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Effects of Eurycoma longifolia Jack (Tongkat Ali) on the Initiation of Sexual Performance of Inexperienced Castrated Male Rats

Hooi Hoon ANG, Hung Seong CHEANG, and Ahmad Pauzi Md. YUSOF

School of Pharmaceutical Sciences, University Science Malaysia, Minden, 11800, Penang, Malaysia

Abstract: *We studied the effects of Eurycoma longifolia Jack, commonly known as Tongkat Ali in Malaysia, on the initiation of sexual performance and the weights of sexual accessories in inexperienced castrated male rats. The doses of 200, 400 and 800 mg/kg body weight, which were extracted from E. longifolia Jack, were orally administered to the rats twice daily for 10 days prior to the tests and continued throughout the test period. Testosterone was used as a positive control after injecting 15 mg/kg daily subcutaneously for 32 days. Results showed that E. longifolia Jack produced a dose-dependent increase in sexual performance of the treated animals, but the E. longifolia Jack groups showed lower sexual performance in mounting, intromission and ejaculation than the testosterone group. Further results also showed that E. longifolia Jack promoted the growth of both ventral prostate and seminal vesicles as compared with the control, but the growth of sexual accessories at 800 mg/kg of butanol, methanol, water and chloroform fractions of E. longifolia Jack was less than that of testosterone treated group. The present study therefore gives further evidence of the folkuse of E. longifolia as an aphrodisiac.*

Key words: *aphrodisiac, Eurycoma longifolia Jack, sexual accessories, sexual performance tests*

Eurycoma longifolia Jack, from the Simaroubaceae family, is found on the sandy soil in both primary, secondary, evergreen and mixed deciduous forests in Southeast Asia [3, 16, 17]. In Malaysia, this plant which is identified as 'Tongkat Ali', is 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 [7]. But, this claim is largely based on subjective opinion rather than scientific verification.

Over the years, this plant has been shown to exhibit antimalarial [1, 2, 4, 5, 10], cytotoxic [8–10, 14, 15], antiulcer [20] and antipyretic [6] activities and these may have been attributed to various quassinoids, squalene derivatives, biphenylneolignans, tirucallane-type triterpenes, canthine-6-one and β-carboline alkaloids.

In this study we therefore further investigated the effects of *E. longifolia* Jack on the initiation of sexual performance of inexperienced castrated male rats.

Sexually inexperienced Sprague-Dawley rats were used as experimental subjects. They were housed in a

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Address corresponding: H.H. Ang, School of Pharmaceutical Sciences, University Science Malaysia, Minden, 11800, Penang, Malaysia

standard wire-mesh cage in animal house under standard condition with *ad libitum* access to food and water. All the rats were castrated 60–90 days before testing. Castration was performed by removing testicular and epidydimal tissues from the experimental male rats under ether anesthesia. A small quantity of chloramphenicol was injected subcutaneously and also applied locally to the operated area to prevent any unwanted infection.

Female rats which were used as stimulus animals were bilaterally ovariectomized via lumbar incisions under phenobarbitone anaesthesia approximately 1 month prior to testing. They were later brought on heat manually with a single subcutaneous dose of 10 μg estradiol benzoate (Sigma Chemical, USA) and 500 μg of progesterone (Sigma Chemical, USA), 48 hr and 4 hr before testing, respectively. Estradiol benzoate induced a specific urge in the ovariectomized rat to seek contact with a sexually active male [12, 13]. Furthermore, only receptive females were chosen in this study and this was indicated by the lordotic reflex in response to manual stimulation of the vaginal region and also confirmed by the vaginal smear. In addition, they were further tested with non-experimental male rats to further ensure receptivity before testing.

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. The roots were then milled and later defatted with petroleum ether before being extracted with methanol. The dried methanol (3%w/w) residue was then partitioned between chloroform and water (2:1) to yield the chloroform extract (0.1% w/w) and the aqueous layer (0.5% w/w). The aqueous layer was then extracted with *n*-butanol (0.45% w/w). The various solvents were then evaporated at reduced pressure to constant weight and stored in a refrigerator.

