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The Evaluation of Inotropic Properties and Antidysrhythmic Effect of Vanillic Acid and Exercise on $Cacl_2$ -Induced Arrhythmia in Young and Aged Rats.

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ABSTRACT

Aging population is rapidly growing in the world. In the elderly people we are confronting with increased frequency of cardiovascular diseases. Due to protective role of antioxidant agents in cardiovascular disease, therefore, many of them are used as medicinal plants in traditional medicine. Vanillic acid (VA), as an antioxidant agent, play an important protective role on some diseases. The beneficial effect of exercise on the cardiovascular system is well documented. The aim was the investigation of inotropic properties and antidysrhythmic effect of VA and exercise in young and aged rats. In this study 64 male Sprague-dawley rats were divided into 8 groups. Young and aged: control, VA (10 mg/kg, gavage, 10 days), exercise (21-days) and exercise+VA. The arrhythmia was induced by i.v injection of $CaCl_2$ (140 mg/kg), and percentage of Ventricular premature beats (VPB), Ventricular fibrillation (VF) and Ventricular tachycardia (VT) were recorded. ANOVA and Fisher`s exact test were used for statistical analysis and $P<0.05$ was considered as significant level. VA and exercise displayed positive inotropic properties and antidysrhythmic effects on $CaCl_2$-induced arrhythmia in both groups in compare with controls, with the highest activity in young groups. Results suggest a protective role of VA and exercise against heart disease.

Keywords: Aging, Vanillic acid, Exercise, Arrhythmia, Inotropic effect, Rat.

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INTRODUCTION

Increasing aging population is a global phenomenon that has become a crisis [1]. The aging is gradual biological phenomena in the life that associated with Biochemical and morphological changes and lead to destructive impacts on organs like the heart [2]. Devastating diseases that occur with aging, includes brain disorders, reducing the activity of the immune system, cancer, cataracts and cardiovascular disorders [3, 4, 5]. Oxidative damage to DNA, proteins and other macromolecules is considered as a major factor in aging [6]. Superoxide, hydrogen peroxide and hydroxyl radicals cause oxidative damage in old age [7].

Heart failure is a common disorder in elderly [5]. Patient with heart failure leading to arrhythmia and cardiac arrhythmias are one of the most common causes of death [8]. Cardiac arrhythmias or dysrhythmia are disturbances in the normal rhythm of the heart beat. Arrhythmias resulted from irregularly at generation of impulse, conduction of impulse in heart or combination of both those [9]. The most common causes of cardiac arrhythmias are congenital heart diseases, myocardial ischemia, electrolyte imbalances, cardiac valvular diseases, metabolic disturbances, acidosis or alkalosis and drugs toxicity [10]. The different types of ventricular arrhythmias including premature ventricular beat (PVB), ventricular fibrillation (VF), and ventricular tachycardia (VT) is the most important causes of mortality rate at industrial communities. VT and VF are two causes of sudden cardiac death (SCD) [11]. VT is organized arrhythmias and manifested with further than 3- 4 beats/min PVB but VF is arrhythmias life-threatening and characterized by undistinguished rhythm [12].

Chemical drugs in according of the Vaughan-Williams are classified into four classes. Sodium channel blockers, calcium channel blockers, beta receptor blockers and potassium channel blockers [13].

Traditional medicines or herbal medicines which have preventive and therapeutic effects in multiple diseases are specially important and valuable. Herbal remedies with respect to the their availability, low cost and side effects have been considered by some researchers as an alternative to chemical drugs [14]. There are many compounds present in the extracts of vanilla. Vanillin is most prominent as the principal flavor and aroma compound in vanilla. Vanillin, a compound widely used in foods, drugs beverages and cosmetics has such multiple function as anti-metastatic [15], anti-melanogenesis [16], and anti-angiogenetic effects [17]. Vanillic acid (VA), a benzoic acid derivative used as an important flavor and aroma agent, is an oxidized form of vanillin [18]. The highest quantity of vanillic acid in plants has been found in the roots of *Angelica sinensis* [19]. Systematical evaluation of the antioxidative properties of vanillic acid, vanillin and ethyl vanillin by multiple assays has provided the evidence that support the superiority of radical-scavenging and antioxidative activity of vanillic acid [20]. Vanillic acid has been shown to have significant protective effects on lipid peroxidation, cardiac troponins, antioxidant system, electrocardiogram, expressions of tumor necrosis factor-alpha gene, interleukin-6 and interleukin-1beta, and also biochemical parameters in the heart of isoproterenol induced cardiotoxic rats [21, 22].

