Research Article / Araştırma Makalesi
doi: 10.18678/dtfd.748816

Duzce Med J, 2020;22(2):124-130
Düzce Tıp Fak Derg, 2020;22(2):124-130

Protective Effects of Ellagic Acid Against Chemotherapy-Induced Hepatotoxicity

Kemoterapi Kaynaklı Hepatotoksisiteye Karşı Ellajik Asitin Koruyucu Etkileri

Alper YALÇIN[1]
0000-0002-8975-1008
Hikmet KELEŞ[2]
0000-0002-2308-0811
Tahir KAHRAMAN[3]
0000-0003-4238-8528
Mehmet Fatih BOZKURT[2]
0000-0002-1669-0988
Hasan AYDIN[4]
0000-0002-1955-6178

[1]Sutcu Imam University Faculty of Medicine Department of Histology and Embryology, Kahramanmaras, Turkey

[2]Afyon Kocatepe University Faculty of Veterinary Medicine Department of Pathology, Afyonkarahisar, Turkey

[3]Karabuk University Faculty of Medicine Department of Medical Biochemistry, Karabuk, Turkey

[4]Adiyaman University Faculty of Pharmacy Department of Pharmaceutical Toxicology, Adiyaman, Turkey

Corresponding Author
Sorumlu Yazar
Alper YALÇIN
alperyalcin0171@hotmail.com

Received / Geliş Tarihi : 06.06.2020
Accepted / Kabul Tarihi : 18.08.2020
Available Online / Çevrimiçi Yayın Tarihi : 25.08.2020

ABSTRACT

Aim: Cyclophosphamide (CP) is a commonly used chemotherapeutic agent despite its toxic adverse effects, including hepatotoxicity. Ellagic acid (EA) is an antioxidant agent and exhibits free radical scavenging activities. In this experimental study, the effects of EA on CP-induced liver injury were investigated.

Material and Methods: Twenty-four Sprague-Dawley rats (180-220 gr) were separated into four equal groups. A single dose of 150 mg/kg CP was given intraperitoneally to generate hepatotoxicity. Different doses (50 and 75 mg/kg) of EA were administered orally 20 minutes before, 4 and 8 hours after CP administration. The histopathological evaluation of kidney tissues and immunohistochemical evaluation for caspase-3 were conducted as well as the serum biochemical analyses.

Results: CP treated group exhibited a significant increase in serum hepatic enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), compared to the control group. Similarly, the total triglycerides (TG) and very-low-density lipoprotein cholesterol (VLDL-C) levels increased significantly. Additionally, the high-density lipoprotein cholesterol (HDL-C) levels decreased, which was not significant, compared to the control group. At both EA doses, VLDL-C, AST, ALT levels decreased significantly while HDL-C level revealed a significant increase. 75 mg/kg EA treatment caused a non-significant elevation in total cholesterol (TC) concentration. Microscopic analysis showed a significant congestion, edema, degeneration and necrosis in the livers of CP administered group. However, edema, degeneration, and necrosis were significantly reduced in animals treated with EA-75. In addition, caspase-3 expression significantly decreased in EA-75 group.

Conclusion: These results indicate the protective effects of EA in CP-induced hepatotoxicity in rats.

Keywords: Caspase-3; cyclophosphamide; ellagic acid; hepatotoxicity; lipids.

ÖZ

Amaç: Siklofosfamid (CP), hepatotoksisite dahil olmak üzere, toksik yan etkilerine rağmen yaygın olarak kullanılan kemoterapötik bir ajandır. Ellajik asit (EA) antioksidan bir ajandır ve serbest radikal süpürücü aktiviteler sergilemektedir. Bu deneysel çalışmada, EA'nın, CP'ye bağlı karaciğer hasarı üzerindeki etkileri araştırılmıştır.

Gereç ve Yöntemler: Yirmi dört adet Sprague-Dawley türü sıçan (180-220 gr) dört eşit gruba ayrıldı. Hepatotoksisite oluşturmak için intraperitonal olarak tek doz 150 mg/kg CP verildi. CP uygulamasından 20 dakika önce ve 4 ila 8 saat sonra oral yolla farklı dozlarda (50 ve 75 mg/kg) EA uygulandı. Serumun biyokimyasal analizlerinin yanı sıra böbrek dokularının histopatolojik değerlendirmesi ve kaspaz-3 için immünohistokimyasal değerlendirme yapıldı.

Bulgular: CP uygulanan grup, kontrol grubuna kıyasla, serum hepatik enzimleri olan aspartat aminotransferaz (AST) ve alanin aminotransferaz (ALT)'da önemli bir artış gösterdi. Benzer şekilde, total trigliserit (TG) ve çok düşük yoğunluklu lipoprotein kolesterol (VLDL-C) seviyeleri önemli ölçüde arttı. Ayrıca, yüksek yoğunluklu lipoprotein kolesterol (HDL-C) seviyeleri, kontrol grubuna kıyasla anlamsız olarak azaldı. Her iki EA dozunda da VLDL-C, AST, ALT seviyeleri önemli ölçüde azalırken, HDL-C seviyesi önemli bir artış gösterdi. 75 mg/kg EA tedavisi, total kolesterol (TC) konsantrasyonunda önemsiz bir artışa neden oldu. CP uygulanan grubun karaciğerlerinde mikroskobik olarak önemli derecede konjesyon, ödem, dejenerasyon ve nekroz gözlendi. Bununla beraber EA-75 grubundaki hayvanlarda ödem, dejenerasyon ve nekroz önemli ölçüde azaldı. Ayrıca kaspaz-3 ekspresyonu EA-75 grubunda anlamlı şekilde azaldı.

