Synergistic effects of Tiger Milk Mushroom and Long Jack on endurance running performance and selected physiological responses in athletes

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ABSTRACT The consumption of ergogenic supplements is a common strategy among athletes to enhance sports performance. Tiger Milk Mushroom and Long Jack are traditional herbal supplements known for their potential health and physical fitness benefits, and their use has recently gained attention in the sports industry. However, there is currently limited scientific evidence regarding the effects of these supplements on endurance running performance. This study aimed to investigate the effects of combined Tiger Milk Mushroom and Long Jack supplementation on endurance running performance and selected physiological parameters in athletes. Eight male athletes participated in this randomized cross-over study. Participants consumed either a combination of Tiger Milk Mushroom and Long Jack or a placebo for six consecutive days prior to the endurance running test. On the day of the experimental trial, the supplement or placebo was administered one hour before the test. The time to complete the running test, heart rate, rate of perceived exertion (RPE), maximal oxygen consumption (VO_2max), blood pressure, and body weight were measured in both trials. A one-week washout period was implemented between trials. Data were expressed as mean ± standard deviation. Repeated-measures ANOVA and paired t-tests were used for statistical analysis. This study found no significant differences in the time to complete the running test, VO_2max, heart rate, RPE, blood pressure and body weight between the two trials ($p>0.05$). Heart rate, RPE and blood pressure significantly increased from resting values in both trials ($p<0.05$). In conclusion, six days of combined Tiger Milk Mushroom and Long Jack supplementation, including an additional dose one hour before the endurance running test, did not produce significant ergogenic effects on endurance running performance and selected physiological parameters in athletes.

KEYWORDS: Tiger Milk Mushroom; Long Jack, Endurance running; Heart rate; Maximum oxygen consumption.
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INTRODUCTION

Tiger Milk Mushroom (*Lignosus rhinocerus*) and Long Jack (*Eurycoma longifolia*) are traditional herbal supplements that have recently gained attention in the sports industry for their potential health and physical performance benefits. Tiger Milk Mushroom has been reported to promote general health and improve physical fitness (Wong *et al*., 2024). Long Jack has adaptogen and anti-aging remedy used to combat stress, improve health, and enhance physical strength (Rehman *et al*., 2016; Talbott *et al*., 2013). Long Jack has been found to provide multiple health benefits, including treatment of skin conditions, dysentery, hypertension, fever, and parasitic infections. It has also been associated with aphrodisiac, antimalarial, cytotoxic, antipyretic and antiulcer properties (Bhat & Karim, 2010).

Supplementation with Tiger Milk Mushroom has been well documented for its health-promoting properties, particularly its antioxidant capacity (Tan *et al*., 2021; Fung & Tan, 2017). This antioxidant activity may help reduce free radical production, which in turn may improve sports performance. Antioxidants play a critical role in protecting the body against oxidative stress, reducing muscle damage and fatigue, and potentially enhancing physical performance (Higgins *et al*., 2020). A study by Tan *et al*. (2021) reported that consuming 300 mg of Tiger Milk Mushroom twice daily for three months increased total antioxidant capacity by approximately 70%. Another study by Fung & Tan (2017) found that daily intake of 500 mg of Tiger Milk Mushroom powder for two weeks among 100 volunteers improved stamina, alertness and respiratory function.

Long Jack is widely marketed for its potential to enhance athletic performance and muscle strength (Brauer *et al*., 2019). Several studies have demonstrated its ergogenic and stress-reducing effects in athletes (Chen *et al*., 2014; Talbott *et al*., 2006; Kraemer & Ratamess, 2005). Talbott *et al*. (2006) reported that the consumption of 100 mg of water-soluble Long Jack extract 30 minutes before intense endurance exercise resulted in a 32.3% reduction in cortisol levels and a 16% increase in testosterone levels compared to a placebo. These findings suggest that Long Jack promotes an anabolic hormonal state during intense exercise, which may enhance performance and mitigate performance decline caused by elevated cortisol and suppressed testosterone levels.

