

LEAD ACETATE: A DANGEROUS ENVIRONMENTAL TOXICANT THAT IS AMELIORATED BY THE AQUEOUS EXTRACT OF *FICUS VOGELII* (FV).

ABSTRACT

To investigate the effects of lead toxicity on the histoarchitecture of the vagina and cervix of adult female Wistar rats and the curative role of Fv in lead toxicity. The experiment lasted for a period of 21 days involving 20 adult female albino Wistar rats with an average weight of 145g. The rats were divided into five (5) groups A, B, C, D and E. A is negative control group that received normal saline, B is positive group that received lead acetate solution, C received Aqueous extract of Fv (100g/kg) and lead acetate solution, D received aqueous extract of Fv (300g/kg) and lead acetate solution group while E received aqueous extract of Fv only. Examination of the vagina and cervix histologically revealed various damages such as, necrosis, oedema and reduction in size suspected to be as a result of lead toxicity. These changes were ameliorated by the administration of Fv extract. Exposure to lead also produced significant reduction in blood supply to the vagina and cervix which caused the mucosa to necrotize. These structural changes correlated with the level of exposure in the vagina. They were mainly oedema, necrosis and denudation of the vaginal walls. These alterations can make the vagina and cervix acidic which have been implicated as one of the cause of infertility in females. The results of this study suggested that treatment with *F. Vogelii* has an ameliorative effect.

Keywords: Vagina, lead, cervix, *Ficus vogelii* and Ameliorate.

INTRODUCTION

Toxicity is very deadly to various part of human body which adversely affects the reproductive system leading to impaired reproduction²². It has been reported that the female reproductive system's functions in vitro can be modified by exposure to a very low level of lead^{1,6}. Longer and more variable menstrual cycles have been found in lead treated female Rhesus monkeys¹². The vagina is an important organ of reproduction in the female. It is also referred to as birth canal where the foetus passes during birth. Its importance in reproduction cannot be over emphasized especially to women. The Semen is deposited in the vaginal vault during intercourse and the spermatozoa make their way into the external os of the cervical canal, pass through the cervical canal into the uterine cavity, and then continue through the uterine cavity into the uterine tubes where fertilization occurs in the ampulla^{3,20}. This led us to examine the effect of lead acetate on the vaginal wall female Wistar rats. The extent of natural fertility that takes place is invariably connected to the motility of the spermatozoa and one of the things that affect sperm motility is vaginal toxicity (acidity). The toxicity of the vagina which can affect its histoarchitecture also causes the death of the spermatozoa and thereby impedes the fertility.

The cervix is a narrow and cylindrical part of the uterus which communicates with the uterine body via the internal os and opens into the vagina at the external os^{3,21}. During the movement of the spermatozoa, it moves from the vagina into the cervix and so anything that affects the histology of the cervix likely to alter the structure of the cervical mucus which protects the sperm from naturally acidic environment of the vagina and get it safely to the ovum. It has been noted that animals have protective mechanism in the form of antioxidant nutrients²⁵, vitamins and several enzymes. Antioxidant may play an important role in abating some hazardous effects of lead⁶. This study seeks to assess the lead acetate induced histological damages on vagina and cervix; and to

ascertain the protective effects of aqueous extract *F. vogelii* on female Wistar rats as an experimental model.

Materials and methods

Collection and Authentication of Plant materials

The leaves of *F. vogelii* was collected from Enyibichiri Ndufu-Alike Ikwo a town where Federal University Ndufu-Alike Ikwo (FUNAI) is located in Ikwo Local Government Area of Ebonyi State and authenticated in Botany Department of the University of Nigeria Nsukka (UNN).

Preparation of the extracts

Those leaves were washed and dried in ventilated room and thereafter, were crushed into powder using pestle and mortar, and passed through mesh sieve to get the fine powders. The powdered form was soaked in water for 48 hours and filtered using a white muslin cloth to remove debris and then re-filtered with filter paper to obtain a homogenous clear filtrate which was concentrated *in vacuo*, using a rotary evaporator at $<40^{\circ}\text{C}$ to yield a sticky paste¹⁶. This was stored under refrigeration until it was required. All preparations were performed at the Department of Anatomy Faculty of Basic Medical Sciences, Federal University Ndufu-Alike, Ikwo (FUNAI), Ebonyi State, Nigeria.

