Indian Journal of Experimental Biology
Vol. 62, February 2024, pp. 103-111
DOI: 10.56042/ijeb.v62i02.4286

Antioxidant efficiency of *Prunus laurocerasus* L. fruit extract on doxorubicin induced hepatic and renal damage

*Selma Cirrik[1], Gulay Hacioglu[2], Emel Kabartan Cokeli[3] & Emine Gulceri Gulec Peker[4]

[1]Department of Physiology, Faculty of Medicine, Ordu University, Ordu, Turkiye

[2]Department of Physiology, Faculty of Medicine, Giresun University, Giresun, Turkiye

[3]Laboratory and Veterinary Health Program, Ulubey Vocational School, Ordu University, Ordu, Turkiye

[4]Department of Basic Sciences, Faculty of Engineering, Giresun University, Giresun, Turkiye

Received 07 March 2023; *revised 23 July 2023*

In Turkish traditional medicine, the leaves, fruit and seeds of *Prunus laurocerasus* L., commonly called Cherry laurel, are used to treat various diseases such as cancer, diabetes, influenza, tonsillitis and scalp dandruff. The medicinal value of this plant can be attributed to its rich phenolic content and high antioxidant capacity. In this study, we investigated the efficacy of *P. laurocerasus* (PL) fruit extract in reducing the hepatorenal side effects of doxorubicin (DOX). Sprague-Dawley rats were divided into 4 groups as Control, DOX, PL500+DOX and PL1000+DOX (n=8). PL-extracts were given perorally for two weeks (500 or 1000 mg.kg^{-1}.day^{-1}). After 48–h of DOX injection (15 mg/kg, i.p.), the animals were sacrificed. Compared to control, in DOX group, we observed lower levels of serum albumin, higher alanine transaminase (ALT), aspartate transaminase (AST) and creatinine levels (P <0.001 for each one); decreased Glomerular filtration rate (GFR) (P <0.01); increased urinary neutrophil gelatinase-associated lipocalin (P <0.01); and kidney injury molecule-1 (P <0.001) levels. DOX-induced hepatorenal oxidative stress was approved by increased malondialdehyde (MDA) and decreased glutathione (GSH) levels and decreased superoxide dismutase (SOD) and catalase (CAT) (P <0.001 for each one) activities. Although PL-treatment did not change serum and urinary parameters, it significantly returned hepatic MDA and GSH levels, SOD and CAT activities (P <0.001 for each one) as well as renal MDA (P <0.001) and GSH (P <0.05) levels and CAT activity (P <0.001) to control levels. While high dose PL provided a more significant (P <0.05) reduction in renal lipid peroxidation, it did not significantly affect other parameters. With these observations, it can be suggested that rather than increasing the dose, a longer duration of PL treatment after DOX induction may be more effective in preventing tissue damage and oxidative stress.

Key words: Albumin, Cherry laurel, Creatinine, Glomerular filtration rate (GFR), Kidney injury molecule-1 (Kim-1), Liver, Neutrophil gelatinase-associated lipocalin (NGAL), Oxidative stress, Reactive oxygen species (ROS), Tissue damage

Doxorubicin (DOX) is a chemotherapeutic drug widely used for many years alone or in combination with other drugs for the treatment of both hematological liquid and solid tumors[1,2]. It slows down the rate of division in tumor cells and causes death by different mechanisms such as increasing production of reactive oxygen species (ROS), preventing replication of DNA and mitochondrial dysfunction[1-3]. However, the effects of DOX are not limited to cancer cells; it also has toxic effects on healthy cells. This situation induces cardiac, hepatic and renal side effects that limit the clinical use of the drug[4-9]. Different strategies, including combined treatment with different bioactive compounds, are suggested to reduce the toxic effects of DOX[10-15]. In most cases, these compounds alleviated oxidative stress and toxic effects by lowering ROS levels and/or increasing antioxidant defense. It has also been reported that anti-apoptotic and anti-inflammatory mechanisms are activated[6,7,11].

The nutritional supplement tested in this study to reduce the toxic effects of DOX is the fruit of *Prunus laurocerasus* L. (PL, synonym *Laurocerasus officinalis*), commonly called as Cherry laurel. PL is a perennial herb that grows in all Southeast Europe and Southwest Asia, especially in the Black Sea coast of Turkiye[16,17]. Local people consume the fruits of the plant fresh, as well as in brine, jam, molasses and marmalade. In Turkish traditional medicine, the leaves, fruit and seeds of PL are recommended for the treatment of different diseases including influenza, tonsillitis, cancer, diabetes and scalp dandruff[18]. The pharmaceutical value of this

*Correspondence:
E-Mail: selmacrrk@yahoo.com

plant seems to depend on its rich phenolic content and high antioxidant capacity[19,20].

The rich phenolic content and antioxidant capacity of the plant have led to research its efficacy in various experimental damage and disease models, including diabetes and gastric damage[21-23]. The results obtained from these studies revealed that inhibition of oxidative stress is an important factor in protective effect of PL. The antioxidant capacity of the plant suggests potential benefits in DOX-induced toxicity; however, to the best of our knowledge, the possible protective effect of PL on DOX toxicity has not been investigated before. Therefore, in the present study, we examined the effects of PL fruit extract on oxidative stress and tissue damage caused by DOX in hepatic and renal tissues.

