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Effects of mixtures of lambda-cyhalothrin and dimethoate on the reproductive competence of adult male Albino rats

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Abstract

The indiscriminate use of pesticide mixtures by farmers in developing countries often results in serious environmental pollution and health problems. This study was designed to investigate the effects of commercial mixture of lambda-cyhalothrin and dimethoate on the reproductive competence of male albino rats. Forty (40) adult male albino rats were divided into four (4) equal groups. Dimethoate group (DM) received: (10.7 mg/kg b.w/orally/daily), Lambda-cyhalothrin group (LC) received: (3.9 mg/kg b.w/orally/daily), Magicforce group (MF) received: (1.2 mg/kg b.w/orally/daily) while the Control group (C) received only food and water daily. The rats were sacrificed at the end of the exposure (28 days). The gonads were excised, weighed and the tissue homogenates prepared for biochemical analysis. The lactate dehydrogenase assay was used to determine the cytotoxic effects on the organ while estimating the gonadal oxidative stress (SOD, GST and MDA). Parameters such as testosterone, sperm count, motility and viability were used to determine the reproductive status. The results showed that the LC-treated group recorded significantly higher values ($p<0.05$) in all investigated parameters when compared with DM and MF groups.

Keywords: Magicforce, Toxic, Gonadal, Dimethoate, Lambda-Cyhalothrin

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1. Introduction

The need to achieve higher efficacy and cut cost has led most farmers in developing countries especially in the rural areas into the use of pesticides mixtures whose health impacts are yet to be determined. Hence, there is urgent need to accurately identify their potential hazards to man and other non-target species which might be beneficial [1].

Dimethoate is a broad spectrum insecticide used mostly in agricultural practice as well as domestic application to control houseflies [2]. It is a class II insecticide with moderate toxicity. The mechanism of dimethoate toxicity is due to its inhibition and accumulation of acetycholinesterase (AChE), at the nerve endings and the neuromuscular junctions [3]. Lambda-cyhalothrin (LC) is a type II pyrethroid, used mostly to control insect pest in agriculture, public facilities, homes and gardens. It is often considered a potent neurotoxic pyrethroid with the capacity to cause hepatotoxicity [4]. Recently, most pesticides available in developing countries are in commercial mixtures and have resulted in an increase in the prevalence of toxicity.

Ngoula *et al.*[5] reported that organophosphate pesticides have adverse effects on the reproductive capabilities of exposed male albino rats. Results from his studies showed decrease in serum total protein, sperm density and motility as well as fertility. Sayim [3] observed that subchronic exposure of mice to dimethoate reduced the animals' testicular weights. Farag *et al.* [6] demonstrated the adverse effects of dimethoate on the reproductive status of male albino rats. Data obtained from his investigation showed reduced sperm viability, motility and density in the dimethoate treated rats.

In recent times, more evidence indicates that pyrethroid insecticides can reduce sperm count and motility, cause deformity of the sperm head, increase the count of abnormal sperm, damage sperm DNA and induce its aneuploid rate as well as affect sex hormone levels and produce reproductive toxicity [7]. Sanhez *et al.*[8] observed reduction in the testicular weights of rats exposed to lambda-cyhalothrin which was perhaps due to decreased tubule size, reduced number of germ cells and enlarged spermatids. Decline in sperm motility after oral administration of lambda-cyhalothrin on rats was either by androgen deprivation of the pyrethroid or low

spermatogenesis [9].Studies on male workers exposed to mixtures of pesticides have found reduction in serum bioavailable testosterone levels and reduced semen volume on ejaculation and sperm counts [10].Non-target species are often exposed to complex and variable mixtures of chemicals in the environment because of the varying levels of interactions of the individual chemicals, hence the need for this study [11].

It is our hypothesis in this present study that Magicforce (DA+LCT) oral administration in adult male albino rats may cause adverse effects on the overall reproductive competence of the animals.

