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RESEARCH ARTICLE

Studies on Some Effect of the Aqueous Extract of the Leaves of *Boerhavia Diffusa* on the Ovaries

Adebajo AO[*1,2], Isah KP[1,3] and Ajayi AJ[1,2]

[1]*Department of Anatomy, University of Lagos, Lagos State, Nigeria*
[2]*Department of Anatomy, Faculty of Basic Medical Sciences, Ogbomosho, Oyo State, Nigeria*
[3]*Department of Anatomy, Olabisi Onabanjo University, Ago Iwoye, Ogun State, Nigeria*

[*]**Corresponding author:** Adebajo AO, Department of Anatomy, College of Medicine, University of Lagos, Lagos State, Nigeria, Tel: +2348131364896, E-mail: adebajoseye@gmail.com

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Abstract

Purpose: This study was designed to investigate some effect the aqueous extract of *Boerhavia diffusa* on the female reproductive system using Sprague-Dawley rats as experimental models.

Methods: A total of seventy-five female rats were used for this study. They were divided into three groups (A-C) of twenty-five rats each, and further subdivided into two groups (X of 20 rats and Y of 5 rats). Group A served as control and groups B and C received 50 and 100 mg/kg of *Boerhavia diffusa*. At the end of the administration, regularity of oestrous cycle was observed. Five rats were exposed to coitus and sacrificed after each trimester to determine litter parameters.

Results: A significant dose dependent decrease in ovarian weight was recorded when treatment group was compared to control. Sections of the treatment groups showed normal histology, however, an irregular pattern in the oestrous cycle of the group administered with 100 mg/kg of the extract was observed as zero number of litters was obtained.

Conclusion: Results obtained from this study showed that oral administration of aqueous extract of the leaves of *Boerhavia diffusa* to female Sprague-Dawley rats altered the oestrous cycle based on dose administered.

Keywords: *Boerhavia diffusa*; Ovary; Oestrous cycle; Sprague-Dawley rats

Introduction

The environment of man is endowed with plants and fruits which overtime have been found to be of great nutritional and health importance to humans. Such plants and fruits constitute sources of spices in food, stimulants and some micronutrients [1]. Historically, plants have provided sources of inspiration for drug compounds, as plant-derived medicines have made large contributions to human health and well-being [2].These plants may have become the basis for the development of new medicine, that is, a natural blue print for the development of new drugs,or a phytomedicine to be used for treatment of disease [2,3].

Boerhavia diffusa is a plant known for its medicinal properties employed in folkloric medicine in Nigeria and Ayurvedic medicine system in India [4]. It grows as a common weed whose roots, leaves and seeds are parts used for medicinal purposes [2]. The plant is consumed as vegetable as it is believed to be a rich source of vitamins, minerals, proteins and carbohydrate [5]. It has been shown to contain a large number of compounds such as flavonoids, saponins, steroids and alkaloids [6]. Traditionally, *Boerhavia diffusa* has been evaluated for its diuretic, anti-fibrinolytic, anti-scabies, anti-bacterial, hepatoprotectant, anti-helmintic, anti-leprotic, anti-asthmatic, anti-urethritis, anti-convulsant, cardiotonic, immunosuppressant, anti-viral and anti-oxidant. Anti-inflammatory, anti-diabetic and anti-cancer properties have been validated pharmacologically [6]. In the light of the potential role of the plant *Boerhavia diffusa*, in the treatment of human ailments including abdominal tumour, jaundice, dyspepsia and menstrual disorders, this study aim to investigate the effect of aqueous extract of *Boerhavia diffusa* on the female reproductive system using Sprague-Dawley rats as experimental models [2].

Materials and Methods

Plant Extract

The plant was obtained from Oja Ajagu, a local market in Ogbomosho, Oyo state. It was examined and identified by a plant taxonomist in the department of pure and applied Biology, Ladoke Akintola University of Technology, Ogbomosho, Oyo State. The fresh leaves were shade dried for two weeks, pulverized to powder and finely sieved [7,8]. About 300g of the fine powder was soaked in 5L of distilled water for 24 hours after which it was filtered using Whatman filter paper [7,8]. The extract was evaporated to dryness in a vacuum desiccator at 40 °C and 56g of the substance was obtained. Aliquot portions of *Boerhavia diffusa* were weighed and dissolved in distilled water (at room temperature) for use every day for the duration of administration.

