Iranian Journal of Basic Medical Sciences
ijbms.mums.ac.ir

Nerolidol attenuates cardiac hypertrophy and fibrosis in mice: Modulation of collagen type I, apoptotic and endothelial gene expression

Bushra Sadaf [1, 2], Muhammad Naveed Mushtaq [1]*, Waqas Younis [3], Benu George [2], Irfan Anjum [4], Abdul Aleem [1]

[1] Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan
[2] Department of Experimental and Clinical Pharmacology, University of Minnesota, College of Pharmacy, 3-120 Weaver Densford Hall, 308 Harvard St SE, Minneapolis, MN 55455, United States
[3] Division of Endocrinology, Diabetes and Metabolism, New York University, Grossmann School of Medicine, New York, USA
[4] Department of Basic Medical Sciences, Shifa College of Pharmaceutical Sciences, Shifa Tameer-e-Millat University, Islamabad, Pakistan

ARTICLE INFO

Article type:
Original

Article history:
Received: Nov 10, 2025
Accepted: May 5, 2026

Keywords:
Cardiac hypertrophy
Echocardiography
Endothelial nitric oxide - synthase
Isoproterenol
Nerolidol

ABSTRACT

Objective(s): Cardiac hypertrophy is a major pathological feature of cardiovascular disease worldwide. The beneficial effects of terpenes on cardiovascular diseases are well known. In the current study, the cardioprotective effects of nerolidol (NRD), a sesquiterpene alcohol, were evaluated using an isoproterenol (ISO) induced cardiac hypertrophy mice model.

Materials and Methods: This study was performed by using the chronic subcutaneous infusion model of ISO. Male mice were administered NRD (100 mg/kg) orally for 7 days. On the 8th day with the continuation of NRD dosing, mice received subcutaneous (SC) with ISO (10 mg/kg) for the next 14 days. Cardiac functions, including ejection fraction, cardiac output, and fractional shortening were evaluated by trans-thoracic echocardiography, one day after the final treatment. Then, animals were euthanized and hearts were isolated. The mRNA and protein expressions of cardiac hypertrophy, apoptosis, and fibrosis markers in mice hearts were determined.

Results: Results showed an increased heart-to-body weight ratio. NRD reduced cardiac hypertrophy by down-regulating the hypertrophic (ANP), apoptotic (Bax/Bcl-2), and fibrotic (Col1a1) markers and prevented cardiac remodeling by up-regulating expression of endothelial nitric oxide synthase (eNOS) and anti-apoptotic (Bcl-xL) protein compared to ISO-treated mice. Combining PCR and western blotting data results demonstrated that NRD reverted the ISO-induced cardiac hypertrophy.

Conclusion: These findings suggest that NRD prevents ISO-induced cardiac hypertrophy possibly by elevating the levels of eNOS and Bcl-xL and reducing expression of hypertrophic, apoptotic, and fibrotic markers. Thus, NRD may be used to treat ISO-induced cardiac hypertrophy.

▶ Please cite this article as:
Sadaf B, Mushtaq MN, Younis W, George B, Anjum I, Aleem A. Nerolidol attenuates cardiac hypertrophy and fibrosis in mice: Modulation of collagen type I, apoptotic and endothelial gene expression. Iran J Basic Med Sci 2026; 29: 1100-1106. doi: https://dx.doi.org/10.22038/ijbms.2026.92642.20001

Introduction

Cardiovascular diseases (CVDs) bring modifications in the heart and blood vessels. Due to their higher rate of morbidity and mortality, CVDs are a major worldwide health issue. They are the major cause of death globally (1, 2). During 2019, 58% of global 18.6 million deaths from CVDs occurred in Asia (3).

Cardiac hypertrophy (CH) contributes in the pathological development of cardiovascular disorders and sustained CH eventually leads to heart failure. Several therapeutic strategies have been widely employed in the treatment of ventricular hypertrophy and heart failure. There is growing evidence that various pathophysiological factors, such as neurohumoral activation, including angiotensin II and sympathetic stimulation, ischemic heart conditions, myocarditis, and diabetic cardiomyopathy, contribute to CH and heart failure by promoting the excessive production of oxidative stress (4). Among a range of contributing factors, the reduction or absence of nitric oxide (NO) synthesis emerges as an important element in the initiation, onset and advancement of conditions such as stroke, hypertension (HTN), atherosclerosis, myocardial fibrosis, myocardial infarction, and diabetes. NO, which is produced in endothelial cells from the amino acid L-arginine, has a basal tonic vaso-relaxing effect on the vascular wall (5).

ISO-induced CH, a well-known animal model of CH that occurs without systolic HTN, stimulates the β-adrenoceptors. Regression of left ventricular hypertrophy (LVH) is a significant intermediate goal of antihypertensive therapy; several published studies have found that antihypertensive medications enhance ventricular hypertrophy regression and myocardial healing (6). Research is necessary to

*Corresponding author: Muhammad Naveed Mushtaq. Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan. Email: muhammad.mushtaq2@pharm.uol.edu.pk

investigate drugs that possess the ability to confer protective advantages to the cardiovascular system. The preference of medicinal plants and derived phytochemicals to treat diseases has been increased recently.

