Resveratrol Attenuates Cardiac Remodeling and Vascular Occlusion in Rats

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Abstract: Background: The increased incidence of cardiovascular diseases has encouraged substanceresearch that could improve cardiovascular health.For cardiovascular safety,plant extracts and phytochemicals such as resveratrol have been screened. Resveratrol, a polyphenol compound found predominantly in grapes, was involved in the "French paradox" phenomenon described as low incidence of cardiovascular diseases in the French population despite a high intake of saturated fats,in conjunction with moderate consumption of red wine. Despiteof the numerousresearches on the influence of resveratrol on heart health, yet more studies are urgent aiming to discover morewonders of resveratrol.**Objective:**The purpose of this study was to investigate resveratrol's protective effect on the cardiac muscle.**Methods:**For 3 weeks four groups of rats were treated. Control, resveratrol, dimethylnitrosamine (DMN), and resveratrol then DMN.**Results:** Resveratrol treatment evidently prevented the histopathological remodeling changes in cardiac muscle caused by DMN treatment. In other words, resveratrol decreased interstitial collagen deposition in the myocardium, protected against hypoxia, ischemia and necrosis, inhibited cardiomyocyte apoptosis and significantly regulated myocardiac hypertrophy. Resveratrol remarkably attenuated obstruction of blood vessels and enhanced myocardiocytes regeneration. **Conclusion:** Resveratrol canprevent vascular occlusion, cardiac hypertrophy and remodeling indicating a protective powerof resveratrol. Resveratrol may prevent heart failure.
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Key word: Blood vessels, cardiomyocytes, apoptosis, cardiac remodeling.

1. Introduction

Heart failure (HF) is the world's leading cause of death[1].It is a complicated disease that results from many factors, making it impossible for the heart to pump enough blood into the body.Heart failure develops due to hypertension, ischemic heart disease, or cardiomyopathy. Furthermore, heart hypertrophy always precedes heart failure, thisresults inheart enlargementin response to stress (volume overload or pressure). This happens in the initial stages asa compensatoryphase and it is considered as an adaptation to the stressed heart. If hypertrophy persists, the heart attainsa decompensatoryphase. The transition between these phasesresults in cardiac fibrosisand apoptosis, ultimately leads to failure of the heart[2-5]. Treatments for heart failure patients include the use of β-adrenergic receptor blockers, angiotensin-receptor blockers and enzyme-converting angiotensin inhibitors [6, 7]. However, these treatmentsresult in adverse sideeffects[8].Accordingly, it is urgent to explore alternativetherapies.

Recently, numerous plant extracts and phytochemicals were investigated for antihypertensive and anti-hypertrophic, and anti-fibrotic propertiesto provide effective and safe therapy for heart failure.Previous studies have shown that regulationof intracellular reactive oxygen species (ROS) can stop or slow pathological processes in HF[9]. Anotherstudy revealed that increased fruits and vegetables intake reduced blood pressure in hypertensive patients, loweredcardiovascular disease rate, and enhanced myocardial infarction survival[10].

Resveratrol (RES) (trans-30,40,5-trihydroxystilbene), a polyphenol found mainly in grapes and berries, was studied in manycases of hypertension,infarction of myocardium, and HF [5, 11]. RES has beenshown to have powerful effects as anti-oxidative, anti-inflammatory, anti-apoptotic, and anti-fibrotic agent[12-16].Furthermore, resveratrol has been proved to reverse pressure overload (PO)-induced cardiac hypertrophy[11, 17], decrease cardiovascular mortality [18], improve the left ventricular performance and decrease interstitial fibrosis[19].

Several mechanisms were implied in RES protection against HF, including oxidative stress reduction as well as inflammation[20, 21], inhibition of pathological hypertrophic signaling [22], improvement of Ca2+ handling [23], modification of autophagy and decreasing apoptosis through different intracellular pathways [24].

