REVERSIBLE ANTI-FERTILITY EFFECT OF BENZENE EXTRACT OF *OCIMUM SANCTUM* LEAVES ON SPERM PARAMETERS AND FRUCTOSE CONTENT IN RATS

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**ABSTRACT**

Treatment of albino rats with a benzene extract of *Ocimum sanctum* leaves (250 mg/kg body weight) for 48 d decreased total sperm count, sperm motility, and forward velocity. The percentage of abnormal sperm increased in caudal epididymal fluid, and the fructose content decreased in the caudal plasma of the epididymis and the seminal vesicles. The results suggest that such effects are due to androgen deprivation, caused by the anti-androgenic property of *O. sanctum* leaves. The effect was reversible because all parameters returned to normal 2 wk after the withdrawal of treatment.

**KEYWORDS**

spermatozoa, *Ocimum sanctum*, seminal vesicle, epididymis, albino rat

**INTRODUCTION**

*Ocimum sanctum* (Linn), commonly called tulsi, is an important medicinal plant and has long been recognized for its unique properties. *O. sanctum* is held sacred by Hindus all over India and is
frequently cultivated in gardens, courtyards, and temples. The plant is used as an anti-bacterial and insecticidal agent, as a diaphoretic in malarial fever, and as an antiperiodic in gastric and genitourinary system /1−4/. Additionally, the leaves of *O. sanctum* significantly
- alter the weight of the testis, with no significant effect on epididymis, seminal vesicle, prostate, and vas deferens,
- reduce sperm count and motility /5/;
- decrease the pH, hypertonic environment, and concentration differences of chemical substances of biological importance like mucoprotein, alkaline and acid phosphatases /6/; and
- reduce the mating behavior of both male and female albino rats /7−9/.

The epididymis, an important component of the male reproductive tract, is highly androgen-dependent and plays a role in male fertility. Androgenic hormones reach the epididymis via the blood stream and also via the fluid that accompanies the spermatozoa from the testis /10/. Hence, the purpose of the present study was to investigate in male albino rats the effect of a benzene extract of *O. sanctum* leaves on certain parameters of spermatozoa, namely, total sperm count, sperm motility, forward velocity, abnormal sperm; and the fructose content in the cauda epididymal fluid and the seminal vesicle.

**EXPERIMENTAL**

Fresh *O. sanctum* leaves were dried in the shade and subjected to a soxheltation process to produce a benzene extract. The extract thus obtained was allowed to dry then was stored at 4 °C in a dessicator.

Adult male albino rats of the Wistar strain, 3 to 4 months old and 190 to 200 g body weight, were acclimatized to laboratory conditions and received a standard rat pellet diet (Gold Mohar, Hindustan Lever Ltd., Hyderabad) and water *ad libitum*. The rats were divided into four groups comprising five animals each.

The animals of group I, which served as a control, received 1 ml propylene glycol daily for 48 d and were autopsied 24 h later. Groups II,
III. and IV received a benzene extract, 250 mg/kg body weight, of *O. sanctum* leaves in 1 ml propylene glycol/rat daily for 48 d. After withdrawal of treatment, the animals of group II were autopsied on day 49 and those of groups II and IV on days 8 and 16, respectively. The dose of 250 mg/kg used in the present study was based on standardization in preliminary studies. The treatment period of 48 d is fixed based upon the duration of 3 spermatogenic cycles in the albino rat. The cauda epididymis was chopped in phosphate-buffered glucose saline (PBGS) containing 50 mM NaCl, 200 mM Na₂HPO₄, 200 mM glucose, and 26 mM KH₂HPO₄. The debris was removed and a clear suspension of the epididymal plasma was used for the analysis of total sperm count, sperm motility, forward velocity of the sperm, and the relative percentage of abnormal sperm. Cauda epididymal fluid and seminal plasma were analyzed for fructose. The total sperm count and motility were calculated according to the method of Besley et al. /12/, using a Neubauer hemocytometer. The forward velocity of the sperm was calculated according to the method of Ratnasoorya (modified from Daunter et al.) /13/ by examining 10 sperm from each sample. In 10 samples from each group, the relative proportion of abnormal sperm was determined by examining smears stained with Ziehl Nielson’s carbol fuchsin and counterstained with Loeffer’s methylene blue. Fructose determination was according to the method of Bauer et al. /14/.

The data are presented as mean±SEM. The data were compared for statistical significance using the Student’s *t* test; a probability level of *P* < 0.001 was considered significant.

**RESULTS**

The results are presented in Table 1. Relative to the control group, the animals that received the *O. sanctum* extract showed a significant decrease in the total sperm count, the total number of motile sperm, and the forward velocity of the sperm. A significant increase in the percentage of abnormal sperm and a significant decrease in the fructose level was observed. In group III, this effect was partially reversed as seen the total sperm count, the total number of motile
sperm, and the forward velocity of the sperm, and the fructose content, with a significant decrease in the percentage of abnormal sperm. Group IV showed a complete recovery of all parameters, which were comparable to those of the control group.

