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# Aqueous Extract of *Newbouldia laevis* Abrogates Cadmium-Induced Ovarian Dysfunction in Adult Wistar Rats

## Running Title: *Newbouldia laevis* Ameliorates Ovarian Disruption

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**Abstract:** Background: The extract of *Newbouldia laevis* has been demonstrated to show antioxidant, antimicrobial, sedative, anticonvulsant, analgesic, anti-inflammatory, antinociceptive, hepatoprotective, anticancer, antiulcer, antihypertensive and antidiarrhea properties. However, its reproprotective effect is not well known. The present study was designed to investigate the ameliorative effect of *Newbouldia laevis* in cadmium- induced ovarian dysfunction. Method: Adult female Wistar rats were randomly allotted into groups; Vehicle (received distilled water), cadmium-treated (received 5 mg/kg *b.w.*), *N. laevis*-treated (received 200 mg/kg *b.w.*) and cadmium + *N. laevis*-treated groups. Cadmium sulphate was administered for 3 days (i.p.) followed by oral administration of *N. laevis* for 28 days. The body weight change was monitored using animal weighing balance (Olympia SCL66110 model, Kent Scientific Corporation, Torrington, CT06790, USA), biochemical assay and histology of ovaries were performed as previously described. Results: The results showed weight loss, severe disruption of ovarian follicles and significant reduction of gonadotropic hormones (FSH and LH) in cadmium-treated group compared with vehicle-treated group. These alterations were not associated with inflammatory response. However, concomitant administration of aqueous extract of *N. laevis* and cadmium sulphate significantly ameliorated ovarian disruption. Conclusion: The study demonstrates that administration of aqueous extract of *N. laevis* during treatment with cadmium sulphate preserves ovarian function, suggesting that possibly daily intake of aqueous extract of *N. laevis* prevents the onset of ovarian disorders.

**Key words:** Cadmium, hypertrophy, *Newbouldia laevis*, ovarian dysfunction, reproprotective.

## 1. Introduction

Infertility is a worldwide problem, development in the investigation, treatment and the dissemination of new knowledge has meant a new hope for infertile couples [1]. Infertility is one of the major gynaecological complications that affect women, and the majority of the focus has always been on expensive in-vitro fertilization rather than treating the cause of the infertility [2]. Studies have shown that 25-30% of infertile women suffer from ovarian dysfunction [3], which does not occur only with disorders of the ovaries, but anything that affects the communication between the brain and ovary through hypothalamic-pituitary-gonadal axis [4, 5].

Heavy metals such as cadmium are pollutants generated mostly through human activities, and they have high toxicological impact on humans and animals since they are likely ingested through food like seafood, meat offal, cereals, vegetables and fruits [6, 7]. Cigarette smoke is by far the greatest source of

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cadmium exposure while diet remains a primary source of exposure in non-smokers [8]. Cadmium is a transitional metal that exists in different oxidational or transitional states. Acute exposure to cadmium in vivo causes dysuria, polyuria, chest pain, fatigue, headache, and hepatooxidative, where as chronic exposure through contaminated food or air results to organ dysfunction because of cell death through disruption of cellular mitochondrial function [9, 10]. Cadmium toxicity is associated with several clinical complications, renal dysfunction, bone diseases, hepatic dysfunction, testicular and ovarian dysfunction [11, 12].

*Newbouldia laevis* is a medium sized angiosperm in the Bignoniaceae family. Phytochemical analysis of the root, root bark, stem and leaf of *N. laevis* revealed the presence of alkaloids, phenylpropaniod, glycosides, flavonoids, tanins, saponins, phenols, essential oils, terpenoids, triterpenoids, quinoids, ceramides among others [13]. The roots and leaves are used in the treatment of dysentery, elephantiasis, migraines and seizures [14]. An extract of the leaves of *N. laevis* and mostly used as mouthwash has been shown to be bactericidal in dental caries [15].

Pharmacological studies of extracts of different parts of *N. laevis* have revealed the antioxidant [13], free radical scavenging [15], antimicrobial [16], sedative, anticonvulsant [17, 18], analgesic, antinociceptive [19], hepatoprotective [13], anticancer [20], uterine contraction [21], wound healing and antiulcer [22], antisickling [23], hypoglycemic, antihypertensive and entomocide activities [24, 25]. However, its reproprotective effect is not well known. The current study attempted to investigate the ameliorative effect of *N. laevis* on cadmium-induced ovarian dysfunction in adult female rats.