Materials and Methods

Groups and drug applications

Spraque-Dawley male rats aged 2.5-3 months were used in the study and all applications were approved by the Institutional Animal Ethical Committee (IAEC Registration No: 2020/3/12). The subjects were divided into 4 groups as Control, DOX, PL500+DOX and PL1000+DOX (n=8). During the two-week experimental period, 500 mg/kg PL extract was given to the PL500+DOX group, and 1000 mg/kg PL extract to the PL1000+DOX group was given daily perorally. Drinking water was given to the control and DOX groups by gavage every day for two weeks. All groups except the control group were administered a single intraperitoneal (i.p.) injection of DOX (15 mg/kg) two days before sacrifice[24,25]. The control group was injected with saline the same way. One day before the end of the study period, the subjects were placed in the metabolic cages, and urine samples were collected after 24 h. All subjects were sacrificed under anesthesia (Ketamine: Xylazine; 80:10 mg/kg, i.p.) and then blood, hepatic and renal tissue samples were collected. All biological samples were stored in a deep freezer at −80°C until they were studied.

Preparation of PL extract

The collected fresh PL fruits were dried and ground. Then, 5 mL of 80% (v/v) ethanol solution was mixed with 1 gram of ground fruit. This mixture was stirred for two days at room temperature (22°C) in a shaking water bath. At the end of the 2nd day, the mixture was filtered through filter paper and the fruit extract was obtained by evaporating the solvent under reduced pressure in the evaporator at 50°C[21]. Concentrated extracts were diluted with drinking water and given to the PL500+DOX and PL1000+DOX groups by gavage for two weeks as defined above.

Determination of total phenolic compounds

The total phenolic content of the samples was determined using Folin-Ciocalteu reagent[26]. About 0.5 mL of extract sample (10 mg/mL) was mixed with the reagent and incubated in the dark for 120 min. The absorbance was measured at 650 nm. A calibration curve was used to calculate the total phenolic content and the results were expressed as a μg gallic acid equivalent per g extract.

Determination of total flavonoid content

The total flavonoid content of the samples was determined by modified aluminum nitrate method[27]. On the 0.5 mL of sample (10 mg/mL), 0.1 mL sodium acetate solution was added and the mixture was left for 1 min. Then, the solution of 0.1 mL of 10% (w/v) $Al(NO_3)_3$ was added and the final volume of the mixture was completed to 5 mL with 96% (v/v) ethanol. The mixture was incubated at the room temperature for 40 min and then the absorbance was measured at 450 nm. The total flavonoid content was calculated from the calibration curve and the result was expressed as equivalent of μg quercetin per g extract.

Reduction power determination

The reduction power detection was carried out according to the method previously described[27]. In brief, 50 μL sample (1 μg/mL) volume was completed to 1 mL with distilled water. About 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide (1%) were added and incubated at 50°C for 20 min. At the end of the incubation, 2.5 mL trichloro acetic acid (TCA, 10%) was added to each tube and centrifuged at 3000 rpm for 15 min. The supernatant (1.25 mL) was mixed with distilled water (1.25 mL) and 0.1% FeCl3.6H2O solution (0.25 mL), and incubated at the room temperature for 24 h in the dark. Then, the absorbance values were read at 700 nm. Butylated hydroxytoluene (BHT) was used as a standard and the reduction power was calculated according to the following formula:

$$\text{Reduction power (\%)} = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100$$

where A_0 for control absorbance value and A_1 for absorbance value of the sample or standard.

Measurement of 1,1-diphenyl-2-picryl-hydrazyl radicals (DPPH) radical scavenging activity

DPPH radical scavenging activity of the samples was determined using radicals of 1,1-diphenyl-2-

picrylhydrazyl (DPPH)[28]. In short, 0.75 mL of samples (20 mg/mL) were added to 1.5 mL DPPH solution (20 mg/L), and absorbances were read at 517 nm after waiting for 5, 10, 15 and 30 min in the dark. Trolox was used as a standard and the same procedures were applied. DPPH radical scavenging activity was calculated according to the following formula:

$$\text{DPPH Radical scavenging activity (\%)} = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100$$

where A_0 for control absorbance value and A_1 for absorbance value of the sample or standard

Determination of 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity

About 7.4 mM ABTS and 2.6 mM potassium persulfate were mixed and kept at the room temperature for 14 h. One mL of this mixture was taken and 60 mL of methanol was added to it. The absorbance of this solution was read against methanol in a spectrophotometer at 734 nm. 2850 µL of the prepared methanolic ABTS solution was taken, 150 µL of the prepared sample or standard solution (trolox) at a concentration of 10 mg/mL was added, and the absorbance values were read at 734 nm, after being kept in the dark for 2 h. Calculations were made according to the formula below[29]:

$$\text{ABTS Radical scavenging activity (\%)} = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100$$

where A_0 for control absorbance value and A_1 for absorbance value of the sample or standard

Serum and urinary markers

Measurement of serum albumin, ALT and AST activity

Albumin level in serum samples was detected colorimetrically by bromocresol green binding method[30]. In order to evaluate the liver functions, alanine transaminase (ALT) and aspartate transaminase (AST) activity in serum was measured by ELISA method using suitable commercial kits (Elabscience Biotechnology).

Creatinine and GFR estimation

Creatinine analysis in serum and 24 h urine samples was performed spectrophotometrically according to the Jaffe method[31]. The Glomerular filtration rate (GFR) was estimated using serum (SCr) and urinary (UCr) creatinine concentrations and urine flow rate (V) values with the following formula:

$$\text{GFR} = \frac{(\text{UCr} \times \text{V})}{\text{SCr32}}$$

Measurement of Kim-1 and NGAL levels in urine

As kidney damage markers, urinary *Kidney injury molecule-1* (Kim-1) and *Neutrophil gelatinase-associated lipocalin* (NGAL) levels were measured by ELISA using commercial kits (Elabscience Biotechnology).

