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Biochemical and Metabolic Changes in New Zealand Rabbit *(Oryctolagus Cunniculus)* Induced By Chloropyrifos

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Abstract

Chloropyrifos induced changes in some enzymes and metabolites activities of New Zealand rabbit *(Oryctolagus cunniculus)* were assessed. The probe organism's mean weight, 1.8-21.0kg were acclimatized to laboratory conditions for 14 days in the department of biological sciences, Niger Delta University, Wilberforce Island, Bayelsa State Nigeria. The organisms were exposed to varying sublethal concentrations of the toxicant. The concentration were prepared by pipetting 7.5mls, 15mls, and 22.5mls respectively of the original concentration of the toxicant and making it up to 1.5 litre bore hole water in a metal container to make $1.0gml^{-1}$, $2.00mg^{-1}$, and $3.00mg^{-1}$. Aspartate amino transferase (AST) and alanine amino transferase (AST) were examined in the kidney while acid phosphatase (ALP) and alkaline phosphatase (ALP) were assessed in the liver. A clear inhibition occurred in the kidney AST, while elevation of values characterized kidney ALT, Liver ACP and ALP values were significant ($p<0.05$). Metabolites (total protein and creatinine) were assayed in the kidney, Diminutive values down the experimental group were recorded in the kidney total protein (in a dose dependent pattern), while creatinine values accelerate in the experimental group compared to the control (not in a dose dependent pattern). It is concluded that phosphatase and transferases as well as total protein and creatinine are useful biomarkers of sublethal effect of chloropyrifos on the probe organism. Additionally, the toxicant presence in the environment may pose a threat to aquatic organisms at high concentrations.

Keywords: *Oryctolagus cunniculus*; Chloropyrifos; Phosphatase; Transferases.

1. Introduction

The curiosity about contamination of the environment by xenobiotics has become a global issue. Xenobiotics contamination in the aquatic and terrestrial environment has attracted attention of researchers globally because of the lethality and possible death associated with organisms exposed to it. Pesticides constitute one of the major pollutant in the environment. Pesticide by their nature are toxic compounds, and besides controlling pest they also have potentialities of affecting the life and environment adversely [1].

Pesticides enter aquatic environments through direct application, aerial drift or runoff from application or accidental release and become rapidly distributed through the action of wind and water [2]. Some pesticides or their transformation products may also move back into atmosphere by volatilization [3].

Organophosphate and carbamates constitute two classes of pesticides, widely used in the last decades on a variety of crops and as indoor protection management products [4]. The authors added that exposure of the general population to these compounds has been documented through occupation-related pathways (through the domestic use of pesticides and food consumption). Organophosphate insecticides do not bioaccumulate in tissues and organisms or accumulate in the environment as most other pesticides such as organochlorine [5]. Chloropyrifos is one of the most widely used organophosphate insecticides until 2000 when the United States Environmental Protection Agency restricted some of its domestic uses due to its toxicity [6]. Chloropyrifos remains one of the most popular organophosphate insecticide fully embraced by farmers and non-farmers in the Niger Delta States of Nigeria. It is also available in small bottles in South-South States of Nigeria with a trade name called *Otapiapia*, used domestically in killing insects. The toxicant has a slightly skunky odour, like rotten eggs or garlic [7]. Chloropyrifos is a well-known acetylcholinesterase inhibitor akin to other organophosphates e.g diazinon, parathion and monoclotophos. According to Leudke and Bartley [8], diazinon disrupts the nervous system of an organism by disrupting the activity of acetylcholinesterase (ACTH), a neurotransmitter at nicotic or muscurinic receptors.

Pesticides have been reported to have negative ecological consequences on biota and abiotic environment [9]. Ojezele and Abatan [10], reported a clear alteration of haematological and biochemical parameters of young chickens exposed to chloropyrifos and methidathion. Leudke and Bartley [8], also reported the disruption of nervous system by diazinon (an organophosphate insecticide) when fishes were exposed to the toxicant. Inyang [11], reported a clear manifestation of diazinon effect on common African wetland fish, *Clarias gariepinus. Elelaimy, et al. [6]* , also reported a significant reduction in lymphocytes viability in chloropyrifos intoxicated rats.

