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The combination effect of treadmill exercise and valerian hydroalcoholic extract on the heart of type 2 diabetic rats

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Objectives: Diabetes mellitus is a metabolic disorder recognized for inducing endothelial dysfunction in coronary arteries. This study aimed to investigate the synergistic effects of valerian hydroalcoholic extract and exercise in diabetic rats.

Methods: Thirty-five Wistar rats were randomly divided into five groups: control, diabetes, diabetic rats undergoing treadmill exercise, diabetic rats administered valerian extract (200 mg/kg, oral), and diabetic rats receiving a combination of valerian extract and treadmill exercise for 8 weeks. Antioxidants and lipoproteins were assessed via blood sampling at the study's conclusion. Hemodynamic parameters and the response of the coronary artery bed to constrictors and dilators were evaluated by connecting the heart to a Langendorff device.

Results: Diabetes was associated with a diminished vascular vasodilator response, which was enhanced by the combined treatment of valerian extract and 8 weeks of exercise. Moreover, elevated levels of glutathione peroxidase (GPx) and superoxide dismutase (SOD) were observed in groups receiving both valerian extract and exercise.

Conclusions: The findings suggest that the combination of treadmill exercise and hydroalcoholic extract may hold promise in managing cardiovascular complications in diabetic patients by leveraging their antioxidant properties.

Keywords: antioxidants, hemodynamic factors, treadmill exercise, valerian extract

Introduction

The World Health Organization (WHO) considers diabetes to be the most common endocrine disease globally, resulting in 4 million deaths annually. Each year, 10% of global health expenditures are allocated to combating this disease. Every 10 seconds, one person dies from diabetes worldwide, while two persons are diagnosed. The rate of increase in type 2 diabetes is attributed to lifestyle changes such as decreased physical activity, consumption of high-calorie foods, and rising obesity rates, surpassing the expected increase in type 1 diabetes[1,2]. Hyperglycemia in diabetes and insulin resistance cause oxidative stress[3]. Research has demonstrated that diabetes leads to elevated levels of ROS free radicals and markers like membrane lipid peroxide, along with a reduction in antioxidant levels[4]. The extent of these markers correlates with blood glucose and glycosylated hemoglobin levels. These alterations occur through multiple mechanisms, including the overproduction of superoxide by the mitochondrial electron transport chain. Another contributing mechanism is the heightened production of advanced glycation end products (AGEs)[5,6]. To reduce the oxidative damage effects of ROS, cells establish an antioxidant system. In obese and diabetic individuals, the balance between ROS production and antioxidant defense is disrupted due to the reduction of antioxidants[7]. Vascular endothelial cells are the primary target of chronic hyperglycemic vascular damage, and oxidative stress is the key element that significantly contributes to the pathogenesis of vascular complications[8]. The production of ROS together with the disruption of the mitochondrial electron transport system plays an essential role in the pathogenesis of ischemia/reperfusion in the heart[9] [10]. In patients with uncontrolled diabetes, the level of antioxidants is reduced, indicating that the lack of the catalase enzyme is associated with an increased risk of diabetes[11].

Physical exercise can result in weight loss and reduced body fat, which can lead to lower blood glucose and triglyceride levels, as well as increased sensitivity of cells to insulin and improved glucose homeostasis[12] [13]. Exercise is considered the primary approach in managing the complex risk factors associated with type 2 diabetes and coronary artery disease. The benefits of physical activity extend beyond blood sugar control, as it likely improves certain cardiovascular imbalances caused by diabetes, such as left ventricular dysfunction, vascular endothelial dysfunction, and systemic inflammation[14] [15].

Valeriana officinalis (Valerian), belongs to the Valerianaceae family and is mainly used to treat mild anxiety, insomnia, and

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reduce muscle tension[16,17]. Additionally, preventive effects against hypertension and coronary artery spasms have been observed. Valerian extract has shown coronary dilation and anti-arrhythmic effects in animals[18]. In the current study, the authors aimed to investigate the effects of *V. officinalis* (valerian) extract and exercise training on cardiac function, antioxidant enzyme activity, and lipid profile in streptozotocin-induced diabetic rats. However, limited information is currently available on the impact of Valerian on type 2 diabetes.

Materials and methods

Animals and grouping

All experimental protocols for rats were carried out by adopting the procedures for the precaution and usage of laboratory animals accepted by the National Institutes of Health guide (NIH Publications No. 8023, reviewed 1978). All experimental procedures involving animals were conducted with guidelines for ethical conduct in the care and use of experimental animals approved by LUMS Institutional Animal Care and Use Committee (Animal Ethics Approval code: LUMS.REC.1398.137). In this study, there were 35 male Wister rats (250–300 g weight). Animals were kept under standard light conditions (12 hours of light and 12 hours of darkness) and had access to food and water. Rat food pellets and clean tap water were provided ad libitum.

