ORIGINAL ARTICLE

DOI: 10.4274/nkmj.galenos.2026.07088
Nam Kem Med J 2026;14(2):134-143

Polydatin Attenuates Isoproterenol-induced Pathological Cardiac Hypertrophy via *miR-214/FOXO3/NFAT* Axis

Polidatinin İzoproterenol-indüklenen Patolojik Kardiyak Hipertrofiyi *miR-214/FOXO3/NFAT* Ekseni Üzerinden Azaltıcı Etkisinin İncelenmesi

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ABSTRACT

Aim: Pathological cardiac hypertrophy precedes heart failure and alters myocardial structure, function, and molecular signaling. miRNAs, particularly *miR-214*, regulate key remodeling and hypertrophic pathways. Although polydatin has reported cardioprotective effects, whether these involve miRNA-mediated mechanisms is unclear. This study investigates the potential role of the *miR-214*/*Forkhead box O3* (*FOXO3*)/nuclear factor of activated T-cells (*NFAT*) axis in mediating polydatin's effects.

Materials and Methods: Male Wistar rats were divided into three groups: Control, isoproterenol (ISO), 5 mg/kg/day, s.c.), and ISO + polydatin (100 mg/kg/day, oral). Following four-weeks treatment, cardiac hypertrophy was assessed by measuring heart weight, heart weight/body weight ratio, and heart weight/tibia length ratio. For molecular analyses, the expression levels of miR-1, *miR-214*, and miR-133b, as well as the mRNA levels of *FOXO3*, *NFAT*, atrial natriuretic peptide (*ANP*), and brain natriuretic peptide (*BNP*), were measured by real-time polymerase chain reaction.

Results: Morphological parameters were significantly increased in the ISO group compared with controls ($p<0.001$) and were markedly suppressed by polydatin ($p<0.05$). Among the miRNAs, only *miR-214* was significantly upregulated by ISO ($p=0.006$), and this effect was attenuated by polydatin ($p=0.030$). ISO downregulated *FOXO3* ($p=0.027$) and upregulated *NFAT* ($p<0.001$), effects that were reversed by polydatin. Expression of fetal gene markers *ANP* ($p=0.029$) and *BNP* ($p=0.002$) was markedly elevated in the ISO group, but these increases were abolished with polydatin treatment ($p>0.05$ vs. control).

Conclusion: Polydatin demonstrates cardioprotective effects against ISO-induced pathological cardiac hypertrophy. These effects involve not only morphological improvements but also molecular regulation via the *miR-214*/*FOXO3*/*NFAT* axis and suppression of the fetal gene program. These findings suggest that polydatin may have potential as a pharmacological agent for preventing pathological cardiac remodeling.

Keywords: Polydatin, cardiac hypertrophy, *miR-214*, *Forkhead box O3* (*FOXO3*), nuclear factor of activated T-cells (*NFAT*)

ÖZ

Amaç: Patolojik kardiyak hipertrofi, kalp yetersizliğinin erken bir habercisidir ve miyokardın yapısını, işlevini ve moleküler sinyal yollarını değiştirir. miRNA'lar, özellikle *miR-214*, yeniden şekillenme ve hipertrofi ile ilişkili temel yolları düzenler. Polidatinin kardiyoprotektif etkileri daha önce bildirilmiş olsa da, bu etkilerin miRNA-bağımlı mekanizmalarla gerçekleşip gerçekleşmediği belirsizdir. Bu çalışma, polidatinin etkilerinde *miR-214*/*Forkhead box O3* (*FOXO3*)/nükleer faktör aktivasyon T-hücreleri (*NFAT*) ekseninin olası rolünü araştırmayı amaçlamaktadır.

Gereç ve Yöntem: Erkek Wistar sıçanları üç gruba ayrıldı: Kontrol, İzoproterenol (ISO); 5 mg/kg/gün, cilt altı) ve ISO + polidatin (100 mg/kg/gün, oral). Dört haftalık tedavi sonrası kalp ağırlığı, kalp ağırlığı/vücut ağırlığı oranı ve kalp ağırlığı/tibia uzunluğu oranı ölçülerek kardiyak hipertrofi değerlendirildi. Moleküler analizler için miR-1, *miR-214* ve miR-133b düzeyleri ile *FOXO3*, *NFAT*, atriyal natriüretik peptit (*ANP*) ve beyin natriüretik peptidi (BNP) mRNA düzeyleri gerçek zamanlı polimeraz zincir reaksiyonu yöntemiyle ölçüldü.

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Received: 18.11.2025 **Accepted:** 20.01.2026 **Publication Date:** 16.06.2026

Cite this article as: Yamasan BE, Yıldız M, Bal K, Avkıran M. Polydatin attenuates isoproterenol-induced pathological cardiac hypertrophy via *miR-214/FOXO3/NFAT* axis. Nam Kem Med J. 2026;14(2):134-143

Bulgular: Morfolojik parametreler, ISO grubunda kontrol grubuna kıyasla anlamlı düzeyde arttı (p<0,001) ve bu artışlar polidatin tedavisi ile belirgin şekilde baskılandı (p<0,05). İncelenen miRNA'lar arasında yalnızca *miR-214* düzeyi ISO grubunda anlamlı olarak yükseldi (p=0,006), polidatin tedavisi ise bu artışı azalttı (p=0,030). ISO uygulaması *FOXO3* ekspresyonunu baskıladı (p=0,027) ve *NFAT* ekspresyonunu arttırdı (p<0,001). Polidatin ise bu değişiklikleri tersine çevirdi. Fetal gen belirteçleri olan *ANP* (p=0,029) ve *BNP* (p=0,002) düzeyleri ISO grubunda anlamlı şekilde yükselirken, polidatin tedavisi sonrasında bu artışlar ortadan kalktı ve düzeyler kontrol grubundan farklı bulunmadı (kontrole göre p>0,05).

