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Hesperidin attenuates cisplatin-induced cardiotoxicity in rats through regulating autophagy

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Abstract

Objective: Cisplatin is a well-known anticancer drug whose use has been limited due to a variety of side effects. Evidence has confirmed that cisplatin consumption is associated with cardiac toxicity. Unfortunately, no drug has yet been found to treat cardiac toxicity or reduce the side effects caused by cisplatin. However, hesperidin has been identified as an antioxidant agent which has protective effects on the heart tissue. This study explored the impact of hesperidin on cisplatin-induced cardiac toxicity through its antioxidant effect and adjustment of autophagy.

Materials and Methods: It included male Wistar rats allocated into five groups: sham, cisplatin: animals treated with cisplatin (7.5 mg/kg, intraperitoneally) on the 4th day; and four groups which received hesperidin (50, 100, and 200 mg/kg, gavage, 1 week) along with cisplatin on the 4th day. A mixture of ketamine and xylazine was used to anesthetize all animals on day 8 to obtain blood and tissue samples and to record ECG.

Results: The findings indicated that cisplatin led to an increase in creatine kinase-MB, lactate dehydrogenase, and malondialdehyde levels, and a reduction in total antioxidant capacity in heart tissue. Moreover, it reduced the expression of microtubule-associated protein light chain (*LC3-II*) and *beclin-1*. ECG parameters included: Heart rate (HR) and QRS voltage complex which were reduced by cisplatin. However, hesperidin (100, and 200 mg/kg) reversed these changes.

Conclusion: Hesperidin could protect cardiac tissue against cisplatin administration by exerting its antioxidant effect and regulating autophagy in a dose-dependent manner.

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Introduction

Cisplatin (Cis-dichlorodiamine platinum) is an anti-cancer drug widely applied for patients with solid tumors (Herradón et al. 2017). The use of cisplatin is often limited by its most common complication renal injury (Bennis et al. 2014). Recently, some studies have shown that cisplatin treatment is associated not only with nephrotoxicity but also with cardiovascular dysfunction, including hypertension, hyperlipidemia, myocardial infarction, and cardiac toxicity, potentially leading to heart failure (Pai and Nahata 2000; Pretnar-Oblak et al. 2007). Evidence also confirms that cardiac toxicity induced by cisplatin is increasing (Ishioka et al. 2008). The cisplatin-induced toxicity pathway is very complex and not fully understood (Manohar and Leung 2018). However, several pathways have been suggested for the mechanism of cisplatin-induced toxicity, including the production of toxic metabolites of cisplatin, mitochondrial damage, oxidative stress, disruption of ion homeostasis, inflammation, and apoptosis (Manohar and Leung 2018). Oxidative stress is an imbalance between production of reactive oxygen species (ROS) and antioxidant agents in the body, overcoming antioxidant agents (Manohar and Leung 2018). Oxidative stress is the main reason for cardiac toxicity by cisplatin (Manohar and Leung 2018).

Autophagy is a recycling pathway that degrades damaged organelles and proteins, among other cell components, to promote cell survival under stress condition. Autophagy can be induced by conditions such as oxidative stress, hypoxia, and cell starvation (Gatica et al. 2022). Autophagy has a critical role in the development of normal cardiac tissue (Gatica et al. 2022). Thus, dysregulation of autophagy signaling can contribute to tissue dysfunction including heart disease and renal failure (Gatica et al. 2022).

Nowadays, herbal medicines have earned popularity in the prevention and treatment of diseases (Ganeshpurkar and Saluja 2019). Phytochemicals have commonly been acknowledged for their positive outcome on human being. Flavonoids have a protective role in many types of diseases (Ganeshpurkar and Saluja 2019). Hesperidin is well known as a flavanone that has the highest bioavailability among polyphenol compounds (Morand et al. 2011). Evidence suggests that hesperidin is abundantly found in citrus fruits (Morand et al. 2011). Hesperidin exhibits anti-inflammatory, anti-oxidant, and anticancer properties (Xiong et al. 2019). Moreover, it protects the cardiovascular system by regulating blood pressure and cholesterol level (Xiong et al. 2019). A previous study showed that hesperidin (200 mg/kg) could protect the cardiac tissue in myocardial ischemia/ reperfusion injury through regulating autophagy pathway (Li et al. 2018). Moreover, hesperidin (100 and 300 mg/kg, intraperitoneally) has been found to improve cardiac function by mitigating apoptosis in cisplatin-induced cardiotoxicity (Jia et al. 2022). While hesperidin has demonstrated beneficial effects in various tissues (Tirkey et al. 2005), effective treatments for cisplatin-induced cardiotoxicity remain limited. Therefore, the present study investigated how different doses of hesperidin affect cisplatin-induced cardiotoxicity, focusing on the involvement of autophagy.

Materials and Methods

Chemicals

Hesperidin and cisplatin (Sigma Aldrich, Germany) were used in this study. Kits from Kushan Zist, Iran, were used to measure total antioxidant capacity (TAC) and malondialdehyde (MDA). Kits for molecular parameters were obtained from Ana Cell, Iran.

