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Diminazine protects against cardiac aging through the improvement of mitophagy and apoptosis in aging rats induced by D-galactose

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Abstract

Background Mitochondrial dysfunction is a main feature of the aged heart. However, there is still no effective treatment against cardiac aging. Diminazine (DIZE) is an anti-infective agent for animals. It is effective against cardiac disorders. The present study aimed to investigate the effects of DIZE on age-related cardiac dysfunction.

Methods and results Wistar rats were randomly divided into four groups, with eight rats per group: control rats (CONT), control rats treated with DIZE (CONT + DIZE), aged rats induced by D-galactose (D-GAL), aged rats treated with DIZE (D-GAL + DIZE). Rats received intraperitoneal (IP) injection of D-GAL at 150 mg/kg daily for 8 weeks to induce aging. The aging animals in the D-GAL + DIZE group were treated with subcutaneous injection of DIZE at 15 mg/kg daily for 8 weeks. Heart tissues were harvested to assay molecular parameters. Our results exhibited cardiac hypertrophy and a significant increase in the expression of cardiac BCL2-associated X (Bax) along with a significant decrease in the expression of cardiac Mitofusin 2 (Mfn2), Phosphatase, and tensin homolog (PTEN)-induced putative kinase 1 (PINK1), Dynamin-related protein 1 (Drp1), and B-cell lymphoma 2 (Bcl2) in the aged rats compared with the control animals. DIZE treatment improved cardiac hypertrophy and the expression of genes.

Conclusions Overall, DIZE treatment significantly reversed the downregulation of PINK1, Mfn2, and Drp1. Moreover, DIZE significantly inhibited apoptosis though improving the gene expression of Bax and Bcl-2 in the heart. DIZE is effective in reducing cardiac hypertrophy induced aging through regulating mitochondrial dynamics, mitophagy and apoptosis.

Keywords Aging, Apoptosis, Cardiac hypertrophy, Diminazine, Mitophagy

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Introduction

High blood pressure, adrenal disorders, folate deficiency, and aging are risk factors for several cardiovascular diseases, including cardiac hypertrophy and heart failure [1, 2]. Common cardiovascular disorders, such as left ventricular hypertrophy and heart failure can lead to pulmonary hypertension, pulmonary edema, and even death [3, 4]. It has been indicated that increased load or pressure can cause cardiac hypertrophy [5]. Cardiac dysfunction and consequently enhanced energy consumption cause cardiac hypertrophy through increasing mitochondrial activity. Previous studies have indicated that increased mitochondrial reactive oxygen species (ROS) and reduced endogenous antioxidant enzymes are associated with cardiac hypertrophy. Age related-cardiovascular diseases are associated with damaged mitochondria, fibrosis, and cardiac hypertrophy. Several molecular and intracellular mechanisms, including changes in DNA and increased oxidative stress involve in cardiac aging [6]. Cardiac hypertrophy is associated with abnormal mitochondrial structure in aged mice [7].

Mitochondrial quality control (MQC) is a main mechanism for controlling mitochondrial function in the heart. It affects the structure and function of mitochondria and modulates the life process of cardiac cells [8]. MQC disorders lead to cardiac hypertrophy [9]. MQC encompasses several key processes, including mitochondrial biogenesis, mitochondrial dynamics (fusion and fission), and mitophagy [10]. Mitochondrial dynamics and mitophagy are involved in regulating mitochondrial responses to different stressors. Dysfunction of mitochondrial dynamics and impaired mitophagy are associated with cardiovascular disorders, including cardiac hypertrophy, heart failure, diabetic endothelial dysfunction, and cardiac ischemia/reperfusion [11–13]. Mitochondrial survival is maintained by the mitochondrial dynamics of fusion and fission [14]. Mitochondrial dynamics is modulated by fission protein, including Dynamin-related protein 1 (Drp1) and Mitofusin 2 (Mfn2). DRP1 is an essential regulator of mitochondrial fission. Impaired mitochondrial fission causes mitochondrial dysfunction and disrupted mitophagy. Furthermore, Drp1 dysfunction leads to progressive cardiac pathology and cardiac dysfunction in mice [15, 16]. Downregulation of Mfn2 causes mitochondrial dysfunction and cardiac hypertrophy [17]. Cardiomyocyte deletion of both Mfn2 and Drp1 lead to pathological cardiac hypertrophy in mice [18]. Mitophagy is a process that selectively removes damaged mitochondria [19]. This process efficiently removed damaged mitochondria during cardiac stress. Phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1), an important mitophagy regulator promotes the mitophagy process by Mfn2 phosphorylation [20]. Knockout of PINK1 causes pathological cardiac hypertrophy through increasing inflammation and reducing mitophagy in mice [20]. On the other hand, Mfn2 deficiency results in cardiac dysfunction through disrupting PINK1 pathway and increasing impaired mitochondria [21]. We have recently indicated that downregulation of Drp1 reduces mitophagy and results in cardiac cell death [22]. Our previous study has revealed that PINK1-mediated mitophagy reduces cardiac apoptosis in an aging model induced by D-galactose (D-GAL) [23].

