Antiovulatory and abortifacient potential of the ethanolic extract of roots of *Momordica cymbalaria* Fenzl in rats

R. Koneri, R. Balaraman*, C.D. Saraswati

Objective: To study the antiovulatory and abortifacient activity of the ethanolic extract of roots of *Momordica cymbalaria* Fenzl.

Materials and Methods: Female Wistar albino rats (150 to 200 g) with at least three regular estrous cycles were administered ethanolic extracts of roots of *Momordica cymbalaria* Fenzl, at two doses 250 and 500 mg/kg orally for 15 days. Control group received vehicle (tween 80 1%, p.o. daily). Animals were sacrificed on 16th day. One ovary was subjected to histopathological studies and the other for biochemical studies. Abortifacient study was done in another set of three groups of animals. The extracts at doses of 250 and 500 mg/kg were administered orally through gastric gavage from the day 6 to day15 of pregnancy (the period of organogenesis). The animals were laparotomised under light ether anesthesia and semi-sterile conditions on day 19th of pregnancy. Both horns of the uterus were observed for the number of implantation sites, resorptions, dead and alive foetuses.

Results: Highly significant (P<0.001) decrease in the duration of estrous cycle and metaestrous phase and increase in proestrous phase was seen, but diestrous phase was unchanged in both 250 and 500 mg treated group when compared to untreated group. Significant decrease in the ovarian weight and a highly significant increase in serum cholesterol with 250 mg/kg dose were seen. Histology of ovary showed an increase in preovulatory and atretic follicles. Ethanolic extract showed a dose dependent abortifacient effect in pregnant rats during organogenesis period. At 250 mg/kg ethanolic extract did not show any abortifacient activity but reduced the number of viable foetuses and resorptions with no change in the foetal weight when compared with control group. At 500 mg/kg ethanolic extract showed highly significant (P<0.001) abortifacient activity.

Conclusion: The ethanolic extract at both doses (250 and 500 mg/kg) showed antiovulatory activity. It is abortifacient at 500 mg/kg but not at 250 mg/kg.

KEY WORDS: Antifertility, contraception, ovulation.

Introduction

Synthetic estrogens and progesterones, in combination or alone, are extensively used as contraceptives. Although they are highly effective, they are associated with high incidence of side effects. Therefore, the search for new antifertility molecules with minimal side effects continues. Many plant preparations are used to control fertility.

Material and Methods

Plant collection and preparation of the extract

Fresh roots of *Momordica cymbalaria* were collected from Gadag district, Karnataka. They were identified and authenticated by a botanist in the Department of Botany, Bangalore University, Bangalore. A specimen sample of the same was preserved at the herbarium of the Department of Botany, with the voucher no. 18122003, for future reference.

The roots were isolated and chopped into small pieces. Next, they were dried under shade at room temperature for seven days. The dried roots were powdered, passed through sieve (coarse 10/44), and extracted with 95% v/v ethanol using Soxhlet extractor. The combined extracts were concentrated at 40°C to obtain dark brownish yellow residue. The yield obtained from this process was found to be 21.5% w/w.

Visveswarapura Institute of Pharmaceutical Sciences,
Vokkaligara Sangha Campus, K R Road, Bangalore 560004

*Department of Pharmacy, Faculty of Science and Technology, M.S. University of Baroda, Baroda.

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Correspondence to:
Raju Koneri
E-mail: rajukoneri@rediffmail.com

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for ved as control. Group II and on the duration of the different phases of the estrous cycle in rat yxhibited three regular cycles.

+ xtracted at 250 and 500 mg/kg, y exhibited thick clumps of spermatozoa in the vaginal smear were separated, and that day was designated as day one of pregnancy. The pregnant rats were divided into three groups of six animals each. Group I received vehicle only (1% Tween 80, p.o. daily) and served as control. Group II and group III received ethanolic extract at 250 and 500 mg/kg, p.o. daily, respectively. The extracts were administered orally through gastric gavage from the 6th to the 15th day of pregnancy (period of organogenesis). The animals were laparotomised under light ether anesthesia on the 19th day of pregnancy. Both horns of the uterus were observed for the number of implantation sites, resorptions, and dead and alive fetuses.39,410

The results are expressed as mean±SEM. The statistical significance between groups was analysed using the one-way ANOVA test and the Tukey Kramer multiple comparison post-test. P<0.05 was considered significant.

