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Effects of *Moringa Oleifera* leaf powder infusion on a 20km cycling time-trial performance and cardiorespiratory responses in active people

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Abstract

Moringa Oleifera (MO) is a tropical plant with roots, seeds, and bark that is used as food and in traditional medicine. The MO has already been shown to exert antioxidant, anti-inflammatory, and analgesic properties. On the other hand, the ergogenic properties of MO remain unclear. Our study aimed to compare the energy and time required for participants to complete the required work (cycling 20 km) before and after receiving an infusion of the MO leaf. The performance achieved by 34 participants (19 M, 15 W, Age: 25.9 ± 4.5 years, Weight: 71.4 ± 16.5 kg, Height: 170.4 ± 10.0 cm, VO_2max: 40.1 ± 8.4 ml.kg.min) at the end of a 20 km time trial cycling time trial was recorded. During the protocol, the load was set at a power of 114.1 ± 42.8 Watts (corresponding to the ventilatory threshold 1+10%: VT1+10% of the participants). The heart rate (HR) was measured during the test, both at rest, during effort, and recovery. Also, blood lactate levels, rate of perceived exertion (RPE), and muscle oxygenation were measured. Total work (Δ 293.6 ± 75.3 vs 290.1 ± 77.4 KJ, p=0.012, d=0.5) and time (Δ 50.0 ± 22.3 vs 49.33 ± 21.9 min, p=0.021, d=0.4) were significantly different. HR (Δ 172.4 ± 22.5 vs 175.9 ± 23.1 beats per minute (bpm), p=0.02, d=-0.9) and RPM (Δ 87.6 ± 18.5 vs 93.3 ± 15.8, p=0.008, d= -0.5) were also significantly different. Our findings indicate that the infusion of *Moringa Oleifera* leaf powder enhances the pedaling rate, total work output, and exercise duration when achieving a 20 km cycling time trial.

Keywords: Moringa Oleifera; Cardiorespiratory endurance; Heart rate; VO_2max; Performance; Effort exertion; Blood lactate; Muscle oxygenation

Introduction

A proper diet and regular physical training have a positive impact on health and physical performance. Current knowledge has led to a deeper understanding of the various physiological mechanisms underlying performance. In particular, the roles of various macro- and micronutrients have been highlighted in energy supply, metabolic regulation, and muscle contraction [1].

The various research studies that have focused on Moringa Oleifera (MO) have suggested the potential effect this plant could have on the diet of athletes. Indeed, the powder of MO leaves is a Phyto biotic, well known for its medicinal use [2]. It contains phytochemicals, such as alkaloids and flavonoids, which provide immunomodulatory and antimicrobial properties [3]. Moringa Oleifera leaf also contains many natural antioxidants (vitamin E and selenium), minerals (calcium, phosphorus, and magnesium), and phytochemicals such as caffeic acid [4], which decrease fatigue and the perception of exertion during physical activity.

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Gbedinhessi DGE, et al., J Food Sci Nutr Res 2025
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The beneficial impact of this part of MO on athletic performance can be attributed to its high content of (a) calcium, which plays a crucial role in neuromuscular excitability, (b) potassium, essential for muscle contraction control and regulation of water balance between intracellular and extracellular environments, and (c) protein intake, which aids in the maintenance and growth of muscle mass [5-7]. The presence of flavonoids in the plant's leaf could also contribute to improved athletic performance. They can modulate the activity of certain enzymes and modify the behavior of several cellular systems, suggesting that they could exert a multitude of biological activities, including significant antioxidant, vascular protective, anti-hepatotoxic, antiallergic, anti-inflammatory, anti-ulcer, and even antitumor properties [8]. It has also been reported that a flavonoid concentrate from MO leaf extended exhaustive swimming time by improving mice's energy metabolism and antioxidant capacity [9]. It has also been reported that the aqueous extract of the leaves of this plant improves the swimming ability of rats by delaying the accumulation of blood lactate and urea nitrogen in the blood [10]. The anti-fatigue potential of MO may be attributed to its antioxidant activity [10]. This potential could be associated with increased hemoglobin concentration and enhanced hepatic and muscle glycogen stores. Not to mention, there is a reduction in lactic acid tissue accumulation by extracting this plant's leaf [10]. FitnoxT, which consists of 50% MO, has an antioxidant and vasodilatory effect on men aged 18 to 55. Hence, it also increased red blood cell count and dopamine levels (36% - from 225 mg/24h to 354 mg/24h in the blood, respectively, before and after exercise) in the same participants [11].

Therefore, the present study aimed to determine the effect of supplementation with MO leaf infusion on 20km cycling time-trial performance and cardiorespiratory responses in active participants.

Materials and Methods

Subjects

Thirty-nine participants, comprising 22 men and 17 women, aged 18 to 35, were included. They were healthy and physically active, engaging in more than 150 minutes per week of fast walking, running, cycling, and other physical activities in the fitness room, and were recruited through posters and mass emails. The first participants were also asked to bring the information to their loved ones. Smokers, alcohol users (more than two drinks per day), and individuals taking medications such as stimulants, opiates, hormone antagonists, and modulators were excluded from the study. The study received approval from the institutional ethics committee of the Université du Québec à Montréal (UQÀM) (2023-4759, multi-faculty CERPE). All participants were allowed to ask questions to ensure a thorough understanding of the study's requirements, benefits, and risks before providing their informed consent. Participants agreed not to use medications, including vitamins and caffeine, for several days before the study's commencement and throughout the experiment. They also decided not to change their eating and sports training habits during the study.

