Effects of Dichlorvos Oral Treatment on Cardio protective and Atherogenic Indices of New Zealand White Rabbits.

Abstracts

Aim: To assess the Effects of Dichlorvos Oral Exposure on Cardio protective and Atherogenic Indices of New Zealand White Rabbits.

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 0.05 mg/m3, while 10% of the median lethal dose of dichlorvos which was 0.005 mg/dl was diluted with 1.0 milliliter of distilled water. It was administered by oral route daily for the stipulated periods of 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 of thirty, sixty, and ninety days. The analysis of Lipid profile was carried out using autolab chemistry analyser, while Cardioprotective and Atherogenic Indices were calculated. Data generated were expressed as mean ± SD. ANOVA and Tukey's multiple comparison tests was used for result analysis. Variation in mean of parameters were considered statistically significant at $P<0.05$.

Results: At day 30 oral exposure to dichlorvos cardio protective parameter AAI (anti Atherogenic index) showed significant decrease while TG/HDL-C, CRI-I, CRI-II, AIP, AC showed no significant differences when compared with the control. Between day 60 and 90 days of exposure, significant elevations were observed on CRI-I, CRI-II with significant decrease in AAI level as shown: AAI control-(39.16±5.79), day 30-(21.70±3.59), day 60-(20.09±5.19), day 90-(11.93±1.69); CRI-I control-(6.44±1.18), day 30-(6.85±1.56), day 60-(7.16±1.31), day 90-(11.38±0.77); CRI-II control-(4.46±0.85), day 30-(5.25±1.52), day 60-(5.83±1.18), day 90-(9.83±0.74)

Conclusion: Dichlorvos oral exposure caused changes (decrease) in cardioprotective index (AAI) with significant alterations or increases in the Atherogenic indices of treated rabbits and the severity was more pronounced at day 90 exposure.

INTRODUCTION

Dichlorvos is widely used in the control of household and agricultural pests. *However, their indiscriminate use has led to great environmental pollution, contaminating air, soil, water and farm lands resulting in health effects (Davies et al*., 2016*).*

The toxicity of dichlorvos on the heart is based on its ability to inhibit acetylcholinesterase enzyme leading to the accumulation of acetylcholine in the presynaptic space. This can be associated with various health issues such as muscle incoordination, tremors, myosis, chest discomfort, decreased heart irregularities, loss of reflexes, muscular paralysis, autonomic overstimulation and cardiorespiratory failure (Arthur *et al.,* 2017) .

Studies have shown that dichlorvos poisonings are associated with cardiovascular complications with changes on electrocardiography (ECG), conduction and ventricular arrhythmias (Mostafalou& Abdollahi, 2013).

Measurement of cardiovascular indices has become the major instrument of cardiovascular disease risk assessment. Several lipoprotein ratios or atherogenic indices have been defined in order to optimize the predictive capacity of the lipid profile.

Low density lipoprotein (LDL) cholesterol level has been the most useful index of cardiovascular disease risk and the major diagnostic tool for therapy.

The total cholesterol/high density lipoprotein cholesterol ratio (TC/HDL); and the LDL/HDL cholesterol are the important components and indicators of cardiovascular risk, their predictive value is higher than the isolated parameters. The TC/HDL ratio is considered as a more sensitive and specific index of cardiovascular risk than the traditional total cholesterol. The LDL/HDL cholesterol ratio is also as useful as the TC/HDL ratio for predicting cardiovascular risk because approximately two-thirds of plasma cholesterol are found in LDL. The LDL/HDL ratio appears to have more predictive value if triglyceridemia is taken into consideration.

Considering the indiscriminate use of dichlorvos in the environment coupled with its mechanism of toxicity on humans; the study was aimed at chronically exposing the rabbits to subtle doses of dichlorvos in order to observe its effects on the heart, using cardioprotective and atherogenic indices which are central indicators of cardiovascular damage.

2. Materials and methods

2.1 Experimental Animals

For this investigation, twenty-four (24) two-month-old New Zealand white rabbits (Oryctolagus cuniculus) with an average weight of 1.0 kg were employed. The rabbits were bought from the Port Harcourt animal shelter at Rivers State University's Department of Biological Science. Oral and oral chronic experiments were conducted using them. The rabbits were housed for fourteen (14) days to acclimate, in a roomy, well-ventilated cage that was kept at room temperature and in accordance with their natural circadian cycle. 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

A one-litre concentrated solution of the insecticide dichlorvos (DDVP) 1000EC, which contains one thousand milligrams of the compound 2-2 dichlorovinyl dimethyl phosphate, was bought in Nigeria from Swiss-Nigeria Chemical Company, the exclusive distributor of dichlorvos in Nigeria. For the duration of the 30, 60 and 90 days specified in the chronic oral study, the rabbits received 1.0 milliliter of distilled water mixed with 10% of the lethal dose of dichlorvos, or 0.005 mg/kg. The matched control rabbits received only feed and water *ad libitum* during the study. Whilst, for the chronic oral study, 10% of the LD50 dose of dichlorvos which is equivalent to 0.005mg/m^3 dose of dichlorvos was mixed with 1.0ml of distilled water administered orally for the stipulated period.

