

The effects of olive leaf extract and 28 days forced treadmill exercise on electrocardiographic parameters in rats

Somayeh Javidanpour, Mahin Dianat¹, Fatemeh Ramezani Aliakbari,¹ Alireza Sarkaki¹

Student Research Committee, Science and Religion Work Jundishapur University of Medical Sciences, ¹Department of Physiology and Persian Gulf Physiology Research Centre, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Background: There is evidence that regular activity can prevent of cardiovascular diseases. There are many reports that exercise and the consumption of olive leaf extract (OLE) have a positive effect on cardiovascular parameters. This study was conducted to compare the effects of exercise and OLE alone and together on electrocardiographic parameters in rats. **Materials and Methods:** Male Sprague–Dawley rats were randomly divided into six groups ($n = 8$ rats in each): Control, exercise, OLE (100, 200, and 400 mg/kg, orally for 14 days), and exercise + OLE (200 mg/kg of extract, orally for 14 days). Exercise training in rats was performed using treadmill for 28 days (1 h/day). Electrophysiological parameters including heart rate, PR interval, QT interval, QT corrected (QTc), RR interval, QRS voltage, and duration were obtained from lead II electrocardiogram (ECG) recorded by a PowerLab system. Statistical evaluation was done by one-way analysis of variance followed by Fisher's least significant difference test and $P < 0.05$ was considered statistically significant. **Results:** The amounts of QT ($P = 0.0009$) and QTc interval ($P = 0.0004$), RR interval ($P < 0.0001$), QRS duration ($P = 0.004$), and QRS voltage ($P = 0.003$) in the exercise group were significantly higher than those of the control group. However, there were no significant differences in PR interval in comparison with the control group. Exercise ($P < 0.0001$) and OLE (400 mg/kg, $P = 0.043$) alone and both in combination ($P = 0.007$) reduced heart rate and increased the amount of QRS voltage ($P = 0.003$, $P = 0.047$, and $P = 0.046$, respectively) and RR interval ($P < 0.0001$, $P = 0.046$, and $P = 0.0009$, respectively). **Conclusion:** Results of this study indicated that administration of OLE alone and in combination with exercise has negative chronotropic and positive inotropic effects and also it can prevent of prolongation of QT and QTc interval induced by severe exercise.

Key words: Electrocardiogram, exercise, olive leaf, QRS, QT interval

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INTRODUCTION

Studies have shown that exercise and physical activity are important in the prevention of cardiovascular diseases and sudden cardiac death.^[1] Human and experimental research has demonstrated that exercise training increases survival rate after myocardial infarction.^[2] This effect may be somewhat described by an increase in cardiac vagal activity, which decreases the susceptibility to arrhythmias and sudden death.^[3] Research also indicates that regular physical activity and exercise training has a positive effect on cardiovascular function in patients with chronic heart failure.^[4] The

resting heart rate in athletes is lower compared with nonexercise individual because their heart muscle becomes stronger as a result of exercise, so with less effort, their hearts can pump up more blood volume in each heartbeat.^[5] Therefore, proper and regular exercise plays an important role in preventing and increasing the survival of cardiovascular disease.^[6]

Electrocardiography (ECG) is an important noninvasive clinical test for determination of cardiac contractility and electrical conductivity status.^[7] ECG patterns vary from person to person and even in a person under different physiological conditions. Few studies have been done on the effects of exercise on ECG parameters.^[8]

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Address for correspondence: Dr. Mahin Dianat, Department of Physiology, Persian Gulf Physiology Research Centre, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. E-mail: dianat@ajums.ac.ir

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The Mediterranean diet is known as a highly valid diet for the treatment and prevention of cardiovascular disease.^[9] Olive oil is one of the main components of this diet and its protective effects on heart and blood vessels have been attributed to the oleic acid and polyphenols.^[10,11] In recent years, olive tree leaves (*Olea europaea*) have been used to treat cardiovascular disease.^[12-14] The medicinal effect of olive leaves is related to polyphenols, especially oleuropein that is found in large amounts in olive leaves.^[15] It has been shown that this polyphenol has beneficial effects such as antioxidant, anti-inflammatory, antihypertensive, antiatherogenic, hypoglycemic, and hypocholesterolemic.^[16] It has been reported that this polyphenol can prevent the oxidation of membrane lipids and cardiovascular disease.^[17] Moghaddasi *et al.* reported that the 2-week administration ethanolic extract of olive leaves in three doses of 100, 200, and 300 mg/kg can improve the changes in ECG parameters induced by cerebral hypoperfusion in rats.^[18] A common method of exercise that researchers use to test the effects of exercise training on their desired parameters in animals is forced treadmill.^[19]

Since it has been reported that both exercise and consumption of olive leaf extract (OLE) alone have beneficial effects on cardiovascular parameters, the aim of this study was to determine whether the OLE alone has a better effect on electrocardiogram parameters or exercise alone and also does exercise along with extract can exert synergistic effects on heart rate and electrocardiographic parameters in rats?

