

The effects of moringa seed supplements on oxidative stress and 10 km running performance

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ABSTRACT

Athletes use herbal supplementation to increase physical fitness, reduce the potentially negative consequences of strenuous training, and gain a competitive edge. The goal of this study was to identify the effects of Moringa seed supplements on oxidative stress and 10 km running time. A double-blind, placebo-controlled clinical trial was conducted with 26 male runners. The participants were randomly divided into two groups: the experimental (supplemented) group (n = 15), which received 3% of the athlete's body weight of moringa seed powder added to juices (orang without sugar 300 ml) in the evening after the training, three days a week for 8 weeks. The control (placebo) group (n = 11) received a placebo (orang without sugar 300 ml) at the corresponding times. After the intervention period, malondialdehyde levels were significantly reduced and 10 km running time was improved in the experimental group. No significant changes were observed in catalase, superoxide dismutase and glutathione concentrations in the two groups. In conclusion, moringa seeds were effective in enhancing endurance performance and reducing oxidative stress in a group of young endurance athletes.

Keywords: Sport medicine, Moringa seeds, Oxidative stress, 10 km running.

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INTRODUCTION

Oxygen is one of the greatest biological paradoxes. It is essential for life, yet it produces free radicals, elements harmful to the cell. During breathing, 95% of oxygen is used by cells for energy production, while the remaining part causes free radicals. (Kelvin 2017). Oxidants, although chemically very unstable and highly toxic to cells, are produced under normal conditions inside cells. It is estimated that 5% of all the oxygen we consume in the final stages of oxidative metabolism follows the so-called univalent pathway. Several of the intermediate metabolites that are generated are free radicals. Therefore, normal metabolism is a source of free radicals (Seifried et al. 2007).

Physical exercise is associated with a 10-to-20-fold increase in total oxygen consumption. The oxygen flow in the active fibres of the muscle can increase 100 to 200 times during exercise, thus during intense exercise, the production of reactive oxygen species is high and can result in the generation of oxidative stress (Sen, 2001). These free radicals are neutralized by the antioxidant defence system that consists of both enzymatic antioxidants such as catalase, superoxide dismutase, glutathione peroxidase and non-enzymatic antioxidants such as vitamins A, C, and E among others.

Oxidative stress is a condition in which there is an imbalance between free radicals and antioxidants in the body, leading to damage to cells and tissues. This can result in inflammation, accelerated aging, and increased risk of chronic diseases. Various human studies have shown that the number of free radicals increases during exercise. Running 10 km is an aerobic exercise that increases oxidative stress in the body due to increased oxygen consumption and free radical production. Consuming antioxidant-rich foods and supplements can also help boost the body's defences against oxidative damage. (Santos-Silva et al. 2001).

Athletes use herbal supplementation to increase physical fitness, reduce the potentially negative consequences of strenuous training, and gain a competitive edge (Haskell and Kiernan, 2000). Although herbal supplements are generally safe, there is little scientific evidence to support their claims of improving athletic performance. A well-known herbal supplement that is said to improve muscle activity is Moringa, often referred to as the miracle tree or the tree of life. It is available in a variety of forms including dried leaves, powder, capsules, and seeds. (Ganguly et al. 2005). Moringa is considered a superfood for its many nutritional properties (Giacopo et al. 2017). It is recommended for athletes because it is plant-derived and completely natural. Moringa seeds are rich in antioxidants that help fight oxidative stress caused by intense exercise, ensuring faster recovery times and reducing muscle soreness (Randriamboavonjy et al. 2017). The healthy fats in these seeds also help boost energy levels, allowing athletes to persevere throughout their workout. Although there are numerous reports demonstrating the benefits of moringa in improving athletic performance, many of the studies are still in the early stages or have only been tested on animals rather than humans, and further well controlled research with competitive athletes seems necessary. (Bonoy, et al. 2016).

Therefore, further research is fully justified to clarify the potential benefits of moringa seed supplements on oxidative stress and endurance performance among distance runners to identify its scientific basis.

MATERIAL AND METHODS

Participants

A double-blind, placebo-controlled clinical trial was conducted in 26 male runners. Informed consent was obtained from all athletes. Additionally, formal approved by the Egyptian Athletics Federation. The participants were randomly divided into two groups: the experimental (supplemented) group (n = 15), which

received moringa seeds powder for three days (Sunday- Tuesday- Thursday) a week for 8 weeks, in the evening after the training session. It was consumed as a powder added to juice (orang without sugar 300 ml). The control (placebo) group (n = 11) received a placebo (orang without sugar 300 ml juice without any powder) at the corresponding times.

Experimental design

The experimental design consisted of a 7-day control period, followed by an 8-week intervention of moringa seed powder supplementation at an average dose (2.5 - 3 g) daily according to body weight (4% of body weight). The participants were instructed to maintain their usual diet and activity profile throughout the study period. Side effects such as vomiting, headaches, and diarrhoea were inquired upon daily. It was noted that most of the participants suffered from diarrhoea, so the dose was reduced to 3% of the athlete's body weight. Ten millilitres of blood were collected in the morning before breakfast in EDTA-anticoagulant tubes. Plasma was separated by centrifugation at 4,000 rpm for 5 minutes to determine the oxidative stress markers: malondialdehyde, catalase, superoxide dismutase, and glutathione. Superoxide dismutase and malondialdehyde were assayed by ELISA kits from LifeSpan BioSciences. Catalase was assayed by the human ALCAM (CD166 antigen) ELISA kit (Wuhan Fine Biotech Co., Ltd).