Cardiomyocyte apoptosis is involved in cardiac hypertrophy [24, 25]. Several studies have indicated that herbal compounds, antioxidants, and drugs exert protective effects against cardiomyopathies and myocardial ischemia by improving MQC, increasing mitophagy, and reducing apoptosis in the heart [26–30].

Diminazine (DIZE) has been used as the important trypanocidal drug to treat animals for more than 6 decades [31]. Moreover, DIZE exhibits cardioprotective effects on cardiac hypertrophy, myocardial infarction and hypertension models [32]. Previous studies have shown that DIZE has protective effects against oxidative stress and inflammation [33]. Angiotensin-converting enzyme 2 (ACE2) is a fundamental mechanism for negative modulation of the renin-angiotensin system (RAS) by metabolizing angiotensin II (AngII) into the protective peptide angiotensin (1–7) [Ang (1–7)]. Previous study has exhibited that loss of ACE2 enhances cardiac hypertrophy [2]. In addition, it has been demonstrated that AngII involves in activating pro-apoptotic pathways, relating to cardiac hypertrophy [3]. ACE2 exerts vasodilator and antihypertrophic effects through activating Mas receptor (MasR). Therefore, modulating ACE2/Ang (1–7)/MasR axis seems an effective therapy of cardiovascular diseases [34]. It has been reported that AngII stimulates downregulation of Mfn2, while increased expression of Mfn2 inhibits cardiac hypertrophy induced by AngII [35, 36]. Our previous study has revealed that DIZE improves cardiac hypertrophy by increasing mitophagy through upregulation of Mfn2 in hyperthyroid rats induced by levothyroxine [37]. Thus, DIZE may inhibit AngII-mediated cardiac hypertrophy through upregulation of Mfn2.

D-GAL injection is regarded as a common method for induction of normal aging models in rodents. D-GAL leads to cardiac hypertrophy through inflammation and oxidative stress in the heart [38, 39]. This study was conducted with the purpose of evaluating effect of DIZE on cardiac hypertrophy through mitochondrial dynamics, mitophagy, and apoptosis pathways in aged heart induced by D-GAL in male rats.

Methods

In this study, 32 male Wistar rats (three months old, 250–300 g) were purchased from the animal house of the Hamadan University of Medical Sciences (Hamadan,

Iran). The animals were transferred to the animal house one week before the experiment. They were kept under a 12-hour light-dark cycle and had free access to food and water. The rats were randomly allocated into four groups with eight rats in each group: (1) control rats (CONT) (2) control rats treated with DIZE (CONT + DIZE) (3) aged rats induced by D-GAL (D-GAL) and (4) aged rats treated with DIZE (D-GAL + DIZE) [40, 41]. All experimental protocols were carried out in accordance with the ethical guidelines set by the Animal Experiment Committee and the Guide for the Care and Use of Laboratory Animals (IR.UMSHA.REC.1402.027).

Induction of senescence in rats

Senescence induced by D-GAL at 150 mL/kg via intraperitoneal (IP) injection daily for eight weeks [40]. They received DIZE at 15 mg/kg by subcutaneous injection daily for eight weeks [41]. After 8 weeks of aging induction, the rats were anesthetized with an IP injection of sodium pentobarbital (60 mg/kg). Once the animals were fully anesthetized, their abdomens were opened, approximately 5 ml of blood was collected from the renal vein, and the hearts were quickly removed. The ratio of heart weight (g) to total body weight (g) (HW/BW) and heart weight (g) to tibia length (cm) (HW/TL) were used as indices of cardiac hypertrophy [37].