MATERIALS AND METHODS

A total number of 48 adult (6 months) male Sprague–Dawley rats weighing 200–250 g were provided from the Animal House of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. The animals were housed in standardized conditions (25°C ± 2°C, 12-h light/dark cycle). During the study period, the animals had access to food and water *ad libitum* and were handled to minimize stress of substance administration. The experiments were done in accordance with the ethical guidelines, and the protocol was approved by the Ethics Committee for Animals at Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (No: 93S30).

Extract preparation

The fresh leaves of the olive tree (*O. europaea*) were collected from the campus of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, and were authenticated by Department of Pharmacognosy (Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences). The OLE was washed and dried under shadow. Then, they were

powdered by a mill. In order to provide hydroalcoholic extract, 50 g of the powder was mixed with 300 ml of ethanol (70%). Seventy-two hours later, the solution was filtered through filter paper. The OLE was concentrated in oven at 40°C and then dried.^[20]

Forced exercise

Exercise-included 60-min running treadmill with an incremental slope and a speed of 16 m/min is performed 5 days a week for 4 weeks. Animals received a shock when stationary.^[21]

Experimental protocols

The rats were randomly divided into six groups of 8 each:

1. Control: The rats were put on a treadmill with the motor off for 1 h daily for 28 days and received normal saline orally in the last 15 days of the study
2. Exercise: The rats were put on a treadmill for 28 days, did daily exercise for an hour, and received normal saline orally in the last 15 days of the study^[22]
3. OLE 100
4. OLE 200
5. OLE 400

Rats in groups 3–5 were put on a treadmill with the motor off for 28 days and for the last 15 days of study received 100, 200, and 400 mg/kg of OLE orally, respectively^[18]

6. Exercise + OLE 200: The rats were put on a treadmill for 28 days and for the last 15 days of the study received moderate dose of OLE (200 mg/kg) orally.

Electrocardiographic (ECG) recording method

Five minutes after anesthesia (with 50 mg/kg ketamine and 10 mg/kg xylazine hydrochloride, intraperitoneally), standard bipolar limb lead II was recorded by BioAmp and monitored by a PowerLab system (ADInstruments, Australia) using needle-shaped electrodes, which were located under the skin. ECG parameters such as PR interval, QT and QT corrected (QTc) interval, RR interval, and QRS voltage and duration were recorded. In order to calculate heart rate during ECG recording, the average number of QRS complexes in a suitable time interval (1 min) was measured as the heart rate for each animal. To determine QTc interval, Bazett's formula was used as follows:^[23]

$$QT_c = QT \text{ interval} / \sqrt{RR \text{ interval}}$$

Statistical analysis

Statistical evaluation was done by one-way analysis of variance followed by Fisher's least significant difference test. Normality assumption of data was checked by Kolmogorov–Smirnov test and homogeneity of variances assessed using by Levene's test. Results were expressed as mean ± standard error of the mean and $P < 0.05$ was considered statistically significant.

RESULTS

The effects of exercise and olive leaf extract on ECG parameters

Heart rate

As illustrated in Figure 1a, the amount of heart rate in exercise, OLE 400, and exercise + OLE 200 groups reduced significantly compared to control group ($P < 0.001$, $P < 0.05$, and $P < 0.01$, respectively).

PR interval

No significant difference was found in the PR interval between the studied groups.

QT interval

As shown in Figure 1b, the times of QT interval in exercise group significantly increased in comparison with control group ($P = 0.0009$). The other groups did not show any significant difference with the control group.

QT corrected interval

As demonstrated in Figure 1c, the level of QTc interval in exercise group was higher than that in control group ($P < 0.0004$). However, there was not any difference between the other groups and the control.

RR interval

The amount of RR interval in OLE 400 ($P = 0.046$), exercise ($P < 0.0001$), and exercise + OLE 200 ($P < 0.0001$) groups was higher than that in control group [Figure 1d].

QRS voltage

The voltage of QRS complex in OLE 400, exercise, and exercise + OLE 200 groups significantly increased compared to the control group ($P = 0.047$, $P = 0.003$, and $P = 0.046$, respectively) [Figure 1e].

QRS duration

As observed in Figure 1f, the QRS duration only in exercise group significantly increased in comparison with control group ($P = 0.004$). There was no significant difference between exercise + OLE200 and control groups.

DISCUSSION

In this study, we found that exercise and OLE both in combination and alone reduced heart rate and increased the amount of QRS complex voltage and RR interval. Furthermore, exercise training alone was found to lead to QT and QTc interval prolongation and increased QRS duration.