Chen *et al*. (2014) further demonstrated that six weeks of daily Long Jack supplementation (400 mg) resulted in a testosterone-to-epitestosterone (T/E) ratio of 0.74:1, well below the 4:1 cut-off established by the International Olympic Committee (IOC) Medical Commission. This indicates that while Long Jack supplementation may elevate testosterone levels, it remains within legal and safe limits for competitive athletes. A simultaneous rise in cortisol and decline in testosterone is a known early indicator of performance deterioration and may contribute to increased injury risk (Kraemer & Ratamess, 2005). Additional studies have also reported ergogenic effects of Long Jack supplementation on athletic performance (Henkel *et al*., 2014; Hamzah & Yusof, 2003). According to Rehman *et al*. (2016), Long Jack root may enhance energy levels and athletic performance, and its extracts exhibit significant antioxidant activity by neutralizing and scavenging free radicals.

Multi-ingredient pre-workout supplements (MIPS), a relatively new category of dietary supplements, have gained substantial popularity in recent years due to their claimed benefits on athletic performance. Typically consumed prior to exercise, these supplements contain blends of ingredients believed to exert synergistic effects, enhancing both immediate performance and long-term training adaptations compared with single-ingredient supplements (Harty *et al*., 2018). Despite the individual potential of Tiger Milk Mushroom and Long Jack as ergogenic aids, there remains limited evidence on the combined effects of these two supplements on endurance running performance. It is hypothesized that their combination may produce synergistic effects, even with short-term supplementation. This approach may therefore be more economical and practical for athletes. Therefore, the objective of this study was to investigate the synergistic effects of combined Tiger Milk Mushroom and Long Jack supplementation on endurance running performance and selected physiological parameters in athletes.

METHODOLOGY

Selection of Participants

The sample size was calculated using PS: Power and Sample Size Calculation software. The study power was set at 80% with a 95% confidence interval. The assumed standard deviation was 0.2, the expected difference between populations was 0.23, and the calculated sample size was eight subjects. Eight male subjects aged between 19 and 29 years were recruited for this study through random sampling from a population of athletes in Kota Kinabalu. The subjects participated in a randomized cross-over study design. Participants with a history of hypertension, asthma, diabetes, bronchitis, anaemia, cardiac conditions, kidney or liver disease, or any other major medical conditions were excluded from the study. The full experimental protocol was explained to each subject prior to participation. Each subject completed a set of questionnaires, including demographic information, medical history, lifestyle factors, and the International Physical Activity Questionnaire (IPAQ). Anthropometric measurements were obtained during the pre-trial session.

Experimental Design

Each subject completed a 2.4 km endurance running test on two separate occasions. For the experimental trial, subjects consumed 250 mL of water mixed with 5 grams of a powdered supplement containing Tiger Milk Mushroom and Long Jack daily for six consecutive days. In the control trial, subjects consumed 250 mL of plain water under the same conditions. The two trials (supplementation vs. placebo) were separated by a seven-day washout period. The order of treatments was randomized for each participant.

Preparation of Subjects

Subjects were instructed to record their food intake for three days prior to the first trial and to replicate the same dietary pattern for the three days preceding the second trial. This procedure was implemented to minimize variability in pre-exercise muscle glycogen levels. Additionally, participants were requested to refrain from any strenuous physical activity for at least 24 hours before each test to ensure adequate rest and readiness for the running trials.

Trial Procedure

Upon arrival on the test day, each subject underwent a physical assessment that included measurements of body weight, resting heart rate, and blood pressure. A heart rate monitor was secured to the subject's chest to continuously record heart rate during the test. Each participant was then administered either 250 mL of the supplement drink or plain water one hour before the running test in a randomized cross-over trials. Subjects subsequently performed a 2.4 km endurance running test, and the time taken to complete the test was recorded. Rate of perceived exertion (RPE), heart rate, systolic and diastolic blood pressure, and body weight were measured pre- and post-trial. Maximum oxygen consumption (VO_2max) values were estimated using a field-based 2.4 km running test. VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$) was calculated using the equation: $VO_2max = 3.5 + (483 / \text{time in minutes})$ (ACSM, 2021).

