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CARDIOPROTECTIVE EFFECT OF DICHLOROMETHANE VALERIAN (VALERIANA OFFICINALIS) EXTRACT ON ISCHEMIA-REPERFUSION-INDUCED CARDIAC INJURIES IN RATS

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Abstract

Background. Valepotriate is an active ingredient of valerian (*Valeriana officinalis*) with strong antioxidant activity that is effective for numerous cardiovascular diseases.

Objective. The aim of this study was to investigate the effect of an active ingredient of *V. officinalis* extract on ischemia-reperfusion-induced cardiac injuries in male rats.

Methods. Thirty-two male rats were subjected to ischemia for 40 minutes and reperfusion for five days. The rats were divided into 4 groups of 8 each; group 1 (control) was given normal saline, and groups 2-4 were gavaged with 0.2, 0.1, 0.05 mg/kg of valepotriate extract, respectively, and received extract (0.2 mg/kg ip) two weeks before ischemia induction.

Results. Dichloromethane *V. officinalis* (valepotriate) extract exerted a protective effect against ischemia-reperfusion-induced injuries. So that infarct size and number of ventricular arrhythmia and ventricular escape beats decreased compared to the control group. Moreover, ST segment amplitude, QTC interval, and heart rate decreased in the injured hearts and serum levels of antioxidant enzymes glutathione peroxidase, catalase, and superoxide dismutase increased. Biochemical markers malondialdehyde and lactate dehydrogenase also decreased on day 5 after the onset of reperfusion.

Conclusion. *V. officinalis* extract may have a protective effect against myocardial ischemia-reperfusion by producing antioxidant effects.

Keywords: Valepotriate, Antioxidants, Ischemia, Reperfusion, Cardiac injurey, ST segment, QTC interval.

INTRODUCTION

Cardiovascular diseases (CVDs) including coronary artery disease (CAD), hypertension, congenital heart disease and myocardial infarction (MI) are the leading cause of death internationally (1). The reasons for CVDs include hypertension, hypoxia, elevated total cholesterol and low-density lipoprotein (LDL), decreased serum high-density lipoprotein (HDL) level, diabetes mellitus, and aging. Oxidative stress and inflammation are also important risk factors for CVDs (2). Cardiac ischemia causes tissue damage and dysfunction by increasing the production of free radicals, nitric oxide (NO) and decreasing the activity of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPO), and catalase (CAT) (3). MI is the most common cause of heart failure. MI is associated with changes such as cardiomyocyte hypertrophy, myocardial arrhythmia, left ventricular systolic and diastolic dysfunction, decreased left ventricular contractile strength, increased fibrosis and apoptosis, and decreased capillary density (4). Inducing reperfusion in the ischemic heart is one of the therapeutic methods that, in addition to assisting in treatment, can cause a widely varied tissue damage (5,6). On the other hand, reperfusion potentially might lead to lethal ventricular arrhythmias (VAs) such as tachycardia and ventricular fibrillation (VF) (7).

One of the known ways to reduce the side effects

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of reperfusion is preconditioning. Preconditioning (PC) refers to the preparation of the tissue using a physical stimulus such as inducing short-term ischemic durations [ischemic PC (IPC)] or pharmacological stimulus before induction of ischemia-reperfusion, so that tissue resistance is increased and the severity of ischemia-reperfusion-induced tissue injuries are decreased by stimulating and creating endogenous defense mechanisms (8).

Today, CVD imposes a huge economic burden on society and patients and many companies invest numerous resources on new drugs development for these diseases. The medicinal plants, besides ischemia-reperfusion, could be considered as a good candidate for CVD treatment with low cost and fewer side effects (9). Studies demonstrates that, antioxidants play an important role in creating IPC properties. Antioxidants by scavenging oxygen-derived free radicals have a stunning effect on cardiac contractility (9).

Among the most popular herbal medicines used for their antioxidant effects, different species belonging to the "*Valerianaceae*" family are in the spotlight of research. *Valeriana officinalis* is one of the members of this family and consists of about 150-200 chemical constituents including flavonoids, triterpenes, lignans and alkaloids but the roots and rhizomes of this plant, consist of two main groups of compounds include sesquiterpenes of volatile oil and valepotriates (10,11). In ancient medicine, Valerian exhibits vasodilatory and antiarrhythmic properties. Studies revealed that these properties effects on mice, rabbits and cats are associated with valepotriates (12). In this study we evaluate the impact of preconditioning with valepotriate extract on ischemia-reperfusion-induced cardiac injuries in male rats.

MATERIALS AND METHODS

Animal models

In this experimental study, 40 male Wistar rats (assigned to 5 groups of 8 each) weighing 250-300 g were used. The rats had ad libitum access to water and food under standard laboratory conditions [12-hour light/12-hour dark cycle and 22±2˚C temperature].

