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Effects of *Eurycoma longifolia* on Natural Killer Cells and Endurance Running Performance

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Abstract This study aims to investigate the effects of *Eurycoma longifolia* (EL) supplementation on immune responses following endurance running in the heat. Nine healthy male recreational athletes (23.8 ± 4.1 years old) were recruited in this randomised, double blind, placebo-controlled, cross over study. Participants performed two trials in the heat (31°C, 70% relative humidity) on separate days. Before each trial, participants consumed 2 capsules of either EL (75 mg of EL per capsule) or placebo (P) for 7 days and another 2 capsules 1 h before the experimental trial. During each trial, participants performed warm-up on a treadmill at 50% VO_2max for 5 min and followed by endurance running for 60 min at 60% VO_2max. This was immediately followed by a time trial for 20 min. During each trial, blood samples were collected before the supplementation period, pre-exercise trial, and post-exercise trial. The data was analysed by using Two-way ANOVA with repeated measures. As a result, natural killer (NK) cells count was significantly higher ($P = 0.02$) at the end of the EL trial compared to the P trial (4.8 and 4.0 x 10^3.dL^{-1} respectively). However, endurance running performance was not significantly different ($P = 0.139$) between EL and P trials (2.9 and 2.7 km respectively). Total white blood cells, monocytes, basophils, neutrophils, and eosinophils counts were also not significantly different ($P > 0.05$) between both trials. These observations suggest that EL may enhance immune functions after a bout of endurance running in the heat by increasing NK cells count.

Keywords Exercise, Immune function, Hot environment, Herb

1. Introduction

It is well established that exercise-induced immunodepression depends on the intensity and duration of exercise [1]. In general, moderate exercise enhances immune function whereas prolonged high intensity exercise may impair immune function. As reported previously, one study found a positive correlation between exercise and natural killer (NK) cells count and function [2]. A separate study supports the later finding where they found that lymphocyte count increases after moderate exercise and decrease after heavy exercise [3]. In addition, another study also reported that heavy exercise decreases NK cell's function [4].

Since exercise-induced impaired immune function is always associated with decrease performance, athletes are very concern about their health status during heavy training and tournament. However, there are several factors that have been demonstrated to have a profound influence on the immune system, including age, inflammatory or autoimmune disease, exercise, psychological stress, and nutrition [5]. Certain nutritional strategies have been shown to limit exercise-induced immune dysfunction and are thus recommended to athletes engaged in heavy training and competition. These nutritional strategies include ensuring adequate intake of macronutrients (carbohydrate, protein, and fat), avoidance of deficiencies of micronutrients (e.g. the minerals iron, zinc, copper, and the vitamins A, B12, C, D, E and Folic acid), and taking supplements such as antioxidants, probiotics, herbs, and bovine colostrum. Using herbs as ergogenic aids in exercise and sport has been practiced worldwide. Ginseng, caffeine, ephedrine, and combination of both caffeine and ephedrine are the most popular herbs used in exercise and sports [6]. People believed that these herbs can help to improve their performance, indirectly or directly, thus having an ergogenic effect.

Eurycoma longifolia is one of the famous herbs found in Malaysia. It is commonly known as 'Tongkat Ali' in Malaysia and is also referred to as 'Malaysian Ginseng' since it is well-known among various ethnic groups in Malaysia for treating various diseases and enhancing health [7]. *Eurycoma longifolia* is a tall, single-stemmed [8], slender shrubby, slow growing tree, found on sandy soil [9]. It belongs to the *Simaroubaceae* family and grows wildly in Southeast Asian countries, i.e. Malaysia, Indonesia, Thailand, Myanmar, Laos, and Cambodia [7, 10]. Its active ingredients called quassinoids [9] are concentrated in taproot and reach its reproductive age after five years or more [8].