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Forced treadmill running is a common method of training used by researchers investigating physiological adaptations produced by exercise [23]. Beneficial effects of exercise on the cardiovascular system are well known. There is a direct relation between physical inactivity and cardiovascular-associated mortality, and physical inactivity is an independent risk factor for the development of coronary artery disease (CAD). Most beneficial effects of physical activity on cardiovascular disease mortality can be attained through moderate activity. Some studies have shown that regular exercise reduces mortality from cardiovascular disease [24]; improve muscle function [25] and delayed cell damage and physiological abnormalities in aging [26].

Due to the growing elderly population in the world, the high rate of heart disorder in this course, the unknown effects of vanillic acid on ventricular arrhythmias induced by $CaCl_2$ and the beneficial effect of exercise on the cardiovascular system, we decided to evaluate the possible effects of vanillic acid and exercise in the prevention of $CaCl_2$-induced ventricular arrhythmias in young and aged rats.

MATERIALS AND METHODS

Chemicals

Vanillic acid (Sigma), $CaCl_2$ 2.5% solution (Merk Co. Darmstadt, Germany), normal saline (N/S), ketamine hydrochloride and xylazine (Alfasan Co, Woderen- Holland).

Animals

In this study Male Sprague-Dawley rats (aged: 18-22 months old / young: 4-6 months old) were purchased from Ahvaz Jundishapur University of Medical Sciences animal lab. Animals used in this study were treated in accordance with principals and guidelines on animals care of AJUMS and were kept at 20-24ºC under 12 hr light/dark cycle and were allowed free access to tap water and commercial chow. The investigation was approved by the Animal Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (No. ajums. REC.1392.279).

Procedures

Animals were divided into two (young and aged) groups. Each groups randomly divided into four subgroups [control, vanillic acid (VA), exercise (EX), vanillic acid + exercise (VA+EX)].

The 21- days exercise training protocol consisted of running on a motorized treadmill (1 h at speed 17 m/min, six times per week). Very few shocks were administered during a training session and occurred within the first week of training.

The sedentary young and aged rats in control groups were handled identically to the treadmill-trained rats. At the same time of day, the sedentary rats were placed on a stationary treadmill, with the shock grid turned off, six days per week for the duration of the treadmill training session [27] and received normal saline (1 ml), by gavage, once a day for last 10 consecutive days.

In VA groups, the young and aged rats were placed on a stationary treadmill, with the shock grid turned off, six days per week for the duration of the treadmill training session and received vanillic acid (10 mg/kg), by gavage, once a day for last 10 consecutive days.
In VA+EX groups, the young and aged rats were trained to run on a motor-driven treadmill (21-days) and received vanillic acid 10 mg/kg, by gavages, once a day, during the last 10 days.

In Stanely Mainzen Prince Study the effective dose of vanillic acid was (10 mg/kg), therefore we decided to use one dose (10 mg/kg) in this research [22].

QRS complex recording

The animals were operated under anesthesia with combination of xylazine (10 mg/kg) and ketamine (50 mg/kg) via intraperitoneal (ip) route [28]. Lead II electrocardiogram (ECG) was recorded by Bio Amp and monitored by a Power Lab system (ADInstruments, Australia). QRS complex (as an inotropic property) was calculated from ECG recording in first day and 21 days after performance.

The manner of induced and recording of arrhythmias

After anesthesia, ECG was recorded in all groups for 15 min, before the induction of chemical- arrhythmia to allow hemodynamic equilibration. prep & drep with alcohol were done. Then, an incision was created in area of groin; a poly ethylene catheter was inserted in femoral vein [28]. In this study, arrhythmia was induced by intravenously injection of $CaCl_2$ (140 mg/kg), and Percentage incidence of Premature ventricular beats (PVB), Ventricular tachycardia (VT) and Ventricular fibrillation (VF) were calculated after injection of $CaCl_2$ [29].