Histological examination

Heart tissues were fixed in neutral buffered formalin. After preparing paraffin blocks and sectioning them into 4 µm slices, the sections were dewaxed with xylene, gradually hydrated with an alcohol gradient, and then stained with hematoxylin and eosin (H&E). The sections were washed with an alcohol solution and cleared with xylene. A LABOMED light microscope (California, United States) equipped with a LABOMED digital camera, was used to capture images of the stained sections. For the analysis of H&E images, 10 random sections from each animal were selected and studied at 150 µm intervals [42]. Three animals from each group (eight rats per group) were randomly selected for histological examination. At least 15 non-overlapping fields of view from each group were examined (400X magnification, the scale bar: 50 µm). For blind evaluation, the samples were delivered to the pathologist without labeling the groups. The intercellular space was qualitatively measured. According to similar articles, the extent of disorganization is qualitatively reported. Cardiomyocyte disarray was graded as mild if its extent was 1–25% of the myocardial area on the microscopic slide, moderate if 26–50%, and severe if > 50% [43]. The size of cardiomyocytes was measured by Image J software.

Measuring the gene expression in heart tissue by real-time PCR

The heart tissue was kept in a freezer at -70 °C for molecular analysis. Expression levels of PINK1, Mfn2, Drp1, BCL2-associated X (Bax), and B-cell lymphoma 2 (Bcl2) were measured by real-time PCR using a Sina SYBR Blue NO ROX HSqPCR 100T 1 ml-MM2171 kit (Catalog No.: MM2171, Parstous, Iran). Before testing, the tissues were homogenized. RNA was extracted from the left ventricle of the heart using RNX-plus reagent (Catalog No.: EX6101, Sinaclon, Iran) according to the manufacturer's protocol. Specific sequences of PCR primers (Table 1) were designed based on the published sequences in related studies [37, 44]. A NanoDrop spectrometer was used to evaluate the concentration and purity of total isolated RNA at 260 and 280 nm. Then, 1 µg of total RNA was reversely converted to cDNA using a cDNA synthesis kit (Catalog No.: A101161, Parstous, Iran, Easy cDNA Ultra-TM Synthesis Kit) in a gradient thermal cycler. Finally, the resulting cDNA was analyzed by Real-Time PCR Quantification using a Light Cycler 96 from Roche (Mannheim, Germany) under the following conditions: 95 °C for 15 min (pre-denaturation), followed by 40 cycles

Table 1 Sequence of the primers

Genes	Sequence	Reference sequence	Primer size	Product size
PINK1 F	GGAGATCCAGGCAATTTTTACAC	NM_001106694.1	23	276
PINK1 R	TTGATGGCAAAGGGGAAGGC		20	
Mfn2 F	AGTGTCAAGACCGTGAACCA	NM_130894.4	20	202
Mfn2 R	ACACATCAGCATCCAGGCAA		20	
Bax F	TTTTGCTACAGGGTTTCATCC	NM_017059.2	21	147
Bax R	TATTGCTGTCCAGTTCATCTC		21	
Bcl-2 F	TGGTACCTGCAGCTTCTTTC	NM_016993.2	20	131
Bcl-2 R	ATCTCCAGTATCCCACTCGTA		21	
Drp1 F	GCTAGATGTGCCAGTTCCAGT	NM_053655.3	21	249
Drp1 R	TGTGCCATGTCCTCGGATTC		20	
Beta-actin F	ATCAGCAAGCAGGAGTACGAT	NM_031144.3	21	94
Beta-actin R	AAAGGGTGTAAAACGCAGCTC		21	

PINK1 (PTEN-induced putative kinase 1), Mfn2 (Mitofusin-2), Bax (Bcl-2-associated X protein), Bcl-2 (B-cell lymphoma 2), Drp1 (Dynamin related protein 1)

