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Investigation of the Effects of Chrysin Against Azithromycin-Induced Heart Damage in Rats

Sıçanlarda Azitromisin ile Oluşturulan Kalp Hasarına Karşı Krisin'in Etkilerinin Araştırılması

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ABSTRACT

Azithromycin (AZM) is macrolide antibiotic used to treat infections of the upper and lower respiratory tract. In addition to its therapeutic effects, it has adverse effects such as cardiac and oxidative damage. Chrysin (CHR), which is found in propolis and various plants, is a natural flavonoid known for its antioxidant properties. In this study, we investigated the protective effect of CHR against cardiac damage caused by AZM, a broad-spectrum antibiotic. For this purpose, twenty-eight female rats were divided into four groups: Control, CHR, AZM, AZM+CHR. AZM (200 mg/kg) and CHR (50 mg/kg) were administered orally once daily for seven days. Cardiac markers and oxidative stress parameters were analyzed to determine heart tissue damage. Histopathological analyses were performed to detect tissue damage and structural changes. According to the data obtained from these analyses, AZM increased lactate dehydrogenase (LDH) and creatine kinase-myocardial band (CK-MB) activities and cardiac troponin-I (cTn-I) levels in the heart tissue. AZM toxication significantly increased malondialdehyde (MDA) levels while reducing the activities of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) activities and glutathione (GSH) levels. AZM+CHR treatment decreased cardiac tissue cardiac markers (LDH, CK-MB, and cTn-I). In addition, CHR treatment together with AZM decreased MDA levels and increased GSH levels and GPx, SOD, and CAT activities. When the findings were evaluated together, it was determined that AZM caused heart damage by increasing cardiac markers and oxidative stress, while CHR supplementation reduced the damage by bringing these parameters closer to normal.

Keywords: Azithromycin, chrysin, heart, oxidative stress, rat

ÖZ

Azitromisin (AZM), üst ve alt solunum yolu enfeksiyonlarının tedavisinde kullanılan bir makrolid antibiyotiktir. Terapötik etkilerinin yanında kardiyak ve oksidatif hasar gibi olumsuz etkilere sahiptir. Propoliste ve çeşitli bitkilerde bulunan Krisin (CHR), antioksidan özelliğiyle bilinen doğal bir flavonoiddir. Bu çalışmada, geniş spektrumlu bir antibiyotik olan AZM'nin neden olduğu kalp hasarına karşı CHR'nin koruyucu etkisi araştırıldı. Bu amaçla, yirmi sekiz dişi sıçan Kontrol, CHR, AZM, AZM+CHR olmak üzere dört gruba ayrıldı. AZM (200 mg/kg) ve CHR (50 mg/kg) yedi gün boyunca günde bir kez oral yoldan uygulandı. Kalp dokusunda hasarı belirlemek için kardiyak belirteçler ve oksidatif stres parametreleri analiz edildi. Doku hasarını ve yapısal değişiklikleri tespit etmek için histopatolojik analizler yapıldı. Bu analizler sonucunda elde edilen verilere göre AZM, kalp dokusunda laktat dehidrogenaz (LDH), kreatin kinaz-miyokardiyal bant (CK-MB) aktiviteleri ve kardiyak troponin-I (cTn-I) seviyesini artırdı. AZM toksikasyonu, süperoksit dismutaz (SOD), glutatyon peroksidaz (GPx), katalaz (CAT) aktiviteleri ve glutatyon (GSH) seviyeleri gibi antioksidan enzimlerin aktivitelerini azaltırken, malondialdehit (MDA) seviyelerini önemli ölçüde artırmıştır. AZM+CHR tedavisinin kalp dokusu kardiyak belirteçlerinde (LDH, CK-MB, cTn-I) azalma gösterdiği tespit edildi. Ayrıca, CHR tedavisinin AZM ile birlikte uygulanması MDA düzeyini düşürmüş ve GSH düzeyini ve GPx, SOD ve CAT aktivitelerini artırmıştır. Elde edilen bulgular birlikte değerlendirildiğinde, AZM'nin kardiyak belirteçleri ve oksidatif stresi artırarak kalp hasarına neden olduğu, CHR destekleyici tedavisinin ise bu parametreleri normale yakınlaştırarak hasarı azalttığı tespit edildi.