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Traditionally, people believed that this herb can be used as an anticoagulant for complications during childbirth, treatment for dysentery [10], aphrodisiac [11], antimalarial [12], antibacterial [13], anticancer [14], antihyperglycemic [8], anxiolytic [15].

The effects of *Eurycoma longifolia* on exercise performance have not been extensively studied. However, previous studies found that *Eurycoma longifolia* did not affect cycling [16] and running performances [17]. Hence, the present study intended to examine the effects of *Eurycoma longifolia* on running performance and immune responses. To our knowledge, this is the first study carried out to investigate the effects of *Eurycoma longifolia* on immune responses. The present study was conducted in an environmental chamber in the laboratory which had been maintained hot (31°C) to mimic Malaysia's daily temperature which varies between 30 – 32°C. It is important to investigate effects of supplement when exercising in the heat because it was reported that exercise was significantly impaired in the heat [18].

2. Methods

2.1. Participants

Nine recreational athletes were recruited in this randomised, double blind, placebo-controlled, cross over study. Sample size calculation was carried out based on a study which was carried out by (Ooi et al., 2001). It was calculated by using PS Power and Sample Size Calculation version 2.1.30. The power of the study was set at 80% with 95% confidence interval. The standard deviation (σ) observed was 0.4 minute of cycling time, and difference in population means (δ) was set at 0.9 minute of cycling time. The calculated sample size was 8. For the drop out occurrence, total sample size recruited was 9 participants in the present study.

Participants recruited were healthy males, aged between 20 and 40 years old and performed jogging at least twice per week, with duration of at least 30 min per session. Informed consent was obtained from all the participants prior to their participation in this study. Throughout this study, they were requested to refrain from ingesting any products containing *Eurycoma longifolia*. There are no potential conflicts of interest to participants involved in the present study. This study was approved by the Human Research and Ethics Committee of School of Medical Sciences, Universiti Sains Malaysia, Malaysia and the protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

2.2. *Eurycoma longifolia* and Placebo Supplementation

Eurycoma longifolia was provided by Phytes Biotek Sdn. Bhd., Malaysia in the form of capsules. It was a water extract and was approved and registered by the Ministry of Health, Malaysia and is available in the market. As for placebo, it contains flour which was inserted into capsules similar to the *Eurycoma longifolia* capsules in terms of color and shape.

2.3. Preliminary Tests

Participants performed a sub-maximal test and a maximal oxygen uptake (VO_2max) test on a motorised treadmill (Quinton 18-60, USA). These two tests were carried out on the separate days and the results obtained from these tests were used to calculate the appropriate running speed during the actual trial (running at 60% of their respective VO_2max). Then, a familiarisation trial was carried out to familiarise participants with the actual trials' protocol. In this familiarisation trial, participants running in the heat (31°C, 70% relative humidity) at 60% of their respective VO_2max for 40 min and immediately followed by a 10 min time trial. Following preliminary tests, participants were randomised equally into either arms of this study: exercise trial after placebo supplementation first followed by exercise trial after *Eurycoma longifolia* supplementation or vice versa.

2.4. Pre-Experimental Trial Protocol

The first blood sample was collected before they consumed the supplement. Participants consumed two capsules per day for seven days of either *Eurycoma longifolia* (75 mg/capsule) or placebo. Another two capsules of the supplement were consumed on the subsequent day which was on the experiment trial day. This study was a double blind study, it means that both researchers and participants were not aware of the type of the supplement taken before each trial. This is because both placebo and *Eurycoma longifolia* capsules are similar in shape, color, and taste. A lab technology was in charge to randomise the supplementation order and also to give the supplements to the participants before each trial.

Three days before the trial, participants were asked to record their food intake into a food diary. They were requested to repeat the same diet before the subsequent trial to minimise variation in pre-exercise muscle glycogen status. One day before the trial, participants were asked not to engage in any strenuous physical activity and to start fasting from 10 pm onwards. However, they were permitted to drink plain water during fasting.

