

The ameliorative effects of *Eurycoma longifolia* Jack on testosterone-induced reproductive disorders in female rats

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SUMMARY

The objective of this research was to study the ameliorative effects of a standardized quassinoid-rich extract (TAF 273) of *Eurycoma longifolia* root on some reproductive disorders in female rats. An irregular estrous cycle and ovarian cystic follicles were induced in 21-day-old females by the daily administration of testosterone (10 mg/kg, sc) for three weeks. The hormone-treated rats exhibited persistent diestrus as well as ovaries containing cystic follicles. Upon treatment with TAF 273, fewer animals showed irregular estrous cycles and there was less follicular morphological damage. The reversal effect may be derived from the anti-estrogenic properties of the plant quassinoids. *Reproductive Biology* 2012 **12** 2: 247–255.

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INTRODUCTION

Amenorrhea is a gynecological disorder affecting the fertility of women and arises from several causes, including polycystic ovary syndrome and oligomenorrhea. Ovarian follicular development, ovulation, and luteal function are completely prevented in amenorrheic women, resulting in low levels of estrogen and progesterone [10]. Polycystic ovary syndrome is a major cause of female infertility and is characterized by ovaries studded with fluid-filled cysts developed from the follicles failing to rupture completely or from corpora lutea failing to degenerate [4]. Oligomenorrhea is an unnaturally long menstrual cycle, which lasts for more than 35 days [7]. Consequently, oligomenorrheic women may have difficulty conceiving due to irregular menstrual cycles and predicting the next ovulation.

Several medicinal herbs including *Labisia pumila* (kacip fatimah), *Eurycoma longifolia* (tongkat ali), *Angelica sinensis* (dong quai), *Trifolium pretense* (red clover), *Eleutherococcus senticosus* (Siberian ginseng) and *Oenothera biennis* (evening primrose oil) have been used, both individually and as mixtures with other herbs, in ethno-botanical medicine to treat or relieve gynecological disorders of infertility, amenorrheal pain and to improve the physiological function of the female reproductive system [1, 5]. Amongst the listed herbs, *E. longifolia* Jack is the least described except for the improvement of health after childbirth [1]. In contrast, the roots have been traditionally prescribed as an aphrodisiac for males because of claims of increased libido and sexual prowess [8]. Recently, the potential beneficial effect of eurycomanone and 13 α ,21-dihydroeurycomanone from a standardized root extract TAF 273 of *E. longifolia* on female disorders was observed. These quassinoids reduced significantly an ethynyl estradiol-induced increase in the uterine weight of rats [9]. Prior to clinical studies, the female rats are frequently used as experimental models because their reproductive cycle resembles in many aspects that of humans. The estrous

cycle occurs during the reproductive age, between puberty and menopause [3]. Hormonal fluctuations during the estrous cycle are also similar in humans and rats [3, 10]. Both species are spontaneous ovulators with gonadotropins triggering comparable follicular and oocytic maturational changes within the ovaries. Furthermore, the rats with polycystic ovaries mimic those of humans with irregular ovarian histology and menstrual cycle [2]. The present study therefore evaluated the ameliorative effects of the standardized root extract TAF 273 of *E. longifolia* on testosterone-induced irregular estrous cycles and ovarian unhealthy/cystic follicles of rats.

MATERIALS AND METHODS

The roots of *Eurycoma longifolia* Jack were purchased in Perak (Malaysia) and identified by a pharmaceutical company, Hovid Berhad (Ipoh, Malaysia). A voucher herbarium specimen of the plant (No. 785–117) was deposited at the Penang Botanical Garden (Penang, Malaysia). The root extract TAF 273 was obtained by extraction and chromatographic separation following the method previously described [6]. The profile and content were determined by high-performance liquid chromatography (HPLC) using a C18 Metaphase Crestpak KR100–5 column of 250×4.6 mm, 5 µm particle size [6]. The mobile phase of acetonitrile and water (5:95 v/v) was filtered through a 0.45 µm nylon membrane filter and degassed for 10 min by sonication. The flow rate was maintained at 1.4 mL/min and detection of the quassinoids was at 238 nm.