NRD commonly known as peruviol, is an aliphatic sesquiterpene alcohol (3,7,11-trimethyl-1,6,10-dodecatrien-3-ol), with two distinct isomers. It is found in various plants, such as lavender, lemon grass, and ginger. NRD is reported safe for human consumption by FDA (7). It has been proven to have antioxidant, anticancer, antinociceptive, anti-inflammatory (8–10) antibacterial, anti-neurodegenerative, and anticancer effects. NRD has been shown to have neuroprotective, hepatoprotective, and genoprotective activities (11). Prolonged use of NRD has a cardioprotective impact on myocardial injury in an animal model. It has been observed that NRD dramatically lowered myocardial infarct size and oxidative stress in order to lessen myocardial infarction (MI) induced by ISO (12). However, effect of NRD on coronary heart issues has not been investigated yet. This study focused on assessing the inhibitory effects of NRD in ISO-induced CH in a mouse model, exploring its potential role in mitigating CH.

Materials and Methods

Drugs and chemicals

Nerolidol (Catalog# W277207) and isoproterenol (Catalog# I6379) were purchased from Sigma-Aldrich (USA). Drugs and chemicals of analytical grade were used.

Animals

8- to 9-week-old male mice, were purchased from Charles River Laboratories. Each mouse was housed in a group of three and each cage was kept under a specialized pathogen-free (SPF) environment and 12:12 light-dark (LD) cycle, maintained at a temperature of 21.2 °C. Mice were provided unlimited access to food and drink.

Treatment protocol for ISO-induced CH

Mice were randomly grouped into three study groups (n= 7–8 mice per group): Group I (Control), Group II (ISO), and Group III (NRD + ISO). To produce protective effects of NRD, group III were treated daily orally via oral gavage with NRD (100 mg/kg) and groups I and II received an equivalent volume of vehicle (VEH) for 7 days. On the 8th day the below mentioned 14 days' regimen was followed for mice treatment. Group I (Control) mice were treated with an equivalent volume of VEH orally via oral gavage following SC injection of saline; group II (ISO): the mice received a daily oral dose of an equivalent volume of VEH following SC injection of ISO 10 mg/kg; group III (ISO+NRD): the mice received NRD 100 mg/kg orally daily following SC injection of ISO 10 mg/kg for 14 days (Figure 1). To assess dose tolerability, mice were kept under observation for 30 min following each ISO injection. No morbidity or death was seen in any of the experimental groups. One day after the completion of the ISO injection, the mice were carefully sacrificed by the decapitation method using isoflurane anesthesia. Hearts of the mice were collected, washed with phosphate-buffered saline (PBS), immediately frozen using liquid nitrogen, and stored at -80 °C for further analysis.

Echocardiography

Cardiac functions were assessed using echocardiography. One day after the last dose of ISO or sterile saline solution injection, the response to NRD with extended ISO administration (n = 8 per group) was assessed. Vevo 2100 system (Visual Sonics, Inc., Toronto, Ontario, Canada) with an MS400 transducer was used to do echocardiography. Isoflurane (3%) and oxygen were used to induce anesthesia and maintained at 1–2% of isoflurane. A heated physiologic monitoring stage was used to place the anesthetized mice in a supine position. M-Mode parasternal short-axis images of the left ventricle were acquired at the level of the papillary muscles. To compute cardiac output, fractional shortening, and ejection fraction, the Vevo 2100's Visual Sonics cardiac measurement program was used to manually trace the endocardial and epicardial borders throughout three to four cardiac cycles.

Isolation of RNA and quantification by real-time polymerase chain reaction (qPCR)

According to instructions of the manufacturer, TRIzol reagent (300 µl) was utilized to extract total RNA from frozen heart tissue (20 mg). Concentrations of RNA were determined by Nanodrop 8000 spectrophotometer by checking the absorbance at wavelength of 260 nm. Then, a high-capacity cDNA reverse transcription kit was used to synthesize first-strand cDNA from 1.5 µg of total RNA. Real-time PCR using SYBR Green (Applied Biosystems) was employed to quantify specific mRNA expression on an ABI 7900HT system (Applied Biosystems), utilizing 384-well optical reaction plates. Applied thermocycler conditions were: Denaturation was carried out at 95 °C for 10 min, followed by 40 cycles of annealing/extension at 60 °C for 1 min and denaturation at 95 °C for 15 sec. Primer-BLAST online tool was employed to validate primers used in this

