**Cukurova Medical Journal**
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2024;49(2):353-362
DOI: 10.17826/cumj.1429969

**RESEARCH**

# Effect of Cucumis melo var. agrestis Naudin on doxorubicin-induced cardiotoxicity in rats

## Cucumis melo var. agrestis Naudin'in sıçanlarda doksorubisin kaynaklı kardiyotoksisite üzerine etkisi

Emine Sarman[1]

[1]Afyonkarahisar Health Sciences University, Afyonkarahisar, Türkiye

**Abstract**

**Purpose:** Doxorubicin (DOX), a chemotherapeutic antibiotic, induces toxicity by also targeting non-cancerous cells. Cucumis melo var. agrestis Naudin (CM), a plant belonging to the Cucurbitaceae family with high antioxidant content, is examined in this study for its potential impact on DOX-induced cardiac damage at different doses.

**Materials and Methods:** 30 male rats were randomly divided into 5 groups, with 6 animals in each group: Control group, which received distilled water by gavage for 10 days, and intraperitoneal (i.p.) normal saline application on the 5th day of the experiment. The DOX group consisted of rats receiving a single i.p. dose of 15 mg/kg DOX on the 5th day of the experiment. Rats receiving a single intraperitoneal dose of 15 mg/kg DOX on the 5th day of the experiment were subjected to gavage for 10 days with doses of 100 mg/kg (DOX+CM100), 250 mg/kg (DOX+CM250), and 500 mg/kg (DOX+CM500) of CM, respectively. 24 hours after the last drug administration, the experimental animals were sacrificed under anesthesia. Heart tissue was examined histochemically and immunohistochemically.

**Results:** At the end of the experiment, histopathological examination of the heart tissue; Compared to the control group, histolopathological findings such as degeneration of muscle fibers, vacuole-like structures between muscle fibers, congestion in vessels, and edema between collaterals were observed in the DOX group. These findings significantly decreased in the DOX+CM250 treatment group. While an increase in Caspase-3, HSP 70 and NF-ϰB-p65 immunoreactivities was observed in the DOX group (+++); In the DOX+CM250 group, these findings decreased significantly (+).

**Conclusion:** DOX accelerated the apoptotic process, increased intracellular and oxidative stress, and triggered

**Öz**

**Amaç:** Kemoterapötik bir antibiyotik olan doksorubisin (DOX), kanserli olmayan hücreleri de öldürerek toksisiteye neden olur. Cucumis melo var. agrestis Naudin (CM), Cucurbitaceae familyasına ait olan ve yüksek antioksidan içeriğe sahip bir bitkidir. Bu çalışmanın amacı, DOX kaynaklı kalp hasarına karşı farklı dozlarda uygulanan CM etkisini araştırmaktır.

**Gereç ve Yöntem:** 30 adet erkek rat, rastgele her grupta 6 hayvan olacak şekilde 5 gruba ayrıldı: 10 gün gavajla distile su verilen sıçanlara, deneyin 5. gününde intraperitoneal (i.p.) serum fizyolojik uygulaması yapılan kontrol grubu. Deneyin 5. Günü 15 mg/kg tek doz i.p. DOX uygulanan sıçanlardan oluşan DOX grubu. Deneyin 5. Günü 15 mg/kg tek doz i.p. DOX uygulanan sıçanlara 10 gün gavajla sırasıyla 100 mg/kg: (DOX+CM100), 250 mg/kg: (DOX+CM250), 500 mg/kg: (DOX+CM500) CM uygulanan gruplar. Son ilaç uygulamasından 24 saat sonra deney hayvanları anestezi altında sakrifiye edildi. Kalp dokusu histokimyasal ve immunohistokimyasal olarak incelendi.

**Bulgular:** Deney sonunda kalp dokusunun histopatolojik incelenmesinde; kontrol grubuyla karşılaştırıldığında DOX grubunda kas liflerinin dejenerasyonu, kas lifleri arasında vakuol benzeri yapılar, damarlarda konjesyon, kollateraller arasında ödem gibi bulgular görülmüştür. Bu bulgular tedavi grubu olan DOX+CM250 grubunda önemli derecede azalmıştır. DOX grubunda Caspase-3, HSP 70 ve NF-ϰB-p65 immunreaktivitelerinde bir artış görülürken (+++); DOX+CM250 grubunda ise bu bulgular önemli derecede azalmıştır (+).