Oxidative stress parameters

Determination of MDA and GSH in tissue

Malondialdehyde (MDA) level in tissues homogenized in TCA was studied spectrophotometrically by thiobarbituric acid (TBA) reagent formation method[33]. Tissue homogenates prepared for MDA determination were also used for tissue reduced glutathione (GSH) analysis. The modified Ellman method was applied for the determination of GSH, and the measurement was made spectrophotometrically at a wavelength of 412 nm[34].

Determination of SOD and CAT activity

For the determination of superoxide dismutase (SOD) activity in tissue, a spectrophotometric method based on the development of red colour produced by superoxide radical was used and the measurement was performed at a wavelength of 505 nm[35]. Catalase (CAT) takes part in catalyzing the dismutation of hydrogen peroxide (H_2O_2) to form water and molecular oxygen. CAT activity was measured using a spectrophotometric method that monitors the decrease in H_2O_2 concentration per unit time at 240 nm[36].

Statistical evaluation

Results are presented as mean ± standard deviation. Statistical evaluation was made with one-way analysis of variance and Tukey was used as a post-hoc test. $P < 0.05$ values were considered statistically significant.

Results

Total phenolic and flavonoid content

In the PL extract, total content of phenolic substance was found to be as 83.331±0.53 µg gallic acid/g sample; and the amount of flavonoids was 3.243±0.06 µg quercetin/g sample in the PL extract.

Reduction power

The reducing power of the samples at 50 µg/mL concentration was found to be 0.181±0.005% for the PL extract and 0.195±0.002% for the standard BHT.

DPPH and ABTS radical scavenging activity

The DPPH radical scavenging activity of the samples (at 20 mg/mL concentration) was 49.621±0.321% for PL extract and 88.504±0.098% for Trolox as standard. ABTS radical scavenging activity of the samples at 10 mg/mL concentration was found to be as 55.27±1.06% for PL extract and 94.3±0.34% for Trolox as standard.

Serum and urinary markers

The relevant damage markers and functional parameters for the liver and kidney were presented in Table 1. In DOX group, serum albumin level, which can be defined as an indicator of the synthetic activity of the liver, significantly decreased (P <0.001), while serum ALT (P <0.001) and AST (P <0.001) activities significantly increased. Although PL treatments at different doses alleviated DOX-induced changes in the serum ALT and AST activities and albumin levels, the outcome in PL-treated groups were not significantly different from DOX group (Table 1).

Renal functions were evaluated by measuring serum creatinine levels and calculating GFR. As seen in Table 1, DOX administration caused a significant increase in serum creatinine level (P <0.001) and a significant decrease in GFR (P <0.01). Although the effects of DOX were relieved by PL treatments, no significant improvement was observed in PL-treated groups comparing to DOX group. Kim-1 and NGAL levels, which are urinary markers of kidney damage, increased in the DOX group. PL extracts given at different doses attenuated the DOX-induced effects, but there was no significant change (Table 1).

Oxidative stress parameters in liver

Hepatic MDA and GSH level

In the control group, hepatic MDA level was observed as 101.84±7.74 nmol/g tissue and found to be increased to 657.22±36.23 nmol/g tissue in the DOX group (P <0.001). The MDA value was 483.92±44.25 in the PL500+DOX group (difference from control P <0.001; difference from DOX group P <0.001) and 460.87±49.95 nmol/g tissue in the PL1000+DOX group (difference from control P <0.001; difference from DOX group P <0.001). The effects of 500 and 1000 mg/kg PL treatments were not significantly different [Fig. 1A(i)].

The GSH level in the liver was determined as 6.08±0.28 µmol/g tissue in control subjects. This value was observed to decrease significantly in the DOX group (1.13±0.167 µmol/g tissue, P <0.001). GSH level was 2.33±0.35 µmol/g tissue in PL500+DOX group (difference from control P <0.001, and difference from DOX group P <0.001) and 2.44±0.38 µmol/g tissue in PL1000 + DOX group (difference from control P <0.001, and difference from DOX group P <0.001). In the PL500 + DOX and PL1000 + DOX groups, GSH were at similar levels [Fig. 1A(ii)].

Hepatic SOD and CAT activity

Hepatic SOD activity was 4.12 ± 0.32 U/mg tissue in the control group and decreased to 1.06±0.14 U/mg tissue level in the DOX group (P <0.001). SOD activity in the PL500+DOX group (1.51±0.30 U/mg tissue) was higher than in the DOX group (P <0.05), but still lower than the control (P <0.001). It was observed that SOD activity (1.71±0.28 U/mg tissue) in the PL1000 + DOX group changed as in the PL500 + DOX group (difference from control P <0.001, and difference from DOX group P <0.001). Therefore, the effects of the extracts at different doses on SOD activity were similar [Fig. 2A(i)].

CAT activity in the liver of control subjects was found to be 24.06±1.74 µmol.$H_2O_2^{-1}$.min^{-1}.mg $tissue^{-1}$. DOX administration caused a significant decrease in hepatic CAT activity (10.81±1.14 µmol.$H_2O_2^{-1}$.min^{-1}.mg $tissue^{-1}$, P <0.001). This value was 15.19±1.73 in the PL500+DOX group (difference from DOX group P <0.001) and 15.40±1.13 µmol.$H_2O_2^{-1}$.min^{-1}.mg $tissue^{-1}$ in the PL1000+DOX group (difference from DOX group P <0.001). Although PL treatments increased CAT activity, the values were still lower than the control. The effects of different doses of PL extracts were similar [Fig. 2A(ii)].

