

Aphrodisiac Evaluation in Sexually Naive Male Mice after Chronic Administration of *Eurycoma longifolia* Jack

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Abstract – *Eurycoma longifolia* Jack was evaluated for aphrodisiac property on sexually naive male mice using the electrical copulation cage. Optimum condition was provided for this study and the male mice were treated with 500 mg/kg of either chloroform, methanol, water or n-butanol fractions from *E. longifolia* Jack. However, the mice in the yohimbine and control groups received 30 mg/kg and 3 ml/kg of yohimbine and normal saline respectively. The male mice were then conditioned to seek either an estrous female, sexually vigorous male or no mouse, a measurement of right, wrong or no choice respectively. Besides this, hesitation time which was the time spent before the sexually naive male mice crossed the electrical grid (maintained at 0.12 mA) was also determined. Results showed that *E. longifolia* Jack possesses aphrodisiac property on the sexually naive male mice as shown by the slow and transient reduction in hesitation time and also a similar manner in the increase in the % of sexually naive male mice scoring right choice throughout the investigation period. Hence, this further supports the folkuse of this plant as aphrodisiac.

Key words – *Eurycoma longifolia* Jack, Simaroubaceae, aphrodisiac, electrical grid, hesitation time, preference of choice.

Introduction

Eurycoma longifolia Jack (Simaroubaceae), identified by its local name as Tongkat Ali, is a tall, slender, shrub-tree up to 10 m tall and is commonly found as understorey in the lowland forests at up to 500 m above sea level (Goh *et al.*, 1995). Pharmacological evaluations on the various compounds obtained from this plant showed that it exhibited antimalarial (Ang *et al.*, 1995, 1995, 1995a; Chang *et al.*, 1986, 1989; Kardono *et al.*, 1991), cytotoxic (Itokawa *et al.*, 1992, 1993;

Kardono *et al.*, 1991; Morita *et al.*, 1990, 1993), antiulcer (Tada *et al.*, 1991) and antipyretic (Chan *et al.*, 1995) properties and these may have been attributed to various quassinoids, squalene derivatives, biphenylneolignans, tirucallane-type triterpenes, canthine-6-one and β -carboline alkaloids.

But in Malaysia it has gained notoriety as a male aphrodisiac since it is reputed to increase male virility and sexual prowess (Goh *et al.*, 1995; Gimlette and Thomson, 1997) but this claim is largely based on subjective opinion rather than scientific verification.

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Hence in this paper, we further investigate the effect of different fractions of *E. longifolia* Jack on hesitation time and preference of choice of the sexually naive male mice until 10 days post-treatment using the electrical copulation cage.

Materials and Methods

Test animals – Male albino mice (35-40 g), naive to heterosexual behaviour and drugs, were subjected to a standardized training procedure daily for 10 consecutive days. The purpose was to habituate the animals to the test apparatus. Training period was carried out at the beginning of the dark phase of the light dark cycle and the amount of visible light in the environment was just enough to see the animals. The mice were then caged singly, with food and water available *ad libitum*.

Female mice used as mating stimuli were later brought to heat artificially with a single intramuscular injection of 0.3 mg/kg estradiol benzoate (Sigma Chemical, USA) and 3 mg/kg of progesterone (Sigma Chemical, USA), approximately 3 days and 3 hours before testing, respectively. It was shown that estradiol benzoate induced in the ovariectomized mouse a specific urge to seek contact with a sexual active male (Meyerson and Lindstrom 1971, 1973).

Furthermore, only receptive females were chosen in this study and this was shown by the lordotic reflex in response to manual stimulation of the vaginal region and also confirmed by the vaginal smear. Lordosis is a posture in which the feet are planted and extended, and the rump region elevated. Besides these, they were further tested with non-experimental male mice to further ensure receptivity before testing.

Test compounds – *E. longifolia* Jack roots were obtained from Langkawi Island in Malaysia. This plant was identified by comparison with an authentic sample previously depo-

sited at the School of Pharmaceutical Sciences, University of Science Malaysia, Malaysia (Chan *et al.*, 1986). The roots were then milled and were subsequently defatted with petroleum ether before being extracted with methanol. The dried methanol residue was then partitioned between chloroform and water (2:1) to yield the chloroform extract and the aqueous layer. The aqueous layer was then extracted with *n*-butanol.

Test extracts were given daily with an appropriate oral needle and each male mouse in the respective groups received 500 mg/kg of one of the following fractions: chloroform, methanol, water or *n*-butanol. The mice in the yohimbine and control groups received 30 mg/kg and 3 ml/kg of yohimbine (Sigma Chemical, USA) and normal saline respectively.