**Sonuç:** DOX'un apoptotik süreci hızlandırdığı, enflamatuvar yanıtı tetikleyerek hücre içi stresi ve oksidatif stresi arttırdığı histokimyasal ve immunohistokimyasal olarak görülmüştür. 250 mg/kg dozunda uygulanan CM kardiyak yeniden yapılanmayı hızlandırmıştır.

Address for Correspondence: Emine Sarman, Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Histology and Embryology, Afyonkarahisar, Turkey E-mail: emine.sarman@afsu.edu.tr
Received: 01.02.2024 Accepted: 08.04.2024

an inflammatory response, as demonstrated histochemically and immunohistochemically. CM administered at a dose of 250 mg/kg expedited cardiac remodeling.
**Keywords:**. Doxorubicin, Cucumis melo var. agrestis Naudin, Caspase-3, HSP 70, NF-κB-p65

**Anahtar kelimeler**: Doxorubicin, Cardiotoxicity, Cucumis melo var. agrestis Naudin, Caspase-3, HSP 70, NF-κB-p65

## INTRODUCTION

Doxorubicin (DOX) is a chemotherapeutic drug commonly used in the treatment of various malignancies; however, its use is limited due to cardiotoxic effects. The mechanisms by which doxorubicin induces cardiomyocyte death are not yet fully understood[1].

The molecular mechanisms related to DOX-induced cardiotoxicity primarily arise from the generation of reactive oxygen species during the drug's metabolism in the liver. This condition leads to oxidative stress, low levels of antioxidant enzymes, various adverse effects such as apoptosis and inflammation, and results in mitochondrial dysfunction[2]. It is presumed that mitochondrial dysfunction plays a crucial role in DOX-induced cardiotoxicity, resulting in cardiac myocyte death. Mitochondrial damage leads to cardiac injury through oxidative phosphorylation, and the decreased levels of antioxidant defense mechanisms (such as catalase, superoxide dismutase, and peroxidase) make cardiomyocytes more susceptible to cardiotoxicity compared to other organs like the liver and kidneys[3].

Studies have indicated the potential mitigating effects of medicinal plant extracts and natural products against doxorubicin-induced toxicity[4]. *Cucumis melo* var. *agrestis* Naudin (CM) is a garden plant belonging to the Cucurbitaceae family, widely distributed in many regions worldwide, and its fruit is commonly used[5,6]. Apart from its reported antidiabetic[7,8], anti-hyperlipidemic[9] and antioxidant properties[10], members of the gourd family are also suggested to be effective against ulcers, bronchitis, eye infections, and fever[8].

In a study evaluating the phytochemical analysis and antioxidant activity of different parts of *Cucumis melo* (leaves, stems, fruits, seeds, flowers, and roots), the fruit and flower extracts were noted for their strong antioxidant activity[11].

Ayadi et al.[12], demonstrated the therapeutic effects of the plant's seeds against various diseases, comprising various compounds such as phenols, flavonoids, and tannins. Research has shown that polyphenols, particularly flavonoids, reduce oxidative stress and play an antioxidant role[13].

In a study investigating the polyphenol-rich extract of *Cucumis melo* for its anti-diabetic, antioxidant, and anti-hyperlipidemic effects, Wistar albino rats were divided into groups: control (non-diabetic), diabetic control (alloxan-induced), and treatment groups (250 and 500 mg/kg CM doses). The ethanolic extract of CM was orally administered at different doses for 45 days, and biochemical and histopathological examinations were conducted. The research demonstrated that CM exhibited a hypolipidemic effect along with potent anti-diabetic and antioxidant activities[14].

There have been studies on the toxicity of DOX on the cardiovascular system, but no study on the effect of CM on the cardiac damage caused by DOX has been found in the literature. The aim of this study is to investigate the effects of CM administered at different doses in the cardiotoxicity induced by doxorubicin (DOX).

## MATERIALS AND METHODS

Ethical approval for the research project or animal experiments was received from Afyon Kocatepe University Animal Experiments Local Ethics Committee on 08.11.2023 with the decision numbered 49533702/112 with reference number AKUHADYEK-91-23. All ethical rules were complied with throughout the study.

### Experiment animals

Rats were housed in an environment with a temperature maintained between 21-22 degrees Celsius, subjected to a 12-hour light and 12-hour dark cycle. They were provided with an ad libitum feeding regimen. Thirty (30) adult male Wistar albino rats, weighing between 200-300 g, were randomly divided into 5 (five) groups, each containing 6 (six) rats:

Control Group (Group 1): Rats received distilled water via gavage for 10 days, with intraperitoneal (i.p.) saline (SF) administration on the 5th day of the experiment. A power analysis was conducted to determine the number of group samples required for the experiment[15].

