Journal of Ethnopharmacology 126 (2009) 308–313

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

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

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ARTICLE INFO

Article history:
Received 12 May 2009
Received in revised form 13 August 2009
Accepted 15 August 2009
Available online 22 August 2009

Keywords:
Eurycoma longifolia
Male rat
Sexual behavior
Mating test
Partner preference test
Testosterone

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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doi:10.1016/j.jep.2009.08.021

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).

Table 1
Effect of acute treatment with *Eurycoma longifolia* roots on sexual behavior of sluggish rats.

Treatment (mg/kg)	ML	%M	IL	EL	%E	PEI
Vehicle	372.5 ± 82.5	25	418.5 ± 76.5	1065.0 ± 195.5	25	305.0 ± 33.0
Eurycoma longifolia 250	318.8 ± 112.5	62.5	509.2 ± 133.7	887.0 ± 423.0	25	321.4 ± 19.1
Eurycoma longifolia 500	281.2 ± 99.7	62.5	337.0 ± 113.1	368.0 ± 20.9*	62.5	309.5 ± 5.5
Eurycoma longifolia 1000	139.2 ± 60.6	75	143.6 ± 83.7	326.8 ± 82.7*	62.5	307.4 ± 18.9

Values are mean ± S.E.M. obtained by groups of 8 animals each.
ML = mount latency, %M = percentage of mounting rats, IL = intromission latency, EL = ejaculation latency, %E = percentage of ejaculating rats, PEI = post-ejaculatory interval.
* ANOVA followed by Dunnett's post-hoc test: $p < 0.05$ vs vehicle group.

2.5. Testosterone assays

Sprague–Dawley male rats (250–300 g b.w.), which were not used for sexual behavior study, were randomly divided in two groups of 8 animals each: one was subacutely administered with the dose of 500 mg/kg/day of *Eurycoma longifolia* root, and the other with vehicle. Animals were sacrificed 24 h after the last dose. Trunk blood was collected into centrifuge tubes and serum was prepared by centrifugation (3000 r.p.m., 20 min and 4 °C) and stored frozen until assays.

Testosterone (T) concentrations were determined in duplicate using Testosterone Enzyme Immunoassay kit (Oxford Biomedical Research Inc., Oxford, MI, USA) according to manufacturer's instructions, by a multiskan MCC 340 system (Lab System, Helsinki, Finland). Detection limit for T assay was 10.0 pg/ml; cross-reactivity with corticosteroid and other androgens was minimal (<1%).

2.6. Statistical analysis

The results are expressed as mean ± S.E.M. obtained by groups of 8 rats each. One-way ANOVA followed by Dunnett's *t* test was used for statistical comparison of treatment groups and controls; where appropriate, Student's *t* test was used. The percentages of mounting and ejaculating rats in treated and control groups were compared using Fisher's test. In any case the statistical significance was set at $p < 0.05$. All statistical analyses were performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, California).

3. Results

3.1. Effect of Eurycoma longifolia on sexual behavior of sluggish rats

The acute administration of *Eurycoma longifolia* root powder at the lowest dose (250 mg/kg) did not influence the different parameters of rat copulatory behavior (Table 1). On the other hand a significant reduction in EL ($p < 0.05$ vs control group) was observed in rats treated with the dosages of 500 and 1000 mg/kg. No significant difference in other evaluated parameters (ML, IL and IPE) was recorded between control and treated animals, even if a tendency to a reduction in ML and IL was observed following the administration of the highest dose. An increased percentage of mounting and ejaculating rats was detected in treated rats but the difference in comparison with controls was not statistically significant.

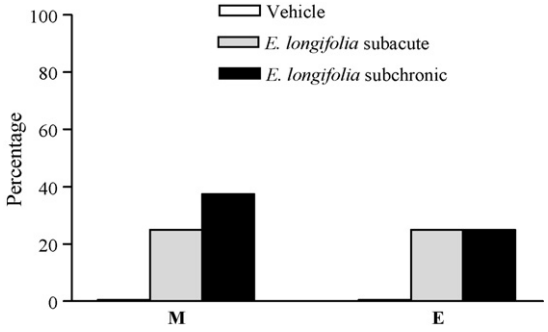

Fig. 1. Percentage of mounting (M) and ejaculating (E) animals after the subacute (6 days) and subchronic (12 days) administration of *Eurycoma longifolia* in impotent rats in comparison with control ones treated with vehicle. Data are obtained by groups of 8 animals each.

