

# Antiimplantation and Antiestrogenic Activity of *Boerhaavia Diffusa* Root Extract in Female Albino Rats

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**Abstract** Disturbance of the natural steroid hormone balance can successfully disorganize the co-ordinate events involved in ovulation, ovum transport and implantation. Thus, compounds possessing estrogenic, progestation and anti-estrogenic, anti-progestational or anti-implantation activity may also exhibit anti-fertility activity. The present study was conducted to investigate the antiimplantation and antiestrogenic activity of *Boerhaavia diffusa* root extract in female rats. Female albino rats were taken and divided into three groups. Group I served as control, group II received methanolic extract at dose of 200 mg/kg body weight and group III received methanolic extract at dose of 400 mg/kg body weight. The root extract of *Boerhaavia diffusa* evaluated for anti-implantation activity, The methanolic extract at a dose of 200 mg/kg body weight inhibited pregnancy with mean number of implants  $5.58 \pm 0.34$  [ $P < 0.001$ ]. The same extract at a dose of 400mg/kg body weight significantly inhibited pregnancy with mean number of implants  $4.47 \pm 0.23$  [ $P < 0.001$ ]. The oral administration of the *Boerhaavia diffusa* methanolic root extract (BDME) at dose of 200 mg/kg and 400 mg/kg body weight caused a significant increase in the uterine weight in immature rats when compared to control, [ $P < 0.001$ ]. These results indicate that the methanolic root extract of *Boerhaavia diffusa* contain bioactive compounds which may cause antiestrogenic activity. We can conclude that the methanolic extract of *Boerhaavia diffusa* root showed significant antifertility activity by means of potent antiestrogenic and anti-implantation, in a dose-dependent manner.

**Keywords:** *Boerhaavia diffusa*, Antiimplantation, Antiestrogenic activity, Antifertility

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## 1. Introduction

Plants have been used globally across varied cultures as a safe natural source of medicines. From time immemorial, humans have relied on plants that could meet their basic necessities such as food, shelter, fuel and health. The knowledge of the healing powers of plants was initially passed down orally through generations, and as civilizations grew written records were prepared for the benefit of the population. A wide majority of herbal plants possess pharmacological principles, which has rendered them useful as curatives for numerous diseases. World Health Organization reports that 70% – 80% of the world population confide in traditional medicine for primary health care [1].

The search for an effective, safe, and reversible female antifertility agent with minimum side effects remains a challenge. To date, a number of compounds having antifertility effects have been isolated from higher plants but most of them are metabolically toxic. In recent years there has been considerable interest in plants with potential contraceptive properties. Research on Indian medicinal plants with contraceptive property has been exhaustively reviewed by so many researchers and

scientists, but so far no single plant is available, which can safely use to prevent pregnancy and reproductive disorders. Although few plants have shown promising results in preventing pregnancy but they have failed in the course of other investigations [2].

With all these consequences, the research is still continued to search out potent contraceptive plants. Hence, the present study has been undertaken to evaluate the effect of *Boerhaavia Diffusa* Linn on physiology of female reproductive system and the other parameters including the biochemical and hematological in female rats. *Boerhaavia diffusa* Linn. commonly known as 'Punarnava' is an abundant creeping weed found all over India. The plant has drawn lot of attention due to its uses in Indian Traditional Medicine. The plant was named in honor of Hermann Boerhaave, a famous Dutch physician of the 18th century. The Plant in whole or its peculiar parts (Aerial parts and Roots) have a numerous medicinal properties and are used by endemic and tribal people in India and Unani medicine in Arab countries to show Anti-bacterial, Anti-nociceptive, hepato-protective, hypo-glycemic, anti-proliferative, anti-estrogenic, antiinflammatory, anti-convulsant, anti-stress and anti-metastatic activities and also in treatment of stress, dyspepsia, abdominal pain, inflammation, jaundice etc. [3]. Saini (1996) reported that root paste of *B. diffusa* is taken with cow's milk to induce

abortion in Central India [17]. As scientific data regarding the antifertility activity of this plant is not available, hence we have selected this plant to scientifically evaluate its rationale to use as potential antifertility agents.

The aim of the present study was, therefore, to carry out pharmacological screening, efficacy and safety studies on reproductive apparatus on one of the traditionally plant *Boerhaavia diffusa* (Linn) because *Boerhaavia diffusa* (Linn) is a plant with diverse medicinal uses.