Sonuç: Bu sonuçlar sıçanlarda CP'nin neden olduğu hepatotoksisitede EA'nın koruyucu etkisi olduğunu göstermiştir.

Anahtar kelimeler: Kaspaz-3; siklofosfamid; ellajik asit; hepatotoksisite; lipidler.

INTRODUCTION

Cyclophosphamide (CP) is a widely used alkylating chemotherapic agent and has myelosuppressive, immunosuppressive, and cytotoxic effects (1). However, excessive toxic side effects often limit the therapeutic uses of this drug (2). The most common adverse effects of CP include hemorrhagic cystitis, hepatotoxicity (3), lung injury, nephrotoxicity (4), testicular toxicity (5), cardiomyopathy (6) as well as damage to the islets of the pancreas (7). Treatment with CP causes toxicity through over production of reactive oxygen species (ROS) leading to increased oxidative stress (8).

As it is the most important detoxification organ (9), liver is frequently exposed to the adverse effects of CP (10). This drug triggers hepatotoxicity and lead damage (11) as liver is an easier target for the effects of oxidative stress (12). Phosphoramide mustard and acrolein constitute two main active metabolites of CP. Phosphoramide mustard has been reported to have antineoplastic property, while acrolein is responsible for CP induced liver injury (13) as it interrupts tissue antioxidant system and forms very reactive oxygen free-radicals (14). Preventive strategies for CP-induced hepatotoxicity are limited and new strategies should be developed to preserve healthy organs and cells against detrimental effects of CP metabolites (15). It has been reported that antioxidants may control the response of tissues to chemotherapy and reduce the adverse effects of antineoplastic agents (16).

Herbal cures have gained importance due their efficiency, safety and lower costs (17). Ellagic acid (EA), a phenolic compound, has an important value among antioxidant substances of vegetable origin and is found in high concentrations in fruits such as raspberries, strawberries, cloudberry, rose hip, and sea buckthorn (18). More recently EA is of interest against liver toxicity due to its pharmacological properties (19). EA has been reported to be effective in protecting cells against oxidative damage (20) and increasing the activity of the antioxidant defense system (21) as it has strong antioxidant potential (22,23).

In the current study, possible protective effects of EA on CP-induced hepatotoxicity were investigated by evaluating histopathological, immunohistochemical and biochemical parameters.

MATERIAL AND METHODS

Chemicals

EA (≥95%, CAS Number 476-66-4) was purchased from Sigma-Aldrich. CP was purchased as a commercial preparation (Endoxan®, Eczacibasi-Turkey).

Animals, Diets and Experimental Protocols

The ethical guidelines for the care of laboratory animals (Afyon Kocatepe University, Animal Experiments Local Ethics Committee, protocol no: 38-19 and date: 19.02.2019) were followed throughout the experiments. Twenty-four Sprague-Dawley rats (180-220 gr) were separated into four groups (n=6). The experiment was initiated after two days of acclimatization in ambient conditions, ensuring 12 h light/dark cycle with ad libitum standard rodent pellet diet and water at room temperature (25±3 °C). The first group was named as control group and the animals were not treated with anything other than isotonic saline by intragastric gavage (i.g.). The second group was named the CP group and the animals received a single dose of 150 mg/kg CP at the beginning of the study via intraperitoneal (i.p.) route in accordance with the previous studies (24) to induce hepatotoxicity. The animals in the other two experimental groups, named the EA50 and the EA75 groups, received a single dose of 150 mg/kg CP by i.p. and a total of three EA treatments which were administered 20 minutes before CP, and 4 and 8 hours after the CP administration with 50 mg/kg and 75 mg/kg doses, respectively by i.g. route of administration. The concentrations of EA were selected in accordance with previous studies (20,25). The animals were fasted 12 hours before anesthesia.

Biochemical Analysis

After 48 hours of treatments, blood samples were collected from the animals under anesthesia (ketamine HCl, 80 mg/kg, i.p. and xylazine HCl, 10 mg/kg, i.p.) by intracardiac puncture and then centrifuged at 3500 rpm to separate sera. The obtained sera were stored at -20 °C until examination. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and very-low-density lipoprotein cholesterol (VLDL-C) levels were measured to evaluate the degree of hepatocellular damage, using an auto analyzer (Cobas 6000, Roche, Switzerland).

Tissue Preparation and Histopathologic Evaluation

The animals were euthanized and quickly necropsied after blood sampling. Liver tissues were taken and a sufficient portion was fixed in buffered 10% formalin solution. After routine processing, samples were placed in paraffin and sectioned. Following stained with hematoxylin-eosin (HE), sections were blindly analyzed. Passive hyperemia, periportal edema, degenerative and/or necrobiotic changes in hepatocyte and possible mononuclear cell clusters in the sinusoids and/or periportal areas in the liver sections were scanned under a light microscope (CX41 attached Kameram® Digital Image Analyze System; Olympus, Tokyo, Japan).

