Evaluation of Cardiac Markers and Lactate Dehydrogenase in Blood of New Zealand white Rabbits exposed to Dichlorvos by Inhalation.

Abstracts

Aim: To Evaluate Cardiac Markers and Lactate Dehydrogenase in Blood of New Zealand white Rabbits exposed to Dichlorvos by Inhalation.

Study design: This is an experimental study.

Methodology: A total of twenty four male New Zealand white rabbits, two months old weighing between 1.0 and 1.2 kg, were used for the study. They were divided into three (3) groups, each consisting of four (4) rabbits and a corresponding number of matched controls, for long-term toxicological effects of dichlorvos on the rabbits (30 days, 60 days and 90 days).The rabbits received ten (10%) of the LD50 dose . The LD50 dose was 0.5 mg/m3, while 10% of the median lethal dose of dichlorvos which was 0.05 mg/m^3 was diluted with 1.0 milliliter of distilled water. It was administered by spraying in a closed cage \containing the rabbits every day for thirty, sixty, and ninety days. At the end of each month, a set of rabbits in the experimental group with their matched control were sacrificed using chloroform. Five milliliters (5 mls) of blood was collected from each rabbit at the stipulated period for CK-MB, Troponin I, and lactate dehydrogenase using ELISA machine. Data generated were expressed as mean +SD. ANOVA and Tukeys multiple comparison test were used to compare the results between means of groups. Variation in mean of parameters were considered statistically significant at $P<0.05$.

Results: The results showed significant increases at $p<0.05$ in the levels of CK-MB, troponin I and lactate dehydrogenase from day 30-90-days in the experimental groups as shown respectively: (394.50±10.08IU/L), (0.96±0.10IU/L) and (2443.80±10.01IU/L); (515.300±30.04IU/L), (1.78±0.05IU/L)and (281.00±6.68IU/L); (822.50±31.44IU/L), (5.90±0.86IU/L) and (320.00±11.60 IU/L) when compared with the control groups (210.50±21.84 IU/L), (0.41±0.03 IU/L) and (176.50±13.50 IU/L) respectively.

Conclusion: From the results, it can be concluded that dichlorvos raised the levels of the cardiac markers - CK-MB, troponin I and lactate dehydrogenase. This is an indication that dichlorvos is capable causing tissue injury and cardiac damage. The effects were proportional to the duration of dichlorvos exposure on therabbits.

Keywords: Chronic toxicological effects, 2, 2-dichlorovinyl dimethyl phosphate (Sniper), Rabbits.

1. INTRODUCTION

Organophosphate insecticide and pesticide dichlorvos (2- 2- dichlorovinyl dimethyl phosphate) are sold in Nigeria under the trade name "sniper," [2]. Dichlorvos has a boiling point of 140^oC at 2.7 Kpa and is a colorless to amber liquid. The chemical formula of dichlorvous is ($C_4H_7Cl_2O_3P$); at 200 degrees Celsius, its molecular weight is 220.98, its vapor pressure is 1.2 x 10-2 mmHg, and at 250 degrees Celsius, its density is 1.415 g/ml [3]. The World Health Organization categorizes dichlorvos as a chemical that is "very hazardous" and belongs to class "B" [4].

Suicidal and homicidal deaths in Nigeria have been linked to the abuse and misuse of dichlorvos, according to reports (3). To distinguish between dichlorvos-intoxicated deaths and homicidal deaths, there is insufficient information on the toxicological results of the deceased. Understanding the toxicity mechanism of dichlorvos intoxication requires measuring biochemical markers. An investigation into the long-term toxicological effects of dichlorvos on rabbit kidneys exposed orally and inhaled could aid in the public health evaluation of potential risks related to dichlorvos exposure. Therefore, the purpose of this study was to evaluate the chronic toxicological effects of 2, 2-dichlorovinyl dimethyl phosphate (Sniper) on the kidney function parameters of New Zealand white rabbits.