## 2. Materials and Methods

### *2.1 Chemicals*

The entire chemicals used in the study were of AR grade, which were obtained from Sigma Chemical, St. Louis, MO, USA.

### *2.2 Preparation of the Extract*

Samples of *N. laevis* were locally obtained. The leaf of the plant was botanically authenticated by Mr Bolu in the Department of Plant Biology, University of Ilorin, Ilorin. Authentication number was issued and the plant was deposited at the herbarium. The plant was air-dried and pounded into powder using pestle and mortar and kept in an air-tight container. 600 g of the sample was percolated in distilled water for 48 hours and stirred intermittently with magnetic stirrer. It was filtered and the filtrate was evaporated in steam bath until substantial water has been removed. It was later dried in the oven at 37 ℃ to make the extract concentrated.

### *2.3 Animals, Grouping and Protocol*

Twenty adult female Wistar rats weighing 110-250 g were obtained from the animal house, College of Medicine and Health Sciences, University of Ilorin, Ilorin, Nigeria. The rats were housed in wire mesh cages and maintained in a well ventilated room at 25 ± 2 ℃, on a 12-h light/12-h dark cycle. Rats had unrestricted access to standard rat chow and tap water. After acclimatized for two weeks, the rats were randomly allotted into groups ($n$ = 5 each); Vehicle (received distilled water), cadmium-treated (received 5 mg/kg *b.w.*), *N. laevis*-treated (received 200 mg/kg *b.w.*) and cadmium + *N. laevis*-treated groups. Cadmium sulphate was administered for 3 days (i.p.) followed by oral administration of *N. laevis* for 28 days. The investigation was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Review Board of University of Ilorin, Ilorin, and every effort was made to minimize both the number of animals used and their suffering. Initial and final body weights were monitored using animal weighing balance (Olympia SCL66110 model, Kent Scientific Corporation, Torrington, CT06790, USA) and the body weight change was estimated.

*2.4 Sample Preparation and Biochemical Analysis*

At the end of treatment, the rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p). Blood was collected from the apex of the heart into EDTA and heparinized bottle and centrifuged at 3,000 rpm for 15 minutes using a bench centrifuge and the plasma was stored frozen until it was needed for biochemical assay. Biochemical analysis of plasma gonadotropic hormones (Follicle stimulating hormone; FSH, Luteinizing hormone; LH) were performed using ELISA kits obtained from Randox Laboratory Ltd. (Co. Antrim, UK). The blood collected with EDTA bottles was used for the analysis of hematological parameters (RBC; red blood cell, Hb; hemoglobin, HCT; hematocrit, PLT; platelet, WBC; white blood cell, PMN; polymorphonuclear, LYM; lymphocyte). Ratios of PLT/LYM and PMN/LYM were calculated as pro-inflammatory markers.

*2.5 Histology*

The ovaries were excised, blotted and weighed. After weighing, ovarian tissues were fixed in 10% buffered formol saline for histological examination using hematoxylin and eosin (H&E) staining techniques and examined microscopically.

*2.6 Statistical Analysis*

All data were expressed as means ± SEM. Statistical group analysis was performed with SPSS, version 22 of statistical software. One-way analysis of variance (ANOVA) was used to compare the mean values of variables among the groups. Bonferroni's test was used to identify the significance of pair wise comparison of mean values among the groups. Statistically significant differences were accepted at $p < 0.05$.

## 3. Results

*3.1 Effects of Newbouldia laevis on Body Weight in Cadmium-Treated Adult Female Rats*

Table 1 depicts the effect of administration *N. laevis* and cadmium on body weight. The results showed significant loss in body weight during treatment with cadmium sulphate alone when compared with vehicle-treated group. However, concomitant treatment with aqueous extract of *N. laevis* during treatment with cadmium sulphate significantly improved the body weight ($p < 0.05$).

*3.2 Effect of Newbouldia laevis on the Histology of Ovary in Cadmium-Treated Adult Female Rats*

Histopathological changes in the ovaries have been shown to influence the function of this organ. H&E stained section of ovaries of vehicle-treated rat, shows normal consistent proliferation of the follicles and normal arrangement of the ovarian epithelium (Fig. 1A), cadmium-treated rat, shows severe pathological lesions evident as hypertrophy of granulosa cells, haemolysis and severe deterioration of ovarian follicles (Fig. 1B), *N. laevis*-treated rat, shows moderate hypertrophy of ovarian follicles (Fig. 1C) and cadmium + *N. laevis*-treated rat, shows mild cellular hypertrophy (Fig. 1D).

