

The Protective And Curative Effect Of Low And High Doses Of Ethanolic Extract Of Ficus Vogelii (Fv) On Lead Acetate Induced Reproductive Toxicity Of An Adult Wistar Rats.

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ABSTRACT: Ficus vogelii is one of the locally used vegetables to cook soup. The herb has also been used as an herbal remedy for some ailments such as malaria, seizure, etc. This research aimed at finding out the effects of lead toxicity on the histology and physiology of the reproductive organs (vagina and cervix) using adult female Wistar rats and also to ascertain the role of Ficus vogelii can play in environmental lead toxicity. This research lasted for a period of 35 days involving 35 adult female Wistar rats weighting an average of 250g. The rats were randomly assigned into seven (7) groups and the groups numbered as follow 1, 2, 3, 4, 5, 6 and 7. Group 1 served as negative control which received tap water ad libitum, group 2 is positive control that received lead acetate solution, groups 3 and 5 received ethanolic extract of Fv (low dose, 100g/kg) and lead acetate solution, groups 4 and 6 received aqueous extract of Fv (high dose, 300g/kg) and lead acetate solution each while group 7 received ethanolic extract of Fv only. Microscopical examination of the organs showed various alterations which include necrosis, oedema and denudation suspected to be as a result of lead toxicity. The lead effects on the organs were either protected or cured with the administration of ethanolic extract of Ficus vogelii from these changes caused by induced lead acetate. The organs especially group 2 presented a significant reduction in blood supply which would have been the cause of mucosa necrosis. These distortions to the walls of reproductive organs (vagina and cervix) can lead to female infertility. The groups that received higher doses (4 and 6) appeared to be more vascularized suspected to be as a result of blood supply restored by the extract. The extract also helped in restoring the integrity of the walls of the lower reproductive organs. The results of this study suggest that F. vogelii leave extract can serve as an herbal remedy that can be very effective in treating reproductive toxicity especially that of lead. In conclusion, lead is very poisonous to all parts of the animal body and Ficus vogelii can go a long way to either protect or cure the organs against its deleterious effects and Ficus vogelii acts better in its high dose.

Keywords: Ficus vogelii, infertility, lead acetate, reproductive toxicity, Vagina.

Introduction

Lead, among all the heavy metals is seen as pollutant in Kosovo, and Mitrovica Region [17], this is because it does not play any physiological role in the body and considered toxic even in small doses. A study has shown that blood lead level was found to be higher than the permissible limits in the animals when they were checked under environmental conditions due to its non absorbent by the body [13], [9], [16]. It is well known that majority of lead usually enters the body through dermal contact, oral ingestion, inhalation and children are mostly affected because of their higher sensitivity to lead [5], [25]-[26]. Cases of high levels of lead exposure can be found in industrial areas and are more common in developing countries [2]. Lead affects various parts of the body including the cardiovascular, gastrointestinal, urinary, nervous, and reproductive systems [6], [25]. Even in small dose, lead causes alterations to the walls of the reproductive organs such as ovaries, uterus, cervix and vagina. The eroded cervical wall will affect the mucous secretion that helps the motility of the sperm and causes an increase in the acidity of vagina which can contribute to the death of sperm [4]. The available toxicology information about the effects of lead on the mammalian female reproductive system is sparser than the male reproductive system [6]. The differences in the effects of

lead on these systems are significant, particularly in terms of gametogenesis and the cyclic nature of the female reproductive function [1]. As a protective measure against lead for the developing fetus, European Union (EU) and United State of America (USA) enacted laws regarding lead industry workers include lower exposure criteria for women in 'reproductive capacity' with the EU having a maximum permissible limit of lead in the blood as 100mg L⁻¹, and the USA 28 mg L⁻¹[6]. With the alterations in the walls arises the need to better comprehend how vulnerable the vagina and cervix can be to lead acetate toxicity. The purpose of the research was to investigate the histological changes following 28 days of exposure to lead acetate in the vagina and cervix and also to observe the consequent role of ethanolic extract of F. vogelii.

