ORIGINAL ARTICLE

Effect of *Alchronea cordifolia* ethanolic root extract on Malathionsub-acute toxicity in sperm and testicular tissue of male Albino rats (*Rattusnorvegicus*

ABSTRACT

Introduction: Modern agricultural practices are becoming indispensably and heavily reliant on the use of pesticides or insecticides for crop yield enhancement. With respect to sperm and testicular tissues, this study design examines the effect of *Alchornea cordifolia* ethanolic root extract dose dependently on malathion sub-acute toxicity in albino rats.

Method: A nest of thirty male albino rats (*Rattus norvegicus*) were divided in six groups of five rats each. The control group (Group 1) was treated to 0.5ml Carboxyl-Methyl-Cellulose (CMC) buffer solution only. Groups II was exposed to 1/25 of LD_{50}25mg/kg body weight of malathion only, while group III received 250mg/kg *Alchornea cordifolia* alone. Subsequent groups, (IV, V and VI) were co-administered 25mg/kg body weight of malathion with varied. Harvested testicular tissues were subjected to biochemical assay and histological study while the sperm cells were microscopically examined.

Results: The malathion and *Alchornea cordifolia* treated rats experienced nasal redness, ocular discharge and lethargy as physical signs of sub-acute toxicity. Malathion treatment alone significantly decrease ($p<0.05$) body weight change, absolute and relative weights of the testis when compared with the control and extract co- treated groups. In a dose dependent manner, co-treatment of malathion with *Alchornea cordifolia* ethanolic root extract tend to maintain body weight, absolute and relative weights of the testis to near normal. After 42days, malathion administration alone significantly increased ($p<0.05$) MDA level and nitric oxide (NO^{-}) activity when compared with extract treated group and control which were significantly reduced ($p<0.05$). Co-administration of malathion with *Alchornea cordifolia* indicated a significant change ($p<0.05$) in sperm motility, sperm count, sperm morphology and sperm volume when compared with malathion administration alone. The damaged and atrophic seminiferous tubule in the testis with edema fills in intertubular space as observed with malathion treatment alone, was reduced upon co- treatment with *A. cordifolia* at 500 and 750mg/kg body weight.

Conclusion: Aside the attenuating potential of *Alchornea cordifolia* in radical formation, *Alchornea cordifolia* dose dependently decline malathion induced toxicity in sperm and testicular tissue of *Rattus norvegicus*.

Keywords: Malathion, Farmers, *Alchornea cordifolia*, Testicular tissue

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INTRODUCTION

In Nigeria, plant produce as well as crop yields are highly influenced by the level of pest infestation on agricultural farm land. Pest control is of great importance in the turnover rate of farm products. Farmers employ the use of pesticide to control, as well as moderate pest infestation on agricultural plants.[1] In addition to preservation of agricultural produce, product quality and quantity are maintained through pesticide usage. Basically traditional, biological and chemical methods are three major avenues of controlling the activities of pest by both local and modern farmers. Chemical method as compared to other methods is widely the most adopted inpest control recently. Organophosphorus (OP) such as malathion, is seasonally employed in controlling pest in livestock and agricultural farming. Since organophosphorus is commonly used in agriculture and households, residents close to agricultural farms as well as farm workers are usually victims of OP pesticide exposure.[1, 2]The major identifiable (whether acute or chronic) exposure channel include, contact of OP contaminated soil and plant with skin through accidental spill, consumption of OP through contaminated food and drinking water, nasal inhalation during production, handling and application of pesticides [3]. In accordance to WHO report, 3 million cases of OP intoxication and poisoning occurs worldwide.[4, 5] Acute malathion poisoning with concomitant muscle dysfunction could lead to death in living organism especially humans as a result of mitochondrial dysfunction.[6]Observation from previous studies has shown that chronic exposure to OP pesticides has multiplied the risk factor of various chronic diseases on living cell.[7]Organophosphorus pesticides are majorly neurotoxic and portray neuro symptoms that are muscarinic, cholinergic and nicotinic in nature.[8]

Studies indicated that the mechanism of action of malathion induced toxicity is by inhibiting acetylcholinesterase (AchE), cholinergic receptors activationand generation of oxidative stress.[9]