Animals

Forty male Wistar rats (200 ± 20 g) were obtained from the Animal

Reproduction Center of Ahvaz Jundishapur University of Medical Sciences (AJUMS), Iran. They were kept under a standard condition (22°C temperature, 50% humidity, 12-hr dark–light cycle) and were provided with water and standard pellet diet *ad libitum*.

Study design

This research involved forty rats assigned to five groups of 8. The sham rats received distilled water (1 ml, gavage) for seven days plus the fourth day (intraperitoneally). The cisplatin group: the rats received distilled water (1 ml, gavage) for one week plus cisplatin 7.5 mg/kg given intraperitoneally on the 4th day (Akomolafe et al. 2014). Other groups included rats receiving hesperidin (50, 100 and 200 mg/kg by gavage, daily) for one week along with cisplatin (7.5 mg/kg, intraperitoneally) on day 4 (Kaur et al. 2006). On the eighth day, all animals were anesthetized using an anesthetic consisting of a ketamine (100 mg/kg) and xylazine (10 mg/kg) mixture (Wellington et al. 2013), and their biochemical, molecular and electrocardiogram parameters were investigated. Blood samples were collected from the heart. Heart tissue was collected for pathological and oxidative stress, as well as molecular assays.

Electrocardiogram recording

On day 8, 20 min after anesthesia, electrocardiograms (ECGs) were recorded using lead II and 26-gauge needle electrodes. The ECG signal was amplified (Bio Amplifier) and digitized using a PowerLab A/D converter (AD Instruments, Australia). Data were acquired and analyzed using Chart software (AD Instruments). The calibration was 25 mm/sec at 10 mm/mV. After 20 min of recording, heart rate (HR), PR interval, QRS voltage, QT, and QTc were checked. The QTc (corrected QT interval) was calculated using the Bazett formula (Vandenberk et al. 2016).

Myocardial injury parameters assay

Following cardiac blood collection, samples were centrifuged at 3000 rpm for 15 min to isolate serum which was stored at -80°C until analysis of cardiac injury markers including creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) activities. Auto Analyzer was used to evaluate these parameters spectrophotometrically.

Oxidative stress parameters assay

On day 8, heart tissue (100 mg) was homogenized in 1 ml of cold phosphate-buffered saline. After centrifugation at 4000 g for 15 min, the supernatant was collected and used to measure total antioxidant capacity (TAC) and malondialdehyde (MDA) levels. MDA and TAC were measured colorimetrically at 535 nm and 420 nm, respectively.

Quantitative real-time PCR analysis

Total RNA was extracted from the frozen heart tissue, using RNA extraction kit. After that, the cDNA was synthetized using cDNA synthesis kit. Gene expression (*Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Microtubule-associated protein light chain (LC3-II), and beclin-1*) was explored using real-time PCR (RT-PCR). The primers were obtained from (Homa gene, Iran). The primers utilized in this study are listed in Table 1. The PCR reaction (10 µL total volume) included 2 µl of cDNA, 2 µL of primers, and 6 µl of SYBR green master mix (without ROX; Primer design, Denmark). Amplification involved an initial denaturation at 95°C for 15 min. After that, there were 45 cycles of 95°C (30 sec), 52°C (30 sec), and 72°C (30 sec). Also, H_2O was considered the negative control. The mRNA expression was explored by an internal control gene. Gene expression was calculated using the $2^{-\Delta\Delta CT}$ formula.

Histology assay

After separation of heart tissues, they were washed by normal saline. Subsequently, they were maintained in 10% formaldehyde. The heart tissue was then cut to slices in 5 μm and kept in paraffin. After that, the heart tissues were stained using H&E (hematoxylin and eosin) method to explore the alterations of the heart.

Statistical analysis

The statistical analyses were performed using one-way ANOVA with Tukey's post hoc comparison test in GraphPad Prism v6.0 (GraphPad Software, San Diego, CA, USA). The results are shown as mean ± standard error (SEM). $p<0.05$ was interpreted as statistically significant.

Table 1. Sequences of primers used.

mRNA	Reverse primer	Forward primer
Beclin-1	GTTTCAATAAATGGCTCCTC	CTAAAGAATGGAGGGGTCTA
LC3-II	AAATAGCTTAGTTTAGGAGGC	GATTCCTCTACATGGTCTAT
GAPDH	CGGAGATGATGACCCTTTTGG	TGCTGGTGCTGAGTATGTCGTG

Results

Histological effects of hesperidin administration

The findings of histology showed normal cardiac tissue with no inflammation, pyknosis, or hyperemia (Figure 1a). By contrast, cisplatin treatment induced several cardiac tissue abnormalities including inflammation, pyknosis, and hyperemia (Figure 1 b and c). In addition, inflammation, pyknosis, and hyperemia were observed in the group receiving hesperidin (50 mg/kg) plus cisplatin (Figure 1d). However, hesperidin (100 mg/kg) plus cisplatin showed slight hyperemia and pyknosis (Figure 1e). Hesperidin (200 mg/kg) plus cisplatin improved cardiac alterations following cisplatin injection (Figure 1f). The results of quantitative analysis are illustrated in Figure 1g. Scoring criteria for morphological findings are presented in Table 2.

