The Effect of *Eurycoma Longifolia* on the Regulation of Reproductive Hormones' in Young Males

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SUMMARY

Eurycoma longifolia supplementation increases testosterone levels in humans via activation of the hypothalamic-pituitary-gonadal axis and/or the hypothalamic-pituitary-adrenal axis mainly in older adults and non-healthy populations. This study aimed to assess the impact of *Eurycoma longifolia* on the hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal axes in healthy young males since this might promote functional testosterone prowess. Thirty-two males (24.4±4.7 years; 1.74±0.07 m; 73.7±8.4 kg) in a placebo-controlled, double-blind, matched-paired study, received 600 mg/day *Eurycoma longifolia* or placebo for two weeks. Blood analysis using repeated measures analysis of variance showed significant interaction and time effects for testosterone ($F_{1,30}$=9.04, p=.005), free testosterone ($F_{1,30}$=7.13, p=.012) and estradiol ($F_{1,30}$=8.07, p=.008) levels in favour of the treatment group, while luteinizing hormone, follicle-stimulating hormone, and sexual hormone-binding globulin did not. The lack of changes in luteinizing hormone and follicle-stimulating hormone levels suggest that a lesser role played by *Eurycoma longifolia* in activating the hypothalamic-pituitary-gonadal axis in the young adults. The raised testosterone level may be due to a greater rate of hormone production via the hypothalamic-pituitary-adrenal axis. The supplementation of *Eurycoma longifolia* for two weeks demonstrates steroidogenic effects on young men were dose-related. Consequently, the raised testosterone following *Eurycoma longifolia* supplementations could benefit muscle and strength gain in young adults.

INTRODUCTION

Consumption of *Eurycoma longifolia* (EL) can elevate testosterone production in humans (Udani *et al*., 2014; Tambi *et al*., 2012; Henkel *et al*., 2014; Talbott *et al*., 2006; Ismail *et al*., 2012; Chen *et al*., 2014). The increase in testosterone levels is attributed to the synergistic effect of bioactive compounds in EL (including eurycomanone) which demonstrate the ability to alleviate sexual dysfunction and hypogonadism among older adults (Bhat and Karim, 2010; Kotirum *et al*., 2015; Ismail *et al*., 2012) or enhance testosterone steroidogenesis and improve fertility in rats (Low *et al*., 2013a). In general, testosterone levels are regulated via the hypothalamic-pituitary-gonadal (HPG) axis and the hypothalamic-pituitary-adrenal (HPA) axis. However, research examining the effect of EL extract (standardized to eurycomanone content) on the activation of the HPG and HPA axis in a young adult population is scarce.

Apart from exercise stimulus, consuming EL alone has been shown to be effective in restoring testosterone levels in older adults (Tambi *et al*., 2012; Henkel *et al*., 2014; Talbott *et al*., 2013; Udani *et al*., 2014) and those with late-onset hypogonadism (LOH) (Tambi *et al*., 2012), hypertension and diabetes (Henkel *et al*., 2014) as well as individuals that are moderately stressed (Talbott *et al*., 2013). However, several studies have reported a lack of change in testosterone levels in healthy males aged between 19 to 55 years old when given EL (Ismail *et al*. 2012; Chen *et al*., 2014; Chen *et al*., 2019). The low dose of EL administered (between

200-400 mg) may be insufficient to increase the testosterone levels in a relatively younger population with normal testosterone levels.

Physiologically, testosterone levels in the body are regulated predominantly via the HPG axis and to a lesser extent the HPA axis. It has been proposed that, in animals, the increased testosterone levels when given eurycomanone, could be attributed to elevated gonadotropins; luteinizing hormone (LH) and follicle-stimulating hormone (FSH) which stimulate the Leydig cells to produce testosterone (Low *et al*., 2013b) and inhibition of aromatase, a key enzyme converting testosterone to estrogen (Low *et al*., 2013a). Hence, eurycomanone could potentially down-regulate the estrogen-mediated feedback of LH and FSH secretions in the HPG axis by preventing negative feedback (Prakash, 2007), in other words, stimulating the pituitary gland to secrete gonadotropins which subsequently enhance testosterone production. Meanwhile, stimulation of the HPA axis in general increases cortisol production which reduces HPG activity (Sherman *et al*., 2016; Viau, 2002; Chen *et al*., 1997). However, it is suggested that EL extract lowers cortisol and increases testosterone levels through the conversion of testosterone precursors in the HPA axis (Talbott *et al*., 2006, 2013). Collectively, the studies suggest that EL supplementation could potentially increase testosterone levels in males; however, whether EL triggers the HPG or/and HPA axis in human remains to be determined.

