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A Study on the Effects of *Eurycoma longifolia* and *Polyalthia bullata* on Reproductive Organs and Androgens of Male Sprague-Dawley Rats

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Abstract: The aims of this study were to determine the similarity and difference of *Eurycoma longifolia* (EL) and *Polyalthia bullata* (PB) on body weight, reproductive organs weight and regulation of potent androgen, testosterone and prohormone, De Hydro Epi Androsterone (DHEA) in Sprague-Dawley rats. Adult male Sprague-Dawley rats weighing 150-250 g were divided into 3 groups (n = 6) where control group received purified water, EL group and PB group received 400 mg/kg of treatment, respectively. The daily body weight of each rat was recorded during the treatment period. After 14 days of treatment (day 15), all rats were anaesthetised using ketamine/xylazine followed by cardiac puncture for blood collection. Rats were dissected and target organs were removed and weighed. Serum was obtained to determine testosterone and DHEA levels. Data was analysed using SPSS Version 23.0. There was a significant increase in mean percentage changes of body weight of EL compared to control group ($p = 0.003$) while a significant decrease in PB 400 mg/kg group compared to control group ($p = 0.04$). Treatment of EL has caused a decrease in right testes weight compared to control ($p = 0.026$) while treatment of PB has caused a decrease in right epididymis weight compared to control ($p = 0.04$). Both treatments have no effect on prostate and seminal vesicles weight of SD rats. Treatment of EL has caused a significantly increased serum testosterone concentration compared to control and PB ($p = 0.04$, $p = 0.03$), while there is no difference in serum DHEA concentration between all groups. In comparison of the *in vivo* effect of known aphrodisiac EL and PB, it is concluded that EL has more prominent effect in percentage of body weight changes, testis weight and serum testosterone level compared to PB. Further study is essential to determine other effects of these two plants on testes histology, androgens level and expression levels of steroidogenic enzymes.

Key words: *Eurycoma longifolia*, *Polyalthia bullata*, reproductive system, androgens, testis, seminal vesicles

INTRODUCTION

Aphrodisiac can be defined as a substance that can enhance sexual performance, increase libido and treat sexual dysfunction (Mathur, 2012). There are several plants that have been claimed to contain aphrodisiac property such as *Eurycoma longifolia* (EL), *Polyalthia bullata* (PB), *Rafflesia* sp., *Terminalia catappa*, *Labisia pumila* and *Smilax myosotiflora* (Asiah *et al*., 2007). *Eurycoma longifolia* Jack or Tongkat Ali (Simaroubaceae family) is a tropical herbal plant found in many South-East Asian countries (Bhat and Karim, 2010). Tongkat Ali has three different species including EL, PB and *Goniothalamus* sp. with EL is the commonly used species for traditional medicines. It is popular for its medicinal values such as an antipyretic, antimalarial, antidiabetic and also to cure sexual dysfunction in male. It is said to be able to increase the testosterone level which will lead to an increase in sexual desire (Ulbricht *et al*., 2013). Besides that it is used to treat malaria, high blood pressure, fatigue, migraine, fever, arthritis and libido. Generally, it is consumed to elevate energy level, increase endurance and stamina, enhance physical and mental performance, enhance immune system and also improve skin and muscle tone (Tambi *et al*., 2012). The compounds found in EL is reported to be able to activate sexual excitement in male rats and decrease the hesitation time for male rats to reach sexually receptive female rats (Ho and Tan, 2011). EL also is the most commonly used by men as aphrodisiac to enhance sexual performance (Mohamed *et al*., 2015). For testes histology, EL has been shown to cause

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concentrated seminiferous tubule with spermatogenic cells and the spermatozoa-filled lumen. The motile sperm are also high in the testes treated with EL (Wahab *et al*., 2010). The well-known claimed benefits of EL resulted in the manufacturing of products in the form of supplements and beverages. The products have high consumer demand as there are about over 202 products registered with the Malaysian regulatory body, National Pharmaceutical Control Bureau (NPCB) (George and Henkel, 2014).

Polyalthia genus consists of 120 species of shrubs and trees commonly found in tropical and subtropical regions. *Polyalthia bullata* or 'Tongkat Ali Hitam' is a small tree that can be found in Peninsular Malaysia and Borneo. It comes from the family of Annonaceae (Asiah *et al*., 2007). This plant is one of the aphrodisiac plants traditionally used in Malaysia (Connolly *et al*., 1996). It stimulates sexual desire and the root decoction is consumed as a male tonic (Ang and Lee, 2006). *Polyalthia bullata* shares the same local name 'Tongkat Ali' that has lead the locals to believe it can be used as an aphrodisiac for men (Burkill, 1966). The leaf, flower and root of PB plant have been reported to treat high blood pressure and diabetes. It is also used to increase male sexual drive, treat skin problems and as a general tonic for both men and women. It is also claimed to treat liver disease (Chee, 2013). However, there is no accurate information regarding its claimed medicinal uses (Chee, 2013). To our knowledge, the mechanism of action of PB on the reproductive system has not been extensively studied.