Preparation of Valeriana officinalis extract

Following described protocol by Backlund *et al.* in 1998 we extracted valepotriates from *V. officinalis* rhizomes with some modifications. *V. officinalis* rhizomes was purchased from a grocery in the center of Lorestan province and then shade dried at 20˚C and powdered. Twenty grams of rhizome powder was extracted with 300 mL of dichloromethane solvent for 24 hours and then filtered. The liquid under the filter was evaporated using a rotary apparatus under vacuum at 30 ˚C, and the resulting dichloromethane extract was wrapped in foil and stored in the refrigerator at 4˚C for the next steps. Extraction using dichloromethane causes the extraction of various derivatives of valepotriate from plant rhizomes (13).

Experimental design

1. Control group: given normal saline by gavage (Control)

2. Valepotriate extract at 0.05 mg/kg by gavage (VT0.05)

3. Valepotriate extract at 0.1 mg/kgby gavage (VT0.1)

4. Valepotriate extract at 0.2 mg/kg by gavage (VT0.2)

5. Valepotriate extract at 0.2 mg/kg by intraperitoneal injection (IP) (VT0.2+ IP).

Animal preparation

The animal was first anesthetized by IP sodium thiopental (60 mg/kg), its neck and chest were shaved, and then it was placed on the surgical table. A small lamp was placed on the surgical table to keep the animal's body temperature at approximately 37˚C. The animal was then connected to a small animal ventilator [Harvard Model 683-USA (60-70 breaths per minute and current volume of 15 ml/kg)] and saliva was suctioned. The electrodes were then connected to record the lead II electrocardiogram of the PowerLab.

Inducing a temporary MI

First, an incision was made between the third and fourth ribs of the chest to expose the heart. It should be noted that the incision should be carefully made so as not to damage the lungs or heart. The pericardium was then gently ruptured and the 0.6 silk thread was carefully passed under the left anterior descending (LAD) artery. The coronary artery closed approximately 2 mm below the left atrium. Loosening the knot and pulling the suture induces temporary ischemia. Rats were subjected to ischemia for 40 minutes and reperfusion for five days. Electrocardiography changes were monitored with the PowerLab to ensure the infarction in rats. The layers of muscle and skin were then sutured. At the completion of the procedure, the animal was placed under pure oxygen to regain consciousness. The animal's body temperature was maintained at 37±1˚C using a heat pad

during the procedure. After the animal regained full consciousness, it was transferred to the cage, provided with water and food, and taken to an animal house.