Taking in consideration the above-mentioned results obtained after the acute administration we choose only the dose of 500 mg/kg for the subacute (6 consecutive days) and subchronic (12 consecutive days) treatments.

The data reported in Table 2 show that the subacute administration of *Eurycoma longifolia* root powder was able to significantly decrease EL ($p < 0.05$) and PEI ($p < 0.01$) in comparison with controls. The extension of the daily treatment until the 12th day did not show any further advantage since no significant difference between control and treated groups of rats was observed (Table 3).

3.2. Effect of Eurycoma longifolia on sexual behavior of impotent rats

The sexual behavior of impotent rats was not affected by the acute treatment with the plant root powder even if administered at the highest dose: no control or treated animal showed mount or intromission behavior in the presence of a receptive female (therefore the data are omitted in the paper). On the other hand the repeated administration of 500 mg/kg daily for 6 and 12 days produced an increase in the percentage of mounting rats from 0% assessed in control group to 25% observed in rats treated for 6 days and 37.5% observed in rats treated for 12 days. The percentage of ejaculating animals increased from 0% (control rats) to 25% in both groups of rats treated with *Eurycoma longifolia* (Fig. 1).

Table 2
Effect of subacute treatment with *Eurycoma longifolia* roots on sexual behavior of sluggish rats.

Treatment (mg/kg)	ML	%M	IL	EL	%E	PEI
Vehicle	234.7 ± 95.6	37.5	271.0 ± 117.1	560.3 ± 58.9	37.5	338.0 ± 21.0
Eurycoma longifolia 500	84.8 ± 31.2	50	267.3 ± 155.9	307.3 ± 36.0*	50	255.5 ± 4.3**

Values are mean ± S.E.M. obtained by groups of 8 animals each.
ML = mount latency, %M = percentage of mounting rats, IL = intromission latency, EL = ejaculation latency, %E = percentage of ejaculating rats, PEI = post-ejaculatory interval.
* Student's *t* test: $p < 0.05$.
** Student's *t* test: $p < 0.01$ vs vehicle group.

Table 3
Effect of subchronic treatment with *Eurycoma longifolia* roots on sexual behavior of sluggish rats.

Treatment (mg/kg)	ML	%M	IL	EL	%E	PEI
Vehicle	145.8 ± 31.7	50	255.3 ± 53.8	284.3 ± 79.6	37.5	298.0 ± 23.6
Eurycoma longifolia 500	99.8 ± 57.4	75	192.8 ± 121.1	230.6 ± 37.2	62.5	320.0 ± 19.9

Values are mean ± S.E.M. obtained by groups of 8 animals each.
ML = mount latency, %M = percentage of mounting rats, IL = intromission latency, EL = ejaculation latency, %E = percentage of ejaculating rats, PEI = post-ejaculatory interval.

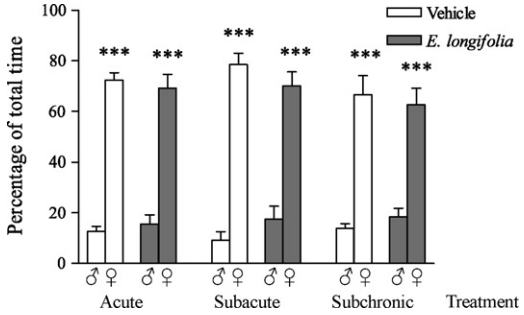

Fig. 2. Percentage of total time spent by rats after the acute (1000 mg/kg), subacute (500 mg/kg/day for 6 days) and subchronic (500 mg/kg/day for 12 days) treatments with *Eurycoma longifolia*, in proximity to a male (♂) or a receptive female (♀). Data are mean ± S.E.M., obtained by groups of 8 animals each. Student's *t* test: ***$p < 0.001$ vs time spent in the male incentive zone for each experimental group.

