Research Paper

Preliminary study on the antiimplantation activity of compounds from the extracts of seeds of *Thespesia populnea*

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ABSTRACT

Objective: To evaluate the preliminary antiimplantation activity of isolated pure principles from successive extracts of petroleum-ether (PE) and ethyl acetate (EAc) and subsequent crude alcoholic extract of seeds of *T. populnea* in female albino rats.

Material and Methods: Graded doses of the active principles and the crude alcoholic extract (in 1% gum acacia suspension) were tested for possible antiimplantation activity in Sprague-Dawley female rats of normal estrus cycle after overnight cohabitation with males of proven fertility. The day when spermatozoa was detected in vaginal smear was treated as 1st day of pregnancy. The compounds were administered to female rats from the 1st day to the 7th day of pregnancy. On the 10th day, the rats were laparotomized under light anesthesia and the numbers of implantation sites and corpora lutea were noted.

Results: Chromatographic pure principle from PE extract showed significant antiimplantation activity (60 %) at the dose of 110 mg/kg, b.w while that from EAc extract showed 48.6 % effect at the same dose. In contrast, the final alcoholic extract showed no such significant action.

Conclusion: The active principles from PE and EAc extracts showed significant antiimplantation activity and they were found to be a mixture of two groups of long-chain fatty acids from GLC.

KEY WORDS: Fatty acids, female contraceptive.

Introduction

Rapid rise in population has caused serious problems in the economic growth and all-round human development in developing countries like India. Family planning has been promoted through several methods of contraception, but due to serious adverse effects produced by synthetic steroidal contraceptives,1-3 attention has now been focused on indigenous plants for possible contraceptive effect.

The plant, *Thespesia populnea* (Linn.) Soland ex Correa of the Malvaceae family is a fairly large, quick-growing evergreen tree distributed mainly along the coastal regions throughout India. The fruit, leaves and root of this plant are used externally in scabies, psoriasis and other skin diseases. The plant is astringent, acrid, depurative, haemostatic, antidiarrheal and antibacterial.4,5

The floral extract of *T. populnea* exhibited antisteroidogenic activity in mouse ovary.6 The flowers contained kaempferol, kaempferol-7-glucoside and gossypetin. The fruit kernels were reported to contain β-sitosterol, ceryl alcohol and a yellow pigment, thepesin.7,8 (+)-Gossypol was reported to be present in the bark and fruit of the plant and it was found to be optically active.2 It was found that oral administration of (+)-gossypol in dose levels of 10, 30 and 100 mg/kg showed 33%, 63% and 79% antiimplantation activity in female albino rats respectively.10

In the present work, we have undertaken the preliminary investigation of the antiimplantation activity of different extracts of seeds of *T. populnea* in rats, which has not been carried out so far, and also the chemical analysis of the extract.

Material and Methods

Plant materials

The seeds of *T. populnea* were procured from United Chemicals & Allied Products, Kolkata, India and authenticated at Botanical Survey of India, Howrah (West Bengal). The thoroughly air-dried seeds were pulverized to powder. About 450 g of powdered seed was successively hot extracted with petroleum-ether (60-80°C), ethyl acetate (EAc) and finally...
alcohol. The solvents were removed by distillation under reduced pressure using rotary vacuum evaporator. The seeds yielded 4.5, 4.0 and 5.5% w/w dried extract of PE, EAc and alcohol respectively.

**Phytochemical analysis**

The dried PE extract was column chromatographed over silica-gel (60-120 mesh). Elution of the column with n-hexane gave a colorless oil, C-1 (3.4 g) which was found to be homogeneous from TLC. From spectral analyses (IR and 1H-NMR), C-1 was found to be a mixture of long-chain fatty acids. This was esterified with ethereal diazomethane at 0°C to give fatty acid methyl ester (FAME) and purified by column chromatography twice to afford a pure waxy solid. This solid was then analyzed for fatty acid ester compositions by GLC.

The dried EAc extract was similarly column chromatographed over silica-gel and elution with petroleum-ether (60-80°C) gave a waxy solid, C-2 (3.2 g). This solid was characterized as a mixture of fatty acids from spectral analyses and hence converted into methyl esters and analyzed by GLC as before. The alcohol extract gave positive test for tannins.

**Experimental animals**

Colony-bred albino rats (Sprague-Dawley strain, weighing 150-200 g) were procured from Indian Institute of Chemical Biology (CSIR), Kolkata, India. All the animals were acclimatized in normal laboratory conditions (ambient temperature: 25±3°C; relative humidity: 50-55%; 12:12 dark: light cycle) with access to food (Hindustan Lever Ltd., Mumbai, India) and water ad libito. The initial body weight of each animal was recorded. The vaginal smears of the female rats were studied microscopically for the estrus cycle every morning. Only females with a normal estrus cycle were selected for the experiment. The experimental protocol was approved by the Institutional Animal Ethics Committee.

**Experimental design**

Antimplantation activity was determined following the method of Khanna and Chowdhury.11 Female rats of proestrus phase were kept with male rats of proven fertility in the ratio of 2:1. The females were examined the following morning for evidence of copulation. The animals which showed thick clumps of spermatozoa in vaginal smears were segregated from the male partner and divided into two groups: control and treated, with five animals each. The day when spermatozoa were detected in the vaginal smear was considered as day 1 of pregnancy.

