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THE EFFECT OF *EURYCOMA LONGIFOLIA* JACK (TONGKAT ALI) ON SEXUAL BEHAVIOUR AND SPERM QUALITY IN RATS

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Eurycoma longifolia (Tongkat Ali) has a lot of benefits and it has been used in traditional medicine to cure many diseases. The objective of this research was to study the effects of its aqueous extract at four different doses; low (30 mg/kg body weight), moderate (60 mg/kg body weight), high (90 mg/kg body weight) and very high (150 mg/kg body weight) doses respectively on sexual behaviour of male rats and the sperm quality. Control group was treated with normal saline. All treatments were by force feeding. Sexual behaviour study was carried out by sexual attraction test, while parameters analysis include sperm count, motility, viability and morphology. The sexual study showed that the E. longifolia water extract increased the sexual activities in the treated rats. From the sperm quality analysis, the treated rats showed an increase in sperm count, motility and viability. The increase in sexual activities and sperm quality were found to be dose dependent. The treatment, however, did not show any effect on the sperm morphology. Therefore, the above study further supports previous findings that E. longifolia can increase sexual behaviour of male rats and the sperm quality.

Keywords: *E. longifolia, Sexual activity, Sperm quality*

INTRODUCTION

Eurycoma longifolia Jack is a plant from the family of Simaroubaceae, known locally as "Tongkat Ali". In South East Asia, all parts of the plant, in particular the roots have long been used medicinally (Jaganath and Ng 2000). People in some regions of Sumatra and Kalimantan, Indonesia use the roots as anti-pyretic (Chan *et al.* 1995). In the Malay Peninsula and Thailand, the roots are used to cure fever, ulcers in the mouth, and intestinal worm (Satayavivad *et al.* 1998; Jaganath and Ng 2000). It is also used as a tonic after childbirth. However, locally, this plant owes its popularity to its aphrodisiac effect. Several studies have been done on the aphrodisiac properties of this plant (Ang *et al.* 1995; Ang and Sim 1998). In this paper, several sperm quality parameters such as sperm count, motility, mortality and morphology will also be determined. We hope both

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data, aphrodisiac and sperm quality analysis, can be used to further strengthen our belief that *E. longifolia* root extract can be used to enhance the male reproductive system.

METHODS

Animals

Adult Sprague-Dawley male rats (250–350 gram), fed standard laboratory food and tap water ad libitum were used in this study. They were kept under standard conditions, on constant dark-light schedule (light 0700-1900 h). Thirty male and three female rats were used in this experiment. All males were divided into five groups including control with 6 rats per group. The groups were respectively fed with 30 mg/kg, 60 mg/kg, 90 mg/kg and 150 mg/kg water extracts of *E. longifolia* roots, and 3 ml/kg normal saline (for control group) for 28 days. These doses were chosen based on a rat sexual behaviour study by Murphy *et al.* (1998) on American ginseng *(Panax quinquefolium).*

Test Compound

Water extract of *E. longifolia* roots was supplied by FRIM (Forest Research Institute Malaysia). Extract solution was prepared by dissolving the extract powder at certain amount accordingly in 3 ml normal saline, respectively. The extracts were given once daily using appropriate oral needle for 28 days prior to test (Murphy *et al.* 1998). Each male rat in the respective groups received 30, 60, 90 and 150 mg/kg of the extracts, whilst the control group received 3 ml/kg normal saline.

Aphrodisiac Activities

These tests were performed between 10.00 to 13.00 h weekly. Two hours prior to the experiment, the animals were weighed, transferred to a dark room, put in an individual cage, and left for at least one hour before the commencement of the sexual behaviour study. Orientation activities of the male rats toward the receptive females were observed according to the method previously described by Meyerson *et al.* (1973).

Female rats used as mating stimuli were brought to heat with a single intramuscular injection of 0.125 mg/kg estradiol benzoate (Sigma

Chemical, USA) and 1.25 mg/kg of progesterone (Sigma Chemical, USA), 48 hours and 4 hours before testing, respectively. 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.

