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Research

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Effect of moringa seed (*Moringa oleifera* Lam.) infusion on stamina performance in male mice

Hanifati Husna[1], Andiyanto Andriyanto[2], Wasmen Manalu[3], Kusdiantoro Mohamad[4]*

[1] Study Program of Veterinary Medicine, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia
[2] Division of Veterinary Pharmacology and Toxicology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia
[3] Division of Physiology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia
[4] Division of Anatomy, Histology, and Embryology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia

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Abstract

Background Individuals experience varying levels of fatigue and stamina. *Moringa oleifera* Lam. Seed extract relieves fatigue due to its high levels of antioxidants and glucosinolate *glucomoringin*, which reduces intracellular oxidative stress.

Objectives This study aimed to determine the effectiveness of infusion of *M. oleifera* seed as a stamina stimulator in mice.

Methods This experiment used 25 male mice divided into five groups (five mice in each group), consisting of *M. oleifera* seed infusion at doses of 0, 1, 3, and 5 g/kg BW as the negative control and treatment groups, and caffeine at a dose of 6.5 mg/kg BW as the positive control group. Stamina effectiveness was evaluated using a swimming endurance test that measured swimming duration and physiological parameters every 15 min for 2 h after administration. Data were analyzed using analysis of variance (ANOVA) followed by Tukey's test.

Results *M. oleifera* seed infusion at a dose of 3 g/kg BW had the shortest swimming duration difference (Δ) value, with no difference compared to the 5 g/kg BW and caffeine groups, but was significantly different ($P<0.05$) compared to the 0 and 1 g/kg BW groups. *M. oleifera* seed infusion at a dose of 3 g/kg BW also showed a lower delta value for body surface temperature and heartbeat rate compared to the negative control, and a lower delta value for the respiratory rate compared to the 5 g/kg BW group.

Conclusion *M. oleifera* seed infusion potentially maintains stamina performance in mice at a dose of 3 g/kg BW.

Keywords: anti-fatigue, infusion, Moringa oleifera, stamina performance, mice

Introduction

Fatigue is a complex physiological condition characterized by a decline in the body's ability to initiate or sustain voluntary activities, primarily due to the depletion of physical or mental energy reserves (Gao *et al.*, 2018). It is broadly classified into physical (peripheral) and mental fatigue, with physical fatigue often manifesting as reduced performance capacity and endurance (Lamou *et al.*, 2015). According to exhaustion theory, fatigue results from a combination of energy substrate depletion and metabolite accumulation (Huang *et al.*, 2012).

***Corresponding author** Email: kusdiantoro@apps.ipb.ac.id

Enhancing exercise tolerance is a key indicator of stamina improvement and is typically measured by the ability to sustain prolonged physical activity (Ding *et al.*, 2011). In recent years, functional foods and natural products have gained prominence as promising non-pharmacological interventions for fatigue management given the limited availability and potential side effects of conventional pharmacological agents (Shimizu *et al.*, 2019).

Traditional Indonesian herbal medicines have long been valued for their role in health maintenance and disease management owing to their perceived safety compared to synthetic drugs (Sumarni *et al.*, 2019). The World Health Organization (WHO) reports that over 80% of the global population relies on traditional medicine for primary healthcare needs (Atta *et al.*, 2014). Notably, plants rich in polyphenols and polysaccharides serve as potent antioxidants, which may enhance endurance and reduce fatigue by extending exercise time to exhaustion. These antioxidant effects are hypothesized to act through the modulation of the Nrf2 pathway, a key regulator of cellular defense against oxidative stress, thereby preventing exercise-induced muscle fatigue (Martins *et al.*, 2018; Chen *et al.*, 2014). Additionally, fatigue resistance has been associated with the preservation of glycogen stores, regulation of oxidative enzymes, and activation of the PGC-1α pathway (Kim *et al.*, 2020).