Hesperidin role in attenuating oxidative stress

According to Figure 2a, in comparison with the sham group, cisplatin significantly elevated MDA levels ($p<0.01$). In hesperidin (100 and 200 mg/kg) plus cisplatin group, there was a significant decrease in MDA levels as opposed to the group receiving cisplatin ($p<0.01$, Figure 2a). In groups treated with hesperidin (100 and 200 mg/kg) plus cisplatin, a significant reduction was also seen in MDA levels in comparison with the group receiving hesperidin (50 mg/kg) plus cisplatin ($p<0.05$, Figure 2a). In addition, the level of TAC significantly decreased in the cisplatin group compared to the control group ($p<0.001$, Figure 2b). Hesperidin (100 and 200 mg/kg) significantly increased TAC levels as opposed to the animals receiving cisplatin ($p<0.01$, $p<0.001$, respectively, Figure 2b). Hesperidin (200 mg/kg) plus cisplatin significantly increased the level of TAC compared to the group receiving hesperidin (50 mg/kg) plus cisplatin ($p<0.01$, Figure 2b).

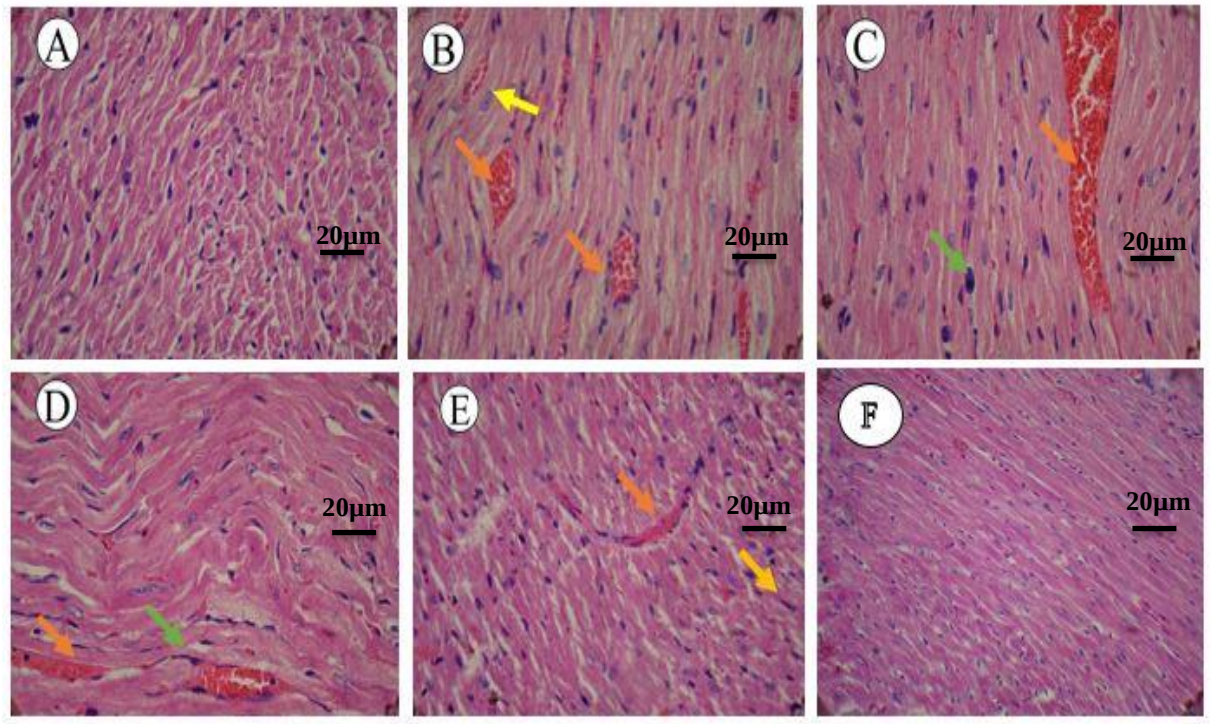
Hesperidin effect on CK-MB and LDH

The levels of CK-MB and LDH significantly increased in cisplatin rats as opposed to the sham group ($p<0.001$, $p<0.01$, respectively, Figure 3a and b). However, hesperidin (100 and 200 mg/kg) plus cisplatin significantly decreased the levels of CK-MB and LDH in comparison with the group receiving cisplatin ($p<0.001$, $p<0.01$, Figure 3a and b). Moreover, hesperidin (100 and 200 mg/kg) plus cisplatin significantly reduced CK-MB and LDH levels ($p<0.001$) compared to hesperidin (50 mg/kg) group (Figure 3 a and b).

