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Antidysrhythmic, Inotropic and Chronotropic Effects of Ellagic Acid and Forced Exercise in Rat.

Mahin Dianat[1*], Negin Amini[2], Mohammad Badavi[1], and Yaghoub Farbood[1]

[1]Faculty member, Physiology Research Center and Department of Physiology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
[2]Ph.D. Student, Department of Physiology, Physiology Research Center, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

ABSTRACT

Mortality and morbidity sudden cardiac death is one of the main causes in dustrial countries. Medicinal plants, due to their low side effects, have been used as alternative to chemical drugs. Exercise is an important factor for preventing cardiovascular risk and has been associated with several cardiovascular benefits. The aim of the present study was to evaluate the cardiopective effects of Ellagic acid and exercise against $CaCl_2$ induced arrhythmia in rats. In this study, 32 male Sprague Dawley rats (200-250 gr), were used for experiments during the period of 21 days. Rats were divided into 4 groups; group I: Control (received saline for 10 days), group II: received Ellagic acid (15 mg/kg, gavage, 10 days), group III: received forced exercise (treadmill,1 hour/day, 21 days), group IV: received Ellagic acid plus forced exercise. After anesthesia, lead II electrocardiogram was recorded for calculating heart rate and QRS complex. The arrhythmia was produced by injection of $CaCl_2$ solution (140 mg/kg, iv) and percentage incidence of Ventricular premature beats (VPB), ventricular fibrillation (VF) and ventricular tachycardia (VT) were recorded. Results were analyzed by using one-way ANOVA and Fisher`s exact test. $P<0.05$ was considered as significant level. The result shows that a positive inotropic and negative chronotropic effect for Ellagic acid and exercise as compare with the control group. Oral pretreatment of Ellagic acid and exercise prevents $CaCl_2$ induced arrhythmia. This substance is suggested as an antiarrhythmic which showed a protective function of Ellagic acid in heart.

Keywords: Ellagic acid, Exercise, Arrhythmias, Chronotropic, Inotropic, Rat.

Corresponding author

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INTRODUCTION

Among mortality and morbidity, sudden cardiac death is one of the main causes in dustrial countries. What is caused by arrhythmias, could be the patients with heart problems whom are diagnosed with cardiac arrhythmias and the high rate of mortality in the world [1- 2]. Evidently free radicals are probably important mediators of ventricular tachycardia and ventricular fibrillation [3-4]. The role of oxidative stress in cardiovascular disease has been characterized [5]. ROS, involving hydrogen peroxide, superoxide anions and hydroxyl radicals provide the genesis of arrhythmias that are associated with ventricular tachycardia and ventricular fibrillation, and antioxidant therapy plays an important role in blockade of focal VT under the conditions of myocardial ischemia [6].

$CaCl_2$ induces arrhythmias through a direct and also indirect action mediated through the sympathetic nervous system [7- 8].

Much research has been done in the field of exercise and physical activity on the health benefits of exercise. Studies have shown that the risk of various diseases such as heart disease, hypertension, infection, obesity and diabetes was reduced by regular exercise [9]. Aerobic exercise avoids cardiovascular risks functional adaptations of the cardiovascular system have an important role in the aerobic performance improvement during exercise. It is associated with an improvement of heart rate, stroke volume, cardiac output, and coronary blood stream [10]. Previous study has shown that exercise leads to the production of atrial myosin light chain in ventricle myocardium can lead to an increase in their ability to do work [11]. In another study has been demonstrated that moderate exercise vasoconstrictor responses to norepinephrine by increasing the bioavailability of nitric oxide reduces [12].

In recent years, the role of polyphenols compounds is known in cardiovascular disease [13]. Ellagic acid is a polyphenol compound. It is found in vegetables, nuts and fruits such as pomegranate, black berry, grapes, apples, strawberries and kiwi [14-15]. Previous studies indicated that Ellagic acid showed free radical scavenging action and anti inflammatory effect [16-17]. Since effect of Ellagic acid on arrhythmias have not been studied and the importance of moderate exercise in heart disease protection for health is expressed, therefore forced exercise protocols, chronotropic and inotropic properties of Ellagic acid and arrhythmia induced by $CaCl_2$ in rats was designed.

