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Effects of Artichoke Against Cyclophosphamide-Induced Nephrotoxicity and Oxidative Stress in Rats *

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This study was undertaken to investigate how artichokes affects the oxidative stress and nephrotoxicity caused by cyclophosphamide (CP) in rats. The first group, known as control group, was given no medication; second group received artichoke treatment for seven days; third group received a single dose of CP by injection; and fourth group received artichoke treatment for five days following administration of single dose of CP, in addition to seven days of artichoke treatment prior to it. One intraperitoneal dosage of CP (150 mg/kg) was administered and artichoke extract (1 g/kg/day) was given using gavage. GSH level (p<0.001) and CAT (p<0.001) activities were found to be significantly lower in the CP group than in the control group, whereas MDA level (P<0.001), GSH-Px (p=0.002), SOD (p<0.001), and GST (p<0.001) activities were found to be significantly higher. When the CP group was compared with the artichoke+CP group, significant changes were determined in other markers except CAT activity. No statistical difference was observed when the control and only artichoke groups were compared. All values except the MDA level approximated to the control group values when artichoke+CP group was compared to the control group. It was found that the decline in MDA levels was statistically different in the artichoke+CP group compared to the CP group, but the average did not reach the control group values statistically. Histopathologically, in CP group, dilatation in distal tubulin, glomerular atrophy in a few areas and tubulin of foamy appearance were encountered. In CP+artichoke group was observed less frequently dilatation in distal tubule. These findings suggest that unique natural antioxidant artichoke may protect rats from oxidative stress and CP-induced nephrotoxicity.

Key Words: *Cyclophosphamide, artichoke, malondialdehyde, antioxidant*

Enginarın Ratlarda Siklofosfamid Kaynaklı Nefrotoksisite ve Oksidatif Strese Karşı Etkileri

Bu çalışmada, enginarın ratlarda siklofosfamidin (CP) neden olduğu oksidatif stres ve nefrotoksisiteyi nasıl etkilediği araştırıldı. Kontrol grubu olarak bilinen ilk gruba hiçbir ilaç verilmedi; ikinci gruba yedi gün boyunca enginar tedavisi verildi; üçüncü gruba enjeksiyon yoluyla tek doz CP verildi; ve dördüncü gruba tek doz CP uygulamasından önce 7 gün boyunca, CP uygulamasından sonra ise 5 gün boyunca enginar tedavisi uygulandı. Bir intraperitoneal CP dozu (150 mg/kg) uygulandı ve enginar ekstraktı (1 g/kg/gün) gavaj yoluyla verildi. GSH düzeyi (P<0.001) ve CAT (P<0.001) aktivitelerinin CP grubunda kontrol grubuna kıyasla istatistiksel olarak anlamlı derecede daha düşük olduğu, MDA düzeyi (p<0.001), GSH-Px (p=0,002), SOD (p<0.001) ve GST (p<0.001) aktivitelerinin ise istatistiksel olarak anlamlı derecede daha yüksek olduğu bulundu. CP grubu, enginar+CP grubuyla karşılaştırıldığında, CAT aktivitesi hariç diğer belirteçlerde istatistiksel olarak anlamlı değişimler belirlendi. Kontrol ve sadece enginar grupları karşılaştırıldığında istatistiksel olarak fark gözlenmedi. Enginar+CP grubu kontrol grubuyla karşılaştırıldığında MDA düzeyi hariç tüm değerler kontrol grubu değerlerine yakındı; MDA düzeylerindeki düşüşün enginar+CP grubunda, CP grubuna göre istatistiksel olarak farklı olduğu, ancak ortalamanın kontrol grubu değerlerine istatistiksel olarak ulaşmadığı bulundu. Histopatolojik olarak CP grubunda distal tübülinde dilatasyon, birkaç alanda glomerüler atrofi ve köpüklü görünümde tübülin görüldü. Enginar+CP grubunda distal tübülde dilatasyon daha az sıklıkla görüldü. Çalışmanın bulguları, eşsiz doğal antioksidan enginarın ratları oksidatif stresten ve CP kaynaklı nefrotoksisiteden koruyabileceğini düşündürmektedir.