Materials and methods

Collection and Preparation of the extract

The leaves of *F. vogelii* were collected from Federal University Ndufu-Alike Ikwo (FUNAI) in Ikwo Local Government Area of Ebonyi State. The leaves were dried in a ventilated room and thereafter, crushed into powder and passed through mesh sieve to get the fine powders. The powdered form was soaked in ethanol for 48 hours to allow for proper extraction. The mixture and the container were kept in a refrigerator for those 48 hours. The mixture was 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°C to yield a sticky paste [16]. 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

Thirty five (35) adult female Wistar rats with average weight of 250g 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 two week (7days).

Animal grouping and administration

The rats used in this experiment were randomly assigned into seven groups as follow: 1, 2, 3, 4, 5, 6 and 7 containing five (5) animals per group. All the rats received standard rat's diet containing 0.5% NaCl, 22% protein and 4-6% dietary fat and tap water ad libitum. The extracts and lead acetate solution were administered by oral intubation using oral gavage (cannula). Group 1 received only water ad libitum, Group 2 received 2mg/kg of Lead acetate solution daily for 14days which served as the positive group. Group 3 and 5 received lead acetate (2mg/kg) for 21days and 24 hours later treated with ethanolic extract of *F. vogelii* (100g/kg) for the next 7 days. Group 4 and 6 received lead acetate (2mg/kg) for 21days and 24 hours later treated with ethanolic extract of *F. vogelii* (300g/kg) for the next 7days. Group 7 received 300mg/kg of ethanolic extract of *F.vogelii* only daily for 21days. The experiment lasted for a period of 35days.

Histological Study

After thirty (35) days, the animals were starved overnight and anaesthetized with chloroform and decapitated before sacrificing them for histological studies [20]. The rats were dissected and the organs (vagina and cervix) were harvested and quickly fixed in bouin's fluid for normal histological examinations. The tissues were processed, embedded in paraffin wax and 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 reproductive organs (vagina and cervix) of the adult female

Wistar rats' at the end of 35days are presented in the histological slides below.

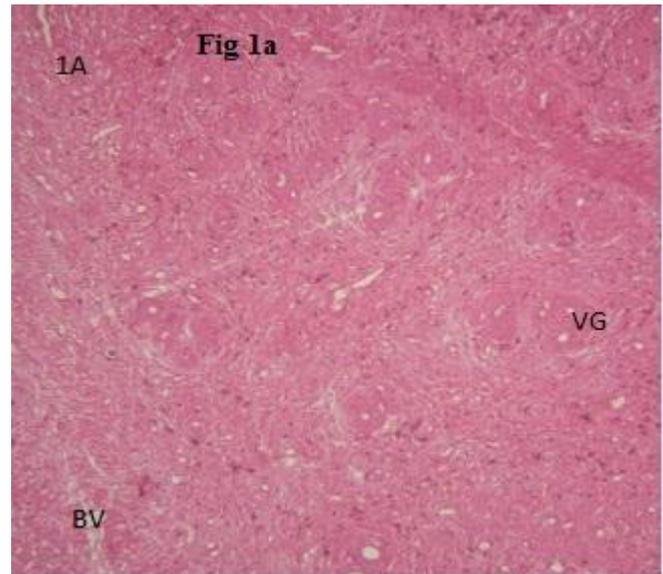


Fig.1 (a) vagina of negative control group showing the presence of a NM-normal mucosa, BV-Blood vessel and VG-Vagina gland.