Report have suggested that most OP insecticides (malathion inclusive) are endocrine receptors (ED) thereby possessing the ability of interfering with hormonal levels through binding and activation of androgen, estrogen and other hormone receptor site.[10]Male reproductive system and spermatogenesis in animal epithelium with decrease body weight, has being reported to be affected by malathion toxicity. [11]A study recently carried out, showed that malathion induced decrease level of testosterone, reduced the performance level in reproductive system of male mice and furthermore inhibited AchE.[12]

Herbal or traditional medication has being on the increase in Nigeria in recent times. This is not to say that folk medicine has not being in existence, it's an age long practice; however the quest to obtain cure for the increasing number of aliments has led to publicity and awareness of herbal and traditional medicine.

Alchornea cordifolia (Schum and Thonn.), popularly known as Christmas Bush is one herbal plant employed in folk medicine. It belongs to the Euphorbiaceae family which is predominant in the coastal region of West Africa. Most counties in Western Africa have ascribed local nomenclatures to this plant. For example, in Sierra Leone it is known as "*Susubolonta*", "*Casamancebugong*" in Senegal, and "*Bondji*" in Cameroon. Similarly, Christmas Bush in Nigeria hasdifferent names according toits medical applications[13]. In Ukwani it is called "Epin-tin", in Akwa-Ibom is called "Irie", in Izon it is called "esin", while in the northern Nigerian precisely in Hausa dialect it is called "Bambari". The multiple biological properties of this plant have culminated into diverse medical relevance of this plant.[13, 14] Surprisingly, its medicinal versatility translates almost all parts of the plant. Although the leaf are readily used as leaf meal for ruminants and non ruminants alike, other parts such as the stem bark, root bark, roots, fruits have locally shown medicinal potentials and principles.[13] The flavonoids extracted from the of *Alchornea cordifolia* (ethanolic) has being shown to posses' sedative, and spasmolytic principles in addition to

its smooth muscle relaxing potential.[15]Other medicinal applications of which *Alchornea cordifolia* has being implicated includes; anti-plasmodial, antibacterial, antimicrobial, anti-inflammatory, anti-diarrhoeal[14, 16, 17,]. Furthermore, its antioxidant ability has exhaustively being investigated [18].

Currently, the indispensible use of Organophosphorus (OP) pesticides such as malathion by male farmers in rural areas of Nigeria has being on the rising and have led to farmers exposures to the health consequences of malathion toxicity. One of such health complicates associated with the use of Organophosphorus (OP) is infertility. Although female infertility has being investigated with Christmas Bush, little or no information has being established on testicular activity, spermatogenesis and male fertility. Hence this study, attempts to demonstrates the possible health benefits and ameliorating property of *Alchornea cordifolia* against malathion induced toxicity in sperm and testicular tissues of *Rattus norvegicus* using the sperm count, motility, morphology and sperm volume as index of measurement.

MATERIALS AND METHOD

Plant identification

Alchornea cordifolia (the experimental plant) was collected from the Botanical Garden of Botany Department, Delta State University, Abraka (Delsu) Nigeria. Botanical identification and authentication was carried out by Mr. Michael Iwunze of the Forestry Research Institute (FHI) of Nigeria, Ibadan. The plant was given a voucher number of FHI 180187 and hence deposited at the herbarium.

Plant Extraction:

The method as described by Ezeokeke et al. [13] was employed. Apparently healthy root parts of *Alchornea cordifolia* were collected and sun-dried between 3 to 4 weeks until a constant weight was derived. The dried root sample was converted into uniform powder by using an electric grinding processor - Thomas Contact Mill (Pyeunicam,

Cambridge, England). For 72 hours and at 100 °C, 100g of the ground root sample was soxhletly extracted using 600 mL ethanol of analytical grade.WhatmanNo.1 filter paper was used to filter the solution and the filtrate further concentrated in adigital water-bath at 45°C (Premiere HH-42). The concentrated extract was weighed yielding28.9g*Alchorneacordifolia* extract. The obtained extracted concentrate was put in stoppered containers and preserved in a refrigerator between 0- 4°Cuntil required.

