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Abstract

In search of new well-acceptable safe anti-fertility agent, the present study examined the effect of crude extract of the flowers of Hibiscus-rosa-sinensis as an antifertility agent in oral route on male albino rats. 84 healthy male albino rats were chosen, out of which 24 rats were kept as control group and designated as group I and remaining 60 rats were grouped into five (5) groups having 12 rats in each group and designated as groups II to VI. Each group was subdivided into two (2) subgroups having six (6) rats in each and marked them as ‘a’ & ‘b’. In ‘a’ subgroup of each group, the dose of crude extract was 150mg/kg and in ‘b’ subgroup the extract dose was 300mg/kg. The duration of the extract treatment were gradually increased from group II to group V, starting from 15 days in group II, 30 days in group III, 45 days in group IV and 60 days in group V. The rats were sacrificed accordingly after the last oral dose, their testes were weighed and biopsies were taken for histological studies using H&E stain. In the last group (group VI), the extract was given for 60 days and rats were sacrificed 30 days after the last oral dose and biopsies were taken accordingly for histological examination. The testes of the control groups were similarly collected at regular interval and histological examination done accordingly. Our study definitely revealed that the crude extract of the Hibiscus-rosa-sinensis flowers when fed orally to male albino rats in definitive dosage for 30, 45 & 60 days lead to changes in germinal epithelium of the testes ranging from mild damage to near total sloughing depending upon the duration of the treatment. The study definitely points towards the potential role of the plant as anti-fertility agent.

Key words: Anti-fertility agents, Anti-spermatogenic Agents, Hibiscus Rosa Sinensis, Spermatogenesis.
STUDY OF THE EFFECTS OF HIBISCUS-ROSA-SINENSIS FLOWER EXTRACT ON ...  

Introduction

In India, today the problem of over-population has been of serious concern. There has been phenomenal increase in population within the last few decades. As per 2011 census, the total population of India is 1.210,193,422 with decadal growth rate (2001-2011) is 17.64% and fertility rate is 2.62 children born per woman. So fertility control programme has gained tremendous importance in medical science at present. There are so many contraceptive devices or procedure advocated in control of fertility; but each of these devices or procedures has its own merits and demerits. Our goal should be such to identify an ideal antifertility agent whose action is easily reversible without having any obvious adverse effects on other systems of the body and of course, should be socially acceptable.

Different literatures show that several indigenous plant products have antifertility activity; e.g. cotton seed oil extract, popularly known as gossypol, and also piper longus, Olmium sandum (Batta and Santhakumari, 1971) and other Ayurvedic ingredients (Rajeswan et al, 2012).

Many studies also showed the anti-fertility effects of Hibiscus-rosa-sinensis as anti-estrogenic and progesterone-lowering-agent in female mice, leading to termination of pregnancy and also anti-spermatogenic agent in male animals (Kholkute and Udupa, 1976; Kholkute, 1977; Kholkute et al,1977; Pakrashi et al,1986).

In our present study, the crude extract of the flowers of Hibiscus-rosa-sinensis (Linn) is used as an experimental agent to determine whether it possesses antifertility activity. The plant Hibiscus-rosa-sinensis is an evergreen woody glabrous, snowy shrub 1.5-2.4m height and cultivated as ornamental plants.

Materials and Methods

The test animals used were post-pubertal healthy male albino rats weighing from 175g to 250g average being 200g each. The animals were kept in standard rat cages and maximum of six rats were kept in one cage. The animals were maintained under uniform condition with free access to standard animals-pellets and water \textit{ad libitum} throughout the experiment. Total number of rats were 84.

The rats were grouped into two broad groups – the control group (24 rats) and the experimental group (60 rats). The animals of the experimental group were sub-grouped subsequently. Each subgroup consists of 6 rats. The prepared extract solution were fed by intra-gastric rubber catheter tube at the following doses and duration. The groups were labeled as follows:

- Group I : the control group (24 rats in 4 cages) – the rats were fed with standard animal pellets and water throughout the experiment.
- Group IIa : 6 rats; the rats received the extract in the dose of 150mg/kg (0.5ml) for 15 days.
- Group IIb : 6 rats; the rats received the extract in the dose of 300mg/kg (1ml) for 15 days.
- Group IIIa : 6 rats; the rats in this group were fed with flower extract in the dose of 150mg/kg (0.5ml) for 30 days.
- Group IIIb : 6 rats; dose of this group was 300mg/kg (1ml) for 30 days.
- Group IVa : 6 rats; dose is 150mg/kg (0.5ml) for 45 days.
- Group IVb : 6 rats; dose is 300mg/kg (1ml) for 45 days.
- Group Va : 6 rats; dose is 150mg/kg (0.5ml) for 60 days.
- Group Vb : 6 rats; dose is 300mg/kg (1ml) for 60 days.
- Group VI a&b : for the recovery study, in this group the drug treatment in the dose of 150mg/kg (VIa) and 300mg/kg (VIb) were given for 60 days and the animals were sacrificed 30 days after the discontinuation of treatment.