Pilot studies (n=2) with 150 mg/kg of C-1 showed 100% inhibition of implantation sites in uteri horns. However, a lower dose of 120 mg/kg also showed 100% inhibitory action while with a dose of 100 mg/kg, there was 55% inhibition of implants. Four doses of 50, 75, 90 and 110 mg/kg were chosen for the experiment (n=5). The same dose regimens were used for C-2 and the alcoholic extract to study their comparative activities.

Specified doses of test compounds and alcohol extract were administered orally to rats after making a suspension in the vehicle of 1% gum acacia in distilled water from 1st day to 7th day of pregnancy. The volume was restricted to 0.2 ml/100 g. A parallel control group received vehicle only during the same period of treatment. All the animals were sacrificed under light anesthesia and laparotomy was performed to determine the number of implantation sites on the two uteri horns and the number of corpora lutea on the two ovaries. The fertility rate was calculated by the percentage of implantations per number of corpora lutea (representing the number of eggs ovulated).

**Statistical analysis**

The data are presented as mean±SEM. The results were statistically analyzed using one-way ANOVA followed by two-tailed Dunnett’s multiple comparison test. *P* values <0.05 were considered to be statistically significant.

**Results**

Both C-1 and C-2 exhibited a dose response pattern in the dose range 50-110 mg/kg vide Table 1. It was found that the C-1 fed to female rats orally at the dose of 110 mg/kg from Days 1-7 of pregnancy showed an average of 60 % inhibition of implantation in uterine horns compared to an average of 48.6 % with C-2 at the same dose.

The alcoholic extract did not show any significant antimplantation activity up to a dose of 110 mg/kg. The inhibitory pattern of implantation with a dose range from 50 to 110 mg/kg was more or less similar and not dose-dependent (Table 1).

The fatty acid compositions of C-1 and C-2 are shown in Table 2. From GLC, C-1 and C-2 were found to contain two groups of fatty acids (FAs) of different compositions, with palmitic acid (59.0%) and oleic acid (22.8%) as predominant FAs of C-1 compared to 46.9% of palmitic acid and 43.2% of oleic acid in case of C-2. Also, GLC showed the presence of other fatty acids in low concentration both in C-1 and C-2 (Table 2). Chemically, C-2 contains FAs with higher level of unsaturation compared to C-1.

**Discussion**

Many crude extracts and active principles derived from medicinal plants were evaluated for their antifertility effects in animal models.12,13 The floral extract of a plant, *Thespesia populnea* was studied by Kavimani et al and was reported to have antisteroidogenic activity in female albino mice.6 Chemical examination of flowers revealed the presence of flavone and its glycoside viz. kaempferol, gossypetin and kaempferol-3-O-glucoside. Furthermore, the seeds of this plant contained thespesin.7,8 Gossypol was reported to be present in the bark and fruit9 and this has already been found to have antimplantation activity in female rats.10

The present work reports the preliminary antimplantation activity of two different groups of fatty acids, C-1 and C-2, present in the seeds of *T. populnea* extracted successively with petroleum-ether and then with ethyl acetate. Autopsy on day 10 revealed that all the control rats (treated with a vehicle of 1% gum acacia) were pregnant and had a normal number of...
implantations and a normal duration of diestrus. On treatment with C-1, it was found that the number of implants on uteri horns decreased as the doses increased from 50 to 110 mg/kg. Similar inhibitory activity of C-2 was found in a dose-dependent pattern. A decrease in the diestrus phase with concomitant increase in estrus was observed with C-1 at the dose of 110 mg/kg from day 1-7 of pregnancy leading to significant inhibition of implantation sites in uteri horns (60%) compared to 48.6% with C-2 at the same dose. The alcoholic extract was found to have no such effects on the number of implantation

