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The mechanism of vascular adhesion molecule-1 in an ischemia-reperfusion cardia model

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Abstract

BACKGROUND: Vascular adhesion molecule-1 (VCAM-1) is involved in promoting inflammation within blood vessels, activating endothelial cells, and is a key factor in the progression of diabetic vasculopathy in rats with diabetes, contributing to the underlying pathophysiological processes. This study focused on the expression level of VCAM-1 in diabetic rats subjected to a six-week schedule of aerobic training and valerian supplements.

METHODS: Fifty male Wistar rats' hearts were removed under deep anesthesia and were studied using Lutgendorf's apparatus. They were divided into five groups (10 each): Healthy control (C), Diabetic control (DC), Diabetic with valerian (DV), Diabetic with exercise (DE), and Diabetic with valerian and exercise (DVE). Diabetes was induced in the animals by administering a shot of STZ (50 mg/kg) in their abdominal area. Following confirmation of diabetes in the animals, moderate exercise five days a week, combined with intraperitoneal administration of 200 mg/kg/day of valerian, was maintained for six weeks. Heart tissue was obtained from diabetic cardiac ischemia-reperfusion model (CI/RM) injury (n=40) and control rats (n=10).

RESULTS: VCAM-1 expression and histological parameters were not observed when comparing experimental and control groups. However, the exercise/valerian treatment (E + V) notably reduced the irregularity in cardiac tissue and increased the size of cardiomyocytes.

CONCLUSION: These findings suggest that E + V extract could diminish the levels of diabetic cardiac complications. Also, it had a dual effect: it corrected cardiac tissue abnormalities and increased the size of cardiomyocytes, enhancing the overall structure and function of the heart. More research is needed to understand non-pharmacological complementary treatments in this area.

Keywords: Cardiomyocytes; Cardiac Rats; Diabetic Vasculopathy; Vascular Cell Adhesion Molecule-1; Diabetes Mellitus; Endothelial Cells; Valerian; Myocardial Ischemia

Introduction

Heart disease is the leading cause of mortality worldwide. The term "heart disease" encompasses multiple heart conditions, with coronary artery disease (CAD) being the most common, which can ultimately lead to heart attacks. Individuals with diabetes have double the risk of developing heart disease or experiencing a stroke compared to those without diabetes. Diabetes adversely impacts blood vessels, leading to a decrease in blood flow to crucial organs like the heart and brain. This is particularly true for individuals with uncontrolled blood sugar levels, who face an elevated risk of developing heart failure, heart attacks, strokes, and cardiomyopathy.

Diabetes also disturbs the heart muscle, triggering both systolic and diastolic heart failure. Activation of the arterial endothelium might play an important part in the progression of an atherosclerosis-prone vascular wall in diabetes.

Over the past few years, numerous biomarkers have been investigated to determine their relationships with various cardiovascular diseases. This is because biomarkers may help in arriving at a precise diagnosis or predicting the prognosis of cardiovascular diseases[1]. Vascular adhesion molecule-1 (VCAM-1) is a protein classified within the immunoglobulin (Ig) superfamily. It is expressed in the cell membrane as a type I transmembrane sialoglycoprotein, which contains multiple Ig-like domains characterized by disulfide-linked loops[2]. VCAM-1 plays a significant role in ischemic diseases; however, its precise function within the pathology of these conditions.

Valerian's antioxidant activity is attributed to its rich content of polyphenols, tannins, and flavonoids. These compounds are responsible for valerian's ability to exhibit antioxidant properties. Numerous assays, including free radical scavenging, reducing power, and total antioxidant capacity, have demonstrated these observations. Nevertheless, the consumption of this traditional plant is widespread globally. Physical activities lead to the accumulation of oxidative stress in various tissues, accompanied by an increase in serum glucocorticoids. This process appears to reduce the activity of antioxidant enzymes, causing an imbalance in the body's response to reactive oxygen species (ROS). As a result, ROS and toxins spread through tissues, but the underlying mechanism remains unclear.

The phytonutrient activity of valerian can be attributed to its polyphenol, tannin, and flavonoid content. In various assessments, including free radical scavenging capacity, reducing power, and overall activity, valerian has shown moderate efficacy compared to other medicinal plants[3]. However, the consumption of this traditional plant is very common worldwide.

Oxidative stress accumulates in different tissues following physical activities. Additionally, an increase in serum glucocorticoid levels is observed post-exercise. It seems that such activities alter and reduce the function of antioxidant enzymes, which leads to an imbalance in the systemic manifestations of reactive oxygen species (ROS). As a result, toxins and various forms of ROS spread throughout tissues; however, the pathway of this process remains unclear[4].