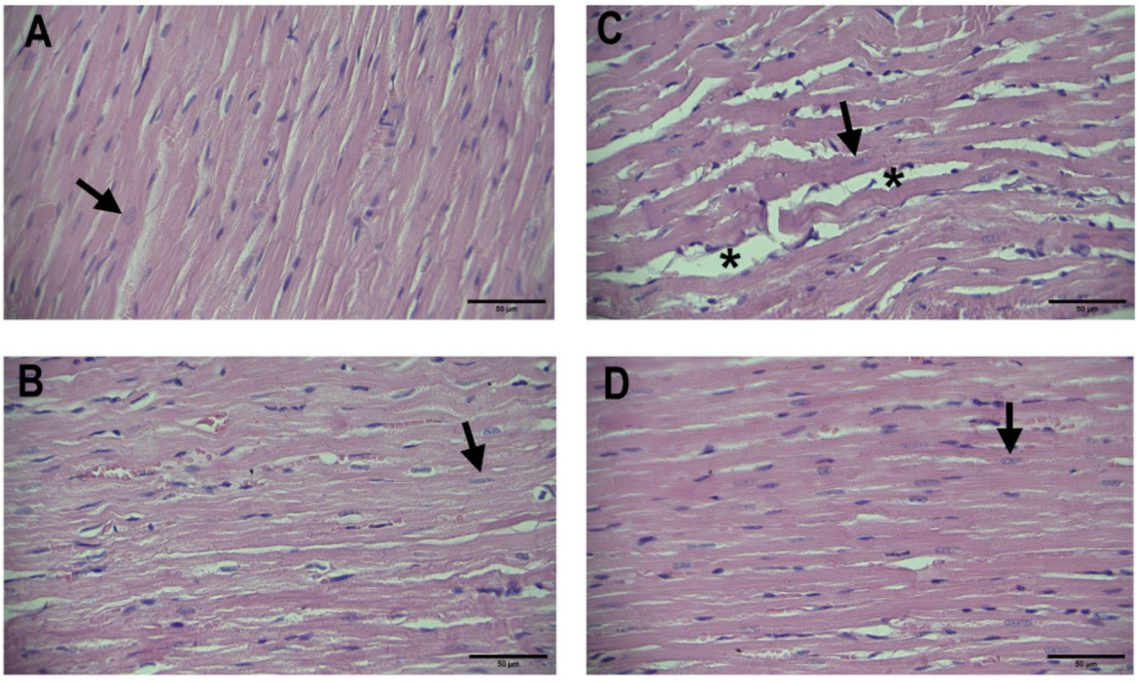

Fig. 1 Effect of DIZE on cardiac histology in aged rats induced by D-GAL. Cardiac tissue architecture in (**A**) the control group, (**B**) the control group receiving DIZE, (**C**) the aging group induced by D-GAL injections, and (**D**) the group co-treated with D-GAL and DIZE. The images are presented at 400X magnification. The scale bar is 50 µm. Arrowheads indicate cardiomyocytes. ***** indicate intercellular spaces. D-GAL: D-galactose; DIZE: Diminazine

of 95 °C for 15–30 s (denaturation), 55–60 °C for 30 s (annealing), and 72 °C for 30 s (extension). The expression levels of genes were normalized to the expression of beta-actin as an internal control. The relative expression of Drp1, Mfn2, PINK1, Bcl2, and Bax were calculated through the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the groups, and Tukey's post hoc test was used to determine the differences between them. SPSS 16.0 software was used to statistical calculation. Data are expressed as mean ± standard error of the Mean (SEM), and $P < 0.05$ is typically considered to be statistically significant.

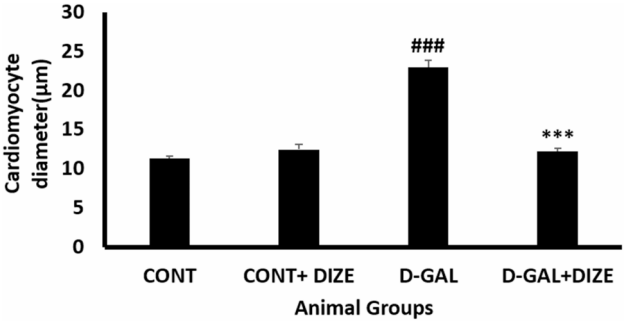

Fig. 2 Effect of DIZE on cardiomyocyte diameter. Data were analyzed by the Tukey-Kramer post hoc test following a one-way ANOVA and are expressed as mean ± SEM ($n = 6–7$). ###$P < 0.001$ compared to the control group and **$P < 0.01$ compared to the D-GAL group. CONT: control; D-GAL: D-galactose; DIZE: Diminazine

