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Opposite effect of acute and subchronic treatments with *Ferula hermonis* on copulatory behavior of male rats

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Sexually potent and sluggish/impotent male rats were orally treated with an extract of *Ferula hermonis* (30 and 60 mg/kg). The acute administration stimulated sexual motivation in potent rats and improved copulatory performance in sluggish/impotent rats. This last effect was elicited only by the higher dose, which, in parallel, increased serum testosterone levels in rats. On the contrary, when the extract was subchronically administered (10 days) a marked reduction in the percentage of rats achieving ejaculation was detected, together with a general impairment of the copulatory pattern. Furthermore, the repeated administration of the extract (6 mg/kg/day for 10 days) resulted in a significant reduction of testosterone levels in comparison with controls. The present results discourage a repeated assumption of *F. hermonis,* while suggesting its acute administration to improve the performance in sexual dysfunctions.
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Introduction

Erectile dysfunction, defined as the 'inability to achieve or maintain a penile erection adequate for sexual satisfaction' has always been associated with negative effects on quality of life.[1] Ancient traditional medicines treated sexual imbalance such as impotence or reduced desire by natural remedies, among which medicinal plants have played, in the course of time, a primary role. In the medicinal systems of different countries, indigenous botanical species were used as aphrodisiacs.[2,3]

Among Lebanese, Syrian, Arabian and Jordanian populations, *Ferula hermonis* has been used against frigidity and impotence and to increase sexual energy.

F. hermonis is a herbal plant that grows on Mount Hermon in Lebanon. Its roots, harvested from August to October, are commercialized under the name 'sex root' or 'Lebanese Viagra' and are suggested to cure erectile dysfunction, as well as menopausal disturbances in women.

Few studies were carried out on the chemical composition of *Ferula* roots. Fitochemical investigations performed up to now showed the presence of aromatic esters of the sesquiterpene alcohol, ferutinol (jaeschkeanadiol), among which ferutinin (ferutinol *p*-hydroxybenzoate) and teferdin (ferutinol benzoate) are the major components.[4] By a simple HPLC method, the presence of the major sesquiterpene esters was quantified in the crude root of *F. hermonis* and in two commercial products (teabags and capsules). The development of the method could be of importance in the quality control of products containing *F. hermonis*, which are emerging in the dietary supplement market.[5]

A recent study performed with the aim of clarifying the efficacy and the safety of this plant was published by El-Thaher *et al*.[6] The authors demonstrated the capacity of the crude oil extracted from the seeds of *F. hermonis*, when administered acutely in rats, to enhance erectile function. On the contrary, when chronically administered, it exerted unwanted and toxic effects such as decrease in total body weight, hepatomegaly and atrophic testis.

In the current study we carried out further investigation on the effect of *F. hermonis* on the copulatory behavior of male rats. Furthermore, *Ferula* effect on the testosterone serum level was

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assessed following acute and subchronic administration in order to elucidate the mechanism of action of the plant extract in terms of its ability to influence male copulatory behavior.

Materials and methods

Animals

Sprague–Dawley rats of either sex, weighing from 160 g (females) to 220 g (males), were purchased from Harlan Italy (Udine, Italy). They were housed in groups of four, males and females separately, in plexiglass cages, and were maintained under controlled laboratory conditions ($22 \pm 1^{\circ}C$, 60% humidity) on a reversed 12 h light/dark cycle, with lights off at 0700. Food in pellet and tap water were provided *ad libitum*. The animals were accustomed to our housing conditions for at least 2 weeks before being used.

The females were ovariectomized under ketamine hydrochloride plus xylazine hydrochloride anesthesia and brought into estrous by sequential subcutaneous injections of 30 μg estradiol benzoate and 500 μg progesterone, 48 and 4 h before the copulatory studies, respectively. They were screened with nonexperimental sexually experienced males and only those exhibiting good sexual receptivity (solicitation behavior and lordosis in response to mounting) and no rejection behavior were used.

All procedures involving animals were performed in accordance with the guidelines of the National (D.L. n. 116/1992) and European legislation (EEC n. 86/609) and of the National Institute of Health (USA) on the use and care of laboratory animals.

