

## Toxicology

# Countering the effects of lead as an environmental toxicant on the microanatomy of female reproductive system of adult wistar rats using aqueous extract of *Ficus vogelii*

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## ABSTRACT

**Background:** Lead without nutritional value is a widely studied occupational and environmental toxicant. Leads' toxic effects on female reproduction are decreased fertility, inability to sustain pregnancy and reduced pregnancy.

**Objective:** This study aimed at examining the effect of oral administration of lead acetate (1.5 mg/kg) on the histology of female albino Wistar rats' ovary and Uterus and the extracts' protective role against toxicity.

**Methods:** The experiment took 28 days involving 25 female Wistar rats divided into 5 groups A, B, C, D and E. A is an untreated group that received normal saline, D lead acetate group that received lead acetate solution, E received aqueous extract, B and C low and high dose of aqueous extract respectively and lead acetate solution.

**Results:** The positive control group showed a significant increase in SOD at  $P \leq 0.01$  compared to the negative control. Group E showed significant decrease ovarian SOD. The organs weights were significantly reduced in group D. The changes seen in the organs include oedema, necrosis, optical empty spaces, denudations and fatty changes. Administrating the extract protected the organs against the lead acetate. These alterations are shown to cause infertility in female rats.

**Conclusion:** The results suggested that the extract has protective role against lead reproductive toxicity.

## 1. Introduction

The environment consists of various levels of heavy metals some of which are deleterious to human health while others can be nutritionally important. Lead has been described as pervasive environmental pollutant whose mechanism of toxicity is currently under investigation by many researchers and it is widely dispersed in the environment and remains for a long period of time [1]. Cases of high level of lead exposure have been found in industrial areas and are more prevalent in the developing countries [2–5]. Lead does not play any physiological role in the body and is considered toxic even in minute doses. According to Patrick [6], lead has very wide negative reproductive consequences; it affects almost all aspects of reproduction [7]. From high to low doses of lead exposure, the different responses of lead reported include reduced fertility [8–11], spontaneous abortions, low birth weight, impaired folliculogenesis and even damage to the ovaries [12]. Lefere [13], reported that low level of lead accumulation in the reproductive system (ovaries and uterus) could impede folliculogenesis.

A low lead concentration in the body of female mouse caused ovarian and uterine dysfunctions with fewer primordial follicles and increased atretic antral follicles [14]. At some stage in life, lead was used to induce abortion as stated by Paul [15]. Oral administration of lead acetate even in low doses led to reduction in the number of ovarian follicles revealing a strong correlation between blood lead level and atresia of ovarian follicles of albino Mice [16]. It is reported that the quality of female gametes in vitro are modified by exposure to very low levels of lead acetate [17]. The effects of lead is not only seen in the reproductive system rather it is felt all over the systems of the body such as cardiovascular system, gastrointestinal system, urinary system, nervous system and reproductive system [18]. Exposures to lead usually occur via dermal contact, oral ingestion or inhalation [1]. High level exposure to lead in female (at levels above  $15 \text{ mg l}^{-1}$ ) either occupationally or environmentally is associated with increase risks of spontaneous abortion and still birth [1].

In Africa, especially south of Sahara, the use of plants and their extracts for treatment of diseases have been in existence since ancient times [19].

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With factors such as poverty and illiteracy still militating against availability and accessibility of conventional medical services, there is a need to explore some other means of treatment or management of illnesses without causing more problems. Hence, the reason for investigation into the deliberate exposure of female Wistar rats to lead acetates (Figs. 1–9).

## 2. Materials and methods

### 2.1. Plant Collection and Preparation of extract

Fresh leave of *F. vogelii* was collected from Enyibichiri Ndufu-Alike Ikwo in Ikwo Local Government Area of Ebonyi State and authenticated at the Botany Department of the University of Nigeria Nsukka (UNN). The leaves were washed and dried in ventilated room. Thereafter, it was crushed into powder using an electric blender and then passed through mesh sieve to get the fine powders. Five hundred grams (500 g) of the powder was weighed using an electronic weighing balance and soaked in 1200 ml of water (powder/solvent) respectively. The mixture was agitated to enhance proper mixing of the solvent with the powder and then poured into air-tight plastic container. The container with the mixture was kept in a refrigerator for 72 h. The mixtures were filtered, first, with cheese cloth and then with Whiteman No 1 filter paper (24 cm). The filtrates were separated and concentrated in vacuum using Rotary Evaporator to 10% of their original volumes at 37°C - 40°C. These were concentrated using a water bath until it yielded a sticky paste. The paste was stored in a refrigerator at a very low temperature until it is required for use.

