

Cypermethrin and chlorpyrifos raises serum urea level and causes abnormal sperm morphology in Wistar rats

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ABSTRACT

Chlorpyrifos (organophosphate) and cypermethrin (pyrethroid) are insecticides, which are widely used for agricultural as well as for domestic purposes. This study investigated the toxicological effect of chlorpyrifos and cypermethrin on selected organs and tissues of male Wistar rats. Nine (9) male Wistar rats were randomly grouped into three and were orally given chlorpyrifos or cypermethrin, while the control group was given distilled water for 28 days. The results revealed a significant increase ($p < 0.05$) in rat serum AST activity for the chlorpyrifos and cypermethrin groups. Also, there was significant elevation in serum urea following oral exposure to either chlorpyrifos or cypermethrin. Conversely, a reduction in the rat liver ALP activity for treatment with cypermethrin or chlorpyrifos was recorded. The histology results revealed that the administration of chlorpyrifos but not cypermethrin for 28 days has no significant effect on the biochemical properties and sperm morphology of the rats. Taken together, findings indicate that cypermethrin and chlorpyrifos exposure in rats predisposes to renal injury, while altering sperm morphology.

Keywords: *biochemical toxicology; medicinal biochemistry; pesticides; reproductive biochemistry.*

1. INTRODUCTION

Pesticides are chemical substances used for preventing and controlling pests, including vectors of human or animal diseases. They are used to control unwanted species of plants or animals that interfere with agricultural commodities [1]. Although, pesticides are beneficial in improving food production, reducing manpower farm needs and improving public health, they adversely affect human, animal health and environmental sustainability [2]. Pesticides could include herbicides, insecticides, fungicides, disinfectant, and rodenticides [1]. Among the various pesticides, insecticides are the most widely used with cypermethrin (CY) and chlorpyrifos (CH) been the dominant chemicals applied [3, 4]. CY is a synthetic, pyrethroid insecticide used to control many pests on crops. It is used in agriculture, forestry as well as in public and animal health programs [4]. CH is a chlorinated organophosphate (OP) insecticide with a broad spectrum of activity against arthropod pests of plants, animals, and humans [5]. Although considered nontoxic to mammals, recent studies have shown the adverse effect of cypermethrin on the nervous system [6], hepatic and renal system [7], as well as on male reproductive system in laboratory animals [8, 9, 10]. Thus, with the frequent usage of these chemicals, the environmental implication of these insecticides has become a public health concern [4]. Studies have reported a significant sperm abnormality including reduction in rat testis and epididymis weights, testicular sperm head counts, sperm motility and live sperm counts following exposure to these

insecticides (4). Additionally, serum testosterone (T), follicle stimulating hormone (FSH), luteinizing hormone (LH), reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx) and total protein (TP) contents were decreased while lipid peroxidation (LPO) level was increased on cypermethrin exposure in rat models.

In separate studies, chlorpyrifos induced genotoxicity [11, 12]. Although some studies reported no change in sperm motility and sperm morphology at any of the dose level used, it was observed that there was a decrease in sperm counts at a high dose [13]. There is an evident that chlorpyrifos causes changes in some hematological and biochemical parameters in experimental animals [14, 15]. Pesticide exposure has also been associated with elevation of cancer risks, and reproductive dysfunctions in agricultural workers [16]. Thus, the reports of biochemical and reproductive toxicity of cypermethrin and chlorpyrifos are a major concern because human spermatogenesis may be vulnerable to chronic exposure to pesticides at very low concentrations. The present study investigated the effects of cypermethrin and chlorpyrifos on some biochemical indices and sperm morphology in male Wistar rats.

2. MATERIALS AND METHODS

Experimental Animals.

Male Wistar rats of range 110 ± 35 g were obtained from the animal holding unit of the Department of Biochemistry, University of Ilorin, Ilorin, Kwara State. They were acclimated for two weeks in a well-ventilated animal house prior to commencement of experimental treatments. The rats were handled in accordance with the NIH guide for the care and use of laboratory animals [17]. Their beddings were regularly changed and they were fed with commercial rat pellets with unrestricted access to clean drinking water.

Test chemicals.

Cypermethrin (10%) was a product of Gharda Chemical Limited, Thane Maharashtra State, India and Chlorpyrifos (48%) was product Redsun Group Corporation, Nanjing 211300, China.