2.5. Experimental Trial Protocol

On the trial day, participants came to the laboratory early in the morning after an overnight fast. Firstly, they were given a standardised breakfast which was a piece of bread (Gardenia®, Malaysia) and 300 ml of cool plain water. Thirty minutes after having their breakfast, participants' nude body weight was measured by an electronic body composition and analyser (Tanita® TBF-410, Japan). Then, participants were cannulated for the blood drawing purposes. The second blood sample was drawn from participants just before the warm-up.

After that, participants warmed-up for 5 min at 50% of their respective VO_2max followed by endurance running at 60% of their respective VO_2max for 60 min in the hot

environment (31°C, 70% relative humidity). This was immediately followed by a 20 min time trial where, participants were allowed to adjust the speed by themselves via a remote control. During this 20 min time trial, participants were encouraged to run as fast as possible to cover longer running distance. At the end of the trial, the third blood sample was drawn from the participants. Room temperature and relative humidity were recorded before exercise and at every 20 min during exercise.

2.6. Blood Sample Analysis

All the blood samples collected were placed in the ethylenediamide tetra-acetic (EDTA) tube and then were immediately analysed by the hematology counter machine (Sysmex XS800i, USA) to determine the total white blood cells, monocytes, basophils, neutrophils, and eosinophils counts. Blood samples were also analysed by the flow cytometer machine (BD FACSCanto II, UK) to determine NK cells count [19].

2.7. Statistical Analysis

Data were analysed using the Statistical Package for Social Sciences (SPSS) version 20 (SPSS Inc., USA). All the data were examined for normality through the Kolmogrov-Smirnov test. Descriptive statistics was used to analysed participants' physical characteristics and physiological profiles. Two-way ANOVA with repeated measures was used to measure significant differences between and within the trials. The accepted level of significance was set at $P < 0.05$. Results are expressed as means (standard deviation).

3. Results

Participants' physical characteristics and physiological profiles were tabulated in Table 1. There were no significant differences between EL and P trials on mean temperature and relative humidity ($P = 0.44$ and 0.13 respectively) where, mean room temperature and relative humidity for both EL and P trials were 31.2 (0.3)°C; 70.4 (1.3) % and 31.1 (0.3)°C; 71.7 (1.5)% respectively. Natural killer cells count was significantly higher ($P = 0.02$) at the end of the EL trial (4.8 (0.4) $10^3.dL^{-1}$) compared to the P trial (4.0 (0.3) $10^3.dL^{-1}$) (Figure 1). However, endurance running performance (Figure 2) was not significantly different ($P = 0.139$) between EL (2.9 (0.5) km) and P (2.7 (0.5) km) trials. Similarly, there were also no significant differences ($P > 0.05$) in total white blood cells, monocytes, basophils, neutrophils, and eosinophils counts following the running trial within each trial and also between both trials (Table 2).

Table 1. Physical Characteristics and Physiological Capacities of the Participants

Parameters	Means (standard deviation)
Age (years)	23.8 (4.1)
Body weight (kg)	66.1 (7.3)
Standing height (m)	1.7 (0.6)
Body mass index ($kg.m^{-2}$)	23.1 (2.4)
Maximal oxygen uptake ($mL.kg^{-1}.min^{-1}$)	47.1 (8.3)