Rats were randomly (simple randomization) divided into five main groups as follows (Fig. 1):

1. Healthy control (CO): in this group, animals were kept without intervention (n = 7).
2. Diabetic control group (SD): in this group, streptozotocin (STZ) at a dose of 60 mg/kg was injected and 15 minutes later, nicotinamide at a dose of 120 mg/kg was injected intraperitoneally (n = 7).
3. Diabetic group + valerian extract (D + ValE): in this group, after the induction of diabetes, the animals received 200 mg/kg of valerian hydroalcoholic extract (gavage) for 8 weeks (n = 7).
4. Diabetes group + treadmill exercise (D + EX): in this group, after the induction of diabetes, the animals were given treadmill exercise on an electric treadmill (treadmill) especially for rodents (n = 7).
5. Diabetic group + valerian extract and treadmill exercise (D + ValE + Ex): in this group, after the induction of diabetes, the animals were given treadmill exercise and administration of valerian hydroalcoholic extract for 8 weeks (n = 7).

Confounders were controlled by randomizing the order of treatments.

Type 2 diabetes induction

Rats were deprived of food 12 hours before the beginning of type 2 diabetes induction, while they were not deprived of water. Then, administration of streptozotocin (60 mg/kg, MO, Louis, St, Sigma, STZ) dissolved in fresh citrate buffer (pH: 4.5 mol/L) and nicotinamide (120 mg/kg, IP) has been proposed to induce type 2 diabetes in the rat. 48 hours after the induction of diabetes, the blood glucose level was measured by a glucometer device (Glucotrend 2, German company). Blood glucose that was higher than 250 mg/dL was considered diabetic animals.

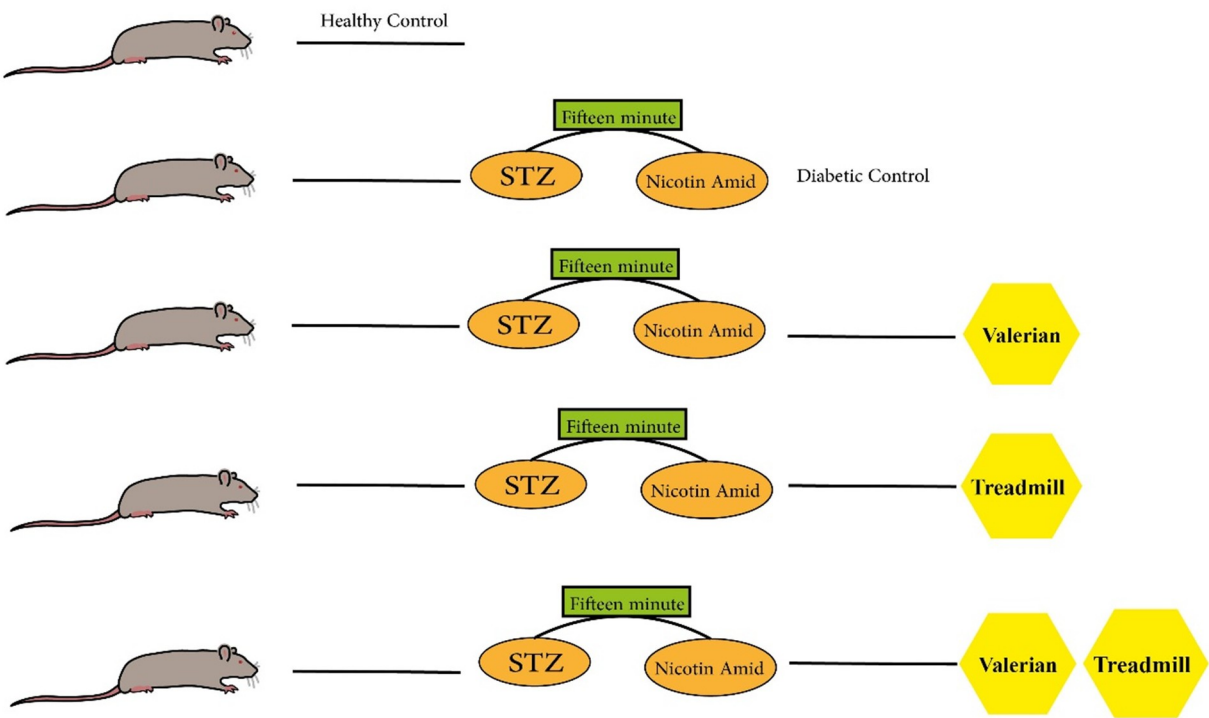

Figure 1. Animals design.

Preparation of hydroalcoholic extract of valerian by maceration method

To prepare the hydroalcoholic extract of valerian, the roots of the valerian plant were first finely ground into a powder. This powder was then placed in a container, and 70% ethanol alcohol solvent was added until it completely covered the surface of the powder. The container was sealed and left in this state for 72 hours. After this period, the solution was filtered through filter paper. The filtered solution was then transferred to a petri dish and placed in an incubator at 37°C until it dried completely and transformed into a powder. Finally, the powder was stored in a refrigerator at 4°C until needed.

