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Original Article

Impact of an Eight-Week Moderate-Intensity Aerobic Training Program, Combined With Supplementation of the Aqueous Extract of *Cynodon dactylon*, on Hemorheological Indices in Young Non-athletic Men

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Abstract

Background: Cardiovascular diseases are the leading cause of death in developing countries and hemorheological indices are the main factors in developing these diseases.

Objectives: The main objective of this study was to investigate the impact of eight weeks of moderate intensity aerobic training combined with supplementation of aqueous extract of *Cynodon dactylon* L. Pers on hemorheological parameters in non-athletic young men.

Methods: A total of 40 young non-athletes (with a mean age of 35.63 ± 6.43 years and a body mass index (BMI) of 23.55 ± 2.13 kg/m^2) were randomly assigned to one of four groups, including control (C), aerobic exercise (E), the *C. dactylon* extract (CD), and aerobic exercise + *C. dactylon* extract (E + CD). Changes in fibrinogen, blood and plasma viscosity, and hematocrit were measured at two stages (baseline and 24 hours after the intervention ended).

Results: The blood fibrinogen level in three groups of E, CD, and E + CD showed a significant decline compared to the baseline status ($P < 0.05$). Additionally, there were significant decreases in blood viscosity levels in groups E ($P = 0.006$), CD ($P = 0.048$), and E + CD ($P = 0.001$) after eight weeks of intervention. The plasma viscosity also showed a significant reduction only in group E after eight weeks of intervention ($P = 0.004$). Moreover, hematocrit level significantly decreased in group E + CD ($P = 0.018$).

Conclusion: The findings of this study suggest that eight weeks of moderate intensity aerobic exercises alone and in combination with the consumption of the aqueous extract of *C. dactylon* L. Pers could improve hemorheological indices and reduce the risk of cardiovascular diseases.

Keywords: Aerobic exercise, *Cynodon dactylon* rhizome, Fibrinogen, Viscosity, Hematocrit

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Background

Despite significant advancements in healthcare methods, cardiovascular disease remains the leading cause of death globally (1). The Ministry of Health in Iran reports that heart attack is the second most common cause of death in the country after road traffic accidents (1). Multiple factors and mechanisms contribute to the development of cardiovascular diseases, including lipid disorders, smoking, high blood pressure, mental stress, inflammatory diseases, and coagulation disorders (2).

Blood rheology is the study of the flow properties of the blood and its components in plasma and cells. Inadequate tissue perfusion and oxygen delivery due to disturbances in the rheological properties of blood are among the main causes of cardiovascular disorders. Blood viscosity is the main determinant of blood rheology, and elevated levels can have negative impacts on blood flow and oxygen delivery to tissues (3). A linear logarithmic relationship exists between the volume percentage of red blood cells (hematocrit) and blood viscosity (4). Among coagulation markers, fibrinogen is considered the most reliable indicator for evaluating the likelihood of cardiovascular impairments. It is the largest plasma protein, accounting for approximately 5.5% of total plasma protein concentration (5). It is the final substrate of the coagulation system and is

converted into fibrin by thrombin. Fibrinogen has multiple functions, including converting to fibrin, which is a cofactor for platelet aggregation, determining blood rheology, and facilitating leukocyte adhesion (4). Epidemiological studies have shown that high plasma fibrinogen levels are associated with an increased risk of cardiovascular disorders (6). During inflammation, fibrinogen levels increase alongside interleukin-6 plasma concentration. The relationship between high fibrinogen levels and increased blood and plasma viscosity is well-known and can significantly affect hemorheological properties.

Today, the most commonly recommended solutions for reducing and controlling cardiovascular diseases are behavioral and lifestyle changes. These changes typically involve increasing physical activity and utilizing medicinal or natural supplements (7). Both physical activity and regular medical check-ups are key factors in extending life expectancy and improving overall quality of life. Numerous studies have shown that regular aerobic exercise can effectively inhibit the inflammatory responses that contribute to cardiovascular disease risk (2).

