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Research Article

Biochemical and Histopathological Investigation of the Protective Effect of Lutein in Rat Kidney Exposed to Cisplatin

Sisplatin'e Maruz Bırakılan Sıçan Böbreğinde Lutein'in Koruyucu Etkisinin Biyokimyasal ve Histopatolojik Olarak İncelenmesi

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Abstract

Aim: The aim of this study was to determine the protection of lutein (L) (100 mg/kg, orally) against kidney damage caused by cisplatin (Cis) (10mg/kg, intraperitoneal).

Materials and Methods: Twenty-eight rats aged 8 weeks were used and divided into four equal groups (n=7): Control, L, Cis, and Cis + L.

Results: As a result of biochemical findings, serum Cr and BUN levels of the Cis group were significantly increased compared to the control group ($p<0.05$). Serum Cr and BUN levels of the Cis + L group were found to be significantly reduced compared to the Cis group ($p<0.05$). In addition, a decrease in MDA level and an increase in GSH, SOD and CAT levels were detected in the Cis + L group compared to the Cis group ($p<0.05$). In the histopathological examinations, in the Cis group kidney tissues; pathological changes such as interstitial mononuclear leukocyte infiltration, tubular degeneration, and tubular necrosis were observed. When the Cis + L group is compared with the Cis group, it can be said that there is a decrease in oxidative stress, an increase in antioxidant activity and a decrease in histopathological changes with the effect of L.

Conclusion: These results show that L is effective in preventing Cis-induced kidney injury. It is also emerging that L is a pharmacological agent with the potential to be used in this damage.

Keywords: Antioxidant, kidney, lutein, cisplatin, rat

Öz

Amaç: Çalışmamızda, sisplatin (Cis) (10mg/kg, intraperitoneal) kaynaklı böbrek hasarına karşı lutein'in (L) (100mg/kg, oral) koruyucu etkisi incelendi.

Materyal ve Metot: Bu çalışma, 8 haftalık 28 adet sıçanla gerçekleştirildi. Oluşturulan 4 grubun her birinde 7 tane sıçan yer aldı: Kontrol, L, Cis ve Cis + L.

Bulgular: Biyokimyasal bulgulara göre, Cis grubunun kontrol grubu ile kıyaslanmasında serum Cr ve BUN seviyelerinin anlamlı düzeyde arttığı görüldü ($p<0.05$). Cis + L grubunda serum Cr ve BUN düzeylerinin Cis grubuna kıyasla anlamlı düzeyde azaldığı tespit edildi ($p<0.05$). Ayrıca Cis + L grubunun Cis grubu ile kıyaslanmasında MDA seviyesinde düşüş, GSH, SOD ve CAT seviyelerinde ise artış kaydedildi ($p<0.05$). Yapılan histopatolojik incelemelerde Cis grubu sıçanların böbrek dokularında interstisyel mononükleer lökosit infiltrasyonu, tubuler dejenerasyon ve tubuler nekroz gibi patolojik değişiklikler gözlendi. Cis + L grubu Cis grubu ile kıyaslandığında L'nin etkisi ile oksidatif streste bir azalma, antioksidan aktivitede bir artma ve histopatolojik değişikliklerde bir azalma olduğu söylenebilir.

Sonuç: Bu sonuçlar L'nin Cis kaynaklı böbrek hasarının önlenmesinde etkili olduğunu göstermektedir. Ayrıca L'nin bu hasarda kullanılma potansiyeli olan bir farmakolojik ajan olduğuda ortaya çıkmaktadır.