In a 2013 in-vitro study documented chromosomal aneuploidy/polyploidy, mitotic arrest, and disruption of mitotic division in the proliferation of the cell population in human cell culture caused by dichlorvos. Dichlorvos inhibits neural acetyl cholinesterase, which is how it causes toxicity in both humans and animals (3). There have been reports of neurological effects following acute oral exposure in animal studies [7]. Nigrostriatal neuronal death was observed in rats after a prolonged 2.5 mg/kg daily dichlorvos exposure [8]. According to the study nigrostriatal dopaminergic degeneration brought about by long-term exposure to dichlorvos was accompanied by a 60–70% decrease in striatal dopamine and tyrosine hydrolase levels [9]. Male Fischer 344 rats exposed to dichlorvos by olive oil gavage exhibited severe lacrimation, fasciculation, irregular breathing, and prostration, according to an LD50 study [11]

The aim of this study was to assess the levels of CK-MB and Troponin in blood of New Zealand white rabbits exposed to 2, 2-dichlorovinyl dimethyl phosphate (Sniper), by inhalation route of exposure.

2. MATERIALS AND METHODS

2.1 Experimental Animals

A total of twenty-four (24), two-month-old New Zealand white rabbits (*Oryctolagus cuniculus*) that weighed averagely 1.0kg were used for this study. The rabbits were purchased from Department of Biological Science, Rivers State University, and Port Harcourt animal house. They were used for oral and inhalation chronic studies. The rabbits were kept in a spacious and well-ventilated cage at room temperature, under natural circadian rhythm and were allowed to acclimatize for fourteen (14) days. They were housed in standard cages and allowed access to feed (Top Feed Finisher Mash, Sapele, Nigeria) and water *ad libitum* from the animal house, department of animal and environmental science, Rivers State University, Port Harcourt. All the animals received humane treatment according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Institute of Health.

2.2 Procurement and administration of Dichlorvos

1 litre of concentrated solution of dichlorvos (DDVP) insecticide 1000EC (which contains 1000mg of 2-2 dichlorovinyl dimethyl phosphate compound was purchased in Nigeria from Swiss–Nigeria chemical company which is the sole marketing company for dichlorvos in Nigeria). For the chronic inhalation study, 10% of the LD50 dose which is 0.05mg/kg dose of dichlorvos, mixed with 1.0ml of distilled water was administered to the rabbits daily for the stipulated period of 0-30, 0-60 and 0-90 days. The matched control rabbits received only feed and water *ad libitum* during the study. The experimental dose of dichlorvos was mixed with

1.0ml of distilled water, sprayed in the closed cages. The rabbits were transferred into the closed cage that has been flirted with dichlorvos to spend 4 hours daily before returning them back to their normal cages.

2.3 Summary of Method

The rabbits were divided into three (3) groups of four (4) rabbits each with four (4) matched controls.

Table 1- Chronic inhalation study

Duration	Chronic inhalation study	Matched control
0-30 days	4	4
0-60 days	4	4
0-90 days	4	4

2.4 Sample Collection, Storage and Analysis

2.4.1 Sample Collection

At day 30, 4 rabbits were sacrificed each from the study group, and the matched control group. Blood specimens were collected at each stage, 5.0mls of blood was collected for estimation of cardiac markers and lactate dehydrogenase.

2.4.2 Laboratory Investigation of Parameters

2.4.2.1 Procedure for Determining CK-MB (ELISA method) [11]

Test Method: ELISA

Test Principle: This is based on the principle of a solid phase ELISA (Enzyme Linked Immunosorbent Assay. This assay system uses a monoclonal antibody directed against a distinct antigenic determinant on the Ck-MB molecule. A goat anti-Ck-MB antibody conjugate to horseradish antibodies, leading to the CK-MB Molecules bring sandwiched between the solid phase and enzyme- linked antibodies. After it has been incubated for 1 hour at room temperature, the wells are washes with water to remove unbound labeled antibodies. A solution of TMN Reagent is added and incubated at room temperature for 20 minutes, resulting in the development of a blue colour. The colour development is stopped with the addition of stop solution which makes the colour change to yellow. CK-MB concentration is directly proportional to the colour intensity of the test sample: The Absorbance is measured spectrophotometrically at 450 nm.