Design

This clinical study employed a crossover experimental design with the same subjects serving as their controls. The time a subject was involved in the trial was divided into three periods. The first period was one day and consisted of welcoming the participant to the laboratory to explain the project's purpose and the progress of the experiments, followed by submission to the VO_2max test. During the remaining two periods, each participant received a double-blinded different treatment. The order of treatment created two groups with a size equal to half the number of participants included in the trial. Each participant's order of application of the treatments (MO-Placebo or Placebo-MO sequence) was randomly allocated. At mid-term, subjects in the treatment group (M, MO) were crossed over to join the control group (P, placebo) and vice versa. Ultimately, all patients were assigned to one of the treatment groups (Figure 1).

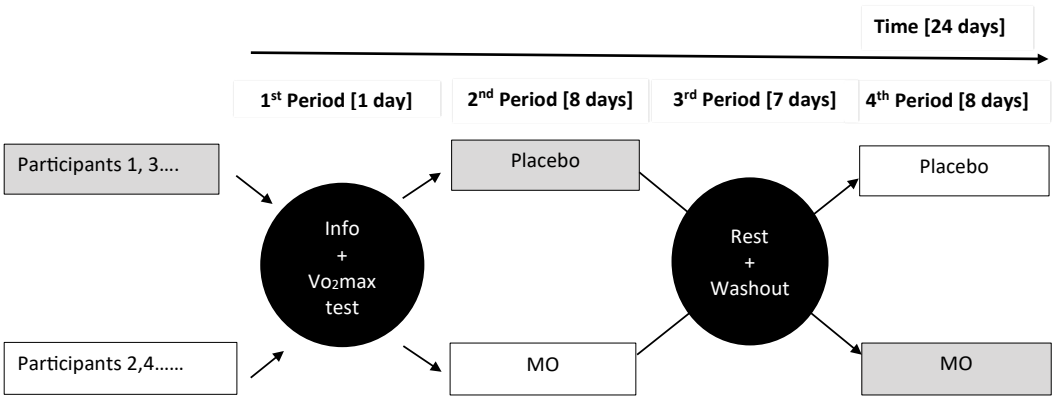

Figure 1: Schematic representation of the test.

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Gbedinhessi DGE, et al., J Food Sci Nutr Res 2025
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1st period (1 day): The participants were invited to the laboratory to learn about the experiments' progress and then subjected to a VO2max test.

2nd period (8 days): Both groups, after taking two doses of 200 ml of water (Placebo) or two doses of 200 ml of MO leaf tea (Treatment) per day for seven days, were subjected to a sustained effort on the eighth day.

3rd period (7 days): After the second period, participants had a 7-day rest and weaning period before the fourth period.

4th period (8 days): During this period, we changed the order of treatment administration. After taking two doses of 200 ml of water (Placebo) or two doses of 200 ml of MO leaf tea (Treatment) per day for seven days, both groups were subjected to sustained effort on the eighth day.

The study was double-blind. Opaque green containers were used to administer the various treatments to the participants. The study lasted 24 days.

Methodology

Obtaining the aqueous extract of MO

Olson et al., Loomis and Hayes demonstrated that MO leaves at a specific dose were non-toxic [12,13]. We were inspired by the infusion method used by Coz-Bolaños et al., who used 240 mL of boiling water and 3 g of powder [14]. We administered a dose of 4g/50kg of body weight to our participants. The powder of MO leaves (organic moringa leaf powder, purchased in Montreal at Yupik, certified by Ecocert Canada) was infused for 5 minutes in 200 milliliters (ml) of hot water, cooled to 80 °C.

Tests and Measurements

Anthropometric measurements: The following parameters were measured: height was measured using a stadiometer (Stadler), and body weight was measured using a Bioimpedance scale (InBody 270).

Heart rate measurement: We measured the participants' heart rate (HR) using a Polar H10 monitor. The resting heart rate (HR) was measured for five minutes in a seated position before the start of exercise. Then, it was recorded during both events (VO_2 max and endurance) and for five (5) minutes after stopping the endurance exercise (post-exercise recovery period).

Muscle oxygenation and total hemoglobin measurement: We measured the SMO_2 and THb of participants using a near-infrared spectroscopy (NIRS) device (Moxy monitors, USA) placed on the quadriceps muscles of the right leg. The measurement was taken from the beginning until the end of the endurance test.

Lactate measurement: At the end of the endurance test, we measured blood lactate using a lactometer (Lactate Pro, Japan). Blood was taken from the participant's finger to perform the measurement.

Physical effort exertion measurement: We measured physical exertion using the rate of perceived exertion (RPE) scale, ranging from 6 to 20. The measurement was taken 20 minutes after the endurance test concluded.

Measurement of the maximum volume of oxygen consumed (VO_2 max): The primary objective of this test was to determine the VO_2max and metabolic thresholds (ventilatory threshold 1 and ventilatory threshold 2) using a progressive test until exhaustion on a stationary bike (Excalibur, Lode, SE) coupled with a metabolic cart system that provided breath-by-breath analysis (MetaMax, Cortex, DE). Indeed, each participant had a mask attached to their face and connected by a tube to the gas exchange measuring device. The exercise test was conducted as described by Lalonde et al. Before each test, the gas analyzers were calibrated with calibration gases (25% O_2 and N_2 balance and 16% O_2, 5% CO_2, and N_2 balance) and the air volume turbine with a 3 L syringe. The manufacturer provided the software to display VO2max and other parameters (ver. 7.2.0.52, 2001-2011, Medgraphics Corporation, St-Paul, MN) [15].