Among the various medicinal plants, the genus *Moringa* (family Moringaceae) has been traditionally employed for wound healing and the treatment of diverse ailments (Abd Rani *et al.*, 2018). *Moringa oleifera* Lam., commonly known as the drumstick tree, horseradish tree, ben oil tree, or kelor (in Indonesia), is native to India but is now widely distributed across Asia and Africa (Minaiyan *et al.*, 2014; Lamou *et al.*, 2015; Raja *et al.*, 2016). A growing body of *in vivo* and *in vitro* evidence supports the pharmacological potential of *M. oleifera*, including its antibacterial, anti-inflammatory, immunomodulatory, hypocholesterolemic, antihypertensive, neuroprotective, and anti-fatigue properties (Shimizu *et al.*, 2019). *M. oleifera* is a rich source of β-carotene, proteins, vitamin C, calcium, potassium, and other bioactive compounds, making it a good natural antioxidant supplement.

The utilization of seeds as waste material can create zero waste so that all parts of the plant can be utilized. The seeds of *M. oleifera* are particularly notable for their content of oleic acid (ben oil), the antibiotic pterygospermin, and various fatty acids, such as linoleic, linolenic, and behenic acids. Additionally, they contain a diverse range of phytochemicals, including tannins, saponins, phenolics, flavonoids, terpenoids, and lectins, as well as essential nutrients, such as dietary fiber, proteins, and vitamins A, B, and C (Gopalakrishnan *et al.*, 2016). Recent studies have demonstrated significant antioxidant activity in raw *M. oleifera* seeds, with levels reaching 1531.36 mg TE/100 g dry matter, and have shown that the phenolic content in sprouts can be enhanced through optimized germination conditions (Coello *et al.*, 2020). Experimental models have also confirmed the stamina-enhancing effects of *M. oleifera* leaf and seed extracts in animal endurance tests such as forced swimming trials in rats (Lamou *et al.*, 2015; Shimizu *et al.*, 2019).

Different extraction methods can interfere with the total amount of active compounds (Zhang *et al.*, 2018). The infusion method is the simplest and most widely used method in everyday life when consuming herbal medicines. This method is easy to dissolve and is easily absorbed by the body (Studzińska-Sroka *et al.*, 2021). This study aimed to evaluate the efficacy of *M. oleifera* seed infusion as a stamina-enhancing agent in mice by assessing swimming endurance and associated physiological parameters. The results were intended to identify the optimal effective dose and provide preliminary data supporting the potential use of *M. oleifera* seed infusion as a safe, affordable, and effective herbal alternative to synthetic stamina enhancers or anti-fatigue agents. Furthermore, this study seeks to contribute to a deeper understanding of the comparative effectiveness of herbal and non-herbal stamina-enhancing interventions.

Methods

Animals

This study was approved by the Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences (SVMBS), IPB University (number 003/KEH/SKE/VI/2022). This study was conducted from November 2021 to April 2022 in the Pharmacology Laboratory and Laboratory Animals Management Unit (UPHL) SVMBS, IPB University, Bogor, Indonesia. The experiment used 25 male mice of the DDY strains weighing 20–30 g body weight. The 5 mice cage was made of plastic materials (55 cm × 37 cm × 17 cm), a water supply container, wood shaving as the base, a modified wire with a wooden frame to close the cage, and labels following dosage treatment. Each cage was filled with DDY from five male mice.

The mice were fed a basic diet once daily with unlimited access to water. The feed was an HI-PRO BR12 (PT Charoen Pokphand, Indonesia) composed of water (14%), ash (8.0%), crude protein (19%–23%), crude fat (5%), crude fiber (6%), calcium (0.8%–1.1%), phosphorus (0.45%), urea and amino acids (1.05%), methionine (0.4% M+C 0.75%), tryptophan (0.18%), and threonine (0.65%). Distilled water was used as the drinking water.

For two weeks prior to the experiment, mice were acclimated under standard laboratory conditions, which included a temperature range of 20–30°C, a relative humidity of 45%–55%, and a 12-hour light/dark cycle. Anthelmintics were administered a few days after placement. The feed was administered daily in the morning.