Animal grouping and treatment.

Nine (9) Wistar rats weighing between 75-140g (110 ± 35 g) were randomly grouped into three (3) groups of three rats each. The rats in groups 1, 2, and 3 were orally administered distilled water (control), 3 mg/kg bw of chlorpyrifos and 10 mg/kg bw of cypermethrin respectively for 28 days.

This study was conducted following the guidelines on the care and use of laboratory animals of the Ethical Committee of the Department of Biochemistry, University of Ilorin, Ilorin, Kwara State, Nigeria.

Sperm Morphology Assay.

Induction of sperm abnormalities was done according to previous studies [18, 19]. Briefly, male Wistar rats of 110 ± 35 g were used for the study. The animals were divided into three groups ($n = 3$).

3. RESULTS

Average weight of animals.

The oral administration of chlorpyrifos and cypermethrin to Wistar rats for 28 days consistently did not alter the mean body weight when compared with the control group (table 1). The rat organ weight showed a slightly significant difference between the rats administered cypermethrin and the control group, while the chlorpyrifos showed no changes when compared with the control (table 2). Organ/body weight ratio also showed a minor change in the group administered with cypermethrin, which was also statistically significant (table 3).

Biochemical indices.

In order to access the toxicity of cypermethrin and chlorpyrifos, a span of biochemical indices such as enzyme activities and homeostatic parameters was determined in rat serum and tissues (table 4). Cypermethrin and chlorpyrifos had an inconsistent effect on the biochemical parameters assayed.

The exposure to cypermethrin elevated the rat serum AST when compared with the control. Additionally, there was a significant increase in the concentration of total bilirubin in rat serum for cypermethrin and chlorpyrifos groups when compared with the control group. Furthermore, cypermethrin and chlorpyrifos

A 28-day period of exposure was considered, while 3 mg/kg rat body weight concentration of chlorpyrifos and 10 mg/kg rat body weight concentration of cypermethrin were used. Sperm was sampled from the caudal epididymis after 30 days of exposure. After mild anaesthetization with diethyl-ether, the rats from each group were sacrificed by jugular puncture method according to standard procedures by Institutional Animal Care and Use Committee. Their caudal epididymis was removed; sperm suspensions were then prepared from the caudal of each testis by mincing the caudal in physiological saline. The slides were air-dried and coded for subsequent microscopic examination at $\times 1000$ magnification. For each rat, $\times 1000$ sperm cells were assessed for morphological abnormalities according to the criteria set by [18].

Biochemical assays.

Biochemical indices for hepatic and nephrotic functions such as the ALP, AST, ALT, urea, creatinine, bilirubin, protein and albumin were determined using commercial assay kits (RANDOX Laboratories Ltd., Crumlin, Antrim, United Kingdom) and following manufacturer's guidelines.

Statistical analysis.

The Statistical Package for Social Science (SPSS®) version 16.0 was used for data analysis. Data obtained were expressed as percentage frequency and mean \pm standard error of mean (SEM). Significance at different concentration-level was tested using one-way analysis of variance (ANOVA) test and Duncan's New Multiple Range Test (DMRT). Significant level was kept at $p < 0.05$.

treatments elevated ($p < 0.05$) rat serum urea when compared to the control group.

Histopathological examination.

Rat semen was prepared and observed under the microscope for any morphological changes following exposure to the insecticides.

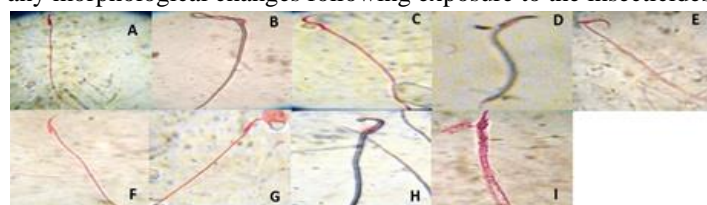


Figure 1. A – Normal sperm; B Cypermethrin – Folded; C – Cypermethrin – Amorphous; D – Cypermethrin – Quasinormal; E – Cypermethrin – Wrong tail attachment; F – Chlorpyrifos – Hook at wrong angle; G – Chlorpyrifos – Kink tail; H – Chlorpyrifos – Coil tail; I – Chlorpyrifos – Amorphous head with two tails. ($\times 1000$).