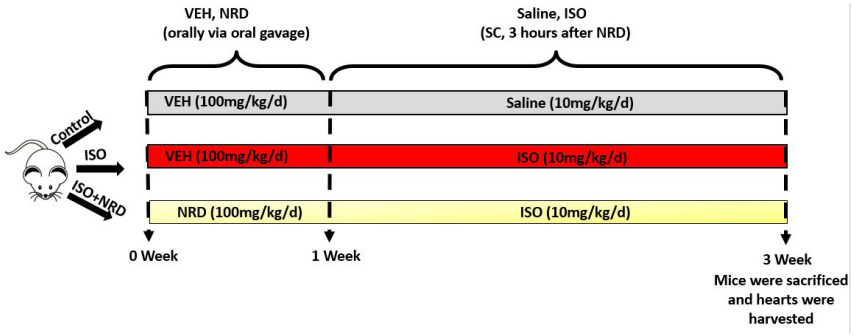

Figure 1. Experimental study design and treatment timeline to evaluate the antihypertrophic effect in C57BL/6N juvenile mice induced by isoproterenol
Mice were divided into three groups (n=8); Control, ISO, and ISO + NRD. Control group received VEH in first week and VEH + saline for next 2 weeks. ISO group received VEH for the 1st week and VEH + ISO for the next 2 weeks, while ISO + NRD group received NRD for 1st week and NRD+ ISO for the next 2 weeks. Abbreviations; NRD: Nerolidol; ISO: Isoproterenol; VEH: Vehicle; SC: Subcutaneous

Table 1. Primer sequence applied to check the mRNA expression in heart tissues of mice

Gene	Forward primer (5'-3')	Reverse primer (3'-5')	Ref.
ß-actin	TATTGGCAACGAGCGGTTCC	GGCATAGAGGTCTTTACGGATGTC	(13)
ANP	GGAGCCTACGAAGATCCAGC	TCCAATCCTGTCAATCCTACCC	(14)
Bax	CGAATTGGAGATGAACTGGACAG	CTAGCAAAGTAGAAGAGGGCAAC	(15)
Bcl-2	TTGTAATTCATCTGCCGCCG	AATGAATCGGGAGTTGGGGT	(16)
eNOS	TCAGCCATCACAGTGTTCCC	ATAGCCCGCATAGCGTATCAG	(17)

study, which are provided in Table 1. The mRNA expression levels were normalized to β-actin and expressed relative to the control group. Using the ΔΔCT method, relative gene expression was calculated. To ensure the specificity of the primers and purity of the final PCR product, melting curve analysis was performed.

Extraction of protein and western blotting

In accordance with the manufacturer's instructions, homogenization of frozen heart tissues was performed as described earlier, and concentration of protein was measured utilizing the Pierce Bicinchoninic Acid (BCA) Protein Assay Kit (Pierce, Thermo Fisher Scientific, Rockford, IL, USA). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was employed to separate the protein (30 µg) from each sample. Then, electroblotting was used to transfer protein onto nitrocellulose membrane. Bcl-xL (Catalog# 2764S, 1:1000 dilution), Collagen-1 (Catalog# PA5-29569, 1:1000 dilution), and eNOS (Catalog# 32027S, 1:1000 dilution), primary rabbit antibodies (Catalog# ab137015 and ab151544; 1:1000 dilution) were procured from Abcam (Cambridge, MA, USA). Primary rabbit antibody against alpha-tubulin (Catalog# 2144; 1:1000 dilution) was procured from Cell Signaling Technology (Danvers, MA, USA). Secondary anti-rabbit antibodies conjugated to HRP were procured from Jackson Immuno Research (Catalog# 111-035-144; 1:10,000 dilutions; West Grove, PA, USA). ImageJ software (National Institutes of Health, Bethesda, MD, USA) was employed to quantify band intensities. Alpha-tubulin protein levels served as loading controls to normalize the band intensities.

Statistical analysis

GraphPad Prism (version 7.01, La Jolla, CA, USA) was employed to analyze the data. Data was analyzed using the Shapiro-Wilk normality test. Parametric data was analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison *post hoc* test and non-parametric data was analyzed using Kruskal-Wallis, followed by Dunn's test. Statistical significance was set as $P \leq 0.05$.

Results

Cardioprotective effects of NRD on echocardiographic parameters

Cardiac function in mice was evaluated using echocardiography after 14 days of treatment: Control group received vehicle, the ISO group received ISO, whereas the ISO+NRD group was treated with NRD along with ISO. Echocardiographic images of representative M-Mode from each group are shown in Figure 2A. Ejection fraction, a precise indicator of systolic function, and fractional shortening were found to be significantly higher in the ISO+NRD group as compared to the ISO group. Ejection fraction was improved by 16% in mice of ISO+NRD group (Figure 2B). In the ISO-treated group, there was a notable reduction of 10% in fractional shortening. Conversely, the ISO+NRD group demonstrated an improvement in fractional shortening by 9% (Figure 2C). This observation highlights a potential ameliorative effect of the ISO+NRD