Results
Mortality in the acute toxicity test was not seen in the limit test at a dose of 5000 mg/kg. Therefore, 1/10th & 1/20th of the dose were selected for this study.

Ethanolic extract at 250 and 500 mg/kg caused a significant (P<0.001) decrease in the duration of the estrous and metestrous phases, no change in the duration of the diestrous phase.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>No. of days in proestrous</th>
<th>No. of days in estrous</th>
<th>No. of days in metestrous</th>
<th>No. of days in diestrous</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control (1% Tween 80)</td>
<td>—</td>
<td>2.08 ± 0.53</td>
<td>3.33 ± 0.33</td>
<td>4 ± 0.28</td>
<td>5.58 ± 0.37</td>
</tr>
<tr>
<td>II</td>
<td>Ethanolic extract</td>
<td>250</td>
<td>7.08 ± 0.5*</td>
<td>1.25 ± 0.28*</td>
<td>1.41 ± 0.2*</td>
<td>5.16 ± 0.47</td>
</tr>
<tr>
<td>III</td>
<td>Ethanolic extract</td>
<td>500</td>
<td>7.41 ± 0.8*</td>
<td>0.75 ± 0.4*</td>
<td>0.75 ± 0.35*</td>
<td>6.16 ± 1.23</td>
</tr>
<tr>
<td>One-way</td>
<td>F</td>
<td></td>
<td>20.853</td>
<td>15.969</td>
<td>34.973</td>
<td></td>
</tr>
<tr>
<td>ANOVA</td>
<td>df</td>
<td></td>
<td>15.2</td>
<td>15.2</td>
<td>15.2</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.0001</td>
<td></td>
<td>0.0002</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM. n=6 in each group. *P<0.001 when compared with control.

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Ovarian weight in mg/100 g body weight</th>
<th>Cholesterol level in ovary (mg/50 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control (TWEEN 80, 1%)</td>
<td>—</td>
<td>40.80±1.13</td>
<td>0.33±0.05</td>
</tr>
<tr>
<td>II</td>
<td>Ethanolic extract</td>
<td>250</td>
<td>31.88±0.9*</td>
<td>1.23±0.15**</td>
</tr>
<tr>
<td>III</td>
<td>Ethanolic extract</td>
<td>500</td>
<td>34.26±1.16*</td>
<td>0.55±0.03</td>
</tr>
<tr>
<td>One-way</td>
<td>F</td>
<td></td>
<td>19.131</td>
<td>17.671</td>
</tr>
<tr>
<td>ANOVA</td>
<td>df</td>
<td></td>
<td>15.2</td>
<td>15.2</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.0001</td>
<td></td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM. n=6 in each group. *P< 0.01, ** P< 0.001 when compared with control.
Table 3
Abortifacient effects of the ethanolic and the aqueous extracts of *Momordica cymbalaria* in rats when fed orally between days 6 and 15 of pregnancy

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>No. of foetus in individual rat</th>
<th>No of resorptions in individual rat</th>
<th>No of foetus</th>
<th>Foetus weight</th>
<th>No. of rats aborted</th>
<th>Abortion in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Tween 80, 1%)</td>
<td>—</td>
<td>13,11,9,12,8,11</td>
<td>0,0,0,0,0,0</td>
<td>10.66±0.16</td>
<td>1.35±0.03</td>
<td>0/6</td>
<td>0.0%</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>250</td>
<td>10,4,12,2,10,8</td>
<td>3,0,3,1,2,0</td>
<td>6.16±1.24*</td>
<td>1.32±0.04</td>
<td>0/6</td>
<td>0.0%</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0,0,0,0,0,7</td>
<td>0,0,0,0,0,7</td>
<td>—</td>
<td>0</td>
<td>6/6</td>
<td>100%</td>
</tr>
</tbody>
</table>

One-way ANOVA

F = 15.899

df = 15.2

P <0.0002

Values are mean±SEM. n=6 in each group. *P<0.05 when compared with control.

