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Attenuating cardiac fibrosis and apoptosis by exercise is associated with modulation of the circSlc8a1/miR-133a axis in D-galactose-treated rats

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Abstract

Purpose

Circular RNAs and microRNAs have been identified as key regulators of aging mechanisms and cardiovascular disorders. This study investigated the effects of moderate-intensity endurance training (MIET) and high-intensity interval training (HIIT) on cardiac fibrosis- and apoptosis-related markers and their relationship with the circSlc8a1/miR-133a axis in a D-galactose-induced aging model of male rats.

Method

Forty-two male Wistar rats were randomly assigned to six groups: control (CTL), MIET, HIIT, D-galactose (D-gal), D-gal+MIET, and D-gal+HIIT. Exercise training was conducted for 8 weeks, five days per week, concurrently with daily D-galactose injections. Expression levels of circSlc8a1 and miR-133a were quantified by real-time PCR, and protein levels of fibrosis (TGF-β, CTGF) and apoptosis (Bim, BMF) markers were measured via western blot analysis.

Results

Results: Both MIET and HIIT attenuated the D-gal-associated changes in circSlc8a1 and miR-133a expression, characterized by increased circSlc8a1 and decreased miR-133a expression. Moreover, both exercise interventions were accompanied by lower levels of the fibrosis- and apoptosis-related markers TGF-β, CTGF, Bim, and BMF compared with the D-gal-treated group.

Conclusion

The present findings suggest that exercise training may attenuate D-galactose-induced cardiac changes and is associated with modulation of the circSlc8a1/miR-133a axis, accompanied by lower levels of markers related to myocardial fibrosis and apoptosis. These results support the potential role of exercise as a non-pharmacological strategy for mitigating aging-associated cardiac changes; however, further studies are needed to clarify the underlying mechanisms and functional implications.

Background

Aging represents one of the most significant global public health challenges. The number and proportion of individuals aged 60 years and older are increasing rapidly across the world (Fried, 2016). Between 2015 and 2050, this proportion will nearly double from 12% to 22%. By 2050, the global population of people aged 60 and older is increased to 2.1 billion (Organization, 2024).

With aging, the incidence of cardiovascular diseases increases. Cardiac aging is characterized by accumulation of damaged proteins, microvascular dysfunction, hypertrophy, fibrosis, left ventricle diastolic

dysfunction, arrhythmias, and heart failure (Lakatta & Levy, 2003; Zhou et al., 2018).

Non-coding RNAs (ncRNAs) are a diverse class of functional RNA molecules that lack protein-coding capacity. Major categories of ncRNAs include microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), all of which play essential roles in the regulation of gene expression and various physiological and pathological processes (Guttman & Rinn, 2012). Numerous studies have shown that overexpression or downexpression of key ncRNAs plays a role in various diseases, including cardiovascular diseases (Sessa et al., 2023; Wang et al., 2019). Other studies indicate that ncRNAs have significant effects on cardiac aging under various pathophysiological conditions (Kinser & Pincus, 2020).

Next-generation sequencing (NGS) identified age-associated alterations in gene expression profiles across humans and a wide range of other organisms (Knupp & Miura, 2018). Many studies have examined changes in microRNAs and lncRNA expression during aging. More recent studies have shown changes in the expression of circRNAs associated with age (Zhao et al., 2024). CircRNAs, initially identified in plant viruses, are predominantly derived from protein-coding genes and have been detected across a wide range of eukaryotic organisms. These covalently closed RNA molecules regulate gene expression at both transcriptional and post-transcriptional levels, primarily by functioning as molecular sponges that bind and sequester specific microRNAs (Kolakofsky, 1976; Olson & Graveley, 2014). Because covalently closed circular structure, circRNAs exhibit a longer half-life compared with messenger RNAs (mRNAs) and are thus resistant to degradation by exonucleases (Enuka et al., 2016). RNA sequencing (RNA-Seq) analysis has shown that the expression of some circRNAs changes with age in fruit flies. Subsequent studies demonstrated that this changes also occurs in other animals (Knupp & Miura, 2018). The studies also verify that circRNAs express in the heart, expression changing play a role in physiological and pathological conditions (Bibi et al., 2025).