2.4.2.2 Determination of Troponin I (ELISA) [12]

Test Method: ELISA

Test Principle: In this format the sample migrates from the sample pad through the conjugate pad where any target analyte present will bind to the conjugate. The sample then continues to migrate across the membrane until it reaches the capture zone where the target/conjugate complex will bind to the immobilized antibodies producing a visible line on the nitro-cellulose membrane. The sample then migrates further along the strip until it reaches the control zone, where excess conjugate will bind and produce a second visible line on the membrane. This

control line indicates that the sample has migrated across the membrane as intended. The optical system of the meter detects the two lines and measures the intensity of the signal line. The integrated software converts the signal intensity to a quantitative result and shows it on the display.

1 Determination of Lactate Dehydrogenase (LDH) (ELISA Method)(Stevens et al.,1983).

Principle:

Lactate dehydrogenases (LDH) catalyzes the conversion of L – Lactate to pyruvate, NAD is reduced to NADH in the process. The initial rate of the NADH formation is directly proportional to the catalytic LDH activity. It is determined by photometric measurement of the increase in absorbance.

Procedure:

50ul of serum was transferred into 1cm cuvettes, 950ml of working reagent was added to the blood sample. The content was properly mixed. The Absorbance was read immediately at OD 565nm wavelength (OD_{SO}). The absorbance was again read at 25 minutes (ODs25). 1ml of water and the calibrator were also read at wavelength of 565nm (ODH_{20}) and (OD_{CAL}). The results were calculated as shown:

$$\text{LDH Activity} = \frac{\text{ODs25}-\text{ODso}}{\text{Emit.1}} \text{ x } \frac{\text{Reaction vol (ul)}}{\text{Time .Sample vol (ul)}}$$

$$= 43.68 \text{ x } \frac{\text{ODS25 ODso}}{\text{ODCAL}-\text{ODH20}} \text{ x n(iu/l)}$$

ODS25 and ODso are ODS65nm values of sample at 25min and O min – Emit is the molar absorption coefficient of reduced MTT. L is the light path length which is calculated from the calibrator. ODCAL and ODH_{20} are OD565nm values of the calibrator and water. Reaction vol and sample vol are 200nl and 10ml, respectively. N is the dilution factor. Unit definition: 1 unit (iv) of LDH will catalyze the conversion of 1Nmol of lactate to pyruvate per min at PH 8.2

2.5 Statistical Analysis

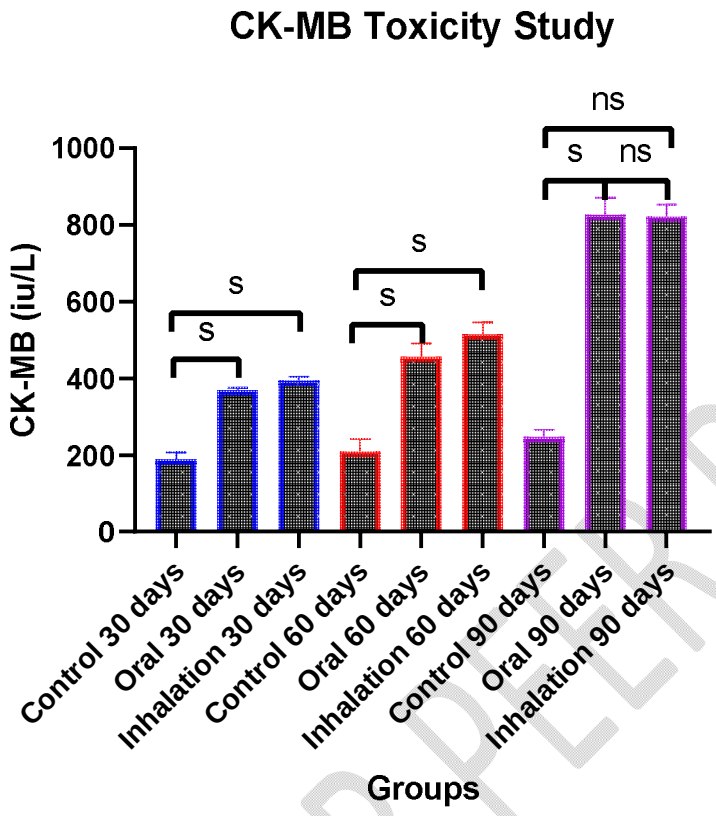
SPSS version 22.0 of windows statistical package was used to analyze the data generated. The mean ± standard deviation was determined. One-way analysis of variance (ANOVA) with Tukey's Post Hoc test was also done to compare the mean values of the parameters among the different groups. Bar charts were also done using the same statistical package. From the values obtained statistical decision and inferential evaluation were made. A probability (p) value of less than or equal to 0.05 was considered statistically significant.