Infusion preparation

Fresh *M. oleifera* seeds were harvested from the Kepung District, Kediri Regency, East Java, in November 2021. The seeds were cleaned immediately after harvesting and dried in the sun for five days. The dried material was ground into a powder. Powder (30 g) was added to 100 mL distilled water, boiled until the temperature reached 90°C, waited for 15 min, and allowed to cool to room temperature. The infusion was obtained by filtering the final solution using filter paper. The initial concentration of the infusion (0.3 g/mL) was obtained. The final concentration for treatment was prepared by adding distilled water according to the experimental doses will be given. The infusion was stored in a glass bottle and refrigerated prior to use.

Experimental design and treatment

The experimental design was completely randomized. The mice were divided into five groups (n= 5 per group in each test) for treatment: (1) negative control using 1 mL of aquadest or 0 g/kg BW dose, (2) 1 g/kg BW dose infusion of *M. oleifera* seeds, (3) 3 g/kg BW dose infusion of *M. oleifera* seeds, (4) 5 g/kg BW dose infusion of *M. oleifera* seeds (treatment doses was chosen according to Anaba *et al.,* 2021), and (5) positive control using caffeine (Sigma-Aldrich, Cat. C7050-100G) 6.5 mg/kg BW dose (according to Lee *et al.,* 2012). All groups used a 1 mL volume of treatment.

M. oleifera was homogenized before force-feeding treatment. Mice were handled by holding the back of the neck and stretching the body by attaching a tail between the fingers. Force-feeding administration of *M. oleifera* infusion used a gastric probe directly to the distal esophagus entering the stomach (Anaba *et al.,* 2021) following the dose of each group. After administration, the stamina test was initiated.

Stamina tests used exhaustive swimming exercises to determine the endurance of each mouse, measured as the swimming time recorded from the beginning of the time to exhaustion by observing uncoordinated movements (Huang *et al.,* 2016) and tiredness of mice. Mice were placed inside a box filled with distilled water. The swimming duration from the start of entering the water after exhaustion was measured using a stopwatch, and physiological changes (surface body temperature, heartbeat, and respiration frequency) were measured manually. The surface body temperature was measured using an infrared thermometer gun (Omron, MC-720, Indonesia) in the forehead area of the mouse, the heartbeat was measured using finger palpation, and the respiration rate was measured by inspecting the thorax/abdomen movement.

Observation and data collection

The observed parameters of the experiment were swimming duration (s), surface temperature (°C), heartbeat (beats per minute, bpm), and respiration rate (bpm) before swimming. Experimental mice were placed in water tanks for exhaustive swimming exercises. The swimming duration was recorded from the start time of the mice to swim until immobilization inside the water. All parameters were measured every 15 min for 2 h after administration. The delta (Δ) values of swimming duration, surface body temperature, heartbeat, and respiration rate were calculated by comparing the parameter results of each minute with the results of 0 min. The average of each group (0 g/kg BW [negative control], 1 g/kg BW dose, 3 g/kg BW dose, 5 g/kg BW dose, and caffeine 6.5 mg/kg BW [positive control]) at each minute and repetition were calculated and compared.

$$\Delta \boldsymbol{P} = P_t - P_0$$

ΔP = Delta (Δ) value of parameter.
P_t = Parameter of certain minute
P_0 = Parameter of 0 minute

Data analysis

The experimental data were analyzed using *Microsoft Excel* and *Minitab* 19 software. The data result from each minute is compared with that at 0 min and then converted into delta (Δ) or difference values to obtain a better data reading. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by *Tukey* test. Statistical significance was set at $p < 0.05$.

Results

The swimming duration, surface body temperature, respiratory rate, and heart rate at minute 0, along with their delta values (i.e., the difference between measurements at specific time points and at minute 0), are presented in **Table 1**. At baseline (minute 0), swimming duration and surface body temperature did not differ significantly among the treatment groups. In contrast, respiratory and heart rates showed significant differences, with the 5 mg/kg BW infusion and caffeine groups exhibiting significantly higher values ($P<0.05$) than the other groups. These baseline variations may reflect individual or inter-group variability or possibly an immediate physiological response to 5 mg/kg BW infusion and caffeine administration.

