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Protective Effects of the Combination of Exercise and Gallic Acid Supplementation on Cardiac System in $CaCl_2$-Induced Arrhythmias Model in rats

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Introduction: Cardiovascular disease is currently the most common global disease. Cardiac arrhythmia is one of the most common types of heart diseases and main cause of mortality. Gallic acid (GA), as an antioxidant agent, has many preventive effects on diseases, and its Cardioprotective activity in myocardial infarction has been also reported. On the other hand, it is well documented that exercise-induced increase in myocardial manganese antioxidant activity is vital for protection against cardiac injuries. Thus, the present study investigated the antiarrhythmic activity of exercise training and GA supplementation alone or combined, on $CaCl_2$-induced arrhythmia in rats.

Materials and Methods: In this study, 32 male Sprague-Dawley rats (4-6 months old and weighing 200-250 g) were randomly assigned to four groups: Control, GA (50 mg/kg, gavage, 10 days), Exercise (21-days) and Exercise+GA. Then, lead II electrocardiogram was recorded to calculate HR and voltage of QRS complex. Also, the percentage of ventricular premature beats (VPB), ventricular fibrillation (VF) and ventricular tachycardia (VT) were recorded after the injection of $CaCl_2$ (140 mg/kg, i.v) in all groups. Results were analyzed by using ANOVA and Fisher's exact test and $P<0.05$ was considered as significant level.

Results: The data gathered in this study showed negative chronotropic and positive inotropic effects in the exercise and GA groups as compared to the control group. Also, co-administration of GA and exercise showed that incidence of ventricular premature beat, ventricular tachycardia and ventricular fibrillation reduced significantly as compared to the control group.

Conclusions: The results suggest that the combination of exercise and GA supplementation has a protective role in preventing and treating cardiac disorders by decreasing incidence of cardiac arrhythmias.

Introduction

The electrocardiograph (ECG) demonstrates a very organized and reproducible sequence of electrical events that occur within the heart. Abnormal electrical events in the electrocardiogram may result in abnormal mechanical events which could compromise cardiac output and normal delivery of oxygen to the brain, heart and other tissues (Israel, 2005).

Cardiac arrhythmia is one of the most common types of heart disorders causing a lot of mortality (approximately 17 million people worldwide each year). Cardiac arrhythmias occur when the electrical impulses in SA node that coordinates heartbeats do not work properly, causing heart to beat too fast, too slow or irregularly. The drugs used in the treatment of cardiac arrhythmia are called antiarrhythmic drugs. Propafenone, amiodarone, quinidine, veramapril and lignocaine are some examples of such drugs (Sharma et al., 2011). Common arrhythmias, particularly atrial fibrillation, ventricular tachycardia and fibrillation are major public health concerns, as some of them can cause sudden cardiac death. While Arrhythmias occurs due to the complex interaction of several factors, increased production of free radicals plays a major role in arrhythmias-induced cardiac injury (Keating and Sanguinetti, 2001).

A radical is any molecule that contains one or more unpaired electrons. Radicals are normally generated in many metabolic pathways. Some of these radicals can exist in a free form and subsequently interact with various tissue components resulting in dysfunction. An antioxidant can be considered as a molecule that, when present at low concentrations compared with those of an oxidisable substrate, significantly inhibits oxidation of that substrate (Brown et al., 2010).

The drugs for treating cardiac arrhythmias can often control or eliminate fast or irregular heartbeats. But, troublesome heart arrhythmias are often made worse or are even caused by a weak or damaged heart. However, adopting a heart-healthy lifestyle may lead to lower incidence of arrhythmias (Quindry and Hamilton, 2013). It is not always possible to avoid an arrhythmia developing, even though a healthy lifestyle can lower the risk of developing a heart condition (Perk et al., 2012).

Exercise is, in turn, an important part of a healthy lifestyle. It is well established that an exercise-induced increase in myocardial manganese antioxidant activity is vital for protection against cardiac injuries (Roshan et al., 2012). Hence, it is not surprising that overexpression of antioxidants enzymes in myocardium or the introduction of a mitochondrial-targeted antioxidant minimizes arrhythmias-induced myocardial injury. Moreover, dietary supplementation with antioxidants has been shown to lower arrhythmias-induced myocardial oxidative injury and the magnitude of infarction (Dianat et al., 2014a).

Herbal medicines or traditional medicines which have preventive and therapeutic effects on numerous disorders are specially valuable and important. Herbal therapies due to the availability, low cost and their mild side effects have been considered by several researchers as an alternative to chemical drugs (Parsaeyan and Rezvani, 2014).