Oxidative stress parameters in the kidney

Renal MDA and GSH level

While the MDA level in the kidney was 94.67± 4.31 nmol/g tissue in the control group, it was determined to increase to 205.50±7.98 nmol/g tissue in the DOX group (P <0.001). The MDA level was 164.92±8.33 nmol/g tissue in the PL500+DOX group (difference from control P <0.001; difference from DOX group P <0.001) and

Table 1 — Serum and urinary parameters in all groups

	Control	DOX	PL500+DOX	PL1000+DOX
Alanine transaminase (ALT) (U/L)	37.5±10.8	88.07±14.89***	71.47±10.35***	68.85±12.43**
Aspartate transaminase (AST) (U/L)	83.02±11.98	139.12±15.64***	120.88±12.01***	116.73±16.71**
Albumin (g/dL)	3.17±0.11	2.78±0.10***	2.90±0.13**	2.92±0.15*
Serum creatinine (mg/dL)	0.84±0.04	1.21±0.09***	1.09±0.18**	1.03±0.16*
Glomerular filtration rate (GFR) (mL/min)	1.74±0.50	1.07±0.33**	1.29±0.27*	1.23±0.31*
Urinary Kim-1 (ng/mL)	1.10±0.16	1.40±0.13***	1.31±0.15*	1.29±0.11*
Urinary NGAL (ng/mL)	1.48±0.47	2.29±0.40**	2.09±0.35*	2.05±0.43*

[Difference from control *P <0.05, **P <0.01, ***P <0.001]

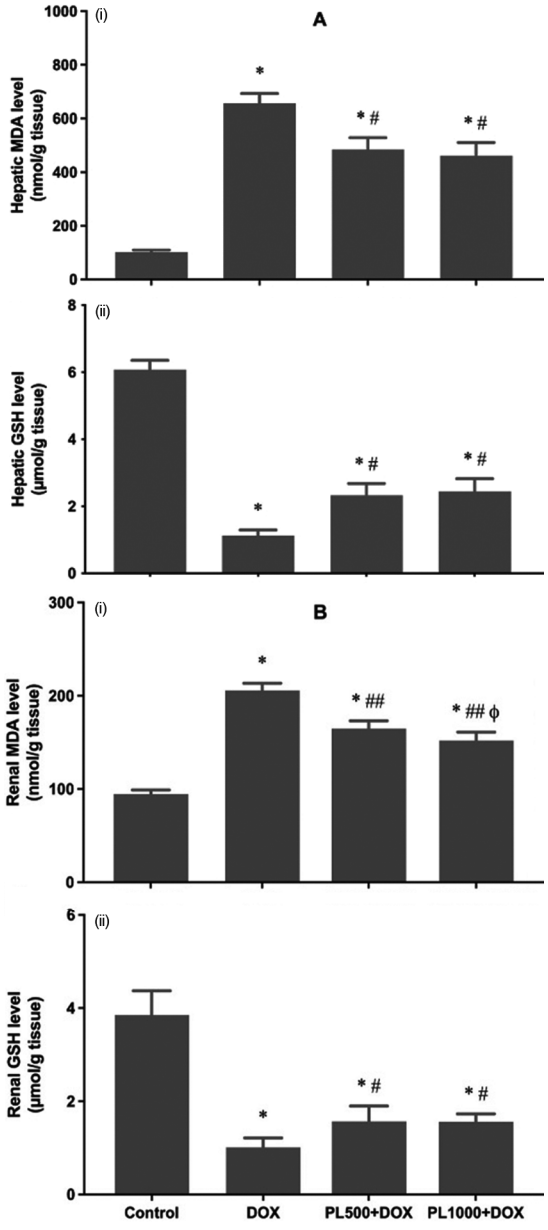

Fig. 1 — Malondialdehyde (MDA) and Glutathione (GSH) levels in (A) liver; and (B) kidney tissue. [Data are presented as mean ± standard deviation (n=8). Difference from control group **P* <0.001; difference from DOX group #*P* <0.001. difference from DOX group #*P* <0.05, ##*P*<0.001, difference from PL500+DOX group ϕ*P* <0.05]