Subsequent analyses were based on delta values to minimize bias associated with baseline differences. These values were calculated by subtracting the minute 0 measurement from each subsequent time point to provide a more accurate basis for comparison across groups. A smaller delta value reflects a better endurance or physiological stability relative to the initial state.

As shown in **Table 1**, the 3 mg/kg BW infusion group demonstrated the most favorable endurance profile, characterized by the smallest change (delta) in the swimming duration. This was comparable to that in the positive control group (5 mg/kg BW infusion and caffeine). In contrast, the delta values for surface body temperature, respiratory rate, and heart rate in the 3 mg/kg BW group were lower and statistically similar to those of

Table 1 The total difference (Δ) value of swimming duration and physiological parameter after treatment of various dosage of *M. oleifera* seed infusion in mice

Treatment group	Total values (average ± SEM) of							
	Stamina parameter		Physiological parameters					
	Swimming duration (sec)		Surface body temperature (°C)		Respiration rate (bpm)		Heartbeat (bpm)	
	At 0 min (n=5)	Difference (Δ) (n=40)	At 0 min (n=5)	Difference (Δ) (n=40)	At 0 min (n=5)	Difference (Δ) (n=40)	At 0 min (n=5)	Difference (Δ) (n=40)
Negative control	100.6 ± 33.3^{a}	-64.2 ± 9.7^{bc}	36.3 ± 0.1^{a}	-0.4 ± 0.1^{b}	167.2 ± 3.2^{ab}	6.4 ± 2.9^{a}	131.2 ± 4.6^{ab}	27.5 ± 3.1^{a}
1 mg/kg BW infusion	130.4 ± 22.8^{a}	-71.0 ± 5.6^{c}	36.5 ± 0.1^{a}	-0.3 ± 0.1^{ab}	136.8 ± 11.8^{a}	1.7 ± 3.1^{ab}	100.0 ± 4.2^{a}	18.0 ± 3.6^{a}
3 mg/kg BW infusion	82.8 ± 11.7^{a}	-30.0 ± 6.2^{a}	36.3 ± 0.1^{a}	-0.1 ± 0.1^{a}	167.2 ± 9.8^{ab}	-1.6 ± 4.0^{ab}	160.0 ± 13.7^{bc}	-5.7 ± 4.2^{b}
5 mg/kg BW infusion	100.6 ± 25.5^{a}	-37.9 ± 9.0^{ab}	36.5 ± 0.2^{a}	-0.5 ± 0.1^{b}	191.2 ± 18.7^{b}	-11.9 ± 6.9^{b}	197.6 ± 27.5^{c}	-19.5 ± 8.5^{b}
Caffeine	108.4 ± 8.9^{a}	-53.8 ± 4.6^{abc}	36.1 ± 0.1^{a}	-0.2 ± 0.1^{ab}	205.6 ± 5.3^{b}	0.7 ± 2.3^{ab}	208.0 ± 3.4^{c}	-2.9 ± 1.7^{b}

P-values were obtained using one-way ANOVA with Tukey's post-hoc test. Data are presented as mean ± standard error of the mean (SEM). Different superscripts (a–d) in the same column indicate significant differences (P<0.05). Caffeine: 6.5 mg/kg BW.

the negative control, but significantly different from those in the 5 mg/kg BW infusion and caffeine groups. These results suggest that the 3 mg/kg BW infusion provided stamina-enhancing benefits comparable to those of higher doses and caffeine, but with more stable physiological responses, indicating a potentially safer and more favorable pharmacological effect.

