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·*Original Article*·

Improved sexual behavior in male rats treated with a Chinese herbal extract: hormonal and neuronal implications

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Abstract

Aim: To investigate the influence of an extract obtained from five Chinese medicinal plants on sexual behavior of adult male rats. **Methods:** The extract was administered at doses of 30, 60 and 120 mg/kg by oral gavage, acutely (one time, 45 min before mating test) or subchronically (daily for 10 days) in sexually potent and sexually sluggish/impotent rats. Sexual behavior, serum levels of luteinizing hormone (LH) and testosterone (T) were evaluated in treated rats and compared with controls receiving vehicle. The effect of the extract on central dopaminergic neurotransmission was assessed in the nucleus accumbens using a microdialysis technique. **Results:** In sexually potent rats, both acute and subchronic treatment with the extract dosed at 30 and 60 mg/kg reduced mount latency and intromission latency. In sluggish/impotent rats, the acutely administered extract at the dose of 60 mg/kg shortened ejaculation latency, whereas subchronically administered at the doses of 30 and 60 mg/kg, reduced mount, intromission and ejaculation latencies, increasing also the percentage of mounting and ejaculating rats. The extract dosed at 60 mg/kg significantly increased LH and T following acute and subchronic administration and increased 3,4-dihydroxyphenylacetic acid levels in the nucleus accumbens, 30 min after the acute administration. **Conclusion:** The improvement in both appetitive and consummatory components of sexual behavior observed in male rats treated with the extract could be ascribed to increased serum T level in parallel with the activation of the central dopaminergic system. *(Asian J Androl 2008 Nov; 10: 937–945)*

Keywords: sexual behavior; rats; testosterone; luteinizing hormone; microdialysis; dopamine; 3,4-dihydroxyphenylacetic acid

1 Introduction

Over the past ten years different drugs have been used in the treatment of male erectile dysfunction (ED). Even if all of them have therapeutic actions, most of them are associated with serious side effects, including headache, facial flushing, dizziness, myalgia and dyspepsia [1]. Therefore, the use of natural products obtained from traditional herbs is appealing.

The present study was undertaken to investigate the ability of a Chinese herbal extract to influence sexual behavior in experimental animals. The components of the extract are: *Lycium barbarum L.* (*L. barbarum L.*) fruits, *Epimedium koreanum Nakai* (*E. koreanum Nakai*) leaves, *Morinda officinalis How* (*M. officinalis How*)

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roots, *Cinnamomum cassia* (*C.cassia*) bark and *Eugenia caryophyllata* flower buds. In traditional Chinese medicine, these medicinal plants are used for many therapeutic purposes and some of them are used in the treatment of reproductive impairments.

The fruits of *L. barbarum L.* (Solanaceae) have been used by Chinese physicians to treat male infertility, even though the active components and the mechanism(s) of action responsible for their fertility-facilitating effect are unknown. It has been demonstrated that Lycii barbarum polysaccharides (LBP) inhibits time-induced and hyperthermia-induced structural damage and degeneration in the murine seminiferous epithelium, *in vitro* [2]. Luo *et al.* [3] confirm the protective effect of LBP against heat-induced damage of rat testicular tissue and demonstrate its ability to improve copulatory performance and reproductive function in hemicastrated male rats. The authors found increased testosterone (T) levels and raised accessory sexual organ weights in addition to improvements in sperm quantity and quality, hence supporting the folk reputation of *L. barbarum L.* fruits as sexual stimulants.

According to traditional Chinese medicine, *E. koreanum Nakai* (Berberidaceae) is used to treat infertility, impotence and senile sexual dysfunctions. The different effects on cardiovascular, immunological and genital systems are reviewed by Ye and Chen [4]. The leaves and stems of the plant contain polysaccharides, flavonoids, alkaloids and terpenic compounds. The flavonoid icariin is considered to be the major pharmacologically active component [5]. Anti-fatigue, anti-hepatotoxic and immuno-regulatory effects of icariin have been reported [6, 7]. Its therapeutic effect on ED in castrated rats is demonstrated by Liu *et al.* [8]. In addition, its T mimetic property has been assessed in chemically-induced hypoandrogenic male rats [9]. Icariin has a selective and dose-dependent inhibitory effect on cGMP-specific phosphodiesterase (PDE5) [10]. The consequent increase in cGMP levels could be responsible for the corpus cavernosum muscle relaxation previously described *in vitro* [11, 12] and *in vivo* [13]. The inhibitory effect of icariin on the purified PDE5 isoforms as well its enhancing effect on cGMP levels in cavernous smooth muscle cells have been recently confirmed [14]. Among different Epimedium species tested by Shen *et al.* [15] for their estrogenic properties, *E. koreanum Nakai* exhibited high estrogenic activity on both ERα and ERβ receptors, probably related to its content in prenylflavonoids. Specifically, two icariin metabolites, icaritin and desmethylicaritin, have been reported to possess estrogen-like activity *in vivo* [16].

