

Eurycoma longifolia JACK and Orientation Activities in Sexually Experienced Male Rats

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The effects of *Eurycoma longifolia* JACK were studied on the orientation activities of sexually experienced male rats towards receptive females (mounting, licking, anogenital sniffing), environment (exploration, raring, climbing), themselves (genital grooming, non-genital grooming) and mobility (unrestricted, restricted) after dosing them with 200, 400 and 800 mg/kg body weight twice daily for 10 d prior to the test. The results showed that *E. longifolia* JACK modified the orientation activities of the treated male rats in that they significantly displayed more frequent and vigorous mounting, licking and anogenital sniffing towards the receptive females, and it further intensified self orientation as indicated by the increased grooming of the genitals compared to the controls ($p < 0.05$). In addition, rats treated with 800 mg/kg of methanol, water and butanol extracts of *E. longifolia* JACK continued to show confinement to a particular area of the cage (around the female), thus showing restriction in movement as compared to the controls ($p < 0.05$). However, the treated males possessed a lack of interest in the external environment as indicated by a reduction in exploration, raring and climbing on the cage wall. Hence, the present study further supports the folk use of *E. longifolia* JACK as an aphrodisiac.

Key words *E. longifolia* JACK; orientation activity; experienced male rat

Eurycoma longifolia JACK (Simaroubaceae), identified by its local name as Tongkat Ali, is commonly found along the hilly jungle slopes of Malaysia. Over the years, pharmacological evaluations of the various compounds isolated from this plant showed that it possessed antimalarial,^{1–5} cytotoxic,^{3,6–9} antiulcer,¹⁰ and antipyretic,¹¹ properties. In addition to these, it has gained notoreity as a male aphrodisiac since it is reputed to increase male virility,¹² and it is usually taken as a decoction of roots in water.

Hence, this present study was undertaken to further investigate the effects of *E. longifolia* JACK on the orientation activities of sexually experienced male rats after treating them with different fractions of *E. longifolia* JACK for 10 d.

MATERIALS AND METHODS

Animals Inbred male albino rats, 3–4 months old, were used in this study. They were kept under conditions of controlled temperature and relative humidity, with water and standard laboratory food available *ad libitum*. Each male received at least 3 mating tests with a non-experimental female to gain sexual experience, and they were acclimated to laboratory conditions before the start of the test.

Test Compounds *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 of Science Malaysia, Malaysia.² The roots were then milled and were 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 the chloroform extract (0.1% w/w) and the aqueous layer (0.5% w/w). The latter was then extracted with *n*-butanol (0.45% w/w).

Test extracts were given twice daily, using an appropriate oral needle, for 10 d prior to the test. Each male rat in the respective groups received 200, 400 or 800 mg/kg of the above

fractions whilst the control group received 3 ml/kg of normal saline.

Orientation Activities This test was performed at the beginning of the dark phase of the light–dark cycle (2000–2400 h), with subdued light in a quiet room with adequate ventilation. After dosing the sexually experienced male rats with their respective dose for 10 d, they were then tested on the 11th day by visual observation for the orientation activities in the above conducive environment.

The orientation activities of the male rats towards the receptive female (mounting, licking, anogenital sniffing), the environment (exploration, raring, climbing), themselves (genital grooming, non-genital grooming) and mobility (unrestricted, restricted) were tested and rated, respectively, according to the methods previously described.^{13,14}

Female rats used as mating stimuli were made receptive by bilateral ovariectomy *via* lumbar incisions under phenobarbitone anaesthesia approximately 1 month prior to testing. They were later brought into behavioural estrus with a single subcutaneous injection of 10 µg estradiol benzoate (Sigma Chemical, U.S.A.) and 500 µg of progesterone (Sigma Chemical, U.S.A.), 48 and 4 h before testing, respectively. It was shown that estradiol benzoate induced in the ovariectomized rat a specific urge to seek contact with a sexually active male.^{15,16}

Furthermore, only receptive females were chosen in this study; this was shown by the lordotic reflex in response to manual stimulation of the vaginal region and was also confirmed by a vaginal smear. In addition, they were further tested with non-experimental male rats to ensure receptivity before testing.

