

Research Article

Gul Fatma Yarim, Filiz Kazak, Mahmut Sozmen, Ilkay Koca,
Harun Albayrak, Murat Yarim*, Sena Cenesiz and Emre Ozan



Investigation of the effect of cornelian cherry (*Cornus mas* L.) fruit extract against cisplatin-induced renal cell injury in vitro

Kızılcık (*Cornus mas* L.) meyva ekstraktının sisplatin indüklü in vitro böbrek hücre hasarına etkisinin araştırılması

DOI 10.1515/tjb-2016-0136

Received August 26, 2016; accepted June 13, 2017; previously published online July 19, 2017

Abstract

Objective: The aim of this study was to evaluate the protective effects of cornelian cherry fruit extract against cisplatin-induced nephrotoxicity in vitro.

Materials and methods: African green monkey kidney epithelial cells (Vero) were incubated with 100 mg/mL of cornelian cherry fruit extract, 50 μ mol/L of cisplatin or 50 μ mol/L of cisplatin plus 100 mg/mL of cornelian cherry fruit extract for 4 h. The wells containing cells without any supplementation served as control. Cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoliumbromide assay. Culture mediums were collected, centrifuged and analyzed for malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD) and glutathione peroxidase (GPx).

Results: The cell viability was 59% in cells co-treated with cisplatin and cornelian cherry fruit extract simultaneously and 42% in cisplatin treated cells. The cellular damage ratio was elevated in cells treated with cisplatin. However, when cisplatin combined with cornelian cherry fruit extract the deleterious effects of cisplatin were significantly decreased. The MDA concentration was significantly higher ($p < 0.05$), GSH concentration and GPx and SOD activities were significantly lower ($p < 0.05$) in cisplatin treated group when compared with control group, cornelian cherry group, and cisplatin+cornelian cherry group.

Conclusion: The present study indicated that cornelian cherry fruit extract exert protective effects on oxidative damage in vitro induced by cisplatin.

Keywords: Cisplatin; Cornelian cherry; Oxidative damage; In vitro; Nephrotoxicity.

Özet

Amaç: Bu in vitro çalışmada, kıızılcık meyva ekstraktının sisplatin indüklü nefrotoksisiteye karşı koruyucu etkisinin değerlendirilmesi amaçlanmıştır.

Materyal ve Metot: Çalışmada Afrika yeşil maymun böbrek epitel hücreleri (Vero), 100 mg/mL kıızılcık meyva ekstraktı, 50 μ mol/L sisplatin veya 50 μ mol/L sisplatin + 100 mg/mL kıızılcık meyva ekstraktı ile 4 saat inkübe edildi. Herhangi bir ekleme yapılmayan, sadece hücre bulunan kuyucuklar kontrol grubu olarak kullanıldı. Hücre canlılığı, 3-(4,5-dimetil-thiazole-2-yl)-2,5-difenil tetrazolium bromide tayini ile belirlendi. Kültür medyumları toplandı ve santrifüj edildi. Malondialdehit (MDA),

*Corresponding author: Murat Yarim, Department of Pathology, Faculty of Veterinary Medicine, Samsun, Atakum, Turkey, e-mail: myarim@omu.edu.tr; muratyarim@hotmail.com

Gul Fatma Yarim and Sena Cenesiz: Ondokuz Mayıs University, Department of Biochemistry, Faculty of Veterinary Medicine, Samsun, Atakum, Turkey

Filiz Kazak: Mustafa Kemal University, Department of Biochemistry, Faculty of Veterinary Medicine, Hatay, Antakya, Turkey

Mahmut Sozmen: Ondokuz Mayıs University, Department of Pathology, Faculty of Veterinary Medicine, Samsun, Atakum, Turkey

Ilkay Koca: Ondokuz Mayıs University, Department of Food Engineering, Faculty of Engineering, Samsun, Atakum, Turkey

Harun Albayrak: Ondokuz Mayıs University, Department of Virology, Faculty of Veterinary Medicine, Samsun, Atakum, Turkey

Emre Ozan: Veterinary Control Institute, Samsun, Atakum, Turkey

glutasyon (GSH), superoksit dismutaz (SOD) ve glutasyon peroksidaz (GPx) analizleri yapıldı.

Bulgular: Sisplatin maruz bırakılan hücrelerde hücre canlılığı %42, sisplatinle birlikte kıvılcık meyva ekstraktı uygulanan hücrelerde ise hücre canlılığı %59 bulundu. Sisplatin uygulanan hücrelerde hücre hasar oranı yüksekti. Bununla birlikte kıvılcık meyva ekstraktı ile kombine sisplatin grubunda, sisplatinin zararlı etkileri anlamlı derecede azalmıştı. Sisplatin grubu, kontrol grubu, kıvılcık grubu ve sisplatin + kıvılcık grupları ile karşılaştırıldığında MDA konsantrasyonu önemli derecede yüksek, GSH konsantrasyonu, GPx ve SOD enzim aktiviteleri anlamlı derecede düşük bulundu ($p < 0.05$).

Sonuç: Bu çalışma sisplatin ile uyarılan in vitro oksidatif hasar üzerine kıvılcık meyva ekstraktının koruyucu etkileri olduğunu göstermiştir.

Anahtar Kelimeler: Sisplatin; Kıvılcık; Oksidatif hasar; In vitro; Nefrotoksisite.

Introduction

Cisplatin {cis-diamminedichloroplatinum (II), $\text{cis-[PtCl}_2(\text{NH}_3)_2]$ } is an inorganic platinum compound that highly effective chemotherapeutic agent for the treatment of many cancer types such as ovary, testis, cervix, lung, head, and neck [1–3]. The most common reported side effects of cisplatin therapy is nephrotoxicity [4–10]. The major renal effect of cisplatin exposure is proximal tubular damage [11]. It has been indicated that cisplatin metabolised in proximal tubule cells cause nephrotoxicity [12]. Knowledge of pathogenic mechanisms of cisplatin in kidney is critical to prevent cisplatin-induced renal impairment.