DOX Group (Group 2): Rats received a single i.p. dose of 15 mg/kg doxorubicin (DOX) on the 5th day of the experiment.

DOX+CM100 Group (Group 3): Rats received 100 mg/kg of *Cucumis melo* var. *agrestis* Naudin (CM) via gavage for 10 days, with a single i.p. dose of 15 mg/kg DOX on the 5th day of the experiment.

DOX+CM250 Group (Group 4): Rats received 250 mg/kg of CM via gavage for 10 days, with a single i.p. dose of 15 mg/kg DOX on the 5th day of the experiment.

DOX+CM500 Group (Group 5): Rats received 500 mg/kg of CM via gavage for 10 days, with a single i.p. dose of 15 mg/kg DOX on the 5th day of the experiment. Twenty-four hours after the last drug administration, the experimental animals were sacrificed under anesthesia, and cardiac tissue samples were collected.

## Procedure

Twenty-four hours after the last drug administration, thirty rats were sacrificed under Ketamine (90 mg/kg; Alfamin, Alfasan IBV) / Xylazine (8-10 mg/kg; Bioveta, Czech Republic) anesthesia, and cardiac tissue samples were collected. For histological assessments, paraffin sections were stained with Hematoxylin-Eosin (HE) and Martius Scarlet Blue (MSB) and evaluated under a light microscope.

Immunohistochemical examinations were conducted to assess the immunoreactivity of Caspase-3, NF-ϰB-p65, and HSP 70 antibodies. Histochemical and immunohistochemical analyzes of the study were carried out by Emine Sarman in the laboratory of Histology and Embryology department of Afyonkarahisar Health Sciences University Faculty of Medicine.

### Preparation of the extract

Fresh fruits of *Cucumis melo* were cut into pieces and dried at room temperature for 24 h. After air drying, they were in a hot air oven at 40°C for 24 hours. Completely dried fruits were turned into powder. 10 grams of dried fruit powder was respectively extracted with 100 ml of water using a soxhlet apparatus and filtered through Whatmann No 1 filter paper and concentrated and stored at -20°C[16].

### Histochemical analysis

For the histological examination, heart tissues obtained from each group were placed in a 10% neutral buffered formaldehyde solution. After fixation in the solution, they were washed under running water overnight, dehydrated through a series of routine histological follow-up solutions (50%-60%-70%-80%-96%-100%), cleared in xylene, and embedded in paraffin. Sections of 5μ thickness were obtained from the prepared paraffin blocks using a rotary microtome. The paraffin sections were stained with Hematoxylin-Eosin (HE) and Martius Scarlet Blue (MSB) and evaluated under a light microscope. Based on the degree and extent of the changes, they were semi-quantitatively assessed as 0=normal, 1=mild, 2=moderate, and 3=severe[17].

### Immunohistochemical analysis

The Avidin-Biotin-Peroxidase Complex method was employed to determine the immunoreactivity of Caspase-3 (E-AB-66940, Elabscience, USA), NF-ϰB-p65 (E-AB-32233, Elabscience, USA), and HSP 70 (sc-32239, Santa Cruz, USA) in heart tissue. Sections of 5μ thickness were obtained from paraffin blocks. Deparaffinized tissues were passed through graded alcohol series and boiled in citrate buffer solution for 10 minutes for antigen retrieval. After treatment with H2O2 (TA-060-HP, Lab Vision Corporation, USA) and Ultra V Block (TA-125-UB, Thermo Scientific, UK) solutions for 5 minutes, they were incubated with the primary antibody (Caspase-3, NF-ϰB-p65, HSP 70) for 60 minutes. Subsequently, they were incubated in a moist environment at room temperature with secondary antibody (Biotinylated Goat Anti-Polyvalent TP-125-BN, Thermo Scientific, UK) and Streptavidin HRP (Horse radish peroxidase) (TS-125-HR, Thermo Scientific, UK) for 30 minutes, and then placed in PBS. After dropping DAB (3,3'-diaminobenzidine) (TA-125 HD, Thermo Scientific, UK) solution, the tissues were counterstained with Mayer's hematoxylin after obtaining the image signal under a light microscope. The tissues were washed in distilled water, dehydrated through reverse alcohols (70%-80%-96%-100-xylene), and mounted with Entellan (HX85172161, Merck, Germany). All preparations were examined and evaluated using Eclipse E-600 Nikon, Japan photomicroscope and image analysis

system (NIS Elements Nikon, Japan), and photographed. The intensity of staining was the main criterion for evaluating immunohistochemical staining. The intensity of immunostaining was semi-quantitatively scored as shown in the table (Table1)[17].

