Turk J Pharm Sci 2022;19(2):132-137
DOI: 10.4274/tjps.galenos.2021.84555

ORIGINAL ARTICLE

Protective Role of *Diospyros lotus* L. in Cisplatin-Induced Cardiotoxicity: Cardiac Damage and Oxidative Stress in Rats

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ABSTRACT

Objectives: Cisplatin is a powerful chemotherapeutic drug that is used to treatment a wide variety of cancers. Despite clinical data demonstrating the cardiotoxic effect of cisplatin, few studies have been carried to improve the cardiotoxicity of cisplatin. In cisplatin-induced toxicity, oxidative stress plays a critical role. This study determined the effect of *Diospyros lotus* L. fruit (DL), a powerful antioxidant plant, on heart damage caused by cisplatin through histological examination and oxidative stress parameters.

Materials and Methods: Twenty eight male rats were randomly divided into four groups. An isotonic solution was given to the control group. A single dose of 7 mg/kg cisplatin was administered intraperitoneally to the cisplatin group. 1.000 mg/kg DL was given by gavage for 10 days to the DL group. Cisplatin and DL were administered together in the same doses to the treatment group. Thiobarbituric acid reactive substances (TBARS) levels, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) activities, and total glutathione (GSH) level were measured in the heart tissue of the experimental rats. Histological examination was also performed to determine any damage to the hearts of the experimental rats.

Results: While TBARS levels in the cisplatin group increased significantly, SOD, CAT, GPx activities, and total GSH level decreased significantly. TBARS levels decreased significantly and SOD, CAT, GPx activities and GSH levels increased with DL treatment. According to the histological examination, histopathological differences were observed in the cisplatin group. Histopathological findings were either absent or decreased in the DL-treated group.

Conclusion: Results of the study showed that DL therapy reduced oxidative stress and histological changes caused by cisplatin. DL could be a potential candidate for reducing cardiac damage caused by cisplatin.

Key words: *Diospyros lotus*, cisplatin, cardiotoxicity, oxidative stress

INTRODUCTION

Cisplatin, an extremely effective chemotherapeutic drug, is a platinum-based drug with strong activity against ovarian, cervical, testicular, bladder, lung cancers, and solid tumors, which are resistant to other treatments.[1,2] Cisplatin shows a cytotoxic effect by cross-linking on DNA with purine bases, causing DNA damage and apoptosis in cancer cells. Notwithstanding the effect stated above, cisplatin provides more than 90% recovery in testicular cancer.[3]

The serious side effects of cisplatin such as neurotoxicity, nephrotoxicity, gastrointestinal disorders, reproductive toxicity, and bone marrow suppression limit its use in therapy.[4] Some studies have suggested that cisplatin treatment may cause cardiotoxicity.[5-7] Heart failure, arrhythmias, myocardial infarction, pericarditis, myocarditis, and congestive cardiomyopathy have been defined as cardiotoxic symptoms caused by cisplatin chemotherapy.[6,7] Cardiotoxicity by cisplatin limits its clinical use. Cisplatin's cardiotoxic mechanism is fully

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Received: 25.03.2021, Accepted: 26.07.2021
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unknown, but it was proposed that cardiotoxicity can result from cisplatin's direct toxic impact on cardiac myocytes and the formation of reactive oxygen species (ROS).[8] Cisplatin reduces the activity of antioxidant enzymes in cardiac tissue, while increasing tissue total oxidant potential and lipid hydroperoxide levels.[9]

The presence of cisplatin in the blood even years after treatment may cause cardiotoxicity later in life. This situation poses a significant risk of cancer patients treated with cisplatin.[6,7,10] The treatment to cisplatin-induced cardiotoxicity is important. Although some cardioprotective strategies are available today, they are insufficient in preventing or reducing cardiotoxicity, especially in clinical practice.[11] According to some studies, cisplatin-induced toxicity is exacerbated by elevated oxidative stress. However, it has been suggested that various antioxidant treatments show beneficial effects by reducing oxidative stress.[5,9,12]

