ORIGINAL ARTICLE

Ficus *vogelii* Stembarks; a Promising Herbal Remedy for Lead Induced Reproductive Toxicity

Obinna O. Uchewa[1], Augustine O. Egwu[2], Chinedu G. Uzomba[3], Emmanuel N. Uchewa[4] Ogbonna E. Egwu[5]

ABSTRACT

Background and Objectives: Toxicity is fast becoming a major cause of infertility in this century unknowingly and researchers can no longer be mute over this situation. In this work, we aimed at exploring the potency of the bark of Ficus *vogelii* as an herbal product in protecting against female toxicity.

Methods: The twenty 25 female Wistar rats used weighed between 140 – 180g and were randomly assigned into five groups of five rats per group with group A servicing as control which received normal saline. Groups B and C received 3.5 mg/kg of Lead acetate 14 days and later received low and high dose of extract respectively. Group D served as lead acetate group while group E received extract only.

Results: There were changes in body, uterine and ovarian weight *(P > 0.01).* Superoxide Dismutase (SOD) enzyme levels were reduced significantly in B and C *(P > 0.01)* group animals while there was an increase in its levels in group D. Several alterations were seen in the ovary and uterus which includes reduced folliculogenesis with a marked increase in the number of atretic follicles, oedema and necrotic zones. These effects were seen to be restored near normal in the groups that were administered with extract.

Conclusions: This work showed that the bark of Ficus *vogelii* could be a good herbal remedy for infertility.

KEYWORDS: Ficus *vogelii*, Bark, Fertility, Herbal, Lead, Toxicity.

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1. Obinna O. Uchewa
2. Augustine O. Egwu
3. Chinedu G. Uzomba,
4. Emmanuel N. Uchewa
5. Ogbonna E. Egwu

1,2,3 Basic Medical Sciences, Alex Ekwueme Federal University Ndufu-Alike, Ikwo (AE-FUNAI), Ebonyi State, Nigeria.

2,4,5 Basic Medical Sciences, Ebonyi State University (EBSU), Abakaliki, Ebonyi State, Nigeria

3,4 Agriculture and Resources Management, Ebonyi State University (EBSU), Abakaliki, Ebonyi State, Nigeria.

Correspondence to:
Uchewa O. Obinna
Department of Anatomy, Faculty of Basic Medical Sciences
Alex Ekwueme-Federal University Ndufu-Alike, Ikwo (FUNAI), Ebonyi State, Nigeria. PMB 1010
E-mail address: euchewa1@gmail.com

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INTRODUCTION

Exposure to environmental toxicants has been implicated in infertility and the female reproductive organ is at the fore front due to its vulnerability.[1,2] Researchers have paid some attention to the toxic environmental factors that cause both ovarian and uterine toxicity and lead is inclusive.[3,4,5] Lead toxicity has been particularly described by Arif et al.[6] as an insidious hazard with the potential to cause irreversible health effects. Reproductive toxicity is the adverse effects of chemicals on gonadal structures and functions, alterations in fertility and impaired gametes functions.[7-12] Infertility is a common challenge that affects perhaps one in every six couples[13]and recent studies have reported that male problems is the single most common cause of infertility

known[8,11,12] even though diagnostically it is difficult to establish the extent to which the females are involved.[14] The use of plant extract as a fertility booster in humans is now on the increase because of the shifting in the attention from synthetic drugs to natural plant products.[15] This new drift in drug use is informed by accessibility, availability and affordability as some of the factors which have prompted over eighty percent of the populace in the developing countries to continue using medicinal plant products in handling primary medical problems.[16]

In Africa, several plants are being identified as a possible fertility regulating herbs with various other properties[17,18] and only very few of them have been tested and proven for such effects while others are under test.[19,20] This has led to explore the need to test the level of antioxidant in the bark of *Ficus vogelii* as a woody shrub that is used as food for both human and animals[21,22]and its potency in reducing uterine and ovarian toxicity. The fruit of most other species are also edible though they are usually of only local economic importance. The leaves of *F. vogelii* are used locally as vegetables while the bark is majorly medicinal. This research aims at investigating the antioxidant potency of the bark of *F. vogelii* as a medicinal plant.

