Journal of Anatomical Sciences

Email:anatomicaljournal@gmail.com

J Anat Sci 5 (1)

The Ameliorating Capabilities of *Croton Zambesicus* Leaf Extract on the Testis of Rats Exposed to Pyrethroid-Based Insecticide

[1]Akunna GG, [1]Saalu LC, [2] Oyewopo AO

[1]Department of Anatomy, College of Medicine, Lagos State University, Ikeja Lagos, Nigeria., [2]Department of Anatomy, University of Ilorin, Ilorin, Kwara State, Nigeria.

Corresponding Author: Saalu LC
E-mail:chiasaalu@hotmail.com

ABSTRACT

The pyrethroid pesticides are swiftly replacing other type of insecticides due to a moderately lower toxicity for mammals. Nevertheless, due to excessive use they have now become an environmental nuisance. We sort to evaluate the attenuating effect of aqueous extract of *Croton zambesicus* (AECZ) on testicular derangement induced by pyrethroid-based insecticide in the rat testis. Thirty five male Wistar rats (10 to 12 weeks old) weighing 230-250 g were where divided into four groups of seven rats each. Group A severed as the control, Group B rats were exposed to pyrethroid-based mosquito coil, Group C, D and E rats were exposed to pyrethroid-based mosquito coil and subsequently treated with 200, 250 and 300 mg/kg body weight of aqueous extract of *Croton zambesicus.* The animals were sacrificed after 10 weeks and results obtained showed a significant ($P<0.001$) decrease in testicular weight, reduction of basal seminiferous epithelial cells, marked testicular atrophy, germinal aplasia and hypospermatozoa formation in the group exposed to pyrethroid-based coil. There was also a significant decrease in testicular activities of superoxide dismutase, catalase, reduced glutathione, gluthathione peroxidase and a significant ($p<0.001$) increase in level of malondialdehye. Also shown in our study is a decrease in the seminiferous tubule diameter and cross sectional area and a significant ($p < 0.001$), decrease in the sperm count and motility but an increase in total abnormal sperm morphology when compared to the values of obtained from control. All these parameters were however ameliorated in a dose dependent matter in the groups that were post-treated with aqueous extract of *Croton zambesicus*, it was concluded that aqueous leaf extract of *Croton zambesicus protect the testis from pyrethroid-based insecticide-induced derangement*.

Key words: Testes, Mosquito coil, Allethrin, Infertility, Croton zambesicus

INTRODUCTION

Pesticides (insecticides, herbicides and fungicides) constitute potential environmental hazards not only to birds, fish, and other animals but also to humans when they become part of the food chains[1] .In 2013, Akunna *et al.* documented the anti-fertility role played by an allethrin-based insecticide in animal models[2]. Annual cases of about 25-77 million pesticide poisoning coupled with the declaration by the World Health Organisation pesticide evaluation scheme (WHOPES) that all pesticides are toxic to humans to some degree has lead to the prohibition of pesticide with acute toxicity and alternative measures being promoted worldwide [3,4,2].

Mosquito coil consist of repellents, and after prolonged exposure, have a potential knock down or killing effect on mosquitoes and other flying insects. The major content (pyrethroids) improves the effectiveness of simple smoke particles and results to a significant enhancement in the biological effect of the coils. Smoke from mosquito coils has been reported contain sub-micron particles coated with considerable amount of heavy metals, allethrin and a wide range of vapours [5].

Due to high efficiency, low toxicity and easy biodegradability, pyrethroids insecticides are preferred to organophosphates, carbamates and organochlorines [6]. However, in excessive dosage and long exposure duration these products have been associated with hematological [7], biochemical [8,9,10], reproductive [11,12] and pathological changes [13,14] .

Focal deciliation of the tracheal epithelium, metaplasia of epithelial cells and morphological alterations of the alveolar macrophages, increased blood brain barrier (BBB) permeability was reported in rats exposed to mosquito coil smoke for 60 days, suggesting a delayed maturity of BBB and biochemical changes [15].

Croton zambesicus is widely distributed in tropical Africa. It is a shrub of about to 16 m high with scaly bark and silvery leaves [16]. It has been shown to have antimicrobial [17,18] anti-inflammatory, analgesic and antipyretic [19] anti-convulsant and antiulcer properties [20].

While the root extract has been reported to possess anti-plasmodial properties [19], infusion of the bark is used locally in cases of malaria [21].

The present study therefore was designed to investigate the protective potentials of difference doses of aqueous leaf extract of *Croton zambesicus* (AECZ) on testicular toxicity induced by pyrethroid-based insecticides in male albino rats.

MATERIALS AND METHODS

Mosquito Coil

Mosquito coils containing 0.2%w/w of d-trans-allethrin, measured 12 cm diameter, 85 cm length and 15.3±3.2 g in weight was purchased from a local outlet located within Bariga, Lagos Nigeria.

Extraction of Plant Material

The fresh leaf of *Croton zambesicus* was purchased from Mile twelve local market in Lagos. The authentication was carried out in the department of Botany, University of Lagos where a voucher specimen number was deposited in the herbarium Department. The fresh leaves of the plant were shaded and dried for 8 days and then it was grounded into a powder using mortar and pestle. 100 g portion of the powder was macerated in 1000 ml of distilled water for 24 hours and then boiled for 15 minutes before it was allowed to cool. Then it was centrifuged at 4000 r.p.m at 40C for 20 minutes. After which, the supernatant part was evaporated to dryness. The dry extract (13.90 g) was stored in a refrigerator at 4°C and used subsequently for the proposed experiment.