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Rabbits are used widely in a variety of research paradigms due their smaller structure, docile demeanor and similar general physiology to humans, second only to mice and rats, they are the next most commonly used species in research [12]. According to Aly and El-Gendy [13], the general physiology of rabbits is similar to humans, therefore the rabbits has been used is a model for human diseases. The authors added that rabbits are large enough to provide adequate quantity of tissue for experimental work without pooling of samples but is small enough to be economical for most studies. Toxic substances such as choropyrifos that affect rabbits, rats, and mice could have a serious effect on humans, hence the choice of this probe organism. Additionally, research work on pesticide effect on rabbits are scarce compared to researches on fish and other aquatic organisms. Therefore, the essence of this present research is an attempt to unveil the effect of commonly used insecticide (chloropyrifos) on some enzymes and metabolites of adults New Zealand rabbit (*Oryctolagus cuniculus*).

2. Materials and Methods

2.1. Source and Acclimatization of the Test Organisms

The probe organism (*Oryctolagus cunniculus,* adults) for this study were obtained from Kester rabbit farm at Mbiama, Rivers State, Nigeria. They were transported individually in a plastic baskets to the Department of Biological Sciences, Niger Delta University, Bayelsa State were the assays were conducted from March to April 2019. A total of twenty four (24) adult New Zealand rabbits (*Oryctolagus cunniculus*) weighing 1.8 -2.0kg per weight were used in the present study. The probe organisms were put inside a well-built rabbitory, one rabbit in a compartment for 14 days acclimation. They were fed with 200g of synthetic growers mash (pelletized) daily. Each compartments were provided with a metal container that contains 1.5L of bore water.

2.2. Range Finding Test

A range finding test (trial test) was carried out to determine the actual concentration of toxicant (choropyrifos 20%EC) to be used for the main experimental run. The trial was grouped into three groups of $1.00mgl^{-1}$, $2.00mgl^{-1}$, and $3.00mgl^{-1}$, three rabbits were exposed to each concentration of chloropyrifos. The test solution was renewed daily akin to feeding.

2.3. Main Bioassay

Sublethal concentration for the main test (Definitive test) was done based on the range finding test. The concentrations were prepared by pipetting 7.5mls, 15mls and 22.5mls respectively of the original concentration of the toxicant and making it up to 1.5 litre bore hole water in a metal container to make $1.00mgl^{-1}$, $2.00mgl^{-1}$, and $3.00mgl^{-1}$ i.e the concentration used. There were four treatment levels with three replicates. The experiment lasted for 14 days. The test solution was renewed daily (renewal bioassay technique).

2.4. Collection of the Parts for Laboratory Analysis

Probe organism were killed and dissected for target organs (kidney and liver), 0.5g each of the organs were macerated with ceramic pestle and mortar. To each sample for metabolic analysis, 5ml of percloric acid were added while physiological saline was used for enzyme analysis. After addition of these diluents, samples were centrifuged the rate of 3000rpn for 10 minutes. The supernatant were then removed and stored in plain bottles at -20^0c for analysis.

2.5. Laboratory Analysis

Activities of AST and ALT were analysed base on the colimetric method of Zimmerman [14], while ALP and ACP was carried using Hafkenscheid and Kohler [15] and Andersch and Szcypinski [16] respectively. Total protein concentration was estimated by the method of Lowry, *et al.* [17], while creatinine was assayed using the method of Witt and Trendeleburg [18], Miller and Harley [19] .

2.6. Statistical Analysis

Analysis of variance (ANOVA) was used to show significant variations, and Duncan multiple range test (DMRT) was used to unveil areas were differences exist ($p<0.05$).