2.6 ETHICAL APPROVAL

Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

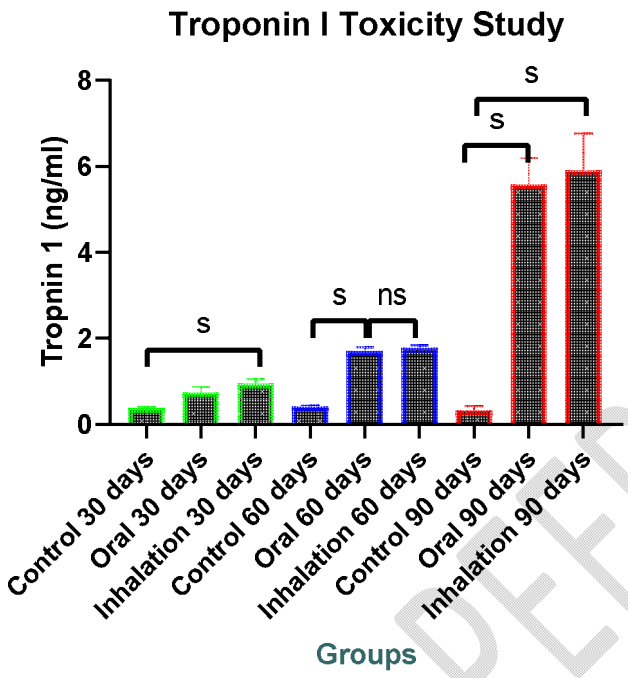
3. RESULTS

Figure 1:Mean ±SD of CK-MB of Rabbits Intoxicated with Dichlorvos by InhalationAdministration for 30 , 60 and 90 days Treatment. Significant increases were observed in the mean level of CK-MB of dichlorvos treated rabbits at day 30, 60 and 90 days when compared with the control at p< 0.05.



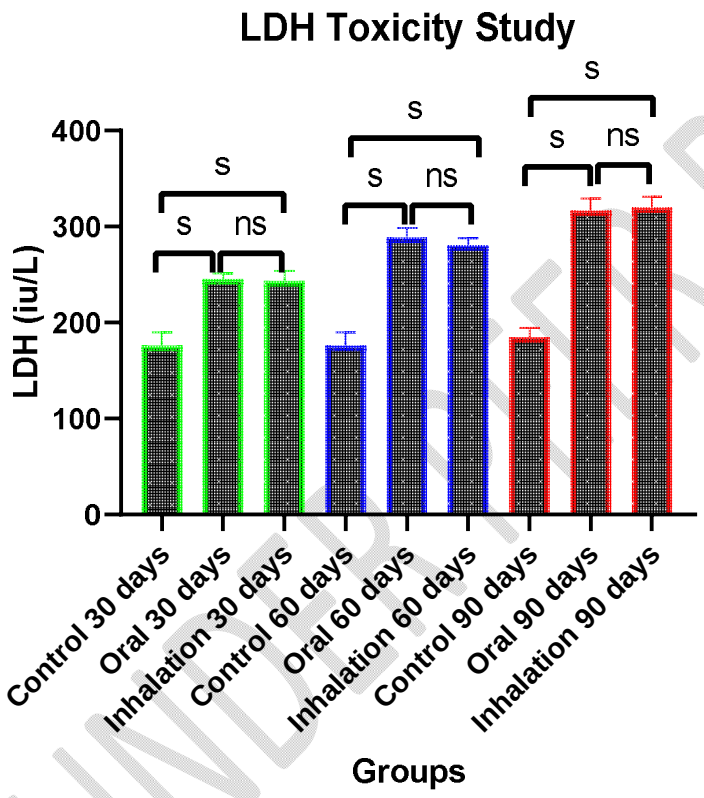
Key: Sniper = 2-2 dichlorovinyl dimethyl phosphate, [a, b] significant when compared with the group