3.3. Effect of Eurycoma longifolia on sexual motivation of sluggish rats

The partner preference test was performed in rats treated with the plant root powder acutely at the highest dose (1000 mg/kg) or daily for 6 and 12 days at the dose of 500 mg/kg. Male rats receiving vehicle spent more time in proximity to the female than in proximity to the male ($p < 0.001$) (Fig. 2). The percentages of time spent in the incentive zones were not modified by the acute, subacute or subchronic treatment with *Eurycoma longifolia*. All treated rats spent more time in the proximity of females than in the proximity of males ($p < 0.001$) (Fig. 2). Consequently, the preference score was not statistically different between treated groups and the corresponding controls (Table 4). In addition to the time spent in the two incentive areas, we recorded the number of visits to both stimulus animals: no difference in the number of visits was detected among the experimental groups (data not shown).

3.4. Effect of Eurycoma longifolia on testosterone serum levels

Considering the results obtained in the behavioral experiments, we determined testosterone serum levels only in animals treated with the dose of 500 mg/kg/day for 6 days. The histograms represented in Fig. 3 show a significant increase of T concentration in the serum of treated rats compared with the control ones ($p < 0.05$).

Table 4
Preference score of sexually sluggish rats following acute, subacute and subchronic treatment with *Eurycoma longifolia* roots.

Treatment (mg/kg)	Preference score
Vehicle	0.85 ± 0.02
Eurycoma longifolia acute (1000)	0.81 ± 0.04
Vehicle	0.88 ± 0.03
Eurycoma longifolia subacute (500)	0.81 ± 0.06
Vehicle	0.81 ± 0.04
Eurycoma longifolia subchronic (500)	0.76 ± 0.05

Values are mean ± S.E.M., obtained by groups of 8 animals each. The preference score was calculated by the ratio: time spent in the sexual incentive zone/(time in this zone + time spent in the social incentive zone).

4. Discussion

As reported in Section 1 the aphrodisiac effect of *Eurycoma longifolia* was already tested in rodents but it must be underlined that most experiments were performed in sexually normal or middle-aged animals. We focused our experiments to the possible therapeutic application of *Eurycoma longifolia* in sexual dysfunctions using sexually sluggish or impotent rats as a more appropriate experimental model. Differently from the previous studies, we used the root powder of the plant instead of the fractions obtained from the root by means of different solvents (methanol, butanol, water and chloroform), avoiding thus any interaction in the pharmacological effect. It is important to underline that we compared the effect of the acute and repeated administration of the powder for 6 or 12 days, in the same experimental conditions. We demonstrated that the oral administration of the root powder was able to improve sexual performance in sluggish rats and partially restore the normal sexual behavior in impotent rats. The percentages of mounting and ejaculating rats were particularly increased in sluggish animals (75% and 62.5%, respectively) by the acute administration of the highest dose (1000 mg/kg), in comparison with the controls (25%). While the acute administration was ineffective in impotent rats, the subacute and subchronic treatments increased the percentage of mounting rats from 0% (controls) to 25% and 37.5%, respectively, and the percentage of ejaculating rats from 0% (controls) to 25% (in both treated groups). Concerning the copulatory parameters, the better results related to ejaculation latency and post-ejaculatory interval were obtained after the subacute administration (6 days) in sluggish rats in comparison with the acute and the subchronic (12 days) administrations. However also a single administration of 500 or 1000 mg/kg of the root powder was able to reduce ejaculation latency. The reduction of EL and PEI is generally suggested to be indicative of an improved copulatory behavior, particularly when observed together with an increase in mounting and ejaculating animals (Bitran and Hull, 1987; Yacubu et al., 2007). The prolonged administration of the root powder for 12 days does not seem to exert a more beneficial effect both in sluggish and in impotent rats,