Exercise protocol

The groups undergoing an exercise program (D + EX and D + ValE + EX) engaged in endurance exercise training for 8 weeks, with five sessions conducted each week on an electric treadmill following a specific training protocol. The intensity and duration of the treadmill exercise gradually increased over the course of the 8-week program (Fig. 2). Starting at 10 meters per minute for 10 minutes in the first week, the speed and duration progressed as follows: 10 meters per minute for 20 minutes in the second week, 15 meters per minute for 20 minutes in the third week, 15 meters per minute for 30 minutes in the fourth week, 17-18 meters per minute for 30 minutes in the fifth week, 17–18 meters per minute for 40 minutes in the sixth week, and finally 20 meters per minute for 40 minutes in the seventh week. In the eighth week, all training variables were maintained at a constant level to ensure adaptation stability. To encourage continued exercise at the end of each treadmill session, a mild electric shock was administered to prompt the animals. Additionally, measures were taken to prevent injury during training by conditioning the animals with a gentle sound or touch to their tail when they reached the start of the treadmill movement. Throughout the 8-week training period, the incline of the treadmill remained at zero.

Langendorff perfused hearts

After the 8-week treatment phase, all animals were anesthetized using ketamine (88 mg/kg) and xylazine (10 mg/kg). Subsequently, the hearts were swiftly excised and connected to a perfusion cannula through the aorta, then positioned on a Langendorff perfusion apparatus. In the Langendorff perfusion method, a Krebs-Hensele bicarbonate buffer solution, enriched with a blend of oxygen and carbon dioxide gas, was utilized to perfuse the heart externally following its attachment to the Langendorff device. This solution closely resembles plasma electrolytes in terms of its composition, containing NaCl (5.118 mmol/L), KCl (7.4 mmol/L), $NaHCO_3$ (25 mmol/L), $MgSO_4$ (2.1 mmol/L), $CaCl_2$ (2.1 mmol/L), glucose (11 mmol/L), and KH_2PO_4 (2.1 mmol/L).

Upon aortic cannulation and heart perfusion, heart contractions and heart rate rhythm were swiftly restored. A specialized balloon was employed to measure the internal pressure of the left ventricle, connected to a pressure transducer via a catheter inserted into the left ventricle. The pressure transducer was linked to the PowerLab device for recording left ventricular pressure. The balloon was introduced into the left ventricle through the left atrium, traversed the mitral valve, and recorded intraventricular pressure fluctuations through the transducer and PowerLab device. The balloon was filled with water to achieve an end-diastolic pressure of approximately 4-8 mmHg in the left ventricle.

Following connection to the Langendorff and PowerLab devices, a 5-minute stabilization period was allowed to establish a steady state. During this period, the pressure transducer and PowerLab electrocardiography recording device electrodes were attached to the animal's heart. Cardiac parameters (pressure, electrocardiography changes, and hemodynamics) were recorded as baseline measurements for 10 minutes. Subsequently, a bolus injection of 10 micromoles of phenylephrine (as a vasoconstrictor response) was administered through the aortic cannula, and heart parameters were recorded for 1 minute. Following this phase, 1 µmol of nitroprusside (as a vasorelaxant response) was injected through the aortic cannula 10 minutes later, with cardiac parameters recorded during the final minute by blinded person.

Antioxidant enzymes

The activity of SOD was assessed using a RANSOD kit from Randox (Crumlin, UK), following the procedure established by previous studies. This assay works by generating superoxide radicals through the reaction of xanthine and xanthine oxidase. These radicals then interact with 2-(4-iodophenyl)-3(4-nitrophenol)-5-phenyl tetrazolium chloride (ITN), resulting in the formation of a red formazan dye. The absorbance of this dye was measured at 505 nm using a spectrophotometer (Pharmacia Biotech, England). The degree of inhibition of this reaction was indicative of SOD activity, which was calculated by comparing the results to a standard curve and expressed as U/mg protein. In addition, the activity of glutathione peroxidase (GPx) was measured based on the method outlined by Paglia and Valentine, utilizing a RANSEL kit from Randox (Crumlin, UK).

Lipid profile assessment

Serum levels of lipoproteins, specifically HDL and LDL, were measured using a calorimetric method. This assessment helps to evaluate the impact of the treatments on lipid metabolism in diabetic rats.

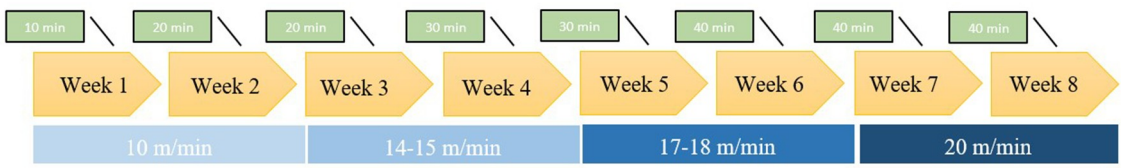

Figure 2. Treadmill exercise protocol.

Table 1

Effect of *V. officinalis* and exercise on heart rate parameter (heart rate per minute) in three basic conditions: baseline, after vasoconstrictor, and vasodilator injection.

