Natural Product Sciences
8(2) : 44-47 (2002)

Effects of *Eurycoma longifolia* Jack on Masculine Copulatory Behaviour in Middle Aged Male Rats - A Comparison Study

Hooi Hoon Ang[1] and Kheng Leng Lee

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

Abstract – The effects of *Eurycoma longifolia* Jack on masculine copulatory behaviour were studied in the middle aged male Sprague-Dawley rats, 9 months old and retired breeders after dosing them with 500 mg/kg twice daily for 10 days prior to test. The test lasted for 30 minutes after a 20 minute adaptation period, was carried out on the 11th day during the dark phase of the light-dark cycle (2000-0700 hours) and in subdued light, using a modified copulation cage but with the presence of a piece of mirror of appropriate size to facilitate observation. Results showed that the mean values of EL-1, EL-2 and EL-3 of the control middle aged male rats were 103.20 sec, 91.21 sec and 80.00 sec but were significantly ($p<0.05$) increased to 118.40-120.20 sec, 101.24-171.28 sec and 100.42-110.21 sec respectively in the methanol-chloroform, methanol-butanol-water and methanol-butanol treated middle aged male rats. However, further results also showed that PEI-1 and PEI-2 of the control middle aged male rats were 182.30 sec and 257.2 sec but were significantly ($p<0.05$) decreased to 100.42-121.31 sec and 40.21-132.31 sec respectively in the methanol-chloroform-butanol-water and methanol-butanol treated middle aged male rats. In conclusion, this study showed that although *E. longifolia* Jack continued to enhance the sexual activity of the middle aged male rats by extending the duration of coitus and decreasing the refractory period between the different series of copulation, but to a smaller degree as compared to sexually active, adult male rats (Ang and Sim, 1997).

Keywords – *E. longifolia* Jack; Copulatory behaviour; Middle aged male rats; Increased the duration of coitus; Decreased the refractory period; Smaller degree

Introduction

Eurycoma longifolia Jack, from the Simaroubaceae family, is identified as 'Tongkat Ali' or Ali's walking stick in Malaysia. It is a symbol of man's ego and strength because 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. Thus, this has enabled the plant to capture the Malaysian market and currently, there are about 200 products, either in single or combined preparations, most of them highlighting the aphrodisiac property (Jagananth and Ng, 2000).

Over the years pharmacological evaluations on this plant showed that it exhibited antimalarial (Ang *et al.*, 1995, 1995a; 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, canthine-6-one and β-carboline alkaloids.

Hence, in this paper, we continue further our investigation on the effects of *E. longifolia* Jack pertaining to masculine copulatory behaviour in middle aged male rats.

Materials and Methods

Animals and surgery – Adult middle aged male Sprague-Dawley rats, 9 months old and retired breeders, were used as experimental subjects. They were housed individually in a standard wire mesh cage in animal house under conditions of controlled temperature of 26±2°C and relative humidity of around 70±5%, with commercial diet and water *ad libitum*.

Female rats used as mating stimuli were made receptive following the methods previously described (Meyerson and Lindstrom, 1971, 1973).

Plant materials – *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.

*Author for correspondence, E-mail: hhang@usm.my

Extraction – 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) which were brown and blackish-brown masses respectively after solvent evaporation. The aqueous layer was then extracted with *n*-butanol (0.45% w/w) and later evaporated to dryness to produce a golden yellow residue. Phytochemical screening (Farnsworth, 1966) carried on these fractions gave positive tests, with different intensities, only for alkaloids, lactones and phenolics.

When required, test compounds were given twice daily at 0800 and 1600 hours with an appropriate oral needle for 10 days prior to test. Each male rat in the respective groups received 500 mg/kg of one of the following fractions chloroform, methanol, water and *n*-butanol whilst the control group received 3 ml/kg of normal saline. Vehicles used were propylene glycol and distilled water for chloroform and non-chloroform fractions respectively.