Three animals from each group were used with a total of three thousand sperm per concentration. Microscopic inspection showed that both cypermethrin and chlorpyrifos caused an abnormal sperm morphology when compared with control with 4.20% and 1.1% abnormal sperm cells for cypermethrin and chlorpyrifos, respectively. Some of the aberrations observed in the sperm's

architecture included amorphous appearance, quasinormal, folded tail, kinked tail, short tail, two tails appearance and others (Fig 1a-i).

Insecticides are widely used pesticides with cypermethrin and chlorpyrifos containing insecticides been the most used. There have been many studies in the literature reporting the adverse effects of such chemical compounds on the environment and human health [19]. Cypermethrin and chlorpyrifos are cytotoxic as reviewed by Idris and coworkers [20]. Cypermethrin has been reported to have the ability to cause DNA damage in the tissues and organs of Swiss albino mice [21]. CPF can induce toxicity and oxidative stress [22, 23]. Experimental data revealed that pesticides such as cypermethrin may possess genotoxic, mutagenic, teratogenic, and carcinogenic properties, inducing mutations, chromosomal alterations, or DNA damage [24]. The prolonged and indiscriminate use of cypermethrin was reported to

cause both acute and chronic toxicity in non- target species including humans [25]. The present study investigated the biochemical and genotoxicity of chlorpyrifos and cypermethrin using biochemical and genotoxicity assays on male Wistar rat. The histology of selected tissues of the experimental Wistar rats was also investigated. The results revealed that the administration of chlorpyrifos at 48% concentration, after 5 weeks of exposure has no significant effect on the biochemical properties and sperm morphology of the rats when compared to cypermethrin. This result is in line with Akhtar *et al.* [13], wherein no change was observed in sperm motility and morphology at low dose. Our result on chlorpyrifos was however, in contrast with the work done by Golec *et al* [26]. They showed that exposure to organophosphate pesticides in agriculture increases the risk of specific morphological abnormalities in sperm as well as decreases the sperm count and the percentage of viable sperm.

Table 1. Average rat weight oral administration with cypermethrin and chlorpyrifos..

Weeks	Control (g)	Cypermethrin (g)	Chlorpyrifos (g)
0	98±2.794 ^a	126±2.579 ^a	97±2.583 ^a
1	114±1.932 ^b	147±2.772 ^b	112±2.161 ^b
2	130±2.242 ^c	164±1.183 ^c	127±1.952 ^c
3	145±1.354 ^d	175±1.558 ^d	137±1.701 ^d
4	152±0.667 ^e	179±1.365 ^e	144±0.694 ^e

Values are expressed as mean ± SEM (n=3). Values in each column with different superscript are significantly different (*p* < 0.05).

Table 2. Average weight of rat organs oral administration with cypermethrin and chlorpyrifos.

Group	Heart (g)	Brain (g)	Liver (g)	Kidney (g)
Control	0.567±0.088 ^a	1.467±0.033 ^a	4.967±1.020 ^a	0.933±0.033 ^a
Cypermethrin	0.667±0.067 ^b	1.600±0.00 ^b	6.800±0.400 ^b	1.033±0.033 ^b
Chlorpyrifos	0.433±0.033 ^c	1.433±0.088 ^a	4.833±0.033 ^a	0.933±0.067 ^a

Values are expressed as mean ± SEM (n=3). Values in each column with different superscript are significantly different (*p* < 0.05).

Table 3. Organ/body weight ratio of rats after oral administration with cypermethrin and chlorpyrifos.

Groups	Heart	Brain	Liver	Kidney
Control	0.390±0.085 ^a	0.983±0.084 ^a	3.487±1.073 ^a	0.630±0.067 ^a
Cypermethrin	0.383±0.063 ^a	0.910±0.055 ^b	3.860±0.200 ^b	0.587±0.032 ^b
Chlorpyrifos	0.300±0.030 ^a	0.987±0.079 ^a	3.320±0.090 ^a	0.640±0.051 ^a

Values are expressed as mean ± SEM (n=3). Values in each column with different superscript are significantly different (*p* < 0.05).

Table 4. Rat biochemical parameters after exposure to cypermethrin and chlorpyrifos.

