Comparatives Evaluation of Antifertility Potential of Leaves of *Bambusa arundinacea* retz. and *Ficus racemosa* Bark Extracts in Female Albino Rats

Vishal Soni¹, Arvind Kumar Jha², Jaya Dwivedi³, Priyanka Soni¹

¹Department of Herbal Drug Research, B.R. Nahata College of Pharmacy, Research Centre, Mhow Neemuch Road, Mandsaur 458001, India
²Faculty of Pharmaceutical Sciences, Shri Sankarachary group of Institution, Shri Sankarachary Technical Campus, Junwani Bhilai Chhattisgarh, 490020, India
³Department of pharmaceutical chemistry Banasthali Vidyapith, Banasthali University, Rajasthan, 304022, India

Abstract

Ethanol and hydroalcoholic (50%) extract of *B. arundinacea* leaves and 50 % ethanol extract of *F. racemosa* barks have been evaluated for antifertility activity in proven fertile rats at a dose of 200 and 400 mg/kg body weight. Plant extracts were tested for their effect on the estrous cycle at two dose level 200 and 400 mg/kg, respectively. Among these three extracts, the hydroalcoholic extracts of *F. racemosa* was found to be most effective in causing significant antiovulatory activity. The extract did not show any significant changes in structure and function of uterus when given alone, but when given along with ethinyl estradiol, it exhibited significant antiestrogenic activity in immature female rats (*P*<0.001). Preliminary phytochemical screening of these two drugs shows the presence of carbohydrates, tannins, steroids, glycosides, flavonoids, phenolic, terpenoids etc. Histopathological studies of the uterus were carried out to confirm the estrogenic activity.

1 Introduction

From dawn of the civilization, humans have relied on plants and their products as a source of drugs for their primary health care. In recent years, their use as a popular alternative to modern medicine has increased considerably even in developed countries.¹ The importance of plants as a source of antifertility drugs has been emphasized by many researchers. Antifertility agents obtained from indigenous medicinal plants would be of immense benefit especially to inhabitants of developing countries, since the cost of these drugs would be within their means.² The antifertility plants with estrogenic property can directly influence pituitary action through peripheral modulation of luteinizing (LH) and follicle-stimulating hormones (FSH) by decreasing the secretion of these hormones and blocking ovulation³. In addition, the plant may also intercept the synchronized development of the ovum and endometrium while others may have abortifacient or antiprogestational effects⁴. Initially the major research efforts were directed towards the discovery of oral contraceptives of synthetic origin and very little attention was paid to the plant kingdom, although the chemical nature of the compounds derived from plants are so diverse that they encompass the prototypes of practically every pharmacological category. In the modern system of medicine, about 25% of prescriptions contain active principle(s) derived from plants. Plant kingdom therefore, holds a great promise for the discovery of new and effective anti-fertility agents. *B. arundinacea* is distributed throughout the moist parts of India. The leaves of *B. arundinacea* are emmenogogue, useful in inflammatory conditions, heals the wound and they are also used to check diarrhea in cattle⁵. Manna is a crystalline substance found inside the bamboo and leaves are also used in Ayurvedic medicine in paralytic complaints. Chemical contents of *B. arundinacea* young shoots have been reported as cholin, betain, urease, cyanogenetic glucosides, oxalic acid, and benzoic acid⁶. Quite a large number of studies have found that different parts of *B. arundinacea* possess medicinal activities like antidiabetic activity⁷, antifertility effect⁸, antibacterial activity⁹, anti-inflammatory and protective effects¹⁰.
Ficus racemosa Linn. (Moraceae) is a popular medicinal plant in India, which has long been used in Ayurveda, the ancient system of Indian medicine, bark, leaves and unripe fruits etc. are used externally and internally to cure many diseases. Root is used in dysentery, pectoral complaints, and diabetes, applied in mumps, other inflammatory glandular enlargements and hydrophobia. The bark is highly efficacious in threatened abortion and also recommended in urological disorders, diabetes, hiccough, leprosy, dysentery, asthma and piles. The leaves are good wash for wounds and ulcers. On the basis of pharmacological investigation and research, it shows antiulcer, anti-diabetic, anti-pyretic, anti-inflammatary, antitussive, hepatoprotective, and antimicrobial activities, anti-diarrhoal activity.