While the effect of EL supplementation (standardized extract) on testosterone regulation is based on the ability to increase testosterone levels in elderly individuals facing hypogonadism, a distinction should be made on how EL could influence testosterone regulation in healthy young males. Hence, the current study aimed to assess the impact of a high dose of EL on the HPG axis, on healthy young males that are not suffering from a decrease in libido and erectile dysfunction.

PARTICIPANTS AND METHODS

Participants

Thirty-two collegiate males aged between 18 to 30 years old were recruited for this study. The participants were healthy with no diagnose of low testosterone levels or hypogonadism. They were excluded if they had any health problems, including diabetes mellitus, heart disease, kidney disease, liver disease, and sleep apnea. In addition, individuals who smoked or were on recreational or prescription drugs were excluded. The study was approved by the Liverpool John Moores University research ethics committee, and all participants were briefed on the study design and protocols and provided their informed consent.

Study design and protocol

The study is a placebo-controlled, double-blind, body weight matched-pairs design where participants were allocated into two groups: *E. longifolia* group (EL) (n = 16); and placebo group (PLA) (n = 16). The EL and PLA groups consumed either

600 mg per day of EL and PLA (maltodextrin), respectively for two weeks. Resting heart rate, blood pressure and anthropometric characteristics were assessed, and blood samples were collected on each visit to the laboratory at pre-intervention (Day 1) and post-intervention (Day 14). The participants were required to fast and refrain from alcohol and performance-enhancing supplements, high-intensity exercise, and sexual activity eight hours prior to each laboratory visit. They completed the 24-hour diet recall and physical activity questionnaire on the first visit and were asked to replicate the same diet and physical activity before the second visit. Participants' adherence to the protocol was assessed using the dietary recall diary where time they took the supplement and how they felt at different times during the day were recorded. Additionally, reminders were sent out via text messages to ensure compliance.

Anthropometric measurements

Height and body weight were determined using a stadiometer and weighing scales (SECA, Hamburg). Body composition was determined using Bioelectrical Impedance Analysis (BIA; Bodystat 1500, Douglas, Isle of Man, UK). Participants were assessed in a supine position, and the self-adhesive disposable electrodes were attached to the right hand, and right foot before the leads were connected.

Venous blood collection and plasma preparation

Fasting venous blood (15 ml) from an antecubital vein was collected using serum separation tubes (BD Vacutainer, New Jersey, USA). The blood was left at room

temperature (20°C) for an hour before centrifugation at 4000rpm (Sigma 3-16KL, Germany). The separated serum was transferred to micro-tubes and stored at -80°C until analysis. Endocrine profiles including testosterone, free testosterone, sexual hormone-binding globulin (SHBG), LH, estrogen, and FSH were assessed by enzyme-listed immunosorbent assay (ELISA; IBL International, Hamburg, Germany) coupled to a spectrophotometer at 490 nm absorbance for detection (Clariostar, BMG Labtech, Offenburg, Germany).

Eurycoma longifolia supplementation

A single batch of EL water extract (Physta®) was acquired from Biotropics Malaysia Berhad (Kuala Lumpur, Malaysia). A capsulated dose contained 600 mg/day of EL (efficacy reported in Tambi, 2005) was administered. The quality of EL was based on the level of eurycomanone, which is the main compound found in EL (Norhidayah *et al*., 2015). The Malaysian Standard (2011) stated that the level should be from 0.8-1.5 w/v (%). The EL used in the present study complied with the standard since eurycomanone content was 1.45 w/v (%). Figure S1 shows the high-performance liquid chromatography (HPLC) analysis of eurycomanone, and Figure S2 shows a calibration curve of eurycomanone at various dosage of EL. A capsule of 600 mg of EL consist of 8.7 mg of eurycomanone based on the 1.45% w/v (Fig. S2).

Statistical analysis

All statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS) for Windows version 22.0 (SPSS Inc., Chicago, USA). Anthropometric baseline data were analyzed using the parametric test (independent *t*-test). All data in text and tables are presented as means ± SD (parametric). Repeated measures analysis of variance (two-way ANOVA) was performed to examine the treatment effect and time. Assumptions of normality, homogeneity of variances, compound symmetry, and homogeneity of regression were checked and fulfilled. A significant level was set at $p < .05$.

RESULTS

Demographic and anthropometric

All participants complied with the supplementation (100% compliance), and no adverse effects were reported. The anthropometric characteristics of the participants in the EL and PLA groups are shown in Table 1. At baseline, there were no significant differences in age, body weight, body fat percentage, and lean body mass between EL and PLA groups ($p > .05$).