The other plants contained in the extract show several pharmacological activities, but as yet their effect on sexual behavior is unknown.

The dried roots of *M. officinalis How* (Rubiaceae) have been extensively used in China over the past two centuries as a tonic, and as an antirheumatic and analgesic agent [17]. In addition, an antidepressant-like activity of the plant has been demonstrated in different animal models [18].

C. cassia (Lauraceae) has been traditionally used to treat gastric disturbances, and cardiovascular and inflammatory diseases. Evidence of its pharmacological activities, including antiulcerogenic, antipyretic, analgesic and anxiolitic ones, has been provided [19]. A herbal Chinese remedy (containing the bark of *C. cassia*) traditionally used in the treatment of gynecological disorder, has been shown to negatively affect plasma levels of lutenizing hormone (LH), follicle stimulating hormone (FSH) and estradiol (E2) in subchronically treated rats [20]. However the role of *C. cassia* in the LH and E2 antagonistic effect has not been clarified.

E. caryophyllata (Syzigium aromaticum) of *Merr & Perry* (*Myrtaceae*), has been demonstrated to exert antimicrobial, antifungal, antiviral and antioxidant activities as well as to possess anti-inflammatory, cytotoxic and anaesthetic properties [21].

In the present study we examined the effect of the acute and subchronic administration of a Chinese herbal extract on male rat copulatory behavior. The evaluation of sexual motivation and performance was carried out both in sexually potent rats and in sexually sluggish/impotent ones. In order to understand the mechanism of action of the tested preparation, its effect on T and LH serum levels was assessed. Furthermore, considering the key role played by dopamine in the regulation of sexual behavior [22–25], we investigated the influence of the Chinese herbal extract on the central dopaminergic system using a microdialysis technique.

2 Materials and methods

2.1 Animals

Sprague-Dawley rats of either sex, weighing from 160 g (female rats) to 220 g (male rats), were purchased from Harlan Italy (Udine, Italy). They were housed in

groups of four, male and female rats separately, in plexiglass cages, and were maintained under controlled laboratory conditions (22 ± 1ºC, 60% humidity) in a reversed 12 h:12 h light : dark cycle, with lights off at 9.00 hours. Commercial rat pellets and water were always available. The animals were accustomed to our housing conditions for at least 2 weeks before being used.

The female rats were ovariectomized under ketamine hydrochloride (Ketavet 10 Farmaceutici Gellini, Latina, Italy) plus xylazine hydrochloride (Rompun; Bayer AG, Leverkusen, Germany) anesthesia and brought into estrous by sequential subcutaneous injections of 30 μg estradiol benzoate (Estradiolo; AMSA, Roma, Italy) and 500 μg progesterone (Prontogest; AMSA) 48 h and 4 h before the copulatory studies, respectively. The female rats were screened with non-experimental sexually experienced male rats 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 Italian law (D. L. n. 116/1992) and European legislation (EEC n. 86/609). The experimental design and procedures received the approval of the Bioethical Committee of the Italian National Institute of Health.

2.2 Copulatory behavior

The sexual behavior of male rats 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 [26, 27]:

• Mount latency (ML): time from the introduction of the female until the first mount

• Intromission latency (IL): time from introduction of the female to the first intromission (vaginal penetration)

• Ejaculation latency (EL): time from the first intromission to ejaculation

• Post-ejaculatory interval (PEI): time from ejaculation to the first intromission of the second copulatory series

• Mount frequency (MF): number of mounts preceding ejaculation

• Intromission frequency (IF): number of intromissions preceding ejaculation.

