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Genotoxic Effects of Mosquito-Cypermethrin Insecticide Exposures on Sprague-Dawley Male Rats Up to Second Filial Generations

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ABSTRACT

Background and Objectives: Cypermethrin insecticide has been used in many countries to control malaria and is known to cause several detrimental features. This work has so far investigated the genotoxic effects of mosquito-cypermethrin insecticide exposures on Sprague-Dawley male rats up to second filial generations. The scope of this study covers evaluation of biomarkers of DNA damages, oxidative stress and antioxidant status of Sprague-Dawley male rats. This study aimed to investigate the genotoxic effects of cypermethrin insecticide exposure on the first (F-1) and second (F-2) filial generations of Sprague-Dawley male rats. This study was conducted at the Human Biochemistry Research Laboratory, Nnamdi Azikiwe University (NAU), Nnewi Campus, Anambra State, Nigeria.
Methods: The research involved 18 male Sprague-Dawley rats, aged approximately 12 weeks and weighing 180-200 g. The rats were divided into control and experimental groups. Key biomarkers, including serum 8-hydroxydeoxyguanosine (8-O-HdG), malondialdehyde (MDA), cortisol, and antioxidant levels, were assessed using spectrophotometric techniques.
Results: Results demonstrated significant increases in 8-OHdG, cortisol, and MDA concentrations in the exposed groups ($P < 0.05$), indicating elevated oxidative stress and hence, deoxyribonucleic acid (DNA) damage. Moreover, total antioxidant capacity (TAC) was significantly reduced ($P < 0.05$) in the F-1 generation, alongside decreased catalase activity, glutathione peroxidase (GPx), and reduced glutathione (GSH) levels in both F-1 and F-2 litters ($P < 0.05$), hence, depleted antioxidant status. Major findings in this work include; oxidative stress induced by cypermethrin exposures, depleted antioxidant levels, and DNA damage.
Conclusions: These findings suggest that cypermethrin exposure might have induced genotoxic effects through damages on DNA induced by oxidative stress in Sprague-Dawley male rats, impacting up to the second filial generation. The exposure may have been dependent on dosage.

Keywords: Antioxidants, cypermethrin, generations, mosquitoes, rats, toxicity

1. INTRODUCTION

Background: Malaria has been wreaking havoc on many human communities worldwide. Plasmodium parasite species have been identified as the causal agents of malarial illness. [1] According to statistics, the WHO-administered region of Africa had a 40 % decline in malaria rates and a 60 % fall in fatality rates between 2000 and 2022. [1] The World Health Organization (WHO) had helped the African region to make significant progress in controlling, preventing, and eliminating malaria. This includes the delivery of mosquito nets, medications, [2] test kits, equipment, and awareness programs. Malaria eradication has long been on the agenda for endemic nations. [3, 4] The primary objective is to eradicate malaria and rid the world of the illness.

Pesticides are playing an important role in agriculture and public health. They play major parts by increasing the manufacture of food consumed and fiber content of food and improving the human health system by decreasing the rate of vector-borne illness. In addition to crop damage induced by pests, these pests can cause negative effects on human health and domestic animals by producing toxic metabolites, which have been known to cause oxidative stress that has been found to cause a lot of human diseases. [5] Today, many pesticides are approved globally for use in various areas of agriculture. However, pesticides represented are of numerous types (examples: insecticides, herbicides, bactericides, nematicides, acaricides, fungicides, molluscicides, and rodenticides). Each is active against specific pests (e.g., insects, weeds, bacteria, nematodes, fungi, snails, and rats). [5]

Mosquitoes are among the pests found in most warm, wet, tropical countries. Mosquitoes play major roles in the transmission of diseases, which include malaria. They are considered a nuisance to humans and other animals.

The use of insecticides is one of the methods employed in most African countries in controlling insects where insect bites, such as mosquitoes, tsetse flies, ants, and scorpions, are prevalent. [5] The use of mosquito insecticides is among the strategies used globally to control mosquito bites, thereby preventing malaria diseases. Many traditional and synthetic insecticide formulations are being used in so many ways in many African countries, including Nigeria, to control and eliminate mosquito bites. Some of these insecticides include organophosphates, organochlorines, carbamates, and pyrethroids. They have been reported to contain active ingredients such as permethrin, N-methyl carbamates, allethrin, cypermethrin, and avermectin, which are highly toxic to the liver and carcinogenic.

Cypermethrin ($C_{22}H_{19}Cl_2NO_3$), a pyrethroid insecticide, is widely used in many countries on farms and in houses in so many formulations. In various houses, it is used to control mosquitoes mainly, ants, cockroaches, and other pests that constitute a nuisance in the houses. In agriculture, cypermethrin is used to control pests which damage crops. According to, [6] cypermethrin pesticide and chloryrifos have shown higher rate of respiratory failure in a mixture containing cypermethrin pesticide and chloryrifos than others. Cypermethrin had also been reported to be nephrotoxic and hepatotoxic in male and female rats. [5, 7] Commercial mixture of cypermethrin had led toxicological effects on Physalaemus gracilis tadpoles. [8] In Agriculture, presence of insecticide residues such as cypermethrin had been known to be toxic to black soldier fly larvae (Hermetia illucens), an insect reared for food and feed. [9, 10] The use of synthetic-mosquito insecticides has been used to control mosquito bites in many countries including Nigeria. Some of these insecticides include cypermethrine-insecticides, mosquito coils, prellethrin insecticides, and pyrethrine insecticides. In some local villages in Nigeria, some people implore the traditional methods of using burnt orange peels and leaves of *Opium vande*, in controlling mosquito bites.

