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The Effects of Aerobic Training and Moringa oleifera on High-Density Lipoprotein and Cardiac Endurance among Males

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ABSTRACT

Aerobic training is a form of physical workout in which heart and lungs work efficiently to supply oxygen to the muscles for improving athletic performance. **Objective:** To investigate the effect of moderate aerobic training and *Moringa oleifera* leaf powder (MOLP) on high-density lipoprotein (HDL) and cardiac endurance among males. **Methods:** One hundred (100) male volunteers of age group 38.9 ± 6.7 years were randomly and divided into four groups i.e. the control group (T_0), the moringa group (T_1), the aerobic group (T_2), and the Combined Group (T_3). *Moringa oleifera* leaf powder (3 gm) was given to the T_1 and T_3 daily for five days a week for 12 weeks, while the T_0 continued a normal diet routine. **Results:** Results indicate an increase in HDL posttest mean values of three interventional groups T_1 (41.80 ±4.81 mg/dl), T_2 (42.08 ± 1.28 mg/dl) and T3 (43.40 ±1.77 mg/dl) as compared to control group T0 (36.28 ±1.86 mg/dl). Beep tests mean values of the post-test of interventional groups T1 (10.1±1.24), T2 (10.2 ± 1.25) and T_3 (12.0 ± 1 .18) and control group T_0 (7.3 ± .67) were recorded. **Conclusions:** It was concluded that moderate aerobic training and MOLP have a positive effect on HDL and cardiac endurance among the interventional groups (T_1, T_2, and T_3), with more significant improvement in T_3 and no improvement in the control group.

INTRODUCTION

Aerobic training is a form of physical exercise in which the heart and lungs work efficiently to supply oxygen to the muscles, improving cardiovascular function and endurance. Regular aerobic exercise lowers resting heart rate, increases blood volume, and enhances overall health by improving body weight, energy levels, sleep patterns, and athletic performance [1]. Importantly, it reduces the risk of chronic diseases such as heart disease, diabetes, and cancer. Insufficiency aerobic activity influences approximately 17% of heart disease, 12% of diabetes, and 10% of breast cancer cases globally [2]. Health professionals recommend engaging in aerobic activities like running, cycling, swimming, and rowing for 20-30 minutes, three to five times per week, to improve physical fitness and cardiac endurance [3]. Several studies concluded that Moringa leaves provide several health advantages, including anti-diabetic, antibacterial, anti-cancer, and anti-inflammatory properties. Moringa has been extensively used as a health-promoting food against various diseases and health issues [4]. The estimates from the World Health Organization (WHO) say that 80% of nations with limited resources and 60% of people worldwide acquire their primary medical assistance from herbal medicine [5]. Moringa is a highly nutritious plant that can help with many health issues, improve mineral deficiency and prevent malnutrition [6]. The combination

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of herbal supplements with regular aerobic exercise can improve physical performance and reduce the harmful effects of synthetic medicines [7]. Research shows that regular exercise and herbal supplements (Moringa) can dramatically increase HDL levels and cardiac endurance, when physical treatment is used along with herbal supplements [8, 9]. A lack of physical activity is a big concern by 2030, nearly 40% of adults in the U.S. could develop heart disease because of a sedentary lifestyle [10]. It was investigated the impact of aerobic training on hemoglobin and cardiovascular efficiency and discovered that hemoglobin levels and heart function significantly increased [11, 12]. Herbal products are abundant in nutrients and phytochemicals that have positive health effects and Moringa supplements are frequently used to treat malnutrition and boost nursing mothers' milk production [13, 14]. Recent studies have highlighted the synergistic benefits of aerobic exercise and *Moringa oleifera* supplementation on cardiovascular performance and metabolic health. Gbedinhessi *et al.*, demonstrated that supplementation with *Moringa oleifera* leaf powder significantly enhanced cardiorespiratory performance and endurance in trained individuals during a 20 km cycling time-trial, suggesting improved aerobic capacity and oxygen utilization [15]. Similarly, Muhammed *et al.*, conducted a randomized controlled trial in patients with type 2 diabetes and found that combining aerobic exercise with *Moringa oleifera* supplementation led to notable improvements in glycemic control and metabolic health markers, indicating a cardioprotective effect [16]. Emerging evidence supports the role of *Moringa oleifera* in enhancing physical endurance through metabolic and antioxidant pathways. Bian *et al.*, investigated the effects of a flavonoid-rich concentrate derived from *Moringa oleifera* leaves and found that it significantly prolonged exhaustive swimming time in mice by boosting energy metabolism and enhancing antioxidant capacity [17]. The ability of the heart and lungs to make sure that muscles get enough oxygen during extended physical activity is known as cardiac endurance and a sedentary lifestyle raises the risk of coronary heart diseases. It is estimated that physical inactivity accounts for over 23 million deaths globally, making it the fourth leading cause of death. A sedentary lifestyle raises the risk of coronary heart diseases [18]. According to scientific research, *Moringa oleifera* is a rich source of macro, micro- nutrients and bioactive compounds that can help prevent several chronic illnesses and health problems [19]. This research could be helpful in examining the pharmacological potential of *Moringa oleifera* and increasing the possibility of developing effective drugs that will enhance human health [20]. Cholesterol is transported throughout the body by lipoproteins and is necessary for cell proliferation, hormone manufacturing, and food digestion. HDL, the "good" cholesterol, dramatically lowers the risk of coronary heart disease by clearing cholesterol from the arteries [21]. The current study aims to ascertain if moderate aerobic exercise or *Moringa oleifera*, either separately or in combination, affects cardiac endurance and high-density lipoprotein (HDL) levels. A Randomized Control Trial (RCT) was set up to evaluate the following hypotheses that were created for the investigation. The study hypothesized that moderate aerobic training and Moringa oleifera has a positive impact on endurance in male adults.