Results

Effect of diminazine on the cardiac tissue

The tissue sections from the control group (Fig. 1A) showed the morphology of cardiomyocytes with oval and bright central nuclei in normal physiological conditions. The DIZE received group (CONT + DIZE) was histologically similar to the control group (Fig. 1B). In the D-GAL group (Fig. 1C), the cardiomyocyte arrangement was disordered and the intercellular space was significantly enhanced. In the D-GAL group, mild Cardiomyocyte disarray was observed. However, treatment with DIZE in aging rats alleviated cardiomyocyte disarray induced by D-GAL in D-GAL + DIZE group. NO reportable fibrosis was identified (Fig. 1). The D-GAL-treated group (Fig. 1C) indicated more eosinophilic staining, and notably, cardiac hypertrophy compared to the control group. However, DIZE treatment (Fig. 1D) significantly reduced cardiac hypertrophy induced by D-GAL compared to the D-GAL group. Moreover, the size of cardiomyocytes was significantly (27.97 ± 0.97 µm vs. 11.3 ± 0.29 µm, $p < 0.001$) increased in the D-GAL rats compared to the control rats. Also, DIZE treatment significantly (12.26 ± 0.34 µm vs. 27.97 ± 0.97 µm, $p < 0.001$) improved the size of cardiomyocytes in the D-GAL + DIZE animals compared to the D-GAL animals (Fig. 2).

Effect of diminazine on cardiac hypertrophy indices

Cardiac hypertrophy indices including HW/BW and HW/TL were significantly higher in aged group compared to the control group (0.32 ± 0.0009 g/g vs. 0.29 ± 0.0008 g/g, $p < 0.05$), and (0.71 ± 0.01 g/cm vs. 0.5 ± 0.05 g/cm, $p < 0.001$, respectively). Cardiac hypertrophy indices were significantly decreased in the D-GAL + DIZE group compared to the aged group (0.29 ± 0.0006 g/g vs. 0.32 ± 0.0009 g/g, $p < 0.05$) and (0.58 ± 0.03 g/cm vs. 0.71 ± 0.01 g/cm, $p < 0.01$, respectively) (Figs. 3 and 4).

Effect of diminazine on gene expression levels

A significant reduction of Drp1 (0.33 ± 0.06 vs. 1 ± 0.06, $p < 0.001$), Mfn2 (0.3 ± 0.02 vs. 1 ± 0.2, $p < 0.05$), PINK1 (0.2 ± 0.06 vs. 1 ± 0.04, $p < 0.01$), and Bcl2 (0.16 ± 0.06 vs. 1 ± 0.06, $p < 0.001$) expression levels in the aged animals compared to the control animals were found. Moreover, a remarkable increase of Bax expression level in aged animals compared to the control animals was observed (3.1 ± 0.1 vs. 1 ± 0.4, $p < 0.001$). Furthermore, we found that DIZE treatment significantly improved Drp1 (0.96 ± 0.2 vs. 0.33 ± 0.06, $p < 0.01$), Mfn2 (0.81 ± 0.05 vs. 0.3 ± 0.02, $p < 0.01$), PINK1 (1 ± 0.04 vs. 0.2 ± 0.06, $p < 0.01$), Bcl2 (0.6 ± 0.02 vs. 0.16 ± 0.06, $p < 0.001$), and Bax (0.4 ± 0.03 vs. 3.1 ± 0.1, $p < 0.001$) expression levels in the D-GAL + DIZE group compared to the aged animals (Figs. 5, 6, 7, 8 and 9).

Discussion

Aging is an important risk factor for cardiac hypertrophy. Cardiomyocyte disarray is a recognized histopathological hallmark of cardiac hypertrophy [45]. Interestingly, we found that all aged rats exhibited increased HW/BW and HW/TL ratios. In addition, our results showed histological alterations in the aged hearts including cardiomyocyte disarray, increased cardiomyocyte sizes, and increased intracellular distance. Our previous studies have demonstrated that D-GAL injection enhances the markers related to cardiac hypertrophy in rats [46–48].

Mitochondrial dysfunction is a major feature of the aging process [46]. Mitochondrial dynamics is tightly regulated by mitochondrial fission and fusion factors [49]. Mfn2 absence inhibits mitochondrial fusion and increases impaired mitochondria, ultimately leading to cardiac hypertrophy [50]. Drp1 depletion reduces mitochondrial fission and disrupts mitophagy, leading to cardiac dysfunction [15, 16]. Disruption of the mitochondrial fission gene Drp-1 or mitochondrial fusion gene Mfn2 causes pathological cardiac hypertrophy and rapid death [15, 51, 52]. Mfn2 dysfunctions have been found to contribute to ventricular wall thickening and cardiac hypertrophy [36, 53, 54]. PINK1 initiates mitophagy by phosphorylation of Mfn2 [55]. Moreover,

