Spermatogenic activity of rhizomes of *Curculigo orchioides* Gaertn in male rats

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**Summary:** The rhizomes of *Curculigo orchioides* Gaertn have been traditionally acclaimed as aphrodisiac. In the present study ethanolic extract of rhizomes was evaluated for its effect on orientation behavior and spermatogenesis in albino rats. A change in orientation behavior was assessed by orientation towards female, towards environment, towards self and type of mobility. Administration of 100 mg/Kg b. w. of ethanolic extract had pronounced effect on orientation of male towards the female rats. Males treated with the extract displayed more frequent and vigorous anogenital sniffing and mounting as compared to untreated animals. The increased spermatogenesis in treated group was confirmed by change in histoarchitecture as evidenced by increase in number of spermatocyte and spermatids. These findings support the folk use of this plant as aphrodisiac.

**Industrial relevance:** Study proposes to elucidate and demarcate the potential of Rasayan herbs in the treatment as well as uprooting the causes of disease; which is the underlying principle of Rasayan therapy. This leads to discovery of newer phytoconstituents with better activities and provide source of new biomolecules for biotechnologists to work on. The study explore the utilization of Rasanya herbs for effective management of sexual dysfunction. It brings out a competent literature on rasyana, validating their utilization. It gives a larger platform for the herbal cultivators by providing scientific support and data to the traditional unvalidated herbal drugs as rejuvenative tonics.

**Keywords:** Aphrodisiac, *Curculigo orchioides*, Rasayana drugs, Spermatogenesis

**Introduction**

Rasayana is a unique concept of Ayurveda which means vital nourishment (Rasa + Ayana) representing a holistic approach, responsible for preventive aspects against ageing as well as curative aspect against diseases (Mehrotra & Ojha 2006). In Ayurveda Vajikarana constitute the medicines which confer upon a man sexual power similar to that of a stallion are called “Vaajkarana” (literally, horse-making) (Sengupta & Nath 1994). There are number of plant derived compounds which are heavily promoted in commercial products for their procedural effects (Rowland & Tai 2003). The rhizomes of *Curculigo orchioides* Gaertn (Amaryllidaceae) are described in Ayurveda as a Vajikarana rasayana (Chunekar & Yadav 2005). Rhizomes of the plant are used as tonic, demulcent, diuretic and restorative (Chopra et al., 1956). The plant is reported to possess estrogenic (Vijayanarayana et al., 2007), pendiculatiry (Thakur & Dixit 2007), hepatoprotective (Rao & Mishra 1996a, b), immunostimulant (Lakshmi et al., 2003; Bafna & Mishra 2006) and antioxidant (Venukumar & Latha 2002) activities. We showed in a preliminary pharmacological study that ethanolic extract increases sexual behavior and mating performance male rats (Chauhan et al., 2007) and investigated antihyperglycemic activity in alloxan induced diabetic rats (Chauhan & Dixit, 2007). Present investigations were undertaken with a view to explain the effect of extract on spermatogenesis and sexual behavior.

**Material and Methods**

**Animal Stock:** The protocol for experimentation was approved by Institutional Animal Ethics Committee (Ref. no: NOEC/DB/365/5) of Dr H. S. Gour University, Sagar, India. Albino rats of either sex weighing 120-150 g were fed on standard diet and water *ad libitum*. The animals were housed at room temperature (24± 2°C) on a reversed day-night cycle (06:00 hrs to 18:00 hrs.).

**Plant material:** Rhizomes of *Curculigo orchioides* Gaertn were collected at Sagar M.P. (India) and taxonomically identified at the Department of Botany, Dr H. S. Gour University, Sagar. A voucher specimen of the same has been deposited (No. NSC-CO-2005) at departmental herbarium centre. The dried powdered rhizomes were defatted by extraction with petroleum ether (60-80°C). The defatted plant material was then extracted with ethanol (95%), and dried under vacuum (4.08 % w/w). The presence of alkaloids (Dragendorff, Mayer, Wagner and Hagers test), phenolic (Ferric
Chloride Test), tannins (Gelatin Solution Test), saponins (Foam test and Haemolysis test) and steroids (Lieberman Burchards Test) were confirmed by qualitative tests (Kokate 2003). The TLC of ethanolic extract in solvent system n-butanol saturated with water and chloroform: acetic acid: methanol: water (16:8:3:2) give 10 and 6 spots respectively (Chauhan 2006).

Preparation of test samples: Ethanolic extract (1%) and sildenafil citrate (0.05%) were suspended in 0.2% of gum acacia and administered orally (p.o) using metal canula. Testosterone propionate was suspended in arachis oil and was administered subcutaneously (s. c.).

