Antiimplantation and Anti-estrogenic Activity of Eugenia jambolana lam. Seed

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ABSTRACT

Petroleum ether, ethyl acetate and ethanol extracts of seeds of Eugenia jambolana Lam. were tested for pre-coital anti-fertility activity in female albino rats. Of these, the ethyl acetate extracts were found to be most effective in causing significant anti-implantation activity. The ethyl acetate extract also exhibited a strong anti-estrogenic activity, when administered alone. It also inhibits the estrogen induced gain in the uterine weight when administered along with ethinyl estradiol. Histological studies of the uterus were carried out to confirm this activity.

Key words: Anti-implantation, Anti-estrogenic, Corpora lutea, Eugenia jambolana Lam.

INTRODUCTION

A large number of plants have been reported to exhibit anti-implantation and abortifacient activity, but a few have been evaluated for such effects in laboratory animals. To date many steroidal and non-steroidal substances have been and are being used as contraceptive agents. Although they act as potent antifertility agents but they are not free from marked side effects. Hence, an approach was pursued to identify new antifertility agents from natural sources. Eugenia jambolana Lam. is one such plant of Myrtaceae family are important ayurvedic medicine in treating diabetes. It has also been reported to have anti-inflammatory (1), hypoglycemic (2), anti ulcerative (3), hypolipidemic (4), antioxidant (5) and several other ailments. The flowers of Eugenia jambolana have also been reported to possess contraceptive activity in male albino rats (6). Phytochemical analysis of the seed of E. jambolana reveals that it has alkaloids, glycosides, flavonoids, steroids, saponins, tannins amino acids, and triterpenoids. Since some of these compounds are known to exhibit antifertility activity in female rats. Hence, the present study was undertaken to find out the unexplored antifertility and hormonal activities of the seed extracts of Eugenia jambolana Lam.

2. MATERIALS AND METHODS

2.1 Plant Collection and Extraction

The fully mature Eugenia jambolana seeds were collected from fields in and around Madikeri district of Karnataka, India, during June – August 2010 from a single tree. The seed was identified and authenticated by Dr. Sudarshana, Professor of the Department of Botany, University of Mysore, Manasagangotri, and Mysore and the plant bearing herbarium number of 1634. The seeds were shade dried, powdered and subjected to Soxhlet extraction successively and separately with petroleum ether (40-60°C), Ethyl acetate (76-77°C) and ethanol (70-75°C). The extracts were concentrated to dryness in a flash evaporator under reduced pressure and controlled temperature (50-60°C). All the extracts were stored in a refrigerator. The extracts were prepared in DMSO (1%) and they were administered orally to the rats at doses of 200 and 600mg/kg body weight.

2.2 ANIMALS

Colony bred female albino rats (Wistar Strain) weighing (150-200g) showing regular estrous cycle were used and were allowed to access to food and water ad libitum. Immature ovariectomized female rats weighing 30-35g were also employed for the study of the estrogenic/antiestrogenic effect of the extract.

2.3 ANTI-IMPLANTATION ACTIVITY

Antimplantation activity was determined following the method of Khanna and Chowdhury (7). Vaginal smears from each rat were monitored daily and the rats found in proestrus phase of cycle were caged with males of proven fertility in the ratio 2:1 and examined the following morning for evidence of copulation. Rats exhibiting thick clumps of spermatozoa in their vaginal smears were separated and the day was designated as day 1 of pregnancy and those rats were divided into seven groups containing six rats in each group. The extract was also administered at 200 and 600mg/kg body weight orally from day 1 to 7 of pregnancy. Control rats received the vehicle (DMSO 1%). On day 10, the females were autopsied under light ether anesthesia and their uteri were examined. The number of pregnant females, number of implantations, and number of live fetus were recorded. Samples of fresh ovary were removed, fixed in Bouin’s fluid for 24h, dehydrated in alcohol, cleared in xylene and embedded in paraffin wax. Routine 5µm sections were then cut and stained using Haematoxylin-Eosin method and count the number of corpora lutea and observe the follicular changes.