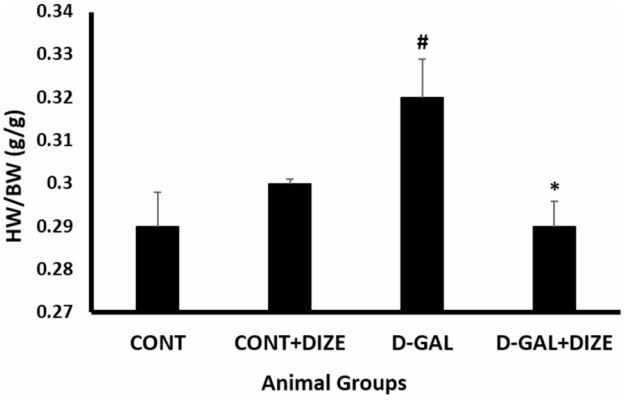

Fig. 3 Effect of DIZE on HW/BW. Data were analyzed by the Tukey-Kramer post hoc test following a one-way ANOVA and are expressed as mean ± SEM ($n = 6–7$). #$P < 0.05$ compared to the control group and *$P < 0.05$ compared to the D-GAL group. CONT: control; D-GAL: D-galactose; DIZE: Diminazine, HW: Heart weight; BW: body weight

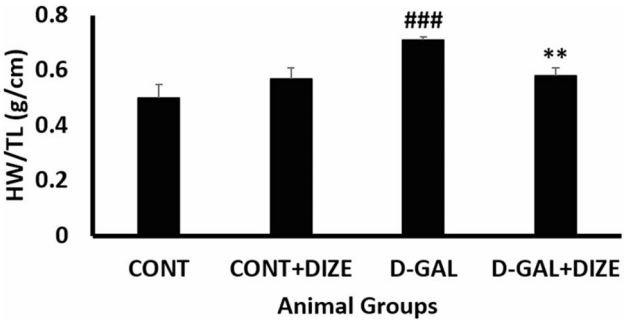

Fig. 4 Effect of DIZE on HW/TL. Data were analyzed by the Tukey-Kramer post hoc test following a one-way ANOVA and are expressed as mean ± SEM ($n = 6–7$). ###$P < 0.001$ compared to the control group and **$P < 0.01$ compared to the D-GAL group. CONT: control; D-GAL: D-galactose; DIZE: Diminazine, HW: Heart weight; TL: tibia length

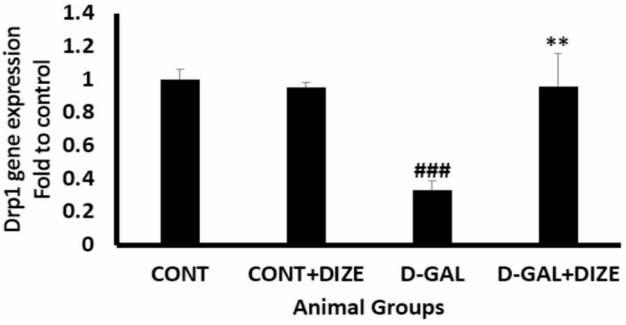

Fig. 5 Effect of DIZE on Drp1 gene expression. Data were analyzed by the Tukey-Kramer post hoc test following a one-way ANOVA analysis and are expressed as mean ± SEM ($n = 6–7$). ###$P < 0.001$ compared to the control group and **$P < 0.01$ compared to the D-GAL group. CONT: control; D-GAL: D-galactose; DIZE: Diminazine

PINK1 downregulation is associated with oxidative stress, apoptosis, and cardiac hypertrophy [56–58]. Drp1 plays a major role in regulating PINK1 expression in the heart and preventing cardiac hypertrophy [59, 60].

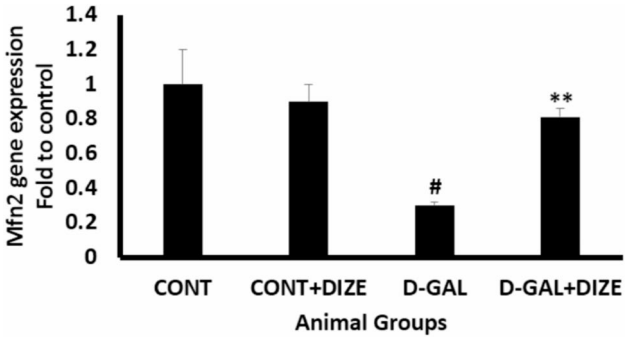

Fig. 6 Effect of DIZE on Mfn2 gene expression. Data were analyzed by the Tukey-Kramer post hoc test following a one-way ANOVA and are expressed as mean ± SEM ($n = 6–7$). #$P < 0.05$ compared to the control group and **$P < 0.01$ compared to the D-GAL group. CONT: control; D-GAL: D-galactose; DIZE: Diminazine