When oxidative stress levels rise in the body, the red blood cell (RBC) membrane triggers activation of the cell's defense mechanisms, including antioxidant enzymes, to counteract the generated oxidative stress. The elevated levels of superoxide dismutase and glutathione peroxidase in erythrocytes appear to align with each other[5]. Given that increased oxidative stress in tissues is a consequence of diabetes, tissues exposed to prolonged oxidative stress must adapt by upregulating their antioxidant systems through enzymatic activity.

Although intense exercise can initially elevate oxidative stress, regular and moderate exercise has been shown to enhance antioxidant defenses. This increased antioxidant activity can ultimately reduce oxidative stress and, consequently, the complications associated with diabetes. This study examines the impact of diabetes on changes in oxidative stress

markers within heart tissue, as well as the effects of prolonged and regular exercise on these parameters. Additionally, the potential benefits of consuming traditional herbs like valerian, which possess moderate antioxidant properties, will be explored. The combined effect of exercise and valerian on VCAM-1 expression and improvement of cardiac complications in animal models of diabetes has not been fully investigated. Therefore, this study aims to provide a better understanding of the protective mechanisms by examining the effects of six weeks of aerobic training and valerian supplementation on VCAM-1.

Methods

Animals

In this experimental study, a total of 50 male Wistar rats weighing between 250g and 300g were procured from the Razi Herbal Medicine Research Center at Lorestan University of Medical Sciences. All animal experiments and protocols were conducted in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The Research Ethics Committee of Lorestan University of Medical Sciences granted approval for the animal studies, assigning the ethics code IR.LUMS.REC.1400.040. Following a two-week acclimatization period in the laboratory setting, the rats were randomly allocated into four groups for treatment and control: Healthy control (C), diabetic control (DC), diabetic valerian (DV), diabetic exercise (DE) and diabetic valerian exercise (DVE). The rats were housed in controlled conditions with an average temperature of 25 ± 1 ◦C, 12:12 h dark/ light cycle, and provided ad libitum access to water and food in specialized polycarbonate cages.

The preparation of the extracts from the roots of valerian root (Valeriana officinalis) (Vf)

For this study, a hydroalcoholic extract of Valeriana officinalis root (Vf) was prepared by Pardis Extract Co., Ltd. The extraction protocol involved dissolving 10 g of Vf in 40 ml of ethanol or 70% ethanol at room temperature for 48 hours. The solution was filtered, and the residue was re-extracted. The filtrates were combined, evaporated, and yielded an extract with a ratio of 3.4%. Each sample was then sonicated in ethanol, centrifuged, and the supernatant was evaporated under nitrogen flow and lyophilized, resulting in an extract with a ratio of 2.9%. The extracts were dissolved in ethanol at a concentration of 100 mg/ml and stored at −20°C until use.

Study design

In the first set of experiments, diabetic control (DC), diabetic valerian (DV), diabetic exercise (DE), and diabetic valerian exercise (DVE) rats received an intraperitoneal injection of Streptozocin (STZ; Sigma-Aldrich, Stockholm, Sweden; 50 mg/kg in standard serum). Blood sugar was monitored weekly. Thereafter, the treadmill exercise regimen was conducted five days a week over a period of six weeks at a moderate intensity level. It was divided into three segments: a warm-up lasting 3 minutes, the main training phase which ranged from 10 to 30 minutes, and a cool-down period of 3 minutes. The intensity and duration of the primary exercise progressively escalated from the first week to the sixth week.

Specifically, during the first week, training was conducted at a speed of 10 m/min for a duration of 10 minutes. In the second week, the speed remained at 10 m/min but was extended to 20 minutes. The third week saw an increase in speed to between 14 and 15 m/min for a duration of 20 minutes. In the fourth and fifth weeks, the speed further increased to between 14–15 m/min and 17–18 m/min, respectively, with each session lasting 30 minutes. Intraperitoneal injection of Vf extract (200 mg/kg daily) was performed for six weeks. Finally, at the end of the sixth week, all five groups underwent surgery. For cardiac ischemia-reperfusion model (CI/RM) injury, the Langendorff heart or isolated perfusion heart assay was used as an ex vivo technique in this research. This technique allows for evaluation of the heartbeat and contraction strength of the heart without harming the animal or human.

Rats were anesthetized by IP injection of 60 mg/kg sodium thiopental containing 500 kg/unit heparin. The hearts were then separated from the chest and transferred to the Langendorff machine, perfused retrograde through the aorta under constant pressure (80 mmHg) with oxygenated Krebs-Hensleit bicarbonate buffer, pH 7.4, at a temperature of 37°C, freshly prepared daily.

