ABSTRACT

Objectives: To investigate the estrogenic effect of *Avicennia alba* (Blume) aerial parts as identified in the folklore recipes as a contraceptive

Methodology: The estrogenic activity study of the methanolic extract was carried out by studying the histopathology of the uterus. The uterine weight and vaginal cornification were also observed. The diameter of uterus, thickness of endometrium and height of the endometrial epithelium were measured using a calibrated ocular micrometer. The estimation of total protein and cholesterol was carried out using a standard method described by Lowely et al.

Results: The presence of alkaloids, anthraquinone glycosides, flavonoids, steroids, polyphenolics were detected in the aerial parts of *Avicennia alba*. The methanolic extract of *Avicennia alba* at 400 mg/kg body weight showed a significant (*p* < 0.05) increase in uterine weight, diameter of uterus, thickness of endometrium and height of the endometrial epithelium compared to the control. There was increase in vaginal cornification status. The histological examination of the uterus also showed estrogenic influence. A significant increase (*p* < 0.05) in total protein and cholesterol content in the uterus of standard and test drug treated rats was also observed. Conclusion: The methanolic extract of *Avicennia alba* at 400 mg/kg body weight showed significant estrogenic activity and the results are in consistent with the folkloric claim reports related to oral contraceptive effect of *Avicennia alba*

Keywords: *Avicennia alba*, contraceptive, estrogenic activity, uterus.

INTRODUCTION

Nowadays many women refuse the hormone replacement therapy such as estrogen replacement, due to adverse effects like vaginal bleeding and increased risk of developing breast and ovary cancer[1]. Therefore, there is a need of an alternative therapy, including herbal drugs. Medicinal plants are used worldwide for the treatment of various ailments, as well as for development of novel drugs. Over 20,000 species of medicinal plants are being used in traditional system of medicine and leading as the potential sources for discovery of new therapeutic agents[2]. Now in developing countries, the search for alternative medication for menopausal and postmenopausal women, based on plant extracts is increasing day by day[3]. Estrogen deficiency during menopause can develop many health problems, such as sleeping disorders, vaginal dryness, joint pain, mood swings, reduced bone density, cardiovascular disease etc[4]. The signaling of estrogens in the cells is mediated through the estrogen receptors like ERa and ERb. Binding of estrogen to ERa or ERb causes a conformational change in the receptor and promotes dimerization of receptors[5].

Phytoestrogens are compounds, which are produced from the natural source, having ability to interfere with estrogen action either interacting with the ERs or modulating the endogenous estrogen concentrations, possessing estrogen-like activity and can be used for the management of menopausal symptoms with few side effects[6]. Many phytoestrogens bind with a higher affinity to ERb than to ERa, suggesting that they may induce physiological effects through this ER subtype.

Since mixture of compounds, present in plant extracts, may exhibit better biological effects than isolated phytoestrogens, therefore, we evaluated the possible

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estrogenic effects of methanolic extract of *Avicennia alba* on immature rat model.

*Avicennia alba* (*A. alba*), belonging to the family- Acanthaceae, is one of the mangrove plants in southeast Asia mangrove forest[7]. The plant has traditionally been used as the remedy for sexual disorders, scabies, rheumatism, paralysis, asthma and snake-bites, skin disease and ulcer[8]. Fruits are plastered on to boils and tumors, a poultice of unripe seed and leaves stop inflammation, and bitter resin used as contraceptive by women[9]. The plant is reported to have a wide spectrum of medicinal properties, such as anti-cancer, anti-inflammatory, anti-microbial, antidiarhoeal and analgesic[10]. It contains high yield of steroids, triterpenes, flavonoids, alkaloids, saponins and tannins[11].

The new naphto-quinines and their analogues named Avicequinone A,B and C and Avicenol A,B and C from *A. alba* were isolated from stem bark of the plant[12]. Due to the absence of scientific reports that corroborate the contraceptive property of *A. Alba*, the present investigation was focused to study the estrogenic effect of *A. Alba* on immature rat.

**MATERIALS AND METHODS**

**Plant Materials**

The aerial parts of plant *Avicennia alba* were collected from Sunderban area, West Bengal in month of October, 2011 and identified by Dr. K. Karthikeyan, Central National Herbarium, Botanical Survey of India, Howrah, West Bengal. A voucher specimen (CNH/128/2011/TECH11/637) has been deposited in the herbarium of the Department of Pharmacognosy, School of Pharmaceutical Sciences, Siksha O’Anusandhan University, Odisha, India.