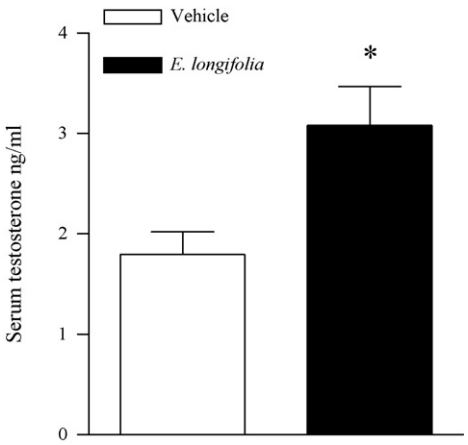

Fig. 3. Testosterone serum levels in rats subacutely treated with vehicle or *Eurycoma longifolia* root powder dosed at 500 mg/kg/day. Values are mean ± S.E.M. obtained by groups of 8 animals each. Student's *t* test: *$p < 0.05$ vs vehicle group.

excluding thus a therapeutic utility of a long period of administration. Mount and intromission latencies were decreased after the acute treatment with the highest dose but not in a significant manner, so that we cannot suggest an influence of the plant on the motivational aspect of sexual behavior. To further investigate this effect, we performed the partner preference test in sluggish animals after acute, subacute and subchronic administration. No alteration was observed in treated rats concerning the time spent with the social or the sexual stimulus and the number of visits. Therefore we can conclude that *Eurycoma longifolia* failed to improve motivation of sluggish rats submitted to the partner preference test. This result disagrees with the ones obtained by Ang and Sim (1998a), Ang and Ngai (2001), Ang et al. (2003a), but it must be stressed that there are differences in the experimental model and in the type of animal (as reported in Section 1).

It has been suggested by other authors that the aphrodisiac property of this plant could be ascribed to a testosterone enhancing property (Ang and Cheang, 1999b; Ang et al., 2000a; Ang and Lee, 2002b) but at our knowledge its demonstration is still lacking. The pro-androgenic effect of *Eurycoma longifolia* has been suggested by the finding of an increased weight of laevator ani muscle in intact male rats treated with the plant extract (Ang and Cheang, 2001), as well increased weights of both ventral prostate and seminal vesicles in castrated male rats (Ang et al., 2000a). The present study shows for the first time that *Eurycoma longifolia* is able to significantly increase testosterone serum levels in rats treated with the powder root daily for 6 days, in parallel with the elicitation of an improved copulatory activity. Testosterone seems to be involved in the complex mechanism regulating the copulatory behavior, acting both centrally and peripherally in concert with other determinants. The ability of different medicinal plants to improve sexual function (e.g. *Tribulus terrestris*, *Panax ginseng*, *Ferula hermonis*) was ascribed to increased levels of testosterone in the serum (Fahim et al., 1982; Gauthaman et al., 2002; Zanoli et al., 2003) or to an enhanced production of the hormone by Leydig cells (e.g. *Ginkgo biloba*) (Ye et al., 2008). Testosterone may facilitate male sexual behavior by increasing dopamine release in the medial preoptic area and potentiating nitrergic neurotransmission (Hull et al., 1999; Putnam et al., 2001).

Our present results confirm the aphrodisiac potential activity of the plant and allow to ascribe, at least partially, the mechanism of action of this plant to a hormonal influence in enhancing male copulatory behavior.

5. Conclusions

In the present study the influence of *Eurycoma longifolia* root on male sexual behavior was evaluated in sexually sluggish and impotent rats as an appropriate model of human sexual dysfunction. Moreover we compared the effects of three different administration schedules (acute, subacute and subchronic) in the same experimental conditions. Our results demonstrated that the root powder of *Eurycoma longifolia* is able to improve the copulatory performance of sexually sluggish rats following acute or subacute administration; no advantage is apparently provided by a longer treatment. In impotent rats only the subacute or subchronic administration of the root plant partially restored the copulation activity. The pharmacological effect of the plant could be mainly ascribed to increased serum levels of testosterone.

Acknowledgement

The authors gratefully acknowledge to Bioera S.p.a. for supplying the material used for the experiments and for the financial support.

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