2.7. Results

Table 1 presents the activities of AST, ALT on the kidney and ACP and ALP in the liver of new Zealand rabbit exposed to sublethal concentration of chloropyrifos for 14 days. Kidney enzymes (AST and ALT) were statistically significant ($p<0.05$). Values of AST in the kidney decreases down the experimental group in a dose dependent pattern. The least value ($33.58\pm0.03\mu/l$) was recorded at the highest concentration ($3.00mgl^{-1}$). Percentage of control also had the least value (5.80%) compared to control that recorded 100%. Kidney ALT values increase down the experimental group quite unlike the AST. Significantly the highest concentration values was slightly higher than the control ($1177.69\pm10.12\mu l$) (Table 1)

Liver ACP fluctuate down the group (not in a dose dependent pattern). A diminutive value was recorded at the control ($23.14\pm00\mu/l$) compared to 140.02 ± 0.31 µ/l at the highest concentration. Liver ALP values decreases down the experimental group in a dose dependent pattern, however the control value was akin to the value recorded at the highest concentration.

Table 2 presents the activities of metabolites (total protein and creatinine) in the kidney of New Zealand rabbits exposed to sublethal concentration of chloropyrifos for 14 days. Total protein values were not significant ($p<0.05$) albeit values decreases down the experiment group in a dose dependent pattern. The percentage of control unveiling a profound deviation from the control. Creatinine values fluctuate down the experimental group (Table 2). A dose dependent pattern unveiled the experimental group. The experimental group values were quite higher than the control. The trend here was akin to total protein.

Table-1. Activities of AST, ALT on the kidney and ACP and ALP in the liver of new Zealand rabbit exposed to sublethal concentration of chloropyrifos for 14 days

Kidney					Liver			
Conc. of Chlor (ppm)	**AST (µ/l)**	**% of control**	**ALT (µ/l)**	**% of control**	**ACP (µ/l)**	**% of contol**	**ALP**	**% of control**
0.00	578.55 ± 2.50^{a}	100.00	1068.81 ± 10.12^{b}	100.00	23.14 ± 0.02^{c}	100.00	280.10 ± 0.35^{b}	100.00
1.00	261.86 ± 1.20^{b}	42.26	582.63 ± 3.50^{c}	54.50	114.88 ± 0.99^{ab}	883.14	373.73 ± 0.40^{a}	133.48
2.00	184.62 ± 0.95^{c}	31.89	1088.16 ± 12.10^{b}	101.79	78.08 ± 0.03^{b}	600.62	308.689 ± 0.09^{ab}	110.24
3.00	33.58 ± 0.03^{d}	5.80	1177.69 ± 12.31^{a}	110.27	140.02 ± 0.31^{a}	1077.07	276.14 ± 0.08^{b}	98.62

Means with the same superscript within column indicate no significant different ($p<0.05$)

Table-2. Activities of metabolites (total protein and creatinine) in the kidney of new Zealand rabbits exposed to sublethal concentration of chloropyrifos for 14 days

Conc. Of chlor (ppm)	Total protein (g/1)	% of Control	Creatinine	% of control (mmol/l)
0.00	32.12 ± 0.90^{a}	100.00	19.69 ± 0.03^{c}	100.00
1.00	17.93 ± 0.08^{b}	56.03	69.37 ± 1.00^{a}	365.10
2.00	16.18 ± 0.01^{b}	50.56	62.01 ± 0.20^{ab}	326.37
3.00	15.77 ± 0.02^{b}	49.28	52.50 ± 0.04^{b}	276.31

Means with the same superscript within column indicate no significant different ($p<0.05$)

3. Discussion

3.1. Total Protein

Albumin, fibrinogen and globulins are part of plasma proteins and they constitute less than 10% of plasma proteins. According to Miller and Harley [19], it is the concentration of these plasma proteins that influences the distribution of water between the blood and extracellular fluid. A decrease in values of total protein down the experimental group was recorded as the concentration of the toxicant increased. The trend also indicate a dose dependent pattern. Decreased total protein concentration have been reported in literatures [11, 20, 21].

Diminutive values of total protein down the experimental group is caused by the toxicant (chloropyrifos) as a result of its toxic effect on protein synthesis. According to Das and Murkherjee [22], exposure of fish for a long time to most toxicants including pesticides interferes with protein metabolism. Sastry, *et al.* [23], also recorded a depletion of total protein when they exposed fishes to quinalphos (an organophosphate insecticide). The occasional decline in protein content as reasoned by Magar and Shaikh [24], could be due to decline in protein synthesis and increased proteolysis, while an increase may be associated with increase in protein synthesis due to enzyme activity involved in protein synthesis. The result of this present research is not in consonance with the result earlier presented by Ovuru [25]. The author reported a clear elevation of albumin and total protein as a result of exposure of rabbit to hydrocarbon. The higher energy demand of the body to counter stress may trigger an increased in protein metabolism, a process in which both blood and structural protein are converted to energy during toxicant induced stress [26].

