Hittite Journal of Science and Engineering, 2022, 9 (4) 275–280
ISSN NUMBER: 2148–4171
DOI: 10.17350/HJSE19030000280

Prunus laurocerasus L. Extracts Prevent Paracetamol-induced Nephrotoxicity by Regulating Antioxidant Status: An Experimental Animal Model

Kaan Kaltalioglu
Giresun University, Espiye Vocational School, Giresun, Turkey

ABSTRACT

Paracetamol is a popular analgesic drug and its overdose may cause toxicity in the kidney. *Prunus laurocerasus* L. (PL) is an important folklore medicinal plant that has antioxidant properties. This study explores effects of PL fruit water and ethanol-water extracts administrations on oxidative stress in paracetamol-induced nephrotoxicity. For this purpose, 30 rats were divided into 5 groups: control, negative control (2 g/kg paracetamol), PL fruit water extract (400 mg/kg PLW+ 2 g/kg paracetamol), PL fruit ethanol-water extract (400 mg/kg PLEW+ 2 g/kg paracetamol) and positive control (150 mg/kg NAC+ 2 g/kg paracetamol). 24 hours after the paracetamol induction, animals were sacrificed and oxidative parameters were analyzed spectrophotometrically in kidney tissue. PLW and PLEW extracts decreased MDA and NOx levels and increased SOD and CAT activities in paracetamol-induced nephrotoxicity. PL fruit extracts can restore the oxidative changes caused by paracetamol.

Keywords:
Antioxidants; Nephrotoxicity; Oxidative stress; Paracetamol, *Prunus laurocerasus*.

Article History:
Received: 2022/08/12
Accepted: 2022/10/04
Online: 2022/12/31

***Correspondence to**: Kaan Kaltalioglu, Giresun University, Espiye Vocational School, 28600, Giresun, Turkey.*
E-Mail: kaan.kaltalioglu@gmail.com
Phone: +90 (454) 310 1430
Fax: +90 (454) 310 1431

INTRODUCTION

Paracetamol (acetaminophen, N-acetyl-p-aminophenol) is an antipyretic and analgesic agent, which is inexpensive, easily accessible, and safe at therapeutic doses. Therefore, it is widely used by humankind [1,2]. However, paracetamol overdose leads to toxicity in various organs, especially in liver and kidney. Paracetamol-induced renal toxicity is usually triggered by hepatotoxicity [3,4]. Excessive usage of paracetamol has been linked to renal failure in patients [5].

The liver is responsible for the majority of paracetamol metabolism, which involves conjugation with glucuronic acid and sulfate. In addition to them, N-Acetyl-p-benzoquinone (NAPQI) is produced by cytochrome P450 enzymes [1,6,7]. NAPQI is a highly reactive oxidant for thiols and pyridine nucleotides in cells [8]. NAPQI detoxification takes place after its reaction with cellular glutathione (GSH) at therapeutic doses, then produced non-toxic metabolites are eliminated in the urine through the kidney [6,9,10]. Consequently, paracetamol overdose causes depletion of hepatic GSH, and excessive NAPQI binds to hepatocyte proteins, resulting in mitochondrial dysfunction, inflammatory response, DNA damage, hepatocellular injury, oxidative stress and lipid peroxidation [8,11,12].
In order to attenuate paracetamol toxicity, N-Acetylcysteine (NAC), a strong antioxidant and precursor of reduced GSH (to replenish the GSH level), is clinically used for a long time [13]. Alternatively, natural products that have antioxidant properties can be used in the treatments of paracetamol toxicity [7].

Prunus laurocerasus L. (PL, Cherry laurel, locally known as taflan) belongs to the Rosaceaceae family. PL is found throughout southeast Europe and southwest Asia, particularly along Turkey's Black Sea coast [14-16]. It has been reported in many studies that phenolics such as chlorogenic acid, hydroxycinnamic acid, protocatechuic acid, syringic acid, p-coumaric acid, vanillic acid are contained in PL fruit and thus it demonstrates protective antioxidant effects [17-19]. Traditionally, PL fruit is utilized for many diseases including asthma, eczemas, stomach ulcer, diabetes mellitus, hemorrhoids and cough. In addition to antioxidant effects, different PL fruit extracts (water or ethanol) can have analgesic, diuretic, antiinflammatory and antifungal effects [18,20].