METHODS

This study followed a Randomized Controlled Trial (RCT) design and was conducted at the Directorate of Sports, Quaid-i-Azam University (QAU), Islamabad, Pakistan. The study utilized the university's playground for moderate aerobic training, the Plant Sciences Laboratory for the analysis of *Moringa oleifera* leaf, and the Armed Forces Institute of Cardiology (AFIC) Pathological Laboratory for blood sample testing. The ethical approval from the Institutional Review Board (IRB) of the University of Lahore and the Bio-Ethics Committee (BEC-FBS-QAU2023-535) of Quaid-i-Azam University, Islamabad, Pakistan. The clinical trial registered under Clinical Trials.gov ID NCT04164771. The study was conducted over a period of 3 months from September to November, 2022. Sample size was determined using G*Power software with a one-way ANOVA analysis comparing four groups: control, aerobic training, *Moringa oleifera*, and their combination. Using medium effect size (f=0.25), alpha of 0.05 and power of 0.80, a sample of 100 (25 per group) was estimated. After accounting for 10% dropout, the final estimate retained 100 (25 in each group). A total of 100 male volunteers aged 38.9 ± 6.7 years were recruited following predefined inclusion and exclusion criteria. Participants were screened based on their lipid profile and cardiovascular endurance, ensuring homogeneity in baseline health conditions. The inclusion criteria for participants were as follows: low-density lipoprotein (LDL) levels ranging from 160 to 189 mg/dL, high-density lipoprotein (HDL) levels below 40 mg/dL, triglyceride levels between 200 and 499 mg/dL, and total cholesterol levels exceeding 200 mg/dL. Additionally, participants were required to have a beep test score of less than 7.9 or a VO_2 max of less than 40%. Individuals with pre-existing cardiovascular diseases, metabolic disorders, or those on lipid-lowering medications were excluded from the study. Prior to participation, all volunteers were fully informed about the study procedure, including pre-testing, aerobic training protocols, and post-testing after the intervention period. Written informed consent was obtained from each participant. The sampling strategy was based on convenient sampling techniques, and data were collected from a census of 100 participants regularly visiting the study settings. Based on participants'