A five-minute warm-up was conducted before the incremental test on a stationary bike at a cadence of 90 revolutions per minute (rpm). The initial load was set at 25 watts (W) and, after two minutes, was increased to 50 W for the remainder of the warm-up period. At the end of the warm-up, the load was initially set at 25 W and then increased by 25 W every minute until exhaustion while maintaining a minimum cadence of 60 RPM (rotations per minute), which varied between 60 and 100 RPM. Participants had to meet two of the following four criteria to confirm VO_2max: an O_2 absorption plateau despite increased workload, a respiratory exchange ratio value >1.15, the predicted maximum heart rate achieved using the age equation 220 – age (men) and 200 – age (women) (if no $\dot{V}O_2$ plateau was reached), or an inability to maintain pedaling cadence above 50 RPM [16-18]. To obtain the moderate and high-intensity range, ventilatory thresholds 1 and 2 were visually determined as the point at which, during the incremental stress test, the ventilatory equivalent for O_2 ($\dot{V}E$ /$\dot{V}O_2$) increased without any change in the ventilatory equivalent for CO_2 ($\dot{V}E/\dot{V}CO_2$) [19]. At the end of the test, participants were asked to rate their perceived effort on a Borg scale [20].

Endurance test: HR and muscle oxygenation were also measured during the endurance event. The perception of the effort during the endurance test (EPR session) was also measured 20 minutes after the test ended. The endurance event began with a warm-up period, during which participants had to pedal at a comfortable cadence (60-120 RPM) for 3 minutes with a resistance of 50W. At the end of their warm-up, the resistance was increased to the level

Gbedinhessi DGE, et al., J Food Sci Nutr Res 2025
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corresponding to their ventilatory threshold (VT) plus 10% (VT + 10%). The participants were then invited to complete a 20km race against the clock as soon as possible. During the race, participants could see how far they had to go but were blinded to all other information. The time needed to complete the 20km race was recorded. The total work, corresponding to the energy supplied (in kilojoules) to perform the exercise, was calculated using the formula:

$$Energy\ deployed\ (kJ) = \frac{Load\ (W)\ \times\ Time\ (minutes) \times 60}{1000}$$

Statistical analysis

The results are presented as mean values ± the standard deviation. The normality of the results was verified using the Shapiro-Wilks test. The t-test for the matched samples was used to compare the mean values of our different variables before and after the intervention. A significant difference was defined as $p< 0.05$. The sample size was calculated using G-Power (ver 3.7) using 80% statistical power and a 5% improvement in endurance test performance. The required sample size was 34 participants. The analyses were conducted using the statistical software SPSS 27.0.

The study received approval from the institutional ethics committee of the Université du Québec à Montréal (UQÀM) (2023-4759, multi-faculty CERPE).

Results

Effects of Moringa Oleifera on heart rate

Figure 2 shows the resting, peak exercise, and recovery heart rate (seven minutes after the end of the test) to perform the 20km cycling time trial test before and after ingestion of the MO leaf infusion. Data are expressed as mean ± SD. Consumption of the MO leaf powder infusion did not induce a significant change in resting and recovery heart rates (71.42 ± 11.7 vs. 70.94 ± 12.1; p=0.764; d=0.05 / 91.4 ± 15.9 vs. 92.4 ± 15.0; p=0.625; d=-0.1 respectively). However, a significant increase in maximum heart rate during exercise was observed (Δ 172.4 ± 22.5 vs 175.9 ± 23.1; p=0.02; d=-0.9).

Effects of Moringa Oleifera on the average pedaling cadence

Figure 3 shows the average pedaling cadence to perform the 20km cycling time trial test before and after the

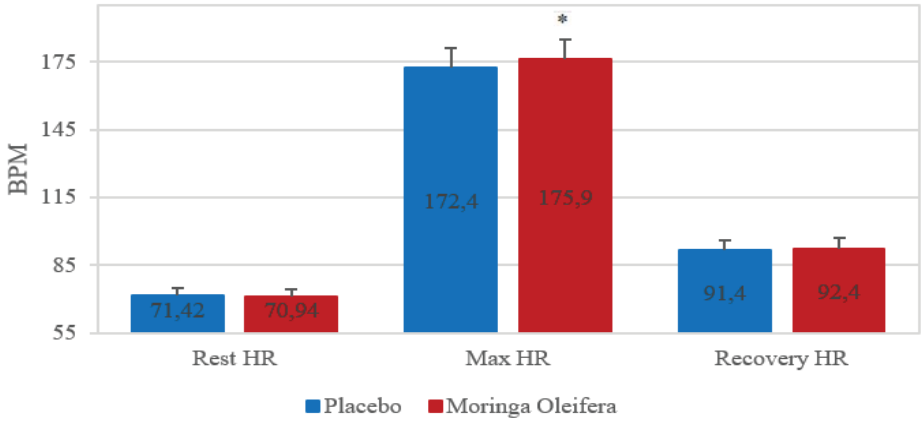

Figure 2: Heart rate (bpm) before and after the infusion of *Moringa Oleifera* leaf.

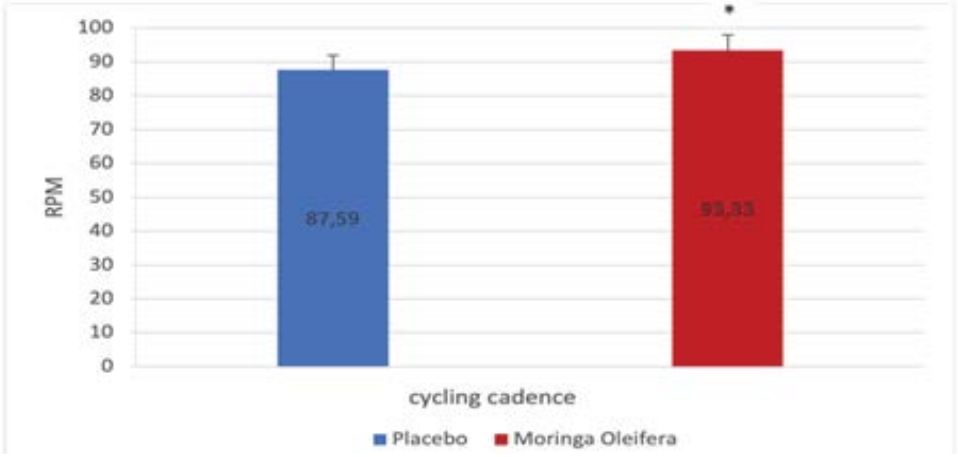

Figure 3: Average of RPM before and after the infusion of *Moringa Oleifera* leaf.