Treatment: The animals were divided in groups of 6 male rats each. Group I animals served as control and received only vehicle i.e. 0.2 % gum acacia suspension. Group II were administered with ethanolic extract of C. orchioides (100mg/kg) daily. Group III animals were given subcutaneously 0.5 mg/Kg dose of Testosterone propionate suspension (Sun Pharma) twice weekly and served as positive control for anabolic and histopathological studies. Animals of Group IV received dose of Sildenafil citrate (Hetro Drugs) 5mg/Kg orally daily and served as positive control for behavior studies.

Effect on spermatogenesis: T The method reported by Saksena & Dixit 1987 as modified by Thakur & Dixit. 2006 was used. In brief, after 30 days of treatment the body weights of animals were taken after which the animals of control as well as treated groups were killed by rapid decapitation. Testis were removed and cut into small pieces, fixed in Bovine’s fixative, dehydrated with varying percentage of ethanol for histological studies. Sections were cut (6µ), stained with eosin and analyzed microscopically. Histometric measurements such as diameter of testes, seminiferous tubules and Leydigs cell nucleus were made by random selections of 30 circular sections by using ocular and stage micrometers. The numbers of different spermatogenic elements were also determined.

Orientation activity: The orientation of male rats towards female (licking, anogenital sniffing), towards self (non-genital grooming, genital grooming), type of immobility (restricted and non restricted) and towards environment (exploration, climbing, raring). The orientation behaviour was observed at 15, 30, 45 and 60 min after treatment to all groups. The severity of response can be scored as 1=presence, 2=moderately severe and 3= intense and continuous action. The cumulative score for each activity in the one-hour was calculated.

Statistical analysis: Results are expressed as mean ± SD. The significance of the data was evaluated using student t-test. The statistical analysis was carried out using Instat 2.1 software.

Results

Histopathological studies: The testis section of control group animals showed normal histological texture. The diameter of seminiferous tubules varied within a range. The tubules having maximum diameter, were not abundant and well within range. The cuboidal germinal epithelium exhibited normal shape and size. Sertoli cells had many cytoplasmic processes which were normal in size. Spermatozoa were embedded in the sertoli cells and showed normal cytoplasmic granularity. Leydigs cells had normal nuclear size. Luminal part of the tubule were normal in number with bundles of spermatozoa. Spermatozoa with long tail with small distinct head were more visible (Figure 1 and 4).

The extract treated group animals showed pronounced effects in terms of testis weight and histological alterations. Since the weight and size of the testis was greater in extract treated groups almost all seminiferous tubules showed greater diameter. The germinal epithelium cells appeared to be hyperactive. Large numbers of different cells at different stages of spermatogenesis were evident. Lumens of every seminiferous tubule had enormous number of spermatozoa. Sertoli cells were enlarged highly processed and rich in nutrients as evidenced by highly granulated cytoplasm. This was the normal response of the sertoli cells when they were in readiness for providing nutritional supplementation to large number of spermatozoa (Majumdar 1995). Almost all leydig cells showed hypertrophy with enlarged nucleus and darkly stained cytoplasm. Increment in the volume of cells and nucleus was strongly suggestive of steroid synthesis under the direct or indirect influence of the drug. Almost all tubules were overcrowded with sperm bundles. In some tubules, spermatids were found scattered amidst spermatozoa. The blood vessels of testis were slightly dilated. Histoarchitecture of testosterone treated group also exhibited similar profile. Increased spermatogenesis was evident by high number of spermatozoa in seminiferous tubules and which is evident by increase in spermatogenic elements as compared to control (Figure 2, 3, 5 and 6 and Table-2).

Orientation activities: Orientation activities (Table - 1) studies show that with treatment of the extract there is increase in attraction of male towards female. Increasing mounting frequency and anogenital sniffing was noticed as compared to control .The attraction towards environment is more in control than drugs treated group. There is also increase in attraction towards female and genital grooming in treated rats which is comparable with standard treatment.

Discussion

The present investigations bring forth the spermatogenic activity of ethanolic extract of rhizomes of C. orchioides in albino rats. Spermatogenesis involves a complex interplay between the structural elements of testis and the endocrine system. Hypothalamic gonadotrophic releasing hormone induces pituitary gonadotrophin (McLachlan 2000).

Abundance of spermatozoa in seminiferous tubule clearly indicates spermatogenesis which is regulated by hormone (Hadziselimovic & Herzog 1997). Hypertrophy of Leydigs cells is also suggestive of steroids synthesis. Earlier phytochemical investigations have shown the presence of glycoside, saponins and sterols (Rao et al., 1978, Xu et al., 1992) in rhizomes. It is likely that these steroidal constituent increase the steroidogenesis and elevate androgen levels which results in observed effect. Involvement of hypothalmo-pitutary axis by way of FSH stimulation can not be ruled out. Rhizome has been demonstrated to have antioxidant properties in-vitro and in-vivo (Bafna & Mishra 2005, 2011).
Table 1. Effect of Curculigo orchioides on Orientation Activities