Experimental animals

Thirty (30) sexually mature male albino rats (*Ratusnorvegicus*) weighing (100-150g) was experimentally used. The rats were obtainedfrom Macdons laboratory Center, Warri and housed in the Animal Unit of Basic Medical Sciences, Delta State University, Abraka in gauzed cages and maintained in standard laboratory condition of room temperature (25±6^{o}C). For the experimental duration, adequate cross ventilation was provided with a 12hours light/12hoursdark cycle. One week period was allowed for acclimatization with uninhibited access to rodent food and clean water before being separated into different groups' weight wise. The animals were treated to a standard rodent diet manufactured by Top Feed, Sapele, Delta State.

Ethical Consideration

Ethical code of conduct governing experimental life animals were strictly observed and adhered to as stipulated by Ward andElsea[19]. For the use and care of laboratory animals, all NIH Guide was employed. Experimental protocol was consented and approved by the Institutions Ethical Committee (IEC) for the maintenance of laboratory animals.

Experimental Design

The 30 animals were randomly shared into groups, with a carrying capacity of six rats per group. Grouping is represented thus **Group I (Normal control group)** :Constituted buffer solution of Carboxyl-Methyl-Cellulose (CMC) at a dose of 0.5 ml was administered via gavage per animal, once a day for the treatment period of 42days, **Group II (Malathion-treated group**

alone): In the same manner and duration, rats were treated with malathion alone at a dose of 25 mg/kg (1/25LD_{50}) body weight per day in 0.5 ml corn oil, **Group III (250mg/kg of *Alchornea cordifolia* treated group alone):** Rats in this group received 250mg/kg of *Alchornea cordifolia* only once as a single daily dose, **Group IV (Malathion plus 250mg/kg of *Alchornea cordifolia* treated group)**: This category of rats received 25 mg/kg of malathionin 0.5ml corn coil once a day thereafter, 250mg/kg of *Alchornea cordifolia* extract (low dose). Extract treatment was done 60 minutes after toxicant administration, **Group V (Malathion plus 500mg/kg of*Alchornea cordifolia* treated group):** Animals assigned here received 25 mg/kg of malathion in 0.5ml corn coil before onward treatment of 750mg/kg of *Alchornea cordifolia* extract (high dose) 60minutes later, **Group VI (Malation plus 750mg/kg of *Alchornea cordifolia* treated group):** 25mg/kg of malathion in 0.5ml corn coil was given to this group before receiving 750mg/kg body weight of α-tocopherol suspension in the same manner as previous groups.

The administration was conducted for the same period of 42daysand animals received orally by gavage. Administered doses of *Alchornea cordifolia* was adopted as carried out from previous study [13].

Sample Collection

At the end of the administration, the rats were subjected to an overnight fast (food and water deprivation). By means of cervical dislocation, the animals were sacrificed followed by laparotomy revealing the internal organs. Sperm sample were culled from Cauda epididymis for biochemical analysis. The harvested testis samples were fixed in 10% formal saline for histological study.

Microscopic Examination:

Sperm Count: The method as adoptedbyOmotosoetal.[20] was used. Spermatozoa numbering was achieved using Neuber's Counting Chamber (haemocytometer).

Sperm Motility: In Tri buffer solution, seminal fluid from the Cauda epididymis was observed

under a light Microscope as descibed by Sonmezetal.[21]

SpermMorphology:

Sperm morphology was determined by the method of Saaluetal.[22] using original dilution factor for motility.

Biochemical Analysis:

Determination of nitric oxide radical inhibition activity: The methods of Greenetal.[23] and Macocci et al.[24]were employed. The production of nitrite ions was measured by Griess reaction which involved the interaction of oxygen with sodium nitroprusside in aqueous solution and at physiological pH to generate nitric oxide. The percentage inhibition of nitric oxide was calculated thus: % of nitric oxide inhibition = [Abc - Abs/ Abc] x 100%; where Abc = absorbance of the control, Abs = absorbance of the sample.

Assessment of lipid peroxidation: This was estimated spectrophotometrically by Thiobarbituric acid- reacting substance (TBARS) method as described by Varshney and Kale [25].