Endocrine hormones responses

There was a significant interaction between group and time for testosterone (F 1, 30 = 9.039, $p = .005$), as well as group (F 1, 30 = 4.01, $p = .05$) and time (F 1, 30 = 4.36, $p = .04$) main effects. Testosterone level was 15 % higher in EL following two weeks of consumption. While for free testosterone, there was a significant main

interaction between group and time (F 1, 30 = 7.13, p = .012), as well as time main effect (F 1, 30 = 11.73, p = .002), where free testosterone was observed 34% higher at post-intervention in EL. As for estradiol, there was a significant interaction between group and time (F 1, 30 = 8.07, p = .008), as well as time main effect (F 1, 30 = 4.22, p = .04), where estradiol was observed 30% higher at post-intervention in EL. Meanwhile, LH, FSH and SHBG analysis did not reveal any differences between and within group effects. The results for all hormone levels are shown in Table 2.

DISCUSSION

The effect of EL on reproductive hormones' regulation among young males in the current study was assessed by measuring testosterone, free testosterone, LH, FSH, estradiol and SHBG levels. The present findings revealed that two weeks of EL supplementation (600 mg/day) lead to significant increases in testosterone (15%), free testosterone (34%) and estrogen (30%) levels. The lack of changes in LH, FSH, and SHBG levels suggest a lesser effect of EL on HPG axis after two weeks of supplementation. However, the increased testosterone level could possibly be explained via activation of the HPA axis. Thus, this study is the first to suggest that EL supplementation increases testosterone level via activation of both HPG and HPA axes.

There is a general consensus that dosages between 200 and 400 mg of EL are able to increase testosterone levels in men and older men (30 to 72 years) with

hypogonadism (Tambi *et al*., 2012; Talbott *et al*., 2013; Henkel *et al*., 2014; Udani *et al*., 2014). However, in young male adults (19 – 35 years), no changes in testosterone levels have been observed when supplemented with a similar dosage of EL (Chen *et al*., 2014; Chen *et al*., 2019). The doses of EL used previously were low, mostly because of concern about possible toxic effects. The dosage of 600 mg EL (8.7 mg of eurycomanone) used in the current study increased testosterone level in young males (18-30 years) and demonstrates the steroidogenic effects of the herb on young males were dose-related when compared with previous studies (Tambi *et al*., 2012; Talbott *et al*., 2013; Henkel *et al*., 2014; Udani *et al*., 2014; Chen *et al*., 2014; Chen *et al*., 2019). In addition, the efficacy of this dosage has not previously been reported despite a dose of up to 600 mg not producing any adverse effects (Tambi, 2005). In the current study, 2-weeks of EL supplementation (600 mg) alone (without strength training), raised the testosterone level (9.24 ng/ml) slightly above the normal human range (2.64 – 9.16 ng/ml) as described by Travison *et al*. (2017). Based on evidence from related studies, none have investigated the effect of higher doses (> 400 mg) on testosterone production in young adults. It is rational that, within a safe limit, according to Binder (2012), the effects of drugs can be amplified by increasing the dosage given. Hence, administration of an increased dosage of EL (600 mg/day) for two weeks, can increase the testosterone level among young male participants and therefore could potentially have a positive impact on sports performance. To date, the use of exogenous testosterone to boost performance is prohibited in sports, where a urinary testosterone to epitestoterone (T:E) ratio of more than 4:1 is considered excessive (World Anti-Doping Agency) and subjected

to further investigation to determine potential use of a banned substance (Aguilera et al., 2001). Interestingly, several studies have shown that T:E ratios were below the threshold limit when supplemented with 300 and 400 mg of EL for 6 and 12 weeks respectively (George *et al*., 2013; Chen *et al*., 2014). Since it is the first time a higher dose demonstrated a significant increase in testosterone levels among young male participants, investigation to understand the mechanism behind the effect of EL on HPG and as well as HPA axes is needed.

Generally, the raised testosterone is converted to estradiol by aromatase (Ishikawa *et al*., 2006; de Ronde & de Jong, 2011) to maintain homeostasis. However, the rise in estradiol observed in this study is still within the normal range of 10 – 82 pg/ml in men (Yamamoto *et al*., 1995). Although, the effect of testosterone on skeletal muscle is well documented, the effect of estradiol on skeletal muscle remains unclear (Ikeda *et al*., 2019). However, positive correlations between estradiol and muscle mass regeneration have been reported by several studies (Ikeda *et al*., 2019; Pöllänen *et al*., 2011; McClung *et al*., 2006; Sitnick *et al*., *2006*; Sugiura *et al*., 2006). More research is needed to understand the roles of testosterone and estradiol that might be increased by EL, in muscular strength and the possible clinical implication.

