The Eurycoma Longifolia Freeze-Dried Water Extract-Physta® Does not Change Normal Ratios of Testosterone to Epitestosterone in Healthy Males

George A1*, Liske E2, Chen CK3 and Ismail SB4

1Biotechs Malaysia Berhad, Lot 21, Jalan U1/19, Section U1, Hicom-Glenmarie Industrial Park, 40150 Shah Alam, Selangor, Malaysia
2Departments of Life Sciences, Technical University of Braunschweig, Germany
3Sports Science Unit, School of Medical Sciences, Universiti Sains Malaysia, Malaysia
4Department of Family Medicine, School of Medical Sciences, Universiti Sains Malaysia, Malaysia

Abstract

Eurycoma longifolia is traditionally known for its adaptogenic properties and often used for the general well-being and increased performance in daily life.

Methods: In a randomized, double blind, placebo-controlled clinical trial, 40 Malaysian men aged 30-55 years performed a battery of physical testings during which the ratio of Testosterone (T) to Epitestosterone (E) was analyzed. The daily intake was 300 mg of the freeze-dried water extract of Eurycoma longifolia root (Physta®, Biotechs Malaysia) or placebo for a period of 12 weeks.

Results: At the end of the study the T/E-ratio in the herbal group ranged from 0.03 to 2.95 and was not significantly different from baseline (p=0.49). Further, the ratio-values for E. longifolia compared to placebo did not change over time. The weight lifting force which measures muscular strength using back & leg strength test, increased significantly from baseline to end of the study in the herbal group by approximately 14 kg (p=0.0166).

Conclusion: The ratios of testosterone to epitestosterone did not change during a 12-weeks E. longifolia intake. The data suggest that this herb does not exhibit "doping"- like effects. Instead, muscular strength improved significantly in the back and leg with E. longifolia supplementation rendering this herb good for physical performance minus the doping effects.

Keywords: Eurycoma longifolia; Strength; Quality of life; Doping; Testosterone; Epitestosterone

Introduction

It is a common human attitude to want to perform better, particularly in sports and tournaments/competitions. The desire to be better with the use of banned performance-enhancing drugs is considered to be "doping". Anti-doping rules and testing have been established as an independent and globally accepted standard by the World Anti-Doping Agency (WADA) [1]. Biochemical measurements for the ratio of Testosterone ("T") to Epitestosterone ("E") are often used in the fight against doping. The hormones are endogenously built in the body and T increases protein-synthesis resulting in growth of cellular tissue, especially in muscles ("anabolic steroids"), whereas E is an inactive isomer of testosterone. In general, the normal ratio of T to E in urine samples is found similar to be 1:1.

Due to the intake of anabolic steroids or products with similar function, testosterone levels increases while E levels remain stable, resulting in a change in T/E-ratio from normal levels. In 2005, a threshold of T/E-ratio higher than 4:1 (>4) was considered by the WADA as a proof of exogenous intake of anabolic substances [2]. The significance of these measures as well as factors which may influence and possibly facilitate false-positive results have been discussed controversially, e.g., non-steroidal products capable of changing T/E-ratio, specified/non-specified stimulants, pathological androgen metabolism, individual genetic variations and enzyme polymorphism [1-4].

Adaptogenic substances, including many herbal medicines have been used traditionally with the goal of increasing physical performance. The history of herbs in sports can be traced back to ancient Greek [5]. Famous examples of herbs for sports purposes are among others Panax ginseng, caffeine, and ephedrine and Eurycoma longifolia E. longifolia, also known as "Tongkat ali" or "Malaysian ginseng", is well-known in Southeast Asian countries and used traditionally for the general well-being and better performance in daily living [5-9]. In a randomized, double-blind placebo controlled trial, healthy male subjects on E. longifolia experienced significant improvement in physical functioning, one of the 9 domains found in the SF-36 Quality of Life questionnaire [9]. The question items in this domain were on moderate and vigorous activities, climbing, bending and kneeling, walking, and bathing/dressing, role physical, and vitality. The supplementation with E. longifolia improved strength in physically active male and female seniors between 57 and 72 years of age in an open pilot study and additionally increased muscle mass and fat free mass in men, in another study [10,11]. The ergogenic properties of the herb appear prevalent in these studies. The proandrogenic effect on the laevator ani muscle by an increase in its weight was also seen in rats [12]. It was shown that E. longifolia contains the presence of a 4.3 kDa peptide which increases testosterone levels in rat leydig cells [13]. However, a few clinical data on the effects of E. longifolia in exercise performance are available, such as endurance bicycling and running which still render non-conclusive the effect of E. longifolia in sports performance [6].