Animals

A total of seventy-five healthy and cycling female Sprague-Dawley rats, weighing 150 ± 40 g were used for this study. They were housed in standard well ventilated wire mesh plastic cages in the Animal House of the Department of Anatomy, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomosho, Oyo State, under standard room temperature ranging between 26 °C-28 °C and relative humidity of 50-55%. The animals were exposed to twelve hours light and twelve hours dark cycle and were left to acclimatize for a period of two weeks before the commencement of the experiment. The animals were cycled for two complete cycles of 8 days so as to ascertain their ability to cycle. The route of administration of the drug was oral, with the use of a feeding tube. The animals were identified by different ear tags. All experimental procedures and techniques were approved by the Health Ethics committee of the Faculty of Basic Medical Sciences of Ladoke Akintola University of Technology, Ogbomosho, Oyo State, Nigeria with strict compliance with the guiding principles for research involving animals.

Experimental Design

The animals were divided into three groups designated as A-C comprising of twenty-five rats each. Group A received 1 ml distilled water (served as control), groups B and C received 50 and 100 mg/kg body weight of *Boerhavia diffusa* extract respectively [9]. The twenty-five animals from each group were further sub-divided into 2 groups designated as X with 20 animals and Y with 5 animals. Animals from sub-group X were administered with extract for 7 weeks. After 2 weeks of administration, they were all mated with mature males. Five animals were randomly selected immediately after 7, 14 and 21 days of administration (to designate first, second and third trimesters) after which the rats were euthanized with the ovaries harvested, fixed in 10% formal saline and litters were grossly observed (litter-size, litter-weights and crown-rump-length), if any. The remaining 5 animals continue to receive the extract till the end of the 35 days, but were not exposed to coitus. They were euthanized and ovaries were harvested and fixed in 10% formal saline. The 5 animals in sub-group Y were administered for 20 days (of 5 oestrous cycles) and were examined daily via vaginal smears and properly documented.

Mating

The female rats were mated with male rats in the ratio 1:1. The presence of spermatozoa in the vaginal smear on the morning of oestrous was a confirmation of pregnancy and was taken as the first day of pregnancy.

Procedure for Vaginal Smearing

Vaginal smears were collected using a small rubber suction manual pipette. Normal saline was introduced into the vaginal canal and released. After 2-3 seconds, the vaginal fluid was suctioned into the tip of the pipette by a means of negative pressure. The fluid was then smeared on a glass slide and examined under the light microscope immediately before drying [11].

Blood Sample Collection

Blood (2ml) was collected from each animal via the retro-orbital sinus with 70µl heparinized capillary tube and put into plain sample bottle for Follicle stimulating hormone (FSH), Luteinizing hormone (LH), Estradiol (E_2) and Estrogen analysis. The sample was centrifuged at 3000 rpm for five minutes. The serum was used to analyse the level of the hormones. Blood samples were collected from the animals at their estrous phase and an enzyme –based immunoassay (EIA) system was used to measure the hormone levels in serum samples collected. The EIA kit was obtained from Immunometrics (London, UK) and contained an EIA quality control sample. A quality control was carried out at the beginning and at the end of the assay to ascertain the acceptability with respect to bias and within batch variation. Also, the blood collected was used for haematological studies.

Statistics

The data obtained from all the groups were compiled and statistically analysed using ONE WAY-ANOVA and Student T-test method on SPSS software version 22. The results of the data were expressed as mean ± SEM (standard error of mean) where $p<0.05$ was taken as significant.

Results

Effect of *Boerhavia diffusa* on Body Weight

There was significant decrease in body weights of the rats in the both treatment groups (50 and 100 mg/kg) when initial was compared to final body weights were compared. This weight loss was in a dose dependent manner (Table 1).

GROUP	INITIAL WEIGHT (g)	FINAL WEIGHT (g)	% WEIGHT DIFFERNCE (g)
Control	114.50 ± 1.50	143.00 ± 2.00*	19.93
Low dose	161.00 ± 2.45	148.00 ± 2.00*	8.78
High dose	193.00 ± 3.74	169.00 ± 4.58*	14.20

Table 1: Effect of *Boerhavia diffusa* on Body Weight

Effect of *Boerhavia diffusa* on ovarian weight

There was statistical difference in the weight of the ovary when treatment group was compared to control. Also, the decrease observed as in a dose dependent manner. When low dose was compared to high dose, a significant decrease in ovarian weights was recorded (Table 2).

