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Mohammed Zohair Allouh, Jordan University of Science and Technology
Nabil A. Khouri, Jordan University of Science & Technology
Haytham M. Daradka, Jerash National University
Ezidin G. Kaddumi, The Hashemite University

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Orchis anatolica Root Ingestion Improves Sexual Motivation and Performance in Male Rats

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Abstract

Orchis anatolica Boiss. is a plant species that grows in the Mediterranean region and assumed to have an aphrodisiac property. This study investigates the effects of ingestion of dried O. anatolica root on the sexual behavior and weights of reproductive organs in adult male rats. Sexually active male rats were divided into two groups (control and experimental). O. anatolica root powder was orally administered to the experimental group in a daily dose of 1g/kg for thirty days. The sexual behavior was then monitored and compared between O. anatolica treated rats and control rats. Weights of reproductive organs and testosterone serum levels were, also, measured for both treated and control rats. O. anatolica root ingestion significantly reduced the mount, intromission, and ejaculation latencies. Also, a significant increase in the copulatory efficacy was reported. Weights of reproductive organs and testosterone serum levels were significantly increased in O. anatolica treated rats compared to controls. The present study concludes that continuous ingestion of O. anatolica root over a period of thirty days can improve the sexual motivation and performance in adult male rats. This effect can be ascribed to increased testosterone level.

KEYWORDS: copulatory, orchids, reproductive organs, sexual behavior, testosterone

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Introduction

Orchidaceae (orchids) is the largest family of the plant kingdom with an estimated 800 genera that include about 24,000-25,000 species (Gravendeel et al. 2004; Fay and Chase, 2009). Several species of this family are considered popular for their supposed aphrodisiac property (Cicero et al. 2001; Bythrow, 2005; Thakur and Dixit, 2007). Special parts of the tuberous roots and fleshy leaves of these species are considered as sexual stimulants and enhancers of male fertility (Bythrow, 2005; Thakur and Dixit, 2007).

*Orchis anatolica* Boiss. (O. anatolica) is a delicate-looking plant that belongs to the Orchidaceae family. It grows mostly in light-shaded pine forests in several countries such as Greece, Turkey and Jordan. This plant blooms in the spring with pinkish to violet flowers that have large lips and long prominent spurs. It has a loosely attached tuberous root that resembles small potato (Fig. 1). Khouri et al. (2006) reported that ingestion of dried *O. anatolica* root bulb can improve the reproductive potential and fertility outcome in adult male mice. They showed that ingestion of *O. anatolica* root by adult male mice increases the number of pregnant females when left to mate.

Until now there is no information about the effects of this plant on the sexual desire and potency. Sexual impotence, recently known as erectile dysfunction, is defined as the “inability to achieve or maintain a penile erection sufficient for sexual satisfaction” (NIH Consensus Development Panel on Impotence, 1993). This problem is associated with adverse effects on the quality of life, specially family and social interrelationships (Laumann et al. 1999; Johannes et al. 2000). The use of herbal medicine, enriched by information from plant research, has always played a primary role in the treatment of this condition (Hollister, 1975).

Based on the previous facts, the current study aims to investigate the effect of *O. anatolica* on the sexual behavior of adult male rats. It tests the hypothesis that daily ingestion of *O. anatolica* root over a period of thirty days can improve the sexual behavior in adult male rats. Also, reproductive organ weights and serum testosterone level are assessed in those animals in order to elucidate the mechanism of action of this plant in influencing the sexual behavior.

Materials and Methods

Experimental model

Adult male albino rats of sprague-dawley strain, weighing about 300gm, produced and raised in Animal House Unit in The Faculty of Medicine at Jordan University of Science and Technology (J.U.S.T.) were used in this study. All animal care
procedures and treatments were conducted with the approval of the J.U.S.T. Committee on animal care, and in accordance with the guidelines of the National Institute of Health on the use and care of laboratory animals (USA).

Rats were allowed for three pre-experimental mating tests with sexually receptive females and those who achieved ejaculations in the three times within a period of less than thirty minutes were chosen for this study. The chosen rats were divided randomly into two groups, control versus experimental, and caged separately. Each group contained twelve male rats. The animals were kept under controlled temperature of 21 ±1˚C and 12 hours light: 12 hours darkness schedule (lights on 06.00–18.00 hr). Food and water were available ad libitum. The rats were allowed for two weeks acclimatization period before starting the treatment.