*Corresponding author: Annie George, Biotechs Malaysia Berhad, Lot 21, Jalan U1/19, Section U1, Hicom-Glenmarie Industrial Park, 40150 Shah Alam, Selangor, Malaysia, Tel: 603-55655600; Fax: 603-55655603; E-mail: annvalu@yahoo.com

Received August 19, 2013; Accepted September 19, 2013; Published September 26, 2013


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Here we report the non-doping effects and the benefits in sport performance during a 12-weeks treatment of Eurycoma longifolia. These data were obtained as an addendum from a randomized, placebo-controlled trial on the quality of life and sexual well-being in men [9].

Methods

Study design and subjects

The randomized, double-blind, placebo-controlled and parallel group study was performed at the Clinical Trial Unit (CTU), Hospital Universiti Sains Malaysia (HUSM), Kubang Kerian, Malaysia. It was conducted in accordance with the Guideline for Good Clinical Practice (ICH-6), Declaration of Helsinki and approved by the Human Research and Ethics Committee for Clinical Studies at HUSM (approval date 24 Dec 2008). An addendum to approve additional endpoints was approved by the Ethics Committee for Clinical Studies at HUSM on 23 Dec 2009 (Ref. USM/PPP/Ethics Com./2009(146)).

One hundred and nine healthy Malaysian men aged between 30 years to 55 years were included. The inclusion/exclusion criteria, randomization and intervention, primary and secondary endpoints as well as sample size estimation and statistical analyses for the study are described in detail in Ismail et al. [9]. Forty subjects agreed for an additional doping test (verum: n=21; placebo: n=19). The subjects were randomly selected and were from the tail end of the ongoing (larger population) study. The additional doping test was added to the protocol at mid-way of the study (larger population) to address possible doping effects upon suggestions by the local Sports Council. The investigators, subjects and study coordinators were double blinded and the products were randomized prior to shipment to the Investigators. The information of the product whether placebo or treatment were sealed in an envelope labeled with the corresponding randomization number and could only be broken upon occurrence of serious adverse event or at the completion of the trial, during data analysis. The envelope containing information of the subjects was checked to ensure there was no breaking of seal.

The subjects were non-athletes and were instructed not to participate in any form of physical training during the study period. A total of four interval visits were scheduled for the entire study: screening (visit 1, week -1 to week-2), measurements taken at baseline (visit 2, day 0: randomisation), at week-6 (visit 3, day 42 ± 3 days) and end-of-study at week-12 (visit 4, day 84 ± 3 days) followed by a follow-up visit (visit 5 at week-14, via telephone or face to face). At screening (visit 1), clinical examinations and interviews were conducted to select subjects who fulfilled the study criteria. Blood samples were collected from the subjects for routine health check (hematological, renal and liver parameters) and Prostatic Specific Antigen (PSA). At each visit (V2, V3, V4) Physical Fitness Testings were performed (see Methods 2.3). The primary endpoints for the ratio of hormonal values for Testosterone (T) to Epitestosterone (E) were taken at baseline (V2) and end of the study (V4). The safety was assessed based on documented Adverse Events, physical examinations, clinical and laboratory measures as published [9].

Study medication

Each subject consumed four capsules per day containing either herbal extract or placebo. One capsule contained 75 mg of freeze-dried water extract of Eurycoma longifolia root (Physta®; Biotropics Malaysia Berhad, Kuala Lumpur, Malaysia) as active ingredients, manufactured under Good Manufacturing Process requirements (GMP) with continuous quality control (DER 20:1). Consumption of 500 to 1000 mg root extracts has been reported traditionally, and clinical data show safe consumption of the water soluble extract between 200 to 600 mg [7,14]. Since the present investigation was the first randomized, placebo controlled clinical trial in a large population, a middle range of 300 mg/day extract was selected. The total daily intake was therefore 300 mg/day herbal extract. The placebo medication (containing maltodextrin) was identical to the active medication however without the herbal extract.

Procedures for the physical fitness tests

Tests for physical fitness were performed: each session of the physical fitness tests contained a battery of different types of tests so that various physiological and muscular parameters could be activated/stimulated, finally leading to an improvement of these fitness activities: flexibility-(type of test: Sit & Reach), muscular strength (Hand grip; Back & Leg), muscular endurance (Sit-up & Push up) and body composition (Bioelectrical Impedance Analysis). All these tests were carried out on the same day for each participants and the duration took approximately two hours for each session of five to eight subjects.

