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Effects of *Eurycoma longifolia* Jack on Laevator Ani Muscle in Both Uncastrated and Testosterone-Stimulated Castrated Intact Male Rats

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It has been reported that *Eurycoma longifolia* Jack commonly known as Tongkat Ali has gained notoreity as a symbol of man's ego and strength by the Malaysian men because it increases male virility and sexual prowess during sexual activities. As such, the effects of 200, 400 and 800 mg/kg of butanol, methanol, water and chloroform fractions of *E. longifolia* Jack were studied on the laevator ani muscle in both uncastrated and testosterone-stimulated castrated intact male rats after dosing them for 12 consecutive weeks. Results showed that 800 mg/kg of butanol, methanol, water and chloroform fractions of *E. longifolia* Jack significantly increased ($p<0.05$) the leavator ani muscle to 58.56 ± 1.22, 58.23 ± 0.31, 60.21 ± 0.86 and 62.35 ± 0.98 mg/100 g body weight, respectively, when compared with the control (untreated) in the uncastrated intact male rats and 49.23 ± 0.82, 52.23 ± 0.36, 50.21 ± 0.66 and 52.35 ± 0.58 mg/100 g body weight, respectively, when compared to control (untreated) in the testosterone-stimulated castrated intact male rats. Hence, the pro-androgenic effect as shown by this study further supported the traditional use of this plant as an aphrodisiac.

Key words: *Eurycoma longifolia* Jack, Laevator ani muscle, Uucastrated, Testosterone-stimulated castrated, Intact male rats, Pro-androgenic

INTRODUCTION

Eurycoma longifolia Jack which is known as tongkat Ali or Ali's walking stick is a plant which grows up to 10 m high and is found in primary and secondary, evergreen and mixed deciduous forests in Southeast Asia. Over the years, this plant has been shown to exhibit antimalarial (Chan *et al.*, 1986, 1989; Kardono *et al.*, 1991; Ang *et al.*, 1995, 1995a), cytotoxic (Kardono *et al.*, 1991; Morita *et al.*, 1990, 1993; Itokawa *et al.*, 1992, 1993), antiulcer (Tada *et al.*, 1991) and antipyretic (Chan *et al.*, 1995) activities and these may have been attributed to various quassinoids, squalene derivatives, biphenylneolignans, tirucallane-type triterpenes, canthine-6-one and β-carboline alkaloids.

However, in Malaysia, this plant has gained notoreity as a symbol of man's ego and strength because it has been claimed by Malaysian men to improve strength and power during sexual activities; it increases male virility and sexual prowess (Gimlette and Thomson, 1977). However, this claim is largely based on subjective opinion rather than scientific verification. Hence, this present study was undertaken to further investigate the effects of *E. longifolia* Jack on the bilateral laevator ani muscle (an indication of the protein anabolic action of androgens) in both uncastrated and testosterone-stimulated castrated intact male rats after dosing them with different fractions of *E. longifolia* Jack for 12 consecutive weeks.

MATERIALS AND METHODS

Plant material

E. longifolia Jack roots were obtained from Langkawi Island in Malaysia. This plant was identified by comparison with an authentic sample previously deposited at the School of Pharmaceutical Sciences, University Science Malaysia, Malaysia.

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Experimental

The experimental animals, both uncastrated and castrated male rats, were randomly divided into groups for daily treatment with 200, 400 and 800 mg/kg of one of the following fractions: chloroform, methanol, water and *n*-butanol. Vehicles used were propylene glycol and distilled water for chloroform and non-chloroform fractions, respectively. These test compounds were given daily using an appropriate oral needle for 12 consecutive weeks. However, the controls received 3 ml/kg of normal saline, 3 ml/kg propylene glycol whilst another control group was untreated.

In addition, testosterone was given subcutaneously 15 mg/kg daily for the castrated male rats. It was dissolved in a small volume of methylene dichloride. Sesame oil was added to the solution and the methylene dichloride was later evaporated off. This was kept at 37°C and whenever, signs of precipitation occurred, a fresh solution was prepared.

At the close of 12 consecutive week, the rats were sacrificed by overexposure to ether. The leavator ani muscle was carefully dissected out by removing the skin from the scrotal area between the base of the penis and the anus. The perineal area is then cleared of fat and connective tissue. Thus the perineal complex is disclosed, including the constriction indicating the point at which the leavator ani muscle joins the bulbocavernosus muscle. The rectum is then transected caudal to the leavator ani muscle. It is then freed from the rectum, removed and the net weight was determined relative to 100 g body weight of rats.

Statistical analysis

Results were statistically evaluated by two-way analysis of variance, completely randomized design followed by one-way analysis of variance, and subsequently by Ducan's multiple test at the 0.05 significance level (Schefler, 1984).

Test samples

The roots were then milled and later, defatted with petroleum ether before being extracted with methanol. The dried methanol residue (% yield: 3% w/w) was then partitioned between chloroform and water (2:1) to yield the chloroform extract (% yield: 0.1% w/w) and the aqueous layer (% yield: 0.55% w/w). The aqueous layer was then extracted with *n*-butanol (% yield: 0.45% w/w). The various solvents were then evaporated at reduced pressure to constant weight and stored in a refrigerator at 4°C.