It has been proved that OLE has antioxidant properties due to the high presence of oleuropein, the main polyphenolic

compound which is found in olive leaves more than in fruit of *O. europaea*. Various studies indicated that oleuropein in addition to its antioxidant activity^[24] has many useful biological effects on animals and human such as anti-inflammatory,^[25] antidiabetic,^[26] anticancer,^[27] hypoglycemic, and hypolipidemic.^[28]

In the present study, the oral administration of OLE, in a dose-dependent manner, was shown to have a negative chronotropic and positive inotropic effects by decreasing the number of heart rate and increasing the amplitude of QRS complex voltage, respectively. It appears that the negative chronotropic effect of OLE to be due to its high oleuropein content. Because in several studies, it has been reported that oleuropein has a blocking effect on L-type Ca^{+2} channel.^[29] It seems to act as a competitive antagonist against Ca^{+2} channels, since the negative chronotropic effect of the OLE was observed only at its maximum dose, not the minimum and moderate doses. Ca^{+2} channels play an important role in pacemaker function in the heart nodal tissue.^[30] Blocking channels of Ca^{+2} , by reducing the electrical conduction velocity along the plateau phase of the action potential, can decrease the number of heart rate.^[30] In addition, the maximum dose of OLE may be via the Frank–Starling mechanism reduces the heart rate. The height of the QRS complex is considered as a marker for cardiac contractility.^[31] This parameter increased significantly in the OLE 400 group compared to the control group. Therefore, administration of OLE increases the cardiac function, followed by an increase in cardiac output and venous return. An increase in the venous return leads to an increase in the ventricular end-diastolic volume or preload. Physiological increase in preload increases the myocardial fiber length, and thus, a stronger contraction occurs.^[32] Therefore, the OLE probably by Ca^{+2} channels blocking and Frank–Starling mechanisms can reduce heart rate. It has been reported that perfusion of isolated rat hearts with oleuropein increases the cardiac contractility.^[17] The results of our study demonstrated that oral administration of OLE for 15 days and exercise training for 28 days alone or both in combination can produce a positive inotropic effect. It has also been demonstrated that exercise training by the mechanism of Frank–starling induces a positive inotropic effect. Therefore, stretching of left ventricular myocardial fibers by more preload causes to a stronger contraction.^[33]

In cardiology, the QT interval is a measure of the time between the start of the Q-wave and the end of the T-wave in the heart's electrical cycle. The prolongation of QT interval is associated with increased risk of ventricular arrhythmia and sudden cardiac death.^[34] Since the QT interval like the RR interval is dependent on the heart rate, the higher heart rate leads to shorter RR and QT interval; therefore, instead of QT interval, QTc interval is calculated based on Bazett's

