Gazi Med J 2025;36(2):152-161

**DOI:** http://dx.doi.org/10.12996/gmj.2024.4162

# Dual Therapy with Ellagic Acid and Carnosic Acid Ameliorates STZ-Induced Diabetic Conditions in Rats by Alleviating Liver Oxidative Stress

## Elajik Asit ve Karnosik Asit ile Yapılan İkili Tedavi Karaciğer Oksidatif Stresini Hafifleterek Sıçanlarda STZ ile İndüklenen Diyabetik Koşulları İyileştirir

Ayşegül Berktaş[1], Elif Naz Gürsoy[1], Kaan Kaltalıoğlu[2], Şule Coşkun Cevher[1]

[1]Department of Biology, Gazi University Faculty of Sciences, Ankara, Türkiye
[2]Department of Occupational Health and Safety, Giresun University Vocational School of Espiye, Giresun, Türkiye

### ABSTRACT

**Objective:** Diabetes, a significant public health issue today, causes serious health problems due to its complications and will become a global epidemic if no action is taken. Diabetic patients experience elevated oxidative stress, which impairs wound healing. The purpose of this study was to investigate the effects of ellagic acid (EA) and carnosic acid (CA) on oxidative events in liver tissue in diabetic rats with wounds.

**Methods:** The rats were divided into 7 groups as control, untreated-3 day, untreated-7 day, topical-3 day, topical-7 day, oral-3 day and oral-7 day. To induce diabetes in the subjects, each group received a single intraperitoneal injection of streptozotocin. Rats were treated topically or orally with EA + CA. On the 3rd and 7th days of recovery, the rats were sacrificed and the levels of nitric oxide (NOx), malondialdehyde (MDA), glutathione (GSH), protein carbonyls (PC), and ascorbic acid (AA) were measured spectrophotometrically to investigate the effects of oxidative stress in liver tissue.

**Results:** Liver tissue MDA, NOx, and PC levels were determined to be statistically decreased in both topical and oral applications compared to the control and untreated groups ($p<0.05$). Liver tissue GSH, AA, and collagen levels were found to be statistically increased in both topical and oral applications when compared to the control and untreated groups ($p<0.05$).

**Conclusion:** These results show that the combination of EA and CA with two different application methods significantly reduces oxidative

### ÖZ

**Amaç:** Önemli bir halk sağlığı sorunu haline gelen diyabet, komplikasyonlarıyla birlikte ciddi sağlık sorunları oluşturmakta ve önlem alınmaz ise küresel bir salgın şeklinde ilerlemektedir. Diyabetik hastalarda oksidatif stres artmakta ve yara iyileşmesi bozulmaktadır. Bu çalışmanın amacı, yaralı diyabetik sıçanlarda elajik asit (EA) ve karnosik asidin (CA) karaciğer dokusundaki oksidatif olaylar üzerindeki etkilerini araştırmaktır.

**Yöntemler:** Sıçanlar kontrol, tedavisiz-3 gün, tedavisiz-7 gün, topikal-3 gün, topikal-7 gün, oral-3 gün ve oral-7 gün olmak üzere 7 gruba ayrıldı. Tüm gruplara diyabet modeli oluşturmak amacıyla tek doz streptozotosin enjekte edildi. Sıçanlar EA + CA ile topikal veya oral olarak tedavi edilmiştir. İyileşmenin 3. ve 7. günlerinde sıçanlar sakrifiye edilmiş ve karaciğer dokusunda oksidatif stresin etkilerini araştırmak için nitrik oksit (NOx), malondialdehit (MDA), glutatyon (GSH), protein karbonilleri (PC), askorbik asit (AA) seviyeleri spektrofotometrik olarak ölçülmüştür.

**Bulgular:** Karaciğer dokusu MDA, NOx ve PC düzeyleri hem topikal hem de oral uygulamalarda kontrol ve tedavisiz gruplara kıyasla istatistiksel olarak düşük bulundu ($p<0,05$). Karaciğer dokusu GSH, AA ve kollajen düzeyleri hem topikal hem de oral uygulamalarda kontrol ve tedavisiz gruplara kıyasla istatistiksel olarak yüksek bulundu ($p<0,05$).

**Sonuç:** Bu sonuçlar, EA ve CA'nın iki farklı uygulama yöntemiyle kombinasyonunun karaciğer dokusundaki oksidatif olaylara etki ederek ilaç metabolizmasındaki oksidatif stresi önemli ölçüde azalttığını ve

**Cite this article as:** Berktaş A, Gürsoy EN, Kaltalıoğlu K, Coşlun Cevher Ş. Dual therapy with ellagic acid and carnosic acid ameliorates stz-induced diabetic conditions in rats by alleviating liver oxidative stress. Gazi Med J. 2025;36(2):152-161

**Address for Correspondence/Yazışma Adresi:** Şule Coşkun Cevher, Prof, MD, Department of Biology, Gazi University Faculty of Sciences, Ankara, Türkiye
**E-mail / E-posta:** sule@gazi.edu.tr
**ORCID ID:** orcid.org/0000-0001-6204-2845

**Received/Geliş Tarihi:** 12.03.2024
**Accepted/Kabul Tarihi:** 06.06.2024
**Publication Date/Yayınlanma Tarihi:** 15.04.2025

## ABSTRACT

stress associated with drug metabolism by affecting oxidative events in liver tissue, and has the potential to prevent possible complications of diabetes.

**Keywords:** Ellagic acid, carnosic acid, oxidative stress, liver, inflammation

## ÖZ

diyabetin olası komplikasyonlarını önleme konusunda potansiyeli olduğunu göstermektedir.

**Anahtar Sözcükler:** Elajik asit, karnosik asit, oksidatif stres, karaciğer, enflamasyon

## INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic endocrine disorder that affects carbohydrate, protein, and lipid metabolism due to insufficient or absent insulin secretion and/or action (1). Recent data published by the International Diabetes Federation shows that there are approximately 9 million people with diabetes in Türkiye between the ages of 20-79 years, which represents approximately 14.5% of the total adult population (2). All types of diabetes are linked to the dysfunction and destruction of beta cells. Factors such as autoimmunity, inflammation, insulin resistance, obesity, an aging population, and physical inactivity can lead to the loss of pancreatic beta cells (1).

Uncontrolled diabetes can lead to various macrovascular complications, including macrovascular complications such as coronary artery disease, and microvascular complications such as neuropathy, nephropathy, and retinopathy. These complications occur in the long term due to oxidative stress caused by increased reactive oxygen species (ROS) production (3). Additionally, diabetes can impair wound healing due to infection, local circulatory disturbance caused by angiopathy, and peripheral neuropathy. The cause of diabetic wounds is not certain, but it may be due to accelerated atherosclerosis and infections induced by decreased perfusion, caused by neuropathy, slow collagen synthesis and deposition, decreased angiogenesis, and weaker tensile strength of the wound (4). As normal wound healing cannot occur, free radicals or oxidants continue to increase tissue damage by targeting DNA, proteins, and lipids (5,6). Biological systems have an integrated antioxidant defence system, including catalase (CAT), glutathione (GSH), and superoxide dismutase (SOD), to counteract these molecules (7).