Herbal preparation & animal treatments

*O. anatolica* plant was harvested from northern Jordan mountainous region during the spring (March-April). The harvested plant was identified by a specialized botanist (Dr. Ahmad El-Oqlah, Yarmouk University). Certain specifications for collection were adopted so that the tuberous parts of the root are preserved intact. *O. anatolica* roots were separated and air dried for two weeks. Dried roots were grinded into powder to facilitate their oral administration to the animals in a dose of 1g/kg/day as one single dose in the morning for thirty days. This dose was chosen based on previous pilot studies (unpublished) in which we administered four different doses; 0.5, 1, 1.5 and 2 g/kg/day for four weeks to determine the effects on sexual behavior. The dose of 1g/kg was the minimum dose to show significant differences in the sexual behavior parameters. This dose is considered comparable with the human dose, since an average human usually consumes between 1-3g/kg daily of dried orchis root in the form of a hot beverage known as salep (Tamer et al. 2006; Tekinsen and Güner, 2010). Each dose was mixed with 4ml distilled water before being administered by oral gavage to facilitate its ingestion. Control rats received the same volume of distilled water only.

Female rats of the same strain were used in this experiment. Each female was brought into estrous by sequential subcutaneous injections of 30μg estradiol benzoate (Intervet International B.V., Holland) and 1mg progesterone (Schering AG, Germany) 48 hrs and 4 hrs before testing, respectively (Ågmo, 1997). The females were screened with non-experimental males and the ones that show good sexual receptivity (solicitation and Lordosis in response to mounting) were used.

Sexual behavior test

The sexual behavior of male rats was monitored by two trained observers unaware of the experimental design in a sound-attenuated room according to the standard
procedure (Ågmo, 1997). The test was performed after the last administration during the dark phase of the light/dark cycle (at 19:00 hr). Single male rat was placed in a rectangular Plexiglas observation chamber (45 x 40 x 30 cm height) and allowed to acclimate for 5 min. Then a sexually receptive female rat was introduced in the chamber. The following parameters of sexual behavior were measured as described by Ågmo (1997):

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 until 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.

Also, the following parameters were calculated on the basis of the above data:

7. Inter-intromission interval (III): 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 post-ejaculatory intromission.

Weights of reproductive organs

Four rats from each group, which were not submitted to mating tests, were used to analyze the weights of reproductive organs and serum testosterone level. Animals were weighed and euthanized by ethyl ether 24 hrs after the last dose. Trunk blood was collected into centrifuge tubes for serum testosterone assay (see below). The reproductive organs were then dissected out and trimmed free of fat. Both sides of each organ were weighed together on an electronic balance scale and the results were then compared between treated and control groups.

Testosterone assay

Serum was prepared by centrifugation of the collected trunk blood at 3000 r.p.m. for 30 min, and then stored frozen (-20°C) until testosterone assay. The testosterone concentration was determined in triplicate experiments using the Testosterone Enzyme Immunoassay test kit (BioCheck Inc., Foster City, California, USA) according to the manufacturer’s instructions. The minimum detectable concentration of this assay was estimated to be 0.05 ng/ml and cross reactivity with other corticosteroids was minimal (<0.05%).
Statistical analysis

After applying Levene's test to determine the homogeneity of variance, data were evaluated at 5% and 1% levels of significance by using t-test for independent samples. The data are presented as mean ± standard deviation (SD). All statistical tests were performed using SPSS program (standard version 13.0, SPSS Inc., Illinois, USA).

Results

Sexual behavior

The effect of treatment with O. anatolica dried roots on the sexual behavior of adult male rats is shown in Table 1. Treatment with O. anatolica root induced significant (P<0.01) reductions in mounting, intromission and ejaculation latencies in comparison with corresponding values of control rats. In addition, there were significant (P<0.05) reductions in both postejaculatory and inter-intromission intervals between O. anatolica treated and control rats. Also, a significant (P<0.01) increase in the copulatory efficacy was detected in O. anatolica treated rats (85%), compared with the controls (73%).

Weights of reproductive organs

Total body and reproductive organ weights were calculated and compared between O. anatolica treated and control rats (Table 2). The weights of testes and epididymides were found to be increased significantly (P<0.05) in O. anatolica treated rats compared to controls. However, there were no significant variations in the weights of total body, seminal vesicles and vasa deferentia.

Testosterone serum levels

Treatment with O. anatolica dried roots significantly (P<0.01) increased serum testosterone level in comparison with vehicle treated animals (Fig. 2). Serum testosterone level in O. anatolica treated rats was 3.82 ± 0.58 ng/ml while in control rats 2.97 ± 0.73 ng/ml.

Discussion

This study is the first to reveal the ability of O. anatolica plant in improving the sexual desire and performance in adult male rats. We show that ingestion of the dried roots of O. anatolica, over a period of thirty days, significantly reduces the
mounting, intromission and ejaculation latencies in those animals. These parameters are considered inversely proportional to sexual motivation and desire (Beach, 1956). In addition, the copulatory efficacy is significantly increased in these animals due to *O. anatolica* treatment. Copulatory efficacy represents the efficiency of erection and penile orientation, and is considered an indication to sexual potency and performance (Agmo, 1997).