CircRNAs contribute to myocardial diseases and cardiac aging by regulating inflammation, oxidative stress, autophagy, fibrosis, and apoptosis. Through these mechanisms, they influence cardiac function and the development of cardiovascular diseases (Bibi et al., 2025; Farazi et al., 2024). For example, it was shown the expression of the circSlc8a1 increases in response to reactive oxygen species (ROS) in the H9c2 myoblast cell line and promotes apoptosis in cardiomyocytes by sponging miR-133-a (M. Li et al., 2018). This circRNA has a complementary sequence for miR-133-a and inhibits miR-133-a activity through the sponging process. Knockdown of circSlc8a1 in an ischemia-reperfusion experimental model reduces apoptosis by decreasing the expression of the cell death-inducing protein (CDIP1), which is a target of miR-133-a. Studies have shown that miR-133a suppresses angiogenesis, inflammation, cardiomyocyte apoptosis, cardiac hypertrophy, and fibrosis. It also participates in the early pathological events of ischemic heart disease and contributes to later cardiac remodeling.(Xiao et al., 2019). MiRNAs regulate many biological and pathological processes, including aging and lifespan (Kinser & Pincus, 2020; Suh, 2018). The expression of many miRNAs changes over the lifespan, and their use as biomarkers of aging has been proposed (Kinser & Pincus, 2020). Therefore, miRNAs may be a therapeutic target for cardiac aging.

Another target of miR-133-a is the connective tissue growth factor (CTGF). It has been shown that miR-133-a inhibits CTGF expression, which is associated with cardiac fibrosis. Targeting CTGF via miR-133-a may reduce age-related cardiac fibrosis and improve cardiac function (N. Li et al., 2018). MiR-133-a also

modulates the transforming growth factor-beta (TGF-β) pathway, which is often dysregulated in aged hearts and leads to fibrosis and cardiac hypertrophy (N. Li et al., 2018).

MiR-133-a is associated with regulating pro-apoptotic genes including Bcl-2 interacting mediator of cell death (Bim) and Bcl-2 modifying factor (BMF) involved in cell death during cardiac stress (Izarra et al., 2014).

Regular physical activity and exercise training are linked to a lower risk of cardiovascular diseases, strokes, and cardiovascular-related deaths (Valenzuela et al., 2023; N. N. Wu et al., 2019). Regular exercise training is also widely recognized as a powerful intervention for mitigating the declines in physiological functions associated with aging. Numerous studies demonstrate that consistent physical activity, particularly aerobic and resistance training can attenuate the risk of chronic diseases such as cardiovascular disorders, diabetes, and osteoporosis (Ciolac, 2013; Mcleod et al., 2019).

In our previous study, exercise was shown to reduce cardiac fibrosis and histopathological damage and improve cardiac function in a D-galactose-induced aging model (Askarimoghadam et al., 2025). Building on these findings, we hypothesized that exercise-related changes in cardiac circular RNAs, microRNAs, and fibrosis- and apoptosis-related proteins may be associated with the cardiac effects of exercise in this aging model. Therefore, this study aimed to investigate the effects of moderate-intensity endurance training (MIET) and high-intensity interval training (HIIT) on the expression of circSlc8a1 and miR-133a, as well as the levels of fibrosis-related proteins (TGF-β and CTGF) and apoptosis-related proteins (Bim and BMF), in the hearts of male rats subjected to D-galactose-induced aging.

Material and methods

This study was performed on 42 male Wistar rats (220 ± 20 g) obtained from the animal breeding center of the same university and maintained in the physiology research animal facility. Animals were kept under standard laboratory conditions with ad libitum access to food and water and a 12-hour light/dark light–dark cycle. The study included six experimental groups (n=7 per group) including the control (CTL): a group receiving normal saline as a vehicle, moderate intensity exercise training (MIET): a group undergoing moderate endurance exercise training, high-intensity interval training (HIIT): a group performing high-intensity interval training, D-galactose (D-gal): a D-galactose-treated group (150 mg/kg/day for 8 weeks), D-gal+MIET: a group receiving D-galactose along with MIET; and D-gal+HIIT: a group receiving D-galactose along with HIIT.

Induction of Aging

Aging was induced by daily subcutaneous administration of D-galactose at a dose of 150 mg/kg for 8 weeks (W. K. Chen et al., 2018).