<table>
<thead>
<tr>
<th>Orientation Towards</th>
<th>Control group</th>
<th>Extract group</th>
<th>Standard group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mounting</td>
<td>21</td>
<td>74</td>
<td>90</td>
</tr>
<tr>
<td>Licking</td>
<td>20</td>
<td>48</td>
<td>49</td>
</tr>
<tr>
<td>Anogential smelling</td>
<td>27</td>
<td>36</td>
<td>48</td>
</tr>
<tr>
<td>Environment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exploration</td>
<td>44</td>
<td>23</td>
<td>27</td>
</tr>
<tr>
<td>Rarring</td>
<td>58</td>
<td>28</td>
<td>11</td>
</tr>
<tr>
<td>Climbing</td>
<td>52</td>
<td>26</td>
<td>25</td>
</tr>
<tr>
<td>Self</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nongential grooming</td>
<td>26</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td>Gential grooming</td>
<td>24</td>
<td>40</td>
<td>58</td>
</tr>
<tr>
<td>Mobility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Restricted</td>
<td>16</td>
<td>31</td>
<td>60</td>
</tr>
<tr>
<td>Not restricted</td>
<td>24</td>
<td>21</td>
<td>16</td>
</tr>
</tbody>
</table>

Scored Point 1=presence, 2=moderately severe and 3= intense and continuous action.

Table 2. Effect of ethanolic extract of C. orchioides on spermatogenic element in male rats

<table>
<thead>
<tr>
<th>S. No</th>
<th>Size of Seminiferous tubules (µm)</th>
<th>Number of spermatogenic elements</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length</td>
<td>Breadth</td>
</tr>
<tr>
<td>Control Group</td>
<td>240.2±48.9</td>
<td>110.4±29.4</td>
</tr>
<tr>
<td>Extract Group (100 mg/kg)</td>
<td>308.8±41.5**</td>
<td>108.0±29.9**</td>
</tr>
<tr>
<td>Testosterone Group (0.5 mg/kg)</td>
<td>336.8±76.5**</td>
<td>116.0±23.0**</td>
</tr>
</tbody>
</table>

Values are Mean ± S.D; p < .001; All treated groups are compared with control group.

Figure 1: Histoarchitecture of testis section of control group X 100. LC = Leydig’s cell; SGM = Spermatogonia; SB = Sperm bundles; ST = Seminiferous tubules S = Space
Aphrodisiac activity of *Curculigo orchioides*

Figure 2: Histoarchitecture of testis section of Ethanolic extract treated group at X 100. LC = Leydig’s cell; GE = Germinal epithelium; SGM = Spermatogonia; SB = Sperm bundles; ST= Seminiferous tubules

Figure 3: Histoarchitecture of testis section of Testosterone treated group at X 100. LC = Leydig’s cell; GE = Germinal epithelium; SGM = Spermatogonia; SB = Sperm bundles; ST= Seminiferous tubules

Figure 4: Histoarchitecture of testis section of control group X 450. GE = Germinal epithelium; SGM = Spermatogonia; SB = Sperm bundles; 1PS = Primary spermatocyte; 2PS = Secondary spermatocyte; ST= Seminiferous tubules
Figure 5: Histoarchitecture of testis section of *C. orchioides* ethanolic extract treated group at X 450. LC = Leydig’s cell; BV = Blood vessels; GE = Germinal epithelium; SGM = Spermatogonia; SC = Serotoli cells; SB = Sperm bundles; 1PS = Primary spermatocyte; 2PS = Secondary spermatocyte SS = Spermatids

Figure 6: Histoarchitecture of testis section of Testosterone treated group at X 450. LC = Leydig’s cell; SGM = Spermatogonia; SB = Sperm bundles; 1PS = Primary spermatocyte

Venukumar & Latha 2002, Tang et al., 2004). The phenol and phenolic glycoside shows antioxidative property (Wu et al., 2005). These antioxidant defence systems are of major importance because peroxidative damage is currently regarded as the single most important cause of impaired testicular function underpinning the pathological consequences of a wide range of conditions from testicular torsion to diabetes and xenobiotic exposure (Aitken & Roman 2007). In a normal situation, the antioxidant mechanisms present in the reproductive tissues and their secretions are likely to quench these reactive oxygen species (ROS) and protect against oxidative damage to gonadal cells and mature spermatozoa (Sikka SC 2001). Antioxidant compounds also alter androgen level (Islam et al., 1991) and change in androgen level like testosterone may be responsible for spermatogenesis.

Reduced number of spermatozoa, mal formed spermatozoa or their reduced or insufficient motility are the leading causes of disturbed fertility or infertility in patient. The drug may thus provide an alternative for management of infertility due to reduced spermatogenesis. Further studies are necessary to elucidate the compounds of the ethanolic extract is responsible for enhances spermatogenesis in rats.

**Acknowledgments**

Authors gratefully acknowledge Hetro Drugs for providing Sildenafil citrate as a gift sample. One of the authors Nagendra S.Chauhan is also thankful to UGC Major Research Project for providing financial support.

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