

Aphrodisiac evaluation in non-copulator male rats after chronic administration of *Eurycoma longifolia* Jack

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Keywords

aphrodisiac,
Eurycoma longifolia Jack,
hesitation time,
noncopulator male rats

ABSTRACT

The aphrodisiac effect of *Eurycoma longifolia* Jack (0.5 g/kg) was evaluated in noncopulator male rats using an electrical cage. Fractions of *E. longifolia* Jack decreased the hesitation time of noncopulator male rats, throughout the investigation period. Furthermore, it possessed a transient increase in the percentage of the male rats responding to the right choice, more than 50% of the male rats scored 'right choice' after 3 weeks post-treatment and the effect became more prominent after 8 weeks post-treatment (only 40–50% of the control male rats responded to the right choice) using the electrical copulation cage. Hence, this study lends further support to the use of the plant by indigenous populations as a traditional medicine for its aphrodisiac property.

Received 18 April 2001;
revised 25 June 2001;
accepted 25 June 2001

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INTRODUCTION

Eurycoma longifolia Jack (Simaroubaceae), which is known as 'Tongkat Ali' or Ali's walking stick in Malaysia, is a plant that grows to a height of 10 m with a branch diameter of 10 cm. It is found in primary and secondary, evergreen and mixed deciduous forests in Burma, Indochina, Thailand, Malaysia, Sumatra, Borneo and the Phillipines. It is popularly used, singly or as an essential component, in herbal remedies, for various illnesses including fevers, aches, sexual insufficiency, hypertensive, tuberculosis, vermifuge and as health supplement. It has not been indicated strongly for any specific illness. Hence, it is well-known among various ethnic groups in Malaysia for treating disease and enhancing health. It is sometimes referred to as 'Malaysian ginseng' [1].

Over the years, pharmacological evaluations of this plant showed that it exhibited antimalarial [2–6] cytotoxic [4,7–9] antiulcer [10] and antipyretic [11] activities. In Malaysia, it has gained a reputation as a male aphrodisiac because it is claimed to increase virility and sexual prowess [12] when taken as a decoction of roots in water. Thus, this plant has long captured the Malaysian market and currently, there are about 200

tongkat Ali products, most of them focusing and emphasizing on its aphrodisiac property [1].

In this paper, we investigated the aphrodisiac effects of different fractions of *E. longifolia* root in noncopulator male rats using an electrical copulation cage after treating the male rats daily with different fractions of *E. longifolia* Jack and observing them for 12 consecutive weeks.

METHODS

Test compounds

E. longifolia roots, collected in January 2000 from Langkawi Island in Malaysia and 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 subsequently defatted with petroleum ether before being extracted with methanol. The dried methanol (3% w/w) residue was then partitioned between chloroform and water to yield a chloroform extract (0.1% w/w) and the aqueous layer (0.5% w/w). The latter was extracted with *n*-butanol (0.45% w/w). Phytochemical screening [13] carried out on these fractions gave positive tests with different

intensities but only for alkaloids, lactones and phenolics. The various solvents were then evaporated at reduced pressure to constant weight and stored in a refrigerator.

Animals

Sexually sluggish old adult male Sprague-Dawley rats, weighing around 300 g, were used. They were housed individually in a standard wire-mesh cage and maintained under standard environmental conditions and fed with commercial diet and water ad libitum. Male rats were tested with receptive female rats for copulatory behaviour, each test lasting for 1 h daily for 4 consecutive weeks. Males that failed to show any copulatory behaviour during this observation period were selected as noncopulator and hence, used in this study.

Female rats, 2–3 months old (225–275 g), were bilaterally ovariectomized via lumbar incisions under phenobarbitone anaesthesia approximately 1 month prior to testing. They were later brought on heat with single subcutaneous doses of estradiol benzoate (10 µg) and progesterone (500 µg), 48 and 4 h before testing, respectively. Estradiol benzoate induced a specific urge to seek contact with a sexual active male in the ovariectomized rat [14,15]. The sexual receptivity of the females was tested by the lordotic reflex (feet planted and extended with the bump region elevated in response to manual stimulation of the vaginal region) (16–19) and confirmed by a vaginal smear. In addition, they were further tested with nonexperimental male rats to further ensure receptivity before testing.