## 2. Materials and Methods

### 2.1. Collection and Identification of Plant Material

Plant of *Boerhaavia diffusa* (Linn) was collected from National Research Institute for Ayurveda-Siddha Human Resource Development (Under CCRAS, New Delhi), Aamkho, Gwalior, Madhya Pradesh, India. The following plant material were submitted to the Department of Pharmacognosy, B.R.Nahata College of Pharmacy, Mandsaur and identified by Prof. (Dr.) Gyanendra Tiwari, Scientist Botanist, KNK College of Horticulture, Mandsaur (M.P.) as Voucher Specimen number, BRNCP/BD/006/2010).

### 2.2. Extraction of Plant Material

The roots of the plant were collected in the month of September and October because the plant of *Boerhaavia diffusa* (Linn) grows profusely in the rainy season. The roots were cut into the pieces and were dried in shade at room temperature and finely grinded powder was serially extracted by using Soxhlet apparatus with methanol for 6 hrs at 60°C. The solvent was removed by distillation and semisolid mass was dried by using hot water bath at 40-50°C and the % yield of the methanolic extract of *Boerhaavia diffusa* root was calculated [4,5].

### 2.3. Pharmacological Evaluation

#### 2.3.1. Animals

Female albino rats (wistar strain weighing 180–200 g) were used for pharmacological evaluation of BDME. The animals were housed in standard environmental conditions of temperature ( $21 \pm 2^\circ\text{C}$ ), humidity ( $55 \pm 10\%$ ) and a 12-h light–dark cycle. Rats were supplied with standard pellet diet and water ad libitum. The animals were acclimatized to laboratory hygienic conditions for 10 days before starting the experiment. Animal study was performed in the Division of Pharmacology and Toxicology, B R Nahata College of Pharmacy, Mandsaur, with due permission from the Institutional Animal Ethics Committee (NO. 161/Ph.D./10/IAEC/BRNCP/09-10/Mandsaur).

#### 2.3.2. Acute Toxicity Study

The acute toxicity study of the extract was determined according to the Organization for Economic Co-operation and Development (OECD) guidelines no. 420. Female rats (180–200 g) were used for this study. After the sighting study, a starting dose of 2,000 mg/kg (p.o.) of the test sample was given to five animals in group. The treated

animals were monitored for 14 days for mortality and general behavior.

#### 2.3.3. Dose Fixation for Animal Study

Dose fixation for animal study was carried out on albino rats (180–200 g). All the extracts were homogenized and dissolved in distilled water, and they were administered to rats orally. It was observed that none of the extracts were found to be lethal even at the dose of 2000 mg/kg, so that 200 mg/kg body weight of the crude extracts was fixed as therapeutic dose and double of this dose i.e. 400 mg/kg was also chosen to ascertain the response of animals and also to study dose dependency.

#### 2.3.4. Antioviulatory Activity

The antioviulatory activity was carried out in female albino rats weighing (180–200 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. The selected rats were divided into 3 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, p.o. daily) and served as control. Groups II to III received methanolic extracts of *Boerhaavia diffusa* root 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 [6, 7].

#### 2.3.5. Antiimplantation Activity/Abortifacient Activity

Anti-implantation activity was performed as per method described by Londonkar Ramesh L et al, 2009, Sharrangouda et al, 2008 and Vishnu N. Thakare, Pankaj S. Kothavade et al, 2009 [2,6,7] with some modification and used to evaluate the anti-fertility activity of *Boerhaavia diffusa*. Proven fertile female albino strain rats, with normal estrous cycle were selected for this study. Antifertility activity was determined in female albino rats. Rats found in the estrous phase of the cycle were caged with males of proven fertility in the ratio of 2:1. Animals, which showed thick clumps of spermatozoa in the vaginal smear on the next day, were separated for the experiment and that day was designated as day 1 of pregnancy. These animals were divided into 3 groups consisting of 6 animals in each group. The group I received vehicle only and served as control. Groups II and III received methanolic extract at doses of 200 and 400 mg/kg body weight respectively. All the above treatments were given from day 1 to 7 of pregnancy and on day 10, laparotomy was performed under light ether anesthesia using aseptic condition and uteri were observed for number and size of implantation sites.

