Estrogenic Activity of *Bauhinia racemosa* Extract in Female Albino Rats: An Investigational Study

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**Abstract**

Objective: The objective was to evaluate the effect of petroleum ether, ethanol, and water extract of *Bauhinia racemosa* in female albino rats. Materials and Methods: Plant extracts were tested for their estrogenic activity using ovariectomized method at two dose level: 200 mg/kg and 400 mg/kg, respectively. Further, plant extracts and standard drug ethinyl estradiol (1 µg/kg) combination were tested for synergistic estrogenic activity. Results: *In-vivo* investigation revealed that ethanol extract at dose of 200 mg/kg and 400 mg/kg shows significance increase 325.23 ± 5.23, 328.84 ± 4.56 and 3.252 ± 0.47, 3.288 ± 0.27 in uterine wet weight and uterine weight ratio, respectively, in female albino rat as compared to control and petroleum ether and water extracts. Simultaneous administration of standard drug ethinyl estradiol (1 µg/kg) with ethanol extract at dose of 200 mg/kg and 400 mg/kg potentiates (327.36 ± 5.78, 330.95 ± 6.21 and 3.273 ± 0.64, 3.309 ± 0.49 uterine wet weight and uterine weight ratio respectively in female albino rat) the estrogenic activity as compared to individual administration of ethanol extract as a synergistic effect. Conclusion: It was observed that ethanol extract of *B. racemosa* produced significance estrogenic activity.

**Keywords:** *Bauhinia racemosa*, ethinyl estradiol, estrogenic activity, ovariectomy

**Introduction**

The plant *Bauhinia racemosa* Lam. (Caesalpiniaceae) commonly known as APTA in Indian system of medicines widely distributed throughout India, Ceylon, China, and Timor. The genus *Bauhinia* L. is often called as “Orchid tree” ornamental value.¹ *B. racemosa* Lam. Fabaceae *Caesalpinioidae* is a small deciduous tree with a dark brown scabrous bark, distributed in Asian countries like India, Pakistan, and Sri Lanka.² It is one of the sacred plants of Hindus, worshipped during the great festival of Dussehra. Leaves are exchanged along with *Acacia fereigna* as a token of love and respect. The bark is astringent and is used for inflammation, chronic dysentery, diarrhea, glandular inflammations, ulcer also effective for goiter, and some skin diseases.³⁴ Decoction of leaves is given to relieve headache in a malarial fever, flowers are used to treat hemorrhage, piles, cough, and as laxative.³ A new tetracyclic phenol and other constituents reported from the roots⁵ and structural determination of seed polysaccharide by mythylation studies.⁶ Methanol extract of stem bark has anti-inflammatory, analgesic and antipyretic,⁷ antitumor and antioxidant,⁸ antimicrobial,⁹ and hepatorenal functions in mice.¹⁰ The roots of the plant have found to consists of new tetracyclic 2,2-dimethylchroman derivative, de-omethylracemosol compound (Compound-3).¹¹ Furthermore, the various extracts of *B. racemosa* lam. roots belonging to family *Caesalpiniaeaceae* were found to contain 1,7,8,12b-tetrahydro-2,2,4-trimethyl-2H-benzo[6,7] cyclohepta[1,2,3-de][1] benzopyran-5,10,11-triol.¹² Earlier investigations on this species have resulted in isolation of two compounds from the heartwood, a new dibenzoxepin derivative, pacharin (Copound-1), and a novel tetracyclic phenol, racemosol (Copound-2). Besides this, Stillene (resveratrol) was also isolated from the heartwood of this plant.¹³ Many plants have proved to possess significant estrogenic activity, for example, *Azadirachta indica*, *Citrus medica*, *Ruta graveolens*, *Achyranthes aspera*, *Jasminum officinale*, *Mansonia gagei*, *Cissampelos pareira*, *Carica papaya*, *Moringa oleifera*, *Mimosa pudica*, *Nelumbo nucifera*, *Crataeva nurvala*, *Leonotis cymifolia*.¹⁴⁻²⁶ In the present investigation, estrogenic activity of *B. racemosa* was investigated using experimental *in vivo* models.
MATERIALS AND METHODS

Plant material

The stem bark of fully matured plant was identified with the help of local botanist and then collected in the month of June from Toranmal, Dist. Nandurbar near to Satpuda Hills, Maharashtra, India. The plant material further authenticated by Botanical Survey of India (B.S.I) vide voucher specimen (No. BADIBS) Pune, Maharashtra, India.

Experimental animals

Healthy colony breed immature female Wistar rats weighing 100 g were housed in groups of six, under standard laboratory conditions of temperature (25 ± 2°C) and 12/12 h light/dark cycle. Animals had free access to standard pellet diet and water ad libitum. All animal experimentation was carried out after approval of the protocol by the Institutional Animal Ethical Committee vid no. NIB/IAEC/09-10/86 dated 15/01/2010.

Chemical and reagents

5% polyethylene glycol (PEG-400), ethinyl estradiol (German Remedies, India), petroleum ether (60-80°C), ethanol (95%). All Chemical and reagents were of analytical grade.

Statistical analysis

The data were presented as mean ± Standard error mean. The statistical significance between the groups has been tested by analysis of variance followed by Dunnett’s test. A P < 0.05 were considered as significant.