Figure 1. Section of rat ovary treated with Tween 80 1% (control) shows matured graffian follicle (GF), corpus luteum (CL) and developing follicles (DF). H x E 100x

Figure 2. Section of rat ovary treated with ethanolic extract (250 mg/kg, body weight) shows many primary developing follicles (DF), atretic follicles (AF) and disorganised stroma cells. H x E 100x

Figure 3. Section of rat ovary treated with ethanolic extract (500mg/bdy weight) shows many primary developing follicles (DF), atretic follicles (AF) and disorganised stroma cells. H x E 100x

and a significant (P<0.001) increase in the duration of the proestrous phase, compared with the control group. [Table 1]

Ethanolic extract at 250 and 500 mg/kg significantly (P<0.01) decreased the weight of ovaries, compared with the control group. [Table 2] An increase in preovulatory follicles and atretic follicles was seen in the treated groups (Figure 2 and 3). A highly significant (P<0.001) increase in ovarian cholesterol level was observed at 250 mg/kg dose, compared with the control group. [Table 3]

At 500 mg/kg, ethanolic extract showed a highly significant (P<0.001) abortifacient effect. At 250 mg/kg, ethanolic extract did not show any abortifacient activity, but reduced the number of viable fetuses and resorptions (6.16±1.24). There was no change in the fetal weight, compared with the control group. [Table 4]

Discussion

Many morphological, histological, physiological, and biochemical changes occur in the ovary during the estrous cycle. During the maturation of preovulatory follicles, ovulation
takes place under the combined and balanced influence of ovarian and extra ovarian hormones. Imbalance in these hormones leads to irregularity in the ovarian functions and duration of the estrous cycle.[12][14]

The estrous cycle in the rats treated with extract (250 and 500 mg/kg) showed a decrease in the duration of estrous and the metestrous phases. It was also characterised by a prolongation of the proestrous phase. The prolongation of the proestrous phase indicates that maturation of the follicle in the preovulatory phase was delayed, leading to non-maturation of graffian follicle. Non-availability of matured graffian follicle was indicated by reduction in the estrous and the metestrous phases. Therefore, ovulation was inhibited. This result was further supported by our histopathological studies (Figure 2 and 3) in which the transverse section of the ovary showed the presence of primary or developing follicles.

Ovary can be considered an aggregate of three endocrine tissues, the stroma, the follicle and the corpus luteum. The weights of these tissues constitute the net weight of the ovary. During the estrous cycle the weight of the ovarian tissue increases under the influence of gonadotrophic and steroidal hormones. The decrease in the weight of ovaries of the rats treated with extract indicates a decrease in the activity of the stroma, the follicle, and the corpus luteum in the ovary. This decrease may be due to the non-availability of gonadotrophic or steroidal hormones or both.[19]

Atretic follicles are degenerating preovulatory follicles. The degeneration of preovulatory follicles takes place due to non-availability of steroidal hormones (essential for their maturation and differentiation), non-availability of local estrogen produced by granulosa cells, or imbalance in endogenous steroid, protein and hormones. The presence of increased atretic follicles in the rats treated with ethanolic extract, compared with control rats, indicates that the extract promotes the degeneration of preovulatory follicles. Cholesterol is the precursor for the steroidogenesis of ovarian endocrine tissues. The significant increase in ovarian cholesterol in the treated group (250 mg/kg) indicates that cholesterol is not used for steroidogenesis.[18] But 500 mg dose did not show a similar effect indicating that it occurs only at a lower dose.

Abortion refers to the premature expulsion of the products of conception from the uterus. Abortion may be due to maternal exposure to chemicals, which can disrupt pregnancy and cause detachment of the embryo.[10] Ethanolic extract at 500 mg/kg showed 100% abortifacient activity, while 250 mg/kg did not show abortifacient activity. However, it reduced the number of viable fetuses.

To conclude a highly significant decrease in the duration of the estrous and the metestrous phases and increase in the duration the proestrus phase was seen. In addition, a highly significant decrease in ovarian weight and increase in cholesterol level, compared with the control group, was noted. These findings indicate that extract *Momordica cymbalaria* produces temporary inhibition of ovulation. The result of administration of extract to the pregnant rats during organogenesis shows that the extract is abortifacient only at the higher dose of 500 mg. These findings could explain the traditional use of *Momordica cymbalaria* as abortifacient and antiovulator.

References