

Evaluation of Extract Fractions of *Vernonia calvaona* on Hormonal Parameters and Histopathological Changes in Male Albino Wistar Rats Exposed to Heavy Metals Laden Water

Emmanuel Peter Ufort^{1*}, A.A. Uwakwe¹, E.B. Essien¹

¹Department of Biochemistry, University of Port Harcourt, Choba Rivers State, Nigeria.

How to cite:

Ufort et al. 2023. "Evaluation of Extract Fractions of *Vernonia calvaona* on Hormonal Parameters and Histopathological Changes in Male Albino Wistar Rats Exposed to Heavy Metals Laden Water". *Journal of Applied Health Sciences and Medicine* 3(6): 28 – 46.

<https://doi.org/10.58614/jahsm364>

Received:

May 25, 2023

Accepted:

June 18, 2023

Published:

June 28, 2023

Corresponding author:

Emmanuel, Peter Ufort
ufort.peter@gmail.com

Abstract: Antioxidative potential of *Vernonia calvaona* on heavy metals induced toxicity in male albino wistar rats. thirty (30) albino rats weighting 90-120g were divided into six groups of five rats each. With the exception of the normal control (NC) group, all other groups were exposed to heavy metals laden water twice daily. Groups 1 and 2 served as normal (NC) and Positive control (PC) controls. Groups III were administered heavy metals laden water and vitamin C (0.0167g/g.b. w). Groups IV were administered heavy metals laden water and 400mg/kg b.w of aqueous crude extracts of *Vernonia calvaona*. Group V were administered the heavy metals laden water and 400mg/kg b.w of methanol extracts of *Vernonia calvaona*. Group VI were administered heavy metals laden water and 400mg/kg b.w of n-Hexane fraction of *Vernonia calvaona*. Administration of treatment was done once daily for a period of 30 days. Rats were sacrificed and samples collected for analysis. There was increase ($p < 0.05$) in the concentration of testosterone in PC group (3.60mg/ml) when compared to NC (1.80mg/ml). Groups III, IV, VI, showed significant ($P > 0.05$) decreases in serum testosterone concentration when compared to PC group. There was increase in the conc. Of PSA in the PC group II, when compared to the NC. FSH Conc in group II compared to NC groups show a significant decrease. Groups III showed an ($P > 0.05$) increases in serum FSH concentration when compared to PC group. The PC group showed decreases in LH concentration compared to Normal Control. In conclusion, the changes observed in this study revealed that heavy metals adversely affected the gonads, causing alterations in the levels of major hormones. Furthermore, the extracts were able to exert varied degrees of ameliorative effects with the crude extract showing greatest potential at managing the heavy metals induced irregularities, and at reversing testicular damage.

Keywords: heavy metals, testosterone, luteinizing hormone, follicle stimulating hormone, testes, vernonia calvaona, histopathological indices.

1. Introduction

The physical, biological, and chemical components of the atmospheric system can be contaminated in such a way as to interfere with regular environmental processes. Additionally, it refers to environmental contamination that negatively impacts living



© 2023

by the authors. The terms and conditions of the Creative Commons Attribution (CC BY) licence apply to this open access article

things. The extraction of various important minerals from subsurface deposits is a time-honored technique that has been used for a very long time. Many quarried materials were employed by the ancient Egyptians and Romans to build the pyramids, temples, and monuments that are still standing today. Numerous other human activities also had a negative impact on the ecosystem. However, these activities produce a significant amount of particle pollution, which can be harmful to animals, plants, and water. The majority of these substances build up in living things' body tissues, and their concentration rises as they move from the lower tropics to the higher tropics (referred to as "bio magnifications") of the ecosystem (Langer and Bode, 2011).

During their production, transportation, and sale, heavy metal emissions from industry and vehicles may be deposited on the surfaces of plants. High quantities of heavy metals have also been found in plants, according to AL-Jassir et al. (2005) (Sharma et al. 2008). When heavy metal concentrations in plants and animals reach a particular level, they are considered hazardous. Despite playing vital roles in plant and animal cells, heavy metals like cobalt, copper, iron, molybdenum, nickel, and zinc can be extremely harmful when their concentrations surpass a predetermined level (WHO acceptable limit) (IOSHIC, 1999; Klaus, 2010). The amount of heavy metals in the soil varies according to its age, composition, and location, according to studies (Udosen et al. 1990; Haluschak et al. 1998; Odukoya et al. 2000).

Lead, zinc, and cadmium concentrations in all the vegetables analysed above the maximum allowable concentration, according to another study on the assessment of heavy metals concentration in urban-grown vegetables in Thikakown, Kenya. In accordance with the World Health Organization's acknowledged human health limit of 0.3 mg/kg, lead concentration in vegetables was reported to be over that level (WHO, 2004). In rural areas across Africa, Asia, and South America, medicinal plants are an essential part of the traditional healthcare systems. According to statistics, many people use medicinal plants, and around 80% of those in developing nations rely directly or indirectly on traditional medicine to treat the majority of their medical issues (Bodeker 1999; Joyce 1992; King 2000; WHO 2000). It is also well known that more than 75% of Nigerians living in rural areas rely on traditional medications made from herbal remedies for their medical requirements (Tolu, 2008). Traditional medicines based on herbal cures have long been a significant part of the healthcare systems of many African nations, including Nigeria, since the dawn of civilization.

The creation of medications in both poor and developed nations can benefit from research on medicinal plants. More than half of the population of Africa today relies on animal and plant-based medicine to meet their health needs (Ainslie, 1973). In complementary or alternative medicine, a variety of herbs with hypolipidemic, hypoglycemic, antiplatelet, antimalarial, antiviral, or immune stimulating and modifying activities may be helpful (Borchers et al. 1997). It is important to be aware that plants have long been thought of as sources of therapeutic substances for the treatment of infections and disorders like chicken pox and hepatitis (Bramstedt, 2006). The indigenous inhabitants of the Central Senatorial District of Cross River State, Nigeria, commonly refer to *Vernonia calvaona* as "ekeke leaves" (Igile et al. 2013). It is a little shrub that grows to less than one metre in height, with petiolate leaves that

are about 10mm wide. It is utilised both as an ethnomedical remedy and as a green leafy vegetable (Igile et al. 2013). With or without palm oil, it is a favourite local delicacy to be consumed raw or fresh. Among native customers, it also forms part of a classic salad. Additionally, it can be used to make native soups, stews, and porridge with potatoes, yams, and plantains (Igile et al. 2013). Its use is justified by the notion that the entire plant treats heart conditions, blindness, diabetes, malaria, and functions as an anti-helminthic agent. In South Western Cameroun and the South Eastern region of Nigeria, the plant is widely dispersed, just like its near cousins *V. teniana* and *V. amygdalina*. Although these three species share many morphological characteristics, it is possible to distinguish between them based on specific anatomical characteristics, such as the height and width of the leaves and the potency of the plant's bitter flavour. *Vernonia calvaona* is the least bitter and has the shortest height of the three species (Igile et al. 2013). In order to assess the biochemical safety and antioxidative potential of *Vernonia calvaona*'s crude extract (CE) and extract fractions, as well as their impact on male sex hormones and histopathological changes in male albino wistar rats fed with heavy metal-polluted water, the study examined CE and extract fractions of *Vernonia calvaona*.