Hepatic enzymes such as aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) and non-enzyme marker such as bilirubin are among the biomarkers of hepatotoxicity. [11-12] Reactive oxygen species can damage DNA, RNA and other macromolecules in the body such as bilirubin, total proteins, carbohydrates, lipids and antioxidants (GSH, GPX, CAT and TAC). [13] Some of these effects cause some damages in some genes in the body and can be transferred from one generation to the other over a period of time. [14] Cytochrome p450 genes and other genes of the body such as Tp53, BAX and BCL are prone to oxidation by reactive oxygen species and therefore among the genetic markers used to access genotoxicity in experimental animals. [15-16]

Significance of study: The indiscriminate use of mosquito insecticides such as cypermethrin in homes, offices, and the environment without considering the health implications is a serious concern to public health. There is, therefore, a clear need to assemble a set of tests that cover different complexity levels so that we can have a more accurate approximation of the underlying mechanism of action of genotoxic potentials of insecticides inside the mitochondria of animals. This will go a long way in helping to design drugs for the effective treatment of genotoxic effects of insecticides. Literature abounds on the toxic effects of insecticides on many organs of the body, such as the liver, kidney, lungs, heart, and eyes, and also on the gastrointestinal effects of insecticides but the information is still scanty on the genotoxic effects of cypermethrin-insecticide exposures on animals at various doses up to the second filial generation. It is, therefore, necessary to increase the scientific evidence regarding the toxicity and genotoxicity effects of chemical substances applied in our country, Nigeria and the inheritable effects from one generation to the other. This work therefore seeks to close this gap. Aim: This work aimed at evaluating the genotoxicity of mosquito-cypermethrin insecticide exposures on Sprague-Dawley male-rats up to the second filial generation (F2). This will help in understanding the underlying mechanism of reactions of cypermethrin on DNA inside the mitochondria in the cells of Sprague-Dawley male rats.

2. MATERIALS & METHODS

2.1. Materials

All of the analytical-grade chemicals used in this project were obtained from British Drug House Ltd. in Poole, England, through her sales representative in Ikeja, Lagos State, Nigeria. We purchased the 8-hydroxy-2-deoxyguanosine (8-OHdG) kit from Elabscience Biotechnology Inc. in Wuhan, Hubei, China. The source of the distilled water used was obtained from Citadel Project Hub (Research Laboratory), Okofia-Otolo, Nnewi, Anambra State, Nigeria. In Lagos State, Nigeria, SC. Johnson, West Africa Ltd. provided the cypermethrin that was used. Rat feed used was obtained from UAC Foods Ltd., Jos, Plateau State, Nigeria, through her sales representative in Nnewi, Anambra State, Nigeria. The Sprague-Dawley male rats used were obtained from the Animal Facility Unit of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe Univ

2.1.1. Overview of the Research Area

This study took place at the Department of Human Biochemistry Laboratory, Faculty of Basic Medical Sciences, College of Health Sciences, NAU, Nnewi Campus, Anambra State, Nigeria.

2.2. METHODS

2.2.1. Animal Handling

The Sprague-Dawley male rats were fed on a rat pellet diet. Animal care and handling were done according to guidelines given by, [17] the National Health Research Ethics Committee of Nigeria (NHREC), and Animal Research Ethics Committee (AREC) of Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. The ethical

approval was given with reference number as NAU/AREC/2024/0101. The rats were kept in metal cages, given water and feed ad libitum. They were acclimatized for two weeks, housed under the same conditions of temperature, humidity and a 12 h of light and dark cycle.

2.2.2. Experimental Design

A total of 18 adult Sprague-Dawley male rats of about 12 weeks-old weighing 180-200 g each were used for this study. The male rats were made to mate with the female counterparts after exposure to cypermethrin at 4 mg/kgbw/d and 100 mg/kgbw/d for 28 d to produce the first filial generation respectively. The female rats became pregnant and delivered after 25 d. This became first filial generation (F1). The pregnancy was checked for by observing for abdominal increase, mammary development, increase in nipple size, body weight and food consumption at day 13 and 14 respectively. The male pups (progenys) were grouped into three groups of three rats each as follows:

Group A: Control (First filial generation of rats given feed and water only for 28 d)

Group B: First filial generation of rats exposed to 4 mg/kgbw/d of cypermethrin for 28 d

Group C: First filial generation of rats exposed to 100 mg/kgbw/d of cypermethrin for 28 d

After 12 weeks, blood samples were taken from the rats via orbital sinus for onward analysis.

The rats were allowed to stay 4 weeks to become stable after taken blood samples from them.

The rats were then put in the same cages with the females for mating and copulation to take place. Again, the female rats became pregnant which were confirmed using the method described above and the males separated from them. The pregnant rats delivered after 26 d. This became the second filial generation (F2).