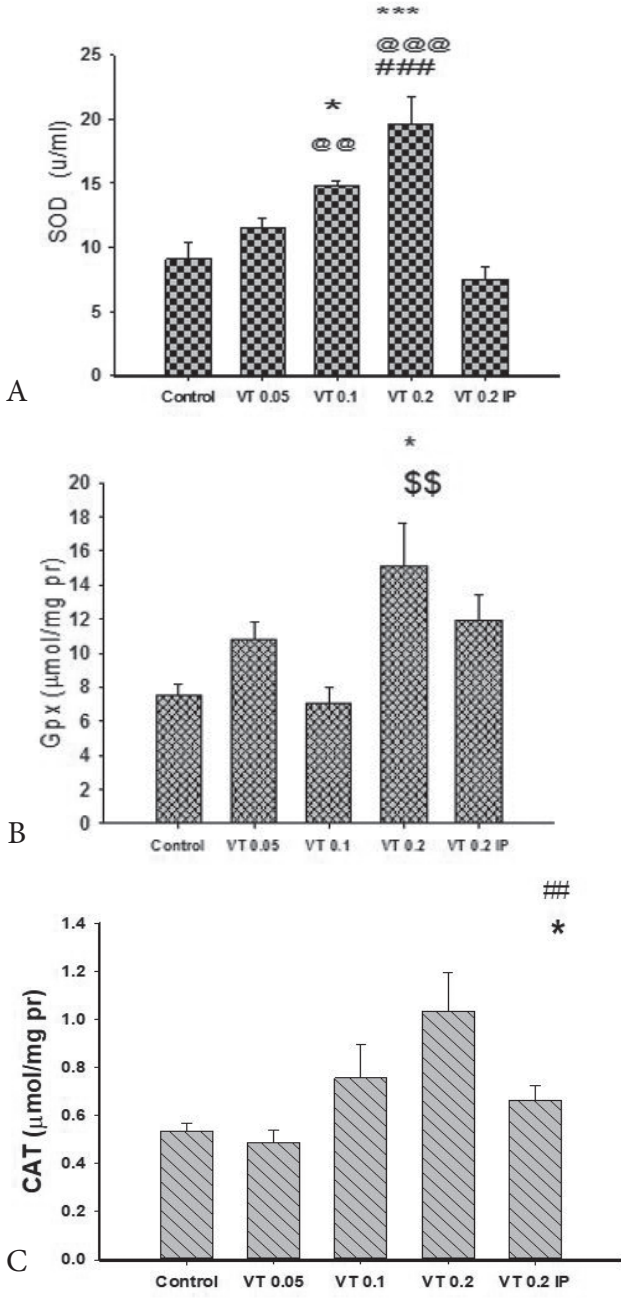

Figure 1. Effect of Valeriana officinalis extract on oxidative stress markers. Results are expressed as mean±SEM. (a) glutathione peroxidase (GPx), (b) superoxide dismutase (SOD), (c) catalase (CAT), (d) malondialdehyde (MDA). VT0.05, V. officinalis extract at 0.05mg/kg; VT0.1, V. officinalis extract at 0.1 mg/kg; VT0.2, V. officinalis extract at 0.2 mg/kg; VT0.2 IP, V. officinalis extract at 0.2 mg/kg by peritoneal injection. *P<0.05 and ***P<0.001, significant difference compared to control group; ##P<0.01 and ###P<0.001, significant difference compared to VT0.05 group; $$P<0.05, significant difference compared to the VT0.1 group; @@P<0.01 and @@@P<0.001, significant difference compared to the VT0.2 IP group.

Determining infarct size

At the end of the study and completion of reperfusion period, the heart was removed under deep anesthesia. First, the heart was rinsed with distilled water, and its atria, vascular roots and additional appendages were separated, and then it was wrapped in an aluminum foil and left in the freezer until the next day. After removing the heart from the freezer, 2 mm sections of the left ventricle (from the base to the apex) were prepared using graduated molds. Next, they were stained with 2,3,5-triphenyltetrazolium chloride at 37˚C for 20 minutes, fixed in formalin 10% and then the percentage of infarct size was calculated by image J software (National Institute of Health, Bethesda, MD, USA).

Measurement of cardiac enzymes and antioxidants

At the end of reperfusion, blood samples were collected from the carotid artery and centrifuged at 5000 rpm for 15 minutes. The serum was frozen and stored at -70˚C until subsequent measurements. Finally, as the markers of myocyte necrosis, the serum levels of lactate dehydrogenase (LDH) were measured by LDH assay kit (Pars Azmoon Co., Iran) and troponin I (CTnI) measured by Zellbio kit (Germany) via autoanalyzer apparatus (Roche Hitachi Modular DP Systems, Mannheim, Germany).

Plasma MDA level, as an indicator of oxidative stress, was measured based on thiobarbituric acid reaction by spectrophotometry method. Measurements of antioxidant enzymes include SOD, GPx, and CAT were performed according to the kit's instructions and a spectrophotometer apparatus (Shimadzu, Tokyo, Japan) (All kits provided from Pars Azmoon Co., Iran) (14).

RESULTS

Effect of V. officinalis extract on serum antioxidant enzymes activity

The level of SOD activity in serum is shown in Figure 1a. In comparison to the control group, SOD activity significantly increased after administration of *V. officinalis* extract in VT0.1, VT0.02 and VT0.2 IP groups in dose dependent manner (P<0.01). In VT0.2 group SOD activity increased 2.1-fold rather than control group but in inter peritoneal injected group SOD showed 0.4-fold lower activity.

Figure 2b showed activity of GPX. In comparison with control group, GPX activity increase significantly in VT0.2 group (1.9-fold).

As you seen in Figure 2c, CAT activity increases 1.6-fold in 0.2VT group rather than control.

V. officinalis extract alter biochemical factors

The serum LDH, MDA and cTnI levels in rats in different groups were shown in Figure 2. Five days after reperfusion, rather the control group cTnI decreased in all 4 groups but this reduction is not statistically significant (P>0.05).