In recent years, there has been an increase in attention towards the application of herbal medicines due to their minimal side effects. It is noteworthy that approximately 25% of all medicines available in America are derived from medicinal plants (8). *Cynodon dactylon*, which belongs to the Graminaceae family, has been recognized as a medicinal plant in certain regions of Iran, including Azerbaijan, since ancient times (9). Studies have revealed that the extract of this plant contains a number of beneficial compounds, including beta-carotene, beta-sitosterol, vitamin C, palmitic acid, flavonoids, saponins, triterpenoids, alkaloids, furfural, glucose, fructose, and selenium (10). The presence of these compounds in *C. dactylon* has been reported to result in various effects, including anti-cancer, anti-seizure, anti-heart irregularity, anti-pain, anti-fever, anti-microbial, anti-inflammatory, and immune modulatory effects (11).

Previous studies have shown that regular and appropriate physical activities can result in "hemorheological fitness," which is characterized by a reduction in hematocrit, red blood cell (RBC) aggregation, whole blood and plasma viscosity, and an improvement in RBC deformability (12,13). A meta-analysis study conducted by Romain et al (14) also highlighted that regular exercise can improve hematocrit and RBC aggregation. On the other hand, studies have suggested that plasma factors, especially fibrinogen level (15,16) and blood/plasma viscosity (17,18), can be used to evaluate RBC aggregation and deformability, respectively. Therefore, in this study, we investigated the impact of eight weeks of aerobic exercise combined with *C. dactylon* extract supplementation on fibrinogen level, blood viscosity, plasma viscosity, and hematocrit in sedentary young men, considering the potential advantages of physical activity and this extract in preventing atherosclerosis.

Materials and Methods

Study Population

The current double-blind study was conducted with the approval of the Ethics Committee of Urmia University (IR.UMSU.REC.1397.4). The study focused on sedentary healthy men aged 30 to 55 years old, who had not engaged in regular exercise for the past six months and had similar anthropometric characteristics. All participants provided written consent before being randomly divided into four groups: control (C, n = 10), aerobic exercise (E, n = 10), *C. dactylon* extract supplementation (CD, n = 10), and aerobic exercise with *C. dactylon* extract supplementation (E + CD, n = 10).

Herbal Extraction Preparation

The *C. dactylon* plant was collected from the Anzal area of Gurchin Qala village in Urmia city. An expert botanist from Urmia University determined the species of the plant. To prepare the aqueous extract from the rhizome of the plant, Soxhlet extraction method was used as previously reported (19). In brief, the rhizome of the plant was shade-dried at a temperature of 25 °C, and it was ground into powder using a mechanical mill. Then, 10 g of plant powder was added to 150 mL of distilled water in a Soxhlet extraction apparatus (Soxhlet Electrothermal, Rochford, UK). After 12 hours, the extract was dried using a rotary evaporator (Electrothermal, Rochford, UK). The recommended dose was 100 mg/kg/d, which equates to approximately 5 to 7 mL/d. The groups with no extract supplementation (C and E groups) were given a placebo consisting of 5 mg/kg of Maltodextrin (Zar Fructose, Hashtgerd, Iran) dissolved in distilled water.

Exercise Program

The participants performed aerobic exercises three times a week at a gym. In the first week, the activity level was set at 50%-60% of their reserve heart rate for 50 minutes. Over the following eight weeks, the intensity of the exercises increased by 3% every two weeks, and the session duration increased by 5 minutes. From the second to the eighth week, the participants ran at an intensity of 60%-75% of their reserve heart rate for 60 minutes. The subjects warmed up and cooled down before and after each session with stretching and softening movements, lasting 10-20 minutes each.

Sample Collection and Analyses

Prior to each blood sampling, participants were instructed to complete a 24-hour dietary recall questionnaire. They were also advised to avoid strenuous activities and the consumption of fat-burning or anti-inflammatory supplements, such as ibuprofen or ginger. Blood samples were taken 48 hours following the last day of training and/or supplementation. In order to measure blood rheology indicators in plasma, 4 mL of each sample was collected into anticoagulant-containing tubes, while the remaining samples were centrifuged to obtain serum. All plasma

and serum samples were stored in separate tubes at a temperature of -20 °C for subsequent testing.