When required, test compounds were given twice daily with an appropriate oral needle for 10 days prior to the sexual performance test and continued throughout the test period. Vehicles used were propylene glycol and distilled water for chloroform and non-chloroform fractions, respectively. Each male rat in the respective group received 200, 400 and 800 mg/kg of one of the above fractions, whereas the controls received 3 m*l*/kg physiological saline.

Testosterone was given subcutaneously 15 mg/kg daily for 32 days during the sexual performance test. 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.

Substances extracted from *E. longifolia* Jack were administered 50–80 days post castration. Sexual performance tests were then began 10 days after the start of treatment with *E. longifolia* Jack but immediately after the start of testosterone treatment. They were performed in a copulation cage [11] on alternate days. Furthermore, they were performed during the dark phase of the light-dark cycle (2000–7000 hr), with subdued light in a quiet room with adequate ventilation.

After a 3 min period of adaptation of the experimental male rat in the copulation cage, a sexually receptive female rat was introduced. Every 5 min, the receptive female was replaced by a new one, thus providing the experimental male rat with optimal sexual stimulation.

The normal copulatory behaviour of the male rat consists of bouts or series of mounts (without intromission) and vaginal intromissions, with each complete series terminated by an ejaculation [18].

The sexual performance tests were terminated when one of the following conditions was fulfilled: 15 minutes after the presentation of the female to the male, if by that time no intromission had occurred, 30 min after the first intromission if no ejaculation had occurred, 15 min after ejaculation, if no intromission had occurred, or after the first intromission after ejaculation.

After the final test, the rats were sacrificed by overexposure to ether. The seminal vesicles and ventral prostate were carefully dissected and weighed to the nearest 0.1 mg.

Results were statistically evaluated by two-way analysis of variance, completely randomized design followed by one-way analysis of variance, and subsequently by Duncan's multiple test at the 0.05 significance level [19].

The cumulative percentages of animals responding to mounting, intromission and ejaculation each day are summarized in Table 1. The results showed that *E. longifolia* Jack produced a dose-dependent increase in the sexual performance of the treated animals with 400 mg/kg of butanol, methanol, water and chloroform frac-

Table 1. Cummulative percentage of animals responding to mounting, {intromission} and [ejaculation] on each day

Treatment \ Day	0	4	8	12	16	20	24	28	32
@Butanol (mg/kg)									
200	0{0}[0]	0{0}[0]	5{0}[0]	10{0}[0]	10{5}[0]	15{20}[0]	30{30}[15]	35{35}[35]	35{40}[40]
400	0{0}[0]	0{0}[0]	15{0}[0]	15{10}[0]	15{10}[0]	20{25}[0]	35{30}[20]	40{40}[35]	50{45}[45]
800	0{0}[0]	0{0}[0]	20{5}[0]	20{10}[0]	30{20}[0]	35{30}[0]	40{35}[30]	55{45}[35]	65{55}[50]
@Methanol (mg/kg)									
200	0{0}[0]	0{0}[0]	5{0}[0]	5{5}[0]	15{5}[0]	20{15}[0]	35{30}[20]	35{35}[30]	40{40}[40]
400	0{0}[0]	0{0}[0]	15{0}[0]	15{10}[0]	25{10}[0]	30{20}[0]	35{35}[25]	40{40}[35]	45{45}[40]
800	0{0}[0]	0{0}[0]	20{0}[0]	20{10}[0]	30{20}[0]	35{30}[0]	45{35}[30]	60{45}[40]	60{50}[45]
@Water (mg/kg)									
200	0{0}[0]	0{0}[0]	5{0}[0]	5{5}[0]	15{5}[0]	20{20}[0]	25{25}[20]	35{35}[30]	50{50}[40]
400	0{0}[0]	0{0}[0]	10{0}[0]	10{5}[0]	20{10}[0]	30{20}[0]	40{30}[25]	60{40}[35]	70{55}[45]
800	0{0}[0]	0{0}[0]	15{0}[0]	15{10}[0]	30{15}[0]	40{25}[0]	50{30}[30]	70{45}[40]	75{60}[50]
@Chloroform (mg/kg)									
200	0{0}[0]	0{0}[0]	5{0}[0]	5{5}[0]	15{5}[0]	20{20}[0]	30{20}[25]	35{30}[25]	50{50}[40]
400	0{0}[0]	0{0}[0]	5{0}[0]	10{5}[0]	20{10}[0]	30{20}[0]	40{25}[25]	50{40}[30]	60{55}[40]
800	0{0}[0]	0{0}[0]	10{0}[0]	15{5}[0]	30{15}[0]	35{25}[0]	45{30}[25]	60{45}[35]	80{65}[55]
Control (ml/kg)									
	0{0}[0]	0{0}[0]	0{0}[0]	5{5}[0]	10{5}[0]	15{10}[0]	15{15}[5]	20{15}[10]	25{20}[15]
Testosterone (mg/kg)									
15	0{0}[0]	0{0}[0]	25{10}[0]	25{20}[0]	35{30}[0]	45{40}[30]	75{50}[40]	90{75}[40]	90{75}[70]