Several hypotheses have been proposed to explain the mechanism of cisplatin-induced nephrotoxicity. These include tubular cell toxicity, reactive intermediates, inflammation and apoptosis-related mechanisms [11, 13–16]. Studies have suggested that exposure to cisplatin induce oxidative stress in the kidney [17–21]. Reactive oxygen species, specifically superoxide radical, has been stated in the mechanism that underlies cisplatin-induced renal injury [22]. Because oxidative stress plays a crucial role in cisplatin-induced hepatic toxicity, antioxidant supplements may be useful in the prevention and treatment of cisplatin-induced renal injury. Plants particularly horticulture section are raw material and used by people for food, either as edible products, or for culinary ingredients, for medicinal use or ornamental and aesthetic purposes. They are genetically very diverse group and play a major

role in modern society and economy. Fruits and vegetables are an important component of traditional food, but are also central to healthy diets of modern urban population [23–27]. It is well documented through in vivo and in vitro studies that natural products provides powerful protection against cisplatin-induced nephrotoxicity through reduced oxidative stress [28–34]. Studies have shown that the strong antioxidant properties of cornelian cherry are attributed to specific compounds, such as ascorbic acid, anthocyanins, and polyphenols [35–42]. *Cornus mas* L. has been reported to reduce oxidative stress and related damage [41, 43]. However, much less is known with regard to protective effect of cornelian cherry fruit extract against cisplatin-induced nephrotoxicity. The aim of this study was to evaluate the protective effect of cornelian cherry fruit extract on cisplatin-induced nephrotoxicity in vitro.

Materials and methods

Materials

Cornelian cherry fruits were collected from Samsun, Turkey in September 2014, and kept frozen at -20°C until analysis (about 5 months). Some properties of the materials can be seen in Table 1.

Extractions

For extraction, about 100 g of frozen cornelian cherry fruits were thawed in refrigerator. The fruits were crushed for 5 min in a Waring blender after removing the stones. An aliquot of 50 g pulp was mixed with 300 mL of deionized water. The mixture was sonicated (Bandelin Sonorex, Germany) for 30 min at 20°C and then filled up to the final volume of 500 mL with the water. The extract was centrifuged at 1550 g for 15 min at 4°C . The supernatant was used for analysis.

Determination of some physical and chemical properties of cornelian cherry fruits

Fruit weight was determined by using a digital balance with a sensitivity of 0.001 g, and flesh/stone ratio was calculated using $[(\text{fruit weight} - \text{stone weight}) / \text{stone weight}]$ formula. Length and width of the fruit were measured by using caliper with a sensitivity of 0.01 mm. The colour of the cornelian cherry fruits was recorded by a Minolta

Table 1: Some properties of the cornelian cherry fruits analysed.

Parameters	Values
Length (mm)	15.11
Width (mm)	11.20
Average fruit weight (g)	3.52
Flesh/stone ratio	5.29
L^a	25.04
$+a^a$	21.57
$+b^a$	6.59
Dry matter (%)	22.14
Soluble solids (%)	13.0
Titrateable acidity (%)	1.22
pH	3.67
Total sugar (%)	8.20
Reducing sugar (%)	7.56
Unreducing sugar (%)	0.61
Total phenolics (μg gallic acid equivalents) ^a	83.01
Oleanolic acid (mg/kg)	45.60
Ascorbic acid (mg/kg)	215.62
Total anthocyanins (μg cyanidin 3-glucoside equivalents) ^a	27.70
Individual anthocyanins (%) ^b	
Cyanidin-3-glucoside	45.20
Pelargonidin-3-glucoside	47.78
Cyanidin-3-rutinoside	3.50
FRAP ($\mu\text{mol Fe}^{+2}$) ^a	1031.08
EC ₅₀ (mg/mL) ^a	1.10

^aIn 100 mg/mL of the extract, ^bIndividual compounds presented as percentage of total peak area monitored at 515 nm.

CR-400 (Japan) Chromameter and reported on the Hunter $L^*a^*b^*$ method, where lightness L^* and two colour coordinates, a^* and b^* . The dry matter content of the fruits was conducted by heating in a vacuum oven at 70°C until a constant weight. The total soluble solid content of the fruits was assessed by refractometry, using an Abbe refractometer (Atago, Japan). Titrateable acidity was measured with 0.1 M NaOH up to pH 8.2 and expressed as percentage of malic acid. The pH value was measured using a pH meter (Eutech Cyberscan, Singapore) for a 10% (w/v) solution of the fruit prepared in deionized water. Sugar contents were determined according to the Lane-Eynon method [44].

Determination of antioxidant properties of the extracts

Total phenolics was quantified using Folin-Ciocalteu reagent [45]. The oleanolic acid was quantified using HPLC according to the procedure described by Zhang et al. [46]. Ascorbic acid content was performed by high performance liquid chromatography (HPLC) [47]. Total anthocyanin content was carried out using the pH differential

absorbance method [48]. The anthocyanin profile of the fruit was carried out by means of HPLC–UV as mentioned by Tural and Koca [42]. The antioxidant activity test was performed by two different methods. One was based on the evaluation of the free radical scavenging capacity (DPPH) of the extracts [42], and the other measured their ferric-reducing antioxidant power (FRAP) [49].