Exercise Training Protocol

To reduce stress and facilitate acclimatization to treadmill running, animals underwent low-intensity exercise (10 m/min for 10 min) for 4 consecutive days. After that, maximal oxygen uptake (VO_{2max}) was

determined using an incremental treadmill test, in which rats began running at 5 m/min and speed was increased by 3 m/min every 3 min until exhaustion. The exercise training protocol lasted 8 weeks (5 days/week). Moderate-intensity exercise was defined as 60–65% of VO_{2max} (Table 1) (F.-H. Li et al., 2019). High-intensity interval training (HIIT) was designed as a table 2 (X. Li & Wang, 2017).

Table 1. High-intensity interval training (HIIT) protocol.

week	Frequency	intervals	HIID (min)	LIID (min)	HIIV (Vmax)	LIIV (Vmax)	TETS (min)
1	5	4	2	1	80	50	12
2	5	4	2	1	80	50	12
3	5	6	2	1	85	50	18
4	5	6	2	1	85	50	18
5	5	8	2	1	90	50	24
6	5	8	2	1	90	50	24
7	5	10	2	1	95	50	30
8	5	10	2	1	95	50	30

HIID: High-intensity interval duration**, LIID:** Low-intensity interval duration, **HIIV**: High-intensity interval velocity, **LIIV**: Low-intensity interval velocity, **TETS**: Total exercise time in a session

Table 2. Moderate-Intensity Endurance Training (MIET) protocol

Weeks	1	2	3	4	5	6	7	8
Speed **Vmax**	50-55%	50-55%	60-65%	60-65%	60-65%	60-65%	60-65%	60-65%
Time (min)	15	15	20	25	40	45	50	50

Tissue Collection

Twenty-four hours after the final exercise session, animals were deeply anesthetized with an intraperitoneal injection of thiopental sodium (60 mg/kg). Adequate anesthesia was confirmed by the absence of withdrawal and corneal reflexes before the animals were euthanized by decapitation using a guillotine to ensure rapid tissue preservation (Rostamzadeh et al., 2024). Samples of the left ventricle (LV) along with the interventricular septum were collected and stored at −80$^{\circ C}$ for molecular analyses.

Real-Time PCR Analysis

Total cellular RNA was extracted using the Total RNA extraction kit (Parstous Biotechnology, Mashhad, Iran). mRNA concentration and purity (A260/A280 ratio) were determined using a Nanodrop spectrophotometer. cDNA was synthesized from RNA using a cDNA synthesis kit (Parstous Biotechnology, Iran). Real-time PCR was performed using SYBR Green dye (RealQ Plus 2X Master Mix Green (Amplicon, Denmark) on a StepOnePlus instrument (Applied Biosystems), following standard protocols. The expression levels of circSlc8a1 and miR-133a were assessed using primers listed in table 3. GAPDH served as an internal control for circular RNA, and RNU6 was used as the internal control for miR-133a. The results were reported as $2^{-\Delta\Delta CT}$

Western blotting

Protein levels were assessed via Western blotting following established protocols [30]. Briefly, heart tissue was homogenized in cold RIPA buffer on ice, and then lysates were centrifuged at 14,000 rpm for 20 min at 4°C to remove debris. Protein concentration was determined using a Bradford assay kit (DB0017, DNAbioTech, Iran).

Samples were mixed with 2X Laemmli buffer, boiled for 5 min, and 20 µg of protein was loaded per lane in SDS-PAGE. Proteins were then transferred to a 0.2 µm PVDF membrane (Cat No: 162-017777; Bio-Rad Laboratories, CA, USA). Membranes were blocked with 5% BSA (Cat No: A-7888; Sigma Aldrich, MO, USA) in 0.1% Tween 20 for 1 h and incubated overnight at 4°C with primary antibodies, including anti TGF-β (1/1000, ab215715, Abcam), CTGF (1/1000, ab6992, Abcam), BIM (1/1000, 2933S, Cell signaling), BMF (1/1000, 50542S, cell signaling), and anti-β-actin (1:2500, ab8227, Abcam). After three TBST washing, membranes were incubated with an HRP-conjugated secondary antibody (1:10,000, ab6721, Abcam) for 1 h at room temperature. Protein bands were visualized using enhanced chemiluminescence (ECL) and quantified via

image j. Band intensities were normalized to β-actin, and relative expression levels were compared between groups.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 10. Data are presented as mean ± standard error of the mean (SEM). Normality was assessed using the Shapiro–Wilk test. Differences among groups were analyzed using two-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. A two-tailed P value < 0.05 was considered statistically significant. Relative gene expression levels were calculated using the 2^−ΔΔCt method.