Second filial generation (F2) was grouped as follows:

A2: Control: 2nd filial generation of rats given feed and water only for 28 d

Group D: 2nd filial generation of rats exposed to 4 mg/kgbw of cypermethrin for 28 d

Group E: 2nd filial generation of rats exposed to 100 mg/kgbw of cypermethrin for 28 d

2.2.3. The measurement of 8-hydroxy-2-deoxyguanosine (8-OHdG)

This was accomplished following the manufacturer's instructions, using a micro plate reader machine (Model: RT-2100C, China) and an Enzyme Linke-immunosorbent Assay (ELISA) kit purchased from Elabscience Biotechnology Inc., USA, in accordance with the procedure outlined by. [18]

Competitive-ELISA is the principle that this ELISA kit employs. 8-OHdG has already been applied to the micro ELISA plate included in this kit. A set quantity of 8-OHdG on the solid phase supporter competes with 8-OHdG in the sample or standard for locations on the Biotinylated Detection Ab that are specific to 8-OHdG during the reaction. After washing away any excess conjugate and unbound sample or standard, each microplate is thoroughly filled with Avidin-Horseradish Peroxidase (HRP) conjugate, and it is then incubated. A TMB substrate solution is then introduced into every well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns from blue to yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of 8-OHdG in tested samples can be calculated by comparing the OD of the samples to the standard curve.

2.2.4. Determination of Serum Cortisol Measurement of Serum Cortisol

Following the manufacturer's instructions, ELISA kits from Elabscience Inc. in the USA were used to accomplish this in accordance with the methodology of. [19]

Competitive-ELISA is the principle that this ELISA kit employs. Cortisol has already been applied to the micro ELISA plate included in this kit. A set quantity of cortisol on the solid phase supporter competes with the cortisol in the sample or standard for locations on the Biotinylated Detection Ab that are specific to cortisol during the reaction. After washing away any excess conjugate and unbound sample or standard, each microplate is thoroughly filled with Avidin-Horseradish Peroxidase (HRP) conjugate, and it is then incubated. A TMB substrate solution is then introduced into every well. The addition of stop solution stops the enzyme-substrate process, and the color turns from blue to yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of Cortisol in tested samples can be calculated by comparing the OD of the samples to the standard curve.

Assay Procedure

Fifty microliters (50 µl) each of diluted reference standard and samples was added to every micro plate well. This was followed by the addition of 50 µl of biotinylated detection Ab working solution. The plate was covered with a sealer. The mixture was incubated for 45 min at 37 ^{O}C using laboratory incubators (Model: DHG 9023A, Techmel and Techmel, Ltd., China). The solution was decanted and plate patted dry against adsorbent tissue. The plate was washed using diluted wash buffer (350 µl). This was repeated three more times, making it a total of four times. Hundred microliter HRP conjugate reagent was added to the micro plate wells and the plate covered with a new sealer. The solution was decanted and the plate patted dry. The plate was washed dry using diluted wash buffer (350 µl). This was repeated for five more times, making it a total of six times. Ninety microliters (90 µl) of substrate reagent was added and the plate covered with a new sealer. The plate was protected from sun light. Incubation was done for 15 min at 37 ^{O}C using a laboratory incubator. Fifty microliters of stop solution was added and the plate covered with a new sealer. The absorbance was read at 450 nm using microplate reader (Model: RT-2000C, China). A standard curve of the serial dilution of the standard prepared was generated. The values of the unknown samples were extrapolated from the graph.

2.2.5. Preparation of rat liver tissue homogenate for various antioxidant assays

This was done according to [20] with some modifications. One gram of liver tissue was weighed into a beaker using electronic weighing balance (Model: BL-PID/20001, China). This was homogenized with 9 ml of phosphate buffer instead of normal saline in [20], using homogenizer. This was centrifuged at 4000 rpm at 4 ^{O}C for 20 min instead of 10 min in [20], the supernatant separated and stored for onward analysis at -80 ^{O}C in a deep freezer (Model: GR-8252 VPL, China).

2.2.6. Determination of antioxidant Status on rat liver homogenate

a. Determination of Superoxide Dismutase (SOD) Activities

Superoxide dismutase was assayed according to the method of. [21]

Principle

The ability of superoxide dismutase (SOD) to inhibit the autooxidation of adrenaline at pH 10.2 makes this reaction a basis for the SOD assay. Superoxide anion ($02^{\cdot-}$) generated by the xanthine oxidase reaction is known to cause the oxidation of adrenaline to adrenochrome. The yield of adrenochrome produced per superoxide anion introduced increased with increasing pH and concentration of adrenaline.

Procedure

Five hundred microliters (500 µl) of reagent 1(R 1-carbonate buffer (pH 10.2)) was added to 40 µl of sample supernatant and blank in a clean test tube. The resulting solution was mixed thoroughly, and allowed to equilibrate by incubating at 37 ^{O}C for 5

min. Thereafter, 300 µl of freshly prepared epinephrine, Reagent 2 (R2) was added and the reaction mixture read at 30 s interval for 150 s at 480 nm. The blank was treated the same way except that 40 µl of distilled water was used instead of serum. The changes in absorbance of both test and blank were determined. The percentage inhibition of auto oxidation of epinephrine by SOD was calculated and the serum SOD activity was expressed as U/ml. One unit of SOD activity was equivalent to the amount of SOD that can cause 50 % inhibition of epinephrine.