**Table 1 Effects of *Newbouldia laevis* on body weight in cadmium-treated Wistar rats.**

| | Vehicle-treated | Cadmium-treated | *N. laevis*-treated | Cadmium + *N. laevis*-treated |
|---|---|---|---|---|
| Initial weight (g) | 120.2 ± 6.2 | 127.5 ± 9.5 | 132.7 ± 5.4 | 130.6 ± 8.3 |
| Final weight (g) | 160.0 ± 11.7 | 121.0 ± 10.5 | 154.1 ± 0.6 | 153.3 ± 11.8 |
| Weight gain (g) | 39.8 ±7.8 | (6.5 ± 0.5)* | 21.4 ± 0.5 | 22.5 ± 6.4# |

Data are expressed as mean ± SEM. $n = 5$. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc test.* (*$p < 0.05$ vs. vehicle; # $p < 0.05$ vs. cadmium).

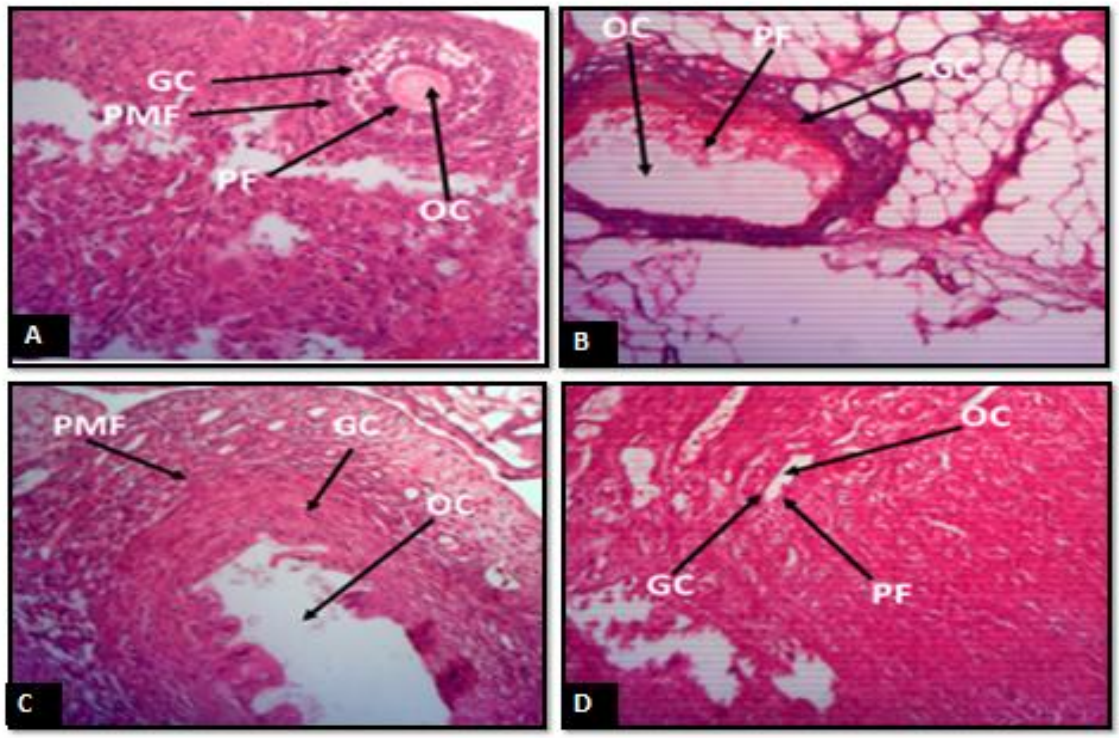


**Fig. 1 Photomicrograph of a section of ovary in cadmium-treated rat. Vehicle-treated rat, shows normal consistent proliferation of the follicles and normal arrangement of the ovarian epithelium (a); Cadmium-treated rat, shows severe pathological lesions evident as hypertrophy of granulosa cells, haemolysis and severe deterioration of ovarian follicles (b); *N. laevis*-treated rat, shows normal moderate hypertrophy of ovarian follicles (c); Cadmium + *N. laevis*-treated rat, shows mild cellular hypertrophy (d).**
(H & E paraffin stain; ×200, transverse section). PMF (primordial follicle); PF (primary follicle); GC (Granulosa cell); OC (OOcyte).

