/kg). The study was completed with a total of 28 rats from 4 groups, each consisting of 7 subjects. Control, L, CS and CS + L. Lung damage induced by CS was a dose-limiting side effect of CS and caused an increase in PCO2 level and a decrease in PO2 and SaO2 levels. In our study, a significant decrease in PCO2 levels and an increase in SaO2 and PO2 levels were observed in L application ($p< 0.05$). In the CS + L group, there was an increase in CAT, SOD and GSH levels and a decrease in MDA levels compared to the CS group. A significant decrease in body weight was observed in the CS-treated group compared to the control. L supplementation in these rats caused a significant increase in body weight, bringing this value closer to normal ($p< 0.05$). It is understood from the study that L alleviates the results of oxidative stress, increases antioxidant functions and positively supports lung functions. It also demonstrates the ability of L to prevent CS-induced lung damage. Ultimately, L appears to be a applicable pharmacological agent in this injury.

1. Introduction

Chemotherapy is a method used in the treatment of cancer (Aktaş and Yahyazadeh, 2022). Cisplatin (CS) is applied as an antineoplastic drug in the treatment of various tumors in cancer patients. However, it also causes side effects on the body's normally functioning organs. CS affects the lungs and other tissues and organs, causing oxidative stress. Side effects include kidney toxicity, nausea, vomiting, as well as fibrosis, interstitial inflammation, structural lung damage (Badreldin and Al Moundhri, 2006). In patients with lung injury, CS chemotherapy increases perioperative complications by inducing bronchitis-associated fibrosis and interstitial inflammation (Leo et al., 2018). As a side effect of CS, the antioxidant system is negatively affected and harmful radicals are produced that cause oxidative damage (Badary et al., 2005). The occurrence of genotoxicity and myelosuppression limits the use of CS therapy. Additionally, there is a significant dose-dependent increase in DNA damage. It interferes with the synthesis of DNA, resulting in cross-linking of DNA that stimulates cell cycle progression. As a result, it induces apoptosis of tumor-forming cells (Bakır et al., 2018). In addition, CS reduces the scavenging of free radicals by binding to the sulfhydryl of glutathione. In addition, the CS-sulfhydryl structure damages enzyme function and cell membranes, ultimately causing damage to mitochondria and increased lipid peroxidation.

It is known that cells in the organism create many mechanisms to prevent oxidative stress-based damages. Studies aimed to eliminate the toxic effects of drugs through antioxidants administered together with the drug (Caffrey and Frenkel, 2000; Aktaş and Armağan, 2019; Aktas and Bayram, 2020; Aktaş and Sevimli, 2020; Aktas and Ozgocmen, 2020). Lutein (L) has been used in many studies due to its antioxidant properties. They are carotenoids (do not contain vitamin A) that cannot be synthesized in the organism and are synthesized by algae, bacteria and plants. It reacts with free oxygen radicals formed in the body due to its hydroxyl structure. Due to this feature, it has a strong free radical scavenging function (Ojima et al., 1999; Krishnaswamy et al., 2010). Additionally, L has anti-cancer and anti-inflammatory effects (Chucair et al., 2007). L also protects against stomach lesions and gastrointestinal ulcers (Jávor et al., 1983; Mozsik et al., 2001). In general terms, its low toxicity makes it advantageous in conventional treatment.

In the literature, it is seen that a sufficient number of antioxidant supporting substances have been studied in order to eliminate the undesirable damages of CS in lung treatment. For this purpose, the fact that the protection of L against the side effects of CS has not yet been investigated makes our study unique. Therefore, in our study, the protection of L against toxic damage caused by CS in the lung tissues of rats was investigated.

2. Material and Methods

2.1. Chemicals

Chemical L in this study was supplied by Solgar (USA). The others were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Animals

This research was conducted with 28 Sprague-Dawley male rats. Rats weighed (W) 210-265 g and were 11 weeks old. Animals were taken from the Experimental Animal Research Center of the institution where we work. The procedures were carried out according to the protocol (Protocol 2022/056) received from the ethics committee of the same center. In the experiment, care was taken to ensure that the humidity and temperature in the environment were at optimal levels (55% ± 5, 22 ± 2°C, respectively). Water and feed supplements were provided to the animals according to their average needs.