Cell culture

An established cell line, African green monkey kidney epithelial cells-Vero (ATCC, CCL-81) (American Type Culture Collection [ATCC], USA) was cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mM nonessential amino acids, and 1.0 mM sodium pyruvate, 90%; and fetal bovine serum (FBS), 10%. No antibiotics were added. Cells were grown to confluence in flasks (75 cm²) over 6–7 days. Cells were subcultured by treatment with 0.25% trypsin containing 0.1 mM EDTA in PBS. Cells were diluted in fresh medium and seeded into 96-well plates (1.5×10^4 cells/well). After incubation for 24 h, the medium was aspirated from all wells and replaced with 100 μL /well extraction mix or control medium and incubated for another 24 h before cytotoxicity was addressed (8 wells/dilution/incubation period).

Cell treatment with cisplatin and cornelian cherry fruit extract

The preliminary assays were performed by adding 50 $\mu\text{mol/L}$, 100 $\mu\text{mol/L}$, 200 $\mu\text{mol/L}$, 300 $\mu\text{mol/L}$, 400 $\mu\text{mol/L}$ and 500 $\mu\text{mol/L}$ for cisplatin and 1 mg/mL, 10 mg/mL, 100 mg/mL, 200 mg/mL for cornelian cherry fruit extract to cultures to obtain a significant effect in cell viability after 4 h without excess cell death at 24 h. The concentration of 50 μM of cisplatin was selected to induce cell injury since it allowed to obtain after 4 h a decrease of at least 42% of both cell lines viability. Although, the higher concentration (200 mg/mL) of cornelian cherry fruit extract was toxic for cell culture, lower concentration of cornelian cherry fruit extract did not show any toxic effect on cell viability. Due to this reason, the concentration of 100 mg/mL of cornelian cherry fruit extract was selected for assay. Vero cells were seeded in 24-well plates at a density of 1×10^5 cells/mL in Dulbecco's modified minimum essential medium containing 10% FBS. After 24 h, they were incubated with 50 $\mu\text{mol/L}$ of cisplatin or

50 µmol/L of cisplatin plus 100 mg/mL of cornelian cherry fruit extract for 4 h. The wells containing cells without any supplementation served as control.

Assessment of cell viability

The colorimetric assay (the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide-MTT) developed by Mosmann and modified by Edmondson et al. was used as a test for cell proliferation and survival [50, 51]. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide dye solution (MTT) (Sigma, St Louis, MO) was prepared as 0.5 mg/mL in PBS at 37°C just before use. A total of 20 µL MTT dye was added to each well and incubated at 37°C in air containing 5% CO₂ and at 95% relative humidity for 4 h in the dark. After incubation, the MTT was aspirated and the formazan product was solubilized in 50 µL dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St Louis, MO, USA). The plates were shaken before the optical densities were measured at 570 nm using a microplate reader (Infinite F50, Tecan Austria GmbH, Grödig, Austria). All assays were repeated at least twice to ensure reproducibility. The absorption value obtained with the control was deemed to indicate 100% viability. The percentage of viable cells was determined using the following formula:

$$\text{percentage of viable cells} = (A/B) * 100,$$

where A = viable cells in the experimental well and B = viable cells in the control well. More than 90 % cell viability was considered as noncytotoxic, 60% to 90% as slightly cytotoxic, 30% to 59% as moderately cytotoxic, and less than 30% cell viability was considered as strongly cytotoxic [50].

Preparation of cell medium for analyses of oxidative-stress biomarkers

The cell culture mediums were centrifuged at 1550 g for 5 min and then supernatants were collected separately for analyses of oxidative-stress biomarkers.

Determination of MDA concentration

Culture medium malondialdehyde concentration was determined according to Yoshioka et al. [52]. Briefly, 0.5 mL of medium samples were mixed with 2.5 mL of 20% trichloroacetic acid and 1 mL of 0.67% thiobarbituric acid then the mixture boiled at 95°C for 30 min. After cooling,

the mixture was extracted in 4 mL n-butanol and centrifuged at 1550 g for 10 min. Then, the n-butanol phase was separated and absorbance of the supernatant was measured at 535 nm by spectrophotometer (Thermo Scientific, Genesys 10S UV-VIS, Madison, WI, USA) against n-butanol. The results were evaluated from the standard curve obtained using 1,1,3,3 tetraethoxypropane of 0.1, 0.5, 1, 5 and 10 nmol/mL. The results were expressed as nmol/mL.

Determination of GSH concentration

The glutathione concentration of culture medium was determined by the method of Beutler et al. [53]. Briefly, a 500 µL aliquot of culture medium was mixed 3 mL of the precipitating solution (1.67 g metaphosphoric acid, 0.2 g EDTA and 30 g NaCl in 100 mL distilled water), kept in ice bath for 5 min and centrifuged at 1550 g for 10 min at 4°C. Then, 500 µL supernatant was mixed with 2 mL of 0.3 M disodium hydrogen phosphate solution and 250 µL of 0.001 M freshly prepared 5,5'-dithiobis (2-nitrobenzoic acid) dissolved in 1% w/v sodium citrate was added in assay tube. The standard solutions containing 0–5 nmol/mL of reduced glutathione was treated similar procedure for a standard curve, simultaneously. A blank tube was prepared with 2 mL buffer, 500 µL of diluted precipitating solution (three parts to two parts of distilled water) and 250 µL of DTNB reagent. The absorbance of test tubes were measured by spectrophotometer at 412 nm against a blank. GSH concentration was calculated from the GSH standard curve and results were expressed as nmol/mL.

Determination of GPx activity

GPx activity of culture medium was measured by the method suggested by Paglia and Valentine [54]. An aliquot of 20 µL culture medium was mixed with 800 µL of the reaction mixture, containing 0.1 M phosphate buffer, pH 7.0, 1 mM ethylene diamine tetra-acetic acid (EDTA), 10 mM GSH, 1 mM sodium azide, 1 unit of glutathione reductase, 1.5 mM nicotinamide adenine dinucleotide phosphate (NADPH). After incubation for 5 min at 37°C, 10 µL of the 30 mM tert-butyl hydroperoxide was added to the assay mixture for initiation of reaction. GPx activity was measured as the rate of NADPH oxidation was monitored in a spectrophotometer at 340 nm for approximately 5 min. The GPx activities were expressed as U/mL.