MATERIAL AND METHODES

Drug and chemical

Ellagic acid and $CaCl_2$ were purchased from sigma chemical.

Experimental animals

All the experiments were carried out male Sprague Dawley rats weighing 200-250 gr, purchased from Animal reproduction center. They were housed in cages, renewed every 24h under a 12 h light/dark cycle at around 22^0 C with 50% humidity. The rats had free

access to water. The rats were fed on a standard pellet diet. The experiment was carried out according to the guidelines of the committee for the purpose of control and supervision of experiments on animals and was approved by the Animal Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (No. ajums. REC.1392.280).

Experimental protocols

The rats were divided randomly into four groups of eight rats each. Group I: normal control rats were given 2 ml of saline orally by gavage daily for a period of 10 days, Group II: normal rats were treated with Ellagic acid (15 mg/kg) in 2 ml of saline orally by gavage daily for a period of 10 days [14], Group III: normal rats received forced exercise (treadmill,1 hour/day for 21 days), Group IV: received Ellagic acid (15 mg/kg, gavage, 10 days) plus forced exercise [18-19].

Forced exercise protocol

Forced exercise was performed by treadmill. Speed and duration of exercise for two groups were kept constant at 17-18 (m/min), 60 min daily for 21 days. Slope was different during 60 min forced exercise. The slope was 0° at first 10 min, 5° at second 10 min, and during next two 20 min periods it was adjusted to 10° and 15° [18-19].

Anesthesia technique

All rats were anesthetized with a mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg) by intraperitoneal injection [20].

Electrocardiographic recording method

After anesthesia, standard bipolar limb lead II was recorded for record the electrocardiogram (ECG). Lead II ECG was recorded by Bio-Amp and monitored by a Power Lab system (AD-Instruments, Australia).

Induction of arrhythmia

At the end of the 21 days in all groups, after $CaCl_2$ injection at a dose of 140 mg/kg, incidence rate of premature ventricular beats (PVB), ventricular fibrillation (VF) and ventricular tachycardia (VT) were calculated. $CaCl_2$ (140 mg/kg) was dissolved in saline and intravenously injected to rats [20].

Assess inotropic and chronotropic effects

In all groups (before and after the 21-days period), to evaluate the effect chronotropic and inotropic, 15 min after anesthesia standard bipolar limb Lead II, to investigate the ECG was recorded. Heart lead II was recorded and heart rate (To assess chronotropic) and voltage of QRS complex (To assess inotropic) were calculated in first day and 21 days after manipulation anesthesia [21].

Statistical analysis

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For comparisons between groups were analyzed using one-way ANOVA statistical method and to compare the data in each of the groups (before and after testing) Paired t-test was used, and Fisher`s exact test to evaluate the data arrhythmias was used. The values P<0.05 were considered significant level.

RESULTS

The Rats that received Ellagic acid (15 mg/kg), showed a significant decrease in heart rate (P<0.05, Figure-1), and their QRS complex voltage significantly increased (P<0.05, Figure-2). Rats that Treadmill forced exercise were performed for 21 days (6 hour per day), their heart rate significantly decreased (P<0.05, Figure-1) and their QRS complex voltage significantly increased (P<0.05, Figure-2). The rats that received Elagic acid (15 mg/kg, gavage, 10 days) plus forced exercise showed a significant decrease in heart rate (P<0.05, Figure-1) and their QRS complex voltage significantly increased (P<0.05, Figure-2).