**Preparation of plant extract**

The air-dried plant materials (1 kg) of *A. alba* were coarsely powdered and extracted in a Soxhlet apparatus with methanol (2L) for 72 h. The methanolic extract was examined chemically to screen the presence of different phytoconstituents. The extract was concentrated to dryness in a flash evaporator (Buchi type) under reduced pressure and controlled temperature 50–60 °C. A total mass of 135 g of crude extract was obtained. The extract was stored in a refrigerator and a weighed amount of the extract was suspended in 10% aqueous Tween 80 solution and used for the present study.

**ANIMALS**

Colony-bred immature female albino rats (Wister strain), 21–23 days old and weighing between 30–45 grams were used. All the animals were maintained under standard husbandry conditions with food and water *ad libitum* in animal house. The temperature was 23±2°c and humidity was 50±5%. The experimental protocol was approved by Institutional Animal Ethics Committee, School of Pharmaceutical Sciences, Siksha O’ Anusandhan University, Odisha, India. (Registration no. 1171/c/08/CPCSEA)

**Acute toxicity study**

Acute oral toxicity of methanolic extract of *A alba* was determined by using female, non pregment rat weighing 160–200 gm. The animals were fasted for 3 hours prior to the experiment. Organization for Economic Cooperation and Development (OECD) guideline 420[13] was adopted for toxicity studies. Animals were administered with single dose of extracts and observed for their mortality during 48 hrs study period. The dose up to 4000 mg/kg body weight was found tolerable as no death was found. Rats were observed individually after dosing for first 30 minutes periodically and daily thereafter, till 14 days for any toxicity sign of gross changes in skin and fur, eyes, mucous membrane, circulatory, central nervous system, autonomic and behavior pattern if any.

**Estrogenic activity study**

The uterine weight and vaginal cornification method was employed for the study[14,15]. Colony-bred immature ovariectomised female albino rats were divided into four groups consisting six animals in each group. The first group served as control and received the vehicles (Tween-80, 1%) only. The second group received a suspension of Ethinyl estradiol in distilled water using Tween-80 (1%) at a dose of 0.02mg/kg body wt. The third group received the methanol extract at 400 mg/kg body weight and Fourth group received methanol extract at 400 mg/kg body weight in an addition to 0.02mg/kg body wt of Ethinyl estradiol. Body weight and vaginal smears were recorded daily. All the above treatments were given orally for 7 days. All the animals were sacrificed on 8th day of the experiment by decapitation under light ether anesthesia and the uterus were dissected out, surrounding tissues removed, blotted on filter paper and weighed quickly on a sensitive balance.

A portion of uterine tissues from control and treated animals were fixed in Bouins fluid for 24 hours, dehydrated in alcohol and then embedded in paraffin. The paraffin blocks were section at 6 μm and stained with haematoxylin-
eosin for histological examination for uterus. The diameter of uterus, thickness of endometrium and height of the endometrial epithelium were measured in 16 randomly selected sections using a calibrated ocular micrometer. The other portion of uterus was homogenized with ice-cold distilled water in a pre-cooled mortar and pestle to contain 10 mg of tissue/ml. The homogenated tissue was centrifuged in cold at 3000 rpm for 15 minutes and the supernatant was used for the estimation of total protein and cholesterol using standard method[16].

**Statistical analysis**

Statistical analysis of the differences between the group were analyzed by one-way analysis of variance (ANOVA) followed by Dunnert's t-test. \( p<0.05 \) was considered as statistically significant. All data are expressed as the mean value \( \pm \) SD.

**RESULTS**

**Phytochemical study**

The preliminary phytochemical screening of the methanolic extract of *A. alba* revealed the presence of alkaloids, anthraquinone glycosides, flavonoids, steroids and polyphenolic compounds.

**Estrogenic activity**

Assessment of estrogenic activity of methanolic extract of *A. alba* was done by taking status of vaginal cornification, uterine weight, uterine biochemical estimation and uterine histology as parameters. The effect of the methanolic extract of *A. alba* on immature rat uterus is shown in Table 1, Table 2 and Table 3.