Determination of SOD activity

Superoxide dismutase activity was measured by the method based on Sun et al. [55]. Briefly, 100 μ L of culture medium was mixed with 2.45 mL of assay reagent which containing 0.3 mM xanthine, 0.6 mM disodium ethylene diamine tetraacetic acid, 0.15 mM nitroblue tetrazolium, 0.4 M sodium carbonate, 1 g/L bovine serum albumin. The reaction was initiated by the addition 20 μ L of xanthine oxidase to the mixture, incubated at 25°C for 20 min. The reaction was terminated by the addition of 1 mL of 0.8 mM copper (II) chloride to the mixture. The SOD activity was monitored at 560 nm by detecting the inhibition of the nitroblue tetrazolium reduction rate. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of NBT reduction to formazan. The SOD activities were expressed as U/mL.

Cytological examination of cell cultures

Vero cell culture samples were fixed for 10 s in absolute methanol. Following fixation cell culture samples stained with Giemsa for 10 s and then rinsed with tap water. Vero cell culture samples were examined under light microscope (Nikon Eclipse E600, Nikon Instruments Inc., Tokyo, Japan).

Cytological evaluation of cell cultures

Cell culture samples stained with Giemsa were scored semiquantitatively for cellular degeneration and damage. Cells were evaluated at x200 final magnification with an eyepiece showing grids of 100 squares from a total of 10 different adjacent fields measuring a total area of 0.15 mm². On average a total of 1100 cells from each sample were scanned for evaluating cellular degeneration and results are reflected as percentage score. Cells showing signs of cytoplasmic basophilia, cellular shrinkage, loosing cellular contours, nuclear condensation, nuclear fragmentation, cytoplasmic budding were considered as degenerated or showing signs of apoptosis.

Statistical analysis

The statistical significance of the differences between groups was analyzed by One-Way ANOVA analysis of variance and Duncan's multiple range test using SPSS Statistics V21.0 (IBM Corporation, Armonk, NY, USA).

Each value was the mean of two replications obtained from 4 independent experiments. Data are expressed as mean $\pm$ standard deviation (SD) and p value of <0.05 was considered statistically significant.

Results

Cytological findings

The cell viability was 59% in cells co-treated with cisplatin and cornelian cherry fruit extract simultaneously and 42% in cisplatin treated cells. In Vero cell culture without any application, and Vero cell culture treated with only 100 mg/mL of cornelian cherry fruit extract, a few cells showing signs of cytoplasmic basophilia, nuclear and cellular condensation were observed (Figures 1 and 2). The cellular damage ratio was elevated with treatment of 50 μ mol/L of cisplatin. Cells showing cytoplasmic basophilia, nuclear and cellular condensation were high in 50 μ mol/L cisplatin treated cells (Figure 3). However, when 50 μ mol/L of cisplatin combined with 100 mg/mL of cornelian fruit cherry extract the deleterious effects of cisplatin were significantly decreased (Figure 4).

Oxidative stress parameters

The results of oxidative stress parameters of the treatment groups were presented in Table 2. The MDA concentration was significantly higher ($p < 0.05$) in the cisplatin

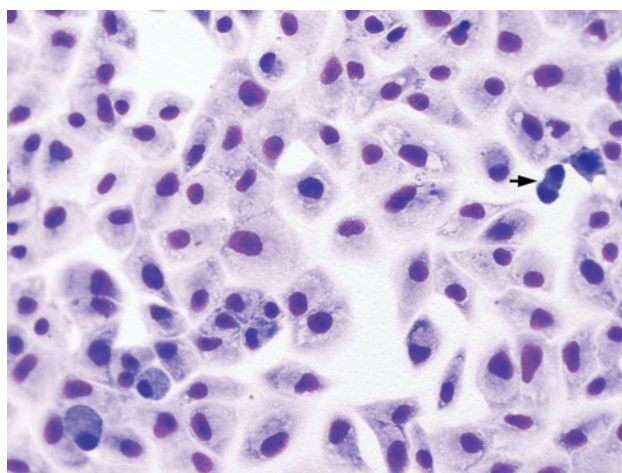


Figure 1: Vero cell culture without any application. A few cells are showing signs of cytoplasmic basophilia, nuclear and cellular condensation (arrow). Giemsa x20.

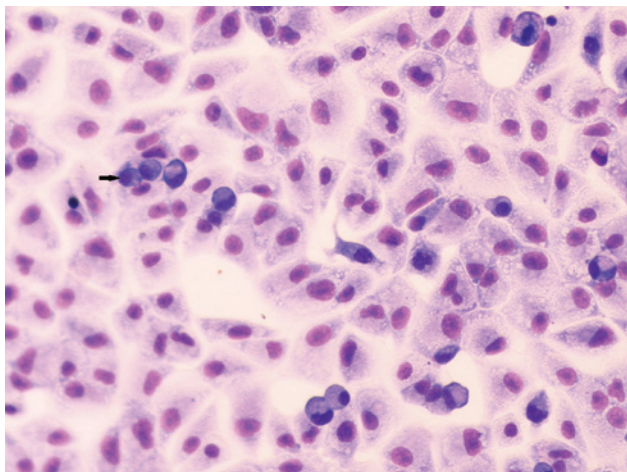


Figure 2: Vero cell culture treated with only 100 mg/mL of cornelian cherry fruit extract for 4 h. A few cells are showing signs of cytoplasmic basophilia, nuclear and cellular condensation (arrow). Giemsa x20.