Patients are cautious about the use of medications to treat diabetic wounds because of the potential negative metabolic effects of the drugs. As the principal organ for drug metabolism, the liver is essential to drug detoxification and the body's removal of chemicals. The genesis of drug-induced hepatotoxicity is an early event that generates radical species, such as ROS (8). Additionally, it contains a higher concentration of antioxidant enzymes such as CAT and glutathione peroxidase than other organs (9).

Endogenous and exogenous drugs, as well as specialised devices and techniques, are used to treat diabetic wounds. However, due to the low success rate of treatment, high cost, and potential side effects to the patient, as well as an increase in oxidative stress during the process, late wound closure or organ loss may occur. Therefore, the use of compounds with natural antioxidant content is necessary in the treatment of impaired wounds. According to the data gathered, ellagic acid (EA) and carnosic acid (CA) have properties that can prevent or treat a variety of diseases, including diabetes, Parkinson's disease, cancer, cardiovascular disease, ulcerative colitis, and Alzheimer's disease. These properties include anti-inflammatory, antioxidant, and antimicrobial effects (10-14). EA exhibits anti-inflammatory activity by reducing mediator molecules such as interleukin-6 and tumour necrosis factor-α. Additionally, it scavenges free radicals through transcriptional activation of nuclear erythroid 2-related factor 2 (Nrf2) and has antioxidant activity against toxic conditions in the liver (12,13). CA is another compound that exhibits antioxidant efficacy by disrupting the formation of the Nox4 enzyme complex through inhibition of the nuclear factor-kappa B (NF-κB) inhibitor signaling pathway (14). It acts as a metal ion chelator and is a potent scavenger of peroxyl radicals. Its effect on membrane lipid peroxidation is higher than that reported for artificial antioxidants (15,16). CA has been demonstrated to protect against lipopolysaccharide-induced liver injury by inducing phase 2 antioxidant enzymes. Moreover, it reduces oxidative/nitrosative stress caused by lipopolysaccharide (LPS) by reducing lipid peroxidation, serum nitric oxide (NOx) levels, and protein carbonylation (17). In combination with other natural compounds, CA has shown beneficial effects in most experimental disease models and is a promising compound in many fields (11,17).

This study hypothesizes that the administration of these two compounds can significantly impact oxidative stress in drug metabolism. To this end, the effects of various routes of administration of EA and CA on oxidative stress in liver tissue were evaluated for the first time.

## MATERIAL AND METHODS

### *Animals*

The Gazi University Laboratory Animal Committee Ethics Committee granted permission for the investigations (approval number: G.U.ET-20.012, date: 14.02.2022). Prior to tissue sample collection, all procedures were carried out at the Animal Breeding and Experimental Research Center (GUDAM) of Gazi University Laboratory. Forty-two male albino Wistar rats weighing 250-300 g were used, and these were utilized in the studies from GUDAM. The rats were fed freely both before and throughout the experiment, and they were housed in separate cages at 20±5 °C with synchronized light cycles.

### *Creation of a Diabetes and Wound Model*

To develop diabetes, rats were given a single dose of 60 mg/kg streptozotocin intraperitoneally (Sigma-Aldrich). After three days, blood glucose levels were tested, and those greater than 250 mg/dL were classified as diabetic. The animals were weighed, and

anaesthetised according to their weight. Intramuscular injections of ketamine (Alfasan, NED 50 mg/kg) and xylazine (Alfasan, NED 5 mg/kg) were administered to induce anaesthesia. Following anaesthesia, the dorsal region of the animals was shaved without damaging the skin. The animals were then restrained face down. With the exception of the control group, six full-thickness excisional wounds were created on both sides of the spine in parallel, in the dorsal region of all animals using a punch biopsy (Acuderm, USA).

### *Experimental Design and Liver Tissue Collection*

The rats were divided into seven groups, as shown in Table 1: control; untreated-3 day; untreated-7 day; topical-3 day; topical-7 day; oral-3 day and oral-7 day.

The topical gel was prepared using Carbopol 974P at a concentration of 2%. CA was dispersed in distilled water (98 g) and mixed at 800 rpm for 60 minutes. A 10% NaOH solution was added dropwise to this mixture to form a transparent gel (18). The dose of EA and CA was adjusted to 10 mg/kg. The gel was stored at +4 °C for 24 hours to eliminate air bubbles. It was then UV sterilised and prepared for *in vivo* topical application. The gel formulation, which contained EA + CA, was spread as a thin layer on a Petri dish and sterilised under a 254 nm UV-C lamp in a laminar flow cabinet for 2 hours. In the topical administration groups, a single daily dose of EA + CA (10 mg/kg), containing gel, was applied topically to each wound. In the oral administration groups, a single daily dose of EA + CA (10 mg/kg) was administered by oral gavage after being dissolved in water.

The experiment weighed the animals on a standard balance and induced general anaesthesia by injecting them with ketamine (alfamine 50 mg/kg) and xylazine (alfazyne 5 mg/kg) in proportion to their weight. Spectrophotometric evaluation was used to measure the levels of malondialdehyde (MDA), GSH, NOx, protein carbonyls (PC), ascorbic acid (AA), and collagen in the liver tissue of the sacrificed animals on days 3 and 7.

### *Biochemical Analysis*

### *MDA, GSH, NO and AA Determination*

The Buege and Aust (19) method was utilized. A homogenizer set at 5000 rpm was used to homogenize liver tissue in 150 mM KCl. The tissue sample was mixed with 15% TCA solution to precipitate the protein. After centrifuging the precipitate, a portion of the supernatant was combined with 0.67% TBA and 1% BHT, and the mixture was heated. The spectrophotometer was used to measure absorbance at 532 nm after cooling.

The Elman method was used to measure GSH levels in tissue samples. The samples were homogenized in 0.15 M cold KCl, and then to deproteinize the homogenate, a metaphosphoric acid-EDTA NaCl combination was added. DTNB solution was added to the 0.3 M $Na_2HPO_4$ supernatant following centrifugation. The samples were measured at 412 nm (20).

The AA determination procedure in tissue involves homogenizing the tissues in a cold PCA/EDTA combination, as described by Roe and Kutherin and as further edited by Berger et al. (21). Next, the homogenate was centrifuged. Two tubes were prepared: one carrying a conventional AA solution and the other containing PCA solution. The samples were then prepared in the tubes holding the supernatant and incubated individually after a color reagent was added. The samples were mixed and sulfuric acid was added to each tube. Then, the mixture was maintained at room temperature. Spectrophotometry was used to analyze the samples at a wavelength of 520 nm.

The NOx level in the tissue was evaluated by detecting the stable end products, nitrite and nitrate, using the Griess reaction. To deproteinize the sample, 0.3 M NaOH and 10% $ZnSO_4$ were used. After the experiment, the mixture was centrifuged, and the supernatants were employed in the Griess experiment (22).