Anahtar Kelimeler: Antioksidan, böbrek, lutein, sisplatin, sıçan

INTRODUCTION

Chemotherapy is known as the most common and effective method for cancer treatment. Although chemotherapeutic drugs help to cure cancer, they have been dedected to cause side effects in healthy organs. Many chemotherapeutic drugs are widely used in the treatment of cancer patients (1). Cisplatin (Cis) is used against various tumor types and malignancies as an anti-

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neoplastic drug. Although Cis is widely used in clinical practice, it has many undesirable side effects such as neurotoxicity, ototoxicity and nephrotoxicity (2,3). It has been reported that the most important side effect limiting the use of this drug is nephrotoxicity (4). Nephrotoxic effects have been reported in 20-40% of patients during treatment with Cis (5). In the investigations, it has been reported that histopathological changes such as tubular degeneration, interstitial mononuclear leukocyte infiltration, congestion in inter-tubular blood vessels, and tubular necrosis are formed as a result of the use of Cis (6). At the same time, it has been determined that this drug causes changes in many parameters in different organs such as liver and kidney (7). In addition, it was determined that Cis negatively affects the antioxidant system and produces many harmful radicals that cause oxidative damage (8). Studies have reported that Cis increases free radicals, causes damage to many tissues, and reduces protective activity against the effects of oxidative damage. Therefore, it is hypothesized that Cis-induced nephrotoxicity may result from increased oxidative stress from the increase of free oxygen radicals (9).

As a result of various studies conducted in recent years, it has been shown that some side effects of Cis can be reduced without changing the effects of chemotherapy by applying antioxidants obtained by different methods (10,11). After the nephrotoxic effect of Cis was determined, many agents and compounds with known antioxidant effects were used experimentally for therapeutic purposes (12). In this context, lutein (L), which has antioxidant properties, has been used in many studies. L is a carotenoid synthesized by plants, bacteria, and algae and is not synthesized in the body. It is also known that L is a carotenoid that does not contain vitamin A. At the same time, L is a member of the xanthophyll group obtained by the consumption of various green leafy plants. It can react strongly with free oxygen radicals through its hydroxyl group in the L structure. In this way, it can show an effective free radical scavenging activity (13). In addition, it has been noted that L has an oxidative stress-reducing effect through its antioxidant properties (14). In addition, L has been found to have many different effects as an anti-inflammatory (15) and anti-cancer agent (16). It has also been proven that L protects against stomach lesions (17) and gastrointestinal ulcers (18). Overall, the low toxicity of L makes it advantageous among conventional therapeutic drugs. Therefore, L can be tested against kidney damage due to chemotherapeutic drugs.

Although many studies have been conducted on agents with antioxidant effects to prevent the nephrotoxic effects of Cis, the fact that the protectiveness of L has not been examined in this regard adds originality to our study. Therefore, it was aimed to reveal the protectiveness of L in order to eliminate the pathological changes caused by Cis in kidneys.

MATERIAL AND METHOD

Chemicals

In our study, all chemicals, including Cis, were obtained from Sigma Chemical Co. (St. Louis, MO). L was obtained from Solgar (USA).

Animals

The rats preferred in this study were 210-255 g in weight and 8 weeks old. At the same time, 28 Sprague-Dawley type male rats were determined as needed. These were purchased from Adıyaman University Experimental Animals Research Center. Our research was conducted in accordance with the rules determined by the Ethics Committee (Protocol 2022/036). The temperature of the experimental environment was kept at 22±2°C and the humidity rate was 55±5%. The animals were given free feed and water as much as they could consume (*ad libitum*).

Experiment Protocol

In the experiment, groups were randomly formed and designed to have seven rats (n=7) in each of the 4 groups.

1. Control group: Rats were given 1 ml of saline orally for 7 days.
2. L group: L (100mg/kg/day, gastric gavage) for seven days (19).
3. Cis group: A single dose of Cis (10mg/kg, i.p.) on the 4th day of the study (20).
4. Cis + L group: A single dose of Cis (10mg/kg, i.p.) on the 4th day of the study. L (100mg/kg/day, gastric gavage for seven days).

All experimental applications in our study were completed within 7 days. Then, the rats were anesthetized with xylazine and ketamine and decapitated under anesthesia. Blood and kidney tissues were taken to be used in biochemical and histopathological examinations. The obtained serum was placed at -85°C for use in biochemical analyzes. One of the taken kidneys was placed at -85°C for biochemical analysis, while the other was immersed in fixation solution for histopathological examinations.