#### 2.3.6. Antioestrogenic Activity

Anti-estrogenic activity was performed on female albino rats weighing 180–200g. Animals were maintained under standard husbandry conditions with food and water ad libitum. Vaginal smear for each rat was monitored daily in the morning. Only rats with normal estrous cycle were selected for the experiment. To study the effect of *Boerhaavia diffusa* methanolic extract (BDME) on the estrous cycle, the selected animals were divided into three groups; group I served as control, group II & III received

methanolic extract at dose of 200 & 400 mg/kg b.wt. respectively. The treatment was continued for 40 days. During this period the vaginal smear of the rats were examined daily in the morning hours. On day 41<sup>st</sup> the rats were sacrificed under light ether anesthesia. Ovary, uterus and vagina were dissected out, weighed and kept at -20°C for biochemical estimations. Blood was collected by cardiac puncture for haematological studies [8].

### 2.3.7. Histopathological Examination

For histopathological examinations, the ovary and uterus were quickly removed and fixed in 10% neutral buffered formalin solution. Following fixation, specimens were dehydrated in graded ethanol, embedded in wax, sectioned to 5 microns thickness. The sections were stained with Haematoxylin and Eosin and examined using light microscope [9].

## 2.4. Toxicological Screening

### 2.4.1. Biochemical and Haematological Investigation

Blood samples of each animal were collected by cardiac puncture for toxicological screening. Total RBC, WBC, haemoglobin and haematocrit, total protein, cholesterol, glycogen, values were recorded by using autoanalyzer and reagent kits.

## 2.5. Statistical Analysis

Statistical difference in the mean analyzed using one-way ANOVA followed by Turkey's multiple comparison tests  $P < 0.001$  was considered as statistically more significant.

## 3. Results

### 3.1. Acute Toxicity Study

No mortality and behavioral changes were observed in the treated groups up to 2,000 mg/kg body weight. It was observed that none of the extracts were found to be lethal even at the dose of 2000 mg/kg, so that 200 mg/kg body weight of the crude extracts was fixed as therapeutic dose and double of this dose i.e. 400 mg/kg was also chosen to ascertain the response of animals and also to study dose dependency.

### 3.2. Anti-implantation Activity

The root extract of *Boerhaavia diffusa* evaluated for antiimplantation activity, The methanolic extract at a dose of 200 mg/kg body weight inhibited with mean number of implants (Table 1)  $5.58 \pm 0.34$  [ $P < 0.001$ ]. The same extract at a dose of 400mg/kg body weight significantly inhibited pregnancy with mean number of implants  $4.47 \pm 0.23$  [ $P < 0.001$ ].

No toxic effect was observed in the animals and their pups either by gross visual examination or in the weight of experimental animals (Table 4). Soon after the parturition all the experimental animals exhibited normal estrous cycle and on breeding they underwent normal pregnancy and delivered normal litters. The antiimplantation activity was reversible on withdrawal of the treatment of the extract and the complete recovery was observed within 20-25 days.

**Table 1. Antiimplantation activities of methanolic extract of *Boerhaavia diffusa* root (BDME)**

Group	Treatment	No. of rats without implantation sites on day 10	Mean no. of implants $\pm$ S.E. on day 10	% of rats having implantation sites on day 10	% rats delivered on full term
I	Control (1% Tween-80 b.w.)	NIL	$11.28 \pm 0.24$	100	100
II	BDME (200mg/kg b.w.)	02	$5.58 \pm 0.34^{**}$	49.46	000
III	BDME (400mg/kg b.w.)	03	$4.47 \pm 0.23^{***}$	39.62	000

M  $\pm$  S.E. = Mean  $\pm$  Standard error, Duration: 07 days, received the treatment from day 1 to 7 and laparotomized on day 10 of pregnancy. Six animals were maintained in each group, \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$  when compared with control.

**Table 2. Effect of methanolic extract of *Boerhaavia diffusa* root (BDME) on uterine wet weight, vaginal opening and cornification of vaginal epithelial cells in bilaterally ovariectomized immature rats**

Group	Treatment	Uterine wet weight (mg/100g)	Vaginal opening and cornification
I	Control (1% Tween-80 b.w.)	$98.25 \pm 7.23$	-/-
II	BDME (200mg/kg b.w.)	$143 \pm 3.23^{***}$	6/6
III	BDME (400mg/kg b.w.)	$147 \pm 3.28^{***}$	6/6

M  $\pm$  S.E. = Mean  $\pm$  Standard error, Duration: 07 days, six animals were maintained in each group, \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$  when compared with control.