**Table 1: Estrogenic activity of the methanolic extract of aerial part of Avicennia alba**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (Dose-mg/kg body weight)</th>
<th>Uterine weight mg/100gm body wt. (mean± SD)</th>
<th>Vaginal status</th>
<th>Vaginal cornification</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>Control (Tween 80, 1% v/v)</td>
<td>60.66±5.57</td>
<td>Closed</td>
<td>Nil</td>
</tr>
<tr>
<td>ii</td>
<td>Ethinyl estradiol (0.02 mg/kg)</td>
<td>146.83±5.26*</td>
<td>Open</td>
<td>+++</td>
</tr>
<tr>
<td>iii</td>
<td>Methanolic extract (400 mg/kg)</td>
<td>133.33±4.88*</td>
<td>Open</td>
<td>+ to ++</td>
</tr>
<tr>
<td>iv</td>
<td>Ethinyl estradiol (0.02 mg/kg) + Methanolic extract (400 mg/kg)</td>
<td>234.50±5.57*</td>
<td>Open</td>
<td>+++</td>
</tr>
</tbody>
</table>

No of animals used each group 6; + Nucleated epithelial cells, ++ Nucleated and cornified cells, +++ Cornified cells; \( * p<0.05 \) when compared to control

**Table 2: Histological changes in the uterus and endometrium after treatment with methanolic extract of aerial part of Avicennia alba**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (Dose-mg/kg body weight)</th>
<th>Diameter of the Uterus (µm) (Mean±SD)</th>
<th>Thickness of the endometrium (µm) (Mean±SD)</th>
<th>Height of endometrial epithelium (µm) (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>Control (Tween 80, 1% v/v)</td>
<td>356.33±7.33</td>
<td>99.83±6.76</td>
<td>19.83±5.26</td>
</tr>
<tr>
<td>ii</td>
<td>Ethinyl estradiol (0.02mg/kg)</td>
<td>684.50±7.17*</td>
<td>280.33±4.92*</td>
<td>35.83±4.35*</td>
</tr>
<tr>
<td>iii</td>
<td>Methanolic extract (400mg/kg)</td>
<td>544.50±3.61*</td>
<td>215.16±3.71*</td>
<td>28±4.33*</td>
</tr>
<tr>
<td>iv</td>
<td>Ethinyl estradiol (0.02mg/kg) + Methanolic extract (400mg/kg)</td>
<td>744±4.60*</td>
<td>341.50±5.46*</td>
<td>46.83±5.45*</td>
</tr>
</tbody>
</table>

No. of animals used each group 6; \( * p<0.05 \) when compared to control
Oral administration of the methanolic extract at 400 mg/kg body weight caused a significant increase ($p < 0.05$) in uterine weight in immature ovariectomised rats. The standard drug, Ethinyl estradiol produced statistically significant ($p < 0.05$), 1.42-fold increase in uterine weight. The methanolic extract of *A. alba*-induced proliferative changes in the uterine endometrium as evidenced by increased height of luminal epithelium, with loose stroma and increased number of glands (Figure 3), compared to control. The control animals presented a typical infantile condition (Figure 1). Ethinyl estradiol induced proliferative changes (Figure 2). Methanolic extract in combination with Ethinyl estradiol also induced similar proliferative changes (Figure 4). It exhibit estrogenic activity as shown by the significant ($p < 0.05$) increase in diameter of uterus and thickness of endometrium and vaginal epithelial cornification in immature rats when compared with control.

The number of cornified cells in the vaginal smears was considerably higher (+ to ++) in methanol extract treated rats and (+ to +++ in rats treated with combination of extract and Ethinyl estradiol than those of the controls (0 to +). Simultaneous administration of Ethinyl estradiol and the methanolic extract caused a highly significant increase in uterine weight (versus control, $p < 0.05$). There was also a significant increase in total protein and cholesterol content in the uterus of standard and test drug treated rats (versus control, $p < 0.05$).