Sonuç: Polidatin, ISO kaynaklı patolojik kardiyak hipertrofiye karşı kardiyoprotektif etkiler göstermektedir. Bu etkiler yalnızca morfolojik düzelmelerle sınırlı değildir; aynı zamanda *miR-214*/*FOXO3*/*NFAT* ekseni üzerinden moleküler düzenlemeyi ve fetal gen programının baskılanmasını da içerir. Bulgular, polidatinin patolojik kardiyak yeniden şekillenmenin önlenmesinde potansiyel bir farmakolojik ajan olabileceğini göstermektedir.

Anahtar Kelimeler: Polidatin, kardiyak hipertrofi, *miR-214*, *Forkhead box O3* (*FOXO3*), nükleer faktör aktivasyon T-hücreleri (*NFAT*)

INTRODUCTION

Cardiac hypertrophy is defined as an adaptive response of the myocardium to an increased hemodynamic load, tissue injury, or a pathophysiological stimulus[1,2]. Initially, it emerges as a compensatory mechanism aiming to preserve cardiac function by reducing oxygen consumption, balancing systolic wall stress, and enhancing ejection performance[3]. However, when this process becomes chronic, it leads to major cellular and molecular changes, including a marked increase in protein synthesis in cardiomyocytes, reorganization of sarcomeres, alterations in the cytoskeletal structure, a metabolic shift from oxidative phosphorylation to glycolysis, and reactivation of the fetal gene expression program[4,5]. Over time, this adaptive response progresses to pathological remodeling, driven by the development of perivascular and interstitial fibrosis, cellular reprogramming, and sustained activation of signaling pathways such as calcineurin-nuclear factor of activated T-cells (*NFAT*)[6,7]. The resulting structural and functional alterations eventually exceed the compensatory capacity of the heart, leading to progressive cardiac dysfunction and, ultimately, heart failure. Therefore, a comprehensive understanding of the molecular mechanisms underlying pathological hypertrophy plays a critical role in the development of targeted and rational therapeutic strategies for the prevention and treatment of heart failure[8].

MiRNAs are small non-coding RNA molecules, 18-25 nucleotides in length, that play a critical role in the post-transcriptional regulation of gene expression[9]. By interacting with complementary sequences in the 3' untranslated region of protein-coding mRNAs, they induce translational repression or mRNA degradation[10,11]. In this way, miRNAs fine-tune cellular protein synthesis and contribute to the regulation of fundamental biological processes[12]. Recent studies have demonstrated that miRNAs occupy central roles in physiological processes such as cell proliferation, differentiation, and cell cycle control[13,14]. However, dysregulation of their expression levels can drive pathological processes such as cardiomyocyte hypertrophy, fibrosis, and apoptosis[15,16]. In the cardiovascular system, specific miRNAs such as miR-1, miR-133, and *miR-214* have been shown to play important roles in the pathogenesis of diseases including pathological left ventricular hypertrophy, myocardial ischemia, and heart failure[9,17-20]. Among these, *miR-214*, which is highly conserved across species, is abundantly expressed in the heart and has emerged as a potent epigenetic regulator. Initially identified in tumorigenesis and cancer progression[21], *miR-214* has more recently been shown to exert key effects in cardiac hypertrophy and heart failure[22-24]. Experimental models demonstrate that inhibition of *miR-214* significantly reduces pressure overload-induced hypertrophic responses, whereas transgenic mice with cardiomyocyte-specific overexpression of *miR-214* exhibit pronounced hypertrophic phenotypes[23,25]. Consistently, *miR-214* has been found to be significantly upregulated in both human heart failure patients and hypertrophic mouse hearts[24,26]. In angiotensin II (Ang II)-induced hypertrophy models, *miR-214* expression was markedly elevated; its overexpression aggravated hypertrophy, whereas its inhibition attenuated hypertrophic responses both *in vitro* and *in vivo*[27]. Collectively, these findings suggest that *miR-214* is a strong regulator of cardiac hypertrophy and that its inhibition may confer cardioprotective benefits[25]. The biological effects of miRNAs are mediated through their direct mRNA targets[28]. In this context, *miR-214* has been identified as a critical upstream regulator of the *sirtuin 3* (*SIRT3*)/*Forkhead box O3 (FOXO3)* signaling axis. Recent evidence demonstrates that *miR-214* directly targets *SIRT3*, whose suppression leads to impaired deacetylation and reduced stabilization of *FOXO3*[27]. Consequently, elevated *miR-214* levels are associated with the functional attenuation of *FOXO3* signaling. The *FOXO* family regulates multiple fundamental processes including cell growth, differentiation, apoptosis, oxidative stress response, and energy metabolism[29,30]. Within the cardiovascular system, *FOXO* proteins serve as protective barriers against pathological hypertrophy[28,31]. Numerous studies have shown that activation of *FOXO* exerts anti-hypertrophic effects and plays key roles in reducing oxidative stress, preserving mitochondrial function, and maintaining cellular homeostasis[32,33]. The regulatory role of *FOXO* is directly linked to the *NFAT* signaling pathway, which is frequently activated in cardiomyocytes[34]. Upon activation through the calcium/calcineurin pathway, *NFAT* translocates into the nucleus and promotes the expression of hypertrophic marker genes such as atrial natriuretic peptide (*ANP*), and brain natriuretic peptide (*BNP*)[30]. Thus, *NFAT* functions as a potent transcription factor driving cardiac hypertrophy[34]. Conversely,

FOXO counteracts excessive *NFAT* activation, thereby preventing pathological cardiomyocyte growth[7,29,35]. This establishes an antagonistic balance between *FOXO* and *NFAT*: when *FOXO* is active, hypertrophic gene expression is restrained, whereas suppression of *FOXO* amplifies *NFAT*-mediated hypertrophic responses[34].

