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Effects of *Eurycoma longifolia* Jack in Maintaining Mating Behavior of Sexually Experienced Castrated Male Rats

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Abstract – The effects of *Eurycoma longifolia* Jack were studied in maintaining mating behavior of sexually experienced castrated male rats after dosing them with 500 mg/kg daily of *E. longifolia* Jack for 10 days prior to test and later continued for two weeks where the rats were then castrated. The similar dosage was then continued for 12 weeks post-castration. However, 400 μg/day of testosterone was administered subcutaneously on the day of castration and lasted for 6 weeks post-castration but later raised to 800 μg/day until 12 week post-castration. Tests were conducted weekly from 2-6 weeks and 8-12 weeks post-castration. Results showed that all the experimental male rats exhibited mating behavior before castration. Further results also indicated that *E. longifolia* Jack successfully maintained mating behavior but less than precastration level from 2-6 weeks and later increased from 8-12 weeks post-castration. Similarly, 400 μg/day of testosterone was effective in maintaining mating behavior from 2-6 weeks post-castration. However, 800 μg/day of testosterone managed to return the male rats to the precastration level with all male rats exhibited mating behavior from 8-12 weeks post-castration. Further results also indicated that testosterone significantly increased the penis weights ($p < 0.05$) as compared to the *E. longifolia* Jack. In conclusion, this study shows that *E. longifolia* Jack continued to maintain mating behavior of sexually experienced castrated male rats, giving further evidence of the folkuse of this plant as aphrodisiac.

Key words – *E. longifolia* Jack; mating behavior; sexually experienced castrated male rats

Introduction

Eurycoma longifolia Jack, from the Simaroubaceae family, is found on the sandy soil up to 10 m tall and commonly found as undestroyed in the lowlands forests at up to 500 m above sea level (Goh *et al.*, 1995). In Malaysia, this plant which is identified as 'Tongkat Ali' or Ali's walking stick, is a symbol of man's ego and strength because it has been claimed locally to improve strength and power during sexual activities; it increases male virility and sexual prowess (Goh *et al.*, 1995; Gimlette and Thomson, 1977) and it is usually taken as a decoction of the roots in water. But, this claim is largely based on subjective opinion rather than scientific verification.

Over the years pharmacological evaluations on this plant showed that it exhibited antimalarial (Ang *et al.*, 1995a, 1995b; Chan *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) activities and these may have been attributed to various quassinoids, squalene derivatives, biphenylneolignans, tirucallane-type triterpenes, canthin-6-one and β-carboline alkaloids. Hence, in this paper, we further investigated the effects of *E. longifolia* Jack in maintaining mating behavior of sexually experienced castrated male rats.

Materials and Methods

Animals and surgery Inbred Sprague-Dawley rats strain of male albino rats were used as experimental subjects. They were housed in standard wire-mesh cage in animal house under standard condition with *ad libitum* access to food and water. Each male rat received at least 3 mating tests with non-experimental female to gain sexual experience, and they were adapted to laboratory conditions before the start of the test. The males were allowed to mate until they had achieved a single intromission following the first ejaculation or for a maximum of 60 minutes if an ejaculation did not occur or if the male failed to resume mating following the first ejaculation. Follow-

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ing three successive pretests during which all males ejaculated, the males were castrated by making an incision through the skin of the scrotum of the anesthetized rats. The testis on one 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. Female rats which were used as stimulus animals were bilaterally ovariectomized via lumbar incisions under phenobarbitone anaesthesia approximately one month prior to testing. They were later brought on heat manually with a single subcutaneous dose of 10 μg estradiol benzoate (Sigma Chemical, Co., USA) and 500 of progesterone (Sigma Chemical, Co., USA), 48 hours and 4 hours before testing, respectively. Estradiol benzoate induced a specific urge to seek contact with a sexually active male in the ovariectomized rat (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 actually a posture consisting of an arching of the back and elevation of the pelvis, frequently accompanied by tail deviation (Diakow, 1975; Komisaruk and Diakow, 1973; Kuehn and Beach, 1963; Sachs and Barfield, 1970). In addition, they were further tested with non-experimental male rats and only those females exhibiting good receptive with no rejection behavior with the non-experimental male rats were used.

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 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 for an oral dose of 500 mg/kg daily of one of the above fractions, butanol, methanol, water and chloroform, with an appropriate oral needle 10 days prior to the sexual performance test and later continued for two weeks where the rats were then castrated. The similar dosage was then continued until 12 weeks post-castration. Vehicles used were propylene glycol and distilled water for chloroform and non-chloroform fractions, respectively. However, 400 μg testosterone which was used as the positive control in this study, was initiated subcutaneously daily on the day of castration and this was given for six weeks post-castration. After this, testosterone was then raised to 800 μg daily and tests were conducted weekly from 2-6 weeks and 8-12 weeks post-castration. Testosterone was dissolved in a small volume of methylene dichloride. Sesame oil was added to the solution and methylene dichloride was subsequently evaporated. This was kept at 37°C and whenever signs of precipitation occurred, fresh solution was prepared.

