

Ameliorative potentials of *Boerhavia diffusa* Ethanolic leaf extract on cypermethrin-induced reproductive dysfunction in Wistar rats

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Abstract

With rising global reports on reproductive and hematological health indicators, numerous animal based researches have more often focused on herbal-based medical remedies. In this study, the ameliorative potentials of ethanolic leaf extract of *Boerhavia diffusa* was investigated on reproductive and selected hematological variables in wistar rats [packed cell volume (PCV), total white blood cell count (TWBCC), total red blood cell (TRBCC) count and total Platelet count (TPC)]. Twenty five (25) female wistar rats (between 150-200 g) were procured and acclimatized for two weeks in the animal house of Ambrose Alli University, Ekpoma, Edo State. The rats were then grouped into five (5) groups of five rats each (n = 5). While group I animals received standard rat chow and water at liberty (control), reproductive dysfunction was orally induced in group II rats, using 3.83 mg/kg bw of cypermethrin for 14 days. Groups III, IV and V received (orally) 200 mg/kg bw, 300 mg/kg bw and 400 mg/kg bw of *Boerhavia diffusa* extract (ethanolic) for five weeks respectively; haven also induced reproductive dysfunction. At the end of the period of administration, animals were euthanized under chloroform anaesthesia and blood sample obtained for hematological analysis, while also harvesting and observing the ovaries for histo-architectural changes. Results from one way analysis of variance (ANOVA) showed a statistically significant (p < 0.05) dose-dependent increase in the PCV TPC and TRBCC levels of all groups compared to control, while TWBCC increased insignificantly across groups. More studies to corroborative the observations from this works highly recommended.

Keywords: Hematology; Reproduction and *Boerhavia Diffusa*

1. Introduction

Boerhavia diffusa (Family- Nyctaginaceae), is a very controversial plant that has drawn numerous attention across the globe as a result of its immune-modulatory effects [1], anti-lymph and anti-proliferative activity, anti-viral effects [2], as well as its anti-biotic actions. The plant has also been reported to be useful in combating anaemia [3, 4], as a purifier of blood [5], speedy healing of wounds [6], in anti-inflammation [7], etc. Its implications in the quick stoppage of bleeding has also been reported [8], not neglecting its diuretic, cardiotoxic [9], antioxidant [10], antihemorrhagic [11], antispasmodic [12], antimicrobial [13], cytotoxic and anticancer roles. *Boerhavia diffusa* is widely dispersed, occurring

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throughout India, the Pacific, and southern United States. Its flowers are small, around 5 mm in diameter, with round pollens of roughly 65 microns in diameter. This wide range is explained by its small fruit, which are very sticky and grow a few inches off the ground, ideally placed to latch on to small migratory birds as they walk by [13]. Although a true and accurate accounting of the native range of *Boerhavia diffusa* has not been determined. However, it is very widespread, and has become naturalized in many places.

The root of *Boerhavia diffusa* acts as an anticonvulsant, analgesic [4], laxative medication that when rubbed in honey can be locally applied for cataract, chronic conjunctivitis and blepharitis. Useful for curing heart diseases, anemia and edema (or oedema), Punarnava is an effective remedy that reduces swelling and foul smell in skin disorders. Apart from the root, Punarnava's leaves are also consumed as a vegetarian dish to reduce oedema. As an Ayurvedic medicine, this herb is said to cure disorders like intestinal colic, kidney disorders, cough, hemorrhoids, skin diseases, alcoholism, insomnia, eye diseases, asthma and jaundice, *Boerhavia diffusa* (Sri Lankan Name Pita Sudu sarana best green medicine for Diabetes [5].

Recently, a growing number of studies have revealed that reproductive disorders in animals may have a negative impact on pregnancy, from implantation until term. In addition, humans with reproductive disorders often require assisted reproductive technologies to affect pregnancy outcomes. Thus, it is a difficult task to distinguish the contribution of specific reproductive disorders relative to the interventions required for pregnancy success [5, 6]. Also, Hormones and inflammatory mechanisms are implicated in the major events of reproductive functions, while increasing evidence shows that hormonal aberrations and a hyper-inflammatory states may lead to derangements in cross talks that predisposes one to reproductive dysfunction.