Anahtar Kelimeler: *Siklofosfamid, enginar, malondialdehit, antioksidan*

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Introduction

About 8 million people die from cancer each year, and this number is predicted to rise to approximately 13 million over the course of the next ten years. Without a doubt, the development of novel cancer treatments or the enhancement of current medications can aid in addressing such fatal emergencies globally (1). Nitrogen mustard is related to the antineoplastic chemical cyclophosphamide (CP). Various cancers, autoimmune illnesses such rheumatoid arthritis, Wegener's granulomatosis, and pediatric nephritic syndrome, as well as immunosuppressive therapy after organ transplants, are all treated with it in clinical settings. Despite CP's wide range of clinical applications, its therapeutic use in clinical settings is limited due to its multiple dose-dependent organ limitations (2).

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CP is a prodrug that undergoes hydroxylation in the liver to become 4-hydroxy-cyclophosphamide, which is the active form. 4-hydroxy-cyclophosphamide and aldophosphamide, its tautomeric counterpart, are in equilibrium. It is often believed that aldophosphamide is converted into the alkylating agent phosphoramide mustard through the spontaneous β-elimination of acrolein. Phosphoramide mustard is thought to stop cell development by alkylating DNA. Apart from the processes triggered by phosphoramide mustard, acrolein and the hydroxylation reaction's byproduct, chloroacetaldehyde, are thought to be the primary sources of CP toxicity (3).

According to reports, the use of CP results in reactive oxygen species (ROS) and free radical generation, which can lead to oxidative stress (4). The metabolic activation of CP by the cytochrome P-450 mixed functional oxidase system results in the formation of two metabolites, phosphoramide mustard and acrolein, which promote oxidative stress (5). Acrolein exerts its effect by passing through the uroepithelium and stimulating some ROS. Overproduction of ROS causes oxidative stress (6). Due to excessive free radical production, administration of CP has been shown to chance glutathione (GSH) levels and superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activity, as well as raise malondialdehyde (MDA) levels in the kidney (7).

Today, prevention or reduction of oxidative stress due to toxications resulting from exposure to chemical toxic substances and carcinogens is one of the most studied subjects. For this purpose, studies on the research and development of substances with antioxidant properties are needed now as in the past, and there have been several in vitro and in vivo models employed (1, 2, 6, 8, 9). Artichoke is one of these ingredients.

Artichoke (*Cynara scolymus L.*) has strong antioxidant properties and it has been stated that it has liver protective, antimicrobial, cholesterol-lowering properties thanks to its flavonoids (luteolin, apigenin) and caffeoyl quinic acid derivatives (cynarin and chlorogenic acid). However, it is also frequently used in conditions such as dyspepsia and intestinal syndrome. Owing to the active compounds' water solubility and bitter flavor, the majority of teas and aqueous extracts of dried leaves are utilized medicinally (10). Numerous research efforts have concentrated on the antioxidant and liver-protective properties of artichoke, demonstrating its efficacy in these areas (11-15).

Thus, early therapies and an understanding of the pathogenic pathways are necessary for the successful prevention of kidney injury. The mechanisms and goals for reducing CP-induced nephrotoxicity are the main topics of this study. Therefore, the purpose of this study was to use certain histopathological and biochemical data to examine how artichokes affected oxidative damage and stress and CP-induced nephrotoxicity in rats.

Materials and Methods

Research and Publication Ethics: This work was approved by the local ethics committee for animal experiments at Fırat University (Approval number: 2014/14-131).

Experimental Animals: Twenty eight male Wistar Albino rats, weighing an average of 250–300 g and 8–10 weeks old, were used in the investigation. The Fırat University, Center for Experimental Research and Application (Elazığ, Türkiye) provided the rats that were bought. Before the application, the rats spent a week getting used to their new surroundings. The outside temperature was 24±1°C with a 45±5% relative humidity. There was also a 12-hour cycle of light and dark in the rats' surroundings. Rats were given a regular pellet diet and unlimited access to tap water.