Procedures

Preparation of moringa seeds

As with all supplements, moringa is not monitored by the US Food and Drug Administration (FDA), so there may be concerns about its safety, and potential side effects. Raw moringa seeds were manually cleaned to remove broken seeds and foreign matters, milled into a fine powder, and passed through a 45 mm mesh size sieve. The cleaned moringa seeds were surface disinfected with 0.1 % (v/v) sodium hypochlorite for 20 min and later rinsed with distilled water to prevent microbial growth. The seeds were soaked in distilled water (1:10 w/v) for 12 h at room temperature (25 ± 2°C). (The soaking water was changed every 2 h to prevent fermentation). The soaked grains were washed twice with water followed by rinsing with distilled water.

After soaking, the water was then drained off, and imbibed seeds were germinated by layering them over a moistened filter paper in germination trays in an incubator chamber equipped with temperature control, where water circulation by capillarity was created. The trays were introduced in the germinator (Seedburo Equipment Company, Chicago, USA, Model NO. 549/A), in darkness for 72h at 20-25°C. The germinated seeds were then dried at 60°C for a constant weight into a hot air oven, milled into a fine powder and passed through a 45 mm mesh size sieve. All flours were kept in sealed plastic pages and stored at -22°C in deep freezer till further analysis (Chinma et al., 2009; Cevallos-Casals and Cisneros- Zevallos, 2010).

Table 1. Proximate chemical composition of raw and germinated Moringa flour (% DW).

	Raw seeds	Germinated seeds (72h)
Moisture	3.08 ± 0.05	4.28 ± 0.03
Ash	3.79 ± 0.04	4.69 ± 0.2
Protein	28.74 ± 1.0	33.37 ± 0.7
Fat	30.94 ± 0.5	33.32 ± 0.6
Carbohydrate*	36.53 ± 1.5	28.62 ± 0.5

Note. Means ± Standard deviation (SD), on dry weight basis. * Calculated by difference.

Proximate chemical composition

Chemical composition analyses of moisture, crude oil, crude protein, and ash contents were conducted according to standard methods (AOAC, 2012): defatting in a Soxhlet apparatus with petroleum ether for lipid analyses; and micro-Kjeldahl for crude protein content (CPC) quantification (N × 6.25). The carbohydrate content was estimated by difference using Equation 1:

$$\text{Carbohydrates [g/100 g]} = 100 - \text{CPC [g/100 g]} - \text{Lipids [g/100 g]} - \text{Ash [g/100 g]}.$$

All determinations were performed in triplicates and the means were reported.

Determination of total antioxidant activity

The 2, 2- Diphenyl-1-picrylhydrazyl (DPPH) test was performed according to the method described by Lee et al. (2003) with some modifications. The stock reagent solution (1×10^{-3} mol L⁻¹) was prepared by dissolving 22 mg of (DPPH) in 50 ml of methanol and stored at -20°C until use. The working solution (6×10^{-5} Mol L⁻¹) was prepared by mixing 6 ml of stock solution with 100 ml of methanol to obtain an absorbance value of 0.8 ± 0.02 at 515 nm, as measured using a spectrophotometer. The extracts of samples were prepared in (methanol water 60:40). Extract solutions of tested samples (0.1 ml) were vortexes for 30 s with 3.9 ml of DPPH solution and left to react for 30 min, after which the absorbance was measured at 515 nm on an UV/Visible 6850 spectrophotometer (UV/Visible 6850, Jenway, UK) and recorded. The BHT was used as reference sample and mixture without sample extract or BHT was used as the control. Scavenging activity was then calculated as follows:

$$\text{DPPH radical scavenging activity (\%)} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100$$

Where Abs is the absorbance at 515 nm.

Table 2. Total antioxidant activity (DPPH %) of raw and germinated moringa seeds (% DW).

Treatment	Total antioxidant activity (DPPH %)
Raw Seeds	17.83 ± 0.42
Germinated seeds (72h)	29.43 ± 0.72

Note. Means $\pm$ Standard deviation (SD), on dry weight basis.

Statistical analysis

All statistical analyses were performed with the SPSS statistical package. The results are reported as means and standard deviations (SD). Differences between two groups were reported as mean difference $\pm 95\%$ confidence intervals ($p < .05$). Student's T-test for independent samples was used to determine the differences in biochemical variables and time of the 10 km running trial between the two groups.

RESULTS

Table 3 shows the basic characteristics of the study participants. No significant differences were observed in age, anthropometric variables, or training experience between the two groups.

Table 3. Basic characteristics of study participants.

Variables	N	Age (year)	Body mass (kg)	Height (cm)	Training experience (years)
Moringa group	15	20.4 ± 1.5	70.9 ± 10.2	175 ± 6	5.2 ± 2.1
Control group	11	21.4 ± 1.7	71.2 ± 11.7	173 ± 6	5.7 ± 1.9

Note. Data are presented as means $\pm$ SD.