Table 3. The primer sequences

Cicular RNA and miRNA	Froward primer 5'-3'	Reverse primer 5'-3'	Reference
circSlc8a1	ATTGAAGGCACAGCCCGAGGTG	CCCATTGAAACATTGGGTGGGAGAC	(M. Li et al., 2018)
miR-133-a	GCGTTTGGTCCCCTTCAAC	AGTGCAGGGTCCGAGGTATT	(M. Li et al., 2018)
miR-133a RT primer	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCAGCTG		
GAPDH	AACGACCCCTTCATTGAC	TCCACGACATACTCAGCAC	(Chandra et al., 2017)
RNU6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT	(Rajabi et al., 2022)

Results

Our results demonstrated that circSlc8a1 expression was significantly elevated in the D-gal group compared with the control group ($P < 0.001$). Both HIIT and MIET markedly attenuated this increase in D-gal-treated animals ($P < 0.001$) (Fig. 1A).

In contrast, miR-133a expression showed a non-significant reduction in the D-gal group compared with controls. However, both MIET ($P < 0.01$) and HIIT ($P < 0.05$) significantly upregulated miR-133a expression in D-gal-treated rats (Fig. 1B).

Regarding apoptosis-related markers, HIIT (P < 0.01) and MIET (P < 0.05) significantly prevented the D-gal-induced increase in BMF protein levels (P < 0.01) (Fig. 2A, C). Similarly, Bim expression was significantly elevated in the D-gal group compared with controls (P < 0.01), whereas both HIIT (P < 0.001) and MIET (P < 0.001) effectively suppressed this increase (Fig. 2B, C).

In terms of fibrosis-related markers, CTGF levels were significantly higher in the D-gal group compared with controls (P < 0.001). HIIT significantly reduced CTGF expression in D-gal-treated rats (P < 0.01) (Fig. 3A, C).

In addition, both HIIT and MIET significantly inhibited the D-gal-induced upregulation of TGF-β expression ($P < 0.001$), suggesting a protective effect of exercise against cardiac fibrotic remodeling (Fig. 3B, C).

Discussion

Our experimental data demonstrated significant alterations in the expression levels of several molecular markers related to fibrosis and apoptosis in response to D-gal administration, with or without concurrent exercise training. D-gal administration was accompanied by increased circSlc8a1 expression, whereas concurrent MIET and HIIT attenuated this increase. In addition, higher miR-133a expression was observed in D-gal-treated rats undergoing either MIET or HIIT. D-gal administration also altered the expression of the fibrosis-related markers TGF-β and CTGF, as well as the apoptosis-related markers Bim and BMF, while these changes were less pronounced in rats receiving concurrent exercise training. Collectively, these findings suggest that endurance and interval exercise training performed concurrently with D-gal administration may mitigate molecular changes related to cardiac fibrosis and apoptosis, with these effects coinciding with modulation of the circSlc8a1/miR-133a axis.

CVDs and aging are closely linked to dysregulated gene expression, oxidative stress, inflammation, and maladaptive remodeling. CircRNAs have emerged as important regulators in these processes due to their ability to modulate molecular pathways involved in cardiac physiology and pathology (Zhang et al., 2020). Previous studies have demonstrated that circSlc8a1 is involved in the progression of cardiac hypertrophy, fibrosis, cardiac remodeling, and heart failure (Lu et al., 2022). This study revealed increase in the expression of circSlc8a1 in heart of D-galactose aging model. The significant increase in circSlc8a1 expression may reflect its involvement in molecular changes associated with D-galactose-induced cardiac aging. D-galactose induces oxidative stress (Z. Chen et al., 2020; Cheng et al., 2021) and mimics aging phenotypes, and elevated circSlc8a1 may reflect a pathological response to oxidative damage. Exercise interventions reducing circSlc8a1 levels suggested modulating its expression could be protective against aging-related cardiac deterioration. The beneficial effects of exercise training were shown in another study. Swimming upregulates circUtrn in cardiomyocytes of mice, which promoted physiological cardiac hypertrophy and conferred protection against myocardial ischemia-reperfusion injury (Zhang et al., 2020). Experimental reduction of circslc8a1 decreases heart damage caused by ischemia–reperfusion injury in animal models. These results suggest that circslc8a1 is a key modulator of cardiac stress responses and a potential therapeutic target for cardiac hypertrophy and injury (Lim et al., 2019). Notably, recent findings have identified SLC8a1-604 as a protein encoded by circSlc8a1 that is implicated in pressure overload-associated cardiac remodeling (F. Li et al., 2025).