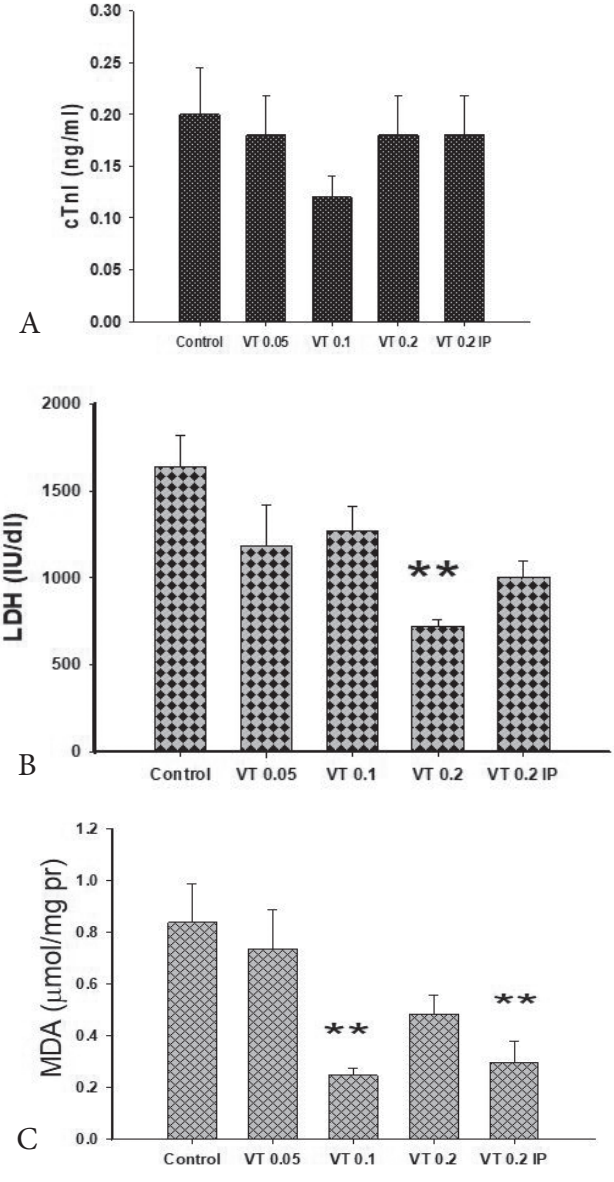

Figure 2. Effect of Valeriana officinalis extract on biochemical markers. Results are expressed as Mean±SEM. (A) troponin I (cTnI), (B) lactate dehydrogenase (LDH), (C) malondialdehyde (MDA). VT0.05, V. officinalis extract at 0.05 mg/kg; VT0.1, V. officinalis extract at 0.1 mg/kg; VT0.2, V. officinalis extract at 0.2 mg/kg by gavage; VT0.2 IP, V. officinalis extract at 0.2 mg/kg by peritoneal injection; **P<0.01, significant difference compared to the control group.

As seen in Figure 2b, LDH in animals that received 0.2 mg/kg *V. officinalis* extract significantly decreased more than 2-fold. MDA in Figure 2c exhibit same manner and decrease 2.2 and 2.7-fold in 0.2 VTIP and VT0.1 respectively.

Infarct size of myocardial tissue

As illustrated in Figure 3, treatment with *V. officinalis* extract caused a significant reduction in the percentage of infarcted tissue at VT0.1, VT0.2 and VT0.2 IP compared to the control group (P<0.001, P<0.01 and P<0.01, respectively) (Fig. 3).

Determination and evaluation of ventricular arrhythmias (VAs)

In this study, VAs including ventricular escape beats (VEBs), ventricular tachycardia (VT) and ventricular fibrillation (VF) were evaluated based on the Lambeth model. VEB refers to premature and flattened QRS complexes that are called bigeminy when they occur one in between with the normal QRS complex and are called trigeminy when one VEB occurs for both natural QRS complexes. In other combination types such as couplets (two VEBs in a row) and triplets (three VEBs in a row), VEBs were counted separately.

VT refers to the generation of more than three VEBs in a row. VFs refer to indeterminate and low voltage QRS complexes that, if they elapse for less than 2 minutes, will be transient, and otherwise, they will be

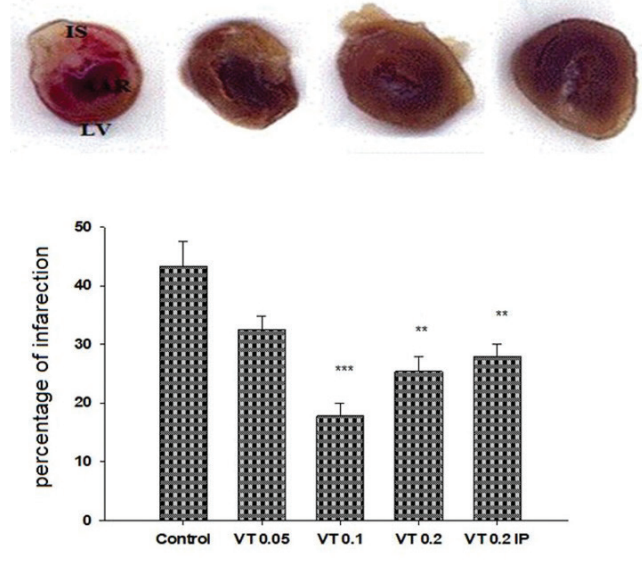

Figure 3. Effect of valepotriate extract on infarct size percentage. The results are expressed as mean±.SEM. VT0.05, valepotriate extract at 0.05 mg/kg; VT0.1, valepotriate extract at 0.1 mg/kg; VT0.2, valepotriate extract at 0.2 mg/kg; **P<0.01 and ***P<0.001, significant difference compared to the control group.

stable. In this study, the incidence of VF, duration and number of VTs, as well as the severity of Vas, were investigated at 30-minute ischemia intervals.