TIME	Control	SD	D + ValE	D + Ex	D + ValE + Ex
Baseline	234 ± 131	228 ± 89	166 ± 114	231 ± 136	172 ± 89
PHE	241 ± 134	289 ± 135[c]	321 ± 91[b]	220 ± 142[a]	148 ± 73[a]
NPS	263 ± 108	204 ± 113[c]	252 ± 85[b]	240 ± 141	199 ± 113

All results were expressed as the mean ± SD and analyzed using one-way ANOVA.
[a]$P < 0.05$ indicates significant difference when compared with D + ValE.
[b]$P < 0.05$ indicates significant difference when compared SD group.
[c]$P < 0.05$ indicates significant difference when compared control group.

Statistical analysis

Statistical analyses are performed using the statistic package for social science (SPSS) software (18.0, SPSS Inc., Chicago, IL). One-way analysis of variance (ANOVA) is used to determine the significance level among the different groups. Post-hoc analysis was performed using Tukey's test. Hemodynamic parameters were analyzed with repeated measures of analysis of variance. Mean ± standard error of the mean (SEM) was used to show the results of each group. $P < 0.05$ was considered statistically significant.

Results

Effect of V. officinalis and exercise on ECG in rats

The impact of treadmill exercise or *V. officinalis* treatment on ECG recordings at three stages, including responses to constrictor, dilator, and baseline, is detailed in Tables 1 and 2. The heart rate of the diabetic control group exhibited a significant variance ($P < 0.05$) in response to constrictor and vasodilator agents compared to the nondiabetic group. Following treatment with *V. officinalis*, a notable increase in heart rate ($P < 0.05$) was observed during the phenylephrine (PHE) phase in comparison to the SD group. Diabetic rats subjected to treadmill exercise in both exercise groups displayed a substantial reduction ($P < 0.05$) in response to phenylephrine when contrasted with the D + ValE group.

The QRS interval of diabetic rats engaged in treadmill exercise exhibited a significant difference ($P < 0.05$) at three instances (baseline, PHE, and NPS) when compared to the D + ValE and SD groups. Additionally, a noteworthy variance in the QRS interval of the D + Ex group was noted at baseline compared to the Control and D + ValE + Ex groups ($P < 0.05$). The QT interval of the D + Ex group demonstrated a significant decrease ($P < 0.05$) in baseline time compared to the D + ValE and D + ValE + Ex groups, while there was a significant increase in the latter two groups compared to the control group ($P < 0.05$). The QTc interval of the D + ValE + Ex group exhibited a notable decrease in response to phenylephrine when contrasted with the other groups ($P < 0.05$). Among diabetic rats that underwent exercise, the ST segment displayed significant differences compared to the SD and D + ValE groups ($P < 0.05$). Furthermore, in the D + Ex group, a significant decrease was observed when compared to the Control and D + ValE + Ex groups. The PR interval in the D + Ex group notably increased in response to phenylephrine compared to other groups except the control group ($P < 0.05$). Lastly, the RR interval in the combination group displayed a significant increase compared to the SD and D + ValE groups ($P < 0.05$).

Effect of V. officinalis and exercise on coronary blood flow (CBF) and perfusion pressure

Table 3 displays the coronary perfusion pressure response of isolated hearts in control and diabetic rats. The diabetic hearts exhibited an increase in perfusion pressure in response to

Table 2

Effect of *V. officinalis* and exercise on cardiac parameter in three basic conditions, after vasoconstrictor and vasodilator injection.

TIME	Variable	Control	SD	D + ValE	D + Ex	D + ValE + Ex
Baseline	QRS (ms)	0.02 ± 0.003	0.02 ± 0.007	0.02 ± 0.005	0.01 ± 0.002[c.a.d.b]	0.02 ± 0.008
	QT (ms)	0.05 ± 0.01	0.04 ± 0.01	0.06 ± 0.01[c]	0.04 ± 0.01[a.d]	0.06 ± 0.02[c]
	QTc (ms)	0.1 ± 0.03	0.1 ± 0.03	0.1 ± 0.03	0.09 ± 0.03	0.1 ± 0.06
	ST (mV)	85 ± 42	51 ± 16	92 ± 85	31 ± 67[c.d.b.a]	82 ± 63[a.b]
	PR (ms)	0.03 ± 0.007	0.03 ± 0.004	0.03 ± 0.004	0.04 ± 0.009	0.03 ± 0.007
	RR (ms)	0.3 ± 0.2	0.3 ± 0.1	0.5 ± 0.3	0.3 ± 0.3	0.5 ± 0.2
PHE	QRS (ms)	0.02 ± 0.003	0.02 ± 0.004	0.02 ± 0.006	0.01 ± 0.005[a.b]	0.02 ± 0.003
	QT (ms)	0.04 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
	QTc (ms)	0.1 ± 0.02	0.1 ± 0.05	0.1 ± 0.03	0.1 ± 0.05	0.08 ± 0.02[c.a.b]^
	ST (mV)	32 ± 15	12 ± 16	56 ± 85	52 ± 67	20 ± 63
	PR (ms)	0.03 ± 0.006	0.04 ± 0.008	0.03 ± 0.007	0.08 ± 0.1[d.b.a]	0.03 ± 0.005
	RR (ms)	0.3 ± 0.2	0.3 ± 0.3	0.2 ± 0.06	0.4 ± 0.3	0.5 ± 0.1[a.b]
NPS	QRS (ms)	0.02 ± 0.003	0.02 ± 0.003	0.02 ± 0.004	0.01 ± 0.001[d.b.a]	0.02 ± 0.003
	QT (ms)	0.04 ± 0.01	0.06 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.2 ± 0.5
	QTc (ms)	0.08 ± 0.02	0.1 ± 0.07	0.1 ± 0.02	0.1 ± 0.04	0.1 ± 0.02
	ST (mV)	16 ± 14	40 ± 32	45 ± 45	48 ± 86	40 ± 46
	PR (ms)	0.03 ± 0.007	0.03 ± 0.008	0.04 ± 0.006	0.04 ± 0.008	0.03 ± 0.01
	RR (ms)	0.3 ± 0.2	0.5 ± 0.3	0.3 ± 0.1	0.3 ± 0.3	0.5 ± 0.2