Figure 2:Mean ±SD of Troponin I of Rabbits Intoxicated with Dichlorvos by InhalationAdministration for 30 , 60 and 90 days Treatment. Significant increases were observed in the mean level of Troponin I of dichlorvos treated rabbits at day 30, 60 and 90 days when compared with the control at p< 0.05.



Key: Sniper = 2-2 dichlorovinyl dimethyl phosphate, [a, b] *significant when compared with the group*

Figure 3: Mean ±SD of LDH of Rabbits Intoxicated with Dichlorvos by InhalationAdministration for 30 , 60 and 90 days Treatment. Significant increases were observed in the mean level of LDHof dichlorvos treated rabbits at day 30, 60 and 90 days when compared with the control at p< 0.05.



Key: Sniper = 2-2 dichlorovinyl dimethyl phosphate, [a, b] significant when compared with the group

4. DISCUSSION

The results from this study show that dichlorvos exposure caused significant increases in the mean CK-MB ($p<0.001$) and Troponin I ($p=0.002$), when the values obtained at 30-90 days were compared with the matched control. Elevation in the mean value of the cardiac markers was dependent on the duration of dichlorvos exposure. Dichlorvos-induced cardio toxicity mainly manifests as myocardial congestion, interstitial edema, ischemia and reversible interstitial inflammation (14). Acute dichlorvos poisoning could cause severe myocardial injury with refractory cardiogenic shock in which the mortality rate may exceed 60% [13], as were observed in the present study.

As observed in this present study, the main mechanism underlying myocardial injury in acute and chronic dichlorvos exposure may be due to the following reasons: direct toxic effects of the dichlorvos on the myocardium by dichlorvos, solvents and impurities; sympathetic and para-sympathetic dysfunction caused by the release of a large quantity of catecholmines and marked sensitivity of the heart to catecholamines, which results in coronary artery spasm, myocardial ischemia and myocardial injury. Again, the accumulation of large amount of acetylcholine and the release of large quantity of cytokines and inflammatory mediators affecting myocardial cells result in toxic myocarditis. Furthermore, complications of respiratory failure, marked electrolyte imbalances and acidosis which lead to internal environmental disturbances, further aggravates myocardial damage. (12).

The results of this study were also supported by the findings of Chen, et al14] who reported increased CK-MB and troponin I values in acute organophosphates pesticide poisoned rats. In a normal cellular system, there is always a balance between the number of free radicals and antioxidant defense molecules for normal cell functions [15].

Lactate dehydrogenase enzyme is present in almost all body tissues but it is present in high concentrations in the muscles, liver, kidney and moderately in red blood cells. Conditions that can lead to increased level of lactate dehydrogenase may include liver diseases, kidney diseases, muscle injury, trauma, heart attack and anaemia. Therefore, the significant increase in LDH level noted in the chronic dichlorvos exposure is a classical signal of adverse effect of dichlorvos on the tissues. LDH elevation in this study is a marker or sign that dichlorvos exposure caused serious tissue injury or damage which led to the release of high concentration of LDH enzyme from the affected or damaged cells of the liver, kidney, heart and muscles into the blood stream.
In other words, the increased serum LDH level observed in this study could be associated with organ destructive effect of dichlorvos leading to cell death that resulted in loss of cytoplasm. The anaemic condition coupled with the renal, liver and cardiac damages observed in the present study could be associated with the significant elevation in the LDH levels in the oral chronic study. This result is consistent with the report of Ogutcu& Kalender, (2008) who observed significant increase in AST, ALT, LDH and total cholesterol at the end of 4thand 7^{th} weeks in the dichlorvos and vitamins C & E treated rabbits

CONCLUSION

Dichlorvos caused an elevation in the levels of the cardiac markers- CK-MB and troponin; and the level of lactate dehydrogenase.Again, the increments in themarkers were proportional to the duration of inhalation exposure to the chemical.