Biochemical indices	Group	Serum
Aspartate transaminase (AST) U/I	Control	30±5.014 ^a
	Cypermethrin	41±11.215 ^b
	Chlorpyrifos	57±7.540 ^c
Alanine amino transferase (ALT) U/I	Control	67±12.418 ^a
	Cypermethrin	32±0.627 ^b
	Chlorpyrifos	44±22.475 ^c
Alkaline phosphatase (ALP) U/I	Control	8020±429.471 ^a
	Cypermethrin	8222±468.664 ^a

Values are expressed as mean ± SEM (n=3). Values in column for each biochemical parameter with different superscript are significantly different (*p* < 0.05).

Cypermethrin induced an increase in abnormal sperm shape and shows a significant difference when compared to chlorpyrifos and negative control. This result is in accordance with several studies of cypermethrin [6, 27, 28]. The toxicity of cypermethrin possibly is due to its stress-causing effect [6, 29]. Stress conditions cause the release of adrenocorticotrophic hormone, triggering the consequent secretion of cortisol by the adrenal cortex [30], which reduces cellular protein stores, except in the liver. The cause of the increase in the frequency of abnormal sperm may be due to an error in DNA packaging in the sperm, it may also occur as a result of point mutation or as result of the incorporation of pesticides in the spermatogenic cycle. Joshi *et al.* indicated that

pesticide-induced reactive oxygen species (ROS) production, can adversely affect sperm motility and increased sperm abnormality [31]. Also, the induction of abnormal sperms was assumed to be as a result of an abnormal chromosome [32, 33]. Bruce and Heddle, 1979 attributed the occurrence of sperm head abnormalities to the chromosomal aberrations that occur during the packaging of genetic material in the sperm head or occurrence of point mutation in testicular DNA [34]. This is consistent with the results obtained from our study as we did not encounter any sperm head abnormality. Several studies also reported that abnormalities may also arise as a consequence of mistakes in the

spermatozoa-differentiating process during spermatogenesis [19, 35, 36].

The increase in the AST serum of cypermethrin group in our study correlates with previous studies [37]. AST is related to mitochondrial damage and increased AST activity in serum reflects hepatocellular damage under cypermethrin stress, leading to leakage of this enzyme into general circulation [37]. The slight increase in the ALP activity in the serum of the cypermethrin group may also have been due to necrosis of hepatic tissue due to the effect of the insecticides on hepatic parenchymal cells [38]. There was a significant increase in the concentration of total bilirubin in the serum and liver of cypermethrin and chlorpyrifos groups in comparison with the control group. Since liver plays a key role in bilirubin metabolism, any damage to liver cells, which probably was inflammation in the present study resulting in disturbed bile excretion, might be responsible for hyperbilirubinemia in cypermethrin intoxicated rats [37, 39, 40].

4. CONCLUSIONS

In conclusion, findings indicate that cypermethrin and chlorpyrifos exposure in rats predisposes to renal injury. Additionally, exposure to cypermethrin and chlorpyrifos altered

There was no significant difference in the concentration of albumin in the serum and liver but there was a significant increase in the concentration of albumin in the kidney. Hyperalbuminaemia has little diagnostic relevance except, perhaps in dehydration [41].

The slight and significant rise in the serum creatinine level of rats may be due to the impairment of the glomerular function and tubular damage to the kidneys [42]. Cypermethrin and Chlorpyrifos groups significantly raised serum urea concentration when compared to the control group. Elevated serum urea correlates with increased protein catabolism in mammalian body or from more efficient conversion of ammonia to urea because of increased synthesis of the enzyme involved in urea production [43]. In kidneys, urea is filtered out of blood by glomeruli and is partially being reabsorbed with water. The increased levels of these end-products in blood especially serum creatinine and serum urea indicate poor clearance of these substances by the kidneys, rather than excessive production [43].

rat sperm morphology. Taken together, the findings suggest caution in the application of these pesticides, particularly for domestic purposes.

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6. ACKNOWLEDGEMENTS

We wish to recognize and acknowledge the following for their contribution to this research; Sanni, Halimah Oziohu; Ushedo, Marie Isioma; Raheem, Omothoso and Sodiq, and Adetunji, Adetutu Mariam of the Department of Biochemistry, University of Ilorin, 2014/2015 Session.



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