Immunohistochemical Examination

For antigen retrieval, following rehydrating procedure, sections were boiled for 5 min in a microwave oven (750 W) seven times in citrate buffer solution, pH 6. Sections were cooled at room temperature for 20 min, washed three times with phosphate-buffered saline (PBS) (P4417; Sigma Chemical Co.) for 5 min. Next sections were incubated for 5 min with hydrogen peroxide block solution (TA-125-HP; Lab Vision Corp. USA) to block endogenous peroxidase activity. The sections, then, were washed with PBS three times for 5 min. After applying Ultra V Block (TA-125-UB; Lab Vision Corp.) for 5 min, in a humid environment, tissue sections were incubated with primary antibodies for caspase-3 (rabbit polyclonal IgG, ab2302; Abcam, London, UK), diluted 1:200, at room temperature for 60 min. The sections then were incubated at room temperature for 30 min in a humid environment with secondary antibody (biotinylated goat anti-mouse/rabbit IgG, TP-125-BN; Lab Vision Corp.) after washing with PBS three times for 5 min each. Afterwards, sections were washed with PBS three times for 5 min and incubated at room temperature for 30 min in a humid environment with streptavidin peroxidase (TS-125-HR;

Lab Vision Corp.), then placed in PBS. 3-Amino-9-ethylcarbazole (AEC) substrate + AEC chromogen (AEC substrate, TA-015 and HAS, AEC Chromogen, TA-002-HAC; Lab Vision Corp.) solution was dripped on the sections. The sections were washed with PBS. For counterstaining, Mayer's hematoxylin was applied, then, sections were passed through PBS, distilled water and mounted with Large Volume Vision Mount (TA-125-UG; Lab Vision Corp). Sections were evaluated and images were taken by using a Leica DM500 microscope (Leica DFC295). The histoscore, which reflects the prevalence of immunoreactivity of caspase-3 on the liver tissues, was calculated according to the rating scale as: 0.1, <25%; 0.4, 26-50%; 0.6, 51-75%; 0.9, 76-100%, and intensity of immunoreactivity: 0, unstained; 0.5, little staining; 1, some staining; 2, moderate staining; 3, strong staining. The histoscore = prevalence x intensity.

Statistical Analysis

Statistical analyses were performed using the SPSS 15.0 version (SPSS Inc., Chicago, IL). The normal distributions of numerical variables were assessed by the Shapiro Wilk test. Levene test was used for the homogeneity test of variances. One-way analysis of variance (ANOVA) was used for the assessment of group comparisons of hepatic enzyme activities and serum lipid profiles. Tukey's multiple comparison test was used to determine the differences between the groups of significant variables. Kruskal-Wallis H test was used for group comparisons of histopathological findings. Mann Whitney U test was used to determine the differences between the groups of significant variables. Bonferroni correction was applied for which p values <0.008. The results were given as mean±SD and median (min-max). The level of significance was considered to be at least $p<0.05$.

RESULTS

Hepatic Enzyme Activities

To establish the therapeutic utility of EA in CP-induced hepatotoxicity, we performed biochemical analyses of liver tissues from animals that were treated with CP and control alone or in combination with EA. There were significant differences between groups both for AST and ALT levels (both p values were <0.001). According to the post hoc test results, a significant increase in the AST and ALT levels were observed in CP group in comparison with saline-treated control group. However, significant decreases were seen of AST and ALT levels in the EA50 and EA75 groups compared to the CP-treatment group (Table 1).

Serum Lipid Profile

TG levels was shown a statistically significant difference in four groups (p=0.033). Post hoc test revealed that TG levels increased in a significant manner in CP group in comparison with the control group. In the EA50 and EA75 groups, the decrease in the levels of TG was not significant compared to the CP group. In terms of TC levels, no significant difference was observed between control, CP, EA50 groups and EA75 groups (p=0.137). A statistically significant difference was found in HDL-C levels of groups ($p<0.001$). EA-treatment at both concentrations increased HDL-C levels compared to CP group, whereas a higher level was detected in the EA75 group. While LDL-C levels were similar in the control, CP, and, EA 50 group, EA75 group exhibited a non-significant increase (p=0.069). There was a significant difference between the groups in terms of VLDL-C levels ($p<0.001$). CP treatment increased the VLDL-C level, which was significantly decreased in the animals treated with EA50 and EA75 mg doses (Table 2).

Liver Histopathology

In terms of histopathological findings, there were statistically significant differences between groups for congestion, edema, degeneration and necrosis (all p values were <0.001). Significant congestion and edema were seen in the CP applied groups compared to control group. Congestion and edema were reduced in the EA75 group, and to a lesser extent, in the EA50 group compared to the CP group. EA treatment at a dose of 75 mg/kg gave very good results against CP-induced degeneration and necrosis; however, administration of 50 mg/kg was insufficient. The success of 75 mg/kg EA in preventing necrosis has been particularly significant. In the CP and EA50 groups, severe degeneration and necrosis in the hepatocytes around the vena centralis was observed in comparison with other groups (Figure 1, Table 3). These results clearly demonstrate that EA significantly minimizes the cytotoxic effects of CP in liver tissue similar to the biochemical findings.