Gbedinhessi DGE, et al., J Food Sci Nutr Res 2025
DOI:10.26502/jfsnr.2642-110000173

consumption of the MO leaf infusion. Data are expressed as mean ± SD. There was a significant increase in the average pedaling cadence (Δ 87.6 ± 18.5 vs. 93.3 ± 15.8; p=0.008; d=-0.5) during the endurance test after consuming an MO leaf infusion compared to the placebo consumption.

Effects of *Moringa Oleifera* on total work

Figure 4 shows the total work to perform the 20km cycling time trial test before and after MO leaf infusion consumption. Data are expressed as mean ± SD. The energy required to perform the endurance test is significantly lower with MO than with placebo (Δ 293.6 ± 75.3 vs. 290.1 ± 77.4; p = 0.012; d = 0.5).

Effects of Moringa Oleifera on the travel time

Figure 5 shows the time to complete the 20 km cycling time trial before and after MO leaf infusion. Data are expressed as mean ± SD. The time to perform the test was significantly longer when the placebo was ingested than when the MO was ingested (Δ 50.0±22.3 vs. 49.33±21.9; p=0.021; d=0.4). It, therefore, seems that the consumption of MO favors the reduction of the time needed to complete the endurance test compared to the consumption of a placebo.

Effects of Moringa Oleifera on the pedaling cadence with the relative travel time

As illustrated in figure 6, pedaling cadence has been expressed as a function of relative time. Given that the time required to complete the 20km time trial varies from participant to participant, the time to complete the course was standardized as a percentage of the total time for each participant. The significance of variations in the data presented in figure 6 was determined by employing a T-test at each percentage of the total time, with T1 representing the first and T8 the last. Standardizing travel time as a percentage of relative time facilitated an objective comparison of the participant's performance in this study.

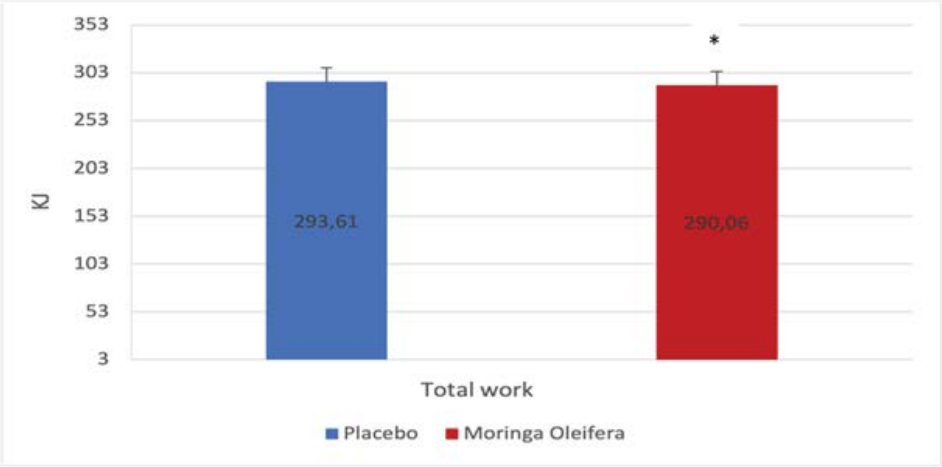

Figure 4: Total work (expressed in kilojoules (KJ)) before and after *Moringa Oleifera* leaf infusion consumption.

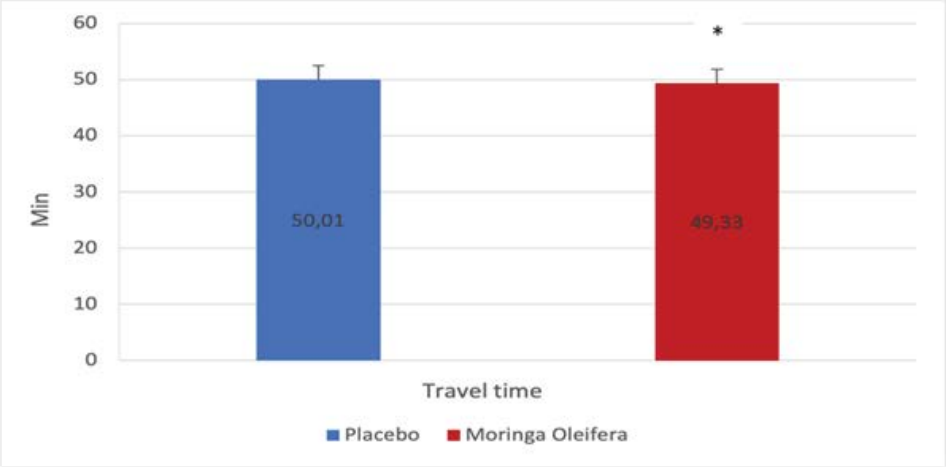

Figure 5: Travel time (min) before and after infusion of *Moringa Oleifera* leaf.