2. Materials and Methods

The following tools, supplies, and chemicals were needed for this study; all of the chemicals utilised were of analytical quality. Dimethyl Sulphur Oxide (DMSO), distil water, cheese cloth, water bath, syringes, EDTA bottles, cages, electronic weighing scale, wood shavings, glass beakers, and methanol were all obtained at a chemical store in Yeagona, Bayelsa State. Syringes (2ml and 5ml), containers for collecting blood samples, and all of these items were purchased from a chemical store in Yeagona, Bayelsa state. The United States' Sigma produced hematoxylin and eosin. FSH, LH, and testosterone hormone testing kits were bought from the mono-bind company in Lake Forest, USA. The Niger Delta University's department of pharmacology's animal house was where the experimental animals were purchased. The laboratory, Divic Medical Laboratory Choba, Port Harcourt, Rivers State, provided the micropipettes, centrifuge, pasteur pipette, refrigerator, atomic emission spectroscopy, dissecting equipment, dissecting board, aluminium foil, methylated spirit, dettol, and gloves that were used for the analysis.

2.1. Collection of Plant Sample

The *Vernonia calvaona* leaves were gathered from a nearby farm in Nigeria's Bayelsa state's Ogbia Local Government Area. A botanist from the Faculty of Science, University of Calabar, Cross Rivers State, Nigeria, validated the plant. Additionally, it matched a specimen that had already been deposited in the department's herbarium (BCH/VC/01). The plant portion (leaves) was thoroughly rinsed with distil water after being thoroughly washed with tap water. After that, the plant material was spread out to air dry for a week.

2.2. Preparation of Material

Using an electronic blender, dry *Vernonia calvaona* leaves were reduced to a fine powder. The powder was measured using an electronic balance and then soaked in a mixture of 80% n-hexane and 20% methanol. For 48 hours, the mixture is stirred with a magnetic stirrer at a controlled temperature of 4–8 °C. Cheese cloth and then filter paper were used to double-filter the extract. A rotary evaporator was used to concentrate the filtrate at a controlled temperature of 40 to 50°C. Finally, the extract was evaporated in a water bath.

At the Niger Delta University in Bayelsa State, Nigeria, the Department of Pharmacology animal house provided thirty (30) albino Wistar rats with weights ranging from 90 to 120g. The Institute for Laboratory Animal Research (ILAR) established standard guidelines for the housing and care of the thirty (30) rats.

The purchased animals were given seven days to adjust at the animal house. They were housed in wire-mesh coverings over plastic cages to improve ventilation. The animals were kept in an environment with a controlled 28 ± 2 °C temperature, 50 ± 5% relative humidity, and a 12-hour light/dark cycle. The animals were housed in an appropriately ventilated animal facility, fed commercial rat pellets, and given unlimited access to water during the trial.

2.3. Experimental Design and Treatment of Animals

Orogastric intubation was used to administer the medication twice daily for a total of 30 days. Thirty (30) albino rats were used in the experiment. Six groups of five rats each were formed from the rat population. Rats in Group 1M, the usual control group, received placebo injections. Animals in Group II M, the positive control group, were given placebo water and water contaminated with heavy metals. Group III M received vitamin C and water contaminated with heavy metals. Treatment for Groups IV M included the administration of water contaminated with heavy metals and crude *Vernonia calvaona* preparations. The heavy metal-filled water and methanol extracts of *Vernonia calvaona* were given to Group V M. Group VI M received water contaminated with heavy metals as well as *Vernonia calvaona*'s n-Hexane fraction.

Table 1. Animal Grouping and Treatments

Experimental groups	Number of animals	Treatments
Group 1M (Normal Control)	5	Normal Saline
Group IIM (Positive Control)	5	Heavy metals laden water +Normal Saline
Group IIIM (VIT. C)	5	Heavy metals laden + Vitamin C
Group IV M (V.C.)	5	Heavy metals laden water + Crude Extract V. C. (400mg/kg b. w. of animal)
Group V M (Methanol)	5	Heavy metals laden water +Methanol Fraction of V. C.
Group VI M (n-Hexane)	5	Heavy metals laden water +n Hexane fraction of V. C

NC-Normal Control, PC-Positive Control, CE-Crude Extract, b.w- body weight, n-HEX=n-Hexane.PSA=Postrate Specific Antigen.VC=*Vernonia Calvaona*.

2.4. Collection of Blood Samples for Analysis

The animals were killed 12 hours after the previous dose, and entire blood was extracted from the heart using a sterile syringe and needle during a cardiac puncture. The blood samples were placed in both standard tubes and tubes containing lithium heparin. The blood samples in the plain tubes were centrifuged with the blood samples in the lithium heparin tubes at 4000 rpm for 10 minutes to separate the serum from the red blood cells after being allowed to clot for two hours at room temperature. Using pasteur pipettes, serum was collected into another plain tube with the appropriate label from each centrifuged plain tube and lithium heparin tube. When required for various biochemical experiments, the distinct sera were then kept frozen in a refrigerator.

Serum Hormonal Assay

Estimation of the concentration of the Biochemical (hormonal) parameters were carried out by ELISA methods using analytical grade laboratory kits from ACCU-BIND as described below

2.5. Determination of Serum Testosterone Concentration

The determination of testosterone was carried out using a competitive enzyme immunoassay approach. An antibody, an enzyme-antigen conjugate, and a native antigen are needed for the determination. The native antigen and the enzyme antigen conjugate compete for a finite number of antibody binding sites when biotinylated antibody, enzyme antigen conjugate, and a serum containing the native antigen are combined.

2.6. Determination of Serum Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) Concentration

An immune-enzymometric assay was employed to measure the levels of serum FSH and LH, and for this purpose, antibodies with high affinity and specificity and separate and distinct epitopes to which an antigenic material binds were used. An immobilisation that occurs at the surface of a streptavidin-coated micro well with exogenously supplied antibody serves as the catalyst for the reaction. When monoclonal biotinylated antibody is combined with enzyme-labeled antibody and serum that contains native antigen and antibodies without steric or competitive interference, a sandwich complex is created.

The high affinity reaction between streptavidin and the biotinylated antibody produced a complex for each hormone that was determined in this study and was then deposited on the micro wells. The antibody-bound fraction is separated from unbound antigen by decantation or aspiration after equilibrium is reached. Each absorbance is evaluated by a microplate reader, and the enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration (Lashansky, 1991). Dose response curves were produced using a number of different serum references with known antigen concentrations, and from these curves extrapolation was used to calculate the antigen content of unknown samples (Odel, 1981).

Data and Statistical Analysis

Data obtained were analyzed using SPSS package version 20 and statistical significance measured by One-way Analysis of variance (ANOVA) with a post hoc Donnett value at $P < 0.05$. Charts were plotted using Microsoft excel 13. All data were expressed as Mean $\pm$ SEM, $n=5$.

3. Result

3.1. Acute toxicity test

The WHO permissible level was compared to the anticipated total mean of cadmium, lead, iron, nickel, and cobalt. Except for copper, mercury, and chromium, the concentration of heavy metals was substantially ($p > 0.05$) below the WHO acceptable level. All other heavy metals concentrations were significantly ($p < 0.05$) over the WHO allowable limit.

Table 2. Heavy Metals Analysis From Underground Water Sample (ppm)

Heavy Metals	Iron	Chromium	Cadmium	Nickel	Copper	Lead	Cobalt	Mercury
UW Values	3.8261 $\pm$ 1.05	0.0434 $\pm$ 0.00	0.0456 $\pm$ 0.00	0.8395 $\pm$ 0.00	0.0924 $\pm$ 0.00	0.0585 $\pm$ 0.00	0.1226 $\pm$ 0.00	0.002 $\pm$ 0.00*
WHO Values	0.300 $\pm$ 0.00*	0.05 $\pm$ 0.00	0.003 $\pm$ 0.00*	0.07 $\pm$ 0.00*	2.00 $\pm$ 0.01*	0.01 $\pm$ 0.00*	0.003 $\pm$ 0.00	0.006 $\pm$ 0.00*

UW: Underground water

WHO: World Health Organization.

*Significant compared with UW @ $p < 0.05$

Whilst the Ld_{50} of *vernonia calvaona* have been determined by previous studies to be greater than 5000mg/kg body weight (Iwara et al. 2015).