Tribal around Salem (Madras) chew leaves of B. arundinacea Retz. Wild. in the morning and evening for 1-3 days to induce abortion of an early conception. The decoction of bark of F. racemosa Linn is traditionally used to cause infertility and induce threatened abortion. However, despite the abortifacient claim of B. arundinacea leaf and F. racemosa Linn bark in folklore medicine of India, there is no published scientific evidence that has either substantiated or refuted this claim. Therefore, we have decided to provide scientific evidence to the acclaimed antifertility potentials of the hydroalcoholic extract of B. arundinacea leaves and F. racemosa Linn in female albino using parameters such as antiovulatory and, estrogenicity.

2 Materials and Methods

2.1 Plant materials

Leaves of B. arundinacea and bark of F. racemosa were collected from garden, Jhalawar district, Raj. in the month of Nov-Dec and positively identify by Dr. S. N. Mishra, Botanist, and K.N.K College of Horticulture, Mandsaur, and Madhya Pradesh (MP). Voucher specimen (BRNCP/B/08/2010) and (BRNCP/F/04/2010) were deposited in the herbarium of the department of Pharmacognosy, BRNCP and Mandsaur for future reference. The material was dried under shade, powdered mechanically and stored in air tight container.

2.2 Preparation of extracts

The shade dried leaves were coarsely powdered. The powdered material was extracted using ethanol and hydroalcohol (50%) for 72 h each in a Soxhlet apparatus. The extracts were evaporated under reduced pressure to solid masses and the percentage yield of extracts was found to be 6.5% and 5.8% w/w, respectively. The bark of F. racemosa was dried, powdered using electrical grinder and extracted in 50% ethanol at 37°C for 72 h. It was filtered and lyophilized. The yield of the lyophilized powder was 7.6% w/w dry bark. The brownish yellow powder was kept in dark clean jar in room temperature for further experiments. For oral administration, the lyophilized powder of plant extract was suspended in 1% Tween 80.

2.3 Preliminary Phytochemical screening

In order to determine the presence of various phytoconstituents, a preliminary phytochemical study (colour reaction) with extracts was carried out by using the standard procedure given by. In order to determine the presence of various phytoconstituents, a preliminary phytochemical study (colour reaction) with extracts was carried out by using the standard procedure given by 15.

2.4 Experimental animals

Female albino rats (Wistar strain weighing 150-200 g) were used for antiovulatory activity and immature female rats of 21-23 days old, were used for estrogenic activity. The animals were housed in standard environmental condition of temperature (21± 2°C), humidity (55 ± 10%) and a 12-h light dark cycle. The rats were acclimatized to laboratory hygienic conditions for 10 days before stating the experiment. Animal study was performed in the Division of Pharmacology, BR Nahata College of Pharmacy. All the experimental protocols were approved by Institutional Animal Ethics Committee for the Purpose of Control and Supervision of experiment on animal (CPCSEA) guidelines.

2.5 Acute Toxicity studies

Ethanol, hydroalcoholic (50%) extracts of leaves of B. arundinacea and 50% ethanol extract of Ficus racemosa were studied for acute oral toxicity according to the guidelines set by Organization for Economic Co-operation and development (OECD) guideline number 420. Female Wistar rats (150-180 g) were used for this study. After the sighting study, a starting dose of 2,000 mg/kg (p.o.) of the test samples were given to various extract groups containing five animals in each groups. The treated animals were observed for 14 days for mortality and general behaviour.