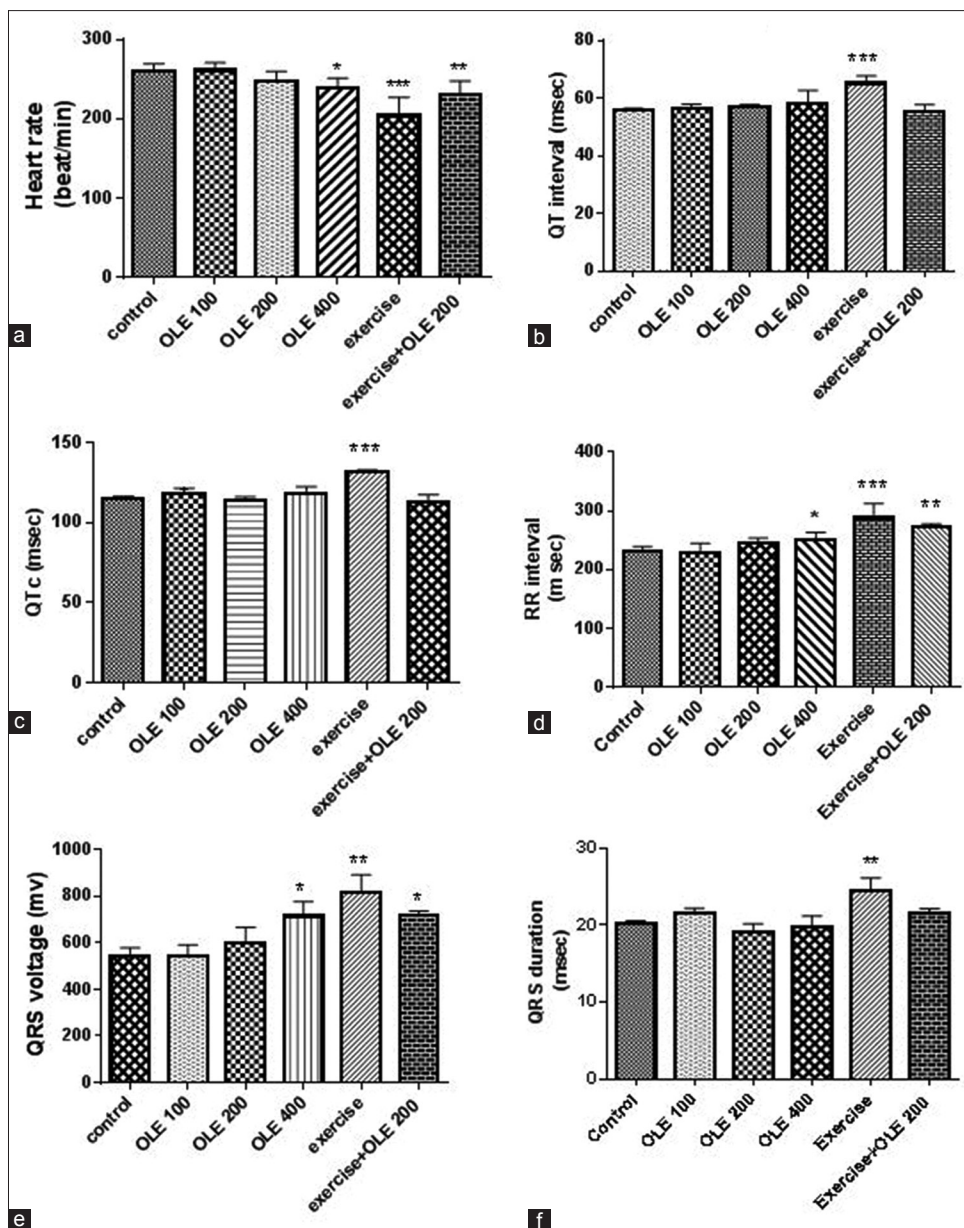


Figure 1: The effects of exercise training and oral administration of olive leaf extract alone and together on heart rate (a), QT interval (b), QTc interval (c), RR interval (d), QRS voltage (e), and QRS duration (f) in different groups. Results are expressed as mean \pm standard error of the mean of 8 rats per group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus control

formula,^[23] and evaluation of QTc parameter makes the identification of patients at increased risk of ventricular arrhythmia more accurate.^[35] In the present study, contrary to our expectation, we found that the time of QT and QTc interval in exercise group significantly increased compared to the control group, but there was not any significant difference in QTc interval in exercise + OLE 200 versus control group. Therefore, it seems that the prolongation of QT and QTc interval in the exercise group was due to exercise-induced hypertrophy. There is evidence that in a number of athletes, left ventricular hypertrophy and QRS prolongation are detected.^[36] Since in the combined group exercise + OLE 200, no QT prolongation was observed, it is

claimed that oral administration of OLE for 15 days may prevent severe exercise-induced hypertrophy. Oxidative stress has been recognized as one of the main factors in the development of cardiac hypertrophy.^[37] There are reports that the generation of free radicals is elevated in severe exercise due to the hyperactivity of skeletal and cardiac muscles.^[38] By increasing the antioxidant capacity of the body, the severity of damage from free radicals can be reduced.^[39] Yavari *et al.* showed that regular consumption of various fresh vegetables and fruits is an effective method to supply all essential antioxidants in physically active persons and athletes.^[40] Therefore, it can be concluded that the oral administration of OLE in exercise + OLE 200 by its potent

antioxidant activity can prevent severe exercise-induced hypertrophy.

In this study, RR interval in the exercise group alone and in combination with the extract as well as in the group receiving the highest dose of OLE (exercise, exercise + OLE 200, and OLE 400) was found to be higher than the control group. These results are fully consistent with the results of heart rate because the heart rate and RR interval have a direct inverse correlation such that an increase in RR interval causes a decrease in heart rate.^[41] Furthermore, in our study, increased QRS duration was observed only in the exercise group, which seems to be related to ventricular hypertrophy described above and this requires more research. This study indicated that forced exercise treadmill is considered a severe exercise in animals because it caused to prolongation of QT and QTc interval. Two-week consumption of OLE can prevent these changes in ECG of animals that have been under severe exercise. It is suggested that the effects of OLE on cardiac hypertrophy and changes in ECG be studied in mild, moderate, and severe exercise in future studies.

CONCLUSION

Results of this study indicated that forced exercise treadmill is a severe exercise training because it caused to QT and QTc prolongation which is an important risk factor for arrhythmias. Two-week oral administration of OLE has negative chronotropic and positive inotropic effects and also it can prevent of prolongation of QT and QTc interval induced by severe exercise.

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Conflicts of interest

There are no conflicts of interest.

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