Statistical Analyses

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 29.0 (SPSS Inc., United States). Data were expressed as mean ± standard deviation (SD). A repeated measures ANOVA and paired t-test were used to determine statistically significant differences, with significance set at $p < 0.05$.

RESULTS AND DICUSSION

The body mass index (BMI) and waist-to-hip ratio (WHR) of the participants indicated that they were within the normal range as shown in Table 1. In terms of running test, there is no significant difference in the time to complete the 2.4 km endurance run between the supplement and placebo trials (11.28 ± 1.07 min vs. 11.79 ± 1.05 min, respectively; $p > 0.05$; Cohen's d = -0.77; 95% CI: −1.60 to 0.06). Similarly, no significant difference was observed in the maximal oxygen consumption (VO_2max) between the two trials (45.13 ± 5.77 kg/min/L vs. 43.88± 4.82 kg/min/L, respectively; $p > 0.05$; Cohen's d = 0.56, 95% CI: -0.28 to 1.39) as shown in Figure 1.

Table 1. Physical characteristics of the participants.

Physical characteristics of the participants (n=8)	Mean ± SD
Height (cm)	171.89 ± 4.53
Weight (kg)	65.30 ± 10.00
BMI (kg/m²)	22.03 ± 2.79
Waist circumference (cm)	82.90 ± 5.32
Hip circumference (cm)	96.97 ± 7.04
Waist-to-hip ratio (WHR)	0.85 ± 0.03

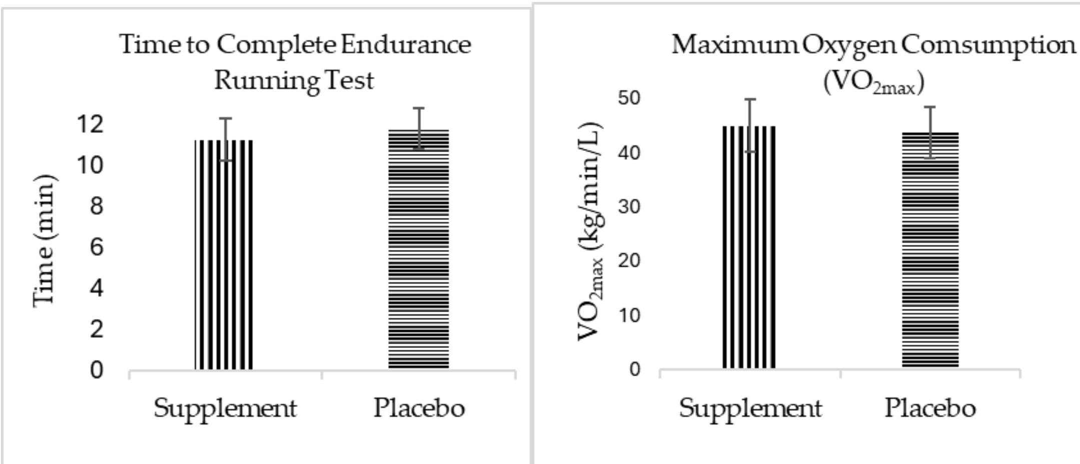

Figure 1. Time to complete endurance running test and maximum oxygen consumption. (VO_{2max}) in the supplement and placebo trials. Data is presented as mean ± SD.