Statistical Analysis An analysis of variance (ANOVA) 2-way layout completely randomized design, followed by an ANOVA 1-way layout completely randomized design, and, subsequently, *post hoc* statistical comparison using Duncan's multiple test at a significance level of 0.05, were tested on the various mean activity levels of the different behavioral

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components against the control in each group. Differences were considered significant when $p < 0.05$.¹⁷⁾

RESULTS AND DISCUSSION

Tables 1 and 2 show that different fractions of *E. longifolia* JACK increased the orientation activities of the treated male rats towards the receptive females (mounting, licking, anogenital sniffing) but decreased the response to the environment (exploration, raring, climbing) as compared to the controls. Further results also showed that the treated male rats exhibited a dose-dependent increase and also significantly displayed more frequent and vigorous mounting, lick-

ing and anogenital sniffing of females as compared to the controls ($p < 0.05$). However, the treated males possessed a lack of interest in the external environment as evidenced by a reduction in exploration (inquisitiveness), raring and climbing on cage wall.

Table 3 shows that males treated with various fractions of *E. longifolia* JACK exhibited significantly enhanced orientation towards themselves, as evidenced by increased grooming of the genitals ($p < 0.05$); however, non-genital grooming remained unchanged ($p > 0.05$). In addition, rats treated with 800 mg/kg of methanol, water and butanol extracts of *E. longifolia* JACK continued to show confinement, with orientation and movement directed to a particular area of the cage

Table 1. Effect of Different Fractions of *E. longifolia* JACK on Anogenital Sniffing, Licking and Mounting towards Receptive Female during Copulation

	10 min			20 min			30 min		
	Anogenital sniffing	Licking	Mounting	Anogenital sniffing	Licking	Mounting	Anogenital sniffing	Licking	Mounting
Control	1.5±0.2	1.0±0.1	1.2±0.2	1.8±0.2	1.8±0.2	1.3±0.2	1.8±0.2	1.8±0.3	1.3±0.2
Chloroform ^{a)}									
200 mg/kg	2.6±0.3	2.0±0.2	1.8±0.3	2.8±0.1	2.3±0.3	1.9±0.2	3.0±0.1	2.5±0.1	2.0±0.3
400 mg/kg	2.9±0.1	2.2±0.1	1.9±0.2	3.1±0.2	2.5±0.2	2.0±0.2	3.3±0.1	2.7±0.2	2.4±0.1
800 mg/kg	3.1±0.2	2.4±0.1	2.5±0.2	3.3±0.1	2.6±0.1	2.6±0.1	3.5±0.1	2.8±0.1	2.7±0.1
Methanol ^{a)}									
200 mg/kg	2.7±0.1	2.4±0.2	2.4±0.3	2.7±0.1	2.6±0.1	2.3±0.1	2.9±0.1	2.8±0.1	2.5±0.1
400 mg/kg	2.6±0.1	2.6±0.1	2.5±0.1	2.9±0.2	2.7±0.1	2.6±0.1	3.0±0.2	2.9±0.2	2.7±0.1
800 mg/kg	2.8±0.1	2.6±0.1	2.7±0.1	3.0±0.1	2.8±0.1	2.8±0.2	3.2±0.1	3.0±0.2	2.9±0.1
Water ^{a)}									
200 mg/kg	2.5±0.1	2.3±0.1	2.2±0.3	3.0±0.1	2.5±0.2	2.3±0.1	3.2±0.1	2.7±0.1	2.5±0.2
400 mg/kg	2.9±0.1	2.6±0.2	2.1±0.2	3.1±0.1	2.7±0.2	2.3±0.1	3.3±0.1	2.8±0.1	2.6±0.1
800 mg/kg	3.1±0.1	2.5±0.2	2.3±0.1	3.3±0.2	2.7±0.1	2.7±0.1	3.5±0.4	2.9±0.1	2.8±0.2
Butanol ^{a)}									
200 mg/kg	2.8±0.1	2.5±0.2	1.7±0.1	3.0±0.2	2.6±0.1	1.8±0.1	3.3±0.1	2.8±0.1	1.9±0.1
400 mg/kg	3.1±0.1	2.6±0.1	2.0±0.1	3.3±0.1	2.8±0.1	2.1±0.1	3.5±0.1	3.0±0.2	2.3±0.1
800 mg/kg	3.3±0.1	3.1±0.2	2.3±0.1	3.5±0.1	3.0±0.1	2.5±0.1	3.7±0.2	3.1±0.1	2.6±0.1

a) Fractions obtained from *E. longifolia* JACK. Each value represents the mean activity±S.E.M. of 20 experiments. $S p < 0.05$ all values are significantly different from the control in each of the orientation activities.