Although numerous researches have been done on resveratrol effect on heart muscle, more studies are still required aiming to explore resveratrol'spotential for heart fibrosis and heart failure.Understandingthe pathogenesisof heart fibrosis will help to understand the fibrosis mechanism and develop new strategies to prevent heart failure and adverse cardiac remodeling.

2. Methods

Animals

Ethic clearance

In accordance with the guidelines and the Research Ethical Committee at King Abdulaziz University, Jeddah, Saudi Arabia. Twenty eight

male Wistar albino rats (90 - 116 g.) were used in the experiment.

Study design:

Group 1(control): control treated orally with saline, with the volumes of saline and 0.5% CMC solution equivalent to those of the resveratrol group, for 3 weeks.

Group 2: resveratrol treated (20 mg/kg body weight/day) orally for 3 weeks.

Group 3: (Fibrotic): induction of fibrosis. Dimethylnitrosamine (DMN) intraperitoneally (i.p.) treated group (10 mg/kg body weight/day, 3 days/week) for 3 weeks.

Group 4: (protective): resveratrol + DMN. RatsreceivedRES, then 2 hours late DMN for 3 weeks [25][26].

The morphological changes were observed. At the beginning of the experiment, and every week, animals were weighed. Animals have been anesthetized and slaughtered one day after the last dose.

For histologic examination: hearts were put in formalin fixative, sliced and embedded in paraffin. Sections (3-5 μm thick) were cut LV and stained.

Stains: Masson Trichromestain (MTS) for collagen fibers (fibrosis).

Heamatoxylin & Eosin (H&E) for general histopathological study.

Digital Light Microscope: Olympus BX51 was used for imaging.

Statistical Analysis

All values of weights are expressed as mean value ± SD. Using SPSS 23.0 (SPSS Inc., USA) software, one - way variance analysis (ANOVA) was used to analyze variations between group means. Statistically significant was considered to be $P<0.05$.

3. Results:

The ratio of total body weight increase in the DMN group ($P\leq0.05$) was significantly low compared to the control. However, resveratrol treatment significantly ameliorated theloss in body weight (table1).

Histopathological investigation revealed severe alterations in DMN treated group such asperivascular and interstitial fibrosis (Fig. 1), blood vessels occlusion andfat deposition (Fig. 2), vascularization, myocardiocyte hypertrophy, inflammation, ischemia, necrosis and apoptosis (Figs. 3 and 4). Ischemic cardiomyocytes resulted in increased apoptosis(Fig. 4B). However, resveratrol treatment significantly reduced these pathological changes reflected by reduced collagen deposition, suppressed inflammatory reaction, impaired cardiomyocytehypertrophy, markedly reduced necrosis and apoptosis. Resveratrolamazingly cleared obstructed blood vessels and brought faster blood flow through vessels. RES treatment remarkably promoted regeneration of cardiomyocytes and reduced the unfavorable alterations (Figs. 1,2,3 and 4).