Gbedinhessi DGE, et al., J Food Sci Nutr Res 2025
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Effects of Moringa Oleifera on the rate of perception of the effort (RPE)

Figure 7 illustrates the participants' perception of the effort required to perform the 20km cycling time trial test before and after consuming the MO leaf infusion. Data are expressed as mean ± SD. The RPE estimated by the participants at the end of the two tests was not significantly different (13.9 ± 2.8 vs. 14.3 ± 2.5; p = 0.357; d = -0.2). The MO did not seem to affect participants' perception of effort.

Effects of Moringa Oleifera on the blood lactate

Figure 8 shows the blood lactate levels following the 20km cycling time trial test before and after the consumption of the infusion of Moringa Oleifera leaf. Data are expressed as mean ± SD. There was no significant difference between MO or placebo (7.2 ± 4.2 vs 7.4 ± 3.5; p=0.635; d=-0.1).

Effects of *Moringa Oleifera* on muscle oxygenation

Figure 9 shows muscle oxygen saturation (SmO_2) and the relative travel time percentage during the 20km cycling time trial test before and after the consumption of the MO leaf infusion. Data are expressed as mean ± SD. We compared the values at 25%, 50%, 75%, and 100% of the participants' travel time before and after MO consumption. The figure shows that at 25% of the travel time, the SmO_2 was almost the same with and without MO. At 50% and 100%, there was a decrease in SmO_2 before MO consumption, while it increased after consumption. However, at 75%, muscle oxygen saturation was higher before than after MO consumption. The data did not reveal any difference between the MO and placebo groups.

Effects of *Moringa Oleifera* on the total oxygenated hemoglobin

Figure 10 shows the total oxygenated hemoglobin (THb) level and the percentage of relative travel time during the 20-km cycling time trial test before and after the consumption of the MO leaf infusion. Data are expressed as mean ± SD. We compared the values at 25%, 50%, 75%, and 100% of the participants' travel time before and after MO consumption. The figure shows that the THB before MO consumption decreased by 25% to 50%. It increased slightly from 50% to 75%, then decreased slightly from 75% to 100%. However, after MO consumption, there is a gradual drop in THB from 25% to 100% of the travel time. Compared to each other, we observed that at 25% and 50%, the THB before consuming MO was lower than that after consumption. At 75% and 100%, the THB before consumption of MO remained higher than that after consumption. However, these differences were not significant and practically did not show any change in THb between the conditions of consumption and the absence of MO during the test.

Comparing figure 9 and figure 10, for the test performed in the absence of MO, the only increase in SmO_2 observed

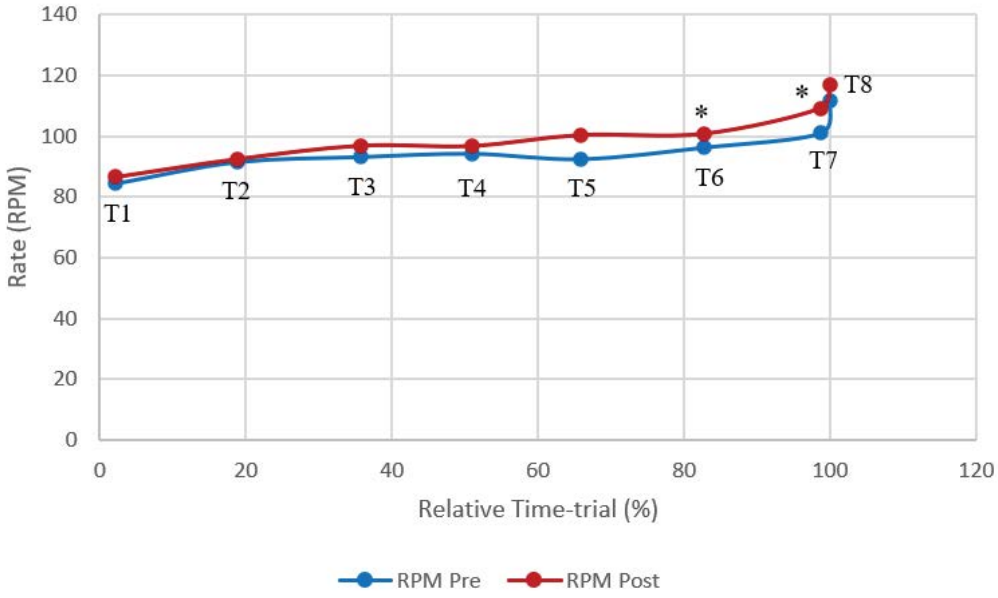

Figure 6: Illustrates the pedaling cadence, along with the relative travel time (expressed as a percentage of total time) to complete the 20km cycling time trial test before and after consuming the MO leaf infusion. Data are presented as mean ± SD. The data shown in the graph represents data collected at regular intervals. T represents the relative time when the information was collected. The first collection was made at T1, while the last was at T8. By analyzing Figure 6, the strategy adopted by the participants appears to be the same before and after consuming MO: a low cadence at the beginning, followed by a gradual increase in pace. From T4 onwards, we observed a slight difference in the curve profiles, but this was not significant. Thus, the difference was significant between the two groups from T6 to T7, where we noticed an increase in the pace.

