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Evaluation of Extract Fractions of *Vernonia calvaona* on Some Biochemical Parameters and Histopathological Changes in Albino Wistar Rats Exposed To Domestic Insecticides

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Abstract: Despite widespread use, little is known about the risk associated with insecticides exposure to human health hence, this study. 48 wistar rats (90-120g) were randomly grouped into six (n=8). All except Normal control (NC) were exposed to domestic insecticide fumes once every 48 hours. Group 2, insecticide control (IC) was exposed to (20% of 81.55mg/m^3) 0.20mg/ml of DAKSH insecticide. Group 3, positive control (PC) was treated with a standard testosterone drug-Nugenix. Groups 4, 5 and 6 received 400mg/kg b.w each of crude extract (CE), ethylacetate and n-hexane fractions of *Vernonia calvaona* (VC) daily via orogastric intubation for 21 days. After administration, animal sacrifice and blood collection was done via cardiac puncture for analysis. The result presented a significant decrease ($p<0.05$) in testosterone of IC group compared to NC. Groups 3 and all treated groups effected progressive increase in testosterone concentration compared to IC group. FSH was significantly reduced ($p>0.05$) in IC and n-hexane fraction of VC compared to control. However, nugenix treated and ethylacetate fraction caused a remarkable increase in FSH compared to IC. WBC was significantly decreased ($p<0.05$) in IC and n-hexane groups compared to control. The Crude Extract treated group significantly increased WBC count compared to IC group. Histopathological studies of the testes showed varied levels of histological derangements in IC while Ethyl acetate treated group showed highest regenerative ability. This study revealed that insecticides adversely affected male gonads while the extracts showed ameliorative effects with the highest effect seen in ethyl acetate fraction in managing the insecticide induced damage.

Keywords: Insecticides, Testosterone, Luteinizing hormone, Testes, *Vernonia calvaona,* haematological indices.

INTRODUCTION

There have been increasing incidences of epidemics ravaging the world, mostly the developing countries. Some of these epidemics are caused or spread by insects or other pathogens. This has resulted in increase demand for insecticides.

Insecticides are a subclass of pesticides formulated to kill, cause harm or repel insects [1]. They may be targeted at destroying insect eggs (Ovicides), killing insect larvae (Larvicides) and/or killing adult insects (Adulticides).

Different insecticides are sold in the market place with little or no control over their quality due to inadequate and inefficient regulatory framework and weak legislation. This has led to the entry of all forms of chemical products into the Nigerian market place and sold as insecticides. Some of these substances have been banned by the United Nations Environment Projects (UNEP) in the Basel, Stockholm and Rotterdam Conventions [2].

Exposure to insecticides has been associated in animals and humans with occurences of spontaneous abortion, low birth weight, birth defects, change in male female sex ratio of offspring, inhibition of spermatogenesis and ovogenesis, destruction of seminiferous epithelium, hydrocele resulting to reduction in fertility [3-5]. Medicinal plants constitute a fundamental component of traditional healthcare system in Asia, South America and rural communities throughout Africa. Statistics have shown that medicinal plants are widely used; and about 80% of people in the developing world rely directly or indirectly on traditional medicine for most of their healthcare problems [2,6]. Also well known is the fact that over 75% of residents of rural communities in Nigeria used traditional herbal remedies for their medicinal needs.

One of such plant which has captured great medicinal interest is *Vernonia calvaona.* This plant is a small shrub of less than 1 metre tall with petiolate leaves of about 10mm wide which serves as a green-leafy vegetable and also used for ethnomedicinal purposes. The plant is widely distributed in South Western Cameroun and South Eastern part of Nigeria. It is popularly eaten raw or fresh as a local delicacy with or without palm oil, and also serves as a component of traditional salad among the indigenous consumers. It may also be used in preparation of native soups and stews and in preparation of potatoes, yam and plantain porridge. Its consumption is based on the belief that the plant as a whole cures heart diseases, blindness, diabetes, malaria and act as an anti helminthic agent [7].