For evaluating the effect of the extract of Hibiscus-rosa-sinensis on the experimental animals, the animals were sacrificed accordingly after the last oral dose, their testes were removed and weighed and recorded and then fixed for...
histological slides. Similarly the testes of the control groups were collected at regular interval along with experimental sub-group and were processed for microscopic studies using H&E stain.

Results

Table 1: Weight of testes (in gm) after therapy.

<table>
<thead>
<tr>
<th>Sl no.</th>
<th>Groups</th>
<th>Weight of testes (in gm)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>1.02 – 1.20</td>
<td>1.10</td>
</tr>
<tr>
<td>2</td>
<td>IIA</td>
<td>1.01 – 1.14</td>
<td>1.08</td>
</tr>
<tr>
<td>3</td>
<td>IIb</td>
<td>0.95 – 1.12</td>
<td>1.06</td>
</tr>
<tr>
<td>4</td>
<td>IIIa</td>
<td>0.93 – 1.10</td>
<td>1.04</td>
</tr>
<tr>
<td>5</td>
<td>IIib</td>
<td>0.94 – 1.08</td>
<td>1.02</td>
</tr>
<tr>
<td>6</td>
<td>IVa</td>
<td>0.92 – 1.06</td>
<td>1.00</td>
</tr>
<tr>
<td>7</td>
<td>IVb</td>
<td>0.90 – 1.00</td>
<td>0.96</td>
</tr>
<tr>
<td>8</td>
<td>Va</td>
<td>0.82 – 0.98</td>
<td>0.88</td>
</tr>
<tr>
<td>9</td>
<td>Vb</td>
<td>0.82 – 0.92</td>
<td>0.86</td>
</tr>
<tr>
<td>10</td>
<td>VIa</td>
<td>0.98 – 1.18</td>
<td>1.10</td>
</tr>
<tr>
<td>11</td>
<td>VIb</td>
<td>0.96 – 1.14</td>
<td>1.02</td>
</tr>
</tbody>
</table>

During observation, it was found that there was loss of weight of the testes of the experimental animals with the use of extract of Hibiscus and the loss of the weight was maximum in the animals of the group Vb, when 300mg/kg of the extract solution was given for 60 days.

The weight of the control group (Group I) was 1.02 – 1.20g average being 1.10g. the weight of the testes of Group VIb (withdrawal group) varied from 0.96 to 1.14g, average being 1.02g.

The effect of the drug on the other organs viz. stomach, duodenum, liver, kidney and heart were also noted following treatment with the extract in the dose of 300 mg/kg of body weight for 30 days and 60 days. Microscopic findings of these organs showed no alteration in their structures.

Discussion

Ancient literature mentions the use of a number of plants/preparations for fertility regulation. Some local contraceptive agents have also been described in Ayurvedic and Unani texts. Documented experiments or clinical data are, however, lacking. Therefore, some previous studies were undertaken to explore the antifertility and estrogenic activity of *Hibiscus rosa-sinensis* Linn. A strong anti-implantation (inhibition 100%) and uterotropic activity was observed at the dose level of 400 mg/kg body weight. Histological studies were carried out to confirm this effect to show the Post-Coital Antifertility Activity of *Hibiscus rosa-sinensis* Linn. roots.(Vasudeva and Sharma, 2008; Kabir et al,1984).

In one study the effects of oral administration of aqueous and alcoholic extracts of flowers of H. r. sinensis (250 mg/kg BW/day, 30 days) on the reproductive organs of male rats was examined. The results indicated that the organ weights were unaffected by the extracts: weights of the testis, epididymis, ventral prostate, and seminal vesicle of the treated animals were not significantly different from those of the controls. The testis and epididymis of the rats also showed normal histological features, irrespective of treatment. No apparent toxicity of the extracts was discernible.(Tan,1983).

In our present study the crude extract of the flowers *Hibiscus rosa-sinensis* was studied to know its effect as an antifertility agent in oral route on male albino rats. 84 healthy male albino rats were chosen, out of which 24 rats kept as control group and designated as group I and remaining 60 rats were grouped into five (5) groups having 12 rats in each group and designated as group II to VI. Each group was subdivided into two (2) subgroups.
having six (6) rats in each and marked them as ‘a’ & ‘b’. in ‘a’ subgroup of each group the dose of crude extract was 150mg/kg and in ‘b’ subgroup the extract dose was 300mg/kg. the duration of the extract treatment were gradually increased from group II to group V, starting from 15 days in group II, 30 days in group III, 45 days in group IV and 60 days in group V. the rats were sacrificed accordingly after the last oral dose, their testes were weighed and biopsies were taken for histological studies using H&E stain. In the last group (group VI), the extract was given for 60 days and rats were sacrificed 30 days after the last oral dose and biopsies were taken accordingly for histological examination.