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Gbedinhessi DGE, et al., J Food Sci Nutr Res 2025
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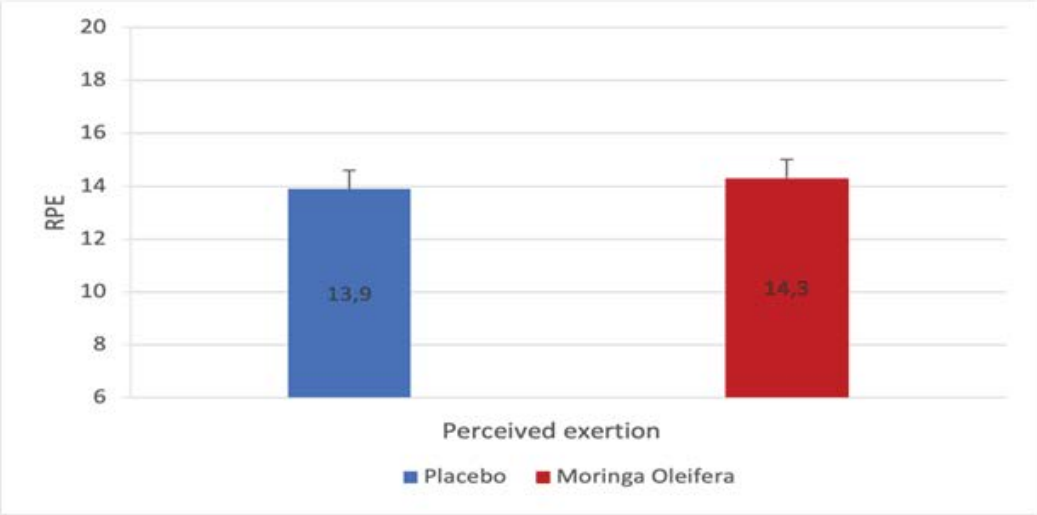

Figure 7: Rate of perceived exertion (RPE) of pre- and post- *Moringa Oleifera* leaf infusion consumption.

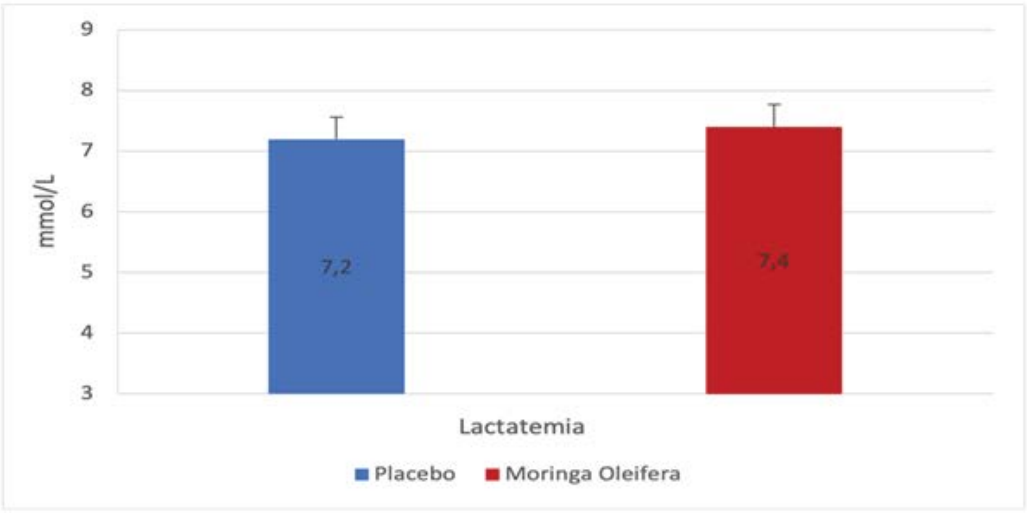

Figure 8: Blood lactate (mmol/L) before and after consumption of the infusion of *Moringa Oleifera.*

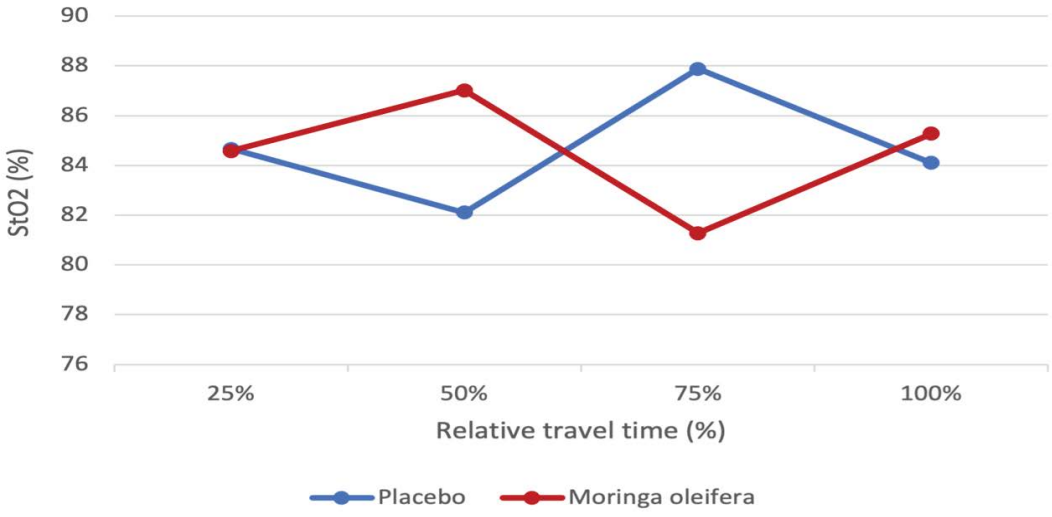

Figure 9: Muscle oxygen saturation (SmO2) as a percentage of relative travel time before and after Moringa oleifera leaf infusion consumption.