3.2. Creatinine

Urea and creatinine have been used as important indices for evaluation of the effects of chemicals on the kidney using a variety of both invivo and invitro methods [27]. Creatinine is an anhydride of creatinine. Creatinine and creatine phosphate undergo non-enzymatic spontaneous cyclization at a slow but constant rate to form creatinine, which is excreted in urine [28]. The experimental values recorded in this present research were much higher than control value. Additionally, the concentration of creatinine decreases down the experimental group in a dose dependent pattern. According to Ajeniyi and Solomon [29], urea and creatinine levels increased which may result either from increased breakdown of tissue or dietary or impaired excretion or increased synthesis or decreased urinary clearance by the kidney or decreased dehydration of these compounds. Creatinine concentration also increases as kidney function decreases [29]. Elevation of values recorded in this research unveiled kidney dysfunction. The presence of increasing concentration of urea and creatinine as reasoned by Calbreath [30] suggested the inability of the kidney to excrete these products, which further indicated a decrease in glomerular filtration rate. Inyang and Thomas [31], also recorded the same acceleration of values of creatinine when they exposed *Clarias gariepinus* to fluazifop-p-butyl (a phonoxy herbicide).

3.3. Enzymes

Enzymes are imperative during metabolic processes of all cells. Physiological process in cells cannot proceed effectively without enzyme input. Hence, any xenobiotic that affect their function means disruption of smooth

metabolic process(s) in that cell or tissue. Phosphatase (ALP and ACP) and transferase (AST and ALT) test are part of standard laboratory test to detect health abnormalities in animals [32, 33].

Kidney AST values were absolutely significant ($p<0.05$) in a dose dependent pattern characterized the trend of concentration down the experimental group. Values retrogresses down the group as concentration increased. Decrease in concentration of AST have been reported [11, 13, 34]. The disruption of transaminases as recorded by Aly and El-Gendy [13] from the normal values denotes biochemical impairment and lesions of tissues and cellular function because they are involved in the detoxification process, metabolism and biosynthesis of energetic macromolecules for different essential function. Additionally, the AST trend simply indicates absolute inhibition of the enzymes by toxicant (chloropyrifos), an organophosphate insecticide. Similar report was also unveiled by Simeon [35]. The author unveiled the offshoot of exposure of *Clarias gariepinus* to monoclotophos (a well known organophosphate insecticide), that the inhibition of activities of the enzyme (AST and ALT) infers a breakdown or slowing down in the kreb's cycle (TCA) intermediates since transmission which provides the keto acid (α-ketoguterate) is inactive and α-ketoglutarate is one of the intermediates in the tricarboxyic cycle.

A clear elevation of values were observed in liver ACP and ALP compared to control. Elevation of values was observed at the first two concentrations and almost similar value at the least concentration. Kaur and Dhanju [36] also reported a significant increase in the activities of AST, ALT and ALP in the liver of albino rats exposed to monoclotophos methyl parathion and dimethoate given orally for 90 days and inferred that such increase is an indication of cellular toxicity of these organophosphates causing the release of these enzymes into the blood. Elevation of ALP activity is attributed to liver damage [32].

Organophosphate insecticides effect on organism's biochemical activities have been reported in literature [34, 37]. One of the key effect on organism's chemistry is the inhibition of enzymes e.g acetylcholinesterase. Increased or decrease in concentration away from the control depicts that the biochemical activities of the organism will experience a serious aberration, which may lead to serious physiological problems of the affected organism. Our results in this present research has confirmed this fact.

4. Conclusion

The findings of this present study demonstrate that chronic exposure of rabbits to chloropyrifos at 1.00ppm, 2.00ppm and 3.00ppm can lead to serious aberration in the biochemical and metabolic indices in New Zealand rabbit. The use of this common insecticide (chloropyrifos) close to rabbittory, should be done with caution.

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