Regarding the number of VEBs, treatment with *V. officinalis* extract at 0.1 mg/kg (VT0.1) and 0.2 mg/kg (VT0.2) significantly reduced the number of arrhythmias at the 30-minute ischemia interval compared to the control group (P<0.01 for both) (Fig. 4a). Furthermore, the number of VTs observed at the 30-minute ischemia interval significantly decreased at 0.2 mg/kg (VT0.2) compared to the control group and at 0.05 mg/kg (VT0.05) and 0.1 mg/kg (VT0.1) (P<0.05 for all, Fig. 4b).

Electrocardiography parameters

HR

HR at baseline interval was significantly different between all groups. Treatment with *V. officinalis* extract significantly reduced the number of HR at 0.1 mg/kg (VT0.1) at the 30-minute ischemia interval compared to the control group (P<0.05). Furthermore, the number of HR at 60-minute reperfusion interval at 0.1 mg/kg (VT0.1) and 0.2 mg/kg (VT0.2) significantly decreased compared to the control group (P<0.05). Comparison of different intervals of the control group showed that the 30-minute ischemia interval and 60-minute reperfusion interval significantly increased compared to the baseline (P<0.01 and P<0.001, respectively). Besides that, the 60-minute reperfusion interval in the control group significantly increased compared to the 30-minute reperfusion interval (P<0.05). After intraperitoneal injection of 0.2 mg/kg extract (VT0.2 IP), the 60-minute reperfusion interval significantly increased compared to the baseline (P<0.05, Table 1).

QTc length (ms)

The duration of QTc in different groups was not significantly different at baseline. Pretreatment with *V. officinalis* extract at the 30-minute ischemia interval significantly decreased during QTc at 0.05 mg/kg (VT0.05) and 0.1 mg/kg (VT0.1) compared to the control group (P<0.05 and P<0.01, respectively). QTc duration at the 60-minute reperfusion interval at 0.05 mg/kg (VT0.05) and 0.01 mg/kg (VT0.1) significantly

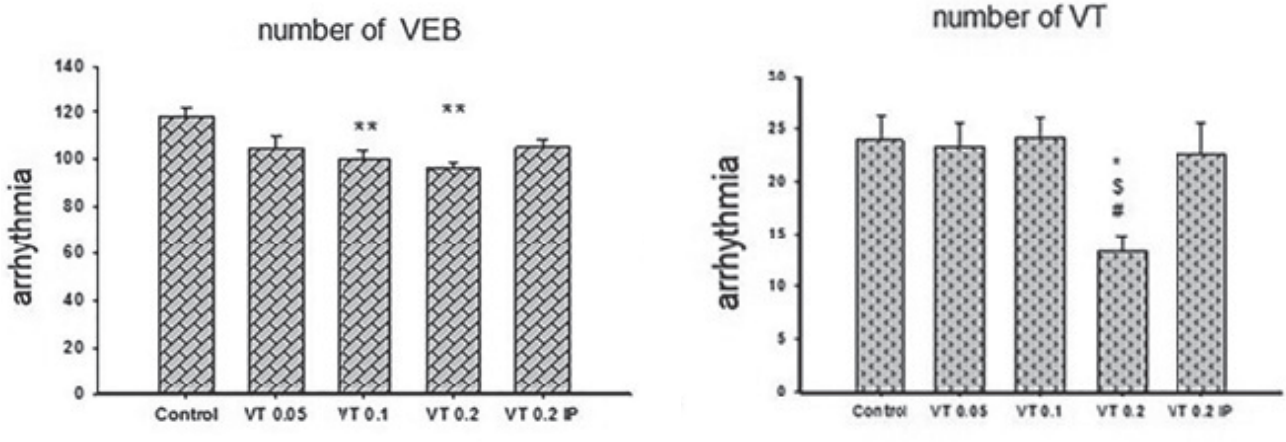

Figure 4. Effect of valepotriate extract on the number of arrhythmias recorded on electrocardiography. Results are expressed as Mean±SEM. (a) Number of ventricular escape beats (VEBs); (b) Number of ventricular tachycardia (VT); VT0.01, valepotriate extract at 0.1 mg/kg; VT0.2, valepotriate extract at 0.2 mg/kg; VT0.2 IP, intraperitoneal injection of valepotriate extract at 0.2 mg/kg; *P<0.05 and **P<0.01, significant difference compared to control group; #P<0.05, significant difference compared to VT0.05 group; $P<0.05, significant difference compared to VT0.1 group.