The swimming duration across 15-minute intervals over a 2-hour observation period were illustrated in **Figure 1**. The 3 mg/kg BW group consistently exhibited the smallest change in swimming duration compared with the 1 and 5 mg/kg BW infusion groups, although at certain time points, the 1 and 5 mg/kg BW infusion groups demonstrated comparable or slightly improved values. Overall, all treatment groups (1, 3, and 5 mg/kg BW infusion) showed prolonged swimming duration compared to the negative control, supporting the stamina-enhancing potential of Moringa oleifera seed infusion. The observed initial decrease in swimming duration at 15 min was likely due to fatigue from early swimming activity, while subsequent increases after 30 min suggested the onset of infusion effects. Notably, the 3 mg/kg BW infusion group outperformed the negative control, 1, and 5 mg/kg BW infusion groups, as well as the caffeine group, in terms of overall stamina performance. Statistical analysis confirmed that the 3 mg/kg BW infusion group had significantly improved swimming duration compared to the negative control and 1 mg/kg BW infusion groups (P<0.05), although no significant difference was found when compared to the 5 mg/kg BW infusion or caffeine groups (P>0.05).

Figure 1 Swimming duration difference (Δ) from 0 minute (seconds, average ± SEM) after treatment of *M. oleifera* seed infusion in interval of 15 minutes for two hours in mice. *M. oleifera* seed infusion at dose 3 g/kg BW was the shortest swimming duration difference (Δ) value, significantly different compared to negative control and infusion at dose 1 g/kg BW groups (P<0.05), but no difference compared to infusion at dose 5 g/kg BW and caffeine (6.5 mg/kg BW) groups (P>0.05).

The surface body temperature delta values every 15 min for 2 h were showed in **Figure 2**. The 3 mg/kg BW infusion group exhibited the smallest decrease in surface temperature. This reduction was not significantly different from that in the 1 mg/kg BW infusion and caffeine

groups (P>0.05), but was significantly different from that in both the negative control and 5 mg/kg BW infusion groups (P<0.05). Elevation in stamina performance may be related to improved muscle activity, which typically elevates body temperature. However, immersion in water likely causes thermal dissipation, resulting in a lower net surface temperature.

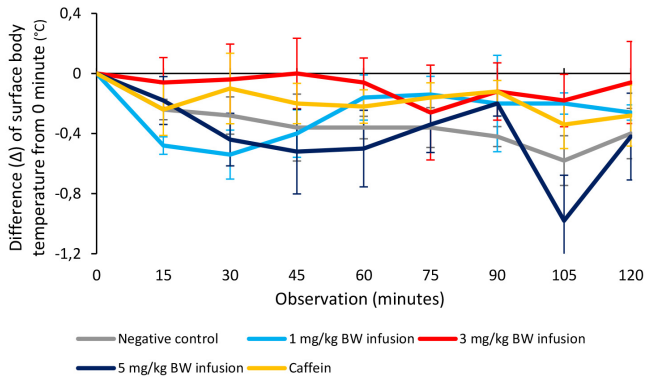

Figure 2 Surface body temperature difference (Δ) from 0 minute (°C, average ± SEM) after treatment of *M. oleifera* seed infusion in interval of 15 minutes for two hours in mice. The lowest mice surface body temperature difference (Δ) value was infusion at dose 3 g/kg BW group, significantly different compared to infusion at 0 and 5 g/kg BW groups (P<0.05), but no difference compared to infusion at 1 g/kg BW and caffeine (6.5 mg/kg BW) groups (P>0.05).

The respiratory rate delta values across the 2-hour swimming endurance test were presented in **Figure 3**. In the first 60 min, respiratory rates varied among the groups, while a more consistent pattern emerged during the second hour. The 3 mg/kg BW infusion group exhibited smaller decreases in respiratory rate than the other groups. On average, the 1 and 3 mg/kg BW infusion groups maintained the lowest delta values, with no statistically significant differences observed when compared with the negative control and caffeine groups (P>0.05).