### *Assay Methods of PC and Collagen*

Reznick and Packer (23) developed a method for measuring the amount of reactive carbonyl groups in protein oxidation, which indicates the existence of oxidative stress. We employed the hydrazone formation method to measure the PC groups, where hydrazone is produced by the interaction of 2,4-dinitrophenylhydrazine with protein carbonyl groups. We next examined the PC groups spectrophotometrically at 370 nm and calculated the total protein content at 280 nm (24). The modified Lowry method (25) was used to measure collagen concentrations in tissues.

### *Statistical Analysis*

For all values, the arithmetic mean ± standard error was used. One-way ANOVA and the Tukey's multiple comparison test were used to

**Table 1.** Group specifications

| Groups | Specifications |
|---|---|
| **Control** | STZ injected, not wounded, no treatment (n=6). |
| **Untreated 3 day** | STZ injected, wounded, no treatment, sacrificed at post wounding day 3 (n=6). |
| **Untreated 7 day** | STZ injected, wounded, no treatment, sacrificed at post wounding day 7 (n=6). |
| **Topical 3 day** | STZ injected, wounded, a single daily dose EA + CA (10 mg/kg) was applied topically to wounds, sacrificed at post wounding day 3 (n=6). |
| **Topical 7 day** | STZ injected, wounded, a single daily dose EA + CA (10 mg/kg) was applied topically to wounds, sacrificed at post wounding day 7 (n=6). |
| **Oral 3 day** | STZ injected, wounded, a single daily dose EA + CA (10 mg/kg) was applied orally, sacrificed at post wounding day 3 (n=6). |
| **Oral 7 day** | STZ injected, wounded, a single daily dose EA + CA (10 mg/kg) was applied orally, sacrificed at post wounding day 7 (n=6). |

STZ: Streptozotocin, EA: Ellagic acid, CA: Carnosic acid.

assess the values (SPSS 16.0 for Windows, SPSS Inc., USA). P values less than 0.05 were regarded as statistically significant.

## RESULTS

### *MDA Levels of Groups*

The MDA levels in the liver tissue of the various groups are displayed in Figure 1 and Table 2. MDA levels were significantly lower in the oral and topical application groups than in the control and untreated groups ($p<0.05$). Because of enhanced lipid peroxidation, the liver MDA levels were greater in the untreated groups observed at 3 and 7 days.

### *GSH Levels of Groups*

Liver tissue GSH levels were significantly higher in the topical and oral treatment groups compared to the control and untreated groups (Figure 2 and Table 2) ($p<0.05$). A significant increase was found in the 7-day oral treatment group when compared to the 3-day oral treatment group ($p<0.05$) (Figure 2). The administration of both substances, orally and topically, resulted in an increase in GSH production in liver tissue, due to their antioxidant capacity against oxidative stress. It is believed that EA and CA possess synergistic antioxidant properties. The decrease in oxidative stress occurred due to the antioxidant capacity of the compounds used in the application (EA and CA), which reduced lipid peroxidation and oxidative damage.

### *NOx Levels of Groups*

According to the study, the untreated 3-day and 7-day diabetic groups had substantially higher NOx levels than the control group (Table 2) ($p<0.05$). Figure 3 shows that all oral and topical application groups had significantly lower NOx levels than the untreated groups ($p<0.05$). Figure 3 and Table 2 show that the 3-day oral application

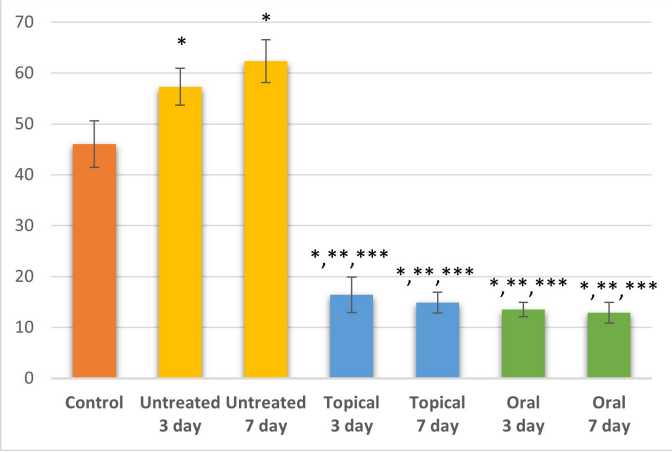


**Figure 1.** MDA level in the livers of groups.

*p<0.05 compared to control, **p<0.05 compared with untreated 3 day group, ***p<0.05 compared with untreated 7 day group.

*MDA: Malondialdehyde.*

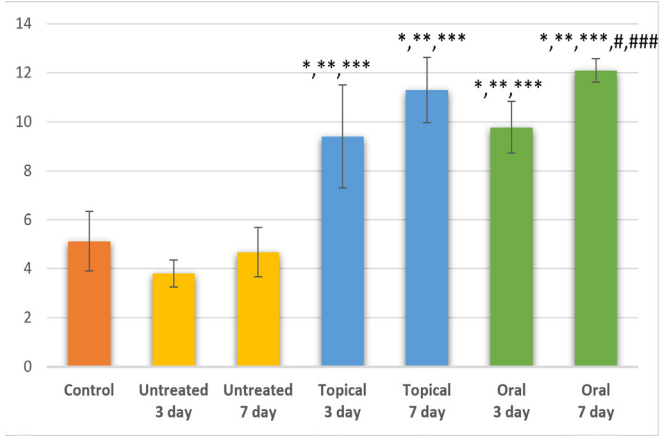


**Figure 2.** GSH level in the livers of groups.

*p<0.05 compared to control, **p<0.05 compared with untreated 3 day group, ***p<0.05 compared with untreated 7 day group, #p<0.05 compared with topical 3 day group, ##p<0.05 compared with topical 7 day group, ###p<0.05 compared with oral 3 day group.

*GSH: Glutathione.*

**Table 2.** MDA, GSH, NOx, AA, PC and collagen levels in the liver tissue of rats

| Groups | MDA (nmol/g tissue) | GSH (µmol/g tissue) | NOx (nmol/g tissue) | AA (µg/g tissue) | PC (nmol/g tissue) | Collagen (mg/g tissue) |
|---|---|---|---|---|---|---|
| Control | 46.02±4.57 | 5.13±1.21 | 27.24±3.58 | 14.51±0.73 | 13.59±1.63 | 42.80±2.00 |
| Untreated 3 day | 57.29±3.60* | 3.81±0.55 | 32.79±1.8* | 8.33±0.22* | 14.82±2.41 | 26.13±4.21* |
| Untreated 7 day | 62.37±4.22* | 4.68±1.01 | 35.06±3.8* | 8.03±0.52* | 28.38±3.52*,** | 20.82±1.80*,** |
| Topical 3 day | 16.39±3.5*,**,*** | 9.39±2.10*,**,*** | 11.39±3.30*,**,*** | 14.26±0.55**,*** | 23.52±2.54*,**,*** | 27.18±2.77*,*** |
| Topical 7 day | 14.83±2.03 *,**,*** | 11.29±1.33*,**,*** | 6.72±1.65*,**,*** | 14.13±0.41**,*** | 12.02±1.81***,# | 31.73±1.94*,**,***,# |
| Oral 3 day | 1.48±1.40*,**,*** | 9.78±1.06*,**,*** | 4.94±1.97*,**,***,# | 11.71±0.45*,**,***,#,## | 18.01±2.04*,***,#,## | 30.23±1.61*,*** |
| Oral 7 day | 12.88±2.02*,**,*** | 12.10±0.48*,**,***,#,### | 3.81±1.08*,**,***,# | 14.26±2.10**,***,### | 10.14±1.43**,***,#,### | 34.28±1.95*,**,***,# |