Animal Groupings and Mosquito Coil Exposure

Thirty male Wistar rats (8 to 10 weeks old) weighing 230-250 g were obtained from the animal house of the Lagos state college of medicine. The study was done in five (A, B, C, D and E) undisturbed cages of size 5 m^3 with cross ventilation. The rats in group A served as the control group and were treated orally with 2.5 ml/kg body weight/daily of distilled water for 16 weeks. The rats in group B was exposed via whole body inhalation to the commercially available mosquito coil smoke for 8 hours (7am-3pm) everyday for 16 weeks consecutively. The rats in group C, D and E rats were exposed to mosquito coil smoke via whole body inhalation for 8 hours (7am-3pm) and subsequently treated with 200, 250 and 300 mg /kg body weight of aqueous extract *of Croton zambesicus* for 16 weeks consecutively. Experimental procedures involving the animals and their care were conducted in conformity with International, National and institutional guidelines for the care of laboratory animals in Biomedical Research and Use of Laboratory Animals in Biomedical Research as promulgated by the Canadian Council of Animal Care [22].

Further the animal experimental models used conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals [23].

Animal Sacrifice, Sample Collection and Parameter determination

The rats were first weighed and then anaesthetized by inserting them in a clogged jar which contains chloroform anaesthesia. The abdominal cavity was opened up through a midline abdominal incision to expose the reproductive organs. The testes were excised and trimmed of all fat. The testicular weights of each animal were evaluated with an electronic analytical and precision balance (BA 210S, d=0.0001-Sartoriusen GA, Goettingen, Germany). The testes volumes were measured by water displacement method. The two testes of each rat were measured and the average value obtained for each of the two parameters was regarded as one observation. One of the testes of each animal was fixed in 10% formol saline for histological and stereological examination. Serum and the remaining testes of each animal were stored at –25°C for subsequent biochemical assays.

Epididymal Sperm Characteristics (ESC)

As described by Akunna *et al*, the testes from each rat were carefully exposed and removed [24]. They were trimmed free of the epididymides and adjoining tissues. From each separated epididymis, the cauda part was removed and placed in a beaker containing 1 mL physiological saline solution. Each section was quickly macerated with a pair of sharp scissors and left for a few minutes to release its spermatozoa into the saline solution. Sperm motility, concentration and progressive motility were determined as earlier described by Saalu *et al,* [25]. Semen drops were placed on the slide and two drops of warm 2.9% sodium citrate were added. The slide was covered with a cover slip and examined under the microscope using X40 objective for sperm motility. Sperm count was done under the microscope using improved Neubauer haemocytometer.

Histological Analysis

The organs were cut in slabs of about 0.5 cm thick and fixed in Bouin's fluid for a day after which it was transferred to 70% alcohol for dehydration. The tissues were passed through 90% alcohol and chloroform for different durations before they were transferred into two changes of molten paraffin wax for 20 min each in an oven at 57° C. Serial sections of 5 µ m thick were obtained from a solid block of tissue and were stained with haematoxylin and eosin stains, after which they were passed through a mixture of equal concentration of xylene and alcohol. Following clearance in xylene, the tissues were oven–dried. Light microscopy was used for the evaluations.

Three-dimensional (3D) Evaluations

Histological slides were prepared from the formol-saline fixed testes. However, before embedding, it was ensured that the sections were orientated perpendicular to their long axes, and chosen as "vertical sections". For

each testis, five vertical sections from the polar and the equatorial regions were sampled [26] and an unbiased numerical estimation of the following morphometric parameters was estimated using a systematic random scheme [27]. The diameter of seminiferous tubules with profiles that were round or nearly round were estimated for each animal and a mean, *D*, was determined by taking the average of two diameters, *D1* and *D2* (Perpendicular to one another). *D1* and *D2* were taken no more than when $D1/D2 \geq 0.85$. The cross-sectional areas of the seminiferous tubules was estimated from the formula $AC = \pi D2/4$, (where π is equivalent to 3.142 and *D* the mean diameter of the seminiferous tubules).

The Number of profiles of seminiferous tubules per unit area was determined using the unbiased counting frame anticipated by Gundersen (1977). Using this frame, in addition to counting profiles completely inside the frame we counted all profiles with any part inside the frame provided they do not intersect the forbidden line. This is the number of profiles per unit volume and was determined by using the modified Floderus equation: $NV = NA/(D + T)$ [28] where, *NA* is the number of profiles per unit area, *D* is the diameter and *T* the average thickness of the section. The evaluation of the diameter was done with calibrated eyepiece and stage grids mounted on a light research microscope. Estimation of volume density of testicular components and number of seminiferous tubules were done on a computer monitor onto whom a graph sheet was superimposed and on which slides were projected from a research light microscope (Model N -400ME, CEL-TECH Diagnostics, Hamburg, Germany).

Determination of Testicular Enzymatic Antioxidants

Catalase (**CAT**) activity was estimated based on the method of [29]. 0.1 ml of the testicular homogenate (supernatant) was pipetted into cuvette containing 1.9 ml of 50 mM phosphate buffer, pH 7.0. Reaction was started by the addition of 1.0 ml of freshly prepared 30% (v/v) hydrogen peroxide (H_2O_2). The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of enzyme was expressed as units /mg protein. Superoxide dismutase (**SOD**) activity was studied according to the method described by [30]. The principle of the assay was based on the ability of SOD to inhibit the reduction of nitro-blue tetrazolium (NBT).

The reaction mixture contained 2.7 ml of 0.067M phosphate buffer, pH 7.8, 0.05 ml of 0.12mM riboflavin, 0.1 ml of 1.5mM NBT, 0.05 ml 0f 0.01M methionine and 0.1 ml of enzyme samples. Uniform illumination of the tubes was ensured by placing it in air aluminum foil in a box with a 15W fluorescent lamp for 10 minutes. Control without the enzyme source was included. The absorbance was measured at 560nm. One unit of SOD was defined as the amount of enzyme required to inhibit the reduction of NBT by 50% under the specific conditions. It was expressed as u/mg protein. Glutathione peroxidase (**GPx**) activity was evaluated by the method described by [31]. The reaction mixture contained 2.0 ml of 0.4M Tris- HCl buffer, pH 7.0, 0.01 ml of 10mM sodium azide, 0.2 ml of enzyme. 0.2 ml of 10 mM glutathione and 0.5 ml of 0.2mM. H_2O_2. The contents were incubated at 370C for 10 minutes followed by the termination of the reaction by the addition of 0.4 ml 10% (v/v) TCA, centrifuged at 5000 rpm for 5 minutes. The absorbance of the product was read at 430 nm and expressed as nmol/mg protein.

