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Influence of *Eurycoma longifolia* on the copulatory activity of sexually sluggish and impotent male rats

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ABSTRACT

Aim of the study: The root of *Eurycoma longifolia* Jack, native to South East Asia, has long been used as a male aphrodisiac remedy to treat sexual disorders.

In the study we evaluated the influence of *Eurycoma longifolia* Jack on sexual behavior (including both motivation and copulatory performance) of sexually sluggish and impotent male rats.

Materials and methods: The root powder of the plant was orally administered to adult Sprague–Dawley male rats, classified as sexually sluggish or impotent taking in account their behavior in pre-experimental tests. Groups of 8 animals each were submitted to three different types of treatment: (1) acute at 3 dose levels (250, 500 and 1000 mg/kg); (2) subacute (daily for 6 days) at the dose of 500 mg/kg and (3) subchronic (daily for 12 days) at the same dose (500 mg/kg). Mount, intromission and ejaculation latencies and post-ejaculatory interval were recorded during the mating test in order to evaluate sexual performance. In addition the partner preference test was used to assess sexual motivation. Testosterone serum levels were measured in subacutely treated rats and compared with the values of controls receiving vehicle.

Results: Concerning the copulatory activity of sexually sluggish rats, both acute (dosed at 500 and 1000 mg/kg) and subacute treatments with the root powder significantly reduced ejaculation latencies, increasing also the percentage of mounting and ejaculating animals; in addition the subacute administration reduced post-ejaculatory interval. In impotent rats both subacute and subchronic treatments increased the percentage of mounting and ejaculating rats. The motivational behavior of sluggish rats during the partner preference test was not affected by the treatments. Testosterone serum levels were increased in rats subacutely treated in comparison with controls.

Conclusion: Eurycoma longifolia root improved sexual performance but not motivation in sluggish rats after acute or subacute administration. The effect could be mainly ascribed to increased testosterone levels.

1. Introduction

Eurycoma longifolia Jack (Simaroubaceae family), locally known as "Tongkat Ali", is a small evergreen shrub tree commonly found in the tropical forests of South East Asia (Indonesia, Thailand, Malaysia and the Philippines). It is a dioecious plant, with male and female flowers produced in large panicles, on different trees. The pinnate leaves, 20–40 cm long with ovate–lanceolate leaflets, are spirally arranged. The fruit is ovoid, 1–2 cm long and 0.5–1 cm broad; its colour moves from green to blackish-red when it ripes. Phytochemical studies on this plant revealed the presence of various quassinoids, squalene derivatives, biphenylneolignans, tirucallane-type triterpenes, canthine-6-one and β-carboline alkaloids (Chan et al., 1989; Itokawa et al., 1992; Ang et al., 2000b). In South East Asia all the parts of *Eurycoma longifolia*, in particular the roots, have long been used medicinally for the treatment of different illness such as fever, intestinal worms, mouth ulcers, headache and many other general pains (Perry and Metzger, 1980). A tea prepared by cooking 20–50 g of roots for about half an hour is commonly used as a health tonic and antistress remedy, so that the plant is also called "Malaysian ginseng". The antianxiety effect, as well as the antiulcer, antidiabetic, antimalarial and cytotoxic activities was demonstrated by pharmacological studies (Kardono et al., 1991; Tada et al., 1991; Ang and Cheang, 1999a; Husen et al., 2004). In Malaysia the plant represents a traditional remedy for preventing or treating erectile dysfunction in men (Gimlette and Thomson,

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1977; Ong and Nordiana, 1999; Low and Tan, 2007). There is no clinical evidence to support specific doses of *Eurycoma longifolia*; however a dosage of 100 mg/day of a water soluble extract of the plant was reported to have ergogenic effects in men after 5 weeks of supplementation (Hamzah and Yusof, 2003). Several experimental studies were performed in rodents showing the ability of *Eurycoma longifolia* to improve sexual behavior, but they were mostly carried out in sexually normal male rats (Ang and Sim, 1997), in sexually naïve male rats and mice (Ang and Sim, 1998a, 1998b), in middle-aged rats and mice (Ang and Cheang, 2002; Ang and Lee, 2002a; Ang et al., 2003a, 2003b). At our knowledge only one study was performed in sexually sluggish old rats, showing an increased number of yawning and stretching episodes (Ang et al., 2004). In addition non-copulator male rats were used to demonstrate a decreased hesitation time using an electrical copulation cage and a high level of intromissions during mating test (Ang and Sim, 1998c; Ang and Ngai, 2001). In our opinion the utilization of sluggish and impotent male rats, mimicking human sexual disorders, seems to represent the most suitable animal model for studying the pharmacological activity of an aphrodisiac remedy. In addition, the commercial interest in the products containing *Eurycoma longifolia* for the treatment of male sexual dysfunction is widespread in Malaysia and South East Asia, nevertheless clinical trials are still lacking. Therefore the aim of the present study was to accurately investigate the effect of the oral administration of *Eurycoma longifolia* root powder in sluggish and impotent rats, concerning: (1) the copulatory performance during the mating test; (2) the sexual motivation in the partner preference test and (3) testosterone serum levels.