Animal Procurement and Housing

Twenty (20) adult female Wistar rats with average weight of 145g 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 (7 days).

Animal Grouping and Experimental Design

The rats used in this experiment were randomly assigned into five groups as follow: A, B, C, D and E containing four (4) animals per group.

Group A-Control Group (negative): The rats of this group received standard rat's diet containing 0.5% NaCl, 22% protein and 4-6% dietary fat and tap water *ad libitum*.

Group B: Lead acetate solution (positive control): The rats in this group received 1.5mg/kg of Lead acetate solution daily for 14days.

Group C: Lead acetate solution & aqueous *F. vogelii* extract (lower dose): The rats in this group received lead acetate (1.5mg/kg) for 7 days and 24 hours later exposed to aqueous extract of *F. vogelii* (100g/kg) till the end of experiment. The dosage was calculated based on the rats' weight.

Group D: Lead acetate solution & aqueous *F. vogelii* extract (higher dose): The rats in this group received lead acetate (1.5mg/kg) for 7 days and 24 hours later exposed to aqueous extract of *F. vogelii* (300g/kg) till the end of experiment. The dosage was calculated based on the rats' weight.

Group E: Aqueous extract of *F.vogelii* only: The rats in this group received 300mg/kg of aqueous extract of *F.vogelii* only daily for 14days.

NOTE: The extracts and lead acetate solution were administered by oral intubation using oral gavage (cannula). The experiment lasted for a period of 21 days.

Histological Study

At the end of the experiment, the rats were starved overnight and anaesthetized with chloroform and then decapitated¹⁷. The animals were dissected, and the vagina and cervix were quickly harvested and fixed in bouin's fluid for routine histological procedures. The tissue was processed and embedded in paraffin wax. Thin sections (5-6 μm) were obtained and stained using

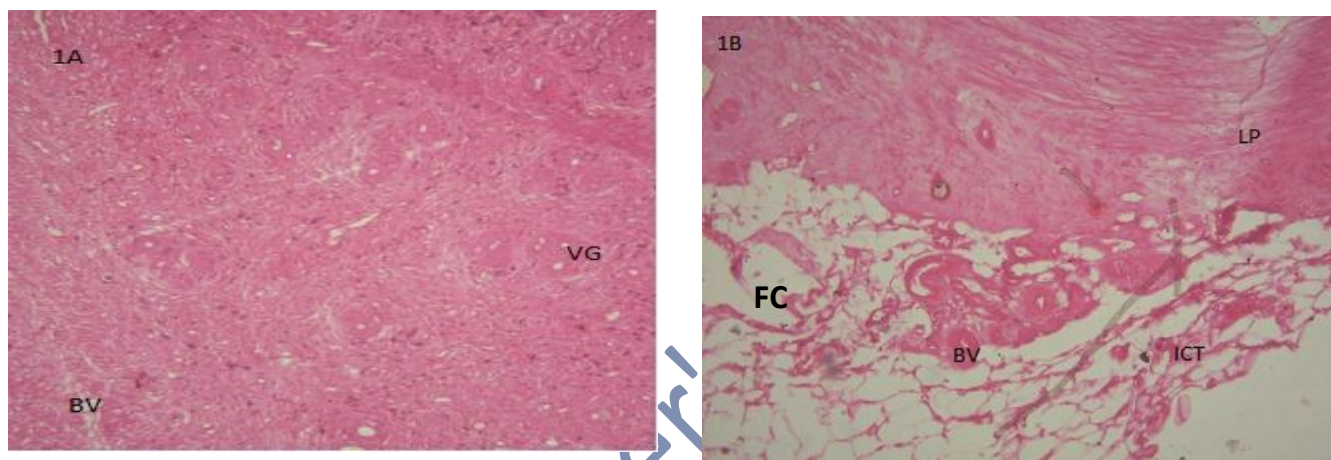
haematoxylin and eosin (H&E) and were examined under light microscope to determine the histological changes that occurred.