All results were expressed as the mean ± SD and analyzed using one-way ANOVA.
[a]$P < 0.05$ indicates significant difference when compared with D + ValE.
[b]$P < 0.05$ indicates significant difference when compared SD group.
[c]$P < 0.05$ indicates significant difference when compared control group.
[d]$P < 0.05$ indicates significant difference when compared D + ValE + Ex group.

Table 3

Effect of *V. officinalis* and exercise on coronary perfusion pressure in three basic conditions: baseline, after vasoconstrictor, and vasodilator injection.

TIME	Control	SD	D + ValE	D + Ex	D + ValE + Ex
Baseline	20.7 ± 8.5	48 ± 9.5	19.8 ± 3	50.6 ± 3.1[d,a]	19.8 ± 3.1
PHE	21 ± 8.3	51 ± 9.8[c]	19.3 ± 9.7[b]	51.2 ± 3.2[c,d,a]	18.9 ± 38[b]
NPS	20 ± 9	49.9 ± 9.8[c]	18.9 ± 1[b]	55.8 ± 2.9[c,d,a]	19.7 ± 42[b,a,e]

All results were expressed as the mean ± SD and analyzed using one-way ANOVA.
[a]$P < 0.05$ indicates significant difference when compared with D + ValE.
[b]$P < 0.05$ indicates significant difference when compared to SD group.
[c]$P < 0.05$ indicates significant difference when compared to the control group.
[d]$P < 0.05$ indicates significant difference when compared to D + ValE + Ex group.
[e]$P < 0.05$ indicates significant difference when compared with D + Ex.

phenylephrine and nitroprusside compared to the control group ($P < 0.05$). Among the groups that received interventions individually, a significant difference in heart rate response was observed only in the D + ValE and D + ValE + Ex groups compared to the SD group ($P < 0.05$). Additionally, in the combination group, there was a notable variance in heart responses to vasodilators when contrasted with the D + ValE and D + Ex groups ($P < 0.05$).

Table 4 presents the cerebral blood flow (CBF) for the control group and other groups at three different time points in this study. CBF demonstrated a significant increase in response to nitroprusside in the groups that received *V. officinalis* and a combination of treatments compared to the control group ($P < 0.05$). CBF remained relatively stable in the hearts of the other groups throughout the investigation.

Effect of V. officinalis and exercise on cardiac antioxidant enzyme activities

In assessing the blood plasma antioxidant enzyme response to *V. officinalis* and exercise in diabetic rats, we measured the activities of GPx, CAT, and SOD (Fig. 3). Trained diabetic rats, whether individually or in combination, exhibited a notable increase in SOD activity compared to non-trained diabetic rats ($P < 0.05$). The activity of GPx was significantly higher in groups that received *V. officinalis* alone or in combination with exercise ($P < 0.05$). However, there was no significant variance in CAT activity observed among the experimental groups.

Effect of V. officinalis and exercise on changes in serum levels of HDL and LDL

As depicted in Fig. 4, diabetic rats exhibited a significant variance in HDL levels compared to control rats ($P < 0.05$). Similarly, there was a notable difference in serum LDL levels ($P < 0.05$) in diabetic rats when contrasted with the control group (Fig. 4). However, the daily administration of *V. officinalis* and the 8-week treadmill exercise regimen did not lead to alterations in serum HDL and LDL levels in treated diabetic rats compared to untreated diabetic rats.