2.3.2 Determination of Total Cholesterol (8)Randox Kit Method)

Principle

Free cholesterols are determined through the hydrolysis of the enzyme, cholesterol esterase. The free cholesterol obtained gets oxidized to form hydrogen peroxide which reacts further with phenol and 4-aminoantipyrine by the catalytic action of peroxides to form a red colouredquinoneimine dye complex. The intensity of the colour developed is directly proportional to the concentration of cholesterol present in the sample read colourimetrically at 500nm.

2.3.3 Determination of Triglycerides (8)(Randox Kit Method)

Principle

Triglycerides are determined after the complex enzymatic hydrolysis with lipases. Under the catalytic initiation of peroxidases, an indicator of a quinoneimine formed from hydrogen peroxide, 4- aminophenazone and 4-chlorophenol will be obtained.

2.3.4 Determination of High-Density Lipoprotein (Abell Kendall, 1952) [8] (Randox Kit Method)

Principle

The presence of the chylomicrons, low density lipoproteins as well as the very low-density lipoprotein fractions initiate the precipitation of the high-density lipoprotein as supernatant on the addition of phosphotungstic acid and magnesium chloride. The precipitated fraction of the high-density lipoprotein is subjected to the test for cholesterol following an enzymatic end point reaction.

2.3.5 Determination of Low-Density Lipoprotein-Cholesterol (Friedwald Equation) [9]

Determination of low-density lipoprotein (LDL) was calculated using Friedwald equation [9]

LDL= Total cholesterol – Triglyceride- High density lipoprotein

2.3.6 Determination of Very Low-Density Lipoprotein Cholesterol (VLDL-C)

Determination of very low-density lipoprotein cholesterol (VLDL-C) was calculated using formular stated below (Gentile et al., 2020) [10]

VLDL-C (mmol/l) = $\frac{\boldsymbol{Triglyceride}}{\mathbf{2.2}}$

Calculated markers

Castelli risk index 1 = (TC/HDL-C)

Castelli risk index 2 =(LDL-C/HDL-C)

TG/HDL-C

Atherogenic coefficient (AC) = (TC –HDL-C/HDL-C)

Atherogenic index of plasma (AIP) = LOG TG/HDL-C

Antiatherogenic index (AAI) = 100 x HDL-C/TC-HDL-C

2.4 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, and 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 0.*05* was considered statistically significant.

2.5 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.

RESULTS AND DISCUSION

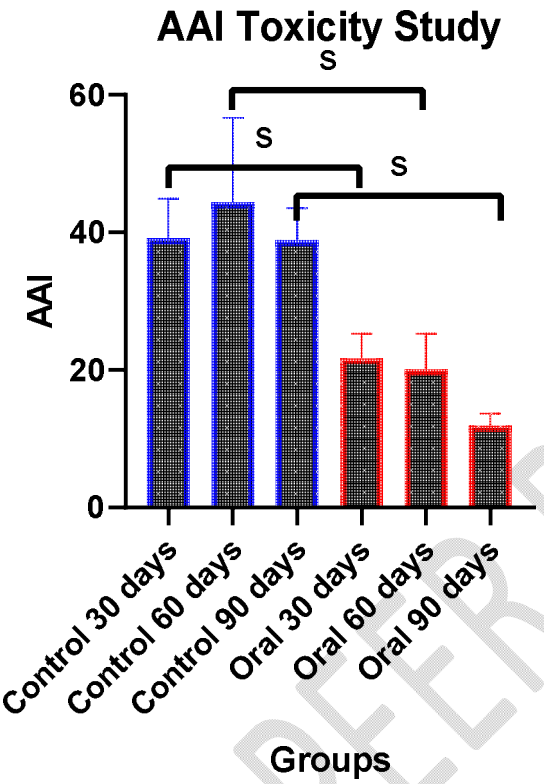

Figure 1: Mean ± SD Analysis of AAI (Anti Atherogenic Index) in the serum of rabbits treated with dichlorvos by oral administration