Table 2. Effect of Different Fractions of *E. longifolia* JACK on Climbing, Raring and Exploration towards Environment during Copulation

	10 min			20 min			30 min		
	Climbing	Raring	Exploration	Climbing	Raring	Exploration	Climbing	Raring	Exploration
Control	0.5±0.1	0.6±0.2	1.0±0.3	0.3±0.2	0.5±0.2	0.8±0.1	0.3±0.1	0.5±0.1	0.8±0.2
Chloroform ^{a)}									
200 mg/kg	0.5±0.2	0.5±0.1	0.8±0.1	0.4±0.1	0.4±0.2	0.3±0.1*	0.3±0.1	0.4±0.1	0.3±0.1*
400 mg/kg	0.5±0.1	0.4±0.1	0.8±0.2	0.4±0.1	0.3±0.1	0.5±0.1	0.3±0.1	0.3±0.1	0.4±0.1*
800 mg/kg	0.3±0.1	0.4±0.1	0.8±0.1	0.2±0.1	0.3±0.1	0.6±0.1	0.2±0.1	0.2±0.1	0.6±0.1
Methanol ^{a)}									
200 mg/kg	0.6±0.1	0.6±0.1	0.5±0.1*	0.4±0.1	0.5±0.2	0.6±0.1	0.3±0.1	0.4±0.1	0.7±0.1
400 mg/kg	0.5±0.2	0.4±0.1	0.5±0.2*	0.4±0.2	0.3±0.1	0.6±0.1	0.3±0.2	0.3±0.1	0.7±0.1
800 mg/kg	0.4±0.1	0.4±0.1	0.8±0.2	0.3±0.2	0.4±0.1	0.7±0.2	0.3±0.1	0.2±0.1	0.6±0.1
Water ^{a)}									
200 mg/kg	0.5±0.1	0.6±0.1	0.6±0.1	0.4±0.1	0.4±0.1	0.7±0.1	0.3±0.2	0.3±0.1	0.7±0.1
400 mg/kg	0.4±0.1	0.5±0.1	0.6±0.1	0.3±0.1	0.4±0.1	0.6±0.1	0.3±0.1	0.3±0.1	0.6±0.1
800 mg/kg	0.4±0.1	0.4±0.2	0.8±0.2	0.3±0.1	0.3±0.1	0.5±0.1	0.2±0.1	0.1±0.1	0.3±0.1*
Butanol ^{a)}									
200 mg/kg	0.5±0.1	0.5±0.2	0.7±0.2	0.4±0.3	0.4±0.2	0.7±0.1	0.3±0.1	0.4±0.2	0.7±0.1
400 mg/kg	0.5±0.1	0.5±0.2	0.7±0.1	0.4±0.1	0.5±0.1	0.5±0.2	0.3±0.1	0.4±0.2	0.5±0.2
800 mg/kg	0.3±0.1	0.3±0.1	0.7±0.1	0.3±0.1	0.3±0.1	0.6±0.1	0.2±0.1	0.3±0.1	0.4±0.1*

a) Fractions obtained from *E. longifolia* JACK. Each value represents the mean activity±S.E.M. of 20 experiments. * $S p < 0.05$ significantly different from a) with the control in each of the orientation activities. NS $p > 0.05$ not significantly different for the rest of the comparisons with the control in each of the orientation activities.

Table 3. Effect of Different Fractions of *E. longifolia* JACK on Non-genital Grooming, Genital Grooming towards Themselves and Restricted, Unrestricted towards Mobility during Copulation

