Male antifertility effect of *Ocimum sanctum* Linn.: A study in albino rat

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Summary

Experiments were carried out to study the effect of high dose of “tulsi” (*Ocimum sanctum* Linn.) pellets on testis and epididymis in male albino rat. Wheat flour, oil and honey pellets of tulsi leaves were fed to albino rat, at 400mg/100g body weight per day, along with normal diet, for a period of 72 days. One group of tulsi-fed rats was left for recovery, after the last dose fed on day 72, up to day 120. This high dose of tulsi was found to cause duration-dependant decrease of testis weight and derangements in the histo-architecture of testis as well as epididymis. The diameter of seminiferous tubules decreased considerably, with corresponding increase in the interstitium. Spermatogenesis was arrested, accompanied by degeneration of seminiferous epithelial elements. Epididymal tubules regressed, and the luminal spermatozoa formed a coagulum. In the recovery group, testis and epididymis regained normal weights, where as spermatogenesis was partially restored. Thus, high dose of tulsi leaf affects testicular and epididymyal structure and function reversibly.

Key words: Antifertility, epididymis, *Ocimum sanctum*, testis.

Introduction

Attempts are being made to find a safe and effective oral contraceptive for males to control the tremendously increasing human population. Many phytochemicals as well as Indian medicinal plant extracts (Kholkute, 1977; Bhargava and Singh, 1981; Bansal and Davies, 1986; Adhikary et al., 1989; Akbarsha et al., 1990; Bhiwgade et al., 1990; Awari and Bhiwgade, 1992; Akbarsha and Murugaian, 2000; Mishra and Singh 2005; Sethi et al., 2010; Mathur et al., 2010) have been tried for antifertility effect in laboratory animals. A review of herbal medicine research on potential antifertility activity has thrown light on the use of plant drugs as abortifacient and as contraceptive agents both in male and female animal models (Priya et al., 2012; Singh et al., 2012). More than fifty plant species, including tulsi, are believed to possess these uses.

A review of literature on the medicinal properties of tulsi (*Ocimum sanctum* Linn.), commonly called holy basil, reveals that it is a well-known sacred plant of Hindu mythology and has a variety of medicinal properties (Ahmed et al., 2002; Prakash and Gupta, 2005; Singh et al., 2012). It has been shown to have chemo-preventive (Prashar et al., 1994; Jai Prakash et al., 2000), antioxidant (Adhikary et al., 1989; Devi et al., 1999), anticonvulsant (Jaggi et al., 2003), antiulcerogenic and ulcer curative (Mandal et al., 1993; Dharmani et al., 2004), hypoglycemic and antidiabetic properties (Sarkar and Pant, 1989; Sarkar et al., 1990; Chattopadhyay, 1993) and can reduce the blood and urinary uric acid levels (Sarkar et al., 1990). It’s antinociceptive and cognitive effects (Khanna and Bhatia, 2003; Joshi and Parle, 2006), wound healing property (Udupa et al., 2006) and radio-protective effect at high doses (Devi et al., 1995; Ganasoundari et al., 1997; Bhartiya et al., 2006) etc., were also elucidated. Singh et al. (2012) made an elaborate study on the medicinal properties of tulsi and found it to be antidiabetic, hepatoprotective, cardioprotective and neuroprotective. Above all, it was also shown to have anti-inflammatory and antipyretic activities (Godhwani et al., 1987), immunoregulatory property and adaptogenic action (Godhwani et al., 1988; Udupa et al., 2006) and to facilitate humoral immune response (Mediratta et al., 1988). Eugenol (1-hydroxy-2-methoxy-4-allylbenezene) (Sen et al., 1992) is the active compound in tulsi extract that has been shown to be largely responsible for the medicinal properties of this plant (Liu et al., 1987; Singh et al., 2002; Prakash and Gupta, 2005).

Apart from all the above medicinal properties, oral feeding of tulsi leaf to female albino rat has been shown to impair fertility as revealed in the pregnancy failure as well as impairment of oogenesis (Khanna et al., 1986). Short-term treatment of tulsi leaf to male rat affected...
reproduction (Kantak and Kogate, 1992). Similar treatment to male mouse produced a slight impairment in spermatogenesis (Kasinathan et al., 1972). Long-term administration of tulsi leaf affected mating behavior in male as well as female albino rats with decreased sperm counts, sperm motility and weight of male reproductive organs in the experimental animals (Khanna et al., 1986).

Therefore, the present study was aimed at monitoring the histo-architectural alterations in the cells and tissues of testis as well as different segments of epididymis after administration at high dose (400mg /kg body weight) of tulsi leaf for up to 72 days in adult male albino rat. It was also the aim of this study to find if effect if any is transient or long-lasting.

Materials and methods

Animals

The study was conducted under approval from the Institutional Animal Ethics Committee. Ninety day old male albino rats (Wistar strain), weighing 200-250 g, were divided into two groups, control (C) and treatment (T). Animals in the treatment group were again divided into four sub-groups of ten each which received pellets of tulsi leaf along with standard pellet feed (Hindustan Lever Ltd., Mumbai) and maintained under standard uniform husbandry conditions (27 ± 2°C and 14:10 hr L:D cycle).