STATISTICAL METHODS

Results were analyzed using SPSS and expressed as Mean ± SEM. Comparisons among groups were performed using paired t-test, FISHER's exact test and two- way ANOVA. $P< 0.05$ was considered significant statistically.

RESULTS

Effect of exercise on inotropic property of heart in aged and young rats

The obtained results in this study showed that, in both young ($P<0.001$, Figure-1) and aged exercise groups ($P<0.05$, Figure-2), inotropic effect significantly were increased compared to control groups. Increase in cardiac contractility in young group was shown that to be more than aged group ($P<0.01$, Figure-3).

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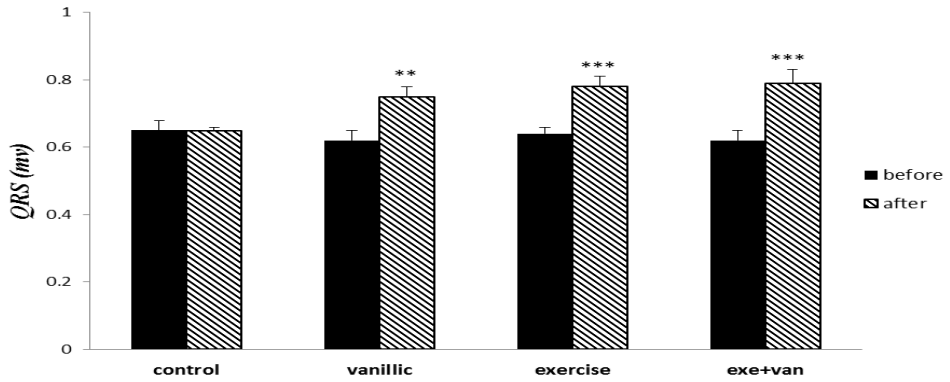

Figure 1: Effect of vanillic acid, exercise and vanillic acid+exercise on voltage of QRS complex in young rats. Results are expressed as Mean ± SEM of 8 rats per group, t-test was used; **P<0.01, ***P<0.001 were compared in each group.

Effect of vanillic acid on inotropic properties of heart in aged and young rats

The obtained results in this study showed that, in both young (P<0.01, Figure-1) and aged (P<0.05, Figure-2) groups which received VA, inotropic effect significantly were increased compared to control groups. Increase in cardiac contractility in young group was shown that to be more than aged rats (P<0.05, Figure-3).

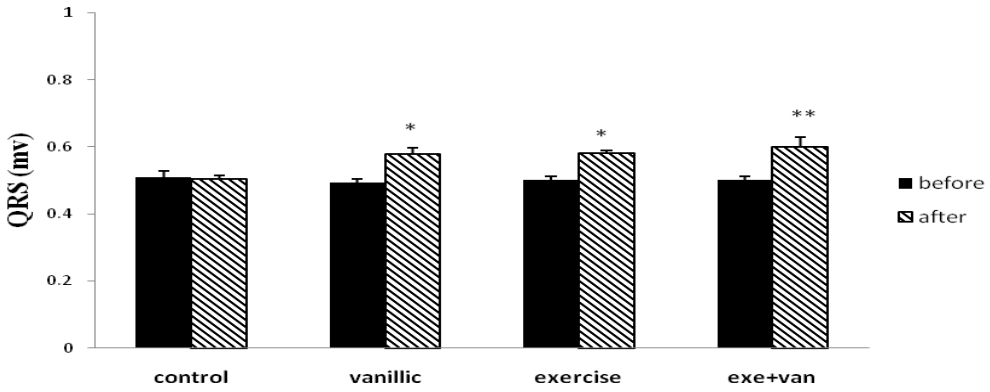

Figure 2: Effect of vanillic acid, exercise and vanillic acid+exercise on voltage of QRS complex in aged rats. Results are expressed as Mean ± SEM of 8 rats per group, t-test was used; *P<0.05, **P<0.01 were compared in each group.