4. Effect of Heavy Metal Laden Water, Vitamin C, Crude Extract and Its Fractions on Serum Hormonal Parameters

4.1. Hormonal Profile

Follicle Stimulating Hormone (FSH)

Results reveal that serum concentration of FSH for animals in group PC (2.95 $\pm$ 0.005) and group n-Hexane (2.70 $\pm$ 0.20) were significantly ($P < 0.05$) lowered compared with that of group NC (4.45 $\pm$ 0.45) while those of group VIT C (4.15 $\pm$ 0.25), caused an increase in the levels of the hormone concentration when compared to that of the normal control. Whereas results for the VC (6.05 $\pm$ 0.55), Methanol (5.00 $\pm$ 0.00) treated groups were all significantly ($P < 0.05$) higher compared with that of group PC (2.95 $\pm$ 0.05).

Leuteinizing Hormone (LH)

The serum LH concentration of animals in group PC (1.45 $\pm$ 0.44) was significantly ($P < 0.05$) decreased compared with that of Normal Control (NC) (6.05) while those of groups VIT C (4.60 $\pm$ 0.20), VC (5.65 $\pm$ 2.55), methanol (4.80 $\pm$ 0.10) and n-hexane (4.10 $\pm$ 0.62) were all significantly ($P < 0.05$) higher compared with that of group PC (1.45 $\pm$ 0.44).

Testosterone

The serum testosterone concentration of animals in group PC (3.60 ± 0.00) and n-Hexane (2.90 ± 0.20) were significantly ($P < 0.05$) higher compared with that of group NC (1.80 ± 0.40) while other groups compare well with that of group NC. Crude extracts (CE) of *Vernonia calvaona* (VC) showed the best ameliorative potentials in normalizing the testosterone concentration levels.

Prostrate Specific Antigen

The serum concentration of PSA in group PC was significantly higher when compared to NC. Crude Extract (1.05 ± 0.15) Vit C and Methanol (1.55 ± 0.85) treated groups were significantly ($P < 0.05$) decreased compared with that of group NC (3.40 ± 0.40). n-Hexane treated group show a significantly increase in PSA conc level when compare to the NC Group.

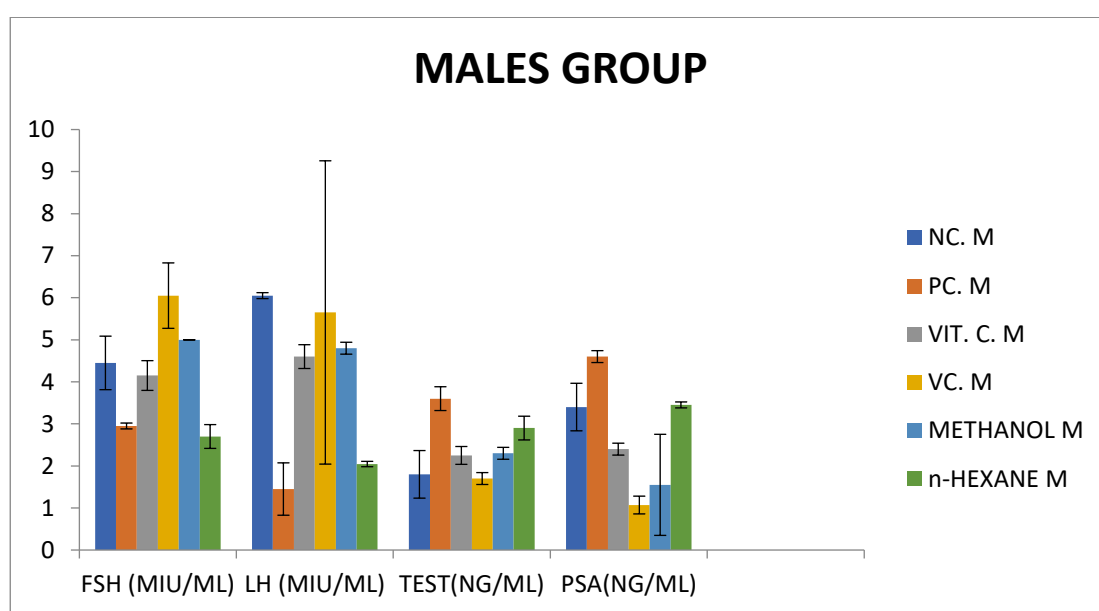


Figure 1. Males group

Effect of Treatment on Histology of Testes

Testis with dispersed seminiferous tubules and uneven basement membranes (BM) containing disorganised loosely atrophic spermatogonia cells were visible in histopathological photomicrographs of testes sections for the group exposed to heavy metal-laden water. Photomicrographs of the heavy metal exposed water exposed rats treated with n-hexane and crude extracts of *vernonia calvaona* showed partial recovery in spermatogenesis, but much better recovery was observed for the vitamin C and methanol group. Testes from normal control rats had closely packed seminiferous tubules lined by developing spermatogonia cells at various stages of development.

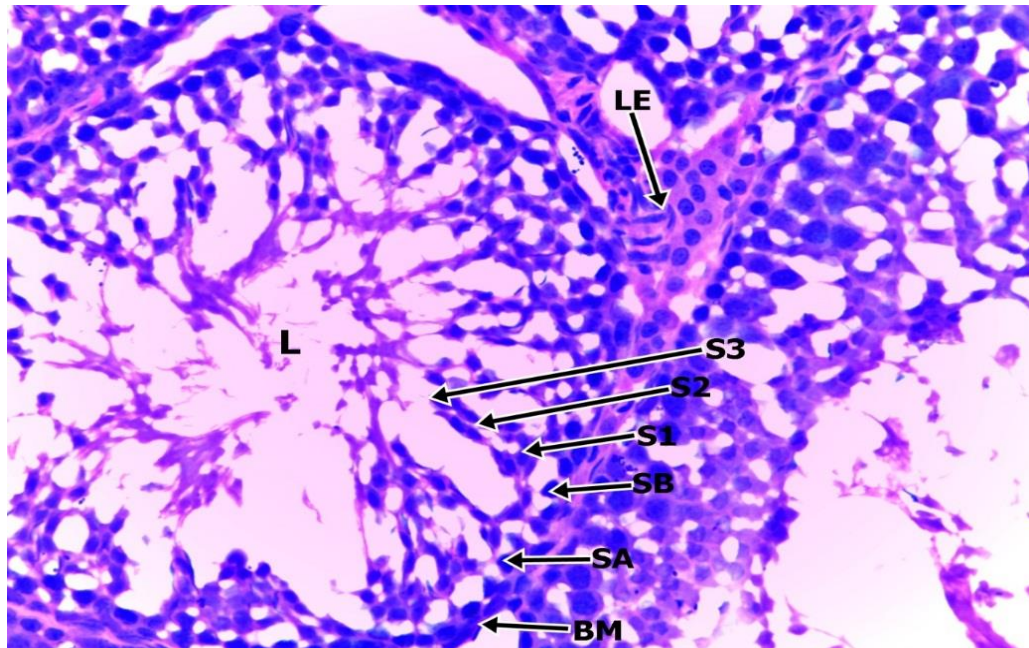


Figure 2. Photomicrographs (x 400) of Testis of Normal Control rats given placebo treatment (Stained with Gomori Aldehyde Fuschin stain).

Intact basement membranes (BM) can be seen in sections of the testis, along with tightly packed seminiferous tubules that contain three to five layers of spermatogonia in various stages of development that are held in place by sertoli cells. The cytoplasm is mild, and the cells feature round to oval basophilic nuclei. The leydig cells in the interstitial space between them are tightly packed and sparse. Spermatogonia A and B, primary spermatocyte (S1), secondary spermatocytes (S2), and spermatid (S3) made up the cell. Stem cells SA and SB; spermatogonia cells S1, S2, S3, S4; basement membrane BM; leydig cells LE; lumen.