This study aims to investigate the impact of PL fruit extracts (both water and ethanol-water) on oxidative stress in paracetamol-induced nephrotoxicity.

MATERIAL AND METHODS

Chemicals and drugs

NAC was bought from Sigma-Aldrich (USA) and prepared in distilled water. Paracetamol was purchased from the Atabay Drug Company (Istanbul, Turkey) and a solution was dissolved in 1% carboxymethyl-cellulose (CMC) in phosphate-buffered saline. All additional chemicals were bought from Sigma-Aldrich (USA) and were of analytical quality.

Plant materials and extraction techniques

PL fruit was obtained from a local producer in Giresun province in Turkey and dried at 40 °C. The samples (20 g) were homogenized using a blender and were extracted in water or ethanol-water solvents with Soxhlet apparatus for 7 days. The obtained extracts were filtered using Whatman paper and a rotary evaporator was used to evaporate the solvents until they were completely dry.

Experimental design

The local animal experiment ethics committee at Giresun University granted consent for this investigation (2021/04). 30 male Wistar-Albino rats were utilized in the experiment (250-300 g). All rats were kept in conditions compliant with international standards. The rats were divided up into five groups as shown in Table 1 (n=6). Twenty-four hours following inducing paracetamol toxicity, all animals were sacrificed with high dose anesthetics (ketamine and xylazine). After sacrifice, the kidney tissue was taken quickly and frozen at -80 °C for further analysis.

Determination of MDA levels

The levels of MDA, an important oxidant product and an important marker in lipid peroxidation, in the collected kidney tissue samples were determined spectrophotometrically. In this method, thiobarbituric acid (TBA) reacts with MDA to produce a color compound. [21]. In order to homogenize the kidney tissues, ice-cold 0.15 M KCl was used. After centrifugation at 2000 g, the supernatant (1 mL) was taken and mixed with 0.67% TBA and 1% butylated hydroxytoluene.

Table 1. Group specifications

No	Groups	Specifications
Group 1	Control	Healthy, no administration (n=6)
Group 2	Negative control (Paracetamol)	Received a single dose paracetamol (2 g/kg) by oral gavage (n=6)
Group 3	PLW (PL fruit water extract + Paracetamol)	Received a single dose PL fruit water extract (400 mg/kg) by oral gavage. After 1h, the paracetamol (2 g/kg) was administrated by oral gavage (n=6)
Group 4	PLEW (PL fruit ethanol-water extract + Paracetamol)	Received a single dose PL fruit ethanol-water extract (400 mg/kg) by oral gavage. After 1h, the paracetamol (2 g/kg) was administrated by oral gavage (n=6)
Group 5	Positive control (NAC + Paracetamol)	Received a single dose NAC (150 mg/kg) by oral gavage. After 1h, the paracetamol (2 g/kg) was administrated by oral gavage (n=6)

Following that, the mixture was subjected to a 10-minute heating session at 100 °C. Absorbance was recorded at 535 nm.

Determination of GSH levels

The levels of GSH were evaluated as defined by Aykaç et al. [22]. 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) interacts with reduced GSH to create yellow color in this method. In brief, kidney samples were homogenized as MDA method. The homogenate was mixed with deproteinization solution (sodium chloride/ethylenediaminetetraacetic acid/metaphosphoric acid). After centrifugation at 4000 rpm, the supernatant (0.5 mL) was added into 0.3 M $Na_2HPO_4.2H_2O$ solutions (2 mL). Following the addition of 0.2 mL of Ellman reactive (DTNB and sodium citrate), the absorbance at 412 nm was determined.

Determination of SOD activities

Method proposed by Sun et al. [23] was used to evaluate the SOD enzyme activity that relies on the xanthine-xanthine oxidase system, a superoxide producer, to reduce nitro blue tetrazolium (NBT). First, kidney tissues were homogenized in ice-cold 0.9% NaCl. After centrifugation at 7000 rpm, the supernatant (1 mL) was mixed with ethanol–chloroform (3:5, v/v). Then, assay reagent (xanthine, EDTA, NBT, Na_2CO_3) was added to mixture. 50 µL xanthine oxidase was used to initiate the reaction and reduce the NBT. At 560 nm, absorbance was observed. One unit of SOD is equal to the quantity of enzyme that suppresses NBT by fifty percent.