Immature female Sprague Dawley rats (40–50 g; 21 days old) were obtained from the Animal Research and Service Centre, University of Science Malaysia (USM). The rats were housed under ambient temperature (23±2°C) with 12:12 h light-dark cycle at the Pharmacology Department of the School of Pharmaceutical Sciences, USM. The rats were fed with commercial rat diet (Gold Coin Sdn. Bhd.) and water *ad libitum*. The animal care and experimental protocols were approved by the Animal Ethics Committee of the university.

The experiment followed the method of Beloosesky et al. [2], and involved subcutaneous injections (sc) of immature female rats

with 1.0 ml/kg testosterone (10 mg/ml) in sesame oil daily for 21 days. In Experiment 1, 28 immature female rats (38–45 g, 21-day old) were divided into three groups. The testosterone-treated group (n=10) received 10 mg/kg (sc) of testosterone from day 21 to day 42, and 10 ml/kg of distilled water (orally) from day 36 to day 56. The testosterone- plus TAF 273-treated group (n=8) received 10 mg/kg (sc) of testosterone from day 21 to day 42, and 50 mg/kg of TAF 273 (orally) from day 36 to day 56. The control group (n=10) received 1.0 ml/kg (sc) of sesame oil from day 21 to day 42 and 10 ml/kg of distilled water (orally) from day 36 to day 56. The rat vaginal smear was obtained daily between 9:00 and 10:00 am [12] to monitor microscopically the cytological changes following the different phases of the cycle in rats from all groups [3, 11]. For normal rats, the length of the estrous cycle was about four to five days. The percentage of rats displaying regular estrous cycles was calculated from the number with regular estrous cycle over the total number in the group multiplied by 100. In Experiment 2 for ovarian histological evaluation, only 18 immature female rats of six per group were used following the same treatment schedule as in Experiment 1. The animals were sacrificed at the end of treatment on day 56. The whole ovaries were excised and fixed in 10% formalin. The tissues were initially dehydrated with increasing concentrations of ethanol, followed by immersion in xylene, and then embedded in paraffin wax. The paraffin-embedded ovary sections (5 μ m) were stained with hematoxylin and eosin, and then analyzed under a conventional light microscope of 100 \times magnification. To establish a number of healthy, unhealthy and cystic follicles at least three of the ovarian sections were examined for each rat. A healthy Graafian follicle was defined to contain the ovum surrounded by abundant granulosa cells. An unhealthy follicle had very few granulosa cells surrounding the degenerating ovum. A cystic follicle was fluid-filled without any ovum. The number of healthy follicles in the whole ovary section for the control group (n=6), testosterone-treated group (n=6) and testosterone plus the extract TAF 273-treated group (n=6) was determined and then analyzed by one-way ANOVA followed by Tukey test. The level of significant difference was determined at $p < 0.05$. The statistical tests were derived from the SPSS[®] ver. 15 software.

RESULTS AND DISCUSSION

In Experiment 1, 100% of the animals from the control group (vehicle-treated) displayed a regular estrous cycle. From the testosterone-treated group, the number of animals which displayed a normal estrous cycle was 0% after first week, 10% after second week and 20% after third week of the experiment. The rats which did not exhibit a normal estrous cycle exhibited a persistent diestrous phase. In contrast, the testosterone- and TAF 27-treated group showed gradual reversal to a normal estrous cycle of 12.5% after first week, 37.5% after second week and 62.5% after third week, when compared to those given testosterone alone (fig. 1). The changes in the rat estrous cycle may be linked to alterations in the circulating concentrations of the sex steroid hormones and gonadotropins [11]. These hormones control the ovarian function, including follicular maturation [3] and hormonal imbalance may