After complete aortic cannulation and initiation of perfusion, the heart was allowed 15 minutes to reach a steady state. Then, 10 micromoles of phenylephrine (as a vasoconstriction response) were injected as a bolus. Ten minutes after the injection of phenylephrine, 1 micromole of nitroprusside was injected (as a vasorelaxation response in Hensleit). After 10 additional minutes, the animal's heart was completely removed from the device[4].

Determination of cardiac functions

The rats were anesthetized and fixed in the supine position. M-mode echocardiography was obtained using a small-animal ultrasound probe. Measurements of the left side of the heart were taken as follows: the size of the heart chamber when it is full of blood (LVIDd), the size of the heart chamber when it is empty (LVIDs), the amount of blood in the heart when it is full (LVEDV), the amount of blood in the heart when it is empty (LVESV), how well the heart pumps blood (ejection fraction, EF), how much the heart shrinks when it pumps (fractional shortening, FS), the thickness of the heart wall when it is full (LVPWd), and the thickness of the heart wall when it is empty (LVPWs)[5].

Histology

Following surgical procedures, heart tissue was harvested, and the body weight and heart measurements of the rats were recorded. The hearts were preserved in 10% formalin, and samples were taken from the border area of myocardial infarction in the left ventricle of each heart. Subsequently, 5 µm-thick sections were stained using the hematoxylin and eosin (H&E) method and analyzed using stereological (physical dissector method) and immunohistochemical techniques. The diameter of cardiomyocytes (µm) was determined using ImageJ software (NIH, USA) on H&E-stained slides (refer to Fig. 1), and cardiomyocyte diameter was assessed by counting six cells in each of six fields (totaling 30 cells per rat's heart).

Additionally, the number and diameter of vessels, as well as histological characteristics of myocardial infarction—such as cardiac fibrosis and myofiber waviness—were quantified using ImageJ software based on color histograms. Mast cell concentration and degranulation were examined by staining histological sections using the toluidine blue method[6,7]. The histological features of the heart were encoded for analysis using ImageJ software. A transparent lattice line was created to outline the ischemic region, with the area and perimeter representing this specific zone.

Immunohistochemistry

Detection of several tissue antigens was performed using the primary antibody VCAM-1 for endothelial cells (Biorbyt, UK-orb348962), as determined during technical optimization and validation of the IHC test according to the diagnostic kit protocol. The slides were placed in TBS solution (1X, Sigma-T5912) inside a microwave, and after reaching boiling point, the microwave was turned off and the samples remained in the solution for 20 minutes. The samples were then washed with PBS (P4417, Sigma) in three steps at 5-minute intervals. H_2O_2 (Sigma-7722-84-1) and methanol were mixed in a ratio of 1:9 and applied to the samples for 10 minutes. After washing with PBS, the primary antibody diluted 1:100 in PBS was added to the samples and left at room temperature for one hour. Subsequently, the samples were washed three times with PBS, each wash lasting 5 minutes, and 100 µl of linker (Diagnostic BioSystems-PVP1000D), used as the secondary antibody in the kit, was added to the samples for 15 minutes[8]. The samples were again washed three times with PBS, and 100 µl of polymer

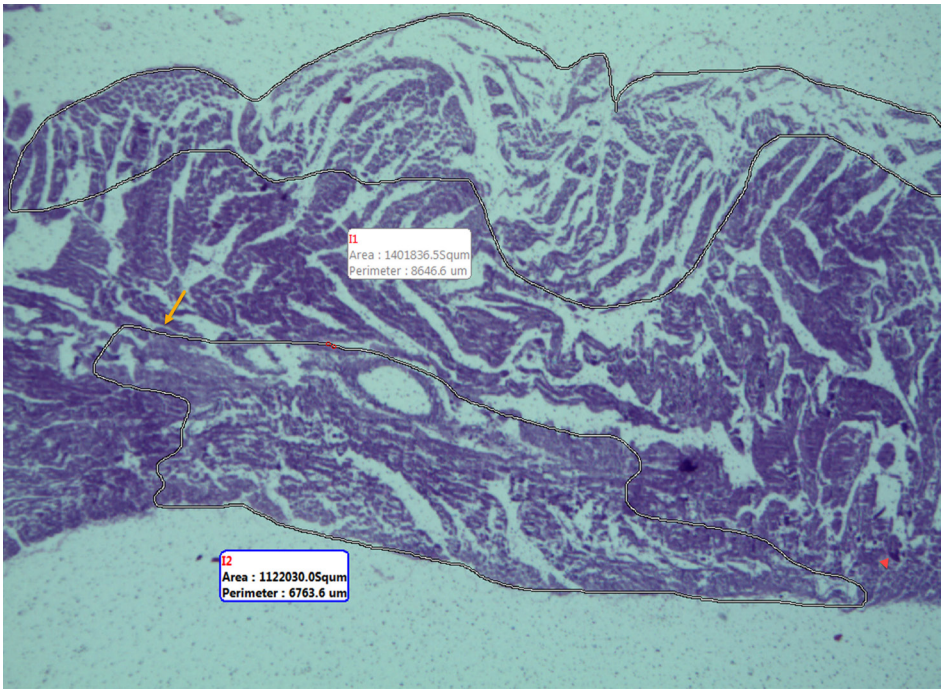

Figure 1. Coding of the histological structures of the heart for software image j estimation. A transparent lattice-bearing line was constructed to serve as an ischemic area. The area and perimeter correspond to an Ischemic area. Longitudinally and obliquely sectioned cardiomyocyte, vessels, and connective tissue, the arrow indicates the line