METHODS

Plant Collection and Preparation of the Extracts

The barks of *F. vogelii* were gotten from the stem of *F. vogelii* plant at Ikwo Local Government Area of Ebonyi State, Nigeria. These were dried in ventilated room for three weeks and thereafter, were crushed into powder and passed through mesh sieve for fine powders. The powder was soaked in a container with water and the mixture was agitated using an electric blender to enable the solvent mix properly with the powder and then poured into air-tight plastic container which was kept for 72 hours.[12] The mixture was first filtered with cheese cloth and then with Whiteman No 1 filters paper. The filtrates were separated and concentrated in vacuum using Rotary Evaporator to 10% of their original volumes at 37ºC – 40ºC. This was heated using water bath until a paste was formed and stored until required for use.

Experimental Design and Grouping

The twenty-five (25) female Wistar rats were procured and maintained in the animal house of the Department of Anatomy, Faculty of Basic Medical Sciences of Alex Ekwueme Federal University Ndufu-Alike, Ikwo (AE-FUNAI). After acclimatization period of fourteen (14) days, the animals were randomly assigned into five groups (A, B, C, D and E). They were housed in netted cages, fed with standard rat's diet and allowed water *ad libitum* throughout the period of the study.[23,24] The untreated Group received normal saline to expose them to the same stress as other groups. Groups B and C received Lead acetate solution (3.5 mg/kg) daily and 24 – hours later the aqueous extract was administered. Group B received 100 and 300 mg/kg and served as the extract's low dose high doses respectively. The lead acetate Group D which served as the positive control received 3.5 mg/kg of lead acetate.[25] Group E received only the extract which served to ascertain efficacy of the extract in the organs.

Ethical Approval

It was ensured that we strictly adhered to the International Guidelines for the use of animals in researches according to the Directive 2010/63/Eu,[26] of the European parliament and the European Council passed into law on 22nd September, 2010 as it concerns the use and protection of animals use for experimental purposes and the Organization of Economic Co-operation Development, Paris, guideline for testing of chemical usage in Experimental animals; OECD[27].The ethical approval for this research was given by the institutional committee of Alex Ekwueme Federal University Ndufu-Alike, Ikwo, Ebonyi State, Nigeria vide Letter No. 521/b/2019/AE-FUNAI.

Ovarian SOD activities

This experiment lasted for 28 days after which the animals were sacrificed. One of the ovaries was taken and homogenized with mortar and pestles[2,12] and then preserved with phosphate buffer. This homogenate was centrifuged and refrigerated overnight before it was sent to a laboratory for antioxidant check. Superoxide dismutase activity

was measured according to the method of Winterbourn[28] as described by Rukmini and colleagues[29] and Bi, Lim and Henry.[30]

Histological Study

After a period of 28 days of the experiment including the acclimatization period of fourteen days, the rats were starved overnight and anaesthetized with chloroform and then decapitated.[31] The animals were sacrificed, dissected then 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 to obtain a thin section for microscopical examinations.

STATISTICAL ANALYSIS

The weights and SOD values from the present study were analyzed using descriptive statistics and presented as mean ± standard error of mean (SEM). Inferential statistics of one-way Analysis of Variance (ANOVA) was adopted and the statistical significance level was established at a value of *P≤0.05 (*)* or *P≤0.01 (**)* with the aid of Statistical Package for Social Sciences (SPSS) version 20.0.

RESULTS

The results as presented inTable-1 show the weight changes as recorded by the animals during the period under review. During the experiment, group A (control) showed significant increase in weight at *P≤0.05* and *P≤0.01* while group D showed a significant reduction in weight which was also recorded. The mean weight of the uterus also shows that group D animals had a significant uterine weight loss. All the other groups when compared to group D (untreated lead group) showed a statistically significant uterine weight gain. The table also shows the mean weight of the ovaries suggesting that group D (untreated lead group) recorded a significant ovarian weight loss *(P≤0.01)* when compared to control. All the other groups when compared to untreated lead group showed a statistically significant *(P≤0.01)* ovarian weight gain.

Table-1: The body, uterine and ovarian weight changes as measured during the experiment.