2.4 HORMONAL PROFILE

2.4.1 Estrogenic/Anti-estrogenic activity

The ethyl acetate extract was found to be the most active of the extracts of Eugenia jambolana seed. Hence, they were subjected to a detailed investigation for potential estrogenic and antiestrogenic activity. Colony bred immature female albino rats (Wistar strain), 21-23 days old, weighing between 30-35g were bilaterally ovariectomised by dorsolateral approach under light ether anesthesia and sterile conditions. They were divided into six groups consisting of six rats each. Group I received the control (DMSO 1%), Group II received 1µg ethinyl estradiol/ rat/day in olive oil subcutaneously. Group III and IV received the ethyl acetate seed extract at 200 and 600mg/kg body weight respectively. Group V and VI received in addition to 1µg ethinyl estradiol a test dose of the ethyl acetate seed extract at 200 and 600mg/kg body weight respectively. All the above treatments were given for 7 days. On the 8th day, the rats were sacrificed by ether anesthesia, the uteri dissected out and separated from the adherent tissues and weighed up to the nearest milligram on sensitive balance. Estrogenic activity was assessed according to the method of taking uterine wet weight, opening of the vagina, and cornification of vaginal epithelial cells as the points of evaluation (8). Additionally, the uterine tissue of
rats from each group was fixed in Bouin’s fluid and processed for histological preparation using Haematoxylin-Eosin method. The diameter of the uterus, thickness of the endometrium and the height of the endometrial epithelium were measured in 20 randomly sectioned sections using an ocular micrometer.

25. Statistical analysis
Results were expressed as mean ± standard error. The data was analyzed using One-Way analysis of variance (ANOVA). The significance level considered was P<0.05.

3. RESULTS

3.1. Effect of the extract on the implantation and estrogenic/antiestrogenic Activity
Of the three extracts of Eugenia jambolana seeds evaluated for anti-implantation activity, the ethyl acetate extract at 200 and 600mg/kg b.w. significantly inhibited pregnancy in two of 6 rats with a mean number of implants of 4.16 ± 1.56 (P<0.05) and four of 6 rats with a mean number of implants of 1.16 ± 1.21 (P<0.001) respectively (Table 1). Ethyl acetate seed extract was also showed reduction (P<0.05) in the number of corpora lutea of pregnancy as well as the postcoitum fertility index. However, both the doses of the petroleum ether and ethanol extracts were found to be ineffective and the number of implantation sites and the number of corpora lutea in these cases were comparable with the control rats.

No toxic effects were observed either by gross visual examination or in the weight of animals. After discontinuation of treatment, all the animals were mated. This resulted in pregnancy and delivery of normal litters, indicating that the action of the extracts was reversible.

The antiestrogenic effects of the ethyl acetate extract are shown in Table 2 and 3. When given to immature mice, ethyl acetate seed extract at 200 mg/kg body weight induced a significant decrease (P<0.05) and at 600 mg/kg body weight, caused a highly significant decrease (P<0.001) in the weight of the uterus when compared with the control. The uterine weight is still more decreased when administered the ethyl acetate extract along with ethinyl estradiol, indicating the high antiestrogenic activity of the ethyl acetate seed extract. The ethyl acetate extract at both the dose level either along with ethinyl estradiol or alone has caused highly significant (P<0.001) decrease in the diameter of uterus, thickness of myometrium, thickness of endometrium and surface epithelial cell height, in overiectomized immature rats. Also all the experimental rats treated with ethyl acetate extract alone or in combination with ethinyl estradiol were showed closed vagina and the absence of cornified epithelial cells in the vagina.

Table 1: Antimplantation activity of E. jambolana seed in female rat when fed orally from day 1 to 7 of pregnancy. (6 animals were used in each group).