Table 4 shows significant changes that were observed in malondialdehyde levels and time of the 10 km run in the experimental group. No significant changes were observed in catalase, superoxide dismutase, and glutathione in both groups.

Table 4. Biochemical variables and 10 km running performance for the control and experimental groups.

Variables	Control			Experimental			p
	Pre*	Post*	Change%	Pre*	Post*	Change%	
Malondialdehyde (nmol/ml)	18.5 ± 3.4	18.4 ± 3.4	0.4	17.3 ± 3.2	15.3 ± 2.9	11.5	.05
Catalase (pg/ml)	64.2 ± 10.5	65.2 ± 9.6	1.4	62.7 ± 10.2	66.1 ± 10.9	5.4	Ns
Superoxide dismutase (ng/ml)	66.7 ± 13.2	67.4 ± 11.2	1.0	65.9 ± 11.7	69.3 ± 11.9	5.1	Ns
Glutathione (μmol/L)	62.4 ± 5.7	64.4 ± 6.3	3.1	63.0 ± 6.7	67.8 ± 7.5	7.6	Ns
10 km running time (min)	30.5 ± 0.1	29.4 ± 0.3	3.5	30.2 ± 0.1	28.5 ± 0.3	5.5	.05

Note. *Data are presented as means ± SD.

DISCUSSION AND CONCLUSIONS

The goal of this study was to identify the effects of moringa seed supplements on oxidative stress and 10 km running performance. The results indicated that moringa seed supplements reduced malondialdehyde levels by 11.5% and improved 10 km running time by 5.7%. Although the changes were not statistically significant, there were increases in plasma concentrations of catalase (5.4%), superoxide dismutase (5.1%), and glutathione (7.6%). These results support the hypothesis that moringa seeds could be used in conjunction with normal athletic training to improve oxidative stress and 10 km running performance in male endurance athletes.

Our study revealed a noteworthy decrease in blood malondialdehyde levels following supplementation with moringa seeds. These results underscore the antioxidant properties of moringa seeds. Marcela et al. (2017) pointed out that moringa is abundant in antioxidants like quercetin and chlorogenic acid, which can aid in alleviating oxidative stress caused by intense exercise. By decreasing inflammation and supporting quicker recovery, athletes may discover that adding moringa supplements to their diet enables them to engage in more rigorous and frequent training without the usual physical strain associated with intense exercise. (Piyush et al. 2022).

Araneda et al. (2014) reported that in physically active individuals, running a 10 km race leads to an increase in pro-oxidative substances derived from oxygen and nitrogen, without causing early peroxidation. This differs from the results observed in amateur runners. A study by Hamidie et al. (2023) has found that moringa oleifera may enhance human performance by amplifying the impact of exercise on increasing VO₂max. Moreover, the study suggests that moringa oleifera potentially boosts local muscle endurance, thereby improving anaerobic capacity. Additionally, the capacity of moringa to stabilize blood sugar levels can be especially beneficial for endurance athletes. Maintaining balanced blood sugar enables sustained energy levels during prolonged exercise, ultimately improving overall performance and endurance. (Karina et al. 2019).

Several studies have shown that Moringa supplementation significantly increases catalase levels (Wafa et al. 2017; Mohamed et al., 2020), superoxide dismutase (Woranan et al. 2013), and glutathione (Wafa et al. 2017; Adebayo et al. 2017). (Romano-Ely et al. 2006; Ponce-Gonzalez, et al. 2021) determined that adding antioxidant vitamins to the diet has positive effects on lipid peroxidation and muscle damage caused by exercise. Elsayy et al. (2014) observed that certain antioxidant vitamins have been found to reduce the exercise-induced rise in lipid peroxidation, potentially aiding in the prevention of muscle tissue damage. Another crucial aspect of moringa supplementation is its contribution to hydration and electrolyte balance. The tree serves as a natural source of potassium, calcium, and magnesium — essential nutrients for sustaining muscle function and preventing cramps during extended training sessions or competitions.

Practical implications

Endurance athletes often encounter challenging conditions during their training periods. In response to these challenges, they are exploring different dietary approaches to enhance their endurance performance and promote better metabolic health. Moringa seeds could be used in conjunction with normal athletic training to improve oxidative stress and 10 km running performance in male endurance athletes.

The first week of using Moringa seed powder may cause diarrhoea in addition to headache and slight dizziness. These side effects end after the first week of use. A percentage of 3% of body weight is very suitable for the age and training stage of athletes.

AUTHOR CONTRIBUTIONS

The contribution of the authors has been as follows in each of the sections: Nasser Abouzeid, writing - original draft; Mohamed Saad, data curation; Bogdan-Constantin Ungurean, visualization; Alin Larion, supervision; Adam Zajac, funding acquisition; Walaa Kobacy, fundings; Usama Elgazzar, materials; Gehad Mahmoud, design; Hachim Shani, critical review; Hatem Ibadi, performed the analysis; Amr Hamza, conception.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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