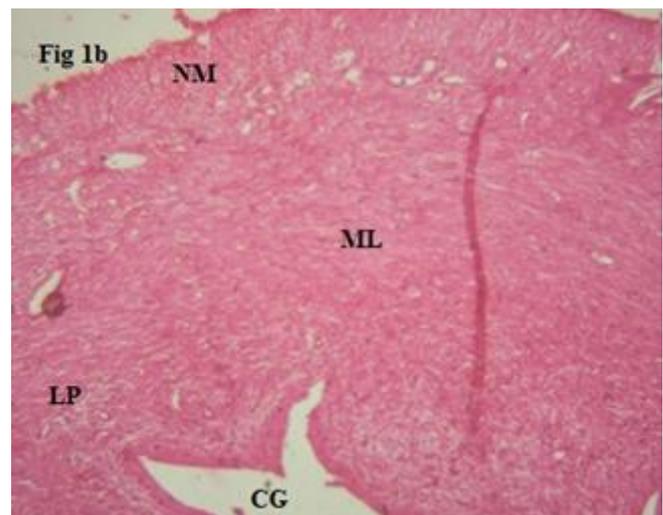


Fig.1 (b) The cervix of negative control group showing the presence of NM-normal mucosa, ML- Muscular layer, LP- Lamina propria and CG-Cervical gland.

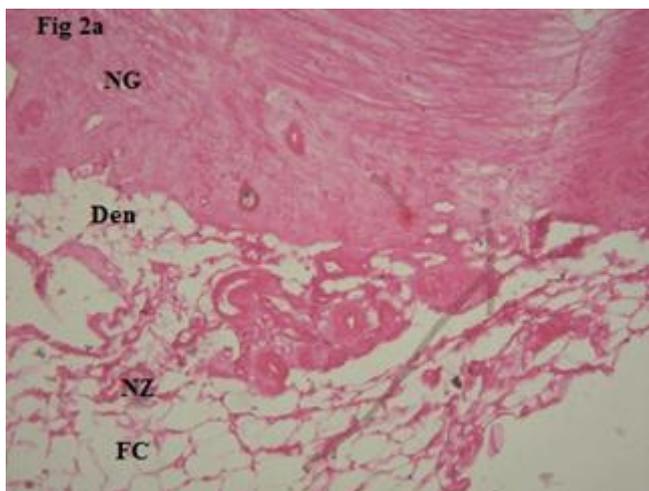


Fig.2 (a) Vagina following administration of lead acetate (2mg/kg) presents NG-necrotic gland, Den-denudation, NZ-necrotic zone and FC-Fatty change.

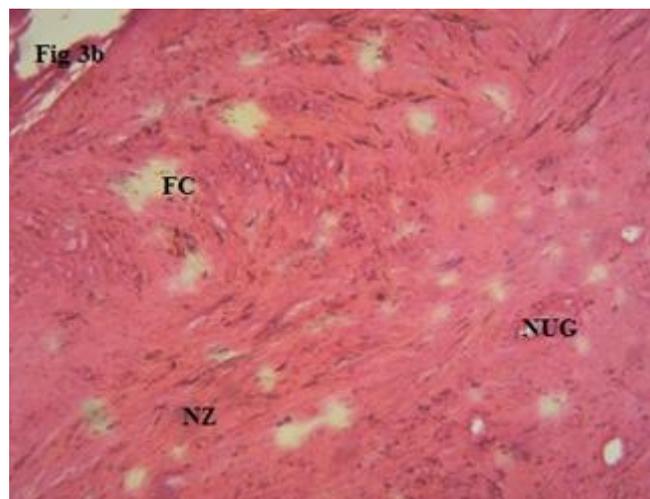


Fig.3 (b) Cervix following administration of lead acetate (2mg/kg) and 100mg/kg of extract presents FC-Fatty change, NZ-necrotic zone and NG-necrotic gland.