2.3. Experimental protocol

In this research, 4 groups, each containing 7 rats, were randomly created (n = 7).

1. Control group: This group was offered 1 ml of physiological saline orally daily.

2. L group: L was given to subjects daily by gavage (100 mg/kg/day) (Katyal et al., 2013).

3. CS group: CS was administered as a single dose (by i.p., 10 mg/kg) on the 4th day of the experiment (Capasso et al., 1990).

4. L + CS group: In the experiment, L and CS were administered as in their respective groups.

The experiment was completed in 7 days. Then, anesthesia (xylazine and ketamine) and decapitation procedures were performed on the rats. Blood and lung samples were taken for biochemical study.

2.4. Body weight and relative lung weight

The animals were weighed on the 1st and 7th days of the study. Lung tissues were washed with saline, dried and weight. The W of lungs/body weight ratio was determined by this formula (Aktaş and Yahyazadeh, 2022):

$$\text{Relative Organ W (\%)} = \frac{\text{Organ W} \times 100}{\text{Body W}}$$

2.5. Arterial blood gas analysis

For blood gas analysis, 2 ml of blood was taken from the carotid artery and centrifuged (3000 rpm, r: 15 cm) (Abbott, USA). The amount of protein in the obtained serum was measured by the bicinchoninic acid method (Liu et al., 2015).

2.6. Biochemical analysis

Homogenization of the samples was carried out in a cold environment for 1-2 minutes at 12,000 rpm (IKA, Germany). The ideal temperature for targeted procedures is 4°C. Homogenates were designed to be 0.5-1.0 g in size to be used in analyses. Lung tissues were prepared for use in the analysis of glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA). Protein values were determined using the method of Lowry et al. (1951).

MDA was evaluated using the Uchiyama and Mihara (1978) method. Therefore, the thiobarbituric acid reaction was established at pH 3 and 95°C for 15 min. Then, pink pigment was obtained as a result of maximum absorption and measurements at 532 nm.

GSH was assessed by the method of Ellman (1959). Chemicals were included in the samples to cause the reaction (yellow green color). The results were obtained by spectrophotometer with 410 nm absorbance.

SOD was measured by the method of Marklund and Marklund (1974). This process occurred by preventing the autoxidation of pyrogallol. Enzyme activity was measured at a wavelength of 440 nm for 180 seconds. Results were defined as U/mg Hb.

To detect CAT activity, 0.9% NaCl was mixed into 10% homogenate using phosphate buffer. Afterwards, hydrogen peroxide hydrolysis was examined at pH 7.0. Results were reported as nmol/mg protein measured with maximum absorbance at 240 nm (Aebi, 1984).

2.7. Statistical analysis

The analyses in this study were carried out with SPSS version 20.0. Data are expressed as mean ± SEM. Body weight analyses were performed by applying paired samples t-test to the groups at both the beginning and end of the study. Comparing the means of more than two sample groups was done using analysis of variance (ANOVA), and comparing the means of two sample groups was done using t-tests. Intergroup and intragroup comparisons of parametric values in antioxidant parameters were made with one-way ANOVA and LSD post-hoc test. Non-parametric values were analyzed using the Kruskal-Wallis test. A p-value of ≤ 0.05 was considered statistically significant.

3. Results

3.1. Body and organ weights

The effects of L treatment on lung and body weight before and after CS application are in Table 1. According to the available data, rats treated with CS alone exhibited a significant reduction in final body weight compared with the control and L treatment group ($p< 0.001$). L supplementation to CS-treated animals resulted in a significant increase in final body weight ($p< 0.001$). A significant ($p< 0.001$) increase in lung weight was observed in CS-treated rats compared with the control and L treatment groups. L treatment significantly corrected the fluctuations in lung weight, in contrast to the CS-only treated group.

Table 1. Effect of CS and L treatment on body and lung weight of rats

	Group Body weight (g)		
	First	Last	Lung weight
Control	221.1 ± 0.781	263.9 ± 0.483	0.627 ± 0.05
L	216.3 ± 0.479	264.1 ± 0.572	0.745 ± 0.10
CS	220.8 ± 0.485	227.3 ± 0.589	0.959 ± 0.04
CS + L	218.9 ± 0.714	249.3 ± 0.585	0.721 ± 0.014
Statistical comparison (Initial of study vs final of study) (p)			
Control			0.002
L			0.005
CS			0.024
CS + L			0.030

Changes in the body and lung weight of experimental rats. Values are expressed as mean ± SEM. The groups were compared with the paired-samples T-test at the beginning and end of the treatment p <0.05.