Assay of Testicular Non-Enzymatic Antioxidants

Lipid peroxidation (**MDA**) in the testicular tissue was studied colorimetrically by thiobarbituric acid reactive substances TBARS method of [32]. A principle component of TBARS being malondialdehyde (MDA), a product of lipid peroxidation. In brief, 0.1 ml of tissue homogenate (Tris-Hcl buffer, pH 7.5) was treated with 2 ml of (1:1:1 ratio) TBA-TCAHCl reagent (thiobarbituric acid 0.37%, 0.25 N HCl and 15% TCA) and placed in water bath for 15 min, cooled. The absorbance of clear supernatant was measured against reference blank at 535nm. Concentration was calculated using the molar absorptivity of malondialdehyde which is 1.56 x105 M-1 cm-1 and expressed as nmol/mg protein.

Statistical Analysis

The obtained data were expressed as mean ± SD of number of experiments (n = 10). A homogenic level among the groups was tested using Analysis of Variance (ANOVA) [33]. Where heterogeneity occurred, the groups were separated using Duncan Multiple Range Test (DMRT). A value of $p < 0.05$ and $p<0.005$ was considered to indicate a significant difference between groups.

RESULTS

Body Weight, Testes Weights and Volume

The control group of rats had a significant increase in body weight when compared to rats in group B that loss significant ($p<0.001$) amount of weight when compared to their initial weight. ($P<0.05$) when compared with group B rats that were not treated. There was a significant ($p < 0.001$) decrease in the testis weight, testis weight/body weight ratio and testis volume in B rats compared to the control group. The group of rats that were treated with AECZ had a significantly improved body weights testis weight, testis weight/body weight ratio and testis volume which were dose dependent

Table 1: The changes in gross anatomical parameters of Experimental rats

Gross Anatomical Parameters	Initial Bod y Weight (g)	Final Body Weight (g)	Body Weight. Differences	Testicular Weight. (g)	Testicular Volume (ml)	Testicular Wt./Body Wt. Ratio
Group A	245.3±0.3	270.1±05	24.8	1.85±2.1	1.82±0.2	0.008
Group B	240.6±1.2	190.1±0.4	50.5 **	1.33±0.5 **	1.30±3.0 **	0.005 *
Group C	230.6±0.1	200±5.3	30.6 *	1.50±0.1 *	1.51±1.1 *	0.006 *
Group D	245±3.4	230±0.2	15 **	1.67±8.2 *	1.63±3.1 *	0.006 *
Group E	249.4±8.1	245±3.2	4.4 **	1.78±2.0 *	1.77±0.3 *	0.007 *

Values are expressed as mean ± SD for n=8; *p < 0.05, **p < 0.001 significantly dissimilar from control A: 2.5 ml/kg body weight of distilled water

B: Pyrethroid-based mosquito coil
C: Pyrethroid-based mosquito coil and treated with 200 mg/kg body weight of AECZ
D: Pyrethroid-based mosquito coil and treated with 250 mg/kg body weight of AECZ
E: Pyrethroid-based mosquito coil and treated with 300 mg/kg body weight of AECZ

Epididymal Sperm Characteristics

As shown in Table 2, the group of rat that was exposed to allethrin-based mosquito coil had a marked (P<0.005) reduction in sperm count when compared to the control groups. The exposed group treated with AECZ however, showed an improved sperm concentration (P<0.05) which were comparable to that of the control. The percentage sperm motility of the treated groups (C,D an E) were significantly (P<0.05) higher when compared to the untreated model group (B) and comparable with that of the control value. The results of the sperm progressivity, live/dead ratio and morphology were consistent with sperm count and motility.

Table 2: Epididymal sperm concentration, motility, morphology and Live/Dead ratio of Experimental rats

Treatment Groups	Sperm Count x10^6/ml	Sperm Motility %	Sperm Progressivity	Sperm Morphology % Normal	% Abnormal	Live/Dead ratio
Group A	127 .6±0.5	86 .5±0.2	a 1	66.2 ±1.6	33.8±1.5	89 .2 ± 1.6
Group B	90.2±1.5 **	59.2±0.5 **	b1*	45.1±1.3*	54 .9±1.3 **	59.0 ±1. 0**
Group C	117.5±2.4 *	74±1.3 *	b1 *	50.2±0.5*	49.8 ±2.7 *	70.2±0.5*
Group D	123±1.3 *	81.0 ±1.5*	a1	62 .2±0.5	37.8 ±1.3*	62 .2±0.5 *
Group E	126.4 ± 0.3	78.2 ± 0.1*	a1	70.2 ± 3.2*	29.9 ± 0.3*	73.3 ± 2.2*

Values are expressed as mean ± SD for n=8; *p < 0.05, **p < 0.001 significantly dissimilar from control**.**

a1 = rapid linear progressive motility, b1 = show sluggish linear or non-linear motility.
In this study, a spermatozoon was considered abnormal morphologically if it had one or more of the following features: rudimentary tail, round head and detached head.
A: 2.5 ml/kg body weight of distilled water
B: Pyrethroid-based mosquito coil
C: Pyrethroid-based mosquito coil and treated with 200 mg/kg body weight of AECZ
D: Pyrethroid-based mosquito coil and treated with 250 mg/kg body weight of AECZ
E: Pyrethroid-based mosquito coil and treated with 300 mg/kg body weight of AECZ

Testicular Geometry

As shown in Table 3, there was a significant (*P*<0.001) reduction in the mean seminiferous tubular diameters, cross sectional area, number of profiles per unit area and the mean numerical density of seminiferous tubules of the exposed mosquito coil alone group when compared to the control groups. However, there was a significant (*P<0.05*) increase in the tubular diameter of animals treated with AECZ as compared to tubular diameter of the control groups. There was a significant (*P*<0.005) increase in the cross-sectional area of the tubules, the number of tubular profiles per unit area and the mean numerical density of seminiferous tubules of the models treated with varying doses of AECZ.