Table 1. Effect of valepotriate extract on heart rate in the last five days of reperfusion

Groups	Baseline	End of ischemia 30'	End of reperfusion 60'
Control	211.15±10.56	270.40±7.98##	311.08±13.59###$
VT 0.05	213.53±17.85	239.79±16.86	253.84±8.83
VT 0.1	201.4±13.25	205.11±15.55*	249.77±16.17*
VT 0.2	215.23±16.05	220.54±13.31	243.19±16.60*
VT 0.2 IP	223.05±14.44	249.8±14.29	285.44±#18.65

Results are expressed as Mean±SD. VT0.05, valepotriate extract at 0.05 mg/kg; VT 0.1, valepotriate extract at 0.1 mg/kg; VT0.2, valepotriate extract at 0.2 mg/kg; VT0.2 IP, intraperitoneal injection of valepotriate extract at 0.2 mg/kg. *P<0.05, significant difference compared to the control group in the corresponding interval; #P<0.05, significant difference compared to baseline; ##P<0.01, significant difference compared to baseline; ###P<0.001, significant difference compared to baseline; $P<0.05, significant difference compared to 30-minute reperfusion interval.

decreased compared to the control group (P<0.05 for both). In comparison to the control group at different intervals, the 30-minute ischemia interval and the 30-minute reperfusion interval significantly increased compared to the baseline interval (P<0.001 and P<0.01, respectively) (Table 2).

R wave amplitude (μv)

R wave amplitude was not significantly different at baseline in different groups and at the 60-minute reperfusion interval in the control group and the VT0.2 IP group (0.2 mg/kg), but treatment with *V. officinalis* extract at 0.1 mg/kg (VT0.1) reduced R wave amplitude compared to 0.2 mg/kg (VT0.2) at the 30-minute ischemia interval (P<0.01). The comparison of the intervals showed that the R wave amplitude at 0.05 mg/kg (VT0.05), 0.1 mg/kg (VT0.1) and 0.2 mg/kg (VT0.2) significantly decreased at the 60-minute reperfusion interval compared to the baseline interval (P<0.05, P<0.01 and P<0.05, respectively) (Table 3).

T wave amplitude (μv)

T wave amplitude in different groups was not significantly different at baseline, 30-minute ischemia interval and 60-minute reperfusion interval, but significantly decreased at the 60-minute reperfusion interval after intraperitoneal injection of 0.2 mg/kg (VT0.2 IP) compared to the baseline interval and at the corresponding dose (P<0.05, Table 4).

ST-segment amplitude (μv)

ST-segment amplitude in different groups was not significantly different at baseline. Pretreatment with *V. officinalis* extract reduced ST segment amplitude at the 30-minute ischemia interval at 0.1 mg/kg (VT0.1) and after intraperitoneal injection of 0.2 mg/kg (VT0.2 IP) compared to the control group. In addition, ST-segment amplitude significantly decreased at the 60-minute reperfusion interval at 0.05 mg/kg (VT0.05), 0.1 mg/kg (VT0.1) and after intraperitoneal injection of 0.2 mg/kg (VT0.2 IP) compared to the control group (P<0.05, P<0.01 and P<0.05, respectively). Comparison between different intervals in the control group showed that the 30-minute ischemia interval significantly decreased compared to the baseline interval (P<0.05). Moreover, the extract at 0.2 mg/kg by gavage (VT0.2) significantly increased at the

Table 2. QTc alterations in the last five days of reperfusion

Groups	Baseline	End of ischemia 30'	End of reperfusion 60'
Control	0.113±0.003	0.197±0.007###	0.184±0.019##
VT 0.05	0.123±0.013	0.152±0.011*	0.119±0.010*
VT 0.1	0.121±0.08	0.138±0.015**	0.124±0.012*
VT 0.2	0.130±0.010	0.158±0.009	0.138±0.009
VT 0.2 IP	0.125±0.013	0.159±0.009	0.129±0.011

Results are expressed as Mean±SD. VT0.05, valepotriate extract at 0.05 mg/kg; VT0.1, valepotriate extract at 0.1 mg/kg; VT0.2, valepotriate extract at 0.2 mg/kg; VT0.2 IP, intraperitoneal injection of valepotriate extract at 0.2 mg/kg. *P<0.05 and **P<0.01, significant difference compared to control group at the corresponding interval; ##P<0.01 and ###P<0.001, significant difference compared to the baseline interval.

Table 3. R wave amplitude on the last five days of reperfusion

Groups	Baseline	End of ischemia 30'	End of reperfusion 60'
Control	0.519±0.05	0.477±0.066	0.338±0.093
VT 0.05	0.509±0.09	0.511±0.034	0.337±0.079#
VT 0.1	0.516±0.041	0.335±0.026**	0.264±0.053##
VT 0.2	0.634±0.030	0.581±0.051	0.450±0.084#
VT 0.2 IP	0.519±0.041	0.513±0.018	0.482±0.041

The results are expressed as mean±SD. VT 0.05, valepotriate extract at 0.05 mg/kg; VT0.1, valepotriate extract at 0.1 mg/kg; VT0.2, valepotriate extract at 0.2 mg/kg; VT0.2 IP, intraperitoneal injection of 0.2 mg/kg valepotriate extract. **P<0.01, significant difference compared to the control group at the corresponding interval; #P<0.05 and ##P<0.01, significant difference compared to the baseline interval.