RESULTS

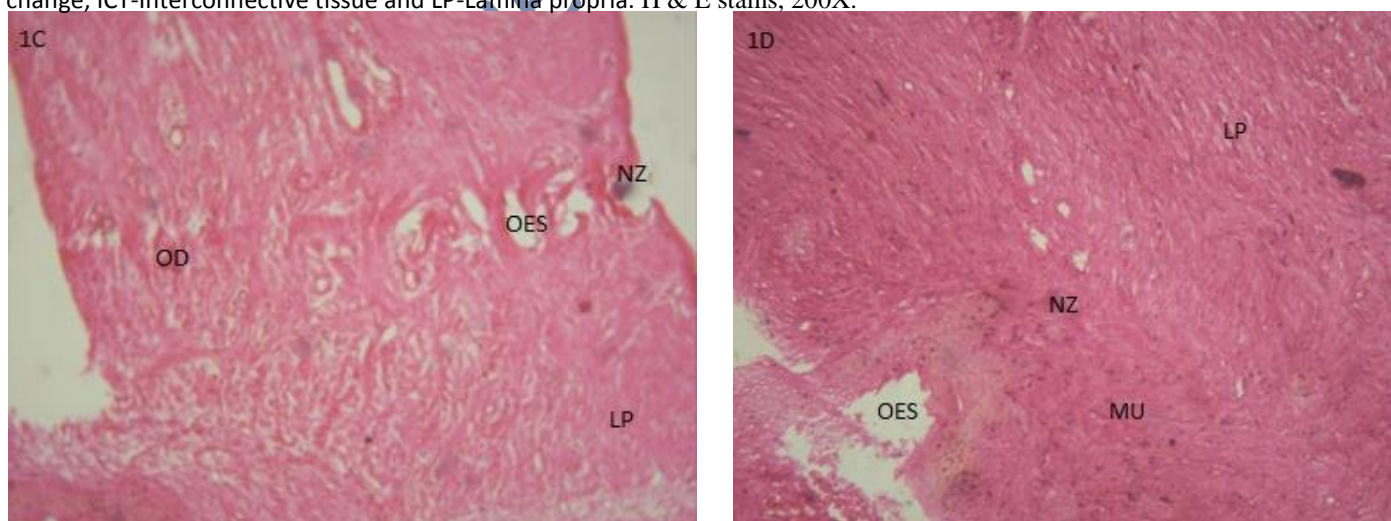
The results of the histological studies carried out in adult female Wistar rats' vagina and cervix at the end of 21 days are presented in the figures below as histological slides.

The vagina

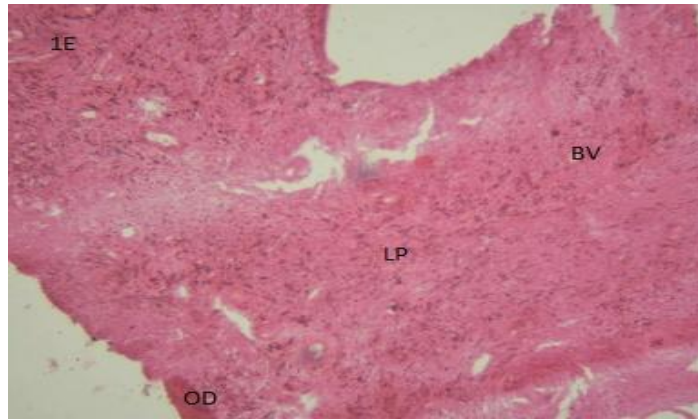
When the vagina was microscopically examined, the negative control and extract group revealed the presence of a normal structure of vaginal mucosa and glands with no alterations (Figures 1A and 1E). Following exposure to 1.5mg/kg lead acetate (positive control), some areas of the vagina presented optically empty spaces in the tissue, as well as diffuse oedemas and vaginal wall denudation (Figure 1B), necrotic zones, diffused oedemas and vaginal glands necrosis were also visibly present. After the animals were treated with the extract of Fv, there were signs of recovery revealed by the vagina. This is presented in Figures 1C and 1D below.