Effect of exercise and vanillic acid on inotropic properties of heart in aged and young rats

The obtained results in this study showed that, in both young (P<0.001, Figure-1) and aged (P<0.01, Figure-2) exercise+vanillic acid groups, inotropic effect significantly were increased compared to control group. Increase in cardiac contractility in young rats was shown that to be more than aged group (P<0.01, Figure-3).

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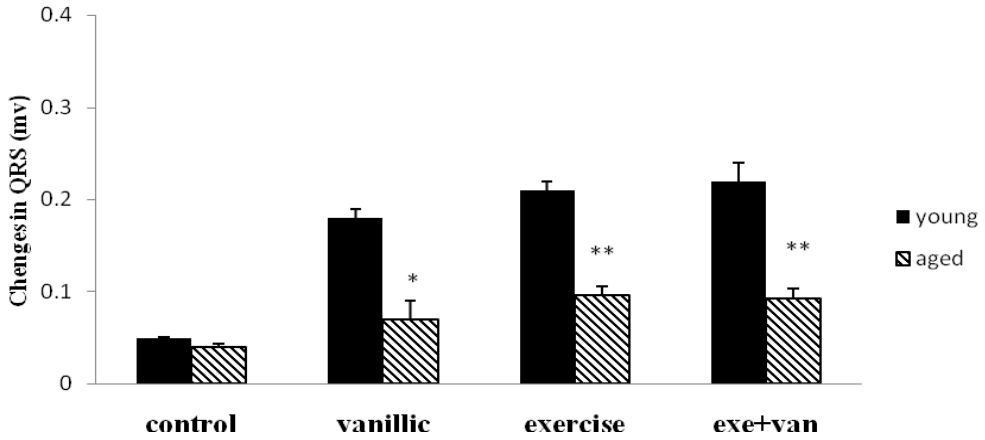

Figure 3: Comparison of the changes in voltage of QRS complex in young and aged groups (control, vanillic acid, exercise and vanillic acid+exercise)**.** Results are expressed as Mean ± SEM of 8 rats per group, ANOVA was used, followed by LSD test; *P<0.05, **P<0.01 were compared between young and aged rats in each group.

Effect of exercise on $CaCl_2$ -induced arrhythmia in aged and young rats

Evaluation of effects of 21-days exercise showed that in both young (Figure-4 A) and aged (Figure-4 B) groups incidence of premature ventricular beat (PVB), ventricular tachycardia (VT) and Ventricular fibrillation (VF) significantly were reduced compared to control groups. Comparison of the reduction effects on arrhythmias in young and aged rats was shown that to be more effective in young rats (Figure-5).

