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PROTECTIVE ROLE OF SELENIUM ON THE HEART OF RATS EXPOSED ACRYLAMIDE

AKRİLAMİDE MARUZ KALAN SIÇANLARIN KALP DOKUSU ÜZERİNDE SELENYUMUN KORUYUCU ROLÜ

Nasıf Fatih KARAKUYU[1], Halil AŞCI[2], Mehtap SAVRAN[2], Kanat GÜLLE[3]

[1] Süleyman Demirel Üniversitesi, Eczacılık Fakültesi, Farmakoloji Ana Bilim Dalı, Isparta, TÜRKİYE
[2] Süleyman Demirel Üniversitesi, Tıp Fakültesi, Tıbbi Farmakoloji Ana Bilim Dalı, Isparta, TÜRKİYE
[3] Süleyman Demirel Üniversitesi, Tıp Fakültesi, Histoloji-Embriyoloji Ana Bilim Dalı, Isparta, TÜRKİYE

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Öz

Amaç
Akrilamid (ACR), farklı endüstriyel alanlarda yaygın olarak kullanılan zararlı organik reaktif bir bileşiktir. Selenyum (SEL), hem hayvanlarda hem de insan organizmalarında meydana gelen bazı kimyasal ve enzimatik reaksiyonlarda yer alan ve eksikliği durumunda kardiyovasküler sistem gibi birçok sistemde bazı rahatsızlıklara neden olabilen bir eser elementtir. Bu çalışmanın amacı, sıçanlarda deneysel olarak oluşturulan ACR toksisitesinde SEL'in kalp dokusu üzerinde koruyucu bir etkisinin olup olmadığını araştırmaktır.

Gereç ve Yöntem
Toplam 28 sıçan rastgele ve eşit olarak dört gruba ayrıldı: Kontrol, SEL, ACR, SEL + ACR. Çalışma sonunda kan örneklerinden kreatin kinaz MB (CKMB), laktat dehidrojenaz (LDH) ve iskemi modifiye albümin (IMA) gibi kardiyak belirteçler ile kalp dokusundan toplam oksidan durumu (TOS), toplam antioksidan durumu (TAS) ve oksidatif stres indeksi (OSI) gibi oksidatif stres belirteçlerinin analizleri yapıldı. Ayıca kalp kesitlerinde immünohistokimyasal yöntemler kullanılarak indüklenebilir nitrik oksit sentaz (iNOS) aktiviteleri belirlendi.

Bulgular
ACR + SEL grubunda, ACR grubuna göre TOS, OSI, CKMB, LDH, IMA düzeylerinde anlamlı düşüş ve TAS düzeyinde önemli artış gözlemlendi. Kalp dokusu kesitlerinin histopatolojik ve immünhistokimyasal değerlendirmesinde; ACR grubunda kalp kası liflerinde düzensizlik, kas lifleri arasında bağ dokusu artışı ve kas liflerinde düzensiz eozinofili tespit edildi. SEL uygulanan gruplarda ACR grubuna göre histopatolojik bulgularda azalma gözlendi. iNOS immünreaktiviteleri; ACR grubunda orta düzeyde ekspresyon gözlenirken, ACR + SEL grubunda daha zayıf bir ekspresyon gözlendi.

Sonuç
ACR'nin sıçanların kalp dokusunda çeşitli metabolik yollarla hasara yol açtığını ve bu hasarın SEL verilen gruplarda tersine döndürüldüğünü belirledik.

Anahtar Kelimeler: Akrilamid, Antioksidan, Selenyum, Sıçan

Abstract

Objective
Acrylamide (ACR) is a harmful organic reactive compound widely used in different industrial fields. Sele-

Sorumlu yazar ve iletişim adresi /Corresponding author and contact address: N.F.K. / fatihkarakuyu03@gmail.com
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ORCID IDs of the authors: N.F.K: 0000-0002-2249-4668; H.A: 0000-0002-1545-035X; M.S: 0000-0002-7933-0453; K.G: 0000-0002-6337-8962

nium (SEL) is a trace element that takes part in some chemical and enzymatic reactions in both animals and human organisms and can cause some disorders in many systems such as the cardiovascular system in case of deficiency. The aim of this study is to investigate whether SEL has a protective effect on cardiac tissue in experimentally induced ACR toxicity in rats.

