THE ANTIESTROGENICITY OF HIBISCUS ROSA SINENSIS FLOWERS ON FEMALE RATS

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The effect of alcoholic extract of Hibiscus rosa sinensis flowers was studied on the reproductive system of female wistar rats. The extract was administered subcutaneously at the dose level of .4 ml/day/rat and .2 ml/day/rat for 7 days and 15 days respectively. At the said dose levels the drug caused antifertility effects via antiestrogenicity which was well marked by histological and biochemical observations.

Hibiscus rosa sinensis (flower) commonly known as Gurhal (Family-Malvaceae) are demulcent and used for fevers and its fresh root juice for gonorrhoea. The flowers are attributed to possess contraceptive properties in Ayurvedic literature, Materia medica and folklores. Prevention of pregnancy by the alcoholic extract of H. rosa sinensis flowers has been reported on female rats and mice by Singh et al.1, Kabir et al.2 and Bhattacharya3. The present study was aimed to examine the histological, biochemical and morphological changes in the reproductive organs of female rats after the administration of alcoholic extract of the flowers of the said plant.

MATERIALS AND METHODS

Hibiscus rosa sinensis flowers collected from Pantnagar (G.B. Pant University, Pantnagar) were shade dried and coarsely powdered plant material was successively extracted with petroleum ether (B.P. 60°-80°C) and rectified spirit according to Khanna et al.1. Female healthy wistar rats (3 to 5 months old), weighing from 120 to 150 gms used in the experiment were fed with standard rat feed and ordinary tap water. The rats were divided into three groups. Animals of the 1st group served as controls, 2nd and 3rd group received alcoholic extract at the dose level of .4 and .2 ml for 7 to 15 days respectively (15.04 mg
powder dissolved in 1 ml of 50% ethanol). Animals were autopsied 24 hrs after the last dose of the extract. The reproductive tract was removed and cleaned of adherent tissues. The ovaries, uterine horns and vagina were excised out and their wet weights were recorded. For routine histological test, the tissues were fixed in Bouin’s fluid and after sectioning (5μ to 8μ) stained with Harris Haematoxylin and eosin. The right uterine horn and the remaining part of the vaginal tissues were frozen and total RNA, sialic acid and glycogen were determined as stated by Munro and Fleck, Warren and Montgomery. Vaginal smear was checked on every alternate day to determine the sexual cycle.

RESULTS AND DISCUSSION

Weight response: After administration of the alcoholic extract the weights of ovary, uterus + cervix and vagina did not change significantly, although in general, the organ weights were decreased, particularly in uterine horns + cervix and in vagina (Table 1).

Table 1—Changes in the weights of the ovaries, uterus + cervix and vagina and RNA, sialic acid in uterus and vagina and glycogen in the uterus after administration of the alcoholic extract of Hibiscus rosa-sinensis flowers.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Genital organ weight (mg)</th>
<th>In uterus</th>
<th>In vagina</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ovary</td>
<td>Uterus + cervix</td>
<td>Vagina</td>
</tr>
<tr>
<td>Group I</td>
<td>24.8</td>
<td>138.5</td>
<td>62.77</td>
</tr>
<tr>
<td>Control</td>
<td>± 3.06</td>
<td>± 27.83</td>
<td>± 2.35</td>
</tr>
<tr>
<td>Group II</td>
<td>24.5</td>
<td>103.07</td>
<td>53.7</td>
</tr>
<tr>
<td>.4 ml</td>
<td>± .81++</td>
<td>± 2.6+++</td>
<td>± 2.09++</td>
</tr>
<tr>
<td>Alcoholic-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>21.6</td>
<td>123.0</td>
<td>62.02</td>
</tr>
<tr>
<td>.2 ml</td>
<td>± 0.34+++</td>
<td>± 2.03+++</td>
<td>± 0.44+++</td>
</tr>
<tr>
<td>Alcoholic-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 days</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