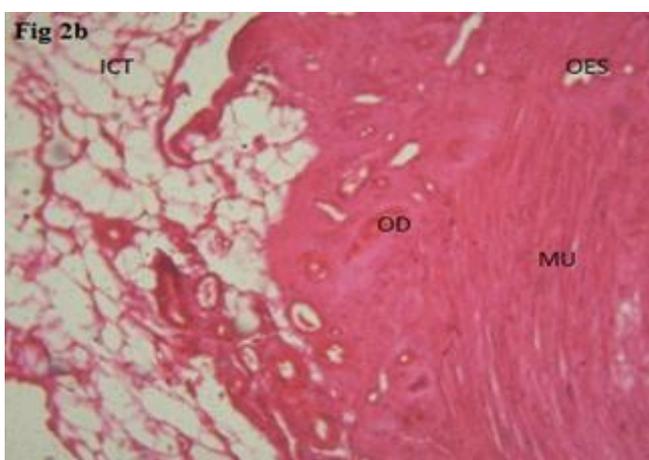


Fig.2 (b) Cervix following administration of lead acetate (2mg/kg) presents OD-Oedema, FC-Fatty change, ICT-Interconnective tissue, MU-Muscle and OES-Optically empty space.

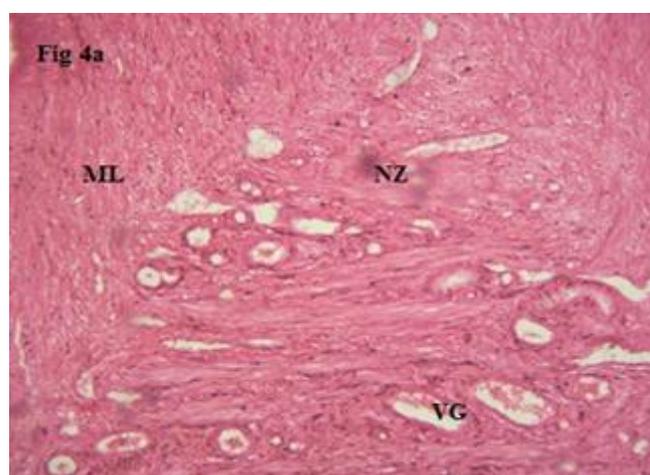


Fig.4 (a) Vagina following administration of lead acetate (2mg/kg) and 300mg/kg of extract presents ML-muscular layer, NZ-necrotic zone and VG-vaginal gland.

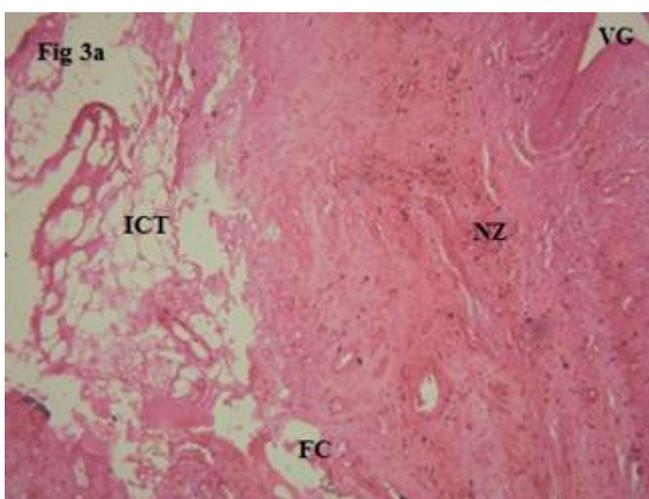


Fig.3 (a) Vagina following administration of lead acetate (2mg/kg) and 100mg/kg of extract presents ICT-Interconnective tissue, NZ-necrotic zone and NG-necrotic gland.