**Table 3. Histopathology changes in the uterus due to the administration of methanolic extract of *Boerhaavia diffusa* root (BDME) in bilaterally ovariectomized immature rats**

Group	Treatment / Dose (mg/kg b.w.)	Diameter of uterus ( $\mu$ m)	Thickness of myometrium ( $\mu$ m)	Thickness of endometrium ( $\mu$ m)	Epithelial cell height ( $\mu$ m)
I	Control (1% Tween-80)	$265.23 \pm 3.45$	$26.23 \pm 2.14$	$42.68 \pm 2.23$	$23.74 \pm 1.86$
II	BDME (200)	$423.45 \pm 2.86^{**}$	$48.23 \pm 3.24^*$	$83.23 \pm 2.78^{**}$	$32.34 \pm 1.23^*$
III	BDME (400)	$573.34 \pm 5.49^{***}$	$67.37 \pm 2.56^{***}$	$173.3 \pm 2.54^{***}$	$42.21 \pm 3.38^{***}$

M  $\pm$  S.E. = Mean  $\pm$  Standard error, Duration: 07 days, six animals were maintained in each group, \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$  when compared with control.

### 3.3. Estrogenic/antiestrogenic Activity of the Methanolic Extract

The oral administration of the BDME at dose of 200 mg/kg and 400 mg/kg body weight caused a significant increase in the uterine weight in immature rats when compared to control (Table 2), [P<0.001]. The uterotrophic changes such as the diameter of the uterus [P<0.001] and

thickness of the endometrium [P<0.001] were significantly increased when compared with control rats (Table 3). The treated rats showed an open vagina (Table 2) and an estrous smear. The number of cornified cells in the vaginal smears was considerably higher than that of controls rats. These results indicate that the methanolic extract of *Boerhaavia diffusa* root contain bioactive compounds which can show estrogenic activity.

**Table 4. Effects on organs and body weight of female albino rats after the administration of methanolic extract of *Boerhaavia diffusa* root.**

Groups	Body weight (g)		Reproductive organs weight (mg)		
	Initial	Final	Ovary	Uterus	Vagina
Group I: Control	192.30 ± 1.85	198.70 ± 2.43 ns	42±3.73	217±0.003	218±0.002
Group II: BDME(200mg/kg) treated	197.60±1.32	206.70±2.67 ns	26±3.23*	212±0.006*	187±0.007*
Group III: BDME(400mg/kg) treated	201±1.28	214.34±2.87ns	27±3.45*	216±0.009*	186±0.005*

(Mean ±SEM of 6 animals), Group II & III compared with group I: \* = significant (p≤0.01), ns = non-significant.

**Table 5. Effects on estrous cycle of female albino rats after the administration of methanolic extract of *Boerhaavia diffusa* root.**

Groups	Phases				
	Estrous cycle	Proestrous phase	Estrous phase	Metestrous Phase	Di-estrous Phase
Group-I, Control	4.23±0.16	0.87±0.07	0.83±0.17	0.86±0.13	1.85±0.26
Group-II, BDME (200mg/kg)	5.58±0.48*	0.48±0.13*	0.87±0.19	0.47±0.27*	3.68±0.43**
Group-III, BDME (400mg/kg)	5.85 ± 0.39**	0.26 ± 0.11 *	0.83 ± 0.65	0.66 ± 0.33*	4.10 ± 0.51**

(Mean ±SEM of 6 animals), Group II & III compared with group I: \*\*=highly significant (p≤0.001), \* = significant (p≤0.01) ns = non-significant.

**Table 6. Haematological changes in female albino rats after the administration of methanolic extract of *Boerhaavia diffusa* root.**

Groups	RBC Count (million/ mm <sup>3</sup> )	WBC Count (per.cu.mm)	Haemoglobin (gm %)	Haematocrit (%)	Blood Sugar (mg/dl)
Group-I, Control group	3.62±0.43	7.3±0.46	13.46±0.58	48.38±1.48	82.36±0.67
Group-II, BDME (200mg/kg)	3.78±0.003ns	7.5±0.36 ns	13.27±0.47 ns	42.19±0.89 ns	79.42±0.82 ns
Group-II, BDME (400mg/kg)	3.92±0.003ns	7.2±0.36 ns	13.37±0.47 ns	42.23±0.89 ns	79.45±0.82 ns

(Mean ±SEM of 6 animals), Group II & III compared with group I: ns = non-significant.