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characteristics and inclusion criteria of the study, 100 participants were found eligible, which were divided into 4 equal groups. Participants who met the inclusion criteria were randomly assigned into four groups (n = 25 per group) using a computer-generated randomization protocol to reduce selection bias. The groups were as follows: T0 (control group), which received no intervention and continued their usual diet; T1 (Moringa group), which received 3 g/day of *Moringa oleifera* leaf powder; T2 (aerobic group), which engaged in moderate aerobic exercise at 45–60% intensity for 30 minutes per day, five days per week; and T3 (combined group), which received both Moringa supplementation (3 g/day) and aerobic training as outlined in the T1 and T2 protocols. Several recent studies reinforce the beneficial role of *Moringa oleifera* in enhancing lipid metabolism, physical endurance, and muscular function. Khan *et al.*, reported that *Moringa oleifera* leaf powder significantly improved lipid profiles in male rabbits by lowering total cholesterol and triglycerides while increasing HDL levels, suggesting strong potential for cardiovascular risk reduction [22]. Similarly, Ray *et al.*, highlighted the broader application of *Moringa oleifera* in improving human physical performance, attributing its effects to its rich nutritional and adaptogenic profile [23]. From a muscular perspective, Nayak *et al.*, found that *Moringa oleifera* extract counteracted exercise- and dexamethasone-induced skeletal muscle impairment, implying protective effects on muscle function under stress conditions [24]. Moreover, Tsuk *et al.*, in a pilot study, observed improved physical fitness outcomes in young adults consuming a commercial Moringa supplement, adding early clinical support for its ergogenic use [26]. Collectively, these studies highlight *Moringa oleifera*'s multifaceted benefits in enhancing HDL, preserving muscle integrity, and improving endurance, making it a promising natural adjunct in athletic and cardiovascular health interventions for males. The randomization ensured equal distribution of participants across groups, reducing potential confounding variables. The study followed a parallel-group design, with interventions applied independently to assess their isolated and combined effects on lipid profile and cardiovascular endurance. Qualified technical officials conducted the beep test to assess cardiac endurance. Blood samples were collected by trained paramedical staff under sterile conditions at AFIC, Rawalpindi. A 5 ml venous blood sample was drawn from the antecubital vein of each participant after an overnight fast of 10–12 hours. The collected samples were immediately stored in EDTA tubes and transported in a temperature-controlled container to the laboratory for lipid profiling. Data analysis was conducted using SPSS (Version 23.0) and Minitab (Version 16). Appropriate statistical methods were employed to ensure a rigorous evaluation of the intervention effects. The Shapiro-Wilk test was used to assess the normality of pre- and post-intervention data. Within-group differences were evaluated using paired samples t-tests. One-way ANOVA was performed to compare mean differences across the four groups, contingent on the assumption of normality. Post-hoc analysis was carried out using Tukey's pairwise comparisons to identify significant inter-group differences. Additionally, scatter plots were generated to visualize data trends and relationships. The randomization, strict inclusion criteria, and statistical approach ensured internal validity and minimized bias, allowing for an accurate assessment of the effects of *Moringa oleifera* supplementation and aerobic training on lipid profiles and cardiovascular endurance.

RESULTS

The study was conducted on 100 male adult participants. Due to non-normal distribution, the Shapiro-Wilk test was applied to report pre-and-intervention data of HDL and Beep test of all experimental groups and control groups, and the results are given in table 1.

Table 1: Group Comparison of HDL and Beep Pre-Test

Variables	Group	Statistic	df	Significance (p-Value)	CI
HDL Pre-Test	Control group T_0	0.998	25	>0.073	0.996-1.00
	Moringa group T_1	0.995	25	>0.069	0.992-0.998
	Aerobic group T_2	0.997	25	>0.091	0.994-1.00
	Combined groupT_3	0.997	25	>0.085	0.994-1.00
Beep Pre-Test	Control group T_0	0.975	25	>0.080	0.965-0.985
	Moringa group T_1	0.953	25	>0.068	0.940-0.966
	Aerobic group T_2	0.987	25	>0.063	0.978-0.996
	Combined group T_3	0.963	25	>0.079	0.950-0.976

The result indicated that the significance values in all cases were greater than the selected significance value (0.05), drawing the inference that the data was symmetrical and represented a normal distribution. The data were thus amenable to parametric analysis and application of relevant statistical tests which were applied. Box plots were drawn for explanatory data analysis, as depicted in figure 1.

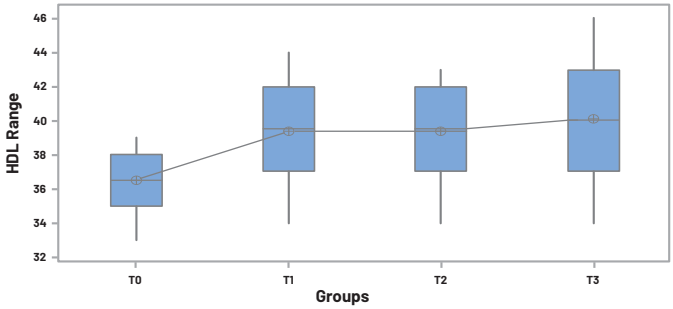

Figure 1: Box Plot of HDL data of all groups (T_0, T_1, T_2, and T_3)

Box plots of four groups showed that the data of HDL values in all groups (T_0, T_1, T_2, and T_3) were normally distributed, with no outliers. The interventional groups (T_1, T_2, and T_3) had a higher spread compared to the control group (T_0). Scattered plots were drawn for the Beep test for all groups (T_0, T_1, T_2, and T_3), with the results depicted in figure 2.