## Evaluation process

Structural changes in heart tissue sections from the experimental and control groups were assessed according to the scoring system established by (Table 1)[18].

**Table 1. Scoring table**

| | | |
|---|---|---|
| (-) score | eNegative score | It means the absence of any structural changes. |
| (+) score | 1 positive score | It refers to a slight structural change. |
| (++) score | 2 positive score | It represents moderate structural change. |
| (+++) score | 3 positive score | It represents a serious structural change. |

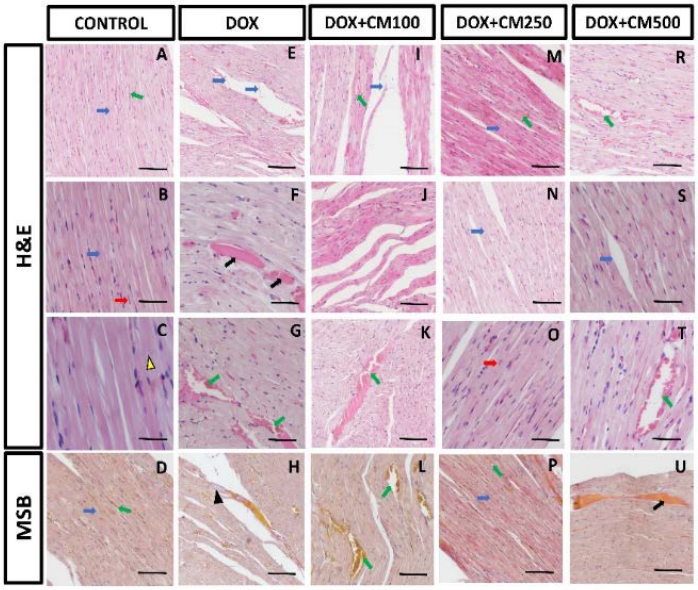


**Figure 1. Histopathological appearance of the cardiac tissue between the groups**

## Statistical analysis

Histochemical and immunohistochemical results were compared between the groups. The Shapiro-Wilk W test and Kruskal-Wallis H test were used to determine the normality of distributions. Tukey's test was used for normally distributed variables, while the Mann Whitney-U test was used for non-normally distributed variables. All data were analyzed using SPSS v. 24 (SPSS Inc., Chicago, IL, USA) statistical program and a significance level of $p < 0.05$ was considered statistically significant[17,19].

## RESULTS

The Control Group sections exhibit the normal histology of heart tissue. In the doxorubicin (DOX) group, degeneration of muscle fibers, vacuole-like structures between muscle fibers either individually or in groups, vessel congestion, and edema between collaterals are observed. In the DOX+CM100 group, separation and deterioration of muscle fibers and collaterals, as well as vessel congestion, are noted. It was observed that there was no significant change in

this treatment group, and the effect of DOX persisted. In the DOX+CM250 group, muscle fibers, blood vessels, euchromatic nuclei, and central cardiomyocyte nuclei appear close to normal. The most favorable effect was observed in this group, with the structures of heart tissue largely preserved compared to the DOX group. In the DOX+CM500 group, separation and disruption of congested muscle fibers and a vacuole-like structure were observed within the vessels (Figure 1, Table 2).

Groups; Control (A, B, C and D), DOX (E, F, G and H), DOX+CM100 (I, J, K and L), DOX+CM250 (M, N, O and P), DOX+CM500 (R, S, T and U). Histopathological appearance of the cardiac tissue between the groups. No histopathological findings were found in the control group. In the DOX group, degeneration of muscle fibers (blue arrow), vacuole-like structures between muscle fibers either singly or in groups (black arrow), congestion in blood vessels (green arrow), and edema between collaterals (black arrowhead) are observed. In the DOX+CM100 group, separation and disruption of muscle fibers and collaterals (blue arrow), and congestion in blood vessels (green arrow) are observed. In the DOX+CM250 group, muscle fibers (blue arrow), blood vessels (green arrow), euchromatic and central nucleus of cardiomyocytes (red arrow) exhibit a near-normal appearance. In the DOX+CM500 group, congestion in blood vessels (green arrow), separation and disruption of muscle fibers (blue arrow), and vacuole-like structures (black arrow) are observed. A, E, I, M, R, D, H, L, P, U Scale bar = 100 μm, x 200. B, C, F, G, J, K, N, O, S, T Scale bar = 50 μm, x 400.