The testes of the control groups were similarly collected at regular interval and histological examination done accordingly. Our study definitely revealed that the crude extract of the Hibiscus-rosa-sinensis flowers when fed orally to male albino rats in definitive dose for 30, 45 & 60 days lead to changes in germinal epithelium of the testes ranging from mild damage to near total sloughing depending upon the duration of the treatment.

### Table 2: The structural changes in the testes after therapy.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Basement membrane</th>
<th>Spematogenic cells</th>
<th>Mature spermatozoa</th>
<th>Leydig cells</th>
<th>Sertoli cells</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Intact</td>
<td>Organized stages of maturation seen</td>
<td>Adequate, with normal morphology</td>
<td>Present</td>
<td>Present</td>
<td>Multinucleated giant cells - few Multinucleated giant cells seen</td>
</tr>
<tr>
<td>IIa</td>
<td>Intact</td>
<td>Disorganized but stages of maturation seen</td>
<td>Few matured spermatozoa with prominent tails seen</td>
<td>Present</td>
<td>Present</td>
<td></td>
</tr>
<tr>
<td>IIb</td>
<td>Break in different places</td>
<td>Disorganized but stages of maturation seen</td>
<td>Scanty</td>
<td>Absent</td>
<td>Present</td>
<td></td>
</tr>
<tr>
<td>IIIa</td>
<td>Discontinuous at places</td>
<td>Disorganized</td>
<td>Extremely scanty</td>
<td>Absent</td>
<td>Granular &amp; fragmented</td>
<td></td>
</tr>
<tr>
<td>IIIb</td>
<td>Discontinuous at places</td>
<td>Disorganized totally, scattered throughout the field</td>
<td>Extremely scanty</td>
<td>Absent</td>
<td>Fragmented</td>
<td></td>
</tr>
<tr>
<td>Iva</td>
<td>Total disruption</td>
<td>Spermatogenic cells, identification of individual cell is difficult</td>
<td>Extremely scanty</td>
<td>Absent</td>
<td>Fragmented</td>
<td></td>
</tr>
<tr>
<td>IVa</td>
<td>Total loss of basement membrane in few tubules</td>
<td>Total disorganization of cells in field</td>
<td>Extremely scanty</td>
<td>Absent</td>
<td>Fragmented</td>
<td></td>
</tr>
<tr>
<td>IVb</td>
<td>Tubular structures are lost</td>
<td>Total disorganization of cells</td>
<td>Extremely scanty</td>
<td>Absent</td>
<td>Fragmented</td>
<td></td>
</tr>
<tr>
<td>Va</td>
<td>Tubular structures are totally lost</td>
<td>Total disorganization of cells</td>
<td>Extremely scanty</td>
<td>Absent</td>
<td>Fragmented</td>
<td></td>
</tr>
<tr>
<td>Vb</td>
<td>Beginning of basement membrane, tubular luminal structure begins to appear</td>
<td>Total disorganization of cells</td>
<td>Absent</td>
<td>Absent</td>
<td>Fragmented</td>
<td></td>
</tr>
<tr>
<td>Vla</td>
<td>Central part of the cells show spermatogenic cells</td>
<td>Absent</td>
<td>Few</td>
<td>Normal Sertoli cells are seen</td>
<td>Few giant cells</td>
<td></td>
</tr>
<tr>
<td>Vlb</td>
<td>Spematogenic cells are seen at different stages of maturation</td>
<td>Few mature spermatozoa seen</td>
<td>Present</td>
<td>Normal cells are seen</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Though the anti-testicular effect of the agent was proved still the mechanism of action is not clear (Kholkute et al., 1972). The antispermatic effect of the plant is possibly mediated via the pituitary (Kholkute and Udupa, 1974).

The effect of the extract on recovery group (group VI) suggests that the flower’s extract on the spermatogenesis were however temporary and completely reversed in 30 days of discontinuation of the treatment. The extract also did not produce any significant change on other vital organs like liver, kidney, brain etc.

**Conclusion**

In our study, it has been demonstrated that there was definite antifertility effect of crude extract of *Hibiscus rosa-sinensis* in causing degenerative changes in the germinal epithelium of male albino rats. The effect was temporary and reversible, and the extract had no deleterious effect on other vital organs of the body. The flowers are easily available in many parts of the globe, so it can be used as an easily available potential antifertility agent. Though further study and trials on higher mammals are needed to reach the goal.

**References**