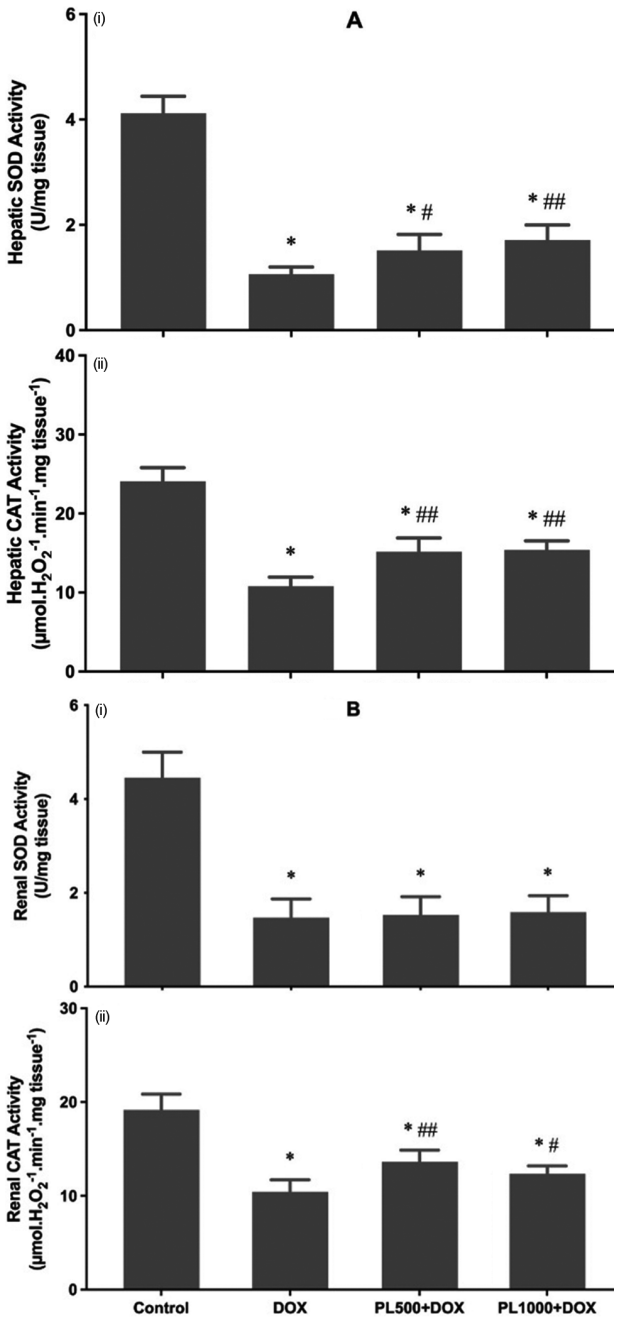

Fig. 2 — Superoxide dismutase (SOD) and Catalase (CAT) activities in (A) liver; and (B) kidney tissue. [Data are presented as mean ± standard deviation (n=8). Difference from control group **P* <0.001;. Difference from control group **P* <0.001]

151.97±9.09 nmol/g tissue in the PL1000+DOX group (difference from control *P* <0.001; difference from DOX group *P*<0.001). When the two extract groups were compared, it was observed that the MDA level in the PL1000+DOX group was lower than the PL500+DOX group [*P* <0.05, Fig. 1B(i)].

Renal GSH level was 3.85±0.52 µmol/g tissue in the control and decreased to 1.01±0.20 µmol/g tissue in the DOX group (*P* <0.001). The effects of different doses of PL extracts on renal GSH levels were similar, with 1.57±0.33 µmol/g tissue in the PL500+DOX (difference from control *P* <0.001, and

difference from DOX group P <0.05) and 1.56±0.17 µmol/g tissue in PL1000 + DOX (difference from control P <0.001, and difference from DOX group P <0.05) groups [Fig. 1B(ii)].

Renal SOD and CAT activity

The SOD activity in the kidney was determined as 4.46±0.54 U/mg tissue in the control group. Values in the DOX group (1.47±0.40 U/mg tissue, P <0.001), PL500 + DOX group (1.53±0.39 U/mg tissue, P <0.001) and PL1000 + DOX group (1.59±0.35 U/mg tissue, P <0.001) were lower than the control. There was no significant difference among the DOX, PL500 + DOX and PL1000 + DOX groups [Fig. 2B(i)].

Similarly, the CAT activity in kidney tissue was 19.17±1.69 $\mu mol.H_2O_2^{-1}.min^{-1}.mg\ tissue^{-1}$ in the control and decreased significantly in the DOX group (10.43±1.28 $\mu mol.H_2O_2^{-1}.min^{-1}.mg\ tissue^{-1}$, P <0.001). In the PL500 + DOX group, this value was 13.64±1.23 $\mu mol.H_2O_2^{-1}.min^{-1}.mg\ tissue^{-1}$ (difference from control P <0.001, and difference from DOX group P <0.001) and in the PL1000 + DOX group CAT activity was 12.35±0.84 $\mu mol.H_2O_2^{-1}.min^{-1}.mg\ tissue^{-1}$ (difference from control P <0.001, and difference from DOX group P <0.05). CAT activities were similar in the PL500+DOX and PL1000+DOX groups [Fig. 2B(ii)].

Discussion

DOX is an anticancer drug, which is widely used in the treatment of different types of cancer. However, the toxic side effects of DOX limit its clinical use. This study focused on a new therapeutic solution to minimize the toxic effects of DOX in the liver and kidney tissues.

The role of oxidative stress in DOX-induced cell damage has been demonstrated in many studies[1-3]. ROS and reactive nitrogen species such as superoxide radical, hydroxyl radical, hydrogen peroxide, nitric oxide and peroxynitrite, play a role in DOX-mediated oxidative damage[37]. These radicals induce lipid peroxidation and oxidative damage especially in cell membrane structures, and also harm other macromolecules of the cell, such as nucleic acids and proteins. It is known that DOX weakens the antioxidant defense of the cell by causing a decrease in the activity of antioxidant enzymes such as SOD, CAT and glutathione peroxidase (GPx) and a decrease in GSH[4,6,7].

In the present study, DOX was given at a single dose of 15 mg/kg and the animals were sacrificed 48 hours after the injection. The evaluation of damage and functional status of the tissues were made by using standard markers in serum and urine samples. In the DOX group, decreased serum albumin level showed weakened hepatic synthesis function, and increased serum ALT and AST activities showed liver damage. Increased serum creatinine level and decreased GFR were indicators of DOX-induced renal functional impairment. Besides, increased urinary NGAL and Kim-1 levels in the DOX group indicated kidney damage. The toxic effects of DOX in the liver and kidney are in accordance with the literature[7-10,38]. Additionally, elevated levels of MDA in the liver and kidney tissues indicate the oxidative stress and lipid peroxidation in DOX-injected rats. Together with MDA levels, enzymatic (SOD and CAT activity) and non-enzymatic (GSH level) antioxidant capacity of hepatorenal tissues were also evaluated in this study. Comparing to control rats, significant reductions in SOD and CAT activities and GSH level in hepatic and renal tissues were observed in DOX group. These results, in consistence with previous findings, exhibited that DOX induces reduction of antioxidant capacity of the cell[6-10].