*p<0.05 compared to control, **p<0.05 compared with untreated 3 day group, ***p<0.05 compared with untreated 7 day group, #p<0.05 compared with topical 3 day group, ##p<0.05 compared with topical 7 day group, ###p<0.05 compared with oral 3 day group.
MDA: Malondialdehyde, GSH: Glutathione, NOx: Nitric oxide AA: Ascorbic acid, PC: Protein carbonyls.

group had a lower NOx level (p<0.05) than the 3-day non-oral application therapy group.

### *AA Levels of Groups*

In all treated groups where EA and CA together were administered, AA levels in liver tissue were statistically higher than in the untreated groups (Figure 4) (p<0.05). In the groups that did not receive treatment, the AA level was significantly lower than in the control group (Table 2). The AA level was significantly higher in the 7-day oral treatment group than in the 3-day oral gavage treatment group, while it was lower in the 3-day oral treatment group compared to the 3-day topical treatment group (Figure 4) (p<0.05).

### PC Levels of Groups

The 7-day untreated group had substantially greater PC levels compared to the control and 3-day untreated groups (Figure 5; p<0.05). A comparison of topical treatment groups found that the 3-day group had significantly higher PC levels than the control and 7-day groups. When compared to the untreated group, the 3-day oral therapy group considerably increased in effect, while the 7-day oral therapy group dramatically reduced in effect (p<0.05) (Figure 5 and Table 2).

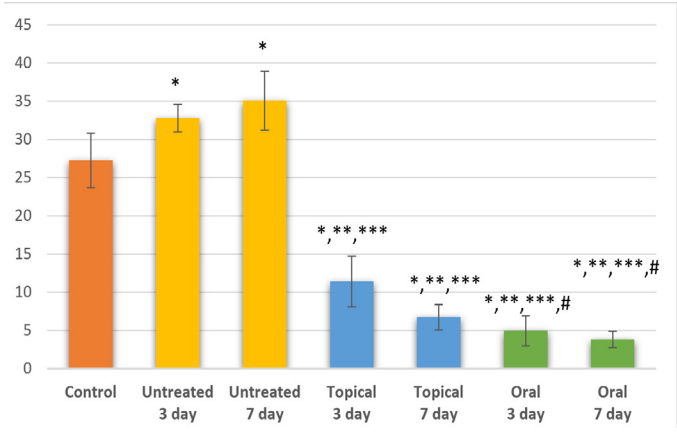


**Figure 3.** NOx level in the livers of groups.

*p<0.05 compared to control, **p<0.05 compared with untreated 3 day group, ***p<0.05 compared with untreated 7 day group, #p<0.05 compared with topical 3 day group.

*NOx: Nitric oxide.*

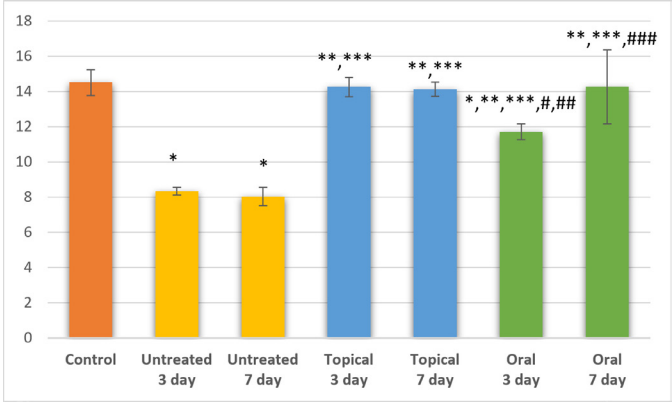


**Figure 4.** AA level in the livers of groups.

*p<0.05 compared to control, **p<0.05 compared with untreated 3 day group, ***p<0.05 compared with untreated 7 day group, #p<0.05 compared with topical 3 day group, ##p<0.05 compared with topical 7 day group, ###p<0.05 compared with oral 3 day group.

### *Collagen Levels of Groups*

Collagen levels in liver tissue were found to be significantly higher in all groups treated with both EA and CA than in the untreated 7-day group (Figure 6). Furthermore, a significant decrease was observed in all treatment groups, including the untreated group, when compared to the control group. There was no significant difference observed between the 3-day topical and 3-day oral groups, or between the 7-day topical and 7-day oral application groups (p>0.05). In all groups that were treated, both topical and oral gavage applications of EA and CA resulted in an increase in collagen levels in liver tissue (Figure 6 and Table 2).

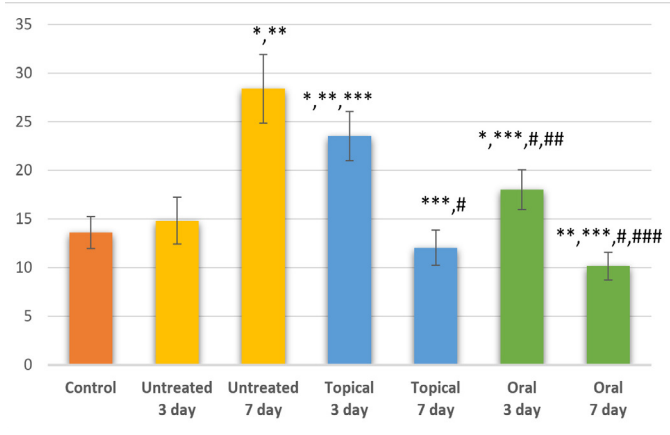


**Figure 5.** PC level in the livers of groups.

*p<0.05 compared to control, **p<0.05 compared with untreated 3 day group, ***p<0.05 compared with untreated 7 day group, #p<0.05 compared with topical 3 day group, ##p<0.05 compared with topical 7 day group, ###p<0.05 compared with oral 3 day group