Sexual behavioural test was conducted by depositing a treated male rat in a cage, which led to a choice selection with two doors at the far end. Each door led into a separate goal cage. The receptive female was placed in one of the goal cages. The male rat was expected to reach receptive female within 3 min. The time that the male started to mount the receptive female was recorded and considered as positive sexual behaviour. Males which failed to respond within 3 min after being placed in the cage were considered as having negative sexual behaviour.

Sperm Analysis

The males were killed by an overdose of sodium pentobarbital (i.p) on day 29, after the fourth sexual behaviour test. Sperm from the cauda epididymis were collected in the Biggers, Whitten and Whittingham (BWW) medium (Biggers *et al.* 1971) as described by Ellis *et al.* (1985). The sperm sample preparation was then used for the following analysis. Sperm count, motility or progression, viability and morphology were determined according to the WHO criteria (WHO 2000). Sperm morphology was assessed by haematoxylin and eosin (H & E) staining method (Humason 1979).

Statistical Analysis

One way analysis of variance (ANOVA) test was employed for statistical comparison and $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Effect of the Water Extract of *E. longifolia* on Aphrodisiac Activity

Table 1 shows the aphrodisiac response of the male rats after consuming certain doses of water extract of *E. longifolia* (for treated group) and 3 ml/kg of normal saline (for control group). The extract (at 150 mg/kg) treated male rats displayed the shortest period of time to reach the female

as compared to other treated (30 mg/kg) and control group ($p < 0.05$) (Table 1). The pattern can be seen from the first week of treatment. The aphrodisiac activity, however, was not significant in animals treated with 60 and 90 mg/kg ($p > 0.05$). Overall results showed that this sexual behaviour was dose dependent manner. The present investigation reveals that the water extract of Tongkat Ali can enhance male sexual behaviour.

Generally, sexual behaviours are enhanced by elevated testosterone levels (Subramoniam *et al.* 1997). Aphrodisiac activity measured in this study suggests that the *E. longifolia* affect testosterone production and the sensitivity of the target organs to the hormone. This belief is further supported by the results from sperm analysis (sperm count).

Table 1: Mean of time for males (n = 6) to reach and subsequently mount the receptive female after being treated with E. longifolia extract at different doses.

Dose (mg/kg)	Mean time ± SE (sec)			
	Weeks			
	1	2	3	4
Control	NR	NR	NR	NR
30	NR	188 ± 56	182 ± 48	179 ± 13 [a]
60	NR	242 ± 47	169 ± 54	166 ± 26 [ab]
90	NR	227 ± 33	158 ± 40	156 ± 19 [ab]
150	332 ± 53	168 ± 30	123 ± 40	144 ± 16 [b]

NR No response even at 360 sec.
[a,b] Values with different superscipts within columns are significantly different (ANOVA, $p < 0.05$)

Effect of the Water Extract of *E. longifolia* on Sperm Quality

Oral administration of the water extract of *E. longifolia* resulted a significant increased ($p < 0.05$) in the sperm count and viability. This was clearly showed in Table 2 when comparing between rats treated with 150 mg/kg and control, as well as the other treated groups. The mean sperm count measured in rats treated with 150 mg/kg was $43.33 \times 10^6 \pm 6.09$, approximately 1.5 times higher than sperm count in rats treated with 30 mg/kg, and two times higher than control group during 28 days

treatment (Table 2). The increase in sperm count indicates the effect of *E. longifolia* on testicular spermatogenesis. Testicular histology studies are underway to verify this conclusion.

Sperm viability was also increased with *E. longifolia* doses. For example, at lower dose (30 mg/kg), the viability was 46.33% ± 11.50; and at higher dose (150 mg/kg), the viability was 53.47%. Similarly to the sperm count parameter, result in the sperm viability between 30 mg/kg, 60 mg/kg and 90 mg/kg was not significant ($p > 0.05$) (Table 2). Sperm morphology by H & E staining revealed that the rats from treated and non-treated groups are having normal sperm morphology (data not shown).