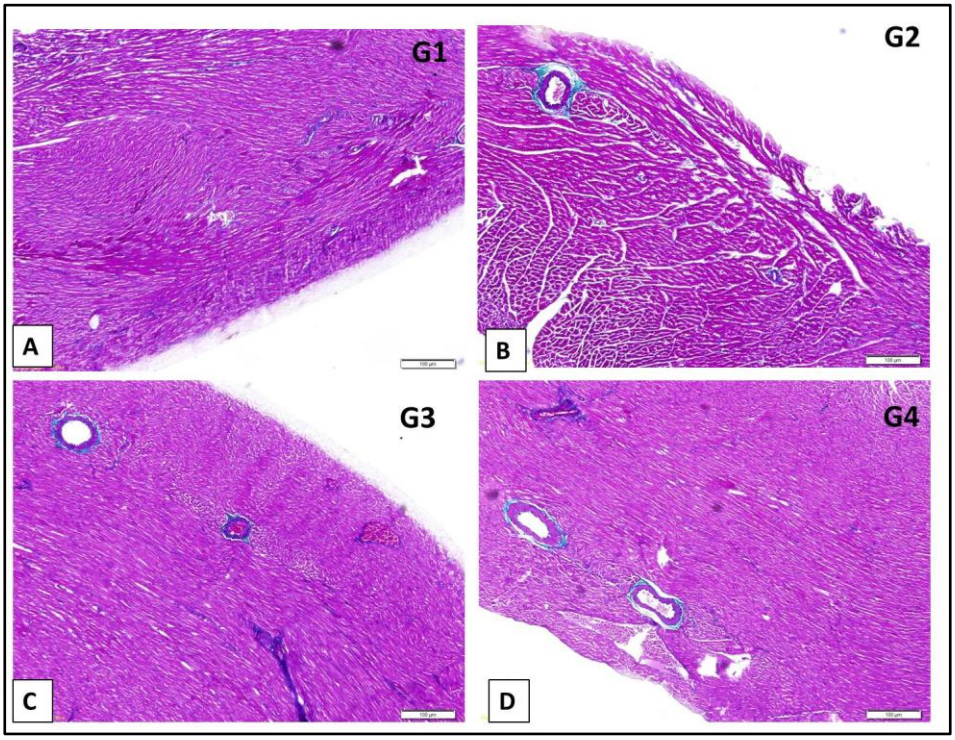

Figure1: **Resveratrol (RES) moderates collagen deposition:** (A) control; (B) RES; (C) DMN, increased collagen content compared to the control reflected as perivascular and interstitial fibrosis (D) RES and DMN, reduced collagen deposition. Masson's trichrome. Scale bar: 100 µm.

Table 1. Body weight of the groups treated for 21 days.

Body weight G	Control	RES	DMN	RES+DMN
Day 0	99.80±3.83	105.40±5.98	110.80±4.96	93.20±2.58
Day 7	120.60±12.72	134.40±5.72	121.40±8.96	122.20±11.41
Day 14	153.40±9.71	164.80±9.17	148.80±12.67	143.00±13.01
Day 21	188.20±18.55	193.20±11.12	160.60±8.38*	178.80±13.55**
Ratio of total increase in B.wt.	88.5%	83.3%	44.9%*	91.8%

Statistical analysis of body weightof rats during DMN treatment, and RES pre-treatment. Body weight was measured weekly during the study. Results are analyzed by one way anova and presented as mean±SEM $P \leq 0.05$. *refers to a significant difference between DMN treated rats and control. **Indicates significance between rats treated RESplus DMN with DMN.