Heart rate, rate of perceived exertion (RPE), blood pressure and body weight are presented in Figures 2 and 3. There was a significant main effect of time on heart rate (F=91.46 df=1; p<0.05), perceived of exertion (F=213.35 df=1; p<0.05), systolic blood pressure ((F=64.09 df=1; p<0.05), diastolic blood pressure (F=5.08 df=1; p<0.05) and body weight (F=13.92 df=1; p<0.05). In the supplement trial, heart rate increased from 69.88 ± 10.83 bpm at rest to 130.38± 30.55 bpm post-run, while in the placebo trial, heart rate increased from 68.13 ± 3.18 bpm to 136.38 ± 19.28 bpm. The rate of perceived exertion (RPE) also increased in both trials, from 6.75 ± 1.17 to 17.38 ± 1.99 in the supplement trial, and from 7.00 ± 1.07 to 17.38 ± 2.06 in the placebo trial. Pre-exercise blood pressure in the supplement trial was 129.75 ± 7.21 mmHg (systolic) and 85.63 ± 11.82 mmHg (diastolic), rising to 157.38 ± 15.97 mmhg (systolic) and 91.25 ± 11.41 mmHg (diastolic) post-run; in the placebo trial, blood pressure increased from 128.13 ± 11.79 mmhg (systolic) and 84.00 ± 10.88 mmHg (diastolic) to 162.00 ± 10.30 mmhg (systolic) and 89.00 ± 6.55 mmHg (diastolic). Body weight showed a slight reduction, with the supplement trial showing 65.44 ± 9.76 kg pre-run and 65.11 ± 9.79 kg post-run, and the placebo trial showing 65.55 ± 9.83 kg pre-run and 65.18 ± 9.72 kg post-run. All four parameters showed significant differences from respective pre-test value at p<0.05. Overall, no statistically significant differences were observed between supplement and placebo trials for any measured parameter (p > 0.05).

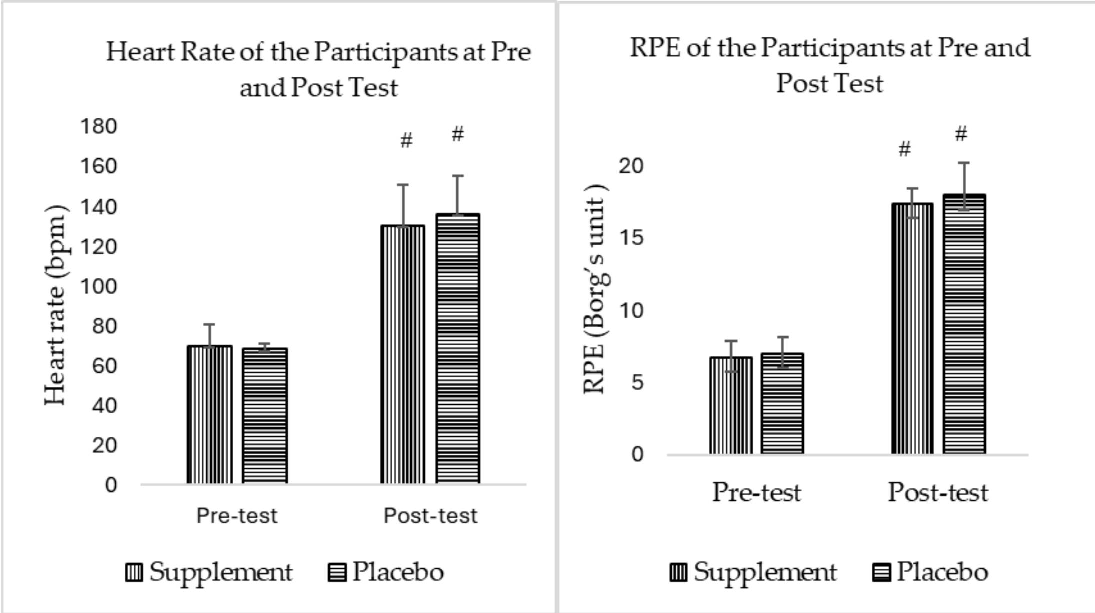

Figure 2. Heart rate and rate of perceived exertion in the supplement and placebo trials. Data is presented as mean ± SD. # denotes significant difference from respective pre-test values at p<0.05.