Copulatory behaviour test – The copulatory behaviour test was carried out following previously described method (Ang and Sim, 1997) and was performed in a modified copulation cage (Mendelson and Gorzalka, 1987) but with the presence of a piece of mirror of appropriate size to facilitate observation. This observation, lasted for 30 minutes after a 20 minute adaptation period, was carried out during the dark phase of the light-dark cycle (2000-0700 hours) and in subdued light.

The normal copulatory behaviour of the male rats consists of bouts or series of mounts (without intromission) and vaginal intromissions, each complete series terminated by an ejaculation (Sachs and Barfield, 1970). In this study, ejaculation latency (EL) which is defined as the period from the first intromission of a series until the ejaculation which terminates the series and postejaculatory interval (PEI) which is defined as the period from the occurence of ejaculation until the initiation of a new

Table 1. Effects of *E. longifolia* Jack (500 mg/kg; p.o) on mean latency of copulatory behaviour in rats

	Sexually active adult male rats (Ang and Sim, 1997)					
	EL-1[a]	% ▲ [b]	EL-2[a]	%▲ [b]	EL-3[a]	% ▲ [b]
Normal saline (control)	192.80±5.60	–	167.60±9.72	–	162.50±4.92	–
E. longifolia Jack fractions						
Methanol	292.60±3.60	+51.8	242.60±2.06	+44.7	262.00±8.50	+61.2
Chloroform	249.00±3.67	+29.1	56.20±2.00	–66.5	94.00±4.12[c]	–42.2
Butanol	82.00±7.55	–57.5	255.80±1.02	+52.6	210.50±2.45	+29.5
Water	60.00±1.90	–68.9	364.40±9.52	+117.4	92.80±9.57[c]	–42.9
	Middle aged male rats, 9 months old and retired breeders					
	EL-1[a]	% ▲ [b]	EL-2[a]	% ▲ [b]	EL-3[a]	% ▲ [b]
Normal saline (control)	103.20±8.72	–	91.21±8.23	–	80.00±5.31[d]	–
E. longifolia Jack fractions						
Methanol	118.40±2.15	+14.7	101.24±1.45	+11.0	110.21±2.53	+37.8
Chloroform	120.20±1.50	+16.5	40.24±2.15	–55.8	75.21±1.28	–6.00
Butanol	75.20±1.20	–27.1	123.51±1.25	+35.4	100.42±2.31	+25.5
Water	54.31±1.40	–47.4	171.28±3.15	+87.8	80.00±4.32[d]	0.00

[a]Readings expressed as mean latency ± s.e.m. (sec); [b]Percentage difference when compared to the controls on each parameter; $n_{each\ group}$=20; NS $p>0.05$ for [c] and [d] on EL-3; S $p<0.05$ for comparisons for all test compounds on each parameter.

Table 2. Effects of *E. longifolia* Jack (500 mg/kg; p.o) on mean interval of copulatory behaviour in rats

	Sexually active adult male rats (Ang and Sim, 1997)				Middle aged male rats, 9 months old and retired breeders			
	PEL-1[e]	% ▲ [b]	PEL-2[e]	%▲ [b]	PEL-1[e]	% ▲ [b]	PEL-2[e]	%▲ [b]
Normal saline (control)	139.60±8.44	–	215.00±9.23	–	182.30±1.23	–	257.20±2.34	–
E. longifolia Jack fractions								
Methanol	61.20±4.63	–56.2	121.67±8.50	–43.4	102.30±2.31[h]	–43.9	132.31±1.21	–48.6
Chloroform	56.20±2.04	–59.7	244.00±4.23	+13.5	100.42±1.41[h]	–44.9	266.31±2.31	+3.54
Butanol	73.80±9.75[g]	–47.1	20.00±4.54	–90.7	121.31±2.91[i]	–33.5	40.21±0.43	–84.4
Water	72.00±4.35[g]	–48.4	287.6±5.87	+33.8	120.42±1.41[i]	–33.9	293.41±1.42	+14.1

[e]Readings expressed as mean interval ± s.e.m. (sec); [b]Percentage difference when compared to the controls on each parameter; $n_{each\ group}$=20; NS $p>0.05$ for [g,h] and [i] on PEI-1; S $p<0.05$ for comparisons for all test compounds on each parameter.

series, as indicated by the next intromission were considered. Individual series are designated by a hyphen and the appropriate number, eg. EL-1.