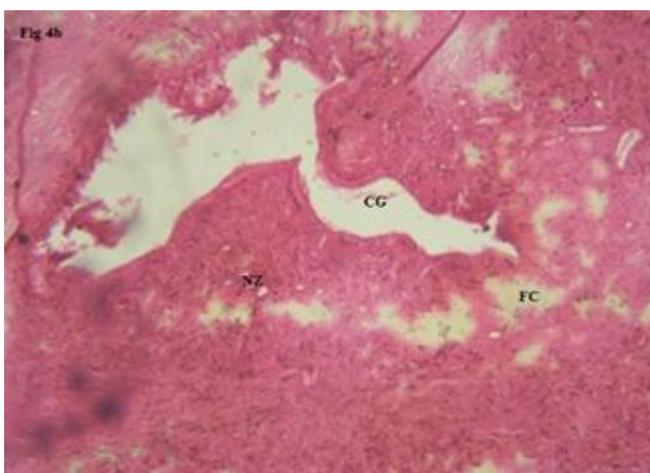


Fig.4 (b) Cervix following administration of lead acetate (2mg/kg) and 300mg/kg of extract presents CG-cervical gland, NZ-necrotic zone and FC-fatty change.

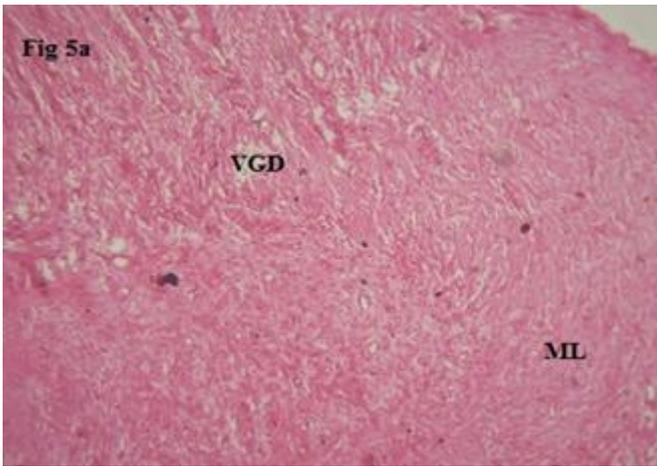


Fig.5 (a) Vagina following administration of 100mg/kg of extract and lead acetate (2mg/kg) presents VGD-vaginal gland denudation and muscular layer.

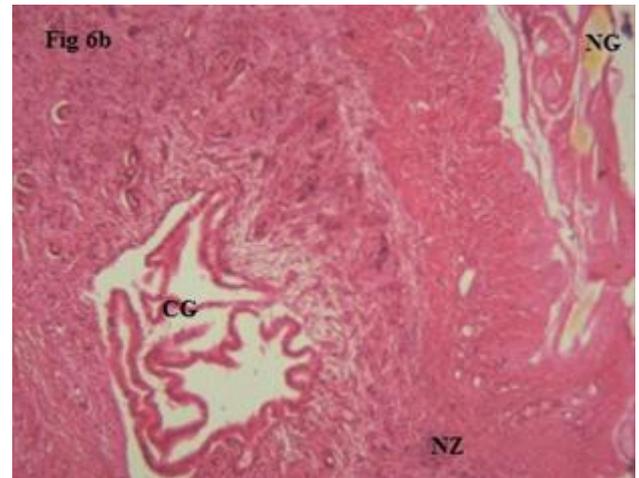


Fig.6 (b) Cervix following administration of 300mg/kg of extract and lead acetate (2mg/kg) presents CG-vaginal gland, NZ-necrotic zone and NG-necrotic gland.

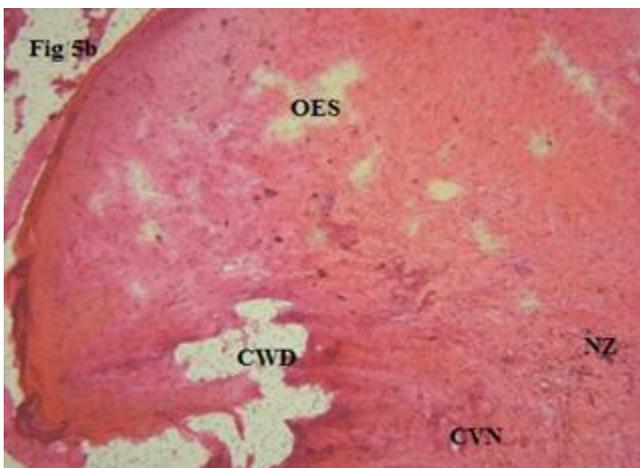


Fig.5 (b) Cervix following administration of 100mg/kg of extract and lead acetate (2mg/kg) presents OES-optical empty space, CWD-cervical wall denudation, CGN-cervical gland necrosis and NZ-necrotic zone.