The date palm tree is a member of Ebenaceae family, which is grown in many regions such as Asia, Southern Europe, and Turkey. It has_a nutritious fruit used in traditional Chinese medicine.[12,13] *Diospyros lotus* L. fruits (DL) have significant antioxidant activity due to their phenolic content. Therefore, it is an effective source of natural antioxidants. DL is thought to be an important food product and its ingredients have health benefits.[13,14] A previous study suggested that sperm toxicity caused by cisplatin can be reduced with DL fruits.[12]

The purpose of this study was to investigate the easing effect of DL, which is thought to have high antioxidant potential, on the cardiotoxic effects of cisplatin. Hence, histopathological and biochemical changes caused by cisplatin in the heart were examined and the effects of DL treatment were investigated in this study. In this study, a single extract was prepared from the plant. Since the amount of phenolic substances in the extracts prepared with water, acetone, and methanol was higher in the water part, the aqueous extract was used in our study. Phenolic substance measurement was not performed.[12]

MATERIALS AND METHODS

Chemicals

Cisplatin (10 mg/10 mL, code 1876A) was purchased from Faulding Pharmaceuticals Plc (Warwickshire, UK). DL fruits were obtained from Kumludere village (Trabzon, Turkey) in autumn of 2020. All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Plant material and preparation of the extract

DL was collected in autumn in 2020 around Trabzon. The DL was dried at 38°C for 5 days in an oven and milled to 2-3 mm in size. Extract was obtained using distilled water for 24 h using the percolation method. The extract was filtered through filter paper. The filtrated part was freeze-dried until a crude solid extract was obtained.[15] Identification of the plant was performed by Assoc. Dr. Mustafa Karaköse from Department of Medicinal and Aromatic Plants, Espiye Vocational School, Giresun University (Turkey).

Twenty-eight adult male Spraque Dawley rats, 2-3 month-old and weighing 250-300 g, were used for the experiments. Rats were obtained from the Institute of Experimental Animals in Pamukkale, Turkey and maintenance was performed in these centers.

The rats were housed in a 12-hour daylight/12-hour dark cycle, ventilated, constant temperature (21 ± 1°C) rooms and sterile cages according to the standards. Their feeding was provided by standard rat pellet feed and tap water. No special diet was applied. All experimental applications were performed according to the "Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health". The experimental protocol was approved by the Ethical Committee on Animal Research of Pamukkale University (PAUHDEK -2021/09).

Drug administration

The 28 rats were divided into 4 groups. A single dose of cisplatin at 7 mg/kg was administered intraperitoneally (*i.p.*). Previous studies showed that a single dose of 7 mg/kg injection of cisplatin causes testicular damage in rats.[16,17]

The phenol content of the extract of DL in water is higher than that of extracts from methanol or acetone. Because of the antioxidant effect of the aqueous extract, DL was suspended in distilled water in this experiment. 1.000 mg/kg DL extract was administered for 10 days. The effective dose was determined by reference to the study of Rashed et al.[18] A control group was accepted as the negative control. Isotonic saline *i.p.* and distilled water was administered gavage. 7 mg/kg of cisplatin was given once to the cisplatin group. DL extract was given to DL group without cisplatin by gavage for 10 days. Cisplatin and DL were administered to the treatment group with the specified protocol. Because of the procedure, tissue and blood samples were taken. The heart tissue, which was quickly removed, was sliced on cold glass. Blood samples were centrifuged at 3000 rpm for 20 min at 4°C and blood serum was obtained. Tissue and serum samples were stored at -80°C.

Biochemical analysis

Care was taken to protect the cold chain in all homogenization processes. 1:10 (w/v) dilution was made by adding 150 mM KCl (pH 7.4) to homogenize the heart tissue. Homogenization was done in a teflon glass homogenizer. The examples were centrifuged for 40 min at 3500 rpm at +4°C using a refrigerated centrifuge. Catalase (CAT), thiobarbituric acid reactive substances (TBARS), and total glutathione (GSH) activities were analyzed in the homogenate. Superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were determined in the supernatant obtained after centrifugation.

Determination of thiobarbituric acid reactive substances activity

TBARS activity in homogenized tissue as a lipid peroxidation marker was determined by the Yagi[19] method. Under aerobic conditions and pH: 3.5, the tissue homogenate was precipitated with 10% trichloroacetic acid (TCA) and kept in a water bath for 15 min, then cooled and centrifuged at 3000 rpm for 10 min. The

supernatant obtained was incubated with TBA in a 95˚C water bath for 50 min and cooled. The absorbances of the pink-colored complex formed because of the reaction of malondialdehyde, the secondary product of lipid peroxidation, with TBA were measured at 532 nm using a spectrophotometer.