Table 3: Seminiferous tubular diameter (μm), cross sectional area Ac (×103μm2), numerical densities of seminiferous tubules NA (×10-8μm-2) and number of profiles per unit area Nv (×10-10μm-3) of Experimental rats

Treatment Groups	D (μm)	A_c (×10^{3} μm^{2})	N_A (×10^{-8} μm^{-2})	N_v (×10^{-10} μm^{-3})
Group A	160.6±6. 3	31.2±1. 2	30.2±1.3	20.3±4. 3
Group B	110±8.1**	18.4± 2.4**	17.2±3.3**	8.7±5. 7**
Group C	130.1±1. 2*	38.1±5. 2	32. 2±4.1	20.5±4. 3
Group D	166.3 ±7.1	40.1±8. 3*	35. 1±5.13	19.4±4. 5
Group E	169.9 ±0.3*	51.8 ±3.3*	40.2 ±2.1*	28.1 ±2.1*

Values are expressed as mean ± SD for n=8; *p < 0.05, **p < 0.001 significantly dissimilar from control.

A: 2.5 ml/kg body weight of distilled water
B: Pyrethroid-based mosquito coil
C: Pyrethroid-based mosquito coil and treated with 200 mg/kg body weight of AECZ
D: Pyrethroid-based mosquito coil and treated with 250 mg/kg body weight of AECZ
E: Pyrethroid-based mosquito coil and treated with 300 mg/kg body weight of AECZ

Testicular Oxidative Stress

Activities of testicular enzymes SOD, CAT and GPx:
As shown in Table 4, the group of rat exposed to mosquito coil alone had a significant decrease in SOD, CAT activity level when compared to the control group. The groups that were treated with varying doses of AECZ showed a significantly increase in testicular SOD activity which is comparable to the control values. The testicular activities of CAT and GPx of the rat treated with AECZ approximated (P<0.05) that of the control groups. The group that was exposed only to mosquito coil however, had a markedly decreased GPx activity compared to that of control values.

Testicular Content of Malondialdehyde (MDA)

The rat that were exposed to mosquito coil had a significantly (P<0.001) elevated testicular MDA as compared to the control value. Administration of AECZ caused a remarkable dose dependent reduction in the testicular MDA level when compared to rats that was exposed to only mosquito coil.

Table 4: Testicular enzymatic and testicular non-enzymatic antioxidants of Experimental rats

Treatment Groups	SOD (u/mg protein)	CAT (u/mg protein)	GPx (nmol/mg protein)	MDA nmol/ng protein
Group A	9.4±3.1	386±0.1	0.92 ±0.1	15. 3±0. 1
Group B	5.5±2. 5*	33 9±2.4 **	0. 4 2±0.1 **	43.1 ±0.4 **
Group C	6.7±4. 5*	372±3. 5*	0.70± 0. 5*	15.2±0. 5
Group D	8.5 ±0.4	382±2. 4	0. 81±0. 6*	12.1±0. 3*
Group E	7.5 ±0. 1*	388 ±2.5	0.81±0.2 *	6.3±0. 3**

Values are expressed as mean ± SD for n=8; *p < 0.05, **p < 0.001 significantly dissimilar from control.
A: 2.5 ml/kg body weight of distilled water
B: Pyrethroid-based mosquito coil
C: Pyrethroid-based mosquito coil and treated with 200 mg/kg body weight of AECZ
D: Pyrethroid-based mosquito coil and treated with 250 mg/kg body weight of AECZ
E: Pyrethroid-based mosquito coil and treated with 300 mg/kg body weight of AECZ

Testis Morphology

Rats that were exposed to mosquito coil alone showed destructive changes in their seminiferous tubules and interstitial tissues. The spermatocytes and spermatids showed degeneration in the shape of vacuolated cytoplasm in uneven arrangement at the basal portion of the germinal epithelium (Fig 2). There was hypo spermatozoa formation in the tubules when compared to the control group of rat (Fig 1). Although a few of the sections of the testis of rat treated with the plant extract showed mild atrophy, these groups exhibited a lesser pathological alteration to the tubular epithelium when compared to the group B rats. As shown in Fig 3, 4 and 5, the seminiferous tubules showed near-normal architecture, with diverse layers of spermatogenic cells up to the spermatids.

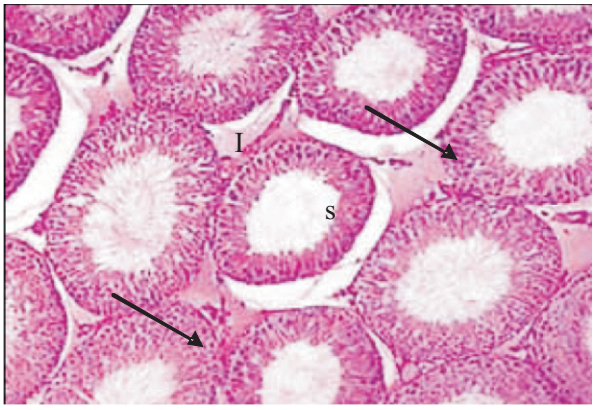

Figure 1 Photomicrograph of the testis of rat *treated with 2.5 ml/kg body weight of distilled water* (Group A) showing normal seminiferous tubular profile (Haematoxylin & Eosin, × 10)