+ significant (p < .01), +++ Non significant (p < .01) when compared with control.
Fig. 1-9: 1. Ovary of the control rat. 2. Ovary of the rat after treatment with the
alcoholic extract of the H.r.s. at the dose level of 0.4 ml for 7 days. Well marked degeneration in the ovary, HE × 100. 3. Treated ovary at the dose level of 0.2 ml for 15 days. Atrophic granulosa cells seen, HE × 100. 4. Uterus of the control rat. 5. Treated uterus after 0.4 ml for 7 days (H.r.s.) showed reduced lumen lined by low columnar epithelium, typical dioestrous condition, HE × 100. 6. Treated uterus after 0.2 ml for 15 days (H.r.s.), the oovascular, atrophic uterus showing typical dioestrous condition. HE × 100. 7. Control vagina of the rat. 8. Treated vagina after 0.4 ml for 7 days (H.r.s.) showed sloughing of the vaginal epithelium, HE × 100. 9. Treated vagina after 0.2 ml for 15 days (H.r.s.) keratinization does not occur, HE × 100.

Ovary: The ovary of control rat sacrificed at oestrus stage showed normal features which includes the graffian follicles in different stages of development (Fig. 1). When the alcoholic extract of *H. rosa sinensis* was administered, remarkable changes in the histoarchitecture of the ovary were observed. In 7 days treated ones (0.4 ml/day/rat), degeneration of graffian follicles were seen (Fig. 2). When the extract was administered for 15 days (at the dose level of 0.2 ml/day), too much disorganization in the graffian follicles as well as in corpora lutea were observed and not only this, the growth of primary follicles were also found arrested.

Uterus: The histoarchitecture of uterus of control rats at oestrus stage showed typical stimulated features comprising well developed single layered endometrial epithelium with basal nuclei and numerous uterine glands (Fig. 4). After 7 days treatment (0.4 ml/day/rat), there were disorganization in stromal cells and uterine glands. The lumen was obliterated and the luminal epithelium was ruptured at number of places (Fig. 5) and in 15 days treated animals (0.2 ml/day/rat) induced toxic effects in uterus was seen and uterine glands showed atrophy and stroma was detached from circular muscles. The uterine glycogen, RNA and sialic acid were reduced significantly (Fig. 6 & Table 1).

Vagina: The control vagina was lined by low columnar epithelial cells (Fig. 7). After alcoholic extract administration, atrophic changes were seen in the vagina. The oestrous cycle interrupted after one week treatment (Fig. 8 & 9) and RNA and sialic acid were also reduced significantly after the treatment of the extract (Table 1).
In the present investigations the alcoholic extract of the flowers of *Hibiscus rosa-sinensis* can have antifertility effects showing anti-estrogenicity since

1. The wet weight of the gonads after the treatment of the said flowers extract were found slightly reduced (Table 1) and shows statistically significance in both 7 and 15 days.

2. Degeneration of graffian follicles and growth of primary follicles arrested after administration of the extract in both short and long term treatment i.e. 7 and 15 days (Fig. 2).

3. Disorganization in stromal cells and uterine gland and obliteration of lumen as well as luminal epithelium was ruptured at number of places under 7 and 15 days duration of treatment (Fig. 5).

4. Oestrus cycle was interrupted after one week treatment of the extract.

5. Atrophic changes were seen in the uterine glands (Fig. 6) and vagina (Fig. 8 & 9), and RNA, sialic acid and glycogen were reduced significantly in both uterus and vagina (Table 1) showing anti-estrogenic effects of the said flowers extract.

The present findings of the antifertility effects of *H. rosa sinensis* flowers extract can also get support from the work of many investigators. As Kholkute et al. reported the benzene extract of *Hibiscus rosa sinensis* flowers having antifertility efficacy; and Batta and Santha Kumari also reported the antifertility effects of the ethanolic extract of the flowers of *H. rosa sinensis*. Kholkute and Udupa also reported anti-estrogenic activity of benzene and ethanolic extract of the said flowers in rats. In the present study also RNA, sialic acid and glycogen level of both uterus and vagina were observed to decrease after treatment of the ethanolic extract of the *H. rosa sinensis* flowers, having found antiestrogenic effects as Paksash et al. and Tewari et al. observed that estrogen elevate RNA, sialic acid and glycogen concentration in the uterine and vaginal tissue, and antiestrogens decrease them significantly.

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REFERENCES

Antiestrogenicity of Flower Gurhal