Figures 1A Vagina from group A (negative control) revealed the presence of a normal mucosa, BV-Blood vessel and VG-Vagina gland. **(1B)** Vagina following administration of lead acetate (1.5mg/kg) for 14 days. BV-Blood vessel, FC-Fatty change, ICT-Interconnective tissue and LP-Lamina propria. H & E stains, 200X.



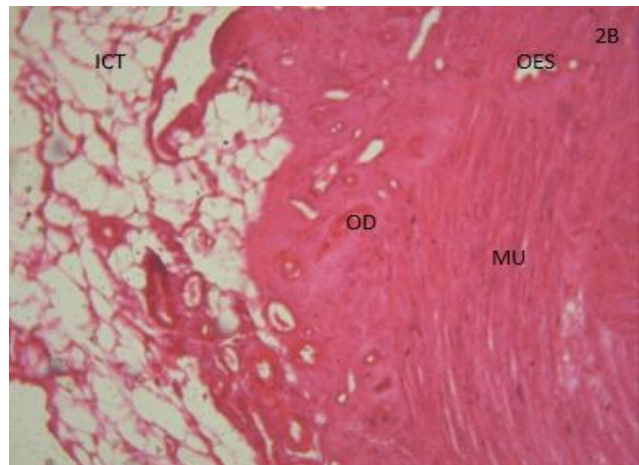
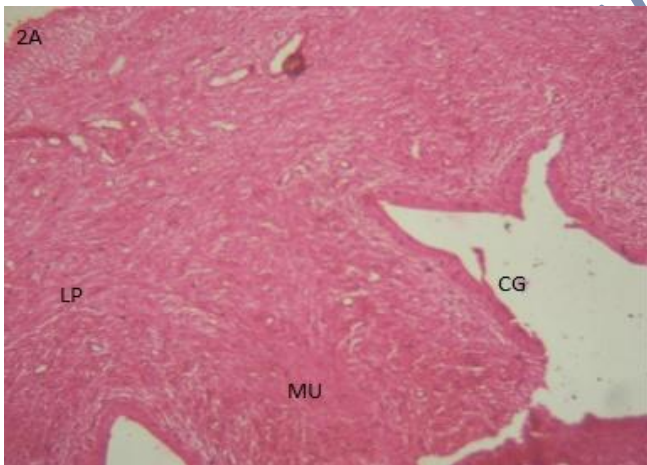
Figures (1C) Vagina following administration of lead acetate (1.5mg/kg) for 7 days and 24 hours later exposed to aqueous extract of *F. vogelii* (100g/kg) for another 7 days. OD-Oedema, LP-Lamina propria, OES-Optically empty space and NZ-Necrotic zone. **(1D)** Vagina following administration of lead acetate (1.5mg/kg) for 7 days and 24 hours later exposed to aqueous extract of *F. vogelii* (300g/kg) for another 7 days. OES-Optically empty space, MU-Muscle, NZ-Necrotic zone and LP-Lamina propria. H & E stains, 200X.



Figures: (1E) Vagina following administration of aqueous extract of *F. vogelii* (300g/kg) for another 14 days. OD-Oedema, LP-Lamina propria, and BV-Blood vessel. (H & E stains, 200X.)

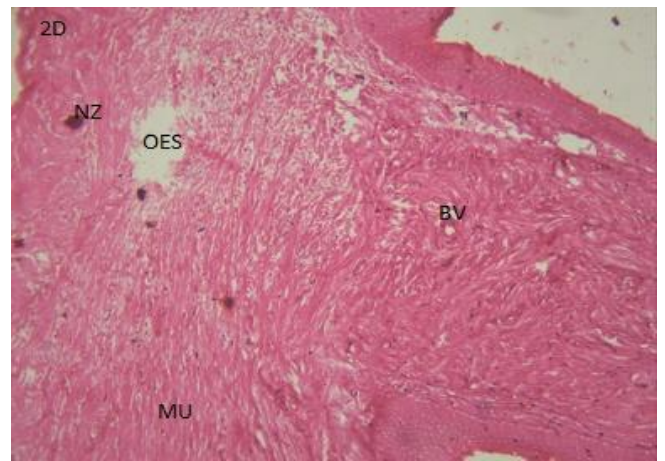
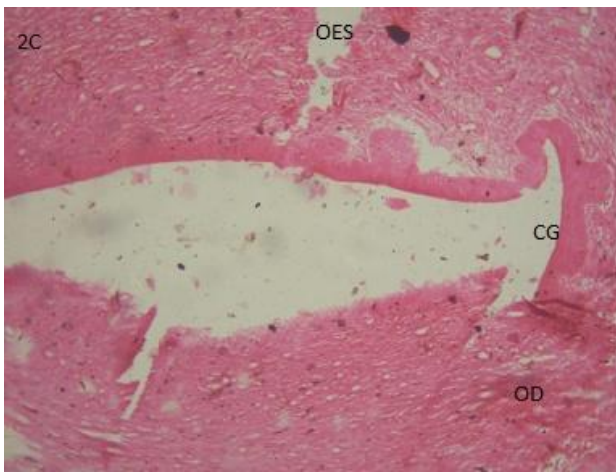
The cervix

