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# Effects of Circuit Resistance Training with *Crocus Sativus* (Saffron) Supplementation on Plasma Viscosity and Fibrinogen

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## **BSTRACT**

A limited number of studies has been carried out concerning the combined effects of resistance training and saffron supplementation on cardiovascular risk factors. The aim of this study was to assess the effects of circuit resistance training with *Crocus sativus* (saffron) supplementation on plasma viscosity and fibrinogen. For this purpose, 44 healthy male subjects, based on individual characteristics and after homogenization, were divided into four groups, including water-training (WT; n=11), petal sweat-training (PST; n=10), bottom part of flower-training (BFT; n=11), and upper part of flower-training (UFT; n=12). Resistance training consisted of 12 stations (each station for 30 seconds with 40% of one repetition maximum) for 2 weeks (5 sessions per week). Saffron in the amount of 500 mg was used twice daily, i.e. in the morning and immediately after exercise. Blood samples were taken before and 48 hours after the last exercise session and were analyzed for fibrinogen and plasma viscosity. Significant differences were observed between groups in plasma levels of fibrinogen ( $P=0.01$ ). The post hoc test showed significant differences between the UFT and PST groups and the UFT and BFT groups (respectively,  $P=0.04$  and  $P=0.014$ ). In the post-test, plasma fibrinogen had significantly decreased in the WT ( $P=0.005$ ), PWT ( $P=0.003$ ), and UFT ( $P=0.001$ ) groups compared with pre-test data (within group difference). Moreover, plasma viscosity was significantly changed among groups ( $F_{3, 37}=3.52$ ,  $P=0.024$ ). The post hoc test showed significant differences between the UFT and WT groups ( $P=0.037$ ). In post-test data, plasma viscosity had significantly decreased in the WT ( $P=0.015$ ) and UFT ( $P<0.001$ ) groups compared with pre-test data. The present results show that circuit resistance training with saffron supplements can reduce cardiovascular risk factors (fibrinogen and plasma viscosity).

**Key Words:** Circuit resistance training, Saffron supplementation, Viscosity, Fibrinogen.

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## INTRODUCTION

Thrombus and atherosclerosis play important roles in cardiovascular and peripheral arterial system diseases and are of the most effective factors in mortality due to cardiovascular diseases in the industrialized world (1). Impairment of the rheological properties of normal blood is as an independent risk factor for coronary heart disease, especially viscosity which can increase coronary obstruction and blood pressure (2, 3). Increased blood viscosity may have adverse effects on blood flow and oxygen transport. Changes in levels of blood proteins, such as albumin and fibrinogen, increase according to the shape and size of the protein; the relationship between high levels of fibrinogen and increased blood and plasma viscosity is well understood. Fibrinogen is the largest plasma protein which includes an approximately 5.5% concentration of total plasma protein (4). Recent evidence has shown the role of fibrinogen in the pathogenesis of atherosclerosis vascular diseases and possibly as a risk factor for cardiovascular diseases (5). The role of fibrinogen is considered in blood homeostatic mechanisms and as a determinant of blood rheology through its influence on the red blood cell aggregation process (4). Increased plasma fibrinogen may cause adverse effects to the atherosclerosis process by increasing the interaction of platelets with the vessel wall, increasing blood viscosity through non-rheological routes such as blood coagulation, or by a direct effect on the vessel wall (6).