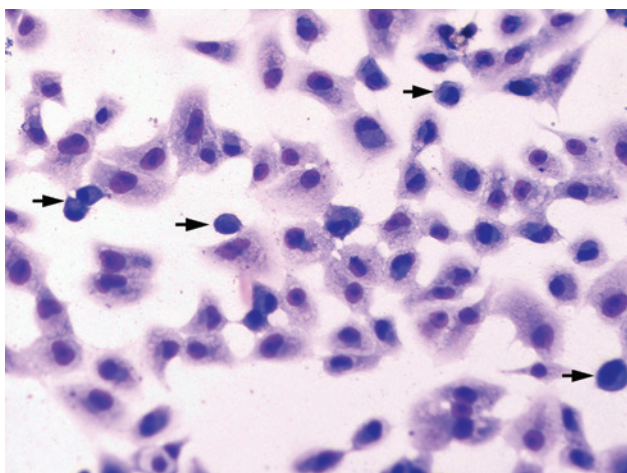


Figure 3: Vero cell culture treated with 50 µmol/L of cisplatin for 4 h. Increased number of cells are showing cytoplasmic basophilia, nuclear and cellular condensation (arrows). Giemsa x20.

treated group (4.49 ± 0.31 nmol/mL) when compared with control group (0.96 ± 0.07 nmol/mL), cornelian cherry group (0.90 ± 0.07 nmol/mL) and cisplatin+cornelian cherry group (1.09 ± 0.26 nmol/mL). The concentration of GSH was significantly lower ($p < 0.05$) in the cisplatin treated group (0.73 ± 0.08 nmol/mL) compared to control group (2.51 ± 0.37 nmol/mL), cornelian cherry group (2.64 ± 0.39 nmol/mL) and cisplatin+cornelian cherry group (2.33 ± 0.42 nmol/mL). The activities of GPx and SOD were significantly lower ($p < 0.05$) in the cisplatin treated group (0.31 ± 0.07 U/mL and 1.16 ± 0.13 U/mL, respectively) when compared with control group (0.96 ± 0.13 U/mL and 2.42 ± 0.30 U/mL, respectively), cornelian cherry

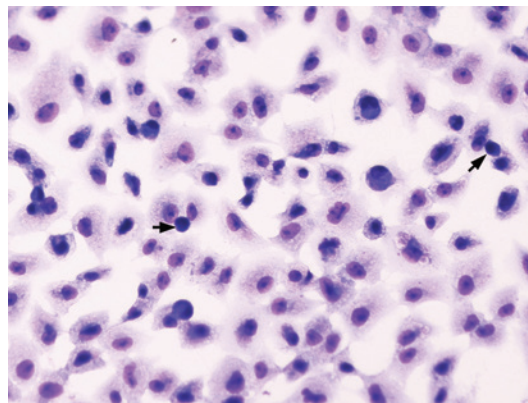


Figure 4: Vero cell culture co-treated with 50 µmol/L of cisplatin and 100 mg/mL of cornelian cherry fruit extract simultaneously for 4 h. Decreased number of cells are showing cytoplasmic basophilia, nuclear and cellular condensation (arrows). Giemsa x20.

group (1.09 ± 0.15 and 2.78 ± 0.34 U/mL, respectively), and cisplatin+cornelian cherry group (0.83 ± 0.15 U/mL and 2.18 ± 0.32 U/mL, respectively).

Discussion

It is well known that cisplatin causes nephrotoxicity [4–10]. In cisplatin therapy, pathological changes in kidney have been described as focal acute tubular necrosis, affecting primarily the distal convoluted tubules and collecting ducts, dilatation of convoluted tubules and formation of casts [7]. Previous studies have demonstrated that exposure to cisplatin caused renal injury through oxidative stress [17, 19, 21, 56].

The potential beneficial effects of natural antioxidants in the treatment and prevention of various disease have been well established. Growing body of evidence indicate that natural substances derived mainly from plants may play a crucial role to alleviate cisplatin-induced renal oxidative stress. Badary et al. (2005) reported the protective effect of naringenin, a natural dietary antioxidant, against cisplatin-induced alterations in renal cortex antioxidant defense system in rats [28]. Tangeretin reduced renal oxidative stress due to cisplatin by decreased lipid peroxides with concomitant augmentation of glutathione and glutathione peroxidase, so that attenuated cisplatin-induced renal injury in rats [57]. Quercetin, a common bioflavonoid in fruit and vegetables, has been shown to reduce cisplatin toxicity in cultured tubular epithelial cells by scavenging of free oxygen radicals [31]. It has been demonstrated that anthocyanin-rich *Seoritae* extract may ameliorate renal oxidative stress via activation of adenosine

Table 2: Oxidative stress parameters of the treatment groups (n=4).

Groups	MDA (nmol/mL)	GSH (nmol/mL)	GPx (U/mL)	SOD (U/mL)
Control	0.96 ± 0.07 ^a	2.51 ± 0.37 ^a	0.96 ± 0.13 ^{a,b}	2.42 ± 0.30 ^{a,b}
Cornelian cherry	0.90 ± 0.07 ^a	2.64 ± 0.39 ^a	1.09 ± 0.15 ^a	2.78 ± 0.34 ^a
Cisplatin	4.49 ± 0.31 ^b	0.73 ± 0.08 ^b	0.31 ± 0.07 ^c	1.16 ± 0.13 ^c
Cisplatin + cornelian cherry	1.09 ± 0.26 ^a	2.33 ± 0.42 ^a	0.83 ± 0.15 ^{a,b}	2.18 ± 0.32 ^b

^{a,b,c}Means with different superscripts within a column differ significantly (p < 0.05, Duncan's multiple range test).

monophosphate-activated protein kinase [58]. Sahu et al. (2013) demonstrated that hesperidin attenuates cisplatin-induced acute renal injury by decreasing oxidative stress which the major patho-mechanisms of cisplatin induced nephrotoxicity [59]. Natural antioxidant extract supplementation was also shown to prevent cisplatin-induced kidney failure through strengthening the renal antioxidant system and eliminating oxidation reactions [60]. These studies may suggest that natural antioxidants have a potential nephroprotective role.