*PC: Protein carbonyls.*

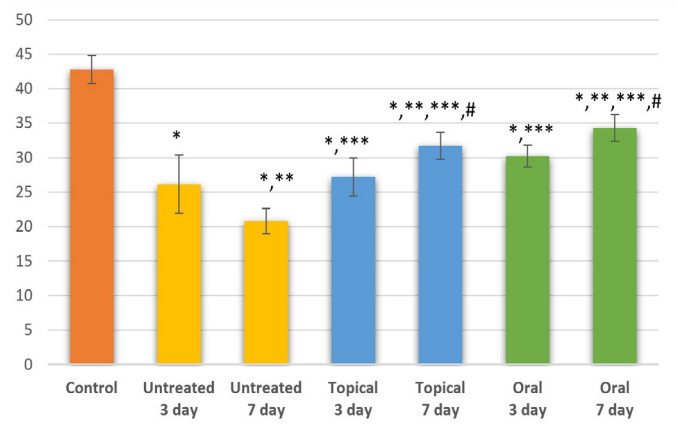


**Figure 6.** Collagen level in the livers of groups.

*p<0.05 compared to control, **p<0.05 compared with untreated 3 day group, ***p<0.05 compared with untreated 7 day group, #p<0.05 compared with topical 3 day group, ##p<0.05 compared with topical 7 day group, ###p<0.05 compared with oral 3 day group

## DISCUSSION

Wound healing is a dynamic and complex process that involves hemostasis, inflammation, proliferation, and maturation phases. Local and systemic factors, such as DM, play a crucial role in wound healing. The chronicisation of diabetic wounds involves various factors, including an impaired immune system due to decreased perfusion caused by accelerated atherosclerosis and neuropathy, decreased angiogenesis, slower collagen synthesis and deposition, weaker tensile strength of wounds, and impaired oxidative balance (26). Diabetes disrupts the redox equilibrium, leading to oxidative stress brought on by either a reduction in the activity of antioxidant systems or an increase in the production of free radicals. The liver is the primary organ where specific enzymes accumulate and perform a two-stage detoxification process to scavenge free radicals (27). When diabetes and its complications are not effectively controlled, diabetic wounds can become chronic, leading to impaired oxidative balance and potentially severe consequences. Numerous studies have demonstrated the antioxidant and anti-inflammatory properties of EA and CA, which help to balance oxidative stress. This work investigates the effect of different applications of EA and CA on oxidative damage in diabetic wounds, since the liver is the most important organ for detoxification enzymes that scavenge free radicals.

Abdelkader et al. (28) carried out research on liver toxicity and found that oral gavage administration of EA had an inhibitory effect on oxidative stress. This was demonstrated by measuring liver MDA, NOx, and GSH levels. Our study also showed a significant decrease in MDA and NOx levels in the groups that received both EA and CA, indicating a reduction in oxidative stress. Although no significant difference was observed in the MDA level of the treated groups with oral and topical application, the significant decrease in NOx in the oral application groups suggests that the antioxidant effect of these two substances may be more effective in the gastrointestinal tract. Furthermore, Xiang et al. (16) discovered that the harmful effects of NOx, cytokines, and free oxygen radicals were all inhibited by CA. Furthermore, by raising liver GSH levels, it markedly improved the body's cellular antioxidant defense mechanism.

NOx is a molecule that contributes to every stage of wound healing, including angiogenesis, epithelial cell migration, keratinocyte proliferation, and collagen secretion by fibroblasts. Although it has a protective effect at physiological doses, at high levels, it exhibits a cytotoxic effect and reacts with ROS in the body to form radicals (29). The significant increase in NOx in the untreated groups and the significant decrease in NOx in the orally treated groups indicate that the two substances reduce nitrosative stress. These findings are consistent with those of Xiang et al. (16). Bagheri Tadi et al. (30) investigated the gene expression of antioxidant and oxidative stress factors in chronic wounds of diabetic rats by applying adipose-derived stem cell-associated photobiomodulation. The study found that this application strongly facilitated the inflammatory and proliferative phases of the wound healing process, by reducing the inflammatory response, NOx1, and NOx4. According to this study, the reduced NOx ratio in liver tissue resulting from the various EA and CA applications used in our study may have hastened the inflammatory and proliferative phases of wound healing processes, leading to increased formation of granulation tissue.

Enzymatic catalysis and chemical processes can both be used to create MDA from polyunsaturated fatty acids. It is one of the end products of lipid peroxidation metabolism (31). The low levels of MDA in both the oral gavage and topical treatment groups suggest that EA and CA reduce oxidative stress by decreasing lipid peroxidation in the liver. Zhu et al. (32) conducted a study on brain tissue, which showed that EA scavenges free radicals, reduces ROS production, and exhibits anti-inflammatory effects by regulating Nf-κB, MAPKs, and JAK/STAT pathways. This supports our own findings. The liver is the primary organ for harboring detoxification enzymes and the scavenging of endogenous and exogenous free radicals (8). In this study, we found no significant difference in MDA levels in liver tissue between the topical and oral-treated groups. This lack of difference may be attributed to the poor water solubility and low bioavailability of EA. In the gastrointestinal tract and other places, such as the liver, phase I and phase II enzymes metabolize EA, converting it into more water-soluble metabolites that may be eliminated in the urine or held in tissues (33). In their study on Iberian pigs, Espín et al. (34) discovered that the urolithin metabolite resulting from EA was only present in high concentrations in the gallbladder and urinary bladder, but not in any of the tested tissues, including muscle, liver, heart, and adipose tissue. The untreated groups showed that hydroxyl and peroxyl radicals were responsible for initiating and promoting lipid peroxidation, resulting in an increase in MDA. The low levels of MDA in the EA-treated and CA-treated groups may be due to EA's ability to inhibit lipid peroxidation even at very low concentrations, as demonstrated in Priyadarsini et al. (35).

Our study revealed a significant reduction in oxidative effects in liver tissue following the administration of 10 mg of EA and CA. CA was used in addition to EA due to its antioxidant and anti-inflammatory effects, which have been shown to be beneficial in treating various health disorders (10,11). Furthermore, we assessed oxidative events in the liver tissue of subjects with diabetic wounds for the first time by using both EA and CA, taking into account, the low bioavailability of EA and high bioavailability of CA (33,34,36). In a recent work by Chen et al. (36), the distribution of CA in various tissues, including the liver, stomach, and digestive contents, was determined to investigate its absorption and transport mechanisms. The researchers concluded that CA is both locally and systemically accessible in the digestive tract after it was administered orally for seven days. Additionally, the study raises the possibility that the gut microbiota is essential for the breakdown and assimilation of CA. In the study by Xu et al. (37), the high MDA level in the liver tissue of the CA-treated groups was reduced, and lipid peroxidation was minimized in the other groups. One possible explanation for the low level of MDA in the groups where EA and CA were administered together is that CA may act as a metal ion chelator, function as a strong scavenger of peroxyl radicals, and have a significant effect on lipid peroxidation (14).

