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Negative Chronotropic and Antiarrhythmic Effects of Ginger (*Zingiber officinale*) and Intermittent Fasting on $Cacl_2$ Induced-Arrhythmia.

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ABSTRACT

The aim of this study was to investigate the chronotropic and antiarrhythmic effect of ginger and intermittent fasting (IF) on $CaCl_2$ induced arrhythmia in rat. Adult male Wistar rats (body weight, 250-300 g) were randomly divided to five experimental groups (n = 8) as follow: control group, ginger group (received 100 mg/kg of ginger for 15 days); IF group (15 days undergoing IF); ginger + IF group (received 100 mg/kg of ginger simultaneously with IF); Amiodarone group (received 10 mg/kg of amiodarone). White Ginger powder was suspended in 2 ml normal saline and administered to rats via an oral gavage needle. The arrhythmia was induced by i.v injection of CaCl2 (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. Ginger and IF exerted significant negative chronotropic and antidysrhythmic effects on CaCl2-induced arrhythmia with the highest activity in ginger + IF group. It may be concluded that ginger and IF alone and especially simultaneously can reduced the incidence of arrhythmia and protect heart.

Keywords: Ginger, Intermittent Fasting, Arrhythmia, Chronotropic, Rat

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INTRODUCTION

Sudden cardiac death, that is a main cause of mortality in patients with cardiovascular diseases, is caused by the arrhythmias [1]. Arrhythmias resulted from irregularity at impulse generation, conduction or combination of both in the heart [2]. The different and lethal types of ventricular arrhythmias including, premature ventricular beat (PVB), ventricular fibrillation (VF), and ventricular tachycardia (VT) [3]. The mechanisms of arrhythmogenesis are not fully understood so far, but some molecular mechanisms known to contribute to arrhythmias include genetic alterations of ion channels and structural remodeling of the left ventricle [4]. There are many evidence suggesting that altered ion homeostasis and structural remodeling are highly associated with increased reactive oxygen species (ROS) [5]. ROS can be produced through electron leakage from mitochondria that occupy approximately 30% of the volume of ventricular cardiomyocytes, during oxidative phosphorylation and through the activation of several cellular enzymes including the xanthine oxidase, NADPH oxidase, and uncoupled nitric oxide synthase (NOS) [6]. It has been established that during oxidative stress, increased ROS production is associated with antioxidant reserve reduction [7-9]. The limited efficacy and proarrhythmic potential of conventional antiarrhythmic drugs has generated interest in new approaches to the treatment of arrhythmias. Due to the deleterious role of ROS in arrhythmogenesis and also to the diminished endogenous antioxidant reserve, it would be reasonable to suppose that the administration of antioxidants might be effective for treatment of arrhythmias. Ginger (Zingiber officinale), well known and widely used herb, contains a number of antioxidants such as ascorbic acid, beta-carotene, terpenoids, alkaloids, and polyphenols such as flavonoids, flavones glycosides, rutin and etc [10]. Previous studies have shown that ginger has good free radical scavenging ability and can be used as a radical inhibitor or scavenger, acting possibly as a primary antioxidant [11]. Ginger has been used in herbal medicine for the treatment of a range of diseases such as hypercholesterolemia, rheumatoid arthritis, neurological diseases, asthma, stroke, constipation, diabetes and cancer [10,12,13]. Dietary restrictions, either a diminished energy intake or intermittent fasting (IF), a dietary regimen in which food is available ad libitum but only every other day, have been proven to enhance life span and to decrease the incidence of age-associated diseases, including diabetes, cancer and kidney disease in animal models [14]. The anti-inflammatory properties and antioxidant potential of dietary restriction have been reported [15]. Post-ischemic oxidative stress and inflammatory response have been significantly reduced in the myocardium of calorie-restricted rats [16]. In addition, dietary restriction could reduce the infarct size, the number of apoptotic myocytes and the inflammatory response after coronary artery ligation, in the IF group compared with control diet group [17]. These results suggest that dietary restriction protects myocardium against oxidative and ischemic damage. The objectives of the present study are to investigate the antiarrhythmic effect of ginger, as an antioxidant, and IF alone and simultaneously and also to comparison the antiarrhythmic effect of these two treatments in $CaCl_2$ induced arrhythmias in rat.

There are three methods to induce arrhythmias including, ischemia-reperfusion, electrical and chemical (such as $CaCl_2$) [18]. $CaCl_2$ induces arrhythmias through a direct and also indirect action mediated through the sympathetic nervous system [19].

