Effect of *Tinospora cordifolia* supplementation on sexual behaviour and semen production in Muzzafarnagari rams

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Received: 07-09-2012 Accepted: 22-05-2013 doi: 10.5958/0976-0555.2015.00029.1

ABSTRACT

The present study was undertaken to assess the effect of dietary *Tinospora cordifolia* supplementation on sexual behaviour, semen production and serum testosterone level of Muzzafarnagari rams. Twelve rams were divided into two groups in which one group served as control while the other group was supplemented with *T. cordifolia* at the rate of 1g/kg body weight. Reaction time, sexual behaviour, seminal parameters and serum testosterone level of rams were evaluated. No significant changes were observed in reaction time, sexual behaviour and serum testosterone level of ram due to *T. cordifolia* supplementation in the diet.

Key Words: Muzzafarnagari rams, Seminal parameters, Serum testosterone, Sexual behaviour, *Tinospora cordifolia*.

*T. cordifolia* is basically an immunemodulator (Nair *et al.*, 2004) and has adaptogenic effects to help the body to adapt the stress. *Tinospora* may also have anti-cancer (Singh *et al.*, 2005), nerve cell protecting (Rawal *et al.*, 2004), anti-diabetic (Stanley *et al.*, 2003), cholesterol-lowering (Stanley *et al.*, 2003) and liver-protective (Bishayi *et al.*, 2002) actions. *Tinospora* has also been used to decrease the tissue damage caused by radiation (Subramanian *et al.*, 2002) and the side effects induced by chemotherapeutic drugs (Mathew and Kuttan, 1998). *T. cordifolia* is widely used in Indian ayurvedic medicine for treating diabetes mellitus.

*T. cordifolia* have been reported to contain a wide variety of antioxidants and have been in use in Indian system of medicine for various disorders. *T. cordifolia* has antioxidant property and has reduced reactive oxygen and nitrogen species (ROS/RNS), generated by photosensitization/peroxynitrite (Veena *et al.*, 2002).

There is scarcity of information on effect of *T. cordifolia* supplementation on semen quality, sexual behaviour and testosterone production in domestic animals. The present study was undertaken to study the effect of *T. cordifolia* supplementation on semen quality, sexual behaviour and serum testosterone level of rams.

Experimental Animals: The present study was conducted on twelve Muzzafarnagari rams of 1.5-2 years with good body condition (score 5-6) and average body weight of 38.3±1.23kg maintained at Animal Nutrition Division, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India. Therams were maintained as per the standard criteria fixed for maintenance of breeding rams during the entire course of investigation.

These twelve rams were randomly divided into two groups having six rams in each group. All these animals were fed with standard basal diet consisting of concentrate mixture and roughage (wheat straw) to meet their nutrient requirement according to NRC (1985). Semen and blood was collected from the animals after two months of feeding.

Formulation of diet and supplementation of *T. cordifolia* were according to body weight and total dry matter intake. All the animals were offered concentrate at 1% of body weight and wheat straw was *ad libitum*. The dietary regimen of the two groups was as follows: Group I (Control): Basal diet alone and Group II (Treatment): Basal diet + Guduchi (*T. cordifolia*) @ 1g/kg body weight.

The calculated quantity of *T. cordifolia* whole plant powder was added to the concentrate mixture of the treatment group so as to ensure its consumption at 1g/kg body weight.

Libido and sexual behaviour of rams: Six rams used for semen collection were closely observed for their libido and sexual behaviour at the time of semen collection. The scoring
was done as per the method of Anzar et al. (1993) in buffalo bull with modification. The behaviour of sexual aggressiveness of rams during approach towards the teaser was assessed visually as

Aggressive: Extremely eager to mount and approached the teaser with full vigor.

Active: Approached the teaser with less vigor and aggression

Dull: Proceeded with a dull expression and took a longer time to mount.

Shy: Exhibited mild sexual interest and was reluctant to mount.

Libido scoring (%) of ram was estimated as follow

\[
\text{Libido score} = \frac{([\text{RT score} + \text{SA score}] + 0.2 \times \text{TS})}{10} \times 100
\]

RT - reaction time, SA - sexual aggressiveness, TS - tactile stimulation

The mating ability score was determined in successful attempts as follows

\[
\text{Mating ability} = \frac{([\text{PE} + \text{MO} + \text{ET} + \text{PM} + \text{GT} + \text{TW} + \text{SM} + \text{E}])}{10} \times 100
\]

PE - penile erection, MO - mounting ability, ET - ejaculatory trust, PM - penile movement, GT - grasping the teaser firmly, TW- throwing whole body forward, SM- sudden backward movement of head, E - ejaculation

The sexual behaviour score was calculated from the net scores of libido and mating ability as follow

\[
\text{Sexual behaviour} = \frac{(\text{Libido score} + \text{mating ability})}{2}
\]

Collection of semen: The semen was collected once a week from all the 12 rams for 6 weeks using artificial vagina, (AV). The collections were obtained by exposing the ram to the oestrus ewe. Immediately after collection, the tubes containing semen were placed in a small thermo-flask having water at 37°C for further processing. From each ram 6 ejaculates were collected for assessment overall 72 ejaculates were assessed.