Table 2. Histopathological changes in the heart

	Sham	Cis	Cis+Hes50	Cis+Hes100	Cis+Hes200
Inflammation	0	++	0	0	0
Pyknosis	0	+++	+	+	0
Hyperemia	0	+++	+	+	0

Sham group: Normal heart tissue, Cisplatin (Cis), Hesperidin (Hes 50 mg/kg) + Cis, Hesperidin (Hes 100 mg/kg) + Cis, Hesperidin (Hes 200 mg/kg) + Cis. *None (0), Mild (+), Moderate (++), and Severe (+++)*



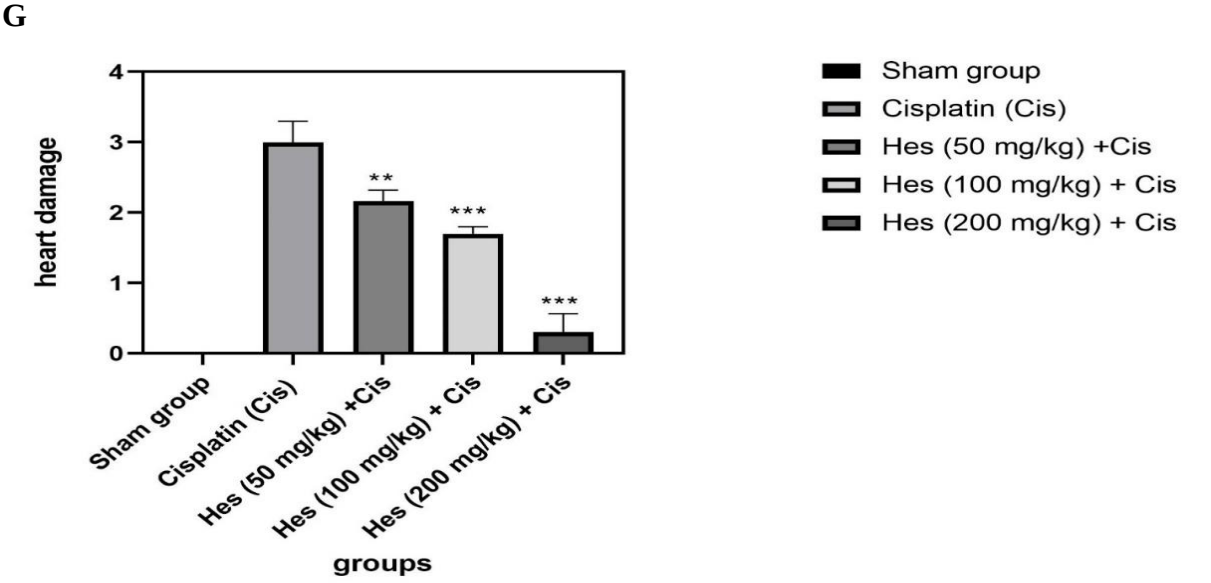


Figure 1. Effects of hesperidin (Hes) on histological evaluation of heart tissue in different groups. (a) Sham group: Normal heart tissue (200×). (b, c) Cisplatin (Cis) group: Indicating pyknosis (Green arrows), Hyperemia (orange arrows), and inflammatory cell infiltrations (Yellow arrows) (200×). (d) Rats receiving Hes (50 mg/kg) + Cis: indicating indicating pyknosis (Green arrows), and inflammatory cell infiltrations (Yellow arrows) (200×). (e) Rats receiving Hes (100 mg/kg) + Cis: indicating pyknosis (Green arrows), and inflammatory cell infiltrations (Yellow arrows) (200×). (f) Rats receiving Hes (200 mg/kg) + Cis: indicating normal structure (200×). Tissue samples were stained with H&E dyes. **p<0.01 and ***p<0.001 compared to sham rats.

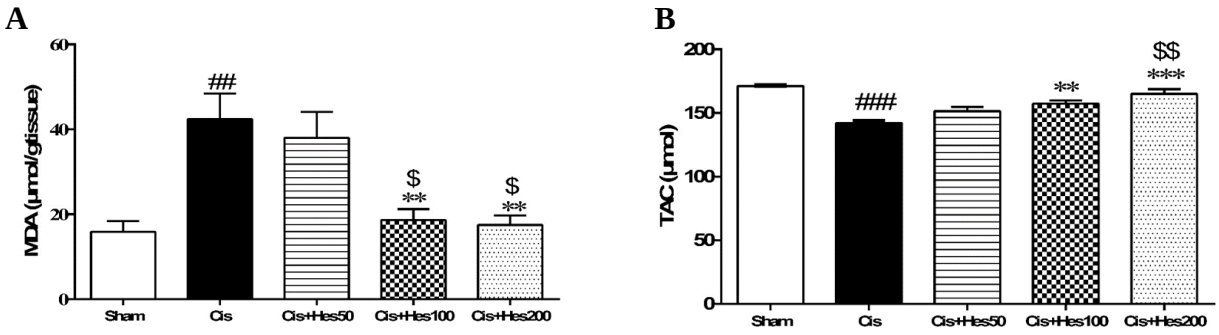


Figure 2. Effects of Hes on heart tissue MDA (a) and TAC (b) in Cis nephrotoxicity (mean ± SEM, n=5). Sham, Cis (7.5 mg/kg, i.p.), Hes (50 mg/kg, gavage, seven days) +Cis (7.5 mg/kg, i.p.), Hes (100 mg/kg, gavage, seven days)+Cis (7.5 mg/kg, i.p.), and Hes (200 mg/kg, gavage, seven days)+Cis (7.5 mg/kg, i.p.). One way ANOVA followed by Tukey's post hoc test. ###p<0.001 and ##p<0.01, vs. sham group, **p<0.01 and ***p<0.001, vs. Cis group. $p<0.05 and $$p<0.01, vs. Hes (50 mg/kg) + Cis group.