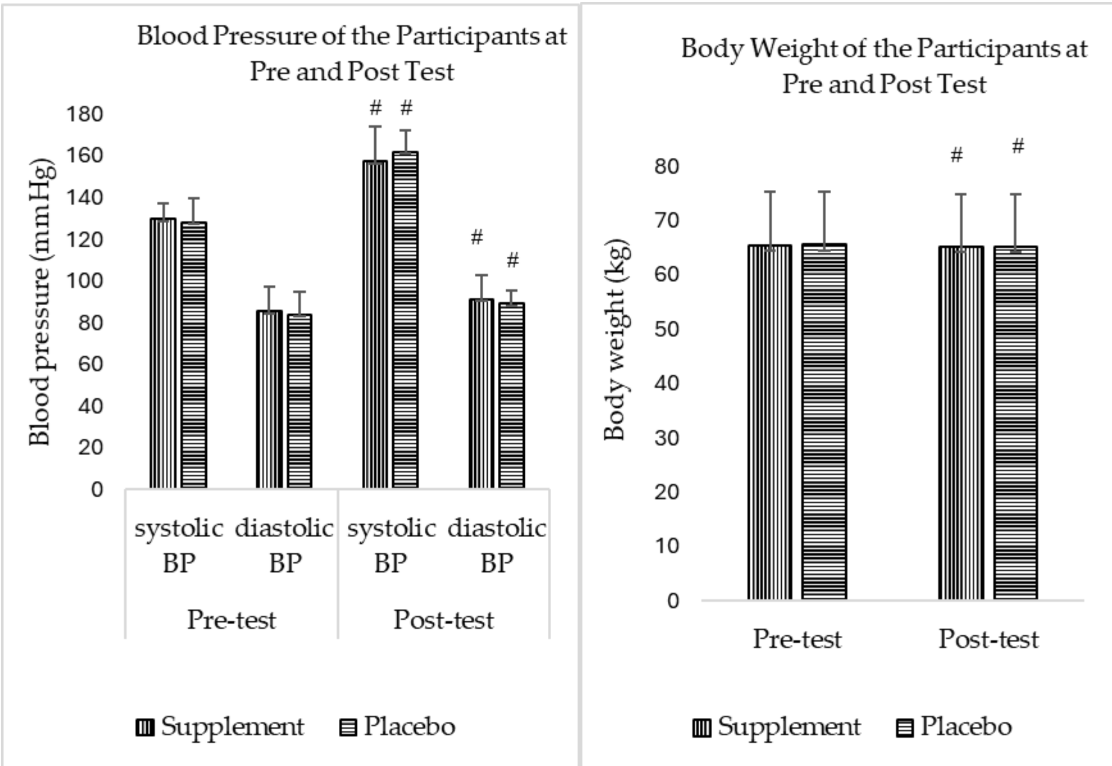

Figure 3. Blood pressure and body weight in the supplement and placebo trials. Data is presented as mean ± SD. # denotes significant difference from respective pre-test values at p<0.05.

The increases in heart rate and blood pressure during running can be attributed to the physiological demands of exercise, which require increased delivery of oxygen and nutrients to the working skeletal muscles to produce adenosine triphosphate (ATP) for energy. Participants reported their perceived exertion using Borg's rating of perceived exertion (RPE) scale. RPE values increased significantly as the exercise progressed in both trials, indicating that participants exerted maximal effort and approached exhaustion by the end of the running test. No significant difference in RPE values was observed between the trials. Body weight decreased after running compared with the respective pre-test values in both trials; however, this reduction did not differ significantly between the trials ($p > 0.05$).

The observed weight loss is most likely attributable to fluid loss through sweating during the endurance running test. Changes in body weight were similar in both trials, suggesting that the supplementation did not act as a diuretic and did not affect hydration status during endurance running. The main finding of the present study was that supplementation with a combination of Tiger Milk Mushroom and Long Jack for six days, including a single dose taken one hour prior to the endurance trial, did not significantly affect endurance running performance or selected physiological parameters in athletes. To the best of our knowledge, this is the first study to investigate the synergistic effects of Tiger Milk Mushroom and Long Jack on endurance running performance using a field-based endurance running test among Malaysian athletes. This finding is consistent with several previous studies reporting no significant effects of these supplements on sports performance (Zakaria *et al*., 2023; Chen *et al*., 2016; Muhamad *et al*., 2010; Ooi *et al*., 2001).