Grp	Initial Weight	Final Weight	WC	UW	OW
A	139.25 ± 12.07	152.83 ± 13.85**	13.58 ± 1.78	521.00 ± 0.50	60.10 ± 0.50
B	145.90 ± 12.91	144.60± 16.12+	1.3 ± 3.21	511.71 ± 1.50[a]	52.40 ± 0.50
C	111.78 ± 5.50	116.03± 9.02**	4.25 ± 3.52	510.13 ± 5.00[a]	53.50 ± 0.50
D	163.65 ± 12.15	158.78± 5.71+	-4.87 ± 6.44	463.50 ± 5.00**	47.10 ± 2.00[y]
E	109.13 ± 0.87	114.13± 0.96	5 ± 0.09	527.11 ± 5.00[b]	67.43 ± 0.51***

WC means weight change, UW means uterine weight, OW means ovarian weight,
Grp means Group, Mean ± SEM, Number of animals per group is five (5)
**significant weight gain at P≤0.01; + significant decrease in weight at P≤0.01
[x]Significant decrease in uterine weight compared to A (P≤0.01 and P≤0.05)
[a]Significant uterine weight gain compared to D (P≤0.01)
[b]Significant uterine weight gain compared to A (P≤0.01)
***Significant rise in ovarian weight compared to A (P ≤0.01)
[y]Significant decrease in ovarian weight compared to A (P≤0.01)

Ovarian SOD activities

After checking the superoxide dismutase (SOD) level, the results as presented inTable-2s how that untreated lead group recorded a significant increase in SOD (27.15 ± 5.22) activity when compared to the control (20.00 ± 0.38).The low and high dose groups showed an increased SOD of 26.00 ± 1.77 and 26.89 ± 1.73 respectively while the extract group E showed a near normal activity level (21.16 ± 0.60).

Table-2: Result for activities of ovarian enzyme Superoxide Dismutase (SOD) of the experimental and control groups.

S/No	Groups	Mean ± SEM (µg/mg)
1.	A	20.00 ± 0.38
2.	B	26.00 ± 1.77
3.	C	26.89 ± 1.73
4.	D	27.15 ± 5.22***
5.	E	21.16 ± 0.60

***Significant increase (P ≤0.01 and P≤0.05)
**Significant decrease SOD compared to untreated (P≤0.01)

Histological Studies

The tissues harvested from the animals after sacrifice were histologically processed to get thin sections that were viewed under microscope for histoarchitectural damage. Microscopical examinations of the tissues showed that the main histological alterations included necrosis, oedema, reduced folliculogenesis, fatty changes and inflammation as presented in Fig.1 to Fig.10 below.