### 2.2. Ethical clearance

We respected and strictly observed the following councils directive on the use of experimental animals such as the Directive 2010/63/Eu of the European parliament and the European Council as passed on 22 September 2010 on the use and protection of experimental animals [20] and the Organization of Economic Co-operation Development, Paris, guideline for testing of chemical usage in Experimental animals, OECD, [21]. We also sort for and obtained the ethical clearance from the Faculty of Basic Medical Sciences University of Nigeria Enugu Campus.

### 2.3. Animal procurement and housing

Twenty five (25) adult female Wistar rats were procured from the animal house of the Department of Pharmacology, University of Nigeria Enugu Campus (UNEC) and maintained in the Animal House of Anatomy Department of Faculty of Basic Medical Sciences of the same University. The animals were housed in netted cages, fed with grower's mash and allowed water *ad libitum* with acclimatization period of one week (7days) was observed. They were randomly assigned into six groups (A, B, C, D and E).

### 2.4. Experimental Design and Drug Administration

Wistar rats in this experiment were grouped into five (5) of A, B, C, D and E groups and they received standard rat's diet containing 0.5% NaCl, 16.8%

**Table 1**

Table showing the weight (g) of animals that received aqueous extract of *F. vogelii* and lead acetate solution during the period of the research.

| S/No | Groups | No of Animals | Weeks (Mean ± SEM) |                            |                            |
|------|--------|---------------|--------------------|----------------------------|----------------------------|
|      |        |               | First              | Second                     | Third                      |
| 1    | A      | 5             | 139.25 ± 12.07     | 150.05 ± 12.52*            | 152.83 ± 13.85**           |
| 2    | B      | 5             | 119.18 ± 7.51      | 122.55 ± 9.08**            | 125.15 ± 9.93 <sup>+</sup> |
| 3    | C      | 5             | 119.58 ± 6.07      | 120.00 ± 6.47 <sup>+</sup> | 119.03 ± 5.58              |
| 4    | D      | 5             | 163.65 ± 12.15     | 165.55 ± 5.14              | 158.78 ± 5.71 <sup>+</sup> |
| 5    | E      | 5             | 109.13 ± 0.87      | 111.75 ± 0.66              | 114.13 ± 0.96              |

\* Significant increase in weight at  $P \leq 0.05$ .

\*\* Significant increase in weight at  $P \leq 0.01$ .

<sup>+</sup> Significant reduction in weight at  $P \leq 0.01$ .

**Table 2**

Mean weight (mg) of uterus of control and experimental groups of adult female Wistar rats.

| S/NO | Groups | No. Animals | Mean ± SEM (mg)            |
|------|--------|-------------|----------------------------|
| 1    | A      | 5           | 521.00 ± 0.50              |
| 2    | B      | 5           | 514.32 ± 5.00 <sup>†</sup> |
| 3    | C      | 5           | 517.80 ± 1.50 <sup>†</sup> |
| 4    | D      | 5           | 463.50 ± 5.00**            |
| 5    | E      | 5           | 527.11 ± 5.00***           |

\*\* Significant reduction in uterine weight compared to A ( $P \leq 0.01$  and  $P \leq 0.05$ ).

<sup>†</sup> Significant uterine weight gain when compared to D ( $P \leq 0.01$ ).

\*\*\* Significant uterine weight gain when compared to D ( $P \leq 0.01$ ).

protein and 46.5% dietary fat, 35.7%, vitamins, other micro nutrients and tap water *ad libitum* [22] throughout the period of the study. Group A is an untreated group which received rats' feed with water *ad libitum* and formol saline. Group B and C received 100 mg/kg and 300 mg/kg of aqueous extract of *F. vogelii* respectively and later exposed to lead acetate (1.5 mg/kg) till the end of experiment. Group D is the Lead acetate group (positive control) and the rats in this group received 1.5 mg/kg of Lead acetate solution daily for the period of the experiment [23]. Group E received aqueous extract of *F. vogelii* only throughout the experiment. The extracts and lead acetate solution were administered by oral intubation using oral gavage (cannula). Administration lasted for a period of 21 days and 24 h after the last administration the animals were sacrificed and the uterus and ovaries were removed and preserved for histological studies.