Blood viscosity generally depends on the blood concentration. In fact, hematocrit, viscosity, and blood viscosity are directly related to each other and inversely related to plasma volume (7). When hematocrit increases, blood viscosity increases; consequently, the blood flow rate decreases and thus, the supply of oxygen to tissue is reduced (7). The effects of different sports and physical activities on blood viscosity and

fibrinogen levels were examined in the current study. The results demonstrated the relationship between maximal oxygen uptake and a higher level of physical fitness (8, 9). Conversely, some studies have reported no change, and some have reported an increase of 28% after resistance activity. Some other studies have reported an increase of 38% after aerobic activity (10). Long-term training is not usually associated with significant changes in hematocrit, but it is associated with an increase in total plasma protein, which is one of the mechanisms causing plasma viscosity to increase (9). Although it seems that plasma viscosity increases in response to long-term training, not getting an increase in hematocrit in response to such training may show slight changes in total viscosity (8). Despite assessing the effect of light circuit resistance exercises on hemorheologic parameters such as the volume of platelets, white blood cells, and red blood cells (11), there is little information about the effects of sports activities, especially resistance activities on blood viscosity, plasma, and fibrinogen. Moreover, the effect of resistance exercises, particularly short-term and sequential circuit with food-drug supplementation has not yet been clarified. In one study using resistance exercises, however, it was shown that viscosity immediately increased and then decreased again during a 30 minute recovery period. Similar changes in levels of fibrinogen, plasma total protein, and albumin were seen, but at the end of the recovery period, these levels returned to the baseline (12). Considering the conflicting findings about blood viscosity, plasma, and fibrinogen as significant indices in cardiovascular diseases, the simultaneous use of a food-drug before or after physical exercise, especially sequential circuit resistance exercises, can undoubtedly provide a new area to manage the correct usage of food supplementation with training. *Crocus sativus*, or saffron, is

one of the considerable plants for Iran and other nations and is known as an expensive but pleasant flavor for everyone. General characteristics of the plant include it containing 10% moisture, 12% protein, 5% fat, 5% minerals, 5% crude fiber, and 63% carbohydrates (starches, restored sugars, pentoses, riboflavin and thiamine) (13).

The effect of saffron tablets on the short-term safety and tolerability of healthy adults was investigated. In this study, a dose of 200 mg of saffron reduced the platelet count, international normalized ratio (INR), and the time of bleeding (14, 15). The watery essence of saffron inhibited the platelet aggregation induced by ADP, epinephrine, and collagen. Chives of saffron have a protein which compresses the platelets (16). It is said that saffron has antioxidant properties that increase glutation levels and prevent lipid oxidation (17). Studies have reported the effects of saffron on atherosclerosis (18-20); it decreases lipid deposits in artery walls and consequently decreases atherosclerosis by reducing adhesive vascular molecules (20). Thus, considering the influence of resistance training on permanent increases in blood viscosity and plasma induced by increased fibrinogen, albumin, total protein, and hematocrit following resistance activities (21), this study sought to answer the

questions of whether taking all parts of saffron confirm or reject the possible effects of saffron with short-term resistance training and whether supplementation with various parts of the saffron flower strengthens the anticoagulant effects of physical exercise, thereby having a protective effect. The present study was designed to examine the effects of circuit resistance training while simultaneously taking various parts of the saffron flower (upper part, bottom part, and petal sweat) on viscosity and fibrinogen.

## MATERIALS AND METHODS

**Participants.** Forty-four students at Mazandaran University participated voluntarily in this study. Before participation, the whole methodology was explained to them, they completed a medical questionnaire, and they gave written consent. Inclusion criteria were no addiction to drugs, lack of regular physical activity for at least 6 months, no history of kidney, liver, cardiovascular diseases or diabetes or any injury or physical problem. Subjects were divided homogeneously into 4 groups:

- 1) Water + training (WT: n = 11)
- 2) Petal sweat + training (PST: n = 10)
- 3) Bottom part of flower + training (BFT: n = 11)
- 4) Upper part of flower + training (UFT: n = 12).