The microscopic examination of the negative control and extract group revealed the presence of a normal structure of uterine cervix mucosa and glands with no alterations (Figures 2A and 2E). as the animals were exposed to 1.5mg/kg lead acetate (positive control), some areas of the cervix presented optically empty spaces, as well as diffuse oedemas and cervical wall denudation (Figure 2B), necrotic zones, and cervical glands necrosis were also visibly. Following the administration of the aqueous extract of *F. vogelii*, the cervix showed signs of recovery as presented in Figures 2C and 2D below.

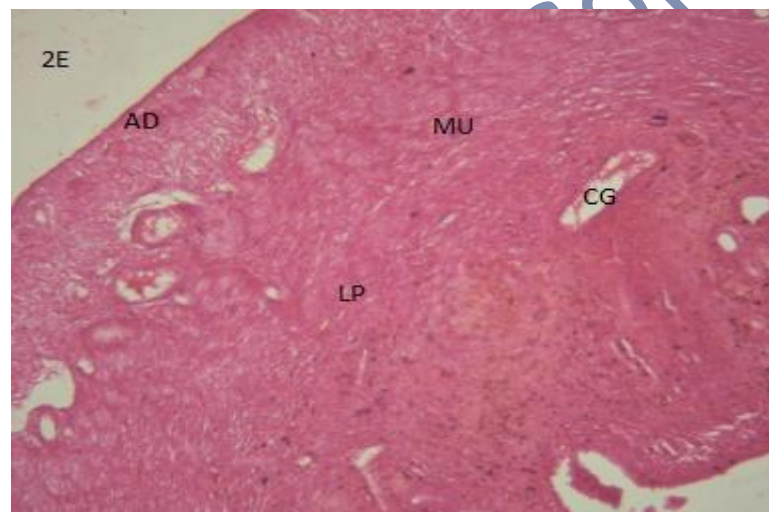


FC

Figures 2A The cervix of group A (negative control) revealed the presence of a normal mucosa, LP-Lamina propria and CG-Cervical gland. **(2B)** Cervix following administration of lead acetate (1.5mg/kg) for 14 days. OD-Oedema, FC-Fatty change, ICT-Interconnective tissue, MU-Muscle and OES-Optically empty space. H & E stains, 200X.



Figures (2C) Cervix following administration of lead acetate (1.5mg/kg) for 7 days and 24 hours later exposed to aqueous extract of *F. vogelii* (100g/kg) for another 7 days. OD-Oedema, CG-Cervical gland and OES-Optically empty space. **(2D)** Cervix following administration of lead acetate (1.5mg/kg) for 7 days and 24 hours later exposed to aqueous extract of *F. vogelii* (300g/kg) for another 7 days. OES-Optically empty space, MU-Muscle, NZ-Necrotic zone and Bv-Blood vessel. H & E stains, 200X.



Figures: (2E) Cervix following administration of aqueous extract of *F. vogelii* (300g/kg) for another 14 days. MU-Muscle, CG-Cervical gland, LP-Lamina propria, and AD-adventitia. (H & E stains, 200X).