The fibrinogen levels were assessed utilizing a commercial fibrinogen assay kit (Mahsa Yaran Kit, Tehran, Iran) based on the Clauss method (20). Additionally, the hematocrit was determined using a Sysmex KX-21N cell counter (Sysmex Corporation, USA). To measure blood viscosity, a Cone Plate Wells-Brookfield Micro Viscometer model LVT (Brookfield Engineering, California, USA) was utilized.

Statistical Analysis

The distribution and homogeneity of the data were assessed using the Shapiro-Wilk test and independent *t* test, respectively. After confirming the normality of the data, statistical analyses were conducted using paired *t* test, repeated analysis of variance, and Bonferroni's post hoc test, with a significance level of 0.05. SPSS version 26.0 was utilized for the analyses.

Results

Table 1 presents the descriptive information of all groups, including age, height, weight, and body mass index (BMI). The results showed that there was no significant difference in these factors among the four studied groups ($P>0.05$).

Table 1. Characteristics of sedentary young men participated in the study

	C (n=10)	E (n=10)	CD (n=10)	E+CD (n=10)
Age (y)	34.71±6.8	38.3±8.41	33.4±2.95	36.3±6.18
Height (m)	1.71±0.1	1.72±0.1	1.73±0.1	1.73±0.2
Weight (kg)	69.96±1.87	71.6±2.06	70.07±2.06	69.13±2.5
BMI (kg/m^2)	23.87±0.47	24.15±0.7	23.38±0.64	23.03±0.91

BMI: body mass index; C: control; E: aerobic exercise; CD: *Cynodon dactylon* extract supplementation; E+CD: aerobic exercise+*Cynodon dactylon* extract supplement.
Data are shown as mean±standard deviation. No significant difference was observed among the groups ($P>0.05$).

Table 2 displays the results of blood rheology indices. We observed a significant reduction in blood fibrinogen levels in the three groups [E ($P<0.001$), E+CD ($P<0.001$), and CD ($P=0.016$)] compared to the baseline status. However, the comparison among the groups did not show a significant difference ($P>0.05$). The blood viscosity was also significantly decreased in groups E ($P=0.006$), CD ($P=0.048$), and E+CD ($P=0.001$) after the 8-week intervention. However, the plasma viscosity showed a significant decline only in group E after the 8-week intervention ($P=0.004$), with no significant change among other groups ($P>0.05$). Additionally, hematocrit significantly decreased compared to baseline only in group E+CD ($P=0.018$).

Discussion

The findings of this study demonstrated that the blood fibrinogen level was significantly reduced after eight weeks of aerobic exercise, *C. dactylon* extract supplementation, or a combination of both. These results are consistent with previous research indicating that aerobic exercise could lower fibrinogen levels (12, 21-24). For example, Soltani et al (25) found a decrease in fibrinogen following intense intermittent exercise in patients with high blood pressure. Given that fibrinogen is a major independent risk factor for atherosclerosis and heart attacks, even a slight decrease in its concentration could indicate a reduction in the risk of atherosclerosis. Regular aerobic exercise may reduce fibrinogen levels by modulating catecholamine stimulation and increasing blood flow to the muscles (26). A cross-sectional study has revealed a negative correlation between regular physical activity and fibrinogen levels

Table 2. Comparison of Rheology Indices among Study Groups

	Groups	Time of evaluation		*P* value (before vs. after the intervention)	*P* value (among groups)
		Bassline	After eight weeks		
Fibrinogen (mg/dL)	C	225.7±11.16	230.4±9.87	0.062	0.73
	E	229.33±11.6	217.11±11.29	<0.001	
	CD	235.0±9.51	229.8±8.85	0.016	
	E+CD	226.3±9.81	207.4±9.12	<0.001	
Blood viscosity (centipoise)	C	4.76±0.20	4.93±0.21	0.11	0.241
	E	4.80±0.24	4.60±0.19	0.006	
	CD	5.07±0.23	4.91±0.20	0.048	
	E+CD	4.60±0.21	4.28±0.15	0.001	
Plasma viscosity (centipoise)	C	1.63±0.10	1.64±0.09	0.823	0.098
	E	1.62±0.12	1.50±0.11	0.004	
	CD	1.64±0.39	1.63±0.38	0.427	
	E+CD	1.95±0.25	1.63±0.10	0.133	
Hematocrit (%)	C	45.49±1.61	45.18±1.37	0.302	0.084
	E	45.52±1.07	48.06±0.04	0.186	
	CD	44.54±1.10	44.68±0.88	0.730	
	E+CD	44.62±0.98	41.07±0.86	0.018	