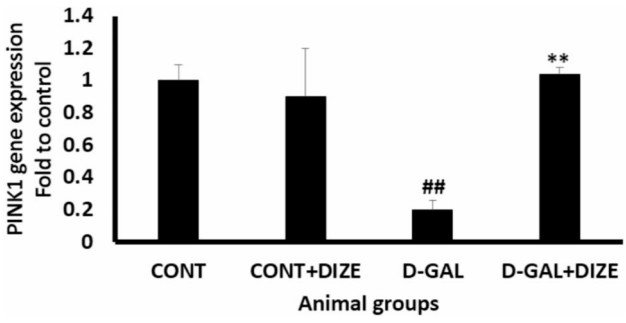

Fig. 7 Effect of DIZE on PINK1 gene expression. Data were analyzed by the Tukey-Kramer post hoc test following a one-way ANOVA and are expressed as mean ± SEM ($n = 6–7$). ##$P < 0.01$ compared to the control group and **$P < 0.01$ compared to the D-GAL group. CONT: control; D-GAL: D-galactose; DIZE: Diminazine

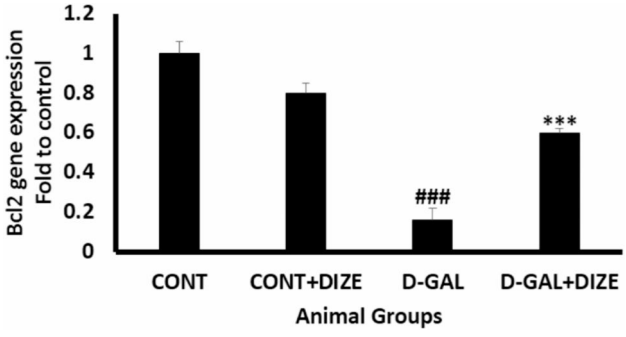

Fig. 8 Effect of DIZE on Bcl2 gene expression. Data were analyzed by the Tukey-Kramer post hoc test following a one-way ANOVA and are expressed as mean ± SEM ($n = 6–7$). ###$P < 0.001$ compared to the control group and ***$P < 0.001$ compared to the D-GAL group. CONT: control; D-GAL: D-galactose; DIZE: Diminazine

Drp1 ameliorates mitochondrial dysfunction and cardiac hypertrophy induced by obesity [61]. The integrity of the heart and the brain is maintained by Drp1 expression in the mammals [62]. A reduction in Drp1 expression leads to the inhibition of mitophagy, mitochondrial dysfunction, oxidative stress, and ultimately apoptosis [63]. Our

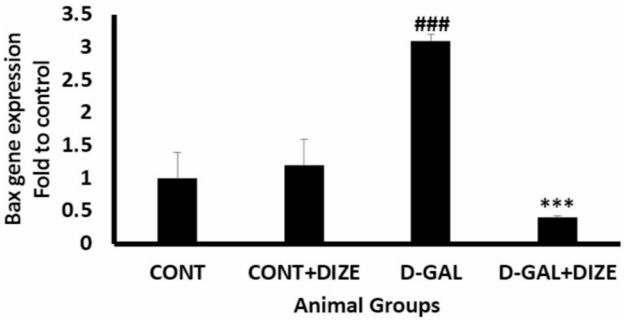

Fig. 9 Effect of DIZE on Bax gene expression. Data were analyzed by the Tukey-Kramer post hoc test following a one-way ANOVA and are expressed as mean ± SEM ($n = 6–7$). ###$P < 0.001$ compared to the control group and ***$P < 0.001$ compared to the D-GAL group. CONT: control; D-GAL: D-galactose; DIZE: Diminazine

results showed downregulation of PINK1, Mfn2 and Drp1 in the aged animals. Therefore, impaired mitophagy induced by aging contributes to the cardiac hypertrophy.