Number of animals used in each group=20; @Fractions obtained from *E. longifolia* Jack; Sexual performance test was done on alternate days but the results were tabulated every 4 days.

tions eliciting 50%, 45%, 70%, 60% mounting, 45%, 45%, 55%, 55% intromission and 45%, 40%, 45%, 40% ejaculation, and 800 mg/kg further increased the figures to 65%, 60%, 75%, 80% mounting, 55%, 50%, 60%, 65% intromission and 50%, 45%, 50%, 55% ejaculation, respectively on the 32nd day of observation. However, 15 mg/kg testosterone elicited 90%, 75% and 70% mounting, intromission and ejaculation, repectively, on the 32nd day of observation and controls exhibited 25%, 20% and 15% mounting, intromission and ejaculation, respectively.

The mean weights of the ventral prostate and seminal vesicles in both the *E. longifolia* Jack and testosterone are summarized in Table 2. The results showed that *E. longifolia* Jack promoted the growth of sexual accessories continuously with 800 mg/kg of butanol, methanol, water and chloroform fractions significantly ($p<0.05$) increasing the ventral prostate to 350.4 ± 0.3 mg, 360.5 ± 0.4 mg, 340.2 ± 0.3 mg and 370.2 ± 0.1 mg and seminal vesicles to 340.2 ± 0.4 mg, 340.3 ± 0.4 mg, 320.1 ± 0.1 mg and 360.2 ± 0.1 mg, respectively. In contrast, testosterone treated male rats had a 400.9 ± 0.1 mg ventral prostate and 380.2 ± 0.2 mg seminal vesicle and the controls had a 245.3 ± 0.1 mg ventral prostate and 239.2 ± 0.3 mg seminal vesicle.

Table 2. Effect of *E. longifolia* Jack and testosterone on accessory sex organ development

Treatment	Dose (mg/kg)	Ventral prostate (mg)	Seminal vesicles (mg)
@Butanol	200	250.2 ± 0.2*	240.4 ± 0.3
	400	300.1 ± 0.4*	280.2 ± 0.2*
	800	350.4 ± 0.3*	340.2 ± 0.4*
@Methanol	200	280.7 ± 0.4*	270.7 ± 0.1*
	400	290.5 ± 0.3*	280.2 ± 0.5*
	800	360.5 ± 0.4*	340.3 ± 0.4*
@Water	200	270.2 ± 0.3*	260.1 ± 0.1*
	400	290.4 ± 0.4*	280.2 ± 0.2*
	800	340.2 ± 0.3*	320.1 ± 0.1*
@Chloroform	200	290.4 ± 0.2*	280.1 ± 0.4*
	400	320.3 ± 0.3*	310.4 ± 0.2*
	800	370.2 ± 0.1*	360.2 ± 0.1*
Control	-	245.3 ± 0.1	239.2 ± 0.3
Testosterone	15	400.9 ± 0.1*	380.2 ± 0.2*

Results are expressed as the mean ± s.e.m, *S $p<0.05$ compared with the control, $n_{each\ group} = 20$.

Generally, these results showed that there was not

much difference between the results of the sexual performance tests or the growth of sexual accessories after dosing the male rats with 800 mg/kg of various fractions of *E. longifolia* Jack, and this may be attributable to the presence of active constituents in more than one fraction.

In conclusion, this study shows that *E. longifolia* Jack enhanced the initiation of sexual performance of inexperienced castrated male rats, thus providing further evidence of the folkuse of this plant as aphrodisiac.

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