The Percentages of incidence of ventricular tachycardia (VT), ventricular fibrillation (VF) and ventricular premature beats (VPB) in four groups were shown in Figure 3, 4 and 5. Arrhythmias in all groups were created by injection of $CaCl_2$. The rats that received Ellagic acid (15 mg/kg), showed significant decrease in percentages of incidence of VT (P<0.05), VF (P<0.01), and VPB (P<0.001). Rats that Treadmill forced exercise were performed for 21 days (6 hour per day), showed significant decrease in percentages of incidence of ventricular tachycardia (P<0.05), ventricular fibrillation (P<0.01) and ventricular premature beats (P<0.01). Rats that treated with Ellagic acid plus Treadmill forced exercise were performed for 21 days (6 hour per day), showed significant decrease in percentages of incidence of ventricular tachycardia (P<0.01), ventricular fibrillation (P<0.001) and ventricular premature beats (P<0.01).

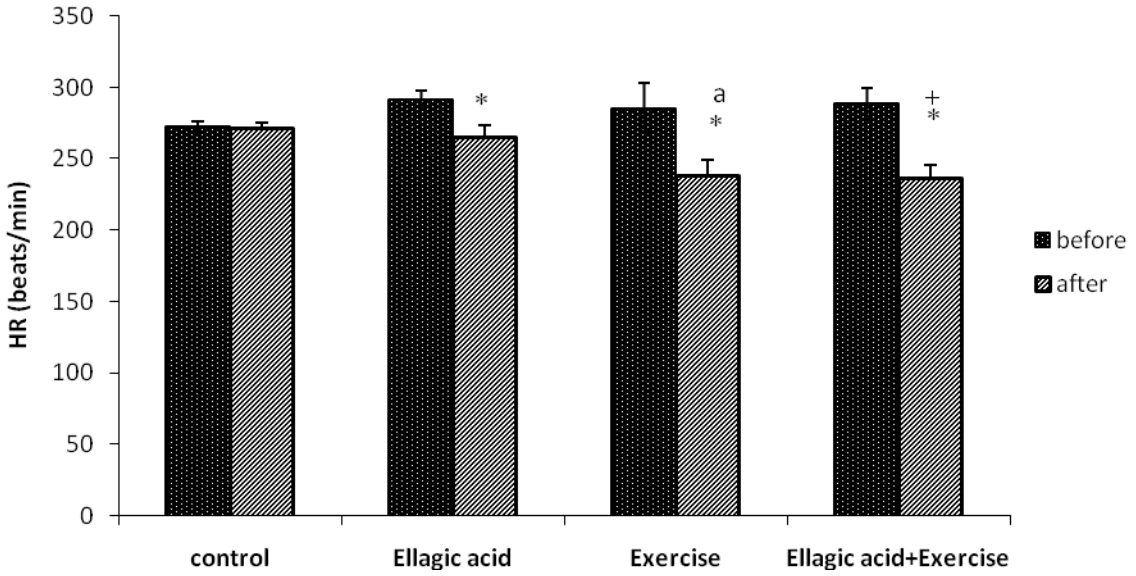

Figure1: Comparison of Heart rate in different groups [Control, Ellagic acid (15 mg/kg), Exercise (21 days), Ellagic acid (15 mg/kg)+Exercise (21 days)]. *P<0.05 were compared in each group (Paired t-test was used), +P<0.05 were compared vs. control group and Ellagic acid group, and a p<0.05 Significant differences with Ellagic acid group (n=8, Mean±SEM, One-way ANOVA followed by LSD).

