Assessment of ‘Tentex royal’ for sexual activity in an experimental model

Gopumadhavan, S., Mohamed Rafiq, Venkataranganna, M.V., Kala Suhas Kulkarni, and Mitra, S.K.
R&D Center, The Himalaya Drug Company, Makali, Bangalore-562 123, India

ABSTRACT
A study was conducted to assess the efficacy of Tentex royal, a polyherbal formulation, in enhancing male sexual activity in an experimental model. The study involved virgin female rats, in oestrous state induced by administering oestrogen, and male rats, randomised into five groups and classified into control, sildenafil citrate reference standard group and Tentex royal-treated groups (125, 250 and 500 mg/kg) respectively, for 5 days. Parameters such as total sexual behaviour, mounting frequency, mounting latency, ejaculation frequency, ejaculation latency, serum testosterone levels and sperm count were carefully monitored. A significant improvement in all the parameters of sexual indices was observed in the Tentex royal group. Treatment with Tentex royal also showed an increase in sperm count and testosterone levels. Histological evaluation of the anterior pituitary revealed an increase in FSH-LH-producing basophils and a decrease in ACTH producing cells. The study revealed that Tentex royal improves erectile capacity. Considering the limitations of sildenafil citrate in clinical practice, Tentex royal is a safe and alternative treatment for correction of erectile dysfunction.

INTRODUCTION
Erectile dysfunction (ED) defined as the inability of a man to achieve or maintain an erection sufficient to satisfy his sexual needs or the needs of his partner, is a common problem found among men and increases with age. While there is an estimated prevalence across all ages in about 10%, the prevalence rises to over 50% in men between 50 and 70 years of age. A few men experience chronic, complete erectile dysfunction, and others achieve partial or brief erections. Frequent erectile dysfunction can cause emotional and relationship problems, which often leads to diminished self-esteem. According to the Massachusetts Male Aging Study (MMAS), a community-based survey of men aged 40-70 years, 52% of the respondents reported some degree of erectile difficulty. Complete ED, defined as the total inability to obtain or maintain erections during sexual stimulation as well as the absence of nocturnal erections, occurred in 10% of the respondents. Lesser degrees of mild and moderate ED occurred in 17% and 25% of respondents.

Penile erections involve an integration of complex physiologic processes involving the central nervous system (CNS), peripheral nervous system and hormonal and vascular systems. Any abnormality involving these systems, whether from medication or disease, has a significant impact on the ability to develop and sustain an erection, ejaculate and experience orgasm. The vascular filling of the corpora cavernosa relies upon neural and hormonal
mechanisms operating at various levels of the neural axis. This is unique among visceral functions, as it requires central neurological input. The penile element of the process leading to erections represents only a single component. The ability to achieve and maintain a full erection also depends on the status of the peripheral nerves, integrity of the vascular supply and biochemical events within the corpora cavernosa.

Many plant extracts are traditionally employed among different cultures in order to improve sexual performance. The modern view of management of erectile dysfunction subscribes to a single etiology, i.e. the mechanism of erection. A large number of pharmacological agents are orally consumed and vasoactive agents inserted intra-urethrally or injected intrapenially to regain good erection, but these drugs are not free from side effects. In the present study, Tentex royal, a polyherbal formulation containing extract of Tribulus terrestris and powders of Asteracantha longifolia, Prunus amygdalus, Blepharis edulis and Crocus sativus, has been evaluated for its effects on sexual performance in experimental animals.

**MATERIAL AND METHODS**

**Experimental animals**

Sexually matured male and female Wistar rats were used for the study. Female rats were housed in polypropylene cages with free access to standard feed (Amruth Laboratories, Mumbai) and water. The rats were maintained at a temperature of 22 ± 2°C and relative humidity of 55-60%. A reversed twelve hour light-dark cycle was employed with fluorescent ceiling light. A dim red light was provided during the dark cycle. All the rats were allowed 2 weeks to adjust to the environment prior to the experiment. The experiments were carried out between 9.00 AM – 12.00PM.

**Preparation of females**

Female albino rats were brought to oestrous by single intramuscular injection of estradiol valerate at a dose of 2 µg/kg intramuscularly, 48 h before the test.

**Training of rats**

Male rats were trained individually with active female rats in oestrous in a transparent mating arena (60 x 30 x 18 cm) for 15 min. A male rat was considered sexually active if it attempted to mount any active female rat introduced into the cage. Only such male rats were used for subsequent experiments.