Male hypogonadism that is not widely being diagnosed is characterized by the symptoms such as sexual dysfunction and erectile dysfunction that requires treatment. This problem can be diagnosed when level of testosterone in serum shows an abnormally low result. Testosterone Replacement Therapy (TRT) is the common treatment for this problem (Nieschlag *et al*., 2004) which also increases the risk of getting prostatic cancer. Therefore, there are erectile dysfunction's sufferers that seek for alternative or herbal supplement to treat their problem but without the recommendations by the clinicians as there is insufficient scientific evidence on the efficiency and safety of alternative treatment (Shah *et al*., 2012). EL is a natural alternative for TRT as it has been proven to elevate serum testosterone level (George and Henkel, 2014) and the extract can be used as an alternative treatment without causing the side effects of TRT (Effendy *et al*., 2012). Therefore, the present study aimed to identify whether EL and PB plant extract influences the regulation of serum androgens (i.e., testosterone and DHEA). This study will be able to make potency comparison between the plants and provide the mechanism of action on androgen synthesis pathway. We also made comparison of body weight and reproductive organ weight. To our knowledge, no studies had made a comprehensive comparison between these two plants and its complete effect on the male reproductive system.

MATERIALS AND METHODS

EL and PB extract (in powdered form) was obtained from LJack Sdn Bhd., Malaysia. Testosterone ELISA kit (Item No. 582701) was purchased from Cayman Chemical Company, MI USA. Rat DHEA ELISA kit (Catalog No. E-EL-R0326) was purchased from Elabscience Biotechnology Co., Ltd, Wuhan PRC. All other ingredients used were of analytical grade.

Animal and treatment: Total 18 male Sprague-Dawley (SD) rats, each weighing 150-250 g were purchased from KL Rodentry Sdn Bhd., Malaysia and kept in separate Individual Ventilated Cages (IVC) at the Laboratory Animal Facility and Management (LAFAM), University Teknologi Mara (UiTM) Malaysia. The rats were fed with pellets and water ad libitum. Table 1 shows animal grouping and treatment received. All treatments were administered daily between 8.00-11.00 a.m. for 14 days. Body weights of the rats were recorded daily.

Sacrifice and organ/blood collection: At day 15, all rats were anesthetized with ketamine and xylazine. Sacrifice was performed via. cardiac puncture. Animals were dissected and reproductive organs were removed and weighed. Blood collected were centrifuged at 3000 rotation per minute (rpm) at 4°C for 10 min. Serum samples were stored at -80°C until hormonal assay assessment.

Hormone assay: Enzyme Linked-Immuno Sorbent Assay (ELISA) method was performed according to manufacturer's protocol to quantify the level of DHEA and testosterone.

Statistical analysis: All data were recorded and statistically analyzed by one way ANOVA, Mann Whitney and Kruskal Wallis test (SPSS Version 23.0). The value of $p<0.05$ was considered as significant. The results are presented as mean±SD.

Table 1: Animal group and treatment

Groups (400 mg/kg)	Quantity (n)	Treatment
Control	6	Purified water
EL	6	Eurycoma longifolia
PB	6	Polyalthia bullata

RESULTS AND DISCUSSION

Body weight and body weight changes: Following the treatment of animals, no changes could be found for the body weight of rats at day 15 as shown in Fig. 1-6. However, EL has caused a significantly higher mean percentage body weight changes compared to control ($p = 0.003$) while PB has caused a significantly decreased body weight changes compared to control ($p = 0.0046$). Increased body weight was reported for rats treated with other aphrodisiac plants such as rafflesia cantleyi (Erasmus, 2012). However, there is a study that has reported that body weight of rats was decreased with EL treatment (Husen *et al*., 2004).

Reproductive organs weight: Treatment of EL on rats has caused a decrease in right testes weight compared to control ($p = 0.026$). Similar right testes weight is observed on PB group but this is not significant as compared to control. Whilst both EL and PB showed a trend of decrease testes weight compared to control, statistical analysis showed no significant results. Previous research has shown no changes in testes weight with EL treatment (Solomon *et al*., 2014).

Treatment of EL and PB showed a trend of decreased epididymis weight with only the right epididymis from PB group showed a significant value ($p = 0.04$). This is supported by treatment with other aphrodisiac Hibiscus sabdariffa calyx which shown a decreased in epididymis weight for treatment of extract (Orisakwe *et al*., 2004).