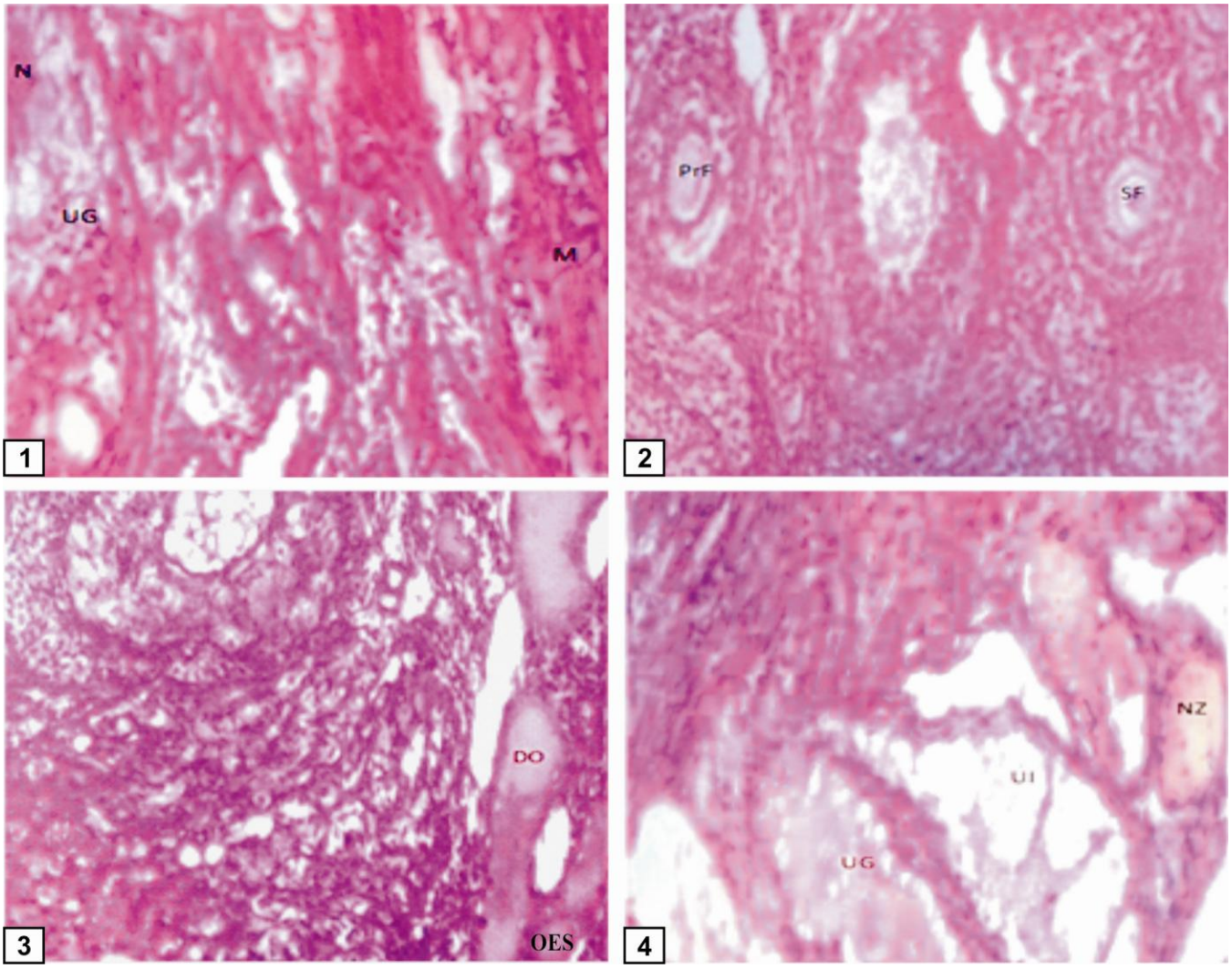

Fig.1: Photomicrograph of the uterus from the control showing N- Normal mucosa structure, M-Myometrium and UG-uterine glands). H & E 200X. **Fig.2:** Photomicrograph of the ovaries control showing ovarian follicles at different stages of development (AnF-antral follicles, PrF-Primary follicle and SF-Secondary follicle). H & E, 200X. **Fig.3:** Photomicrograph of group B ovary after receiving of 3.5mg/kg of lead acetate and aqueous extract of *F. vogelii* (100mg/kg) showing DO – Diffused oedema, OES – Optical empty spaces, H & E, 200X. **Fig.4:** Photomicrograph of group B uterus following administration of 3.5mg/kg of lead acetate and aqueous extract of *F. vogelii* (100mg/kg). It shows NZ-Necr4otic zone, UI-Uterine injury and UG-uterine glands, H & E, 200X.