Disrupted mitochondrial dynamics and impaired mitophagy induced by aging lead to ROS production, mitochondrial damage and cytochrome C release [64]. This is a vicious cycle of ROS production and mitochondrial dysfunction that ultimately leads to apoptosis. Apoptosis is a critical pathway that leads to cardiac hypertrophy and cardiac dysfunction [65, 66]. It is controlled by two clusters of Bax and Bcl-2 proteins [67]. Increasing evidence also shows that a reduction in mitophagy induces apoptosis [68]. Cardiac deletion of Mfn2 is accompanied by an increase in the expression of Bax and a reduction in the expression of Bcl2. Mfn2 and PINK1 inhibit apoptosis through downregulation of Bax and upregulation of Bcl2 [21, 22]. In addition, Mfn2 and PINK1 play a critical role in reducing oxidative stress [35]. oxidative stress can stimulate inflammatory pathways. Moreover, inflammation causes enhanced ROS levels which can induce oxidative stress. Oxidative stress and inflammation are directly involved in cardiac aging [69].

In the present study, the aged rats exhibited a significant increase in Bax expression and a significant reduction in Bcl-2 expression in the heart, indicating increased apoptosis in aged animals. However, DIZE treatment ameliorated age-related cardiac hypertrophy through improving cardiac hypertrophy indices, decreasing cardiomyocyte disarray, cardiomyocyte size and intracellular distance. DIZE treatment improved disrupted mitochondrial dynamics and impaired mitophagy induced by aging through increasing expression of Mfn2, Drp1, and PINK1in the heart. Moreover, DIZE treatment ameliorated cardiac apoptosis through reducing Bax expression and increasing Bcl-2 expression. AngII produces ROS, thus involving in the induction of cardiac hypertrophy. It has been shown that AngII induces cardiac hypertrophy

through reducing Mfn2 expression. In addition, upregulation of Mfn2 suppresses Ang II-induced myocardial hypertrophy [35, 36]. ACE2 is an important member of the RAS responsible for stimulation of Ang (1–7)/MasR axis and counteracting pathophysiological effects of AngII [70]. Therefore, DIZE, as an ACE2 activator, may also suppress Ang II-induced cardiac hypertrophy and exert its effects through Mfn2 upregulation in the heart. It has been reported that DIZE enhances structural remodeling and alleviates cardiac hypertrophy [34]. It can exert cardioprotective effects through ameliorating mitochondrial function, ATP generation, and modulating electrical alterations [71]. Moreover, our previous study has indicated that DIZE inhibits apoptosis by improving the expression of Bax and Bcl-2 in the heart. DIZE effectively ameliorates cardiac hypertrophy induced by hyperthyroidism by regulating the mitophagy, reducing apoptosis, and alleviating oxidative stress [37]. The natural compounds, antioxidants, Vitamin D, and interventions such as exercise, have also exhibited similar effect on age-related cardiac hypertrophy compared to DIZE [23, 47, 72–74].

Conclusion

The results of this study provide evidence supporting for the protective effects of DIZE on ameliorating cardiac hypertrophy and improving cardiac damage induced by aging. The findings propose that the action mechanism of DIZE on cardiac hypertrophy might be associated with mitophagy and mitochondrial-related apoptosis. However, the causes of cardiac hypertrophy induced by aging are complex; therefore more experimental and clinical investigations are needed in the future on DIZE and the its role in cardiac aging.

Limitations

The present study has limitations. The absence of oxidative stress and inflammatory indicators in the heart and cardiac mechanical and electrical parameters may limit a comprehensive understanding of cardioprotective effects of DIZE in aging. We agree that further experimental and clinical researches are necessary to explore the protective effects of DIZE on cardiac aging.

Abbreviations

ANOVA	Analysis of variance
ACE2	Angiotensin-converting enzyme 2
AngII	Angiotensin II
Bax	BCL2 associated X
Bcl2	B-cell lymphoma 2
BW	Body weight
CONT	Control
DIZE	Diminazine
D-GAL	D-galactose
Drp1	Dynamin-related protein 1
HW	Heart Weight
H&E	Hematoxylin and eosin
IP	Intraperitoneal
MasR	Mas receptor
MQC	Mitochondrial Quality Control
Mfn2	Mitofusin 2
PINK1	Phosphatase and tensin homolog (PTEN)-induced putative kinase 1
RAS	Renin-Angiotensin System
ROS	Reactive oxygen species
SEM	Standard Error of the Mean

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Author contributions

EV and FRA conceived the project. EV, FRA, AS, and MZ participated in data extraction and analysis, prepared figures, and wrote the manuscript. FRA and AK revised the manuscript. All the authors read and approved the final manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

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Data availability

The data used and analyzed in this study are available from the corresponding author on reasonable request.

Declarations

Ethical approval

Ethical approval was granted by the Ethics Committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1402.027).

Consent for publication

None.

Competing interests

The authors declare no competing interests.

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