Test animals

Ninety male Sprageu-Dawley rats, approximately 30 days of age at the beginning of the study, were used in this study. In another set of experiment, another ninety 20-day-old male Sprague-Dawley rats were castrated under ether anesthesia.

Castration was perfomed by making an incision through the skin of the scrotum of the anesthetized rats. The testis on the side is separated from the surrounding tissue by blunt dissection. A second incision is then made through the transparent *tunica vaginalis* which enabled the tunica to be retracted and the testis to be exposed. The gland is then removed by a cut close to the ligature. The second testis is then removed in the same manner and the skin incision is closed with two or three sutures. Care must be taken not to interfere with the function of rectum and anus.

A small quantity of chloramphenicol was injected subcutaneously and also applied locally to the operated area to prevent any unwanted infection (Zarrow *et al.*, 1964).

All the above experimental rats, both intact and castrated male rats, were housed individually in a standard wire-mesh cage in animal house in standard conditions and fed with commercial diet and water *ad libitum*.

RESULTS AND DISCUSSION

The average weights of the leavator ani muscle in both the uncastrated and testosterone-stimulated castrated male rats are summarized in Tables I and II. Results

Table I. Effects of *E. longifolia* Jack on the growth of leavator ani muscle in uncastrated intact male rats

Treatment	n	leavator ani muscle (mg/100 g)
Control (3 ml/kg normal saline)	6	50.27 ± 1.20
Control (3 ml/kg propylene glycol)	6	50.81 ± 1.21
Control (untreated)	6	50.52 ± 1.30
E. longifolia Jack		
Methanol		
200 mg/kg	6	57.21 ± 0.28
400 mg/kg	6	57.96 ± 0.29
800 mg/kg	6	58.23 ± 0.31*
Chloroform		
200 mg/kg	6	59.29 ± 0.95*
400 mg/kg	6	61.23 ± 0.93*
800 mg/kg	6	62.35 ± 0.98*
Butanol		
200 mg/kg	6	55.23 ± 1.12
400 mg/kg	6	58.33 ± 1.23*
800 mg/kg	6	58.56 ± 1.22*
Water		
200 mg/kg	6	58.23 ± 0.58*
400 mg/kg	6	59.23 ± 0.59*
800 mg/kg	6	60.21 ± 0.86*

Results are expressed as average ± s.e.m; *$p<0.05$ significantly different for comparisons with control (untreated)

Table II. Effects of *E. longifolia* Jack on the growth of leavator ani muscle in testosterone-stimulated castrated intact male rats

Treatment	n	leavator ani muscle (mg/100 g)
Control (3 ml/kg normal saline)	6	45.23 ± 0.20
Control (3 ml/kg propylene glycol)	6	45.21 ± 0.31
Control (untreated)	6	45.14 ± 0.15
E. longifolia Jack		
Methanol		
200 mg/kg	6	49.23 ± 0.83*
400 mg/kg	6	51.23 ± 0.92*
800 mg/kg	6	52.23 ± 0.36*
Chloroform		
200 mg/kg	6	49.29 ± 0.15*
400 mg/kg	6	51.23 ± 0.33*
800 mg/kg	6	52.35 ± 0.58*
Butanol		
200 mg/kg	6	46.23 ± 0.13
400 mg/kg	6	48.21 ± 0.43*
800 mg/kg	6	49.23 ± 0.82*
Water		
200 mg/kg	6	48.23 ± 0.78*
400 mg/kg	6	49.23 ± 0.57*
800 mg/kg	6	50.21 ± 0.66*

Results are expressed as average ± s.e.m; *$p<0.05$ significantly different for comparisons with control (untreated)

showed that *E. longifolia* Jack promoted the growth of the sexual accessory continuously with 800 mg/kg of butanol, methanol, water and chloroform fractions significantly increased ($p<0.05$) the leavator ani muscle to 58.56 ± 1.22, 58.23 ± 0.31, 60.21 ± 0.86 and 62.35 ± 0.98 mg/100 g body weight, respectively, when compared with the controls (untreated) in the uncastrated intact male rats. Similarly, 400 mg/kg of the above fractions significantly increased ($p<0.05$) the leavator ani muscle to 48.21 ± 0.43, 51.23 ± 0.92, 49.23 ± 0.57 and 51.23 ± 0.33 mg/100 g body weight, respectively, whilst 800 mg/kg of the extracts further increased them to 49.23 ± 0.82, 52.23 ± 0.36, 50.21 ± 0.66 and 52.35 ± 0.58 mg/100 g body weight, respectivley, when compared to controls (untreated) in the testosterone-stimulated castrated intact male rats.

Results from this study showed that not much difference was observed among the different fractions of *E. longifolia* Jack and this may be attributed to the presence of the active compounds in more than one fraction. In fact, the synergistic pro-androgenic effect as indicated by the enlargement of the leavator ani muscle which was observed in the various fractions of *E. longifolia* Jack may responsible for the male virility and sexual prowess while taking the whole plant as a decoction of the roots in water by the Malaysian community. Although a definite conclusion pertaining to the mechanism of action cannot be drawn from the available data and this study failed to determine which fraction is the most superior, nevertheless, it lends further support the traditional use of this plant by the indigenous population as an aphrodisiac.

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