In this study, we compared the treatments we applied with other groups, using PC levels, as well as MDA and NOx levels. Free radicals not only affect compounds such as lipids and nucleic acids through oxidative reactions, but they also react with important compounds such as proteins, causing various biological problems. One kind of oxidative protein alteration that is considered irreversible is protein carbonyl modification. They contain products of lipid peroxidation that disappear in a matter of minutes. Since protein oxidation happens very early during oxidative stress and is not caused by a

particular oxidant, we employed PC levels as a marker of protein oxidation in this work (38). In this study, the untreated groups showed a significant increase in PC levels after 7 days. This indicates that protein carbonyls remain in the bloodstream for a longer period than other oxidative stress markers. Without treatment, oxidative stress increases over time and protein dysfunction worsens. In a study conducted by Balabanlı and Balaban (39), where endotoxaemia was induced in rats, PC levels were found to be high in the endotoxin-treated group, which supports our finding. Our study revealed significant differences of protein carbonyl levels, depending on timing rather than application methods. A significant decrease was determined in the 7-day treatment group compared to both the untreated 7-day group and the topical 3-day group, regardless of the application method used. The significant decrease in PC levels in the tissue with EA and CA, is thought to be due to the protective impact of these two active substances on proteins from carboxylation. They also play an effective role in antioxidant metabolism in longer applications, both topical and oral. Although no significant difference was observed between the 7-day treatment groups, the strongest antioxidant effect was observed in the 7-day oral gavage treatment. Xiang et al. (16) found that oral administration of CA reduced protein carbonyls and showed antioxidant effects in LPS-induced liver damage. Karimi et al. (40) demonstrated that EA administration at 30 mg/kg orally significantly decreased elevated MDA, NOx, and protein carbonyl levels in toxin-induced hepatotoxicity, reversing oxidative damage. Moreover, the outcomes show that the inhibitory impact changes according to concentration. Therefore, further research should be conducted on the method, and duration of use of the CA and EA combination.

Antioxidant defence systems are crucial for protecting cells and tissues from damage caused by free radicals generated by reactive oxygen and nitrogen. GSH, an antioxidant synthesised from glutamate, cysteine and glycine in the liver, plays a vital role in this protection (29). In this study, GSH levels were significantly higher in all therapy groups compared to the control and untreated groups, indicating an increase in antioxidant capacity. Nisari (41) investigated the protective effect on antioxidant enzymes in the liver tissue of diabetic rats, and found a significant decrease in GSH activity. The study found that GSH production increased in liver tissue as a result of both oral and topical application of the two substances due to its antioxidant capacity against oxidative stress. The most significant increase in GSH was observed in the 7-day oral treatment group compared to the 3-day topical and 3-day oral treatment groups. This suggests that the two substances may be more effective in terms of bioavailability when administered orally, over a longer duration. The results of Shendge et al. (42) support our findings regarding the favourable effects of EA on oxidative stress in hepatotoxicity caused by iron overload. In their in vivo study, after determining the in vitro antioxidant capacity of EA, they found that ROS levels decreased, and SOD, CAT, GST, and GSH increased in the groups treated orally with EA, in liver tissue samples. The results of MDA and PC in EA-treated groups in this study are also consistent with our results previously discussed. Similarly, Das et al. (43) found results similar to ours regarding CA treatment. They showed that CA treatment (10 and 20 mg/kg) increased lipid peroxidation and protein carbonylation in hepatic tissue to levels close to normal. It also increased GSH and other endogenous antioxidant enzymes.

Another parameter used in the study was AA, an acronym for an important substance with non-enzymatic antioxidant properties similar to GSH. The statistically significant increase in AA levels in liver tissue in all treated groups, indicates an increase in antioxidant capacity. Devipriya et al. (12) conducted a study that yielded results similar to ours. They administered three different concentrations of EA intragastrically to evaluate its effects against alcohol-induced damage. The study found that AA in plasma increased along with other antioxidants, and oxidative stress was reduced by a decrease in liver marker enzymes. It is believed that CA may have an antioxidant effect, particularly by scavenging lipid peroxyl radicals, as reported in previous studies (14). The significant increase in GSH levels in all treated groups, administered via both topical and oral gavage applications, compared to the untreated groups, indicates an increase in antioxidant capacity. Previous studies have also reported a positive and significant correlation between AA supplementation and GSH levels (44). When evaluating the treated groups separately, no significant correlation was found among the topically treated groups. However, in the orally treated groups, the 7-day orally gavaged group showed a significant increase in AA compared to the 3-day orally gavaged group. This suggests that the two antioxidants may be more effective in terms of bioavailability when administered orally over a longer period of time. The results indicate a significant decrease in the 3-day oral group compared to the topically treated groups and the control group, suggesting that AA can effectively eliminate oxidative stress. Additionally, AA is used in all stages of wound healing, including apoptosis and phagocytosis of neutrophils, synthesis, and secretion of collagen. Therefore, it is possible that the level of AA was lower on the 3rd day of injury with oral application compared to topical application. The impact of EA and CA on AA in liver tissue may be more closely linked to the method of administration when examining our findings.

AA is essential for tissue formation and collagen synthesis. It converts proline, a non-essential amino acid found in collagen, into hydroxyproline (45). Collagen is a crucial matrix component that plays a significant role in cell proliferation and migration during the normal wound healing process. The number of myofibroblasts increases during the fibrosis period when collagen synthesis, which is necessary for extracellular matrix (ECM) formation, occurs. Various cellular subgroups and mechanisms in the liver, kidney, lung, heart, and skin contribute to extracellular matrix formation during fibrogenesis. The liver is a significant source of ECM production, with numerous cell types, including hepatic stellate cells, portal myofibroblasts, resident fibroblasts, and bone marrow-derived myofibroblast precursors. We investigated the effect of EA + CA applications on collagen levels in the liver because hepatic stellate cells and portal myofibroblasts, which perform collagen synthesis, originate from the liver (46). Previous studies have investigated the connection between epidermal adhesion and collagen as well as the migration of fibroblasts in the skin. Duckworth et al. (47) investigated the effect of EA on collagen and elastin production by administering retinoic acid to human dermal fibroblasts for varying periods. They found a significant increase in production and concluded that EA can improve fine wrinkles by enhancing elastin and collagen production. Using CA together with other chemicals, Darawsha et al. (48) investigated the protective impact of polyphenols, estradiol, and carotenoids on cutaneous fibroblasts under oxidative stress.

The compounds were pre-incubated in dermal cells, resulting in a decrease in the ROS level generated by $H_2O_2$, inhibition of cell death, reversal of the increase in MMP-1 secretion, and reversal of the decrease in pro-collagen levels caused by oxidative stress. In this study, we observed a significant increase in collagen levels in the treatment groups compared to the untreated groups, which is consistent with the findings of Duckworth et al. (47) and Darawsha et al. (48). The activation of the Nrf2 transcription pathway, which increases the production of antioxidant enzymes that lower ROS, is responsible for the increase in collagen in the EA + CA treated groups (48).