Determination of CAT activities

Aebi (1984) method [24] based on hydrogen peroxide (H_2O_2 dismutation) was used to measure CAT activity. In brief, H_2O_2 was utilized as the substrate, and the reduction in H_2O_2 amount in phosphate buffer at 20 °C was measured using spectrophotometrically at 240 nm. CAT activity was described as the quantity of enzyme necessary to reduce 1 µmol of H_2O_2 per minute.

Determination of NOx levels

The NOx levels were assessed using method described by Miranda et al. [25] that relies on the Griess reaction. NOx act as the stable end product of nitric oxide. First, kidney samples were homogenized in phosphate buffer (pH 7). After centrifugation at 3500 rpm, the supernatants (0.5 mL) were added to 0.3 M NaOH (0.25 mL). Then, vanadium trichloride was added to mixture. Following incubation, Griess reagents were added. Sodium nitrite was used as standard. Absorbances were recorded at 540 nm.

Statistical analysis

The Prism statistical package was used for all analyses (v.7, GraphPad Software, USA). The data was shown using a mean and standard deviation format. To determine statistical significance at the 0.05 level, one-way ANOVA (with Tukey's multiple comparison test) were used.

RESULTS

Paracetamol administration decreased antioxidant capacity and increased oxidative stress in kidney compared to control group

In our study, it was determined that the MDA and NOx levels in the negative control group, which was administered only paracetamol, increased approximately 2 times when compared to the control group ($p<0.05$) (Table 2). The negative control group had a significant reduction in SOD and CAT activity compared to the control group ($p<0.05$). Similarly, it was discovered that the GSH level reduced in the paracetamol group compared to the control group ($p<0.05$) (Table 2).

Effects of PLW, PLEW and NAC on MDA levels in paracetamol-induced nephrotoxicity

As shown in Fig 1, the PLW, PLEW and NAC administrations statistically decreased the MDA levels in kidney when compared to the negative control group ($p<0.05$). While PLEW administration was more effective than PLW administration ($p<0.05$), it showed a similar effect to NAC administration ($p>0.05$) (Table 2 and Fig 1).

Effects of PLW, PLEW and NAC on GSH levels in paracetamol-induced nephrotoxicity

The PLW, PLEW and NAC administrations caused statistically significant elevations in kidney GSH levels when compared to the negative control group ($p<0.05$). Moreover, in the PLEW and NAC groups, the GSH level was found to be significantly higher as compared to the PLW group ($p<0.05$) (Table 2 and Fig 2).

Effects of PLW, PLEW and NAC on SOD activities in paracetamol-induced nephrotoxicity

The decreased SOD activities in negative control group significantly elevated after the PLW, PLEW and NAC administrations ($p<0.05$). No statistically significant difference was founded between the PLEW and PLW groups ($p>0.05$). Considering the SOD activities, the effect of PLEW administration was found to be similar to NAC administration ($p>0.05$) (Table 2 and Fig 3).

Table 2. The effects of PLW, PLEW and NAC administrations on the MDA, GSH, SOD, CAT and NOx parameters in paracetamol-induced rats

Groups	MDA (µmol/g tissue)	GSH (µmol/g tissue)	SOD (unit/mg tissue)	CAT (µmol/H_2O_2/min /mg protein)	NOx (nmol/g tissue)
Control	41.99 ± 4.39a	5.90 ± 0.23a	4.06 ± 0.27a	25.48 ± 1.58a	17.41 ± 1.05a
Negative control (Paracetamol)	95.73 ± 6.33d	3.89 ± 0.27b	1.88 ± 0.14b	15.42 ± 1.41b	41.46 ± 2.27c
PLW (PL fruit water extract + Paracetamol)	75.08 ± 6.76c	4.83 ± 0.36c	3.09 ± 0.11c	23.07 ± 2.07a	21.93 ± 1.64b
PLEW (PL fruit ethanol-water extract + Paracetamol)	60.35 ± 6.24b	5.56 ± 0.33a	3.24 ± 0.22 c,d	20.10 ± 1.74c	21.85 ± 1.99b
Positive control (NAC + Paracetamol)	61.44 ± 2.22b	5.49 ± 0.28a	3.47 ± 0.27d	20.09 ± 1.18c	22.35 ± 0.46b