Mosquito and other pathogenic infestation and the resultant use of insecticides of various types to kill them may have exposed populations in sub-tropical Africa, including Nigeria to all forms of chemical toxicities. This may be responsible for some toxicity presentations clinically diagnosed as hepatotoxicities, cardiotoxicities, gonadotoxicity, hemogglobinopathies and respiratory complications. The extent and damage caused by these toxicities in man have not yet been evaluated and quantified.

Because of their ability to mitigate the unwanted activities of pests, insecticides have enjoyed wide use in such sectors as agriculture, medicine, industry, environment and household. Therefore, the present study aimed to investigate the adverse effects of insecticide exposure on reproductive hormones, gonadal architecture, and the ability of extracts of V.Calvaona to reverse any identifiable toxicity.

MATERIALS AND METHODS

Collection of plant materials and preparation of crude extract

Vernonia calvaona leaves were harvested from Ugep, Yakurr Local Government Area of Cross River State. The plant was identified by a Botanist with the Department of Botany, University of Calabar and was constant with an already deposited specimen in the herbarium of the Department (BCH/VC/01). The leaves were then thoroughly washed under running tap water to rid of dirt and allowed to drain. The leaves were there after shaded dried for seven days and blended into powdered form. 3kg of blended leaves were extracted by means of cold maceration using ethanol as the solvent to powdered leave ratio being 1:4 for 48 hours. The extract was then filtered first with Chess cloth then with filter paper (Whatman No.1 filter paper). The filtrate was concentrated to dryness using a water bath at 40^0C. A paste yield of 220g was obtained from which a portion was used for fractionation.

Fractionation of Plant Extract

Portions of the ethanolic crude plant extract were subjected to graded serial solvent fractionation using n-hexane and ethylacetate solvents respectively. The crude extract was chromatographed on silica gel (60-120 mesh size) packed in a glass chromatographic column and eluted in succession using the aforementioned solvents in order of increasing polarity. Fractions of each solvent system were collected in beakers and evaporated to dryness.

Acute Toxicity Testing

Inhalation toxicity test of the insecticide and LD_{50} of the plant extracts were carried out using the method described by Lorke [8].

Experimental Animal and protocol

Forty eight wistar rats weighing 90-120g were randomly groued into six (n=8). With the exception of Group 1 (Normal control, NC), all other groups were exposed to domestic insecticide fumes once every 48 hours. Group 2 served as insecticide control (IC) and exposed to (20% of 81.55mg/m^3) 0.20mg/ml of DAKSH insecticide. Group 3 served as positive control and treated with a standard testosterone drug (Nugenix). Groups 4, 5 and 6 were treated with 400mg/kg body weight each of crude extract (CE), ethylacetate and n-hexane fractions of VC daily via orogastric intubation for 21 days. As seen in the table below, the dose used was based on LCT_{50} study of the insecticide (20% of 81.55mg/m^3) and LD_{50} of the plant as determined using Lorke's method.

Table-1: Animal Grouping and Treatments

Experimental Groups.	Number of animals	Treatments
Group 1 (NC)	8	0.20mg/ml of10% DMSO (placebo).
Group 2 (IC)	8	(20% of 81.55mg/m^3) 0.20mg/ml of DAKSH insecticide.
Group 3 (NUG)	8	Insecticide+ Nugenix. (5mg/kg b.w of animal)
Group 4 (CE)	8	Insecticide + Crude Extract VC.(400mg/kg b.w of animal)
Group 5	8	Insecticide+n-Hexane fraction .(400mg/kg b.w of animal)
Group 6 (Ethyl acetate)	8	Insecticide + Ethyl Acetate Fraction VC.(400mg/kg b.w of animal)

NC- Normal Control, IC- Insecticide Control, CE- Crude Extract of *Vernonia Calvaona*, b.w- body weight; NUG = Nugenix; n-HEX = n=Hexane

Process of exposure and treatment

Animals were placed in a $1m^3$ exposure chamber thirty minutes after the chamber had been fleeted with a specific quantity of *daksh* insecticide and subsequently allowed exposure duration of 30minutes after which animals were placed in their normal wooden cages with adequate ventilation and allowed *ad libitum* access to feed and water. Animals in the insecticide exposed treated groups were administered crude and fractional extracts of *Vernonia calvaona* at 400mg/kg b.w respectively.