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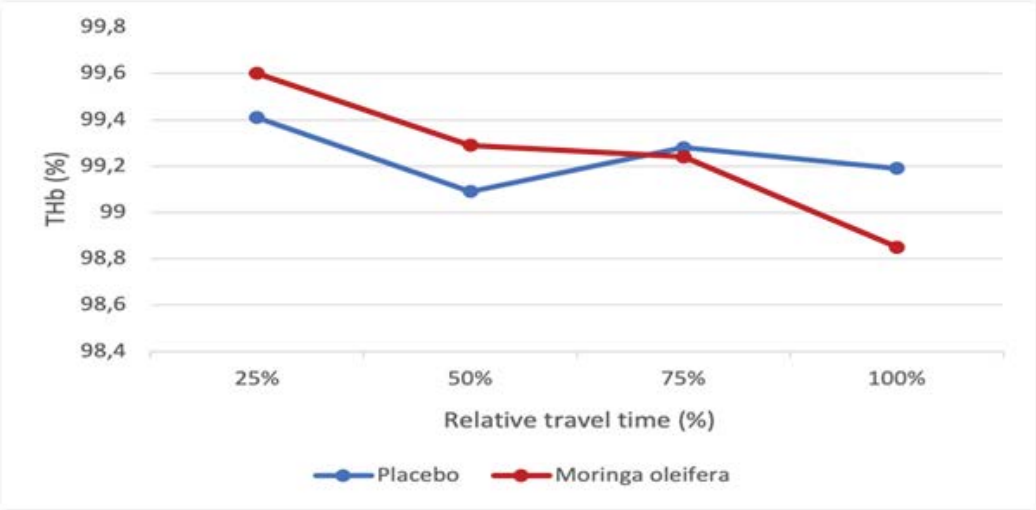

Figure 10: Total oxygenated hemoglobin (THb) as a percentage of relative travel time before and after Moringa oleifera leaf infusion consumption.

from 50% to 75% was accompanied by the rise in total oxygenated hemoglobin in the same period. The same was true for all instances where a decrease in SmO_2 was observed, accompanied by a reduction in THb (from 25% to 50% and from 75% to 100%). However, the test performed in the presence of MO shows that, despite a constant decrease in total oxygenated hemoglobin, muscle oxygen saturation (Figure 9) increased from 25% to 50% and from 75% to 100% of the time the test was performed. This indicates that MO can extract oxygen more effectively.

Discussion

The objective of the present study was to compare the performance of volunteers before and after MO consumption during an endurance test. The results enable testing the hypothesis that the MO leaf enhances endurance performance.

This study suggests that the infusion of MO leaf over seven days improves endurance performance in active individuals. Indeed, with seven days of treatment, the maximum heart rate and pedaling cadence are significantly higher when MO is used than with the placebo. Additionally, the total work and time required to complete the 20km journey are significantly reduced when the MO is used. Our results align with those of Dong W, et al. [21], who reported improved performance in treadmill exhaustion and push-up tests in young adults who received an MO supplement for 30 days compared to the placebo group.

The MO allowed the participants to achieve a higher maximum heart rate during the endurance test. This contradicts our hypothesis. However, this could be attributed to the participant's higher pedaling rate during the endurance test. Indeed, during physical exertion, total peripheral vascular resistance (TPVR) decreases due to the vasodilation of arterioles in active muscles, mediated by the orthosympathetic nervous system [22]. Cardiac output (CO), which is dependent on stroke volume (SV) and heart rate (HR), also increases with exercise. The increase in heart rate during exercise is explained by the fact that the duration of the cardiac cycle decreases, with diastole time being shorter than systole time. Therefore, ventricular filling time is reduced [22]. Then, the MO may act as a vasodilator, allowing for a higher effort and thus causing an increase in heart rate to perform the same type of time trial (our protocol is described in the Methodology section). The study by Aekthammarat et al. on MO demonstrated the plant leaf's vasodilatory effect, which corroborates our observations [23].

As anticipated in our hypothesis, the total work (energy expended to complete the test) and performance time were significantly reduced. This suggested that the infusion of the MO leaf may decrease the energy required to perform the exercise, thereby improving energy efficiency. In other words, participants needed less energy to achieve the same exercise goal (e.g., traveling 20km with a fixed load), indicating that the MO leaf might help reduce fatigue. Moreover, the participant's pedaling cadence (RPM) increased significantly with the MO infusion. This set of acute adaptations supports the hypothesis that MO leaf infusion may have direct anti-fatigue properties on activated skeletal muscle, leading to enhanced energy efficiency by requiring less energy for the same work. Scientifically, this could be explained by the fact that the effort made by the participants after consuming the MO infusion was more intense, as Indicated by an Increased pedaling cadence. Therefore, the fact that the RPM has increased, coupled with a decrease in total work required, possibly indicates a direct ergogenic effect on muscle performance. All of this is correlated with the results of several studies that have demonstrated the anti-fatigue effect

Gbedinhessi DGE, et al., J Food Sci Nutr Res 2025
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of the plant [10,24,25]. However, it cannot be ruled out that consuming an MO leaf infusion has a cognitive placebo effect. Nevertheless, our cross-referenced experimental design partially corrects the placebo effect.

Barodia et al. found that MO leaf extracts improved endurance and locomotor activity in rats [22]. Our study enables us to confirm a similar effect in humans, as we observed a significant decrease in travel time and total work with the consumption of the infusion. It would also seem that the MO had a motor effect that favored the efficiency of the movement. This is justified by the significant increase in the pedaling cadence after consumption.

When the time trial was compared with the cadence (RPM), a significant difference was observed in RPM at 80% and 100% of the distance. Thus, Moringa Oleifera appears to increase the pace towards the end of an endurance trial, thereby improving overall endurance.