Determination of total glutathione levels

The supernatant was obtained from the homogenates centrifuged at 3500 rpm for 10 min with 10% TCA (0.2 M, pH: 8.9). Tris-ethylenediamine tetraacetic acid (EDTA) buffer solution and 0.01 M 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB) were added to the supernatants. DTNB is reduced by sulfhydryl compounds and forms a yellow complex, which is a disulfide compound. The optical density of this yellow compound was determined at 412 nm with a spectrophotometer using the Sedlak and Lindsay[20] method.

Determination of SOD activity

SOD enzyme activity was determined as described by Sun et al.[21] SOD determination method is based on nitroblue tetrazolium (NBT) reduction of superoxide produced by xanthine/xanthine oxidase system. This reaction was stopped by adding copper (II) chloride. The colored formazan product formed by the reduction of NBT by superoxide radicals was measured using a spectrophotometer at 560 nm.

Determination of CAT activity

The CAT activity of the heart tissue was analyzed using the Aebi[22] method. The catalytic activity of CAT decomposed H_2O_2 was added to the sample into water and oxygen. In the CAT determination, hydrogen peroxide showed maximum absorbance at 240 nm as measured by a spectrophotometer. The breakdown of H_2O_2 by CAT was followed by an absorbance reduction. This decrease in absorbance is directly proportional to the enzyme activity. CAT activity was determined by the absorbance difference *per* unit time.

Determination of GPx activity

GPx activity was analyzed according to the method of Paglia and Valentina.[23] GPx is an enzyme that converts H_2O_2 to water using reduced glutathione. GPx catalyzes the conversion of GSH to oxidized glutathione disulfide (GSSG) in the presence of H_2O_2. GSSG formed by GPx in the presence of H_2O_2 is converted back to GSH with the help of glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH). For this purpose, NADPH, GSH reductase, and sodium azide were prepared separately in buffered EDTA solution. The prepared solutions were added to the supernatant maintained and kept at room temperature for 30 min. GSH-Px activity was measured using a spectrophotometer at 340 nm, the absorbance difference that occurred in the optical density with the conversion of NADPH in the experimental environment to $NADP^+$.

Determination of total protein amount

The amount of protein in heart tissue was measured using $CuSO_4$, Folin-Ciocalteu reagent, and bovine serum albumin according to the Lowry et al.[24] Analysis procedure is as follows; alkaline copper (Cu^{+2}) forms a complex with peptide bonds and each 7 or 8 amino acid residues bind 1 atom of copper. When the Folin-Cieolteu reagent is added to the copper-treated mixture, a violet-blue color is formed, which is measured by a spectrophotometer at 700 nm.

Histological analysis

Heart tissue samples were fixed in 10% formaldehyde solution. Then, the samples embedded in paraffin were cut into sections of 5 µm thickness. Sections were stained with hematoxylin-eosin on a coverslip. Tissue samples were examined for necrosis, mononuclear cell infiltration, bleeding, vascular occlusion using a "Leica DFC280 light microscope" and a "Leica Q Win Image Analysis system (Leica Micros Imaging Solutions Ltd., Cambridge, UK)". The histopathological damage score was calculated according to the findings.

Statistical analysis

Biochemical and histological data are presented as mean ± standard deviation. The normal distribution of biochemical data was analyzed using the Shapiro-Wilk test. One-Way ANOVA and Tukey tests were used for biochemical statistical analysis. Kruskal-Wallis were used for histological results. For statistical significance, $p<0.001$ was accepted. "SPSS 13.0 (SPSS Inc., Chicago, Ill., USA)" and "MedCalc 11.0 (Belgium)" statistical programs were used for analysis.

RESULTS

Biochemical evaluation

TBARS levels, SOD, CAT, GPx activities, and total GSH levels in heart tissue was measured by spectrophotometric methods. The results showed that cisplatin administration significantly ($p<0.01$) increased TBARS levels and decreased SOD, GPx, CAT activities, and total GSH levels compared to all groups. There was no statistically significant difference between the control group and the DL-only groups in terms of biochemical parameters. TBARS levels increased with cisplatin in the treatment group and decreased significantly ($p<0.01$) with DL treatment. SOD, GPx, CAT activities, and total GSH levels decreased with cisplatin but increased again with DL treatment. DL treatment reduced the negative effects of cisplatin on biochemical parameters, as shown in (Table 1).