	10 min				20 min				30 min			
	Non-genital grooming	Genital grooming	Restricted	Unrestricted	Non-genital grooming	Genital grooming	Restricted	Unrestricted	Non-genital grooming	Genital grooming	Restricted	Unrestricted
Control	0.5±0.1	1.2±0.2	1.2±0.3	0.8±0.2	0.8±0.2	1.8±0.1	1.2±0.1	0.8±0.1	1.0±0.2	1.8±0.1	1.3±0.1	0.7±0.2
Chloroform ^{a)}												
200 mg/kg	0.4±0.1	2.3±0.2*	1.2±0.1	0.8±0.1	0.5±0.1	2.5±0.2*	1.3±0.2	0.8±0.1	0.6±0.1	2.6±0.1*	1.4±0.1	0.8±0.1
400 mg/kg	0.4±0.1	2.5±0.1*	1.3±0.1	0.8±0.2	0.5±0.2	2.7±0.2*	1.5±0.1	0.8±0.2	0.6±0.1	2.8±0.2*	1.6±0.2	0.8±0.2
800 mg/kg	0.3±0.2	2.6±0.1*	1.4±0.2	0.8±0.1	0.6±0.1	2.8±0.1*	1.6±0.1*	0.8±0.1	0.7±0.1	3.0±0.2*	1.8±0.2*	0.9±0.3
Methanol ^{a)}												
200 mg/kg	0.4±0.1	2.6±0.2*	1.3±0.3	0.7±0.1	0.6±0.1	2.8±0.1*	1.5±0.1	0.7±0.1	0.7±0.1	3.0±0.1*	1.6±0.1	0.8±0.1
400 mg/kg	0.6±0.1	2.7±0.1*	1.4±0.1	0.7±0.2	0.7±0.1	2.8±0.1*	1.6±0.2*	0.7±0.2	0.8±0.1	3.0±0.1*	1.8±0.2*	0.8±0.1
800 mg/kg	0.6±0.1	2.9±0.1*	1.6±0.1*	0.7±0.1	0.7±0.1	3.1±0.2*	1.8±0.1*	0.7±0.2	0.9±0.2	3.2±0.2*	2.0±0.1*	0.9±0.2
Water ^{a)}												
200 mg/kg	0.5±0.1	2.7±0.2*	1.2±0.1	0.7±0.1	0.6±0.1	2.9±0.1*	1.3±0.2	0.8±0.1	0.7±0.1	3.1±0.2*	1.5±0.1	0.9±0.1
400 mg/kg	0.5±0.2	3.0±0.2*	1.3±0.2	0.7±0.1	0.7±0.2	3.1±0.2*	1.5±0.1	0.8±0.1	0.8±0.1	3.3±0.3*	1.7±0.1*	0.9±0.1
800 mg/kg	0.5±0.2	3.2±0.1*	1.7±0.1*	0.7±0.2	0.7±0.1	3.3±0.1*	1.9±0.1*	0.7±0.1	0.8±0.2	3.5±0.1*	1.9±0.1*	0.9±0.2
Butanol ^{a)}												
200 mg/kg	0.6±0.1	2.3±0.2*	1.3±0.2	0.7±0.2	0.7±0.1	2.5±0.1*	1.6±0.1*	0.7±0.1	0.5±0.1*	2.6±0.1*	1.7±0.2*	0.8±0.1
400 mg/kg	0.6±0.1	2.5±0.1*	1.6±0.1*	0.7±0.1	0.7±0.1	2.7±0.1*	1.7±0.1*	0.8±0.2	0.6±0.1	2.8±0.2*	1.8±0.1*	0.9±0.1
800 mg/kg	0.5±0.1	2.7±0.2*	1.7±0.2*	0.8±0.1	0.6±0.1	2.9±0.1*	1.9±0.2*	0.8±0.1	0.7±0.1	3.0±0.3*	2.0±0.1*	0.9±0.2

a) Fractions obtained from *E. longifolia* JACK. Each value represents the mean activity±S.E.M. of 20 experiments. * $p < 0.05$ significantly different from a) with the control in each of the orientation activities. NS $p > 0.05$ not significantly different for the rest of the comparisons with the control in each of the orientation activities.

(around and on every side of the female), thus showing confined and limited movement as compared to the controls during the test period ($p < 0.05$). Furthermore, it was found that not much difference was obtained 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 general, this study showed that *E. longifolia* JACK modified the orientation activities of treated male rats, one of the criteria in determining male sexual behavior.^{13,18} Furthermore, *E. longifolia* JACK also enhanced the orientation activities of treated males towards females by causing them to display vigorous anogenital investigatory behaviour, and it further intensified self orientation as evidenced by increased grooming of their own genitals, which both suggest improved sexual performance.^{19,20} Thus, the present results further support the folk use of this plant as an aphrodisiac.

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