Studies on the Anxiolytic Activity of *Eurycoma longifolia* Jack Roots in Mice

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ABSTRACT—The anxiolytic effect of *Eurycoma longifolia* Jack in mice was examined. Fractions of *E. longifolia* Jack extract produced a significant increase in the number of squares crossed (controls=118.2 ±10.2 squares), but significantly decreased both the immobility (controls=39.4±4.0 sec) and fecal pellets (controls=12.3±2.1 fecal pellets) when compared with control mice in the open-field test; they significantly increased the number of entries (controls=6.7±0.5 entries) and time spent (controls=42.9±0.1 sec) in the open arms, but decreased both the number of entries (controls=13.2±0.7 entries) and time spent (controls=193.4±0.7 sec) when compared with the control mice in the closed arms of the elevated plus-maze test. Furthermore, fractions of *E. longifolia* Jack extract decreased the fighting episodes significantly (controls=18.0±0.4 fighting episodes) when compared with control mice. In addition, these results were found to be consistent with anxiolytic effect produced by diazepam. Hence, this study supports the medicinal use of this plant for anxiety therapy.

Keywords: Open-field test, Elevated plus-maze test, Anti-fighting effect

Eurycoma longifolia Jack, from the Simaroubaceae family and identified locally as '*Tongkat Ali*', is found in primary and secondary, evergreen and mixed deciduous forests in Burma, Indochina, Thailand, Malaysia, Sumatra, Borneo and the Philippines. It is popularly sought after as a singly or an essential component for the treatment of fevers, aches, sexual insufficiency and also as health supplements, but has not been indicated strongly for any specific illness. However, traditional medicinal users usually take a decoction of the roots in water as a health tonic and antistress remedy.

As such, the antistress effect of *E. longifolia* Jack may account for its observed efficacy in stress-related disorders like ulcer (1). Other documentated medicinal uses of this plant are based on its antimalarial (2–6) and cytotoxic (4, 7–9) 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 investigated the antianxiety effect of various fractions of *E. longifolia* Jack using the open-field, elevated plus-maze and antifighting tests.

Inbred adult albino mice (35–40 g) were used as experimental subjects. They were housed individually in a standard wire-mesh cage in an animal house under conditions of controlled temperature of 26±2℃ and a relative humidity of around 70±5%, with commercial diet and water ad libitum.

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 methanol extract was then evaporated to dryness under reduced pressure to give a thick dark brown viscous residue (yield: 3%). It was then partitioned between chloroform and water (2:1) to yield the chloroform extract (yield: 0.1% w/w) and the aqueous layer (yield: 0.5% w/w) which were brown and blackish-brown masses, respectively, after solvent evaporation. The aqueous extract was further extracted with *n*-butanol and later evaporated to dryness to produce a golden yellow residue (yield: 0.45% w/w). The resulting four fractions of different polarities were then stored in a refrigerator at 4℃. Phytochemical screening carried out on these fractions produced positive tests with different intensities, only for alkaloids, lactones and phenolics.

During the tests, these compounds were given twice daily using an appropriate oral needle for 5 days. The

mice were then tested 4 hr after the final administration of the test compounds. Vehicles used were propylene glycol and distilled water for chloroform and non-chloroform fractions, respectively. Each animal in the respective experimental groups received 0.3 g/kg of one of the following fractions: chloroform, methanol, water and *n*-butanol and the control group received 3 ml/kg of saline. Diazepam (1 mg/kg, i.p.) was used as the standard anxiolytic agent (10) and was administered 15 min before experimentation.

The open-field test is considered to be an indicator of the emotional state of the test animals. This test was performed at a low noise behavioral room, with subdued light in a quiet room but adequate ventilation for at least 1 hr before the experiment. They were tested by visual observation for anxiolytic activity in the above conducive environment. After oral administration of *E. longifolia* Jack, each mouse was put in a corner of the open-field with dimensions of 92 cm × 92 cm with 16 squares division and observed for 15 min in three 5-min periods (11). During the test period, the number of squares crossed, the time spent in total immobility (freezing) and number of fecal pellets were recorded. Between tests, the apparatus was thoroughly cleaned.

The elevated plus-maze test is a rapid and selective technique. It is capable of detecting anxiolytic and anxiogenic drug effects under identical conditions. The plus-maze is in the shape of a cross or plus with two closed arms, measuring 25 × 5 cm, running along a north-south axis and two open arms (similar dimensions with the closed arms) running east-west. The height of the apparatus is 30 cm and was elevated 25 cm from the floor in a dimly illuminated room.

Male mice were placed individually in the center of the maze, facing an enclosed arm, and the time spent on the open and closed arms were recorded during the next 5 min (11). An arm entry was defined as all four feet in the arm. The apparatus was cleaned after each use.

Pairs of male mice were placed under a glass beaker on a grid constructed of stainless steel rods. Footshocks of 2-mA intensity were delivered for 3 min, and the frequency of fighting episodes was noted. Mice that showed 5 or more fighting episodes were selected for this study. The mice pairs were re-tested after drug treatments, and the fighting episodes were recorded during the 3-min observation period (12).

Results 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 (13).

Table 1 shows the effects of normal saline, diazepam and fractions of *E. longifolia* Jack extract on the open-field test in mice. Results showed that mice administered with 3 ml/kg of normal saline and 1 mg/kg of diazepam exhibited crossings of 118.2±10.2 and 170.3±6.3 squares, immobility of 39.4±4.0 and 10.2±3.1 sec, 12.3±2.1 and 2.3±1.5 fecal pellets, respectively, in the open-field test. Results also indicated that fractions of *E. longifolia* Jack extract significantly increased the number of squares crossed, 145.3−152.2 squares, but also significantly decreased immobility, 12.3−14.3 sec, and fecal pellets, 3.3−4.0 fecal pellets, when compared with controls ($P<0.05$) in the open-field test.