GROUP	WEIGHT (g)
Control	0.46 ± 2.10
Low dose	0.31 ± 1.09[a]
High dose	0.20 ± 0.74[ab]

Table 2: Effect of Boerhavia diffusa on ovarian weight

Effect of *Boerhavia diffusa* on Hormonal levels

There was statistical difference in the levels of FSH, LH, Estradiol and Estrogen when treatment group was compared to control. Also, the decrease observed as in a dose dependent manner. When low dose was compared to high dose, a significant decrease in the level of estrogen was recorded (Table 3).

GROUP	FSH (Pg/ml)	LH (Pg/ml)	ESTRADIOL (Pg/ml)	ESTROGEN (Pg/ml)
Control	34.20 ± 0.41	32.31 ± 0.11	36.30 ± 1.26	37.56 ± 1.11
Low dose	20.61 ± 0.30[a]	26.30 ± 0.42[a]	27.04 ± 2.30[a]	29.49 ± 2.33[a]
High dose	14.28 ± 2.11[ab]	20.67 ± 2.31[ab]	16.23 ± 0.19[ab]	19.10 ± 2.51[ab]

Table 3: Effect of *Boerhavia diffusa* on Estrogen levels

Effect of *Boerhavia diffusa* on Oestrous Cycle

Similar and consistent pattern of oestrous cycles was recorded when the low dose group was compared to control throughout the duration of the experiment as an irregular pattern was observed in the oestrous cycle of the high dose group (Table 4).

Group / Day	Control	Low dose	High dose
1	Proestrous	Proestrous	Metestrous
2	Estrous	Estrous	Diestrous
3	Metestrous	Metestrous	Diestrous
4	Diestrous	Diestrous	Diestrous
5	Proestrous	Proestrous	Metestous
6	Estrous	Estrous	Metestrous
7	Metestrous	Metestrous	Metestrous
8	Diestrous	Diestrous	Diestrous
9	Proestrous	Proestrous	Diestrous
10	Estrous	Estrous	Diestrous
11	Metestrous	Metestrous	Diestrous
12	Diestrous	Diestrous	Diestrous
13	Proestrous	Proestrous	Estrous
14	Estrous	Estrous	Estrous

Day \ Group	Control	Low dose	High dose
15	Metestrous	Metestrous	Estrous
16	Diestrous	Diestrous	Metestrous
17	Proestrous	Proestrous	Diestrous
18	Estrous	Estrous	Estrous
19	Metestrous	Metestrous	Estrous
20	Diestrous	Diestrous	Estrous

Table 4: Effect of *Boerhavia diffusa* on Oestrous Cycle

Effect of *Boerhavia diffusa* on the Parameters of Reproductive outcome

There were no significant differences in the parameters of the litters in when low dose dams were compared to control. On the other hand, the low dose group recorded zero number of litters (Table 5).

Group	1st Trimester			2nd Trimester			3rd Trimester		
Parameters	LW (g)	LS (cm)	CRL (cm)	LW (g)	LS (cm)	CRL (cm)	LW (g)	LS (cm)	CRL (cm)
Control	1.53 ± 1.92	7.80 ± 0.86	1.30 ± 0.45	2.03 ± 0.02	7.11 ± 1.21	1.90 ± 0.10	6.20 ± 1.02	5.46 ± 0.30	4.80 ± 0.18
Low dose	1.44 ± 5.61	7.42 ± 0.37	1.25 ± 0.02	1.95 ± 0.01	7.02 ± 1.00	1.82 ± 0.05	6.18 ± 0.06	5.20 ± 0.90	4.23 ± 0.16
High dose	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL

Table 5: Effect of *Boerhavia diffusa* on Reproductive outcome

Average litter-size (LS), Average litter-weights (LW) and Average crown-rump-length (CRL)

Effect of *Boerhavia diffusa* on Haematological changes

There was no significant difference in the haematological levels when treatment group was compared to control (Table 6).