**Table 2. Histological damage scores of heart tissues**

| | Control (n=6) | DOX (n=6) | DOX+CM100 (n=6) | DOX+CM250 (n=6) | DOX+CM500 (n=6) |
|---|---|---|---|---|---|
| Myofibril degeneration | 0.17 ± 0.389 | 2.83 ± 0.389 [a] | 2.75 ± 0.452 [b] | 1.25 ± 0.452 [c,e] | 2.33 ±0.492 [d] |
| Vacuole-like structures between myofibrils | 0.08 ± 0.289 | 2.83 ± 0.389 [a] | 2.75 ± 0.452 [b] | 1.17 ± 0.389 [c,e] | 2.50 ± 0.522 [d] |
| Congestion in the vessels | 0.08 ± 0.289 | 2.83 ± 0.389 [a] | 1.92 ± 0.515 [b] | 1.83 ± 0.389 [c,e] | 2.67 ± 0.492 [d] |
| Degeneration of collaterals | 0.17 ± 0.389 | 2.75 ± 0.452 [a] | 2.67 ± 0.492 [b] | 1.17 ± 0.389 [c,e] | 2.17 ±0.389 [d] |
| Edema between collaterals | 0.08 ± 0.289 | 1.83 ± 0.389 [a] | 2.83 ± 0.389 [b] | 1.33 ± 0.492 [c,e] | 2.83 ± 0.389 [d] |

DOX: Doxorubicin, CM: Cucumis melo var. agrestis Naudin, a:Control vs. DOX ($p < 0.001$). b: Control vs. DOX+CM100 ($p < 0.01$) c: Control vs. DOX+CM250 ($p < 0.01$) d: Control vs. DOX+CM500 ($p < 0.01$) e: DOX vs. DOX+CM250 ($p < 0.001$).

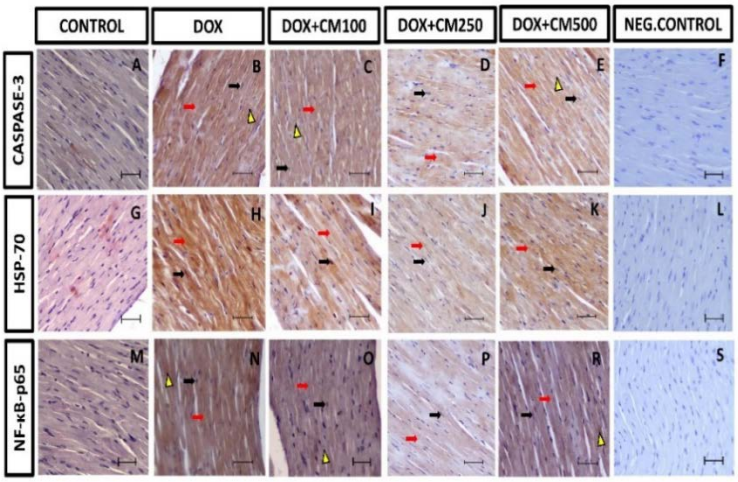


**Figure 2. Immunoreactivities of Caspase-3, HSP 70 and NF-*κ*B-p65 on cardiac tissue**

### Caspase-3 immunoreactivity

No staining was observed in the heart tissue of the Control Group. In the DOX group, intense staining was observed in myofibrils, intercalated discs and cardiomyocytes. Moderate staining was observed in myofibrils, discus intercalaris and cardiomyocytes in the DOX+CM100 and DOX+CM500 groups. In the DOX+CM250 group, minimal staining was observed in myofibrils and cardiomyocytes (Figure 2, Figure 3, Table 1).

### HSP 70 immunoreactivity

No staining was observed in the heart tissue of the Control Group. Intense staining was observed in myofibrils and cardiomyocytes in the DOX Group. Moderate staining was observed in myofibrils, intercalated discs and cardiomyocytes in the DOX+CM100 and DOX+CM500 groups. In the DOX+CM250 group, slight staining was observed in myofibrils and cardiomyocytes (Figure 2, Figure 3, Table 1).

### NF-κB-p65 immunoreactivity

No staining was observed in the heart tissue of the Control Group. In the DOX Group, intense staining was seen in myofibrils, intercalated discs and cardiomyocytes. Moderate staining was observed in myofibrils, intercalated discs and cardiomyocytes in the DOX+CM100 and DOX+CM500 groups. Light staining was observed in myofibrils and cardiomyocytes in the DOX+CM250 group (Figure 2, Figure 3, Table 1).