2. Materials and Methods

2.1. Materials

2.1.1. Insecticides

Dimethoate (DM) (40% EC; Jiangsu Tenglong Biological and Medicinal Co. Ltd. China.) and Lambda-cyhalothrin (LC) (2.5% EC; Bretmont Ltd. England) insecticides were used in the present research. A Mixture of both insecticides (20:1) was contained in MagicForce; a commercial EC formulation produced by Anhui Zhongshan Chemical Industries Co. Ltd. China. These chemicals were purchased from Alaba International market in Owerri, Imo state, Nigeria.

2.1.2. Experimental animals

Forty male albino rats (3–4 months old; 160–180g b.wt.) were obtained from the breeding animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were housed in individual cages and allowed to acclimatize under laboratory conditions at room temperature for one week prior to commencement of the experiment. The animals were kept under hygienic and favorable conditions, and maintained under a 12 h light/12 h dark cycle, with pelletized rat feeds (UAC Vital Feeds, Nigeria) and water available ad libitum.

2.2. Methods

2.2.1. Experimental design

Sub-lethal doses of the insecticides (5% of the LD_{50}) were used in this research and administered through oral gavage. The animals were divided into 4 groups of 10 animals each with the mean weights equalized as nearly as possible. Dimethoate group (DM) received 10.7 mg/kg body weight. Lambda-cyhalothrin group (LC) received 3.95 mg/kg body weight. Magicforce group (MF) received 1.2 mg/kg body weight while the control group (C) received food and distilled water only. The animals were given normal diet and water ad libitum throughout the 28 days period of the experiment. At the end of the 28 days feeding period, an overnight fast was imposed on the animals before sacrificing.

2.2.2. Blood and tissue sampling

At the end of the post feeding fast, the rats were subjected to light diethyl ether to induce anesthesia. Blood was collected by cardiac puncture into clean and dry test-tubes without anticoagulant, allowed to stand to separate serum and then preserved in a refrigerator until used for analyses. Then the testes was collected, cleaned, weighed (absolute weight) and homogenized in 5mL cold buffer (0.1 M-phosphate buffer, pH 7.4) per gram tissue. The homogenates were further vortexed for 15 minutes, allowed to stand and the supernatant collected into sterile tubes and preserved in a refrigerator until used for biochemical analyses.

2.2.3. Relative organ weight of the rats

The relative organ weight of each animal was calculated as follows:

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Final body weight of the rat (g)}} \times 100$$

2.3. Biochemical analyses of samples

2.3.1. Determination of serum lactate dehydrogenase (LDH)

Serum LDH was determined according to the method of McQueen, (1975) as reported by Sherif *et al.*[12].

2.3.2. Determination of superoxide dismutase (SOD) activity in the samples

SOD activity was determined as reported by Sherif *et al.* [12].

2.3.3. Determination of glutathione-S-transferase (GST) in the samples

Glutathione-S-transferase (GST) was measured by the method of Habig et al., [12] as reported by Sherif *et al.* [13].

2.3.4. Determination of lipid peroxidation

The levels of lipid peroxidation in samples were measured as malondialdehyde (MDA) according to the method of Buege and Aust (1978) as reported by Sherif *et al.* [12].

2.4. Sperm Evaluation

Rat spermatozoa were obtained by the method of Cancel *et al.* [13]. A 5g of testes was minced in 2 ml of physiological saline and incubated at 370˚C for 45 minutes to allow dispersion of spermatozoa.

Sperm Motility: Sperm motility was evaluated by placing a drop of the sperm suspension on a microscopic slide and observed for sperm motility at 400x magnification using a light microscope. Ten microscopic fields and a total of 200 sperms were observed in each case. Sperm motility was expressed as a percentage of motile sperm relative to the total sperm count according to World Health Organization [14].

Sperm Count: Ten micro litre of the diluted sperm suspension made by adding 5µl of diluted buffer (0.35% formalin containing 5% $NaHCO_3$ and 0.25% trypan blue) was transferred to each counting chamber of the haemocytometer and was allowed to stand for 5 min.