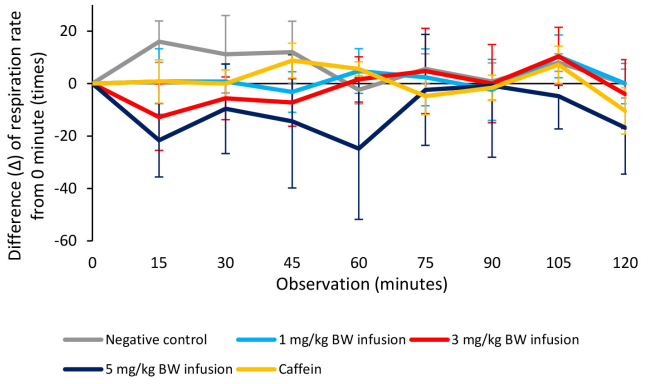

Figure 3 Mice respiration rate difference (Δ) from 0 minute (times, average ± SEM) after treatment with *M. oleifera* seed infusion in interval of 15 minutes for two hours in mice. *M. oleifera* seed infusion at dose 3 g/kg BW group was lower than infusion at 5 mg/kg BW group (P<0.05), but no different with negative control, infusion at dose 1 mg/kg BW, and caffein (6.5 mg/kg BW) groups (P>0.05).

The heart rate delta value every 15 min during the 2-hour swimming test were displayed in **Figure 4**. The higher doses (3 and 5 mg/kg BW) and caffeine groups exhibited distinct response patterns compared with the lower doses (negative control and 1 mg/kg BW infusion). Notably, the 3 mg/kg BW-infusion group closely mirrored the response observed in the caffeine group. Among all groups, the 3 mg/kg BW infusion group demonstrated the lowest change in heart rate relative to baseline. Statistical analysis revealed significant differences between the 3 mg/kg BW infusion group and the negative control and 1 mg/kg BW infusion groups (P<0.05); however, no significant differences were observed between the 5 mg/kg BW infusion and caffeine groups (P>0.05).

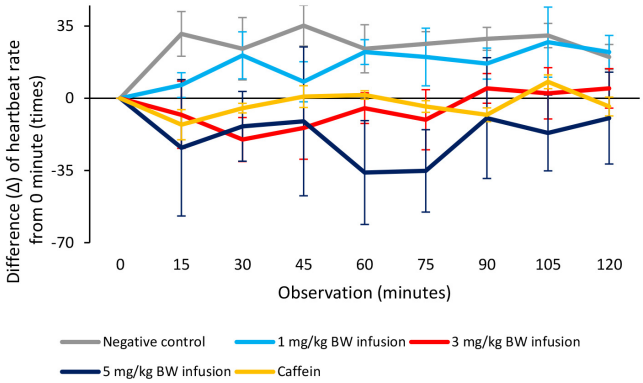

Figure 4 Mice heartbeat difference (Δ) from 0 minute (times, average ± SEM) after treatment with *M. oleifera* seed infusion in interval of 15 minutes for two hours in mice. *M. oleifera* seed infusion at dose 3 g/kg BW group was lower than negative control and infusion at dose 1 g/kg BW groups (P<0.05), but no difference compared to infusion at dose 5 g/kg BW and caffeine (6.5 mg/kg BW) groups (P>0.05).

Discussion

This study was conducted to evaluate the effects of *M. oleifera* seed infusion on swimming endurance in mice using swimming duration, body temperature, respiration rate, and heart rate as key parameters. The swimming endurance test revealed that pharmacological agents can modulate movement coordination by influencing the central nervous system (CNS) activity (Lukman & Vivi, 2013). Immobility during the test was considered indicative of fatigue, exhaustion, and diminished stamina, with the endpoint defined as the moment when the mice could no longer swim and began to drown (Adkar *et al.*, 2014). Fatigue arises from multiple physiological mechanisms, including (i) proton accumulation in muscle cells that lowers pH and inhibits key enzymes, such as phosphofructokinase; (ii) depletion of energy reserves, such as phosphocreatine and glycogen; (iii) ammonia accumulation in blood and tissues; (iv) oxidative stress; (v) muscle tissue damage; and (vi) neurochemical alterations, such as increased serotonin and decreased dopamine levels, which together contribute to fatigue and reduced physical performance (Coqueiro *et al.*, 2019).