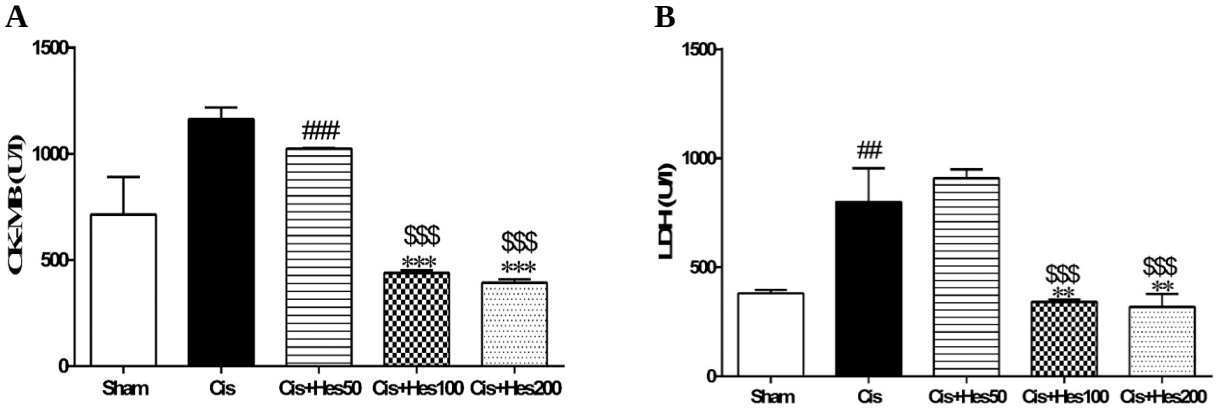


Figure 3. Effects of Hes on serum levels of CK-MB (a), and LDH (b) in Cis cardiotoxicity (mean ± SEM, n=5). Sham, Cis (7.5 mg/kg, i.p.), Hes (50 mg/kg, gavage, seven days) +Cis (7.5 mg/kg, i.p.), Hes (100 mg/kg, gavage, seven days) +Cis (7.5 mg/kg, i.p.), and Hes (200 mg/kg, gavage, seven days) +Cis (7.5 mg/kg, i.p.). One-way ANOVA followed by Tukey's post hoc test. ###p<0.001 and ##p<0.01 vs. sham group, **p<0.01 and ***p< 0.001, vs. Cis group. $$$p<0.001, vs. Hes (50 mg/kg) + Cis group.

The impact of hesperidin on molecular parameters

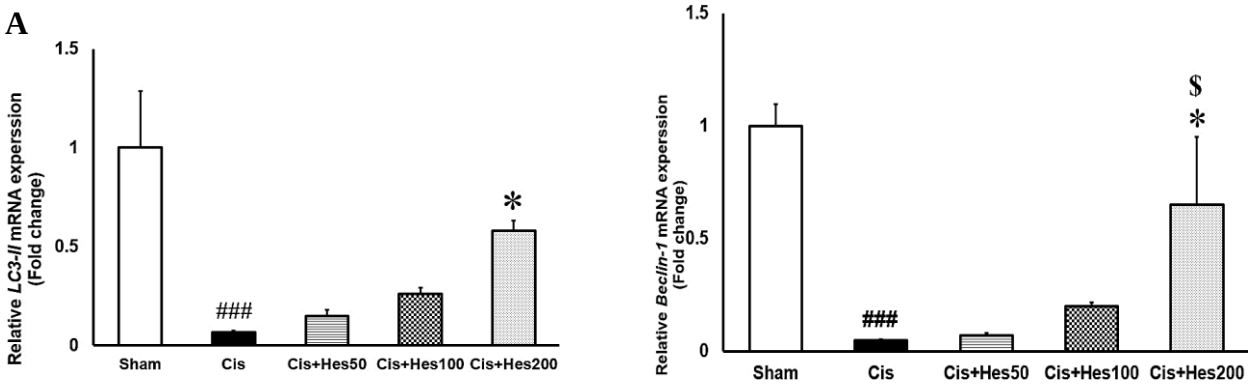
The mRNA *LC3-II* expression significantly decreased in cisplatin group in comparison with sham rats (p<0.001, Figure 4a). Compared with the group treated with cisplatin, hesperidin (200 mg/kg) plus cisplatin remarkably increased mRNA *LC3-II* expression (p<0.05, Figure 4a). Also, cisplatin significantly reduced the mRNA *beclin-1*expression compared to sham group (p<0.001, Figure 4b). However, hesperidin (200 mg/kg) plus cisplatin significantly elevated the mRNA *beclin-1*expression in comparison with the rats receiving cisplatin (p<0.05, Figure 4b). Additionally, hesperidin (200 mg/kg) plus cisplatin significantly raised the mRNA *beclin-1*expression compared to hesperidin (50 mg/kg) plus cisplatin (p<0.05, Figure 4b).