Copulatory behavior

The sexual behavior of males was monitored by trained observers unaware of the experimental design in a sound-attenuated, air conditioned room lit with a dim red light according to the standard procedure.[7] Single male rats were placed in rectangular glass observation cages ($40 \times 50 \times 40$ cm) and allowed to acclimate to the test chamber for 5 min. Then a sexually receptive female rat was introduced in the cage. The following parameters of sexual behavior were measured as described by Agmo:[8]

(1) *mount latency (ML):* time from the introduction of the female until the first mount;
(2) *intromission latency (IL):* time from introduction of the female until the first intromission (vaginal penetration);
(3) *ejaculation latency (EL):* time from the first intromission to ejaculation;
(4) *postejaculatory interval (PEI):* time from ejaculation until the next intromission;
(5) *mount frequency (MF):* number of mounts preceding ejaculation;
(6) *intromission frequency (IF):* number of intromissions preceding ejaculation;

The following parameters were calculated on the basis of the above data:

(7) *intercopulatory interval (ICI):* average interval between successive intromissions (calculated as ejaculation latency divided by intromission frequency);
(8) *copulatory efficacy (CE):* a measure of intromissive success (calculated as intromission frequency divided by mount frequency + intromission frequency).

Tests were normally ended immediately after the first postejaculatory intromission; or if intromission did not occur within 15 min; or if ejaculation latency exceeded 30 min; or in the case where postejaculatory interval exceeded 15 min. Rats were trained with sexually receptive females seven times before the experimental test. After the seventh pre-experimental training test, rats that concluded the performance in the last three tests were defined as sexually potent. The remaining rats, who failed to achieve ejaculation in one, two or all the last three pre-experimental tests, were considered sexually sluggish or impotent.

Treatments

F. hermonis acetonic extract, supplied by Indena Spa (Milan, Italy), was solubilized in Tween 80 (10%) and water, and administered in the volume of 5 ml/kg, by oral gavage. The control animals received the same volume of the vehicle media.

Sexually potent and sexually sluggish/impotent rats were randomly assigned to one of the following treatment groups: (1) vehicle, (2) *F. hermonis* 30 mg/kg, (3) *F. hermonis* 60 mg/kg. Treatments were carried out 45 min before the test. In the subchronic experiments, rats were administered once a day for 10 consecutive days with the extract or the vehicle. In the first series of experiments *Ferula* extract was administered at doses of 30 and 60 mg/kg/day, while in the second series, it was administered at a lower dose, 6 mg/kg/day. The copulatory test was performed 45 min after the last administration.

Testosterone assay

Sprague–Dawley male rats (250–300 g b.w.), which were not submitted to mating tests, were treated with a single dose (30 and 60 mg/kg) or with a repeated dose (6 mg/kg/day for 10 days) of *Ferula*

extract. Animals were killed by decapitation 45 min after the acute administration or 24 h after the last dose, when the extract was subchronically administered. Trunk blood was collected into centrifuge tubes and the serum was prepared by centrifugation (3000 r.p.m., 20 min, 4°C) and stored frozen until testosterone assay.

The testosterone concentration was determined in duplicate using the Testosterone Enzyme Immunoassay kit (Assay Design Inc., Ann Arbour, USA) according to the manufacturer's instructions. The detection limit for this assay was 3.82 pg/ml; cross-reactivity with corticosteroid and other androgens was minimal (<1%).

Data analysis

The data, expressed as mean ± s.e.m., obtained by groups consisting of 8–10 animals, were analyzed using one-way analysis of variance (ANOVA). *Post hoc* comparisons between individual treatment groups and controls were made with Dunnett's test. Student's *t* analysis was used where appropriate. Percentages of ejaculating rats in treated groups of animals *vs* vehicle controls were evaluated with Fisher's exact probability test. In all cases, $P<0.05$ was taken as the level of significance.

Results

The doses of *F. hermonis* administered to rats were chosen following the results obtained in dose–effect preliminary experiments. From these data, it appears that doses over 120 mg/kg reduced motor activity and induced absence of sexual orientation towards the female followed by marked sedation and sleep, observed in rats dosed at 500 mg/kg. Hence, for the present experiments we chose two dose levels 30 and 60 mg/kg, since there was apparently no advantage in using higher doses in order to enhance sexual performance.

Copulatory behavior following acute treatment

The effect of an acute treatment with *F. hermonis* extract on the copulatory behavior of sexually potent rats is shown in Table 1. The treatment with both doses of the extract was able to induce a significant reduction in mount and intromission latencies in comparison with the corresponding values of vehicle-treated rats. The other parameters used to evaluate the copulatory behavior of rats were not significantly affected by the treatments with the plant extract.