Copulatory behaviour test

During the study, test compounds were given daily by gavage for 12 consecutive weeks and animals were observed for copulatory behaviour for the above dur-

ation. Each noncopulator male rat in the respective groups received 0.5 g/kg of one of the test *E. longifolia* whilst the control groups received 3 mL/kg of saline. The vehicles used were propylene glycol for the chloroform fraction, and distilled water for the other fractions.

Test apparatus

Tests were performed on selected sexually sluggish male rats with an electrical copulation cage during the dark phase of the light-dark cycle (20:00–07:00 h) and in subdued light. An electric grid, maintained at 0.10 mA was used as an obstruction in the electrical copulation cage in order to determine how much an aversive stimulus (crossing an electric grid) the noncopulator male rat was willing to overcome to reach the estrous receptive female in the goal cage. Non-copulator male rat was separated from the electric grid by a plexiglass door and this door was automatically opened when the male rat was placed in a starting cage. Once the door was opened, the male rat crossed the electric grid to the goal cage, which housed either an estrous receptive female, a sexually vigorous male or no rat at all, with a measurement of 'right', 'wrong', or 'no' choice, respectively. The time spent before the sexually naive male rat crossed the electric grid is considered to be hesitation time. However, the contact between the sexually sluggish male rat and the caged animals was restricted by a wire mesh screen, preventing direct sexual intercourse. The intensity of the grid current was maintained at 0.10 mA and this was the intensity at which the noncopulator male rats in the control group failed to cross over to reach the goal cage.

Unless stated otherwise, all copulation tests lasted for 30 min, prior to 20 min adaptation. This was observed daily for 12 consecutive weeks.

Table I Effect of different fractions of *E. longifolia* Jack and normal saline on hesitation time of the noncopulator male rats until 12 weeks post-treatment using the electrical copulation cage.

	Time (week post-treatment)											
	1	2	3	4	5	6	7	8	9	10	11	12
Hesitation time (s)												
chloroform*	966 ± 10	964 ± 9	954 ± 8	940 ± 5	930 ± 4	925 ± 7	918 ± 8	910 ± 8	890 ± 8	850 ± 8	830 ± 8	800 ± 7
metanol*	960 ± 12	952 ± 12	942 ± 15	940 ± 10	936 ± 10	934 ± 7	930 ± 8	910 ± 9	890 ± 9	854 ± 9	800 ± 7	780 ± 8
water*	920 ± 7	910 ± 8	908 ± 10	904 ± 9	900 ± 10	894 ± 11	890 ± 12	888 ± 15	885 ± 9	850 ± 10	845 ± 8	700 ± 12
butanol*	960 ± 10	912 ± 8	910 ± 11	906 ± 7	900 ± 5	890 ± 6	880 ± 8	875 ± 8	870 ± 8	858 ± 9	845 ± 7	800 ± 8
control	1000 ± 8	984 ± 7	960 ± 8	950 ± 5	944 ± 5	940 ± 5	920 ± 2	916 ± 5	900 ± 5	860 ± 4	850 ± 5	840 ± 5

*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.

Statistical analysis

The mean values of the hesitation time for both the treated and control groups were statistically analysed by analysis of variance (ANOVA) two-way layout completely randomised design, followed by ANOVA one-way layout completely randomised design and subsequently, Duncan's multiple test at 0.05 significant level (20).

RESULTS

Table I shows the effects of different fractions of *E. longifolia* Jack and normal saline on hesitation time of the male rats until 12 weeks post-treatment using the electrical copulation cage. Results showed that *E. longifolia* Jack decreased the hesitation time of noncopulator male rats as compared to controls, with the various fractions of