Preparation of tulsi and treatment to rats

Tulsi leaves and soft stems, collected locally, were dried in shade and mixed with equal amount of wheat flour and made into pellet and suspended in equal amounts of groundnut oil and honey. Each experimental rat was fed tulsi pellet weighing 400mg/100g body wt/ day. Control rats (Group C) received only pellets of equal quantity of wheat flour and oil + honey. The experimental rats (T) were administered the tulsi pellet through oral route for different durations- 24 days (T1), 48 days (T2), 72 days (T3) and the fourth sub-group was treated for 72 days and then left untreated for up to 120 days (T4). Recovery, if any, was recorded in this group of rats.

Methods

Body weight (g) of the animals and organ weight of testis and caput and cauda epididymides (mg/100g body wt) were recorded after sacrificing the animals under anesthesia. The histo-architecture of the organs was studied adopting the routine hematoxylin - eosin (H & E) staining of paraffin sections. Diameters of the seminiferous tubules and epididymal duct were measured with the help of an ocular micrometer. Statistical analyses for minimizing the variations were made by calculating the arithmetic means (x) and the standard deviations (SD).

Results

The body weight of the animals increased gradually with age, and this increase was higher in the treated than in the control animals from 48 day treatment group onwards (Table 1). The wet weight of the paired testicles decreased in duration-dependent manner (Table 2). The wet weight of caput epididymides also showed duration-dependent decrease where as the wet weight of cauda epididymides decreased in 24 and 72 day treatment groups and there was no difference with the control in 48 day treatment group (Table 2). The diameters of seminiferous tubules, caput epididymal duct and cauda epididymal duct decreased in duration-dependent manner (Table 3).

Graded degenerative histological changes were observed in the testicles of O. sanctum treated rats as compared to control rats. The prominent changes included shrinkage of tubules resulting in apparent increase in the surrounding interstitium that got filled with an edematous fluid (Plate 1, Fig. G). Spermatogenesis was arrested at secondary spermatocyte stage after 32 days and it becomes more evident after 48 days and 72 days of treatment, resulting in ill-defined spaces and vacuoles in the spermatocytes (Plate 1, Fig. F, H). The nuclei of spermatocytes were pyknotic (PN) and many multinucleated giant cells (GC) appeared towards the lumen of the tubules (Plate 1, Fig. F, H). The Sertoli cells were lifted off from the basal lamina of degenerating seminiferous tubules in the 72 day treatment group (Plate 1, Fig. 7). The Leydig cells were also affected and showed degenerative changes.

Alterations were observed in both the caput and cauda regions of the epididymis. The basal lamina, epithelium, luminal sperm and interstitial connective tissue revealed severe damage. The epithelium revealed vacuolization of cytoplasm and pyknotic nuclei (Plate 3, Fig. 6). Epithelial height decreased and the lumen increased in the 48 day treatment group and thereafter (Plate 2, Fig. 5 - 8; Plate 3, Fig. 4 - 8). Lumina of some
### Table 1.

**Body weight of control and *O. sanctum* treated experimental rats**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Duration of treatment in days</th>
<th>Control: Body wt in g</th>
<th>Experimental: Body wt in g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>210 ± 1.80</td>
<td>212 ± 1.98</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>248 ± 2.03</td>
<td>255 ± 2.96</td>
</tr>
<tr>
<td>3</td>
<td>48</td>
<td>273 ± 1.36</td>
<td>302 ± 1.33</td>
</tr>
<tr>
<td>4</td>
<td>72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>After termination of treatment up to 120 days</td>
<td>320 ± 2.38</td>
<td>378 ± 3.40</td>
</tr>
</tbody>
</table>

Values are means ± SD of 5 animals each.

### Table 2.

**Wet weights of organs in mg/100 g body weight**

<table>
<thead>
<tr>
<th>Organs →</th>
<th>Dose Group ↓</th>
<th>Testicles</th>
<th>Caput epididymides</th>
<th>Cauda epididymides</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 day</td>
<td>943.25 ± 5.38</td>
<td>--</td>
<td>176.96 ± 2.46</td>
<td>-</td>
</tr>
<tr>
<td>24 days</td>
<td>928.54 ± 6.67</td>
<td>798.17 ± 3.49</td>
<td>172.00 ± 2.46</td>
<td>137.20 ± 5.80</td>
</tr>
<tr>
<td>48 days</td>
<td>824.50 ± 4.364</td>
<td>685.20 ± 5.24</td>
<td>165.09 ± 2.40</td>
<td>140.25 ± 2.80</td>
</tr>
<tr>
<td>72 days</td>
<td>815.73 ± 4.20</td>
<td>603.00 ± 5.42</td>
<td>159.75 ± 2.40</td>
<td>132.01 ± 3.70</td>
</tr>
<tr>
<td>After termination of treatment up to 120 days</td>
<td>880.53 ± 6.80</td>
<td>701.87 ± 5.21</td>
<td>135.75 ± 3.80</td>
<td>129.48 ± 4.70</td>
</tr>
</tbody>
</table>

Values are means ± SD; Number in paranthesis, number of animals.