Hesperidin effect on ECG parameters

Lead II electrocardiogram was recorded to explore the effect of cisplatin on cardiac tissue. Heart rate, PR interval, and QRS voltage were measured in all groups. At the end of experiment, cisplatin treatment significantly decreased both heart rate and QRS voltage compared to sham group (p<0.001, p<0.05, respectively, Figure 5 a and b). Nevertheless, hesperidin (100 and 200 mg/kg) plus cisplatin significantly elevated heart rate compared to cisplatin rats (p<0.05 and p<0.01, respectively, Figure 5a). Remarkably, hesperidin (100 and 200 mg/kg) plus cisplatin increased HR

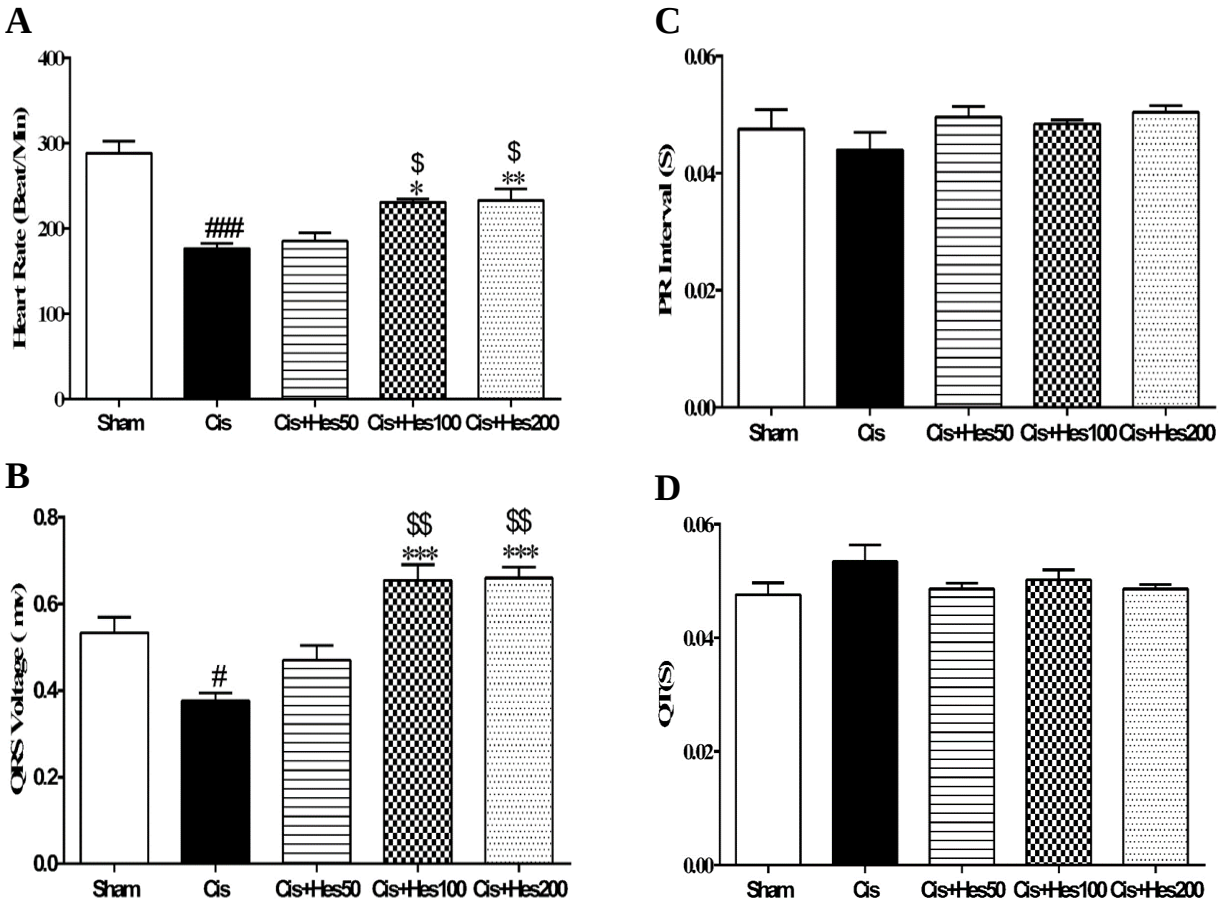
compared to hesperidin (50 mg/kg) plus cisplatin ($p<0.05$, Figure 5a).

Hesperidin (100 and 200 mg/kg) plus cisplatin significantly increased QRS voltage compared to cisplatin group ($p<0.001$, Figure 5b). Hesperidin (100 and 200 mg/kg) plus cisplatin caused a significant increase in QRS voltage as opposed to hesperidin (50 mg/kg) plus cisplatin ($p<0.01$, Figure 5b). PR interval, QT, and QTc did not change in any group (Figure 5c-e).