Amiodarone, that is a class III antiarrhythmic agent used for various types of cardiac dysrhythmias, both ventricular and atrial [20], has been used in this study as a standard antiarrhythmic drug.

MATERIALS AND METHODS

Chemicals

Ginger and Amiodarone (sigma), $CaCl_2$ (Merk Co. Darmstadt, Germany), ketamine hydrochloride and xylazine (Alfasan Co, Woderen- Holland).

Animals and treatments

Adult male Wistar rats (body weight, 250-300 g) were randomly divided to five experimental groups (n = 8) as follow: control group, ginger group (received 100 mg/kg of ginger for 15 days); IF group (15 days undergoing IF); ginger + IF group (received 100 mg/kg of ginger simultaneously with IF); Amiodarone group (received 10 mg/kg of amiodarone). All groups were maintained under the same condition (temperature 22 ± 2°C and 12-hours dark-light cycle) supplied with food and water ad libitum. White Ginger powder was suspended in 2 ml normal saline and administered to rats via an oral gavage needle for 15 days. Control group

received 2 ml of saline orally for the same duration and for doing IF, animals received food every other day for 15 days. Ginger + IF group, were under IF and simultaneously received ginger with the same dose and duration as other groups.

This study was approved by the animal care and ethical committee of the Shiraz University of Medical Sciences, Shiraz, Iran.

Preparation of animals

Rats were anesthetized with intraperitoneal injection of xylazine (5 mg/kg) and ketamine (50 mg/kg). After anesthesia, prep & drep with betadine were done. Then, a longitudinal incision was made in area of groin. A poly ethylene catheter was inserted in femoral vein, and fixed around it, for IV injections [6]. Lead II electrocardiogram (ECG) was recorded by Bio Amp and monitored continuously by a Power Lab system (AD Instruments, Australia) for calculating and interpretation of electrophysiological parameters.

Inducing and recording of arrhythmias

ECG was recorded in all groups for 15 min, before the induction of chemical- arrhythmia to allow hemodynamic equilibration. Heart rate (HR) was calculated from ECG recording in the first day and 15 days after administration of ginger or IF. In the 15^{th} day of experiment, arrhythmia was induced by intravenous injection of $CaCl_2$ (140mg/kg), and percentage of incidence of ventricular premature beats (VPB), ventricular tachycardia (VT) and ventricular fibrillation (VF) were calculated after injection of CaCl2 [21].

Statistical analysis

Between groups comparisons were analyzed by one-way ANOVA while for within group comparisons Paired t-test was used. Fisher`s exact test was also employed to evaluate the arrhythmias data. The value $p<0.05$ were considered significant level.

RESULTS

Effect on HR

The effects of administration of ginger and undergoing IF for 15 days and simultaneous effect of these two interventions on HR of male Wistar rats are presented in Figure1. In ginger group, HR significantly ($p<0.05$) decreased after 15 days receiving ginger. Figure 1.shows that in rats subjected to 15 days IF, HR significantly decreased ($p<0.05$) in comparison to first day. Rats in ginger + IF group showed more significant reduction of HR ($p<0.01$) after receiving of ginger and simultaneously subjected to IF in comparison to first day. To test the difference between experimental groups with control group, one-way ANOVA carried out. Based on this statistical analysis, HR significantly decreased in Ginger and IF groups ($p<0.05$), and also reduced in ginger + IF group ($p<0.001$) in comparison to control group.

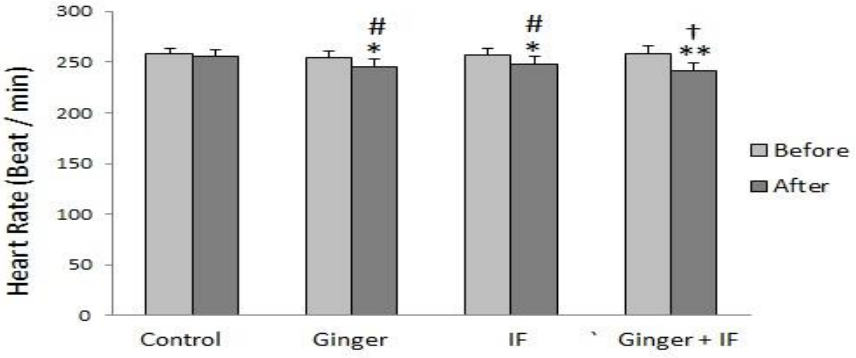

Figure 1: Effect of ginger and IF on HR. Results are expressed as Mean ± SEM of 8 rats per group, within and between group comparison done by paired-t test and one-way ANOVA respectively. *P<0.05, **P<0. 01 shows within group difference and # P<0.05, † P<0. 01 shows difference with control group.