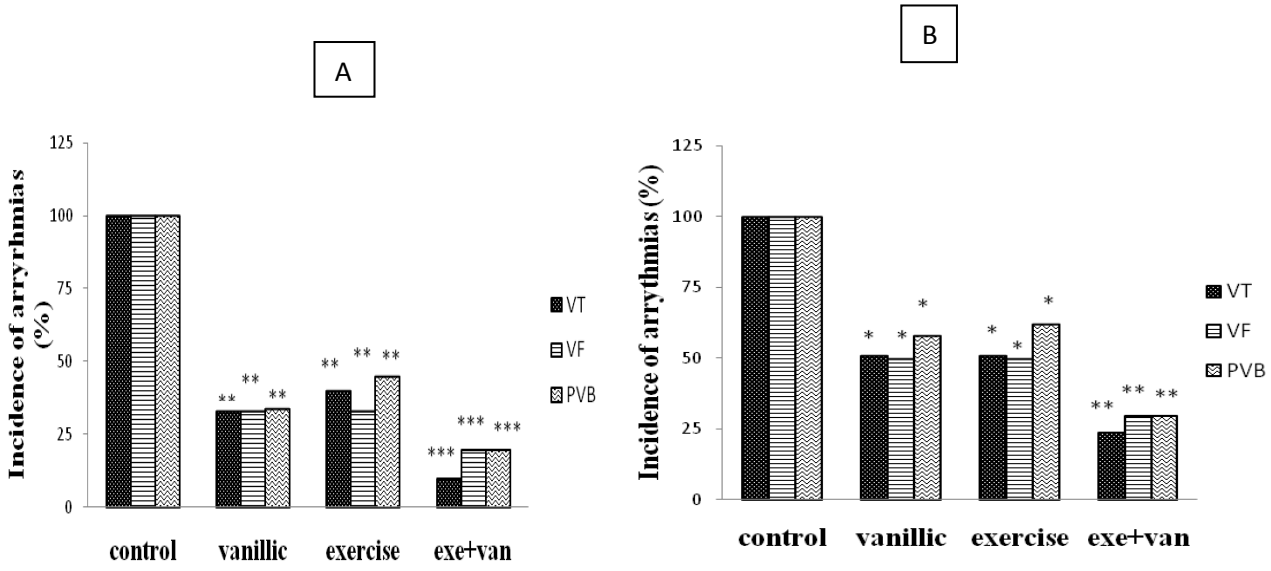

Figure 4: Effect of vanillic acid, exercise and vanillic acid+exercise on $CaCl_2$-induced arrhythmias. The data from the control group was considered as 100 % and the results were compared to those of control group and are expressed as a percentage data. (A: young, B: aged, VT: Ventricular tachycardia, VF: Ventricular fibrillation; PVB: Premature ventricular beats); FISHER's exact test was used; *P<0.05, **P<0.01, ***P<0.001 vs control group).

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Effect of vanillic acid on $CaCl_2$ -induced arrhythmia in aged and young rats

Evaluation of antiarrhythmic effects of chronic doses of vanillic acid (10 mg/kg) showed that incidence of Premature ventricular beat, Ventricular tachycardia and Ventricular fibrillation significantly were reduced in both young (Figure-4 A) and aged (Figure-4 B) groups compared to control groups. Comparison of the reduction effects on arrhythmias in young and aged rats was shown that to be more effective in young rats (Figure-5).