Material and Method
A total of 28 rats were randomly and equally divided into four groups: Control, SEL, ACR, SEL + ACR. At the end of the study, cardiac markers such as creatine kinase MB (CKMB), lactate dehydrogenase (LDH) and ischemia-modified albumin (IMA) from blood samples were measured. Oxidative stress markers such as total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI) in heart tissue were analyzed. In addition, inducible nitric oxide synthase iNOS activities were determined in heart sections using immunohistochemical methods.

Results
The ACR + SEL group showed a significant decrease in TOS, OSI, CKMB, LDH, IMA levels and significant increase in TAS level compared to the ACR group. In histopathological and immunohistochemical evaluation of the heart sections; Disorganization in cardiac muscle fibers, increased connective tissue between muscle fibers and irregular eosinophilia in muscle fibers were detected in ACR group. A decrease in histopathological findings was observed in the SEL treated groups compared to the ACR group. iNOS immunoreactivities; moderate marking was observed in the ACR group sections, while poor marking was observed in the ACR + SEL group.

Conclusion
We determined that ACR caused damage to the heart tissue of rats through various metabolic pathways and that these damages were reversed in the groups given the SEL.

Keywords: Acrylamide, Antioxidant, Rat, Selenium

Introduction

Industrialization, with the development of technology, causes the pollution of natural resources and can cause health problems on various living things, especially human beings. Acrylamide (ACR) is an organic reactive compound that is widely used in different industrial areas and forms polyacrylamide by polymerization. Edible foods are main sources of ACR. Especially it found in heated foods because of the reaction between reduced sugars and amino acids. High concentrations have been observed in products such as bread, coffee, and potatoes (1). In addition, the ACR has been classified as "Group 2A carcinogenic" by the International Agency for Research on Cancer (IARC) and it has been reported that the major tobacco derivatives are caused by fumes and environmental pollutants in water sources (2). Studies on ACR have become widespread in recent years due to its toxic effects on animals and humans (1).

There are very few in vivo studies conducted in experimental animals on heart tissue sensitive to exposure of environmental pollutants such as ACR (3). ACR is metabolized by the CYP2E1 enzyme within the cytochrome P450 enzyme family in the liver, conjugated with glutathione or glydamide (4). Glutathione (GSH) has an important role in preventing toxicity caused by ACR in various systems (5). Oxidative stress and inflammation are among the basic mechanisms of this toxicity. Reactive oxygen species (ROS) formed by these two mechanisms and the increase in the production of cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β) are responsible (4, 6).

Selenium (SEL) is a trace element involved in some chemical and enzymatic reactions as glutathione peroxidase (GSH-Px) synthesis that occur in both animals and human organisms, and in case of deficiency, it can cause some disorders in many systems such as cardiovascular system (7, 8). In addition, in some studies, it has been shown that SEL has a protective effect in cases of toxicity caused by some substances that are toxic to the organism, called xenobiotics, including heavy metals (7).

When damage occurs in the body for any reason, the antioxidant enzyme system is activated. This system causes the use of antioxidant enzymes. For this reason, there is a decrease in the levels of these enzymes in the blood and tissues. Failure to replace this activity with exogenous antioxidant supplementation results in insufficient antioxidant activity, and the oxidant-antioxidant levels in balance shift towards oxidant. For example, increased oxidative stress as a result of the decrease in GSH-Px enzyme activity, of which SEL is a cofactor, damages the cell membrane due to lipid peroxide accumulation and makes the cell vulnerable

(9). Antioxidant activity of selenium has been proven in many studies (10, 11).

In addition to the antioxidant effect of SEL, there are studies showing that it has an anti-inflammatory effect by reducing the level of some inflammation markers such as interleukin-6, TNF-α and C-reactive protein in the treatment of many chronic inflammatory disease (12). Orally administered SEL shows its anti-inflammatory effect by preventing the adhesion of monocyte to the endothelium (13). As known, monocyte adhering to endothelial cells infiltrates into tissue spaces and transforms into macrophages, which play a major role in inflammation (14). For these reasons mentioned above, it is known that SEL has cardioprotective effects against the cardiotoxic effects of some xenobiotic substances (15).