Determination acetylcholinesterase activity: This was carried out by Colorimetric determination (Ellman's assay Method).[26]

Photomicrography

After tissue fixation and staining, images obtained from stained tissue were captured using digital microscopic eyepiece 'Scoptek' Dcm 500, 5.0mega pixels connected to Usb 2.0 computer.

Statistical Analysis

Analyzed data and results were expressed as Mean ± SD. Treatment effect was analyzed by one way analysis of variance (ANOVA), ensued by post Hoc LSD for multiple comparison using SPSS version 21 windows software. A significant value was set at $p< 0.05$.

RESULTS

The result of malathion administration and ethanolic root extract of *Alchornea cordifolia* treatment on body weight change in male adult rats is represented in Table 1. Malathion treated groups significantly decrease ($p<0.05$) body weight change when compared with the control and extract treated groups. There was no

significant difference ($p>0.05$) in body weight change of *Alchornea cordifolia* alone administration when compared with the control. In a dose dependent manner, co-treatment of malathion with *Alchornea cordifolia* ethanolic root extract showed a significant increase ($p<0.05$) in body weight change when compared with malathion treated group alone, however no statistical difference ($p>0.05$) was observe in the absolute and relative weights of the testis between the control and malathion treated groups. Malathion administration alone as reflected in Table 2, significantly increased ($p<0.05$) lipid peroxidation (MDA) and nitric oxide activity when compared with the control and other groups. While malathion plus *Alchornea cordifolia* ethanolic root extract was significantly different ($p<0.05$) in lipid peroxidation and nitric oxide activity, no significant difference ($p>0.05$) was observable with acetylcholinesterase activity when compared with other groups. The effect of co-administration of malathion with ethanolic root extract of *Alchornea cordifolia* on some sperm characteristics was shown in Tables 3. Result showed significant change ($p<0.05$) in sperm motility, sperm count, sperm morphology and sperm volume upon malathion administration and its co-administered with *Alchorneacordifolia*.

Photomicrographs of the testis with respect to control, malathion plus *Alchorneacordifolia* administration is shown in Plate 1- 4. The seminiferous tubules of the testis in the control group were observed to be normal with no edema. Malathion administered group showed damaged and atrophic seminiferous tubules in testicular tissue with edema fills in intertubular space. The treatment of malathion with 250mg/kg, 500mg/kg and 750mg/kg *A. cordifolia* revealed slight atrophic seminiferous tubules in the testis with reduced edema fills in intertubular space.

Table 1: Body weight change after co- treatment with Malathionand *A. cordifolia* Extract

Treatment Groups	Initial body weight (g)	Final body weight (g)	Change in body weight	Absolute Testis weight (g)	Relative testis weight (%)
Control(Group I)	144.69±7.60	154.61±12.79	6.42±5.67	0.69±0.02	0.83±0.01
Group II (Malathion alone)	142.02±7.74	142.63±11.02	0.43±9.22**	0.61±0.01	0.70±0.03
Group III (250mg/kg *A.cordifolia* alone)	139.11±5.33	147.50±15.17	5.69±6.03	0.65±0.03	0.83±0.02
Group IV (Malathion plus 250mg/kg *A.cordifolia*)	135.25±6.88	138.50±15.17	2.64±8.94**	0.60±0.04	0.80±0.03
Group V (Malathion plus 500mg/kg *A.cordifolia*)	147.15±8.87	161.41±4.90	5.56±3.68*	0.65±0.03	0.83±0.01
Group VI (Malathion plus750mg/kg*A.cordifolia*)	139.59±12.14	139.33±9.80	5.62±5.40*	0.67±0.02	0.81±0.02

Values are expressed as Mean±SD, n = 5 rats per group.* = significant difference ($p<0.05$) with respect to control. **= significant difference ($p<0.05$) with respect to control and other groups.