Table 1. Effects of diazepam and *E. longifolia* Jack on the open-field test in mice

Treatment (dose)	Squares crossed (n)	Immobility (sec)	Fecal pellets (n)
Control (3 ml/kg)	118.2±10.2	39.4±4.0	12.3±2.1
Diazepam (1 mg/kg)	170.3±6.3*	10.2±3.1*	2.3±1.5*
E. longifolia Jack fractions			
Chloroform (0.3 g/kg)	145.3±2.3*	13.4±2.3*	3.3±0.9*
Methanol (0.3 g/kg)	152.2±2.1*	12.3±1.8*	3.9±0.2*
Water (0.3 g/kg)	148.4±2.6*	14.3±1.3*	3.4±1.2*
Butanol (0.3 g/kg)	151.3±1.9*	12.3±0.6*	4.0±1.2*

Values represent the mean±S.E.M.; number of mice used in each group=20; *$P<0.05$, compared with the control in each of the parameters tested.

Table 2 shows that mice administered with normal saline and diazepam made 6.7±0.5 and 15.3±0.7 entries and also spent 42.9±0.1 and 99.7±0.7 sec, respectively, in the open arms of the elevated plus-maze test. In addition, fractions of *E. longifolia* Jack extract significantly increased the number of entries, 12.2−13.1 entries, and also time spent, 96.2−97.3 sec, in the open arms when compared with controls ($P<0.05$) in the similar test. In contrast, control and diazepam-treated mice made 13.2±0.7 and 5.5±0.5 entries and spent 193.4±0.7 and 110.2±0.5 sec, respectively, in the closed arms of the similar test. In addition, fractions of *E. longifolia* Jack extract significantly decreased both the number of entries, 5.8−6.1 entries, and time spent, 120.3−124.5 sec, in the closed arms when compared with controls ($P<0.05$) in the elevated plus-maze test.

Table 3 shows that normal saline and diazepam treated rats produced 18.0±0.4 and 3.5±0.2 fighting episodes respectively. Results also indicated that fractions of *E. longifolia* extract significantly inhibited the fighting behavior, 5.3−6.2 fighting episodes, when compared with controls ($P<0.05$) in the antifighting test.

Various behavioral tests have been used to measure anxiolytic activity. In the open-field test, where animals are taken from their home cage and placed in an unfamiliar environment, normally they showed anxiety and

Table 2. Effects of diazepam and *E. longifolia* Jack on the elevated plus-maze test in mice

Treatment (dose)	Number of entries (n)		Time spent (sec)	
	open arms	closed arms	open arms	closed arms
Control (3 ml/kg)	6.7±0.5	13.2±0.7	42.9±0.1	193.4±0.7
Diazepam (1 mg/kg)	15.3±0.7*	5.5±0.5*	99.7±0.7*	110.2±0.5*
E. longifolia Jack fractions				
Chloroform (0.3 g/kg)	12.3±0.1*	5.8±0.3*	96.3±0.2*	124.5±0.4*
Methanol (0.3 g/kg)	13.1±0.2*	6.1±0.4*	97.3±0.5*	120.3±0.2*
Water (0.3 g/kg)	12.5±0.2*	5.9±0.1*	96.2±0.2*	121.1±0.4*
Butanol (0.3 g/kg)	12.2±0.4*	5.8±0.4*	96.4±0.2*	123.4±0.2*

Values represent the mean±S.E.M.; number of mice used in each group=20; *$P<0.05$, compared with the control in each of the parameters tested.

Table 3. Effects of diazepam and *E. longifolia* Jack on fighting episodes in mice

Treatment (dose)	Fighting episodes (n)
Control (3 ml/kg)	18.0±0.4
Diazepam (1 mg/kg)	3.5±0.2*
E. longifolia Jack fractions	
Chloroform (0.3 g/kg)	6.2±0.1*
Methanol (0.3 g/kg)	5.6±0.2*
Water (0.3 g/kg)	5.3±0.4*
Butanol (0.3 g/kg)	5.9±0.3*

Values represent the mean±S.E.M.; number of mice used in each group=20; *$P<0.05$, compared with the control.

fear by remaining immobile, decreasing ambulation, exploration and freezing, but increasing defecation due to heightened autonomic anxiety. This phenomena will be attenuated by classical anxiolytics such as diazepam that is used in this study but augmented by anxiogenic agents (14).

Likewise, the elevated plus-maze is another widely-used test model. Normally, mice spend most of their time in the closed arms and avoided the open arms (afraid, possibly, of falling off). As such, administration of anxiolytic drugs such as diazepam increases the time spent in the open arms and also increases the mobility as evidenced by the frequency of crossing the transection (15) as compared to the controls. Unlike both the above experimental test models that are dependent upon fear and anxiety, the fighting behavior in paired rodents represents hyperemotionality and inherent aggressive behavior.

In general, it is found that not much difference was obtained after dosing the mice with various fractions of *E. longifolia* Jack, and this may be attributed to the presence of active components in more than one fraction. In conclusion, this study shows that *E. longifolia* Jack attenuated anxiety parameters in the open-field and plus-maze tests and also inhibited footshock-induced fighting behavior, thus further supporting the medicinal use of this plant.

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