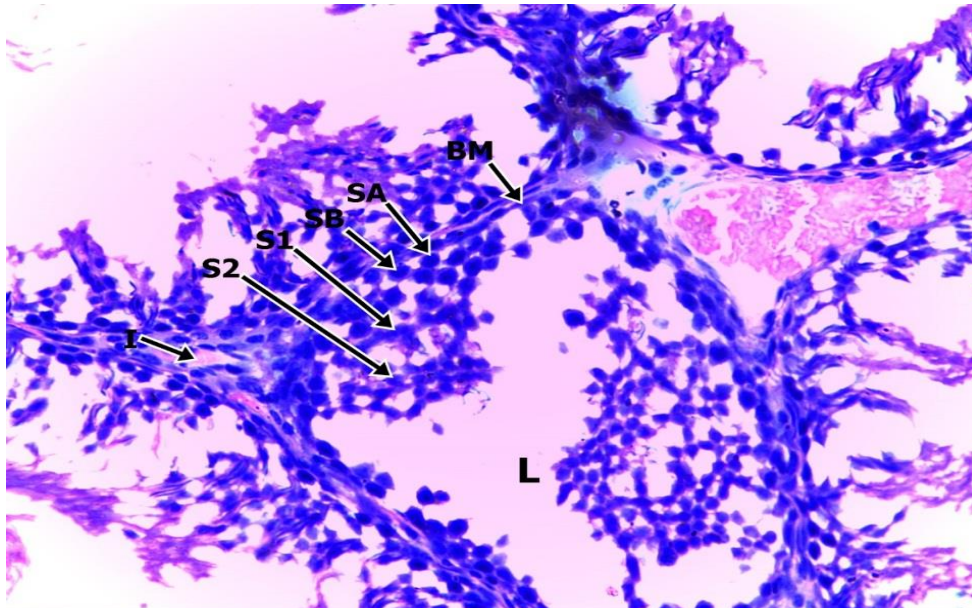


Figure 3. Photomicrographs (x 400) of Testis of rats exposed to heavy metal laden water (PC group) and given placebo treatment (Stained with Gomori Aldehyde Fuschin stain).

In a section of the testis, spermatogonia cells are scattered and loosely atrophic, with disorganised seminiferous tubules and uneven basement membranes (BM). The cells' nuclei are spherical and basophilic. The interstitial space in between is thin and packed with leydig cells, which have clogged blood arteries. Spermatogonia A and B, primary spermatocyte (S1), secondary spermatocytes (S2), and spermatid (S3) made up the cell. Stem cells SA and SB; spermatogonia cells S1, S2, S3, S4; basement membrane BM; leydig cells LE; lumen.

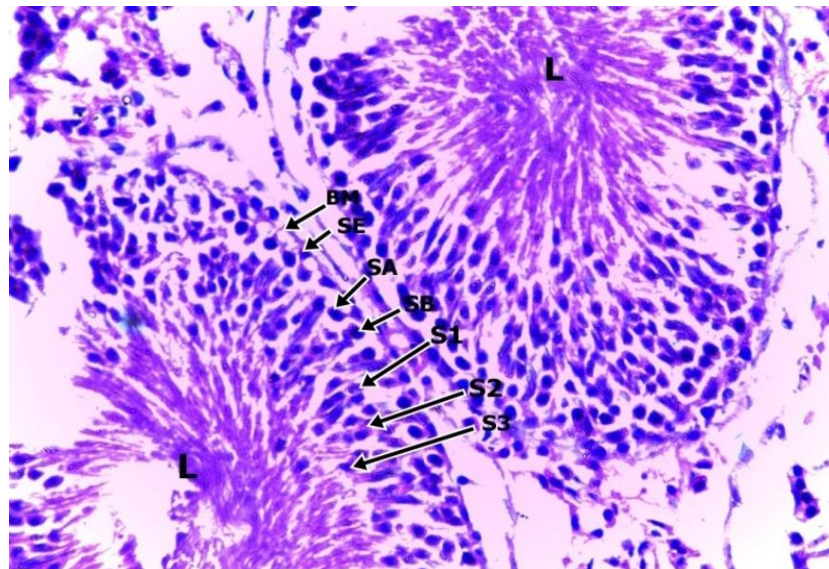


Figure 4. Photomicrographs (x 400) of Testis of rats exposed to heavy metal laden water and treated with Vitamin C. (Stained with Gomori Aldehyde Fuschin stain).

Intact basement membranes (BM) are seen in well-packed seminiferous tubules in sections of the testis, which contain three to layers of spermatogonia in various stages

of development that are held in place by sertoli cells. The cytoplasm is mild, and the cells feature round to oval basophilic nuclei. The leydig cells in the interstitial space between them are tightly packed and sparse. Spermatogonia A and B, primary spermatocyte (S1), secondary spermatocytes (S2), and spermatid (S3) made up the cell. Stem cells SA and SB; spermatogonia cells S1, S2, S3, S4; basement membrane BM; leydig cells LE; lumen.

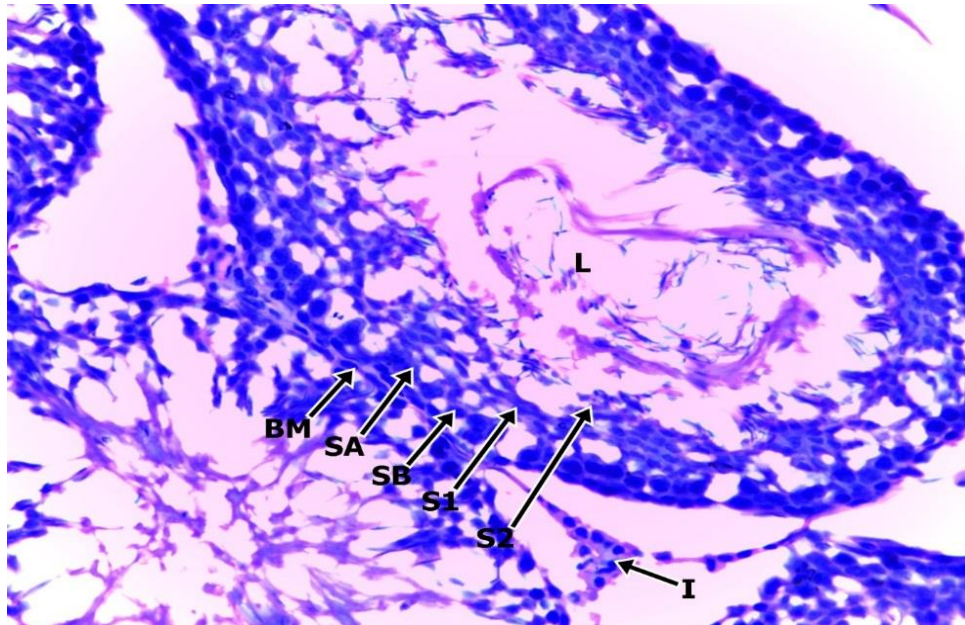


Figure 5. Photomicrographs (x 400) of Testis of rats exposed to heavy metal laden water and treated with Crude Extract of *Vernonia calvaona*. (Stained with Gomori Aldehyde Fuschin)

A section of the testis reveals tightly clustered, atypical seminiferous tubules with intact basement membranes (BM), less than five layers of compact spermatogonia cells, which are kept in place by sertoli cells, at various stages of development. Spermatogonia cells are sparsely distributed within the lumen. There are only a few tightly packed leydig in the interstitial in between. The leydig cells in the interstitial space between them are tightly packed and sparse. Spermatogonia A and B, primary spermatocyte (S1), secondary spermatocytes (S2), and spematid (S3) made up the cell. Stem cells SA and SB; spermatogonia cells S1, S2, S3, S4; basement membrane BM; leydig cells LE; lumen.