Experimental Protocol: There were four different groups of rats, each including seven rats. There are three experimental groups and a control in the experimental design. The first group, referred to as the control group, did not receive any medicine; second group received artichoke treatment for seven days; third group received a single dose of CP by injection; and fourth group received artichoke treatment for five days following administration of single dose of CP, in addition to seven days of treatment prior to it. One intraperitoneal dosage of CP (Cas No: 6055-19-2, C3250000, Merck, Germany) (150 mg/kg) (16, 17) was administered and artichoke extract (1 g/kg/day) (Arı Engineering, Ankara/Türkiye) (15, 18) was given using oral gavage and artichoke was prepared by dissolving in distilled water. The rats were decapitated 24 hours following the final administration, and their blood kidney tissues were subsequently collected for biochemical and histopathological analyses.

Determination of MDA, GSH Levels and Some Antioxidant Enzyme Activities: Following the applications, the rats were killed, and samples of kidney tissue were collected. A Potter-Elvehjem homogenizer was used to homogenize the kidney tissues, which were then diluted 1:10 with distilled water, and cleaned with physiological serum before to the analyses. The homogenate was centrifuged for 15 min at 3.500 rpm in a refrigerated centrifuge (NÜVE NF 800R) for MDA, GSH, CAT and GST analyses and the supernatants were taken (18, 19).

This study used a spectrophotometer and a modified Placer et al. (20) method to detect the levels of MDA in tissue samples. The process involves the interaction of MDA, an LPO byproduct, with thiobarbituric acid. The method described by Ellman et al. (21) was used to quantify the GSH levels. Spectrophotometric study of the yellow coloration that results from the introduction of 5,5'-dithiobis (2-nitrobenzoic acid) to sulfhydryl groups is the basis for the procedure. Since hydrogen peroxide (H_2O_2) can absorb light at 240 nm, the methodology established by Aebi in 1974 was employed to assess the activity of CAT (22). This method uses spectrophotometry to measure the rate of H_2O_2 breakdown by the CAT enzyme. The Beutler

method (23) was used to calculate the GSH-Px activity. This method entails using H_2O_2 to catalyze. The conversion of GSH to its oxidized form, known as oxidized glutathione (GSSG), which is done by GSH-Px. The glutathione reductase (GR) reaction was used to calculate the rate at which GSSG formed. The Habig et al. (24) technique was used to measure GST activity. GSH and 1-chloro-2,4-dinitrobenzene (CDNB) were used to measure the quantity of enzyme catalyzing 1 µmol of 1-(S-gluthathionyl)-2,4-dinitrobenzene produced at 340 nm at 37°C per minute in order to evaluate enzyme activity using spectrophotometry. Using a modified version of Sun et al.'s (25) approach, the SOD activity was measured by measuring the color of the reduction product, which was obtained by reducing nitroblue tetrazolium with the superoxide anion (O_2^-) generated by the xanthine oxidase (XO) system. The Lowry et al. (26) technique was used to calculate the protein concentration. Protein levels were used to calculate the specific activity of enzymes.

Histopathological Analyses: At the end of experimental study, kidney tissue specimen of all group were removed and fixed in 10% formaldehyde solution. The samples were serially sectioned at 5 µm after being embedded in parafin. Sections of tissue stained using Masson's Trichrome and Hematoxylin & Eosin methods to determine structıral changes due to CP. All stained tissue sections were examined with an Olympus BH2 photomicroscope (27).

Statistical Analysis: The SPSS 22 program was used to assess the statistical significance between the different groups. To determine if the raw values of all measured parameters displayed a normal distribution, the Shapiro-Wilk normality test was employed. The outcomes of the test demonstrated that every parameter value was normally distributed. Based on the outcomes of this test, group differences were assessed utilizing *post hoc* Tukey testing and one-way analysis of variance (ANOVA), it was utilized to compare the groups. All values were derived using the mean and the standard error of the mean. The mean and standard error were used to illustrate the study's conclusions. *p*-values that fell below 0.05 were considered to be statistically significant.