solution (Diagnostic BioSystems-PVP1000D) was applied for 30 minutes. After another PBS wash, 100 µl of DAB solution (ScyTek-ACV999) was added to the sample. Following a 5-minute incubation, the samples were washed with water and then placed in hematoxylin dye for 10 seconds. A final water wash was performed, and after dehydration and clarification steps, the slide was affixed and examined under an optical microscope (LABOMED).

Photography was performed. After preparing the immunohistochemical samples by the described method, the samples were observed using a digital microscope at 400× magnification. The number of VCAM-1-positive blood vessels and heart muscle cells in a 2 mm^2 measurement area was evaluated. Six random fields from three random slices of each tissue were used to assess the presence of positive cells[9,10].

Statistical analysis

All results were reported as the mean ± standard deviation, based on one-way analysis of variance (ANOVA). For comparison of heart function between groups, the Student t-test was utilized, and for pairwise comparisons, the Tukey post hoc test was applied in the remaining analyses.

Results

Exercise (E) and Valerian (Vf) effectively improved cardiac function in CI/RM Rats

EF, FS, LVIDd, LVIDs, LVPWd, LVPWs, LVEDV and LVESV recorded in postoperatively (Table1).

E and V effectively improved Cardiomyocyte Size in CI/RM rats

The inclusion criteria for cardiomyocytes were: (1) presence of a nucleus, clear cell boundary, and rectangular or rounded shape (length/width ratio < 1:5%). A one-way analysis of variance (ANOVA) revealed a significant difference in the average size of cardiomyocytes among the groups ($P < 0.001$). Multiple t-tests showed that the mean size in the C group was significantly smaller than that in the DC group ($P = 0.002519$), but not significantly different from the DV ($P = 0.703275$), DE ($P = 0.168590$), or DVE ($P = 0.142805$) groups ($P < 0.05$) (Fig. 2A) (Table 2).

Table 1. Echocardiographic and anatomical data were obtained from the 5 experimental groups at the end of the experimental protocol

Groups number &Rats Parameters	Healthy control (C)	diabetic control (DC)	diabetic valerian (DV)	diabetic exercise (DE)	diabetic valerian exercise (DVE)	p-Value
N	*10*	10	10	10	10	
Bodyweight (g)	230± 16	209 ± 13	247± 14	248± 18	245± 12	
Heart weight (g)	1.24 ± 0.04	1.44 ± 0.12	1.054 ± 0.07	1.06 ± 0.05	1.057 ± 0.01	<0.0001
Heart rate (bpm)	234± 27	237.1 ± 8	229.1± 18	234`± 1	233`± 2	<0.0001
Fractional shortening (%)	35.41± 1.2	37.3 ±0.3	18.05±0.8	16.02± 0.3	17.02± 0.2	<0.0001
Ejection fraction (%)	52±0.12	59.98± 0.6	24.07±0.4	29.11±5.0	30.11±4.0	<0.0001
Systolic blood pressure (mmHg)	85.6± 6	89.1±5	87.7± 3	89.1±3	90.1± 4	<0.0001
Diastolic blood pressure (mmHg)	46.12± 1.1	41.22± 3	41.71± 3	41.22±2	42.22± 3	<0.0001
Left ventricular internal diameter in diastole (mm)	4.3 ± 0.5	4.441± 0.4	4.9± 2	6.11 ± 0.5	6.13 ± 0.2	<0.0001
Interventricular septum in diastole (mm)	0.24 ± 0.1	0.87± 0.4	0.67± 0.4	0.7± 0.3	0.8 ± 0.1	0.0006