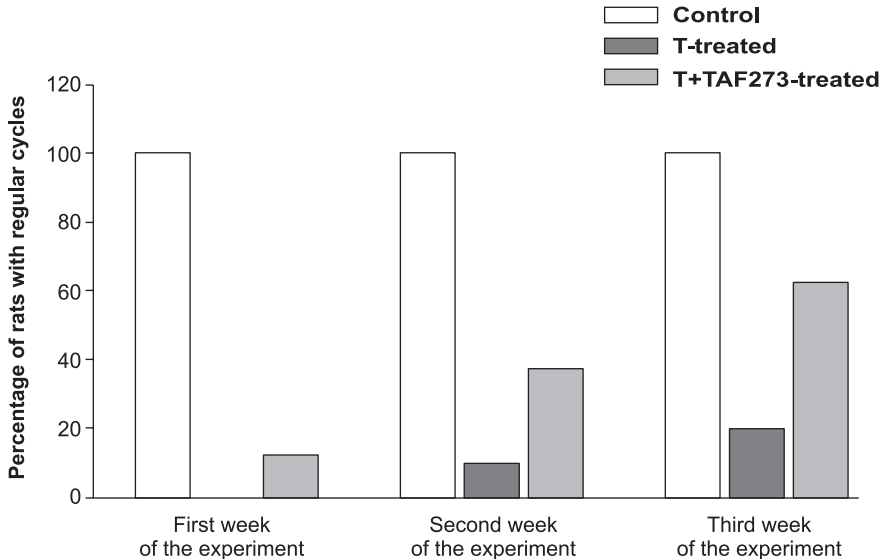


Figure 1. The percentage of rats displaying regular estrous cycles after treatment with vehicle (control; n=10), testosterone (T; n=10) and T plus standardized root extract TAF 273 of *E. longifolia* (T+TAF 273; n=8) after first (days 36–42), second (days 43–49) and third week (days 50–56) of the TAF 273 treatment.

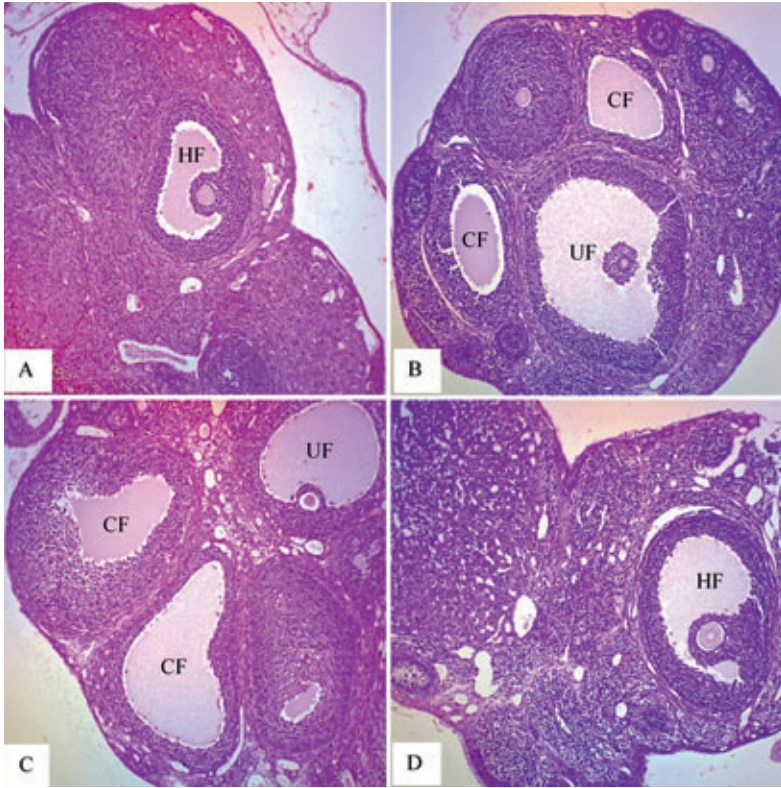


Figure 2. The photomicrographs of the ovarian sections from treated and untreated rats. A/ representative section of a control rat; B/ representative section of a T-treated rat (day 42); C/ representative section of a T-treated rat (day 56); D/ representative section of a T+TAF 273-treated rat (day 56); HF: healthy follicles; UF: unhealthy follicles, CF: cystic follicles; TAF 273: standardized root extract of *E. longifolia*. Magnification: 100×

lead to irregular estrous cycle thereby affecting the ovarian function. Most of the testosterone-treated rats (80–100%) showed a persistent diestrous phase during the three weeks of treatment when compared with those of control rats. In contrast, 62.5% of the animals in the testosterone plus TAF 273 group exhibited regular estrous cycles after three weeks of treatment.