Cornelian cherry has been indicated as valuable source of substances including ascorbic acid, anthocyanins and polyphenols with high antioxidant activity [35–42, 61]. It is increasingly thought that cornelian cherry may play preventive and therapeutic roles in oxidative stress-related diseases. In a recent study, freeze-dried cornelian cherry fruit addition to diet has been evaluated to verify the hypothesis of protective impact of cornelian cherry as an antioxidant. Researchers demonstrated elevated catalase activity upon cornelian cherry supplementation in the brain tissue of Wistar rats [62]. Cornelian cherry treatment ameliorated myocardial injury and enhanced the antioxidant defense against carbon tetrachloride-induced cardiotoxicity in rats and exhibited cardioprotective properties. Treatment of cornelian cherry in carbon tetrachloride-treated rats significantly decreased the increased levels of myocardial lipid peroxides and significantly increased the myocardial endogenous antioxidants (glutathione peroxidase, superoxide dismutase and catalase) levels [63]. The protective effect of cornelian cherry fruit extract in carbon tetrachloride-induced hepatotoxicity has been demonstrated by decreased liver MDA concentration in male rats [43]. Es Haghi et al. (2014) reported that treatment of rats with 300 mg/kg and 700 mg/kg doses of cornelian cherry fruit extract improved the level of antioxidant enzymes such as SOD, CAT and GPx, protected kidney from oxidative stress induced by carbon tetrachloride and also they suggested the protective effects of cornelian cherry fruit extract in attenuating carbon tetrachloride induced morphological abnormalities in the glomerular and tubular compartments [64]. Furthermore,

cornelian cherry has been shown to decrease MDA and increase GSH concentrations in the liver of hypercholesterolemic rabbits [41]. However, there is, as yet, no evidence about the effect of cornelian cherry fruit extract on cisplatin-induced nephrotoxicity. In the present in vitro study, we have demonstrated that cornelian cherry fruit extract supplementation significantly reduced cisplatin-induced oxidative stress in Vero cell culture. There was a significant increase of MDA concentration in cisplatin-exposed group as compared to the control group, cornelian cherry group and cisplatin + cornelian cherry-exposed group. The administration of cornelian cherry fruit extract to cisplatin-exposed Vero cell culture significantly increased the GSH concentration. In this study, decreased activities of GPx and SOD in culture medium exposed to cisplatin indicates that these enzymes were consumed due to increased oxidative stress. However, GPx and SOD activities significantly elevated following cornelian cherry administration.

Conclusions

In conclusion, the present study indicate that cornelian cherry fruit extract supplementation significantly alleviates the cisplatin-induced oxidative stress in Vero cell culture. Cornelian cherry could be an effective strategy to prevent cisplatin-induced renal damage. However, further experimental studies are prerequisite on this issue before clinical application can be recommended.

Conflict of interest statement: The authors have no conflict of interest.

References

- Cooper JS, Pajak TF, Forastiere AA, Jacobs J, Campbell BH, Saxman SB, et al. Postoperative concurrent radiotherapy and chemotherapy for high-risk squamous-cell carcinoma of the head and neck. *N Engl J Med* 2004;350:1937–44.