Anahtar Kelimeler: Azitromisin, kalp, krisin, oksidatif stres, sıçan

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INTRODUCTION

Azithromycin (AZM) is a effectively used broad-spectrum antibiotic of the macrolide group.[1,2] It has been reported have broad antimicrobial activity against both aerobic and anaerobic bacteria.[3,4] The Food and Drug Administration (FDA) reported that it can be used in respiratory infections.[2] In 2012, the FDA warned that AZM carries fatal cardiovascular risks. In 2013, it stated that AZM could cause potentially irregular heart rhythms in electrical activity of the heart.[1,5] Wei et al.[6] They examined the effects of AZM and reported that it caused cardiovascular malformations as a result of their findings. Different studies have stated that AZM causes heart damage, but the reasons underlying this damage mechanism have not been fully elucidated.[1,7] In addition, it has been reported that the risk of cardiovascular death increases in patients treated with AZM.[2] AZM administration is believed to increase the production reactive oxygen species (ROS) in heart tissue, and increased ROS triggers oxidative stress and myocardial tissue apoptosis, which increases heart damage.[1,2,8] The increase in ROS production is balanced by glutathione (GSH).[9] Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) also contribute to the process by neutralizing ROS and strengthening the defense system. Insufficiency of the mentioned antioxidants or constantly triggered production of ROS leads to disruption of the antioxidant-oxidant balance.[3] It has been reported that plant-based flavonoid and phenolic compounds, which are frequently used in alternative medicine and included in treatment protocols, prevent the development of many diseases.[10]

Chrysin (CHR) is a natural flavonoid found in plants including propolis, honey, shell of some walnut species, passion flower, wild Himalayan pear and bitter melon, used for therapeutic purposes.[11,12] Due to the presence of hydroxyl groups in the seventh-fifth positions, it is effective in eliminating free radicals and preventing the formation of oxidative stress.[13-15] In different studies where CHR was used as a supportive treatment, its antioxidant, antiapoptotic and anti-inflammatory properties have been determined and it has been stated it is effective in reducing or preventing many tissue damage thanks to these properties.[16-18]

The aim of the study was to investigate the effects of CHR on cardiac damage caused by AZM, which is frequently used in treatment.

MATERIALS AND METHODS

Chemicals

AZM (Azitro Tablet 500 mg, Tekirdağ) and CHR (Sigma, Cas. No: 480-40-0, 97% purity) were obtained commercially. Other chemicals were of analytical grade and were supplied by Sigma.

Ethical Approval

Ethical approval was obtained from the Atatürk University Animal Experiments Local Ethics Committee (Date: 31.01.2025, Approval No: 2025/01/22).

Groups and Experimental Procedures

Twenty-eight female *Sprague-Dawley* rats (220-250 g) were obtained from Atatürk University Experimental Research and Application Center (ATADEM) (Erzurum/Türkiye). The rats were housed in cages at 24-25 °C with a 12-hours light-dark cycle. Groups (n = 7):

1) Control: Orally administered saline for seven days.
2) Chrysin (CHR): 50 mg/kg CHR administered orally for seven days.[16]
3) Azithromycin (AZM): 200 mg/kg AZM administered orally for seven days.[19]
4) Azithromycin+Chrysin (AZM+CHR): 50 mg/kg CHR administered half an hour after 200 mg/kg AZM for seven days.

After the treatments were completed (day 8), rats were decapitated under light sevoflurane (Sevorane®; Queenborough, UK) anesthesia and heart tissue was collected. A portion of the heart tissue was stored at −80 °C for biochemical examination, while the rest was stored in 10% formaldehyde solution for histological examination.

Analysis of Cardiac Markers

The lactate dehydrogenase (LDH) and creatine kinase-myocardial band (CK-MB) activities and cardiac troponin-I (cTn-I) level were determined by using rat ELISA kit of Sunred Biological Technology (Shanghai, China, LDH Cat. No: 201-11-0531, CK-MB Cat. No: 201-11-0312 and cTn-I Cat. No: 201-11-0640). Analysis was performed by ELISA Plate Reader (Bio-Tek, Winooski, VT) according to the instruction of manufacturer.