Histological evaluation

In the control (Figure 1A) and DL (Figure 1B) groups, the heart tissue was in normal histological appearance. Necrosis (Figure 2A), mononuclear cell infiltration (Figures 2B and E), hemorrhage (Figures 2C and D), vascular occlusion (Figure 2F) were determined in the cisplatin group. The damage score calculated according to histopathological findings increased significantly in the cisplatin group (2.29 ± 0.10) compared to the control (0.49 ± 0.08) (Table 2). Although histological changes decreased in the treatment group compared with the cisplatin group, small-vessel occlusion (Figure 3A) and mononuclear cell infiltration (Figure 3B) were observed. The level of damage decreased significantly in the treatment group (0.86 ± 0.10) (Table 2).

Table 1. The activities of TBARS, total GSH, CAT, SOD and GPx and in rat heart tissue (mean ± SD)

Groups	TBARS (nmol/g tissue)	Total GSH (nmol/mL)	CAT (U/mg protein)	SOD (U/mg protein)	GPx (U/mg protein)
Control	9.28 ± 0.92[a]	92.7 ± 3.41[a]	0.035 ± 0.0007[a]	34.8 ± 2.14[a]	256.5 ± 23.9[a]
CIS	15.5 ± 1.12[b]	71.1 ± 3.59[b]	0.022 ± 0.0005[b]	22.4 ± 2.48[b]	158.9 ± 25.2[b]
DL	8.92 ± 0.89[a]	97.3 ± 4.19[a]	0.036 ± 0.0005[a]	35.2 ± 3.61[a]	265.3 ± 23.6[a]
CIS + DL	12.4 ± 1.06[c]	85.1 ± 3.16[c]	0.029 ± 0.0008[c]	28.7 ± 2.47[c]	204.2 ± 28.1[c]

Values with different superscripts in the same column are statistically significantly different from each other ($p \leq 0.01$). a: Indicates that the control and DL group is different from the other groups, b: Indicates that the CIS group is different from the other groups, c: Indicates that the CIS + DL group is different from the other groups. TBARS: Thiobarbituric acid reactive substances, GSH: Glutathione, CAT: Catalase, SOD: Superoxide dismutase, GPx: Glutathione peroxidase, CIS: Cisplatin, DL: *Diospyros lotus*, SD: Standard deviation (n= 7)

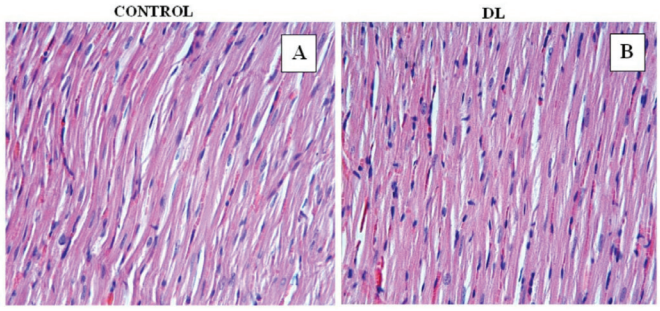


Figure 1. Control and DL group. In control and DL groups, heart tissue showed a normal histological appearance. (A) H-E; X40, (B) H-E; X40, DL: *Diospyros lotus* group, H-E: Hematoxylin-eosin

Table 2. Comparison of histopathological heart damage score between groups

Groups	Histopathological heart damage score (mean ± SD)
Control	0.49 ± 0.08[a]
CIS	2.29 ± 0.10[b]
CIS + DL	1.41 ± 0.12[c]
DL	0.86 ± 0.10[a]

a: Indicates that the control and DL group is different from the other groups, b: Indicates that the CIS group is different from the other groups, c: Indicates that the CIS+DL group is different from the other groups. The mean differences of values with different superscript letters in the same column are statistically significant ($p \leq 0.001$). CIS: Cisplatin, DL: *Diospyros lotus*), SD: Standard deviation (n= 7)