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>No of rats with implantation sites</th>
<th>No of rats without implantation sites</th>
<th>No of Corpora lutea</th>
<th>No of implants/rat</th>
<th>Mean number of implants ± SE</th>
<th>Fertility index(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>DMSO(1%)</td>
<td>6</td>
<td>0</td>
<td>15.56</td>
<td>11,10,8,12,10,10</td>
<td>10.16</td>
<td>100</td>
</tr>
<tr>
<td>Petroleum Ether</td>
<td>200</td>
<td>6</td>
<td>0</td>
<td>13.83</td>
<td>11,11,12,11,10,13</td>
<td>11.33</td>
<td>100</td>
</tr>
<tr>
<td>Petroleum Ether</td>
<td>600</td>
<td>6</td>
<td>0</td>
<td>14.27</td>
<td>9.10,12,11,10,11</td>
<td>10.5</td>
<td>100</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>200</td>
<td>4</td>
<td>2</td>
<td>7.79***</td>
<td>0,4,8,5,0,8</td>
<td>4.16***</td>
<td>66.7***</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>600</td>
<td>2</td>
<td>4</td>
<td>4.24***</td>
<td>0,4,0,3,0,0</td>
<td>1.16***</td>
<td>33.4***</td>
</tr>
<tr>
<td>Ethanol</td>
<td>200</td>
<td>6</td>
<td>0</td>
<td>12.15</td>
<td>11,8,10,10,12,10</td>
<td>10.16</td>
<td>100</td>
</tr>
<tr>
<td>Ethanol</td>
<td>600</td>
<td>5</td>
<td>1</td>
<td>10.22</td>
<td>10,8,8,10,10,7,7</td>
<td>7.16</td>
<td>83.4</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM, ***P<0.001, **p<0.01, *p<0.05, when compared with control

Table 2: Estrogenic/antiestrogenic activity due to the administration of ethyl acetate extract of E. jambolana seeds in immature bilaterally overiectomized rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Uterus wt (mg/100g b.w.)</th>
<th>Vaginal status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>DMSO(1%)</td>
<td>67.01</td>
<td>Vagina not open</td>
</tr>
<tr>
<td>Ethinyl estradiol</td>
<td>1µg/rat/day</td>
<td>104.24***</td>
<td>Opened</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>200</td>
<td>45.16***</td>
<td>Not opened</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>600</td>
<td>33.24***</td>
<td>Not opened</td>
</tr>
<tr>
<td>Ethyl acetate +Ethinyl estradiol</td>
<td>1µg/rat/day + 200mg</td>
<td>38.04***</td>
<td>Not opened</td>
</tr>
<tr>
<td>Ethyl acetate +Ethinyl estradiol</td>
<td>1µg/rat/day + 600mg</td>
<td>30.44***</td>
<td>Not opened</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM, ***P<0.001, **p<0.01, *p<0.05, when compared with control

Table 3: Histological changes of the uterus due to the administration of ethyl acetate extract of E. Jambolana seeds in bilaterally overiectomized immature rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Diameter of uterus (µm)</th>
<th>Thickness of myometrium (µm)</th>
<th>Thickness of endometrium (µm)</th>
<th>Epithelial cell height (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>DMSO(1%)</td>
<td>514.64±3.42</td>
<td>69.48 ± 2.90</td>
<td>289.70 ± 2.60</td>
<td>27.36 ± 1.03</td>
</tr>
<tr>
<td>Ethinyl estradiol</td>
<td>1µg/rat/day</td>
<td>1013.07 ± 4.2</td>
<td>101.35 ± 2.13</td>
<td>592.21 ± 4.13</td>
<td>39.25 ± 0.63</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>200</td>
<td>436.61 ± 4.2</td>
<td>48.22 ± 1.70**</td>
<td>203.41 ± 3.44***</td>
<td>20.16 ± 1.05**</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>600</td>
<td>302.04 ± 6.33***</td>
<td>40.46 ± 2.24***</td>
<td>169.66 ± 3.30***</td>
<td>16.94 ± 0.66***</td>
</tr>
<tr>
<td>Ethyl acetate +Ethinyl estradiol</td>
<td>1µg/rat/day + 200mg</td>
<td>406.03 ± 3.76**</td>
<td>42.94 ± 1.98***</td>
<td>171.89 ± 2.98***</td>
<td>13.11 ± 0.25***</td>
</tr>
<tr>
<td>Ethyl acetate +Ethinyl estradiol</td>
<td>1µg/rat/day + 600mg</td>
<td>277.32 ± 6.09***</td>
<td>37.34 ± 2.67***</td>
<td>134.16 ± 3.12***</td>
<td>9.38 ± 1.43***</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM, ***P<0.001, **p<0.01, *p<0.05, when compared with control