Table 2: Effect of Malathion and *Alchorneacordifolia*administration onmalodialdehyde,acetylcholinesterase and nitric oxide activity on testicular tissue

Treatment Group	MDA (µM)	Nitric Oxide (µmol/min/g)	AChE (µmol/min/g)
Group I (Control)	41.33±3.45	02.88±0.75	22.17±0.57
Group II (Malathion alone)	20.47±9.20**	06.52±3.42**	20.02±2.93
Group III (250mg/kg *A.cordifolia* alone)	38.27±4.06	3.01±3.45	21.83± 1.50
Group IV (Malathion plus 250mg/kg *A.cordifolia*)	22.01±3.54**	5.77±1.06*	20.99±1.41
Group V (Malathion plus500mg/kg *A.cordifolia*)	29.63±5.18*	5.34±1.01*	21.05±0.67
Group VI (Malathion plus 750mg/kg *A.cordifolia*)	30.33±3.45*	5.01±0.05*	22.33±1.22

Values are expressed as Mean±SD, n = 5 rats per group. * = significant difference ($p<0.05$) with respect to control. **= significant difference ($p<0.05$) with respect to control and other groups

Table 3: Sperm characteristics after treatment with malathion and *Alchornea cordifolia* in male adult rats

Treatment Groups	Sperm motility (%)		Sperm counts (Milllion/mm^3)	Sperm morphology (%)	Sperm Volume (ml)
	Cauda epididymis		Cauda epididymis	Cauda epididymis	Cauda epididymis
	Progressive	Non progessive			
Group 1 (Control)	66.99±5.22	28.11±5.45	9.01±7.03	3.70±0.51	0.90±0.10
Group II (Malathionalone)	50.32±3.13**	37.55±2.36**	6.60±2.58**	1.30±0.67**	0.84±0.21
Group III (250mg/kg *A.cordifolia* alone)	63.17±5.26	25.38±5.41	8.80±4.09	2.80±0.85	0.80±0.85
Group IV (Malathion plus 250mg/kg *A.cordifolia*)	58.30±2.99*	33.06±2.74*	7.99±3.61*	2.40±0.20*	0.89±0.16
Group V (Malathion plus500mg/kg *A.cordifolia*)	60.20±2.37	31.42±2.99*	7.10±2.17*	2.80±0.85	0.88±0.25
Group VI (Malathion plus750mg/kg *A.cordifolia)*	60.60±4.31	30.11±4.28	8.60±2.95	3.0±0.99	0.91±0.18

Values are expressed as Mean±SD, n = 5 rats per group * = significant difference ($p<0.05$) with respect control. **= significant difference ($p<0.05$) with respect control and other groups

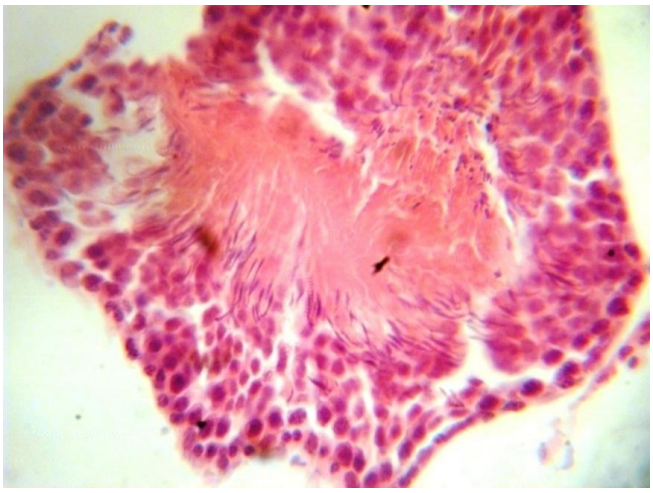

Plate 1 (Pho tomicrograph of Control**):** Seminiferous tubules in the testis were observed normal. Edema not present in intertubular space.