**Treatment**

Forty sexually active male rats were randomised and divided into 5 groups of 8 each. Rats of group I received 10 ml/kg b.wt. p.o. of water as vehicle and served as control. Group II rats received sildenafil citrate at a dose of 25 mg/kg b.wt. p.o. and served as the reference group. Rats from groups III, IV and V received Tentex royal at a dose of 125, 250 and 500 mg/kg p.o. respectively. Figure 1 shows the HPTLC finger print pattern of Tentex royal. To ensure and maintain quality control, standardisation was carried out from batch to batch. All drugs were administered in the form of aqueous suspension for a period of 5 days. On day 5, 1 hour after the respective assigned drug administration, the following parameters were observed.
a) **Mounting frequency:** Number of mounts in a given period of time (15 min).
b) **Mounting latency:** Time taken for the first mount after the introduction of the male rat into the mating arena.
c) **Ejaculation frequency:** Number of ejaculations in a given period of time (15 min).
d) **Ejaculation latency:** Interval between the first mount to first ejaculation.
e) **Total sexual behavior (TSB):** Male sexual behavior such as genital grooming and sniffing at females was visually monitored and recorded.

At the end of the trial, blood samples were collected from the retro-orbital plexus for the estimation of serum testosterone levels using RIA methods\(^5,6\). The rats were then euthanized under ether anesthesia and the cauda of epididymis was collected to perform the sperm count\(^7\). Pituitary glands were taken for all animals and stained for basophil cells producing LH, FSH and ACTH\(^8\).

**Extraction and TLC development procedure:**
- Weigh accurately 3.0 gms powder of capsules in 250 ml flat bottom flask.
- Extract with 20.0 ml of dichloromethane on water bath for 30 minutes at 45\(^\circ\)C.
- Filter the extract and evaporate to dryness.
- Reconstitute the residue in 3.0 ml of dichloromethane.
- Spot 15 µl of the filtered residue on pre-coated thin layer silica plate 60F 254, 20 x 20 cm.
- Develop the plate up to 15.5 cm in the system dichloromethane : methanol (97 : 3).
- Time for pre-saturation with solvent vapor is 2.0 hours.
- Dry the plate and expose to saturated iodine vapors for 10 minutes (as shown in the Fig. 1).

**Statistical analysis**
The data for all the groups were expressed as mean ± SEM. The results were analysed using ANOVA followed by Dunnett’s multiple comparison test. The level of confidence was fixed at 95%.
RESULTS

The total sexual behavior, mounting frequency and ejaculation frequency were significantly higher with a decrease in mounting latency and ejaculation latency in the Tentex royal-treated group as compared with the control group. The effect of Tentex royal was comparable to reference standard (Figs. 2 and 3). Tentex royal treatment also showed significant increase in testosterone production and sperm count as compared to control (Table 1). The same was not observed in the reference group, thus indicating a different mode of action by the reference drug.

**Table 1: Effect of Tentex royal on serum testosterone levels and sperm count in rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum testosterone (ng/dl)</th>
<th>Sperm count (10^6/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>236.50 ± 8.33</td>
<td>115.00 ± 5.13</td>
</tr>
<tr>
<td>Reference standard</td>
<td>230.75 ± 7.20</td>
<td>125.88 ± 5.86</td>
</tr>
<tr>
<td>(25 mg/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tentex royal</td>
<td>329.00 ± 17.07*</td>
<td>170.63 ± 8.65*</td>
</tr>
<tr>
<td>(500 mg/kg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05 as compared to control.

Histological evaluation of the anterior pituitary revealed an improvement in the cellular morphology after treatment with Tentex royal. The cell counts of LH-FSH producing basophils were higher, while there was a reduction in the population of ACTH-producing cells in the Tentex royal-treated groups when compared with the control group.

DISCUSSION

Male sexual behaviour depends on the circulating levels of testosterone in the blood. Improvement in mounting frequency, ejaculation frequency and sperm count in the Tentex royal-treated animals indicate that the drugs probably act by raising testosterone levels.

The gonadotropin-releasing hormone (GnRH) from the hypothalamus acts on the anterior pituitary to release both the FSH, which stimulates gametogenesis and LH, which stimulates androgen secretion. The histological evaluation in this study also revealed an increase in FSH-LH-producing basophil cells in anterior pituitary thus indicating a possible role of the hypothalamo-pituitary-gonadal axis.
The constituents such as *Prunus amygdalus*, *Crocus sativus* and *Tribulus terrestris* present in Tentex royal have been used in traditional medicine as aphrodisiacs. *Prunus amygdalus* particularly increases the sperm motility and sperm contents in the epididymis and vas deferens without producing any spermatotoxic effects. A phytochemical agent derived from *Tribulus terrestris* has been clinically proven to improve sexual desire and enhance erection via the conversion of protodioscine to dehydroepiandrosterone. *Tribulus terrestris* also relaxes the corpus cavernosal smooth muscles. This effect could be probably due to the increase in the release of nitric oxide from the endothelium and nerve endings, which may account for its aphrodisiac activity.

Thus, Tentex royal, a polyherbal formulation revealed potent activity in improving sexual performance in experimental animals. The pharmacodynamic activity of Tentex royal was comparable to that of the reference standard. No side effects were observed in the treated animals.

Clinical trials in patients with erectile dysfunction have shown that Tentex royal in recommended doses improves the erectile capacity significantly along with improvement in sperm count. Considering the limitations of using sildenafil citrate in clinical practice, Tentex royal is a safe and alternative treatment to correct erectile dysfunction.

REFERENCES