Collection of Blood and Tissue samples for Analysis

After 21 days exposure and treatment period, the animals were allowed an overnight fast but were allowed unrestricted access to water. The rats were subsequently sacrificed after being euthanized using chloroform vapour and blood samples for analysis were collected by cardiac puncture. Blood samples for hematological assays were placed in EDTA bottles and gently inverted a couple of times each. Whole blood for hormonal assays were collected in 10ml plain tubes, allowed to stand and centrifuged at 3000rpm for 10mins causing a distinct separation of the serum from the packed clotted cells. The serum was then separated into distinctly labeled plain tubes and refrigerated until they were used. The testes were surgically removed for histological studies. The tissue was immediately suspended in 10% formal saline prior to preparation of slides.

Serum Hormonal Assay

Estimation of serum luteinizing hormone and testosterone concentrations were done using the Microwell FSH EIA enzyme immunoassay kit (Synton Bioresearch, Inc. USA).

Analysis of haematological parameters

Automated blood cell analyser (Model PCE 210, Japan) was used. The machine was switched on with its specific diluents and haemolysate and allowed to boot. When the machine indicated readiness, each sample bottle was raised for the probe of the machine to suck the required volume of the blood for the test. The machine automatically began to measure the blood parameters. The results were displayed on the screen and were subsequently printed out.

Analysis of Data

Data obtained were analyzed using SPSS package version 20 and statistical significance measured by One-way Analysis of variance (ANOVA) with a post hoc Donett value at $P<0.05$. Charts were plotted using Microsoft excel 13. All data were expressed as Mean ± SEM, n=8.

RESULTS

Acute toxicity test

The Ld_{50} of Daksh insecticide was determined and was found to be about $81mg/m^3$/30mins whilst the LD_{50} of crude extract of *Vernonia calvaona* have been determined by previous studies to be greater than 5000mg/kg body weight [9].

Serum testosterone levels

Values obtained for testosterone concentrations in serum indicate a decrease in all exposed groups compared to that of the normal control (4.52±0.09). Of these, the insecticide control (IC) group which was exposed but not treated was observed to be significantly ($P<0.05$) lower in testosterone concentrations (3.51±0.41) as compared to that of the normal control (NC). Treatment using a known testosterone booster (Nugenix) yielded a significantly ($P>0.05$) increased level of the hormone, bringing its concentration close to normal (4.33±0.23). Extracts (CE) and fractions (ethylacetate and n-hexane) of Vernonia calvaona (VC) showed varied ameliorative potentials respectively as they raised the testosterone levels (3.80±0.49; 4.90±0.31; 3.81±0.32) though not significant.

Comparison of Serum FSH and LH concentrations in the different experimental groups

Results for the assay for serum FSH concentrations showed a significant decrease ($P<0.05$) in the IC group and in the n-hexane extract fraction treated group (1.57±0.13, 1.46±0.15 respectively) when compared to the normal control (2.52±0.53) whereas the standard drug, Nugenix caused an increase in the levels of the hormone (2.12±0.12). Concentrations when compared to that of the normal. Whereas results for the crude and ethyl acetate fraction treated groups showed an increased levels of this hormone concentration (1.66±0.14, 1.88 ±0.14 respectively), although none was significant ($P<0.05$). Meanwhile, no significant change was seen in LH amongst the groups.