* Same letters in each column were not significantly different ($p>0.05$). Each value is the mean ± SD (n=6 for each group)

Effects of PLW, PLEW and NAC on CAT activities in paracetamol-induced nephrotoxicity

Our results demonstrated that the PLW, PLEW and NAC administrations increased CAT activities of kidney tissue as compared to negative control ($p<0.05$). While PLEW administration was more effective than PLW administration ($p<0.05$), it showed a similar effect to NAC administration ($p>0.05$) (Table 2 and Fig 4).

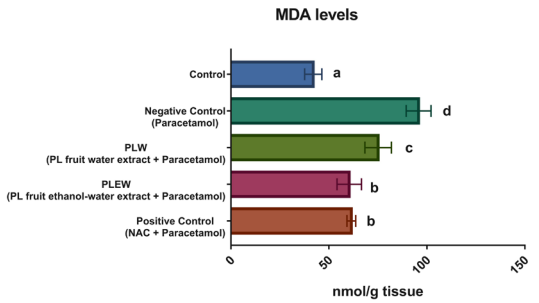

Figure 1. Comparison of PLW, PLEW, NAC administrations and negative control groups in terms of MDA levels

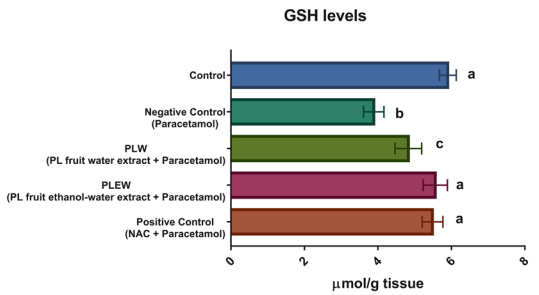

Figure 2. Comparison of PLW, PLEW, NAC administrations and negative control groups in terms of GSH levels

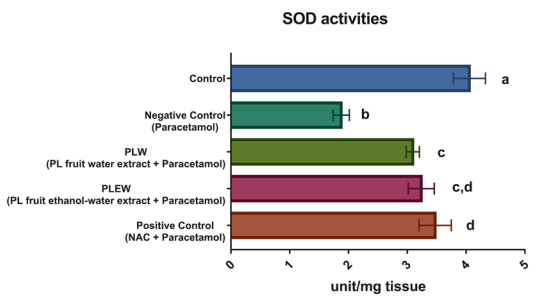

Figure 3. Comparison of PLW, PLEW, NAC administrations and negative control groups in terms of SOD activities

Effects of PLW, PLEW and NAC on NOx levels in paracetamol-induced nephrotoxicity

As shown in Fig 5, a significant decrease was detected in the NOx level of PLW, PLEW and NAC administration groups as compared to the negative control group ($p<0.05$). When both the PLW, PLEW and NAC administration groups were compared, no statistically difference was found in terms of kidney tissue NOx levels ($p>0.05$) (Table 2 and Fig 5).

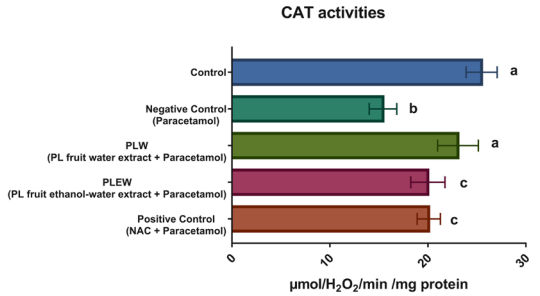

Figure 4. Comparison of PLW, PLEW, NAC administrations and negative control groups in terms of CAT activities

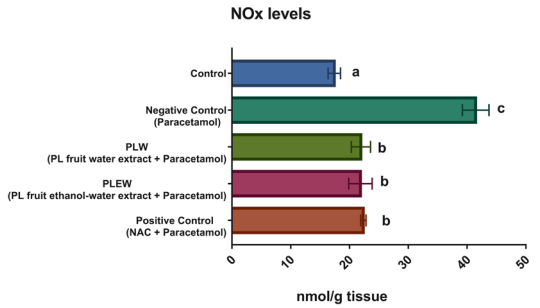

Figure 5. Comparison of PLW, PLEW, NAC administrations and negative control groups in terms of NOx levels

DISCUSSION

Although paracetamol is a strong analgesic drug, it shows harmful side effects when used excessively. Oxidative stress is one of these side effects [3,4]. It damages DNA, lipids and proteins in cells and thus it deteriorates tissues. As a result, it causes toxicity in liver and kidney [8,9]. This study aimed to attenuate nephrotoxicity through P. laurocerasus L. fruit extract, which has various bioactivities.