Calculation:

$$\% \text{ inhibition} = \frac{\text{OD blank} - \text{OD test}}{\text{OD blank}} \times 100\ (\%) \quad 1$$

Enzyme Unit (U/ml) = (% inhibition/50) X dilution factor.
OD= Optical density

b. Estimation of Glutathione Peroxidase (GPx) Activities in Rat Liver Homogenate

This was done using UV-VIS spectrophotometer (Model 752G, China) according to the method of [21].

Principle

Glutathione peroxidase in the presence of hydrogen peroxide (H_2O_2) oxidizes reduced glutathione (GSH) to form H_2O. The amount of GSH consumed is directly proportional to the activities of GPx and it is expressed as U/ml (µmol of GSH consumed/minute). The GSH remains after the reaction is allowed to react with 5'-5' dithiobis-2-nitrobenzoic acid (DTNB) to form a yellow complex that absorbs maximally at 412 nm.

$$2GSH + H_20_2 \xrightarrow{GPx} GSSG + 2H_20$$

Procedure

The reaction mixture contained 50 µl of reagent 1 (R1 (phosphate buffer 40 µl, pH 7.0, and phosphotungstic acid, 10 µl), 20 µl of each of the sample supernatant, calibrator (standard) or blank, and 10 µl of reagent 2 (R2 (H_2O_2 peroxide reagent). The resulting solution was thoroughly mixed and incubated at 37 ^{O}C for 10 min. The reaction was arrested by the addition of 40 µl of reagent 3 (R3, 10% trichloroacetic acid (TCA)). The tubes were centrifuged at 4000 rpm for 5 min using Ultra-Modern Centrifuge machine (Model: 800DM, China). Thereafter, 50 µl of the supernatants shall be added into a cleaned test tubes followed by the addition of 250 µl of reagent 4 (phosphate buffer, pH 7.0 and 50 µl of 40 mM DTNB)). The solution shall be thoroughly mixed and the resulting yellow colour was read at 412 nm. A blank was treated the same way except that it contained 50 µl of distilled water instead of sample. Twenty milligrams per hundred mills of reduced glutathione (GSH) standard (0.651µmol/ml) was also used. The activity of glutathione peroxidase was expressed as U/ml of plasma (µmoles of GSH utilized/ minute).

Calculation:

Actual Test OD = OD Test–OD Blank
Actual Std OD = OD Std–OD Blank.
GPx activity = Actual OD Test/Actual OD STD X STD Concentration (U/mL). Equation 2

c. Determination of reduced glutathione (GSH)

The level of reduced glutathione in rat liver homogenate was determined using UV-VIS spectrophotometer (Model 752G, China) according to the method of. [22]

Principle

This method was based on the development of yellow colour when 5; 5'-dithio-bis-2-nitrobenzoic (DTNB) acid was added to compound containing sulphydryl groups. The colour developed was read at 412 nm in a spectrophotometer.

Procedure

Two hundred microlitre of sample supernatant was mixed with reagent 1(R1, 180 µl of EDTA solution and 300 µl of phosphotungstic acid). This was mixed thoroughly and kept for 5 min before centrifugation at 4000 rpm for 20 min. After this, 200 µl of the filtrate, 400 µl of R 2 (0.3 M disodium hydrogen phosphate solution and 100 µl of DTNB reagent) were added and the colour developed was read at 412 nm in a spectrophotometer. Calibration (Standard) solutions containing 10 mg/dl of reduced glutathione were treated similarly. The values were expressed as mg/dl.

Calculation:

Conc of GSH (umol/l) = OD test / OD std X conc of standard 3

d. Estimation of Total Antioxidant Capacity (TAC) By Ferric Antioxidant Properties (FRAP) Assay

Total antioxidant capacity of fish shall be determined by using UV-VIS spectrophotometer (Model 752G, China) according to the method described by. [23]

Principle

At low pH, antioxidant power causes the reduction of ferric tripyridyl triazine (Fe III -TPTZ) complex to ferrous form (which has an intense blue colour) that can be monitored by measuring the change in absorption at 593 nm. Ferric reducing ability of plasma (FRAP) values shall be obtained by comparing the absorbance change at 593 nm in mixture (test) with those containing ferrous ion in known concentration (Standard).

Procedure

Initially, a working reagent comprising acetate buffer (pH 3.6), ferric chloride and tripyridyltriazine in the ratio of 10:1:1 respectively was prepared. Six microlitres each of samples and standard was added into test tubes for samples and standard respectively. This was followed by the addition of 180 µl of the working reagent to all the test tubes. The reaction mixture was mixed thoroughly, and incubated at 37 ^{O}C for 10 min. The resulting blue coloured solution developed was then read at 593 nm. The blank was treated the same way except that 60 ul of distilled water was added instead of tissue homogenate.

Calculation:

$$\text{Total antioxidant capacity (µmol/l)} = \frac{\text{Abs test}}{\text{Abs STD}} \text{ X Conc of STD (1000) µmol/l)} \quad 4$$

STD= standard

e. Estimation of catalase activities

The activity of catalase was determined on the rat liver homogenate according to the standard method described by [24] by using UV-VIS spectrophotometer (Model 752G, China).