Similarly, hematology, which studies the numbers and morphology of the cellular elements of the blood – the red cells (erythrocytes), white cells (leucocytes), and the platelets (thrombocytes) and the use of these results in the diagnosis and monitoring of disease [5, 6] is useful in the diagnosis of many diseases as well as investigation of the extent of damage to blood [7]. Hematological parameters are good indicators of the physiological status of animals. Hematological parameters are those parameters that are related to the blood and blood forming organs.

1.1. Aim of Study

This study investigated the histological and hematological changes in *Boerhavia diffusa* extract treated, reproductive dysfunctional wistar rats. Specifically, the study;

Determined the effects of ethanolic extract of *Boerhavia diffusa* leaf on body weights.

Examined the changes in selected hematological variables [packed cell volume (PCV), total white blood cell count (TWBCC), total red blood cell (TRBCC) count and total Platelet count (TPC)], following administration of ethanolic leaf extract of *Boerhavia diffusa* to wistar rats.

Investigated the histo-architectural changes in the ovary of *Boerhavia diffusa* treated rats.

2. Material and methods

2.1. Study Area

This study was conducted within the animal unit of the faculty of basic medical sciences, college of medicine, Ambrose Alli University, Ekpoma, Edo State.

2.2. Study Design

Study was designed to be experimental in nature, procuring a total of Twenty five (25) wistar rats (between 150-200 g), and acclimatizing them for two weeks within the animal house of Ambrose Alli University, Ekpoma, Edo State. The rats were then grouped into five (5) groups of five rats each (n = 5). While group I animals received standard rat chow and water at liberty (normal control), reproductive dysfunction was orally induced in group II rats, using 3.83 mg/kg bw of cypermethrin for 14 days (negative control). Groups III, IV and V received (orally) 200 mg/kg bw, 300 mg/kg bw and 400 mg/kg bw of *Boerhavia diffusa* extract (ethanolic) for five weeks respectively; haven also induced reproductive dysfunction with 3.83 mg/kg bw of cypermethrin.

2.3. Extract Preparation

After obtaining *Boerhavia diffusa* leaves from local farms within the study area, they were then sun-dried for days and weighed continuously with electronic balance. Dry leaves were then homogenized in pestle and mortar and then filtered with Wattmann filter paper. Next, graded doses of *Boerhavia diffusa* [high, medium and low] were estimated from previously established lethal dose. About 2g, 3g and 4g were dissolved in 200ml, 300ml and 400ml of ethanol to make their respective stock solutions. The body weights of the animals were then taken and the dose of test drugs in millilitre to be administered was calculated using;

$$\% \text{ yield} = \frac{\text{weight of extract X 100}}{\text{weight of plant sample used}}$$

2.4. Body Weight Determination

Using the electronic weighing scale (Cardinal Scale Manufacturing Co., Webb City, MO), body weights of animals were obtained and recorded weekly (in grams (g)) throughout the period of administration (five weeks).

2.5. Inducing Reproductive Dysfunction

Reproductive Dysfunction was induced by the oral administration of cypermethrin (3.83 mg/kg bw) for 14 days according to the procedure of Hu *et al.*, (2013).

2.6. Samples Collection

Following period of administration, animals were euthanized by cervical decapitation, then, collection of blood sample from the superior vena cava followed, with the aid of a 5ml syringe. Collected samples were then centrifuged at 3000rpm for 15minutes, while refrigerating collected sera for hematological analysis [packed cell volume (PCV), total white blood cell count (TWBCC), total red blood cell (TRBCC) count and total Platelet count (TPC)] at -20°C. Also, animals were dissected to remove the ovaries; which were then cleared of adherent tissues for histological studies.

2.7. Ethical Clearance

Ethical approval was sought from the Research and Ethics Committee of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

2.8. Statistical Analysis

Obtained results were represented as mean standard deviation. Statistical analysis was done using the one-way analysis of variance (ANOVA) and post-hoc (tukey) test. Statistics was carried out with a graph pad prism software (version 8.0). A p-level less than 0.05 was considered as statistically significant.