There are 3 forms of nitric oxide synthase which are inducible nitric oxide synthase (iNOS), endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS). iNOS contributes to the pathophysiology of inflammatory diseases and septic shock, and increased levels of iNOS in inflammation have been shown to cause endothelial dysfunction (16-21).

In this study, we aimed to investigate whether SEL has a protective effect on cardiac tissue in the ACR toxicity created experimentally in rats.

Material and Method

Animals and Ethical Approval

All experiments were performed under the guidelines for animal research from the National Institutes of Health and were approved by the Committee on Animal Research of Suleyman Demirel University (Approval No: 21/10/2020 08-06).

Twenty-eight male Wistar Albino rats, weighing 250–350 g, were placed in a room-controlled temperature (21°C -22 °C) and humidity (60% ± 5%) conditions, in which a 12:12 h light/dark cycle was maintained. All rats were fed a standard commercial chow diet (Korkuteli Yem, Antalya, TURKEY).

Experimental Design

The rats were distributed into the following four groups each containing seven rats: control, SEL, ACR and ACR+SEL groups.

1-Control group, from the 0th day until the 27th day, 1 mL saline per day were administered orally.

2- In the SEL group, 0.5 mL 0.1 mg/kg SEL (Sodium selenate, NS412130207, Alfa Aesar, USA) was dissolved in 0.5 mL saline once a day from the 0th day until the 27th day and administered orally (5).

3- In the ACR group, from the 0th day until the 27th day, 0.5 mL 20 mg/kg/day ACR (329747349 Sigma-Aldrich,) was dissolved in 0.5 mL saline and administered orally (22, 23).

4- In the ACR+SEL, from day 0th to day 27th, 0.5 mL 20 mg/kg/day ACR and 0.5 mL 0.1 mg /kg/day SEL was given orally.

24 h after the last application of ACR, all rats were anesthetized by an intraperitoneal injection of 90 mg/kg ketamine (Alfamin, Alfasan IBV) and 10 mg/kg xylazine (Alfazin, Alfasan IBV). After abdominal incision, blood samples were taken from vena cava inferior to analyses biochemical markers of cardiac damage. Then cardiac tissues were removed and half of the cardiac specimens were placed into liquid nitrogen and stored at -20 °C until the analysis of assayed for biochemical parameters. The remaining tissues were collected during necropsy and fixed in 10% buffered formalin for histopathological and immunohistochemical analyses.

Measurement of cardiac damage parameters in blood An auto analyzer (Beckman Coulter AU680, Pasadena, California, USA) was used to determine the creatine kinase MB (CKMB), lactate dehydrogenase (LDH) and ischemia-modified albumin (IMA) levels in the blood samples.

Measurement of Oxidative Stress Parameters in the Heart Tissue

The heart tissues were homogenized in a motor-driven tissue homogenizer (IKA UltraTurrax T25 Basic; Labortechnic, Staufen, Germany) and a sonicator (UW 2070 Bandelin Electronic, Germany) with phosphate buffer (pH 7.4). Unbroken cells, cell debris, and nuclei were sedimented by centrifugation at 10.000 g for 10 min. Protein levels in the homogenate and supernatant were determined according to the Bradford method (24). Total oxidant status (TOS) (RL0024, Relassay, Turkey) and Total antioxidant status (TAS) (RL0017, Relassay, Turkey) levels were determined using an automated colorimetric kit. The color intensity was related to the total amount of oxidant molecules, and the change in absorbance at 660 nm was related to the total antioxidant level of the sample, which was measured spectrophotometrically, as shown in the sample. The results are expressed as hydrogen peroxide equivalent per gram liter

(mmol H_2O_2 equiv./L, mmol H_2O_2 equiv./mg protein) for TOS levels and mmol Troloxequiv./mg protein for TAS levels. TAS and TOS levels were measured spectrophotometrically by the automated chemistry analyzer Beckman Coulter AU5800 (Japan) (25, 26).