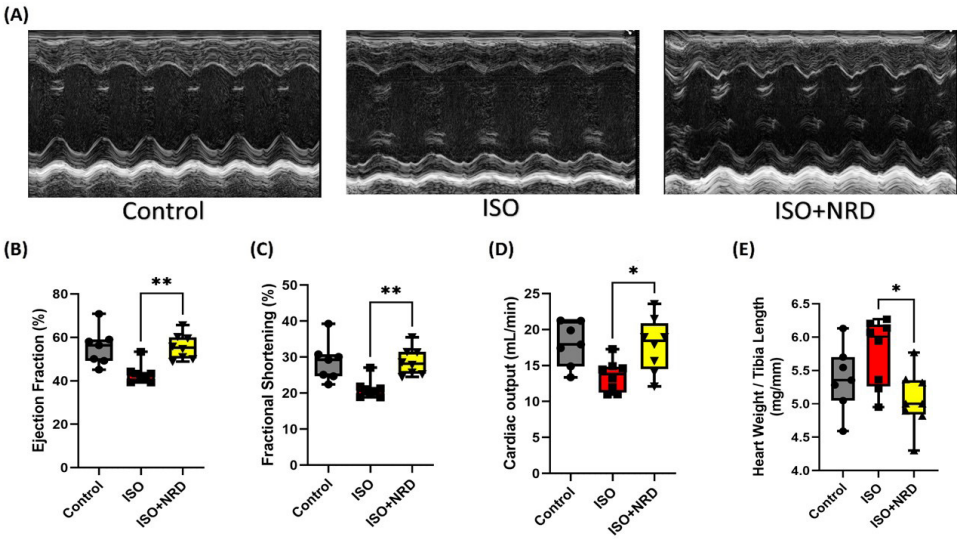

Figure 2. Ameliorating effect of NRD on ISO-induced mouse cardiac dysfunction and hypertrophy
(A) Representative M-mode echocardiographic images of control, ISO, and ISO+NRD groups; (B-C) Ejection fraction and fractional shortening was shortened by ISO treatment but increased by NRD administration; D) Cardiac output was reduced due to ISO treatment but increased due to NRD administration; (E) HW/TL was increased due to the ISO treatment but reduced by NRD administration. Values are represented as means ± SEM. *$P<0.05$, **$P \leq 0.01$.
NRD: Nerolidol; ISO: Isoproterenol

intervention on fractional shortening. Cardiac output was increased by 30% in the mice of the ISO+NRD group as compared to that of the ISO group (Figure 2D). Mice treated with ISO+NRD exhibited a significant reduction in the heart weight/tibia length (HW/TL) ratio as compared to those treated with ISO, as shown in Figure 2E. One-way ANOVA showed a significant effect of NRD on cardiac output, ejection fraction, fractional shortening, and HW/TL ratio.

Co-administration of NRD in prolonged ISO-treated mice restored the mRNA and protein expression levels of markers related to cardiac apoptosis, hypertrophy, and fibrosis in the heart

Increased mRNA expression levels of ANP and Coll1a1 (1.5-fold and 2-fold, respectively) were observed in ISO group mice hearts as compared to the control group (Figures 3A and 3B), and ANP and Coll1a1 reversed the trend (1.1-fold and 1.2-fold, respectively), observed with co-treatment of NRD. The mRNA expression of eNOS was significantly elevated up to 1.5-fold for the ISO+NRD group as compared to control, and an increasing trend was observed between ISO+NRD and ISO groups (Figure 3C).

Meanwhile, in the ISO+NRD group, the expression of Bax was reduced while Bcl-2 was increased as compared to the ISO group, resulting in a significant decrease in the Bax/Bcl-2 ratio in ISO+NRD group relative to ISO group (Figure 3D).

Protein expression of eNOS was significantly elevated after treatment with NRD, with a notable 2-fold elevation in eNOS levels noted in the ISO+NRD group as compared to the ISO group (Figure 3F). Further evaluation of reversal of ISO-induced apoptosis by NRD treatment was done using western blotting. Western blotting data demonstrated a significant increase (P<0.01) in Bcl-xL in the ISO+NRD group (Figures 3E and 3G) as compared to the ISO group.

Discussion

Medicinal plants contain phytochemicals that are utilized in the development of novel medications, offering health benefits with fewer side effects (18). NRD showed multiple pharmacological activities, i.e. antiparasitic, analgesic, antioxidant, and anticancer activities (7, 19). Further, it has been reported to produce cardioprotective (18), neuroprotective, and renoprotective effects (19, 20). It has been reported that NRD reduces myocardial infarct size significantly and suppresses oxidative stress to attenuate MI induced by isoproterenol (20). Prior to this research, there were no documented instances of antihypertrophic effects of NRD. The current study evaluated the cardioprotective effects of NRD in ISO-induced CH model of mice.