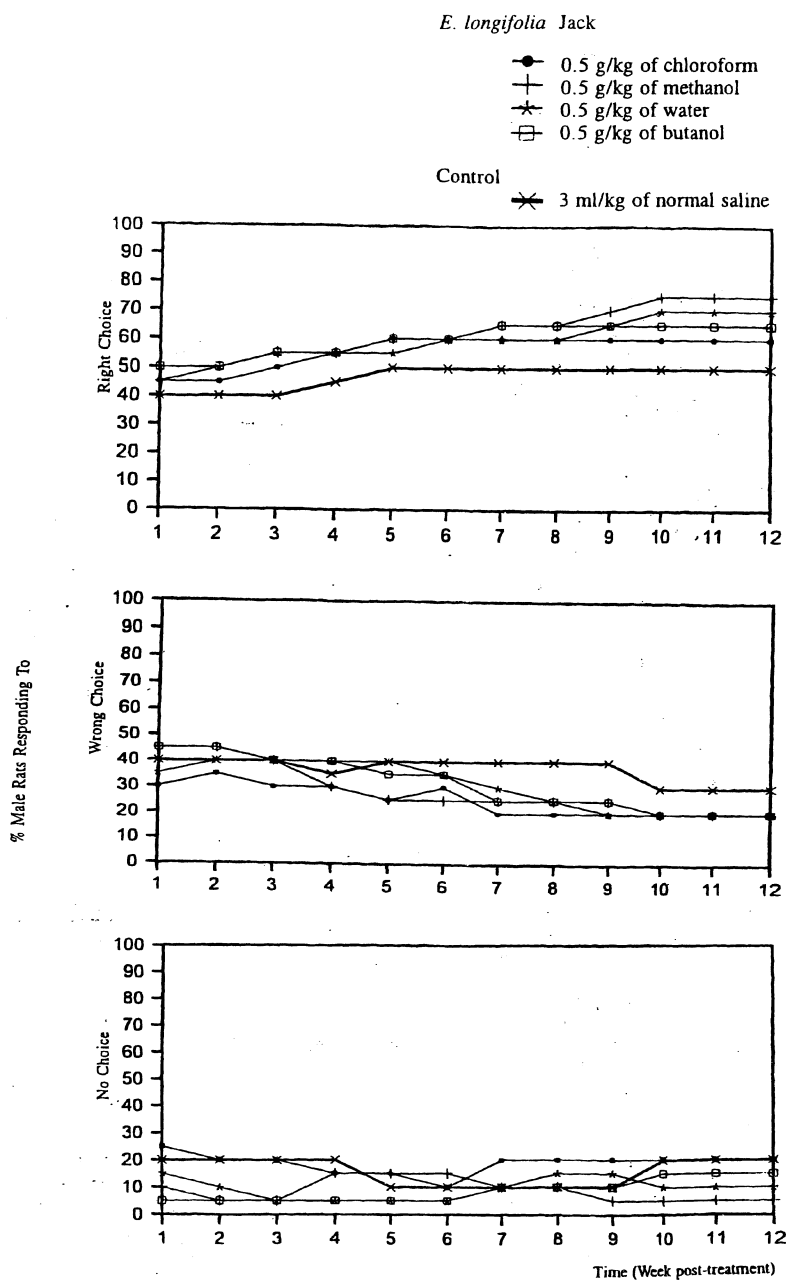


Figure 1 Effects of different fractions of *E. longifolia* Jack and normal saline on male rats ($n_{\text{each group}} = 20$) until 12 weeks post-treatment.

E. longifolia Jack producing the following ranges of hesitation time for each consecutive week throughout the treatment: 920–966 s hesitation in week 1, 910–964 in week 2, 908–954 in week 3, 904–940 in week 4, 900–936 in week 5, 894–934 in week 6, 880–930 in week 7, 875–910 in week 8, 870–890 in week 9, 850–858 in week 10, 800–845 in week 11, 700–800 in week; this was in contrast to controls which produced 1000, 984, 960, 950, 944, 940, 920, 916, 900, 860, 850, 850 sec, respectively, throughout the investigation period.

Figure 1 also shows that there was a transient increase in the percentage of the male rats responding to the right choice after chronic administration of 0.5 g/kg *E. longifolia* Jack. In addition to this, more than 50% of the male rats scored the right choice after 3 weeks post-treatment and the effect became more prominent after 8 weeks post-treatment using the electrical copulation cage. However, there was no sexual enhancement of the noncopulator male rats that consumed normal saline (control), because only 40–50% of the male rats responded to the right choice throughout the investigation period.

DISCUSSION AND CONCLUSION

In general, results from this study showed that little 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. Although this study fails to determine which fraction of *E. longifolia* Jack is more efficient, it lends further support to the use of the plant by the indigenous population as a traditional medicine for aphrodisiac property. Hence, further studies should be conducted to determine if this plant has similar quality in aged humans.

ACKNOWLEDGEMENTS

This study was financed by a short-term research grant from University Science Malaysia, Penang Malaysia.

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