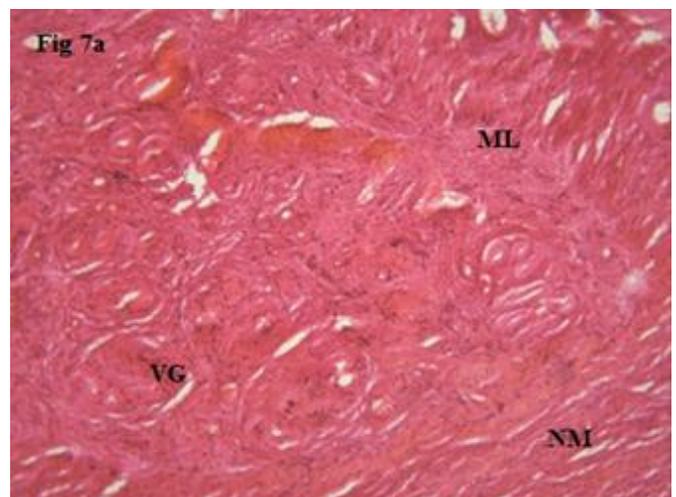


Fig. 7 (a) Vagina following administration of 300mg/kg of extract presents ML-muscular layer, VG-vaginal gland and NM-normal mucosa.

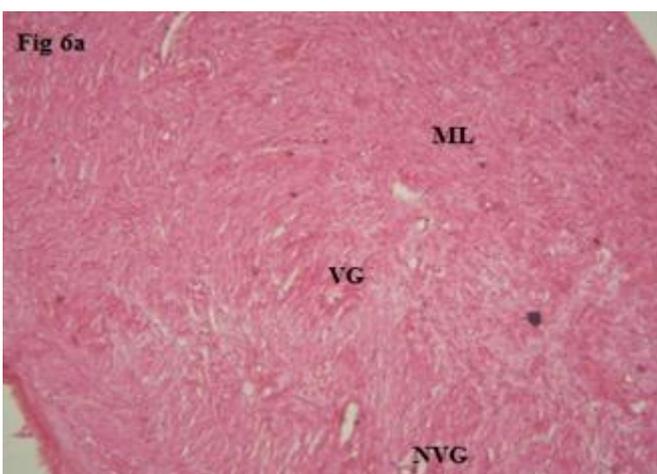


Fig.6 (a) Vagina following administration of 300mg/kg of extract and lead acetate (2mg/kg) presents VG-vaginal gland, NVG-necrotic vaginal gland and muscular layer.

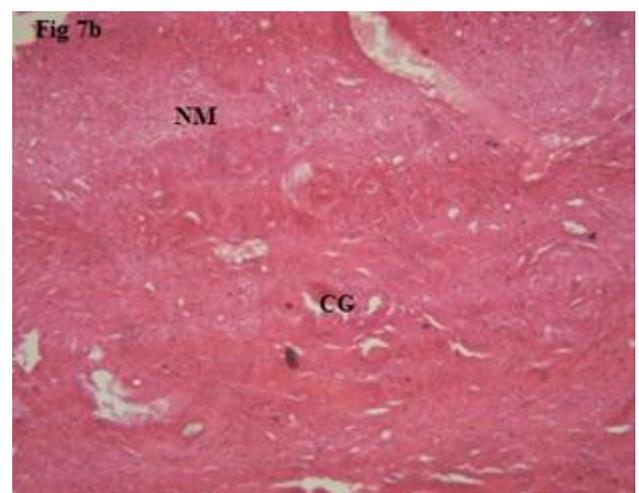


Fig.7 (b) Cervical wall following administration of 300mg/kg of extract presents NM-normal mucosa and CG-cervical gland.