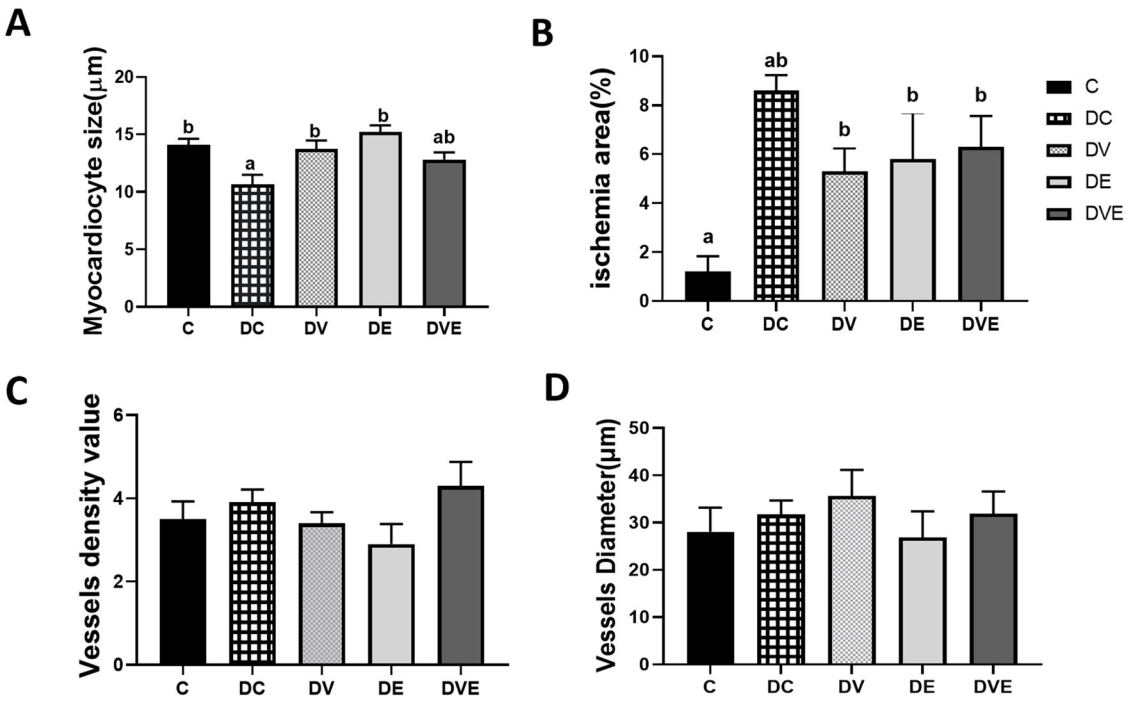

Figure 2 A and B. The size of cardiomyocyte and percentage of cardiac fibrosis as an ischemic area; **C and D**. vessel density value and diameter in studied groups expressed as the mean ± SD and compared with one-way ANOVA and Tukey tests. Scale bar 20µm. Each letter in the graphs means the comparison of the control with the treated groups. On- equal letters indicate no statistically significant difference between the groups.

EX and Vf reduced the myocardial ischemia area

The amount of heart tissue scarring was measured with Image J software by looking at a color chart., taking into account the following criteria: (a) myofiber waviness; (b) interstitial edema; (c) hyper eosinophilia and coagulative necrosis of cardiomyocytes; (d) extensive granulocyte infiltration with karyorrhexis; (e) macrophage and lymphocyte infiltration accompanied by early removal of necrotic debris; (f) granulation tissue with the development of microvessels; (g) fibroblast proliferation and

Table 2. Histopathology data were obtained from the 5 experimental groups at the end of the experimental protocol

Groups number &Rats Parameters	Healthy control (C)	diabetic control (DC)	diabetic valerian (DV)	diabetic exercise (DE)	diabetic valerian exercise (DVE)	p-Value
N	*10*	10	10	10	10	
Cardiomyocyte Size(µm)	14.08± 0.53	10.67± 0.81	13.73± 0.74	15.21± 0.57	12.81± 0.62	P<0.001
Myocardial ischemia area	8.95±0.2	1.23±0.2	1.04±0.3	2.08±0.6	1.61±0.4	P=0.0375
Vessel's diameter	28.04± 5.13	31.66± 3.01	35.65± 5.50	26.85± 5.56	31.89± 4.69	P=0.726
Number vessels value	3.5± 0.42	3.9± 0.31	3.4± 0.26	2.9± 0.48	4.3± 0.57	P=0.212
Total density of mast cells	3.6± 0.26	3.9± 0.4	5.4± 1.1	4.1± 0.23	4.9± 0.56	P= 0.2140
Density of degranulated mast cells	1.8± 0.32	0.58± 2.4	2.4±0.87	2±0.47	2±0.42	P=0.923
protein expression	25.63± 2.7	85.6±2.15	58.36±3.24	73.73±1.21	37.8±4.13	