Analysis of seminal parameters: The routine semen parameters such as volume, colour, pH, mass activity, individual motility, concentration (hemocytometer) (Tomar, 1997), livability (Eosin-Nigrosin stain) (Yavuz, 2001), abnormality (Rose Bengal stain) (Herman and Madden, 1953), acrosomal integrity (Giemsa stain) (Watson, 1975) and in vitro fertility test (Hypo-osmotic swelling test (HOST) (Jeyendran et al., 1984) were conducted as per standard procedure.

Serum testosterone level: Blood samples were collected from both the groups of rams at monthly interval by vein puncture. Over all 48 blood samples were collected. Serum was separated and stored at -20°C in sterilized plastic vials till assayed. Total testosterone in serum samples was quantitatively determined by the testosterone IMMUNOTECH radio-immunoassay kit.

Statistical analysis: The results were analysed statistically after arc sine transformation of percentage data. Means were analysed by one-way ANOVA followed by the Tukey's post hoc test to determine significant differences between the months of testosterone production for a particular group and Student’s ‘t’ test was used to assess the difference between the treatment and control group for different seminal parameters. The data were expressed as mean ± SEM. Differences were considered significant if \( p < 0.05 \) (Snedecor and Cochran, 1989).

Effect of T. cordifolia supplementation on sexual behaviour and physicomorphological actions has been presented in Table 1.

The mean reaction time (seconds) during semen collection in two groups were recorded as 22.36 ± 2.12 (control) and 21.97 ± 2.12 (guduchi). No significant differences were observed in reaction time between control and guduchi supplemented group.

The mean sexual behaviour percentage during semen collection was recorded as 83.00 ± 1.77 (control) and 82.97 ± 1.77 (guduchi). There was no significant difference between control and guduchi supplemented group.

The observed values of routine seminal parameters such as volume, colour, 

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (36)</th>
<th>Guduchi (36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction time (second)</td>
<td>22.36±2.12</td>
<td>21.97±2.12</td>
</tr>
<tr>
<td>Sexual behaviour (%)</td>
<td>83.00±1.77</td>
<td>82.97±1.77</td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>0.68±0.02</td>
<td>0.67±0.02</td>
</tr>
<tr>
<td>pH</td>
<td>6.58±0.03</td>
<td>6.51±0.03</td>
</tr>
<tr>
<td>Mass activity (0-5 scale)</td>
<td>4.08±0.09</td>
<td>4.01±0.08</td>
</tr>
<tr>
<td>Individual motility (%)</td>
<td>73.06±1.43</td>
<td>76.39±1.33</td>
</tr>
<tr>
<td>Livability (%)</td>
<td>76.89±1.10</td>
<td>78.64±1.16</td>
</tr>
<tr>
<td>Sperm concentration (x10⁶/ml)</td>
<td>2667.89±1.53</td>
<td>2674.75±6.22</td>
</tr>
<tr>
<td>Total Abnormality (%)</td>
<td>3.86±0.16</td>
<td>3.96±0.13</td>
</tr>
<tr>
<td>Head Abnormality (%)</td>
<td>0.66±0.04</td>
<td>0.65±0.05</td>
</tr>
<tr>
<td>Mid piece Abnormality (%)</td>
<td>0.72±0.05</td>
<td>0.67±0.04</td>
</tr>
<tr>
<td>Tail Abnormality (%)</td>
<td>0.85±0.06</td>
<td>0.73±0.06</td>
</tr>
<tr>
<td>Protoplasmic droplets</td>
<td>1.63±0.11</td>
<td>1.92±0.11</td>
</tr>
<tr>
<td>Acrosomal integrity (%)</td>
<td>89.65±0.44</td>
<td>89.95±0.30</td>
</tr>
<tr>
<td>HOST (%)</td>
<td>65.72±0.89</td>
<td>66.92±0.96</td>
</tr>
</tbody>
</table>

TABLE 1: Effect of Tinospora cordifolia (Guduchi) supplementation on sexual behaviour and physicomorphological attributes of Muzzafarnagari rams
TABLE 2: Effect of *Tinospora cordifolia* (Guduchi) supplementation on serum testosterone level in Muzzafarnagari rams

<table>
<thead>
<tr>
<th>Group</th>
<th>October</th>
<th>November</th>
<th>December</th>
<th>January</th>
<th>Overall mean ± S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.12±0.18a</td>
<td>1.59±0.24b</td>
<td>0.82 ±0.18b</td>
<td>1.61±0.16b</td>
<td>1.28±0.11</td>
</tr>
<tr>
<td>Guduchi</td>
<td>1.05±0.20ab</td>
<td>1.57±0.27b</td>
<td>0.73±0.21b</td>
<td>1.27±0.25ab</td>
<td>1.15±0.13</td>
</tr>
</tbody>
</table>

Means with different superscripts in a row (ab) differ significantly (p < 0.05).

The mean serum testosterone level (ng/ml) in two groups were recorded as 1.28 ± 0.11 (control) and 1.15 ± 0.13 (guduchi). Non-significant differences were observed between control and guduchi supplemented groups.

**CONCLUSION**

It was concluded that supplementation of *T. cordifolia* didn’t have significant effect on semen production, *in-vitro* fertilizing potential of ram semen, sexual behaviour and serum testosterone level of ram.

**REFERENCES**