The effect of acute treatment with *Ferula* extract on the copulatory behavior of sluggish/impotent rats is shown in Table 2. The treatment with the dose of 60 mg/kg of *Ferula* extract significantly reduced the ejaculation latency of sexually sluggish/impotent rats, in comparison to control animals ($P<0.01$). In the same treated rats a significant decrease in ICI was detected, since intromission frequency was not modified; moreover, a higher percentage of ejaculating animals was observed (80 *vs* 57%) in comparison to the control group (data not shown).

Table 1 Influence of acute administration of *F.hermonis* extract on copulatory behavior of sexually potent male rats

Treatment (mg/kg)	*ML (s)*	*IL (s)*	*MF (No)*	*IF (No)*	*EL (s)*	*PEI (s)*	*CE*	*ICI (s)*
Vehicle	247.1 ± 66.3	275.7 ± 68.4	2.2 ± 0.7	17.7 ± 3.8	450.3 ± 119.9	421.3 ± 24.4	0.88 ± 0.03	24.5 ± 2.4
Ferula 30	72.3 ± 25.8*	83.5 ± 24.4**	1.1 ± 0.1	18.7 ± 1.6	381.1 ± 72.2	410.7 ± 28.5	0.93 ± 0.01	21.7 ± 5.2
Ferula 60	45.2 ± 12.7**	65.5 ± 21.0**	1.5 ± 0.2	17.1 ± 1.8	424.2 ± 62.8	424.6 ± 42.2	0.91 ± 0.02	25.2 ± 3.3

Treatments were performed 45 min prior to the test. Each value is the mean ± s.e.m. obtained by groups of 10 rats per group. *$P<0.05$, **$P<0.01$ compared with vehicle-treated rats (ANOVA followed by Dunnett's test). ML = mount latency; IL = intromission latency; MF = mount frequency; IF = intromission frequency; EL = ejaculatory latency; PEI = post-ejaculatory interval; CE = copulatory efficacy; ICI = intercopulatory interval.

Table 2 Influence of acute administration of *F. hermonis* extract on copulatory behavior of sexually sluggish impotent male rats

Treatment (mg/kg)	*ML (s)*	*IL (s)*	*MF (No)*	*IF (No)*	*EL (s)*	*PEI (s)*	*CE*	*ICI (s)*
Vehicle	311.7 ± 75.6	475.0 ± 71.9	4.3 ± 1.3	19.0 ± 2.5	1471.0 ± 216.9	473.5 ± 14.0	0.88 ± 0.02	58.1 ± 4.4
Ferula 30	455.0 ± 52.4	507.5 ± 59.6	1.5 ± 0.5	16.5 ± 1.5	1121.0 ± 139.2	399.8 ± 25.8	0.87 ± 0.02	69.5 ± 8.4
Ferula 60	518.8 ± 41.9	581.3 ± 50.7	2.2 ± 0.5	18.0 ± 1.2	368.0 ± 54.4**	413.0 ± 33.1	0.88 ± 0.02	20.7 ± 3.4**

Treatments were performed 45 min prior to the test. Each value is the mean ± s.e.m. obtained by groups of 10 rats per group. **$P<0.01$ compared with vehicle-treated rats (ANOVA followed by Dunnett's test). ML = mount latency; IL = intromission latency; MF = mount frequency; IF = intromission frequency; EL = ejaculatory latency; PEI = postejaculatory interval; CE = copulatory efficacy; ICI = intercopulatory interval.

Copulatory behavior following subchronic administration

When *Ferula* extract, dosed at 30 and 60 mg/kg, was administered daily for 10 days, copulatory behavior of sexually potent rats, tested 45 min after the last treatment, was negatively affected since an increase in mount, intromission, ejaculation latencies and postejaculatory interval was found in comparison with controls (data not shown). In particular, a very significant decrease in the percentage of ejaculating rats was detected as shown in Figure 1 (panel a). In sexually sluggish/impotent rats subchronically treated with the same dosages of *Ferula* extract, no influence on copulatory behavior was observed. However, it must be stressed that a reduction in the percentage of ejaculating rats, treated with the higher dose of the extract, was still evident as it occurs in sexually potent rats (Figure 1, panel b).