### 2.5. Ovarian Superoxide dismutase (SOD) activities

The procedure to estimate the Superoxide dismutase activity was measured according to the method described by Rumini et al., [24]. The principle of the assay was based on the ability of SOD to inhibit the reduction of nitro-blue tetrazolium (NBT). Briefly, the reaction mixture contained 2.7 ml of 0.067 M phosphate buffer, pH 7.8, 0.05 ml of 0.12 mM riboflavin, 0.1 ml of 1.5 mM NBT, 0.05 ml of 0.01 M methionine and 0.1 ml of enzyme samples. Uniform illumination of the tubes was ensured by placing it in air aluminum foil in a box with a 15 W fluorescent lamp for 10 min. Control without the enzyme source was included. The absorbance was measured at 560 nm [25]. One unit of SOD was defined as the amount of enzyme required to inhibit the reduction of NBT by 50% under the specific conditions.

### 2.6. Histological Study

At the end of the experiment, the rats were starved overnight and anaesthetized with chloroform and then decapitated [26]. The animals were dissected and the uterus and ovary removed, weighed and quickly fixed in bouin's fluid for routine histological procedures. The tissues were processed and embedded in paraffin wax. Thin sections (5–6 µm) were obtained and stained using haematoxylin and eosin (H&E) and

**Table 3**  
Mean weight (mg) of Ovaries of control and experimental groups of adult female Wistar rats.

| S/NO | GROUPS | No. of Animals | Mean ± SEM (mg) |
|------|--------|----------------|-----------------|
| 1    | A      | 5              | 60.10 ± 0.50    |
| 2    | B      | 5              | 57.51 ± 1.00*   |
| 3    | C      | 5              | 57.00 ± 1.00*   |
| 4    | D      | 5              | 47.10 ± 2.00 +  |
| 5    | E      | 5              | 67.43 ± 0.51**  |

\*\* Significant increase in ovarian weight when compared to group A (P ≤ 0.01).

+ Significant reduction in ovarian weight when compared to group A (P ≤ 0.01).

\* Significant increase in ovarian weight when compared to group D (P ≤ 0.01).

**Table 4**  
Results of activities of ovarian enzyme (SOD) of the experimental groups.

| S/NO | GROUPS | No. Animals | Mean ± SEM (µg/mg) |
|------|--------|-------------|--------------------|
| 1    | A      | 5           | 20.00 ± 0.38       |
| 2    | B      | 5           | 22.66 ± 1.48**     |
| 3    | C      | 5           | 20.00 ± 0.54**     |
| 4    | D      | 5           | 27.15 ± 5.22***    |
| 5    | E      | 5           | 21.16 ± 0.60       |

\*\*\* Significant increase in SOD when compared to group A (P ≤ 0.01 and P ≤ 0.05).

\*\* Significant reduction in SOD activity when compared to group D (P ≤ 0.01).

were examined under light microscope to determine the histological changes in the Ovaries and Uterus.

2.7. Statistical Analysis

Data from the experiment were analyzed by the use of one-way ANOVA and the values were expressed as mean ± S.E.M. The statistical significance level was established at a value of P ≤ 0.05 (\*) or P ≤ 0.01 (\*\*) by comparing the data obtained.

3. Results

The results of the experiment which include the animals, uterine and ovarian weights, SOD activities, phytochemical screening and the histological studies are summarized below.

The effects of lead exposure and *Ficus vogelii* extract treatments on the variation (gain/loss) of body weight are show in Table 1. During the experiment, the untreated group showed significant increase in weight at P ≤ 0.05 and P ≤ 0.01 in second and third weeks respectively. The lead group recorded weight increase in the second week but a significant reduction in third week. There was a progressive weight loss in group B but only significant in third week.

The effect of lead exposure and the extract on the uterus is as shown in Table 2. The lead group when compared to untreated group recorded a significant weight loss in their uterine weight as shown in the table below. All the other groups when compared to positive control group showed significant uterine weight gain.

The result of lead acetate and the extract on the ovarian weights for the period of the experiment are presented in Table 3. The lead acetate