*3.3 Effects of Newbouldia laevis on Gonadotropic Hormones (FSH and LH) in Cadmium-Treated Adult Female Rats*

Plasma levels of gonadotropic hormones (FSH and LH) significantly decreased in cadmium-treated group when compared with vehicle-treated group. However, treatment with *N. laevis* significantly restored FSH and LH levels (Fig. 2).

*3.4 Effects of Newbouldia laevis on Hematological Parameters in Cadmium-Treated Adult Female Rats*

Treatment with cadmium sulphate significantly reduced red blood cells, hematocrit, platelet and white blood cells compared with vehicle-treated group. Whereas cadmium + *N. laevis*-treated group showed a significant increase in red blood cells, hematocrit, platelet and white blood cells compared with cadmium-treated group (Table 2). Hemoglobin, lymphocyte and polymorphonuclear remained unchanged in all the treated groups compared with vehicle-treated group.

*3.5 Effects of Newbouldia laevis on Pro-inflammatory Biomarkers (PMN/LYM and PLT/LYM) in Cadmium-Treated Adult Female Rats*

PMN/LYM and PLT/LYM are pro-inflammatory markers. Treatment with cadmium and cadmium + *N. laevis* did not significantly alter PMN/LYM and PLT/LYM when compared with control group (Fig. 3).

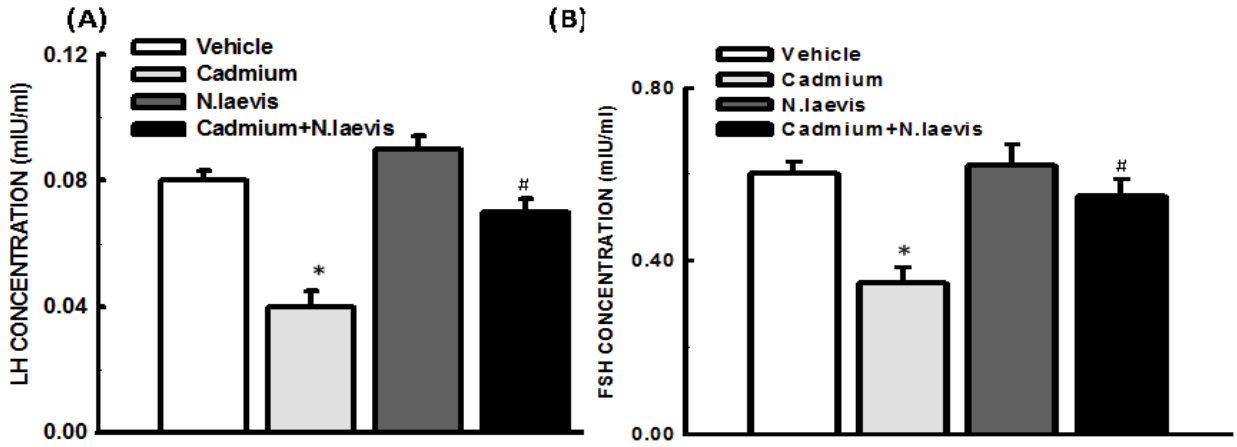


**Fig. 2 Effect of *N. laevis* on circulating luteinizing hormone; LH (a) and follicle stimulating hormone; FSH (b) in cadmium-treated Wistar rats. Data are expressed as mean ± SEM. *n* = 5. Data were analysed by one-way ANOVA followed by Bonferroni post hoc test.**

*$p < 0.05$ vs. vehicle; # $p < 0.05$ vs. cadmium.

**Table 2 Effects of *Newbouldia laevis* on hematological parameters in cadmium-treated Wistar rats.**

| | Vehicle-treated | Cadmium-treated | *N. laevis*-treated | Cadmium + *N. laevis*-treated |
|---|---|---|---|---|
| RBC ($10^{12}$ cells/L) | 5.2 ±0.1 | 3.2 ±0.5* | 5.7 ±0.4 | 5.0 ±0.3# |
| Hb (g/L) | 12.8 ±0.9 | 11.4 ±0.2 | 12.7 ±0.9 | 11.7±0.5 |
| HCT (%) | 40.8 ±0.7 | 34.2 ±0.5* | 39.3 ±0.6 | 38.3 ±0.8# |
| PLT ($10^9$cells/L) | 365.8 ±18.3 | 332.3 ±8.4* | 383.5 ±29.4 | 371.0 ±20.3# |
| WBC ($10^9$ cells/L) | 9.9 ±2.4 | 4.6 ±0.4* | 8.4 ±1.8 | 7.6 ±0.7# |
| PMN ($10^9$ cells/L) | 3.1 ±0.2 | 3.4 ±1.2 | 3.2 ±1.1 | 3.3 ±0.4 |
| LYM ($10^9$ cells/L) | 7.5 ±6.0 | 7.0 ±3.3 | 7.2 ±3.9 | 7.3 ±4.1 |