Histopathological Analyses

Heart tissues obtained from animals were taken into 10% neutral formaldehyde solution and fixed. After the fixation was completed, the tissue samples were taken to the washing process. Washed samples, respectively; it was passed through alcohol batches, dehydrated, made transparent with xylol and embedded in paraffin. 4-5µm sections were taken from the obtained paraffin blocks (RM2125RTS, Leica, Germany). Samples belonging to all groups were stained with Hematoxylin-Eosin (H-E) for histopathological evaluation. Histopathological findings were evaluated under four headings; disorganization of muscle fibers, increased connective tissue between muscle fibers, irregular eosinophilia and mononuclear cell infiltration. Histopathological scoring was performed in terms of findings by examining different areas from each section with 40x objective. Structural changes in the hearth tissue sections of the experimental and control groups were evaluated according to the scoring made by Nezic et al (27) (Table 1). The images of the sections were obtained with imaging-supported binocular light microscope (ECLIPSE Ni-U, Nikon, Tokyo, Japan).

Immunohistochemical Analyses

In heart sections, iNOS activities were determined using immunohistochemical methods. Sections 4-5 µm thick were first deparaffinized and dehydrated. Then the sections were incubated with 3% hydrogen peroxide, Ultra-V Block (ThermoFisher), primary antibodies (Bioss), secondary antibody (ThermoFisher), streptavidin peroxidase (ThermoFisher), respectively. Core staining was done with hematoxylin, labeling with DAB solution (Vectorlab). Sections immunoreactivities, semi-quantitative scoring method were evaluated according to their degree of staining and sections were evaluated by imaging-assisted light microscope (ECLIPSE Images were acquired with Ni-U, Nikon, Tokyo, Japan) as shown in Table 1.

Statistical Analyses

Variables were presented as mean ± standard deviations. One way ANOVA (post hoc LSD) tests were used to compare biochemical, histopathological and immunohistochemical scores between the groups. Statistical calculations were made using the SPSS 18.0 program pack (SPSS Inc., Chicago, IL, USA). $p<0.05$ was set as the value for significance.

Results

Results of Cardiac Damage Parameters in Blood

CKMB, LDH and IMA levels increased significantly in the ACR group compared to the control group ($p\leq0.001$, $p\leq0.001$ and $p=0.001$, respectively) and compared to the SEL group ($p\leq0.001$, $p\leq0.001$ and $p=0.020$, respectively). CKMB and LDH levels increased significantly in the ACR + SEL group compared to the control group ($p=0.016$ and $p=0.015$, respectively). The ACR + SEL group showed a significant decrease in all parameter levels compared to the ACR group ($p\leq0.001$, $p=0.001$ and $p=0.025$) (Table 2).

Results of Oxidative Stress Parameters in the Heart Tissue

TAS levels decreased significantly in the ACR group compared to the control group ($p=0.023$) and a significant increase was observed in TAS levels in the SEL and ACR+SEL group compared to the ACR group ($p=0.016$ and $p=0.033$; respectively).

TOS and OSI levels increased significantly in the ACR group compared to the control group ($p\leq0.001$ for both) and SEL group ($p \leq 0.001$ for both). When

Table 1 Scoring table of histopathological and immunohistochemical evaluations

Symbol	Mean	Histopathological scores	Immunohistochemical scores
(-) score	negative score	no finding	no marking
(+) score	1 positive score	low level of finding	low marking
(++) score	2 positive scores	moderate finding	moderate marking
(+++) score	3 positive scores	severe finding	strong labeling

Table 2 Biochemical changes in blood of the experiment groups.