The results (**Figure 1**) demonstrated that *M. oleifera* seed infusion enhanced swimming endurance over a two-hour period, particularly in the 1, 3, and 5 g/kg BW infusion groups, compared to the negative control group. Stamina are closely linked to muscular strength and have broader implications for immune function (Nopitasari *et al.*, 2022). Previous studies have shown that ethanolic extracts of *M. oleifera* seeds can modulate immune responses, including suppression of both cellular and humoral immunity, and inhibit macrophage phagocytic activity (Mahajan and Mehta, 2010). Although various parts of the *M. oleifera* plant have been traditionally used, the seeds are especially rich in bioactive compounds with anti-inflammatory, antioxidant, hypotensive, antibacterial, and chemopreventive properties. The key phytochemicals include glucosinolates (GLSs), isothiocyanates (ITCs), nitriles, carbamates, and thiocarbamates, with glucomoringin (GLS-1) being the predominant GLS in the seeds (Jaja-Chimedza *et al.*, 2017).

Glucomoringin is unique among glucosinolates due to its additional saccharide residue, which may account for its distinct biological activities. Both glucosinolates and isothiocyanates are well-recognized for their anticancer and antioxidant properties, notably their ability to induce phase II detoxification enzymes and inhibit phase I activation enzymes (Maldini *et al.*, 2014).

Although this study did not directly assess biochemical markers of fatigue, it is important to consider the underlying mechanisms. Muscle fatigue typically occurs when aerobic metabolism is insufficient, forcing reliance on anaerobic pathways to produce lactic acid. Elevated serum urea nitrogen (SUN) levels reflect increased protein and amino acid catabolism during prolonged exercise, whereas enzymes such as lactate dehydrogenase (LDH) and creatine kinase (CK) serve as biochemical markers of muscle fatigue and cellular damage (Huang *et al.*, 2019).

Oxidative stress plays a central role in physical fatigue because strenuous exercise generates excessive reactive oxygen species (ROS) (Huang *et al.*, 2019). Endogenous antioxidant defenses, including superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), mitigate oxidative damage by neutralizing ROS levels. SOD catalyses the conversion of superoxide radicals to hydrogen peroxide, which is subsequently reduced by GSH-Px. Malondialdehyde (MDA), a byproduct of lipid peroxidation, is a commonly used biomarker of oxidative stress in anti-fatigue studies. Previous research has suggested that *M. oleifera* seed extract mitigates fatigue primarily through its antioxidant effects (Shimizu *et al.*, 2019).

Under normal conditions, ROS levels are regulated tightly. However, excessive ROS generation can overwhelm antioxidant defenses, leading to oxidative stress and cellular damage, which contributes to inflammation and fatigue. Therefore, antioxidants have been proposed as therapeutic agents for the recovery (Shimizu *et al.*, 2019). *M. oleifera* seeds exhibit substantial antioxidant capacity (1531.36 mg TE/100 g dry matter), and environmental factors such as temperature and germination time can modulate the accumulation of bioactive phenolic compounds (Coello *et al.*, 2020).

The seeds contain 8–10% glucomoringin, which has a special structure with extra sugar part (Galuppo *et al.*, 2013). Glucomoringin from Moringa seeds changes into moringin with an enzyme and activates PPAR β/δ, which may help improve muscle endurance and could explain Moringa's stamina benefits (Shimizu *et al.,* 2019).

Interestingly, the results also showed that *M. oleifera* seed infusion slightly lowered body temperature during the endurance test (**Figure 2**). This effect may partly result from the use of distilled water at room temperature. Effective thermoregulation is essential during exercise, as metabolic heat production increases dramatically, with only ~20% of the muscle energy used for mechanical work, and the remainder converted to heat (Takeda and Okazaki, 2018). Blood circulation and cutaneous vasodilation play critical roles in dissipating heat, particularly in cooler environments, whereas evaporative cooling through sweating becomes the dominant mechanism (Takeda and Okazaki, 2018).