In recent years, the therapeutic potential of natural products has been increasingly investigated, with particular attention given to the protective effects of polyphenolic compounds on the cardiovascular system[36]. One such natural product is polydatin, a resveratrol glucoside isolated from Polygonum cuspidatum, which is also naturally found in foods such as red wine and grapes[37]. Numerous studies have demonstrated that polydatin possesses a broad spectrum of pharmacological activities, including antioxidant, anti-inflammatory, anti-apoptotic, and neuroprotective effects[38]. Experimental findings have revealed that polydatin exerts notable benefits on the cardiovascular system[39,40]. Specifically, it has been reported to exert preventive effects against Ang II-induced cardiac hypertrophy, protect cardiomyocytes from ischemia-reperfusion injury, and alleviate cardiac dysfunction in diabetic models[39-42]. In addition, polydatin has been shown to inhibit the calcineurin-*NFAT* signaling pathway, thereby decreasing hypertrophic gene expression and directly influencing the process of cardiac remodeling[43]. However, despite these cardioprotective and antioxidant activities, the precise mechanisms by which polydatin improves cardiac remodeling and the extent of these effects remain incompletely understood[39].

Based on this background, the primary aim of the present study was to investigate the role of the *miR-214*/*FOXO3*/*NFAT* signaling pathway in an isoproterenol (ISO)-induced cardiac hypertrophy model and to determine whether polydatin modulates this axis. Furthermore, we sought to evaluate whether polydatin modulates the *miR-214*-mediated signaling axis in the same model, thereby clarifying its potential cardioprotective mechanisms during cardiac hypertrophy and remodeling. In this way, the study aims to contribute both to the understanding of the molecular basis of pathological cardiac hypertrophy and to the identification of polydatin as a potential therapeutic candidate.

MATERIALS AND METHODS

Experimental Animals and Tissue Source

Cardiac tissues were obtained from eight-week-old male Wistar rats (n=12) provided by the Trakya University Laboratory Animal Research Unit as part of a previously approved project. All experimental procedures, including animal housing and treatments, were carried out in compliance with institutional ethical standards and were approved by the Trakya University Animal Experiments Local Ethics Committee (protocol number: TÜHADYEK-2024/08, date: 05.11.2025). Cardiac tissues became available for the present study following the completion of that project, thereby avoiding additional animal use.

The original project protocol involved three experimental groups: Control, ISO, 5 mg/kg/day, s.c.), and ISO + polydatin (100 mg/kg/day, oral gavage), with treatments administered for four weeks. During the experimental period, animals were kept under standard laboratory conditions (22±1 °C, 55% relative humidity, 12-hour light/dark cycle) with ad libitum access to food and water. Solutions were freshly prepared in 0.9% saline on a daily basis. Body weights were monitored weekly throughout the experimental period. The experimental design, treatment duration, and dosing regimen were established following a comprehensive review of the relevant literature[39,44].

Morphological Analysis of the Hearts

At the end of the four-week treatment period, body weight, heart weight, lung weight, and tibia length of the animals were measured to evaluate the presence of cardiac hypertrophy. To provide a more accurate assessment of morphological changes, the following ratios were calculated: heart weight/body weight, heart weight/tibia length, lung weight/body weight, and lung weight/tibia length. These parameters served as indices of structural alterations and hypertrophy.

Determination of mRNA Expression Levels

The mRNA expression levels of *FOXO3*, *NFAT*, *ANP*, and *BNP* genes in cardiac tissue were determined using quantitative real-time polymerase chain reaction (RT-qPCR). First, total RNA was isolated from left ventricular tissues using the mirVana™ miRNA Isolation Kit (Thermo Fisher Scientific, USA). The purity and concentration of the isolated total RNA were measured by evaluating the A260/280 ratio with a microplate reader (Tecan Infinite m1000 Pro Microplate Reader with Tecan Quant Plate). Subsequently, 300 ng/µL of total RNA from each sample was converted into cDNA using the High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. RT-qPCR analysis was then performed with gene-specific primers (*FOXO3*, *NFAT*, *ANP*, *BNP*), cDNA samples, and SYBR Select Master Mix (Thermo Fisher Scientific, USA) on the 7500 Fast Real-Time PCR system (Applied Biosystems, USA). The PCR conditions consisted of an initial denaturation at 95 °C for 2 minutes, followed by 40 cycles of 95 °C for 15 seconds, 60 °C for 15 seconds, and 72 °C for 15 seconds. Cycle threshold (Ct) values obtained during PCR amplification were used to evaluate gene expression levels. Relative gene expression changes were calculated using the $2^{-\Delta\Delta Ct}$ method, with *glyceraldehyde-3-phosphate dehydrogenase* serving as the reference gene for normalization. All samples were analyzed in technical duplicates. The results were expressed as relative fold changes compared to the Control group. The primer sequences used in the reactions are provided in Table 1.