It was revealed circSlc8a1 by sequencing miR-133a reduce cardiac hypertrophy in pressure overload model (M. Li et al., 2018; N. Wu et al., 2023). CircRNAs have emerged as important modulators of gene expression by sequestering microRNAs and providing structural platforms for the assembly of protein complexes, thereby contributing to the regulation of cellular functions (Kameda et al., 2023; Panni et al., 2020). MiR-133a is a muscle-specific microRNA that plays a crucial role in cardiovascular disease by regulating cardiac remodeling, apoptosis, and fibrosis. Our data indicated both types of exercise intervention increase the miR-

133a expression in D-gal group. Since, circSlc8a1 acts as an endogenous sponge for miR-133a in cardiomyocytes, the reduction of circSlc8a1 in response to exercise may increase the levels of miR-133a.

CircRNAs play a significant role in regulating apoptosis in CVDs, such as atherosclerosis, acute myocardial infarction (AMI), and myocardial I/R injury. They primarily modulate cardiomyocyte apoptosis by acting as sponges for specific microRNAs and influencing signaling pathways such as TGF-β1 and NF-κB (Pan et al., 2025). Our findings showed that changes in circSlc8a1 and miR-133a expression coincided with changes in apoptosis-related markers, including Bim and BMF, suggesting a potential relationship between this regulatory axis and molecular changes associated with cardiac apoptosis. This suppression leads to decreased activation of apoptotic pathways, such as caspase-3 activity, enhancing cell survival under stress conditions like oxidative stress (Izarra et al., 2014). In accordance with mentioned studies, the finding of this study also indicated HIIT and MIET by modifying circSlc8a1 and miR-133a expression targets Bim and BMF mRNA to suppress its translation, reducing apoptosis and preserving cardiac cell viability during aging and stress. It was demonstrated that circslc8a1 is upregulated in cardiomyocytes under oxidative stress and contributes to cell death by binding and inhibiting miR-133a, a microRNA that normally protects against cardiac hypertrophy and apoptosis.

MiR-133a inhibits hypertrophic growth of cardiomyocytes and reduces collagen deposition by targeting fibrotic mediators like CTGF and TGF-β signaling pathway, which are central drivers of fibrosis and stiffening in aging hearts ref. Our finding also indicated the relationship between the expression of circSlc8a1 and miR-133a and CTGF and TGF-β levels in heart of aged animal model. Both type of exercise intervention also able to alleviate fibrosis markers. Both aerobic and resistance exercise training have been shown to alleviate cardiac fibrosis by inhibiting the TGF-β1/Smad2/3 signaling pathway, which is central to fibrotic processes (Ma et al., 2021). Elevated circslc81a1 may bind miR-133a, preventing it from downregulating CTGF and TGF-β signaling components, thus promoting fibrosis. In mice models of MI, aerobic exercise led to an upregulation of miR-133a-3p, which was associated with improved cardiac function and reduced fibrosis. This effect is mediated through the downregulation of CTGF, a key player in cardiac fibrosis (Ma et al., 2021).

This study was performed using a male Wistar rat model of D-galactose-induced aging; therefore, the findings should be extrapolated to other species, particularly humans, with caution. Although this model reproduces several features of age-related cardiac alterations (Azman & Zakaria, 2019), differences in metabolism, lifespan, and molecular regulation between rodents and humans may limit the direct translation of these findings. Future studies using additional animal models and clinical populations are required to further clarify the relevance of the circSlc8a1/miR-133a axis in human cardiac aging.

Conclusion

The results of this study showed that both MIET and HIIT, performed concurrently with D-galactose administration, were associated with lower circSlc8a1 expression, higher miR-133a expression, and reduced levels of fibrosis- and apoptosis-related markers. These findings suggest that exercise may be related to modulation of the circSlc8a1/miR-133a axis and attenuation of molecular changes associated with cardiac fibrosis and apoptosis.