GROUP	RBC Count (million/mm³)	WBC Count (per. cu.mm)	HAEMOGLOBIN (gm %)
Control	5.41 ± 9.12	9.03 ± 0.75	16.77 ± 3.12
Low dose	5. 60 ± 7.72	9.21 ± 0.81	16.83 ± 2.84
High dose	5.79 ± 5.32	9.38 ± 0.66	16.91 ± 2.04

Table 6: Effect of Boerhavia diffusa on Haematological changes

Effect of *Boerhavia diffusa* on Histology of Ovary

Sections of ovary the treatment groups showed normal histology when compared to control. Cortex of the ovaries showed differential maturation of the follicles from the early primary, late primary to secondary and tertiary follicles (Figure 1,2 and 3).

Effect on Control

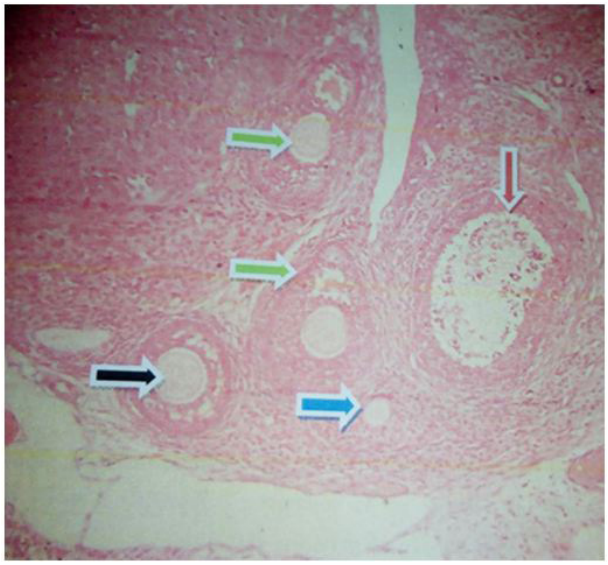

Figure 1: Effect on Control

A photomicrograph of control showing normal ovary. There is differential maturation of the follicle. Primordial follicle (blue arrow), primary follicle (black arrow), secondary follicle (green arrow) and tertiary follicle (red arrow) in the ovary cortex

Effect on Low dose

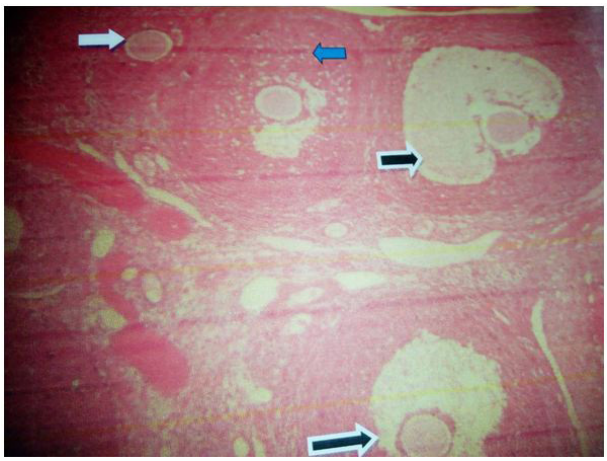

Figure 2: Effect on Low dose

A photomicrograph of low dose showing normal ovary. There is differential maturation of the follicle. Primary follicle (white arrow), secondary follicle (blue arrow) and Graafian follicle (black arrow)

Effect on High dose

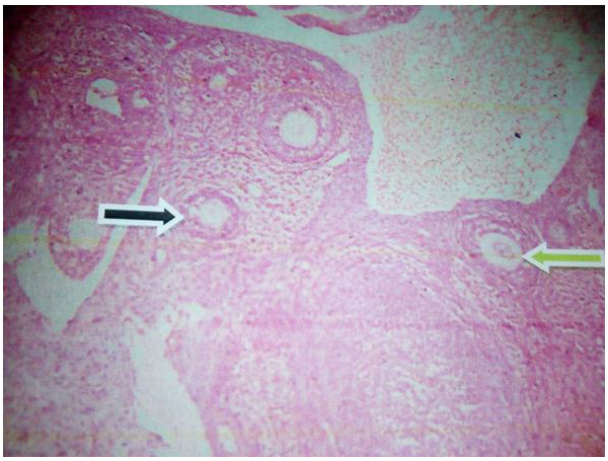

Figure 3: Effect on High dose

A photomicrograph of a high dose showing normal ovary. There is differential maturation of the follicle. Primary follicle (black arrow) and secondary follicle (green arrow) in the ovary cortex

Discussion

The increased incidence of infertility in females due to the frequent use (or abuse) of a number of medicinal plant or extracts have made efforts to study their side effects on the female reproduction. Various substances including herbal mixtures or plant(s) extracts have been reported to affect fertility in females as infertility is a major problem for 15-20% couples and about 30-45% of infertility is related to problems associated to females [11,12]. One of the several factors contributing to the increase in female infertility is the use (or abuse) of medicinal plants based on folkloric uses, as availability and accessibility of the plants or herbs cannot be checked. This study was designed to determine the antifertility effect of aqueous extract of *Boerhavia diffusa* on the female reproductive system using Sprague-Dawley rats as experimental models.