The study found a significant decrease in AA levels in the 3-day topical group, which showed the least increase in collagen levels. This suggests that AA, which is necessary for collagen synthesis, is used to eliminate oxidative stress, resulting in a low increase in collagen levels (45). Abdelkader et al. (28) conducted a study on VPA-induced liver toxicity. The treatment groups showed a significant decrease in the content of hydroxyproline, one of the collagen components, following the oral gavage administration of EA. In this study, we found that collagen levels in the liver tissue of the control group were higher than those of the wound-treated groups, which may be due to hyperglycaemic conditions. This suggests that EA and CA helped to eliminate hyperglycaemic conditions and maintain normal cell conditions. Numerous studies have investigated the effect of EA on collagen levels in various contexts, including hypertrophic scars, liver tissue affected by schistosoma, and myocardial infarction. The findings indicate that EA inhibits the proliferation and migration of fibroblasts by inhibiting the expression of collagen I, collagen III, MMP-2, and MMP-9, and ECM deposition. As a result, collagen fibre deposition is significantly reduced (49). Zhang et al. (50) conducted a study on the effect of CA on liver fibrosis. The study found that administering CA, intragastrically to rats, which were induced with bile duct ligation resulted in a reduction of α-smooth muscle actin and collagen 1 expression in the liver.

When considering the two different application methods of EA and CA in total, particularly in 7-day oral gavage and 7-day topical application, it can be inferred that EA and CA applications reduce lipid peroxidation and protein modification, increase antioxidant capacity, and achieve oxidative balance. This is supported by the decrease in MDA, NOx, and PC, as well as the increase in GSH, AA, and collagen. EA and CA may have a synergistic effect when used together, reducing inflammation and influencing cell behaviour under normoglycaemic conditions. The study suggests that EA and CA have a positive effect on liver tissue by reducing oxidative stress and inflammation, which is important for overall metabolic control. These findings contribute positively to the existing literature. EA and CA work together to reduce oxidative stress and inflammation by acting through the same pathway. The study shows that EA and CA increased antioxidant activity by upregulating Nrf2 expression in liver tissue. The Nrf2/Keap1 system regulates the expression of most endogenous antioxidants. Therefore, it can be concluded that EA and CA enhance antioxidant capacity and reduce oxidative stress by activating the Nrf2 pathway.

### *Study Limitations*

The remarkable limitation of our study is the absence of data specific to enzymatic antioxidants and molecular signaling pathways.

## CONCLUSION

The positive effect of two antioxidant substances, (EA and CA) on oxidative events in liver tissue was revealed, in this study, using a diabetic wound model. Thus, the positive effects of a mixture with potential use in diabetic wounds were revealed.

### *Ethics*

**Ethics Committee Approval:** The Gazi University Laboratory Animal Committee Ethics Committee granted permission for the investigations (approval number: G.U.ET-20.012, date: 14.02.2022).

**Informed Consent:** : Informed consent was obtained from all participants included in the study.

### *Acknowledgement*

This work was supported by the Gazi University Scientific Research Projects Coordination Unit (05/2020-07).

### *Footnotes*

### *Authorship Contributions*

Concept: A.B., Ş.C.C., Design: A.B., Ş.C.C., Supervision: Ş.C.C., Resources: Ş.C.C., Data Collection or Processing: A.B., E.N.G., Ş.C.C., Analysis or Interpretation: A.B., E.N.G., Ş.C.C., Literature Search: A.B., E.N.G., Ş.C.C., Writing: K.K., Ş.C.C., Critical Review: K.K., Ş.C.C.

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** Gazi University Scientific Research Projects Coordination Unit (05/2020-07).

## REFERENCES

1. Soysal G, Akman Yılmaz A. Diyabetli Hastanın Periopratif Yönetimi. In: Akbas E, editor. Sağlık Bilim 2022 Hemşirelik-III, Efe Akademik Yayıncılık, 2022:141.
2. The International Diabetes Federation. Turkey Diabetes report 2000 - 2045. 2021.
3. Hamamcıoglu AC. The The role of oxidative stress and antioxidants in diabetes mellitus. Turk J Diab Obes. 2017; 1: 7-13.
4. Tombulturk FK, Soydas T, Kanigur-Sultuybek G. Topical metformin accelerates wound healing by promoting collagen synthesis and inhibiting apoptosis in a diabetic wound model. Int Wound J. 2024; 21: e14345.
5. Kopal C, Deveci M, Oztürk S, Sengezer M. Effects of topical glutathione treatment in rat ischemic wound model. Ann Plast Surg. 2007; 58: 449-55.
6. Yager DR, Kulina RA, Gilman LA. Wound fluids: a window into the wound environment? Int J Low Extrem Wounds. 2007; 6: 262-72.
7. Jomova K, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Valko M. Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. Arch Toxicol. 2024; 98: 1323-67.
8. Seung K. Yoon. Oleuropein as an Antioxidant and Liver Protect. In: Vinood BP, Rajkumar R, Victor RP, editor. The Liver, Academic Press, 2018:323-335.
9. Galicia-Moreno M, Lucano-Landeros S, Monroy-Ramirez HC, Silva-Gomez J, Gutierrez-Cuevas J, Santos A, et al. Roles of Nrf2 in liver diseases: molecular, pharmacological, and epigenetic aspects. Antioxidants (Basel). 2020; 9: 980.