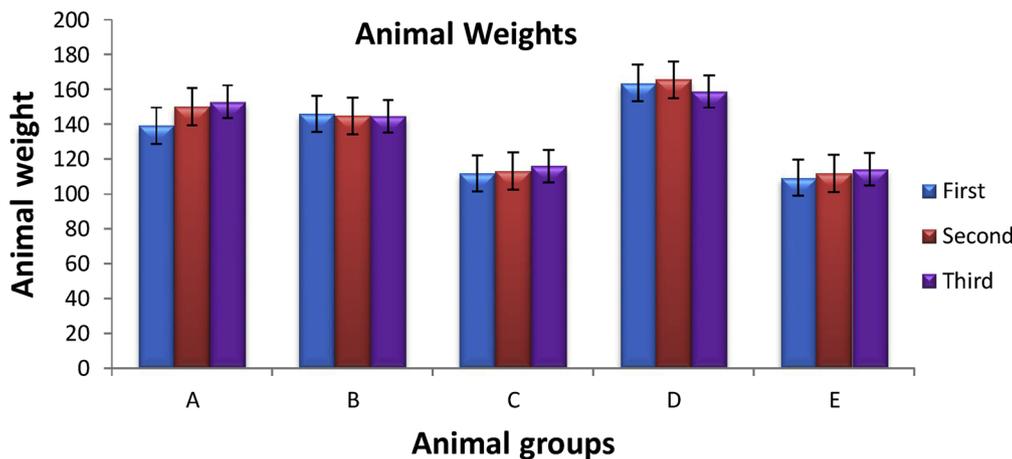


Fig. 1. The weight of the animals that received aqueous extract of *F. vogelii* and lead acetate compared to the control groups (A and D).

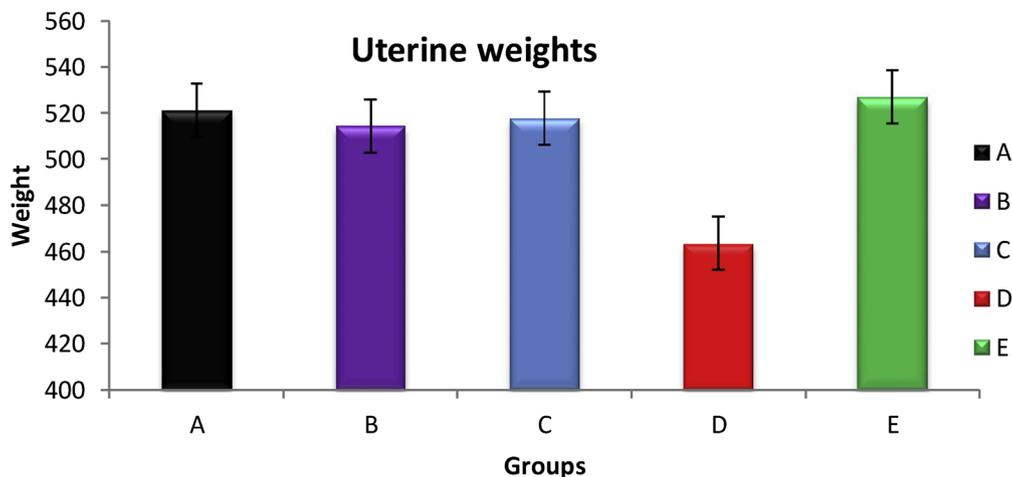


Fig. 2. The Uterine weight (mg) of the control and experimental groups.

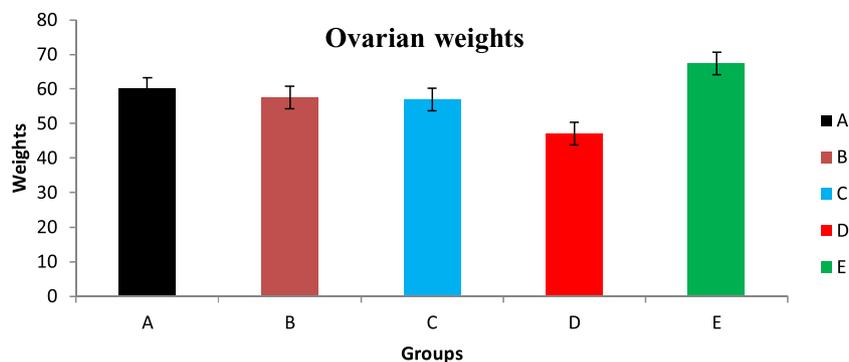


Fig. 3. The Ovarian weight of the control and experimental groups.

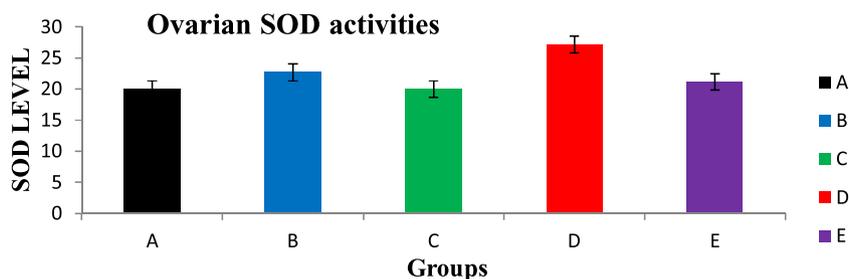


Fig. 4. The mean ± SEM antioxidant activity of the Superoxide Dismutase (SOD) enzyme.