Sexual performance test – Sexual performance tests began 10 days after the onset of treatment of *E. longifolia* Jack in a copulation cage (Mendelson and Gorzalka, 1987) during the dark phase of the light-dark cycle (20:00-07:00), with subdued light in a quiet room with adequate ventilation. After a three minute adaptation period of the experimental male rat in the copulation cage, a sexually receptive female rat was introduced. Every five minute, the receptive female exchanged for a new one, thus providing the experimental male rat with optimal sexual stimulation. The normal copulatory behavior of the male rats consists of bouts or series of mounts (without intromission) and vaginal intromissions, with each complete series terminated by an ejaculation (Sachs and Barfield, 1970). 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 at the time no intromission had occurred, 30 minutes after the first intromission if no ejaculation had occurred, 15 minutes 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 penis was carefully dissected and weighed to the nearest 0.1 mg.

Statistical Analysis – Results of the penis weights

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

Results and Discussion

The effects of various fractions of *E. longifolia* Jack and testosterone in maintaining mating behavior in the castrated male rats are as shown in Figure 1. Results showed that 100% of the experimental male rats exhibited mountings, intromissions and ejaculations before castration. Further results also indicated that butanol, methanol, water and chloroform fractions of *E. longifolia* Jack were effective in maintaining mating behavior but less than precastration level, 60-75%, 65-75%, 65-80%, 65-75% exhibited mountings from 2-6 weeks and later increased to 80-95%, 80-95%, 80-95%, 75-95% from 8-12 weeks post-castration respectively. Subsequently, the above test compounds continued to maintain mating behavior, 50-55%, 60-70%, 60-75%, 60-70% of the test population exhibited intromissions and subsequently increased to 50-60%, 70-80%, 80-90%, 70-90% respectively during the intervals studied. Mating

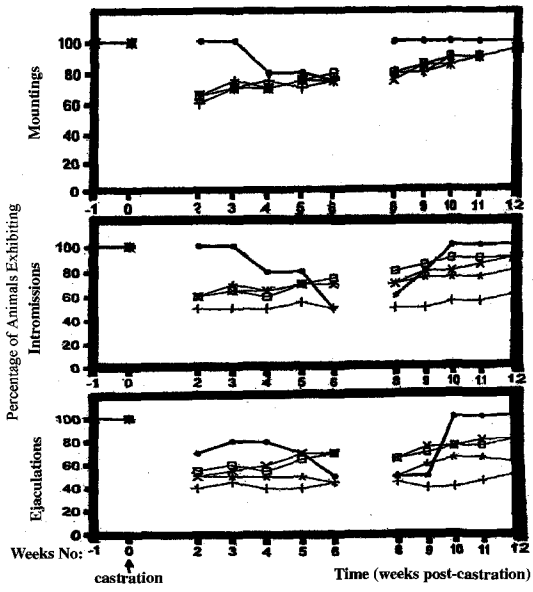

Fig. 1. Percentage of male rats exhibiting mountings, intromissions and ejaculations prior to castration (weeks -1 to 0) and until 12 weeks post-castration. ● testosterone; + butanol; ⊤ methanol; ⊟ water; × chloroform.

Table 1. Effects of *E. longifolia* Jack and testosterone on the weights of penis from castrated male rats

Treatment	n	penis weight (mg)
Testosterone	20	294.6 ± 0.4
E. longifolia Jack fractions		
Butanol	20	285.7 ± 0.3*
Methanol	20	287.3 ± 0.2*
Water	20	273.2 ± 0.1*
Chloroform	20	272.3 ± 0.5*

*$p < 0.05$ compared with testosterone

behavior was further maintained, 40-45%, 45-50%, 55-70%, 50-70% of the experimental male rats exhibited ejaculations, but further increased to 40-50 %, 50-65%, 65-80%, 65-80% respectively at the similar intervals. Similarly, 400 μg/day of testosterone, which was used as a positive control in this study, was effective in maintaining mating behavior, 75-100%, 50-100%, 50-80% of the male rats exhibited mountings, intromissions and ejaculations respectively from 2-6 weeks post-castration. However, 800 μg/day of testosterone managed to return the male rats to the precastration level with all male rats exhibited mountings, 60-100% exhibited intromissions and 50-100% exhibited ejaculations respectively from 8-12 weeks post-castration. Results on the penis weights are as shown in Table 1. Results indicated that testosterone significantly increased the penis weights ($p < 0.05$) as compared to the various fractions of *E. longifolia* Jack because testosterone is potent in stimulating phallic growth and hence, further enhanced the overall sexual performance, providing evidence that the results from the present study were consistent with those previously reported (Beach and Holtz-Tucker, 1949; Davidson *et al.*, 1966; Luttge and Whalen, 1970; Luttge, 1972).

In conclusion, this study shows that various fraction of *E. longifolia* Jack continued to maintain mating behavior of sexually experienced castrated male rats, thus providing further evidence of the folkuse of this plant as aphrodisiac.

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