Boerhavia diffusa has been reported to a have safety margin. The LD50 of the aqueous extract of *Boerhavia diffusa* is well above 2000mgkg-1 had been clinically used for trials [13]. In lieu of this, two doses of 50 and 100 mg/kg body weight were used based on administration for this study.

Changes in body weight or an organ is an indication of toxicity of the substance consumed [14,15]. The dose dependent decrease in body weight of the treatment groups may be due to the toxicity of the plant extract. From our study, blood parameters have been to be within normal rage, showing the non-toxic effect of the extract on the body. Also, a dose dependent decrease in the weights of the ovaries was observed which can be due to the toxicity of the extract (Table 6). This is consistent with a study evaluating the

anti-estrogenic activity of *Boerhavia diffusa* roots in which a dose dependent decrease in ovarian weight of the experimental animal was reported [16].

The histological evaluations of the experimental-treated ovaries in comparison to the experimental-control ovaries show no difference as been observed from the respective photomicrographs, which revealed normal sections of ovaries. This could be due to the antioxidant and anti-inflammatory potentials of the extract [10].

From this study, an irregular pattern in the oestrous cycle was observed in the treatment group that received 100 mg/kg of the extract (high dose group) as compared to the group that received 50 mg/kg of the extract that had regular oestrous cycle similar to the control. This irregular pattern that showed only Metestrous and Diestrous phase of the oestrous cycle could be due to the presence of high level of phytoestrogens like saponins. The inhibitory effect of saponin on the oestrous cycles has been reported [17]. Saponins have been reported to reduce fertility in animals upon continuous administration [17]. Also, the oxytocic effects of a plant that has saponins, steroids and alkaloids on the female rats have been reported, as these compounds are found in the leaves of *Boerhavia diffusa* [18].

Phytochemical screenings have revealed many bioactive compounds as well as toxic agents of plant extracts that can affect the regularity of the oestrous cycle, conception and reproduction. Alkaloids and some other phytochemicals have been shown to reduce plasma concentrations of luteinizing hormone (LH), estradiol and follicle stimulating hormone [19]. It is possible the extract might have exerted its effect on the anterior pituitary since the secretion of FSH is regulated by the gonadotropic releasing hormone secreted by the hypothalamus. The reduction in the serum concentration of estradiol observed in this study may be attributed to a decreased aromatase activity or substrate supplementation during estrogen synthesis [20]. The female reproduction is controlled functionally by estogen level usually when during the estrous phase of the estrous cycle. From this study, decrease in the level of estrogen was observed, this gives an indication that the extract may act on the ovary (the source of estrogen) through altered endocrine functions associated with decrease in estrogen level [21]. The reduction in the levels of the hormone might adversely have affected conception in the females. Similarly, several reports have been demonstrated on LH release surges at the proestrous phase of the oestrous cycle as they are responsible for ovulation [22,23]. In addition it is well established that estradiol is directly responsible for the growth and development of reproductive organs (31), so that a significant decrease in diameter and weight of the ovaries in the experimental group was noticed [24]. Also, any substance capable of inhibiting this release could provoke disruption of ovulation by decreasing the number of mature follicles and ultimaltely reducing the chances of conception [25]. This could actually explain for the zero number of litters recorded in all phases of trimester in the group that received *Boerhavia diffusa* at 100 mg/kg dose.

Conclusion

Results obtained from this study showed that oral administration of aqueous extract of the leaves of *Boerhavia diffusa* to female Sprague-Dawley rats altered the oestrous cycle based on dose administered and this suggests antifertility effect of the extract.

Recommendation

Further research is suggested to be carried out so as to determine the mechanism of action of *Boerhavia diffusa* with respect to antifertility activities in female reproduction.

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