B

Figure 4. Effect of Hes on *LC3-II* (a), and *beclin-1*(b) in Cis cardiotoxicity (mean ± SEM, n=5). Sham, Cis (7.5 mg/kg, i.p.), Hes (50 mg/kg, gavage, seven days) +Cis (7.5 mg/kg, i.p.), Hes (100 mg/kg, gavage, seven days) + Cis (7.5 mg/kg, i.p.), and Hes (200 mg/kg, gavage, seven days) + Cis (20 mg/kg, i.p.). Analysis of the QRT-PCR results showed that the level of *LC3-II* and *beclin-1* expression in Cis group was significantly lower than that in Hes (200 mg/kg) groups. One-way ANOVA followed by Tukey's post hoc test. ###$p<0.001$, vs. sham, *$p<0.05$, vs. Cis group. \$$p<0.05$, vs. Hes 50 mg/kg+Cis.



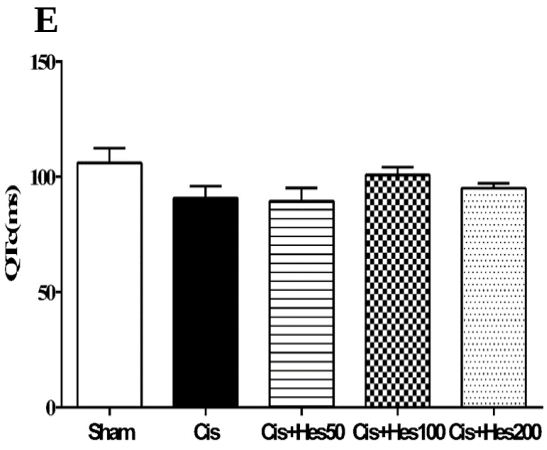


Figure 5. Effect of Hes on Heart rate (A), QRS voltage (B), QT (C), QTc (D), and PR interval (E) in cardiotoxicity (mean ± SEM, n = 5). Sham, Cis (7.5 mg/kg, i.p.), Hes (50 mg/kg, gavage, seven days) +Cis (7.5 mg/kg, i.p.), Hes (100 mg/kg, gavage, seven days) + Cis (7.5 mg/kg, i.p.), and Hes (200 mg/kg, gavage, seven days) + Cis (20 mg/kg, i.p.), One-way ANOVA followed by Tukey's post hoc test. #p < 0.05 and ###p< 0.001 vs. sham group. *p<0.05, **p<0.01 and ***p<0.001, vs. Cis group. $p<0.05 and $$p<0.01, vs. Hes 50 mg/kg+Cis.

Discussion

As the results of this study showed, cisplatin (7.5 mg/kg) treatment increased MDA levels and decreased TAC levels. Moreover, cisplatin promoted CK-MB and LDH serum levels. Cisplatin suppressed autophagy, as evidenced by reduced mRNA expression of *LC3-II* and *beclin-1*. Cisplatin treatment also reduced QRS voltage and heart rate. However, hesperidin administration mitigated these effects. Cisplatin remains a critical drug in treating many solid tumor malignancies (Herradón et al. 2017). However, its use is limited due to significant side effects, particularly nephrotoxicity and cardiovascular complications (Herradón et al. 2017). The exact mechanisms of these detrimental effects have not been explored yet (Herradón et al. 2017). However, inflammation, oxidative stress, and apoptosis signaling have been suggested (Dugbartey et al. 2016). Cisplatin not only depletes antioxidants but also disrupts mitochondrial function, leading to increased oxidative stress (Dugbartey et al. 2016). MDA is considered a lipid peroxidation index, following ROS over-generation (Gaweł et al. 2004). Our findings of increased lipid peroxidation and decreased TAC are in agreement with earlier reports (Ali et al. 2023). Cisplatin has been shown to deplete intracellular antioxidants, disrupt mitochondrial function, and enhance ROS production, leading to oxidative damage and cellular injury (Dugbartey et al. 2016). The current observation of elevated cardiac MDA levels following cisplatin administration supports previous experimental evidence (Ali et al. 2023; Khadrawy et al. 2021). TAC, a comprehensive measure of antioxidant of antioxidant defense status, is considered a reliable indicator of the body's oxidative balance (Kusano and Ferrari 2008). Numerous studies have shown that polyphenol compounds acting as antioxidant agents can increase antioxidant levels in the body (Jia et al. 2022). As our results confirmed, hesperidin (100 and 200 mg/kg) as a polyphenol compound increased TAC in heart tissue, thereby protecting cardiac function. A previous experimental research demonstrated that hesperidin had a profound impact on cardiac tissue following cisplatin injection (Jia et al. 2022). These protective effects are likely mediated through the free-radical scavenging activity of hesperidin and its ability to stabilize cellular membranes and mitochondrial integrity (Jia et al. 2022). Consistent with these studies, our data

indicate that hesperidin supplementation restored TAC levels, reduced MDA accumulation, and normalized serum CK-MB and LDH concentrations (Chen et al. 2021).