Oxidative stress is one of the important indicators of tissue damage and toxicity. In our investigation, paracetamol administration exacerbated oxidative stress (MDA) while decreasing enzymatic (SOD and CAT) and non-enzymatic (GSH) antioxidant capacity. The findings are directly in line with previous findings. They have also found that over doses of paracetamol increase oxidative stress and lipid peroxidation, which is linked with paracetamol toxicity [2,26,27]. MDA, a final product of lipid peroxidation, has been commonly used for long times as a useful marker for oxidative stress [28]. An increased MDA level suggests a lack of antioxidant capability against oxidants, which causes tissue damage [29]. Our results showed that the PLW, PLEW and NAC administrations alleviated oxidative stress by reducing the MDA level compared to the negative control group (Table 2 and Fig 1). This data supports result from previous study by Eken et al. (2017) [29]. Considering results were quite compatible with literature, it can be said that PL

fruit extracts protect the kidney against paracetamol-induced oxidative toxicity. Moreover, PL fruit ethanol-water extract (PLEW) showed close efficacy to the reference drug, NAC. This positive effect may be due to the fact that PL fruit is a rich source of phenolics and therefore shows antioxidant activity.

GSH, comprised of cysteine, glutamic acid, and glycine, is a powerful member of the antioxidant defense mechanism. This popular antioxidant protects cells from oxidants (such as superoxide radicals, hydrogen peroxide) by acting as a radical scavenger [30]. Likewise, GSH also has a scavenging effect on NAPQI [31]. In our study, the PLW, PLEW and NAC administrations restored GSH levels in paracetamol-induced nephrotoxicity (Table 2 and Fig 2). The PLEW demonstrated an impact similar to NAC, which was consistent with the obtained MDA results. Uslu et al. (2018) reported that PL ethanol-water extract increases GSH level at different doses in diabetes-induced kidney tissue [14]. In light of these results, we can say that PL fruit extracts protect the kidney against oxidative toxicity through nephrotic GSH depletion.

Enzymatic antioxidants are essential in the detoxification of free radicals and reactive oxygen species (ROS) [32]. SOD and CAT are enzymatic antioxidants that protect cells from oxidative stress. Our results demonstrated that the PLW, PLEW and NAC administrations elevated SOD and CAT activities as compared to negative control groups (Table 2 and Fig 3,4). Additionally, the effects of water and ethanol-water extracts of PL fruit are similar. Confirming these results, Eken et al. (2017) indicated that cherry laurel fruit methanolic extract administration increased the SOD and CAT activities, and the parameters affected by oxidative stress returned to almost normal levels [29]. SOD converts the harmful superoxide anion into oxygen and hydrogen peroxide, whereas CAT converts hydrogen peroxide into oxygen and water [32]. Our results suggest that PL fruit extracts increase the antioxidant capacity of kidney tissue by increasing SOD and CAT activity in our study.

NO, ROS and their interactions play significant roles in renal function regulation. Renal disorders are linked to oxidative stress and nitric oxide reduction [33]. The reaction between nitric oxide and the superoxide radical produces peroxynitrite. Peroxynitrite is a dangerous oxidant that causes extensive tissue damage, including lipid peroxidation and enzyme inactivation [34]. Our results showed that PLW, PLEW and NAC administrations had a similar effect on NOx levels and reduced them compared to the negative control group (Table 2 and Fig 5). This finding was also reported by Aydin Berktas and Gulec Peker (2022) [35]. Chlorogenic acid is one of the main phenolic compounds of the PL fruit [17-19]. Moreover, Ohno et al. (2012) determined that chlorogenic acid reduces inducible nitric oxide synthase expression and NO generation in rat hepatocytes [36]. As a consequence, the chlorogenic acid in PL fruit can be attributed to its NO-reducing impact in renal tissue.