**TABLE 1**

Effect of *O. sanctum* leaves (benzene extract) on various sperm parameters and fructose content in cauda epididymal and seminal plasma of albino rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Spermatozoa</th>
<th>Plasma fructose (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Count&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Motile&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>I</td>
<td>56.40±1.39</td>
<td>52.40±2.15</td>
</tr>
<tr>
<td>II</td>
<td>32.00±1.22</td>
<td>24.00±1.70</td>
</tr>
<tr>
<td>III</td>
<td>46.40±1.73</td>
<td>34.80±1.44</td>
</tr>
<tr>
<td>IV</td>
<td>55.20±0.51</td>
<td>54.00±1.14</td>
</tr>
</tbody>
</table>

*Group I = control; II = O. sanctum; III = 1 wk recovery; IV = 2 wk recovery; Total no. × 10⁶/ml; P values: ¹ < 0.001; ² < 0.01

**DISCUSSION**

Various plants like *Vinca rosea, Solanum xanthocarpum, Bambusa arundinacea, Ocimum sanctum, Dolichos biflorus, and Amaranthus spinosus, Carica papaya, and Spirulina plantensis* are reported to possess antifertility activity /15–23/. Treatment with such plant material results in reducing the sperm count, motility, fertility, and viability, as well as increasing the amount of abnormal sperms. It has been suggested that their extracts cause an androgen depletion at the target level, particularly in the cauda epididymis, thereby affecting the physiological maturation of sperm /21/. Recently, *O. sanctum* has been reported to have anti-fertility, anti-spermatic activity /5–7/, and it reduces the mating behavior of both male and female rats /7–9/.

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Without a continuous supply of androgen, sperm reproduction does not proceed optimally to completion /24/. Studies involving hypophysectomy, castration, and androgen-replacement therapy have shown that androgen is essential for the physiological maturation and survival of spermatozoa in the epididymis /25, 26/. Sperm possesses two principal attributes—namely, motility and fertilizing ability—that are prerequisite for fertilization. Any negative impact on motility would seriously affect fertilizing ability /16/. A semen sample containing more than 20% abnormal sperm per ejaculate is considered poorly fertile /14/. The occurrence of morphologically abnormal spermatozoa is a diagnostic sign for infertility, besides using other characteristics like motility, density, and viability. A high incidence of abnormality is also associated with infertility /27–30/. The relative distribution of different morphological types of spermatozoa in a sample provides the most significant clue to discriminating between fertile and infertile samples /31, 32/. Sperm movement is different in morphologically normal and abnormal spermatozoa /33/. Assessment of morphology is a good indicator of the fertilizing ability of spermatozoa. The increased amount of headless spermatozoa found in infertile groups has confirmed that this characteristic is associated with infertility /38, 39/.

The role of the epididymis in the maturation process remains controversial. Change in the internal milieu of the epididymis is known to affect sperm maturation and motility /21, 23, 36–38/. Sperm maturation is androgen dependent and is available either from the peripheral blood or from the luminal fluid in the epididymis. The efficacy of cryptopertone acetate in blocking the effect of exogenous testosterone provides additional evidence that testosterone acts on sperm maturation via epididymal tissue /25/. Androgens can affect the sperm either directly or by modifying the epididymal milieu. Several pieces of evidence suggest that the hormones have a direct effect on the organ /39/. Androgens are essential for the survival and motility of spermatozoa in the rat epididymis; with the cauda region being the most favorable site /40–42/.

Sperm analysis is only one step in the investigation of male infertility /43/. Seminal biochemistry is an indicator of the functional
status of the accessory reproductive glands. Glyceryl phosphoral choline, carnitine, and neutral alpha glucosidase have been associated with epididymal function /44/. Very little purpose is served by determining the concentration of such chemicals in semen because their concentrations vary for a number of reasons.

In most mammals, fructose is the only sugar that is present in the semen /45/ and is an important source of energy for spermatozoa. The rate of fructolysis correlates with the number of motile sperm, and a diminished level of fructose has been shown to parallel androgen deficiency /46/. Therefore, diminished fructose content can be an indicator of semen pathology. The finding in several studies that low fructose levels respond to testosterone therapy proves that a direct relation exists between fructose and testosterone levels /14, 21, 23, 46/.

In the present study, the increased percentage of abnormal sperm; the reduced count, motility, and speed of sperm, as well as the diminished fructose level in the seminal vesicle is probably due to androgen deficiency consequent to the anti-androgenic property of O. sanctum leaves. The gradual recovery occurred after the treatment was halted indicates that the extract possesses reversible antifertility effects without any apparent toxic side effects.

REFERENCES