The estrous cycle in the females is associated with numerous histological and morphological changes within the ovary [11]. In Experiment 2, most ovaries

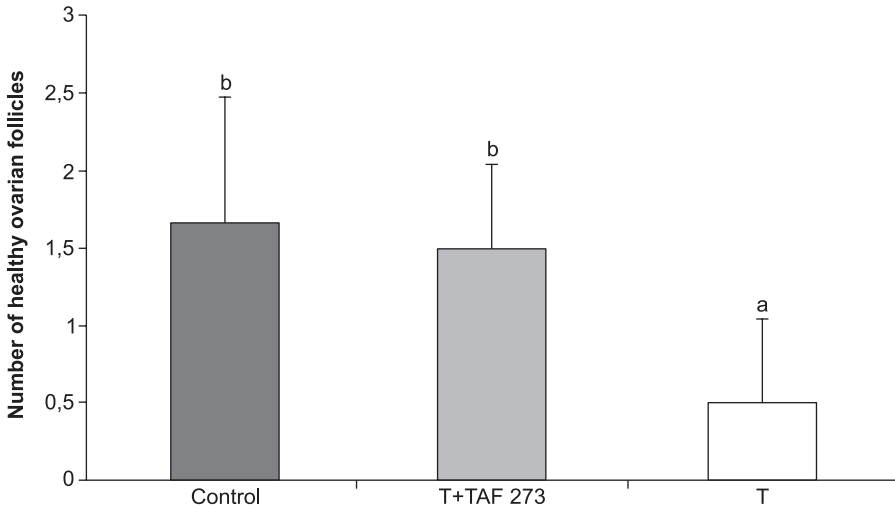


Figure 3. The mean number of healthy follicles (mean±SD) in the ovarian section in the control group (n=6), testosterone -treated group (T; n=6) and testosterone plus standardized root extract TAF 273 of *E.longifolia*-treated group (T+TAF 273; n=6). Bars with different superscripts are significantly different at $p<0.05$.

of control rats contained healthy follicles, with secondary Graafian follicles at various stages of development (fig. 2A). More unhealthy and cystic follicles were observed in the testosterone-treated group (fig. 2BC) and the number of healthy follicles was significantly lower ($p<0.05$) than that of the control group (fig. 3). These findings were consistent with those previously described for abnormal estrous cycle [2]. In contrast, the number of healthy ovarian follicles in testosterone plus TAF 273-treated group was not significantly different from that of the control group, but was significantly higher ($p<0.05$) than that of the testosterone-treated group (fig. 3). The appearance of more healthy follicles in the whole ovarian section of the testosterone plus TAF 273-treated group (fig. 2D) provided further evidence that the *E. longifolia* standardized root extract TAF 273 may be beneficial to some ovarian hormonal disorders. The HPLC analytical results showed that the standardized root extract TAF 273 of *E. longifolia* (mean±SD; n=3) contained four major quassinoids of eurycomanone (23.6%±0.9 w/w), 13 α (21)-epoxyeurycomanone (6.4%±0.1 w/w), 13 α ,21-dihydroeurycomanone (0.8%±0.1 w/w) and eurycomanol

(4.8%±0.2 w/w). Interestingly, eurycomanone, having the highest concentration in the extract, together with 13 α ,21-dihydroeurycomanone, were previously reported to show anti-estrogenic activity against ethynyl estradiol-induced increase in uterine weight of rats [9]. This may be responsible for the ameliorative effects of the TAF 273 extract on the occurrence of irregular estrous cycle and number of ovarian unhealthy/cystic follicles of the testosterone-treated animals. In conclusion, the present study showed that the plant extract normalized the irregular estrous cycle and reduced the follicular morphological damages caused by chronic testosterone administration in the female rats. Further work is required to identify the exact mechanism behind the ameliorative effects of *E. longifolia*.

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