Results

Biochemical Results: Table 1 displays the results of the CAT, GSH-Px, SOD, GST, and MDA and GSH levels. GSH level ($p<0.001$) and CAT ($p<0.001$) activities were found to be statistically significantly lower in the CP group than in the control group, whereas MDA level ($p<0.001$), GSH-Px ($p=0.002$), SOD ($p<0.001$), and GST ($p<0.001$) activities were found to be statistically significantly higher. When the CP group was compared with the artichoke+CP group, statistically significant changes were determined in other markers except CAT activity. No statistical difference was observed when the control and only artichoke groups were compared. When the artichoke+CP group was compared with the control group, it was observed that all values except MDA level approached the control group values, and it was found that the decline in MDA levels was statistically different in the artichoke+CP group compared to the CP group, but the average did not reach the control group values statistically.

Histopathologically, the kidney section of control rats showed normal structural integrity (Figure 1A). In the section of kidneys of artichoke group that received only artichoke group, histological appearances of cortex and medulla closely resembled those of the controls except obvious peritubular congestion (Figure 1B). Hyalin-like material accumulation was observed both of cortical and medullar tubules in the CP administered group (Figure 1C). In addition, distal tubule dilatation and foamy appearanced tubular cells were observed in the renal cortex of CP group (Figure 1D, E).

In the section of kidney of the CP group that recevied artichoke group, foamy appearanced tubular cells were absent. Structural integrity of kidney tissue better than CP group. However, there was peritubular congestion prominently in this group (Figure 1F). Moreover, there were fewer dilated tubules and hyalin-like material containing tubule.

Table 1. Effects of artichoke on kidney MDA, GSH levels and CAT, GSH-Px, GST, SOD activities in CP-applied rats

	Control	Artichoke	CP	Artichoke+CP
MDA (nmol/g tissue)	1.00±0.04^{a}	1.08±0.03^{a}	1.49±0.03^{c}	1.32±0.01^{b}
GSH (µmol/mL)	11.51±0.10^{ab}	11.94±0.24^{a}	10.54±0.10^{c}	11.21±0.10^{b}
CAT (k/g protein)	97.37±3.77^{a}	92.31±7.63^{a}	67.75±2.23^{b}	80.75±4.25^{ab}
GSH-Px (U/g protein)	225.19±11.14^{a}	237.33±15.97^{a}	289.32±12.66^{b}	210.74±2.07^{a}
SOD (U/mg protein)	66.65±2.20^{a}	69.44±1.17^{a}	90.93±2.78^{b}	64.05±6.13^{a}
GST (U/mg protein)	1.31±0.08^{a}	1.31±0.16^{a}	2.60±0.11^{b}	1.41±0.07^{a}

Means with distinct letters (a, b, and c) within rows differ significantly ($p<0.05$).

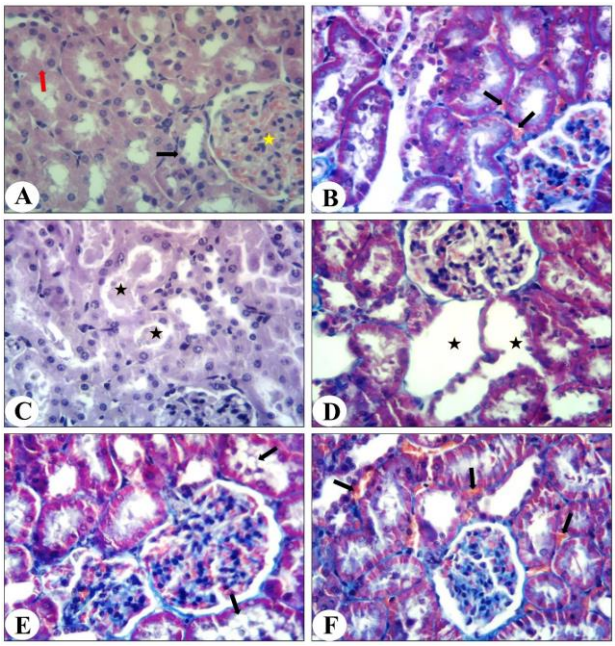

Figure 1. (A) Control Group. Glomerulus (*), distal convoluted tubule (black arrow), proximal convoluted tubule (red arrow) are exhibiting normal structure. (B) Artichoke Group. Peritubular congestion (arrow) in the cortex. (C) CP Group. Cortical distal convoluted tubules containing eosinophilic hyalin-like material. (D) CP Group. Dilatation of distal convoluted tubule (*). (E) CP Group. Foamy appearanced tubules in the cortex (arrow). (F) CP + Artichoke Group. Peritubular congestion (arrow) in the cortex. (A) and (C), Hematoxylin & Eosin x 400. (B), (D), (E), (F), Masson's Trichrome x 400.