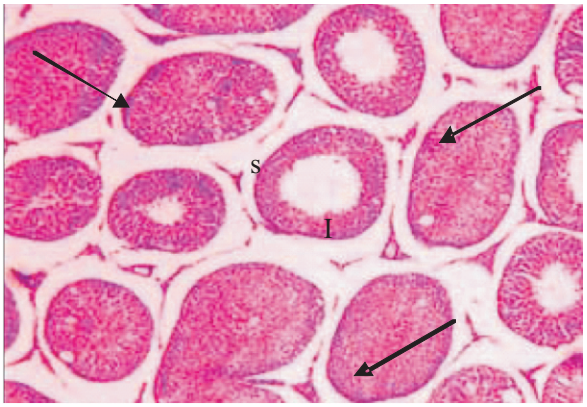

Figure 2 Photomicrograph of the testis of rat exposed to mosquito pyrethroid-based insecticide. Shows necrosis in more than 70% of the seminiferous tubular profiles (Haematoxylin & Eosin, × 10).

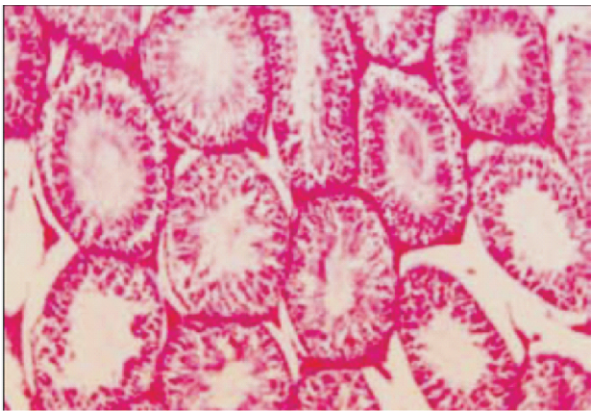

Figure 3 Photomicrograph of the testis of rat exposed to mosquito pyrethroid-based insecticide and treated with 200 mg/kg body weight of AECZ. Showing less than 35% of necrosis in the seminiferous tubular profile (Haematoxylin & Eosin, × 10).

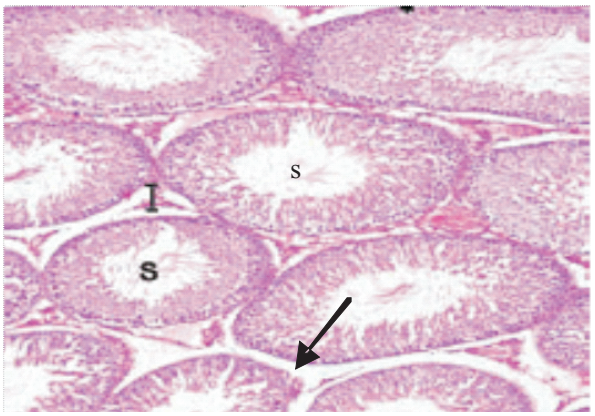

Figure 4 Photomicrograph of the testis of rat exposed to mosquito pyrethroid-based insecticide and treated with 250 mg/kg body weight of AECZ. Showing less than 30% of necrosis in the seminiferous tubular profile (Haematoxylin & Eosin, × 10).

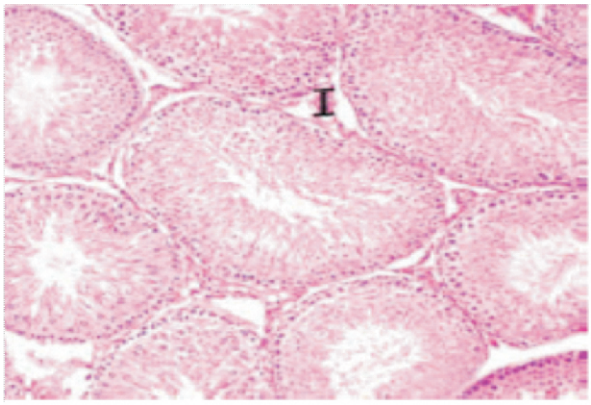

Figure 5 Photomicrograph of the testis of rat exposed to mosquito pyrethroid-based insecticide and treated with 300 mg/kg body weight of AECZ. Showing less than 25% of necrosis in the seminiferous tubular profile (Haematoxylin & Eosin, × 10).

DISCUSSION

Harmful effects of mosquito repellents are now been evaluated by researchers alike. Pyrethroid-based insecticide remains of the most documented reports.

In this study, the mean seminiferous tubular diameters, cross-sectional area of the tubules, the number of tubular profiles per unit area and the mean numerical density of seminiferous tubules of rat exposed to mosquito coil without treatment were significantly reduced. This is consistent with previous reports [34,35].

Sakr and Azeb reported a hyalinised and thickened seminiferous tubules with deformed and poorly developed Leydig cells post pyrethroid treatment [36]. This was also consistent with the report of Alhazza and Bashandy [35]. Such results of studies with pyrethroids in animals have been speculated to be due to mitochondrial membrane damage. Cholesterol itself can be synthesized in the body for use in steroidogenesis, which requires HMG-CoA synthase and HMG-CoA reductase in cytosol of Sertoli and Leydig cells [37]. The reduction in the level of these enzymes on animals models exposed to pyrethroid has been documented [37,38]. Pyrethroid induces toxicity via oxidative stress by generation of free radical and reactive oxygen species (ROS), together with alteration of enzymatic antioxidative systems such as glutathione redox system [39,40].