ISO is a well-known non-selective beta-adrenergic agonist that predominantly activates β_1-adrenergic receptors in the heart and β_2-adrenergic receptors in various tissues, including smooth muscle. β1-adrenergic effects increase the workload on the heart and lead to induced cardiac dysfunction and ventricular hypertrophy by increasing ventricular collagen content (21). Overexpression of β_2-AR has been reported to increase superoxide anion generation, which in turn drives hypertrophic and fibrotic signaling cascades and contributes to cardiac remodeling and failure

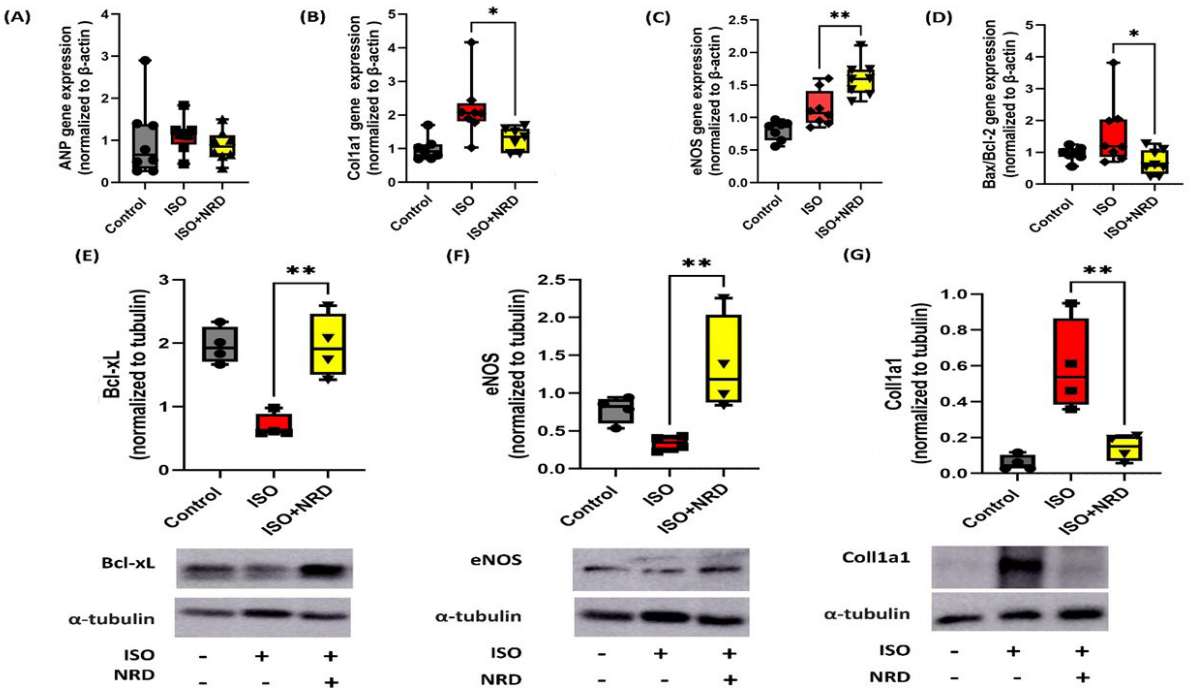

Figure 3. (A, B, C, D) prolonged NRD coadministration with ISO ameliorated ISO- induced fibrosis, cardiac hypertrophy, and apoptosis in mice

Mice of the NRD group were treated for one week orally with 100 mg/kg NRD and ISO while treating the control group with VEH. On the 8th day ISO and NRD groups were injected SC with ISO (10 mg/kg), 3 hr after NRD administration, while the control group received saline for 14 days (n = 7–8). mRNA expression of (A) ANP, (B) Coll1a1, (C) eNOS, and (D) Bax/Bcl-2 ratio in the hearts

VEH: Vehicle; ISO: Isoproterenol; NED: Nerolidol

Results were normalized to β-actin and presented relative to the male control group. Data is depicted as means ± SEM. *P<0.05, **P≤0.01, as compared to the ISO treatment group using one-way ANOVA followed by Dunnett's multiple comparisons test. (E, F, G) NRD coadministration with ISO reduced cardiac hypertrophy, apoptosis, and fibrosis. Expression levels of the antihypertrophic, fibrosis, and apoptosis markers: (E) Bcl-XL, (F) eNOS, (G) Coll1a1 estimated by western blotting. Data is presented as means ± SEM (n = 3-4). Results are depicted as means ± SEM (n = 3-4). Analysis of data was done using one-way ANOVA followed by multiple comparisons tests. Note: *P<0.05, **P<0.01, ***P<0.001.