Determination of miRNA Expression Levels

Total RNA, including small RNAs, was isolated from left ventricular tissue samples using the mirVana™ miRNA Isolation Kit (Thermo Fisher Scientific, USA) according to the manufacturer's protocol. The purity and concentration of the isolated RNA were determined with a Tecan Infinite M1000 Pro Microplate Reader with Quant Plate. For specific reverse transcription of target miRNAs, cDNA synthesis was performed using the TaqMan™ MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific, USA) together with gene-specific stem-loop primers. The synthesized cDNAs were subsequently amplified by RT-qPCR using TaqMan™ Universal PCR Master Mix and TaqMan MicroRNA Assays (Thermo Fisher Scientific, USA) on the 7500 Fast Real-Time PCR System (Applied Biosystems, USA), following the manufacturer's instructions. The probe sets used contained specific sequences for the experimentally investigated miRNAs: miR-1 (assay ID: 002222), *miR-214* (assay ID: 000517), and miR-133b (assay ID: 002247). Ct values obtained during the PCR cycles were used to calculate the expression levels of the target miRNAs. Relative expression changes were analyzed using the $2^{-\Delta\Delta Ct}$ method, with U6 small nuclear RNA (assay ID: 001973) serving as the reference gene for normalization. All samples were analyzed in technical duplicates. The results were expressed as relative fold changes compared to the Control group.

Statistical Analysis

The normality of the data distribution was assessed using the Shapiro-Wilk test. Group differences were analyzed using ANOVA, followed by Tukey-honestly significant difference post hoc test for pairwise comparisons. This approach was applied to analyze miRNA, mRNA, and morphological parameters, as well as *ANP* and *BNP* expression levels. Experimental data were presented as mean ± standard error of the mean (SEM). A p-value of <0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA).

Table 1. Primer sequences used for RT-qPCR analysis

Gene region	Primer sequences
FOXO3	F: 5- CTTCAAGGATAAGGGCGACAGCA -3 R: 5- GCTCTTGCCAGTCCCTTCGTT -3
NFAT	F: 5- CGAGGACGGGGCACCAAC -3 R: 5- AGTAGATGGAGGCGGGTCTACATT -3
ANP	F: 5- ATTGGAGCAAATCCCGTATACAGT -3 R: 5- GCTTCATCGGTCTGCTCGCTCA-3
BNP	F: 5- CTGCTCCTGCTTTTCCTTAATCTGT -3 R: 5- AGCTGTCTCTGAGCCATTTCC -3
GAPDH	F: 5-GCAGCCCAGAACATCATCCCT-3 R: 5-CATGCCAGTGAGCTTCCCGTT-3

FOXO3: Forkhead box O3, NFAT: Nuclear factor of activated T-cells, *ANP:* Atrial natriuretic peptide, *BNP: Brain natriuretic peptide, GAPDH: Glyceraldehyde-3-phosphate dehydrogenase*, RT-qPCR: Real-time polymerase chain reaction

RESULTS

At the end of the four-week treatment period, cardiac hypertrophy was evaluated based on heart weight, heart weight/body weight ratio, heart weight/tibia length ratio, as well as lung weight and lung weight/tibia length ratio. In rats with ISO-induced cardiac hypertrophy, heart weight, heart weight/body weight ratio, and heart weight/tibia length ratio were significantly higher than the Control group ($p<0.001$). In contrast, polydatin administration markedly reduced these parameters and significantly attenuated the hypertrophic effects of ISO ($p<0.05$) (Figure 1). These findings indicate that ISO induced a pathological concentric type of cardiac hypertrophy by increasing myocardial mass, whereas polydatin substantially mitigated this hypertrophic response, exerting a cardioprotective effect.

Furthermore, no statistically significant differences were observed among groups in terms of lung weight or lung weight/tibia length ratio ($p>0.05$) (Figure 1). This suggests that ISO-induced hypertrophy was not accompanied by pulmonary edema or advanced heart failure. Thus, although pronounced cardiac hypertrophy was established in this experimental model, secondary effects of cardiac dysfunction at the pulmonary level were not evident. Overall, these results demonstrate that ISO successfully induced an experimental model of pathological cardiac hypertrophy, while polydatin provided significant morphological protection against this process.

miRNAs are critical regulators of pathological cardiac hypertrophy. In this study, we evaluated the expression of miR-1, *miR-214*, and miR-133b, which have previously been implicated in hypertrophic processes. The findings revealed significant differences among groups specifically in the expression levels of *miR-214* ($p=0.007$). In the ISO-treated group, *miR-214* expression was significantly higher compared with the Control group ($p=0.006$). This increase strongly supports the close association of *miR-214* with pathological cardiac hypertrophy, as previously reported in the literature. In contrast, polydatin treatment significantly reduced *miR-214* expression compared with the ISO group ($p=0.030$), suggesting that one of the mechanisms by which polydatin attenuates cardiac hypertrophy may involve the regulation of *miR-214*. On the other hand, no significant differences were observed among groups in the expression levels of miR-1 and miR-133b ($p=0.860$ and $p=0.554$, respectively) (Figure 2). This indicates that miR-1 and miR-133b did not undergo notable alterations during the development of ISO-induced cardiac hypertrophy and did not contribute meaningfully to hypertrophy pathogenesis under the conditions of this study.

Furthermore, polydatin treatment did not exert a significant effect on these two miRNAs. Overall, these results suggest that the cardioprotective effects of polydatin are not mediated through miR-1 or miR-133b, but rather by modulating the expression of miRNAs directly linked to pathological hypertrophy, particularly *miR-214*. This highlights the possibility that the beneficial effects of polydatin on cardiac hypertrophy are molecularly associated with the regulation of *miR-214*.