Histopathological Analyses

Tissues were placed in 10% neutral buffered formaldehyde for one day and were paraffin embedded after routine histological procedures (21). Tissues cut from 5 µm thick paraffin blocks by microtome (Leica Biosystems RM 2245) were stained with hematoxylin-eosin method after being taken on the slide (22,23). All sections were evaluated using a light microscope (Nikon Eclipse Ni-U), followed by images with a camera (Nikon DS-Fi3).

Biochemical Analysis

Blood Sample Assessment

Creatinine (Cr) and blood urea nitrogen (BUN) (mg/dL) levels were obtained in serum by picric acid method (24). Measurements were performed using Abbott ARCHITECT

c16000 (Abbott Laboratories, Abbott Park, IL) and Roche Diagnostics kits (Mannheim, Germany). BUN and Cr parameters were determined as mg/dl serum.

Renal Oxidative Stress Biomarker Assessment

Our samples were homogenized in ice (IKA, Germany) at 12.000 rpm for 1-2 minutes. Targeted procedures were found to work best at 4°C. Homogenates to be used in biochemical analyzes were prepared as 0.5-1.0g. Kidney tissues were prepared for malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) analyzes and protein level. Results were obtained using the methods of Lowry et al. (1951) to determine protein concentrations (25).

The procedure for evaluating the MDA parameter was performed using the methods of Uchiyama and Mihara (1978). At this stage, thiobarbituric acid reaction was determined to take about 15 minutes at pH 3 and 95°C. Then, as a result of measurements performed at 532 nm and maximum absorption, the formation of pink pigment was achieved (26).

The procedure for the evaluation of the GSH parameter was performed using the Ellman (1959) method. First of all, chemicals were added to the sample and the reaction took place in the environment. After this procedure, a yellow-green color formation was achieved. The evaluation procedure of the GSH parameter was performed using a spectrophotometer at 410nm absorbance (27).

In our study, SOD activity was measured by inhibiting the autoxidation of pyrogallol. These procedures were carried out by applying the method of Marklund and Marklund (1974). Procedures for enzyme activity were performed at 440nm for 180 s(s). In this context, the results obtained are expressed as U/mg Hb (28).

Numerous procedures were used to analyze the CAT parameter. First, 0.9% NaCl was added to 10% tissue homogenates. A phosphate buffer was used to measure the CAT parameter. Analysis of hydrogen peroxide hydrolysis at pH 7.0 was then performed. We observed the maximum absorbance at 240nm. Our results are reported as nmol/mg protein (29).

Statistical Analysis

Statistical analyzes in our study were made with SPSS software (v.21). The data produced were evaluated as mean ± SEM. Normally distributed data were analyzed with the Shapiro-Wilk test. In the biochemical parametric evaluation, comparisons between groups and within groups were made with ANOVA and post-hoc LSD tests. In addition, Kruskal-Wallis test was used for histopathological results. The significance level was determined as 0.05.

RESULTS

Biochemical Serum Parameters

Biochemical analysis results obtained in serum are shown in Table 1. Cr and BUN levels were measured to evaluate kidney function. It was determined that these parameters increased significantly in the rats of the Cis group compared to the control and L groups ($p<0.05$). In the Cis + L group, a significant decrease was observed in Cr and BUN levels compared to the Cis group ($p<0.05$) (Table 1).

Biochemical parameters in tissue

The results of the biochemical parameters in the tissue are shown in Table 1. The kidney tissue MDA parameter results of the control and L groups were similar to each other. The MDA level of the Cis group was significantly increased compared to the control and L groups ($p<0.05$). There was a significant decrease in MDA level in the Cis + L group compared to the Cis group ($p<0.05$) (Table 1).