Although ROS are produced at a minimal concentration by normal aerobic circle, increased levels of **oxidative stress** and failure of inbuilt antioxidant defence system can result to death of a cell [41]. The structure of sperm membrane helps for its flexibility and the functional ability of spermatozoa, but also explains the vulnerability to lipid peroxidation as they present highly specific lipidic composition with high content of polyunsaturated fatty acids (PUFA), plasmalogens and sphingomyelins [2]. These reactive species interact with lipids, proteins and nucleic acids and resulting in the loss of membrane integrity, structural or efficient changes in proteins and genetic mutations, respectively [42].

Evidenced in our study, is a significant ($p < 0.01$) decrease in the activities of SOD, CAT and GPx; in addition to the significant ($p < 0.01$) reduction in the GSH level as well as a significantly ($p < 0.01$) enhanced lipid peroxidation measured as MDA. However, treatment with AECZ ameliorated this derangement and this is evidenced by moderation of antioxidative biochemical markers. In previous reports and evidenced by an increased Catalase activity and reduced Superoxide dismutase, Glutathione preoxidase, Vitamin E and Vitamin C activities in, Vitamin E supplementation have been shown to protect the testis of pyrethroid treated animals [4,6,42,43].

Vitamin E treatment increased the spermatozoal plasminogen activator activity in rams [44]. This only but suggests that pyrethroid induces testicular toxicity via oxidative stress [45]. AECZ could have attenuated the pyrethroid-induced toxicity via a reduction in free radicals dependent lipid peroxidation [46]. Enhancing the antioxidant system levels can favour reproductive potentials [2]. Akunna *et al* reported that at ample levels of antioxidants such as SOD, CAT and probably GPx and reductase, the scavenging potential in the testis can be sustain [2].

Evidenced in this study, was a significant ($P<0.05$) increase in the tubular diameter, profiles per unit area, cross-sectional area and the mean numerical density of seminiferous tubules of group treated with AECZ and Mosquito coil as compared control groups.

In agreement with the report of Garba *et al* and Ahmed *et al* involving mosquito coil exposure to animal models, the findings from this study showed a

significant ($p < 0.01$) decrease in the testis weight, testis weight/body weight ratio and testis volume in rats exposed to only mosquito coil when compared to the controls and the groups that had AECZ post-exposure [47,48]. Pyrethroid exposure in various animals has been reported to decrease sperm parameters [49,50]. A significant decline in sperm characteristic was indicated in the group of rat that was exposed to pyrethroid-based mosquito coil without post treatment with AECZ.

The reduced sperm counts might be caused by a direct effect of the pyrethroids on testicular Leydig and sertoli cells, causing a decrease in testosterone production [51]. It should be noted that acute regulatory protein of steroidogenic origin and enzymes concerned with the biosynthesis of testosterone are essential for smooth functioning of Leydig cells [52]. Pyrethroid causes DNA damage with increase sperm head abnormalities followed by death [11]. Another possibility cause of low sperm count in our study could be attributed to the fact that pyrethroids interact competitively with androgen receptors and sex hormone binding globulins, mimicking estrogen and resulting in a disruption of the endocrine system hence low sperm counts [53]. The abnormal testes might be another factor further exaggerating the fertility loss. Pyrethroids have been documented to cause a reduction in the levels of P450scc (cytochrome P450 side chain cleavage) testicular protein and mRNA levels of steroidogenic acute regulatory (StAR) protein in testes [54].

A spermatozoon has to pass through the epididymis before maturation can occur; this involves an extremely complicated tuned relationship amid spermatozoa and epididymal epithelium [55,56]. Spermatozoa maturation can be halted as a result of hurried spermatozoa in the epididymal epithelium thereby leading to a reduction in viable spermatozoa. Treatment with AECZ post mosquito coil exposure averted the derangement in sperm parameters with the values comparable to that of the control. This could be as a result of the antioxidative properties of *Croton zambesicus* as shown in previous reports [57,19,24].

Vacuolization of the interstitium and hypospermatozoa formation in the seminiferous tubules of rats exposed to mosquito coil alone showed clear degenerative changes characterized by lumen devoid of spermatozoa. The histological evidences herein this study is consistent with several other reports on male infertility [25,46,58], reported vacuolation, necrosis and significantly decreased viable cell counts post cypermethrin exposure.

The degenerative changes in our study could have been as a result of increased oxidative stress. It must be noted however, that the group of rat treated with AECZ post exposure to mosquito coil had a normal epithelia outline with intact interstitium indicating its role as an antioxidant as earlier reported [2].

The overall results obtained in this study suggest that Croton zambesicus leave extract possesses dose dependent protective effect on the testes of rat exposed to insecticide.