Principle

Catalase activity was assessed by incubating the enzyme sample in substrate hydrogen peroxide in the sodium–potassium phosphate buffer, (pH7.4) at 37 ^{O}C for three minutes. The reaction was stopped with ammonium molybdate. Absorbance of the yellow complex of molybdate and hydrogen peroxide was measured at 374 nm against the blank.

Catalase catalyzes the following reaction:

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_20 + 0_2$$

Procedure

Four clean test tubes were provided for: test, control-test, standard and blank respectively. Hundred microlitre of sample supernatant and distilled water were each added into test and control-test tubes. One mill of distilled water was added into control-test tube while one mill of hydrogen peroxide was added into test and standard tubes. The tubes were incubated at 37 ^{O}C for 3 min. This was followed by the addition of 4 ml of ammonium molybdate into all the tubes and the changes in absorbance were

recorded at 374 nm against the reagent blank.

Calculation:

Catalase activity of test in U/L = 2.303/t x (logs S°/S-M) x Vt/Vs. 5

Where: t = time, S° = absorbance of standard tube, S = absorbance of test tube, M = absorbance of control-test (correction factor), Vt = total volume of reagents in test tube and Vs = volume of serum.

f. Determination of lipid peroxidation level on rat liver homogenate

Lipid peroxidation was determined on the rat liver homogenate by determining the level of malondialdehyde MDA level on the rat liver homogenate using UV-VIS spectrophotometer (Model 752G, China) according to the method of. [25]

Principle

Malondialdehyde (MDA) is a product of lipid peroxidation. When heated with 2-thiobarbituric acid (TBA) under alkaline condition, it forms a pink coloured product, which has an absorption maximum at 532 M. The intensity of colour generated is directly proportional to the concentration of MDA in the sample. This is equivalent to the level of lipid peroxidation in the liver homogenate.

Procedure

One mill of thiobarbituric acid dissolved in alkaline medium (sodium hydroxide) was added to 0.1 ml of sample supernatant in the test tube. The mixture was mixed thoroughly, and 1 ml of glacial acetic acid was added to the mixture. The reaction mixture was also shaken thoroughly and incubated in boiling water (100 ^{O}C) using water bath (Model: HH-2, China) for 15 min. It was allowed to cool and the turbidity removed by centrifugation at 3000 rpm for 10 min at 25 ^{O}C. Thereafter, the absorbance of the supernatant was read at 532 nm. The same volume of TBA and glacial acetic acid was added to the blank, but 0.1 ml of distilled water was added to the blank instead of liver homogenate. The level of MDA in the liver homogenate was expressed as nmol/ml using the molar extinction coefficient for MDA (1.56x105 M-1cm-1).

Calculation:

MDA (nmol/ml) = (OD X 1000000)/ E5321

Where E532 = Molar extinction coefficient for MDA (1.56x105 $M^{-1}cm^{-1}$)

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2.2.7. STATISTICAL ANALYSIS

Results obtained were expressed as mean ± standard deviation of triplicate determinations. Students' T-test and one-way analysis of ANOVA were used to analyze results using SPSS statistical software (version 25). Testing of hypotheses was done by testing for significant difference at 0.05 level of significance. This is to know whether there was significance change in values of the tested parameters as a result of the effect of the treatment.

3.0. RESULT

Table 1 shows the results of oxidative stress markers of first filial generation of male Sprague-Dawley rats exposed to cypermethrin for 28 d. Results showed the mean 8-O-HdG had a significant increase in groups B and C compared to A ($P< 0.05$). In addition, group C when compared to B had a significant increase in the mean 8-O-HdG level. The mean MDA level showed significant increase in group C compared to A. Group C showed no significant change when compared to B ($P < 0.05$). Serum cortisol level increased significantly in both groups B and C (those given 4 and 100 mg/kgbw/d of cypermethrin for 28 d) when compared with group A (control, normal rats). Highest increased was observed in those given cypermethrin at 100 mg/kgbw/d of cypermethrin for 28 d).

Table 1: Determination of Level of Oxidative Stress Markers on First Filial Generation of Sprague-Dawley Males Rats Exposed to Cypermethrin for 28 days

Groups	Cortisol(ng/ml)	8-O-HdG(ng/ml)	MDA (umol/ml)
Group A (Control)	3.15±0.03	1.48±0.03	69.33±0.65
Group B (4mg/kgbw/d of cypermethrin)	8.20±0.36*	1.55±0.01	87.54±1.44*
Group C (100mg/kgbw/d of cypermethrin)	15.10±0.43*#	1.56±0.00*	135.86±1.53*#
F-ratio	330.80	3.923	726.38

Values are means of triplicate determination ± SD. *: significant difference when compared with A. #: significance difference when compared with B. Level of significance: P< 0.05

The results of liver tissue levels of superoxide dismutase (SOD), catalase (CAT) activities, glutathione peroxidase (GPx) and total antioxidant capacity levels of first filial generation of rats exposed to cypermethrin are hereby presented on table 2. The results showed that there was no significant change in the values of superoxide dismutase (SOD) among all the groups ($P < 0.05$). Catalase activities decreased significantly in rats given cypermethrin at 4 and 100 mg/kgbw/d for 28 d (groups B and C) ($P < 0.05$). The highest decrease was observed in group B. Also, glutathione peroxidase (GPx) activities and reduced glutathione (GSH) level decreased significantly when compared with the control. There was significant increase in the serum cortisol level in groups B and C compared with A. Also, group C showed a significant increase in serum cortisol level compared with group B. The total antioxidant capacity result presented a significant decrease in groups B and C compared with group A. However, group C had significant decrease compared with B.