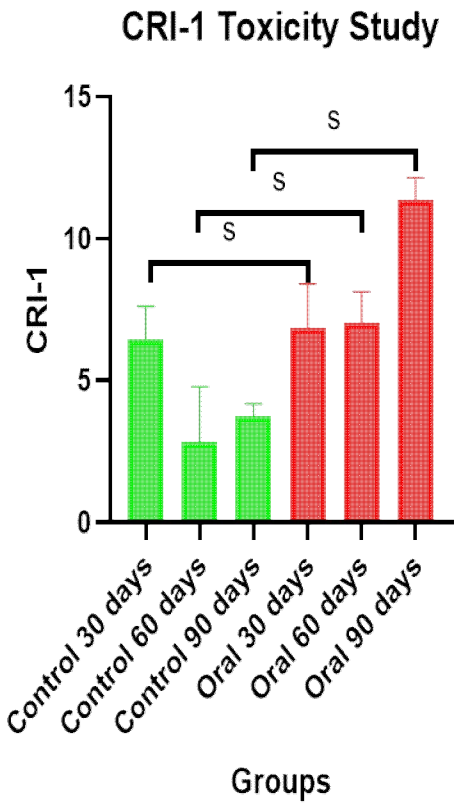

Figure 2: Mean ± SD Analysis of CRI-I in the serum of rabbits treated with dichlorvos by oral administration

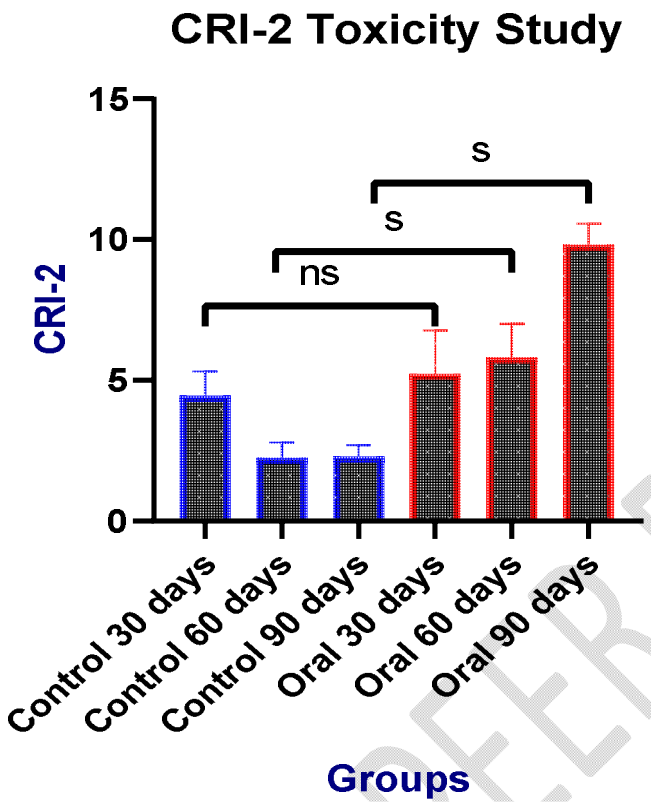

Figure 3: Mean ± SD Analysis of CRI-II in the serum of rabbits treated with dichlorvos by oral administration

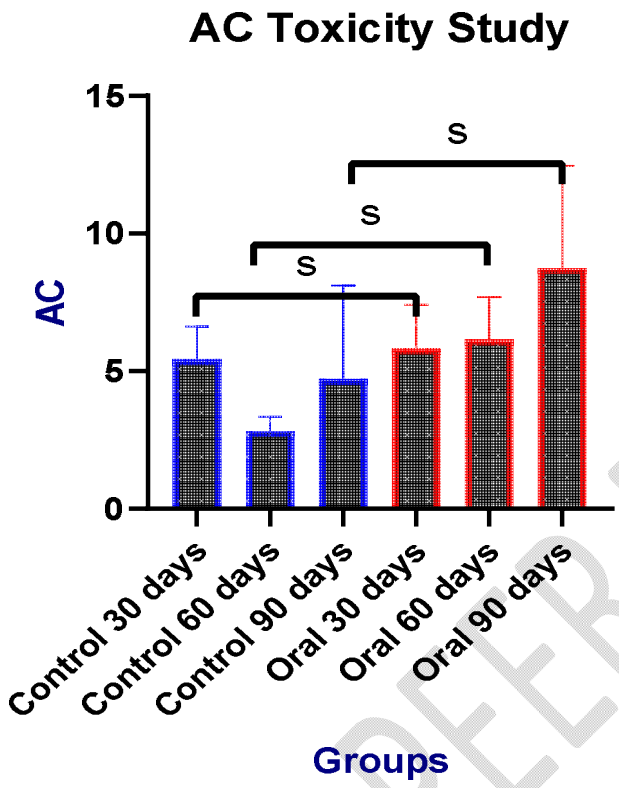

Figure 4: Mean ± SD Analysis of AC in the serum of rabbits treated with dichlorvos by oral administration