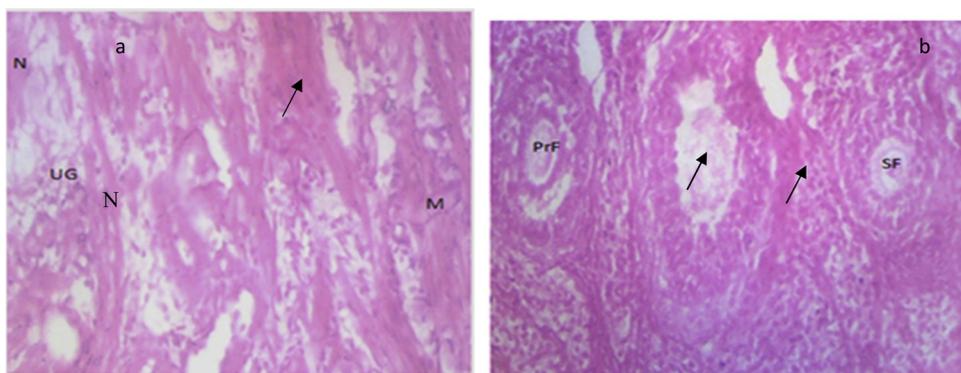


Fig. 5. (a) Uterus of group A reveals N- Normal mucosa structure, M-Myometrium and UG-uterine glands and (b) Ovaries of group A revealed ovarian follicles at different stages of development PF- primordial follicles; AnF- antral follicles, PrF- Primary follicle and SF- Secondary follicle; H & E 200 × .

Histological studies: There were various changes noticeable in the microanatomy of the vagina and the uterus. They include the following necrosis, oedema, fatty changes, optical empty spaces, inflammations, reduced blood supply signified by tissue appearance, etc. The main histological alterations observed in the ovaries and uterus after inducing toxicity with lead acetate solution and treating with aqueous extract of *F. vogelii* are presented in the photomicrographs below as they appear in H&E stain, 200 × .

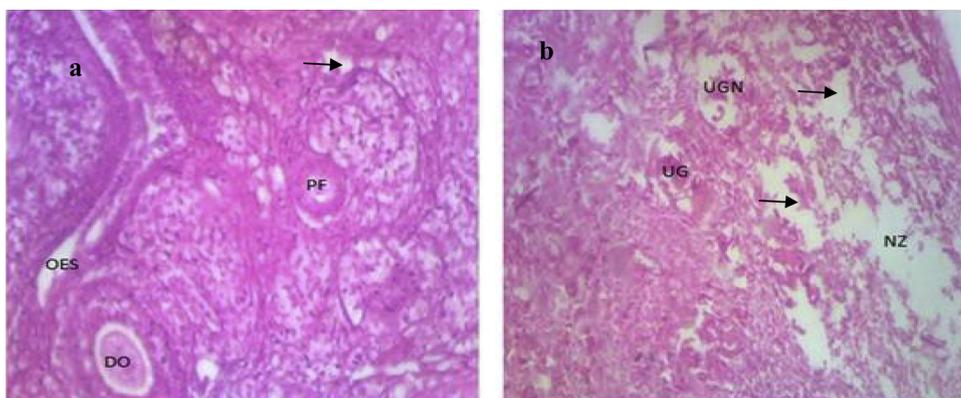
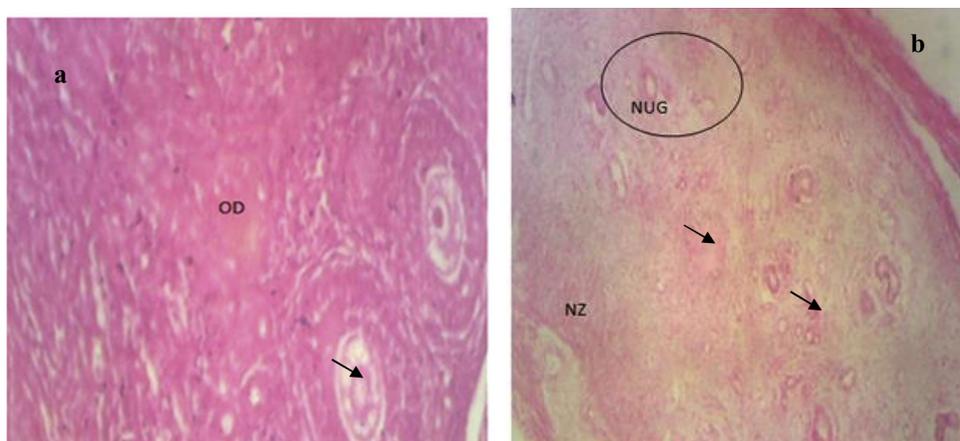
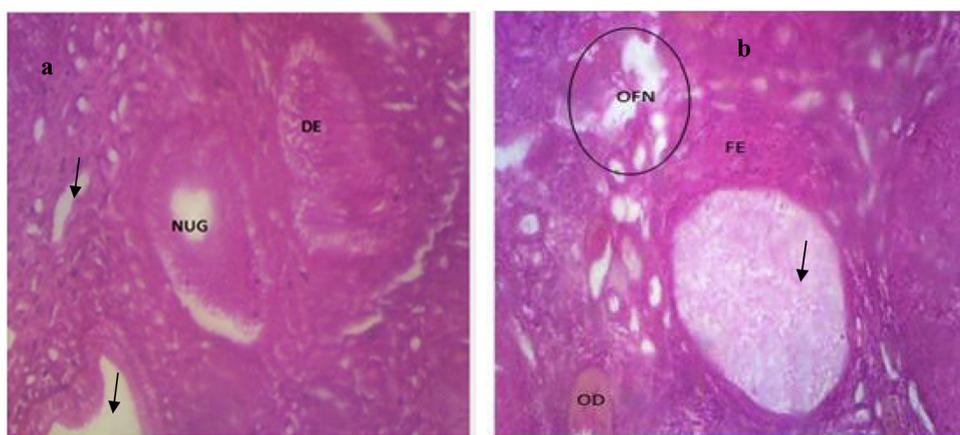