Statistical analysis – The values of the observed parameters of the treated and control male rodents 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

Tables 1 and 2 show the effects of different fractions of *E. longifolia* Jack and normal saline on mean latency and interval of copulatory behaviour in rats after treating them for 10 days. Table 1 shows that the mean values of EL-1, EL-2 and EL-3 of the control middle aged male rats were 103.20 sec, 91.21 sec and 80.00 sec but were significantly ($p<0.05$) increased to 118.40-120.00 sec, 101.24-171.28 sec and 110.42-110.21 sec respectively in the methanol-chloroform, methanol-butanol-water and methanol-butanol treated middle aged male rats. The increase in EL-1, EL-2 and EL-3 shows that *E. longifolia* Jack enhances the sexual activity of the middle aged male rats by extending the duration of coitus (Beach and Whalen, 1959; Ferrari *et al.*, 1985), similarly to previously reported but in sexually active, adult male rats (Ang and Sim, 1997). However, the above fractions managed to cause a minor increase, *viz.* of 14.7-16.5%, 11.0-87.8% and 25.5-37.8% in middle aged male rats in contrast to 29.1-51.8%, 44.7-117.4% and 29.5-61.2% in sexually active, adult male rats (Ang and Sim, 1997) during the observation period.

Besides these, Table 2 also shows that PEI-1 and PEI-2 of the control middle aged male rats were 182.30 sec and 257.2 sec but were significantly ($p<0.05$) decreased to 100.42-121.31 sec and 40.21-132.31 sec respectively in the methanol-chloroform-butanol-water and methanol-butanol treated middle aged male rats. The decrease in both PEI-1 and PEI-2 shows that *E. longifolia* Jack decreased the refractory period between the different series of copulation, similar to what was previously reported in sexually active, adult male rats (Ang and Sim, 1997). However, the above fractions managed to cause a minor decrease of 33.5-44.9% and 48.6-84.4% in middle aged male rats in contrast to 47.1-59.7% and 43.4-90.7% in sexually active, adult male rats (Ang and Sim, 1997) during the observation period.

Although the above study shows that different fractions of *E. longifolia* Jack continues to intensify the sexual activity of the middle aged male rats by extending the duration of coitus and decreasing the refractory period between the different series of copulation, it however, performs at a smaller degree in contrast to what was prefiously reported in sexually active, adult male rats (Ang and Sim, 1997). As such, it is suggested that further studies should be carried out in sexually sluggish, old male rats to further investigate the above matter.

References

Ang, H.H., Chan, K.L., and Mak, J.W., *In Vitro* Antimalarial Activity of Quassinoids from *Eurycoma longifolia* against Malaysian Chloroquine-Resistant *Plasmodium falciparum* Isolates. *Planta Med.* **61**, 177-178 (1995).

Ang, H.H., Chan, K.L., and Mak, J.W., Effect of 7-day Daily Replacement with Culture Medium Containing *Eurycoma longifolia* Jack Constituents on Malaysian *Plasmodium falciparum* Isolates. *J. Ethnopharmacol.* **49**, 171-175 (1995a).

Ang, H.H. and Sim, M.K., Effects of *Eurycoma longifolia* Jack on Sexual Behaviour of Male Rats. *Arch. Pharm. Res.*, **20**, 656-658 (1997).

Beach, E.A. and Whalen, R.E., Effect of Ejaculation on Sexual Behaviour in the Male Rat. *J. Comp. Physiol. Psychol.* **52**, 249-252 (1959).

Chan, K.L., O'Neill, M.J., Phillipson, J.D., and Warhurst, D.C., Plants as Sources of Antimalarial Drugs. Part 3. *Eurycoma longifolia* Jack. *Planta Med.* **52**, 105-107 (1986).