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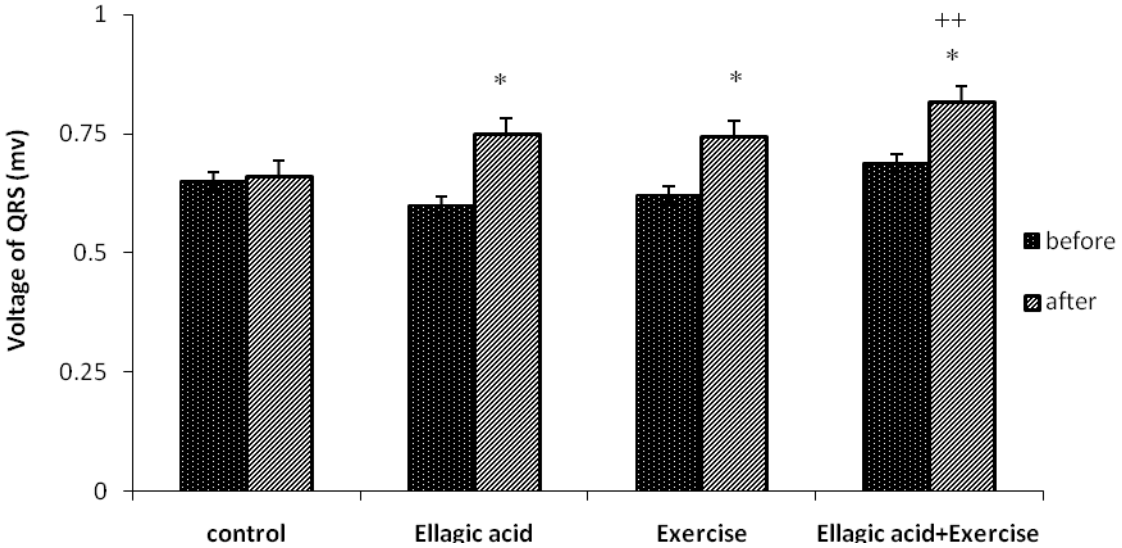

Figure 2: Comparison of Voltage QRS in different groups [Control, Ellagic acid (15 mg/kg), Exercise (21 days), Ellagic acid (15 mg/kg)+Exercise (21 days)]. *P<0.05 were compared in each group (Paired t-test was used), ++P<0.01 were compared vs. control group (n=8, Mean±SEM, One-way ANOVA followed by LSD).

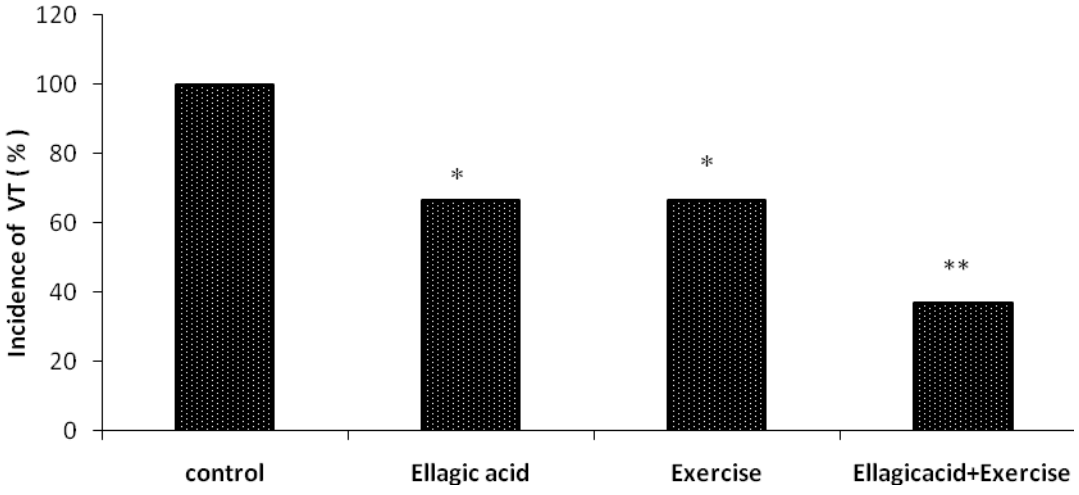

Figure 3: Evaluation of ventricular tachycardia after chemical arrhythmias inducing by intravenous $CaCl_2$ injection (140 mg/kg) in different groups [Control, Ellagic acid (15 mg/kg), Exercise (21 days), Ellagic acid (15 mg/kg) +Exercise (21 days)]. The data from the control group was considered as 100 % and the results were compared to those of control group and are expressed as a percentage data. *P<0.05, **P<0.01 were compared vs. control group (n=8, Fisher`s exact test).