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. Male rats were trained with sexually receptive female rats seven times before the experimental test. After the seventh pre-experimental training test, rats achieving ejaculation in the last three tests were defined as sexually potent. The remaining rats, who failed to achieve ejaculation in one, two or all three pre-experimental tests, were considered sexually sluggish or impotent [28].

2.3 Treatments

The Chinese herbal dried extract, consisting of *L. barbarum* fruits (39.5%), *E. koreanum* leaves (23.7%), *M. officinalis* roots (23.7%), *C. cassia* bark (7.9%) and *E. caryophyllata* flower buds (5.3%), was supplied by Indena S. p. A. (Milan, Italy). The extract, dissolved in Tween 80 (10%) and water, was acutely administered, in the volume of 5 mL/kg body weight, by oral gavage at three dose levels (30, 60 and 120 mg/kg), 45 min before the mating test. In subchronic experiments the extract was administered, at the same dosages, daily for 10 consecutive days and the mating test was carried out 45 min after the last dose. Control animals received vehicle solution (Tween 80 and water).

2.4 T and LH assays

Sprague–Dawley male rats (250–300 g body weight), which were not used for the sexual behavior study, were randomly divided in two groups of eight rats in each: the first one was acutely administered with a dose of 60 mg/kg of the Chinese herbs extract, 45 min before being killed. The second one was treated daily with the same dose of extract for 10 days and killed 24 h after the last dose. Trunk blood was collected into centrifuge tubes and serum was prepared by centrifugation (1 500 × *g*, 20 min, 4ºC) and stored frozen until assayed.

LH and T concentrations were determined in duplicate using LH ELISA (IBL, Hamburg, Germany) and a Testosterone Enzyme Immunoassay kit (Assay Designs, Ann Arbor, MI, USA), respectively, according to the manufacturer's instructions. The detection limit for LH assay was 0.3 ng/mL. The detection limit for the T as-

say was 10.0 pg/mL; cross-reactivity with corticosteroid and other androgens was minimal (< 1%).

2.5 Microdialysis procedure

Male Sprague-Dawley rats (250–300 g body weight) were killed with a short acting barbiturate, equithesin, i.p. injected with a volume of 3.5 mL/kg. Following exposure of the skull, a vertically oriented probe was lowered into the right nucleus accumbens: coordinates relative to Bregma were AP = +1.8 mm, ML = +1.2 mm and DV = –8.3 mm, according to the atlas by Paxinos and Watson [29]. After surgery, all rats were housed individually in transparent plexiglass cages with food and water freely available.

Microdialysis probes, constructed as previously described with minor modifications [30], incorporated a hollow acrylonitrile-sodium methallyl sulfonate fiber (Filtral 12 AN69 HF, Hospal Industrie, Meyzieu, France). The exposed length of dialysis surface area was 2 mm. Probes were perfused at a flow rate of 2 μL/min with a modified Ringer solution containing NaCl (147 mmol/L), KCl (4.0 mmol/L), $CaCl_2 \cdot 2H_2O$ (2.2 mmol/L), $MgCl_2 \cdot 6H_2O$ (1.0 mmol/L), adjusted to pH 7.4 with $Na_2HPO_2 \cdot 12H_2O$. Probes were calibrated *in vitro* prior to implantation by being placing in a standard solution containing dopamine (DA) and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) (1 μmol/L). Dialysate samples were collected over 30 min intervals and the concentrations of DA and DOPAC were quantified by high performance liquid chromatography using an electrochemical detector (HPLC-ED). The system consisted of a Merck-Hitachi model L-6200A pump (Merck-Hitachi, Poole, Dorset, UK), a Rheodyne 7295 injector (Bensheim, Germany), a Lichrosphere RP-C18 column, 4.6 mm × 250 mm, 5 μm (Merck KgaA, Darmstadt, Germany) and an electrochemical detector (Model 5100A, ESA Coulochem, Chelmsford, MA, USA), set at + 300 mV. The mobile phase consisted of 50 μmol/L EDTA, 0.4 nmol/L sodium octylsulphonate, 50 mmol/L citric acid, pH 2.9 (adjusted with 1 mol/L KOH) and 8% methanol (v/v). The flow rate of the mobile phase was set at 1 mL/min. Recovery was calculated as the ratio between the concentration of recovered substance in the dialysate and the concentration in the standard solution, multiplied by 100. Recoveries obtained *in vitro* were 30.0% ± 2.0% (DA) and 41.0% ± 3.0% (DOPAC).