**Table 1. Descriptive characteristics of group samples (Mean  $\pm$ SD)**

Group	UFT	WT	PST	BFT
<b>Age</b> (years)	21.50 $\pm$ 1.93	21.91 $\pm$ 2.34	22.00 $\pm$ 2.35	21.18 $\pm$ 1.72
<b>Height</b> (cm)	175.92 $\pm$ 5.31	178.18 $\pm$ 4.75	175.10 $\pm$ 6.08	175.36 $\pm$ 4.6
<b>Weight</b> (kg)	67.42 $\pm$ 8.46	69.91 $\pm$ 9.40	73.10 $\pm$ 10.51	67.36 $\pm$ 8.21
<b>BMI</b> (kg/m <sup>2</sup> )	21.75 $\pm$ 1.96	22.00 $\pm$ 2.96	23.90 $\pm$ 2.72	21.82 $\pm$ 2.60

**UFT:** Upper part of flower + training, **WT:** Water + training, **PST:** Petal sweat + training, **BFT:** Bottom part of flower + training

**Procedure.** Subjects received 500 mg of saffron in two steps, a 250 mg capsule after

breakfast and then a 250 mg capsule along with 100 ml of water immediately after

exercise. The PST group consumed 200 ml of petal sweat with a placebo, and the WT group consumed 200 ml of water with a placebo. According to the exercise procedure, subjects were familiarized with the working environment before performing circuit resistance exercises, and they came to the exercise location to determine 1RM of

activities during three separate sessions. Values of 1RM of activities such as the squat, bench press, standing calf raise, military press, leg press, rowing, leg extension, leg curl, French press, trunk extension, and sit-ups were calculated using the trial and error and the Berzisky equation methods (22).

**Berzisky equation to calculate 1RM:**

$$1 \text{ RM} = \frac{(\text{kg}) \text{ displaced weight}}{1.0278} - (\text{number of repetitions up to fatigue} \times 0.0278)$$

Subjects performed these activities with 40% of mean 1RM and moderate velocity for 2 weeks (5 sessions per week). Each exercise session consisted of 5 minutes of warm-up and then nonstop exercise between the 12 activity stations. The duration of each station was 30 seconds. The number of repetitions for each subject at each station was recorded. For the first two sessions, one period of exercise was done. From the third session on, subjects did exercises for two periods between which they took an active rest for 3 minutes. Blood was sampled from an arm

vein after 10 to 12 hours fasting, two times: 48 hours before starting exercises and 48 hours after the latest session of exercises. Blood samples were poured into test tubes containing EDTA and centrifuged for 10 minutes at 3000 rounds per minute. Then, the separated plasma was used to analyze fibrinogen, and other factors were used to calculate viscosity. Fibrinogen was measured by a set (LABI TECH) made in Germany. Viscosity was calculated using the following formula (7):

$$\text{Plasma viscosity} = 1.352 + 0.0167 \times \text{total cholesterol (mmol)} + 0.0285 \times \text{fibrinogen (g/l)} + 0.0054 \times \text{triglyceride (mmol)} + 0.00318 \times \text{hematocrit} - 0.03 - c \text{ (mmol)}$$

**Statistical Analysis.** Data was analyzed using SPSS software version 20. Normal distribution of data was confirmed using the Kolmogorov Smirnov test. Repeated measures (4×2) ANOVA was also used to analyze data. Independent t-test was used to find intergroup changes. A level of (0.05) was considered significant.

## RESULTS

Statistical analysis showed no significant differences between plasma levels of fibrinogen in the groups before and after exercise using the two-way repeated ANOVA test, ( $f_{3,40} = 0.206$ ,  $p = 0.89$ ), but

there was a significant difference between various times ( $f_{1,40} = 39.96$ ,  $p < 0.001$ ). Results of the Bonferoni test showed no significant differences between groups before and after exercise ( $P > 0.05$ ). A negative interaction between time and group ( $f_{3,40} = 4.43$ ,  $p = 0.009$ ) was also observed (Fig. 1). Using the dependent t test, a significant difference was observed in pre- and post-test data in the WT ( $t_{10} = 3.54$ ,  $p = 0.005$ ), PST ( $t_9 = 3.06$ ,  $p = 0.003$ ), and UFT ( $t_{11} = 4.52$ ,  $p = 0.001$ ) groups, but no significant difference was seen between pre-test and post-test data of the BFT group ( $t_{10} = 1.52$ ,  $p = 0.15$ ) (Fig. 1).