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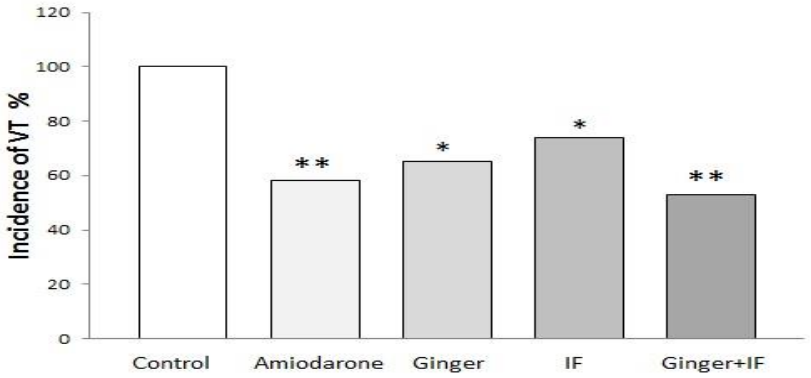
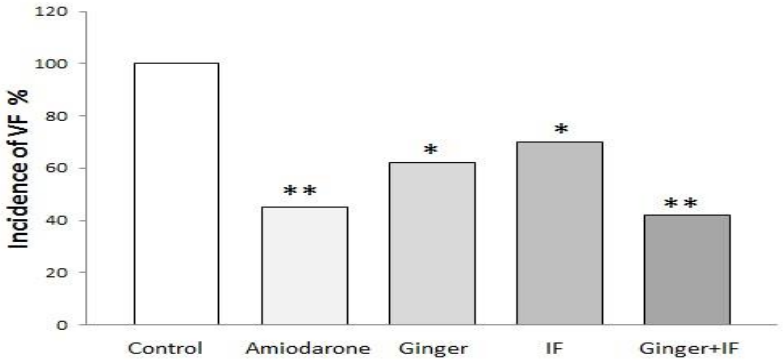
Effect on arrhythmia$CaCl_2$ -induced arrhythmia

The data from the control group were considered as 100. Other data were compared with the control group and the results were expressed as percentages. Data presented in Figure 2, shows significant decrease in the incidence of VT in the amiodarone group ($p<0.01$), ginger group ($p<0.05$), IF group ($p<0.05$) and ginger + IF group ($p<0.01$) compared with control group.



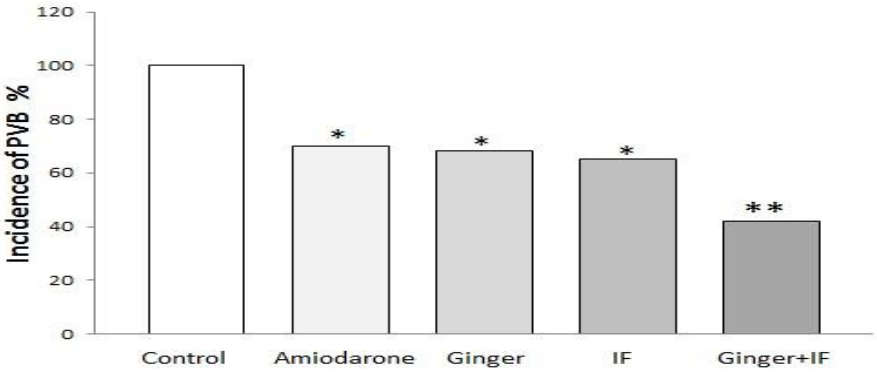
Figurer 2. Effect of ginger and IF on incidence of Ventricular Tachycardia (VT). The data from the control group was considered as 100 % and the results were compared to those of control group and are expressed as a percentage data. *P<0.05, **P<0. 01 vs control group.

The incidence of VF reduced significantly in the amiodarone group ($p<0.01$), ginger group ($p<0.05$), IF group ($p<0.05$) and ginger + IF group ($p<0.01$) compared with control group (Figure. 3).



Figurer 3. Effect of ginger and IF on incidence of Ventricular Fibrillation (VF). 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 vs control group.

The incidence of VPB reduced significantly in the amiodarone group ($p<0.05$), ginger group ($p<0.05$), IF group ($p<0.05$) and ginger + IF group ($p<0.01$) compared with control group (Figure. 4).