	CKMB (U/L)		LDH (U/L)		IMA (ABSU)	
Groups	**Mean±SD**	**P value**	**Mean±SD**	**P value**	**Mean±SD**	**P value**
Control	225.43 ± 27.50		480.83 ± 119.64		0.15 ± 0.03	
SEL	257.32 ± 62.92		600.39 ± 205.33		0.22 ± 0.02	
ACR	460.02 ± 52.81[a,b]	a≤0.001 b≤0.001	961.52 ± 63.38[a,b]	a≤0.000 b≤0.001	0.35 ± 0.14[a,b]	a=0.001 b=0.020
ACR+SEL	303.91 ± 57.02[a,c]	a=0.016 c≤0.001	675.50 ± 61.56[a,c]	a=0.015 c=0.001	0.22 ± 0.07[c]	c=0.025

Values are presented as means ± SD. The relationships between groups and results of biochemical markers are assessed by One-way ANOVA test (post hoc LSD test). CKMB: Creatine Kinase MB, LDH: Lactate Dehydrogenase, IMA: Ischemia-Modified Albumin, ACR: Acrylamide, SEL: Selenium, a: Compared with the control group, b: Compared with the SEL group, c: compared with the ACR group

Table 3 TAS, TOS, OSI values in groups of heart tissue.

	TAS (mmol Trolox Equivalents/L)		TOS (μmol H_2O_2 Equivalents/L)		OSI	
Groups	**Mean±SD**	**P value**	**Mean±SD**	**P value**	**Mean±SD**	**P value**
Control	0.82±0.13		6.30±1.42		0.75±0.19	
SEL	0.84±0.29		6.34±1.56		0.80±0.45	
ACR	0.57±0.11	a=0.023 b=0.016	10.17±1.76	a≤0.001 b≤0.001	1.77±0.35	a≤0.001 b≤0.001
ACR+SEL	0.79±0.15	c=0.033	7.89±1.25	c=0.022	1.07±0.25	c=0.003

Values are presented as means ± SD. The relationships between groups and results of oxidative stress markers are assessed by One-way ANOVA test (post hoc LSD test). TAS: Total Antioxidant Status TOS: Total Oxidant Status, OSI: Oxidative stress index, ACR: Acrylamide, SEL: Selenium. a: Compared with the control group, b: Compared with the SEL group, c: compared with the ACR group.

the ACR+SEL group was compared with the ACR group, it was observed that there was a significant decrease in TOS and OSI levels (p=0.022 and p=0.003, respectively) (Table 3).

Histopathological Results

No histopathological findings were found in the sections belonging to the control group and SEL groups (Figure 1A, 1B). Disorganization in cardiac muscle fibers, increased connective tissue between muscle fibers and irregular eosinophilia in muscle fibers were detected in sections belonging to the ACR group (Figure 1C). A decrease in histopathological findings was observed in the ACR+SEL group compared to the ACR group (Figure 1D). The findings are summarized in Table 4.

Immunohistochemical Results

iNOS immunoreactivities were not observed in heart tissue of control and SEL group (Figure 2A, 2B). Moderate marking was observed in the ACR group sections, while poor marking was observed in the ACR + SEL group (Figure 2C, 2D).

Table 4 Histopathological Findings

Parameters / Group	Control	SEL	ACR	ACR+SEL
Disorganization of muscle fibers	-	-	+++	+
Increased connective tissue between muscle fibers	-	-	+++	+
Irregular eosinophilia in fibers	-	-	+++	+
Mononuclear cell infiltration	-	-	-	-

ACR: Acrylamide, SEL: Selenium.

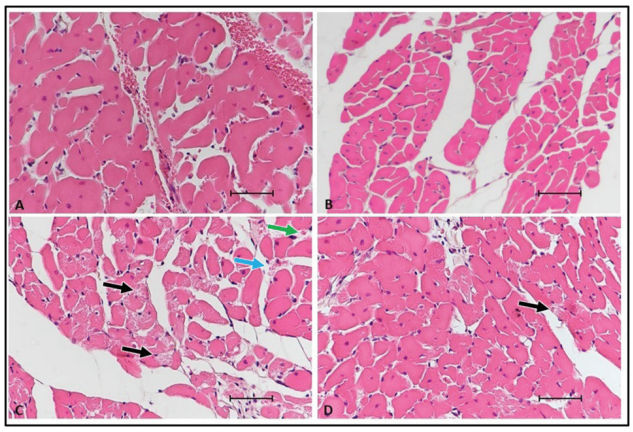

Figure 1:
Heart tissue histopathology between the groups.
(A) Normal heart tissue structure in the control group, (B) SEL group normal heart tissue histology, (C) ACR group disorganization in muscle fibers (black arrow), increase in connective tissue between muscle fibers (blue arrow) and irregular eosinophilia in heart tissue (green arrow), (D) Low level histopathological findings in the ACR+SEL group. ACR: Acrylamide, SEL: Selenium, HE, Bar 50 µm, 40x