Groups; Control (A, G and M), DOX (B, H and N), DOX+CM100 (C, I and O), DOX+CM250 (D, J and P), DOX+CM500 (F, K and R). Negative control (F). Caspase-3 (A, B, C, D and E), HSP 70 (G, H, I, J and K) and NF-κB-p65 (M, N, O, P and R). Caspase-3, HSP 70 and NF-κB-p65 immunoreactivity in the control groups heart tissue, no staining is observed. In the DOX group, intense staining (+++) is observed in myofibrils (red arrow), intercalated discs (yellow arrowhead), and cardiomyocytes (black arrow). In the DOX+CM100 and DOX+CM500 groups, moderate staining (++) is observed in myofibrils (red arrow), intercalated discs (yellow arrowhead), and cardiomyocytes (black arrow). In the DOX+CM250 group, mild staining (+) is observed in myofibrils (red arrow) and cardiomyocytes (black arrow). Scale bar = 50 μm, x 400.

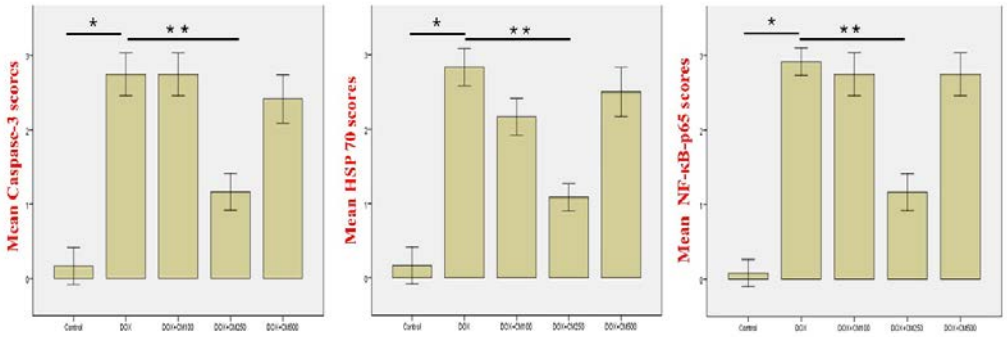


**Figure 3. Immunohistochemical findings for caspase-3, HSP 70 and NF-κB-p65 on cardiac tissue. DOX: Doxorubicin, CM: Cucumis melo var. agrestis Naudin. '*' represents p <0.001, '**' represents p<0.01.**

## DISCUSSION

The conducted studies have mostly focused on the anti-diabetic effects of *Cucumis melo* var. *agrestis* Naudin. However, there has been no prior research on the cardioprotective effects of *Cucumis melo* var. *agrestis* Naudin against the damage caused by Doxorubicin (DOX). Therefore, our study is distinctive as it reveals histochemical and immunohistochemical results in this context.

Doxorubicin, classified under the anthracycline group and used in anticancer therapy, induces cardiotoxic effects[20]. Specifically, it leads to harmful effects such as increased reactive oxygen species

(ROS), DNA damage, lipid peroxidation, irreversible damage to mitochondria, and cell apoptosis[21].

In a rat model examining Doxorubicin-induced cardiotoxicity, one group received 15 mg/kg Doxorubicin for three weeks, while another group received 60 mg/kg/day Doxorubicin + sacubitril/valsartan. After 6 weeks, echocardiography was performed. It was demonstrated that sacubitril/valsartan significantly reduced Doxorubicin-induced cardiac dysfunction, apoptosis, and expression levels of ER stress-related proteins[22]. In our study, a single dose of 15 mg/kg Doxorubicin (DOX) was administered for 10 days, and different doses of *Cucumis melo* var. *agrestis* Naudin (CM) (100 mg/kg - 250 mg/kg - 500 mg/kg) were administered via gavage. Following histochemical and immunohistochemical examinations, it was observed that DOX induced heart damage, while CM administered at a dose of 250 mg/kg mitigated this damage. Thus, our findings align with parallel studies in this field.

In another study evaluating Doxorubicin-induced cardiotoxicity, groups were categorized as acute lymphoblastic leukemia children treated with black seed oil and those treated with Doxorubicin alone. It was observed that black seed oil had a protective effect, showing improved systolic functions and ameliorating certain cardiac side effects[23].

