Validation of Antifertility Activity of Various Rubus Species in Female Albino Rats

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Alcoholic extracts of leaves of various species of Rubus, *Rubus ellipticus*, *R. niveus*, *R. racemosus* and *R. rugosus var. thawaiensis* (Rosaceae) were tested for antifertility activity in female Wistar albino rats. The results indicate decreased implantation sites and increased resorption sites, which denotes antiimplantation and early abortifacient activities of Rubus species. The results are in agreement with the traditional use of this plant as abortifacient by the tribals of Nilgiris.

Use of plant preparations by ancient physicians of India for pregnancy interception is evident from the available reports and reviews. One such plant is *Rubus ellipticus* (Rosaceae) a shrub found in Nilgiri hills. The roots and shoots of *Rubus ellipticus*, commonly known as “Zardanchu” or “Hinasalu” were reported to have therapeutic uses in the conditions like colic pain, diabetes and hyperthermia. A recent report indicates antiprotozoal activity of this plant against *Entamoeba histolytica*. The antifertility activity of *R. ellipticus* has been reported in Ayurvedic and Unani literature. Whereas, Sharma et al reported antiimplantation activity in roots and aerial parts, ethnomedicinally, root decoction of *R. ellipticus* were used to treat diarrhoea and dysentery and also as antifertility agent. Other Rubus species *R. niveus*, *R. racemosus* and *R. rugosus var. thawaiensis* (Rosaceae) has been used widely by tribals of Nilgiris for abortifacient action. Validation of these plants for its activity are not available in the plethora of literature. Since antifertility activity of this genus has been reported in *R. ellipticus*, the present study was undertaken to find out the antifertility activity of other unexplored Rubus species available in Nilgiri hills including *Rubus ellipticus*.

Fresh leaves of various species of *Rubus viz.*, *Rubus ellipticus*, *R. niveus*, *R. racemosus* and *R. rugosus var. thawaiensis*, family Rosaceae were collected from different parts of Nilgiri hills. The leaves were dried under shade, mechanically reduced to a coarse powder and then extracted with Petroleum ether (60-80°) to remove fatty matter. Then subjected to hot continuous extraction in Soxhlet apparatus using ethyl alcohol (90%). Extracts were concentrated below 60° and further drying was carried out under reduced pressure. The dried extracts were used for pharmacological screening. Overnight fasted albino mice of either sex (24-30 g) were used to evaluate behaviour and toxicity studies. Ethanolic extracts were administered orally as a fine suspension in PEG 400 at a graded dose level (1000 mg to 5000 mg/kg). Behavioural changes were observed for 4 h, and mortality rate was recorded after 72 h.

Proven fertile male and female Wistar strain albino rats (150-200 g) were used for antiimplantation and early abortifacient studies. The rats were maintained at room
### TABLE 1: DATA SHOWING ANTIFERTILITY ACTIVITY OF ALCOHOLIC LEAF EXTRACTS OF VARIOUS SPECIES OF RUBUS

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Treatment</th>
<th>Dose</th>
<th>No. of Corpora Lutea</th>
<th>No. of Implantation Sites</th>
<th>No. of resorbed Implantation</th>
<th>% anti-Implantation activity</th>
<th>% early abortifacient activity</th>
<th>% total anti-fertility activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Vehicle (PEG 400)</td>
<td>2 ml/kg</td>
<td>11.16 ± 0.95</td>
<td>10.50 ± 0.43</td>
<td>00</td>
<td>5.91</td>
<td>00</td>
<td>5.91</td>
</tr>
<tr>
<td>2.</td>
<td>Positive control (Ethinyl oestradiol)</td>
<td>0.45 mg/1kg</td>
<td>11.50 ± 0.92</td>
<td>00</td>
<td>00</td>
<td>100</td>
<td>00</td>
<td>100</td>
</tr>
<tr>
<td>3.</td>
<td><em>R. ellipticus</em> extract</td>
<td>200 mg/ml</td>
<td>11.67 ± 1.15</td>
<td>5.33 ± 1.17 aa</td>
<td>4.33 ± 1.02 aa</td>
<td>54.33</td>
<td>37.10</td>
<td>91.43</td>
</tr>
<tr>
<td>4.</td>
<td><em>R. niveus</em> extract</td>
<td>200 mg/kg</td>
<td>11.00 ± 0.97</td>
<td>6.33 ± 1.33</td>
<td>4.67 ± 1.17</td>
<td>42.45</td>
<td>42.43</td>
<td>84.88</td>
</tr>
<tr>
<td>5.</td>
<td><em>R. racemosus</em> extract</td>
<td>200 mg/kg</td>
<td>12.17 ± 1.02</td>
<td>5.00 ± 1.15 a</td>
<td>4.66 ± 0.95 a</td>
<td>58.91</td>
<td>38.30</td>
<td>97.21</td>
</tr>
<tr>
<td>6.</td>
<td><em>R. rugosus</em> extract</td>
<td>200 mg/kg</td>
<td>11.83 ± 0.95</td>
<td>7.67 ± 1.10</td>
<td>3.33 ± 0.99 a</td>
<td>35.16</td>
<td>28.15</td>
<td>63.31</td>
</tr>
</tbody>
</table>

*a, aa, aaa denotes statistical significance in comparison to vehicle treatment at p < 0.05, p < 0.01 and p < 0.001 respectively.*

Temperature with natural day light and fed with pelleted feed and water *ad libitum*. Female rats were divided into 6 groups and paired with males in 2:1 ratio. Group I to group IV received suspension of alcoholic extracts of leaves of *Rubus ellipticus*, *R. niveus*, *R. racemosus* and *R. rugosus* var. *thawaitesii* prepared in PEG 400. Extracts were administered orally at a dose of 200 mg/kg. Mating of the animals was confirmed by observing the presence of sperm in the vaginal smear and considered as day 1 of gestation. Drugs were administered from day 1 of gestation to day 7. Female rats were autopsied on day 10 of gestation and the number of implantation sites, corpora lutea, normal and degenerated fetus including gross abnormalities, if any, were recorded. Data are expressed as mean±SEM and subjected to student’s "t" test for statistical significance at 95% probability level.

The alcoholic extracts of various species of Rubus showed no toxic effect at the tested dose level (5000 mg/kg). However, these extracts produced CNS activity such as analgesia and sedation. Present study reveals antiimplantation activity of Rubus species found in Nilgiris. The results showed significantly decreased implantation sites and increased resorption in female rats received alcoholic extracts of Rubus plants indicating antifertility activity (Table 1). No pups have shown deformities. Similar observations was observed by Rana et al.² in rats treated with *R. ellipticus*. Similarly, other newer species *Rubus ellipticus*, *R. niveus*, *R. racemosus* and *R. rugosus* var. *thawaitesii* which we have selected, also shown antiimplantation activity. No significant differences was noticed among the species tested. Antifertility activity of Rubus species may be due to presence of flavonoids. Further, Rana et al.⁴ reported antiestrogenic activity and anti androgenic activity in *R. ellipticus* and correlated with antiimplantation action of this species. Estrogens inhibit pregnancy by affecting tubular transport of egg or endometrial maturation. Estrogenic substances suppress both follicular stimulating hormone (FSH) and luteinizing hormone (LH) levels. Moreover they produce thickening of cervical mucosa, alter endometrial pattern and prevent the implantation. Thus the follicular growth does not initiate and ovulation doesnot occur.
It may be concluded that the Rubus plants used by the tribal peoples of Nilgiris have shown antiimplantation activity and this observed activity of Rubus may be due to their anti estogenic/androgenic action\textsuperscript{14}.

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