Discussion

The current study delved into exploring the impact of valerian hydroalcoholic extract and an 8-week exercise training regimen, either individually or in combination, on the cardiac tissue of diabetic rats. The findings revealed that the treatment of diabetic rats with valerian extract and exercise led to a reduction in vascular response to vasoconstrictors and an increase in cerebral blood flow (CBF). Moreover, the concomitant administration of valerian extract and exercise training for 8 weeks amplified the response to vasodilators and enhanced CBF in diabetic rats undergoing exercise training and valerian extract treatment together. Analysis of electrocardiographic indicators demonstrated improvements in HR-QTc-QT-QRS-CPP parameters following vasodilator injection (NPS) and QTC-CPP parameters after vasoconstrictor injection (PHE) in diabetic rats treated with valerian extract. Furthermore, in the D + ValE + Ex group receiving a combination of valerian extract and exercise, CBF and CPP parameters post-vasodilator injection, as well as HR and CPP parameters post-vasoconstrictor injection, exhibited enhancements.

Diabetes is associated with a decrease in mean arterial blood pressure (MABP) and a decline in blood pressure response to vasoconstrictors.[19]. In our study, while the HR level in the diabetic group did not exhibit a significant change compared to the control group, a decrease in heart rate was observed in response to vasodilators. Consistent with previous research, our findings suggest that diabetes is not inherently linked to increased blood pressure and heart rate.

Cardiovascular complications are prevalent among individuals with diabetes and are a leading cause of mortality[20,21]. Studies have highlighted the protective effects of regular exercise in mitigating cardiovascular diseases in diabetes by reducing lipid peroxidation and bolstering antioxidant enzyme levels[22-24]. In our study, exercise enhanced the response to NPS in diabetic rats, aligning with existing literature[25]. Furthermore, regular moderate physical activity was shown to enhance the body's enzymatic defense system by elevating antioxidant enzyme levels such as GPx and SOD[26]. The oxidative stress present in diabetic patients due to heightened free radical production may contribute to the development of cardiovascular diseases. Diabetes is also associated with increased lipid peroxidation, which weakens the body's antioxidant system, damages cell organelles and enzymes, and exacerbates insulin resistance, thereby fostering the progression of diabetes complications[27] [28].

Our study revealed a significant decrease in the activities of SOD, CAT, and GPx enzymes in the hearts of STZ-induced diabetic rats. However, following 8 weeks of aerobic exercise, the activity of SOD, CAT, and GPx enzymes increased notably in combination groups, albeit without a significant increase in CAT enzyme activity. Both valerian extract and exercise independently increased antioxidant enzyme levels like GPx and

Table 4

Effect of *V. officinalis* and exercise on coronary blood flow in three basic conditions: baseline, after vasoconstrictor, and vasodilator injection.

TIME	Control	SD	D + ValE	D + Ex	D + ValE + Ex
Baseline	17.1 ± 3.7	26.1 ± 6.6	40.8 ± 12.8[b]	20.8 ± 3.6	11.4 ± 4.5[a]
PHE	18.4 ± 3.7	18.8 ± 1.6	22 ± 5.8	28.1 ± 8.8	22.1 ± 6.6
NPS	12.8 ± 1.9	19.5 ± 3.4	54.8 ± 18.2[b]	22.7 ± 4.5	31.2 ± 9.6[b]

All results were expressed as the mean ± SD and analyzed using one-way ANOVA.
[a]$P < 0.05$ indicates significant difference when compared with D + ValE and SD groups.
[b]$P < 0.05$ indicates significant difference when compared to the control group.