3.2. Blood gas parameters in the artery

Compared to the other groups, a significant increase in arterial carbon dioxide pressure (PCO_2) level and a significant decrease in partial oxygen pressure (PO_2) and arterial oxygen saturation (SaO_2) levels were observed in the CS group (p< 0.05). In the CS + L group, PCO_2 levels significantly decreased, PO_2 and SaO_2 levels significantly increased compared to the CS group (p< 0.05). The results are in Table 2.

3.3. Biochemical parameters in tissue

Tissue biochemical parameter results are in Table 1. Compared with the other groups, a significant increase in MDA level was observed in the CS group (p< 0.05). A significant decrease in MDA level was detected in the CS + L group compared to the CS group (p< 0.05). A significant decrease was found in GSH, SOD and CAT parameters when compared to the CS group, L and control groups (p <0.05). Lung tissue GSH, CAT and SOD levels in the CS + L group were significantly increased compared to the CS group (p< 0.05) (Table 1).

Table 2. Biochemical parameters and tissue oxidative stress parameters in the lung

	Control	L	CS	CS + L
Serum biochemical biomarkers				
Arterial blood gases				
PCO_2 (mmHg)	36.41 ± 1.14^{c}	42.16 ± 1.11	53.58 ± 1.08^{a,d}	44.74 ± 1.12^{c}
PO_2 (mmHg)	88.17 ± 0.42^{c}	94.13 ± 0.41	69.04 ± 0.45^{a,d}	81.3 ± 1.18^{c}
SaO_2 (%)	91.25 ± 1.12^{c}	98.56 ± 1.27	78.08 ± 1.16^{a,d}	82.14 ± 1.54^{c}
Lung tissue oxidative stress biomarkers				
SOD (U/g)	2.99 ± 0.18^{c}	3.22 ± 0.13	1.88 ± 0.24^{a,d}	3.41 ± 2.12^{c}
CAT (µmol H_2O_2/g)	0.61 ± 0.08^{c}	0.68 ± 0.23	0.29 ± 0.12^{a,d}	0.59 ± 0.14^{c}
GSH (µmol/g)	0.59 ± 0.25^{c}	0.62 ± 0.47	0.28 ± 0.26^{a,d}	0.64 ± 0.19^{c}
MDA (nmol/g)	0.41 ± 0.49^{c}	0.51 ± 0.47	0.88 ± 0.74^{a,d}	0.47 ± 0.15^{c}

Data are mean ± SEM, n = 7. CS, cisplatin; L, lutein; MDA, malondialdehyde; GSH, glutathione; SOD, superoxide dismutase; CAT, catalase. a: Significant difference from control, b: Significant difference from L, c: Significant difference from CS, d: Significant difference from CS + L.

4. Discussion

Chemotherapeutic drugs have been found to cause side effects in healthy organs in addition to their therapeutic effects. A large number of chemotherapeutic drugs are widely used in the treatment of cancer patients. CS is an important anticancer drug applied in laboratory and clinical research. More effective drugs must be developed to cure cancer cases (Iraz et al., 2015). Although CS is effective in some tumors, it causes serious side effects in normal organs. Therefore, CS has a strong anti-proliferative effect against tumor cells. CS also stabilizes microtubules, blocks mitosis, and shows its functions by inducing apoptosis. All of these cases limit the use of CS as a chemotherapeutic agent (Tutun et al., 2019; Bilgiç and Aktaş, 2022). The effectiveness of chemotherapeutics increases when used together with vitamins or antioxidants. Therefore, this study demonstrated that CS treatment with L protects against CS-induced damage in the lungs of rats (Geyikoğlu et al., 2017). It was determined that there was a significant decrease in body weight of rats treated only with CS. Additionally, in our study, a significant increase in body weight was reported in animals in which L supplementation was administered along with CS. In a study on this subject, Afsar et al. (2018) aimed to evaluate the protection of Acacia hydaspica (polyphenol-rich ethyl acetate extract) against CS-induced pulmonary toxicity. The decrease in body weight and increase in lung weight due to CS-induced toxicity obtained here coincide with our results (Afsar et al., 2018).