Caspase-3 Immunohistochemistry of Liver

For evaluating the effects of EA on CP-induced apoptosis in liver, caspase-3 antibody was used as a marker. Caspase-3 expression was determined in the cytoplasm of hepatocytes as shown in Figure 2. There was a significant difference in caspase-3 immunoreactivity of the groups (<0.001), and according to the post hoc test results, immunoreactivity of caspase-3 significantly increased in CP-treated group in comparison with those in the control group. However, contrary to CP-treated group, immunoreactivity of caspase-3 decreased in the CP+EA50 and CP+EA75 groups in a significant manner (Table 4). These findings provide evidence that EA significantly reduces the apoptotic effects of CP in liver tissue at both doses.

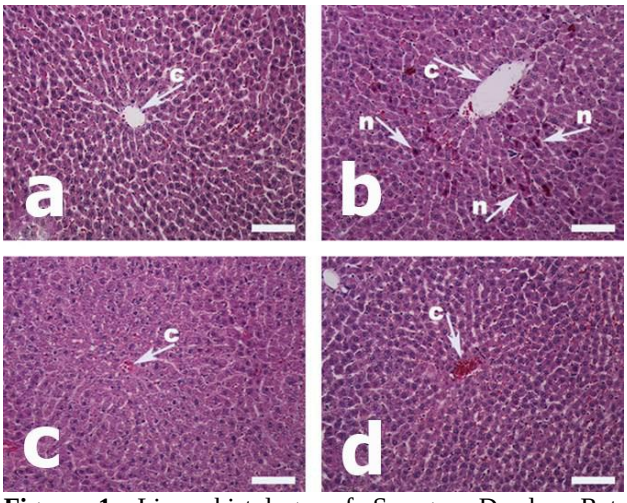

Figure 1. Liver histology of Sprague Dawley Rat. Representative figures were stained with HE. The scale bars represent 100 µm. Arrow pointed events; c: vena centralis, n: necrotic hepatocytes. **a)** Control group (standard rodent diet) **b)** CP group (150 mg/kg CP) **c)** EA50 group (150 mg/kg CP and 50 mg/kg EA) **d)** EA75 group (150 mg/kg CP and 75 mg/kg EA)

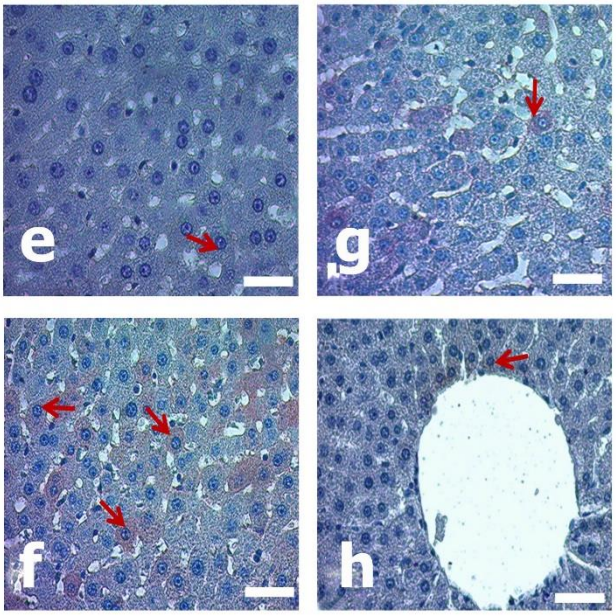

Figure 2. Immunohistochemical staining for caspase-3 (arrow) in liver tissue. The scale bars represent 100 µm. **e)** Control group **f)** Increased caspase-3 immunoreactivity of CP group **g)** Decreased caspase-3 immunoreactivity of EA50 group **h)** Decreased caspase-3 immunoreactivity of EA75 group

DISCUSSION

CP is a widely used chemotherapeutic and immunosuppressive agent for various cancers and autoimmune diseases (26). Although CP exhibit effective cytotoxic activity on malignant cells, it can also cause significant damage in normal tissues (4,5). The liver is one of the mostly affected organs by CP-induced toxicity (3) that leads to significant functional impairments (27). In experimental studies, CP was shown to cause deterioration of the oxidant-antioxidant balance (28) and increase in lipid peroxidation (29) in addition to the macroscopic and microscopic deformities in different tissues. CP applications may alter the serum lipid profile. In CP-treated rats, LDL-C and VLDL-C increased and HDL-C decreased along with changes in lipid metabolizing enzymes (30). Moreover, plasma TG, TC, and LDL-C levels were also increased in rats treated with CP (8). The TC level increased, but the HDL-C level decreased in CP-administered rabbits (31).

Chemotherapeutic agents used currently in cancer treatment usually have some side effects. In this respect, supportive applications that can minimize the side effects of those agents used in cancer patients are needed.

Some fruits and vegetables are a potential candidate in this context through their numerous bioactive components,

Table 1. Hepatic enzyme activities (AST, ALT) of the groups

	Control	CP	EA50	EA75	p
AST	68.28±6.09^{a}	133.58±13.65^{c}	97.28±8.46^{b}	81.45±8.90^{a}	**<0.001**
ALT	22.82±1.23^{a}	43.12±9.57^{b}	25.90±3.79^{a}	23.93±1.21^{a}	**<0.001**

CP: Cyclophosphamide, EA50: Ellagic acid 50 mg/kg, EA75: Ellagic acid 75 mg/kg, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ^{abc}: Different superscripts denote significant difference between groups (p<0.05, Tukey's test)