### Table 3: Biochemical changes in the uterus after treatment with methanolic extract of aerial part of *Avicennia alba*

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (Dose-mg/kg body weight)</th>
<th>Total protein mg/100mg (Mean±SD)</th>
<th>Cholesterol mg/100mg (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>Control (Tween 80, 1% v/v)</td>
<td>3.8±1.26</td>
<td>18.69±3.26*</td>
</tr>
<tr>
<td>ii</td>
<td>Ethinyl estradiol (0.02mg/kg)</td>
<td>7.45±1.09*</td>
<td>25.19±2.85*</td>
</tr>
<tr>
<td>iii</td>
<td>Methanolic extract (400mg/kg)</td>
<td>5.7±1.03*</td>
<td>22.11±3.60</td>
</tr>
<tr>
<td>iv</td>
<td>Ethinyl estradiol (0.02mg/kg) + Methanolic extract (400mg/kg)</td>
<td>9.26±1.63*</td>
<td>30.83±2.35*</td>
</tr>
</tbody>
</table>

No. of animals used each group 6; $p<0.05$ when compared to control

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**Estrogenic activity of methanolic extract of aerial parts of *Avicennia alba***

![Figure 1](image1.png)  
**Figure 1:** Section of immature rat uterus treated with Tween 80, 1% (Control) (H&E,100x), showing intact stroma.

![Figure 2](image2.png)  
**Figure 2:** Section of immature rat uterus treated with (Ethinyl estradiol (0.02mg/kg bw) (H&E,100x), showing loose stroma and increased no. of glands.
DISCUSSION

The ovarian hormone, estrogen causes various physiologic and biochemical changes in uterus and the female reproductive tract. When female rats are ovariectomized, the resultant lack of estrogen causes atrophy of the uterus and administration of estrogenic substances to ovariectomized rats causes uterotrophic effects, vaginal cornification and proliferative changes in uterine endometrium epithelium height\(^{17}\).

Estrogen causes an increase in protein synthesis, uterine weight, water uptake and retention of fluid leading to ballooning of uterus\(^{18}\). Estrogen also causes vaginal opening which is a quality measure of estrogen potency. Presence of cornified cells in vaginal smears also indicates estrogenic activity\(^{19}\). In the ovariectomised rats, the uterine protein concentration in increased by estrogen\(^{20,21}\).

Estrogen like steroid stimulates the synthesis of mRNA, protein and DNA\(^{22}\). In the present study, the increase in total protein concentration in uterus of female rats administered with aerial parts of methanolic extract of Avicennia alba may be attributed to steroid like substances in it. Cholesterol is a precursor molecule in steroidogenesis\(^{23}\). It was previously reported that estrogen administered increase the uterine cholesterol content in ovariectomised rats\(^{24}\). The methanolic extract increases the cholesterol level in ovariectomised rat uterus, which may be due to the presence of steroid like substances in the extract of Avicennia alba.

Methanolic extract of A. alba exhibited estrogenic activity as shown by significant increase in uterine weight, diameter of uterus, thickness of endometrium and vaginal epithelial cornification in immature rats. The histological examination of uterus of extract treated rats showed estrogenic influence, as evidenced by increased height of luminal epithelium with loose stroma and increased number of glands\(^{25}\). This confirms the estrogenic property of A. alba.

Literature review on A. alba showed the presence of steroids, triterpenes, flavonoids, alkaloids, saponins and tannins\(^{11}\). Flavonoids and phenolic compounds are known to possess estrogenic activity\(^{26}\). Phytoestrogens like β-sitosterol, stigenosterol, stigmagmastanol etc possess estrogenic activity due to their affinity with estrogenic receptors leading to infertility in animals\(^{27,28}\). Thus the estrogenic activity shown by the extract A. alba may be attributed to the presence of flavonoids, phenolic compounds and phytosterols.

Simultaneous administration of Ethinyl estradiol and methanol extract caused significant increase in uterine weight when compared with control \((p<0.05)\). The degree of uterotrophic potency was more than that produced by Ethinyl estradiol, when compared with control. These observations have also been confirmed when the uterotrophic changes such as diameter of uterus, thickness of endometrium were compared with control and standard Ethinyl estradiol treatment. This shows that the methanol extract has significant estrogenic activity when given alone. There was no anti estrogenic activity when given...
along with Ethinyl estradiol at the tested dose. So the plant may be beneficial both as a contraceptive as well as in post menopausal hormonal replacement therapy.

Further, isolation of active constituents from the methanolic extract of *Avicennia alba* and estrogenic activity of such isolated compounds are in progress.

REFERENCES