Figurer 4. Effect of ginger and IF on incidence of Ventricular Premature Beat (VPB). 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 vs control group.

DISCUSSION

Developing a strategy to prevent arrhythmias that are the most common cause of sudden cardiac death, without any side effects is still under study. Due to the established and detrimental role of oxidative stress in arrhythmogenesis and proarrhythmic potential of conventional antiarrhythmic drugs, investigating the antidysrhythmic effects of natural antioxidant has been interested. Ginger has been recorded in "Generally Recognized as Safe" (GRAS) document of the US FDA. This widely used herbal spice with a dose of 0.5 – 1.0 g ingested 2-3 times for periods ranging from 3 months to 2.5 years did not cause any adverse effects in human [22]. The aim of this study, was to examine the possible choronotropic and antiarrhythmic effects of ginger and IF individually and together. Significant decrease of HR in all groups, especially in ginger + IF group, exhibits that these treatments exerted negative choronotropic effects. Data showed that receiving ginger for 15 days and also subjecting to 15 days of IF, significantly reduces HR and more significant reduction was seen in ginger + IF group. Acetylcholine, released by the parasympathetic nerve endings, acting through nicotinic receptors in the sinoatrial node (SA node) reduces HR and is degraded by acetylcholinesterase. There is evidence that both red and white ginger especially latter, used in this study, inhibit acetylcholinesterase [23] and negative choronotropic effect of ginger can be attributed to its inhibitory effect on acetylcholinesterase activity. In addition, it has been shown that polyphenol compounds, bonding beta-adrenergic receptors, prevent increasing of heart rate [24]. In pathological conditions, increased ROS production stimulate central sympathetic outflow promoting sympathetic hyperactivity, an effect that has been normalized by antioxidant therapy in experimental hypertension [25]. Presented data of HR shows IF, in addition to ginger, also exerted negative choronotropic effect and interestingly, this effect was more significant in ginger + IF group. Calorie restriction and IF in a wide range of laboratory animals, extended lifespan and decreased the incidence of several age-related diseases [26]. Investigating heart-rate variability in Sprague-Dawley rats after IF showed reduced sympathetic activity associated with increased vagal tone [27]. It has been reported that IF is capable of reducing HR and blood pressure [28]. Regarding this evidenced and considerable HR lowering effect of IF and ginger, these exerted negative choronotropic effect in present study and potentiated this effect of each other to cause more significant negative choronotropy in ginger + IF group than two other groups. To assess antiarrhythmic effect of ginger and IF, we evaluated this effect on $CaCl_2$ induced arrhythmia and showed that these interventions reduce three main lethal type of ventricular arrhythmias including VT, VF and VPB. Although ginger and IF individually did not show the same antiarrhythmic effect as amiodarone, concurrently did. Cardiac myocytes have poor antioxidant defense systems, and therefore are susceptible to the damage caused by ROS [5]. Accumulating ROS may lead to cardiac Ca^{2+} overload and consequently delayed afterdepolarizations (DADs), which is likely to initiate triggered activity and ventricular arrhythmias [29]. Ginger, through its strong antioxidant and radical scavenging effects, have eliminated Cisplatin induced cardiotoxicity and significantly decreased leakage of LDH and CK from cardiac myocytes and increased level of endogenous antioxidant enzymes [30]. Ventricular arrhythmias enhances plasma level of angiotensin II (AngII), which through AT1 receptors and activation of NADPH oxidase increases ROS generation [31,32]. The major ingredient of ginger, [6]-Gingerol, has been identified as a novel AT1 receptor antagonist, which partially clarified the mechanism of ginger regulating blood pressure, reducing oxidative damage and strengthening heart in the cardiovascular system [33]. Nrf2, a transcription factor regulating the expression of antioxidant enzymes in response to oxidative stress, has been upregulated in cell line model treated with 6-Shogaol which is another bioactive component of ginger [34]. There are many reports indicating that IF not only reduces oxidative stress but increases adaptive responses to most stressors [14-16, 28]. Significant reductions of post ischemic oxidative stress and inflammatory responses have been shown in the myocardium of rats subjected to IF [14]. It seems that antioxidant and cardioprotective effects of both ginger and IF could additively reduce incidence of ventricular arrhythmias in this study.

CONCLUSION

The data obtained from this experimental study clearly demonstrate that ginger and IF, due to their antioxidant and cardioprotective effects, alone and specially together exerted negative choronotropic effect, and significantly reduced the incidence of ventricular arrhythmias in $CaCl_2$ induced arrhythmia in rat.

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