The fruits of PL have been reported to be rich in phenolic and flavonoid content[20]. Phenolic acids and flavonoids are common compounds found in plants, which exert antioxidant properties. Consistent with previous studies, our results presented that the PL fruit extract used in this study contains phenolic and flavonoid substances. The reducing power of the extract was similar to the BHT standard, and the DPPH and ABST radical scavenging properties were 55-60% of the Trolox, used as a standard. These results confirmed the antioxidant properties of the PL fruit extract used in the present study. It has previously been reported that different extracts of the PL were protective in various models of damage and diseases[16,20-23]. In this study, the possible protective effect of pretreatment with PL fruit extract on the reduction of DOX-related damage in the liver and kidney was investigated.

According to the current results, DOX-induced changes such as decreased serum albumin levels and increased serum ALT and AST activities, alleviated slightly after two weeks of PL treatment, but these alterations were not statistically significant. The serum creatinine level and GFR values, which we examined as indicators of kidney function, and urinary NGAL and Kim-1 levels, which are markers

of renal damage[39-40], approached to the control values following PL treatment. High dose of PL treatment seemed slightly more effective in approximating serum ALT, AST and urinary NGAL, Kim-1 levels to the control values. This suggests that administration of higher doses of phenolic compounds in PL1000+DOX group might cause a better protection of hepatorenal tissues. However, PL treatment-induced changes in serum and urinary markers did not show any statistical significance at different doses.

When it comes to the changes in tissue level, current results showed that treatment with PL extract produced a significant decrease in DOX-induced hepatic oxidative stress. Lipid peroxidation was found to be lower in groups receiving PL treatment. Moreover, hepatic GSH level, SOD and CAT activities were higher in PL-treated groups, compared to the DOX group. Although better results were obtained with higher levels of PL treatment, the changes in oxidative stress parameters were not statistically different between the low and high extract groups. Uslu *et al*.[16] published a study comparable to the present study in terms of time and dose application using PL leaf extract. In that study, researchers presented the effects of 25 days-treatment with PL leaf extract at different doses (500, 1000 and 1500 mg/kg) in the streptozotocin (STZ)-induced diabetes model. The authors have reported that the increased lipid peroxidation and decreased CAT activity in the liver of diabetic rats were not affected by the extract treatment, but SOD activity elevated compared to the control values. It has also been demonstrated that the effects of the extract given at different doses on hepatic oxidative stress were found to be similar. The similar effectiveness of the extract at increasing doses is consistent with present findings. However, the low efficacy of PL extract in reducing STZ-induced hepatic oxidative stress suggested that the effects of PL may vary in different pathological conditions. The hypothesis that the effects of fruit and leaf extracts of PL might be different, was tested in a different study conducted by the same researchers[21]. In the mentioned study, diabetic rats were treated with an equal dose of PL fruit, seed or leaf extract (500 mg/kg) for 21 days and examined in terms of plasma MDA, GSH and GPx changes. It was reported that the effects of extracts obtained from different parts of the plant were similar[21].

Regarding the effects of PL extract on the kidneys, it was observed that DOX-induced renal lipid peroxidation was significantly reduced, and more importantly, treatment with higher dose of PL extract (1000 mg/kg) was more effective in reducing lipid peroxidation. This might depend on the presence of higher levels of antioxidant flavonoids in PL1000+DOX group. These results and previous study[16] showing that treatment with prunus leaf extract reduced lipid peroxidation in the kidney, but not in the liver of diabetic rats, suggested that kidneys might be more sensitive to the treatment with PL extracts. However, other oxidative stress parameters in kidney did not changed the same way. For example, DOX-induced decrements in renal GSH level and CAT activity were attenuated, but SOD activity was not affected by PL treatment, even the efficiencies of low and high dose of extracts were similar. These results are compatible with previous report[16] showing increased SOD, but unchanged CAT activity in the livers of diabetic rats, and suggest that different variables might differ in susceptibility to PL treatment. Nevertheless, when the oxidative stress parameters are evaluated as a whole, PL treatment can be considered successful in reducing DOX-induced renal oxidative stress.

As seen in the current results, PL treatments reduced the oxidative stress induced by DOX in hepatorenal tissues but, showed a weak efficacy in reducing tissue damage or restoring loss of function. Since the effects of PL on hepatic and renal markers have not been studied in any disease or damage models before, there is no chance to compare present results with the literature findings. Although the results showing oxidative stress and damage status of hepatorenal tissues may seem inconsistent at first glance, present results are actually consistent within themselves, since pretreatment with PL extract reduced, but not completely inhibited the DOX-induced lipid peroxidation in the liver and kidney tissues. Therefore, the inability of adequately benefit from PL treatment in tissue damage induced by DOX, may be attributed to the ongoing high level of oxidative stress in the tissues. It can be suggested that if the treatment had been continued for more than 48 h after the DOX injection, the benefits from the extract would have been greater, and we would have seen more significant reduction in both oxidative stress and tissue damage.

Conclusion

The present study demonstrated that the treatment with the cherry laurel, *Prunus laurocerasus* L. (PL)

fruit extract significantly reduced doxorubicin (DOX)-induced hepatic and renal oxidative stress. The enhancement of enzymatic and non-enzymatic antioxidant protection appears to be a significant contributor to this effect. However, the use of PL did not completely eliminate the oxidative stress induced by DOX. Presumably for this reason, there was only a slight amelioration observed in the functional impairment of the tissues. Nevertheless, PL fruit extract still retains its potential as a promising supplement that can be used to reduce the toxic side effects of DOX. It will be useful to test the efficacy of the extract in studies with different treatment durations.

Acknowledgement

The study was financially supported by Ordu University (A-2101).

Conflict of Interest

Authors declare no competing interests.