**Table 7. Biochemical Changes in the ovary, uterus and vagina of BDME treated female rats**

Group	Treatment (mg/kg b.w.)	Protein content			Glycogen content			Cholesterol content		
		Ovary	Uterus	Vagina	Ovary	Uterus	Vagina	Ovary	Uterus	Vagina
I	Control	182 ±4.35	179 ±3.32	164±5.35	8.6 ±0.32	17.89±1.32	8.78±0.42	8.67±0.79	4.6±0.14	5.32±0.12
II	BDME (200)	136 ±4.22	142 ±4.47	121±3.12	4.56±0.22	12.23±0.92	4.34±0.22	12.38±0.48	8.45±0.38	8.37±0.74
III	BDME (400)	128 ± 5.22	134±3.45	118±2.85	4.23±0.83	11.28±0.87	4.02±0.67	12.89±0.52	8.86±0.27	8.48±0.43

not used for steroidogenesis hence accumulated in the ovary.

## 4. Discussion

The magic of Indian plants in reducing fertility of mammalian species is well established [10]. A number of plants from Indian origin have been experimentally tested using modern techniques for their antifertility activities [11]. The oral administration of the methanolic extract at 200 and 400 mg/kg body weight caused a significant increase in the uterine weight in immature rats when compared to control, [P<0.001]. Data revealed that oral administration of *Boerhaavia diffusa* root extract showed a significant decline in the weight of ovary, uterus, vagina as well as protein and glycogen level, however cholesterol level (Table 4) increased significantly. Cholesterol derived from the different sources is the precursor for the steroidogenesis of ovarian endocrine tissue. The significant increase in the cholesterol level (Table 7) of the group receiving extract indicates that cholesterol was

The decrease in the glycogen content of BDME treated uterus confirms the antiestrogenic nature of the drug. Reduction in protein content (Table 7) of the female genital tract of BDME treated rats suggests an inhibition of estrogen production. The decrease in the weight of ovary and uterus shows antiestrogenic nature of BDME since antiestrogenic substance decreases the wet weight of the uterus [8]. The prolonged estrous cycle and diestrous phase (Table 5) observed with the extract suggests the antifertility effect of BDME.

Blood parameters have been found to be within normal range (Table 6) indicating non toxic action of *Boerhaavia diffusa* on general body metabolism. These results suggest that *Boerhaavia diffusa* has the anti-estrogenic activity without altering the general physiology of the female rats.

Implantation in the rat depends on the completion of basic sequence of events occurring both at the fertilized egg and endometrium. The endometrium needs 48 hours

period of progesterone preparation and presence of estrogen at the end, leading to the formation high sensitive decasualized endometrium [12]. The requirement of progesterone is in milligrams whereas the estrogen requirement is in micrograms. The any imbalance in reproductive hormonal level is results in failure of implantation [13,14,15,16]. The compound of hormonal values usually disturbs the hormonal milieu in the uterus and provokes infertility effects [2].

Plants have the property to inhibit the estrogen surge for implantation. In mice and humans, estrogens play a pivotal role in the implantation since they participate in the estrogen, progesterone balance and thereby in the uterine receptivity to the embryo. Administration of BDME to female rats caused estrogen inhibition due to its antiestrogenic nature. The decrease in the weight of ovary and uterus shows antiestrogenic nature of BDME since antiestrogenic substance decreases the wet weight of the uterus [8]. The prolonged estrous cycle and diestrous phase observed with the extract suggests the antifertility effect of BDME.

## 5. Conclusion

The methanolic root extract of this plant at two different doses of 200 mg/kg and 400 mg/kg b.w. prevented the pregnancy. The methanolic extract of *Boerhaavia diffusa* root at 400 mg/kg were found to possess more significant ( $p < 0.001$ ) antiimplantation and antiestrogenic activities compared to at 200 mg/kg. All these observations suggest that the *Boerhaavia diffusa* root extract has reversible antifertility effect in female albino rats and provide the evidence for the antifertility activity of *Boerhaavia diffusa* as claimed in the traditional use. We can conclude that the methanolic extract of *Boerhaavia diffusa* root showed significant antifertility activity by means of potent antiestrogenic and anti-implantation, in a dose-dependent manner.

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