Since miRNAs exert their biological functions by regulating target gene expression, we next examined the impact of altered *miR-214* levels on genes associated with cardiac hypertrophy. Specifically, we evaluated the mRNA expression of *FOXO3* and *NFAT*, which are downstream effectors functionally regulated within the *miR-214-SIRT3* signaling pathway and are central regulators of hypertrophic remodeling. The analyses revealed significant differences among groups in *NFAT* and *FOXO3* expression levels ($p<0.001$ and $p=0.006$, respectively). In the ISO-treated group, *FOXO3* expression was markedly decreased ($p=0.027$), while *NFAT* expression was significantly increased compared with the Control group ($p<0.001$). These findings indicate that ISO suppresses anti-hypertrophic mechanisms via *FOXO3*, while simultaneously activating hypertrophic signaling through *NFAT* during the development of cardiac hypertrophy. Polydatin treatment reversed these changes, reducing *NFAT* levels ($p<0.001$) and increasing *FOXO3* expression ($p=0.005$) compared with the ISO group. Moreover, no significant differences were observed between the Control and ISO + POL groups in terms of *NFAT* and *FOXO3* expression ($p>0.05$), suggesting that polydatin largely restored gene expression to control levels (Figure 3). These results demonstrate that the cardioprotective effects of polydatin are mediated not only through the regulation of *miR-214* but also via the modulation of hypertrophy-related transcription factors such as *FOXO3* and *NFAT*, which are established targets of *miR-214*.

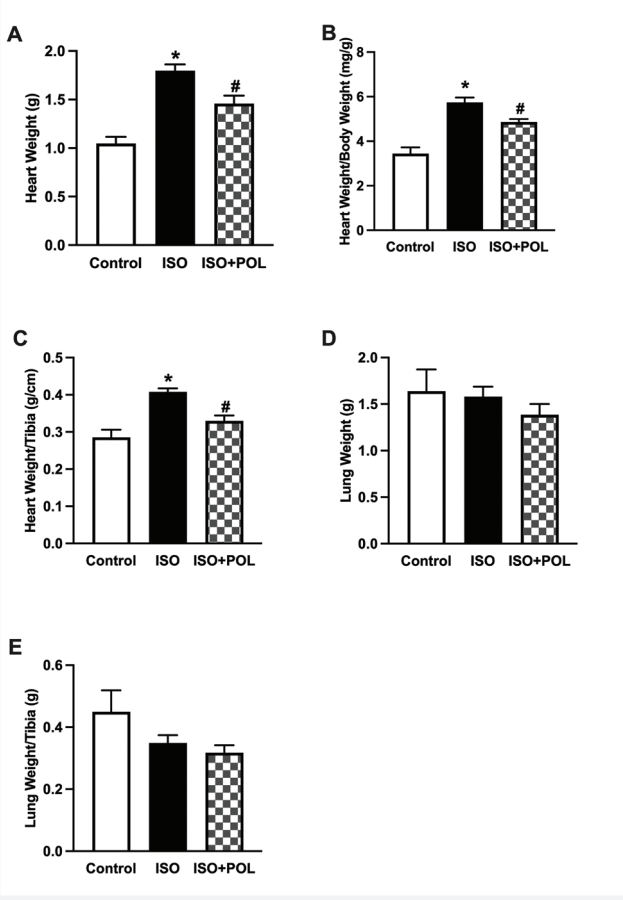

Figure 1. Effects of polydatin treatment on isoproterenol-induced pathological cardiac hypertrophy. The evaluated parameters include heart weight (A), heart weight/body weight ratio (B), heart weight/tibia length ratio (C), lung weight (D), and lung weight/tibia length ratio (E). Values are presented as mean ± SEM (n=4 for Control, ISO, and ISO + POL groups). *$p<0.001$ vs. Control group; #$p<0.05$ vs. ISO group

ISO: Isoproterenol, POL: Propranolol, SEM: Standard error of the mean

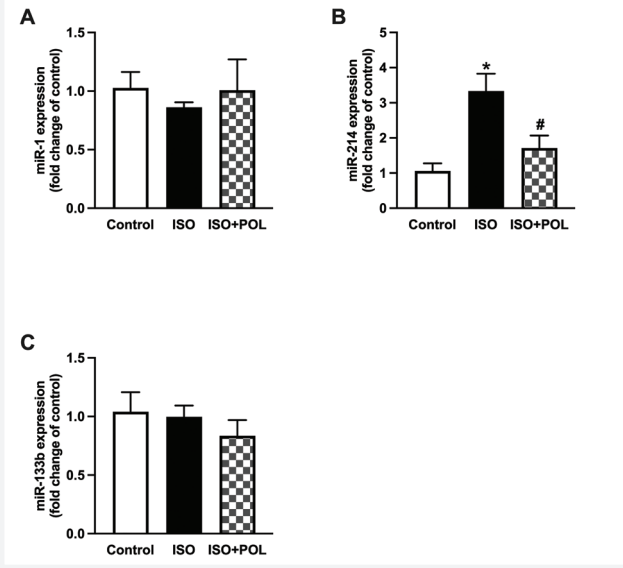

Figure 2. Effects of four-week polydatin treatment on the expression levels of cardiac hypertrophy-related miRNAs: (A) miR-1, (B) miR-214, and (C) miR-133b. Changes in the expression levels of these miRNAs were evaluated in the isoproterenol-induced pathological cardiac hypertrophy model following polydatin administration. Data are presented as mean ± SEM (n=4 for Control, ISO, and ISO + POL groups; each sample analyzed in duplicate). *$p<0.05$ vs. Control group; #$p<0.05$ vs. ISO group

ISO: Isoproterenol, POL: Propranolol, SEM: Standard error of the mean

Consistent with the increase in *NFAT* expression, the expression levels of *ANP* and *BNP* established markers of cardiac hypertrophy were significantly elevated in the ISO group (p=0.029 and p=0.002, respectively). These findings indicate that ISO-induced pathological cardiac hypertrophy involves the reactivation of the fetal gene program. Polydatin treatment markedly suppressed these alterations, significantly reducing *ANP* and *BNP* expression and restoring them to levels comparable with those of the Control group (p>0.05) (Figure 3). This result demonstrates that polydatin exerts a regulatory effect not only at the transcription factor level by inhibiting *NFAT* activation, but also on its downstream targets, the hypertrophic markers. Thus, polydatin attenuates hypertrophic signaling mediated by *NFAT* and prevents the reactivation of the fetal gene program, thereby reinforcing its cardioprotective effect.