Table 2. Serum lipid profiles (TG, TC, VLDL-C, LDL-C, HDL-C) of the groups

	Control	CP	EA50	EA75	p
TG	33.88±3.73^{a}	50.67±17.06^{b}	47.80±9.25^{ab}	38.75±4.80^{ab}	**0.033**
TC	70.15±12.45	76.05±6.53	78.70±19.59	90.62±16.68	0.137
HDL-C	52.37±8.94^{ab}	43.42±5.30^{a}	62.40±10.19^{b}	85.55±11.67^{c}	**<0.001**
LDL-C	24.38±4.91	28.77±7.13	28.87±8.92	39.12±13.62	0.069
VLDL-C	6.78±0.75^{a}	12.63±1.75^{b}	8.73±1.01^{a}	8.42±1,51^{a}	**<0.001**

CP: Cyclophosphamide, EA50: Ellagic acid 50 mg/kg, EA75: Ellagic acid 75 mg/kg, TG: Triglyceride, TC: Total cholesterol, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, VLDL-C: Very low-density lipoprotein cholesterol, ^{abc}: Different superscripts denote significant difference between groups (p<0.05, Tukey's test)

Table 3. Histopathological findings of the groups

	Control		CP		EA50		EA75		p
	Mean±SD	Median (min-max)	Mean±SD	Median (min-max)	Mean±SD	Median (min-max)	Mean±SD	Median (min-max)	
Congestion	0.17±0.41^{a}	0 (0-1)	2.50±0.53^{c}	2.5 (2-3)	2.33±0.52^{c}	2 (2-3)	2.00±0.00^{b}	1 (1-2)	**<0.001**
Edema	0.33±0.52^{a}	0 (0-1)	2.50±0.55^{c}	2.5 (2-3)	2.17±0.42^{c}	2 (2-3)	1.83±0.42^{b}	1 (1-2)	**<0.001**
Degeneration	0.17±0.41^{a}	0 (0-1)	3.83±0.41^{b}	4 (3-4)	3.17±0.75^{b}	3 (2-4)	0.50±0.84^{a}	0 (0-2)	**<0.001**
Necrosis	0.00±0.00^{a}	0 (0-0)	3.33±0.52^{c}	3 (3-4)	2.17±1.17^{b}	2 (0-3)	0±0.00^{a}	0 (0-0)	**<0.001**

CP: Cyclophosphamide, EA50: Ellagic acid 50 mg/kg, EA75: Ellagic acid 75 mg/kg, Descriptive statistics were given as mean±SD and median (min-max), ^{abc}: Different superscripts denote significant difference between groups (p<0,008 was considered significant because of multiple tests, Mann Whitney U test was applied with Bonferroni adjustment), SD: Standard deviation, Congestion; 1 vs 4: p=0.005, 1 vs 3: p=0.003, 1 vs 2: p=0.002, 4 vs 3: p=0.003, 4 vs 2: p=0.002 and 3 vs 2: p=0.118, Edema; 1 vs 4: p=0.005, 1 vs 3: p=0.003, 1 vs 2: p=0.002, 4 vs 3: p=0.003, 4 vs 2: p=0.002 and 3 vs 2: p=0.241, Degeneration; 1 vs 4: p=0.461, 1 vs 3: p=0.003, 1 vs 2: p=0.002, 4 vs 3: p=0.002, 4 vs 2: p=0.002 and 3 vs 2: p=0.083, Degeneration; 1 vs 4: p=1.000, 1 vs 3: p=0.007, 1 vs 2: p=0.002, 4 vs 3: p=0.006, 4 vs 2: p=0.002 and 3 vs 2: p=0.007

Table 4. Caspase-3 immunoreactivity of the groups

	Control	CP	EA50	EA75	p
Caspase-3	0.486±0.186^{a}	1.200±0.300^{b}	0.729±0.198^{a}	0.429±0.180^{a}	**<0.001**

CP: Cyclophosphamide, EA50: Ellagic acid 50 mg/kg, EA75: Ellagic acid 75 mg/kg, ^{ab}: Different superscripts denote significant difference between groups (p<0.05, Tukey's test)

including fiber, vitamins, minerals, and especially polyphenols. Dietary polyphenols could modulate lipid metabolism as well as other positive effects (32). EA is a polyphenol found abundant in fruits and vegetables. In natural conditions, EA is largely taken as free EA or ellagitannins (ETs) by consumption of fruits and vegetables (33).

In the current study liver injury induced by CP was evidenced by severe congestion, edema, degeneration, and necrosis. The mechanism of CP induced cellular injury has been reported to be related with the induction of oxidative stress by the formation of free radicals and ROS (34). Histopathologic abnormalities were ameliorated by EA and decreased to the control group levels in the EA75 group, except congestion. This reveals the hepatoprotective potential of EA against liver toxicity of CP through its antioxidant potential. Similar to our findings, EA supplementation improved hepatic steatosis (35), degenerative, necrotic and inflammatory changes (36) sinusoidal dilatation, degeneration, edema, and lymphocyte infiltration in the liver (37).