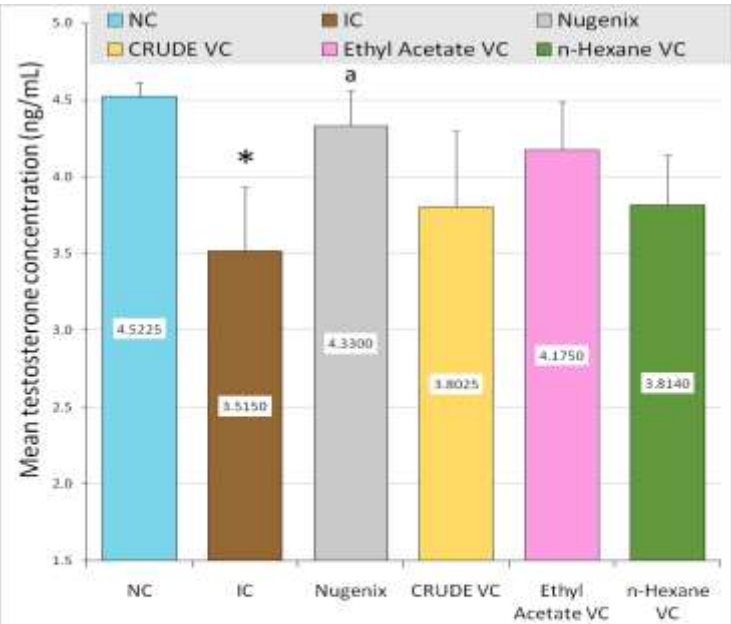


Chart 4.1: Testosterone concentrations of the different experimental groups.
Values are expressed as Mean ± SEM. n=8.
* significantly different from NC at p<0.05
a significantly different from IC at p<0.05

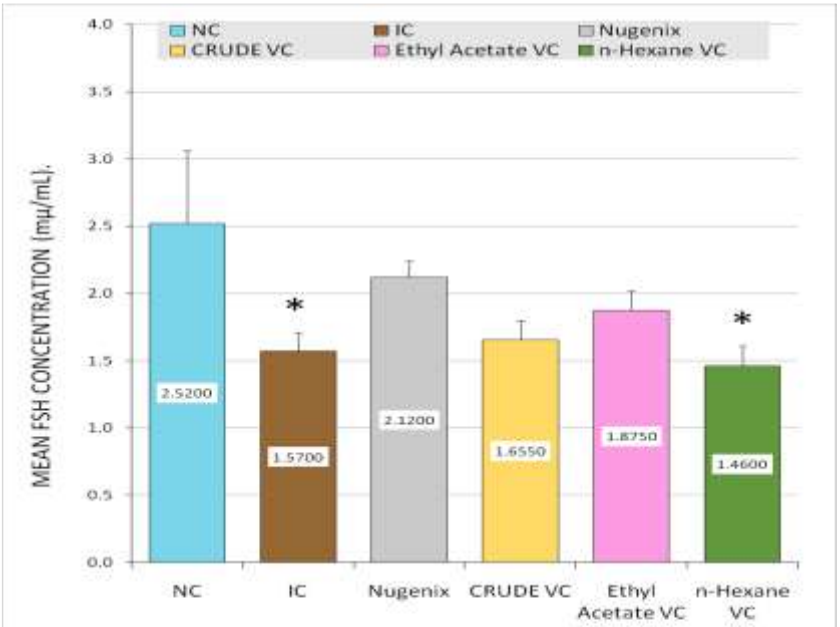


Chart 4.2: FSH concentrations of the different experimental groups.
values are expressed as Mean ± SEM. n=8.
* significantly different from NC at p<0.05

Comparison of hematological indices in the different experimental groups

From table 2 below, Full blood count showed no significant variations in haematolocgical indices except for PCV and WBC. WBC count showed a significant reduction in all groups exposed to insecticide with a much significant decrease ($p<0.05$) seen in the insecticide control (IC) when compared to control. The White Blood Cell Concentration (WBC) IC groups treated with nugenix, crude extract and ethyl acetate fractions of *Vernonia calvaona* showed marked improvement in WBC count compared to insecticide control.

Table-2: Some Haematological indices in the different experimental groups

Variables / **Groups**	WBC $\times10^3/\mu L$	RBC $\times10^6/\mu L$	HGB g/dL	HCT %	MCHC g/dL	PLT $\times10^3/\mu L$
NC	16.77±0.89	7.71±0.18	14.2±0.30	50±1.29	28.48±0.79	778.5±67.22
IC	13.67±1.3*	7.15±0.29	13.53±0.57	47.33±1.71	29.17±0.29	805±108.34
Nugenix	14.73±1.47	7.28±0.53	13.42±0.57	47.13±6.67	27.82±0.21	807.12±53.26
Crude VC	16.0±2.1[a]	7.44±0.58	13.63±0.38	46.77±1.42	27.77±0.13	832.33±46.48
Ethylacetate VC	14.86±3.12	7.18±0.11	13.83±0.67	48.07±0.26	28.8±0.21	778.33±14.33
N Hexane VC	13.53±0.47*	7.19±0.22	13.77±0.52	49.60±1.93	28.6±0.4	820.66±95.89