Fig. 6. Group D: (a) Ovary following the administration of 1.5 mg/kg lead acetate only for 14 days (OES- optical empty spaces; PF- primordial ovarian follicles; DO- diffuse oedema; OFN- Ovarian follicles necrosis; (b) Uterus showing NZ- Necrotic zone; UG- uterine glands and UGN-Necrotic Uterine Gland; the arrows show fatty changes; H & E 200 × .

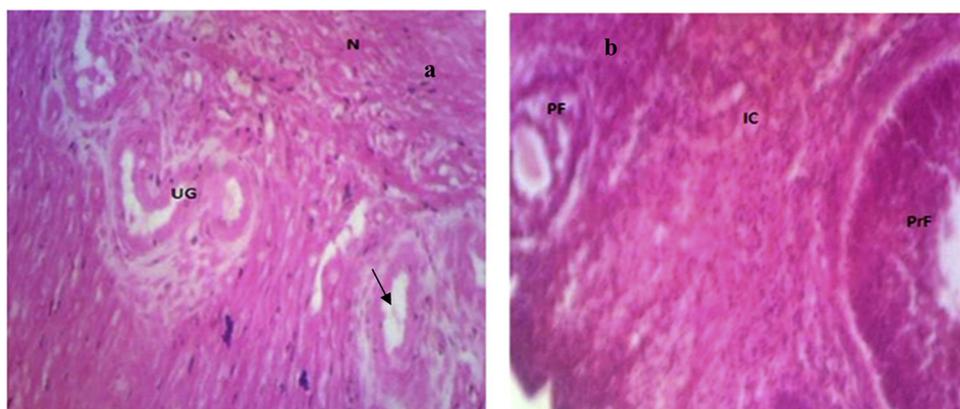
Histological studies: There were various changes noticeable in the microanatomy of the vagina and the uterus. They include the following necrosis, oedema, fatty changes, optical empty spaces, inflammations, reduced blood supply signified by tissue appearance, etc. The main histological alterations observed in the ovaries and uterus after inducing toxicity with lead acetate solution and treating with aqueous extract of *F. vogelii* are presented in the photomicrographs below as they appear in H&E stain, 200 × .



