Anti-steroidogenic activity of methanolic extract of *Cuscuta reflexa* Roxb. stem and *Corchorus olitorius* Linn. seed in mouse ovary

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Methanolic extract (ME) of both *C. reflexa* stem and *C. olitorius* seed arrested the normal oestrous cycle of adult female mouse and significantly decreased the weight of ovaries and uterus. The cholesterol and ascorbic acid contents in ovaries were significantly increased in the treated mice. Two key enzymes, Δ^5^3β-hydroxysteroid dehydrogenase and glucose-6-phosphate dehydrogenase, were decreased significantly in ME of both *C. reflexa* stem and *C. olitorius* seed after 17 days of treatment. High level of substrates and low level of enzymes indicate the inhibition of steroidogenesis in treated mice and may be due to the presence of flavonoids.

**Key words**: *Cuscuta reflexa* seed extract, *Corchorus olitorius* seed extract, Glucose-6-phosphate dehydrogenase, Δ^5^3β-hydroxysteroid dehydrogenase, Mouse ovary, Steroidogenesis.

*Cuscuta reflexa* Roxb. (Family: Convolvulaceae, Swarnalata in Bengali, Amarel in Hindi) is a golden yellow dodder-like parasite. The plant is common throughout India, and is widely distributed in plains of West Bengal. Various parts of this plant were used by tribes in ailments like fits, melancholy and insanity. It is also useful externally against itch and internally in fevers, ‘retention of wind’ and ‘induration of the liver’. On preliminary analysis, *C. reflexa* stem, has been found to contain large quantity of flavonoids and its different extracts on preliminary investigation have been found to possess antifertility effect. *Corchorus olitorius* Linn., (Family: Tiliaceae, Jute) is an annual herb with slender stems. It is cultivated in many parts of India. The seeds are used as purgative and leaves are used as demulcent, diuretic, febrifuge (infusion) and in chronic cystitis and dysuria. *C. olitorius* seed is a traditional tribal medicine for birth control. However, no detailed study has been undertaken on antifertility activity of *C. reflexa* stem and *C. olitorius* seeds. In the present communication, we have assessed the *in vivo* anti-steroidogenic activity of methanolic extract (ME) of *C. reflexa* stem and *C. olitorius* seeds by observing the changes in oestrous cycle, weight of the ovaries and uterus and biochemical parameters in mice. The changes in cholesterol and ascorbic acid content in ovaries were measured as cholesterol is the raw material for estrogen synthesis and ascorbic acid level serves as an index for determining the normal ovarian activity. The effect of *C. reflexa* stem and *C. olitorius* seed extract on the activities of Δ^5^3β-hydroxysteroid dehydrogenase (5 HSD) and glucose-6-phosphate dehydrogenase (G-6- PDH), the two key enzymes involved in ovarian steroidogenesis, were also measured.

**Preparation of extract** — The stems of *Cuscuta reflexa* Roxb. and the seeds of *Corchorus olitorius* Linn., collected locally were authenticated by the Division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Kolkata. The voucher specimens have been preserved in our laboratory. Shade-dried, powdered plant material was soaked extracted first with petroleum ether (40°-60°C) and then with methanol. The methanolic extract was evaporated to dryness. The trace amount of methanol which may be present within the solid mass of methanolic extract was removed by washing with ethyl alcohol. For pharmacological testing, ME of *C. reflexa* stem and *C. olitorius* seed were dissolved in propylene glycol (PG).

**Animal experiments** — Adult female albino mice of Swiss strain (22±2 g body weight) were acclimatized to laboratory conditions (25°-30°C, 75-85% RH,
for one week and given pellet diet (Hindustan Lever) and water ad libitum. Experiments were performed under the guidance of The Ethical Committee, Jadavpur University, Kolkata. The LD₉₀ values of ME of *C. reflexa* stem and *C. olitorius* seed are 435 and 191 mg/kg body weight respectively. Mice showing normal oestrus cycle for a period of 2 weeks were divided into 8 groups of 6 mice each and were given the following treatment. Groups 1 and 2 served as normal saline control (5 ml/kg, 0.9% NaCl w/v, ip) and vehicle control (5 ml/kg, propylene glycol, ip) respectively. Groups 3, 4 and 5 were treated with ME of *C. reflexa* dissolved in propylene glycol at the doses of (25, 50 and 75 mg/kg, ip) respectively and groups 6, 7 and 8 were treated with ME of *C. olitorius* dissolved in propylene glycol at the doses of (15, 20 and 25 mg/kg, ip) respectively. The doses were administered on alternate days for 17 days. Body weight was noted and oestrus cycle was examined everyday in the morning and in the evening by microscopical examination (x 100) of vaginal smear using methylene blue as staining solution.

On the 18th day, after 18 hr of fasting mice were sacrificed by cervical dislocation. Normal saline and PG treated groups were sacrificed in the same oestrus phase of the ME treated groups (dioestrus phase). Uterus was dissected out, freed from fatty materials, weighed and kept on ice for further processing.

Biochemical estimation — Cholesterol in the ovaries was estimated by the method of Kingsley and Roscoe. The absorbance was observed at 680 nm and total cholesterol was quantified from the standard curve. Ascorbic acid in the ovarian tissue was measured by the reduction of dichlorophenolindophenol (DCPIP) fourteen. The optical density of the colour formed was measured at 520 nm against DCPIP reagent as blank. For the estimation of Δ,β-hydroxysteroid dehydrogenase (HSD) ovaries were homogenized with 1 ml normal saline and 1 ml of 0.1 M phosphate buffer (pH 7.4) and centrifuged at 5000 g for 20 min. The supernatant was collected and the enzyme was assayed by the method of Rabin et al. The protein content of tissue determined and the specific activity of HSD activity was expressed as U/mg of protein. Glucose-6-phosphate dehydrogenase (G6PD) in the ovarian tissue was measured by the method of Lohr et al. Glucose-6-phosphate was used as the substrate and the formation of NADPH was monitored at 340 nm against reagent blank for 10 min. The specific activity of G-6-PDH was calculated in terms of U/mg of protein. Protein content of ovaries was estimated with Folin phenol reagent.