References

1 Van der Zanden SY, Qiao X & Neefjes J, New insights into the activities and toxicities of the old anticancer drug doxorubicin. *FEBS J*, 288 (2021) 6095.
2 Syahputra RA, Harahap U, Dalimunthe A, Nasution MP & Satria D, The Role of Flavonoids as a Cardioprotective Strategy against Doxorubicin-Induced Cardiotoxicity: A Review. *Molecules*, 27 (2022) 1320.
3 Yousefian M, Hosseinzadeh H, Hayes AW, Hadizadeh F & Karimi G, The protective effect of natural compounds on doxorubicin-induced cardiotoxicity via nicotinamide adenine dinucleotide phosphate oxidase inhibition. *J Pharm Pharmacol*, 74 (2022) 351.
4 Shi S, Chen Y, Luo Z, Nie G & Dai Y, Role of oxidative stress and inflammation-related signaling pathways in doxorubicin-induced cardiomyopathy. *Cell Commun Signal*, 21 (2023) 61.
5 Zhao X, Tian Z, Sun M & Dong D, Nrf2: a dark horse in doxorubicin-induced cardiotoxicity. *Cell Death Discov*, 9 (2023) 261.
6 AlAsmari AF, Ali N, Alharbi M, Alqahtani F, Alasmari F, Almoqbel D, AlSwayyed M, Alshammari A, Alanazi MM, Alhoshani A & Al-Harbi NO, Geraniol Ameliorates Doxorubicin-Mediated Kidney Injury through Alteration of Antioxidant Status, Inflammation, and Apoptosis: Potential Roles of NF-κB and Nrf2/Ho-1. *Nutrients*, 14 (2022) 1620.
7 Wu Q, Li W, Zhao J, Sun W, Yang Q, Chen C, Xia P, Zhu J, Zhou Y, Huang G, Yong C, Zheng M, Zhou E & Gao K, Apigenin ameliorates doxorubicin-induced renal injury via inhibition of oxidative stress and inflammation. *Biomed Pharmacother*, 137 (2021) 111308.
8 Espírito Santo SG, Monte MG, Polegato BF, Barbisan LF & Romualdo GR, Protective Effects of Omega-3 Supplementation against Doxorubicin-Induced Deleterious Effects on the Liver and Kidneys of Rats. *Molecules*, 28 (2023) 3004.
9 Sirwi A, Shaik RA, Alamoudi AJ, Eid BG, Kammoun AK, Ibrahim SRM, Mohamed GA, Abdallah HM & Abdel-Naim AB, Mokko Lactone Attenuates Doxorubicin-Induced Hepatotoxicity in Rats: Emphasis on Sirt-1/FOXO1/NF-κB Axis. *Nutrients*, 13 (2021) 4142.
10 Nimbal SK, Gadad PC & Koti BC, Effect of ethanolic extract of *Rosa centifolia* against doxorubicin induced nephro-toxicity in albino rats. *J Ayurveda Integr Med*, 12 (2021) 657.
11 Arunachalam S, Nagoor Meeran MF, Azimullah S, Jha NK, Saraswathiamma D, Subramanya S, Albawardi A & Ojha S, α-Bisabolol Attenuates Doxorubicin Induced Renal Toxicity by Modulating NF-κB/MAPK Signaling and Caspase-Dependent Apoptosis in Rats. *Int J Mol Sci*, 23 (2022) 10528.
12 Soltani Hekmat A, Chenari A, Alipanah H & Javanmardi K, Protective effect of alamandine on doxorubicin-induced nephrotoxicity in rats. *BMC Pharmacol Toxicol*, 22 (2021) 31.
13 Yıldızhan K, Huyut Z, Altındağ F & Ahlatcı A, Effect of selenium against doxorubicin-induced oxidative stress, inflammation, and apoptosis in the brain of rats: Role of TRPM2 channel. *Indian J Biochem Biophys*, 60 (2023) 177.
14 Veena RK, Ajith TA & Janardhanan KK, Bioactive extract of mycelia biomass of *Ganoderma lucidum* protects doxorubicin induced cardiomyopathy. *Indian J Exp Biol*, 60 (2022) 587.
15 Varghese R, Dalvi Y B, Vijay N & Gowda V, *Ganoderma applanatum* (Pers.) Pat. augments antitumor activity of doxorubicin and provides chemoprevention to murine tumor model. *Indian J Exp Biol*, 61 (2023) 606.
16 Uslu H, Uslu GA, Özen H & Karaman M, Effects of different doses of Prunus laurocerasus L. leaf extract on oxidative stress, hyperglycaemia and hyperlipidaemia induced by type I diabetes. *Indian J Tradit Knowl*, 17 (2018) 430.
17 Islam A, Hale ORTA, Kaçar YA & Dönmez D, Genetic Diversity of Cherry Laurel (*Laurocerasus officinalis* Roemer) BY SSR Markers. *J Agric Sci*, 29 (2023) 239.
18 Akbulut S, Karaköse M & Şen G, Medicinal plants used in folk medicine of akcaabat district (Turkey). *Fresenius Env Bull*, 31 (2022) 7160.
19 Abanoz YY & Okcu Z, Biochemical content of cherry laurel (*Prunus laurocerasus* L.) fruits with edible coatings based on caseinat, Semperfresh and lecithin. *Turk J Agric Forest*, 46 (2022) 908.
20 Karakaş N, Okur ME, Öztunç N, Çiçek Polat D & Karadağ AE, *Laurocerasus officinalis* Roem. fruit extract induces cell death through caspase mediated apoptosis in gastric cancer cell lines. *Turk J Biochem*, 46 (2021) 213.
21 Uslu H & Uslu GA, Evaluating the effects of Prunus laurocerasus seed, fruit and leaf extracts on hyperglycaemia, insulin sensitivity and anti-oxidative activities in experimental diabetes in rat. *Thai J Vet Med*, 51 (2021) 667.
22 Yegin SQ & Guder A, Laurocerasus officinalis roem: Biochemical parameters and antioxidant components in diabetic rat model. *Indian J Anim Res*, 56 (2022) 290.
23 Aydin Berktas O, Keskin A & Gulec Peker EG, Gastroprotective effects of *Prunus laurocerasus* L. fruit

extracts against the oxidative stress induced by indomethacin in rats. *Eur J Clin Exp Med*, 3 (2022) 284.