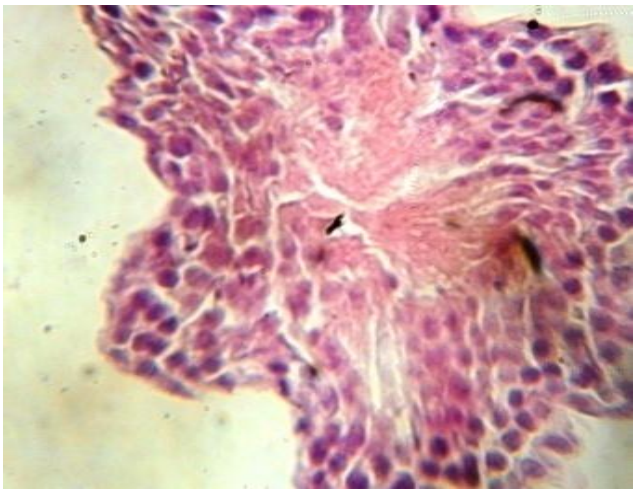

Plate 2 (Photomicrograph of Malathion alone**):** Damaged seminiferous tubules in the testis.Atrophywas noticed in some seminiferous tubules with edema filledintertubular space. Seminiferous lumen indicated sloughing of germ cell.

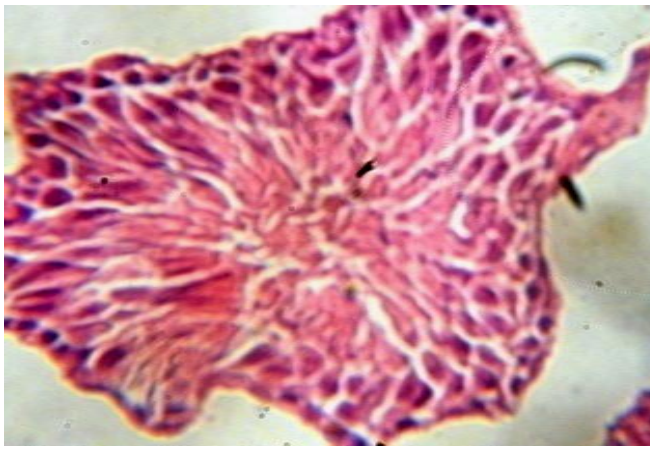

Plate 3 (Photomicrograph of Malathion plus500mg/kg *A.cordifolia*)**:** Atrophic seminiferous tubules in the testis. Reduced edema fills in intertubular space.

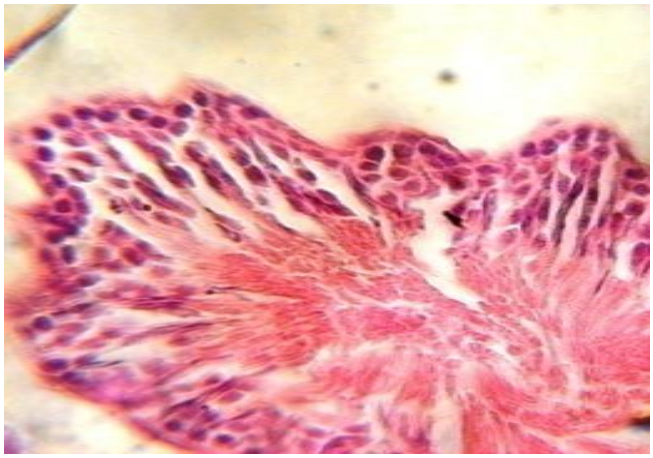

Plate 4 (Photomicrograph of Malathion plus 750mg/kg *A.cordifolia*)**:** Intertubular space revealing almost normal tissues less filled by edema. However, slightly atrophic seminiferous tubules were observed.

DISCUSSION

The use of Organophosphorus (OP) such as malathion as pest regulating agent by both crude and mechanized farmers in Nigeria is escalating and prevalent. Although its application has improved crop yield as pest infestation declines, studies have revealed possible health complications including infertility[5]upon prolong application. Such complications are initiated or further enhanced through ROS release and acetylcholinesterase inhibition there by resulting to tissue toxicity[9]. The compromise in the balance between ROS release and antioxidant generation induces oxidative stress which is capable of inflicting oxidative damage on tissues [27]. The significant decrease ($p<0.05$) in weight change as observed from this study is an indication that malathion enhances ROS toxicity. This however, supports previous report on the effect of malathion on body weight reduction of experimental mice[11]. Excessive generation of ROS can compromises the defence mechanism of the testis thereby initiating oxidative stress.