2.6 Antifertility activity of extracts

2.6.1 Antiovulatory activity

Experiments were carried out in female Wistar rats weighing (150-180 g). The vaginal smear of each rat was examined daily between 9-10 A.M for 15 days to select the animals showing regular cycles (4-5 days). The selected rats were divided into 7 groups of six animals each. The extracts were administered orally for five days to cover one regular estrous cycle. Group I received vehicle (1% Tween 80) and served as control. Group II-V received ethanol and hydroalcoholic leaves extracts of B. arundinacea at 200 and 400 mg/kg body weight. Group VI and VII received hydroalcoholic extract of F. racemosa at 200 and 400 mg/kg body weight. Vaginal smear from each animal was observed every morning between 9-10 A.M for five days of treatment and subsequently for 15 days. [16].
2.6.2 Estrogenic and antiestrogenic activity

The extracts with antiovulatory activity were further evaluated for estrogenic activity and antiestrogenic activity [17]. Immature female Wistar strain rats, 30-35 day old, weighing between 35 and 45 g, were divided into 6 groups of six rats each. The first group served as control and received the vehicle only (1% Tween 80). The second group received ethinyl estradiol (standard) in distil water at a dose of 0.02 mg/kg body weight. The third and fourth group received the active ethanolic (50%) extract of B. arundinacea leaves at two dose level 200 and 400 mg/kg body weight, respectively. The fifth and sixth group received the most active ethanolic (50%) extract of F. racemosa bark at two dose level 200 and 400 mg/kg body weight, respectively. The groups sixth and seventh received ethinyl estradiol in addition to a test dose of the 50% ethanolic extract of B. arundinacea leaves at the same dose. The groups ninth and tenth received ethinyl estradiol in addition to a test dose of the 50% ethanolic extract of F. racemosa bark at the same dose. All the above treatment were given for three days (p.o.).

2.7 Histopathology

On the fourth day, the rats were sacrificed by decapitation, the uteri dissected out and surrounding tissues removed and washed with normal saline. The uteri were blotted on filter paper and weight quickly on a sensitive balance and fixed in Bouin’s solution for 24 h. The paraffin-embedded tissues were cut at 5 mm thickness and stained with hematoxylin-eosin solution. The sections were examined microscopically for histological observation [18].

2.8 Statistical analysis

Statistical analysis was carried out by one-way (ANOVA) followed by Dunnett-test. Results were expressed as mean ± SEM from six rats in each group. P values p<0.001 were considered significant.

3 Results

3.1 Preliminary phytochemical investigation

The phytochemical screening of different extracts revealed the presence of various constituents. Phytochemical screening of ethanolic extract of B. arundinacea leaves shows the presence of carbohydrates, flavanoid, tannins, steroid glycosides, terpenoids whereas hydro alcoholic extract of B. arundinacea leaves shows the presence of carbohydrates, tannins, glycosides, flavonoid, proteins and steroids. Hydroalcoholic extract of F. racemosa bark reveal the presence of phenolics, tannins, glycosides, flavanoid, saponin and triperpenoids.

3.2 Acute toxicity studies

No mortality and changes in the behaviour was observed in the treatment groups up to 2000 mg/kg body weight and from the results 400 mg/kg dose was chosen as maximum dose for further experimentation.

3.3 Effect of extracts of B. arundinacea leaves and F. racemosa bark on the estrous cycle of rats

The present study revealed that the 50% ethanol extract of B. arundinacea and F. racemosa bark showed an antifertility effect. It is observed that in the control group of animals treated with 1% Tween 80 which was used as a vehicle in the present experiment all the six animals manifested normal cyclical oestrous phase throughout the study period. Treatment of rats with 50 % ethanol extract of F. racemosa bark at 400 mg/kg dose level prolonged the estrous cycle more significantly (P > 0.001) as normal estrous cycle lasted for some period of drug treatment and even after discontinuation of the drug.

Hydroalcoholic extract of B. arundinacea leave also showed reduced duration of estrous and metestrous phases, characterized by a prolongation of the diestrous phase (P< 0.05) shown in Table 1. From the above observation it is seen that hydroalcoholic extracts of F. racemosa bark and B. arundinacea caused suppression of the estrous phase in female albino rats in a dose dependent, reversible manner. Since estrous phase in animal is a manifestation of ovulation, it may be presumed that suppression of estrous phase in albino rats is due to suppression of ovulation, suggesting an antiovulatory effect of the drug in the experimental group of animals. Different phases of estrous cycle are shown in Figure 2.