Both EL and PB treatment has no effect on prostate weight of rats although, there is a trend of increased prostate weight by PB as compared to control. While this is supported in one previous study (Solomon *et al*., 2014), another study using reported significant changes in prostate weight of male SD rats treated with EL 10 mg/kg compared with control group and EL 5 mg/kg (Ang *et al*., 2000). This is due to increase in proliferative activity of the prostatic epithelium and histopathological changes of prostate gland that are related to testosterone level.

Similar result was observed for seminal vesicle weight where there is no effect of both EL and PB as compared to control. Graph shows a trend of increased epididymis weight by EL and decreased epididymis weight by PB. EL has been reported to promote the growth of seminal vesicle (Watcho *et al*., 2005). Another aphrodisiac plants mondia white plant is also shown to increase the relative weight of the seminal vesicle in higher dose (100 mg/kg) when compared to control group (Dewan *et al*., 2000).

Solomon *et al*. (2014) reported no changes in male reproductive organ weights (testes, epididymis and prostate) of rats that received low dose 200 mg/kg and high dose 800 mg/kg of EL. Rats undergone treatment with androgen is associated with increased weight of the epididymis, prostate and seminal vesicles (George and Henkel, 2014). This is because growth, sex differentiation of the epididymis, prostate and seminal vesicles are androgen-dependent processes (Dewan *et al*., 2000).

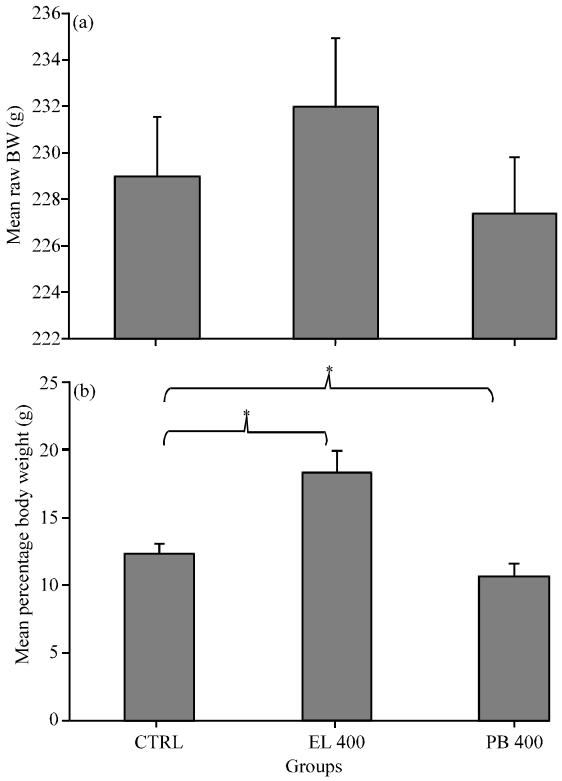

Fig. 1: Mean raw body weight: a) rats at day 15 and mean body changes and b) for control and treatment groups (PB and EL)

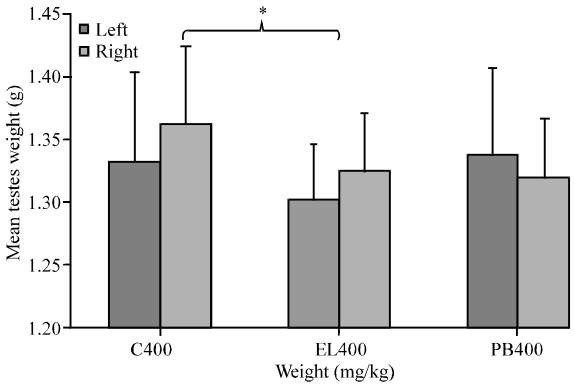

Fig. 2: Mean Left (L) and Right (R) testis weight for control and treatment groups (PB and EL)

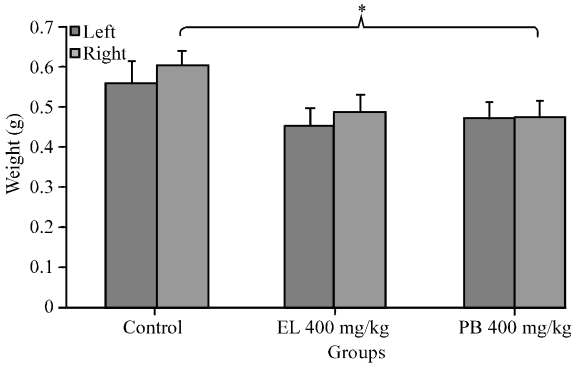

Fig. 3: Mean Left (L) and Right (R) epididymis weight for control and treatment groups (PB and EL)