Muscle oxygenation

The analysis of muscle oxygenation and related to the relative duration of the journey expressed as a percentage of travel time showed that in the presence of MO, it is observed that despite an appearance of a (non-significant) constant decrease in total hemoglobin, muscle oxygen saturation increased at the beginning and end of the effort. This could indicate better oxygen extraction by the muscle in the presence of MO. This observation is corroborated by the work of Eze et al., reporting that MO has the potential to be an ergogenic aid through an improvement of energy metabolism in adult skeletal muscle by increasing the expression of critical metabolic markers (PGC-1α, PPARγ, SDHB, SUCLG1, VEGF, PGAM-2, PGK1 and MYLPF) [9]. These effects may be mediated through the increased expression of critical metabolic markers, including PGC-1α, PPARγ, SDHB, SUCLG1, VEGF, PGAM-2, PGK1, and MYLPF. In contrast, a recent study by Tsuk et al. showed that MO does not physiologically improve endurance in young and healthy participants [26]. However, it should be noted that this is a pilot study in which the dosage, treatment time, and sample size were small compared to the methodology adopted in the present clinical trial.

Although the maximum heart rate was significantly higher with MO than with placebo during the test, the recovery heart rate was not different between the two groups. This could be explained by the cardioprotective properties of MO, conferred by N,α-L-rhamnopyranosyl vincosamide (an alkaloid extracted from its leaves), which allow for the reduction of serum cardiac markers, such as troponin-T and creatine kinase-MB, after isoproterenol-induced cardiac toxicity [27]. Additionally, the aqueous extract of MO leaves exhibits antihypertensive properties, as evidenced by reductions in blood pressure and heart rate in hypertensive rats. It would cause better vasodilation by acting directly on the endothelium and, therefore, seems to be a natural antihypertensive product [23]. This may be due to the arginine content of MO leaf powder [23]. The latter has been shown to improve NO production in mice [28].

The Rate of Perception of Effort (RPE) and Lactatemia

The result of the perception of effort did not verify our hypothesis. Yet, we expected to observe a decrease since Lamou et al.'s study showed that MO had anti-fatigue properties during a swimming test [10]. In our research, it is possible that the increase in rate following the consumption of MO tea contributed to maintaining a high RPE.

Lactate levels were also expected to decrease with MO consumption, as observed in the study by Lamou et al. [10]. However, the blood lactate level after the MO leaf infusion was slightly higher than that obtained after consuming the placebo. This may be due to the high frequency of participants pedaling after consuming MO.

Limits

The possible limits of our study are of several kinds:

A small amount of data was collected for certain variables, specifically muscle oxygenation and lactatemia.

We followed up with the participants regarding compliance with the instructions, based on their honesty in reporting any non-compliance. Perhaps the instructions were not followed entirely by the participants: forgetting to take a one-time dose, being associated with alcohol, and having modified daily habits. However, a few participants informed us of this, and their results were removed from the study.

Some of our participants were student researchers already used to this type of study. This may be a bias since they were not completely blind and could have attempted to adapt to the situation.

The taste of the MO infusion differs from that of water; some participants could tell the difference between the two.

The lack of proper control: using participants as their control can enhance our study's accuracy, efficiency, and sensitivity while minimizing ethical concerns and costs. However, it's essential to consider the potential for carryover effects, where the first condition affects the subsequent one.

Pratical applications

The following practical applications can be considered in the context of the use of moringa oleifera by athletes:

Enhanced Sports Performance: *Moringa Oleifera* leaf powder can be incorporated into diets to improve endurance and overall performance during training.

Gbedinhessi DGE, et al., J Food Sci Nutr Res 2025
DOI:10.26502/jfsnr.2642-110000173

Natural Supplementation: Coaches and sports nutritionists can recommend *Moringa Oleifera* as a natural alternative to synthetic supplements.

Cardiorespiratory Benefits: The study's findings can be used to develop targeted nutrition plans that optimize cardiorespiratory function.

Conclusions

This study indicates that the infusion of MO leaf over seven days enhances pedaling rate, total work output, and exercise duration in young and active participants.

The infusion reduced the energy expended and the time required to cover the 20km by helping participants maintain a higher level of effort. However, it did not significantly affect the other parameters studied, including rating of perceived exertion (RPE), lactate levels, muscle oxygenation, and resting and recovery heart rates.

Given our observations on MO, it would be interesting to characterize more precisely the mechanisms by which MO influences our results and then investigate the potential effects this could have on skeletal muscle and its functioning. Also, check if MO increases products that are considered doping, such as octopamine and many others.

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Conflicts of interest: The authors declare that they have no conflicts of interest.

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Institutional Review Statement: The Research Ethics Board for Student Projects Involving Humans (Multifaculty CEPRE) has reviewed the following research project and deemed it to be in accordance with usual practices and the standards established by UQAM's Policy No. 54 on the Ethics of Research Involving Humans (2020). Project title: EFFECTS OF MORINGA OLEIFERA LEAF POWDER SUPPLEMENTATION ON ATHLETIC PERFORMANCE AND CARDIORESPIRATORY RESPONSES DURING ENDURANCE TESTING. Student name: Dona Géraud Enock Gbedinhessi. Program of study: Master's degree in human kinetics. Research Supervisor(s): Alain Steve Comtois; David H. St-Pierre; Gawiyou Danialou. The study was conducted in accordance with the guidelines of the Declaration of Helsinki. The Institutional Ethics Board of the Université du Québec à Montréal (UQAM) approved it (protocol code 2023-4759, dated October 25, 2022).

Informed consent statement: Informed consent was obtained from all subjects involved in the study.

Data availability statement: The tests' results will be visible only to UQAM researchers associated with this project. The data collected in this study may be used in secondary studies. In this case, requests for access to this data will be evaluated and approved by the UQAM Research Ethics Board.

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