Statistical analysis — Results are expressed as mean ± SE. Statistical analysis was done by Student’s t-test and the difference was considered statistically significant at P < 0.05.

Results are summarised in Tables 1 and 2.

Vehicle control mice showed regular oestrus cycle. Normal cyclical changes of the vaginal smear were examined in mice of groups 3, 4, 5, 6, 7 and 8 throughout the treatment period. After administration of 4th dose in low, medium and high dose level of ME

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Weight of ovaries (mg)</th>
<th>Weight of uterus (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saline</td>
<td>17.9 ± 2.5</td>
<td>27.6 ± 1.9</td>
<td>9.5 ± 1.1</td>
<td>45.1 ± 0.9</td>
</tr>
<tr>
<td>2</td>
<td>Vehicle</td>
<td>18.1 ± 1.9</td>
<td>27.8 ± 1.4</td>
<td>9.1 ± 0.9</td>
<td>46.0 ± 1.3</td>
</tr>
<tr>
<td>3</td>
<td>ME of <em>C. reflexa</em> (5 mg/kg, ip)</td>
<td>17.8 ± 1.2</td>
<td>28.0 ± 1.0</td>
<td>5.8 ± 0.7</td>
<td>30.0 ± 1.1</td>
</tr>
<tr>
<td>4</td>
<td>ME of <em>C. reflexa</em> (25 mg/kg, ip)</td>
<td>17.9 ± 1.3</td>
<td>(+1.45)</td>
<td>5.7 ± 0.8</td>
<td>26.0 ± 1.4</td>
</tr>
<tr>
<td>5</td>
<td>ME of <em>C. reflexa</em> (50 mg/kg, ip)</td>
<td>17.9 ± 1.3</td>
<td>(-5.43)</td>
<td>40.00</td>
<td>(-42.35)</td>
</tr>
<tr>
<td>6</td>
<td>ME of <em>C. reflecta</em> (75 mg/kg, ip)</td>
<td>18.0 ± 1.1</td>
<td>28.1 ± 1.8</td>
<td>5.6 ± 0.5</td>
<td>27.3 ± 1.6</td>
</tr>
<tr>
<td>7</td>
<td>ME of <em>C. olitorius</em> (15 mg/kg, ip)</td>
<td>18.7 ± 1.5</td>
<td>(-4.88)</td>
<td>41.05</td>
<td>(-39.47)</td>
</tr>
<tr>
<td>8</td>
<td>ME of <em>C. olitorius</em> (25 mg/kg, ip)</td>
<td>18.7 ± 1.5</td>
<td>25.7 ± 1.5</td>
<td>5.7 ± 0.9</td>
<td>32.5 ± 1.3</td>
</tr>
<tr>
<td>9</td>
<td>ME of <em>C. olitorius</em> (20 mg/kg, ip)</td>
<td>18.5 ± 1.4</td>
<td>27.9 ± 1.4</td>
<td>5.4 ± 0.8</td>
<td>33.4 ± 1.0</td>
</tr>
<tr>
<td>10</td>
<td>ME of <em>C. olitorius</em> (25 mg/kg, ip)</td>
<td>18.7 ± 1.6</td>
<td>(+2.17)</td>
<td>45.26</td>
<td>(-29.27)</td>
</tr>
</tbody>
</table>

*P < 0.05 when compared with control (Student’s t-test)
of both *C. reflexa* stem and *C. olitorius* seed, the estrus cycle was arrested at the dioestrous stage.

The wet weight of ovaries and uterus was reduced significantly (*P*<0.05) whereas there was no significant change in the body weight of the animal (Table 1). The crude extract of *C. reflexa* stem and *C. olitorius* seed significantly elevated the level of total cholesterol ascorbic acid contents respectively in mouse ovaries as compared to the vehicle control (Table 2). The activities of G-6-PDH and HSD were inhibited significantly (*P*<0.05) by crude extract of both *C. reflexa* stem and *C. olitorius* seed.

Sequencial changes of the vaginal smear in different phases of the oestrus cycle are closely associated with simultaneous secretory patterns of gonadal steroids. Ovarian hypothfion and anoestrus vaginal smears appear to be due to the absence or decrease of circulating gonadotropins. Both the extracts reduced the wet weight of ovaries and arrested the oestrus cycle at dioestrous stage where minimum activity of steroid hormones has been reported. These disturbances in the reproductive cycle and the decrease in the weight of the ovary and uterus in the present investigation are related with the diminution of ovarian steroidogenesis. This was associated with an elevation in the level of cholesterol, which serves as a precursor for the synthesis of steroid hormones in ovaries, suggesting thereby that cholesterol was not utilised. The ovarian dysfunction was evident in the increase in ascorbic acid level after treatment with crude extract of *C. reflexa* stem and *C. olitorius* seed. To substantiate these facts, the activities of G-6-PDH and HSD, the two key enzymes involved in steroidogenesis, were determined. ME of *C. reflexa* and *C. olitorius* inhibited the activity of both enzymes to a significant extent and thus indicating anti-steroidogenic activity of the extracts.

Preliminary phytochemical tests indicate the presence of flavonoids in the ME of *C. reflexa* stem.

Since various flavonoids have been reported to possess antifertility activity, the anti-steroidogenic property of the ME of *C. reflexa* stem may be due to the presence of such compounds.

### References


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