Autophagy plays a critical role in maintaining cardiac hemostasis and function through removing damaged organelles and unfolded proteins (Sciarretta et al. 2018; Mei et al. 2015). The autophagic process is regulated by *LC3-II* and *beclin-1* (Mei et al. 2015). *LC3-II* is known as the most reliable autophagy marker so far. Studies have also confirmed the role of *beclin-1* as an autophagy initiator (Mei et al. 2015, Sciarretta et al. 2018). In this study, cisplatin downregulated the mRNA expression *LC3-II* and *beclin-1*, suggesting suppression of autophagy. This finding agrees with previous experimental data indicating that cisplatin prevents autophagy-related gene expression in cardiac tissue (Salah et al. 2024). Contrary to our study, however, a previous *in vitro* research indicated that *beclin-1* and *LC3-II* proteins increased in a glucose deprivation/reperfusion model (Yi et al. 2020). This contradiction might be due to the different study design and type. Evidence has confirmed that oxidative stress induced by chemical drugs such as cisplatin and doxorubicin can lead to myocardial injury and ECG alterations (Saleh et al. 2015; Puri et al. 2005). Cisplatin treatment significantly increased serum CK-MB and LDH levels. High levels of these chemical factors are indicative of myocardial injury induced by cisplatin, which is consistent with a previous study (Silva et al. 2024). Hesperidin, an antioxidant, recovered these changes, which aligns with an earlier study (Saleh et al. 2015). Other antioxidants can protect cardiac tissue against cardiotoxicity induced by isoproterenol through intensifying antioxidant levels and mitigating apoptosis (Chen et al. 2021).

This study found that cisplatin altered ECG parameters, specifically HR and QRS complex voltage. However, PR interval, QT, and QTc remained unchanged across all groups. Oxidative stress induced by cisplatin led to a reduction in HR, which is consistent with a previous study (Silva et al. 2024). Hesperidin (100, and 200 mg/kg), however, reversed this effect. While our findings did not show changes in the QT and QTc intervals, a previous experimental study reported that long-term cisplatin administration (once weekly for five weeks) was associated with prolongation of both intervals (Silva et al. 2024). This discrepancy might be due to differences in cisplatin dosage and administration schedules (Silva et al. 2024). Interestingly, the current study showed that cisplatin reduced QRS complex voltage. Additionally, our previous work confirmed that oxidative damage induced by renal ischemia-reperfusion injury reduced QRS complex voltage (Amini et al. 2019). However, hesperidin recovered the alteration of QRS voltage. Evidence showed that antioxidant agents can ameliorate ECG parameters such as ST segment in cardiotoxicity induced by isoproterenol (Prince et al. 2011). With regard to ECG alterations, electrolyte disturbances induced by oxidative stress are accompanied by reduced contractility and conductivity disturbances, finally leading to cardiac dysfunction (Lefer and Granger 2000). Additionally, cardiotoxicity may result from alterations in cardiac ion channel activity. Cisplatin has been indicated to inhibit sodium and L-type calcium currents which could reduce myocardial excitability and contractility, leading to the reduced QRS complex voltage (Dugbartey et al. 2016; Orfali at al., 2025). Although cisplatin can interfere with potassium currents responsible for repolarization, the absence of QT and QTc changes in the current study suggests that these channels were not markedly affected by the dosage and duration. Hesperidin likely exerted its protective effect by preserving ionic balance and maintaining ion channel integrity through its antioxidant properties (Ahmed et al. 2021).

Therefore, both oxidative stress and ion current dysregulation appear to contribute to ECG alterations (Dugbartey et al. 2016; Orfali at al. 2025).

The study had several limitations. Notably, Western Blot analysis was not performed due to a lack of available equipment and financial constraints.

The present study demonstrated that cisplatin administration induces marked cardiac toxicity characterized by histological abnormalities, elevated lipid peroxidation, decreased total antioxidant capacity, altered autophagy-related gene expression, and significant ECG disturbances. These alterations indicated that oxidative stress and impaired autophagy are key mechanisms involved in cisplatin-induced cardiotoxicity. Hesperidin (at doses of 100 and 200 mg/kg) alleviated these adverse effects by restoring antioxidant defense systems, enhancing the expression of autophagy markers. Therefore, hesperidin can be considered a promising natural compound for preventing cisplatin-associated cardiac injury. However, further pharmacological and clinical investigations are warranted to confirm its safety, efficacy, and optimal therapeutic dosage in humans before clinical application.

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Conflicts of interest

The authors declare no competing interests

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Ethical Considerations

The current research was confirmed by the Animal Ethics Committee (IR.AJUMS.ABHC.REC.1401.107).

Code of Ethics

IR.AJUMS.ABHC.REC.1401.107

Authors' Contributions

NA Investigation, Project administration, Supervision, Conceptualization, Methodology, Validation, Writing - Original draft. MB Formal analysis, Methodology, Visualization, Data curation. FND Formal analysis, Methodology. MD Supervision, Project administration, Resources. HHK Resource. KHN Resources. All authors have read and approved the final manuscript.

Abbreviations

LC3-II: Microtubule-associated protein light chain, HR: Heart rate, ROS: Reactive oxygen species, TAC: Total antioxidant capacity, MDA: Malondialdehyde, ECG: Electrocardiogram, CK-MB: Creatine kinase-MB, LDH: Lactate dehydrogenase, GAPDH: Glyceraldehyde-3-phosphate dehydrogenase, H&E: hematoxylin and eosin

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