Statements & Declarations

Ethics approval and consent to participate: The experimental protocol was approved by the Ethics Committee of Kerman University of Medical Sciences (IR.KMU.AEC.1403.067).

Conflict of Interest statement: The authors declare that there is no conflict of interest regarding the publication of this article

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Availability of data and materials: The datasets generated and analyzed during the current study are publicly available in the Mendeley Data repository at https://data.mendeley.com/datasets/xdc5m5d69x/1.

Competing interests: The authors declare that there is no conflict of interest regarding the publication of this article

Authors' contributions: SJ and FR designed the research. MMG and HJ trained the animals. FR and MMG recorded hemodynamic and cardiac function indices and collected tissue samples. SJ and MH measured the RNAs expression. FR analyzed the data and wrote the manuscript in collaboration with HJ, SJ and ZS. All authors read and approved the final version of the manuscript.

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Figures

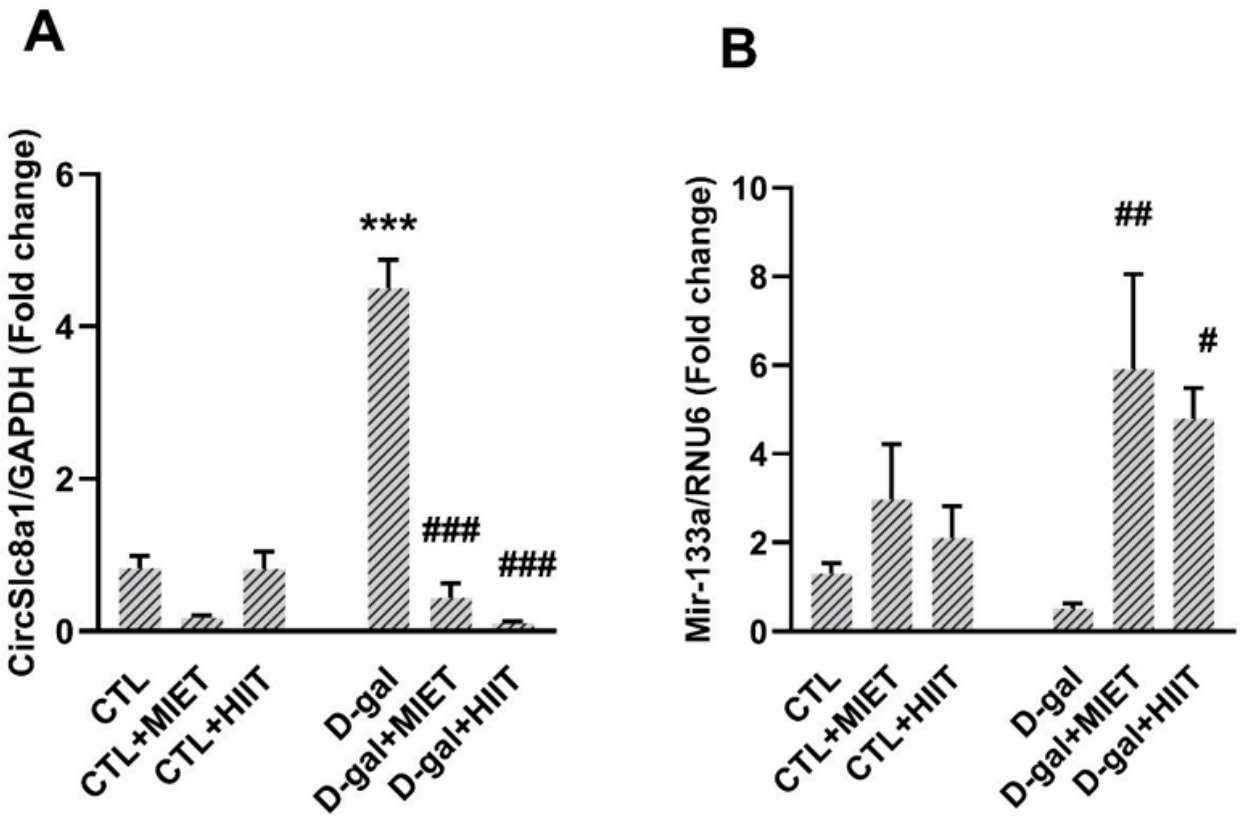


Figure 1

Expression levels of circular *Slc81a1* and miR-133a in cardiac tissue of study groups. The values represent the mean ± SEM (n = 7). ****P* < 0.001, *vs*. CTL and # *P* < 0.05, ##*P* < 0.01 *vs*. D-gal groups.