Sit and reach: The subjects sat on the floor with legs stretched out straight ahead. They were bare-footed and the soles of the feet are placed flat against the the sit and reach box. Both knees were locked and pressed flat to the floor. With the palms facing downwards, and the hands on top of each other, the subjects were told to reach forward along the measuring line as far as possible. Then, the subjects hold that position for at least two seconds while the distance was recorded. The subjects were given three trials and the longest distance to the nearest centimeter reached was noted.

Hand grip: The subjects held the hydraulic hand dynamometer (Jamar, United Kingdom) in the hand to be tested, with the arm at right angles and the elbow by the side of the body. The handle of the dynamometer was adjusted if required and the base was rested on the first metacarpal (heel of palm), while the handle rested on the middle of four fingers. Then, the subjects squeezed the dynamometer with maximum isometric effort, which was maintained for about 5 seconds. No other body movement was allowed. The subjects were strongly encouraged to give a maximum effort. The best result in kilograms from three trials for the dominant hand was recorded, with at least 15 seconds recovery between each effort.

Back and leg strength: The subjects stood upright on the base of the back and leg dynamometer (Vacumed, USA: http://www.vacumed.com/images/12-0403.JPG) with their feet shoulder width apart. They were instructed to hang their arms straight down to hold the centre of the bar with both hands, and with the palms facing toward the body. The chain was adjusted so that the knees are bent at approximately 110 degrees. In this position the subjects’ back are bent slightly forward at the hips, their heads held upright and looking look straight ahead. Then without bending their backs, they pulled as hard as possible on the chain and try to straighten their legs while keeping their arms straight. Additionally, they were told to pull against the weight steadily whilst keeping the feet flat on the base of the dynamometer. The subjects were reminded that maximum performance will result when their legs are almost straight at the end of the lift. The best result in kilograms from three trials was recorded, with at least 15 seconds recovery between each effort.

Sit-up: The subjects lay on a carpeted floor with their knees bent at approximately right angles, with feet flat on the ground. They were instructed to rest their hands on their thighs. Then, they pushed their backs flat and raised high enough for their hands to slide along their thighs to the tops of their knees. They were also reminded not to
pull with their necks or heads and to keep their lower backs on the floor. They were required to do as many sit-ups as possible in one minute. The number of sit-ups performed in one minute was recorded.

**Push-up:** The subjects were instructed to use the standard “military style” push-up position with only the hands and the toes touching the floor in the starting position. They were told to lower the chest down towards the floor, always to the same level each time, either till their elbows was at right angles or their chests touch the ground. They were required to do as many push-ups as possible in one minute. The number of push-ups performed in one minute was recorded.

**Body composition:** A body fat analyzer (Omron HBF 306, Japan) was used to analyze the percent body fat of the subjects. Personal information such as age, gender, weight and height were keyed into the analyzer. Then the subjects were told to grip the device handles and held arms straight out at a 90° angle to the body. Percent body fat, fat mass content and Body Mass Index was calculated automatically in seconds.

**Analysis of testosterone T/epitestosterone E**

The analysis of urine samples was performed by the Doping Control Centre, Penang, a NATA -accredited laboratory (National Association of Testing Authorities, Australia). The quantification of T/E ratio was determined by Gas Chromatography–Mass Spectrometry (GC-MS). The free and conjugated T and E were extracted by solid phase extraction using the Nexus (Varian) column combined with liquid-liquid extraction using tert-Butyl methyl ether, followed by hydrolysis using enzyme β-glucuronidase from Esherichia Coli K12 (Roche Diagnostics Mannheim, Germany); extracts were then derivatized with activated N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA). The calibration of T was performed using concentration levels of between 2 ng/mL and 80 ng/mL while the values for E were between 2 ng/mL to 20 ng/mL. Selected ion monitoring mode with electron ionization on the GC-MS was used for identification and quantification of both T and E.