Gallic acid (GA) (3, 4, 5-trihydroxy benzoic acid) are found in some plants such as tea leaves, grape seeds, walnuts and sumacs that have various properties including anti- fungal, antimicrobial and anti-viral. As GA attenuates visceral fat as well as plasma lipid level and also abates insulin-resistance, it is reported to be an effective factor for weight-loss. Cardio protective effect of GA resulting from its antilipoperoxidative and antioxidant properties has been already shown in myocardial infarction induced by isoproterenol (Dianat et al., 2014b). The present study is accordingly designed to investigate the protective effects of co-administration of exercise and GA against $CaCl_2$-induced arrhythmias.

Materials and Methods

Chemicals

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

Animals

In this study, 32 male Sprague-Dawley rats (weighing 200-250 g) were purchased from the Animal Breeding Lab at Ahvaz Jundishapur University of Medical Sciences (AJUMS). The animals used in this study were treated in accordance with the principles and guidelines on animals care of AJUMS and were kept at 20-24ºC under 12 h light/dark cycle and allowed free access to tap water and commercial chow. The investigation was also approved by the Animal Ethics Committee of AJUMS (No. 93S27).

Exercise training

The 21-day training protocol consisted of running on a motorized treadmill six times per week. Rats were put in a ten-channel treadmill once a day for 60 minutes for a period of 21 days with a constant inclination of zero. Rats were forced to run via a mild electric shock stimulation system installed on the end of the treadmill's channels in the first week of the procedure. All animals ran at a speed of 17 m/min. and few shocks were administered in each training session and continued during the first week of training. The animals were then randomly assigned to: Control, GA (50 mg/kg, gavage, 10 days), Exercise (21-days) and Exercise+GA. The sedentary rats in the control group were handled identically to the treadmill-trained rodent. Then, they were placed on a stationary treadmill, with the shock grid turned on, six days per week for the duration of the treadmill training session and received normal saline (1 ml), by gavage, once a day for the last 10 consecutive days (Dianat et al., 2014c).

Likewise, in GA groups, the rats were placed on a stationary treadmill, with the shock grid turned on, six days per week for the duration of the treadmill training session and received GA (50 mg/kg), by gavage, once a day for the last 10 consecutive days (Dianat et al., 2014c).

In GA+EX groups, the rats were trained to run on a motor-driven treadmill (21 days) and received GA 50 mg/kg, by gavages, once a day, during the last 10 days.
In our previous study, based on animal body weight calculations, the effective dose of GA was (50 mg/kg), therefore, we decided to use the same dose (50 mg/kg) in this research (Dianat et al., 2013).

Heart rate and QRS complex recording

The animals were operated under anesthesia with the combination of ketamine (50 mg/kg) and xylazine (10 mg/kg) via intraperitoneal (ip) manner. Lead II electrocardiogram (ECG) was recorded by Bio Amp and monitored by a Power Lab system (AD Instruments, Australia). Heart rate (as a chronotropic property) and QRS complex (as an inotropic property) were calculated from ECG recorded at the first day and also 21 days after the performance (Dianat et al., 2013).

The manner of induced and recording of arrhythmias

When the rats were anesthetized, to allow hemodynamic equilibration to occur, we recorded ECG for 15 min in all five groups before the induction of chemical-arrhythmia. Prep & drep with alcohol were done. Then, an incision was made; a poly ethylene catheter (18 G) was inserted in femoral vein. In effect, arrhythmia was induced by intravenously injection of $CaCl_2$ (140 mg/kg), and after injection of $CaCl_2$, it is expressed as a percentage of ventricular fibrillation (VF), ventricular tachycardia (VT) and premature ventricular beats (PVB) incidence (Dianat et al., 2014c).

Statistical analysis

Results were analyzed using SPSS software and were expressed as mean ± SEM. Statistical comparisons among groups were performed using t-test, one way ANOVA and Fisher's exact test. P values of less than 0.05 were considered statistically significant.

Results

Effects of exercise on inotropic and chronotropic properties of heart:

Twenty four hours before forced running on treadmill, the ECG was recorded and the baseline inotropic and chronotropic properties showed no significant alteration between all groups. After 21 days running as forced exercise, data showed that in treadmill trained rats QRS complex enhanced significantly (0.76±0.03) compared to that of the control group (0.96±0.02) ($P<0.01$) (Fig 1), while, HR significantly decreased as compared to the control group's (222±4.84, 263±4.57; respectively) ($P<0.01$) (Fig 2).