Table 4. T wave amplitude on the last five days of reperfusion

Groups	Baseline	End of ischemia 30'	End of reperfusion 60'
Control	0.0907±0.0226	0.1748±0.0709	0.0734±0.0294
VT 0.05	0.1103±0.0175	0.1716±0.0236	0.0704±0.0387
VT 0.1	0.1407±0.0224	0.1484±0.0266	0.0531±0.0309
VT 0.2	0.1529±0.0094	0.1713±0.0220	0.1153±0.0191
VT 0.2 IP	0.1364±0.0084	0.1184±0.0747	0.0469±0.0160#

The results are expressed as Mean±SD. VT0.05, valepotriate extract at 0.05 mg/kg; VT0.1, valepotriate extract at 0.1 mg/kg; VT 0.2, valepotriate extract at 0.2 mg/kg, VT0.2 IP; intraperitoneal injection of 0.2 mg/kg valepotriate extract. **P<0.01, significant difference compared to the control group at the corresponding interval. #P<0.05, significant difference compared to the control group and at the corresponding dose.

30-minute ischemia interval compared to the baseline and 60-minute reperfusion intervals (P<0.05 for both, Table 5).

Electrocardiography parameters

Left ventricular end-diastolic volume (LVS) at 0.2 mg/kg (VT0.2) significantly increased compared to the control group (P<0.05), but for other parameters, no significant difference was observed between the treatment groups and the control groups (Table 6).

DISCUSSION

This study showed that dichloromethane *V. officinalis* (valproate) extract has a protective effect against ischemia-reperfusion, which was confirmed by the reduction of infarct size and the number of VTs and VEBs in comparison with the control group. The extract also caused a decrease in ST segment amplitude, QTc interval and the number of HR in the affected heart, an increase in the serum levels of antioxidant enzymes GPx, CAT and SOD, and a decrease in biochemical markers include cTnI, MDA and LDH on day 5 after the onset of perfusion.

For over two decades, the role of oxygen species in heart disease has been studied and findings revealed that there is a balance between the forms of oxygen radicals and antioxidants amount in normal conditions. In some pathophysiological conditions and subsequent reperfusion, disconnection and loss of balance may occur, but the products of free radicals are reduced in the presence of antioxidants (15). ROS play a pivotal role in creating ischemia-reperfusion-induced injuries (16). ROS cause a number of disorders including cardiomyocyte hypertrophy, apoptosis, fibrosis and decreased capillary density, arrhythmia, systolic and diastolic dysfunction, and decreased ventricular contractile strength in the myocardium *in vitro* and *in vivo* (4). According to the previous studies, ROS have a substantially destructive effect on myocardial contractility, which can be prevented during ischemia-reperfusion by antioxidants pretreatment (17,18). Beneficial effects of long-term use of antioxidants in preventing heart failure after reperfusion by inhibiting apoptosis have already been reported (19).

Numerous studies have been performed in different laboratories that show the positive effect of CAT and SOD in ischemia-reperfusion models (20,21). The antioxidant effects of valerian have been confirmed in previous studies (22,23). It has been observed that valerian extract can activate and induce the antioxidant enzymes SOD and GPx (24). Wang *et al.* in 2017 has shown that valepotriates in dichloromethane *V. officinalis* extract reduce the amount of ROS and have strong antioxidant effects (25). Also, it has been shown that valepotriates can exert antioxidant properties via the GABA signaling pathway (26,27). Based on research evidence, valepotriates reduce enzymes and biomarkers such as xanthine oxidase, LDH, MDA and tumor necrosis factor (24). It seems that the protective effect of *V. officinalis* extract in previous studies might

Table 5. ST-segment amplitude on the last five days of reperfusion

Groups	Baseline	End of ischemia 30’	End of reperfusion 60’
Control	0.074±0.032	0.182±0.004##	0.148±0.013
VT 0.05	0.067±0.020	0.099±0.030	0.037±0.036*
VT 0.1	0.066±0.021	0.051±0.015*	0.001±0.024**
VT 0.2	0.061±0.009	0.136±0.027#$	0.063±0.01
VT 0.2 IP	0.021±0.02	0.067±0.048*	0.037±0.033*

Results are expressed as mean±SD. VT0.05, valepotriate extract at 0.05 mg/kg; VT0.1, valepotriate extract at 0.1 mg/kg; VT0.2, valepotriate extract at 0.2 mg/kg, VT0.2 IP; intraperitoneal injection of 0.2 mg/kg valepotriate extract. *P<0.05 and **P<0.01, significant difference compared to the control group at the corresponding interval; #P<0.05 and ##P<0.01, significant difference compared to baseline; $P<0.05, significant difference compared to 60- minute reperfusion interval.