Oxidative Stress Analyses

Heart tissue was homogenized with 1.15% potassium chloride (KCl) for GSH and malondialdehyde (MDA) levels and CAT, glutathione peroxidase (GPx), SOD activities, the supernatant was obtained by centrifugation. The absorbance of the color formed by the thiobarbituric acid reaction at 532 nm was measured to determine the level of lipid peroxidation in heart tissue.[20] GSH levels and GPx, CAT, SOD activities and were analyzed to determine the antioxidant status. CAT activity was analyzed according to the method of Aebi[21], SOD activity by Sun et al.[22], GPx activity by Lawrence and Burk[23], and GSH level by Sedlak and

Lindsay.[24] Total protein content of heart tissue was determined according to the method of Lowry et al.[25]

Histopathological Analysis

Heart tissue from all groups were fixed in 10% neutral-buffered formalin for fixative purposes for 48 h. According to routine paraffin tissue processing procedures, the tissues were dehydrated in increasing alcohol levels and cleared in xylene. Then, 5 µm thick sections were obtained from paraffin-embedded tissues and stained with H&E for histopathological evaluation. Images from stained preparations were evaluated using a binocular Olympus Cx43 light microscope (Olympus Inc., Tokyo, Japan) and EP50 camera (Olympus Inc., Tokyo, Japan).

Statistical Analysis

Data obtained at the end of the study were statistically analyzed using SPSS 20.0 (IBM SPSS Corp., Armonk, NY, USA). Data was presented as the mean ± standard error (SEM). Tukey's post hoc tests and one-way analysis of variance (ANOVA) were used for multiple comparisons. Statistical significance was determined at $P < .05$.

RESULTS

Effect of AZM and CHR on LDH, cTn-I and CK-MB Parameters in Heart Tissue

The effects of AZM on rat heart tissue examined, and the findings are shown in Figure 1. LDH (Figure 1A), CK-MB (Figure 1B) activities and cTn-I (Figure 1C) levels evaluated to determine cardiac markers in the heart tissue. According to the obtained data, it determined that cTn-I levels and CK-MB, LDH activities in the AZM toxication increased compared to that in the control and CHR groups ($P < .05$) and that AZM+CHR treatment was effective in bringing these markers to levels close to that in the control group.

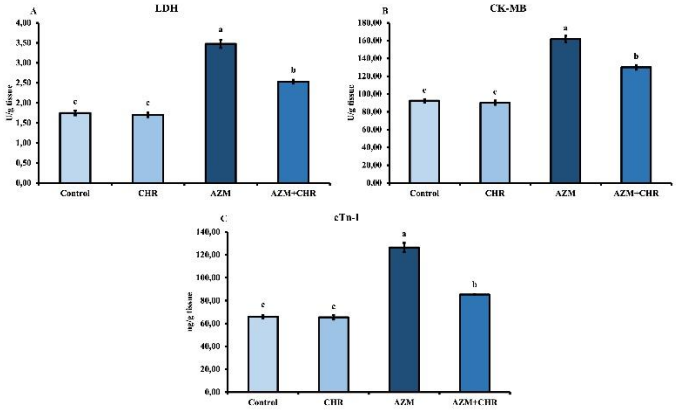

Figure 1: Effects of AZM and CHR applications on LDH (A) and CK-MB (B) activities and cTn-I (C) levels in rat heart tissue. Values for each group are presented as the mean ± SEM. Different letters in columns (a-b-c) indicate differences between groups ($P < .05$). (AZM: Azithromycin, CHR: Chrysin, LDH: Lactate dehydrogenase, CK-MB: Creatine kinase-myocardial band, cTn-I: Cardiac troponin-I).

Effect of AZM and CHR on Oxidative Stress Parameters in Heart Tissue

In the heart tissue, MDA (Figure 2A), GSH (Figure 2B) levels and GPx (Figure 2C), SOD (Figure 2D) and CAT (Figure 2E) activities evaluated. MDA levels were higher in the AZM toxication than in increased compared to the control and CHR groups ($P < .05$), whereas GPx, CAT, SOD activities and GSH levels decreased ($P < .05$). Supportive CHR treatment together with AZM decreased MDA and increased CAT, SOD, GPx activities, GSH levels, thus strengthening antioxidant defense system.