DISCUSSION

Cisplatin is a very potent chemotherapeutic drug. However, its adverse effects on the heart, kidneys, liver, and other organs limit its use in cancer treatment.[4,6] Approaches to reduce the toxic effects of cisplatin will contribute positively to increase the quality of life of cancer patients and to extending drug-dose limits. The toxicity caused by cisplatin is directly related to oxidative stress.[25] In this study, the easing role of DL, which has high antioxidant potential, against cisplatin-induced cardiotoxicity in rats was investigated. Although lipid peroxidation levels in heart tissue increased significantly in the cisplatin group, the antioxidant enzyme activities and total GSH levels decreased. In the group treated with DL, cisplatin-induced reductions in antioxidant enzyme activities and total

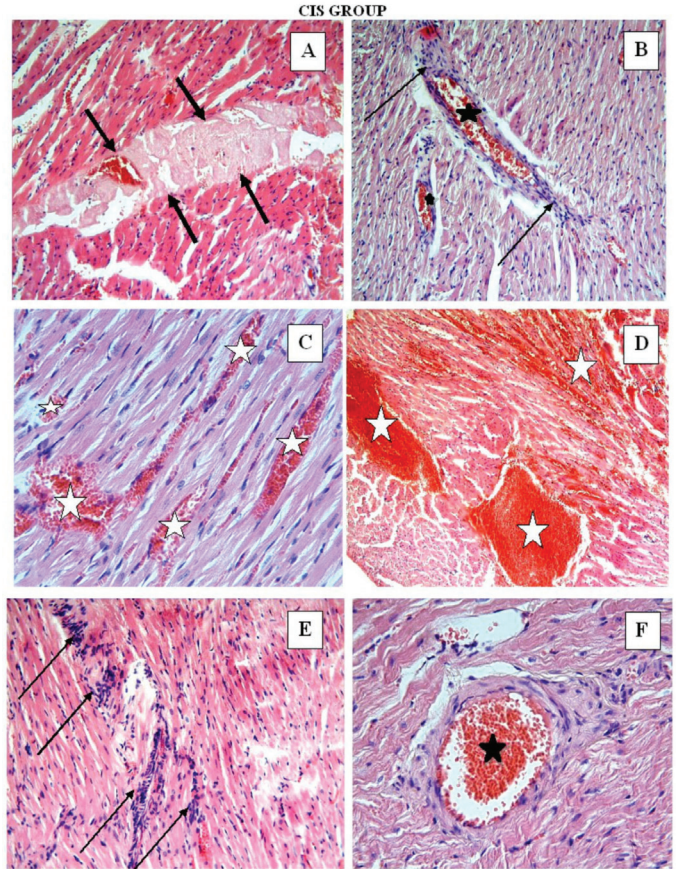


Figure 2. CIS group. Necrosis (black arrows) (A), mononuclear cell infiltration (thin black arrows) (B, E), hemorrhage (white asterisks) (C, D), vascular congestion (black asterisks) (F) were observed in CIS group. (A, B, E): H-E; X20, (D, F): H-E; X10, (C): H-E; X40. CIS: Cisplatin, H-E: Hematoxylin-eosin

GSH levels were improved. Lipid peroxidation levels decreased. According to the findings, DL showed a protective effect by reducing cisplatin cardiotoxicity. histopathological examination confirmed this suggestion.

Impairment of the balance between free radical levels and the antioxidant defense system causes oxidative stress. The increase in free radicals causes increased lipid peroxidation and decreased antioxidant enzymes because of impaired GSH metabolism. SOD, GPx, and CAT enzymes affect the endogenous defense mechanism. These enzymes can reduce

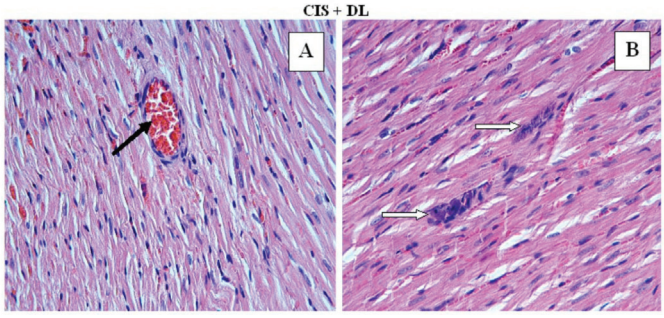


Figure 3. CIS + DL group. Histological changes were decreased in CIS + DL group compared with CIS group. Little vascular congestion (black arrow) (A), mononuclear cell infiltration (white arrow) (B) were observed in CIS + DL group. (A, B): H-E; X40. CIS: Cisplatin, DL: *Diospyros lotus*, H-E: Hematoxylin-eosin