Table 2: Determination of Level of Antioxidant Markers on First Filial Generation of Sprague-Dawley Males Rats Exposed to Cypermethrin for 28 days

Groups	SOD (U/ml)	CAT(U/ml)	GPx(U/ml)	GSH(U/ml)	TAC(U/ml)
Group A (Control)	16.23±0.25	67.34±1.05	0.64±0.02	9.56±0.01	677.18±1.20
Group B (4mg/kgbw/ of cypermethrin)	15.93±0.03	64.81±0.32*	0.58±0.01*	9.46±0.02*	661.74±1.32
Group C (100 mg/kgbw of cypermethrin)	15.89±0.04	64.93±0.38*#	0.56±0.01*	9.45±0.01	647.64±1.58*#
F-ratio	1.59	4.50	5.23	17.53	2.42

Values are means of triplicate determination ± SD. *: significant difference when compared with A. #: significance difference when compared with B. Level of significance: P< 0.05

The results of oxidative stress markers of second filial generation of male Sprague-Dawley rats exposed to cypermethrin for 28 d are hereby presented on table 3. Results showed 8-O-HdG, MDA and cortisol levels had significant increases in groups D and E (second filial generations of those rats given 4 and 100 mg/kgbw/d of cypermethrin for 28 d) compared with group A2 (control for F-2) ($P< 0.05$). In addition, group E when compared with D had a significant increase for 8-O-HdG level when compared with group A (control, normal rats). Highest increased was observed in those given cypermethrin at 100 mg/kgbw/d of cypermethrin for 28 d).

Table 3: Determination of Level of Oxidative Stress Markers on Second Filial Generation of Sprague-Dawley Males Rats Exposed to Cypermethrin for 28 days

Groups	8-O-HdG (ng/ml)	MDA (umol/ml)	Cortisol(ng/ml)
Group A2 (Control)	6.89±0.060.	47±0.030	70.65±1.66
Group D (4mg/kgbw/d of cypermethrin)	10.33±0.12*	0.81±0.05*	92.45±5.6**
Group E (100mg/kgbw/d of cypermethrin)	27.08±1.23*#	0.88±0.04*	92.45±5.5*
F-ratio	225.68	35.51	7.24

Values are means of triplicate determination ± SD. *: significant difference when compared with A. #: significance difference when compared with B. Level of significance: P< 0.05

The results of antioxidant levels of second filial generation of male Sprague-Dawley rats exposed to cypermethrin for 28 d are hereby presented on table 4. The SOD levels in the rats decreased significantly when compared with the control (group A), while in catalase activities there was no significant difference among all the groups. There was significant decrease in the values of GPx ($P < 0.05$) in group E when compared with both group D and A2 respectively. The values of GSH and TAC decreased significantly in rats in groups D and E when compared with group A (control). The highest decrease was shown in second filial generation of rats given 100 mg/kgbw/d of cypermethrin for 28d.

Table 4: Determination of Level of Antioxidant Markers on First Filial Generation of Sprague-Dawley Males Rats Exposed to Cypermethrin for 28 days

Groups	SOD (U/ml)	CAT(U/ml)	GPx(U/ml)	GSH(U/ml)	TAC(U/ml)
Group A (Control)	16.92±0.19	67.38±0.93	1.03±0.11	12.70±0.37	715.77±32.91
Group B (4mg/kgbw/ of cypermethrin)	14.00±0.82*	66.63±0.69	0.91±0.10	8.95±0.70*	378.66±31.68*
Group C (100 mg/kgbw of cypermethrin)	14.39±0.41*	65.14±1.44	0.41±0.08*#	5.12±0.54*#	243.37±7.95*#
F-ratio	8.41	1.13	12.19	46.32	82.59

Values are means of triplicate determination ± SD. *: significant difference when compared with A. #: significance difference when compared with B. Level of significance: P< 0.05