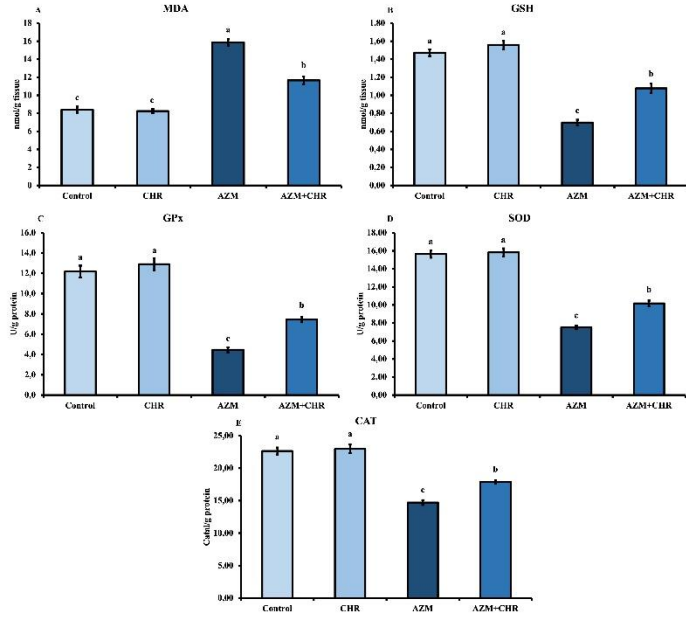

Figure 2: Effects of AZM and CHR applications on MDA (A) and GSH (B) levels and GPx (C), SOD (D) and CAT (E) activities in rat heart tissue. Values for each group are given as mean ± SEM. Different letters in the columns (a-b-c) indicate differences in groups ($P < .05$). (AZM: Azithromycin, CHR: Chrysin, MDA: Malondialdehyde, GSH: Glutathione, GPx: Glutathione peroxidase, SOD: Superoxide dismutase, CAT: Catalase).

Histopathological Results

The histopathological results in the heart tissue sections of the control, CHR, AZM groups are shown in Figure 3. In the control and CHR groups, myocardial muscle fiber branching and organization showed a normal histological alignment. The sarcolemma of cardiomyocytes in the control and CHR groups was regular, single-nucleated, and centrally located. The images of the AZM group showed distinct pathological changes. As a result of AZM application, scattered organization due to separation was observed in the muscle fibers. Eosinophilic changes and pyknotic nuclei were observed in the cytoplasm of cardiomyocytes in this group. Additionally increased vascular congestion and leukocyte infiltration were observed. In the group where CHR was applied together with AZM, cardiomyocytes showed a more

regular alignment and fewer gaps between muscle fibers, indicating that it was comparable to the control. In addition, the number of congested vessels and inflammatory cells was significantly reduced.

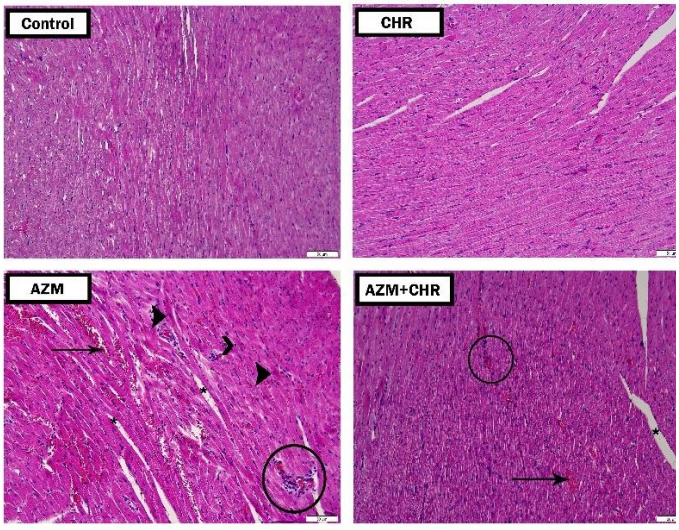

Figure 3. Photomicrographs of heart tissues stained with H&E from the control and experimental groups. The heart tissues from the control and CHR groups showed a normal structure. The heart from the AZM group shows clearly separated muscle fibers (star), inflammatory cells (circle), cardiomyocytes with eosinophilic cytoplasm (arrowhead), cardiomyocytes with pyknotic nuclei (arrowhead), and vascular congestion (arrow) in the myocardial layer. The AZM+CHR group showed recovery similar to that of control group, except for very slightly separated muscle fibers (star), decreased inflammatory cells (circle), and congestion (arrow). (H&E, x200). (AZM: Azithromycin, CHR: Chrysin).

DISCUSSION

AZM, which is used in the treatment of bacterial infections, causes tissue damage, especially in the heart tissue. This damage is associated with the activation of the damage pathways triggered by oxidative stress due to increased ROS production. Therefore, in this study, we investigated the effects of CHR, a flavonoid, on AZM-induced heart damage.