Chan, K.L., Lee, S.P., Sam, T.W., and Han, B.H., A Quassinoid Glycoside from the Roots of *Eurycoma longifolia. Phytochem.* **28**, 2857-2859 (1989).

Chan, K.L., Lee, S.P., and Yuen, K.H., Antipyretic Activity of Quassinoids from *Eurycoma longifolia* Jack. Paper presented at the *11th Chemical Seminar on Natural Products*, 25-28 June, 1995, UNIMAS, Sarawak, Malaysia, Proceedings pp. 197-204.

Farnsworth, N.R., Biological and Phytochemical Screening of Plants. *J. Pharm. Sci.*, **55**, 225-276 (1966).

Ferrari, F., Baggio, G., and Mangiafico, V., The Dopamine Autoreceptor Agonist B-HT 920 Markedly Stimulates Sexual Behaviour in Male Rats. *Experientia* **41**, 636-638 (1985).

Gimlette, J.D. and Thomson, J.W. (eds.), *A Dictionary of Malayan Medicine*, Oxford University Press, Kuala Lumpur, 1977, pp. 183.

Goh, S.H., Chuah, C.H., Mok, J.S.L., and Soepadmo, E., *Malaysian Medicinal Plants for the Treatment of Cardiovascular Diseases*, Pelanduk Publication Sdn Bhd, Selangor, 1995, pp. 95-96.

Itokawa, H., Kishi, E., Morita, H., and Takeya, K., Cytotoxic Quassinoids and Tirucallane-Type Triterpenes from the Woods of *Eurycoma longifolia. Chem. Pharm. Bull.* **40**, 1053-1055 (1992).

Itokawa, H., Oin, X.R., Morita, H., Takeya, K., and Iitaka, Y., Nevel Quassinoids from *Eurycoma longifolia. Chem. Pharm. Bull.* **41**, 403-405 (1993).

Jagananth, J.B. and Ng, L.T., *Herbs the Green Pharmacy of Malaysia*, Vinpress Sdn. Bhd. and Malaysian Agricultural Research and Development Institute (MARDI), Kuala Lumpur, Malaysia, 2000, pp. 45-46.

Kardono, L.B.S., Angerhofer, C.K., Tsauri, S., Padmawinata, K., Pezzuto, J.M., and Kinghorn, D., Cytotoxic and Antimalarial Constituents of the Roots of *Eurycoma longifolia. J. Nat. Prod.* **54**, 1360-1367 (1991).

Mendelson, S.D. and Gorzalka, B.B., An Improved Chamber for the Observation and Analysis of the Sexual Behavior of the Female Rat. *Physiol. Behav.* **39**, 67-71 (1987).

Meyerson, B.J. and Lindstrom, L., In: *Hormonal Steroids James*, V.H.T. and Martini, L. (eds.). Excerpta Medical International Congress, Serial, no. 219 (1971).

Meyerson, B.J. and Lindstrom, L., Sexual Motivation in the Female Rat. *Act. Physiol. Scand.* Suppl 389, 1-80 (1973).

Morita, H., Kishi, E., Takeya, K., Itokawa, H., and Iitaka, Y., New Quassinoids from the Roots of *Eurycoma longifolia. Chem. Lett.* **5**, 749-752 (1990).

Morita, H., Kishi, E., Takeya, K., Itokawa, H., and Iitaka, Y., Squalene Derivatives from *Eurycoma longifolia. Phytochem.* **34**, 765-771 (1993).

Sachs, B.D. and Barfield, R.J., Temporal Patterning of Sexual Behaviour in the Male Rat. *J. Comp. Physiol. Psychol.* **73**, 359-364 (1970).

Schefler, W.C., *Statistics for Health Professionals*, Addison-Wesley Publishing Company, Inc., Reading, Massachusetts, 1984, pp. 251-254.

Tada, H., Yasuda, F., Otani, K., Doteuchi, M., Ishihara, Y., and Shiro, M., New Antiulcer Quassinoids from *Eurycoma longifolia. Eur. J. Med. Chem.* **26**, 345-349 (1993).

(Accepted January 15, 2002)