Apoptosis has a significant role in the pathogenesis of CP induced hepatotoxicity (38). In this study, CP treatment increased caspase-3 expression in liver tissue in accordance with previous publications (23,39). This increase could be attributed to the inductive role of CP on apoptosis in liver (40). On the other hand, EA at the concentration of both 50 mg/kg and 75 mg/kg were able to reduce caspase-3 reactivity significantly due to its anti-apoptotic effects (41) in line with the previous studies (37,41,42). CP treatment causes significant elevations of liver enzymes such as ALT and AST in blood of rats (8). In the current study, serum ALT and AST levels increased in the CP-administered rats. Increased enzyme levels are a marker of liver damage and they indicate CP caused formation of toxic metabolites that result in liver damage. However, in the EA treated groups, especially in the EA75 group, these values returned to normal level as it decreased the leakage of the enzymes. This restoration might be attributed to the protective and free radical scavenging property of EA (35) against CP induced liver toxicity. These findings were consistent with the effects of EA on alcohol-induced liver damage reported by Devipriya et al (25). Moreover, sodium arsenide-induced changes in serum AST and ALT concentrations returned to normal levels with EA supplementation in rats (36).

The regulatory effects of EA on the lipid metabolism are known, and a wide range of positive effects of EA and EA-containing products on chronic metabolic diseases such as type 2 diabetes, dyslipidemia, insulin resistance, and non-alcoholic fatty liver disease have been reported (33). For example, the lipid level (TC, TG, free fatty acids, and phospholipids), which is negatively affected by alcohol consumption in rats, was normalized by EA (24). EA supplementation improved the serum lipid profile in high-fat diet fed mice (35) and in cholesterol fed hyperlipidemic rats (43). Moreover, Kang et al. (44) reported that high-fat and high sugar-induced dyslipidemia was reversed in mice given raspberry seed powder. Ahad et al. (45) showed that oral EA intake significantly inhibited dyslipidemia in high-fat diet/low dose in type 2 diabetic rats. However, the evaluation of serum lipid parameters revealed that EA treatment at both concentrations raised HDL-C level compared to the CP group and control group in this study. EA treatment reduced CP-induced elevations of VLDL-C to a significant level as well as TG levels but not to a significant level. The effects of EA on VLDL-C and TG found in this study are in line with the previous reports (45,46).

Kang et al. (33) reviewed the effects of EA or EA-enriched fraction on obesity and metabolic complications and they reported that EA administration at a dose of 7.5-800 mg/kg for 10 days-16 weeks showed significant improvement in the lipid profiles of mice and rats. As demonstrated in this review, chronic supplementation of EA/ET enriched extracts or pure EA was effective in alleviating metabolic syndrome-associated disorders in rodents.

Studies showed that EA significantly decreases TC and LDL-C in rodents (45,46). However, in the present study EA increased both LDL-C and TC levels, although it was not significant. This inconsistency of results with the previous reports could be due to the difference of disease models and chemical agents used.

EA markedly increased HDL-C levels in dose dependent manner. HDL is a key molecule to remove excess cholesterol from peripheral tissues through a process defined as reversed cholesterol transport (47,48). Beneficial effects of EA on HDL levels have been previously reported (43,49), an important way to eliminate cholesterol.

CONCLUSION

Alterations in the lipid profile observed in this study due to the use of EA showed some consistency and also some inconsistency when compared to other studies. However, most of the studies in literature are designed to evaluate the long-term effect of EA, whereas the present study has established short term activity of EA (48-hour), and is an acute examination compared to other chronic applications. In this context, when the literature is reviewed again, it is seen that the metabolic rate increases due to the EA, and lipid efflux occur shortly after ingestion of EA and EA-containing products. In our study, high lipid level in serum samples may be related to the rearrangement of the lipid balance in the body and the efflux of peripheral lipid deposits compared to other studies. Despite the instability of biochemical lipid results, no cytoplasmic accumulation, degeneration or necrosis of liver hepatocytes in histopathological examination and reduced apoptotic hepatocytes in immunohistochemical examinations demonstrate the protective effect of the EA administration. Moreover, the protective effects of EA on the liver were confirmed by serum liver enzymes which were elevated by CP applications. Our study has demonstrated a significant reduction of these enzymes by 75 mg/kg EA administration. Time-dependent experiments are needed to elucidate this issue. Currently, the cases of liver damage related to polluted water, food, drugs, and other environmental factors continue to rise in the world. Therefore, the consumption of fruits and vegetables containing EA, which have positive effects on health, may be supportive for the liver.

Declaration of Conflict of Interest

The authors report no conflict of interest.