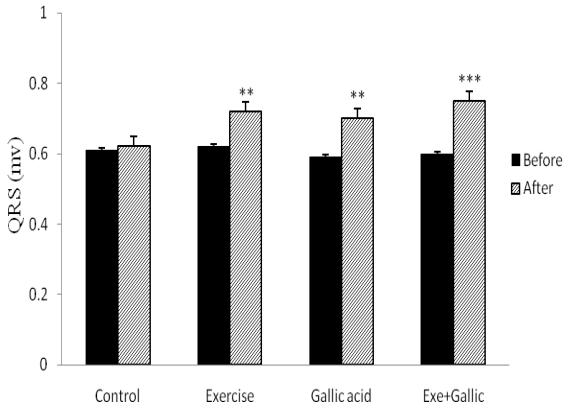

Figure 1: Effects of exercise (21 days), GA (50 mg/kg) and exercise plus GA on voltage of QRS complex in rats. Results are expressed as Mean ± SEM of 8 rats per group, use t-test; **P<0.01, ***P<0.001.

Effects of GA on inotropic and chronotropic properties of heart

Inotropic property enhanced significantly after 10 days treatment with GA (50 mg/kg) as compared to the baseline of the experiment time for all groups (Fig 1). Despite this, comparison between rats received GA (10 days) and those in the control group showed significant decrease in chronotropic properties (227±5.64 & 263±4.57; respectively) (P<0.05) (Fig 2).

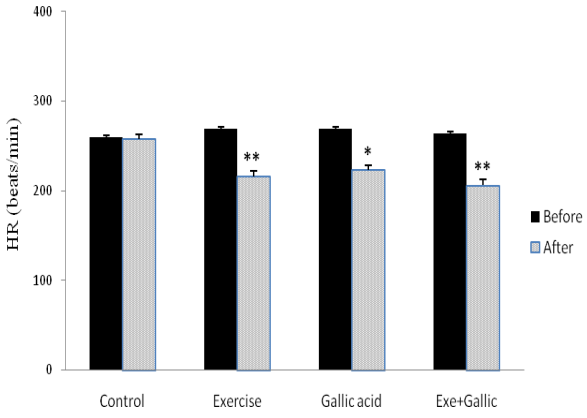

Figure 2: EffectS of exercise (21 days), GA (50 mg/kg) and exercise plus GA on heart rate in rats. Results are expressed as Mean ± SEM of 8 rats per group, use t-test; *P<0.05, **P<0.01.

Effect of exercise plus GA on inotropic and chronotropic properties of heart

This experiment also examined the effects of treadmill exercises in 21 days plus administration of GA (50 mg/kg in last 10 days) on QRS complex and heart rate in rat groups. The obtained results showed that, in exercise + GA groups, inotropic effects were significantly enhanced (0.79± 0.04) (p<0.001) (Fig 1). In contrast, chronotropic properties decreased significantly compared to the control group's (213±6.9& 263±4.57; respectively) (p<0.001) (Fig 2).

Assessment of $CaCl_2$-induced arrhythmia in exercise, GA and exercise plus GA groups

An evaluation of the effects of the 21-day exercise in treadmill trained group showed that incidence of premature ventricular beat (PVB) (P<0.05), ventricular tachycardia (VT) (P<0.05) and ventricular fibrillation (VF) (P<0.01), were significantly reduced (75%, 80% & 45%; respectively) compared to those of the control group (100%). Also, in GA group with daily gavage of GA (50 mg/kg) reduction in the incidence of ventricular beat (PVB) (P<0.01), ventricular tachycardia (VT) (P<0.01) and ventricular fibrillation (VF) (P<0.001) were shown to be (45%, 50% & 20%; respectively) when compared to the sedentary rats. Besides, our results showed that 21 days of treadmill forced exercise plus administration of chronic dose of GA in the last 10 days was more effective reduction effect in the percentage of VT (P<0.01), VF (P<0.001) and PVB (45%, 40% & 15%; respectively) (P<0.01) incidence when compared to the treadmill exercise group (Table 1).

Table 1: Effects of of exercise, GA and exercise plus GA on $CaCl_2$-induced arrhythmia. The data from the control group was considered as 100 % and the results were compared to those of other groups and are expressed as a percentage; VT: ventricular tachycardia, VF: ventricular fibrillation; PVB: ventricular premature beats; *P<0.05, **P<0.01, ***P<0.001 vs. the control group.

Groups	Arrhythmia (%)		
	PVB	VT	VF
Control	100	100	100
Exercise	75*	80*	45**
GA	45**	50**	20***
Exe+GA	45**	40**	15***

Discussion

Based on our results, it can be concluded that training forced exercise on treadmill in 21 days had positive inotropic, negative chronotropic and antidysrhythmic effects. This is consistent with the results of recent studies and also previously overlooked findings, which demonstrated that the Frank-Starling mechanism is thought to be mainly responsible for the observed increases in stroke volume and cardiac output. This is actually due to an increased diastolic filling (i.e. an increased end-diastolic volume (EDV) and an increased stroke volume (due to the Frank-Starling effect). In fact, most of the signal transduction pathways that increase inotropic properties finally involve calcium, either by increasing calcium influx (via calcium channels) during the action potential, or by increasing the release of calcium through the sarcoplasmic reticulum, or by sensitizing troponin-C (TN-C) to calcium (Warburton et al., 2002).