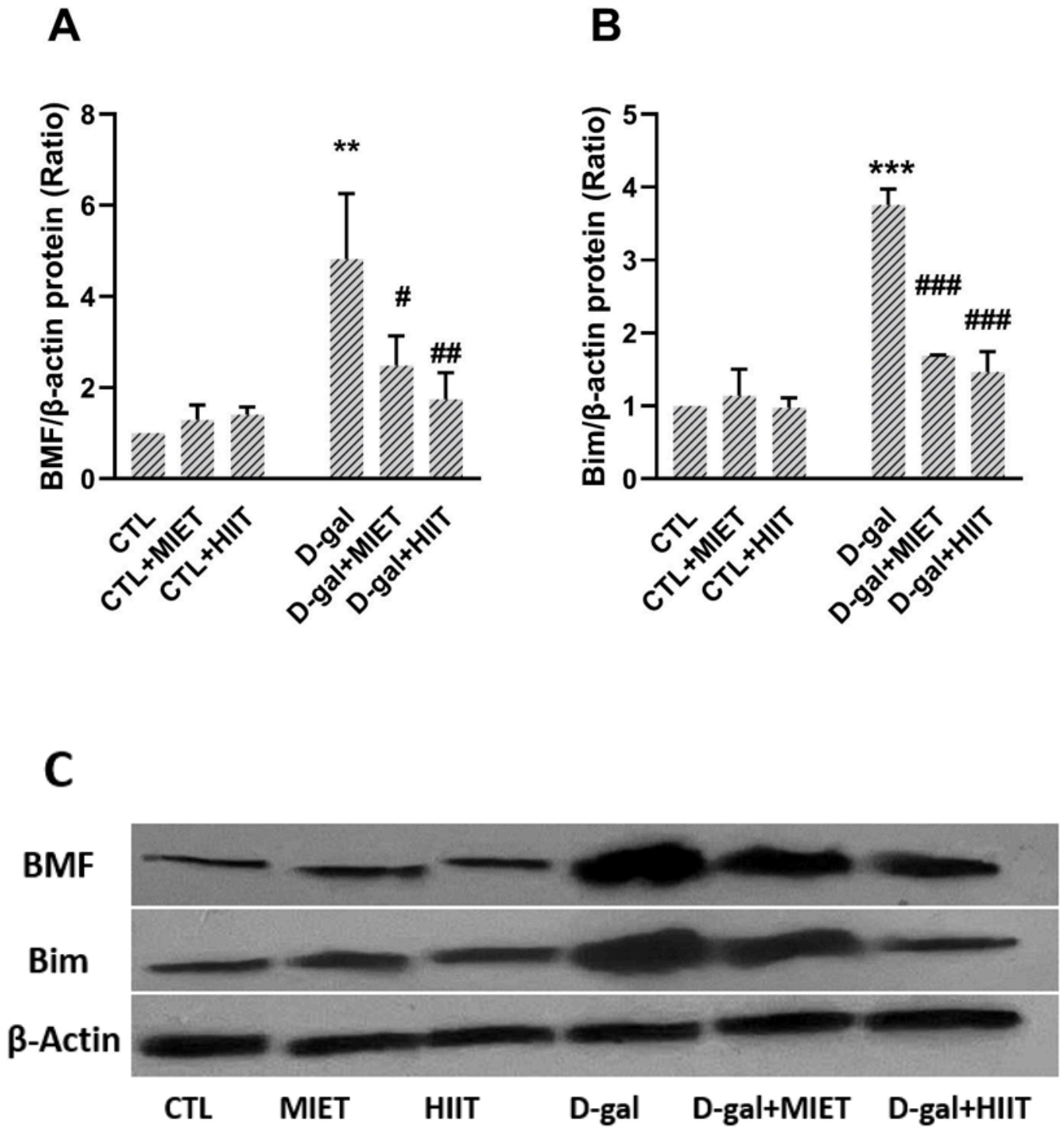


Figure 2

Western blot indicated the levels of BMF (A, C) and Bim (B, C) in cardiac tissue of study groups. The values represent the mean ± SEM (n =4). ** $P < 0.01$, *** $P < 0.001$, vs. CTL and # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ vs. D-gal groups.