The enhancement in sexual desire and performance after ingestion of dried *O. anatolica* root could be ascribed to the significant increase in serum testosterone level. Several previous studies have related the increase in testosterone level to the ability of different herbs in improving the sexual function (Gauthaman et al. 2002; Zanoli et al. 2008; 2009). The effects of testosterone on the sexual behavior and erectile function are well established. Testosterone has been found to enhance the sexual interest, add to the frequency of sexual acts, and increase the incidence of nocturnal erections (Mulligan and Schmitt, 1993). These effects of testosterone are induced through multiple mechanisms that include central and peripheral pathways. Peripherally, testosterone has been found to enhance the erectile response of cavernous nerve and to increase blood flow into the sinuses of corpus cavernosum in the rat (Giuliano et al. 1993; Mills et al. 1998).

The exact mechanism of how *O. anatolica* boosts the testosterone level is not totally clear. Khouri et al. (2006) have reported that the ingestion of *O. anatolica* root results in a significant increase in the number of Leydig cells in adult male mice. They, also, showed a decrease in the number of degenerated Leydig cells in the same animals. It is anticipated that the increase in the population of Leydig cells may lead to additional secretion of testosterone which results in the enhancement of the sexual behavior.

Our finding that *O. anatolica* root ingestion induces significant weight increases in testes and epididymides of the rat coincides with what was previously reported in the mouse model (Khouri et al. 2006). These increases are considered an indication for enhanced physiological functions of those organs, unless other circumstances are present such as inflammations or tumors. Previous studies have strongly correlated between the weight of testes and sperm production inside them (Meistrich and Samuels, 1985; Taepongsorat et al. 2008). In fact, it has been shown that *O. anatolica* root ingestion leads to significant increases in the numbers of all germinal cell types and sperm dynamics within adult male mice (Khouri et al. 2006). It is expected that the increase in testes and epididymides weights occurred as a consequence of elevation in serum level of testosterone, since testosterone has been reported to play a role in increasing reproductive organ weights in a dose dependent manner (Kim et al. 2002; Roijen et al. 1997).

In conclusion, this study provides evidence that continuous ingestion of *O. anatolica* root diet enhances male sexual desire and performance. This
enhancement could be mostly ascribed to increased serum testosterone level. We used the dried root of the plant in this study since it is the traditional way to take this herb, and to avoid any interaction in the pharmacological effect.

Fig. 1 A photograph of two *Orchis anatolica* plants that were harvested from northern Jordan in March, 2009.
Table 1 Influence of thirty days *Orchis anatolica* root ingestion on the sexual behavior of adult male rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ML (sec)</th>
<th>MF</th>
<th>IL (sec)</th>
<th>IF</th>
<th>EL (sec)</th>
<th>PEI (sec)</th>
<th>CE</th>
<th>III (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>74.9 ± 15.6</td>
<td>5.1 ± 1.2</td>
<td>252.7 ± 30.6</td>
<td>14.3 ± 4.0</td>
<td>859.0 ± 109.7</td>
<td>394.9 ± 81.9</td>
<td>0.73 ± 0.04</td>
<td>65.8 ± 24.1</td>
</tr>
<tr>
<td><em>Orchis anatolica</em> (1g/kg/day)</td>
<td>27.0 ± 6.8**</td>
<td>1.9 ± 1.0**</td>
<td>68.9 ± 6.4**</td>
<td>9.5 ± 2.1**</td>
<td>376.1 ± 71.7**</td>
<td>317.4 ± 22.2*</td>
<td>0.85 ± 0.08**</td>
<td>41.7 ± 13.3*</td>
</tr>
</tbody>
</table>

Each value is representing the mean ± standard deviation, obtained for eight rats per group. *P<0.05, **P<0.01 compared with vehicle-treated rats (t-test). ML= mount latency; MF= mount frequency; IL= intromission latency; IF= intromission frequency; EL= ejaculatory latency; PEI= post-ejaculatory interval; CE= copulatory efficacy; III= inter-intromission interval.

Table 2 Influence of thirty days *Orchis anatolica* root ingestion on body and reproductive organ weights (g) of adult male rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight</th>
<th>Testes</th>
<th>Epididymides</th>
<th>Seminal Vesicles</th>
<th>Vasa Deferentia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>305.3 ± 23.7</td>
<td>2.99 ± 0.16</td>
<td>1.09 ± 0.05</td>
<td>1.52 ± 0.31</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td><em>Orchis anatolica</em> (1g/kg/day)</td>
<td>315.8 ± 30.9</td>
<td>3.31 ± 0.07*</td>
<td>1.30 ± 0.16*</td>
<td>1.76 ± 0.26</td>
<td>0.18 ± 0.02</td>
</tr>
</tbody>
</table>

Each value is representing the mean ± standard deviation, obtained for four rats per group. *P<0.05, compared with vehicle-treated rats (t-test).
Fig. 2 Influence of thirty days oral administration of *Orchis anatolica* dried root (1g/kg/day) on serum testosterone level in adult male rats compared to control rats. Each value is the mean ± standard deviation (SD) obtained for four animals in each group. **P<0.01 (t-test).

References