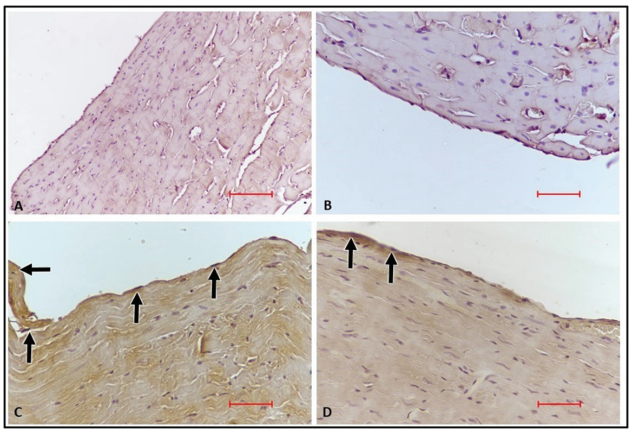

Figure 2:
iNOS immunoreaction of the hearts.
(A) No immunoexpression in the control group, (B) SEL group no immunoreactivity, (C) Moderate immunoreactivity in the ACR group (black arrow), (D) Low level of immunoreactivity in the SEL+ACR group. ACR: Acrylamide, SEL: Selenium, iNOS: Inducible Nitric Oxide Synthase, Scale Bar 50 µm, 40x.

Discussion

ACR is a water-soluble unsaturated amide used in the production of polyacrylamides (28). Orally taken into the body, ACR passes through many organs through the circulatory system and causes toxic effects by reacting with DNA and various enzymes (29, 30). ACR is not a toxic substance by itself, it is oxidized by the CYP2E1 enzyme to activate a genotoxic metabolite, glydamide (31, 32). It has been shown in many studies that ACR, which has become widespread in use with the development of industrialization in recent years, is common in fast-food style foods, has carcinogenesis relationship and genotoxic effects (33, 34).

SEL is an important antioxidant enzyme and has an active role in the immune system (35). It performs this antioxidant function with the GSH-Px enzyme system, by eliminating harmful radicals released as a result of oxidative stress (36, 37). There are many animal studies showing that SEL, which is frequently encountered in foods, especially in grains, meat and seafood, plays an active role in preventing and reducing cancer cases caused by radiation and various chemical carcinogens (38). In the light of this information, we investigated the toxic effects of ACR on heart tissue and whether SEL, known as an antioxidant element, has a protective effect on these tissues.

First of all, we found that CKMB, LDH and IMA levels, which are markers of cardiac damage for the damaged heart tissue, increased significantly in the ACR group compared to the other groups. In the study conducted

by Kushwah et al., it was observed that similar results were found with our study and that ACR increased the levels of cardiac damage markers by causing damage to the heart tissue (39). As a result of these studies, the existence of cardiotoxic effects of ACR on heart tissue can be considered, and our study may be a guide to investigate the cardiotoxic effect of ACR on other living things, especially humans. In addition, when we compared the treatment group with the ACR group, we observed a significant decrease in CKMB, LDH and IMA levels in ACR + SEL group. Güneş et al. investigated the cardioprotective effects of SEL against cyclophosphamide-induced cardiotoxic effects in rats, they found that the activities of these enzyme markers decreased significantly in the SEL treated group (15). In recent studies, the anti-inflammatory activity of selenium with similar markers is in parallel with our study (40,41). Considering these similar results in this study, SEL may have an anti-inflammatory effect against the damage caused by various agents and the inflammation caused by this damage.

In this study, ACR not only led to an increase in biochemical marker levels, but also histopathological, it caused some changes such as disorganization in cardiac muscle fibers, increased connective tissue between muscle fibers and irregular eosinophilia in muscle fibers. Again, in the study of Kushwah et al., increased inflammatory cell infiltration, loss of myocardial tissue and interstitial spaces trying to fill its place, nuclear loss and vacuolization in the heart tissues of rats in the ACR group were shown (39). Considering the histopathological changes caused by ACR on the cardiac tissue in other studies in the literature, especially the cause of myocardial dysfunction shows that similar histopathological findings have emerged through different mechanisms in our study (42, 43). In addition, similar to the result in the study of Soudani et al., a decrease in histopathological findings was observed in the treatment group compared to the ACR group (44).