10. de Oliveira MR. Carnosic Acid as a Promising Agent in Protecting Mitochondria of Brain Cells. Mol Neurobiol. 2018; 55: 6687-99.
11. Bahri S, Jameleddine S, Shlyonsky V. Relevance of carnosic acid to the treatment of several health disorders: molecular targets and mechanisms. Biomed Pharmacother. 2016; 84: 569-82.
12. Devipriya N, Sudheer AR, Menon VP. Dose-response effect of ellagic acid on circulatory antioxidants and lipids during alcohol-induced toxicity in experimental rats. Fundam Clin Pharmacol. 2007; 21: 621-30.
13. Wei YZ, Zhu GF, Zheng CQ, Li JJ, Sheng S, Li DD, et al. Ellagic acid protects dopamine neurons from rotenone-induced neurotoxicity via activation of Nrf2 signalling. J Cell Mol Med. 2020; 24: 9446-56.
14. Nieto G, Ros G, Castillo J. Antioxidant and antimicrobial properties of rosemary (Rosmarinus officinalis, L.): a review. Medicines (Basel). 2018; 5: 98.
15. Boulebd H. Modeling the peroxyl radical scavenging behavior of Carnosic acid: Mechanism, kinetics, and effects of physiological environments. Phytochemistry. 2021; 192: 112950.
16. Xiang Q, Liu Z, Wang Y, Xiao H, Wu W, Xiao C, et al. Carnosic acid attenuates lipopolysaccharide-induced liver injury in rats via fortifying cellular antioxidant defense system. Food Chem Toxicol. 2013;53:1-9.
17. Fuhrman B, Volkova N, Rosenblat M, Aviram M. Lycopene synergistically inhibits LDL oxidation in combination with vitamin E, glabridin, rosmarinic acid, carnosic acid, or garlic. Antioxid Redox Signal. 2000; 2: 491-506.
18. Kim S, Nishimoto SK, Bumgardner JD, Haggard WO, Gaber MW, Yang Y. A chitosan/beta-glycerophosphate thermo-sensitive gel for the delivery of ellagic acid for the treatment of brain cancer. Biomaterials. 2010; 31: 4157-66.
19. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol. 1978; 52: 302-10.
20. Aykaç G, Uysal M, Yalçin AS, Koçak-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. 1985; 36: 71-6.
21. Berger J, Shepard D, Morrow F, Taylor A. Relationship between dietary intake and tissue levels of reduced and total vitamin C in the nonscorbutic guinea pig. J Nutr. 1989; 119: 734-40.
22. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide Biol Chem. 2001; 5: 62-71.
23. Reznick AZ, Packer L. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Methods Enzymol. 1994; 233: 357-63.
24. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, et al. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. 1990; 186: 464-78.
25. Komsa-Penkova R, Spirova R, Bechev B. Modification of Lowry's method for collagen concentration measurement. J Biochem Biophys Methods. 1996; 32: 33-43.
26. Deng L, Du C, Song P, Chen T, Rui S, Armstrong DG, et al. The role of oxidative stress and antioxidants in diabetic wound healing. Oxid Med Cell Longev. 2021; 2021: 8852759.
27. Chaudhary P, Janmeda P, Docea AO, Yeskaliyeva B, Abdull Razis AF, Modu B, et al. Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. Front Chem. 2023; 11: 1158198.
28. Abdelkader NF, Elyamany M, Gad AM, Assaf N, Fawzy HM, Elesawy WH. Ellagic acid attenuates liver toxicity induced by valproic acid in rats. J Pharmacol Sci. 2020; 143: 23-29.
29. Caylak E. Hayvan ve bitkilerde oksidatif stres ile antioksidanlar. Tıp Araştırmaları Derg. 2011; 9: 73-83.
30. Bagheri Tadi F, Noori Mougehi SM, Mostafavinia A, Moheghi A, Amini A, Rezaei F, et al. Photobiomodulation isolated or associated with adipose-derived stem cells allograft improves inflammatory and oxidative parameters in the delayed-healing wound in streptozotocin-induced diabetic rats. Lasers Med Sci. 2022; 37: 3297-308.
31. Tsikas D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. Anal Biochem. 2017; 524: 13-30.
32. Zhu H, Yan Y, Jiang Y, Meng X. Ellagic acid and its anti-aging effects on central nervous system. Int J Mol Sci. 2022; 23: 10937.
33. Gupta A, Singh AK, Kumar R, Jamieson S, Pandey AK, Bishayee A. Neuroprotective potential of ellagic acid: a critical review. Adv Nutr. 2021; 12: 1211-38.
34. Espín JC, González-Barrio R, Cerdá B, López-Bote C, Rey AI, Tomás-Barberán FA. Iberian pig as a model to clarify obscure points in the bioavailability and metabolism of ellagitannins in humans. J Agric Food Chem. 2007; 55: 10476–85.
35. Priyadarsini KI, Khopde SM, Kumar SS, Mohan H. Free radical studies of ellagic acid, a natural phenolic antioxidant. J Agric Food Chem. 2002; 50: 2200-6.
36. Chen X, Huang M, Liu D, Li Y, Luo Q, Pham K, et al. Absorption and transport characteristics and mechanisms of carnosic acid. Biology (Basel). 2021; 10: 1278.
37. Xu T, Zhou J, Zhu J, Zhang S, Zhang N, Zhao Y, et al. Carnosic acid protects non-alcoholic fatty liver-induced dopaminergic neuron injury in rats. Metab Brain Dis. 2017; 32: 483-91.
38. Weber D, Davies MJ, Grune T. Determination of protein carbonyls in plasma, cell extracts, tissue homogenates, isolated proteins: Focus on sample preparation and derivatization conditions. Redox Biol. 2015; 5: 367-80.
39. Balaban T. Balabanlı B, Balaban T. Investigation into the effects of boron on liver tissue protein carbonyl, mda, and glutathione levels in endotoxemia. Biol Trace Elem Res. 2015; 167: 259-63.
40. Karimi MY, Fatemi I, Kalantari H, Mombeini MA, Mehrzadi S, Goudarzi M. Ellagic acid prevents oxidative stress, ınflammation, and histopathological alterations in acrylamide-ınduced hepatotoxicity in wistar rats. J Diet Suppl. 2020; 17: 651-62.
41. Nisari M. Civan percemi (Archilia millefolium)'nin diyabetik sıçanların karaciğer dokusundaki antioksidan enzimler üzerine koruyucu etkisinin araştırılması. Sağlık Bilim Derg. 2019; 28: 172-6.
42. Shendge AK, Basu T, Panja S, Chaudhuri D, Mandal N. An ellagic acid isolated from Clerodendrum viscosum leaves ameliorates iron-overload induced hepatotoxicity in Swiss albino mice through inhibition of oxidative stress and the apoptotic pathway. Biomed Pharmacother. 2018; 106: 454-65.
43. Das S, Joardar S, Manna P, Dua TK, Bhattacharjee N, Khanra R, et al. Carnosic acid, a natural diterpene, attenuates arsenic-ınduced hepatotoxicity via reducing oxidative stress, mapk activation, and apoptotic cell death pathway. Oxid Med Cell Longev. 2018; 2018: 1421438.

44. Lei T, Lu T, Yu H, Su X, Zhang C, Zhu L, et al. Efficacy of vitamin c supplementation on chronic obstructive pulmonary disease (COPD): a systematic review and meta-analysis. Int J Chron Obstruct Pulmon Dis. 2022; 17: 2201-16.
45. Chugaeva UY, Raouf M, Morozova NS, Mahdavian L. Effects of L-ascorbic acid (C6H8O6: Vit-C) on collagen amino acids: DFT study. Amino Acids. 2023; 55: 1655-64.
46. Weiskirchen R, Weiskirchen S, Tacke F. Organ and tissue fibrosis: Molecular signals, cellular mechanisms and translational implications. Mol Aspects Med. 2019; 65: 2-15.
47. Duckworth C, Stutts J, Clatterbuck K, Nosoudi N. Effect of ellagic acid and retinoic acid on collagen and elastin production by human dermal fibroblasts. Biomed Mater Eng. 2023; 34: 473-80.
48. Darawsha A, Trachtenberg A, Levy J, Sharoni Y. The protective effect of carotenoids, polyphenols, and estradiol on dermal fibroblasts under oxidative stress. Antioxidants (Basel). 2021; 10: 2023.
49. Zhao ZJ, Wu DJ, Lv DL, Zhang BD, Chen L, Sun YQ. Ellagic acid inhibits the formation of hypertrophic scars by suppressing TGF-β/Smad signaling pathway activity. Chem Biol Drug Des. 2023; 102: 773-81.
50. Zhang S, Wang Z, Zhu J, Xu T, Zhao Y, Zhao H, et al. Carnosic acid alleviates bdl-ınduced liver fibrosis through mir-29b-3p-mediated ınhibition of the high-mobility group box 1/toll-like receptor 4 signaling pathway in rats. Front Pharmacol. 2018; 8: 976.