3.4 Estrogenic activity and antiestrogenic activity

The effect of estrogenic effect of 50 % ethanol extract of B. arundinacea and F. racemosa bark is shown in Table 2 and Figure 1. Oral administration of 50 % ethanol extract F. racemosa bark extracts at 200 and 400 mg/kg body weight caused significant increases in uterine weight in immature rats (p< 0.001) shown in Figure 6. The thickness of endometrium was significantly increased when compared to the control rats Figure 3. The uteri of these rats were inflated and full of fluid resembling the proestrous/estrous uterus. The epithelium of the endometrium consisted of spindle shaped cells with basal nuclei. The stroma consisted of loose and edematous fibroblast-type cells with edema. The control rats
shows closed vagina whereas the treated rats showed an open vagina. Examination of the vaginal smears of the treated rats revealed predominantly cornified and nucleated epithelium cells. The administration of 50% ethanol extract aggrieved a significant increase in the uterine weight, signifying the estrogenic activity. Oral administration of 50% ethanol extract of *B. arundinacea* at 200 and 400 mg/kg body weight caused a significant increase in uterine weight in immature rats (*p* < 0.01) shown in Figure 5. However, when treated with ethinyl estradiol, it lowers the effect of estrogenic activity produced by ethinyl estradiol (Figure 4) comparatively the ethanol extract of was found to be more active.

**Table 1**: Effect of treatment of extract of *B. arundinacea* leaves and *F. racemosa* bark, on estrous cycle for 5 days in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Duration of cycle (day)</th>
<th>Duration of different phases of estrous cycle (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Proestrous (days)</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>2.53 ±0.42</td>
<td>0.66 ±0.15</td>
</tr>
<tr>
<td>Ba. L-E</td>
<td>200</td>
<td>3.53 ±0.46</td>
<td>0.53 ±0.13</td>
</tr>
<tr>
<td>Ba. L-HA</td>
<td>200</td>
<td>4.13 ±0.49</td>
<td>0.60 ±0.13</td>
</tr>
<tr>
<td>Ba. L-HA</td>
<td>400</td>
<td>4.26 ±0.56**</td>
<td>0.71 ±0.12</td>
</tr>
<tr>
<td>Fr.B-HA</td>
<td>200</td>
<td>5.06 ±0.46**</td>
<td>0.66 ±0.12</td>
</tr>
<tr>
<td>Fr.B-HA</td>
<td>400</td>
<td>5.40 ±0.55***</td>
<td>0.60 ±0.13</td>
</tr>
</tbody>
</table>

Values are expressed in mean ± SEM, n= 6, *p<0.05, **p<0.01, ***p<0.001 vs control (student’s t = test). Ba. L- *Bambusa arundinacea* leaves, Fr.B - *Ficus racemosa* bark, E- Ethanol, HA-Hydroalcoholic (50%)

**Table 2**: Effect of *B. arundinacea* leaves and *F. racemosa* bark extract on uterine weight of immature female rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Uterine weight (mg/100 g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle)</td>
<td>-</td>
<td>58 ± 1.51</td>
</tr>
<tr>
<td>Ethinyl estradiol</td>
<td>0.02</td>
<td>139 ± 3.01</td>
</tr>
<tr>
<td>Ba. L-HA</td>
<td>200</td>
<td>62 ±1.643**</td>
</tr>
<tr>
<td>Ba. L-HA</td>
<td>400</td>
<td>67.80 ±1.68**</td>
</tr>
<tr>
<td>Fr.B-HA</td>
<td>200</td>
<td>81.20 ±1.24**</td>
</tr>
<tr>
<td>Fr.B-HA</td>
<td>400</td>
<td>85.80 ±1.39**</td>
</tr>
<tr>
<td>Ethinyl estradiol + Ba. L-HA</td>
<td>0.02+200</td>
<td>111.4 ±1.288***</td>
</tr>
<tr>
<td>Ethinyl estradiol + Ba. L-HA</td>
<td>0.02+400</td>
<td>118 ±0.707***</td>
</tr>
<tr>
<td>Ethinyl estradiol + Fr.B-HA</td>
<td>0.02+200</td>
<td>132 ±1.00***</td>
</tr>
<tr>
<td>Ethinyl estradiol + Fr.B-HA</td>
<td>0.02+400</td>
<td>136.2 ±0.663***</td>
</tr>
</tbody>
</table>