The opposite results obtained following acute and chronic administrations suggested us to investigate the effect of a low dose (6 mg/kg) on the sexual behavior of potent rats.

The data obtained in the copulatory test performed at the 10th day are presented in Figure 2. A significant increase in mount, intromission, ejaculation latencies and in the postejaculatory interval was detected in treated rats in comparison with controls (panel a). Moreover the percentage of ejaculating rats was significantly reduced from 90% in the control group to 16.6% in the *Ferula*-treated group (panel b). To evaluate the dose dependency of the negative effect exerted by a repeated administration of *Ferula* extract on rat copulatory behavior, the activity of an even lower dosage was studied in the same experimental conditions. The results showed that at a daily dose of 1 mg/kg for 10 days, a worsening in the sexual performance was still evident (data not shown).

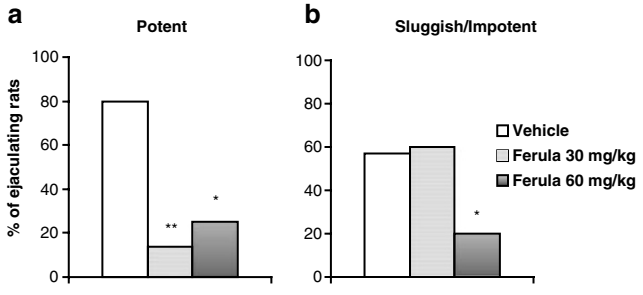

Figure 1 Effect of subchronic administration of *Ferula* extract 30 and 60 mg/kg/day (for 10 days) on the percentage of ejaculating rats in both potent (panel a) and sluggish/impotent animals (panel b). Each value is the mean ± s.e.m. obtained by groups of 10 rats each. $*P<0.05$, $**P<0.01$ compared with vehicle-treated rats (Fisher's exact test).

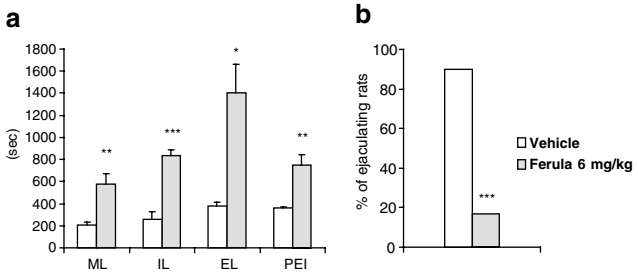

Figure 2 Influence of subchronic administration of *Ferula* extract 6 mg/kg/day (for 10 days) in sexually potent rats on copulatory behavior (panel a) and the percentage of ejaculating animals (panel b). Panel (a) each value is the mean ± s.e.m. obtained by groups of eight rats per group. $*P<0.05$, $**P<0.01$, $***P<0.001$ compared with vehicle-treated rats (ANOVA followed by Dunnett's test). ML = mount latency; IL = intromission latency; EL = ejaculatory latency PEI = postejaculatory interval. Panel b: $***P<0.001$(Fisher's exact test).

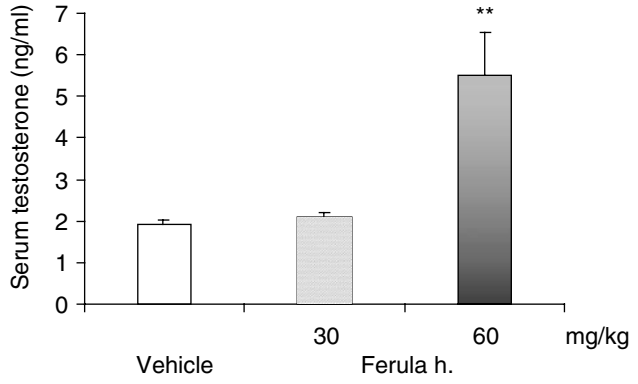

Figure 3 Effect of acute administration of *Ferula* extract (30 and 60 mg/kg) on serum testosterone levels (ng/ml) in male rats in comparison with controls. Each value is the mean ± s.e.m. obtained by groups of eight rats each. $**P<0.01$ compared with vehicle-treated rats (ANOVA followed by Dunnett's test).