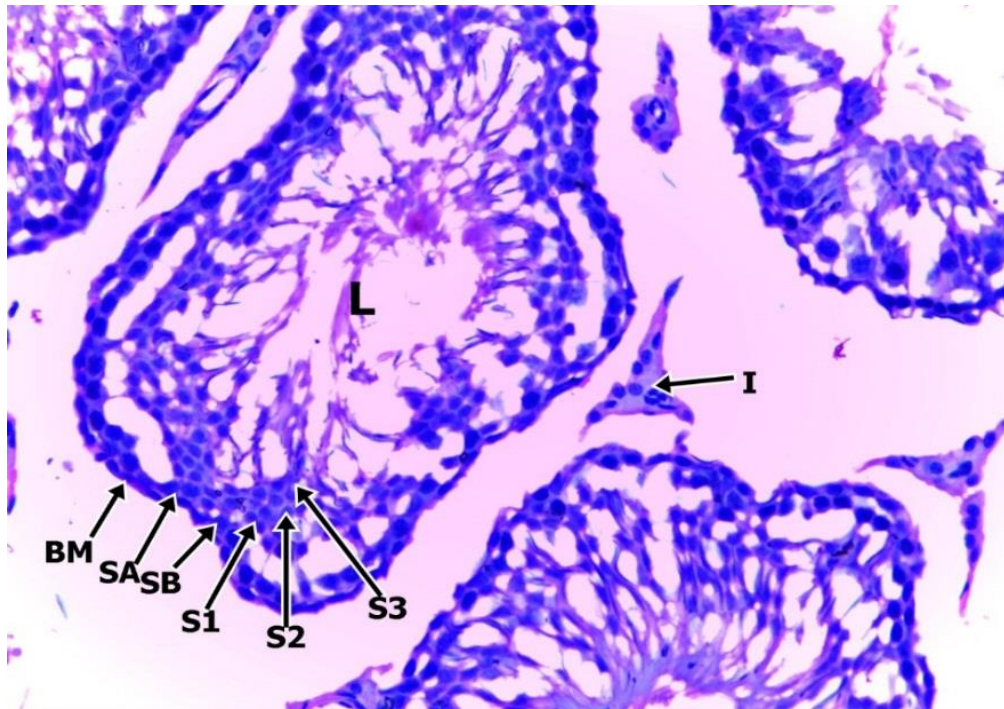


Figure 6. Photomicrographs (x 400) of Testis of rats exposed to heavy metal laden water and treated with Methanol fraction of *Vernonia calvaona*. (Stained with Gomori Aldehyde Fuschin)

A section of the testis reveals irregularly distributed seminiferous tubules with intact basement membranes (BM), three to five layers of atrophic spermatogonia in various developmental stages, and sertoli cells holding the spermatogonia in place. The cytoplasm is mild, and the cells feature round to oval basophilic nuclei. The sparse interstitial in between has leydig cells in it. Spermatogonia A and B, primary spermatocyte (S1), secondary spermatocytes (S2), and spermatid (S3) made up the cell. Stem cells SA and SB; spermatogonia cells S1, S2, S3, S4; basement membrane BM; leydig cells LE; lumen.

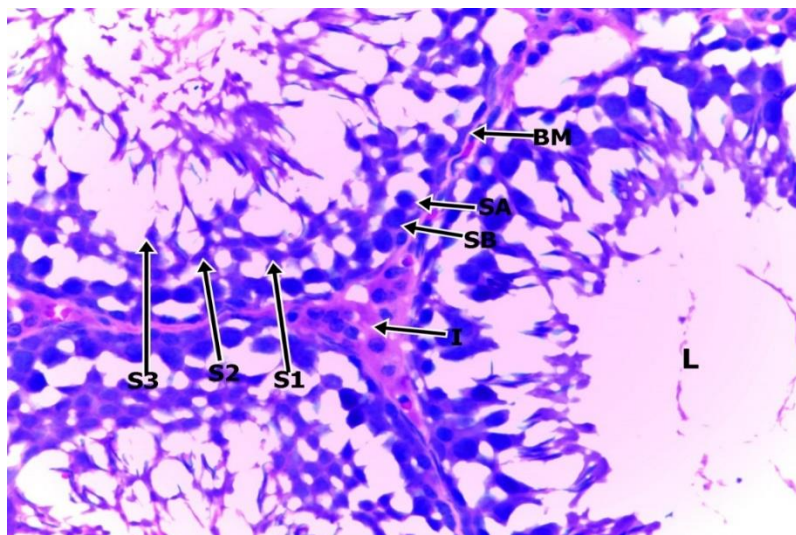


Figure 7. Photomicrographs (x 400) of Testis of rats exposed to heavy metal laden water and treated with n-Hexane fraction of *Vernonia calvaona*. (Stained with Gomori Aldehyde Fuschin)

Intact basement membranes (BM) with less than three layers of spermatogonia in various stages of maturation are seen in a section of the testis, along with tightly packed seminiferous tubules that are held in place by sertoli cells. The cytoplasm is mild, and the cells feature round to oval basophilic nuclei. In the scant interstitial in between, leydig cells are tightly clustered. Stem cells SA and SB; Spermatogonia cells S1, S2, S3, S4; Basement membrane BM; Leydig cells LE; Lumen.

5. Discussion

Heavy metal toxicity has been strongly connected with serious ailments such malignancies, immune system disorders, genotoxicity, skin and eye diseases, and infertility. Human exposure to heavy metals has primarily been by breathing. This investigation evaluated how well they could affect the amounts and operations of the reproductive hormones. (sometimes leading to infertility and erectile dysfunction in men). Plant decoctions have been investigated and found to have a tremendous potential for reversing or improving a variety of health issues over time. As a result of their consistent use for medicinal purposes, several plants are now regarded by traditional medical practitioners as being both safe and beneficial against specific illness conditions (Treasure, 2000). Unlike pharmaceutical medications, which only produce specific effects, medicinal plants produce both the individual and synergistic effects of their constituent phytochemicals, which are almost never harmful. They are known for their capacity to evoke a wide range of physiologic activities. 2000's Treasure.

Effect of Heavy Metal Toxicity on Reproductive Endocrinology

Heavy metal toxicity exposure has a major negative impact on health, including cancer (Ben Rouma et al. 2001), immune system disruption, endocrine disruption, and fertility issues (Colbon et al. 1993, Andersen et al. 2000). Heavy metal exposure can cause hormonal disruptions at any stage of hormonal regulation, including hormone

synthesis, hormone release and storage, hormone transport and clearance, hormone receptor recognition and binding, hormone post-receptor activation, thyroid function, and central nervous system function (Moline et al. 2000). These disruptions can harm spermatozoa and change the functions of Sertoli and Leydig cells (Ngoula et al. 2012).

Effect of Heavy Metals on the Plasma Concentration of Sex Hormones

The gonadotropic hormones (Testosterone, FSH, LH, and PSA) and PSA biochemical assay results significantly differed from those of the normal control (NC). LH and FSH levels were found to be significantly lower in the heavy metals exposed group (PC), and this could have an adverse effect on an individual's reproductive health because these hormones are crucial for the development of secondary sexual characteristics and the production of follicles in males. When compared to the heavy metals exposed group (PC), the groups treated with plant extracts exhibited a significant rise in the levels of sex hormones.

When compared to the normal control group, the heavy metals exposed group (PC) has considerably higher testosterone and PSA levels; a rise in PSA is a marker of post-treatment malignancies. The results of the study unmistakably demonstrated that the plant extract and its fractions may be to blame for the rise in FSH and LH hormone levels in the animal models, with the rise tending towards that of the normal control (NC).