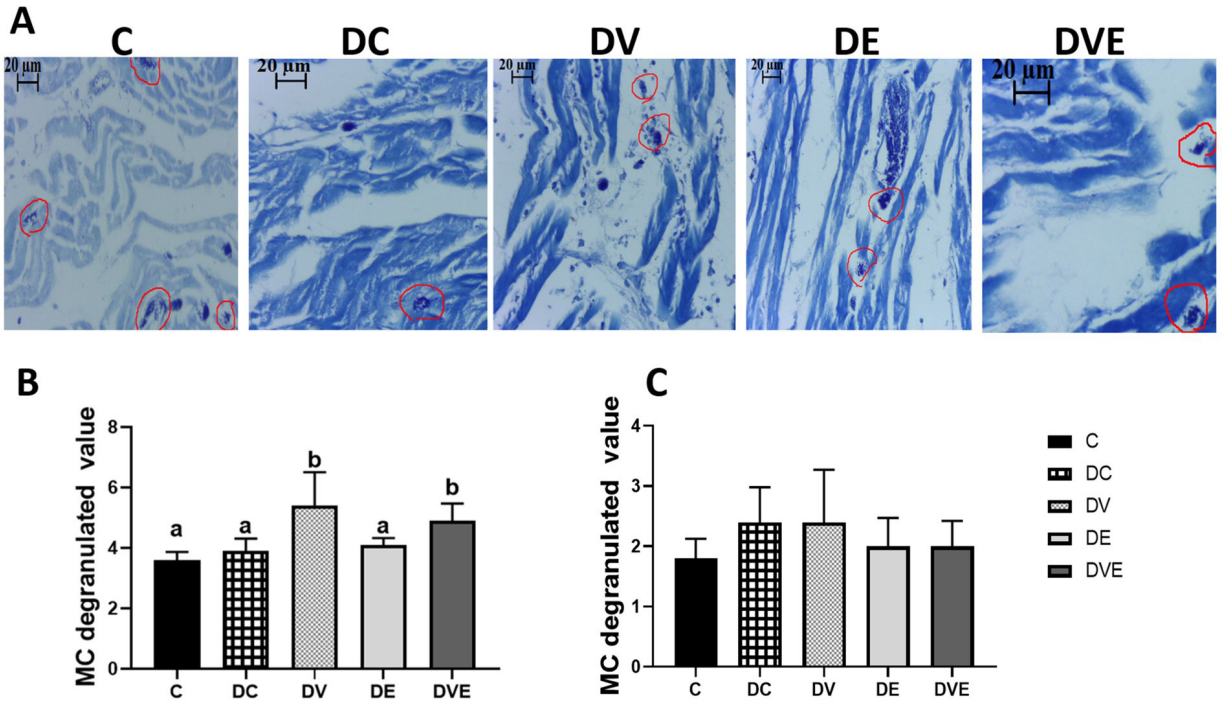

Figure 3A. Presents a histological slide of mast cells in studied groups, stained using the toluidine blue staining method. The red cycle indicates mast cells in heart tissue. **B and C** display the mean ± SD of total mast cell density and degranulation in the studied groups, compared using one-way ANOVA and Tukey tests. On- equal letters indicate no statistically significant difference between the groups.

initial collagen deposition; and (h) dense fibrous scar replacing lost myocytes.

The ANOVA results indicated a significant difference in the mean area of necrosis across the studied groups (P = 0.0375) (Fig. 2B). Multiple t-tests showed that the mean necrotic area in the C group was significantly smaller than those in the DC group (P < 0.000001), and significantly different from the DV (P < 0.01), DE (P < 0.002), and DVE (P = 0.0002) groups (Table 2).

EX and Vf don't affect any pathological changes on vessels number and diameter in myocardium at post- CI/RM

The results of the student test showed that there was no significant difference between the number of vascular sections of the studied groups (P=0.21) (Fig. 2C) and average diameter of the vessels of the studied groups (P=0.72) (Fig. 2D**).** Multiple t-tests showed that the mean vessels number and diameter in myocardium