2. Rose PG, Bundy BN, Watkins EB, Thigpen JT, Deppe G, Maiman MA, et al. Concurrent cisplatin-based radiotherapy and chemotherapy for locally advanced cervical cancer. *N Engl J Med* 1999;340:1144–53.
3. Turrissi AT 3rd, Kim K, Blum R, Sause WT, Livingston RB, Komaki R, et al. Twice-daily compared with once-daily thoracic radiotherapy in limited small-cell lung cancer treated concurrently with cisplatin and etoposide. *N Engl J Med* 1999;340:265–71.
4. Arany I, Safirstein RL. Cisplatin nephrotoxicity. *Semin Nephrol* 2003;5:460–4.
5. Gao S, Chen T, Choi MY, Liang Y, Xue J, Wong YS. Cyanidin reverses cisplatin-induced apoptosis in HK-2 proximal tubular cells through inhibition of ROS-mediated DNA damage and modulation of the ERK and AKT pathways. *Cancer Lett* 2013;333:36–46.
6. Gomez CF, Gonzalez P, Carrillo A, Estelles MC, Rengel M. Cisplatin nephrotoxicity: symptomatic hypomagnesemia and renal failure. *Int J Pediatr Nephrol* 1986;7:151–2.
7. Gonzales-Vitale JC, Hayes DM, Cvitkovic E, Sternberg SS. The renal pathology in clinical trials of cis-platinum (II) diamminedichloride. *Cancer* 1977;39:1362–71.
8. Hamilton CR, Bliss JM, Horwich A. The late effects of cis-platinum on renal function. *Eur J Cancer Clin Oncol* 1989;25:185–9.
9. Laurent G, Yernaux V, Nonclercq D, Toubeau G, Maldague P, Tulkens PM, et al. Tissue injury and proliferative response induced in rat kidney by cis-diamminedichloroplatinum (II). *Virchows Arch B Cell Pathol Incl Mol Pathol* 1988;55:129–45.
10. MacLeod PM, Tyrell CJ, Keeling DH. The effect of cisplatin on renal function in patients with testicular tumours. *Clin Radiol* 1988;39:190–2.
11. Lieberthal W, Triaca V, Levine J. Mechanisms of death induced by cisplatin in proximal tubular epithelial cells: apoptosis vs. necrosis. *Am J Physiol* 1996;270(4 Pt 2):F700–8.
12. Townsend DM, Deng M, Zhang L, Lapus MG, Hanigan MH. Metabolism of cisplatin to a nephrotoxin in proximal tubule cells. *J Am Soc Nephrol* 2003;14:1–10.
13. Faubel S, Lewis EC, Reznikov L, Ljubanovic D, Hoke TS, Somerset H, et al. Cisplatin-induced acute renal failure is associated with an increase in the cytokines interleukin (IL)-1beta, IL-18, IL-6, and neutrophil infiltration in the kidney. *J Pharmacol Exp Ther* 2007;322:8–15.
14. Kaushal GP, Kaushal V, Hong X, Shah SV. Role and regulation of activation of caspases in cisplatin-induced injury to renal tubular epithelial cells. *Kidney Int* 2001;60:1726–36.
15. Ramesh G, Reeves WB. TNF-alpha mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. *J Clin Invest* 2002;110:835–42.
16. Tsuruya K, Ninomiya T, Tokumoto M, Hirakawa M, Masutani K, Taniguchi M, et al. Direct involvement of the receptor-mediated apoptotic pathways in cisplatin-induced renal tubular cell death. *Kidney Int* 2003;63:72–82.
17. Bompard G. Cisplatin-induced changes in cytochrome P-450, lipid peroxidation and drug-metabolizing enzyme activities in rat kidney cortex. *Toxicol Lett* 1989;48:193–9.
18. Hannemann J, Baumann K. Cisplatin-induced lipid peroxidation and decrease of gluconeogenesis in rat kidney cortex: different effects of antioxidants and radical scavengers. *Toxicology* 1988;51:119–32.
19. Matsushima H, Yonemura K, Ohishi K, Hishida A. The role of oxygen free radicals in cisplatin-induced acute renal failure in rats. *J Lab Clin Med* 1998;131:518–26.
20. Sugihara K, Nakano S, Gemba M. Effect of cisplatin on *in vitro* production of lipid peroxides in rat kidney cortex. *Jpn J Pharmacol* 1987;44:71–6.
21. Zhang JG, Lindup WE. Role of mitochondria in cisplatin-induced oxidative damage exhibited by rat renal cortical slices. *Biochem Pharmacol* 1993;45:2215–22.
22. Davis CA, Nick HS, Agarwal A. Manganese superoxide dismutase attenuates cisplatin-induced renal injury: importance of superoxide. *J Am Soc Nephrol* 2001;12:2683–90.
23. Ercisli S. Apricot culture in Turkey. *Sci. Res. Essays* 2009;4:715–19.
24. Erturk Y, Ercisli S, Haznedar A, Cakmakci R. Effects of plant growth promoting rhizobacteria (PGPR) on rooting and root growth of kiwifruit (*Actinidia deliciosa*) stem cuttings. *Biol. Res* 2010;43:91–8.
25. Hricova A, Fejer J, Libiakova G, Szabova M, Gazo J, Gajdosova A. Characterization of phenotypic and nutritional properties of valuable *Amaranthus cruentus* L. mutants. *Turk J Agric For* 2016;40:761–71.
26. Saridas MA, Kafkas NE, Zarifikhosroshahi M, Bozhaydar O, Kargi SP. Quality traits of green plums (*Prunus cerasifera* Ehrh.) at different maturity stages. *Turk J Agric For* 2016;40:655–63.
27. Yazici K, Sahin A. Characterization of Pomegranate (*Punica Granatum* L.) Hybrids and their potential use in further breeding. *Turk J Agric For* 2016;40:813–24.
28. Badary OA, Abdel-Maksoud S, Ahmed WA, Owieda GH. Naringenin attenuates cisplatin nephrotoxicity in rats. *Life Sci* 2005;76:2125–35.
29. Chirino YI, Hernández-Pando R, Pedraza-Chaverrí J. Peroxynitrite decomposition catalyst ameliorates renal damage and protein nitration in cisplatin-induced nephrotoxicity in rats. *BMC Pharmacol* 2004;4:20.
30. Kawai Y, Nakao T, Kunimura N, Kohda Y, Gemba M. Relationship of intracellular calcium and oxygen radicals to cisplatin-related renal cell injury. *J Pharmacol Sci* 2006;100:65–72.
31. Kuhlmann MK, Horsch E, Burkhardt G, Wagner M, Köhler H. Reduction of cisplatin toxicity in cultured renal tubular cells by the bioflavonoid quercetin. *Arch Toxicol* 1998;72:536–40.
32. Nasr AY, Saleh HA. Aged garlic extract protects against oxidative stress and renal changes in cisplatin-treated adult male rats. *Cancer Cell Int* 2014;14:92.
33. Pan H, Mukhopadhyay P, Rajesh M, Patel V, Mukhopadhyay B, Gao B, et al. Cannabidiol attenuates cisplatin-induced nephrotoxicity by decreasing oxidative/nitrosative stress, inflammation, and cell death. *J Pharmacol Exp Ther* 2009;328:708–14.
34. Trujillo J, Molina-Jijón E, Medina-Campos ON, Rodríguez-Muñoz R, Reyes JL, Loredó ML, et al. Curcumin prevents cisplatin-induced decrease in the tight and adherens junctions: relation to oxidative stress. *Food Funct* 2016;7:279–93.
35. Gąstoł M, Krośniak M, Derwisz M, Dobrowolska-Iwanek J. Cornelian cherry (*Cornus mas* L.) juice as a potential source of biological compounds. *J Med Food* 2013;16:728–32.
36. Pantelidis GE, Vasilakakis M, Manganaris GA, Diamantidis Gr. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and Cornelian cherries. *Food Chem* 2007;102:777–83.
37. Pawlowska A, Camangi F, Braca A. Qualitative-quantitative analysis of flavonoids of *Cornus mas* L. (Cornaceae) fruits. *Food Chem* 2010;119:1257–61.