Acute toxicity study

Acute oral toxicity study was performed as per OECD 423 guideline. Extract was administered up to the maximum dose of 2000 mg/kg and animals were observed for mortality.

In vivo assessment of estrogenic activity in female albino rats

Colony-bred immature female albino rats Wistar strain, weighing 100 g were maintained in rat cages in barrier rooms under 12:12-light: Dark cycle, with a temperature of 22 ± 1°C. The animals were divided into six groups consisting six rats in each group.

Group-I: Control, received 0.2 ml 5% (PEG-400) orally.
Group-II: Received 1 µg/kg ethinyl estradiol/rat/day in olive oil s.c.
Group-III: Received test extract of B. racemosa stem bark 200 mg/kg orally.
Group-IV: Received test extract of B. racemosa stem bark 400 mg/kg orally.
Group-V: Received test extract of B. racemosa stem bark 200 mg/kg orally and ethinyl estradiol 1 µg/kg/rat/day in olive oil s.c.
Group-VI: Received test extract of B. racemosa stem bark 400 mg/kg orally and ethinyl estradiol 1 µg/kg/rat/day in olive oil s.c.

All the above treatments were given for 7 days. On the 8th day of the experiment, the animals were sacrificed by cervical decapitation and bilaterally ovariecotomized by the dorsolateral approach under light ether anesthesia. The uteri and adrenals were dissected out and separated from the adherent tissues and weighed up to the nearest mg on an electronic balance. The uterine ratio was calculated by dividing uterine wet weight into milligrams by body weight in grams. The increase in the uterine ratio was associated with the estrogenic effect of the extract as described by Vogel.27

RESULTS AND DISCUSSION

Acute toxicity studies for dose selection

Acute oral toxicity studies of petroleum, ethanol, and water extract revealed that the extract was toxic at dose level of 2000 mg/kg. Two of three animals at both test level of 2000 mg/kg died. Thus, as per OECD 423 guideline it was concluded that LD 50 value for above extract was 1000 mg/kg.

Estrogenic activity in female albino rats

The administration of standard drug ethinyl estradiol to the bilaterally ovariecotomized immature female albino rats (1 µg/kg s.c.) caused increased in uterine wet weight and weight ratio to 331.52 ± 6.94, and 3.315 ± 0.25*, respectively, when compared with the control group. Ethanol extract of B. racemosa Lam. stem bark on administrating orally at the doses of 200 and 400 mg/kg body weight caused a significant increase in the uterine wet weight and weight ratio to 325.23 ± 5.23*, 328.84 ± 4.56* and 3.252 ± 0.47*, 3.288 ± 0.27*, respectively, when

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compared with control. Furthermore, the simultaneous administration of above doses of test extract with that of standard drug have shown a significant increase in uterine wet weight and weight ratio when compared to control the group. The value of significance was found to ($P < 0.05$) which indicates almost significant activity for ethanol extract as shown in Table 1 and Figure 1. Results thus obtained for a test and simultaneous administration of standard and test indicates significant dose dependent estrogenic activity in female albino rats. The present investigation for pharmacological studies of ethanol crude extract confirms the role of plant in controlling the fertility in female albino rats as estrogenic substance may cause the expulsion of ova from the tube, disruption of leuteotrophic activity the blastocyst, disrupt the functional equilibrium which may result in failure in fertility (Figures 2 and 3). In the case of petroleum ether and water extracts, the data obtained were statistically nonsignificant as extracts did not cause increase in uterine wet weight and weight ratio shown in Tables 2 and 3, respectively.

In several animal species, including rats and mice have shown to possess uterotrophic effects on administration of estrogen, and such effects are, therefore, may responsible for the growth and proliferation of endometrial cell number, vaginal cornification. There are various phytoestrogens such as isoflavones, lignans, and resveratrol responsible for anti-fertility activity. Furthermore, flavonoids have found to possess anti-fertility activity. Besides this, experimental evidenced has also shown that, β-sitosterol, a phytosterol

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<th>Table 1: Effect of ethanol extract of B. racemosa stem bark on uterine weight in bilaterally ovariectomized immature female albino rats</th>
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*n=6, Values are in mean±SEM, *P<0.05 when compared with control, SEM: Standard error of the mean, B. racemosa: Bauhinia racemosa

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<th>Table 2: Effect of petroleum ether extract of B. racemosa stem bark on uterine weight in bilaterally ovariectomised immature female albino rats</th>
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*n=6, Values are in mean±SEM, Statistically non-significant data, SEM: Standard error of the mean, B. racemosa: Bauhinia racemosa

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<th>Table 3: Effect of water extract of B. racemosa stem bark on uterine weight in bilaterally ovariectomized immature female albino rats</th>
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**Figure 1:** Effect of ethanol extract of *Bauhinia racemosa* stem bark on uterine weight in bilaterally ovariectomized immature female albino rats.

**Figure 2:** Effect of petroleum ether extract of *Bauhinia racemosa* stem bark on uterine weight in bilaterally ovariectomized immature female albino rats.

**Figure 3:** Effect of water extract of *Bauhinia racemosa* stem bark on uterine weight in bilaterally ovariectomized immature female albino rats.

can exert estrogenic effects in females. The ethanolic extract of stem bark of plant *B. racemosa* lam. has shown content of phytosterol moiety, β-sitosterol as confirmed by high performance thin layer chromatograph analysis which may be the reason responsible for inducing estrogenic effect in female albino rats.
REFERENCES


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