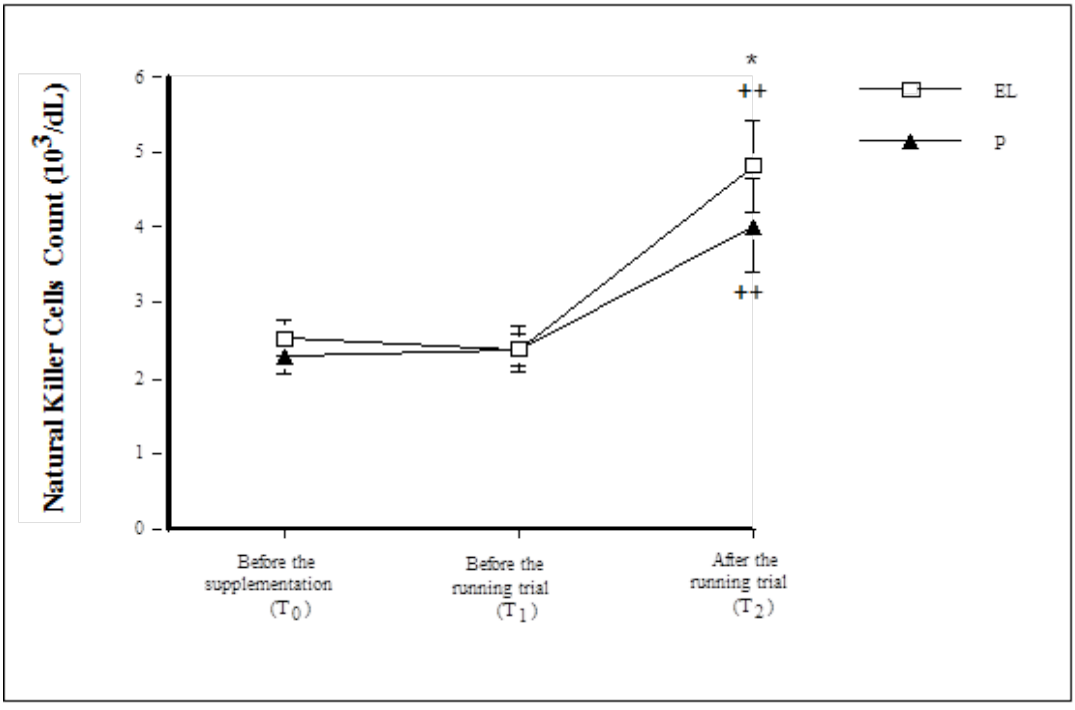


Figure 1. Changes in NK cell count in EL and P trials [Means (standard deviation)]. * Significantly different from corresponding value in P trial ($P < 0.05$). ++ Significantly different respective T_1 value ($P < 0.01$)

Table 2. Changes in Immune Cells in EL and P Trials [Means (standard deviation)]

Immune cells (10^3.uL^{-1})	Trials	T_0 (Before the supplementation)	T_1 (Before the running trial)	T_2 (After the running trial)
White blood cells (WBC)	P	6.01 (0.50)	6.32 (0.30)	10.40 (0.90)
	EL	6.15 (0.50)	6.07 (0.40)	11.72 (0.90)
Monocytes	P	0.35 (0.09)	0.35 (0.09)	0.74 (0.14)
	EL	0.21 (0.09)	0.38 (0.10)	0.57 (0.19)
Neutrophils	P	3.25 (0.38)	3.00 (0.59)	5.47 (0.49)
	EL	3.10 (0.30)	2.67 (0.55)	5.07 (1.06)
Eosinophils	P	0.16 (0.04)	0.17 (0.04)	0.14 (0.04)
	EL	0.13 (0.03)	0.14 (0.03)	0.13 (0.03)
Basophils	P	0.01 (0.01)	0.02 (0.01)	0.03 (0.02)
	EL	0.01 (0.01)	0.02 (0.01)	0.03 (0.01)

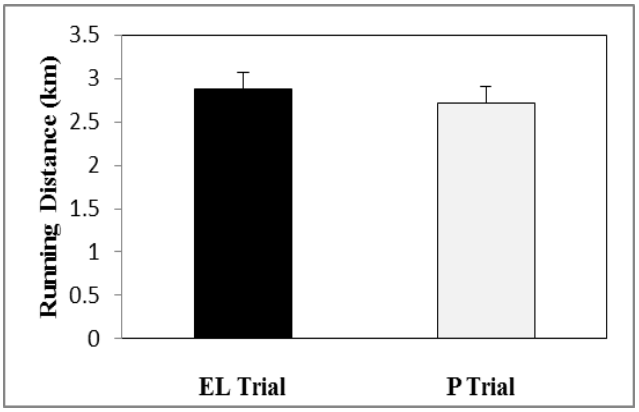


Figure 2. Endurance running time trial performance in EL and P trials [Means (standard deviation)]