**Statistical analyses**

Detailed description on the statistical evaluation was already published elsewhere [9]. Data was explored and descriptive statistical analyses was conducted to measure changes in the parameters between E. longifolia and placebo groups at baseline, week 6 and/or end of treatment. Mean (SEM, standard error of the mean) were used for continuous variables of normality distributed data and median (IQR, inter quartile range) for non-normal distribution. The ratio T/E is visualized by Box-Whisker-Plots, showing the spread of the set of data without any assumption of the underlying data distribution. Independent t-test and non-parametric test (as appropriate, Mann-Whitney) were used for comparison of the two randomized groups. Significant changes in the parameters are referred to a p-value of ≤ 0.05.

**Results**

Forty males agreed for the additional testings: Verum n=21, age (mean ± SD) 45.19 ± 5.37 years (range 30-54 years); Placebo n=19, age 44.63 ± 7.05 years (30-52 years). The flowchart of the study design, bibliographic as well as clinical baseline data of these subjects is listed elsewhere [9].

**Hormones**

During treatment, hormonal values for Total Testosterone (TT), Free Testosterone (FT), Dehydroepiandrosterone-SO4 (DHEA), the metabolic intermediate in biosynthesis of androgens and Sexual Hormone Binding Globulin (SHBG), the testosterone carrier protein were analyzed for the two treatment groups and are shown in Table 1. There were slight increasing trends in almost all of the values during E. longifolia intake from baseline (V2) to end of study (V4), however, none reached significance. TT median V2-V4: 15.3-16.5 nmol/L (p=0.34), FT 31.4-34.9 pmol/L (p=0.24), SHBG 22.6-25.2 nmol/L (p=0.86). The hormone DHEA increased by 13.6% from baseline (4.2 µmol/L) after 6 weeks (p=0.25), however, at the end of treatment, the value dropped back to the basic value (4.23 µmol/L, p=0.87).

In the placebo-group, FT and DHEA showed increasing values compared to baseline, at the end of trial, (after 12 weeks) (V2-V4: 32.4-36.5 µmol/L, p=0.58) and 3.6-4.5 µmol/L, p=0.62 respectively. Comparisons between groups demonstrated no significant deviations over time for all the hormonal values except for SHBG, which already had a significantly higher starting value in the placebo group at baseline (V2: p=0.03).

**Ratio Testosterone to Epitestosterone**

After 12 weeks, the T/E-ratio values in the E. longifolia group ranged from 0.03 to 2.95 with a median of 0.2 (n=21) compared to a median (V2) of 0.16 (n=3) at baseline as shown in Figure 1. This would mean no changes occurred in the hormonal relationship during herbal treatment (p=0.49). At the end of the study, approximately 91%

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Baseline V2</th>
<th>Eurycoma longifolia</th>
<th>Placebo</th>
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<td>Median</td>
<td>IQR</td>
<td>Median</td>
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<tr>
<td>Total Testosterone (nmol/L)</td>
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<td>11.4-19.15</td>
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<td></td>
<td>12.6</td>
<td>10.35-18.35</td>
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<td>19.8</td>
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<tr>
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<td>22.75-40.4</td>
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<td>DHEA (µmol/L)</td>
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<td>4.4 (0.4)</td>
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<td></td>
<td>3.9</td>
<td>2.95-5.9</td>
<td>4.86</td>
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<td></td>
<td>30.8</td>
<td>26.1-37.1</td>
<td>33.99</td>
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Table 1: Testosterone (free and total), Dehydroepiandrosterone (DHEA) and the Sexual Hormone Binding Globulin (SHBG) for the two treatment groups over time.
of the subjects had T/E-ratios of up to 1, which is considered to be a "normal" ratio. Similar data were obtained in the placebo group: after 12 weeks, the T/E-ratio values ranged from 0.05 to 3.3 with a median of 0.81 (n=18). Roughly 72% of the subjects had T/E-ratios of up to 1. At baseline, the median value was 0.55 (n=4), demonstrating no changes from baseline, in middle aged subjects, after an 8-weeks treatment (statistics were not mentioned in the reference) [17]. In another placebo trial compared to baseline mean values (117.21 kg ± 5.62 kg, n=19), barely reaching significance (p=0.045).

**Discussion**

The supplementation of 300 mg per day of Eurycoma longifolia root water extract in healthy middle-aged men, for a period of 12 weeks, was evaluated here for the ratio of Testosterone (T) to Epitestosterone (E) and physical fitness. To the best of our knowledge, this anti-doping measurement, established by the WADA, was applied for the first time in a controlled clinical trial with E. longifolia intake.