Statistical analysis of plasma viscosity levels in pre-test and post-test data using two way repeated ANOVA showed no significant differences between groups ( $f_{3, 40} = 0.997$ ,  $p = 0.4$ ), but there was a significant difference between various times ( $f_{1, 40} = 24.42$ ,  $p < 0.001$ ). Results of the Bonferoni test on pre- and post-test data showed no significant differences in times

between the various groups ( $P > 0.05$ ). There was, however, a significant interaction between time and group ( $f_{3, 40} = 3.19$ ,  $p = 0.034$ ) (Fig. 1).

Using the dependent t test, significant differences between pre-test and post-test data in the WT group ( $t_{10} = 2.93$ ,  $p = 0.015$ ) and the UFT group ( $t_{11} = 5.42$ ,  $p < 0.001$ ) were observed (Fig. 2).

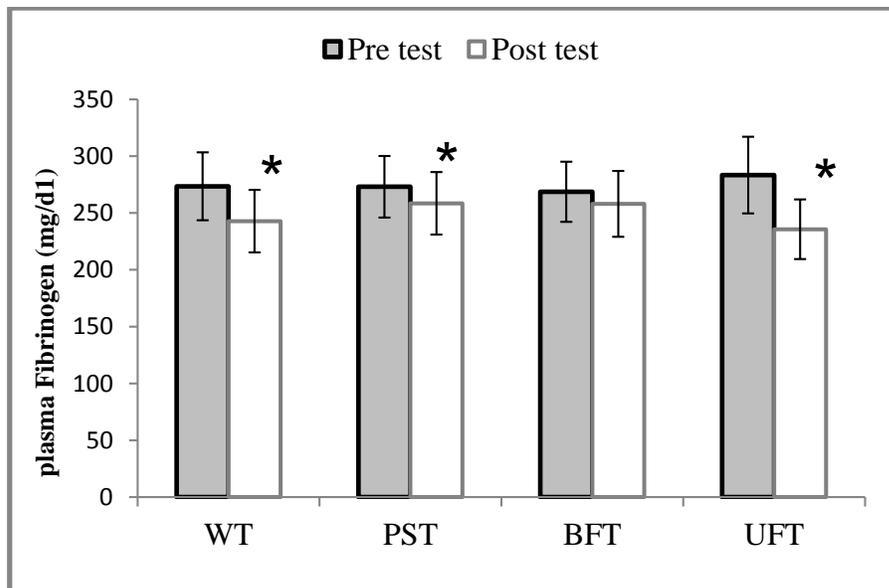


Fig 1. Fibrinogen data. \*shows significant difference between pre-test and post-test data in the groups ( $p < 0.05$ ). WT, water-training; PST, petal sweat-training; BFT, bottom part of flower-training; UFT, upper part of flower-training.