Table 2: Sperm characteristics (count and viability) of rats after 28 days treatment with water extract of *E. longifolia*. (n = 6/group; $p < 0.05$).

Parameters	Control	Dose (mg/kg)			
		30	60	90	150
Sperm count ($x10^6$)	22.50 ± 2.43^c	29.17 ± 3.75^b	30.50 ± 6.06^b	33.67 ± 5.92^b	43.33 ± 6.09^a
Viability (%)	20.23 ± 2.16^c	46.33 ± 11.50^b	48.81 ± 9.59^b	48.34 ± 0.33^b	53.47 ± 9.63^a

^{a,b,c} Values with different superscipts within rows are significantly different (ANOVA, $p < 0.05$)

The effects of *E. longifolia* on sperm motility (progression) are showed in Table 3. There was significant difference between 150 mg/kg and 30 mg/kg *E. longifolia* on the grade of sperm motility during 28 days treatment. At higher dose (150 mg/kg) and 90 mg/kg, 100% of sperm displayed movement grade 'a', whilst at lower dose (30 mg/kg) only 16.66% displayed grade 'a' and while 83.33% displayed grade 'b'. At 60 mg/kg the sperm exhibited equal percentage (50%: 50%) in grade 'a' and 'b'. The increase in grade of sperm motility with the *E. longifolia* doses in this study suggests that the *E. longifolia* also affected sperm maturation process in epididymis. The body weight of the control and treated animals did not show significant changes throughout the course of the investigation (data not shown).

In this study the doses and period of time of *E. longifolia* treatment were chosen based on Murphy *et al.* (1998) and Narayana *et al.* (2002). According to Narayana *et al.* (2002), rat sperm cytotoxicity only increased linearly with increasing concentrations of drug starting from 28 days of administration. However, in this study, the effects of *E. longifolia* were dose dependent only at lower doses (< 30 mg/kg) and showed plateau effect at high doses (> 30 mg/kg).

In general, results of the present study indicated that *E. longifolia* roots extract enhanced both the sexual behaviour and the sperm quality in rats. Although extrapolations from observations made on experimental animals to man has limitations, they can sometimes be indicative. Therefore, further work is necessary to determine whether the present results obtained in rats may apply to man. Furthermore, the effect of *E. longifolia* on fertilizability of the sperm is also need to be investigated. Experiment on sperm functional study has to be carried out to determine if *E. longifolia* may improved or increased fertilization rate. Sperm penetration assay (SPA) using zona free hamster oocytes can be applied to resolve the problem. Freeman *et al.* (2001) have shown that the SPA is a sensitive screening tool for the prediction of fertilizability than sperm analysis (SA) alone. Margalioth *et al.* (1989) demonstrated that the specificity and positive predictive values of the SPA were higher than the SA (77% vs 57%). Therefore, SPA should be applied to address this issue.

Table 3: Sperm characteristics (grade of motility) of rats after 28 days treatment with water extract of *E. longifolia*

Dose (mg/kg)	Grade of motility (a-d)					
	Individual rat					
	1	2	3	4	5	6
Control	c	b	b	c	b	b
30	b	b	b	b	a	b
60	b	a	b	a	b	a
90	a	a	a	a	a	a
150	a	a	a	a	a	a

Notes for the grade of motility WHO (2000).

a – rapid progressive motility (≥ 25 μm/s at 37°C and ≥ 20 μm/s at 20°C)

b – slow or sluggish progressive motility

c – non-progressive motility

d – immotility

CONCLUSION

In conclusion, the present study supports the traditional use of this plant as an aphrodisiac agent. Furthermore, observations on sperm characteristics, i.e., count, viability, motility and morphology made in this study revealed the advantages for male in consuming this plant extract. However, further studies should be conducted to investigate if it also gives similar effect in human system.

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