### Table 3.

**Diameters of seminiferous tubules and epididymal duct**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Duration in days</th>
<th>Tubular diameter in μm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Testis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cont</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>268.5 ± 11.0</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>270.62 ± 10.4</td>
</tr>
<tr>
<td>3</td>
<td>48</td>
<td>273.00 ± 5.40</td>
</tr>
<tr>
<td>4</td>
<td>72</td>
<td>267.88 ± 10.4</td>
</tr>
<tr>
<td>5</td>
<td>After termination of treatment up to 120 days</td>
<td>269.87 ± 7.90</td>
</tr>
</tbody>
</table>

Values are means ± SD of 50 tubules.
T.S. of testis of control rat [Fig. 1 (10x), Fig. 2 (100x)] & experimental animals after 24 days [Fig. 3 (10x), Fig. 4 (60x); 48 days [Fig. 5 (10x), Fig. 6 (60x)] & 72 days [Fig. 7 (10x), Fig. 8 (60x)] of treatment.
T.S. of caput epididymidis of control [Fig. 1 (10 x), Fig. 2 (60 x)] & experimental animals after 24 days [Fig. 3 (10 x), Fig. 4 (60 x), 48 days [Fig. 5 (10 x), Fig. 6 (60 x)] & 72 days [Fig. 7 (10 x), Fig. 8 (60 x)] of treatment.
T.S. of cauda epididymidis of control [Fig. 1 (10x), Fig. 2 (60x)] & experimental animals after 24 days [Fig. 3 (10x), Fig. 4 (60x)], 48 days [Fig. 5 (10x), Fig. 6 (60x)] & 72 days [Fig. 7 (10x), Fig. 8 (60x)] of treatment.
sections of caput epididymal duct were filled with degenerating nuclei, broken tails of disintegrated sperm, and degenerating spermatids, all forming a coagulum (CLM) (Plate 2, Fig. 5, 6). This happened in a majority of cauda epididymal duct in sections as well (Plate 3, Fig. 4, 6). However some sections showed empty lumina (L) (Plate 2, Fig. 8; Plate 3, Fig. 8). Decrease in abundance and size of the Leydig cells and their nuclear diameter was observed.

In the recovery group, the average body weight of all the animals remained unaffected (Table 1). The testicles and epididymides still showed decrease in the wet wt weights (Table 2). But diameters of seminiferous tubules and caput and cauda epididymal ducts increased (Table 3). Histological observations after termination of the treatment revealed restoration of the distorted tissues to normal in more than 80% of the seminiferous tubules, and spermatids and spermatozoa appeared in the lumen (Plate 4, Fig. 2). The interstitial space decreased and the Leydig cells appeared normal (Plate 4, Fig. 1, 2). Both the caput and cauda epididymal ducts indicated recovery (Plate 4, Fig. 3, 4). The epithelium became tall columnar and the cells appeared normal (Plate 4, Fig. 3, 4). Their lumina were filled with fully differentiated normal spermatozoa. Thus, the reproductive organs showed recovery after termination of treatment.
Discussion

A high dose of *Ocimum sanctum* Linn. proves to impair fertility in male rats by affecting the gonads as well as the epididymis. Spermatogenesis was arrested probably at secondary spermatocyte stage likely due to hypogonadotropin levels. Leydig cells were also affected with sustained treatment and became perhaps unable to synthesize testosterone. Decrease in the number of sperm and spermatogenic elements had led to the loss of weight in the testicles and epididymides. Degenerated spermatogenic elements might have accumulated in the cauda epididymidis after 48 days of treatment.

Spermatogenesis occurs in the seminiferous tubules of the testis, followed by spermiogenesis giving rise to functional spermatozoa by polarization of spermatids, formation of acrosomal cap and flagellum and cytoplasmic remodeling after elongation of nucleus (D’Cruz et al., 2010). This process is regulated by testicular steroids. Several medicinal plants have been reported to affect various stages of spermatogenesis (Ogbuewu et al., 2010; Rahim et al., 2010). Most of the plants impair steroidogenesis by targeting the process of hormonal regulation of spermatogenesis via the hypothalamo-pituitary-gonadal axis (Udoh et al., 2005), which hinders the Leydig cell functioning and, finally, arrest spermatogenesis resulting in infertility/subfertility.

Hence, reduction in fertility in the present investigation is attributed to the direct effect of a high dose of *O. sanctum* Linn., which apparently inhibits androgen stimulating hormones like FSH and ICSH. Mammalian epididymis, being a dynamic organ, is also dependent on the testicular androgens for maintenance of its structure and secretory, resorptive, biosynthetic and other metabolic activities. It also responds well to the drug showing degenerative changes in the tubular epithelium which became incapable of keeping the sperm viable, leading to infertility/subfertility in the experimental animals. The recovery study made on day 120 revealed more than 80% reversibility of the antifertility effect. Hence, a high dose of *Ocimum sanctum* (tulsi) can be tried as a potent reversible oral contraceptive without side effects for males, perhaps in combination with other drugs.

References


Effect of Ocimum on spermatogenesis