Malathion toxicity was corroborated with physical appearance of nasal redness, ocular discharge and lethargy.The treatment of malathion with 250mg/kg, 500mg/kgand 750mg/kg of *A. cordifolia* ethanolic root extract (representing low, moderate and high dose respectively) significantly ($p<0.05$) improved the body weight change.

In Table 2, the ameliorating potential of *A.cordifolia* was further assessed through assaying an unstable species such as nitric oxide activity as well as MDA activity. Nitric oxide in the presence of a scavenger decreases in amounts. The extent of decrease reflects the extent of scavenging.[28] The postulation from previous studies that *A. cordifolia* possess antioxidant principles has receive myriads attention and documentations.[13, 14, 29]This study indicates that *A. cordifolia* in a dose dependent manner lowered to a significant level ($p< 0.05$) nitric oxide radical when compared with the toxicant administered group. When compared with the control group, no significant ($p>0.05$) increase in nitric oxide activity was noticed with *A. cordifolia* administration alone. The co treatment of the *A. cordifolia* extract with the toxicant dose dependently exhibited the same trend as the extract alone administered group.

As an index of lipid peroxidation, the concentration of MDA was elevated in testicular tissue of the rats given 25mg/kg malathion, but co-treament with *A. cordifolia* ethanolic root extract decreased its formation. It is scientifically accepted that ROS chain reactions are implicated in lipid peroxidation. Previous studies reveal the activity of malathionin activating a cascade of chain reactions that culminated into peroxidation of cells.[9] It therefore connotes that substances capable of terminating peroxidation chain reaction might possess radical scavenging properties[30]. The significant decrease($p<0.05$) in MDA value upon co-administration of malathion

with A. cordifolia ethanolic root extract(Table 2)is suggestive that the ethanolic extract might have quenched or mopped up malathion generated ROS.

Sperm fertility is affected mainly because of the susceptibility of testicular tissues and sperm parameters (sperm count, motility, morphology and sperm volume) to free radical activities is high. Presumably, the oxidative stress induced by free radical generation contributes significantly to generating reduced sperm count, abnormal sperm as well as sperm DNA fragmentation [27, 31].

The result (Table 3) shows the changes in sperm parameters after treatment with malathion plus *A. cordifolia* in experimental rats. Malathion treatment alone (Group II) shows a significant decrease ($p<0.05$) in the sperm parameters when compared with the control group and *A. cordifolia* treated group. The administration of 250, 500 and 750mg/kg *A. cordifolia* significantly ($p<0.05$)was able to change and restore the sperm parameters to near normal. This biochemical observation was complemented by the histological appearance which indicates invigorated cell features especially for those given 750mg/kg *A. cordifolia*. It can possibly be deduced that the increase in sperm characteristics might as well be as a result of the bioactive constituents present in *Alchorneacordifolia*. Previous experimental studies have further elucidated that *Alchornea cordifolia* possesses a strong antioxidant bioactive ingredient called flavonoids, which alleviates free-radical induced damage in tissuesand gives the plant its protective principle[32, 33].However, this is suggestive on fertility use of *Alchornea cordifolia* which considerably reduced nitric oxide activity in testis, improved sperm motility, sperm count and viability. Histological examination indicated damaged seminiferous tubules in the testis upon malathion treatment and reduce edema fills in intertubular space upon *Alchornea cordifolia*.

CONCLUSION

The use of pesticide and insecticide is increasingly becoming indispensable by farmers to improved crop quantity and quality. However, the chemical composition of these pesticides such as malathion (Organophosphates) if not properly handled might detrimentally generate some health complications to the famers or farm sprayers of

whichmale infertility is a possibility. This study shows that co-administration of malathion with *Alchornea cordifolia* decrease some testicular tissue oxidative markers which culminated in increase sperm viability and motility. However, the efficacy of this herbal drug or extract is subject toherbal pharmacodynamics and species differences; hence this finding cannot be extrapolated directly to humans. This study further suggests that the fertility principle of *Alchornea cordifolia* be investigated with reproductive hormones like testosterone, estrogen, Luteinizing hormone (LH) and the rest.

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