CONCLUSION

In conclusion, this research indicates that the pretreatment of PL fruit extracts has protective properties against paracetamol-induced nephrotoxicity in rats. When the extracts are compared in terms of alleviating oxidative toxicity, it can be said that ethanol-water extract is more effective in terms of MDA and GSH parameters, and water extract is more effective in terms of CAT parameter. However, future investigations are required to better explain therapeutic properties of PL fruit at different doses.

ACKNOWLEDGEMENTS

The author thanks to Dr. O. Aydin Berktas and Dr. E. G. Gulec Peker for their kind support in the study.

CONFLICT OF INTEREST

The author state no conflict of interest.

References

1. Karabağ Çoban F, Kuru B. Effect of boron on trace element level and oxidative stress in paracetamol induced hepatotoxicity model. Journal of Boron 4 (2019) 92–99.
2. Ferah I, Halici Z, Bayir Y, Demirci E, Unal B, Cadirci E. The role of infliximab on paracetamol-induced hepatotoxicity in rats. Immunopharmacology and Immunotoxicology 35 (2013) 373–381.
3. Larson AM, Polson J, Fontana RJ, Davern TJ, Lalani E, Hynan LS, Reisch JS, Schiødt FV, Ostapowicz G, Shakil AO, Lee WM. Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. Hepatology 42 (2005) 1364–1372.
4. Ozkaya O, Genc G, Bek K, Sullu Y. A case of acetaminophen (paracetamol) causing renal failure without liver damage in a child and review of literature. Renal Failure 32 (2010) 1125–1127.
5. Boutis K, Shannon M. Nephrotoxicity after acute severe acetaminophen poisoning in adolescents. Clinical Toxicology 39 (2001) 441–445.
6. Hung OL, Nelson LS. Acetaminophen, in: Tintinalli JE, Kelen GD, Stapczynski JS (Eds.) Tintinalli's emergency medicine: a comprehensive study guide. 6th ed. McGraw-Hill, New York, pp. 1088–1094, 2004.
7. Canayakin D, Bayir Y, Kilic Baygutalp N, Sezen Karaoglan E, Atmaca HT, Kocak Ozgeris FB, Keles MS, Halici Z. Paracetamol-induced nephrotoxicity and oxidative stress in rats: the protective role of Nigella sativa. Pharmaceutical Biology 54 (2016) 2082–2091.
8. Macherey AC, Dansette, PM. Biotransformations Leading to Toxic Metabolites: Chemical Aspects, in: Wermuth CG, Aldous D, Raboisson P, Rognan D (Eds.) The Practice of Medicinal Chemistry. 4th ed. Academic Press, pp. 585–614, 2015.
9. Tejo J. Curcumin, antioxidant activity, and paracetamol toxicity,