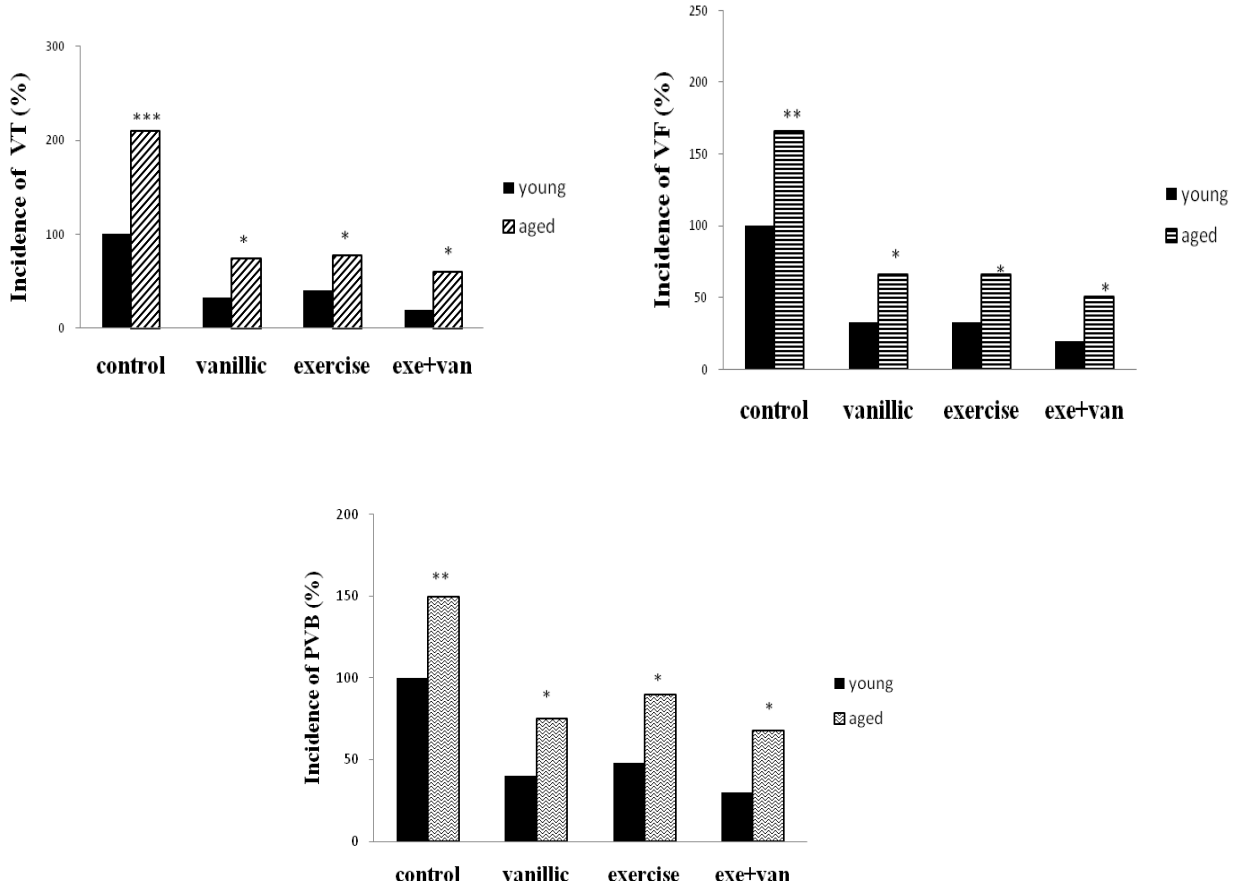

Figure 5: Effect of vanillic acid, exercise and vanillic acid+exercise on $CaCl_2$-induced arrhythmia. The data from the young control group was considered as 100 % and the results were compared to those of other groups and are expressed as a percentage data. (VT: Ventricular tachycardia, VF: Ventricular fibrillation; VPB: Premature ventricular beats); FISHER's exact test was used; *P<0.05, **P<0.01, ***P<0.001 were compared between young and aged rats in each group.

Effect of exercise and vanillic acid on $CaCl_2$ -induced arrhythmia in aged and young rats

Evaluation of effects of 21-days exercise+vanillic acid (10 mg/kg) showed that incidence of Ventricular premature beat, Ventricular tachycardia and Ventricular fibrillation significantly were reduced in both young (Figure-4 A) and aged (Figure-4 B) groups compared to control groups. Comparison of the reduction effects on arrhythmias in young and aged rats was shown that to be more effective in young rats (Figure-5).

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DISCUSSION

The results presented in this study demonstrate antidysrhythmic effects of vanillic acid on $CaCl_2$-induced arrhythmia by decreased VF, PVB and VT in aged and young rats. In according to previous investigations, vanillic acid exerts protective effects in isoproterenol induced cardiotoxic rats due to its free radical scavenging, antioxidant and anti-inflammatory properties [22]. Considerably, previous study was shown that vanillic acid increased activation of non- enzymatic materials such as glutathione which act as a potent agent to scavenging reactive oxygen species (ROS), hydroxyl radicals and superoxide anions [20], these effects may be related to phenolic traits of vanillic acid [30] that eliminated harmful materials such as free radicals and no permission that ROS release. During arrhythmia free radicals cause damage to the sarcoplasmic membrane and increased intracellular calcium, so caused to early and delay after depolarization [31]. Previous study was shown that vanillic acid increased activation of Superoxide dismutase, glutathione peroxidase and catalase and as a scavenger, eliminated free radicals and therefore, vanillic acid exerts protective effects against destructive role of free radicals [22] that these results are agreement with our finding in vanillic acid 10 mg/kg. Considerably, previous study was shown that the amount of free radicals increases with aging [32]. Therefore, we conclude that older subjects have substantially more intrinsic oxidative damage to myocardial than younger subjects. Probably for this reason pretreatment with vanillic acid (10 mg/kg) showed a weaker protective effect in aged rats than young group. This demonstrates the need for application of antioxidants throughout the human lifetime. In the present study, pretreatment with this dose of VA demonstrate preventive effects on cardiac dysrhythmia and suggests that vanillic acid has effects similar to other antioxidants.