Discussion

Most researches on female specimens' exposure to lead has focused on clinically visible effects such as miscarriage, premature delivery, and infant mortality in humans and animals²⁴ and effects of lead on the fallopian tubes, ovaries and uterus^{4,5}. *F. vogelii* is a vegetable that is locally used to treat some illnesses and have also been implicated by so many researchers as an herbal remedy for some human diseases^{7,9,10}. Works on vaginal and cervical toxicity are sparse, yet many authors have identified the toxic effects of lead on the uterus, fallopian tube and ovaries and went ahead to prove that it can hamper reproduction^{14,19,24}.

In this study, we noticed that all the histology of the organs and tissues under investigation were seriously altered. This caused changes in the reproductive system integrity leading to their

deformations. These changes in the vagina make it toxic and increase its acidity making the environment dangerous for the survival of sperm. The major structural changes as found in the vagina were diffuse edema, necrosis in the vagina and cervix, optical empty spaces, denudation of the vaginal and cervical mucosa, and vaginal and cervical gland necrosis as shown in the figures above. The administration of the extract appeared to neutralize the effects of lead on the organs. The fatty change that appeared in the vagina with the oedema disappeared following the administration of the extract as seen in figures 1C and 1D. The main changes in the cervix are diffuse oedema, cervical gland necrosis, optical empty spaces, denudation of the cervical mucosa, and cervical gland necrosis. There was restoration of the muscle and mucosa integrity of the cervix following the administration of the extract of *F.vogelii*. It can be noted that the effects of lead on reproductive systems are complex and sex-specific, and they seem to involve multiple locations on the hypothalamic–pituitary–gonadal axis, confirming our findings on female rats. Women’s occupational exposure to lead is undoubtedly related to reproductive impairments²³. Most impairments in the nervous system, the kidneys, the reproductive system and every other parts of the body as reported in other literatures show the importance and versatility of lead effects in mammals^{2,8,11,13,14,15,18}.

The structural alterations as noticed in the present study in the vagina and cervix adult rats have been able to demonstrate the poisonous effects of lead on the vagina and cervix. With the alterations of the cervical walls, the secretion of cervical mucus that helps the sperm’s move towards the egg will be hampered seriously this in turn leads to infertility in female. With the results of this research, we wish to recommend the use of this leave *Ficus vogelii* as vegetable for families and its inclusion as an herbal medicine for the treatment of reproductive toxicity especially that of lead toxicity in the female laboratory animals.

REFERENCES

1. Avazeri, N. Denys, A. Lefèvre, B. (2006) Lead Cations affect the Control of both Meiosis arrest and Meiosis Resumption of the Mouse oocyte in vitro at least via the PKC pathway. *Biochimie* .88(11); pp. 1823-1829.
2. Borja-Aburto VH, Hertz-Picciotto I, Rojas-Lopez MR, Farias P, Rios C, Blanco J (1999). Blood lead levels measured Prospectively and risk of Spontaneous Abortion. *Am J Epidemiol* 18: 590–597.
3. Drake, R., Vogl, W., Mitchell, A.W.M. (2014). *Grays Anatomy for Students*. 3rd eds. chpt, 5: pp 397-416. Churchill Livingstone (Elsevier’s) Publishers; Saunders.
4. Dumitrescu E; Chiurciu V; Muselin F; Popescu R; Brezovan D; Cristina R. T. (2015) Effects of Long-term Exposure of Female Rats to Low Levels of Lead: Ovary and Uterus Histological Architecture Changes. *Turk J Biol*. 39: 284-289.
5. Dumitrescu E; Cristina RT; and Muselin F. (2014). Reproductive Biology Study of dynamics of Female Sexual hormones: a 12-months exposure to lead acetate Rat model. *Turk J Biol* 38: 581-585.
6. Durgesh N. S. and Lata B. (2014) Role of Vitamin E on Anti Folliculogenesis Effects of Lead Acetate on Diameter of Follicles Containing Ovarian Tissue of Swiss Albino Mice; *G.J.B.A.H.S.,vol.3(1):322-325*.
7. Egbuna, P. A. C.; Joshua, P. E. and Chigbo, M. U. (2011). Antihepatotoxic Effects of *Ficus Vogelii* Ethanol Leaf Extract On The Liver Function Indices of Ccl4–Induced Hepatotoxicity in Rats: *Journal of American Science*. 7(6).
8. Hilderbrand DC, Der R, Griffin WT, Fahim MS (1973). Effect of lead acetate on Reproduction. *Am J Obstet Gynecol* 115: 1058–1065.
9. Igile G.O; Utin I.C.; I.A., Iwara, B.I.A. Mgbeje and P.E. Ebong. (2015^a) Ethanolic Extract of *Ficus vogelii* Ameliorates Dyslipidemia in Diabetic Albino Wistar Rats. *International Journal of Current Research in Biosciences and Plant Biology* 2(8):87-96. www.ijcrbp.com.