Efforts are underway to mitigate the cardiotoxic effects of Doxorubicin[24]. In a study investigating the efficacy of carvedilol in inhibiting anthracycline-induced cardiotoxicity, it was demonstrated that low-dose carvedilol (6.25 mg twice daily) could prevent anthracycline-induced cardiomyopathy and hinder the development of cardiotoxicity[25].

A prospective randomized controlled design with a 10-year long-term follow-up was conducted to evaluate the application of Doxorubicin with a cardioprotective agent. The study involved patients with Hodgkin or non-Hodgkin lymphoma (HL or NHL) receiving metoprolol or enalapril. The long-term follow-up did not reveal any additional benefits from the combined administration of metoprolol and enalapril[20].

In a study examining the effect of Necrostatin-1 (Nec-1) on Doxorubicin-induced cardiotoxicity, the control group exhibited normal heart histology. The Doxorubicin (DOX) group displayed hyalinization, edema, and congestion in muscle fibers. In the Dox + Nec-1 treatment group, a histological appearance close to normal was observed, with pyknotic nuclei and small vacuole-like structures, as demonstrated by hematoxylin-eosin and Masson trichrome staining[26]. Our histopathological examinations in this study revealed degeneration of muscle fibers, vacuole-like structures between muscle fibers, congestion in blood vessels, and edema between collaterals in the DOX group. In the treatment group, a histological appearance close to normal heart histology was observed, aligning with the findings of the conducted study.

In a study conducted by Ibrahim et al., the methanolic extract of *Cucumis melo* L. (Cucurbitaceae) seeds was investigated phytochemically. It was reported that the extract exhibited selective and potent effects on SKOV-3 and MCF-7 cell lines, compared to doxorubicin (IC50 values of 0.05 uM), while showing moderate activity against the HCT-116 cell line[27].

Another study evaluating the antioxidant activity and pharmacological properties of *Cucumis melo* Var. applied 100-200-300 mg of *Cucumis melo* extract to Wistar albino rats. The study revealed that the highest antioxidant and anti-inflammatory content was observed in rats treated with 300 mg of *Cucumis melo* extract, indicating its potential in preventing free radical formation[28]. However, in our study, the group treated with 250 mg/kg of *Cucumis melo* var. *agrestis* Naudin (CM) in combination with doxorubicin (DOX) (DOX+CM250) showed a close-to-normal appearance in muscle fibers, blood vessels, euchromatic, and central cardiomyocyte nucleus. The application of CM at a dose of 100 mg/kg did not demonstrate a therapeutic effect on cardiac tissue. Additionally, the application of CM at a dose of 500 mg/kg appeared to induce cardiotoxic effects, showing discrepancies compared to the referenced study.

In a research evaluating the effects of orally administered *Cucumis melo* var. *agrestis* (CMVA) in diabetic rats for 45 days, the study demonstrated a significant reduction in blood sugar levels in the CMVA-treated group. Oxidative stress and lipid parameters returned to normal levels. Microscopic examinations revealed degeneration, dilation, congestion, and pancreatic asinus cells in the pancreatic tissue of the experimental group. In the CMVA-treated group, protective effects on the regeneration of pancreatic β-cells were attributed to flavonoids and phenolic components, contributing to the improvement of oxidative stress[14].

In a study evaluating the effect of misoprostol on doxorubicin-induced damage, histological examinations revealed necrosis of muscle fibers, mononuclear cell infiltration, and hemorrhagic areas in the doxorubicin (DOX) group. Immunohistochemical examinations indicated intense staining in Caspase-3 and NOX-4 immunoreactivity in the DOX group[29]. In our study, the DOX group exhibited the most intense staining for Caspase-3 immunoreactivity in myofibrils, intercalated discs, and cardiomyocytes. In the DOX+CM100 and DOX+CM500 groups, moderate Caspase-3 immunoreactivity was observed in myofibrils, intercalated discs, and cardiomyocytes, while in the DOX+CM250 group, a decrease in Caspase-3 immunoreactivity was observed in myofibrils and cardiomyocytes. A study evaluating doxorubicin-induced cardiac damage reported a significant increase in Caspase-3 immunostaining in the DOX group compared to the control group[18]. Apoptosis refers to programmed cell death and is executed by intracellular Caspase-3 and Caspase-7. It leads to cell shrinkage, chromatin fragmentation, membrane blebbing, and the formation of membrane-enveloped vesicles called apoptotic bodies [30,31]. Doxorubicin has been shown to induce apoptosis through oxidative stress and inflammation, as well as the Caspase-3 pathway[18]. Our study suggested that CM has a cardioprotective effect, achieved by enhancing the antioxidant defense system, inhibiting inflammatory responses, and suppressing apoptosis.