oxidative stress by removing superoxide, hydrogen peroxide, and hydroxyl radicals.[26] Due to lipid peroxidation, TBARS are produced and are considered indicators of oxidative stress.[27] In this study, cisplatin was shown to increase TBARS levels in heart tissues of rats and cause lipid peroxidation. Similarly, different studies have suggested that cisplatin treatment causes cardiotoxicity with increased lipid peroxidation.[5,7,9] Lipid peroxidation causes cell and organ damage. Cardiotoxicity caused by cisplatin therapy has generally been associated with increased oxidative stress.[25] The glutathione mechanism converts cisplatin into a reactive form that can react rapidly with thiol molecules. Cisplatin reduces glutathione and other antioxidant enzymes, causing ROS accumulation in cells by disrupting the cell's redox mechanism.[5] Increased TBARS levels and decreased endogenous antioxidant enzyme activities are evidence that cisplatin causes oxidative stress. Increased oxidative stress caused damage and histopathological changes in the heart tissue. Previous studies have shown that reducing oxidative stress with antioxidant therapy attenuates cisplatin-induced organ damage.[1,25,28]

DL is a good natural source of antioxidants.[13,14] DL extracts can protect against oxidative stress by eliminating free radicals.[13] Its content of phenolic compounds and flavonoids act as a reducing agent and chelator against free radicals.[14,29] Azadbakhta et al.[30] showed that 1000 mg/kg DL reduced liver, kidney, and heart damage and improved organ morphology in streptozotocin-induced diabetic rats. Saral et al.[12] showed that 1000 mg/kg DL given reduced cisplatin-induced testicular damage. According to the data, in the group treated with DL, TBARS levels decreased significantly while total GSH, CAT, GPx, and SOD activities increased significantly. Observations of this study agree with the results of other previous studies.

Some histological changes such as vascular congestion, hemorrhage, vacuolization in the interstitial area, eosinophilic stained and pyknotic nuclei cells, mononuclear cell infiltration, and necrosis were observed in the cisplatin group. These results are consistent with the histopathological findings of previous cisplatin studies.[12] Histopathological findings were absent or decreased in the groups treated with DL, which alone did not cause any histological changes. Oxidative stress, which decreased with DL treatment, contributed to the recovery of heart tissue.

The results of this study showed that DL antioxidant potential reduces oxidative stress and histological changes caused by cisplatin-induced cardiotoxicity. Increased SOD, CAT, total GSH, and GPx activity as well as decreased TBARS levels suggested that the cardioprotective effect of DL is mediated by its antioxidant content.

CONCLUSION

Oxidative stress caused by cisplatin causes increased lipid peroxidation, decreased antioxidant enzyme activity, and histopathological changes in the heart. The results of this study showed that DL, which has strong antioxidant properties, reduces oxidative stress and histological changes caused by cisplatin treatments. The use of DL may help reduce the cardiotoxic side effects of cisplatin therapy in cancer, thereby increasing the effectiveness of the treatment. These ameliorative effects of DL are attributable to its antioxidant and radical scavenging properties. Therefore, we suggest that DL treatment may be used against CP-induced cardiotoxic toxicity.

ACKNOWLEDGMENTS

We would like to thank Assoc. Dr. Mustafa Karaköse for his contributions to the diagnosis and identification of the plant species used in the study.

Ethics

Ethics Committee Approval: The experimental protocol was approved by the Ethical Committee on Animal Research of Pamukkale University (PAUHDEK -2021/09).

Informed Consent: There is no need.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: N.B.T., D.A.Ö., Concept: Ö.S., Design: O.Ç., Data Collection or Processing: N.B.T., A.T., C.C.G., Analysis or Interpretation: N.B.T., A.T., Literature Search: N.B.T., D.A.Ö., Writing: N.B.T.

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

Financial Disclosure: The authors declared that this study received no financial support.

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