**Fig. 7.** Group B (a) Ovary following administration of aqueous extract of *F. vogelii* (100 mg/kg) and later 1.5 mg/kg of lead acetate solution showing, arrow show follicular oedema, OD-Oedema (b) Uterus showing NUG- necrotic uterine glands, NZ-Necrotic Zone, arrows represent inflammation; H & E 200 × .  
 Histological studies: There were various changes noticeable in the microanatomy of the vagina and the uterus. They include the following necrosis, oedema, fatty changes, optical empty spaces, inflammations, reduced blood supply signified by tissue appearance, etc. The main histological alterations observed in the ovaries and uterus after inducing toxicity with lead acetate solution and treating with aqueous extract of *F. vogelii* are presented in the photomicrographs below as they appear in H&E stain, 200 × .



**Fig. 8.** Group C (a) Uterus following administration of extract of *F. vogelii* (300 mg/kg) and 1.5 mg/kg of lead acetate solution showing NUG- Necrotic uterine glands, DE-Diffused Oedema, arrows represent uterine gland (b) Ovary showing FE-Follicular oedema, OFN-ovarian follicle necrosis and OD-Oedema, arrow shows antra space; H & E 200 × .  
 Histological studies: There were various changes noticeable in the microanatomy of the vagina and the uterus. They include the following necrosis, oedema, fatty changes, optical empty spaces, inflammations, reduced blood supply signified by tissue appearance, etc. The main histological alterations observed in the ovaries and uterus after inducing toxicity with lead acetate solution and treating with aqueous extract of *F. vogelii* are presented in the photomicrographs below as they appear in H&E stain, 200 × .



**Fig. 9.** Group E (a) Uterus following administration of aqueous extract of *F. vogelii* (300 mg/kg) showing UG-uterine glands, M-Myometrium, BV-Blood vessel, uterine gland and N-Normal mucosa (b) Ovary showing PrF-Primary follicle, PF-Primordial follicle and IC-Interstitial cells, H & E 200 × .  
 Histological studies: There were various changes noticeable in the microanatomy of the vagina and the uterus. They include the following necrosis, oedema, fatty changes, optical empty spaces, inflammations, reduced blood supply signified by tissue appearance, etc. The main histological alterations observed in the ovaries and uterus after inducing toxicity with lead acetate solution and treating with aqueous extract of *F. vogelii* are presented in the photomicrographs below as they appear in H&E stain, 200 × .

group (positive control) recorded a significant ovarian weight loss ( $P \leq 0.01$ ) when compared to the untreated group (group A). All the other groups when compared to positive control showed a statistically significant ( $P \leq 0.01$ ) ovarian weight gain.

The SOD results as presented in Table 4 shows that positive control have a significant increase in SOD activity when compared to the negative control (group A). The rats treated with the extract showed a significant decreased ovarian SOD activity when compared to the positive control (group D) .

#### 4. Discussions

Toxicity is a global issue [27] that many researchers have taken interest in more than ever. This is due to the fact that it has been from one lead poisoning to another in some regions of the world such as the case reported by Derek [28]. The positive change in body weight in any research as may be recorded is a very vital indicator of favourable effect of herbal medicine [29,30]. According to OECD [21], European Commission [20] and Ali et al., [30], weight check is also considered as a

vital constituent in the study of safety of therapeutic agent.

The negative control group recorded a significant progressive increase in body weight in the second and third week at  $P \leq 0.01$  and  $P \leq 0.05$  respectively while lead acetate group recorded an insignificant body weight increase in the second week, and a significant weight loss in the third week compared to the second week. The little increase in weight might be due to the fact that lead needed time to cause enough damage that could lead to weight loss, while the reduction in weight in the third week might be as a result of the damage inflicted on the organs by the lead toxicity at this stage. There was also weight gain in the other group E that received aqueous extracts of *F. vogelii*. This weight gain was not significant when compared to positive control which received only lead acetate solution. This increase in weight is one of the major pointers that the leave of *F. vogelii* has a nutritive value as a vegetable. The significant ( $P \leq 0.01$  and  $P \leq 0.05$ ) decrease in the uterine weights of the animals in positive control and an increase in the weight of the other uterus that were treated with the extract compared to negative control as shown in Table 2 agrees with the report of Durgesh and Lata, [17] which stated that “the animals that received only lead acetate experienced a significant reduction in uterine weight.” According to Singh et al., [31], histological examinations of organs are important for anatomical localization of the actions of toxins. Statistically, there was also a significant reduction in the ovarian weight of the animals in positive control (lead acetate) group ( $P \leq 0.01$  and  $P \leq 0.05$ ) and a subsequent increase in the ones treated with the extract compared to negative control (see Table 3.0). The above findings are in agreement with the research carried out by Durgesh and Lata [17]. This weight gain was due to increase in the dose of extracts administered to the rats; the higher the dose the higher the weight gained. This may be as a result of the role of the extracts in restoring the normal physiology of the organs of the body. The groups that received only the aqueous *F. vogelii* recorded a significant ( $P \leq 0.01$ ) uterine and ovarian weight gain. This organ weight gain could be an insight into the therapeutic benefits of *F. vogelii* leaves.