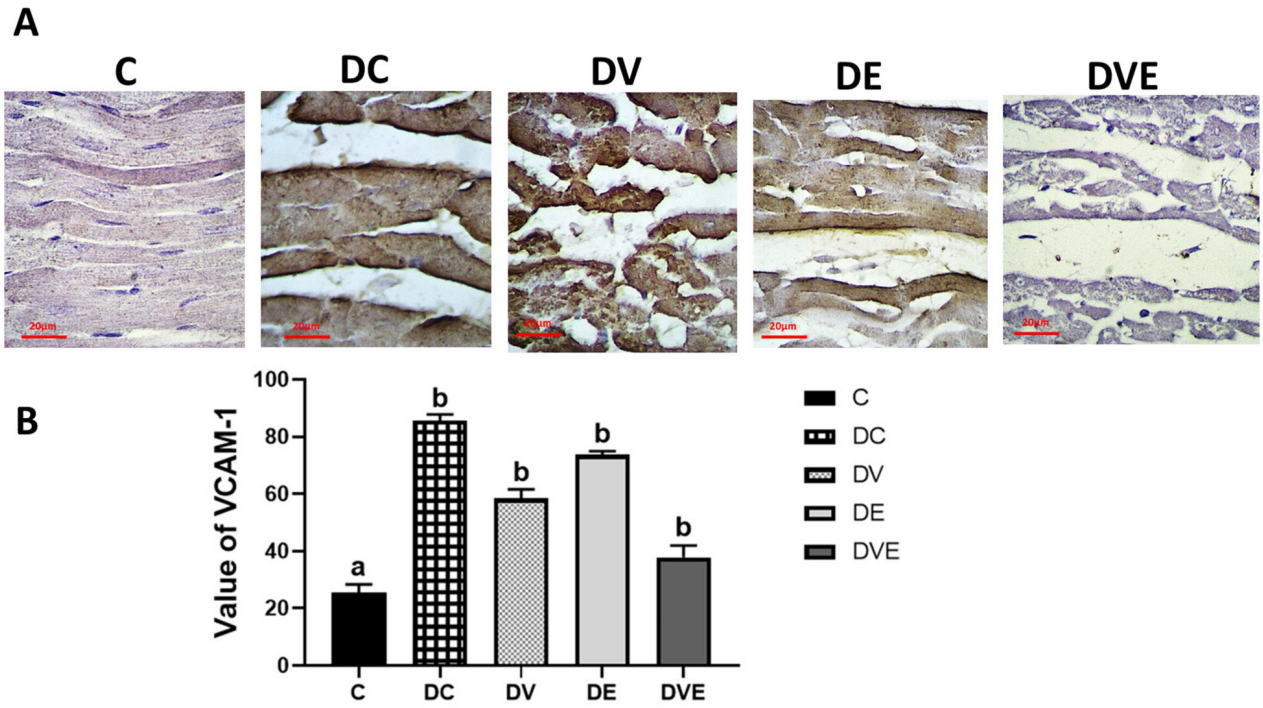

Figure 4A. Shows immunohistochemical micrographs of VCAM-1-positive endothelial cells in studied groups. **B.** Presents the mean ± SD of VCAM-1-positive endothelial cells in the studied group compared made using one-way ANOVA, and Tukey tests. On- equal letters indicate no statistically significant difference between the groups.

respectively of the C group was not significantly from those of the DC (0.46, 0.55) also not significantly different from the DV (0.84, 0.32), DE (0.36, 0.87), DVE (0.28, 0.58) groups, (Table2).

EX and Vf could reduce the number and Degranulated of MC.

Following CIM, an increase in the number of mast cells (MCs) was observed. The administration of EX and Vf resulted of one-way ANOVA revealed a significant reduction in the number of MCs in rats post-CIM. The average total density of mast cells (P=0 .21) (Fig. 3A) and the density of degranulated mast cells (P=0.92) (Fig. 3B) were assessed among the various groups. Multiple t-tests showed that the mean number and Degranulated of MC in myocardium respectively of the C group was not significantly smaller than those of the DC (0.54, 0.38) Also not significantly different from the DV (-, 0.52), DE (0.17, 0.73), DVE (-, 0.71) groups, But significantly in parameter number of mast cells with DV (0.13), and DVE (0.05) groups (Table 2).

EX and Vf decrease VCAM-1 protein expression in heart vessels after CI/RM

Illustrated in Figures 4A and 4B, a notable accumulation of brown-stained affected endothelial cells was observed in the vessels of heart tissues from a CIM rat, which was significantly alleviated by treatment with EX and valerian. One-way ANOVA revealed a significant difference in the average expression of VCAM-1 among the studied groups ($P < 0.001$). Tukey's post hoc comparisons revealed that the mean VCAM-1 expression in the C group was significantly different from the DV, DE, and DC groups ($P < 0.05$), but not significantly different from the DVE group ($P > 0.05$). Additionally, the average VCAM-1 expression in the DC group was significantly different from the DVE, C, and DV groups ($P < 0.05$), but not significantly different from the DE group ($P > 0.05$). Furthermore, the mean VCAM-1 expression in the DV group differed significantly from the C and DVE groups ($P < 0.05$), and a significant difference was observed between the DC and DE groups ($P < 0.05$).

Multiple t-tests showed that the mean VCAM-1 protein expression in the C group was significantly lower than that in the DC group (P = 0.000001), and also significantly different from the DV (P < 0.001), DE (P < 0.01), and DVE (P = 0.02) groups (Table 2).