(22, 23). Furthermore, data suggest that ISO induced overactivation of β_2-adrenergic receptor causes increase in vascular oxidative stress with subsequent endothelial dysfunction, which is linked to eNOS uncoupling (24,25). eNOS regulates the release of NO, which plays an important part in preserving homeostasis of the vascular wall and vascular tone (25). The reduced availability of NO, due to increased degradation by superoxide anions or impaired synthesis, has been recognized as a pivotal factor contributing to endothelial dysfunction in various cardiovascular disorders (26). NO can promote angiogenesis, decrease cardiomyocyte hypertrophy, decrease ability of cardiac fibroblasts to produce extracellular matrix proteins, and impact various steps responsible for the pathophysiology of LV remodeling. Additionally, it causes vasodilation, reducing cardiac afterload and preload. However, findings of this study demonstrate that prolonged ISO administration induced cardiac dysfunction and CH that were restored in NRD-treated mice. Like other biological systems, CH is regulated by both stimulatory and counter-regulatory signaling pathways. Previous *in vivo* studies revealed that eNOS exerts antihypertrophic effects in mice, and NOS1/3 deficient mice develop CH, leading to premature mortality. eNOS improves cardiac function by reducing contractility of heart. Hence, it seems that low amounts of NO produced by eNOS from maladaptive CH lead to adverse remodeling and early death. In short, eNOS plays a crucial part in the onset and advancing of HTN as well as ISO-induced CH. Thus, targeting eNOS and oxidative stress could serve as a potential therapeutic strategy for treating HTN and CH (22). The induction of CH in cardiac myocytes leads to an increase in collagen production. This process is linked to multiple signaling pathway activation leading to fibrotic responses that ultimately contribute to the remodeling of the cardiac extracellular matrix (27). Research has shown that the administration of isoproterenol leads to a significant up-regulation of collagen genes, particularly collagen-I and collagen-III. These changes are associated with fibrotic remodeling in cardiac tissue, which can further compromise cardiac function (28).

This study is the first to propose the potential efficacy of NRD in the treatment CH. Previous research has indicated ISO-induced impairment of cardiac function (29). In our current work, results of echocardiography depict that ISO+NRD treated mice caused improvement in ejection fraction and fractional shortening along with increase of cardiac output. Overexpression of eNOS acts as negative modulator of hypertrophic response to adrenergic stimulation in eNOS-Tg mice model (30). Similar findings in other groups provide evidence in support of these results. Many studies have demonstrated that NO generation or increased eNOS activity help to reduce cardiac abnormalities such ischemic damage and CH (31). PCR and western blotting results of current study revealed that ISO+NRD co-treated mice had decreased expression trend of the cardiac hypertrophic marker (ANP), fibrotic (Coll1a1) and apoptotic (Bax/Bcl-2) along with significant increased expression of antihypertrophic markers (eNOS, Bcl-xL) to produce cardioprotective effects.

It has already been reported that eNOS on myocardial cells would show its distinct role as negative regulator of CH (30). In this investigation, we report that increased eNOS expression generated by NRD treatment prevents ventricular hypertrophy caused by prolonged ISO infusion. When ISO+NRD mice were compared to ISO treated mice, CH was significantly reduced. In general, reactive oxygen species (ROS) are key regulators of apoptosis and an excess of ROS causes apoptosis (32). The results of the current study revealed that in hearts of ISO-treated mice, elevated expression of Bax/Bcl-2 (pro-apoptotic proteins) and decreased expression of Bcl-xL (anti-apoptotic protein) as compared to control mice were observed (33,34) that has been reverted by NRD treatment. Apoptotic markers were down-regulated whereas anti-apoptotic markers were up-regulated with the treatment of NRD which is consistent with earlier findings (35, 36). The apoptotic pathway is regulated by Bcl-2 protein family (37). Bcl-2 proteins are located in different cell organelles such as mitochondria, endoplasmic reticulum, and nuclear membranes. They interact with oxygen-free radicals that are responsible for the induction of apoptosis. In the cell, these proteins are likely to prevent apoptosis by scavenging ROS within the cell. Anti-Bcl-2 gene products have been shown to inhibit programmed cell death in ventricular myocytes (38, 39). This research work demonstrated that NRD exerts a repressive effect on cell death in CH by modulating apoptosis through the Bcl-2 protein family. The mechanisms of the hypertrophic response to adrenergic stimulation have been extensively studied. Previous studies reported that ISO raises the collagen volume of the heart in most animals (30). Our findings show that NRD+ISO treated mice had lower collagen levels than ISO-treated mice. While the use of a single marker per pathway provides preliminary insights into NRD's potential effects on morphological changes and cell viability, incorporating additional markers or complementary methodologies, such as histological or immunostaining in tissue sections, could further substantiate these findings. This approach has been recognized as a limitation of the present study. Nonetheless, echocardiographic, PCR and western blotting results revealed that NRD has the potential to provide a cardioprotective agent against ISO-induced CH.

Conclusion

The findings of the present study highlight the potential of NRD as a promising therapeutic strategy targeting the eNOS pathway for the prevention of CH. These findings suggest that NRD has significant clinical implications in managing CH. However, several challenges must be addressed prior to clinical translation. Further studies are required using different animal models to elucidate underlying mechanisms. Moreover, dosage optimization, safety, pharmacokinetics and long-term efficacy must be evaluated through extensive preclinical studies before going into clinical trials.

Acknowledgment

The results described in this paper were part of a student thesis. The authors acknowledge and thank Dr Beshay Zordoky, Assistant Professor, Experimental and Clinical Pharmacology Department, University of Minnesota, USA, who has given the opportunity to perform cardioprotective study in his lab. The study was funded by the Higher Education Commission (HEC) of Pakistan scholarship grant to Bushra Sadaf. However, HEC had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Ethics Approval and Consent to Participate

The Institutional Animal Care and Use Committee at the University of Minnesota, USA, approved the use of mice in research (Protocol ID: 1807-36187A).