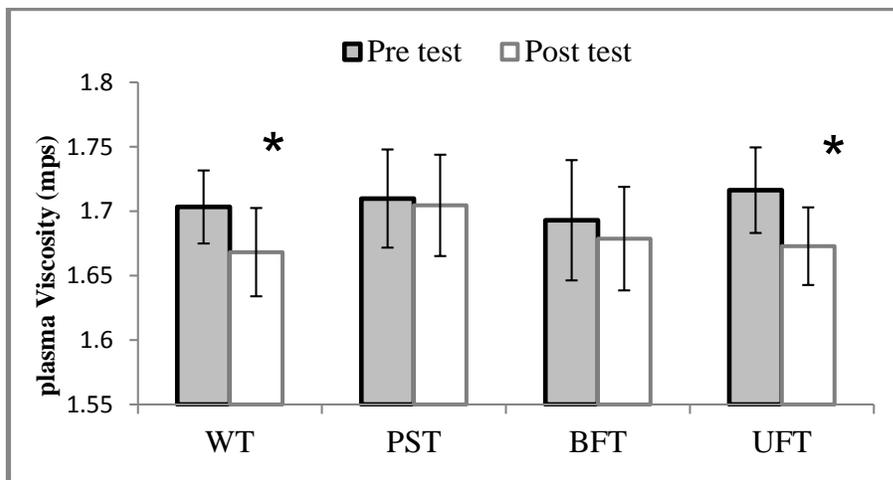


Fig 2. Viscosity data. \*shows significant difference between pre-test and post-test data in the groups ( $p < 0.05$ ). WT, water-training; PST, petal sweat-training; BFT, bottom part of flower-training; UFT, upper part of flower-training.

## DISCUSSION

Sports activities cause changes in blood rheology corresponding with kind, duration, and intensity of the activity as well as an individual's physical fitness (23). Previous studies have indicated that changes in the blood hemorheology are induced by resistance exercises. Intensive resistance activity causes increases in the viscosity and fibrinogen of plasma (12).

In the present study, the effects of short-term circuit exercise on the viscosity and fibrinogen of plasma with and without saffron supplementation were examined. Plasma fibrinogen decreased 10% after two weeks of short-term circuit resistance exercises. In agreement with this study, Ahmadizad *et al.* (12) found that intensive resistance activity increased fibrinogen levels in plasma, yet these levels returned to the baseline during a 30-minute recovery period. They suggested that this mechanism probably occurs because of a decrease in blood plasma levels. Ghanbari Niaki *et al.* (7) found no changes in fibrinogen levels in response to 4 weeks of progressive aerobic exercises. Results of Ghanbari's study contradicts those of the present study. Results of the current study are in agreement with those of Kilic-Toprak *et al.* (24), who indicated a decrease in fibrinogen levels after three weeks of progressive resistance exercises; converse to the current study, however, they reported a return to baseline after twelve weeks (24). This contradiction was induced by the duration, intensity, and entity of resistance exercises used in the present study. Researchers found that circuit resistance exercises are more effective on increasing aerobic capacity and improving cardiorespiratory function than conservative resistance exercises (25, 26). Thus, considering the entity of circuit resistance exercises in this study, a possible mechanism of the decrease in fibrinogen level is the increase in plasma volume and improvement of the cardiovascular system.

Other possible mechanisms to reduce fibrinogen are an increase in nervous system activity and a change in the lipid profile (27, 28). Some studies have indicated an improvement in the lipid profile in response to resistance exercises (29, 30). A decrease in the lipid profile serves to decrease fibrinogen, because fibrinogen has a direct relationship with LDL and an inverse relationship with HDL (7). Increases in aerobic capacity and muscle mass induced by circuit resistance exercises (31) can improve lipid metabolism, and as a result, short-term, two-week circuit resistance exercises lead to reduced fibrinogen by improving lipid profiles and metabolism.

When a supplement from the upper part of the saffron flower was used during an exercise period, the effect of exercise was doubled and plasma fibrinogen was reduced 16%, although there was no significant difference between this group and the others. The plasma fibrinogen in the PST group was reduced 5%, which is a lower reduction compared with the UFT group. Through an examination of the materials of the petal sweat, bottom part, and upper part of the saffron flower, it was found that the upper part had more linoleic acid than its other parts. Moreover, safranal, the main material of saffron, exists only in the upper part. Researchers have indicated that saffron supplements improve lipid profiles (19).

Saffron also has antioxidant properties, and its supplement reduces blood coagulation by inhibiting lipid oxidation (17) and inhibiting platelet aggregation induced by ADP, epinephrine, and collagen (16). A possible reason for the decrease in blood coagulation is a reduction in coagulation factors like fibrinogen. In this study, an upper part of saffron supplement coupled with circuit resistance training reduced fibrinogen 16%. A definite mechanism in this field has not yet been determined; more research is needed to achieve definite results.