Testosterone serum levels

Testosterone serum levels in rats acutely treated with vehicle or *Ferula* extract dosed at 30 and 60 mg/kg. are reported in Figure 3. Only the higher dose of the extract significantly increased testosterone levels in comparison with control animals ($P<0.01$). On the contrary, the daily administration of the extract dosed at 6 mg/kg for 10 days resulted in a significant decrease of the hormone serum level in comparison with vehicle-treated rats ($P<0.05$), as shown in Figure 4.

Discussion

The present results provide, for the first time, information concerning the ability of *F. hermonis* extract to improve male copulatory behavior in rats.

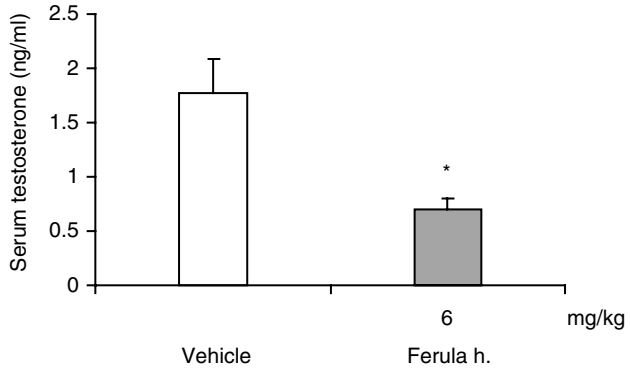

Figure 4 Effect of subchronic administration of *Ferula* extract (6 mg/kg) on serum testosterone levels (ng/ml) in male rats in comparison with controls. Each value is the mean ± s.e.m. obtained by groups of eight rats each. $^*P<0.05$ compared with vehicle-treated rats (Student's *t*-test).

A previous study revealed that *F. hermonis* seed oil was able to stimulate erectile function.[6] In our study, *Ferula* acetonic extract significantly enhanced male sexual appetitive behavior in potent rats, as evidenced by reduced mount and intromission latencies in comparison with controls. These parameters are indeed considered to be inversely proportional to sexual motivation or desire.[9] The acute administration of the extract improves and partially restores the copulatory performance in sluggish/impotent rats, as indicated by a reduced ejaculation latency, which, in laboratory animals, is considered as inversely proportional to copulatory performance.

These findings provide experimental support to the folk traditional use of *F. hermonis* as a sexual stimulant for the treatment of erectile dysfunction and sexual asthenia.

The improvements in copulatory performance after the acute ingestion of the extract dosed at 60 mg/kg could be ascribed to the significant increase in testosterone serum levels.

In the complex mechanism that regulates copulatory behavior, testosterone is considered to contribute to penile erection acting both centrally and peripherally in concert with other determinants. The increased levels of the hormone have been involved in the ability of different medicinal plants to improve sexual function.[10–12]

The effect shown by the acute administration of *Ferula* extract dosed at 30 mg/kg, mainly exerted on the appetitive component, may be ascribed to mechanisms other than testosterone.

In contrast, the repeated treatment of potent rats for 10 days with *Ferula* extract dosed at 30 and 60 mg/kg/day dramatically worsened the copulatory pattern, as indicated by a reduced percent of animals satisfying the criteria of sexual vigor, besides increased mount, intromission and ejaculation latencies associated with a longer postejaculatory interval. The same worsening results were also obtained after low dosages. Reduced testosterone levels observed in rats subchronically treated with the extract (6 mg/kg/day) in comparison with control animals may account for the negative effects observed in mating tests.

An attempt to avoid the negative effect of a repeated treatment with *Ferula* extract by reducing the dose to 1 mg/kg/day failed to reach a dosage lacking unwanted effects.

It must be pointed out that other authors, administering an aqueous extract of *F. hermonis* (3 mg/kg) for 6 weeks in mice, demonstrated that prolonged exposure leads to fertility disturbances.[13] This study moreover showed a reduction in body weight gain and in weights of testes and other sex accessory organs, suggesting an antiandrogenic action of the plant extract. The antifertility effect of *F. hermonis* was recently confirmed by Homady *et al*[14] in male and female mice after intragastric application of 3 mg/kg/day of *F. hermonis* extract for 6 weeks. Such exposure resulted in a significant reduction in male and female mice fertility.

These results taken together are particularly a warning of the potential negative effects following repeated administration of *Ferula* extract. Considering, however, the positive effect of the acute administration of *Ferula* extract on sexual behavior, further experiments are in progress in our laboratory to investigate the role of the different components of *F. hermonis.*

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