Similar results were obtained in SOD, CAT and GSH parameters of the control and L groups. On the other hand, when the Cis group was compared with the control and L groups, a significant decrease was found in these parameters ($p<0.05$). SOD, CAT and GSH levels in the kidney tissues of the Cis + L group were significantly increased compared to the Cis group ($p<0.05$) (Table 1).

Table 1. Serum biochemical and renal tissue oxidative stress biomarkers

	Control	L	Cis	Cis + L
Serum biochemical biomarkers				
BUN (mg/dl)	28.19±0.89^{c,d}	35.78±0.19^{c,d}	55.29±0.01^{a,b,d}	39.13±0.11^{a,b,c}
Cr (mg/dl)	0.39±0.12^{c,d}	0.43±0.14^{c,d}	0.55±0.21^{a,b,d}	0.45±0.23^{a,b,c}
Renal tissue oxidative stress biomarkers				
SOD (U/g)	4.42±0.14^{c,d}	4.65±0.14^{c,d}	3.27±0.32^{a,b,d}	4.58±0.24^{a,b,c}
CAT (K/g)	0.17±0.03^{c,d}	0.19±0.12^{c,d}	0.13±0.14^{a,b,d}	0.18±0.08^{a,b,c}
GSH (µmol/g)	0.30±0.1^{c,d}	0.33±0.14^{c,d}	0.23±0.05^{a,b,d}	0.31±0.15^{a,b,c}
MDA (nmol/g tissue)	0.19±0.09^{c,d}	0.21±0.05^{c,d}	0.26±0.03^{a,b,d}	0.22±0.04^{a,b,c}

Data are means ± SEM, n = 7. Cis, cisplatin; L, lutein; Cr, creatinine; BUN, blood urea nitrogen; MDA, malondialdehyde; GSH, glutathione; SOD, superoxide dismutase; CAT, catalase.. [a] Significant difference from control, [b] significant difference from L, [c] significant difference from Cis, [d] significant difference from Cis + L

Histopathological Results

The histological structure of renal corpuscle, other parenchymal structures, and stroma were normal in the kidneys of control and L group rats. Histopathological changes such as interstitial inflammatory cell infiltration, tubular degeneration, epithelial desquamation, tubular dilatation, hyaline casts in the tubules, tubular necrosis, and segmental glomerular necrosis were observed in the kidneys of Cis group rats. No histopathological changes were observed in the kidney tissues of Cis + L group rats other than a slight focal tubular dilatation and degeneration. The histological structure of rat kidneys in this group was almost the same as in the control group (Figure1).

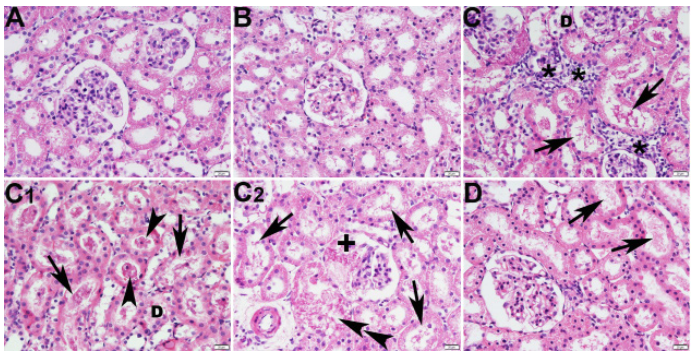

Figure 1. Light microscopic image of kidney sections of control (A), L (B), Cis (C, C1, C2), Cis + L (D) groups. D, tubular degeneration and epithelial desquamation; *, interstitial inflammatory cell infiltration; arrow, tubular dilatation; arrowhead, hyaline casts in the tubules; double arrowhead, tubular necrosis; +, segmental glomerular necrosis. (x 400; H&E)