Experiments were carried out 24 h after probe implantation. Baseline dialysis samples were collected 90, 60 and 30 min before the pharmacological treatment. The animals were orally administered with either the vehicle solution or the Chinese herbal extract dosed at 60 mg/kg: thereafter dialysis samples were collected at 30 min intervals for 150 min and analyzed for DA and DOPAC, as described above.

2.6 Statistical analysis

The results are expressed as means ± SEM obtained by groups of 8–12 rats. For statistical comparison one-way ANOVA followed by Dunnett's test was used in behavioral experiments while unpaired *t*-test was used in biochemical and microdialysis studies. The percentages of mounting and ejaculating rats in the treated and the control group were compared using Fisher's test. In each 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, CA, USA).

3 Results

3.1 Male rat copulatory behavior

The effect of the Chinese herbal extract was evaluated in sexually potent and in sexually sluggish/impotent rats, after acute and subchronic oral administration at three dose levels (30, 60 and 120 mg/kg).

In sexually potent rats, the extract acutely administered at 30 and 60 mg/kg, significantly reduced ML ($F[3, 28] = 5.924$, $P < 0.05$ and $P < 0.01$, respectively), but only at the dose of 60 mg/kg strongly reduced IL in comparison with the controls ($F[3, 28] = 5.355$, $P < 0.01$) (Table 1). In potent rats, EL and PEI were not affected by the treatments. The highest dose (120 mg/kg) did not significantly influence the different parameters. It must be stressed that all treated animals (100%) mounted and ejaculated during the test time, as did the controls.

When the extract was administered daily for 10 consecutive days, a reduction in ML ($F[3, 28] = 8.175$, $P < 0.05$) and IL ($F[3,28] = 6.757$, $P < 0.05$) was detected following the administration of the lowest dosage (Table 2). A more significant reduction in the same parameters (ML: $F[3, 28] = 8.175$, $P < 0.01$; IL: $F[3, 28] = 6.757$, $P < 0.01$) was observed after the ingestion of the extract at the dose of 60 mg/kg. The highest dosage did not affect copulatory behavior, as it happened after the acute administration. Also, in this case the percentage of mounting and ejaculating animals was 100% in all ex-

Table 1. Copulatory behavior of sexually potent rats acutely administered with the extract. Treatments were performed 45 min prior to the test. Values are the mean ± SEM obtained in groups of eight rats each. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with vehicle-treated rats (ANOVA followed by Dunnett's test). ML, mount latency; MF, mount frequency; IL, intromission latency; IF, intromission frequency; EL, ejaculation latency; PEI, post-ejaculatory interval.

Treatment (mg/kg)	ML (s)	MF (*n*)	IL (s)	IF (*n*)	EL (s)	PEI (s)
Vehicle	111.9 ± 9.8	10.4 ± 1.9	121.1 ± 10.5	13.7 ± 1.9	436.6 ± 44.9	400.8 ± 24.8
Extract 30	55.9 ± 16.1*	10.9 ± 2.0	74.5 ± 19.0	13.1 ± 2.1	464.5 ± 87.2	389.5 ± 49.7
Extract 60	36.5 ± 6.3**	9.1 ± 1.2	41.7 ± 6.0**	10.0 ± 1.4	460.4 ± 74.2	360.4 ± 41.1
Extract 120	67.1 ± 17.2	14.1 ± 1.7	82.6 ± 17.0	12.5 ± 1.4	408.5 ± 73.0	349.5 ± 53.5

Table 2. Copulatory behavior of sexually potent rats subchronically administered with the extract. Values are the mean ± SEM obtained in groups of eight rats each. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with vehicle-treated rats (ANOVA followed by Dunnett's test). ML, mount latency; MF, mount frequency; IL, intromission latency; IF, intromission frequency; EL, ejaculation latency; PEI, post-ejaculatory interval.