REFERENCES

1. Tripathi DN, Jena GB. Intervention of astaxanthin against cyclophosphamide-induced oxidative stress and DNA damage: a study in mice. Chem Biol Interact. 2009;180(3):398-406.
2. Papaldo P, Lopez M, Marolla P, Cortesi E, Antimi M, Terzoli E, et al. Impact of five prophylactic filgrastim schedules on hematologic toxicity in early breast cancer patients treated with epirubicin and cyclophosphamide. J Clin Oncol. 2005;23(28):6908-18.
3. El-Naggar SA, Abdel-Farid IB, Germoush MO, Elgebaly HA, Alm-Eldeen AA. Efficacy of Rosmarinus officinalis leaves extract against cyclophosphamide-induced hepatotoxicity. Pharm Biol. 2016;54(10):2007-16.
4. Said E, Elkashef WF, Abdelaziz RR. Tranilast ameliorates cyclophosphamide-induced lung injury and nephrotoxicity. Can J Physiol Pharmacol. 2016;94(4):347-58.
5. Ghobadi E, Moloudizargari M, Asghari MH, Abdollahi M. The mechanisms of cyclophosphamide-induced testicular toxicity and the protective agents. Expert Opin Drug Metab Toxicol. 2016;13(5):525-36.
6. Asiri YA. Probucol attenuates cyclophosphamide-induced oxidative apoptosis, p53 and Bax signal expression in rat cardiac tissues. Oxid Med Cell Longev. 2010;3(5):308-16.
7. Sharma PK, Misra AK, Singh V, Gupta A, Saroha S, Singh S. Cyclophosphamide and epirubicin-induced diabetes mellitus in breast cancer: A rare occurrence. J Pharmacol Pharmacother. 2016;7(3):146-8.
8. Alenzi FQ, El-Bolkiny YE-S, Salem ML. Protective effects of Nigella sativa oil and thymoquinone against toxicity induced by the anticancer drug cyclophosphamide. Br J Biomed Sci. 2010;67(1):20-8.
9. Li QZ, Sun J, Shen HT, Jia SF, Bai DS, Ma D. CdS nanoparticles of different lengths induce differential responses in some of the liver functions of mice. Bratisl Lek Listy. 2018;119(2):75-80.
10. Yuksel S, Tasdemir S, Korkmaz S. Protective effect of thymoquinone against cyclophosphamide-induced genotoxic damage in human lymphocytes. Bratisl Lek Listy. 2017;118(4):208-11.
11. Mahmoud AM, Germoush MO, Alotaibi MF, Hussein OE. Possible involvement of Nrf2 and PPARγ up-regulation in the protective effect of umbelliferone against cyclophosphamide-induced hepatotoxicity. Biomed Pharmacother. 2017;86:297-306.
12. Postaci I, Coskun O, Senol N, Aslankoc R, Comlekci S. The physiopathological effects of quercetin on oxidative stress in radiation of 4.5 g mobile phone exposed liver tissue of rat. Bratisl Lek Listy. 2018;119(8):481-9.
13. Honjo I, Suou T, Hirayama C. Hepatotoxicity of cyclophosphamide in man: pharmacokinetic analysis. Res Commun Chem Pathol Pharmacol. 1988;61(2):149-65.
14. Mythili Y, Sudharsan PT, Selvakumar E, Varalakshmi P. Protective effect of DL-alpha-lipoic acid on cyclophosphamide induced oxidative cardiac injury. Chem Biol Interact. 2004;151(1):13-9.
15. Shokrzadeh M, Ahmadi A, Naghshvar F, Chabra A, Jafarinejhad M. Prophylactic efficacy of melatonin on cyclophosphamide-induced liver toxicity in mice. Biomed Res Int. 2014;2014:470425.
16. Weijl NI, Cleton FJ, Osanto S. Free radicals and antioxidants in chemotherapy-induced toxicity. Cancer Treat Rev. 1997;23(4):209-40.
17. Arora B, Choudhary M, Arya P, Kumur S, Choudhary N, Singh S. Hepatoprotective potential of Saraca ashoka (Roxb) De Wilde bark by carbon tetrachloride induced liver damage in rats. Bull Fac Pharm Cairo Univer. 2015;53(1):23-8.
18. Koponen JM, Happonen AM, Mattila PH, Törrönen AR. Contents of anthocyanins and ellagitannins in selected foods consumed in Finland. J Agric Food Chem. 2007;55(4):1612-9.
19. Liang WZ, Chou CT, Cheng JS, Wang JL, Chang HT, Chen I-S, et al. The effect of the phenol compound ellagic acid on Ca(2+) homeostasis and cytotoxicity in liver cells. Eur J Pharmacol. 2016;780:243-51.
20. Hussein RH, Khalifa FK. The protective role of ellagitannins flavonoids pretreatment against N-nitrosodiethylamine induced-hepatocellular carcinoma. Saudi J Biol Sci. 2014;21(6):589-96.
21. Seeram NP, Adams LS, Henning SM, Niu Y, Zhang Y, Nair MG, et al. In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. J Nutr Biochem. 2005;16(6):360-7.
22. Karimi J, Goodarzi MT, Tavilani H, Khodadadi I, Amiri I. Relationship between advanced glycation end products and increased lipid peroxidation in semen of diabetic men. Diabetes Res Clin Pract. 2011;91(1):61-6.
23. Sarker U, Oba S. Antioxidant constituents of three selected red and green color Amaranthus leafy vegetable. Sci Rep. 2019;9(1):18233.
24. Aladaileh SH, Abukhalil MH, Saghir SAM, Hanieh H, Alfwuaires MA, Almaiman AA, et al. Galangin activates Nrf2 signaling and attenuates oxidative damage, inflammation, and apoptosis in a rat model of cyclophosphamide-induced hepatotoxicity. Biomolecules. 2019;9(8):346.
25. Devipriya N, Sudheer AR, Vishwanathan P, Menon VP. Modulatory potential of ellagic acid, a natural plant polyphenol on altered lipid profile and lipid peroxidation status during alcohol-induced toxicity: a pathohistological study. J Biochem Mol Toxicol. 2008;22(2):101-12.
26. Lawson M, Vasilaras A, De Vries A, Mactaggart P, Nicol D. Urological implications of cyclophosphamide and ifosfamide. Scand J Urol Nephrol. 2008;42(4):309-17.
27. Campbell I. Liver: metabolic functions. Anaesth Intensive Care Med. 2006;7(2):51-4.
28. Tripathi DN, Jena GB. Astaxanthin intervention ameliorates cyclophosphamide-induced oxidative strese, DNA damage and early hepatocarcigonesis in rats: role of Nrf2, p53, p38 and phase-II enzymes. Mutat Res. 2010;696(1):69-80.
29. Ray S, Chowdhury P, Pandit B, Ray SD, Das S. Exploring the antiperoxidative potential of morin on cyclophosphamide and flutamide-induced lipid peroxidation and changes in cholesterol profile in rabbit model. Acta Pol Pharm. 2010;67(1):35-44.
30. Mythili Y, Sudharsan PT, Sudhahar V, Varalakshmi P. Protective effect of DL-alpha-lipoic acid on