This chamber was then placed under a binocular light microscope using am adjustable light source. The calibrated part of the chamber was then focused and the number of spermatozoa counted in five 16-celled squares. The sperm concentration was then calculated multiplied by 5 and expressed as $[X]\times10^6$ ml^{-1}, where [x] is the number of spermatozoa in a 16-celled square. Counting in the microscope was done at 400x magnification [14].

Sperm Viability: To determine the sperm viability, 0.2ml of 1%trypan blue stain was mixed with a drop of the semen in grease free slide for 5 min, then a drop of the solution was pipetted into a Neubauer hemocytometer, covered with a slip and allowed to stand for 2 min. Then observation was under a light microscope at 400X magnification. The numbers of stained and unstained sperms were scored in ten different microscope fields. Dead sperm were stained pink while live ones were not stained.

2.5. Determination of testosterone concentration

The level of testosterone was measured by using ELISA according to the method of Tietz [15].

2.6. Statistical analysis

The data was analyzed using a one-way analysis of variance (ANOVA) using the statistical package for the social science (SPSS) program, version 11 followed by least significant difference (LSD) to compare significance between groups.

3. Results and discussion

3.1. Average testicular weight of the rats

The weight of the testes was significantly reduced. DM, LC and MF groups had the following weights 1.83g, 1.60g and 2.12g, respectively as against the control group with 2.62g. The testicular weights had no significant difference statistically at $p\leq0.05$ as shown in Figure 1. The testicular weight relies on the mean testicular weight. The LC treated group was most reduced compared other groups. Reduction in the testicular weight on of rats exposed to pesticides may be due to decreased tubule size, reduced number of germ cells and enlarged spermatids [8].

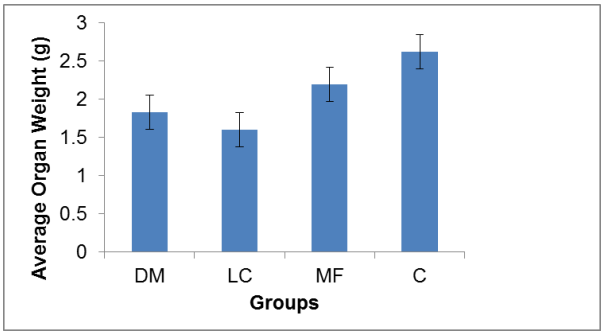

Figure 1: Average Weight of Testes of Male Albino Rats

All values were expressed as mean ±SEM.

LSD* shows the significant difference of treatments compared to control (Group C) at $p\leq0.05$.Dimethoate (DM), Lambda-Cyhalothrin (LC), Magicforce (MF), Control(C)

3.2 Effects of the pesticides and their mixture on testicular lactate dehydrogenase activity

There was significant difference in the lactate dehydrogenase activity in the testes ($P\leq0.05$). The LDH activity in the C group was 261.57µ/L. There was increase in the enzyme activity in pesticide treated groups with highest observed in LC treated group (352.35 µ/L), the MF-treated group was 333.53 µ/L while the least was the DM-treated group (285.58 µ/L) The high lactate dehydrogenase activity suggests that the plasma membrane of the affected cells may have been damaged or ruptured causing the cytosolic enzyme to leak out due to the presence of the toxicants [16]. The toxic effect on the testes were exhibited by the LC and the MF treated groups compared to control group which agrees with reports that man-made pyrethroids adversely affect the testicular functions in experimental animals [17] and that they are potent endocrine disrupters [18].

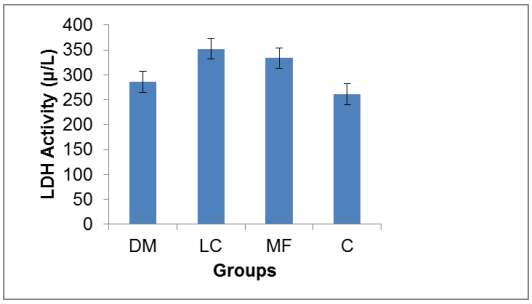

Figure2: Effects of the Pesticides and Their Mixture on Lactate Testicular Dehydrogenase Activity

All values were expressed as mean±SEM

LSD* shows the significant difference of treatments compared to control (Group IV) at p≤0.05 Dimethoate(DM), Lambda-Cyhalothrin(LC), Magicforce(MF), Control(C).