In none of the analyzed subjects were the T/E-ratios greater than 4 observed: a threshold which is considered by WADA as an adverse analytical finding requiring further investigations of the individuals. The maximum T/E-ratio of 2.95 was observed after daily intake of 300 mg of E. longifolia water extract. The majority of subjects (>90%) had T/E-ratios of up to 1, in the treatment group. The highest ratio for subjects on placebo was 3.3 and more than 72% of the subjects had values of up to 1. Since between-group comparisons showed no noteworthy and statistical significance, the results show that a 12 weeks treatment with 300 mg daily of E. longifolia water extract did not interfere with the normal ratio of testosterone to epitestosterone in middle-aged active and healthy men.

Several other hormones involved in the androgenic metabolism were analyzed in this study: total (TT) and Free Testosterone (FT), Dehydroepiandrosterone (-Sulfate) (DHEA) and sex-hormone binding globulin (SHBG). Although increasing values of these parameters ranging from 7% to 11% could be observed during E. longifolia extract intake, it did not reach significance when compared to baseline or when comparing with the placebo-group. However, literature on E. longifolia states conflicting results on its influence in androgenic hormones. It is reported in animal studies that the anabolic action of this herb is in increasing levels of testosterone and human studies suggested that this herb facilitates the production of testosterone by reducing SHBG, thus releasing free circulating testosterone [15,16]. The study, randomized to various doses of standardized E. longifolia extract (200-600 mg daily), showed testosterone and DHEAS at "high normal levels" compared to baseline, in middle aged subjects, after an 8-weeks treatment (statistics were not mentioned in the reference) [17]. In another placebo controlled study, thirty male endurance cyclist, received either 100 mg E. longifolia–water extract (n=15) or placebo (n=15); approximately 30 minutes before and after each of a four 14.91 miles laps, in a high-intensity 24-hrs bicycling program [18]. Supplementation of E. longifolia resulted in testosterone levels 16.4% higher while cortisol levels were 32% lower when compared to placebo (p<0.05). The biochemical profile of the cyclists appeared to be in an anabolic hormonal state. In hypogonadic men who have low serum testosterone levels, E. longifolia supplementation was able to significantly increase testosterone levels to normal levels as well [19].

While the role of testosterone is known to affect muscles [20], the role of E. longifolia may not only be limited to testosterone enhancing. In this study, the muscle strengthening effect did not appear to be
related to a significant increase in testosterone hormones. This is probably due to E. longifolia purportedly being a natural adaptogenic energizer in the maintenance and healthy ageing in men, suggesting E. longifolia’s role in optimizing testosterone levels only when sup-optimal [21]. It can be suggested that E. longifolia supplementation does not indiscriminately increase testosterone levels as do exogenous testosterone supplementation but modulates to optimal levels according to one’s current hormonal levels. Furthermore, the standardized water extract of E. longifolia (Physta) contains >22% protein and peptides possibly adding to the ergogenic benefit despite non-significant elevation of testosterone [13,22]. Hence, future studies on the role of the active ingredients in E. longifolia on the contractile properties of the muscles are warranted.

According to recently published reviews on herbs/botanicals including E. longifolia, dietary supplements and herbal medicines, only little conclusive clinical evidence is available to justify the herbs effects on exercise and sports performance [5,6]. Nevertheless, in this study, the test for muscular strength resulted in significant effects after 12-weeks of Eurycoma-extract intake. There was a highly significant 14 kg improvement (approx. 10% increase) in back and leg strength after 12 weeks (142.78 kg) of E. longifolia intake compared to baseline (128.81 kg; p=0.0166). Placebo-group only reached values of up to 10.8 kg (approx. 8% increase) with a borderline significance of p=0.045. No significant changes in hand grip strength was observed in this study although in physically active male and female seniors aged between 57-72 years of age, increased muscle strength (measured similarly with a hand grip dynamometer) accompanied with significant testosterone elevation, was observed with E. longifolia supplementation of 5 weeks [10]. The ages of the subjects in this study was below 55 years hence the effect of E. longifolia may be more intense in older subjects where sub optimal levels of testosterone normally occurs. An elevation of testosterone in the older subjects may have therefore contributed to the significant improvement of grip strength. The precise mechanism attributing to increase in muscular strength is currently inconclusive since in some studies it is accompanied by testosterone elevation and in others, it either did not increase or was not tested. This is particularly important as strength training can provide functional benefits (ergogenic properties): improve general health and well-being as seen in both groups of this study, however, with an enhanced effect present in the E. longifolia group.