NC-Normal control, IC-Insecticide Control, VC-Vernonia Calvaona

Values are expressed as Mean ± SEM, n=8

* Significantly different from NC at $p<0.05$

[a] significantly different from IC at $p<0.05$

Histolopathological Examination

As shown below, Photomicrographs (x 400) of Testis of NC rats given placebo treatment (Stained with Gomori Aldehyde Fuschin stain) shows closely packed seminiferous tubules with intact basement membranes (BM) containing three to five layers of spermatogonia at various stages of maturation held in place by sertoli cells. The cells have round to oval basophilic nuclei and moderate cytoplasm. The intervening interstitium is sparse and contain closely packed leydig cells.

Photomicrographs (x 400) of Testis of IC rats exposed to Insecticide (IC group) shows dispersed seminiferous tubules with irregular basement membranes (BM) containing disorganized loosely atrophic spermatogonia cells. The cells have round basophilic nuclei. The intervening interstitium is sparse and contain closely packed leydig cells with congested blood vessels.

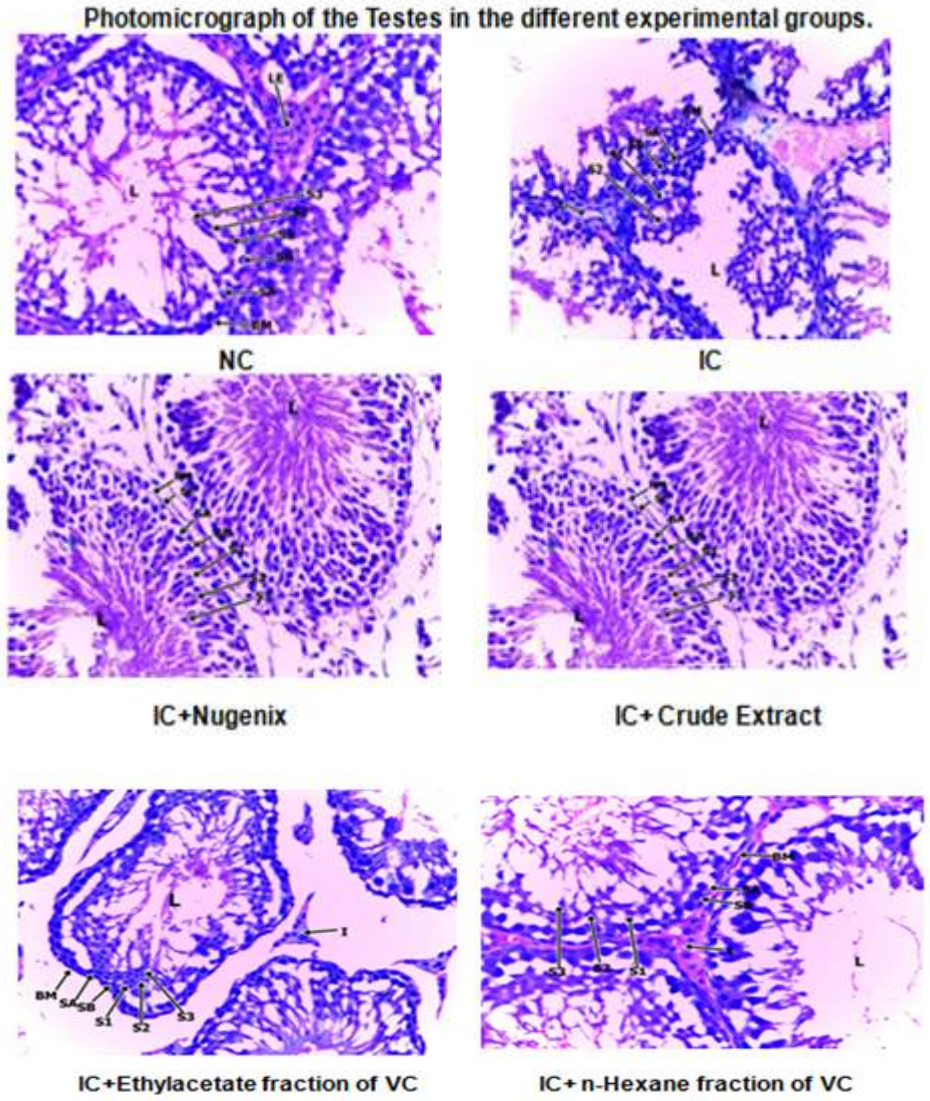
Photomicrographs (x 400) of Testis of rats exposed to Insecticide and treated with Nugenix (a testosterone booster) (Stained with Gomori Aldehyde Fuschin stain) shows well packed seminiferous tubules with intact basement membranes (BM) containing three to five layers of spermatogonia at various stages of maturation held in place by sertoli cells. The cells have round to oval basophilic nuclei and moderate cytoplasm. The intervening interstitial is sparse and contains closely packed leydig cells.