DISCUSSION

It has been proven that Cis causes nephrotoxicity in patients and various animal species. It is known that Cis-induced nephrotoxicity is a dose-limiting side effect. At the same time, this leads to certain losses in kidney functions (30). The rat model of Cis-induced nephrotoxicity is considered a sensitive and reproducible system. In previous studies, there is evidence that Cis exerts its nephrotoxic effects through the formation of free radicals (31). In addition, it has been reported that harmful oxygen radicals are responsible for the pathogenesis of Cis-induced kidney damage (32). It has also been reported that Cis binds to the sulfhydryl groups of reduced glutathione, thereby reducing the scavenging of free oxygen radicals. Another factor is that the cisplatin-sulfhydryl complex can cause lipid peroxidation and mitochondrial damage by damaging cell membrane and enzyme functions (33). It is speculated that Cis-inducing kidney damage is due to selective uptake from proximal tubule cells. It is known that Cis accumulate at certain rates in other parts of the kidney. It was determined that the Cis concentration was 5 times higher than the extracellular concentration in proximal tubule epithelial cells (34).

In addition, it has been proven in many studies that antioxidant compounds prevent oxidative stress and inflammation in Cis-induced kidney damage (35-38). In this study, increased serum BUN and Cr concentrations, increased MDA levels, decreased antioxidant enzyme activities and GSH levels show that kidney damage occurs as a result of Cis administration. In this model, the application of L as a protective antioxidant was preferred. In our study, Cis-induced lipid peroxide accumulation, as well as depletion of GSH and related antioxidant enzymes, indicate the critical role of oxidative stress in Cis nephrotoxicity. In this study, it was also determined that kidney damage, characterized by increased BUN and Cr, occurred as a result of Cis application to animals. It is estimated that Cis causes nephrotoxicity by decreasing the glomerular filtration rate. In the Cis + L group, kidney damage markers such as BUN and Cr were found to be significantly attenuated. In addition, the approximate values of these parameters in the control group show the protective effect of L.

It has been reported in different studies that Cis causes a decrease in antioxidant parameters (SOD, CAT, GSH) in the kidneys (39). In similar studies, it has been proven that Cis causes lipid peroxidation in renal epithelial cells by decreasing the antioxidant capacity of the kidneys and increasing the MDA level (40). In this study, a decrease in GSH and antioxidant enzyme (SOD, CAT) activities in kidney tissue was determined as a result of Cis nephrotoxicity. This may lead to a decrease in the kidney's ability to clear toxic hydrogen peroxide and lipid peroxides. In addition, an increase in antioxidant capacity and a decrease in MDA levels were noted in the Cis + L group. This result indicates that L provides protection against Cis-induced nephrotoxicity in rats.

In the present study, histopathological changes such as tubular degeneration, hyaline cast formation in tubules, interstitial mononuclear leukocyte infiltration, and tubular necrosis were observed in the kidney tissue of Cis group. It is understood that these changes are similar to the results of studies in the literature (6-8) and confirm the nephrotoxic effects of Cis. In the Cis + L group, the nephrotoxic effects of Cis on the kidneys were greatly reduced. These results showed that L is a pharmacological agent that can be used as a preventative against the nephrotoxicity of Cis in kidney tissue.

Previous studies have shown that many pharmacological agents with antioxidant effects can be used successfully to prevent the toxic effects of anticancer drugs (Cis, paclitaxel) in non-target tissues (4-6,22,38). L, which has antioxidant effects, probably protects the kidney tissue from the harmful effects of oxidative stress by preventing the oxidative stress-increasing effects of Cis thanks to these effects. In our study, it was determined that BUN and Cr concentrations decreased, GSH, SOD and CAT levels increased, and MDA level decreased as a result of L administration. Reversal of Cis-induced oxidative stress has also been reported. Therefore, these observations support the hypothesis that the nephrotoxicity mechanism is related to the depletion of the antioxidant defense system.

CONCLUSION

In conclusion, our results showed that L scavenges harmful radicals, activates antioxidant defense systems and reduces histopathological changes, thanks to its strong antioxidant effects. When the results obtained in this study are evaluated together, it can be said that the application of L together with Cis can reduce the undesirable effects of Cis on the kidney tissues, and thus it can be ensured that the cancer treatment continues effectively and uninterruptedly.

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