Oxidative stress occurs due to impaired defense mechanisms in the body and uncontrolled increase in ROS amounts. TAS is a parameter that shows the function of antioxidants in preventing the formation of free radicals in the body. However, TOS is a parameter that shows the severity of oxidative stress in the body and the total peroxide amount of macromolecules at the tissue and cell level (45).

OSI is also defined as a general indicator of oxidative stress showing the ratio of TOS levels to TAS levels (46). In this study, the reason for the decrease of TAS levels may be due to the depletion of antioxidant enzymes as a result of the usage or less production. Similar to this study, Taşkın et al. investigated the protective effect of SEL on Adriamycin-induced damage in rats, and it was shown that the groups given SEL caused an increase in mitochondrial and cytosolic TAS levels compared to other groups with toxic agents causing oxidative stress (47). TOS and OSI levels also increased significantly in the ACR group as a result of the comparison with other groups. In a study conducted by Dönmez et al. on ACR-induced liver toxicity, it was found that TOS and OSI values reached the highest levels in the ACR group (48). In another study by Huang et al. investigating ACR-induced oxidative stress and cardiovascular toxicity in zebrafish embryos, they found that ROS levels increased significantly in the ACR group compared to the other groups (3). It has been supported that ACR causes oxidative stress in different studies (49,50). When we evaluate the results of these studies together with the results of our own study, it can be concluded that ACR has toxic effects on various tissues by increases of oxidative stress parameters and SEL reversed all these parameters.

NO, which is released in excessive amounts as a result of the overexpression of iNOS, which is required in normal physiological processes, plays a role in inflammatory processes and sepsis (51). When we examined the immunohistochemical results of our study, an increase in iNOS immunoreactivity was observed in the ACR group in cardiac tissue similar with literature. For example, in a study by Wei et al. using ACR in female mice, it was shown that iNOS immunreactivity were significantly increased in ovaries (52). In another study examining the levels of NOS subtypes after ACR exposure in rat brain tissue, iNOS levels were found to be higher in the rat group given subacute ACR (53). However, there is no study in the literature about iNOS levels in a rat heart exposed to ACR. Studies on different tissues found that SEL reduced iNOS levels compared to the damage groups (54,55). The decrement of iNOS expressions in SEL treated groups showed that SEL has an anti-inflammatory effect against ACR induced cardiac damage.

In this study, we aimed to examine the toxic effect of ACR on the heart tissue, which has become increasingly widespread in industrial areas and foodstuffs in recent years. According to results, ACR caused damage to the heart tissue through the enhancement of inflammatory and oxidant conditions, SEL treatment reversed all these pathological findings. In the light of the data in the literature, more animal

and human studies are needed to evaluate the cardiotoxic effects of ACR with known carcinogenic and genotoxic effects on rats. In addition, sensitivity should be shown to minimize the use of ACR in the industrial field. However, further studies are needed to support that the SEL element, which has an active role in the immune system, is also protective on the cardiotoxic effects of ACR.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Ethical Approval
Our study was approved by the Animal Research Ethics Committee of Suleyman Demirel University (21/10/2020 08-06).

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Availability of Data and Materials
Authors can confirm that all relevant data are included in the article and/or its supplementary information files. Data sharing not applicable.

Authors Contributions
NFK: Conceptualization; Data curation; Funding acquisition; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing-original draft.

HA: Conceptualization; Formal analysis; Investigation; Methodology; Project administration; Supervision; Writing-review & editing.

MS: Conceptualization; Formal analysis; Investigation; Methodology; Project administration; Validation; Writing-review & editing.

KG: Formal analysis; Investigation; Methodology; Validation; Writing-review & editing.

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Although MS and KG are editorial board members of the journal, they have not taken part in any stage of the publication processes of this article.

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