in: Vinood BP, Victor RP (Eds.) Toxicology. Academic Press, pp. 467–477, 2021.
10. Li J, Chiew AL, Isbister GK, Duffull, SB. Sulfate conjugation may be the key to hepatotoxicity in paracetamol overdose. British journal of clinical pharmacology 87 (2021) 2392–2396.
11. Bessems JG, Vermeulen NP. Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. Critical Reviews in Toxicology 31 (2001) 55–138.
12. Jaeschke H, Bajt ML. Intracellular signaling mechanisms of acetaminophen-induced liver cell death. Toxicological Sciences 89 (2006) 31–41.
13. Aldini G, Altomare A, Baron G, Vistoli G, Carini M, Borsani L, Sergio FN. Acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why. Free Radical Research 52 (2018) 751-762.
14. 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 Journal of Traditional Knowledge 17 (2018) 430–436.
15. Vavilov NI. The origin, variation, immunity and breeding of cultivated plants. Chronica Botanica, Ronald Press, New York, pp. 211, 1951.
16. Elmastas M, Genc N, Demirtas I, Aksit H, Aboul-Enien HY. Isolation and Identification of Functional Components in Seed of Cherry Laurel (Laurocerasusofficinalis Roem.) and Investigation of Their Antioxidant Capacity, Journal of Biologically Active Products from Nature 3 (2013) 115–120.
17. Alasalvar C, Al-Farsi M, Shahidi F. Compositional characteristics and antioxidant components of cherry laurel varieties and pekmez. Journal of food science 70 (2005) S47–S52.
18. Ozturk B, Celik SM, Karakaya M, Karakaya O, Islam A, Yarilgac T. Storage temperature affects phenolic content, antioxidant activity and fruit quality parameters of cherry laurel (*Prunus laurocerasus* L.). Journal of Food Processing and Preservation 41 (2017) e12774.
19. Karabegović IT, Stojičević SS, Veličković DT, Todorović ZB, Nikolić NČ, Lazić ML. The effect of different extraction techniques on the composition and antioxidant activity of cherry laurel (*Prunus laurocerasus*) leaf and fruit extracts. Industrial Crops and Products 54 (2014) 142–148.
20. Baytop, T. Therapy with Medicinal Plants in Turkey (Past and Present), 1st ed.; Istanbul University, Istanbul, pp 178–249, 2001.
21. Casini AF, Ferrali M, Pompella A, Maellaro E, Comporti M. Lipid peroxidation and cellular damage in extrahepatic tissues of bromobenzene-intoxicated mice. American Journal of Pathology 123 (1986) 520–531.
22. Aykac G, Uysal M, Yalcin AS, Kocak-Toker N, Sivas A, Oz H. The effect of chronic ethanol ingestion on hepatic lipid peroxide, glutathione, glutathione peroxidase and glutathione transferase in rats. Toxicology 36 (1985) 71–76.
23. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clinical Chemistry 34 (1988) 497–500.
24. Aebi H. Catalase in vitro. Methods in enzymology 105(1984) 121–126.
25. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 5 (2001) 62–71.
26. Younes M, Cornelius S, Siegers CP. Ferrous ion supported in vivo lipid peroxidation induced by paracetamol – its relation to hepatotoxicity. Research communications in chemical pathology 51 (1986) 89–99.
27. Muriel P, Garciapina T, Perez-Alvarez V, Mourelle M. Silymarin protects against paracetamol-induced lipid peroxidation and liver damage. Journal of Applied Toxicology 12 (1992) 439–442.
28. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative medicine and cellular longevity 2014 (2014) 360438.
29. Eken A, Ünlü Endirlik B, Bakır E, Baldemir A, Yay AH, Cantürk F. Effect of Laurocerasus officinalis Roem. (Cherry laurel) fruit on dimethoate induced hepatotoxicity in rats. Kafkas Universitesi Veteriner Fakultesi Dergisi 23 (2017) 779–787.
30. Schäfer M, Werner S. Oxidative stress in normal and impaired wound repair. Pharmacological research 58 (2008) 165–171.
31. Yayla M, Halici Z, Unal B, Bayir Y, Akpinar E, Gocer F. Protective effect of et-1 receptor antagonist bosentan on paracetamol induced acute liver toxicity in rats. European Journal of Pharmacology 726 (2014) 87–95.
32. Eddaikra A, Eddaikra N. Endogenous Enzymatic Antioxidant Defense and Pathologies. in: Waisundara V. (Eds.) Antioxidants - Benefits, Sources, Mechanisms of Action. IntechOpen, London, pp. 1–646, 2021.
33. Araujo M, Welch William J. Oxidative stress and nitric oxide in kidney function. Current Opinion in Nephrology and Hypertension 15 (2006) 72–77.
34. Virág L, Szabó E, Gergely P, Szabó C. Peroxynitrite-induced cytotoxicity: mechanism and opportunities for intervention. Toxicology letters 140–141 (2003) 113-124.
35. Aydin Berktas O, Gulec Peker EG. Protective effects of *Prunus laurocerasus* extracts against paracetamol-induced hepatotoxicity. Nutrition and Food Processing 5 (2022) 1–5.
36. Ohno N, Yoshigai E, Okuyama T, Yamamoto Y, Okumura T, Sato K, Ikeya Y, Nishizawa M. Chlorogenic acid from the Japanese herbal medicine Kinginka (Flos Lonicerae japonicae) suppresses the expression of inducible nitric oxide synthase in rat hepatocytes. HOAJ Biology 1 (2012) 1–10.