Test apparatus – An electric grid, maintained at 0.12 mA was used as an obstruction in the electrical copulation cage in order to determine how much a negative stimulus (crossing an electrical grid) the male mouse was willing to overcome to reach the sexual contact. Sexually naive male mouse was separated from the electric grid by a plexiglass door and this door would be automatically opened when the male mouse was deposited in a starting cage. Once the door was opened, the male mouse was then crossed the electric grid to the goal cage which house either an estrous receptive female, sexually vigorous male or no mouse, a measurement of right, wrong or no choice respectively. The time spent before the sexually naive male mouse crossed the electrical grid is hesitation time. However, the contact between the sexually naive male mice and the caged animals was restricted by a wire mesh screen preventing direct sexual intercourse.

Statistical analysis – The mean values for the hesitation time of the treated and the control groups were statistically analysed by analysis of variance (ANOVA) 2-way

layout completely randomised design followed by ANOVA 1-way layout completely randomised design and subsequently, Duncan's multiple test at 0.05 significant level (Scheffler., 1984).

Results and Discussion

Table 1 shows the effect of different fractions of *E. longifolia* Jack, yohimbine and normal saline on hesitation time of the male mice until 10 days post-treatment using the electrical copulation cage. Results showed that *E. longifolia* Jack produced a slow and transient decrease in the hesitation time of the sexually naive male mice as compared to yohimbine and control with *E. longifolia* Jack produced 700-750, 685-740, 680-740, 670-735, 660-710, 650-700, 540-685, 530-680, 530-640, 500-620 sec whilst yohimbine and control produced 600, 580, 570, 570, 570, 550, 540, 530, 530, 500 sec and 900, 890, 870, 840, 820, 800, 780, 770, 760 and 750 sec respectively throughout the investigation period. Further results also indicated that

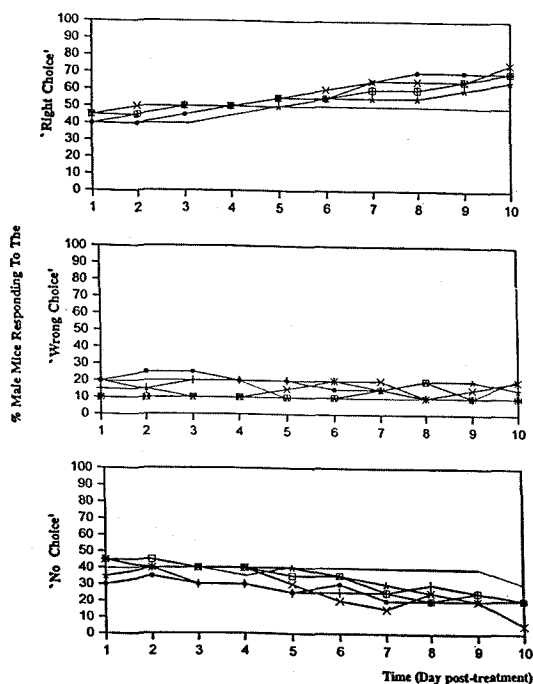


Fig. 1. Effects of different fractions of *E. longifolia* Jack, yohimbine and normal saline on male mice ($n_{\text{each group}}=20$) until 10 days post-treatment.

sexually naive male mice treated with *E. longifolia* Jack and yohimbine were significantly

Table 1. Effect of different fractions of *E. longifolia* Jack, yohimbine and normal saline on hesitation time of the male mice until 10 days post-treatment using the electrical copulation cage

	Time (Day post-treatment)				
	1	2	3	4	5
	Hesitation time (sec)				
@chloroform	700±17	685±32	680±19	675±16	670±18
@methanol	750±17	740±36	740±18	735±17	700±28
@water	700±41	690±23	680±15	670±42	660±10
@butanol	750±22	740±15	730±15	720±12	710±15
yohimbine	600±15	580±12	570±17	570±14	570±18
control	900±12	890±17	870±16	840±18	820±12
	Time (Day post-treatment)				
	6	7	8	9	10
	Hesitation time (sec)				
@chloroform	660±15	650±15	640±15	630±20	620±21
@methanol	690±29	685±37	680±12	640±15	600±19
@water	650±15	630±17	830±18	620±27	620±42
@butanol	700±31	650±15	650±12	620±15	600±21
yohimbine	550±18	540±17	530±25	530±20	500±23
control	800±13	780±14	770±10	760±13	750±19

@Fractions were obtained from *E. longifolia* Jack, results were expressed as mean±standard error mean ($n_{\text{each group}}=20$) and were significantly different ($p<0.05$) when compared with the controls of the same group.

different ($p < 0.05$) from the control.

In addition, Fig. 1 also shows that there was a slow and transient increase in the % of male mice scoring the right choice after chronic treatment of *E. longifolia* Jack. Besides this, more than 50% of the male mice scored right choice after 6 days treatment and the effect became more prominent after 9 days post-treatment using the electrical copulation cage. In contrast, there was no sexual enhancement for the male mice which consumed normal saline because only 40-50% of the male mice scored right choice throughout the investigation period. In general, this study showed that *E. longifolia* Jack possesses aphrodisiac property on the sexually naive male mice as evidenced by the transient reduction in hesitation time but an increase in the % of sexually naive male mice scoring right choice and this definitely further supports the folkuse of this plant as aphrodisiac.

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