Effect of Heavy Metals Exposure on histo-pathological architecture of the testis

The testis' histo-pathological sections revealed varying degrees of abnormalities in the groups exposed to heavy metals, with the heavy metals control group (PC) showing the most damage. Different levels of recovery were seen in the *Vernonia calvaona* extract-treated groups. In the n-hexane treated group, which shown a strong capacity for regeneration, exceptional outcomes were seen.

The study's histopathology findings demonstrated that heavy metals negatively impacted the gonads, altering the levels of two key hormones (FSH and testosterone), as well as the testis' histological architecture. These negatively affect hormone levels and reproductive functions, which leads to infertility in women and impotence in males. They also reduce sperm motility and count, hinder spermatogenesis, and destroy seminiferous epithelium. Additionally, different levels of ameliorative effects were demonstrated by extract fractions of the research plant, *Vernonia calvaona*, with the n-hexane fraction demonstrating the best potential for regulating the heavy metals-induced abnormalities and for reversing testicular damage.

6. Conclusion

There are several ways that heavy metals enter the human body, including inhalation and ingestion. The most significant method of exposure to these substances in the population of humans is by ingestion. Normal hand-to-mouth activity with contaminated hands can expose children to dangerous amounts (Dupler, 2001; Sawicka-Kapusta, 2003). Another kind of exposure is by skin absorption. In industrial, agriculture, and the pharmaceutical industries, heavy metals are most frequently in

contact with people (Roberts, 1999). Numerous health risks, such as tissue damage and abnormalities, have been connected to chronic exposure to hazardous heavy metals. The study went on to say that the formation of follicles in men and the development of secondary sexual characteristics in men depend on levels of key hormones that are significantly depleted in animals subjected to heavy metal exposure. The amounts of hormones rose in the groups given vitamin C and plant extracts, suggesting a potential reversal of the effects of the heavy metal. The research has also revealed associations between exposure to heavy metals and a number of health issues, including gonadotoxicity, haematological abnormalities, and possible infertility.

Recommendation

Occasion by our findings of this research reported above, the following are recommended:

1. The proximate parameters of these plants could be used in identification and standardization of drug.
2. Further purification of phytochemical constituents of *Vernonia calvaona* to produce pure compounds and elucidation of the chemistry of such phytochemicals.
3. Evaluation of teratogenic effects of heavy metals and the ameliorative effects of *Vernonia calvaona* should be study.
4. Isolation and structural elucidation of the components of the phytochemical fractions of *Vernonia calvaona*.

Acknowledgment

Authors hereby acknowledge Dr. Joel Theophilus Johnson of the Department of Biochemistry, Federal University of Otuoke Bayelsa State, for all his support, constructive criticism and counsel throughout the period of these study.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The cost of the reseach was self sponsor.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

Conflict of Interest

The authors affirm that they have on known financial or interpersonal conflicts that would have appeared to have an impact on the reseach described in this publication.

Consent for publication

The paper has been approved for publishing by all authors.

Author Contributions Statement

Emmanuel, peter ufort, Augustine Uwakwe, and Eka Essien all made significant contributions to the idea, planning, and careful revision of the study paper. The manuscript was written by Emmanuel peter ufort, who also helped with the revisions and final approval.

References

- [1] Adebayo, J. O., Adesokan, A. A., Olatunji, L. A., Buoro, D. O., & Soladoye, A. O. (2005). Effect of ethanolic extract of Bougainvillea spectabilis leaves on haematological and serum lipid variables in rats.
- [2] Ainslie, R., Bracht, E., Franz, J., Herring, T., & Todd, E. (1973). U.S. Patent No. 3,715,668. Washington, DC: U.S. Patent and Trademark Office.
- [3] Alexiades, M. and J. Wood Sheldon (1996). Selected Guidelines for Ethnobotanical Research: A Field Manual. New York: New York Botanical Garden Press. ISBN 0893274046
- [4] Al-Sarar, A. S., Bakr, Y. A., Hussein, H. I., & Bayoumi, A. E. (2009). Hematological and Biochemical Alterations in Occupational Pesticides-exposed Workers of Riyadh Municipality, Kingdom of Saudi Arabia. Research Journal of Environmental Toxicology, 3(4), 179-185.
- [5] Andersen, A. G., Jensen, T. K., Carlsen, E., Jørgensen, N., Andersson, A. M., Krarup, T., & Skakkebaek, N. E. (2000). High frequency of sub-optimal semen quality in an unselected population of young men. Human Reproduction, 15(2), 366-372.
- [6] Arbuckle T. E., Lin Z., Mery L. S. (2001). An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population. Environ. Health Perspect. 109: 851-857
- [7] Arbuckle T. E., Savitz D. A., Mery L. S., Curtis K. M. (1999). Exposure to phenoxy herbicides and the risk of spontaneous abortion. Epidemiology, 10:752-760
- [8] Asmathbanu I., Kalwal B. B. (1997). Temporal effect of methyl parathion on ovarian compensatory hypertrophy, follicular dynamics and estrus cycle in hemicastrated albino rats. J. Basic Clin. Physiol. Pharmacol 8: 237-254
- [9] Atangwho, I.J.; Egbon, P.E.; Eteng, M.U. Eyong, E.U. and Obi, A.U. (2007). Effect of V. amaygdalina Del. Leaf on kidney function of diabetic rats. Int. J. Pharm., 3 (2): 142 – 148.
- [10] Azmi, M. A., Naqvi, S. N. H., Akhtar, K., Parveen, S., Parveen, R., & Aslam, M. (2009). Effect of pesticide residues on health and blood parameters of farm workers from rural Gadap, Karachi, Pakistan.
- [11] Balick, M. J. and P. A. Cox (1996). Plants, People and Culture: The Science of Ethnobotany. New York: Scientific American Library. ISBN 071650619
- [12] Barlas, N.E. 1996. Toxicological assessment of biodegraded malathion in albino mice. Bull. Environ. Toxicol. 57:705-712.
- [13] Barlas, Y. (1996). Formal aspects of model validity and validation in system dynamics. System dynamics review, 12(3), 183-210.
- [14] Ben Rhouma, K., Tebouri O., Kirichah R. N. and Sakly M., (2001). Reproductive toxicity of DDT in adult male rats. Human Experimental Toxicology. 20(8). 393-397
- [15] Bodeker, G. (2003). Traditional Medical Knowledge, Intellectual Property Rights & Benefit Sharing. Cardozo J. Int'l & Comp. L., 11, 785.
- [16] Bodeker, G., Kabatesi, D., King, R., & Homsy, J. (2000). A regional task force on traditional medicine and AIDS. The Lancet, 355(9211), 1284.
- [17] Borchers, A. T., Hackman, R. M., Keen, C. L., Stern, J. S., & Gershwin, M. E. (1997). Complementary medicine: a review of immunomodulatory effects of Chinese herbal medicines. The American journal of clinical nutrition, 66(6), 1303-1312.
- [18] Cabello, G., Valenzuela, M., Vilaxa, A., Durán, V., Rudolph, I., Hrepic, N., & Calaf, G. (2001). A rat mammary tumor model induced by the organophosphorous pesticides