Authors' Contributions

B S, MN M, B G, and I A designed the experiments; BS performed experiments and collected data; MN M, W Y, BG, and A A discussed the results and strategy; MN M supervised, directed, and managed the study; B S, MN M, A A, and W Y approved the final version to be published.

Conflicts of Interest

Authors declare no competing interests.

Declaration

We have not used any AI tools or technologies to prepare this manuscript.

References

1. Abdul Ghani MA, Ugusman A, Latip J, Zainalabidin S. Role of terpenophenolics in modulating inflammation and apoptosis in cardiovascular diseases: A review. Int J Mol Sci 2023; 24: 5339.
2. Gaidai O, Cao Y, Loginov S. Global cardiovascular diseases death rate prediction. Curr Probl Cardiol 2023; 48: 101622.
3. Zhao D. Epidemiological features of cardiovascular disease in Asia. JACC Asia 2021; 1: 1–13.
4. Caturano A, Vetrano E, Galiero R, Salvatore T, Docimo G, Epifani R, *et al.* Cardiac Hypertrophy: From Pathophysiological Mechanisms to Heart Failure Development. Rev Cardiovasc Med 2022; 23:165.
5. Cyr AR, Huckaby LV, Shiva SS, Zuckerbraun BS. Nitric oxide and endothelial dysfunction. Crit Care Clin 2020; 36: 307–321.
6. Perez-Bonilla P, LaViolette B, Bhandary B, Ullas S, Chen X, Hirenallur-Shanthappa D. Isoproterenol induced cardiac hypertrophy: A comparison of three doses and two delivery methods in C57BL/6J mice. PLoS One 2024; 19: e0307467.
7. Glumac M, Čikeš Čulić V, Marinović-Terzić I, Radan M. Mechanism of *cis*-nerolidol-induced bladder carcinoma cell death. Cancers (Basel) 2023; 15: 981.
8. Baldissera MD, Souza CF, Riéffel RC, Velho MC, Ramos AP, Nascimento K, *et al.* Protective effect of nerolidol-loaded in nanospheres against cerebral damage caused by Trypanosoma evansi. Naunyn Schmiedebergs Arch Pharmacol. 2018; 391: 753–759.
9. Fonsêca DV, Salgado PRR, de Carvalho FL, Salvadori MGSS, Penha ARS, Leite FC, *et al.* Nerolidol exhibits antinociceptive and anti-inflammatory activity: Involvement of the GABAergic system and proinflammatory cytokines. Fundam Clin Pharmacol 2016; 30: 14–22.
10. Lima DKS, Ballico LJ, Rocha Lapa F, Gonçalves HP, de Souza LM, Iacomini M, *et al.* Evaluation of the antinociceptive, anti-inflammatory and gastric antiulcer activities of the essential oil from Piper aleyreanum C.DC in rodents. J Ethnopharmacol 2012; 142: 274–282.
11. Zhao X, Chinnathambi A, Alharbi SA, Natarajan N, Raman M. Nerolidol, bioactive compound suppress growth of hct-116 colorectal cancer cells through cell cycle arrest and induction of apoptosis. Appl Biochem Biotechnol 2024; 196: 1365–1375.
12. Hammad FT, Al-Salam S, Ahmad R, Yasin J, Hammad AF, Rasheed JA, *et al.* The effect of nerolidol renal dysfunction following ischemia–reperfusion injury in the rat. Nutrients 2023; 15: 455.
13. Arroyo JA, Anthony R V, Parker TA, Galan HL. eNOS, NO, and the activation of ERK and AKT signaling at mid-gestation and near-term in an ovine model of intrauterine growth restriction. Syst Biol Reprod Med 2010; 56: 62–73.
14. Asaikumar L, Vennila L, Akila P, Sivasangari S, Kanimozhi K, Premalatha V, *et al.* Expression of concern: Preventive effect of nerolidol on isoproterenol induced myocardial damage in Wistar rats: Evidences from biochemical and histopathological studies. Drug Dev Res 2019; 80: 814–823.
15. Chan WK, Tan L, Chan KG, Lee LH, Goh BH. Nerolidol: A sesquiterpene alcohol with multi-faceted pharmacological and biological activities. Molecules 2016; 21: 529.
16. Zhang L, Sun D, Bao Y, Shi Y, Cui Y, Guo M. Nerolidol protects against LPS-induced acute kidney injury via inhibiting TLR4/NF-κB Signaling. Phytother Res 2017; 31: 459–465.
17. Taylor PB, Tang Q. Development of isoproterenol-indued cardiac hypertrophy. Can J Physiol Pharmacol 1984; 62: 384–389.
18. Maulik SK, Prabhakar P, Dinda AK, Seth S. Genistein prevents isoproterenol-induced cardiac hypertrophy in rats. Can J Physiol Pharmacol 2012; 90: 1117–1125.