Values are expressed in mean ± n=5, ***p< 0.001 when compared with control.
Fig. 2. Vaginal smear of the rats on 4-day estrous cycle. CXR III camera (x 100)  
Control: 1st day (A), 2nd day (B), 3rd day (C), 4th day (D)  

Fig 3: Showing section of the uterus indicating surface epithelium with no secretory activity (Control group)  

Fig 4: Showing section of the uterus indicating proliferative stage (Standard group)  

Fig 5: Showing section of the uterus indicating stroma consisting of loose fibrous tissues with stimulated uterine glands (hydroalcoholic leaves extracts of B.arundinacea 400 mg/kg)  

Fig 6: Showing section of the uterus indicating loose and edemators stroma with stimulated uterine glands (hydro alcoholic bark extracts of F. racemosa 400 mg/kg)  

3.5 Histopathology  
Histological examination of the uteri were carried out in the 50 % ethanol extract of B. arundinacea leaves and F. racemosa bark treated groups of animals with an idea to substantiate the experimental findings.  

4 Discussions  
Uterus and the female reproductive tract undergo innumerable physiologic and biochemical changes under the influence of ovarian hormones such as estrogen [18]. In the present study, leaves of B. arundinacea and bark of F. racemosa were tested for its antiovulatory and estrogenic activity properties. Ovulation in rat is known to be correlated with the appearance of estrous phase, manifested by the presence of almost 100% cornified cells in the vaginal smear in every four to five days [19]. It was found that both hydroalcoholic extracts of B. arundinacea leave and bark of F. racemosa extract has less or more significant effect on estrous cycle( Table 1) . Hydroalcoholic extracts of bark of F. racemosa at a
dose of 400 mg/kg significantly \((P < 0.001)\) increased the duration of the estrous cycle by directly increasing the diestrous phase, while the 200 mg/kg dose has significantly \((P < 0.01)\) reduced the proestrous phase and on the other hand increased the duration of the diestrous phase as indicated in Table 2. Hydroalcoholic extracts of B. arundinacea leaf at a dose of 400 mg/kg has significantly \((P < 0.05)\) effect on estrous cycle. Hydroalcoholic extracts of B. arundinacea leaf at a dose of 200 mg/kg has not produced any significant change in the estrous cycle. Hydroalcoholic extracts of bark of F. racemosa at a dose of 400 mg/kg was found to be the most significant when compared to the other extracts (Figure 6). If female rats are ovariectomized, the resultant lack of estrogen causes atrophy of the uterus and the reproductive tract; administration of estrogenic substances to ovariectomized rats leads to uterotrophic effects, vaginal cornification, increase in uterine glycogen content and proliferative changes in uterine endometrium\(^{20}\). Estrogenic compounds are known to cause the keratinization and cornification of the vaginal epithelium, causing the superficial cells to be shed into the lumen to form large squamous cells\(^{21}\).

The extract with antiolulatory activity was further studied for their estrogenic and antiestrogenic activity. These extracts also exhibited estrogenic activity as shown by the increases in the diameter of uterus, uterine weight and thickness of endometrium when compared to control (Fig 1).