REFERENCES

1. Abd-Alla EAM, Nassar AM, Neamat-Allah AA and Aly SE. Prevalence of pesticide residues in fish, cheese and human milk. *Assiut Vet. Med. J.,* 2002; 47: 110–124.
2. Akunna GG, Saalu LC, Ogunlade B, Ogunmodede OS, Akingbade AM. Anti-Fertility Role of Allethrin Based-Mosquito Coil on Animal Models. International Journal of Biology, Pharmacy and Allied Science. 2013; 2(2): 192-207.
3. World Health Organization. Tropical Diseases-Progress in International Research 1987-1988, Ninth Programme Report, TDR Special Programme. Geneva: WHO; 1989.
4. Aslam F, A Khan, MZ Khan, S Sharaf, ST Gul and Saleemi MK. Toxico-pathological changes induced by cypermethrin in broiler chicks: Their attenuation with Vitamin E and selenium. Exp Toxicol Pathol, 2010; 62: 441-450.
5. Liu WK, Wong M S and Mui YL. *Toxicol. Lett*., 1987; **39**: 223–230.
6. Sharaf S, Khan A, Khan MZ, Aslam F, Saleemi MK, Mahmood F. Clinico haematological and micronuclear changes induced by cypermethrin in broiler chicks: Their attenuation with vitamin E and selenium. Exp Toxicol Pathol, 2010; 62: 333-341.
7. Hussain R, Mahmood F, Khan A, Javed MT, Rehan S and Mehdi T. Cellular and biochemical effects induced by atrazine on blood of male Japanese quail (Coturnix Japonica). *Pestic. Biochem. Physiol.* 2012; 103: 38–42.
8. Atamanalp M, Keles MS, Haliloglu HI and Aras MS. The effects of cypermethrin (a synthetic pyrethroid) on some biochemical parameters (Ca, P, Na & TP) of rainbow trout (*Oncorhynchus mykiss*). *Turkish J. Vet. Anim. Sci.,* 2002; 26: 1157–1160.
9. Naz S, Rana SA, Javed M and Rehman KU. Effect of two different rodenticides on serum biochemistry of house rats (*Rattus rattus*). *Pakistan Vet. J.*, 2010; 30: 239–241.
10. Hussain R, Mahmood F, Khan MZ, Khan A and Muhammad F. Pathological and genotoxic effects of atrazine in male Japanese quail (*Coturnix japonica*). *Ecotoxicology* 2011; 20: 1–8.
11. Ahmad M, Hussain I, Khan A and Najib-ur-Rehman. Deleterious effects of cypermethrin on semen characteristics and testes of dwarf goats (*Capra hircus*). Exp Toxicol Pathol, 2009; 61: 339-346.
12. Ahmad L, Khan A and Khan MZ. Pyrethroid-inducedreproductive toxico-pathology in non-target species. *Pakistan Vet. J.,* 2012; 32: 1–9.
13. Bhushan B, Saxena N and Saxena PN. Beta-cyfluthrin induced histochemical alterations in the liver of the albino rat. *Scandinavian J. Lab. Anim. Sci*., 2010; 37: 61–66.
14. Ahmad L, Khan A and Khan MZ. Cypermethrin induced biochemical and hepato-renal pathological changes in rabbits. *Int. J.Agric. Biol.,* 2011; 13: 865–872.
15. *Sharma VP*. Health hazards of mosquito repellents and safe alternatives. Current Science, 2001; 80(3): 10.