In a study, male Wistar rats were treated with a single dose of doxorubicin (20 mg/kg, i.p), and immunohistochemical examinations showed that the control group's heart tissue had sparse brownish cytoplasmic areas in NF-κB p65 immunostaining. In the DOX group, there were areas with intense brown staining[32]. Another research indicated that doxorubicin triggered myocarditis and increased the infiltration of inflammatory cells in the left ventricles. NF-κB-p65 was immunohistochemically examined to understand NF-κB activation, revealing a significant reduction in NF-κB-p65 immunoreactivity in the treatment groups [33]. In our study, the DOX group exhibited intense staining for NF-κB-p65 immunoreactivity in myofibrils, intercalated discs, and cardiomyocytes, while the DOX+CM250 group showed a decrease in staining. NF-κB has emerged as a crucial transcription factor in the cell's response to apoptosis, with significant effects on normal development and/or homeostasis in many tissues. The mode of action of NF-κB can be both cytoprotective and pro-apoptotic[34]. In this regard, our study is consistent with previous research, and it was observed that NF-κB contributes to cardiac remodeling and dysfunction[35].

HSP 70 is a crucial protein group with an impact on the regulation of cardiac remodeling[36]. Extracellular HSP 70 released from damaged cardiomyocytes is considered a potential therapeutic target in the treatment of heart hypertrophy and fibrosis[37,38]. In our study, intense expression of HSP 70 was observed in myofibrils and cardiomyocytes in the DOX group. In the DOX+CM100 and DOX+CM500 groups, HSP 70 was moderately expressed in myofibrils, intercalated discs, and cardiomyocytes, while in the DOX+CM250 group, it was least expressed. Inadequate or excessive production of HSP 70 can disrupt cell homeostasis, modulate apoptotic cell death, and activate cellular stress[36]. HSP 70, a heat shock protein, is a group of proteins whose production increases when cells are exposed to high temperatures. Moreover, any factor that can induce stress increases the activation of HSP 70. In our study, DOX increased HSP 70 immunoreactivity by inducing oxidative stress.

In a study investigating the relationship between HSP 70 expression and heart failure and the protective effect of HSP 70 against doxorubicin-induced toxicity, it was noted that doxorubicin administration led to a dose- and time-dependent decrease in the activity of endogenous antioxidant enzymes. However, HSP 70 exhibited a protective effect. Additionally, it is known that HSP 70 prevents the accumulation of misfolded proteins, inhibits myocardial cell death pathways, and regulates ion channels, providing a cardioprotective effect[39,40]. Moreover, in a study demonstrating that treatment with liposomal HSP 70 increased cellular HSP 70 levels in primary macrophages in a dose-dependent manner, it was shown that intracellular HSP 70 negatively modulated pro-inflammatory signaling[41]. No rat study examining HSP 70 immunoreactivity in doxorubicin-induced cardiac damage has been encountered, making our study unique in this regard.

Consistent with other studies in the literature, our study confirms that DOX causes cardiac damage. We evaluated this damage histochemically and immunohistochemically, revealing significant findings. Our study is unique as it demonstrates the protective effect of CM DOX-induced cardiac damage, albeit being preliminary and not evaluated in

terms of biochemical and genetic aspects. This constitutes a limitation of our study.

In conclusion, our study suggests that the administration of CM at a dose of 250 mg/kg exhibits a protective effect against DOX-induced cardiac damage by reducing apoptosis and oxidative stress. As there is no prior research on the cardioprotective effect of CM, our study fills this gap, providing specific histochemical and immunohistochemical results highlighting the cardioprotective potential of CM against DOX-induced heart damage. We recommend further investigations to explore the protective effectiveness of CM using different parameters in future studies.

**Author Contributions:** Concept/Design : ES; Data acquisition:ES; Data analysis and interpretation: ES; Drafting manuscript: ES; Critical revision of manuscript: ES; Final approval and accountability: ES; Technical or material support: ES; Supervision: ES; Securing funding (if available): n/a.
**Ethical Approval:** Ethical approval was obtained by the decision of the Local Ethics Committee of Animal Experiments of Afyon Kocatepe University dated 08.11.2023 and numbered 49533702/112.
**Peer-review:** Externally peer-reviewed.
**Conflict of Interest:** Authors declared no conflict of interest.
**Financial Disclosure:** Authors declared no financial support

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