Discussion

The most important factor limiting the increase in the high therapeutic efficacy of CP is the toxic effects it creates in many tissues and especially in the kidneys (3, 6, 18, 19). The active metabolite of CP, acrolein, is linked to its harmful effects. According to some theories, CP's harmful effects arise from the destruction of acreolin's antioxidant defense mechanisms, which are produced during its metabolism, and the production of large quantities of free radicals (28). In the presented study, it was observed that CP given in a single dose of 150 mg/kg, in accordance with previous studies (29, 30), changed the lipid peroxidation levels, some antioxidant enzyme activities and the normal histopathological structure of the kidney. The biochemical and histopathological findings we obtained were associated with the toxic effect of CP, which started with phosphoramide mustard and was formed by free radicals that emerged later.

Although CP has been used in the treatment of malignancy, cumulative dose-dependent toxicity is the main limiting factor. Many researchers have reported that CP can cause gastrointestinal (31), bone marrow (32) and cardiac (33) toxicity, as well as nephrotoxicity (34) and hepatotoxicity (35). The basic mechanism underlying the kidney damage caused by CP is oxidative stress. It leads to an increase in the levels of H_2O_2, ROS and hydroxyl radicals. Variations in the activity of some antioxidant enzymes and lipid peroxidation were determined by many researchers in rats given CP, and it was determined that antioxidants reversed the process and protected the cell with the addition of antioxidants. In our study, we observed that artichoke, an antioxidant born in rats given CP, improved the biochemical and histological results.

Prior research has demonstrated that CP results in toxicity and oxidative stress by causing changes in some indicators (blood urea nitrogen (BUN) and creatinine (Cr)) MDA, GSH concentration, and antioxidant enzyme activity (e.g., GSH-Px, SOD, CAT) (18, 19, 33, 36). In their studies, researchers have reached results such as increased MDA levels (18, 19, 33, 36), decreased (36) or increased (18, 33) in GSH levels, increased (33) or decreased (18, 19) in CAT activities, decreased (18, 19, 33) in GST activities, increased (19) or decreased (33) in SOD activities, decreased (33) in GSH-Px activities, increased (36) in BUN and Cr levels when compared to the control group after CP application. Our findings showed that CP increases the level of lipid peroxidation (MDA) and changes some antioxidant enzyme activities, which is consistent with the literature. In their investigation into the potential preventive impact of selenium against CP-induced acute nephrotoxicity, Gunes et al. (37), found that after CP applied at the same dose as the current study, creatinine, cystatin C, The levels of the oxidative stress index (OSI) and total oxidant status (TOS) rose, as did the levels of TAS decreased in rats treated only with CP. In addition, the researchers found that Localized shedding of tubular epithelial cells, glomerular compression, hyaline material accumulation in the renal tubules, restricted Bowman's capsule space, inflammatory foci, congested blood vessels, and tiny hemorrhagic regions were all visible in animals treated only with CP. In the current study, significant changes were detected in biomarkers related to oxidative damage and stress and in the histopathology of the kidney.

Many studies have been done to determine whether adding antioxidants to cancer chemotherapy improves treatment outcomes or lessens unwanted side effects. Antioxidants are likely to interfere with a cancer chemotherapeutic agent's anti-neoplastic effectiveness if the drug's production of ROS contributes significantly to its cytotoxicity. Antioxidants, however, can actually lessen the severity of adverse effects without compromising the efficacy of the medication if ROS is the main cause of them. Therefore, it is essential to differentiate between the role that free radicals play in a drug's mode of action and its ability to induce oxidative stress in biological systems. Since CP is an alkylating chemical, DNA alkylation is primarily responsible for its ability to kill tumor cells. Nevertheless, acrolein's generation of free radicals is frequently linked to harmful outcomes that are not desired (18, 19, 33).