16. Adjanohoun EJ, Adjakide V and deSouza S. Contribution to ethnobotanical and floristic studies in Republic of Benin.Vol.1. Agency for Cultural and Technical Cooperation, 1989; 245.
17. Abo KA, Ogunleye A, Ashidi JS. Antimicrobial Potential of Spondias Mombin, *Croton Zambesicus,* and *Zygotoitonia crocea.* Phytother. Res., 1999; 13(6): 494-497.
18. Ofusori DA, Adelakun AE, Ayoka AO, Ibeh JA, Adeeyo AO, Adesanya OA and Ajeigbe KO. Histological investigation of the effects of *Croton zambesicus* on the liver of swiss albino mice. *The Internet Journal of Gastroenterology*, 2008; 7(1): 15-20.
19. Okokon JE, Ofodum KC, Ajibesin KK, Danladi B and Gamaniel KS. Pharmacological screening and antiplasmodial activity of *Croton zambesicus* against *Plasmodium berghei berghei* infection in mice. *Indian Journal of Pharmacology,* 2005; 37(4): 243-246.
20. Okokon JE and Nwafor PA. Antiulcer and anticonvulsant activities of *Croton zambesicus* root extract. *Pakistan Journal of Pharmaceutical Sciences,* 2009; 22(4): 384-390.
21. Boyom FF, Keumdjio F, Dongmo PM, Ngadjui BT, Amvam-Zollo PH, Menut C and Bessiere JM. Essential oil from *Croton zambesicus* Muell. Arg. growing in Cameroun. *Flavour and Fragrance Journal,* 2002; 17: 215-217.
22. Canadian Council of Animal Care (CCAC). Guide to the handling and Use of experimental animals. Ottawa: Ont.; 2 United States NIH publications, 1985; no. 85 – 23: 45-47.
23. American Physiological Society (APS). Guiding principles for research involving animals and human beings. Am J Physiol Regul Integr Comp Physiol., 2002; 283: 281-283.
24. Akunna GG, Saalu CL, Ogunmodede OS, Babatunde O, Bello AJ. Aqueous Extract of Date Fruit (Phoenix Dactylifera) Protects Testis against Atrazine-induced Toxicity in Rat. World J Life Sci. and Medical Research 2012; 2(2):100.
25. Saalu LC, Togun VA, Oyewopo AO and Raji Y. Artificial crypptorchidism and the moderating effect of melatonin in Sprague-Dawley rats. *J.Appl. Sel*., 2006; 6(12): 2889-94.
26. Qin D and Lung MA. Morphometric study on Leydig cells in capsulotomized testis of rats. *Asian J Androl*., 2002; 4: 49-53.
27. Gundersen HJG**,** Jenson EB. The efficiency of systematic sampling in stereology and its prediction. J Microsc., 1987; 147: 229-263.
28. Gilliland KO, Freel CD, Lane CW, Fowler WC, Costello MJ. Multilamellar bodies as potential scattering particles in human age-related nuclear cataracts. *Molecular Vision*, 2001; **7**: 120-130.
29. Aebi H. Catalase. In methods of enzymatic Analysis, Bergmeyer, H.Ed. Verlag Chemical, Weinheim 1983; 3: 273.
30. Rukmini MS, Benedict I, Vivian D. Superoxide dismutase and catalase activities and their correlation with malondialdehyde in Scizophrenic patients. Ind. J. Clin. Biochem. 2004; 19(2): 114-118.
31. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase. Science. 1973; 179: 588-590.
32. Buege JA**,** Aust SD. Microsomal Lipid Peroxidation. In: Fleischer, S., Packer L., (Editors), Methods in Enzymology. New York: Academic Press, 1978; 302-330
33. Snedecor GW, Cochran WG. Statistical Method, 7th edn. Iowa: Iowa State University Press, 215. Tindal HD. Commercial vegetable growing. Oxford Press. London. 1968; 69.
34. Ezeasor DN. Light and electron microscopical observation on the leydig cells of the scrotal and abdominal testis of naturally unilateral cryptorchid in West Mrican dwarf goats. *J. Anat.,* 1990; 141: 27-40.
35. Alhazza LM and Bashandy SA. Influence of vitamin C on the toxicity of Pifpaf (containing permethrin) to gonads of male rats. Saudi J Biol Sci., 1998; 5: 31-37.
36. Sakr, S.A. and A.E. Azab. Effects of pyrethroid inhalation on testes of albino rats. Pak J Biol Sci., 2001; 4: 498-500.
37. Zhang SY, Ito Y, Yamanoshita O, Yanagiba Y, Kobayashi M, Taya K, Li CM, Okamura A, Miyata M, Ueyama J, Lee CH, Kamijima M and Nakajima T. Permethrin may disrupt testosterone biosynthesis via mitochondrial membrane damage of Leydig cells in adult male mouse. Endocrinology, 2007; 148: 3941-3949
38. Jin Y, Wang L, Ruan M, Liu J, Yang Y, Zhou C, Xu B and Fu Z. Cypermethrin exposure during puberty induces oxidative stress and endocrine disruption in male mice. Chemosphere, 2011; 84: 124-130.
39. Sanvidhan GS, Achint K, Rafat SA, Ayanabha C, Tripathi AK, Madiratta PS, Banerjee BD. Protective of melatonin against propoxur-induced oxidative stress and suppression of humoral immune response in tats. *Indian J. Exp. Biol*., 2006; 44: 312-315.
40. Mathur PP, Saradha B, Vaithinathan S. Impact of environmental toxicants on testicular function. *Immunology, Endocrine and Metabolic Agents Med Chem*., 2008; 8: 79-90.
41. Pacher P. FRs induce Chain reaction. *Physiol. Rev*., 2007; 87: 315-424.
42. Raina R, Verma PK, Pankaj NK and Kant V. Ameliorative effects of α-tocopherol on cypermethrin induced oxidative stress and lipid peroxidation in Wistar rats. Int J Med Med Sci, 2009; 1: 396-399.
43. Navayath S and Thiyagarajan D. Fenugreek supplementation imparts erythrocyte resistance to cypermethrin induced oxidative changes *in vivo*. J Complement Integr Med, 2011; 8: 1436-1438.
44. Rekkas C, Kokolis N, Belibasaki S, Tsantarliotou M and Smokovitis A. Effect of α-tocopherol on plasma testosterone and plasminogen activator activity or inhibition in ram spermatozoa. Theriogenology, 2000; 53: 751-760.
45. Yousef MI. Vitamin E modulates reproductive toxicity of pyrethroid lambda-cyhalothrin in male rabbits. Food Chem Toxicol, 2010; 48: 1152-1159.
46. Saalu LC, Osinubi AA and Olagunju JA. Early and delayed effects of doxorubicin on testicular oxidative status and spermatogenesis in rats. *Int. J. Cancer Res*., 2010; 6: 1-9.
47. Garba SH, Shehu MM and Adelaiye AB. Toxicological Effects of Inhaled Mosquito Coil Smoke on the Rat Spleen: A Haematological and Histological Study. *Journal of Medical Sciences,* 2007; 7: 94-99.
48. Ahmed M, Mohamadin, Bassem S, Amany A., Abd El-Aal, Ahmed A. Elberry FA. Al-Abbasi. Protective effects of Nigella sativa oil on propoxur-induced toxicity and oxidative stress in rat brain regions. *Pesticide Biochemistry and Physiology,* 2010; 98: 128-13.
49. Kamijima M, Hibi H, Gotoh M, Taki K, Saito I, Wang H, Itohara S, Yamada T, Ichihara G, Shibata E, Nakajima T and Takeuchi Y. A survey of semen indices in insecticide sprayers. J Occup Health, 2004; 46: 109-118.

50. Perry MJ. Effects of environmental and occupational pesticide exposure on human sperm: a systematic review. Hum ReprodUpdate, 2008; 14: 233-242.
51. Elbetieha A, Da'as SI, Khamas W and Darmani H. Evaluation of the toxic potentials of cypermethrin pesticide on some reproductive and fertility parameters in the male rats. Environ Contam Toxicol., 2001; 41: 522- 528.
52. Wang H, Wang Q, Zhao XF, Liu P, Meng XH, Yu T, Ji YL, Zhang H, Zhang C, Zhang Y and Xu DX. Cypermethrin exposure during puberty disrupts testosterone synthesis via downregulating StAR in mouse testes. Archiv Toxicol, 2010; 84: 53-61.
53. Yousef MI, El-Demerdash FM and Al-Salhen KS. Protective role of isoflavones against the toxic effect of cypermethrin on semen quality and testosterone levels of rabbits (Anglais, abstract in English). J Environ Sc Health B, 2003; 38: 463-478.
54. Farag AT, Goda NF, Shaaban NA and Mansee AH. Effects of oral exposure of synthetic pyrethroid, cypermethrin on the behaviour of F1-progeny in mice. Reprod Toxicol, 2007; 23: 560–567.
55. Mably TA, Moore RW and Peterson RE. In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol App Pharmacol, 1992; 114: 97-107.
56. Moore HD, Samayawardhena LA, Brewis IA. Sperm maturation in vitro: co-culture of spermatozoa and epididymal epithelium. J Reprod Fertil Suppl, 1998; 53: 23-31.
57. Gill SA, Rizvi F, Khan MZ and Khan A. Toxic effects of cypermethrin and methamidophos on bovine corpus luteal cells and progesterone production. Exp Toxicol Pathol., 2011; 63: 131-135.