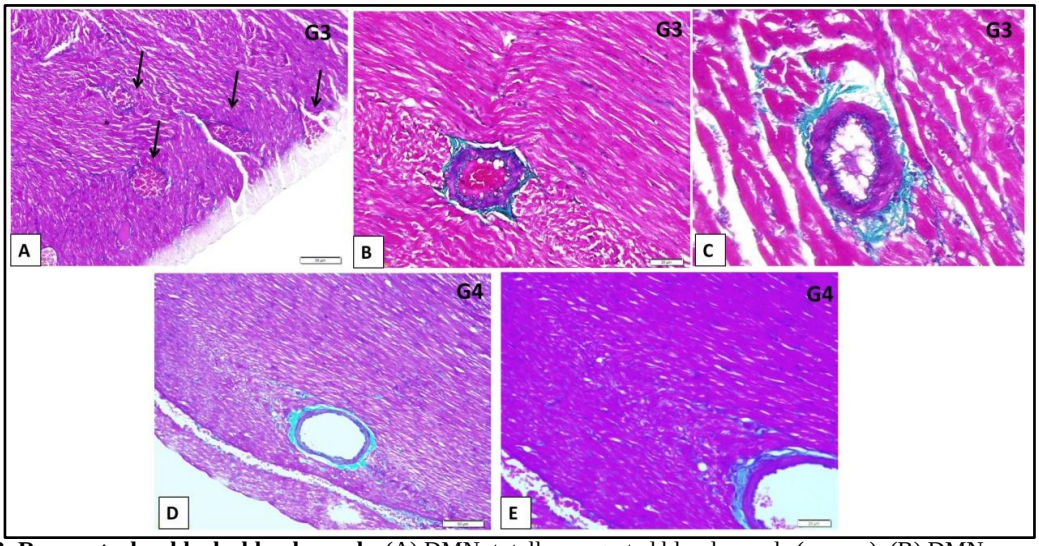

Figure 2: **Resveratrol unblocks blood vessels.** (A) DMN, totally congested blood vessels (arrows). (B) DMN, completely blocked blood vessels. (C) DMN, fatty deposits clogged artery (D& E) RES and DMN, opened and dilated blood vessels. Masson's trichrome. A, D scale bar: 50 µm. B,C and E scale bar: 20 µm.

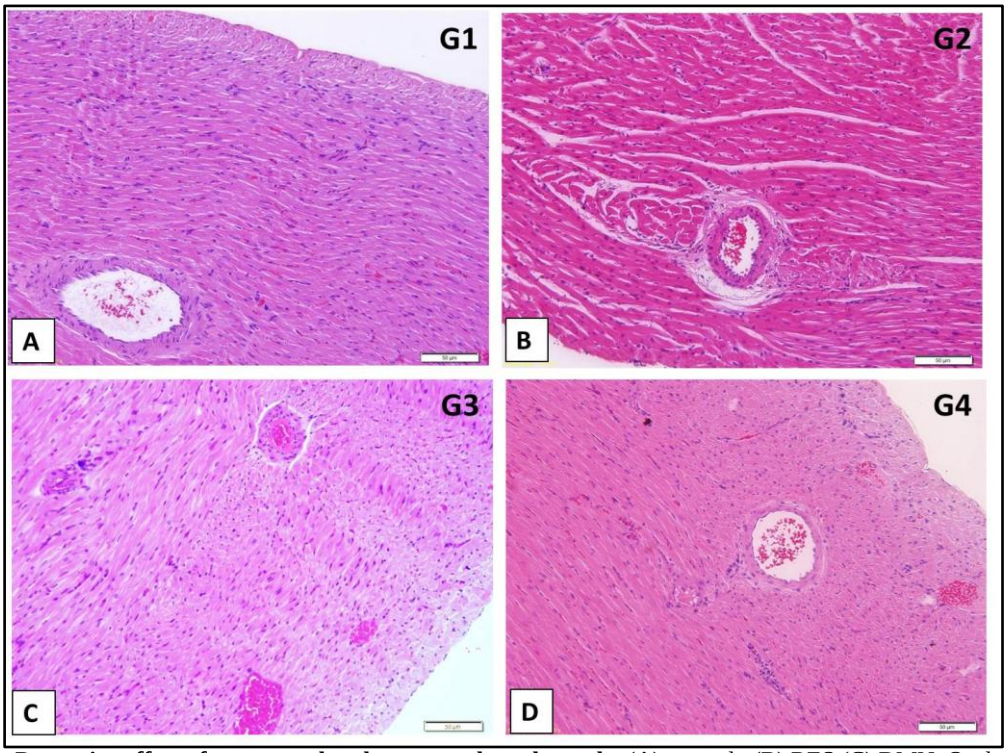

Figure 3: **Protective effect of resveratrol on heart muscle and vessels:** (A) control. (B) RES (C) DMN. Occlusion of blood vessels accompanied by congested spaces between muscle fibers. (D) RES and DMN.H&E scale bar: 50 µm.