Both hydroalcoholic extracts of B. arundinacea leaf and bark of F. racemosa extract had produced significant increase in the uterine weight in immature rats \((P < 0.01)\) \((P < 0.001)\) as shown in Table 2. The 50% ethanolic extract of F. racemosa bark at 200 and 400 mg/kg, shows most significant \((P < 0.001)\) estrogenic activity (Fig 6), than hydroalcoholic extracts of B. arundinacea leaf (Fig 5). The transverse section of uterus of rats treated with extract of F. racemosa bark exhibited increase in the thickness of the epidermal layer also with stroma consisting of the loose fibrous tissue having edema. Extracts provoked significant increase in the uterine wet weight, increase in the thickness of the endometrium and the height of the endometrial epithelium when compared to the control rats (Fig 3). It was observed that the extract suppressed the action of ethinyl estradiol when given alone but, significant anti estrogenic activity was also produced by the extract when given along with ethinyl estradiol. It has significantly \((P < 0.001)\) lowered the effect of estrogenic activity produced by ethinyl estradiol alone (Fig 4). The duration of the estrous cycle in rats is normally 4–5 days. Three cell types are found in the vaginal smear during a normal rat estrous cycle. The presence and absence of these cell types, and the relative proportion of each cell type, determine the stages of the estrous cycle. The prolongation in the diestrous phase explains the remote possibility of the rats getting pregnant. The reversible nature of the antifertility activity of the extract is explained through the observation that there was no significant change in the diestrous and the estrous cycle after withdrawing the extract from those of the control. As a result, the extracts provoked inhibition of the ovulation with consequent reduction of the cyclicity. Estrous cycle and the shift in different stages are mainly governed by the synthesis of ovarian estrogen, which, in turn, is controlled by the secretion of pituitary gonadotropins and hypothalamic-releasing factor. Ethanol extract of B. arundinacea leaves and F. racemosa bark exhibited estrogenic activity by significant increases in uterine weight, diameter of uterus and thickness of the endometrial epithelium, when compared to the control (Fig 3). Estrogen stimulates the content of these in uterus thereby changing the uterine milieu and creating contraceptive condition. The leaves extract acted as estrogen when given alone but when given with ethinyl estradiol it exhibited slight antiestrogenic activity. This shows that the extract acted as competitive antagonist to the much more potent ethinyl estradiol\(^{14}\). It is well known fact that estrogenic substances inhibit pregnancy by suppressing the level of both follicular stimulating hormone (FSH) and luteinizing hormone (LH), which in turn prevent the implantation. Estrogen and progesterone are the hormones responsible for histology and functional modifications of female genital tract. Preliminary phytochemical studies indicated the presence of phenolics, tannins, glycosides, flavonoid, saponin, and triterpenoids in 50 % ethanol extract of F. racemosa bark and carbohydrates, glycosides, tannins, flavanoids, triterpens in hydroalcoholic extract of B. arundinacea leaves. Ethanol extract B. arundinacea leaves shows the presence of carbohydrates, flavonoid, tannins, steroid glycosides, terpenoid but was devoid of activity. According to the literatures, flavonoids, phenolic and saponins are known to exhibit antifertility activity\(^{16, 22-23}\).

Thus the estrogenic activity shown by the extract of B. arundinacea leaf can be attributed to the presence of flavonoids. The synergism produced by flavanoids along with phenolics and saponins could be the reason for the enhanced activity of the hydroalcoholic extract of F. racemosa when compare with other groups. Further, isolation of active constituents and ER selectivity studies of such isolated compounds are in progress. It was found that both hydroalcoholic extracts of B. arundinacea leaf and bark of F. racemosa extract has less or more significant effect on estrous cycle.

5 Acknowledgements

The authors are thankful to Director, B R Nahata College of Pharmacy, Mandsaur College of Pharmacy and Management for providing all necessary facilities to carry out this research work. The authors also sincerely thank Dr. Bagdi, Department of Pathology, and India for providing assistance in histopathological study.

6 Conclusions

The above results indicate that the hydroalcoholic extract F. racemosa bark and B. arundinacea leaves have significant

UK J Pharm & Biosci, 2013: 1(1); 30
antifertility activity. The extract of these two plants can be used for contraceptive purpose. The isolation of extract and identification of lead molecule responsible for activity are under process.

7 References