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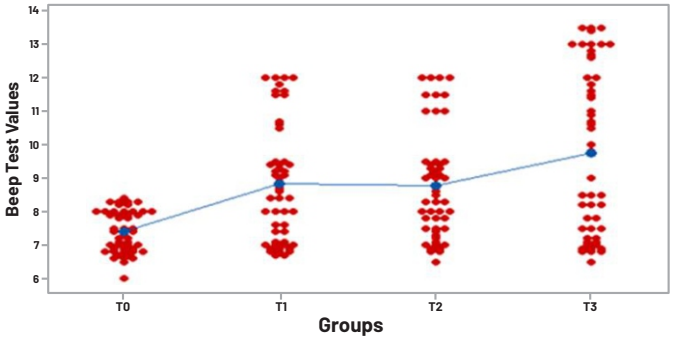

Figure 2: Scatter Plots of the Beep Test for all Groups

The Scatter plots showed that a strong positive relationship existed in the Beep test values in all four groups (T_0, T_1, T_2, and T_3), with higher values in the interventional groups compared to the control group and the highest one in the combined group (T_3). As such, the interventions had a positive effect on cardiac endurance, with more prominence in the combined intervention. A paired samples t-test was used to compare the mean values of pre-tests and post-tests of both variables in the combined group (T_3), results tabulated in table 2.

Table 2: Paired Samples T-Test for HDL and Cardiac Endurance (N= 25)

Variables	Group	Mean ± SD	Diff	p-Value
HDL	Pre-Test	36.96 ± 1.48 mg/dl	6.44	<0.001
	Post-Test	43.40 ± 1.78 mg/dl		
Cardiac Endurance	Pre-Test	7.49 ± 0.69 (VO2 max 38.1%)	4.53	<0.001
	Post-Test	12.03 ± 1.17 (VO2 max 54.6%)		

The findings showed that the p-values for HDL and cardiac endurance in the combined group (T_3) were both less than 0.001, which is well below the 0.05 threshold. This means there was a significant (< 0.001) improvement from the pre-test to the post-test. As a result, the null hypothesis was rejected, confirming that moderate aerobic training and Moringa oleifera had a meaningful positive impact on HDL levels and cardiac endurance. Analysis of Variance (ANOVA) was conducted for comparison of Beep tests for the four groups (T_0, T_1, T_2, and T_3), to evaluate the effect on cardiac endurance, at a significance level (<0.05), with the results tabulated in table 3.

Table 3: Comparison within Group of Beep Comparison Test

Source	DF	Adj SS	Adj MS	F-Value	p-Value
Beep	3	141.7	47.250	15.31	<0.001
Error	196	605.1	3.087		
Total	199	746.8			

The one-way ANOVA revealed that there was a statistically significant difference in mean Beep test scores between at least two groups (F (3, 196) = [15.31], p = 0.001). Given the result of the ANOVA, Tukey's HSD test was conducted to ascertain the Pre and Post-test comparison for the four groups, and pairwise comparisons for the six groups (T_1-T_2, T_1-T_3, T_1-T_0, T_2-T_3, T_2-T_0, T_3-T_0), at significance level (<0.05) (Confidence Interval (CI=95%). The results are compiled in table 4. Tukey's HSD test helps in determining differences of values within group.

Table 4: Tukey's Grouping Information on Pre and Post Beep Test Scores

Group Comparison	Mean Difference (I–J)	Significance	95% CI	Effect Size (Cohen's d)
T0 – T1	3.016*	0.000	3.6355, 2.3965	2.01
T0 – T2	2.844*	0.000	3.4635, 2.2245	1.90
T0 – T3	4.776*	0.000	5.3955, 4.1565	3.18
T1 – T2	1.760 B	0.000	2.3795, 1.1405	1.17
T2 – T3	1.932*	0.000	2.5515, 1.3125	1.29

As reflected in table 4, the groups T_1 and T_2 having a shared alphabet "B" in means, had no significant difference, while groups T_0 and T_3, not having a shared alphabet with any other group, had a significant difference. The results demonstrate that both Moringa supplementation and aerobic exercise are individually effective, but their combination produces a substantially greater effect, as evidenced by the large and statistically significant interaction. This supports the conclusion that the combined intervention (T_3 group) has a synergistic benefit, reinforcing the value of integrating both strategies for maximum effect (Table 5).