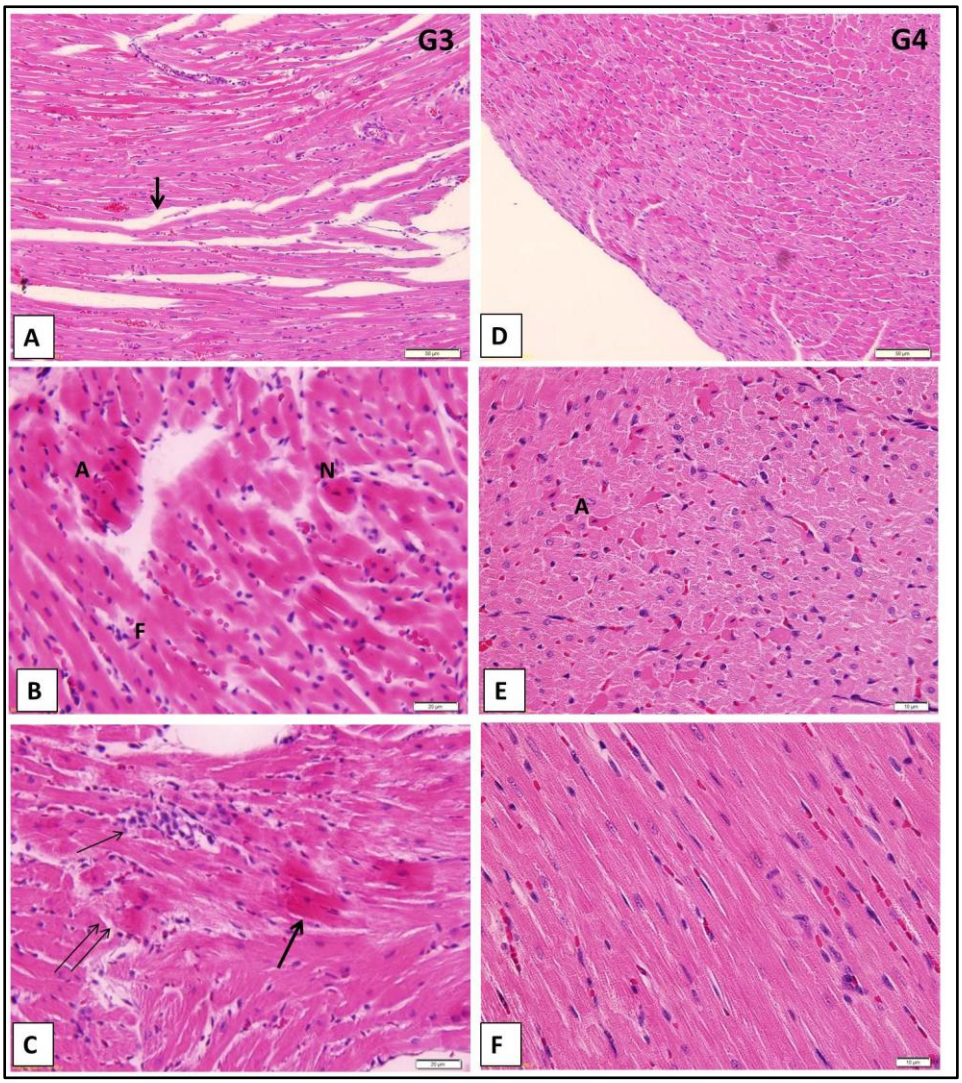

Figure 4: **Resveratrol regulates remodeling of heart muscle:** (A, B, C) DMN. A) Remodeling of heart muscle caused by substitution of myocardium with non-functional fibrotic tissue and vascular tubules (arrow). B) Increased inflammation and apoptosis. Necrotic nucleus (N), apoptotic cells (A), fibroblasts (F). C) Cardiomyocytes hypertrophy (thick arrow), and muscle fiber degeneration (double arrows), areas of myocyte destruction, fibrous connective tissue and mixed inflammatory cells (arrow). (D, E, F) RES and DMN. Resveratrol treatment markedly decreased the remodling process of the heart muscle and induced regeneration of cardiomyocytes. H&E. scale bar: A and D50 µm. B and C: 20 µm. E and F 10 µm.