3. Results

Table 1 Body Weight Changes in *Boerhavia diffusa* Extract Administration

| Treatments Groups | Body Weights | | | | ANOVA (p-value) | Remark |
|-------------------|--------------|----------------------|----------------------|-------------|-----------------|--------|
| | IBW | 14 th day | 28 th Day | FBW | | |
| Control (I) | 154.17±4.54 | 152.52±4.07 | 155.31±5.05 | 157.20±7.50 | 0.2001 | Insig. |
| II | 142.02±6.76 | 140.0±6.29 | 144.80±6.17 | 145.00±6.82 | 0.0001 | Sig. |
| III | 150.61±7.56 | 152.50±7.10 | 151.61±7.58 | 153.60±7.51 | 0.0100 | Sig. |
| IV | 167.01±8.92 | 162.30±8.51 | 161.60±7.76 | 160.32±7.13 | 0.0005 | Sig. |
| V | 170.01±9.12 | 169.11±8.11 | 172.42±9.41 | 175.22±9.97 | 0.0001 | Sig. |

All values are represented as mean ± SEM. From the table above, there was a reduction in body weight of negative control group (II) compared to group I. whereas, administration of varying doses of ethanolic *Boerhavia diffusa* leaf extract caused a significant ($p < 0.05$) weekly recovery in body weights of all experimental animals compared with control.