Table 6. Electrocardiography parameters alterations

Group	Sham	Control	VT 0.05	VT 0.1	VT 0.2	VT 0.2 IP
LVS(mm)	2.10±0.287	1.60±0.059	2.123±0.244	2.193±0.105	2.204±0.032*	1.645±0.142
LVD(mm)	4.150±0.285	3.682±0.182	4.162±0.349	3.850±0.057	4.100±0.204	4.040±0.063
SH.TIME(ms)	0.119±0.006	0.373±0.131	0.127±0.013	0.162±0.010	0.127±0.005	0.358±0.235
SEPD(mm)	1.170±0.084	1.029±0.116	1.483±0.144	1.269±0.144	1.299±0.069	1.160±0.103
FS	71.895±7.079	79.813±2.457	75.809±4.662	66.570±5.975	69.725±3.360	82.795±3.096
EF	48.625±6.539	55.625±2.874	51.823±4.951	42.915±5.301	45.440±2.907	59.183±3.693*

Results are expressed as mean± SD. LVS, left ventricular end-systolic volume; LVD, left ventricular end-systolic volume; SH.time, left ventricule filling up and emptying time; SEPD, the thickness of the wall between the two ventricles (calculated by the formula $\mathbf{FS} = \left(\frac{\mathbf{LVD^2 - LVS^2}}{\mathbf{LVD^2}}\right) \times \mathbf{100}$**FS** $= \left(\frac{\mathbf{LVD^2 - LVS^2}}{\mathbf{LVD^2}}\right) \times \mathbf{100}$); FS, fractional shortening; EF, ejection fraction (calculated by the formula $\mathbf{EF} = \left(\frac{\mathbf{LVD - LVS}}{\mathbf{LVD}}\right) \times \mathbf{100}$)**EF** $= \left(\frac{\mathbf{LVD - LVS}}{\mathbf{LVD}}\right) \times \mathbf{100}$) *P<0.05, significant difference compared to the control group.

be related to antioxidant content of this plant. It has been observed that valepotriates in *V. officinalis* extract have antiarrhythmic properties (28-30).

Previous studies have also shown the vasodilation and vasorelaxation effects of valepotriates (24,28,31,32). In our study, the number of VEBs at 0.1 mg/kg and 0.2 mg/kg extract and the number of VTs at 0.2 mg/kg extract decreased compared to the control group, which confirms the antiarrhythmic effects of valepotriates at the 30-minutes ischemia interval. Valepotriates have positive inotropic and negative chronotropic effects (12). Here, HR at the 30-minute ischemia interval significantly increased compared to baseline, but in comparison with the 30-minute ischemia interval, a significant decrease was observed at 0.1 mg/kg extract compared to the control group, which could confirm the negative chronotropic properties of valepotriate.

Potassium channels play an important role in stimulating the heart and usually serve as a target in determining the effect of antiarrhythmic compounds, and prevent QTc from rising, which results in arrhythmia and death (33,34). Therefore, development of antiarrhythmic therapeutics for reducing QTc has been one of the main goals of many studies. In the present study, a decrease in QTc interval was observed at 0.1 mg/kg and 0.2 mg/kg extract at the 30-minute ischemia and 30-minute reperfusion intervals.

R wave amplitude is one of the electrocardiography parameters of myocardial contractility (35). ST segment and T wave amplitudes are other important markers of ischemia. T-wave is one of the electrocardiography parameters and indicates ventricular repolarization. Decreased T-wave amplitude helps to diagnose heart disease (36). In the current study, ST segment amplitude at 0.1 mg/kg extract and after intraperitoneal injection of 0.2 mg/kg extract significantly decreased compared to the control group at the 30-minute ischemia interval, but the amplitude of R wave and T-wave did not significantly increase in different groups. Therefore, more extensive studies should be done to determine the effects of valepotriate on the electrical activity of the heart.

In our study, LVS significantly increased compared to the control group, but other electrocardiography parameters did not change significantly. The level of ROS increases after the occurrence of ischemia-reperfusion, while ROS decreases if flavonoids are pretreated before ischemia-reperfusion occurs (37). Furthermore, the level of SOD increases after pretreatment compared to before pretreatment and the level of MDA decreases.

In conclusion, given our results and previous studies, it can be concluded that dichloromethane extract of *V. officinalis* rhizome, which mainly contains valepotriates, exerted favorable impacts on antioxidant enzymes (MDA, GPx, SOD and CAT), biochemical factors, infarct size and electrocardiography parameters.

Taken together, valepotriate extract mitigated injuries due to before induction of ischemia-reperfusion. Our study, as with research, showed the strong antioxidant activity of the extract and the role of alkaloids such as valepotriate. Therefore, valerian with strong antioxidant properties may play an important role in preventing cardiac injuries such as arrhythmia and infarct size, and also in activating antioxidant enzymes in heart tissue.

Conflict of interest

The authors declare that they have no conflict of interest.

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