In the normal heart, an imbalance between myocardial oxygen supply and demand during exercise results in the net breakdown of Adenosine Triphosphate and release of Adenosine can increase up to 50-fold [33]. Adenosine also has an important role as an endogenous determinant of ischemic tolerance by modulating several components of cardiac function. Adenosine has potent cardiac electrophysiological effects, including a negative chronotropic action on the sinus node and a predominant negative dromotropic action on the atrioventricular (AV) node, therefore Adenosine is used as an antiarrhythmic agent for the management of ventricular tachycardia and other arrhythmias mediated by reentrant mechanisms involving the AV node. Adenosine also inhibits the electrophysiological effects of increased intracellular cAMP, which occur with sympathetic stimulation. Due to adenosine reduces calcium currents; it can thereby be antidysrhythmic by inhibiting delayed after depolarization elicited by sympathetic stimulation [33]. Thus, an exercise-induced endogenous release of adenosine may provide protection from $CaCl_2$-induced arrhythmia. Also, Adenosine A1 receptor function in rat ventricles has previously been shown to decrease with age [34]. Probably due to this reason protective effect of exercise on the prevention of arrhythmias in aged rats is lower than young rats.

In this study, vanillic acid was shown positive inotropic effect by increased QRS complex amplitude in young and aged groups. Vanillic acid as antioxidant agent probably eliminate factors that reduced the amplitude of QRS complex. Also previous studies were shown that cardiovascular effects of flavonoids and phenolic compound are not caused only by direct antioxidant activity [35]. Vanillic acid is a phenolic compound [30], in Tadano Study

on therapeutic potential of phenolic compound, there have been reported to have cardiotonic activity due to increase myofilament calcium sensitivity [36], that cause to increase contractility of heart. These results are agreement with our finding.

In this study, 21-days exercise was shown positive inotropic effect by increased QRS complex amplitude in young and aged groups. Cardiac output increases or decreases in response to changes in heart rate or stroke volume. Limb movement (muscle pump) during exercise enhances venous return to the heart. Increased venous return, increases the ventricular filling (end-diastolic volume) and therefore preload, which is the initial stretching of the Left ventricular myocytes prior to contraction. As a myocyte is stretched (as occurs with increased ventricular preload), the sarcomeres within the myofibrils are also stretched. With increased sarcomere length, there is an increase in the force of contraction. Changes in sarcomere length are an important mechanism by which the heart regulates its force of contraction. This mechanism enables the heart to eject the additional venous return, therewith increasing stroke volume. This phenomenon is described in mechanical terms by the length-tension and force-velocity relationships for cardiac muscle. Increasing end diastolic volume increases the active tension developed by the muscle fiber and increases the velocity of fiber shortening at a given afterload and inotropic state [37]. The most common mechanism to explain how preload influences contractile force is that increasing the sarcomere length increases troponin C (which serves as a binding site for Ca^{++} during excitation-contraction coupling) calcium sensitivity, which increases the rate of cross-bridge attachment and detachment, and the amount of tension developed by the muscle fiber [38]. Thus, probably, increasing venous return during exercise increasing the cardiac pumping and enhances inotropic properties of heart. Moreover, in senescent, marked shifts occur in the cardiac myosin heavy chain (MHC) isoforms in heart, with the β-isoform becoming predominant; myosin Ca^{2+}-ATPase activity declines with the decline in α-MHC content, thus the heart muscle becomes less efficient with age. Perhaps for this reason the contractile strength of the heart is less affected by vanillic acid or exercise in the aged group [39].

In this study, exercise + vanillic acid was shown antidysrrhythmic effects on $CaCl_2$-induced arrhythmia and positive inotropic effect by increased QRS complex amplitude in young and aged groups. Since the effects of exercise and vanillic acid have acted in a way, probably the combined effect of exercise and vanillic acid produces an additive effect. whereas exercise and vanillic acid alone had less effect in aged than young rats, thus additive effect of these agents had less effect on aged rats too.

CONCLUSION

In conclusion, on the basis of $CaCl_2$-induced arrhythmia studies, the present results approve our hypothesis that exercise and vanillic acid pretreatment improved the dysrhythmia in aged and young rats. Also, positive inotropic properties of exercise and vanillic acid can have important implications for future development of therapeutic strategies in the management of heart failure. Herbal remedies with respect to low side effects, as an alternative to chemical drugs have been considered by this study.

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