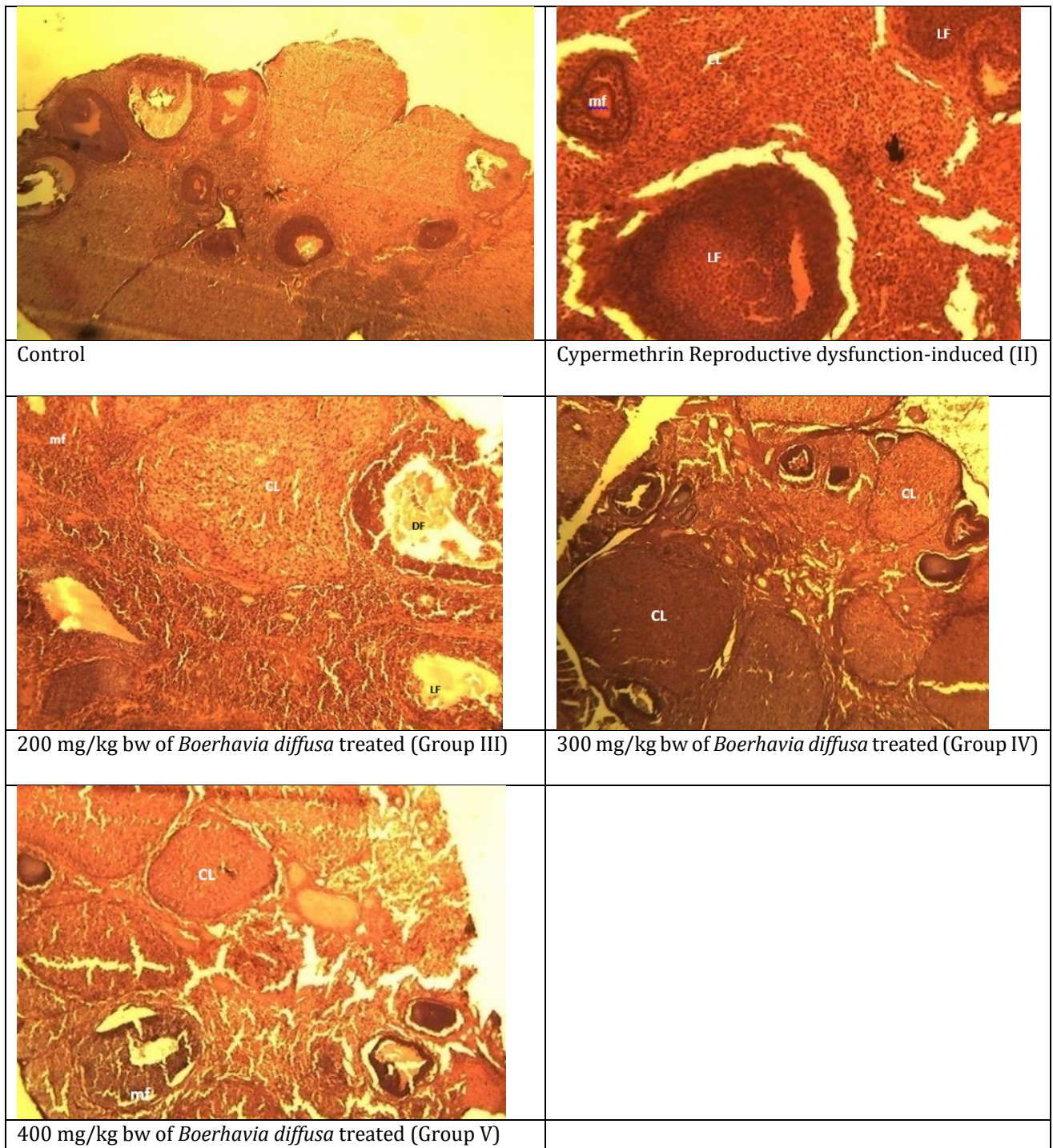


Figure 1 Histo-Micrograph of the Ovary for *Boerhavia diffusa* Extract Treated Rats

Key: CL: corpus luteum, df: degenerative follicles, MF: medium size (secondary) follicles, LF: Large (matured) follicular cells. Hematoxylin and Eosin x 400

Table 2 Comparative Changes in Hematological Parameters in *Boerhavia diffusa* Extract Administration

| Treatment Groups | Hematological Parameters | | | |
|------------------|-----------------------------|--|--|--|
| | PCV (%) | TRBCC (*10 ³ /mm ³) | TWBCC (*10 ³ /mm ³) | TPC (*10 ³ /mm ³) |
| Control | 37.04 ± 1.23 | 3.21±0.07 | 3.62±0.32 | 170.10±21.26 |
| II | 32.42 ± 1.10 * ⁻ | 2.64±0.01 * ⁻ | 3.53±0.12 # ⁻ | 164.24±18.64 * ⁻ |
| III | 41.10 ± 1.61 * ⁺ | 3.46±0.48 * ⁺ | 3.64±0.58 # ⁺ | 168.28±21.04 * ⁻ |
| IV | 43.09 ± 1.82 * ⁺ | 3.72±0.66 * ⁺ | 3.70±0.71 # ⁺ | 174.13±26.27 * ⁺ |
| V | 46.62± 1.94 * ⁺ | 4.37±0.95* ⁺ | 3.81±0.86 # ⁺ | 175.38±29.08 * ⁺ |

PCV: packed cell volume, TWBCC: total white blood cell count, TRBCC: total red blood cell count, and TPC: total Platelet count. All values are represented as mean ± SEM. Also, *⁺ = Significant increase (p < 0.05), *⁻ = Significant decrease, #⁺ = Insignificant increase (p > 0.05), and #⁻ = Insignificant decrease,

From above table, there was a noticeable significant reduction in all assayed hematological parameters for negative control group (II) but for TWBCC which was insignificant compared to control (group I) however, administration of varying doses of ethanolic *Boerhavia diffusa* leaf extract caused a significant improvement in all variables as compared to control; even though these increases were insignificant across groups for TWBCC as compared with control.

Table 3 Histo-Pathological Observations in Ovary of *Boerhavia diffusa* Extract Treated Rats

| Groups/Effects | Control | II | III | IV | V |
|-------------------------------|---------|----|-----|----|----|
| Number of primary follicles | + | ++ | + | ++ | ++ |
| Number of secondary follicles | ++ | ++ | ++ | + | ++ |
| Number of matured follicles | + | + | ++ | + | + |
| Cystic follicles | - | + | - | - | - |

Key: - not observed, + low, ++ moderate, +++ high.