When there is a sudden decrease in coronary blood flow, myocardial damage occurs.[7] LDH, one of the cardiac markers, is released into the blood from the myocardium damaged by AZM by inducing ischemia.[2] It has been reported that the mitochondrial toxicity of AZM is associated with cardiac side effects.[26] CK-MB, LDH activities, cTn-I levels are important markers of cardiac damage.[27] In the current study, it was found AZM administration increased cTn-I levels and LDH, CK-MB activities in the heart tissue of rats, and as a result, caused damage to the heart tissue. This increase was supported by histopathological examination of the heart tissue. Studies have reported that AZM application causes damage by increasing LDH and CK-MB activities.[2,28] Different chemical agents increase cTn-I levels in heart tissue.[29] It was found that the application of CHR, a natural flavonoid, together with AZM reduces cTn-I levels with increasing LDH and CK-MB activities in heart tissue. Studies show that CHR has the ability to increase antioxidant activities against different toxic agents and reduce lipid peroxidation and prevent tissue damage.[12,16]

AZM, is a broad-spectrum antibiotic that, causes death in myocardial tissue via oxidative stress.[2] Oxidative stress occurs through the production of ROS such as superoxide anion radicals and peroxides.[30-32] ROS causes peroxidation of unsaturated fatty acids in cell membranes, damage to nucleic acids and denaturation of proteins. Thus, it leads to deterioration of cell structure and loss of tissue function.[33-37] Free radicals and an impaired antioxidant defense system cause redox imbalance.[38] Non-enzymatic (GSH) and enzymatic (GPx, SOD, CAT) antioxidants are important substances in defense against ROS.[39-41] GSH, a powerful antioxidant compound, helps maintain the redox state in cells.[12,39,42] SOD, GPx, CAT are antioxidant enzymes that provide antioxidant defense in the body.[14,43,44] SOD plays a role in the scavenging of superoxide radicals, CAT plays a role in the decomposition of hydrogen peroxide (H_2O_2) into molecular oxygen and water.[12,45] Another antioxidant defense system, GPx, has been reported play a role in the neutralization of cytotoxic lipid peroxides H_2O_2.[46] When ROS production and lipid peroxidation increase, mitochondrial activity is affected. It has been stated that ATP synthesis in the electron transport chain in mitochondria is negatively affected, causing damage to the cell.[47] MDA is a polyunsaturated fatty acid peroxidation product an indicator of oxidative stress.[27] Antioxidants reduce cellular damage caused by the interaction of protein, DNA, and lipid molecules with ROS.[48] In the present study, it was determined that in the damage induced by AZM in the heart tissue, MDA increased, GSH level, and SOD, GPx, CAT enzyme activities decreased which oxidative stress developed, development of oxidative stress caused tissue damage. El-Shitany and El-Desoky[1] examined heart damage in rats treated with AZM and reported that ROS production increased in the heart tissue and oxidative stress developed, leading to heart damage. In different studies conducted on the subject, it was determined that AZM caused heart damage, and it is stated that one of the most important mechanisms underlying this damage was oxidative stress due to ROS production.[9,28] We found that co-administration of CHR with AZM improved oxidative stress levels in cardiac tissue. Akaras et al.[16] stated that CHR is a powerful antioxidant that, reduces tissue damage, and shows this effect by suppressing ROS production and related oxidative stress. It has been reported that CHR is effective in reducing damage in different toxicity models and different tissues, suppresses ROS production, and strengthens the

antioxidant defense system.[12,17,49]

Histopathological evaluation revealed that eosinophilic changes and pyknotic nuclei in the cytoplasm of cardiomyocytes, increased vascular congestion and leukocyte infiltration confirmed the biochemical changes in AZM-induced cardiac injury. Co-administration of CHR with AZM alleviated the histopathological changes with a more regular arrangement of cardiomyocytes and less space between muscle fibers, indicating the ability of CHR to maintain myocardial structure and integrity. Saleh et al.[50] reported that CHR significantly improved cardiac sections in heart tissue against different toxic agent, with only mild myocardial blood vessel occlusion observed.

In conclusion, AZM caused cardiac marker changes, oxidative stress, tissue structural and structural changes by interfering with the damage pathways in the heart tissue. CHR, a natural flavone, showed healing effects against AZM-induced heart damage. These effects suggest that CHR can be used as a potential therapeutic agent.

Ethics Committee Approval: Ethical approval was obtained from the Atatürk University Animal Experiments Local Ethics Committee (Date: 31.01.2025, Approval No: 2025/01/22).

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