REFERENCES

1. Owoeye, O., Edem, F. V., Akinyoola, B. S., Rahaman, S., Akpang, E. E. & Arinola, G.O. Toxicological Changes in the Liver and Lungs of Rats Exposed to Dichlorvos Before and After Vitamin Supplementation. *EuropeanJournalofAnatomy*, 2019; 6(3): 170 -8.
2. Owoeye, O., Edem, F. V., Akinyoola, B. S., Rahaman, S., Akang, E. E. & Arinola, G. O. Histological Changes in Liver and Lungs of Rats Exposed to Dichlorvos before and after Vitamin Supplementation. *European* Journal *ofAnatomy*, 2012; 16(3): 190 - 8.
3. Okoroiwu, H. C. Dichlorvos Toxicity: A Public Health Perspective. *InterdisciplinaryToxicology,* 2018; 11(2): 129 - 37.
4. Health Organization. *International Programme on Chemical Safety*. WHO Recommended Classification of Pesticide by Hazards & Guidelines to Classification 1994-1995 UNEP/ILO/WHO. 1992.
5. Suchismita, D. (2013). A Review of Dichlorvos Toxicity in Fish Current World Environment. 8(1), 134 - 149.
6. Nazam, N. & Shaikh, D. Assessement of Genotoxic Potential of the Insecticide Dichlorvos using Cytogenetic Assay. *InterdisciplinaryToxicology,* 2013; 6(2): 76 - 82.
7. Yatendra, S., Joshi, S.C., Singah, M, Joshi, A. & Kumar, J. Organophosphorus Poisoning: An Overview: *InternationalJournalofHealthScienceResearch,* 2014; 4: 245 - 57.
8. Binukumar, B. K., Bal, A. & Gill, K. D. Chronic Dichlorvos Exposure: Microglial, Activation, Proinflamatory Cytokines and Damage to NigrostratalDopamingeric System. *Neuro Molecular Medicine,* 2011;13: 251 - 65.
9. Binukumar, B. K. & Gill, K. D. Cellular & Molecular Mechanisms of Dichlorvos Neurotoxicity: Cholinergic, Noncholinergic, Cell Signalling, Gene Expression &Therapentic Aspect. *Indian Journal of Experimental Biology*, 2010; 48: 697 – 709.
10. Owunari, G. U. & Iwu, J. C. (2021). Effect of Chlorpheniramine on Acute Dichlorvos Poisoning in Wister Rats. *GSCBiologicalandPharmacuticalSciences*. 14(1), 48 - 59.
11. Apple, F. S. Acute Myocardial Infarction & Coronary Reperfusion. Serum Cardiac Markers for the 1990's. *American Journal of Clinical Pathology,* 1992; 97(2): 217 - 26.
12. Bhayana, V. & Henderson, A. Biochemical Markers of Myocardial Damage. *ClinicalBiochemistry*, 1995; 28: 1 - 29.
13. Yang, L., Chunshui, C., Xiaolong, L. & Liang, H. Successful Treatment of Severe Myocardial Injury Complicated with Refractory Cardiogenic Shock Caused by Aopp using Extracorporeal Membrane: A case report. *Medicine,* 2021;100(23): 281- 92.
14. Chen, K. X., Zhou, X. H. & Sun, C. A. Manifestations of Risk Factors of Acute Myocardial Injury after Acute Organophosphorus Pesticide Poisoning. *Medicine*, 2019; 98: 1-7.
15. Ustun, K., Taysi, S., Sezer, U., Demir, T., Saricicek, E., Alkis, H., Senyurt S. Z., Tarakcioglu, M. & Aksoy, N. Radio- Protective Effect of Nigella Sativa Oil on Oxidative Stress in Tongue Tissue of Rat. *OralDiseases*, 2014; 20(1): 109-13.