38. Popović BM, Stajner D, Slavko K, Sandra B. Antioxidant capacity of cornelian cherry (*Cornus mas* L.) – comparison between permanganate reducing antioxidant capacity and other antioxidant methods. *Food Chem* 2012;134:734–41.
39. Seeram NP, Schutzki R, Chandra A, Nair MG. Characterization, quantification, and bioactivities of anthocyanins in *Cornus* species. *J Agric Food Chem* 2002;50:2519–23.
40. Sengul M, Eser Z, Ercisli S. Chemical properties and antioxidant capacity of cornelian cherry genotypes grown in Coruh valley of Turkey. *Acta Sci Pol Hortorum Cultus* 2014;13:73–82.
41. Sozański T, Kucharska AZ, Szumny A, Magdalan J, Bielska K, Merwid-Ląd A, et al. The protective effect of the *Cornus mas* fruits (cornelian cherry) on hypertriglyceridemia and atherosclerosis through PPAR α activation in hypercholesterolemic rabbits. *Phytomedicine* 2014;21:1774–84.
42. Tural S, Koca I. Physico-chemical and antioxidant properties of cornelian cherry fruits (*Cornus mas* L.) grown in Turkey. *Scientia Horticulturae* 2008;116:362–6.
43. Alavian SM, Banihabib N, Es HM, Panahi F. Protective effect of *Cornus mas* fruits extract on serum biomarkers in CCl₄-induced hepatotoxicity in male rats. *Hepat Mon* 2014;14:e10330.
44. AOAC. Official Methods of Analysis of the Association of Official Analysis Chemists, 17th ed. AOAC International, Gaithersburg, MD, 2000.
45. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* 1965;16:144–58.
46. Zhang Y, Xue K, Zhao EY, Li Y, Yao L, Yang X, Xie X. Determination of oleanolic acid and ursolic acid in Chinese medicinal plants using HPLC with PAH polymeric C18. *Phcog Mag* 2013;9, S19–24.
47. Lee HS, Coates GA. Vitamin C in frozen, fresh squeezed, unpasteurized, polyethylene-bottled orange juice: a storage study. *Food Chem* 1999;65:165–8.
48. Wrolstad RE. Color and pigment analyses in fruit products. Agricultural experiment station, Oregon State University, Station Bulletin 624, Corvallis, 1976; pp. 1–17.
49. Gao X, Björk L, Trajkovski V, Uggla M. Evaluation of antioxidant activities of rosehip ethanol extracts in different test systems. *J Sci Food Agric* 2000;80:2021–27.
50. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983;65:55–63.
51. Edmondson JM, Armstrong LS, Martinez AO. A rapid and simple MTT-based spectrometric assay for determining drug sensitivity in monolayer cultures. *J Tissue Cult Methods* 1988;11:15–17.
52. Yoshioka T, Kawada K, Shimada T, Mori M. Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygens toxicity in the blood. *Am J Obstet Gynecol* 1979;135:372–6.
53. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. *J Lab Clin Med* 1963;61:882–8.
54. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967;70:158–69.
55. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. *Clin Chem* 1988;34:497–500.
56. Sugiyama S, Hayakawa M, Kato T, Hanaki Y, Shimizu K, Ozawa T. Adverse effects of anti-tumor drug, cisplatin, on rat kidney mitochondria: disturbances in glutathione peroxidase activity. *Biochem Biophys Res Commun* 1989;159:1121–27.
57. Arab HH, Mohamed WR, Barakat BM, Arafa el-SA. Tangeretin attenuates cisplatin-induced renal injury in rats: Impact on the inflammatory cascade and oxidative perturbations. *Chem Biol Interact* 2016;258:205–13.
58. Koh ES, Lim JH, Kim MY, Chung S, Shin SJ, Choi BS, et al. Anthocyanin-rich *Seoritae* extract ameliorates renal lipotoxicity via activation of AMP-activated protein kinase in diabetic mice. *J Transl Med* 2015;13:203.
59. Sahu BD, Kuncha M, Sindhura GJ, Sistla R. Hesperidin attenuates cisplatin-induced acute renal injury by decreasing oxidative stress, inflammation and DNA damage. *Phytomedicine* 2013;20:453–60.
60. Bafna PA, Balaraman R. Antioxidant activity of DHC-1, an herbal formulation, in experimentally-induced cardiac and renal damage. *Phytother Res* 2005;19:216–21.
61. Rop O, Mlcek J, Kramarova D, Jurikova T. Selected cultivars of cornelian cherry (*Cornus mas* L.) as a new food source for human nutrition. *Afr J Infect Dis* 2010;9:1205–10.
62. Francik R, Kryczyk J, Krośniak M, Berköz M, Sanocka I, Francik S. The neuroprotective effect of cornus MAS on brain tissue of Wistar rats. *Sci World J* 2014;2014:847368.
63. Es Haghi M, Zare S, Banihabib N, Nejati V, Farokhi F, Mikaili P. Cardioprotective effect of *Cornus mas* fruit extract against carbon tetrachloride induced-cardiotoxicity in Albino rats. *J Basic Appl Sci Res* 2012;2:11106–14.
64. Es Haghi M, Dehghan G, Banihabib N, Zare S, Mikaili P, Panahi F. Protective effects of *Cornus mas* fruit extract on carbon tetrachloride induced nephrotoxicity in rats. *Indian J Nephrol* 2014;24:291–6.