- parathion and malathion, possibly through acetylcholinesterase inhibition. *Environmental health perspectives*, 109(5), 471.
- [19] Clark, G., Garry, V., Burroughs, B., Holland, S., Chu, M., & Brown, D. (2002). Relationships between exposure to dioxin-like chemicals, testosterone levels, and sex of the children of pesticide applicators. *Organohalogen compounds*, 56, 73-76.
 - [20] Ogg, Clyde L (1997). Pesticide Education Resources web site. Web Master. University
 - [21] Colborn, T., vom Saal, F. S., & Soto, A. M. (1993). Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environmental health perspectives*, 101(5), 378.
 - [22] Costa, L. G., Olibet, G, Murphy S. D. (1988). Alpha 2- adrenoceptorss as a target for formamidine pesticides: in vitro and in vivo studies in mice. *Toxicol. Appl. Pharmacol.* 93: 319- 328
 - [23] Cotton, C. M. (1996). *Ethnobotany: Principles and Applications*. New York: John Wiley and sons. ISBN 047195537X
 - [24] Darren M. R., Ayanthi K., Nick A. B., Gamini M. H., Sheriff R. and Eddleston M. (2003). Influence of pesticide regulation on acute poisoning deaths in Sri-Lanka. *Bulletin of World Health Organization*. 81:112-120
 - [25] Demers, P. A., Vaughan, T. L., Koepsell, T. D., Lyon, J. L., Swanson, G. M., Greenberg, R. S., & Weiss, N. S. (1993). A case-control study of multiple myeloma and occupation. *American journal of industrial medicine*, 23(4), 629-639.
 - [26] Desi, I. (1992). Arsenic contamination of drinking water in south-east Hungary. *Geographia Medica*, 22, 45-53.
 - [27] Dhondup P., Kalicoal B. B. (1997). Inhibition of ovarian compensatory hypertrophy by the administration of methyl parathion in hemicastrated albino rats. *Reprod. Toxicol.* 11: 77-84
 - [28] Donald P. Morgan (1989). *Recognition and Management of pesticide Poisonings*. 4th Edition Document No. 055-000-00359-9. Supt. of Documents, U.S. Government Printing
 - [29] Ebong, P. E., Atangwho, J. I., Eyong E. U. and Egbung G. E. (2008). The Anti-diabetic efficacy of combined extracts from two continental plants: *Azadirachta indica* (A. juss) (Neem) and *Vernonia amygdalina* (Del.) (African Bitter leaf). *American Journal of Biochemistry and Biotechnology*, 4(3), 239-244. <http://dx.doi.org/10.3844/ajbbsp.2008.289.244>
 - [30] Edem, V. F., Akinyoola, S. B., Olaniyi, J. A., Rahamon, S. K., Owioye, O., & Arinola, O. G. (2012). Haematological parameters of wistar rats exposed to 2, 2 dichlorovinyl dimethyl phosphate chemical. *Asian J. Exp. Biol. Sci*, 3(4), 838-841. Yaqub, S. A., Rahamon, S. K., & Arinola, O. G. (2014).
 - [31] Elzawahry, A. M., Khalil, A. E. M., Allam, A. D. A., & Mostafa, R. G. (2015). Effect of the Bio-agents (*Bacillus megaterium* and *Trichoderma album*) on Citrus Nematode (*Tylenchulus semipenetrans*) Infecting Baladi orange and. *Journal of Phytopathology and Pest Management*, 2(2), 1-8.
 - [32] EL Zawahry, E.I. (2004). Assessment of toxicity on chronic treatment with some pesticides on albino rat .*Bull. Egypt. Soc. Physiol .Sci* 24(1).
 - [33] Fattahi, E., Parivar, K., & Jorsaraei, S. G. A. (2009). The effects of diazinon on testosterone, FSH and LH levels and testicular tissue in mice. *Iranian Journal of Reproductive Medicine*, 7(2), 59-64.
 - [34] Ferdinand Ngoula, Omer Bebe, Ngouateu, Jean Rapheal Kana, Henry Fualecfac Defang, Pierre Watcho, Pierre Kamtchoung and Joseph Tchoumboue (2012). *Reproductive and Developmental Toxicity of Insecticides; Insecticides- Advances in Integrated Pest Management*. ISBN: 978-953-307-780-2
 - [35] Focho, D. A., Ndam, W. T., & Fonge, B. A. (2009). Medicinal plants of Aguambu-Bamumbu in the Lebialem highlands, southwest province of Cameroon. *Afr J Pharm Pharmacol*, 3(1), 1-13.

- [36] Garí, J. A. (2003). Agrobiodiversity strategies to combat food insecurity and HIV/AIDS impact in rural Africa. *Advancing grassroots responses for nutrition health and sustainable livelihoods*.
- [37] Gilden, R. C., Huffling, K., & Sattler, B. (2010). Pesticides and health risks. *Journal of Obstetric, Gynecologic, & Neonatal Nursing*, 39(1), 103-110.
- [38] Goulet, L., Theriault, G. (1991). Stillbirth and chemical exposure of pregnant workers. *Scand J Work Environ Health*. 17: 25-31
- [39] Guyton A. C. and Hall J. E. (2010). *Textbook of Medical Physiology*. 11th edition Hajjawi, O. S. (2012). Acetylcholinesterase in human red blood cells. *European Journal of Scientific Research*, 75(4), 510-522.
- [40] Hall, J. E. (2010). *Guyton and Hall textbook of Medical Physiology*. Elsevier Health Sciences.
- [41] Hashim H, Kamali EL, Mohammed Y, (2010). Antibacterial activity and phytochemical screening of ethanolic extracts obtained from selected Sudanese medicinal plants. *Current Research Journal of Biological Science*.
- [42] Hassal, k.A:1990: The bio chemistry and use of pesticides structure metabolism .mode of action and use in crop protection Vclt publishers In C .Werheim and new York.p.263-361.
- [43] Herderson P. T., Borm P. J. A., Kant dri (1995). *Basisboek Arbeidstoxicologic risico-inventansatie en evaluate zeist: Vitgeverij Kerilcebosch*
- [44] Hind A.Jalel, 2012 the effect of malathion on the some hematological parameters of albino mice (Bas.J.Vet.Res.Vol.11, No.1, 2012)
- [45] Ibu, J. O. (2003). *Physiology of blood (54-65)*. Jos: University of Jos press Ltd.
- [46] Igile G. O., Iwara I. A., Mgbeje B. I. (2013). Phytochemicals, Proximate and Nutritional Composition of Vernonia Calvaona Hook (Asteracea): A Green-leafy Vegetable in Nigeria. *Journal of Food research*, Vol 2, No. 6. 1-11; Doi: 10.5539/jfr.v2n6p1
- [47] Igile, G. O., Iwara, I.A., Uboh, F.E., Nwaehujor, C.O., Ebong, P.E (2015,). *In Vitro Antioxidant Properties of Crude Extract and Extract Fractions of Vernonia Calvaona leaves*. *Journal of Medicinal Plants Research (In press)*
- [48] Joshaghani, H., Ahmadi, A., & Mansourian, A. (2007). Effects of occupational exposure in pesticide plant on workers' serum and erythrocyte cholinesterase activity. *International journal of occupational medicine and environmental health*, 20(4), 381-385.
- [49] Joshi, S. C., Mathur, R. and Gulati, N. (2007). Testicular toxicity of chloropyrifos (an organophosphate pesticide) in albino rat. *Toxicol. Ind. Health*; 23: 439-444
- [50] Joyce, C. (1992). Western medicine men return to the field. *BioScience*, 42(6), 399.
- [51] Lashansky, G., Saenger, P., Fishman, K., Gautier, T., Mayes, D., Berg, G., & Reiter, E. (1991). Normative Data for Adrenal Steroidogenesis in a Healthy Pediatric Population: Age-and Sex-Related Changes after Adrenocorticotropin Stimulation*. *The Journal of Clinical Endocrinology & Metabolism*, 73(3), 674-686.
- [52] Lorke, D. (1983) A new approach to practical acute toxicity testing. *Arch in Toxicology*. 544:289.
- [53] Martin G. J. (2004). *Ethnobotany: A Methods Manual*. London Earthscan. ISBN 1844070840
- [54] Minnis, P. E., (2000). *Ethnobotany: A Reader*. Norman, OK: University of Oklahoma Press. ISBN 0806170158
- [55] Narcyan,P.B.S;Sampathraj,e and Vanthakumarr ,G. 1993:Ratoxicity studies with neem oil, world neem conf(Bangalore. India).Astr.p.55.
- [56] New York. Consensus d'actualisation SFAR - Médecine d'Urgence 1996; 18 p. of Nebraska. Lincoln, NE. URL -- /ianr/pat/ephome.htmOffice, Washington, DC 20402-9325of Nebraska.
- [57] Ngoula, F., Defang, H. F., Kana, J. R., Tchoumboué, J., Ngouateu, O. B., Kamtchouing, P., & Watcho, P. (2012). *Reproductive and Developmental Toxicity of Insecticides*. INTECH Open Access Publisher.