Discussion

Reproductive biologists have focused mainly on male reproductive systems toxicity, and even the few that consider the female reproductive system toxicity have concentrated on the ovarian, uterine and fallopian tubes lead toxicity or generally on fertility without considering the lower reproductive system (vagina and cervix). Studies on the vagina or cervix are sparse or even rare, yet so many authors have identified the distortions caused by the presence of lead in the ovaries, fallopian tube, and uterus [6]. This strongly suggests that lead in the body is linked to altered reproductive function [15], [22], [24]. *Ficus vogelii* has been implicated by many authors as an herbal remedy [7] and used locally as anti-malaria¹⁰. Some people with their unconfirmed reports also suggested that it may be used as an anti-seizure herb. The dearth of information concerning the effects of *F. vogelii* as anti-toxicity makes works on reproductive (vaginal and cervix) toxicity rare to come by [14], [22], [24]-[25]. There were serious alterations to the histology of the reproductive organs integrity under consideration (vagina and cervix) which may hamper female reproduction. The toxic effects of lead might have been cushioned by the protective power of the extract. Lead toxicity significantly affects the formation of red blood cells [6], [12] and most systemic impairments such as the nervous, the urinary, the reproductive and every parts of the body as reported in other literatures show the hazardous and versatile effects of lead in mammals [3], [8], [11], [14], [18], [121]. It can be noted that the effects of lead on reproductive systems are complex and sex-specific, and involves multiple locations on the hypothalamic–pituitary–gonadal axis [23]. Microscopical examination showed visible alterations (distortions) to various parts of the organs. These distortions ranged from necrosis, diffused oedema, fatty changes, vaginal glands necrosis, vaginal wall denudations and optically empty spaces. Those changes were more prominent in the positive control group (fig. 2a) which received only lead acetate (2mg/kg). There were positive changes to the integrity of the vaginal wall following administration of the ethanolic extract especially the high doses (300mg/kg) of both the protective and curatives as presented in figures 4a and 6a above. The extract was effective in the low (100mg/kg) dosage as seen in figures 3a and 5a above, but not as effective as the high dose which looked more vascularized may be due to the restoration of the organs blood supply [25]. The changes that were seen in the cervix is not different from that of vagina especially the positive control group (fig. 2b) that received only lead acetate (2mg/kg) while the negative control group (fig. 1b) showed a normal histology of the uterine cervix mucosa and its glands without alterations. After the animals were treated with the extract of *Fv* (curative groups), there were signs of recovery shown in the cervical walls [25] as seen in figures 3b (low dose, 100mg/kg) and 4b (high dose, 300mg/kg). The protective groups (5 and 6), showed less distortions due to the effective role of the extract in preventing damages to the organs as seen in figures 5b (low dose, 100mg/kg) and 6b (high dose, 300mg/kg). The group 7 that received only the extract looked healthier than the negative control group which received water ad libitum and feed. This could be one of the proof that *F. vogelii* leaves are good the body. The histo-architectural changes as observed in the present research demonstrated the deleterious effects of lead to health even the reproductive systems.

Conclusion

Lead is very poisonous to all parts of the animal body and *Ficus vogelii* can go a long way in protecting the organs against its deleterious effects. It can also cure the injuries inflicted on the organs as seen above. The *Ficus vogelii* works better in its high dose.

Recommendations

Having concluded this research with good results, we wish to recommend the use of *F. vogelii* leave as an herbal remedy in treating reproductive toxicity especially that of lead. More so, researchers should balance the attention they have paid to male reproductive system with that of female.

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