4. Discussion

The main finding of this study is that supplementation of *Eurycoma longifolia* (150 mg/day) for 7 days significantly increases NK cells count (Figure 1) following a bout of prolonged exercise in the hot environment among recreational athletes. However, there were no significant effects of *Eurycoma longifolia* on other parameters measured (Table 2) including endurance performance (Figure 2).

To our knowledge, previous studies have not measured the effects of *Eurycoma longifolia* on immune responses. However, a recent related study [20] reported that 4 weeks supplementation of *Eurycoma longifolia* (200 mg/day) significantly improved stress hormone profile in moderately stressed participants where, cortisol level was decreased and testosterone level was increased. Generally, it is known that increased cortisol level is associated with suppressed immune system activity [21], which in turn may lead to high risk of getting respiratory tract infection. Hence, this previous study finding suggests that *Eurycoma longifolia* supplementation may have potential in enhancing immune function by decreasing the stress hormone (cortisol) level.

In addition, a separate study demonstrated that the metanolic extract of the roots of *Eurycoma longifolia* has potent NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) inhibitory effects [22]. It is known that NF-κB is a protein complex which is involved in cellular responses to stimuli such as stress, cytokines, free radicals and bacterial antigens [23]. Hence, it was believed that it may play a key role in regulating the immune response to infection. This new finding adds new evidence regarding possible positive effects of this herb on immune function.

Natural killer ($CD3^-$, $CD16^-$, $CD56^-$) cell is an immune cell that is very important in defense against viral infection and in preventing the development of cancers [5]. It is a large, granular cell that accounts approximately 10 – 20% of lymphocytes. Being one of the components of innate immune system, NK cells are capable of eliminating virally infected host cells at the beginning of infection, without prior sensitization [5].

Numerous studies had reported increase number of NK cells following exercise [2-26] where, intensity of the exercise had influence on the magnitude of NK mobilization into the circulation [24, 25]. However, the magnitude and speed of NK mobilization is unparalleled whereby it peaks upon cessation of a 60-second bout of supramaximal exercise followed by a very rapid recovery [25]. The mechanism of action of large increase in NK cells count during exercise is reported to be attributed to exercise-induced rises in circulating catecholamines [27]. Catecholamines may alter NK cells adhesion, hence may result in mobilization of NK cells into the circulation.

Regarding the effects of *Eurycoma longifolia* on exercise and sports performance, available data in the literature were also limited. Previous studies reported that endurance cycling (0.67 mg) [16] and running (150 mg/day for 7 days) [17] performances were not affected by *Eurycoma longifolia* supplementation. Nevertheless, a few studies discovered that *Eurycoma longifolia* was able to increase muscle strength and size [28]. Furthermore, previous studies also found that *Eurycoma longifolia* root demonstrated ability to accelerate recovery from exercise [20], reduce symptoms of fatigue [29], and improved maximal strength [28]. In addition, it was also found that extracts of the *Eurycoma longifolia* root were able to improve lean body mass [28] and reduced abdominal fat [30].

5. Conclusions

Based on the findings in the present study, *Eurycoma longifolia* may be recommended to athletes as a supplement to improve their immune status, hence in turns may improve their performance and overall health. However, further studies are strongly recommended because there are limited studies regarding effects of Eurycoma longifolia on immune function and sports performance to date. Future studies should consider the appropriate dosage, supplementation period, and study protocol to ensure positive and reliable findings. As a conclusion, supplementation of *Eurycoma longifolia* (150 mg/day) for 7 days significantly increased NK cells count following endurance running in a hot and humid environment among recreational athletes.

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