2. Materials and methods

2.1. Animals

Sprague–Dawley rats of either sex, weighing from 160 g (females) to 220 g (males), were purchased from Harlan Laboratories (Udine, Italy). They were housed, males and females separately, in plexiglass cages, and were maintained under controlled laboratory conditions (22 ± 1 °C and 60% relative humidity) on a reversed 12 h light/dark cycle, with lights off at 9 a.m. Commercial rat pellets (Global Diet 2018, Mucedola s.r.l., Milan, Italy) and water were always available. The animals were accustomed to the housing conditions for at least 2 weeks before being used.

The females were ovariectomized under ketamine hydrochloride (Ketavet 100®, Farmaceutici Gellini, Latina, Italy) plus xylazine hydrochloride (Rompun®, Bayer AG, Leverkusen, Germany) anesthesia and brought into estrous by the administration of a single subcutaneous dose of 30 μg estradiol benzoate (Estradiolo AMSA, Roma, Italy) 48 h before the copulatory tests and 500 μg progesterone (Prontogest®, AMSA, Roma, Italy) 4 h before the copulatory tests. The females were screened with non-experimental sexually experienced males and only those exhibiting good sexual receptivity (solicitation behavior and lordosis in response to mounting) and no rejection behavior, were used.

Animal care, maintenance and surgery were conducted in accordance with the Italian law (D.L. no. 116/1992) and European legislation (EEC no. 86/609). The experimental design and procedures received the approval of the Bioethical Committee of the Italian National Institute of Health (Ministerial Decree 205/2008-B).

2.2. Treatments

Eurycoma longifolia root powder (identification batch no. 2800), supplied by Bioera S.p.a. (Cavriago, Reggio Emilia, Italy), was suspended in water by tragacanth gum and administered by oral gavage, in the volume of 5 ml/kg body weight, acutely at three dose levels (250, 500 and 1000 mg/kg), or daily at the dose of 500 mg/kg for 6 or 12 days. The mating test and partner preference test were carried out 45 min after the single dose or the last dose when repetitively administered. Control animals received vehicle solution (tragacanth gum and water).

2.3. Mating test

The sexual behavior of males was monitored by trained observers, without knowledge of the experimental design, in a sound-attenuated, air conditioned room lit with a dim red light, during the early portion of the dark cycle. Single male rats were placed in rectangular glass observation cages (40 cm × 50 cm × 40 cm) and allowed to become accustomed to the test chamber for 5 min. Then a sexually receptive female rat was introduced in the cage and the copulatory test started. The following parameters of sexual behavior were measured as previously described by Ågmo (1997) and by Zanoli et al. (2003, 2008):

(1) *mount latency (ML):* time from the introduction of the female to the first mount;
(2) *intromission latency (IL):* time from introduction of the female to the first intromission (vaginal penetration);
(3) *ejaculation latency (EL):* time from the first intromission to ejaculation;
(4) *post-ejaculatory interval (PEI):* time from ejaculation to the first intromission of the second copulatory series.

Tests were normally ended immediately after the first post-ejaculatory intromission; or if intromission did not occur within 15 min; or if ejaculation latency exceeded 30 min; or in the case that post-ejaculatory interval exceeded 15 min. Rats were trained with sexually receptive females in a series of seven pre-experimental tests with the aim to classify males as sexually potent, sluggish or impotent. We took in account only the results obtained in the last three pre-experimental tests. Rats achieving ejaculation in all the three tests were defined as sexually potent; those achieving ejaculation in one or two of the last three pre-experimental tests were considered sexually sluggish, while animals which failed to achieve ejaculation in all the three tests were considered sexually impotent (Dewsbury, 1972).

2.4. Partner preference test

The partner preference test, performed according to Ågmo et al. (2004), was used to evaluate sexual motivation in a no-contact condition. The apparatus consisted of an open field arena (100 cm × 50 cm × 40 cm high) with two round cages made of wire meshing (16 cm diameter and 40 cm high) diagonally positioned at the opposite corners of the arena. We used two stimulus animals: a male in one cage and a receptive female in the other one. In addition we defined an incentive area near to each cage: the sexual incentive area near to the female and the social incentive area near to the male. The transmission of visual, olfactory and auditory cues was allowed while mating was avoided. Experimental males were individually placed in the centre of arena for a 5-min adaptation period at the presence of the stimulus animals and thereafter tested for 10 min. The number of visits to the male and the female as well as the time spent near each stimulus animal was recorded. The measure of sexual motivation is expressed by a preference score, i.e. the ratio between the time spent in the sexual incentive area and the total time spent in the two incentive areas (Ågmo et al., 2004).