10. Igile, G.O., Isika, A.I., Essiet, G.A., Iwara, I.A., Mgbeje, B.I.A., Ebong, P.E., (2015^b). Antihypertensive and electrolyte modulatory potential of vernonioside E (steroidal saponin) from *Vernonia amygdalina* (Asteraceae) in adult Wistar rats. *Planta Med. (In press)*.
11. Junaid M, Chowdhuri DK, Narayan R, Shanker R, Saxena DK (1997). Lead- induced changes in ovarian follicular development and maturation in mice. *J Toxicol Env Health* 1997; 50: 31–40.
12. Laughlin, N.K. Bowman, R.E. Franks, P.A. Dierschke, D.J. (1987) Altered menstrual cycles in Rhesus monkeys induced by lead. *Fundam App Toxicol.* 9(4); pp. 722-729.
13. Nampoothiri LP, Gupta S (2006). Simultaneous effect of lead and cadmium on granulosa cells: a cellular model for ovarian toxicity. *Reprod Toxicol* 21: 179–185.
14. Piasek M, Kostial K (1991). Reversibility of the effects of lead on the reproductive performance of female rats. *Reprod Toxicol* 5:45–51.
15. Saritha S, Reddy PS, Reddy GR (2011). Partial recovery of suppressed reproduction by *Withania somnifera* Dunal in female rats following perinatal lead exposure. *Int J Green Pharm* 5: 121–125.
16. Sasidharan S; Chen Y; Saravanan D; Sundram K M; and Yoga L L. (2011) Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts. *Afr J Tradit Complement Altern Med.* 8(1):1-10
17. Schoenwolf, G.C; Bleyl, S.B.; Brauer, P.R.; Francis-west, P.H. and Philipa H. (2015). Larsen's Human Embryology (5thedn). New York; Endinburgh: Churchill Livingstone chapter 16.
18. Shah, A. Mian, M. Khan, S. Tayyab, M. Chaudary, N. Ahmed, N. (2008) Correlation of Blood Lead Levels with Atresia of Ovarian Follicles of Albino Mice. *Ann Pak Inst Med Sci.* 4:188-192.
19. Silberstein T, Saphier O, Paz-Tal O, Trimarchi JR, Gonzales L, Keefe DL (2006). Lead concentrates in ovarian follicle compromises pregnancy. *J Trace Elem Med Biol* 20: 205–207.
20. Snell, R. S. 2012, *Clinical Anatomy by regions*, Lippincott, Williams & Wilkins, London. Chapter 7: pgs 279-297.
21. Standring, S.; Ellis, H.; Healy, J. C.; Johnson, D.; Williams, A. and Collins, P. (2008), *Gray's Anatomy: the Anatomical Basis of Clinical Practice*. 40th edition: Madrid Churchill Livingstone, Chapter 77, pp1530-1720.
22. Sullivan FM. (1993). Impacts of the Environment on Reproduction from Conception to Parturition. *Env. Health Perspec:* 101(Suppl 2):13-18. Pg. 17.
23. Tang N, Zhu ZQ (2003). Adverse reproductive effects in female workers of lead battery plants. *Int J Occup Med Env* 16: 359–361.
24. Taupeau, C. Poupon, J. Nomé, F. Lefèvre, B. (2001) Lead accumulation in the mouse ovary after Treatment-Induced Follicular Atresia. *ReprodToxicol.* 15(4); Pg. 385-391.
25. Young Barbara; James S. Lowe, Alan Stevens; John O. Heath. (2013) Weathers functional Histology A text and colour atlas 6th edition. Churchill Livingstone. Chapter 19, Pg 362-375.