(27). There are several possible mechanisms that could explain this association. Firstly, aerobic exercise may cause a decrease in fibrinogen due to an increase in high-density lipoprotein (HDL) and a decrease in low-density lipoprotein (LDL), as well as a reduction in stress and fat percentage (28). Secondly, regular aerobic exercise could reduce fibrinogen concentration in the blood by modulating catecholamine stimulation and increasing overall blood volume (26). Finally, exercise may also modulate inflammatory processes, leading to a decrease in fibrinogen concentration (29). In contrast to our findings, Amiri Parsa et al (30) and Kushnick et al (31) did not observe a positive impact of aerobic exercise on fibrinogen level. This discrepancy may be attributed to the interplay between genetic factors and environmental factors that regulate plasma fibrinogen levels (32). Previous research on *C. dactylon* extract has shown its ability to regulate immune responses and inflammation in mice (33). Additionally, research has suggested a relationship between inflammation and fibrinogen levels (34). It could be postulated that *C. dactylon* extract might decrease fibrinogen level through modulating inflammatory responses (33,34).

In terms of blood and plasma viscosity, we observed a noteworthy reduction in whole blood viscosity in individuals who participated in aerobic exercise, took *C. dactylon* extract supplements, or did both. However, only those in the exercise group experienced a decrease in plasma viscosity. Studies have reported that regular physical activity may improve blood and plasma viscosity by decreasing erythrocyte aggregation (14) and fibrinogen levels (12,21-24), as seen in the current study. Additionally, regular exercise can increase the production of red blood cells and facilitate the breakdown of old red blood cells. This process enhances the ability of the blood to carry oxygen and reduces its viscosity, leading to better circulation and improved blood flow (35). Consistent exercise has also been linked to reduced inflammation, which can result in an improvement in blood flow and a decrease in blood and plasma viscosity (36). The lack of a significant effect of *C. dactylon* extract on plasma viscosity, while reducing whole blood viscosity, may be attributed to the presence of various bioactive compounds in the extract that decrease whole blood viscosity. In this regard, previous studies on rats and fish have shown that *C. dactylon* extract could affect the quantity and quality of red blood cells which are the main factors determining whole blood viscosity (37,38). Some compounds in herbal extracts, such as flavonoids, have been found to increase red blood cell deformability and reduce their tendency to aggregate, allowing them to change shape easily and flow more smoothly through narrow blood vessels, preventing clumping and increasing blood viscosity (39). However, plasma viscosity is mainly determined by the concentration and quality of proteins and other molecules in the plasma. The *C. dactylon* extract likely does not affect the concentration or composition of plasma proteins, so it does not have an impact on plasma viscosity. The group that received the herbal extract and underwent aerobic exercise had a significant decrease in hematocrit. These findings align with previous research indicating that exercise can reduce hematological factors (40,41). However, some studies have suggested that exercise may increase blood viscosity as a result of elevated levels of hematocrit and RBC (42,43).

Conclusion

Fibrinogen is known to accelerate the accumulation of RBC. Consequently, reducing fibrinogen levels through aerobic exercise and consumption of herbal extract can effectively lower blood viscosity in sedentary men. The study revealed that an 8-week aerobic exercise program, with or without *C. dactylon* extract supplementation, significantly improved the fibrinogen plasma level, blood viscosity, plasma viscosity, and hematocrit in sedentary men. Therefore, it is suggested that exercise combined with the consumption of this extract can be beneficial in mitigating cardiovascular risk factors.

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Authors' Contribution

Conceptualization: Mohammad Reza Zolfaghari, Kamal Khademvatani, Amir Fattahi.
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Competing Interests

The authors declare that they have no conflict of interests.

Ethical Approval

This study was approved by the Ethical Committee of Urmia University (IR.UMSU.REC.1397.4).

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