Discussion

New studies suggest that the number of heart patients will surpass 8 million by 2030. Notably, heart disease remains the leading cause of death worldwide[11]. Given the rise in heart failure cases following acute myocardial infarction, there appears to be a correlation between ventricular remodeling and infarction rates[11]. One of the contributing factors to myocardial infarction is the presence of inflammatory cells within heart tissue during the early post-infarction period, which can disrupt the collagen scaffold[12,13]. Newly formed scar tissue plays a role in preserving ventricular shape and structure. This occurs through fibroblast accumulation at the site of injury, leading to the formation of a new collagen framework. The remaining heart cells have to work harder, they undergo hypertrophy, which ultimately leads to the expansion of the left ventricle. This expansion, in turn, causes the mitral valve to undergo advanced alterations[14]. When the left ventricle doesn't work properly, the amount of blood it pumps out goes down. As a result, echocardiographic measurements of left ventricular diameter and volume can serve as indicators of ventricular remodeling. According to a study, LVIDd, LVIDs, LVEDV, and LVESV were significantly abridged subsequently dealing through exercise and Vf[15]. Notably, our study did not observe a notable decrease in LVIDd following treatment with exercise and valerian.

Furthermore, mast cells, which are associated with inflammation and allergic reactions, are commonly found in elevated numbers in diseases such as cancer. Mast cells are believed to be abundant in heart tissue and release chymase, a substance stored in granules. This release is triggered by a ligand-dependent pathway activated in response to inflammatory processes[15,16]. Research has identified that chymase released from MCs is a significant contributor to tissue fibrosis and repair processes, acting through vasoactive and pro-inflammatory pathways. The chymase and serine protease families possess extensive peptide hydrolysis activities[15,16].

At the onset of cardiac ischemia, the stimulation of the renin-angiotensin-aldosterone system (RAAS) and the catalytic capacity of chymase are reported to be 20 times higher than that of angiotensin-converting enzyme (ACE)[17-19]. Following tissue damage or stimulation by inflammatory cytokines, mast cells (MCs) release their granules, which contain proteases that are subsequently distributed and become inactive[20,21]. Researchers have explored the role of mast cells and chymase in the management of heart failure[22,23]. Interventions aimed at inhibiting chymase activity and reducing mast cell proliferation may contribute to the prevention of myocardial infarction[24-26].

In another study, echocardiographic findings indicated a reduction in ejection fraction (EF) and an enlargement of the left ventricle associated with chronic inflammatory myocarditis (CIM)[27]. In a recent study, the density and degranulation of MCs were found to be altered.

Pathological staining findings indicated the presence of disordered and compromised cardiomyocytes, significant expression of VCAM-1, necrosis, infiltration of inflammatory cells, and pronounced interstitial fibrosis in the cardiac tissues of rats suffering from CIM[27,28]. Immediately after the onset of cardiac ischemia, the rapid conversion of angiotensin factors and activation of the AT1 receptor prompted collagen release, exacerbating cardiac fibrosis and ventricular remodeling[29]. Chymase and mast cells (MCs) played vital roles throughout this process[30]. Additionally, our investigation revealed a notable rise in VCAM-1 expression, particularly prominent in diabetic groups, confirming the widespread presence of VCAM-1 in damaged cardiomyocytes. Various studies have confirmed that Valeriana officinalis extract may exert a protective effect against myocardial ischemia-reperfusion injury through its antioxidant properties.

Following cardiac necrosis, mast cells initiate

an inflammatory response, which triggers self-stimulation of chymase release and the conversion of angiotensin factors through catalytic activity. Notably, the combination of exercise and valerian (Vf) effectively protected cardiac function and deactivated the renin-angiotensin-aldosterone system (RAAS) by reducing Ang II and collagen levels, cardiac fibrosis, increasing left ventricular diameter (LVIDd, LVIDs) and volume (LVEDV, LVESV), and decreasing ejection fraction (EF) and fractional shortening (FS). Additionally, exercise and valerian inhibited mast cell activity, resulting in decreased mast cell numbers and chymase levels. This treatment also demonstrated efficacy in ischemia animal models by significantly reducing infarct size, thereby protecting heart function and preventing complications.

Furthermore, exercise and valerian reduced VCAM-1 expression in myocardial tissue, contributing to the preservation of heart function. The study suggests that exercise and valerian may modulate heart function through a pathway independent of angiotensin-converting enzyme (ACE) following cardiac ischemia, although the precise signaling targets and mechanisms require further investigation.

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Conflict of interests

The authors declare no conflict of interest.

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Author's Contributions

Study Conception or Design: AN; FC
Data Acquisition: FC; SZ
Data Analysis or Interpretation: MB; FC; SZ
Manuscript Drafting: FC; SZ
Critical Manuscript Revision: FC; RK
All authors have approved the final manuscript and are responsible for all aspects of the work.

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