Treatment (mg/kg)	ML (s)	MF (*n*)	IL (s)	IF (*n*)	EL (s)	PEI (s)
Vehicle	92.7 ± 17.5	12.6 ± 2.5	135.5 ± 19.3	11.1 ± 0.7	498.8 ± 78.5	370.6 ± 15.6
Extract 30	40.4 ± 11.8*	9.0 ± 1.7	69.1 ± 15.2*	10.2 ± 1.5	407.8 ± 28.3	364.5 ± 16.9
Extract 60	17.9 ± 3.1**	7.7 ± 1.0	36.6 ± 10.4**	9.0 ± 0.6	358.9 ± 47.2	342.9 ± 10.2
Extract 120	75.0 ± 9.9	12.9 ± 1.5	96.2 ± 18.0	10.9 ± 1.7	537.0 ± 102.7	420.0 ± 28.1

Table 3. Copulatory behavior of sluggish/impotent rats acutely administered with the extract. Treatments were performed 45 min prior to the test. Values are the mean ± SEM obtained by groups of 12 rats each. $^{*}P < 0.05$, compared with vehicle-treated rats (ANOVA followed by Dunnett's test). ML, mount latency; MF, mount frequency; IL, intromission latency; IF, intromission frequency; EL, ejaculation latency; PEI, post-ejaculatory interval.

Treatment (mg/kg)	ML (s)	MF (*n*)	IL (s)	IF (*n*)	EL (s)	PEI (s)
Vehicle	542.8 ± 96.0	14.6 ± 2.0	556.5 ± 92.2	12.4 ± 1.5	1 447.0 ± 133.9	659.0 ± 63.3
Extract 30	437.2 ± 88.2	18.0 ± 3.4	447.0 ± 85.9	15.8 ± 1.9	902.3 ± 196.5	603.4 ± 65.3
Extract 60	390.8 ± 84.6	18.2 ± 2.7	399.1 ± 83.1	13.9 ± 2.2	769.2 ± 151.1*	533.7 ± 60.8
Extract 120	597.3 ± 108.8	20.6 ± 3.7	604.6 ± 106.6	16.6 ± 3.3	1 423.0 ± 165.8	735.9 ± 78.8

Table 4. Copulatory behavior of sluggish/impotent rats subchronically administered with the extract. Values are the mean ± SEM obtained in groups of 12 rats each. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with vehicle-treated rats (ANOVA followed by Dunnett's test). ML, mount latency; MF, mount frequency; IL, intromission latency; IF, intromission frequency; EL, ejaculation latency; PEI, post-ejaculatory interval.

Treatment(mg/kg)	ML (s)	MF (*n*)	IL (s)	IF (*n*)	EL (s)	PEI (s)
Vehicle	526.3 ± 92.6	10.6 ± 1.4	529.7 ± 91.9	12.4 ± 2.2	1 324.0 ± 123.4	609.1 ± 81.3
Extract 30	266.6 ± 86.1*	12.2 ± 1.4	276.5 ± 84.8*	10.0 ± 1.5	1 098.0 ± 134.2	558.7 ± 72.7
Extract 60	94.3 ± 14.5**	11.4 ± 1.2	100.0 ± 13.9**	10.6 ± 1.0	812.1 ± 111.1*	481.9 ± 73.4
Extract 120	697.3 ± 70.3	13.7 ± 2.0	700.5 ± 69.7	14.7 ± 3.0	1 341.0 ± 144.5	695.5 ± 74.7

perimental groups.

In sluggish/impotent rats, the acute administration of the extract, only when dosed at 60 mg/kg, significantly reduced EL ($F[3, 44] = 4.594$, $P < 0.05$, compared with controls), without affecting the other copulatory parameters (Table 3). Interestingly, the percentage of mounting and ejaculating rats increased from 58.3% (controls) to 83.3% (treated rats). No significant effect