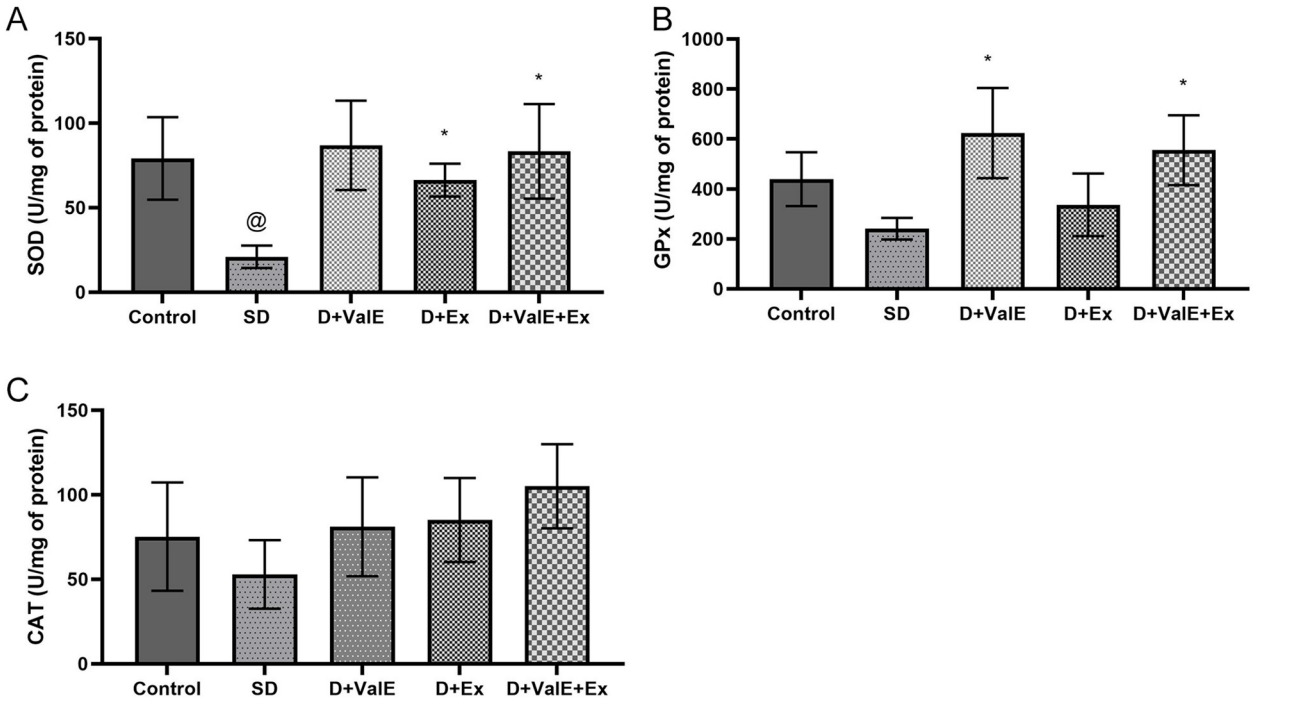

Figure 3. The effect of *V. officinalis* and exercise on the antioxidant enzyme activity levels: (a) SOD activity; (b) GPX activity; (c) CAT activity. Data are shown as mean ± 7 animals, $^{*}P < 0.05$ indicated significantly change compared with SD group. $^{@}P < 0.05$ indicated significantly change compared with interventional groups.

SOD. However, the combination group showed the highest levels of these antioxidants, indicating a synergistic effect. The substantial enhancement of these enzymes following exercise and *V. officinalis* treatment may denote the activation of the primary defense mechanism against oxidative stress induced by exercise. Previous studies on obese diabetic rats engaging in regular moderate-intensity swimming exercises demonstrated reduced oxidative stress and elevated serum SOD enzyme levels[29]. The removal of reactive oxygen species such as hydrogen peroxide ions, which increase in diabetic patients' blood vessels, is facilitated by antioxidant enzymes like CAT, GPx, or SOD. In tissues subjected to prolonged oxidative stress from diabetes, the activity of these enzymes should be heightened to counteract this stress[30]. GPx, an antioxidant that eliminates hydrogen peroxide ions more effectively than other antioxidant enzymes, also prevents the generation of other harmful free radicals like hydroxyl. Studies have indicated that simultaneous consumption of dietary antioxidants and moderate endurance exercise can reinforce the antioxidant defense system by diminishing reactive oxygen species in STZ-induced diabetic rats. The amalgamation of aerobic exercise and antioxidant consumption may enhance the antioxidant defense by curbing reactive oxygen species in diabetic animals with STZ-induced heart issues[31]. Therefore, based on results of other study, the specific molecular pathways involved in the synergistic effects of valerian extract and exercise on antioxidant status are as follows: valerian extract contains compounds like valerenic acid and hesperidin that can directly scavenge ROS and upregulate antioxidant

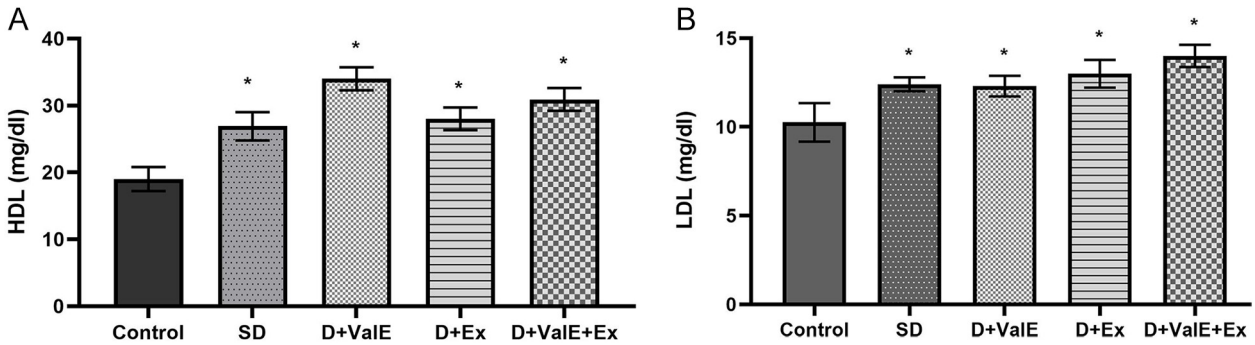

Figure 4. The effect of *V. officinalis* and exercise on HDL and LDL level. Data are shown as mean ± SD for = 7 animals, $^{*}P < 0.05$ indicated significant change compared with control group: (a) HDL levels and (b) LDL levels.

enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. Exercise can also stimulate the activation of the Nrf2 transcription factor, which regulates the expression of numerous antioxidant genes. The combined effect of valerian extract and exercise leads to a more robust antioxidant response, reducing oxidative stress and improving vascular function.