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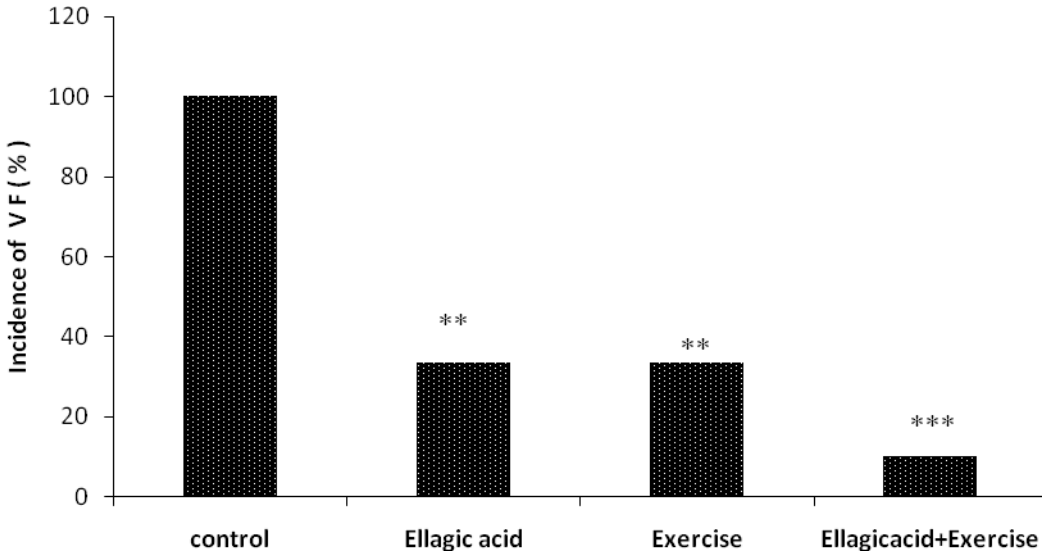

Figure 4: Evaluation of ventricular fibrillation after chemical arrhythmias inducing by intravenous $CaCl_2$ injection (140 mg/kg) in different groups [Control, Ellagic acid (15 mg/kg), Exercise (21 days), Ellagic acid (15 mg/kg) +Exercise (21 days)]. The data from the control group was considered as 100 % and the results were compared to those of control group and are expressed as a percentage data. **P<0.01, ***P<0.001 were compared vs. control group (n=8, Fisher`s exact test).

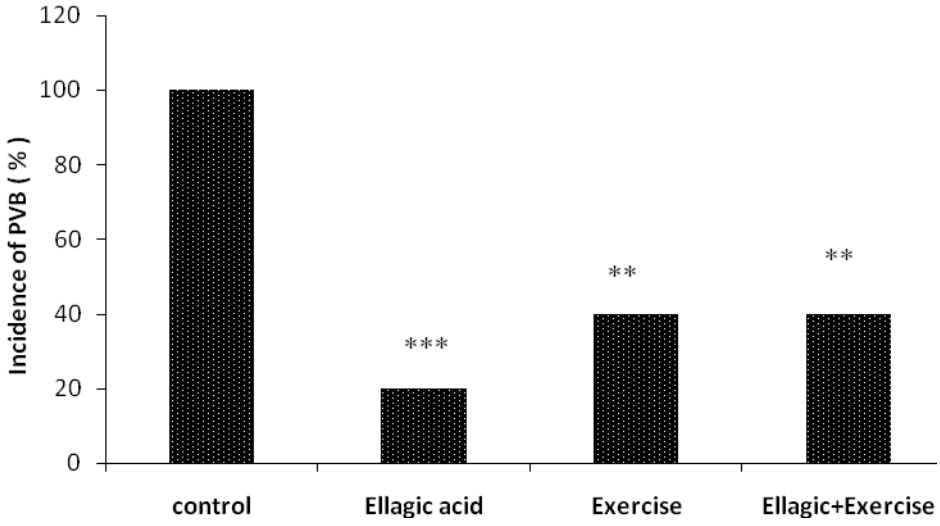

Figure 5: Evaluation of premature ventricular beats after chemical arrhythmias inducing by intravenous $CaCl_2$ injection (140 mg/kg) in different groups [Control, Ellagic acid (15 mg/kg), Exercise (21 days), Ellagic acid (15 mg/kg) +Exercise (21 days)]. The data from the control group was considered as 100 % and the results were compared to those of control group and are expressed as a percentage data. **P<0.01, ***P<0.001 were compared vs. control group (n=8, Fisher`s exact test).