cyclophosphamide induced hyperlipidemic cardiomyopathy. Eur J Pharmacol. 2006;543(1-3):92-6.
31. Ray S, Pandit B, Das S, Chakraborty S. Cyclophosphamide-induced lipid peroxidation and changes in cholesterol content: protective role of reduced glutathione. Iran J Pharm Sci. 2011;7(4):255-67.
32. Amiot MJ, Riva C, Vinet A. Effects of dietary polyphenols on metabolic syndrome features in humans: a systematic review. Obes Rev. 2016;17(7):573-86.
33. Kang I, Buckner T, Shay NF, Gu L, Chung S. Improvements in metabolic health with consumption of ellagic acid and subsequent conversion into urolithins: evidence and mechanisms. Adv Nutr. 2016;7(5):961-72.
34. Ghosh D, Das UB, Ghosh S, Mallick M, Debnath J. Testicular gametogenic and steroidogenic activities in cyclophosphamide treated rat: a correlative study with testicular oxidative stress. Drug Chem Toxicol. 2002;25(3):281-92.
35. Yoshimura Y, Nishii S, Zaima N, Moriyama T, Kawamura Y. Ellagic acid improves hepatic steatosis and serum lipid composition through reduction of serum resistin levels and transcriptional activation of hepatic ppara in obese, diabetic KK-A(y) mice. Biochem Biophys Res Commun. 2013;434(3):486-91.
36. Mehrzadi S, Fatemi I, Malayeri AR, Khodadadi A, Mohammadi F, Mansouri E, et al. Ellagic acid mitigates sodium arsenite-induced renal and hepatic toxicity in male Wistar rats. Pharmacol Rep. 2018;70(4):712-9.
37. Aslan A, Gok O, Erman O, Kuloglu T. Ellagic acid impedes carbontetrachloride-induced liver damage in rats through suppression of NF-kB, Bcl-2 and regulating Nrf-2 and caspase pathway. Biomed Pharmacother. 2018;105:662-9.
38. Caglayan C, Temel Y, Kandemir FM, Yildirim S, Kucukler S. Naringin protects against cyclophosphamide-induced hepatotoxicity and nephrotoxicity through modulation of oxidative stress, inflammation, apoptosis, autophagy, and DNA damage. Environ Sci Pollut Res Int. 2018;25(21):20968-84.
39. Li X, Li B, Jia Y. The hepatoprotective effect of haoqin qingdan decoction against liver injury induced by a chemotherapeutic drug cyclophosphamide. Evid Based Complement Altern Med. 2015;2015:978219.
40. Fouad AA, Qutub HO, Al-Melhim WN. Punicalagin alleviates hepatotoxicity in rats challenged with cyclophosphamide. Environ Toxicol Pharmacol. 2016;45:158-62.
41. Rizk HA, Masoud MA, Maher OW. Prophylactic effects of ellagic acid and rosmarinic acid on doxorubicin-induced neurotoxicity in rats. J Biochem Mol Toxicol. 2017;31(12):e21977.
42. Ding Y, Wang L, Song J, Zhou S. Protective effects of ellagic acid against tetrachloride-induced cirrhosis in mice through the inhibition of reactive oxygen species formation and angiogenesis. Exp Ther Med. 2017;14(4):3375-80.
43. Mali VR, Bodhankar SL, Mohan V, Thakurdesai PA. Subacute toxicity of ellagic acid in cholesterol fed hyperlipidemic rats. Toxicol Int. 2008;15(2):91-5.
44. Kang I, Espín JC, Carr TP, Tomás-Barberán FA, Chung S. Raspberry seed flour attenuates high-sucrose diet-mediated hepatic stress and adipose tissue inflammation. J Nutr Biochem. 2016;32:64-72.
45. Ahad A, Ganai AA, Mujeeb M, Siddiqui WA. Ellagic acid, an NF-κB inhibitor, ameliorates renal function in experimental diabetic nephropathy. Chem Biol Interact. 2014;219:64-75.
46. Kannan MM, Quine SD. Ellagic acid inhibits cardiac arrhythmias, hypertrophy and hyperlipidaemia during myocardial infarction in rats. Metabolism. 2013;62(1):52-61.
47. Huang L-H, Elvington A, Randolph GJ. The role of the lymphatic system in cholesterol transport. Front Pharmacol. 2015;6:182.
48. Yokoyama S. Assembly of high-density lipoprotein by the ABCA1/apolipoprotein pathway. Curr Opin Lipidol. 2005;16(3):269-79.
49. Lei F, Zhang XN, Wang W, Xing DM, Xie WD, Su H, et al. Evidence of anti-obesity effects of the pomegranate leaf extract in high-fat diet induced obese mice. Int J Obes (Lond). 2007;31(6):1023-9.