In an animal model, EL induces testosterone synthesis due to concomitant increases in LH and FSH (Low *et al*., 2013a). While in the same study, the reduction in estrogen level is speculated to be due to inhibition of aromatase by a specific

compound in EL such as eurycomanone, reducing the conversion of testosterone to estrogen (Low *et al*., 2013a; Gunnel & Bloomer., 2014). Studies have shown that an increase in testosterone activates the negative feedback loop on the HPG axis which subsequently reduces testosterone production and maintains homeostasis through inhibition of LH and FSH (McLachlan *et al*., 2002; Weinbauer and Nieschlag, 1993). While supplementation of EL in the current study showed increases in testosterone, free testosterone and estrogen levels, LH and FSH showed no significant changes. Hence the speculated mechanisms shown in the animal studies (Low *et al*., 2013a; Low *et al*., 2013b) may not be applicable since concurrent reduction in LH and FSH levels with increases in testosterone were not observed and therefore an alternative activation pathway may be involved.

Previous studies using animal models showed that gonadal steroids produced mainly from the HPG axis actively regulate the functioning of the HPA axis as well (Viau & Meaney, 1996; Viau, 2002; Seale *et al*., 2004). While our findings concur with previous studies that EL increases testosterone levels, it is plausible that the increase of testosterone could be due to direct stimulation of the adrenal gland in the HPA axis (Talbott *et al*., 2006) to produce testosterone precursors such as dehydroepiandrosterone, dehydroepiandrosterone sulfate, androstenedione, androstenediol and 11β-hydroxyandrostenedione (11βOHA4) (Rege *et al.,* 2013). These precursors could then be converted to more potent androgens such as testosterone and estrogens (Kaufman *et al*., 1990; Luu-The, 2013; Pelletier, 2008; Rosenfield, 2005) by plant compounds such as flavonoids

(Azarneoshan *et al*., 2009), phenolic (Khanam *et al*., 2015) and quassinoids (Low *et al*., 2013a; Rahman *et al*., 2017; Jayusman *et al*., 2018). EL is known to have active flavonoids (Khanam *et al*., 2014; Bashir *et al*., 2017) and quassinoids (Darise *et al*., 1982; Tung *et al*., 2017; Miyake *et al*., 2009), which could potentially increase testosterone levels via the HPG and as well via the HPA axis by converting the testosterone precursors.

Circulating testosterone plays a regulatory role in the activation of HPG and HPA axes to either produce or reduce the production of testosterone via the secretion of LH and FSH, which in turn are governed by estrogen level. Based on the literature, in addition to EL (Low *et al*., 2013a; Low *et al*., 2013b) other herbal extracts such as fenugreek, *Mucuna pruriens*, and *Tribulus terrestris* (Hameid *et al*., 2019; Shukla *et al*., 2009; Rogerson *et al*., 2007) are thought to be capable of increasing testosterone levels by inhibiting aromatase and the conversion of testosterone to estradiol. However, the rise in estradiol level in the current study places the proposed mechanism in dispute. The higher estradiol and testosterone levels reported suggest that EL may not inhibit the aromatase enzyme.

Typically, the HPA and HPG axis are examined independently (Dismukes *et al.*, 2015). Hence the proposed mechanisms interconnecting both axes need further investigation, since the direct comparison is limited. Examining hormones such as gonadotropin-releasing hormone (GnRH), corticotropin releasing hormone, adrenocorticotropic hormone, cortisol, and relevant androgens may be warranted to

understand how EL influences both the HPG and HPA axes. In addition, the increases in circulating testosterone and free testosterone may be due to EL influencing both HPA and HPG axis concurrently and not on HPG axis alone.

In summary, the present study shows that 600 mg/day of EL increases testosterone, free testosterone and estradiol levels among young adult men. Whilst the HPG axis is central to the production of testosterone, the impact of EL on this axis remains unclear due to lack of changes in LH, FSH and SHBG. Meanwhile, contrary to previous suggestions the increase in estradiol suggests that the aromatase may not be inhibited. A plausible alternative explanation on the increase in testosterone could be due to the direct stimulation of the adrenal gland in the HPA axis converting testosterone precursor to testosterone and not entirely from the activation of the HPG axis.

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DATA AVAILABILITY STATEMENT

Author elects to not share data

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Table 1 The anthropometric characteristics of the participants in *Eurycoma longifolia* and placebo groups at baseline

Table 2 Endocrine hormones at pre- (Day 1) and post- (Day 14) of *Eurycoma longifolia* and placebo supplementations.

Fig. S1 High-performance liquid chromatography analysis of a standardized extract of *Eurycoma longifolia* with eurycomanone.

Fig. S2 A calibration curve of eurycomanone (Eurycomanone content % w/w = 1.45)