24 Ikewuchi CC, Ikewuchi JC & Ifeanacho MO, Aqueous leaf extracts of *Chromolaena odorata* and *Tridax procumbens* attenuated doxorubicin-induced pulmonary toxicity in Wistar rats. *BioTechnologia (Pozn)*, 102 (2021) 387.

25 Akin AT, Öztürk E, Kaymak E, Karabulut D & Yakan B, Therapeutic effects of thymoquinone in doxorubicin-induced hepatotoxicity via oxidative stress, inflammation and apoptosis. *Anat Histol Embryol*, 50 (2021) 908.

26 Abdelbaky AS, Mohamed AMHA & Alharthi SS, Antioxidant and Antimicrobial Evaluation and Chemical Investigation of *Rosa gallica* var. *aegyptiaca* Leaf Extracts. *Molecules*, 26 (2021) 6498.

27 Cirrik S, Kabartan Cokel İE, Gulec Peker EG, & Hacioglu G, The effects of propolis on doxorubicin-induced hepatorenal damage: a comparison of ethanolic and oily extracts of propolis. *CyTA J Food*, 21 (2023) 666.

28 Kumar DG, Achar RR, Kumar JR, Amala G, Gopalakrishnan VK, Pradeep S, Shati AA, Alfaifi MY, Elbehairi SEI, Silina E, Stupin V, Manturova N, Shivamallu C & Kollur SP, Assessment of antimicrobial and anthelmintic activity of silver nanoparticles bio-synthesized from Viscum orientale leaf extract. *BMC Complement Med Ther*, 23 (2023) 167.

29 Gotardo LRM, Carvalho FAL, Gomes Quirino DJ, Favaro-Trindade CS, Alencar SM, Oliveira AL & Trindade MA, Study of the oxidative and microbiological stability of nitrite-reduced, vacuum-packed, refrigerated lamb sausages supplemented with red propolis extract. *Foods*, 12 (2023) 4419.

30 Eskew MW, Koslen MM & Benight AS, Ligand binding to natural and modified human serum albumin. *Anal Biochem*, 612 (2021) 113843.

31 Osmic-Husni A, Hukic F & Saric MP, Comparison of Jaffe Method and Enzymatic Method at Measuring Serum Creatinine Level, Creatinine Clearance and Estimated Glomerular Filtration Rate. *Mater Sociomed*, 35 (2023) 108.

32 Chakraborty R & Sen S, Nephroprotective activity *of Pisonia aculeata* L. leaf extract against cisplatin induced nephrotoxicity and renal dysfunction in experimental rodents. *Indian J Exp Biol*, 58 (2020) 770.

33 Fontes GG, Freitas RB, Almeida PP, Lima LM, Cardoso SA, Pizziolo VR, Tabach R, Wanderley AG, Arida RM, Caricati-Neto A, Taha MO, Carvalho CA, Tallo FS & Menezes-Rodrigues FS, Effects of *Calendula officinalis* extract on liver histopathology, lipid profile, and oxidative stress in rats submitted to a diet rich in cholesterol and carbohydrates. *Acta Cir Bras*, 38 (2023) e383723.

34 Aribal P, Alver EN, Kaltalioglu K, Balabanli B, Ebegil M & Coskun-Cevher S, The relationship between experimental 2,4-Dinitrophenol administration and neurological oxidative stress: in terms of dose, time and gender differences. *Mol Cell Biochem*, 478 (2023) 1161.

35 Fatima M, Khan MR, Al-Keridis LA, Alshammari N, Patel M, Adnan M & Sahreen S, *Pleurospermum candollei* Methanolic Extract Ameliorates CCl4-Induced Liver I njury by Modulating Oxidative Stress, Inflammatory, and Apoptotic Markers in Rats. *ACS Omega*, 8 (2023) 25999.

36 Ehigiator BE, Adikwu E & Igweze ZN, Glutamine Supplementation Attenuates Bisphenol A-Induced Testicular Toxicity in Rats. *J Hum Reprod Sci*, 15 (2022) 337.

37 Kong CY, Guo Z, Song P, Zhang X, Yuan YP, Teng T, Yan L & Tang QZ, Underlying the Mechanisms of Doxorubicin-Induced Acute Cardiotoxicity: Oxidative Stress and Cell Death. *Int J Biol Sci*, 18 (2022) 760.

38 Liu X, Qiu Y, Liu Y, Huang N, Hua C, Wang Q, Wu Z, Lu J, Song P, Xu J, Li P & Yin Y, Citronellal ameliorates doxorubicin-induced hepatotoxicity via antioxidative stress, antiapoptosis, and proangiogenesis in rats. *J Biochem Mol Toxicol*, 35 (2021) e22639.

39 Marakala V, Neutrophil gelatinase-associated lipocalin (NGAL) in kidney injury - A systematic review. *Clin Chim Acta*, 536 (2022) 135.

40 Karmakova TA, Sergeeva NS, Kanukoev KY, Alekseev BY & Kaprin AD, Kidney Injury Molecule 1 (KIM-1): a Multifunctional Glycoprotein and Biological Marker (Review). *Sovrem Tekhnologii Med*, 13 (2021) 64.