In general, blood circulation is reduced during sports activities, because the volume of plasma decreases and blood hematocrit increases (4). Blood hematocrit decreases after doing sports exercises because of the increase in plasma volume (32). Hematocrit is one of the main determinants of blood viscosity (4). Fibrinogen, the largest plasma protein and a main determinant of plasma and viscosity (7), was significantly reduced in all groups except BFT. In this study, resistance exercises reduced plasma viscosity by 2% in the WT group. In agreement with the present study, Kilic-Toprak *et al.* (24) indicated an 11% reduction in plasma viscosity after 3 weeks of progressive resistance exercises. This difference in reduction rates probably occurred because of the duration, intensity, and entity of the exercise periods. Plasma viscosity depends on plasma proteins, such as fibrinogen and globulin (33). Fibrinogen is one of the possible mechanisms for decreasing plasma viscosity. Increased levels of fibrinogen in plasma, increased platelet interaction with vessel walls, and intensification of homodynamic disorders lead to increased blood and plasma viscosity (4). It is well understood that increased platelet aggregation results in a rise in blood viscosity (34). In the present study, a 10% reduction in fibrinogen following circuit resistance exercises was associated with a 2% reduction in plasma viscosity. Researchers have indicated that high intensity resistance exercise led to increased platelet aggregation, but regular sports exercises led to increased plasma volume and decreased hematocrit in proportion to blood volume and, consequently, reduced plasma viscosity (35). Furthermore, the utilization of a supplement from the upper part of saffron in this study resulted in a 2.5% reduction in plasma viscosity during circuit resistance exercises. Although there was no significant differences between this group and the other groups, the rate of reduction was considerable in this group. This rate of reduction seems

reasonable regarding the 16% decrease in fibrinogen. Examining the components of the upper part of saffron, it was understood that safranal, one of the main components of saffron, didn't exist in the bottom part or the petal sweat of saffron. Safranal has an antioxidant property which prevents lipid oxidation (36). The upper part of saffron also has more linoleic acid than its petal sweat or bottom part, and it causes a reduction in vascular adhesive molecules and lipid deposits in vessel walls. Saffron supplements lead to increased blood circulation and decreased viscosity (20). Oleic acid causes a reduction in ADP and collagen-stimulating platelets and, consequently, results in a reduction of platelet aggregation (37). It seems, therefore, that an upper part of saffron supplement coupled with short-term circuit resistance training can cause a greater reduction in plasma fibrinogen levels and platelets aggregation and consequently, a slight reduction in plasma viscosity.

Studies have indicated that plasma viscosity has a significant relationship with physical fitness and heart function. In individuals who exercise, some parts of the reduction in viscosity is induced by a reduction in gamma globulin levels. Moreover, plasma viscosity and fibrinogen levels are lower in marathon runners than in individuals who did not exercise. That is because of the increase in plasma volume (4). This study examined subjects who did not exercise and had low physical fitness. Additionally, the duration of exercise and the time of saffron supplement use was short. Possibly, the short duration of exercise resulted in little adaptation in the cardio-respiratory system and, consequently, had little effect on plasma viscosity. It seems that more considerable changes can be observed with a longer duration of saffron supplement use and circuit training. Because there is no determined mechanism for saffron supplement with circuit training, more research is needed.

**CONCLUSION**

The findings of the present study indicated that two weeks of short-term circuit resistance exercises caused a 10% reduction in plasma fibrinogen and a 2% reduction in plasma viscosity in proportion to pre-test data. The upper part of saffron supplement used with circuit resistance caused 16% and 2.5% post-test reductions, respectively, in fibrinogen and plasma viscosity. There was no significant difference between various parts of saffron with resistance training, but the upper part of saffron had more effects in comparison with its petal sweat and bottom part and doubled the effects of circuit training. Considering the results of this study, it can be concluded that saffron supplement use combined with two weeks of circuit resistance training can reduce cardiovascular

risk factors (fibrinogen and plasma viscosity), although supplement with the upper part of saffron in combination with saffron caused a greater reduction in these risk factors.