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DISCUSSION

In This study, in rats that received Ellagic acid (15 mg/kg), decreased heart rate and increased QRS complex voltage were demonstrated. In a previous study, which was performed on the cell surface Ellagic acid with increasing calcium absorption in sarcoplasmic reticulum and increasing Ca-ATPase Pump activity has a positive inotropic effect [22].

Ellagic acid by its prominent antioxidant properties will delete or inhibit numbers of factors in reduction QRS complex voltage and causes positive inotropic effects in heart. In another study showed that Ellagic acid decrease myocardial infarction induced by isoproterenol which causes increase in heart rate, reduces ST segment elevation, decrease in systolic and diastolic blood pressure and exerts a protective effect on the heart [13]. Polyphone compounds reduce harmful levels of cardiac enzymes, such as Troponin I, which causes disorder in structure and function of the heart, and increase non-enzymatic activity in materials in plasma as antioxidant, like Ascorbic acid [13]. It had been suggested that Polyphenol compounds, such as Ellagic Acid, binds with beta-adrenergic receptors and they prevent from increasing heart rate which caused by beta-adrenergic receptors stimulating [23].

This study shows that treadmill forced exercise decrease heart rate and increase QRS complex. In experimental exercise model, the unchanged muscarinic receptor and decreased β adrenergic density in myocardial cell, leads to an increase in the M_2 Ach receptor/β1 adrenergic ratio, and also heart rate variations depend on the sinus node responses to the interaction of parasympathetic and sympathetic activity, exercise increase parasympathetic activity and decrease sympathetic [24]. Previous study has shown that Exercise leads to the production of atrial myosin light chain in ventricle myocardium can lead to an increase in their ability to do work [11]. Our finding is agreement with previous suggested.

In the present study, the rats treated with Ellagic acid at a dose of 15 mg/kg showed percentages of incidence of VT, VF, and VPB were significantly decreased. It had been proved that Polyphenol compounds have negative chronotropic effect and could be prolong the action potential in myocytes. In this way, arrhythmias and increasing in heart rate could be prevented [25]. Pretreatment with Ellagic acid (15mg/kg) significantly decreased the activity of HMGCoA reductase (rate limiting enzyme in cholesterol biosynthesis) in isoproterenol-induced myocardial infarcted rats, compared to isoproterenol -induced non treated rats [26]. Experimental studies reveal that statins, HMG-CoA reductase inhibitors showed antiarrhythmic properties, reducing the recurrence of life threatening arrhythmias in coronary artery disease [27].

Also in forced exercise group and Ellagic acid plus forced exercise group showed significant decrease percentages of incidence of ventricular fibrillation, ventricular tachycardia and ventricular premature beats compared to the control group. The decrease in arrhythmia, in this study, could be due to reduces cardiac sodium-calcium exchanger expression in animal with ventricular fibrillation [28]. As adenosine is released during exercise [29] adenosine inhibits delayed rectifier potassium channels and calcium influx through calcium channels and thereby prevents from arrhythmia [30].

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CONCLUSION

The results of this study showed a positive inotropic effect and negative chronotropic effect for Ellagic acid and exercise as compare with the control group. The present assay provides experimental evidence that the oral pretreatment of Ellagic acid (15mg/kg) was safe and effective in cardio-protection. Also incidence rate of PVB, VF and VT in groups which received Ellagic acid and exercise were decreased, therefore this substance is suggested as an antiarrhythmic factor which showed a protective function of Ellagic acid in heart and from exercise be used as a protective factor in the prevention of heart disease.

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