Data are expressed as mean ± SEM. *n* = 5. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc test.* (*$p < 0.05$ vs. vehicle; # $p < 0.05$ vs. cadmium).

RBC: red blood cell, Hb: hemoglobin, HCT: hematocrit, PLT: platelet, WBC: white blood cell, PMN: polymorphonuclear, LYM: lymphocyte.

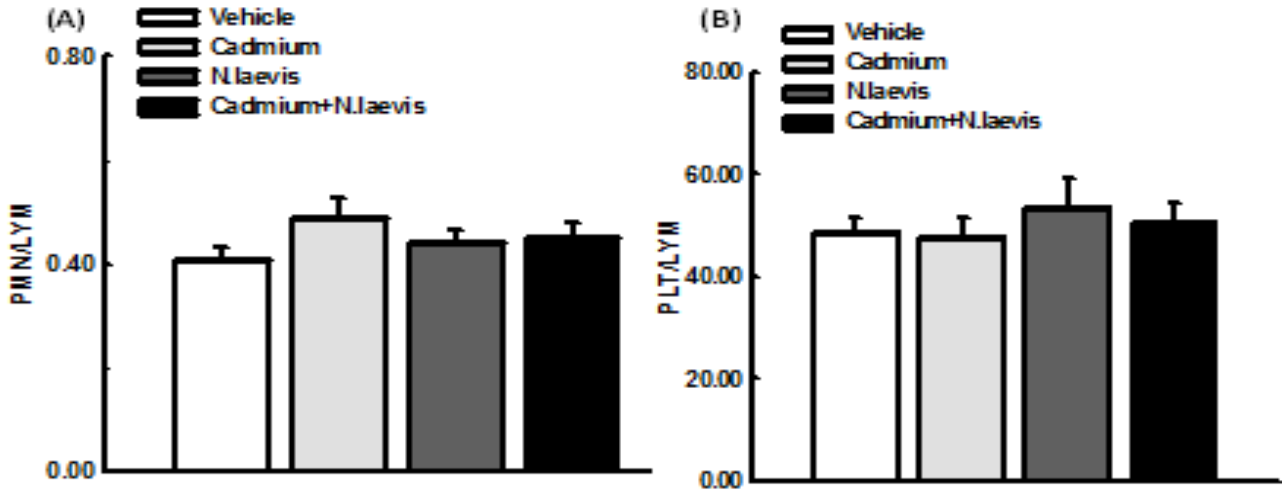


**Fig. 3 Effect of *N. laevis* on pro-inflammatory biomarkers; PMN/LYM (a) and PLT/LYM (b) in cadmium-treated Wistar rats. Data are expressed as mean ± SEM. *n* = 5. Data were analysed by one-way ANOVA followed by Bonferroni post hoc test.**

*$p < 0.05$ vs. vehicle; # $p < 0.05$ vs. cadmium). PMN: polymorphonuclear; PLT: platelet; LYM: lymphocyte.

## 4. Discussion

The primary physiological function of the female reproductive system is to produce ovum necessary for progeny. Ovarian steroid hormones play a vital role in the production of ovum and other functions associated with reproductive behavior. The hormones secreted by the hypothalamus and pituitary also regulate ovarian functions. Cadmium has been documented to target ovary and suppress the synthesis and secretion of hormones and it is evident that cadmium disrupts reproductive endocrine functions by directly and/or indirectly affect the expression of StAR protein and P450scc during progesterone synthesis and thus interfere with progesterone synthesis [26]. The present study demonstrates significant loss in body weight, reduction of erythrocytes, hematocrit, platelet, white blood cells, severe pathological lesions evident as hypertrophy of granulosa cells, haemolysis, severe deterioration of ovarian follicles, and decrease in FSH or LH in cadmium-treated group compared with vehicle-treated group. However, administration of *N. laevis* to the group treated with cadmium significantly increased the body weight, erythrocytes, hematocrit, platelet, white blood cells and led to restoration of ovarian tissues/mild hypertrophy. These were associated with significant increase in FSH or LH but not inflammation when compared with cadmium-treated group.