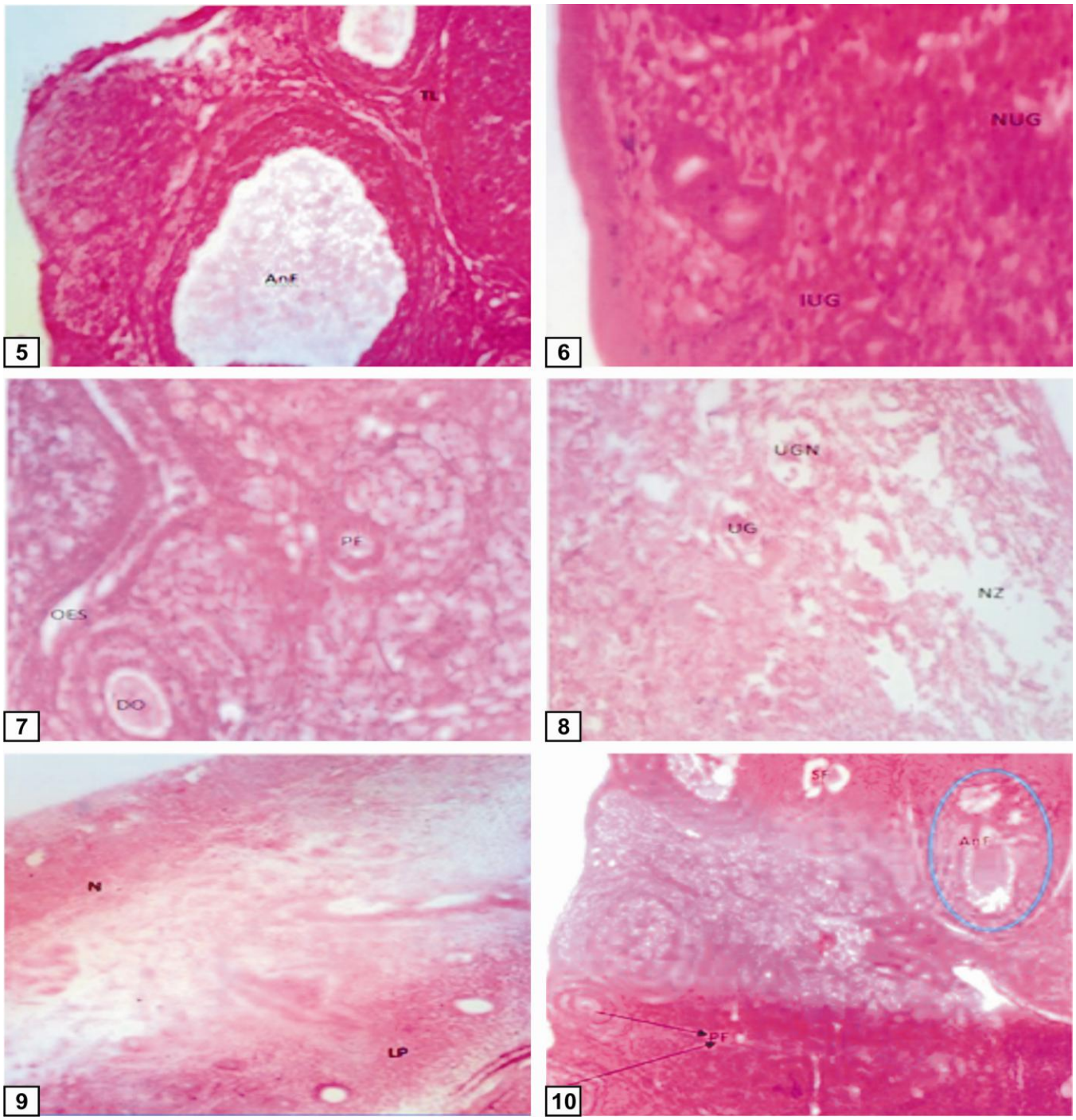

Fig.5: Photomicrograph of group C ovary after receiving 3.5mg/kg of lead acetate and aqueous extract of *F. vogelii* (300mg/kg) showing TL-theca lutea, AnF-Antral follicle and interstitial cells) H & E, 200X. **Fig.6:** Photomicrograph of group C uterus that received 3.5 mg/kg of lead acetate and aqueous extract of *F. vogelii* (300mg/kg) showing NUG-Necrotic uterine glands and IUG-In vaginated uterine gland, H & E, 200X. **Fig.7:** Photomicrograph of group D ovary that received 3.5 mg/kg lead acetate showing OES-optical empty spaces; OFD-ovarian follicle denudation; DO-diffuse oedema; H & E, 200X. **Fig.8:** Photomicrograph of group D uterus that received only 3.5 mg/kg of lead acetate showing NZ-necrotic zone; UG-uterine glands; NUG-Necrotic Uterine Gland, H & E, 200X. **Fig.9:** Photomicrograph of group F uterus that received of only the aqueous extract of *F. vogelii* (300 mg/kg) showing Normal mucosa, LP-Lamina propria, and M-Myometrium, H & E, 200X. **Fig.10:** Photomicrograph of group F ovary after receiving aqueous extract of *F. vogelii* (300mg/kg) showing SF-Secondary follicle, AnF-Antral follicle, PF-primordial follicles. H & E, 200X.