Zakaria *et al*. (2023) found that supplementation with 100 mg of Tiger Milk Mushroom for seven days did not significantly improve peak force, peak power, jump height in countermovement jump (CMJ), drop jump reactive strength index (RSI) and muscle soreness assessed using a 100-mm visual analogue scale. Similarly, Chen *et al*. (2016) reported that combining Tiger Milk Mushroom supplementation with resistance training had no effect on aerobic and anaerobic fitness, isokinetic muscular strength and power, and immune parameters in young male subjects. Muhamad *et al*. (2010) observed that supplementation with 150 mg of Long Jack for seven days did not produce significant improvements in endurance running performance. Likewise, Ooi *et al*. (2001) found no enhancement in endurance cycling performance when cyclists consumed an herbal drink containing Long Jack (3 ml/kg body weight) every 20 minutes during exercise.

However, some studies have reported contradictory findings, suggesting that long-term supplementation with Long Jack may enhance physical performance (Khanijo & Jiraungkoorskul, 2016; Hamzah & Yusof, 2003). For instance, Hamzah and Yusof (2003) demonstrated that supplementation with 150 mg of Long Jack for five weeks significantly increased muscular strength in healthy male adults. Long Jack is rich in bioactive quassinoids, which have been associated with increased muscle strength and reduced anxiety and stress, potentially leading to improved sports performance (Khanijo & Jiraungkoorskul, 2016). These findings suggest that Long Jack may exert more pronounced ergogenic effects in strength and power-based sports rather than endurance or aerobic based sports. Several studies have also highlighted the antioxidant, antihypertensive, anti-inflammatory, and antimicrobial properties of these supplements (Tan *et al*., 2021; Khanijo & Jiraungkoorskul, 2016; Yap *et al*., 2014). Tan *et al*. (2021) reported that Tiger Milk Mushroom effectively improved respiratory health and immune function, which may benefit aerobic athletes. Meanwhile, Long Jack contains glycoproteins and eurycomanone, compounds associated with anti-aging effects and increased androgen levels, including testosterone. Testosterone is known to enhance bone density and muscular strength (Rehman *et al*., 2016; Tambi *et al*., 2012). It is well-established that exercise increases oxidative stress, leading to the production of free radicals that can impair muscle contraction and contribute to fatigue, muscle damage and reduced sports performance (Simioni *et al*., 2018).

Antioxidants play a critical role in neutralizing these free radicals, thereby protecting muscle tissue, reducing fatigue and potentially enhancing sports performance (Higgins *et al*., 2020). Athletes often consume antioxidant supplements to minimize muscle damage, immune dysfunction, and fatigue, with the goal of optimizing performance (Braakhuis & Hopkins, 2015). Based on these

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mechanisms, it was hypothesized that the antioxidant properties of Tiger Milk Mushroom and Long Jack would improve endurance performance. However, the present study did not support this hypothesis. The lack of a significant effect may be attributed to the short duration of supplementation used in this study. Therefore, future research should investigate longer supplementation periods to determine whether more substantial physiological adaptations and improvements in endurance performance can be observed with a larger sample size. In addition, future studies should employ a matched placebo and a double-blind design to better control for expectancy effects and enhance methodological rigor. Future research should also utilize validated laboratory-based protocols with direct gas analysis to obtain more accurate and reliable measurements of maximal oxygen consumption (VO_2max).

CONCLUSION

Although Tiger Milk Mushroom and Long Jack supplements are believed to have synergistic effects on sports performance, the present study found that daily supplementation for six days, including a single dose taken one hour prior to the endurance running test, had no significant effect on endurance running performance in athletes. The supplementation also did not significantly influence maximal oxygen consumption (VO_2max), rate of perceived exertion (RPE), heart rate, blood pressure and body weight in athletes.

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