The data (**Table 1**) indicated that *M. oleifera* infusion affected both respiration and heart rate, with the highest values observed in the 5 g/kg BW infusion group, followed by the negative control and 3 and 1 g/kg BW infusion groups. Swimming imposes additional respiratory challenges owing to hydrostatic pressure across the chest (Wylegala *et al.*, 2006). Furthermore, post-exercise recovery involves elevated respiration rates (oxygen debt) to clear lactate and other metabolic byproducts and to restore homeostasis (Ferretti *et al.*, 2022). The observed decline in respiration rates during recovery aligns with the expected physiological adaptations.

Importantly, the infusion appeared to stabilize both respiration and heart rate ratios over time, particularly in the 3 and 5 g/kg BW infusion groups (**Figures 3** and **4**). *M. oleifera* has been reported to possess cardiostimulant properties that affect both the heart and circulatory system (Trigo *et al.*, 2021). Heart rate regulation is predominantly governed by the autonomic nervous system, with sympathetic nervous system activation during stress or forced exercise modulating the cardiovascular responses (Lakin *et al.*, 2018). Moreover, environmental temperature can influence metabolic and cardiovascular activity in mice, with increased activity observed at lower ambient temperatures (Swoap *et al.*, 2004).

The phytochemical profile of *M. oleifera* seeds includes acetylated isothiocyanates, phenolic glycosides, flavonoids, lipids, fatty acids, proteins, and carbohydrates, all of which contribute to bioactivity (Jaja-Chimedza *et al.*, 2017). While Moringa leaf extracts are rich in antioxidants, the seeds retain considerable therapeutic potential, particularly in combination preparations such as moringa tea infusions (Ilyas *et al.*, 2015).

In this study, the average body weight of mice in the 3 g/kg BW infusion group was the highest, suggesting that body mass, in addition to dosage, may influence swimming endurance. Notably, no prior acclimatization to swimming was performed, which may have induced additional stress or variability. Previous studies have highlighted the importance of familiarization protocols to reduce variability and enhance test reliability, with exercise interventions typically requiring at least six weeks to induce physiological adaptations (Veskoukis *et al.*, 2018). Differences in the extraction methods also caused differences in the metabolite quality of the extract. According to previous research, aqueous infusions have lower antioxidant potency than alcoholic extracts (Rameshvar *et al.*, 2010), which may explain why the effect of aqueous infusions is not very strong.

The future target animal for this infusion application is the sporting horse because it needs a stamina stimulator during horse racing, but should avoid using the drug as a dopant (Fragkaki *et al.*, 2017). The infusion is adjusted to prevent substance damage because the horse digestive system undergoes a fermentation process. The effect of *M. oleifera* seed infusion as a stamina stimulator can be distinguished from that of doping drugs by observing whether it is included in the Prohibited Substances List from the Federation Equestrian International (FEI, 2025).

In this study, we exclusively used male mice. The use of males aimed to minimize the influence of hormonal factors or estrous cycles, which could potentially affect research outcomes. Additionally, the preference for male animals in sports-related contexts, such as horses, serves as another rationale for their selection. Nevertheless, recent studies have shown that using female subjects is not necessarily more complex than using male (Wiseman, 2023). In fact, behavioral research outcomes involving females are encouraged, as they may enrich the data and broaden the interpretation of the findings (Chari *et al.*, 2020). Therefore, the use of only male subjects in this study is a limitation, and the conclusions drawn cannot be generalized to both sexes.

Conclusion

M. oleifera seed infusions at doses of 1, 3, and 5 g/kg BW are available to maintain stamina performance in mice. Each dose used in this experiment was effective in maintaining the stamina performance in mice. The most effective dose was 3 g/kg BW ($P<0.05$), with the shortest swimming duration difference (Δ) average in minutes and the lowest difference (Δ) value in each physiological parameter.

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Conflict of interest The authors declare no conflicts of interest.

Author contribution HH: Investigation, data curation, formal analysis, and writing – original draft; AA: Conceptualization, funding acquisition, and supervision; WM: Supervision, validation, writing – review & editing; KM: Supervision, validation, writing – review & editing.

Preprints This article has no preprint version.

Availability of data Raw data can be accessed upon request from the corresponding author.

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