Photomicrographs (x 400) of Testis of rats exposed to Insecticide and treated with Crude Extract of *Vernonia calvaona* (Stained with Gomori Aldehyde Fuschin) shows closely packed irregular seminiferous tubules with intact basement membranes (BM) containing less than five layers of compact spermatogonia cells at various stages of maturation held in place by sertoli cells. The lumen contains sparsely populated spermatogonia cells.The intervening interstitium is sparse and contain closely packed leydig. The intervening interstitial is sparse and contains closely packed leydig cells.

Photomicrographs (x 400) of Testis of rats exposed to Insecticide and treated with Ethyl acetate fraction of *Vernonia calvaona* (Stained with Gomori Aldehyde Fuschin) shows wildly spaced seminiferous tubules with intact basement membranes (BM) containing three to five layers of atrophic spermatogonia at various stages of maturation held in place by sertoli cells. The cells have round to oval basophilic nuclei and moderate cytoplasm. The

intervening interstitial is scanty and contains leydig cells.

Photomicrographs (x 400) of Testis of rats exposed to Insecticide and treated with n-Hexane fraction of *Vernonia calvaona* (Stained with Gomori Aldehyde Fuschin) shows closely packed seminiferous tubules, intact basement membranes (BM) containing less than three layersof spermatogonia at various stages of maturation held in place by sertoli cells. The cells have round to oval basophilic nuclei and moderate cytoplasm.



Magnification: (x 400); Primary spermatocyte (S1), secondary spermatocytes (S2) and spermatid (S3). SA and SB-Stem cells ; S1, S2,S3, S4 – Spermatogonia cells; BM – Basement membrane; LE- Leydig cells; L- Lumen

DISCUSSION

Chronic exposure to pesticides has been linked to a great deal of health hazards including tissue damage and abnormalities. This study revealed a significant decrease in the concentrations of serum testosterone, follicle stimulating hormone and luteinizing hormone in the insecticide exposed group (IC) compared against those of the normal control (NC). This is in line with the report by Recio *et al*. [10] and Fattahi *et al.* [11].

FSH and LH are known spermatogenesis promoters with LH controlling the normal cell functioning of Leydig cells [12]. A reduction in the promotion of spermatogenesis as a result of significantly lowered FSH concentration would impair testicular function hence, impairing testosterone production which may result in low sperm count, morphology and viability, hence, affecting male sexual function. Full blood count showed no significant variations in haematolocgical indices except for PCV and WBC which showed a significant reduction in their concentrations in the insecticide control (IC). The mechanism through which this insecticide decreases WBC count was not taken in this study but may be

indicative of chemical stress as reported by Al-Sarar *et al.* [13] and Azmi *et al.* [14].

From the result of this study, it is most like likely that chronic exposure to insecticide may induce reproductive dysfunction possible effect on the immune system. This is consequent upon the fact that it reduces testosterone and luteneizing hormonal concentrations and lowers some haematological parameters in rats. The increase in testosterone concentrations in groups treated with standard drug and plant extracts showed possible reversal of the insecticide effects, therefore, plant may be potent in reversing some reproductive dysfunction, hence recommended for further studies.

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