As shown in Table 4 above, positive control group showed a significant ( $27.15 \pm 5.22$ ) increase in SOD activity compared to the normal ( $20.00 \pm 0.38$ ) while those treated with the extract showed significant decrease in ovarian SOD at  $P \leq 0.01$ . These reductions in SOD level could be due to the role of the extract as an antioxidant agent. The groups that received the extract only showed SOD activity level closer to the negative control. This might be a pointer that the leaf of *F. vogelii* is a very good antioxidant capable of removing free radicals produced due to inflammation caused by the presence of toxins. These free radicals cause SOD level in the body to rise above normal [32].

According to Egbuna et al., [33], the leaves of *F. vogelii* contain the following chemicals: saponins, flavonoids, alkaloids, triterpenoids, polyphenols, coumarins, steroids, carotenoids and cardiac glycosides. Various phytosteroids (plant steroids) have been shown to promote fertility [34,35]. Flavonoids present in the leave of plants have been shown to possess many pharmacological properties such as: anti-oxidant activities, anti-inflammatory activities, anti-cancer activities and anti-microbial effects. This is in agreement with the work done by Uche, et al. [36], and Igile et al, [18]. Flavonoids might have contributed to the extracts healing (anti-inflammatory) properties as observed from the histological studies [37,38]. *F. vogelii* is very rich in triterpenoids, a compound which plays a very active role in wound healing [39]. According to Krishnaiah et al., [40], triterpenoids are shown to strengthen the skin, increase the concentration of antioxidants in wounds, and restore inflamed tissues by increasing blood supply.

We also considered the role of the extract in lead induced histological alterations in various parts of the ovary and uterus. On examination of the ovary microscopically, there were damages and reduction in folliculogenesis with a marked increase in the number of atretic follicles. This is in agreement with the work done by Patrick [6], Durgesh and Lata [17], and Igile et al, [18]. According to Taupeau et al., [14], low lead in the ovary caused dysfunction of folliculogenesis with

fewer primordial follicles and increased atretic antral follicles just as we discovered in this study. The major structural changes in the ovaries include diffuse oedema, ovarian follicle necrosis, optical empty spaces, ovarian follicle denudation and atretic follicles [41]. The uterus presented changes that include necrosis, oedema and endometrial denudation. The organs also looked more vascularized in the groups that received the extracts especially the high dose groups which indicated a restoration in the blood supply to the organs that was lost due to the induced toxicity. This work agrees with Shah et al, [16], where they reported that ‘oral administration of lead led to reduced number of ovarian follicles and an increased number of atretic follicles. The effects of lead on reproductive systems of female rats are complex as seen in this study [16]. The uterus of animals in negative control group presented a normal endometrium, intact uterine glands and unaltered stroma [41]. The ovaries also showed normal microanatomy of ovarian follicles at various stages of development. In the groups that received only the extract, the uterus and ovaries looked well perfused with blood and also very healthy when compared to the negative control group. This agrees with the work of Yilmaz et al., [42], which they reported that herbs can also reduce the effects of environmental toxicity on the reproductive systems. The ovaries in this group showed an increased folliculogenesis with ovarian follicles at different stages of development. In this extract group, an improvement was also observed in the organ perfusion. There were no histological alterations in the uterus or ovary in this group signifying the nutritional values of the extract. The results of the present study showed that using *F. vogelii* as an antioxidant interfered with the reactive oxygen species production and resisted lead toxicity as evidenced by the reduction in the ovarian SOD level to near normal.

## 5. Conclusion

The present result shows that the leaves are very good as an alternative herbal medicine. With the effect of the extract on the ovary and uterus, toxicity can be reduced using the herb especially that of lead acetate toxicity on the reproductive organs. The result also signifies the extract’s nutritive value and is good for health.

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## Authors Contribution

Conception: Uchewa OO and Ezugworie JO., Experiment: Uchewa OO., Analysis and interpretations: Uchewa OO and Ezugworie JO., Literature search: Uchewa OO and Ezugworie JO., Write up: Uchewa OO; Final review: Uchewa OO and Ezugworie JO.

## Conflict of Interest

The authors have no conflict of interest to declare.

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