4. Discussion

One of the most frequently used group of insecticides are the synthetic pyrethroids. Recently, their continuous use have reportedly caused increase in the levels of chlorines, phosphates and carbamates from organic sources, possibly rapidly degrading, and affecting mammalian toxicity by its action mechanism. Cypermethrin, being a common pyrethroid is useful agriculture and forestry, also in public and animal health fields. Although Cypermethrin has been considered to be nontoxic to mammals, reports have however shown its adverse effects on the neuro-endocrine, renal, hepatic and reproductive systems [16, 17, 18, and 19] of laboratory animals; with reproductive toxicity reports posing huge concerns due to susceptibility to chronic exposures. Hence, current study was embarked upon to investigate the reproductive toxicity associated with cypermethrin; and the ameliorative potentials of varying *Boerhavia diffusa* extract doses in female Wistar rats. Twenty five (25) wistar rats (between 150-200 g) were first procured and acclimatized (two weeks) for this study; following which they were grouped into 5 of n=5 per group. Group I animals were fed standard chow and water at free will (normal control), while inducing reproductive dysfunction in group II rats by oral administration of 3.83 mg/kg bw of cypermethrin for 14 days (negative control). Groups III, IV and V then received (orally) 200 mg/kg bw, 300 mg/kg bw and 400 mg/kg bw of *Boerhavia diffusa* extract (ethanolic) for five weeks respectively; haven also induced reproductive dysfunction with 3.83 mg/kg bw of cypermethrin.

In this study, Body weight being a valuable indicator of reproductive health was assessed (weekly) across groups (table I). from our result, there was a reduction in body weight of negative control group (II) compared to group I. whereas, administration of varying doses of ethanolic *Boerhavia diffusa* leaf extract caused a significant (p < 0.05) weekly increase in body weights of all experimental animals compared with control. This increase may portend huge recovery across groups following test administration; and may alter serum sex hormone levels as well (though un-assayed for) to implicate test extract as a possible potent repro-degenerative ameliorator. This finding is in concordance previous other

reports, where cypermethrin treatment was associated with body weight recovery and serum Testosterone, Luteinizing and Follicle Stimulating Hormone levels [20, 21, 22, and 23].

Also From table II report of current study, the effect(s) of feeding female wistar rats with varying doses of ethanolic *Boerhavia diffusa* extract was assayed for selected hematological parameters. From the table, there was a noticeable significant reduction in all assayed hematological parameters for negative control group (II) but for TWBCC which was insignificant compared to control (group I). However, administration of varying doses of ethanolic *Boerhavia diffusa* leaf extract caused a significant improvement in all variables as compared to control; even though these increases were insignificant across groups for TWBCC as compared with control. Values obtained from the TPC, TWBCC and TRBCC show that *B. diffusa ethanolic* extract had the most promising effect with increasing doses/regimen; with remarkable improvements in animals' physical and behavioural properties as observed.

Again, a clear look at table III reveals a summary of the Histo-Pathological differences observed in the ovaries of *Boerhavia diffusa* extract treatments to rats at various doses. Here, only moderate number of secondary follicles were noticed in all treatments except group IV; were as, cystic follicles were noticed in Cypermethrin fed, reproductive dysfunctional rats of group II (negative control) as compared to others.

Histologically, this observation showed no noticeable change in the ovaries of treatment groups as against the control and those induced with reproductive dysfunction. However, degenerative follicles of varying sizes were noticed with Hematoxylin and Eosin at a X 400 microscope resolution.

5. Conclusion

In this study, the ameliorative potentials of ethanolic leaf extract of *Boerhavia diffusa* was investigated on reproductive and selected hematological variables in wistar rats. From our results, a statistically significant reduction was observed in body weight of 3.83 mg/kg bw of cypermethrin fed rats (reproductive dysfunctional group) as compared to group I. whereas, administration of varying doses of ethanolic *Boerhavia diffusa* leaf extract caused a significant ($p < 0.05$) increase in body weights of all experimental animals compared with control group. Also, moderate number of secondary follicles were noticed in all treatments except group IV; were as, cystic follicles were noticed in Cypermethrin fed, reproductive dysfunctional rats. Albeit, histological observations showed no noticeable change in the ovaries of treatment groups as against the control.

Compliance with ethical standards

Disclosure of conflict of interest

All authors declare that they have no conflict of interest.

Statement of ethical approval

Ethical approval was sought from the Research and Ethics Committee of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

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