According to the present results and the studies about the effects of aerobic exercise and simultaneous consumption of antioxidants on the function of coronary arteries following diabetes and comparing with the findings of the present study, it can be concluded that the combination of aerobic exercise and consumption of antioxidants can improve coronary artery function by reducing oxidative stress in the heart of STZ-induced diabetic animals[32]. In this study, 8 weeks of exercise training and valerian hydroalcoholic extract could improve inappropriate vascular function caused by diabetes. The synergistic effects of valerian extract and exercise on vascular health involve several key molecular mechanisms: valerian extract has been found to upregulate the expression and activity of endothelial nitric oxide synthase (eNOS), leading to increased nitric oxide (NO) bioavailability. Exercise also stimulates eNOS through shear stress, further enhancing NO-mediated vasodilation, improved blood flow, and reduced vascular inflammation. Also, valerian extract activates the AMP-activated protein kinase (AMPK) pathway, which regulates cellular energy homeostasis and can improve insulin sensitivity, lipid metabolism, and mitochondrial function. Exercise also activates AMPK, leading to synergistic effects on metabolic regulation and vascular protection[33,34].

The outcomes of our study underscored that 8 weeks of exercise training and valerian hydroalcoholic extract administration could ameliorate aberrant vascular function induced by diabetes. Nevertheless, our results did not reveal a significant improvement in the lipid profile of diabetic rats following an 8-week endurance training program with moderate intensity and valerian extract consumption. While regular physical exercise has been shown to reduce LDL levels and prevent cardiovascular diseases when appropriately administered in terms of intensity, duration, and frequency, its impact on decreasing triglyceride and LDL concentrations is limited. Notably, physical activity has been associated with beneficial effects on LDL composite components[35]. HDL plays a crucial role in cholesterol transport, with increased levels often linked to heightened lipoprotein lipase enzyme (LPL) activity post-physical activity. The enzyme LPL's role in converting very low-density lipoprotein (VLDL) to HDL contributes to the elevation of HDL levels alongside increased enzyme activity[36]. In our study, serum HDL levels exhibited a significant increase following valerian extract consumption, consistent with prior research findings[37]. There are some limitations of this study: the study focused on specific cardiac parameters, antioxidant enzymes, and lipid profile markers. Other potential mechanisms, such as inflammation, endothelial function, and mitochondrial function, were not evaluated. The study did not investigate the impact of different doses of valerian extract or varying exercise intensities on the measured parameters. Dose-response relationships and the optimization of treatment regimens were not explored. Despite these limitations, this study provides valuable insights into the potential benefits of combining valerian extract and exercise training in the management of diabetes-related cardiovascular complications. Given the growing prevalence of diabetes and its associated cardiovascular risks, the findings from this study could have implications for preventive strategies targeting high-risk individuals. Incorporating a regimen of valerian supplementation and regular exercise into the management of prediabetes or early-stage diabetes may help mitigate the development of cardiovascular complications and improve overall cardiovascular health. The findings warrant further investigation in larger animal studies and eventually human clinical trials to confirm the therapeutic efficacy and safety of this integrated approach.

Conclusion

In conclusion, the study investigating the combined effect of treadmill exercise and valerian hydroalcoholic extract on the endothelial dysfunction of the coronary artery in type 2 diabetic rats revealed promising results. The findings demonstrated that the concurrent administration of valerian extract and exercise training led to notable improvements in vascular responses to vasoconstrictors and vasodilators, as well as enhancements in cerebral blood flow (CBF) in diabetic rats. Moreover, the electrocardiographic indicators indicated favorable changes in heart rate and cardiac performance parameters following vasodilator and vasoconstrictor injections in rats receiving valerian extract and exercise.

The findings of this study provide valuable insights into the potential therapeutic benefits of combining valerian extract and exercise training in the management of diabetes-related cardiovascular complications. The results suggest that this integrated approach can help improve cardiac function, enhance antioxidant defenses, and modulate lipid profiles in diabetic animals. The study's outcomes support the notion that regular exercise and the use of natural compounds like valerian extract can positively impact antioxidant enzyme activity, vascular responses, and cardiac function in diabetic individuals. Further research and clinical studies are warranted to elucidate the underlying mechanisms and optimize the therapeutic potential of this combined intervention for managing cardiovascular complications associated with diabetes.

Ethical approval

All experimental protocols for rats were carried out by adopting the procedures for the precaution and usage of laboratory animals accepted by the National Institutes of Health guide (NIH Publications No. 8023, reviewed 1978). All experimental procedures involving animals were conducted with guidelines for ethical conduct in the care and use of experimental animals approved by LUMS Institutional Animal Care and Use Committee (Animal Ethics Approval code: LUMS.REC.1398.137).

Consent

Not applicable.

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Author contribution

A.M. and A.K.R., writing original draft; M.M., M.Z., and M.S., data collection; S.Y. visualization, validation; V.Gh. and A.N., project administration, supervision. All these authors have substantial contributions to the final manuscript and approved this submission.

Conflicts of interest disclosure

All the authors declare to have no conflicts of interest relevant to this study.

Research registration unique identifying number (UIN)

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Data availability statement

The data that support the findings of this study are available from the first author, upon reasonable request.

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