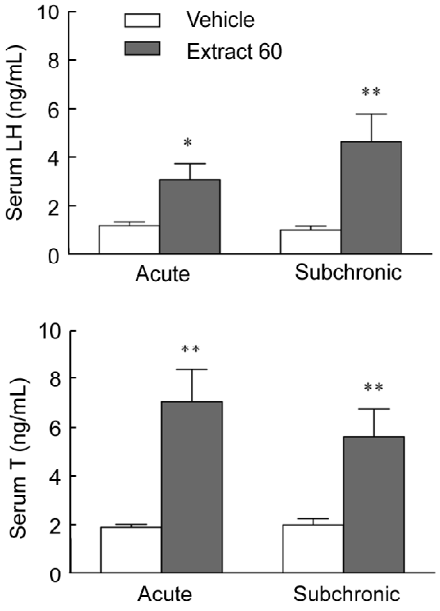

Figure 1. Luteinizing hormone (LH) and testosterone (T) serum levels in male rats acutely or subchronically treated with the extract dosed at 60 mg/kg. Each bar represents the mean ± SEM obtained by groups of 8 rats each. Statistical significance was calculated by unpaired *t*-test: $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the vehicle group.

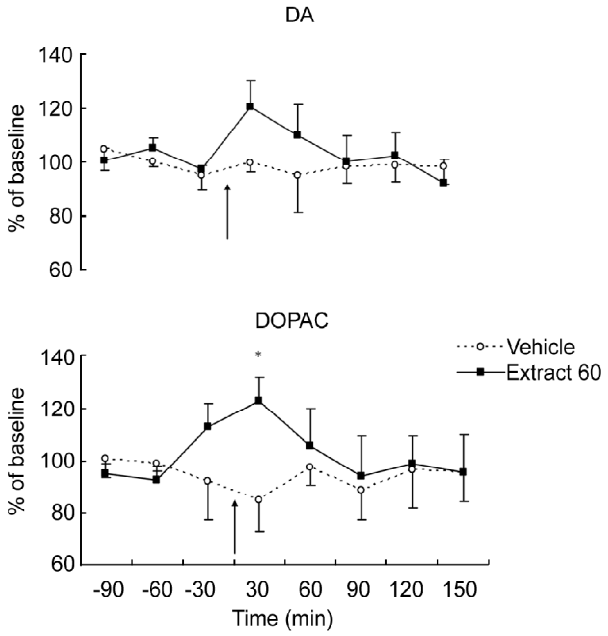

Figure 2. Time course of changes in extracellular dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) in the nucleus accumbens of rats administered with the extract dosed at 60 mg/kg. The extract or vehicle was given at the time point indicated by the arrow. For each subject, DA and DOPAC concentrations at each time point were calculated as a percentage of the mean value of 5 baseline samples. Statistical significance was calculated using unpaired *t*-test: $^{*}P < 0.05$.

was detected following the administration of the other dosages (30 and 120 mg/kg).

In subchronically-treated sluggish/impotent animals, the extract at the lowest dose (30 mg/kg) significantly reduced mount and intromission latencies (ML: $F[3,44] = 13.59$, $P < 0.05$; IL: $F[3, 44] = 13.69$; $P < 0.05$, compared with controls), while at the dose of 60 mg/kg strongly reduced ML ($F[3, 44] = 13.59$, $P < 0.01$), IL ($F[3, 44] = 13.69$, $P < 0.01$) and also EL ($F[3, 44] = 3.679$, $P < 0.05$) (Table 4). It must be stressed that the subchronic treatment with the extract dosed at 60 mg/kg in sluggish/impotent animals normalized the copulatory ability in all animals; therefore, the percentage of mounting and ejaculating rats increased from 66.7% (controls) to 100% (treated rats).

3.2 Serum hormone (T and LH) levels

The Chinese herbal extract acutely administered at 60 mg/kg to male rats significantly increased LH and T serum levels in comparison with the controls ($t[7] = 2.861$, $P < 0.05$; $t[7] = 3.916$, $P < 0.01$, respectively) (Figure 1). The daily administration of the same dosage for 10 days elicited a more consistent increase in LH levels ($t[7] = 3.044$, $P < 0.01$) measured 24 h after the last dose. In parallel, a significant increase in T serum levels was observed in comparison with the controls ($t[7] = 3.119$, $P < 0.01$).

3.3 Microdialysis study

A modest and transient increase in DA levels was observed in the nucleus accumbens of rats during the first hour following the administration of the extract dosed at 60 mg/kg. Significantly higher DOPAC levels, which peaked at 30 min after extract administration, were detected in the same animals ($t[4] = 2.943$, $P < 0.05$, compared with vehicle-treated group) (Figure 2).