DISCUSSION

ISO administration induced pathological concentric cardiac hypertrophy by increasing ventricular wall thickness, whereas polydatin markedly attenuated this hypertrophic response. At the molecular level, ISO was found to increase *miR-214* expression, suppress *FOXO3*, and activate *NFAT*, whereas polydatin reversed these alterations, thereby exerting a cardioprotective effect. Furthermore, elevated *ANP* and *BNP* levels indicated that ISO reactivated the fetal gene program, which was suppressed by polydatin. Taken together, these findings suggest that polydatin confers protection against cardiac hypertrophy by modulating a proposed *miR-214*/*FOXO3*/*NFAT* signaling axis, with *SIRT3* included as an intermediate node based on established literature (Figure 4).

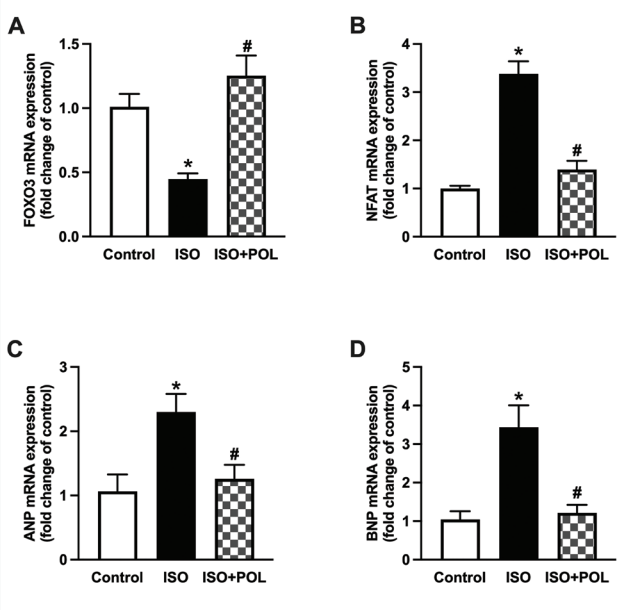

Figure 3. Changes in mRNA expression levels of *FOXO3* and *NFAT*, which are associated with miR-214, as well as the fetal genes *ANP* and *BNP*. (A) *FOXO3*, (B) *NFAT*, (C) *ANP*, and (D) *BNP* mRNA expression levels. Data are presented as mean ± SEM (n=4 for Control, ISO, and ISO + POL groups; each sample analyzed in duplicate). *p<0.05 vs. Control group; #p<0.05 vs. ISO group

FOXO3: FOXO3: Forkhead box O3, NFAT: Nuclear factor of activated T-cells, ANP: Atrial natriuretic peptide, BNP: Brain natriuretic peptide, POL: Propranolol, SEM: Standard error of the mean

Morphometric analysis confirmed the development of pathological hypertrophy in the ISO group, as indicated by marked increases in heart weight indices, while polydatin significantly blunted these effects. This finding is consistent with previous studies reporting that ISO induces cardiac remodeling through β-adrenergic stimulation, leading to increased ventricular wall thickness and hypertrophy[44-46]. The significant reduction of these parameters following polydatin treatment highlights the cardioprotective potential of this compound. This result is in line with earlier research suggesting that polydatin mitigates myocardial injury through its antioxidant, anti-inflammatory, and anti-apoptotic properties[39,42]. Thus, the results of our study reveal that polydatin confers protection not only at the molecular level but also at the structural level, as reflected by organ weight measurements. Another noteworthy aspect of the results is the absence of significant changes in lung weight and lung weight/tibia length ratio despite ISO administration. Notably, the preservation of lung parameters indicates the absence of pulmonary congestion, suggesting that the ISO protocol employed in this study models early to mid-stage pathological cardiac hypertrophy rather than decompensated heart failure. The concordance between robust cardiac hypertrophic remodeling and preserved lung indices supports the interpretation that the observed molecular and structural alterations precede the development of overt hemodynamic congestion. This indicates that the hypertrophic response induced by ISO did not progress to pulmonary edema or advanced heart failure. Therefore, while the model accurately represents pathological hypertrophy, it does not capture the transition to overt heart failure. In this regard, it should be acknowledged that the ISO protocol used in the present study is pharmacologically established to model early to mid-stage pathological cardiac hypertrophy rather than advanced heart failure. Similarly, the literature has reported that ISO predominantly mimics early hypertrophic changes without reproducing long-term cardiac dysfunction[44,45]. Overall, our findings support the validity of ISO as a model of cardiac hypertrophy and demonstrate that polydatin provides meaningful structural protection within this experimental framework. The present study was designed as a focused, exploratory investigation aimed at elucidating molecular and structural alterations associated with pathological cardiac