- [58] Nurminen, T., (1995). Maternal pesticide. Exposure and pregnancy outcome. *J. Occup Environ Med*, 37: 935-940
- [59] Oa eseyin, p ebong, eu eyong, e umoh anda agboke hypoglycaemic activity of ethyl acetate fraction Of the leaf extract of telfairia occidentalis pak. *J. Pharm. Sci.*, vol.23, no.3, july 2010, pp.337-340
- [60] Obute, G. C., & Osuji, L. C. (2002). Environmental awareness and dividends: A scientific discourse. *African journal of interdisciplinary studies*, 3(1), 90-94.
- [61] Padungtod, C., Lasley, B. L., Christiani, D. C., Ryan, L. M., & Xu, X. (1998). Reproductive hormone profile among pesticide factory workers. *Journal of Occupational and Environmental Medicine*, 40(12), 1038-1047.
- [62] Pastore L. M., Hertz-picciotto I., Beaumont J. J. (1997). Risk of still birth from occupational and residential exposures. *Occup. Environ Med*. 54: 511-518
- [63] Rabe T, Van Staden J (2000). Isolation of an antibacterial sesquiterpenoid from *Warburgia salutaris*. *J. Ethnopharmacol*. 73: 171-174.
- [64] Rani, A., Sahai, A., Srivastava, A. K. and Rani (2007). A carbaryl induced histopathological changes in the testes of albino rats. *Journal of Anatomical Society of India*: 56: 4-6
- [65] Ray A., Chatterjee S., Ghosh S., Kabir S. N., Pakrashi A., Deb C. (1991). Suppressive effect of quinalphos on the activity of accessory sex glands and plasma concentrations of gonadotrophins and testosterone in rats. *Arch Environ Contain Toxicol*. 21(3): 383-387
- [66] Roberts, D. M., Karunarathna, A., Buckley, N. A., Manuweera, G., Sheriff, M. H., & Eddleston, M. (2003). Influence of pesticide regulation on acute poisoning deaths in Sri Lanka. *Bulletin of the World Health Organization*, 81(11), 789-798.
- [67] Robinson, H. (1999). Generic and subtribal classification of American Vernonieae. *Smithsonian Contrib. Bot*, (89), 1-116.
- [68] Rupa, D. S., Reddi O. S. (1991). Reproductive performance in population exposed to pesticides in cotton fields in India. *Environ. Res*. 55: 123-128
- [69] Savitz D. A., Whelan E. A., Kleckner R. C. (1989). Effect of parents' occupational on risk of stillbirth, preterm delivery and small-for-gestational-age infants. *Am. J. Empidemiol*. 129: 1201-1218
- [70] Scanlon V. C. and Sander, T., (2007). *Essentials of Anatomy and Physiology*. 5th edition
- [71] Schettler, T., Solomon, G., Kaplan J., Valent, M., (2003). *Geenerations at Risk: How Environmental Toxicants May Affect Reproductive Health in California* Brisbane, CA: George Lithograph.
- [72] Sembullingham K., and Sembullingham P., (2013). *Essentials of Medical Physiology*. 6th edition. Jaypee Brothers Medical Publishers (P) Ltd.
- [73] Stoker T. E., Cooper R. L., Goldman J. M., Andrews J. E. (1996). Characterization of pregnancy outcome following thiram-induced ovulatory delay in the female rat. *Neurotoxico Tertol*. 18: 277-282
- [74] Stoker T. E., Goldman J. M., Cooper R. L. (2001). Delayed ovulation and pregnancy outcome: effect of environmental toxicant on the neuroendocrine control of the ovary (1). 9: 117-129
- [75] Tapsell, L. C., Hemphill, I., Cobiac, L., Sullivan, D. R., Fenech, M., Patch, C. S., & Inge, K. E. (2006). Health benefits of herbs and spices: the past, the present, the future.
- [76] Torell, J., Cillard, J., & Cillard, P. (1986) Antioxidant activity of flavonoids and reactivity with peroxy radical. *Biochem*. 25(2):383-385
- [77] Treasure J. Medical Herb (online) 200; <http://www.herbological.com>
- [78] Van Wyk BE, Gericke N (2000). *People's Plants. A Guide to Useful Plants of Southern Africa*. Briza Publications, Pretoria.
- [79] Wambebe, C. (1998). Development and production of standardised phytomedicines. National Institute for Pharmaceutical.
- [80] Wayland J. Hayes Jr. and Edward R. Laws Jr. Editors. (1991) *Handbook of pesticide Toxicology*. Academic Press, Inc. San Diego, CA.

- [81] WHO (World Health Organization) (1999). Monographs of selected medicinal plants, 1; Pp: 51:20-30.
- [82] World Health Organization. Scientific Group on Epidemiology, & Prevention of Periodontal Diseases. (1978). Epidemiology, Etiology, and Prevention of Periodontal Diseases: Report of a WHO Scientific Group (No. 621). World Health Organization.
- [83] Wyrobeck, A. J., Watchmaker, G., and Gordon L., (1981). Sperm shape abnormalities carbaryl exposed employees. *Environ. Health Perspect.* 40: 255-266
- [84] Yaqub, S. A., Rahamon, S. K., & Arinola, O. G. (2014). Haematological and Immunological Indices in Nigerian Farmworkers Occupationally Exposed to Organophosphate Pesticides. *European Journal of General Medicine*, 11(2).
- [85] IUPAC (2006). "Glossary of Terms Relating to Pesticides". IUPAC. Pg. 2123. Retrieved January, 2014.
- [86] Andersen, A. G., Jensen, T. K., Carlsen, E., Jorgensen, N., Andersson A. M., Krarup, T., Keiding, N. and Skkaek, N. E. Frequence elevee de qualite de sperme inferieure a la moyenne chez une population non selectionnee d' hommes jeunes: *Reproduction Humaine* 2000; 15 (2): 366-372
- [87] Cabello, G., Valenzuela, M. & Vilava, A. A rat mammary Tumor model Induced by the Organophosphorus pesticides Parathion and Malathion, possibly through Acetylcholinesterase inhibition. *Environmental Health perspectives* 2001; 109(5): 471-479
- [88] Colborn, T., Myers, P. J. & Dumanoski, D. *Our Stolen Future*. Penguin Boocks, USA Inc., New York. Consensus d' actualisation SFARK- Medecine d' Urgence 1996; 18p.
- [89] Colborn, T., Vom Saal, F. S. & Soto, A. M. Development effects of endocrine-disrupting chemicals in wildlife and humans. *Environmental Health Perspective* 1993; 7: 17-23.
- [90] Reini, W. B., Chris, M. G. Thomas, Paul T. J. Scheepers, Gerhard A. Zielhuis and Nel R. (2006). Pesticide exposure: the hormonal function of the female reproductive system disrupted.
- [91] Moline, J. M., Golden, A. L., Bar-Chama, N. Smith E., Rauch M. E., Chapin, R. E., Perreault S. D., Schrader S. M., Suk, W. A. and Landrigan P. J. (2000). Exposure to hazardous substance and male reproductive health: A research framework. *Environmental Health Perspectives*: 108 (9): 803-813