LDH = Lactate dehydrogenase

3.3 Oxidative stress effects on the testes

The results from the oxidative state of the testes of male albino rats exposed to 1/20 LD_{50} of the pesticides and their mixtures showed that the activity or levels of SOD, GST and MDA were higher than that of the control. The LC -treated group had more decreased activity of SOD, GST and protein followed by the MF-treated group and DM-treated group. There was an increase in MDA concentration in the LC-treated group followed by the MF, then, the DM. An increase in MDA, the most likely used biomarker of lipid peroxidation, indicates cellular damage, inhibition of several enzymes and cellular functions [19]. This result is in agreement with the previous findings [16]. He reported that decrease in SOD and GST is an indication of some levels of increased generation of reactive oxygen species (ROS) that led to lipid peroxidation in the liver and testes. Since the testes is not the site for metabolism and detoxification, the oxidative stress caused by 1/20 LD_{50} of the pesticides is expected to be lower than that of the liver.

Table 1: Effects on the Oxidative Stress Parameters on the Testes

Parameter	DM	LC	MF	C
SOD(IU/L)	5.33E-05±2.3E-06	3.92E-05±2.6E-06*	4.36E-05±1.1E-06	8.12E-05±2.2E-06
GST(µmol/g.tissue)	3.55E-06±6.E-07	1.01E-05±5.9E-07*	2.31E-05±4.3E-08	2.62E-06±3.5E-07
MDA(nmol/g.tissue)	0.15±0.0	0.51±0.1*	0.17±0.0	0.12±0.0

All values were expressed as mean ±SEM.

LSD* shows the significant difference of treatments compared to control (C) at p≤0.05 Dimethoate(DM), Lambda-Cyhalothrin(LC), Magicforce(MF), Control(C). Dimethoate(DM), Lambda-Cyhalothrin(LC), Magicforce(MF), Control (C). SOD=Superoxide dismutase, CAT=Catalase, GSH=Glutathione, GST=Glutathione-S-transferase, GPx=Glutathione peroxidase, MDA=Malondialdehyde.

3.4 The effects of the pesticides and their mixture on the reproductive status on the male albino rats

Data obtained from the present study revealed that sperm count, sperm motility and sperm viability of the pesticide treated rats were not significantly different from the control. However, Testosterone analysis was significant with much decrease observed in the LC-treated group. The above result is in line with the report of Joshi [9] who stated that serum testosterone levels decreased in male mice following sub lethal exposure to pyrethroid pesticide. The high reduction in testosterone levels might be due to high production of reactive oxygen species (ROS) by the pyrethroid exposure. Similar results were reported previously [20]. The toxic effect was antagonized by dimethoate in their commercial product.

Table 2 : Effects the Pesticide Mixtures on the Reproductive Status of the Rats

Parameter	DM	LC	MF	C
Testosterone (pg/mL)	0.43	0.35*	0.48	0.55
Sperm Count ($x10^6$)	39.77	32.93	35.53	51.21
Sperm Motility (%)	44.03	41.05	42.79	45.32
Sperm Viability (%)	40.75	38.41	38.20	45.67

All values were expressed as mean±SEM

LSD* shows the significant difference of treatments compared to control (C) at p≤0.05 Dimethoate(DM), Lambda-Cyhalothrin(LC), Magicforce(MF), Control(C)

4. Conclusions

It can be concluded that chronic exposure to sub-lethal doses (1/20 LD_{50}) of lambda-cyhalothrin (a synthetic pyrethroid) singly, has the potentials to elicit sterility but in Magicforce (commercial mixture), its toxic effect is antagonized by the presence of dimethoate while maintaining efficacy.

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