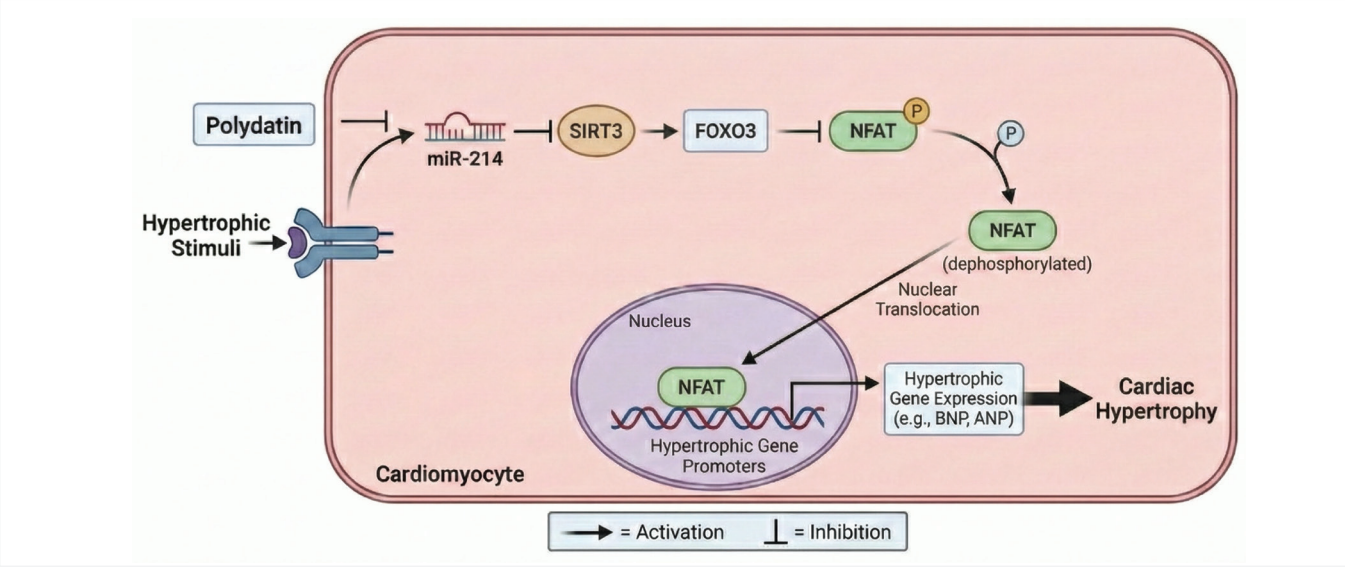

Figure 4. Proposed mechanism illustrating the modulatory effect of polydatin on pathological cardiac hypertrophy via the miR-214/*SIRT3/FOXO3/NFAT* signaling axis in cardiomyocytes. Hypertrophic stimuli upregulate miR-214 expression, which suppresses *SIRT3*, leading to impaired *FOXO3* deacetylation and reduced functional stability. The consequent decrease in *FOXO3* activity relieves its inhibitory control over *NFAT*, facilitating *NFAT* dephosphorylation and nuclear translocation. Activated *NFAT* accumulates in the nucleus and binds to hypertrophy-associated gene promoters, thereby inducing the expression of fetal genes such as *ANP* and *BNP* and promoting the hypertrophic phenotype. Polydatin treatment attenuates this signaling cascade by inhibiting miR-214, restoring the *SIRT3/FOXO3* signaling axis, limiting *NFAT* activation, and consequently suppressing hypertrophic gene transcription. Arrows denote activation, whereas blunt-ended lines indicate inhibitory interactions

SIRT3: *Sirtuin 3*, *FOXO3*: *Forkhead box O3*, *NFAT*: Nuclear factor of activated T-cells, T-cells, BNP: Brain natriuretic peptide, ANP: Atrial natriuretic peptide

hypertrophy, rather than providing a comprehensive functional or electrophysiological characterization. Accordingly, the findings should be interpreted within the framework of a preliminary study that provides a molecular basis for future investigations incorporating expanded experimental groups and functional cardiac assessments.

Expression profiling of miR-1, *miR-214*, and miR-133b revealed that only *miR-214* was significantly upregulated in ISO-induced hypertrophy. The significant upregulation of *miR-214* in the ISO group supports previous reports linking this miRNA to hypertrophy and heart failure[20,27]. This increase highlights the role of *miR-214* in regulating processes such as cardiac remodeling and fibrosis. The observation that polydatin treatment significantly suppressed *miR-214* expression suggests that its cardioprotective effect is at least partly mediated through *miR-214* regulation. In contrast, no significant changes were observed in the expression of miR-1 and miR-133b. Although previous studies have reported potential associations of these miRNAs with cardiac hypertrophy and myocardial remodeling[9,47,48], the lack of changes in our study implies that their involvement may be context-dependent or more prominent in other hypertrophy models. Indeed, several reports have demonstrated significant alterations in miR-1 and miR-133b in pressure overload or ischemia-reperfusion models, suggesting that the regulation of these miRNAs may vary depending on the pathological stimulus or stage of disease. The absence of significant effects of polydatin on these two miRNAs further indicates that polydatin may act selectively through miRNAs more strongly linked to the hypertrophic response, particularly *miR-214*. Taken together, our findings suggest that the protective effects of polydatin against cardiac hypertrophy are not mediated by miR-1 or miR-133b, but rather by modulating the expression of miRNAs directly associated with pathological hypertrophy, such as *miR-214*.

To our knowledge, this study provides initial evidence linking polydatin treatment to the modulation of *miR-214* expression in an experimental model of cardiac hypertrophy. While polydatin has previously been shown to inhibit hypertrophic signaling pathways such as calcineurin-*NFAT*[43], our findings suggest that its cardioprotective effects may also involve upstream regulation at the miRNA level, particularly *miR-214*.