It has been stated that, training-induced bradycardia is widely attributed to the autonomic nervous system, an

increase in vagal tone induced by training (Maron and Pelliccia, 2006). The bradycardia is usually a benign physiological adaptation to maintain blood pressure and normal cardiac output despite the training-induced increase in stroke volume (D'Souza et al., 2014).

In addition, in the exercise-trained group the percentage of arrhythmias incidence decreased significantly. It may be explained, in part, due to the release of endogenous adenosine during exercise (Baravati et al., 2015).

During exercise an imbalance between myocardial oxygen supply and demand results in the net breakdown of adenosine (Roque et al., 2011). In cardiac tissue, adenosine binds to type 1 (A_1) receptors, which are coupled to inhibitory G-proteins (G_i). Stimulation of this pathway opens potassium channels, which hyperpolarizes the cell. Also, activation of the G_i-protein decreases cAMP, which inhibits calcium channels (L-type) and therefore calcium entry into the cell. In cardiac pacemaker cells that are located in the SA node, adenosine acting through receptors (type 1) inhibits the pacemaker current, which decreases the slope of phase 4 of the pacemaker action potential, thereby decreasing its spontaneous firing rate (negative chronotropic) (Headrick et al., 2011). Lastly, adenosine inhibits the release of norepinephrine by acting on presynaptic purinergic receptors that are located on sympathetic nerve terminals. In terms of its electrical effects in the heart, adenosine reduces conduction velocity and decreases heart rate, especially at the AV node, which can work to treat irregular heartbeat and normalize heart rhythm (Burgdorf et al., 2001).

In the present study, GA administration enhanced voltage of QRS complex as an inotropic property and decreased incidence of VT, VF and PVB in rats.

In earlier studies, it has been shown that antioxidant therapy is useful in the management of cardiovascular problems (Myung et al., 2013). Our findings of positive influence of GA on inotropic property and antiarrhythmic effect in $CaCl_2$-induced arrhythmia model, accord with the findings of previous studies on the cardioprotective properties of natural antioxidants.

$CaCl_2$ increased calcium and sodium levels, while decreased potassium levels in blood. Calcium overload leads to an increase in free radicals in $CaCl_2$-induced arrhythmias. Both a great production of free radicals and the insufficiency or depletion of many antioxidant enzymes may reveal exacerbation of the oxidative damage, while the supplementation of many antioxidants generates diverse outcomes (Sharma et al., 2011). The antioxidant defence system includes antioxidant enzymes such as glutathione peroxidase (GPx), superoxide dismutase (SOD), and several non-enzymatic free radical scavengers. The polyunsaturated fatty acids in cardiac tissue are targets to oxidative damage by free radicals due to the unsaturated bonds they contain (Sharma et al., 2011).

Phenolic acids may over the long term provide protection against a number of diseases. Our recent investigation showed that oral administration of GA has antioxidative activity in cardiac ischemia-reperfusion rats and decreased oxidants in cardiac tissue leading to improving hemodynamic and electrophysiological parameters of isolated heart (Dianat et al., 2014B). Furthermore, flavonoids are potent antioxidants that are able to inhibit lipid peroxidation and production of low-density lipoproteins. Alternatively, the protective effects of these compounds may not be entirely due to their antioxidant properties and other mechanisms may also operate. Polyphenols components have specific pharmacologic activities that interact with cell-signalling cascades, influence the cell at a transcriptional level, and down-regulate pathways that lead to cell death, rather than general properties to scavenge ROS and free radicals (Farbood et al., 2013). So, it may be concluded that the oral consumption of GA can improve cardiac function and protect cardiac damage after $CaCl_2$ injection due to its polyphenols components and antioxidant properties. The mechanism underlying these effects will be investigated in future studies.

Besides its antioxidative properties to protect the heart against damage, these effects show that GA may affect the cardiac electrophysiological parameters (involving the contractility) and hemodynamic parameters (heart rate). However, more investigations are needed to determine the mechanism of this probability.

Conclusion

The results suggest that the combination of exercise and GA supplementation positively improves dromotropic and antiarrythmic properties as compared to the use of either of them alone and can be thus used as a part of treatment strategy in heart failure and other cardiac disorders. However, the exact mechanisms for these effects require more attention and investigations.

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Conflict of Interest

There is no conflict of interest to be declared.

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