4 Discussion

The present study provides evidence that the extract containing *L. barbarum* fruits, *E. koreanum* leaves, *M. officinalis* roots, *C. cassia* bark and *E. caryophyllata*

flower buds improves copulatory behavior in male rats.

Both acute and subchronic administration of the extract in potent rats significantly enhanced sexual motivation, as evidenced by reduced mount and intromission latencies. There is, indeed, a general and shared opinion that the shortening of these parameters (ML and IL) points out a stimulation of the appetitive component of the sexual response. In both experimental conditions the major efficacy was obtained by administering the dose of 60 mg/kg.

The most interesting results of our study were obtained in sexually sluggish/impotent rats. The acute administration of the extract, dosed at 60 mg/kg, increased copulatory performance by reducing ejaculation latency, whereas the subchronic administration at the same dose enhanced both sexual arousal and performance, affecting mount, intromission and ejaculation latencies. Particularly noticeable was the restoration of copulatory activity that occurred in sluggish and even in impotent rats, after subchronic treatment.

At this stage, it was crucial to identify which of the five plants contained in the Chinese extract was responsible for the observed pharmacological effect. For this purpose we administered the extract of each plant alone in male rats, applying the dosages equivalent to their percentages in the total formulation. These preliminary experiments demonstrated that at the used dosages each plant administered alone was unable to elicit significant effects (data not shown). Consequently, we believe that only the extract obtained from the combination of the five different plants can exert a stimulatory influence on copulatory behavior, as a result of a synergistic phenomenon. Our findings are not in contrast with the demonstrated aphrodisiac property of some medicinal plants present in the tested extract, such as *L. barbarum* [3] and *E. koreanum* [31]. Differences in the used dosages or the extraction procedure might be responsible for the lack of the pharmacological effect in our experimental conditions.

Our investigation into the mechanism of action of the Chinese herbal extract covered hormonal and neuronal components, which are both involved in sexual behavior.

The serum levels of T and LH hormones, measured 45 min after the single dose or 24 h after the last treatment in the case of subchronic administration, appeared significantly increased in both situations.

It is well known that T is produced by Leydig cells of the testes in response to LH, under the control of the hypothalamic-pituitary-testis axis. It seems reasonable to argue that the elevation in both LH and T serum levels following the acute and subchronic administration of Chinese herbs may be responsible for the observed improvement in copulatory behavior.

Besides the suggested involvement of the pituitary-gonadal axis in the pharmacological effect of the Chinese extract, we hypothesized the possible implication of a particular neurotransmitter system in the brain. Using the microdialysis technique, we evaluated the extracellular concentration of dopamine and its metabolite DOPAC in the nucleus accumbens (NAc), which is the target of mesolimbic fibers coming from the ventral tegmental area. There is evidence of a correlation between dopamine release in the NAc and both appetitive and consummatory components of sexual behavior in male rats [22, 32–34]. In our experimental conditions the increase of extracellular DA in NAc of rats treated with the extract did not reach statistical significance, but there was a significant increase in the concentration of DOPAC in the same area, suggesting either an increased synthesis or release of DA. As suggested by Damsma *et al.* [33], it is possible that a relatively small increase in DA release is not sufficient to overcome the highly efficient intrasynaptic DA uptake system and, consequently, results in a non-significant change in extracellular and dialysate DA concentrations. In such a case, however, altered metabolite levels might better reflect changes in DA transmission.

It has been hypothesized that testosterone primes neural circuits for sexual behavior through enhancement of dopamine release in one or more neural integrative systems that coordinate motivation and behavior [23]. In particular, testosterone may promote copulation through the upregulation of NO synthase activity in the medial preoptic area. As a result, both basal and copulation-induced dopamine release are enhanced [23, 35]. From the present data we can only surmise that the observed increase in testosterone concentration, following the Chinese extract administration, could affect the dopaminergic transmission in the brain.

In conclusion, our study demonstrates that the tested extract was able to improve sexual behavior in rats, particularly after repeated treatments. Interactions with hormonal (LH and T) and neuronal (dopaminergic) pathways have been demonstrated. Although further experiments are needed to elucidate the complex mechanisms

involved in the described stimulation of sexual behavior, particularly noticeable in sluggish/impotent rats, we have provided a reasonable linkage between the described pharmacological effects and some endocrinological and neurochemical correlates.

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