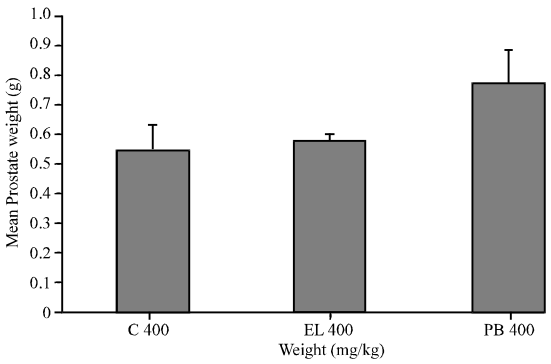

Fig. 4: Mean prostate weight for control and treatment groups (PB and EL)

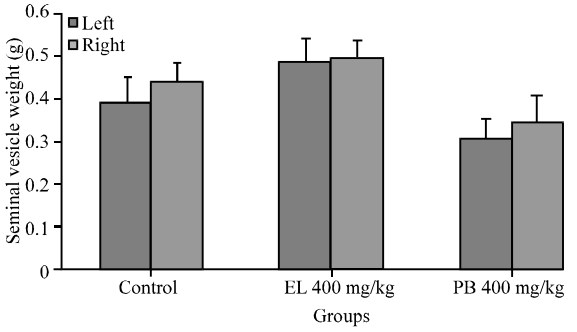

Fig. 5: Mean Left (L) and Right (R) seminal vesicle weight for control and treatment groups (PB and EL)

Hormone analysis: Treatment of EL has caused a significantly increased serum testosterone concentration by 31% compared to control ($p = 0.029$). This is also significantly higher than serum testosterone concentration compared to PB group by 13% ($p = 0.04$). There was in increased trend of serum testosterone as well for PB group but it is not significant. For DHEA serum concentration, no significant different was

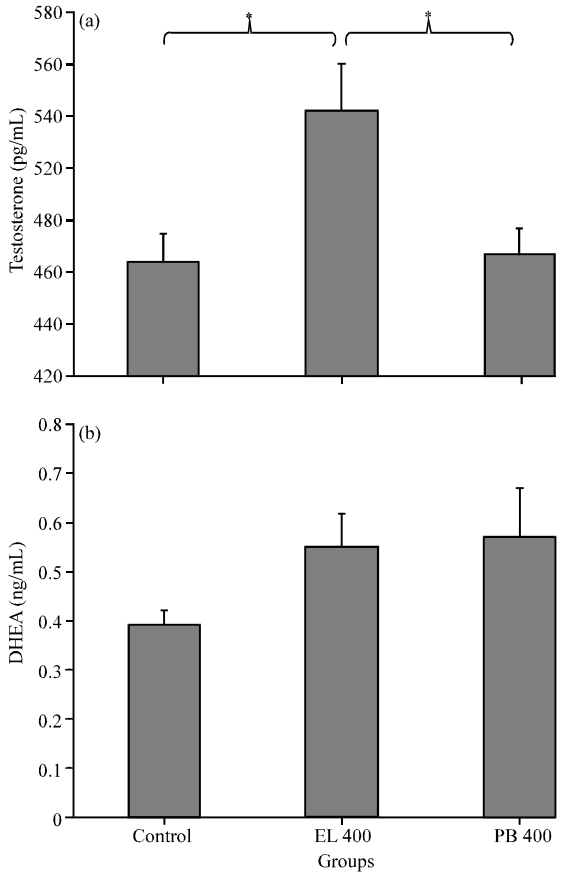

Fig. 6: Mean serum testosterone level: a) serum DHEA level and b) for control and treatment groups (PB and EL)

observed between all groups although, both EL and PB showed an increased pattern of serum DHEA concentration compared to control.

A bioactive peptide, 4.3 kDa found in *Eurycoma longifolia* (EL) roots has been found to be responsible in increasing testosterone levels by enhancing various androgen's biosynthesis (George and Henkel, 2014). The peak of 4.3 kDa was also detected in PB, in which this plant possesses a bioactive peptide similar in EL (Asiah *et al*., 2007). Root extract of EL has the highest concentrated quassinoids, known as eurycomanone. It enhances fertility by increasing testosterones production and spermatogenesis through hypothalamus-pituitary-gonadal axis. Eurycomanone increases steroidogenesis pathway in the leydig cells by inhibiting phosphodiesterase enzyme and aromatase conversion to oestrogen (Tambi *et al*., 2012). DHEA is reported to be enhanced by consuming EL as an adjunct to TRT in hypogonadism men (Low *et al*., 2013).

CONCLUSION

In comparison of the *in vivo* effects of known aphrodisiac EL and PB, it is concluded that EL has more prominent effect by increasing percentage of body weight changes and higher testis weight compared to control. EL also caused increased serum testosterone level compared to both control and PB treatment groups. This is parallel with a vast research that has been done on EL compared to PB although, both plants shared the same common name.

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