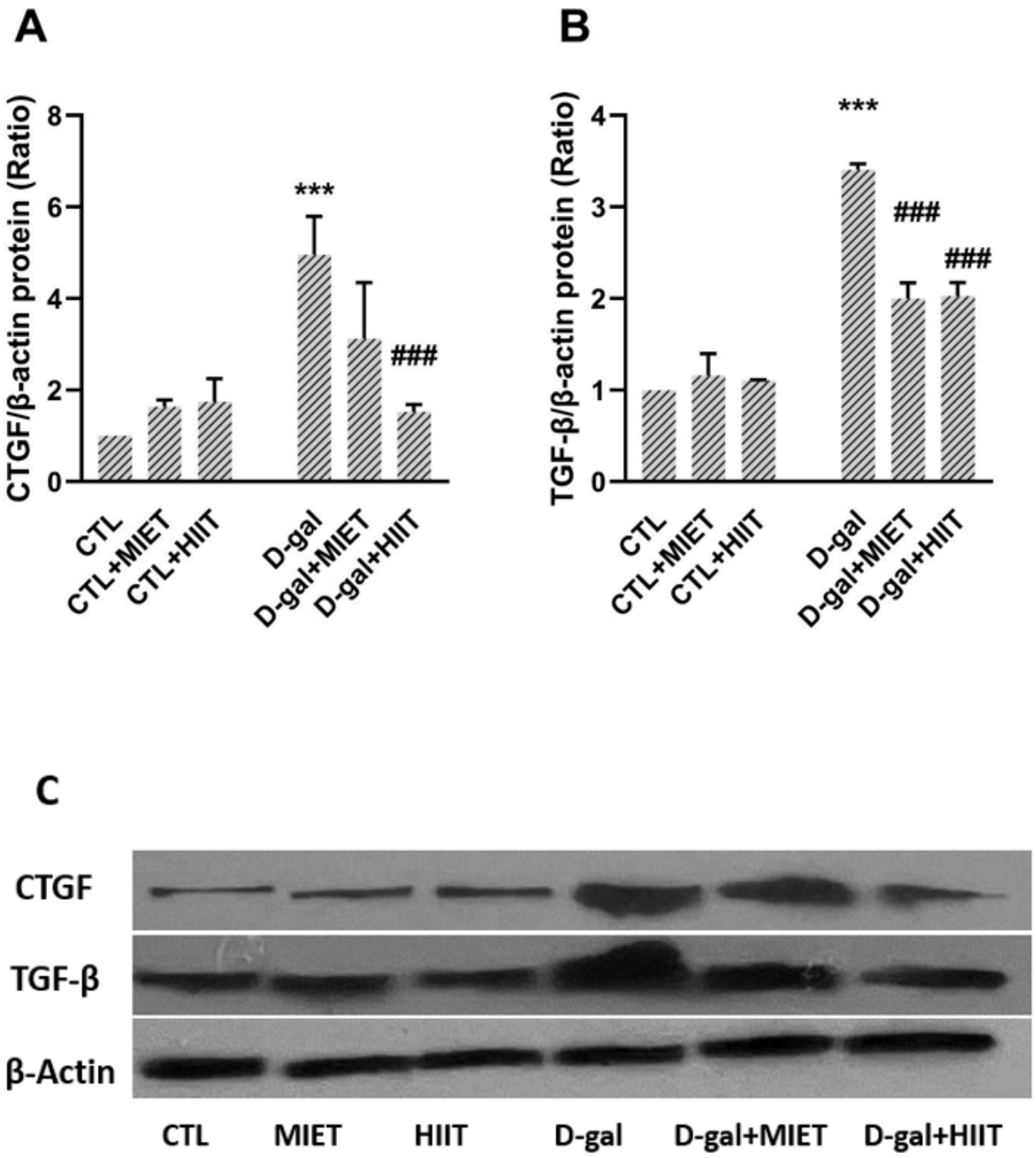


Figure 3

Western blot indicated the levels of CTGF (A, C) and TGF-β (B, C) in cardiac tissue of study groups. The values represent the mean ± SEM (n =4). ***P < 0.001, vs. CTL and ### P < 0.001 vs. D-gal groups.

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