DISCUSSION

Over the years, it has seemed difficult to proffer a solution to the devastating effects of environmental toxicity in the body but with the efforts of researchers it is becoming increasingly easier to achieve, although not completely easy as expected. [1] The wake of 21lst century gave rise to the recognition of herbal medicine more than ever and *F. vogelii* is a vegetable that has been used locally to treat some illnesses and also implicated by so many researchers as an herbal remedy for some human ailments.[12,32,33] The positive change in body weight of animals in any research is a useful indicator of favourable effect of herbal medicine[34-36] and also an important constituent in the study of safety of any therapeutic agent.[25] In the present research, negative control recorded a significant increase in body weight at P≤0.01 and P≤0.05 while untreated lead group showed a significant weight loss as in table-1 agreeing with Dumitrescu et al.[36] This weight loss may have resulted from the deleterious effect of lead on the internal organs. Among the experimental groups, some animals showed a significant increase in body weight at *P≤0.05* and P≤0.01 which agrees with the report of Dumitrescu and colleagues[37] and Durgesh and Lata[38] where they stated that "the animals that received only lead experienced a significant reduction in uterine weight." The extract group only recorded a significant (P≤0.01) uterine and ovarian weight gain when compared to the control as recorded inTable-1 above.

As shown in Table-2, lead group showed a significant *(P < 0.01)* increase in superoxide dismutase (SOD) enzyme level which may have been caused by organ inflammations resulting from the induced toxicity. The extract group only showed a decrease SOD level (21.16 ± 0.60) that is near normal (20.00 ± 0.38) as shown in Table-2. The above might be suggesting the extract is a good antioxidant for removing free radicals as a result of inflammation caused by toxins that causes rise in SOD levelsbeyondnormal.[36,39,40]

Microscopically, the ovary and uterus revealed various alterations which include decreased follicular formation, increased number of atretic follicles which agrees with Serafini and Peluso[31]and Igile and co-workers[32] (Fig. 1-10). The major structural changes in those organs were diffuse oedema, necrosis, optical empty spaces, denudation, folliculogenesis and atretic follicles in accordance with Dumitrescu *et al.*[36] and Durgesh and Lata.[38] From the appearance of the tissues, it is noticed that there was also an improved organ vascularization in the animals that received the extract indicating blood supply restoration to the organs lost due to necrosis (Fig. 3-6).[12] According to Igile,[32] oral administration of high doses of lead causes reduced ovarian follicles and increased atretic follicles (Fig.5-6). The effects of lead on reproductive systems of female rats are complex[34] as also noticed in this study. The uterus of animals in control group (Fig.1) presented a normal endometrium, intact uterine glands and unaltered stroma in agreement with the report of Dumitrescu et al.[36] The ovaries (Fig.2) also showed normal histoarchitecture with ovarian follicles at various stages of development. Group D ovaries that received lead acetate alone (Fig.7) presented a fatty change (a reversible change that occurs in organs due to toxicity) appearance and focal loss of tissue (optical empty spaces).[38] The damage might be as a result of the lead's effect on blood vessels leading to tissue necrosis.[34] Lead causes imbalance between vasoconstrictors and vasodilators which decreases the overall vascular tone.[35] The above changes were also reported by Dumitrescu et al[37] and Durgesh and Lata.[38] The ovary showed some developing follicles with evidence and looked more vascularized than the other groups (Fig.10). The uterus shows a near normal area which might be due to the healing effect of the extract in the body against the toxicity induced by the lead acetate (Fig.9).[37] The antioxidant property of the extract may be implicated in the control or prevention of inflammation.[22]

The author decided to embark on this present study on the stem bark of *F. vogelii*to confirm the claims by traditionalists of the efficacy of the bark and this article if completely different from the previous article by the same author on the leaves of *F. vogelii.* The two studies revealed that *F. vogelii* can be very efficacious in treating lead induced reproductive toxicity.

CONCLUSION

The results of this present research indicate that the bark of *Ficus vogelii* may be a very good

alternative herbal medicine and potent in the treatment of reproductive toxicity especially that of females. As an antioxidant it may be useful in reducing inflammation of reproductive system.

LIMITATIONS OF STUDY

This work was financially limited and only covered a limited enzyme check and so the authors recommend that more research be conducted on the male reproductive system using this extract.

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CONFLICT OF INTEREST

None to declare.

GRANT SUPPORT & FINANCIAL DISCLOSURE

None to disclose.

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Author's Contribution

OOU, AOE: Conception and design.

CGU, OOU: Data acquisition and/or analysis and/or interpretation.

OOU, OEE, ENU: Drafting the article and revising it critically for important intellectual content.

ALL AUTHORS: Approval of the final version of the manuscript to be published.