Our present result that administration of cadmium significantly reduced the body weight was consistent with earlier studies [5, 27]. This has been shown to be due to decrease availability and production of steroid hormone during cadmium administration [26]. However, treatment with aqueous extract of *N. laevis* significantly improved the body weight which implies that aqueous extract of *N. laevis* has the capacity to regulate body weight. Histopathological changes in the cytoarchitecture of ovarian tissue have been used as indicator of ovarian dysfunction. The present results revealed that treatment with cadmium causes severe pathological lesions evident as hypertrophy of granulosa cells, haemolysis and severe deterioration of ovarian follicles (Fig. 1B). This is also in line with previous observation that cadmium treatment induced disruption of ovarian histoarchitecture [28]. Gurel et al. [29] also reported the ovarian follicular cell damage in cadmium-treated female rats. Thus, the present study corroborates the ovo-toxic nature of cadmium. In addition, treatment with aqueous extract of *N. laevis* significantly restored the ovarian structure with mild hypertrophy when compared with cadmium/vehicle-treated rats respectively (Fig. 1D). This implies that aqueous extract of *N. laevis* significantly depletes cadmium-induced ovarian dysfunction. This is the first study to reveal the reproprotective effect of *N. laevis* in cadmium-induced ovarian disruptions.

Furthermore, our current results revealed that the ovarian disruption in cadmium-treated animals was associated with significant decrease in circulating levels of FSH and LH. These observations were also in consonance with previous studies [5]. The decrease in FSH and LH levels may be due to impaired hypothalamic-pituitary-gonadotropin secretions reported in an earlier study [30]. Human reproductive toxicity of cadmium has been suggested by several epidemiologic studies [31-33]. The specific effect of exposure to cadmium on the female reproductive system has been previously associated with a reduction in LH binding and FSH as well as altered steroidogenesis *in vitro* in isolated granulose cells of rats [30]. Nevertheless, in the present study treatment with aqueous extract of *N. laevis* significantly increased circulating levels of FSH and LH in cadmium + *N. laevis*-treated group compared with cadmium-treated group (Fig. 2). This implies that aqueous extract of *N. laevis* significantly stimulates hypothalamic-pituitary-gonadotropin secretions.

Hematological parameters indicate the sub-lethal effects of pollutants [34]. Anemia has been observed in rats, mice, rabbits, and monkeys exposed to cadmium.

The present data on hematological indices indicate that the animals exposed to cadmium were in anemic condition due to significant reduction in erythrocytes and hematocrit. It is reported that oral cadmium treatment reduces gastrointestinal uptake of iron, which can result in anemia [35]. It was well known that anemia reduces the supply of oxygen to tissues by lowering the oxygen-carrying capacity of the blood. This finding is consistent with previous studies of anemia in animals exposed to cadmium [5]. The present study showed that treatment with cadmium leads to decreased platelet and white blood cells but no significant change in hemoglobin, lymphocyte and polymorphonuclear when compared with vehicle-treated rats. However, administration of aqueous extract of *N. laevis* significantly attenuated the anemic condition as observed in cadmium + *N. laevis*-treated group compared with cadmium-treated group through upregulation of erythrocytes and hematocrit.

PMN/LYM and PLT/LYM ratios are pro-inflammatory markers. Our present observation that treatment with cadmium did not significantly alter PMN/LYM and PLT/LYM ratios when compared with vehicle-treated group implies that cadmium-induced ovarian disruption was not associated with inflammation and perhaps through oxidative stress as earlier reported [5, 36]. Administration of *N. laevis* did not also alter PMN/LYM and PLT/LYM ratios in cadmium + *N. laevis*-treated group compared with cadmium-treated group, which means that the ameliorative effect of aqueous extract of *N. laevis* in cadmium-induced ovarian dysfunction may probably be through its antioxidant properties as previously documented [5, 13].

## 5. Conclusions

The study demonstrates that administration of aqueous extract of *N. laevis* during treatment with cadmium sulphate depletes ovarian disruptions, suggesting that possibly daily intake of aqueous extract of *N. laevis* protects against the onset of ovarian disorders.

## Acknowledgement

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## Conflict of Interest

The authors declare that there are no conflicts of interest.

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