APPENDIX 5

PUBLICATION
INTRODUCTION

Stephania hernandifolia belongs to the family Menispermaceae. It is a herb found in West Bengal, Orissa, Assam (Chatterjee, 1996). Tribes of Orissa take approximately 5.5 gm of leaf paste after taking bath on the 4th day of menstruation. Earlier the antifertility effect of rhizomes in albino mice and rats has been observed (Chatterjee, 1996). The roots and rhizomes are reported to contain alkaloids like aknadine, aknadinine, etc. The aerial parts of the plant are reported to contain an amorphous alkaloid C-acetyl aknadine (Chatterjee, 1996). An extensive literature survey from all scientific sources does not validate the antifertility effect of the leaves of the plant. Hence, the present study was planned to investigate the antifertility effect of the leaves of Stephania hernandifolia.

MATERIALS AND METHODS

The fresh leaves of Stephania hernandifolia were collected from Ghatikia Research Garden, Bhubaneswar and authenticated by taxonomists of Botany.

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Department of Revenshaw College, Cuttack. After authentication, the plant materials were washed under running tap water to remove adhering dust, dried under shade and then powdered with the help of mechanical grinder. The resulting coarse powder was then extracted with chloroform in a Soxhlet apparatus. The extract (yield: 1.45% w/w with respect to dried material) was concentrated under vacuum to near dryness. The test samples were prepared by making an emulsion using 0.1% w/v gum acacia.

Adult Sprague–Dawley female rats of proven fertility weighing between 150–200 gms were used for the study. The animals were obtained from the Animal House of Jayadev College of Pharmaceutical Sciences, Naharkanta. The animals were maintained in acrylic cages at room temperature with standard pellet diet and water ad libitum. Before including an animal in the test group, the estrous cycles of the animals were followed for 2 cycles to ensure that the animal has functioning reproductive system. Estrogenic activity of the chloroform extract was assessed in immature rats. The experimental protocols have been approved by the Institutional Animal Ethics Committee.

The vaginal smear of each female rat was examined daily and the rats in proestrus phase of the estrous cycle were left overnight with known fertile males. The vaginal smears of those rats were examined on the following morning to determine the presence of sperm. The day on which sperms appeared in vaginal smear was labeled as day 1 of gestation and the female was considered mated. Mated animals were randomly distributed into four groups of 6 animals each. The extract was fed orally to these pregnant rats at a dose of 100, 200 and 400 mg/kg daily through an intragastric catheter. The control group received only vehicle (0.1% w/v gum acacia). Treatment was given for 10 days and the animals were laparatomised under mild ether anaesthesia on 16th day. The two horns of the uterus were examined for the number of implant and corpora lutea (Ghosh, 1984, Panda et al., 2003, Thompson, 1990).

Estrogenic activity of the extract was assessed in immature female rats weighing 55–65 gm (Vogel, 2002). Eighteen of these rats were bilaterally ovariectomized under mild ether anaesthesia through lateral incisions in the skin just below the last rib and were divided into three groups of 6 each receiving vehicle (0.1% w/v gum acacia, 2 ml/kg) diethylstilbestrol (1.5 mg/kg), chloroform extract (400 mg/kg) respectively once daily for a period of 5 days through oral route. After 24 hours of last dose of treatment the animals were sacrificed and uteri were excised from adhering tissue and weighed. Vaginal opening and vaginal cornification were also recorded and shown in table 1. Statistical analysis of the differences between control and treated groups were carried out using students 't' test. The level of significance was p<0.01.
Table 1: Effect of chloroform extract of *Stephania hernandifolia* on bilaterally ovariectomised immature Sprague-Dawley female rats.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Uterine wt. (mg)</th>
<th>Vaginal opening (%)</th>
<th>Vaginal constriction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group I (control)</td>
<td>2 ml/kg</td>
<td>127.27 ± 10.53</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Group II (Diethylstilbestrol)</td>
<td>1.5</td>
<td>219.37 ± 9.93*</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>Group III (chloroform extract)</td>
<td>400</td>
<td>221.75 ± 11.58*</td>
<td>90</td>
<td>90</td>
</tr>
</tbody>
</table>

Results expressed as Mean ± SD from six observations. All p values are calculated with respect to vehicle control. * denotes statistical significance at p<0.01.

The antifertility effect of chloroform extract with dose, number of implantation sites and number of corpora lutea are shown in table 2.

Table 2: Effect of chloroform extract of *Stephania hernandifolia* on implantation in adult female Sprague-Dawley rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Sample size</th>
<th>Animals showing implantation/no. of implants</th>
<th>% of rats with no implantations sites</th>
<th>Number of corpora lutea.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I control</td>
<td>2 ml/kg</td>
<td>6</td>
<td>6, 8, 10, 9, 7, 9, 11 #</td>
<td>0</td>
<td>9, 12, 9, 9, 10, 12</td>
</tr>
<tr>
<td>Group II</td>
<td>100</td>
<td>6</td>
<td>3/4, 3, 4</td>
<td>50</td>
<td>5, 4, 6</td>
</tr>
<tr>
<td>Group III</td>
<td>200</td>
<td>6</td>
<td>2/2, 3</td>
<td>66.66</td>
<td>3, 4</td>
</tr>
<tr>
<td>Group IV</td>
<td>400</td>
<td>6</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>
Data shows the number of implants and the number of corpora lutea on day 16.

RESULTS

The data shows that the chloroform extract possess anti fertility effect in a dose dependent manner. On laparatomy the uterine horns of rats treated with 100 mg/kg, 200 mg/kg showed reduced number of implantation sites compared to the control group animals. At a dose of 100 mg/kg the drug is found to be 50 % active where as at a dose of 200 mg/kg is 67 % active. Complete resorption of implants was observed at a dose of 400 mg/kg.

The data also shows that the chloroform extract caused opening and cornification of vagina in immature rats. There was also a significant increase in uterine weight in the chloroform extract treated animals.

DISCUSSION

Reproductive cycle in mammals commences with the onset of puberty. In laboratory animals like rats, it is usually judged with the help of vaginal opening at about 38 day of age (Panda et al., 2003). Estrogenic activity is also associated with vaginal cornification and increase in uterine weight of immature female rats (Vogel, 2002). So the anti-fertility effect of chloroform extract of leaves of Stephania hernandifolia is justified.

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REFERENCES