4. Discussion:

Cardiac fibrosis is known as the heart remodeling process, whichtriggers the replacement of myocardium with non-functional fibrotic tissue due to heart injury or stress.This leads to ventricular impairmentand gradualheart failure [27, 28]. Activated fibroblasts are the main determinants of cardiac fibrosis causing excessive extracellular fibrotic matrix and cardiomyocyte hypertrophy (CMs).

Cardiac hypertrophy is regarded as an adjustment to the stressed heart in its initial stages where cardiomyocytes increase in size to perform adequate function in the presence of chronic pathological stress [29].

ROS exert different pathological intracellular signaling pathways ultimately evoking apoptosis and necrosis. Resveratrol employs anti-fibrotic effect through inhibition of oxidative stress. Previous studies have shown that in a variety of pathological models RES has favorable effects on cardiac fibrosis [30, 31]. For instance, in DOCA-salt rats RESrelieved cardiacfibrosis [14] and reducedthe interstitial and perivascular fibrosis of the left ventricle[32].

In this study, RES treatment has inhibited cardiac fibrosis development and deposition of collagen, as well as cardiomyocyte hypertrophy.In addition, RES markedly prevented the disruptionof myocardium as presented by the organized sarcomeres.This can be attributed to cardiomyocyte apoptosis inhibition, autophagy regulation, and oxidative stress reduction [30, 33, 34].Our results are also in accordance with thestudy of [11]which proved that hypertrophy and cardiac dysfunction in hypertensive rats were prevented by RES without a reduction in blood pressure.

In the present study, DMN treatment caused occlusion of blood vessels, which may cause blood hypertension. Occlusion of the coronary artery preceding hypoxia trigger scardiomyocytes necrosis with maximum cellular damage occurring nearby the occlusion area**.**The loss of cardiomyocytesdevelops as necrosis, apoptosis, and autophagy [35].

Current clinical therapy tends to improve blood flow to non-perfused myocardium byrestoring vital blood supply to dying cardiomyocytes [36]. In the present study, resveratrol treatment restored blood flow to cardiomyocytesby endothelial NO synthase upregulation (eNOS) caused vasodilation and prevented congestion and occlusionof blood vessels[37]. Accordingly, RES preventedischemia of myocardial cellswhich reported by [38]. Changing the initial cell responses to ischemia can improve the survival of cardiomyocytes and finally reserve the myocardial function[39].

In an animal model, heart failure of metabolic cardiomyopathy is preceded by apoptosis and fibrosis. [40]. Various studies have confirmed that RES interferes with several pathological pathways in fibrosis and in different cardiovascular illnesses for example, myocarditis [41], cardiac hypertrophy [42], and cardiac failure [9].

In the present study, DMN treatment caused increasedrate of apoptosisof cardiomyocytes. A slight increase in cardiomyocyte apoptosis contributes to the progression of cardiac dysfunction in animals [43]. These cellular changes ultimately end up with cardiomyocyte death caused by necrosis, apoptosis and/or phagocytosis [43].The present study has shown that resveratrol treatment has regenerated cardiomyocytes, which is in agreement with the study of [44] thatRES activates endogenous stem cells of the heart and improves myocardial regeneration after an acute myocardial infarction.

The significant findings of the present study are: daily resveratrol treatment throughout the experiment significantly prevented all the histopathological alterations and cardiac remodeling. Resveratrolmarkedly attenuated cardiac oxidative stress, blood vessels occlusion,inflammation, fibrosis, and apoptosis.RES dramatically accelerates blood flow and relaxes smooth muscle of the blood vessels. Resveratrol has powerful effects on fibrotic hearts induced by DMNindicatingthe therapeutic potential of resveratrol in heart failure.

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Conflicts of Interest

The author declares no conflicts of interest.

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