4. DISCUSSION

Environmental toxicant exposure has been demonstrated to change a number of physiological and biochemical processes in the body, which over time may result in changes to genetic structure. [1] Cypermethrin is a well-known environmental toxin that can cause a number of illnesses, [2] among others. This study examined the impact of cypermethrin exposure on the oxidative and antioxidant stress markers in the F-1 and F-2 generations of Sprague-Dawley male rats. The results showed that cypermethrin exposure in the F-1 and F-2 generations of the male rats had a significant increase in the concentrations of 8-OHdG in groups exposed at 4 mg/kg and 100 mg/kg (Tables 1 and 3). This indicates that 8-OHdG is a biomarker for oxidative stress damage in the DNA, which is primarily caused by an excess of reactive oxygen species (ROS) production in mitochondria, following exposure of cypermethrin. A major contributor to DNA damage and fragmentation, oxidative stress has been shown to negatively impact tissue function and cellular structure. [3] Therefore, elevated 8-OHdG levels are associated with elevated oxidative stress, which leads to changes in DNA function and, ultimately, gene destruction, after elevated lipid peroxidation activities. The study's conclusions were comparable to those of. [26-28] The findings of [4-6] demonstrated an elevated level of 8-OHdG after exposure to cypermethrin poisoning. One possible explanation for this could be oxidative stress-induced DNA damage. It was also shown that P. clarki showed a considerable rise in 8-OHdG levels after being exposed to deltamethrin. [29] The study has similarity with the reports of. [8, 19, 30]

The study findings in the F-2 generation showed similarity to the reports of, [2, 6, 9, 11] which revealed a significant decrease in the levels of SOD activities in rats exposed to cypermethrin at 4 and 100 mg/kgbw/d (Table 4). Antioxidants are important in the repair of tissue damage or cellular impairments and can be endogenous or exogenous, [20, 31] which scavenge free oxygen radical species following environmental toxicants in the body. However, SOD is an important potent antioxidant that is involved in scavenging dioxygen [32] and hydrogen peroxide

following conversion of superoxide anions. [21, 33, 34]

CONCLUSION

This study revealed that exposure of cypermethrin may have resulted in impairment of antioxidant status of the F-1 and F-2 generations of Sprague-Dawley male rats, caused lipid peroxidation as indicated by MDA level, and altered DNA structure as shown by increased 8-OHdG levels. Thus, cypermethrin exposure in Sprague-Dawley male rats may be genotoxic to the rats up to second filial generation and could be dose dependent with 100 mg/kgbw of cypermthrin causing more genotoxic effects.

Highlights

Cypermethrin exposure to rats induced genotoxicity at F-1 and F-2 generations
Cypermethrin exposure to rats induced DNA damage
Cypermethrin exposure to rats caused antioxidant depletion and oxidative stress
The effects of cypermethrin exposure to rats are concentration dependent

Declaration by Authors

Ethical Approval: Approved
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REFERENCES

1. Jiahuan LI, Haragakiza JD, David F et al., Current status of malaria control and elimination in Africa: epidemiology, diagnosis, treatment, progress and challenges. J Epidemiol Glob Hea. 2024. Available from: https://doi.org/10./007/s44197-024-00228-2 (Accessed March, 2024).
2. WHO. Global malaria program report. 2023. Available from: https://www.wo.int/teams/global-malaria-programme/reports/world-malaria-report-2023
3. Wote A, Eyasu M. Efforts made to eliminate drug-resistant malaria and its challenges. Biomed Res Int. 2021. [Online]. Available: https://doi.org/10.1155/2021/5539544
4. Ochomo E, Rund SSC, Mthawanji RS. Mosquito control by abatement programmes in the United States: perspectives and lessons for countries in sub-Saharan Africa. Malar J. 2024; 23(8). Available from: https://doi.org/10.1186/s12936-023-04829-3
5. Abdel-Tawab H, Mossa S M M, Mohafrash NC. Safety of natural insecticides: Toxic effects on experimental animals". Biomed Res Int. 2018. Available from: https://doi.org/101155/2018/4308054
6. Yi-Jan W, Shu-Sen C, Hsien-Yi C et al., Human poisoning with chlorpyrifos and cypermethrin pesticide mixture: Assessment of clinical outcome of cases admitted in a tertiary care hospital in Taiwan. Int J Gen Med. 2023; 16, 4795-4804.
7. Abdullah Y, Canan D. Determination of oxidative stress level and some antioxidant activities in refractory epilepsy patients. Sc Rep. 2023; 14, 6688. Available from: https://doi.org/10.1038/s41598-024-57224-6
8. Natani M, Camila FR, Alexandra F et al., Mortality and toxicity of a commercial formulation of cypermethrin in Physalaemus gralilis tadpols. Sc Rep. 2023; 13, 17826.
9. Meijer N, Zeet I, Rijkers D et al., Toxicity, transfer, and metabolization of the pyrethroid insecticides cypermethrin and deltamethrin by reared black soldier fly larvae, J. Insects Food Feed. 2024; Doi: 10.1163/235245ss-00001167
10. Ravindran J, Pankajshan M, Puthur S. Organochlorine pesticides, their toxic effects on living organisms and their fate in