Table 5: Two-Way ANOVA Summary for Effects of Moringa Supplementation and Aerobic Exercise on Post-Intervention Outcome

Source	Type III Sum of Squares	df	Mean Square	F	p-Value	Partial η^2
Corrected Model	293.491	3	97.830	80.808	<0.001	0.716
Intercept	3923.185	1	3923.185	3240.541	<0.001	0.971
Moringa (main effect)	123.393	1	123.393	101.922	<0.001	0.515
Aerobic (main effect)	89.719	1	89.719	74.107	<0.001	0.436
Moringa × Aerobic (interaction)	285.127	1	285.127	235.514	<0.001	0.710
Error	116.223	96	1.211	-	-	-
Total	10272.190	100	-	-	-	-

Tukey's simultaneous pairwise comparison indicated that the pair T_2-T_1, the two interventions, containing a zero in its confidence interval had no significant difference, whereas pairs T_3-T_1, and T_3-T_2, close to zero but not included, had significant differences, though by a small degree. In other words, the two interventions had individual effects on the Beep score of the same magnitude, but their joint effect was significantly different from their individual effects. The pairs T_1-T_0, T_2-T_0, and T_3-T_0, having confidence intervals distant from zero, were significantly different. The overall position had been that the three interventional groups' pre- and post-Beep test scores were significantly different from the control group, while inter se, the effects of the two inventions individually were alike but significantly different

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from their joint effect, the latter being more pronounced.

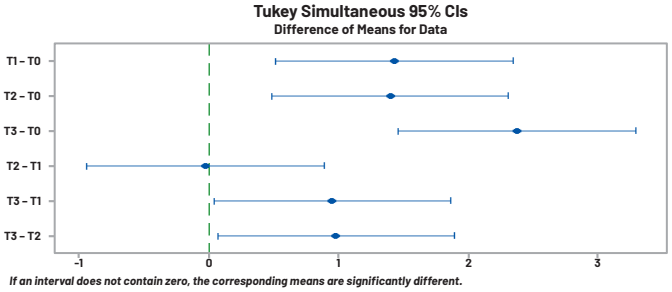

Figure 3: Tukey's Simultaneous Pairwise Comparison

DISCUSSION

The growing interest in natural supplements for enhancing physical fitness and cardiovascular health has led to increased investigation into the potential of *Moringa oleifera* [26]. Another research study was implemented to check the effect of *Moringa oleifera* supplement on physical fitness of young adults and discovered the results that there was no impact on physical activity performance but encouraging signs that the *Moringa oleifera* has beneficial impacts on physiological processes [26]. This study contradicts the current study results in some way because this study carried out with two interventions aerobic training and *Moringa oleifera* as a supplement and elaborated that aerobic training and Moringa leaf powder have significant effects on High density lipoprotein and cardiac endurance. The results of the current study showed that moderate aerobic training and administering *Moringa oleifera* to male adult persons for 12 weeks had a significant positive effect on HDL and Cardiac Endurance. The Beep test used to guess cardiac endurance, analysis showed improvements in the two interventional groups (T1 and T2) but in combined group (T3) showing the most significant improvement. On the other hand, lipid profile was carried out to check the HDL level and paired samples t-test were used to evaluate the HDL of all interventional groups. However, when *Moringa oleifera* leaf powder and moderate aerobic training were provided to combined group (T3), cardiac endurance and HDL increased significantly as compared to other two treatment groups. According to Tukey's pairwise comparisons, and the ANOVA analysis confirmed that the Beep test scores of the groups differed significantly (i.e., <0.05). These findings support other studies showing that regular aerobic exercise improves cardiovascular efficiency [26]. The results of this study are also in line with the larger body of research on the advantages of moderate aerobic exercise and the use of herbal remedies to support cardiovascular health. The present investigation verified that *Moringa oleifera* and moderate aerobic exercise raise cardiac endurance and HDL levels.

CONCLUSIONS

According to the current study's findings, HDL and cardiac endurance are significantly improved by participating in 12 weeks of aerobic exercise and taking a supplement of *Moringa oleifera* leaf powder among adult males.

Authors Contribution

Conceptualization: MSA
Methodology: MSA
Formal analysis: NA
Writing, review and editing: MSA, NA, MKM

All authors have read and agreed to the published version of the manuscript

Conflicts of Interest

All the authors declare no conflict of interest.

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