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose, mg/kg body weight</th>
<th>Pattern of estrus cycle, D1-D7</th>
<th>No. of implantation sites</th>
<th>No. of corpora lutea</th>
<th>% Inhibition of implantation</th>
<th>Fertility rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>All diestrus</td>
<td>14.0 ± 0.8</td>
<td>17.2 ± 0.8</td>
<td>0.0</td>
<td>5.8</td>
</tr>
<tr>
<td>Active component</td>
<td>50</td>
<td>All diestrus</td>
<td>11.8 ± 0.4*</td>
<td>16.2 ± 0.8</td>
<td>15.7</td>
<td>5.2</td>
</tr>
<tr>
<td>from PE extract</td>
<td>75</td>
<td>&quot;</td>
<td>10.2 ± 0.4*</td>
<td>15.0 ± 0.4</td>
<td>27.1</td>
<td>4.8</td>
</tr>
<tr>
<td>(C-1)</td>
<td>90</td>
<td>&quot;</td>
<td>8.0 ± 0.3*</td>
<td>14.2 ± 0.8</td>
<td>42.8</td>
<td>4.0</td>
</tr>
<tr>
<td>(C-2)</td>
<td>110</td>
<td>Duration of diestrus decreases from D1-D3 and estrus increases from D4 onward</td>
<td>5.6 ± 0.4*</td>
<td>12.0 ± 0.4*</td>
<td>60.0</td>
<td>3.3</td>
</tr>
<tr>
<td>Active component</td>
<td>50</td>
<td>All diestrus</td>
<td>12.0 ± 0.3</td>
<td>16.2 ± 0.8</td>
<td>14.3</td>
<td>5.3</td>
</tr>
<tr>
<td>from EAc extract</td>
<td>75</td>
<td>&quot;</td>
<td>10.6 ± 0.4*</td>
<td>15.8 ± 0.9</td>
<td>24.3</td>
<td>4.8</td>
</tr>
<tr>
<td>(C-2)</td>
<td>90</td>
<td>&quot;</td>
<td>8.8 ± 0.3*</td>
<td>14.2 ± 0.5</td>
<td>37.1</td>
<td>4.4</td>
</tr>
<tr>
<td>(C-2)</td>
<td>110</td>
<td>Duration of diestrus decreases from D1-D3 and estrus increases from D4 onward</td>
<td>7.2 ± 0.4*</td>
<td>13.6 ± 0.9*</td>
<td>48.6</td>
<td>3.8</td>
</tr>
<tr>
<td>Crude alcoholic</td>
<td>50</td>
<td>All diestrus</td>
<td>13.0 ± 0.4</td>
<td>16.4 ± 0.7</td>
<td>7.1</td>
<td>5.7</td>
</tr>
<tr>
<td>extract</td>
<td>75</td>
<td>&quot;</td>
<td>12.0 ± 0.8</td>
<td>15.6 ± 0.8</td>
<td>14.3</td>
<td>5.5</td>
</tr>
<tr>
<td>(C-2)</td>
<td>90</td>
<td>&quot;</td>
<td>11.6 ± 0.7*</td>
<td>15.4 ± 0.9</td>
<td>17.1</td>
<td>5.4</td>
</tr>
<tr>
<td>(C-2)</td>
<td>110</td>
<td>&quot;</td>
<td>10.8 ± 0.7*</td>
<td>15.0 ± 0.7</td>
<td>22.8</td>
<td>5.1</td>
</tr>
</tbody>
</table>

One-way ANOVA

F = 21.81, df = 12,52, P <0.01

Values are mean ± SEM, (n = 5).

*P<0.05 in comparison with control.

D1-D7: Day 1-7 of pregnancy.

\( x = \frac{(NI_C - NI_T) \times 100}{NI_C} \), where NI_C and NI_T denote the number of implantation sites in uterine horns for control and drug-treated female rats respectively.

Table 2

Fatty acid (FA) compositions of PE and EAc extracts

<table>
<thead>
<tr>
<th>Fatty acids from PE extract (C-1)</th>
<th>Fatty acids from EAc extract (C-2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fatty acid</strong></td>
<td><strong>R_t</strong></td>
</tr>
<tr>
<td>C14:0</td>
<td>1.01</td>
</tr>
<tr>
<td>C14:1 ( \Delta 9 ) cis</td>
<td>1.30</td>
</tr>
<tr>
<td>C16:0</td>
<td>1.67</td>
</tr>
<tr>
<td>C16:1 ( \Delta 9 ) cis</td>
<td>2.21</td>
</tr>
<tr>
<td>C18:0</td>
<td>2.83</td>
</tr>
<tr>
<td>C18:1 ( \Delta 9 ) cis</td>
<td>3.28</td>
</tr>
<tr>
<td>C18:2 ( \Delta 9,12 ) cis</td>
<td>4.12</td>
</tr>
<tr>
<td>C18:3 ( \Delta 9,12,15 ) cis</td>
<td>4.93</td>
</tr>
<tr>
<td>C22:0</td>
<td>8.70</td>
</tr>
<tr>
<td>C22:4 ( \Delta 9,12,15,18 ) cis</td>
<td>14.69</td>
</tr>
</tbody>
</table>

sites in a similar dose regimen.

This loss of implantation caused by the fatty acids may be due to antizygotic, blastocytotoxic or antimplantation activity as described by Hafez. Further studies such as ovarian hormonal profile and estrogenicity test in immature bilaterally ovariectomised rats are in progress.

In the systematic phytochemical study, the active principles from PE and EAc extracts were found to be a mixture of different FAs which were characterized by GLC after derivatization to methyl esters. Lesser unsaturation of the FAs of C-1 might be a cause of the higher pharmacological effect compared to those of C-2 which contains highly unsaturated FAs. No gossypol and flavones could be detected in any of the three extracts. Upadhyay et al. have reported the immunoc contraceptive property of neem oil from the seeds of *Azadirachta indica* and the active constituents were found to be a mixture of six components which comprised saturated, mono- and di-unsaturated free fatty acids and their methyl esters. In the present investigations also, it was found that two groups of fatty acids (C-1 and C-2) from *T. populnea* seeds exert significant antimplantation activity and further work in this direction for establishing the mechanism of antifertility action is in progress.

**Acknowledgement**

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**References**