the environment, Interdiscip Toxicol. 2016; 9, 90-100.
11. Dike C C, Ezeonu F C, Maduka HCC et al., Hepatoprotective potentials of Sida corymbosa (broom weed) ethanolic leaf extract against carbon tetrachloride (CCl4)-induced hepatotoxicity on male Albino rats, J Pharmacogn Phytochem. 2018a; 7(6): 2166-2175.
12. Dike CC, Ezeonu FC, Maduka HCC et al., Hepatotherapeutic potentials of Sida corymbosa (Wire Weed) ethanolic extract on carbon tetrachloride (CCl4)-induced acute hepatotoxicity on male Albino wistar rats, Eur J Pharm Med Res. 2018b; 5(12): 146-155.
13. Rasheed OS, Liam C, Aldrin VG. A common feature of pesticides: oxidative stress in pesticide-induced toxicity. Oxid Med Cell Longev. 2022; 5563-7559. Doi: 101155/2022/5563759
14. 14. Jone AK, Eligarami S. Pesticide exposure and genotoxic effects as measured by DNA damage and human monitoring biomarkers. Int J Environ Health Res. 2021; 31(7): 805-822.
15. Wang H, Guo M, Wei H et al., Targeting p53 pathways: mechanisms, structures, and advances in therapy. Sig Transduct Target Ther. 2023; 8. Available from: https://doi.org/10.1038/s41392-023-01347-1
16. Patel Y, Stovalt K, Franklin JL. The intrinsic apoptotic pathway lies upstream of oxidative stress in multiple organs. Free Radic Biol Med. 2020; 158: 13-19.
17. WHO. Guidelines for breeding and care of laboratory animals. 1993. Fujikura T, Hovell G J R., Hanninen, O. and Pelkonen, K. Eds. Report No: WHO/ZOON/93.169 DISTR.
18. Yuliani S, Adi H, Indwiani A H. Detection of Urinary 8-hydroxydeoxyguanosine (8-OHdG) Levels as a Biomarker of Oxidative DNA Damage among Home Industry Workers Exposed to Chromium. Procedia Environ Sci. 2015; 23, 290-296.
19. Uzoma RI, Ufearo CS, Njoku-Oji NNet al., Effect of social isolation on serum electrolytes and cortisol Level in Wistar Rats. Asian J Health Sci. 2021; 7(2):24
20. Liu C, Yuan C, Ramashamy H et al., Antioxidant capacity and hepatoprotective activity of myristic acid and acylated derivative of phloridzin. Heliyon. 2019; 5: eo1761
21. Rotruck JT, Pope AL, Ganther H.E et al., Selenium riochemical Role as a component of glutathione peroxidase. Science. 1993; 179 (4073), 588-590.
22. Beutiler E. Enzyme replacement therapy for Gaucher's disease. Bailliere Clin Haem. 1993; 10 (4), 751-763.
23. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power. The FRAP assay. Anal Biochem. 1996; 239(1), 70-76.
24. Hadwan MH, Abed HN. Data supporting the estimation of catalase activity. Data Brief. 2016; 6, 194-199.
25. Alam Z, Fareed UC. A simple spectrophotometric method for the determination of thiobarbitutric acid reactive substance in fried fast foods. J Anal Chem. 2016; 1-5.
26. Singh K., Tiwari MN, Prakash O et al., A current review of cypermethrin-Induced neurotoxicity and Nigrostriatal Dopaminergic Neurodegeneration. Curr Neuropharmacol. 2012; 10(1), 64-71. Available: https://doi.org/10.2174/157015912799362779
27. Arslan H, Özdemir S, Altun S. Cypermethrin toxication leads to histopathological lesions and induces inflammation and apoptosis in common carp (Cyprinus carpio L.). Chemosphere. 2017; 180, 491-499. https://doi.org/10.1016/j.chemosphere.2017.04.057
28. Zhou Y, Huang H, Zhou J, Wang L, Beta-cypermethrin exposure affects female reproduction by enhancing oxidative stress in mice uterine tissue. Regulatory Toxicology and Pharmacology. 2018; 98,284-290.Available from: https://doi.org/10.1016/j.yrtph.2018.08.011
29. Hong Y, Huang Y, Yan G et al., DNA damage, immunotoxicity, and neurotoxicity induced by deltamethrin on the freshwater crayfish, Procambarus clarkii. Environ. Toxicol. 2020; 36(1): 16-23. Available from: https://doi.org/10.1002/tox.23006
30. Wu H, Eckhardt CM, Baccarelli AA. Molecular mechanisms of environmental exposures and human disease. Nat Rev Genet. 2023; 24(5),332–344 [Internet]. Available from: https://doi.org/10.1038/s41576-022-00569-3

31. Ghazouani L., Feriani A., Mufti A., Tir M., Baaziz I., Mansour H. Toxic effect of alpha cypermethrin, an environmental pollutant, on myocardial tissue in male wistar rats. Environ Sci Pollut Res. 2020; 27(6): 5709-5717. Available from: https://doi.org/10.1007/s11356-019-05336-2
32. Martins SG, Zilhão R. Thorsteinsdóttir S. Linking Oxidative Stress and DNA Damage to Changes in the Expression of Extracellular Matrix Components. Front Genet [Internet]. 2021; 12:673002. Available from: https://www.frontiersin.org/journals/genetics/articles/10.3389/fgene.2021.673002
33. Li X, Chen X, Wu Y et al., Investigation on sperm concentration of workers occupationally exposed to pyrethroids. Occup Heal Emerg Rescue. 2022; 40(6):650–654. Available from: http://www.oher.com.cn/en/article/doi/10.16369/j.oher.issn.1007-1326.2022.06.004
34. Oğuz EK. Effects of sublethal concentrations of cypermethrin on the gills of Lake Van Fish (Alburnus tarichi). Chem Ecol. 2022; 38(10):900–12. Available from: https://doi.org/10.1080/02757540.2022.2126462

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