There were also other studies demonstrating possible ergogenic properties of E. longifolia. In one randomized placebo-controlled trial, both groups on either 100 mg E. longifolia-extract or placebo, demonstrated a significant increase in muscle strength after 5 weeks of intensive strength training program. However the increase was larger in the treatment (herbal) group compared to the placebo: 6.78% (p=0.006) vs. 2.77% (p=0.011) respectively [11]. Subjects on E. longifolia extract had improved significantly in fat-free mass, fat mass, maximal strength (1-RM) and arm circumference when compared to the placebo group. This data corresponds with data from our study where a similar increase of muscle strength (approximately 10% compared to 6.78%) demonstrated by the back and leg strength test during E. longifolia intake, was observed. This study however did not measure testosterone levels.

The supplementation with E. longifolia did not significantly affect flexibility of the subjects evaluated through the ‘sit and reach’ activity. The mechanism of action for this herb is likely to be proandrogenic whereby testosterone is unlikely to affect the flexibility of subjects [13].

Other studies evaluating endurance were unable to demonstrate the effect of E. longifolia [23,24]. In a previous trial, 12 male athletes (mean age 23.3 years) who received 2 capsules of 75 mg E. longifolia-extract or placebo for 7 days, 1 hour before a 60 min run on the treadmill, showed no changes in running distance and physical responses compared to placebo [23]. In addition, in a separate study, six male cyclists who received a low-dose of E. longifolia medication (0.67 mg), showed no improvement in cycling performance and physical parameters [24].

In this current study, no significant changes were observed in muscular endurance evaluated through Sit-up and Push-ups. In these three situations, it is possible that supplementation of E. longifolia could not affect sports endurance of the subjects which is also dependent on aerobic fitness levels. Furthermore, a short supplementation period (7 days) and low dosage (0.67 mg) of E.longifolia may have also contributed to the lackluster performance of the subjects. In a trial of endurance cyclists supplemented with 100 mg of E. longifolia in a 24-hr mountain biking event, cortisol levels were 32% lower and testosterone levels were 16% higher in supplemented subjects compared to placebo, indicating a biochemical profile that promotes an “anabolic” hormonal state during the intense endurance exercise [18]. The type of exercise which engage different muscle group, anaerobic or aerobic exercise may play a role in the effectiveness of E. longifolia. In the exercise that engaged the larger muscle group such as quadriceps in intense cycling and back muscles in back and leg strength exercises, a beneficial effect of E. longifolia was invoked. Both strength training and intense cycling are primarily anaerobic. Anabolic activity is primarily during anaerobic respiration and was observed in the intense cycling [18]. Hence, it is possible that E. longifolia affects muscle strength in an anaerobic state than endurance which is influenced by aerobic fitness levels such as running and moderate cycling [24,25].

The body fat composition remained unchanged in this study. This was also the case in the larger population [9]. Only in men with BMB>B25 kg/m2 was fat loss observed. Overall, the size of the population may be too small to reach statistical significance in other parameters within the physical fitness test and hormonal profiles especially the androgenic hormones. The subjects were also non athletes hence may not actually be physically motivated to be evaluated in physical fitness, leading to poorer performance overall in the fitness test. A population of athletes or recreational athletes could be evaluated in the future to gauge E. longifolia as an effective supplement for sports performance. Additionally, it could be that the study duration of 12 weeks may have been too short and the given daily dose of E. longifolia was not optimal for the desired effect in all physical tests.

Besides ergogenic benefits, the supplementation with E. longifolia may have other beneficial effects, e.g. increase in physical performance, vigor and calmer mood [10,25-28]. An overall clarity of mind and mood elevation accompanied by vigor and muscle strength could be an additional useful attribute in a supplement for sports enthusiast in the future.

Conclusion

The ratio of testosterone to epitestosterone was measured for the first time in healthy males during a 12 weeks intake of 300 mg/day Eurycoma longifolia-extract (Physta*), in a randomized, double-blind and placebo-controlled trial. In both treatment groups, the T/E ratio was normal and below 4. A significant increase in muscle strength was observed for the herbal group compared to placebo in the back and leg strength test though muscular endurance, flexibility and body fat composition remained unchanged with 300 mg daily dose of Eurycoma longifolia over 12 weeks.

Acknowledgements

We would like to thank the phyto-pharmaceutical company Biotropics Malaysia Berhad, Kuala Lumpur, Malaysia, for financial support of the clinical trial.
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