Reducing CP side effects may improve medication tolerance and make therapy more efficient and comfortable for those who need it. The antioxidant system in cells reduces the amount of tissue damage

brought on by ROS. Generally speaking, some antioxidants might be helpful in reducing the harmful side effects of anticancer medications. Antioxidants and oxidative stress inhibitors such as selenium (37), propolis (18, 19, 33), artichoke (18), sesamin (17), resveratrol (30), lycopene (31) have been demonstrated to guard against renal damage and oxidative stress brought on by CP.

Studies conducted both in vitro and in vivo have demonstrated that artichokes contain a variety of biological properties, including the ability to scavenge free radicals and act as an efficient antioxidant (38, 39).

Many antioxidants have been tested to prevent CP-induced nephrotoxicity to date, but there is no study in rats where artichoke treatment was applied in CP-induced nephrotoxicity models. In our previous study, artichoke application was encountered in the CP-induced hemorrhagic cystitis model (18). The antitumoral activity of artichoke leaf extract was demonstrated by experimental tests due to its effect on signaling pathways with oncogenic importance. The bladder damage in the study occured due to cell membrane damage by CP metabolites. Because propolis and artichoke, when combined with CP, lower MDA levels and boost antioxidant enzyme activity, bladder tissue were prevented from oxidative injury. Various in vitro studies have shown that the antioxidant effect of artichoke is due to the metallic ion chelating and radical scavenging effects of components such as flavonoids, chlorogenic acid and cynarin. The antioxidant effect of artichoke is associated with the induction of antioxidant enzyme synthesis, which results in the intervention of inflammatory pathways gene expression and reduction of oxidative stress. Thus, the antioxidative function of artichokes is likely linked to CP's protective impact against H_2O_2 toxicity.

When the studies in recent years are examined, it has been observed that the number of studies examining the antioxidant effects of artichoke has increased. In this context, the effects of artichoke against many harmful substances or effects have been examined (13-15).

In a study examining the protective effect of chicory and/or artichoke leaf extracts against chronic nephrotoxicity caused by carbon tetrachloride and gamma irradiation in rats, researchers observed that the levels of lipid peroxidation indicator MDA, which increased after artichoke application, decreased, and the levels of GSH and the activities of antioxidant enzymes SOD and CAT improved (40). Artichoke administration reduced renal histological abnormalities, attenuated renal function, oxidative stress biomarkers, and up-regulated Bcl-2 and p53 mRNA gene expressions in rats treated with diethylnitrosamine (DEN) / acetylaminofluorene (2AAF) in a study designed to estimate the preventive effects of artichoke extracts from Cynara scolymus (41). Artichokes (*Cynara scolymus* L.) were found to improve altered MDA, GSH levels and SOD, GSH-Px activities after high-fat diet application. Additionally, artichoke application improved the kidney histopathologically by reducing the size of Bowman's space and preserving the glomerular structure, according to another study looking at the preventive effect of artichokes against high-fat diet-induced obesity in rats and renal dysfunction (42). In our current study, lipid peroxidation levels (MDA levels) in kidney tissue were lower in the group given artichoke together with CP compared to CP group. Also, GSH levels, GSH-PX, SOD and GST activities also showed changes in the group administered with artichoke+CP compared to the group administered only CP. The change in CAT activities remained statistically insignificant between CP and artichoke+CP groups. Histopathologically, foamy-looking tubular cells were not observed in the kidney section of the CP group receiving artichoke, and the structural integrity of the kidney tissue was better than the CP group. In addition, fewer dilated tubules and tubules containing hyaline-like material were observed. All these are consistent with the results of the studies mentioned above. It was shown that artichoke administration reduced oxidative damage in the kidney tissue of rats administered CP, and these results are consistent with our research hypothesis.

In Wistar rats, artichoke significantly reduces the renal damage caused by CP. According to the results, artichoke may lessen the negative effects of CP, indicating a possible therapeutic use for it in the treatment of drug-induced organ damage. To clarify the particular processes underlying these protective effects and investigate their potential therapeutic importance in human subjects, more research is necessary.

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