Lung injury induced by CS is a dose-limiting side effect of CS and causes an increase in PCO_2 level and a decrease in PO_2 and SaO_2 levels. In our study, blood gas analysis results show that the PCO_2 level of the CS group increased, while the PO_2 and SaO_2 levels decreased. The fact that these results in the CS group are significantly different from the control group proves that CS damages the alveolar-capillary membrane (ACM) and therefore affects gas exchange. Due to the disorder in the barrier function of ACM, permeability increases significantly and the transmembrane transport process of the substance cannot be regulated. Additionally, due to this disorder, water and proteins leak from the capillaries of the cells into the alveolar interstitium and alveolar space. This causes both the thickening of the ACM and the slowing down of CO_2 and O_2 diffusion (Liu et al., 2015). In our study, as a result of L application, there was a significant decrease in PCO_2 level and an increase in PO_2 and SaO_2 levels. In this case, it can be said that with the effect of L, there is a significant improvement in the parameters, the damage caused by CS is reduced, and the disorder in the barrier function of the ACM is improved (Mitchell et al., 2011).

There is evidence from previous studies that lung damage caused by CS occurs as a result of the formation of free radicals. Increased oxidants such as superoxide anion and hydrogen peroxide due to mitochondrial dysfunction caused by reactive oxygen species play a role in the pathogenesis of CS-induced lung injury. It has also been reported that antioxidant enzyme activities decreased (Chen et al., 2020). In addition, CS reduces the excretion of glutathione from the body by binding sulfhydryl groups (free oxygen radicals). Another situation is that CS-sulfhydryl formation damages the enzyme function and the cell membrane. Then, it causes mitochondrial damage as a result of lipid peroxidation (Bilgic et al. 2023). Some studies have also reported that CS reduces lung SOD, CAT enzyme activities and GSH levels (Liu et al., 2015). Other studies have reported that CS reduces antioxidant capacity in the lungs and increases MDA levels, causing lipid peroxidation in lung epithelial cells.

Previous studies have identified undesirable effects of drugs used in anti-cancer treatment on normal tissues. Therefore, many different agents with antioxidant properties have been used to prevent these negative effects (Azirak et al., 2019). In the study, MDA levels increased with the accumulation of CS-derived lipid peroxides, and antioxidant enzymes related to SOD, CAT and GSH were depleted. This situation shows the role of CS-derived oxidative stress in lung

toxicity. CS has been reported in different studies to increase MDA and decrease antioxidant parameters (GSH, SOD and CAT) in the lung (Geyikoğlu et al., 2017; Afsar et al., 2018; Mammadov et al., 2019). In this case, it may cause a decrease in the lungs' ability to cope with peroxides. In the study of Chen et al. (2020), it was found that MDA increased and SOD and GSH decreased in rats administered particulate matter (Tanbek et al., 2017). As a result of L application, it was observed that this situation approached normal values. In addition, these results appear to be compatible with the results obtained from our study (Chen et al., 2020). On the other hand, in our study, the application of L together with CS caused a decrease in the MDA parameter as a result of the increase in antioxidant capacity in rats. This result demonstrates the protection of L against CS-induced lung toxicity. In this case, it can be said that the oxidative stress-increasing effects of CS are prevented through the antioxidant properties of L. In this case, it is understood that L protects the lung from the toxic effects of oxidative stress (Bilgic et al., 2016; Bilgic et al., 2021).

5. Conclusion

As a result, in the current study, It was determined that L removes harmful radicals and activates the antioxidant system with its antioxidant effect. According to the data in the study; It was observed that the undesirable effects of CS on lung tissues could be reduced with the combination of CS + L. Therefore, it can be recommended to apply these two drugs together in order to continue cancer treatment effectively and without interruption.

Declaration of Author Contributions

All authors declare that they have contributed equally to this manuscript, have reviewed the final version, and have approved it for publication.

Declaration of Conflicts of Interest

The authors declare that there is no conflict of interest regarding this study.

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