19. Xu Q, Dalic A, Fang L, Kiriazis H, Ritchie R, Sim K, *et al.* Myocardial oxidative stress contributes to transgenic β_2-adrenoceptor activation-induced cardiomyopathy and heart failure. Br J Pharmacol 2011; 162: 1012–1028.
20. Davel AP, Brum PC, Rossoni LV. Isoproterenol induces vascular oxidative stress and endothelial dysfunction via a Giα-coupled β2-adrenoceptor signaling pathway. PLoS One 2014; 9: e91877.
21. Fleming I, Busse R. Molecular mechanisms involved in the regulation of the endothelial nitric oxide synthase. Am J Physiol Regul Integr Comp Physiol 2003; 284: R1–R12.
22. Xu C, Tang F, Lu M, Yang J, Han R, Mei M, *et al.* Astragaloside IV improves the isoproterenol-induced vascular dysfunction via attenuating eNOS uncoupling-mediated oxidative stress and inhibiting ROS-NF-κB pathways. Int Immunopharmacol 2016; 33: 119–127.
23. Kempf T, Wollert KC. Nitric oxide and the enigma of cardiac hypertrophy. BioEssays 2004; 26: 608–615.
24. Liu Y, Afzal J, Vakrou S, Greenland GV, Talbot CC, Hebl VB, *et al.* Differences in microRNA-29 and pro-fibrotic gene expression in mouse and human hypertrophic cardiomyopathy. Front Cardiovasc Med 2019; 17: 6.
25. Zhao T, Kee HJ, Bai L, Kim MK, Kee SJ, Jeong MH. Selective HDAC8 inhibition attenuates isoproterenol-induced cardiac hypertrophy and fibrosis via p38 MAPK pathway. Front Pharmacol 2021; 20: 12.
26. Grant MKO, Abdelgawad IY, Lewis CA, Seelig D, Zordoky BN. Lack of sexual dimorphism in a mouse model of isoproterenol-induced cardiac dysfunction. PLoS One 2020; 15: e0232507.
27. Ozaki M, Kawashima S, Yamashita T, Hirase T, Ohashi Y, Inoue N, *et al.* Overexpression of endothelial nitric oxide synthase attenuates cardiac hypertrophy induced by chronic isoproterenol infusion. Circ J 2002; 66:851–856.
28. Lin Y, Wang L, Xi Y, Li H, Xiao F, Zhao Y, *et al.* L-arginine inhibits isoproterenol-induced cardiac hypertrophy through nitric oxide and polyamine pathways. Basic Clin Pharmacol Toxicol 2008; 103: 124–130.
29. Lu F, Xing J, Zhang X, Dong S, Zhao Y, Wang L, *et al.* Exogenous hydrogen sulfide prevents cardiomyocyte apoptosis from cardiac hypertrophy induced by isoproterenol. Mol Cell Biochem 2013; 381: 41–50.
30. Gálvez AS, Fiedler JL, Ocaranza MP, Jalil JE, Lavandero S, Díaz-Araya G. Perindopril regulates β-agonist-induced cardiac apoptosis. J Cardiovasc Pharmacol 2005; 46: 255–261.
31. Zimmer HG. Catecholamine-induced cardiac hypertrophy: Significance of proto-oncogene expression. J Mol Med 1997; 75: 849–859.
32. Murtuza B, Suzuki K, Bou-Gharios G, Beauchamp JR, Smolenski RT, Partridge TA, *et al.* Transplantation of skeletal myoblasts secreting an IL-1 inhibitor modulates adverse remodeling in infarcted murine myocardium. Proc Natl Acad Sci U S A 2004; 101: 4216–4221.
33. Takemura G, Fujiwara H. Role of apoptosis in remodeling after myocardial infarction. Pharmacol Ther 2004; 104: 1–16.
34. Adams JM, Cory S. The Bcl-2 protein family: Arbiters of cell

survival. Science (1979). 1998; 281: 1322–1326.
35. Kirshenbaum LA, de Moissac D. The *bcl-2* gene product prevents programmed cell death of ventricular myocytes. Circulation 1997; 96: 1580–1585.
36. Kashina AS. Regulation of actin isoforms in cellular and developmental processes. Semin Cell Dev Biol 2020; 102: 113–121.
37. Agostinucci K, Grant MKO, Melaku W, Nair C, Zordoky BN. Exposure to doxorubicin modulates the cardiac response to isoproterenol in male and female mice. Pharmaceuticals 2023; 16: 391.
38. Zhang L, Ji H, Huang Y, Hu H, Li B, Yang Y, *et al.* Association of BAX hypermethylation with coronary heart disease is specific to individuals aged over 70. Medicine 2019; 98: e14130.
39. Perales S, Alejandre MJ, Morales RP, Torres C, Linares A. Fish oil supplementation reverses the effect of cholesterol on apoptotic gene expression in smooth muscle cells. Lipids Health Dis 2010; 9: 70.