Analysis of *FOXO3* and *NFAT*, which are downstream effectors functionally regulated by *miR-214* signaling, showed that ISO

suppressed *FOXO3* and increased *NFAT* expression. Previous studies have reported that *FOXO3* is a key transcription factor activating anti-hypertrophic gene programs, and its downregulation facilitates the development of hypertrophy[31,34]. Conversely, *NFAT* has been shown to be activated via calcineurin-dependent signaling pathways, promoting the expression of hypertrophic genes[34]. Our findings are consistent with this evidence, indicating that ISO promotes hypertrophy by disrupting the *FOXO3*/*NFAT* balance. Polydatin treatment restored *FOXO3* expression and reduced *NFAT* levels back to control values, suggesting that the cardioprotective effect of polydatin is mediated not only through *miR-214* regulation but also by modulating critical transcription factors that are functionally regulated downstream of *miR-214* signaling. It should be noted that miRNAs classically exert their regulatory effects predominantly through translational repression rather than direct mRNA degradation. However, increasing evidence indicates that miRNA-mediated downregulation of target transcripts can also occur via mRNA destabilization, particularly in chronic pathological settings. In the present 4-week ISO model, the observed reduction in *FOXO3* mRNA may therefore reflect sustained or indirect regulation of *FOXO3* expression downstream of *miR-214* signaling, rather than acute translational inhibition alone. Moreover, given that *FOXO3* was assessed at the transcript level, these findings should be interpreted as indicative of coordinated pathway suppression rather than direct evidence of miRNA-mRNA binding or degradation.

Consistent with the increased *NFAT* activity, we observed significantly elevated expression of *ANP* and *BNP*, established markers of cardiac hypertrophy, in the ISO group. Reactivation of the fetal gene program is considered one of the molecular hallmarks of pathological cardiac hypertrophy[30], and our results support this concept. Importantly, polydatin treatment restored *ANP* and *BNP* expression to levels comparable with the Control group, demonstrating that this compound regulates not only transcription factors but also their downstream targets. Similarly, other natural compounds have also been reported to exert protective effects against cardiac hypertrophy by suppressing the fetal gene program[40,43,49]. Taken together, these findings are consistent with modulation of the *miR-214*/*FOXO3*/*NFAT* axis, whereby both hypertrophic signaling and the reactivation of the fetal gene program are suppressed. These results suggest that polydatin exerts cardioprotective effects against pathological cardiac hypertrophy in this experimental model.

Study Limitations

The present study should be interpreted as a preliminary, hypothesis-driven investigation aimed at elucidating a specific molecular signaling axis rather than providing a comprehensive functional characterization of pathological cardiac hypertrophy. The sample size was modest, which may limit the precision of certain estimates. In addition, only a single dose and treatment duration of polydatin were evaluated; therefore, potential dose- or time-dependent effects could not be assessed. The limited number of experimental groups reflects a design focused on pathway interrogation rather than exhaustive phenotypic stratification. At the molecular level, analyses were restricted to transcript-level measurements; thus, the absence of protein-level validation and assessment of *NFAT* and *FOXO3* nuclear translocation constitutes an important limitation. Furthermore, although our findings support a coordinated regulation of the *miR-214*/*FOXO3*/*NFAT* pathway by polydatin, *SIRT3* was incorporated as an intermediate regulator based on established mechanistic evidence from prior studies. Direct mechanistic validation (e.g., luciferase reporter assays or genetic manipulation of intermediate nodes) was beyond the scope of the present study. Accordingly, the proposed signaling axis should be interpreted as a hypothesis-driven model supported by converging gene expression profiles and established literature[27], warranting future causal investigation. Finally, the lack of electrocardiography or other functional cardiac assessments precludes conclusions regarding electrophysiological alterations and overall cardiac performance, and future studies incorporating functional and electrophysiological endpoints will be required to further substantiate these findings.

CONCLUSION

Polydatin exerted pronounced cardioprotective effects in an ISO-induced hypertrophy model, acting at both structural and molecular levels. Morphologically, it significantly attenuated ventricular wall thickening and the hypertrophic increase in myocardial mass. At the molecular level, ISO was shown to increase *miR-214* expression, suppress *FOXO3*, and activate *NFAT*, leading to elevated *ANP* and *BNP* levels. These findings support the involvement of a *miR-214*/*FOXO3*/*NFAT*-associated signaling axis and the reactivation of the fetal gene program in the progression of pathological hypertrophy. Polydatin treatment reversed these changes by reducing *miR-214* expression, restoring *FOXO3* levels, and suppressing *NFAT* activation. Consequently, the hypertrophic markers *ANP* and *BNP* were maintained at levels comparable to controls. Thus, the cardioprotective effects of polydatin extend beyond its antioxidant and anti-inflammatory properties, encompassing a multilayered mechanism involving miRNA regulation and modulation of transcription factors. Overall, this study identifies polydatin as a strong experimental candidate against pathological cardiac hypertrophy and introduces the *miR-214/FOXO3*/*NFAT* axis as a promising molecular target for the development of therapeutic strategies. Nevertheless, further studies using more comprehensive *in vivo* models, different dosing protocols, and long-term follow-up are required to translate these findings into clinical applications.

Ethics

Ethical Committee Approval: This study was approved by the Trakya University Animal Experiments Local Ethics Committee (protocol number: TÜHADYEK-2024/08, date: 05.11.2025).

Informed Consent: All experimental procedures, including the housing and treatment of animals, were conducted in accordance with institutional ethical standards.

Footnotes

Authorship Contributions

Concept: B.E.Y., Design: B.E.Y., M.Y., Data Collection or Processing: B.E.Y., M.Y., K.B., M.A., Analysis or Interpretation: B.E.Y., Literature Search: B.E.Y., M.Y., Writing: B.E.Y., M.Y., K.B., M.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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