DISCUSSION:
Many plants possessing antiestrogenic property have been reported to possess significant antifertility activity. An active antigestrogen has been reported to decrease the wet uterine weight (8)(9). Moreover, antiestrogenic nature may be designated to those compounds which interfere with any action of the estrogen. (10). The pregnancy interceptive effect of E. jambolana seed extract can be interpreted due to this antiestrogenic nature of the plant. During pregnancy the uterus enlarges in part due to the action of hormones and also due to the stimulus of the concepts. (11). It can be predicted that the estrogen antagonism leads to the development of an inadequate progestational endometrium incapable of supporting pregnancy. Thus, it can be argued that the antiestrogenic nature of the extracts could be embryo toxic and the concept undergoes resorption with a consequent decrease in the wet uterine weight.
The implantation index, reduced corpora lutea and preimplantation loss are useful indices for evaluating the number of blastocyes implanted in the uterus and the underdeveloped (17). The decrease in the number of corpora lutea and graffian follicles and the increase in the number of atretic follicle in treated rats indicate that the development of preovulatory follicles and their conversion of corpora lutea are completely inhibited. The cornification in the vagina is mainly due to the level of stimulation of estrogen which acts directly on the vaginal epithelium (16). But in the present study the absence of cornified epithelial cells in the vagina is due to the decreased level of estrogen which acts directly on the vaginal epithelium. Hence, the anti-implantation action could involve antiestrogenic action, where the estrogen induced uterine hypertrophy is inhibited by Ethyl acetate seed extract.

It is well known that for implantation exact equilibrium of estrogen and progesterone is essential, and any disturbance in the level of these hormones may cause infertility (13). The compound of hormonal milieu in the uterus and provokes an infertility effect. In this study, the histological evidence of the uterus treated with ethyl acetate extract clearly supports an unfavorable uterine milieu. Therefore, the anti-implantation activity may be due to anti-estrogenic activity, since antiestrogen substances decreases the weight of the uterus.

Plant products exhibiting anti implantation and antiestrogenic activity are known in literature. Embelin isolated from dried berries of Embelia ribes inhibited pregnancy and also possesses antiestrogenic and 85.71% antimplantation activity in rats when administered at 50mg/kg body weight for 7 days (14). Similar observation has been reported with Sesquiterpene isolated from roots of Aristolochia indica showed antiimplantation and antiestrogenic activity in female mice (15). The benzene, hexane and alcohol extracts of Echinops echinatus root showed to possess rich antiestrogenic active principle and also inhibited pregnancy (16). The anti-estrogenic quality of neem oil also explains its anti-implantation effect (17). Preliminary phytochemical studies indicated the presence of alkaloids, glycosides, flavonoids, steroids, saponins, amino acids, and triterpenoids. Since some of these compounds are known to exhibit antiestrogenicity and also inhibited pregnancy and also possesses antiestrogenic and 85.71% antimplantation activity in rats when administered at 50mg/kg body weight for 7 days (14). Similar observation has been reported with Sesquiterpene isolated from roots of Aristolochia indica showed antiimplantation and antiestrogenic activity in female mice (15). The benzene, hexane and alcohol extracts of Echinops echinatus root showed to possess rich antiestrogenic active principle and also inhibited pregnancy (16). The anti-estrogenic quality of neem oil also explains its anti-implantation effect (17). Preliminary phytochemical studies indicated the presence of alkaloids, glycosides, flavonoids, steroids, saponins, amino acids, and triterpenoids. Since some of these compounds are known to exhibit antiestrogenicity and also inhibited pregnancy.

Thus, the present investigation suggests that ethyl acetate seed extract of Eugenia jambolana. Lam. exerts antifertility and antiestrogenic activity in female rats.

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REFERENCES:

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