**APPLICABLE REMARKS**

- All parts of *Crocus Sativus* (Saffron) specially upper part could be useful for athletes and non-athletes who want to improve their anti-fibrinolytic capacity.
- Circuit resistance training is a one of training strategies to provide a better condition for cardiovascular functions.

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تازه‌های علوم کاربردی ورزش

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## تأثیر تمرین مقاومتی دایره‌ای همراه با مکمل کروکوس ساتیووس (زعفران) بر فیبرینوژن و ویسکوزیته پلاسما

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### چکیده

در مورد تأثیر ترکیب تمرین مقاومتی با مکمل دهی زعفران بر عوامل خطرزای قلبی عروقی مطالعات محدودی انجام شده است. هدف از تحقیق حاضر بررسی اثرات تمرین مقاومتی دایره‌ای کوتاه مدت با و بدون مکمل زعفران بر ویسکوزیته و فیبرینوژن پلاسما بود. برای این منظور ۴۴ مرد سالم تمرین نکرده بعد از همگن سازی بر اساس ویژگی های فردی به ۴ گروه آب-تمرین (۱۱ نفر)، عرق-تمرین (۱۰ نفر)، ته گل-تمرین (۱۱ نفر) و سرگل-تمرین (۱۲ نفر) تقسیم شدند. تمرین مقاومتی شامل ۱۲ ایستگاه (هر ایستگاه ۳۰ ثانیه با شدت ۴۰٪ یک تکرار بیشینه) به مدت ۲ هفته (۵ جلسه در هفته) بود. روزانه ۵۰۰ میلی گرم زعفران در دو وعده صبح و بلافاصله بعد از تمرین استفاده شد. نمونه خونی قبل و ۴۸ ساعت بعد از آخرین جلسه تمرین گرفته شد و برای آنالیز فیبرینوژن و ویسکوزیته مورد استفاده قرار گرفت. تفاوت معنی داری در سطوح پلاسمایی فیبرینوژن بین گروه‌ها مشاهده نشد ( $F_{3,4} = 0/206, P = 0/89$ ). اما تعامل معنی داری بین زمان و گروه مشاهده شد ( $P = 0/009, F_{3,4} = 4/43$ ). آنالیزهای بعدی نشان داد که فیبرینوژن پلاسما در پس آزمون در گروه‌های آب-تمرین ( $P = 0/005$ )، عرق-تمرین ( $P = 0/003$ ) و سرگل-تمرین ( $P = 0/001$ ) نسبت به پیش آزمون (تفاوت درون گروهی) کاهش معنی داری داشت. همچنین ویسکوزیته پلاسما بین گروه‌ها اختلاف معنی داری نداشت ( $P = 0/4, F_{3,4} = 0/997$ ). اما تعامل معنی داری بین زمان و گروه مشاهده شد ( $P = 0/034, F_{3,4} = 3/19$ ). آنالیزهای بعدی کاهش معنی دار ویسکوزیته پلاسما بعد از تمرین در گروه‌های آب-تمرین ( $P = 0/015$ ) و سرگل-تمرین ( $P < 0/001$ ) را نسبت به پیش آزمون نشان داد. نتایج حاصل از این تحقیق نشان داد که تمرین مقاومتی دایره‌ای به همراه مصرف مکمل زعفران می تواند موجب کاهش عوامل خطرزای مستقل قلبی-عروقی (فیبرینوژن و ویسکوزیته پلاسما) شود.

**واژگان کلیدی:** تمرین مقاومتی دایره‌ای، مکمل زعفران، ویسکوزیته پلاسما، فیبرینوژن.

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