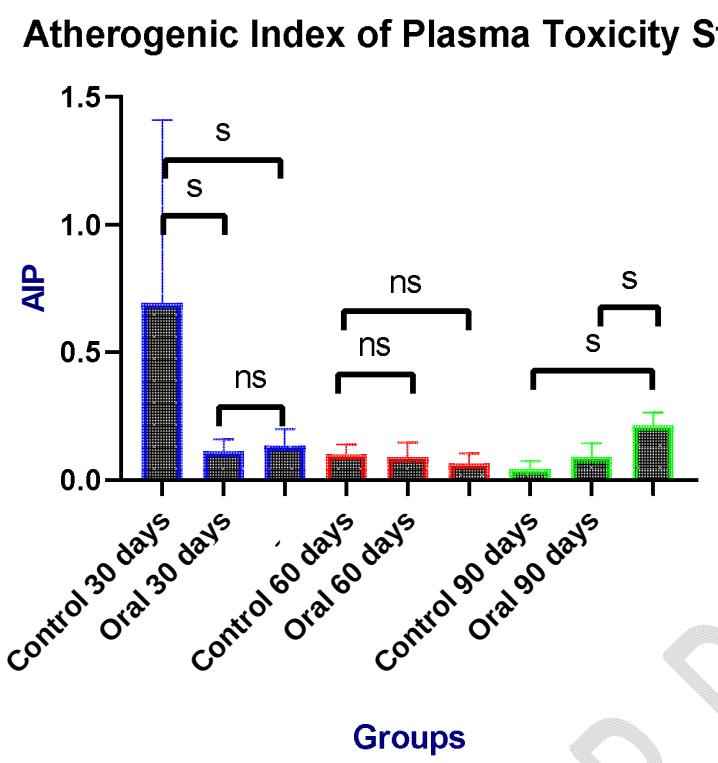

Figure 5: Mean ± SD Analysis of Atherogenic index of plasma in the serum of rabbits treated with dichlorvos by oral administration

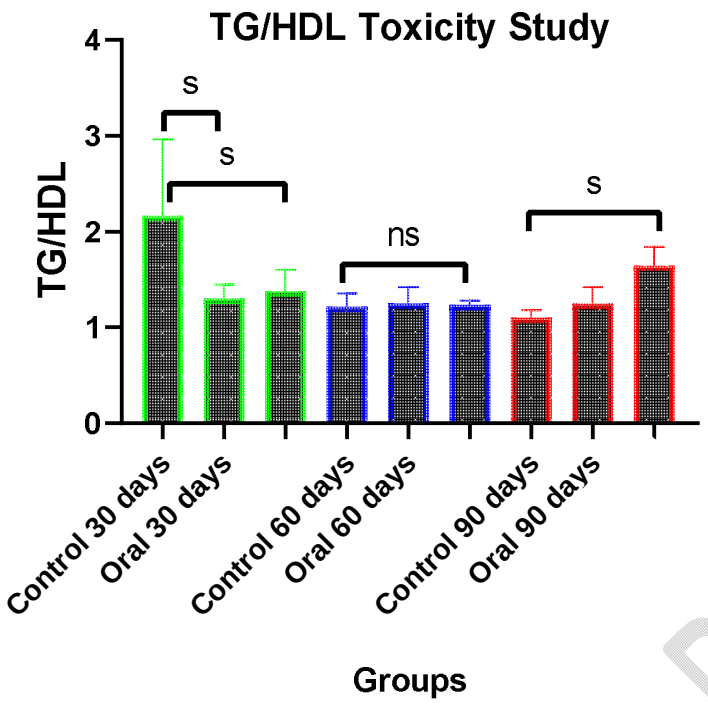

Figure 6: Mean ± SD Analysis of TG/HDL in the serum of rabbits treated with dichlorvos by oral administration

The study has revealed that dichlorvos is capable of inducing changes in the cardioprotective and atherogenic indices of rabbits. Significant decrease was observed in levels of anti atherogenic index (which is a cardiac protective parameter) from day 30 to day 90. The decrease was more pronounced as the duration of exposure increased. No significant statistical difference was observed at day 30 in the levels of TG/HDL, CRI-I, CRI-II, atherogenic index of plasma and atherogenic coefficient. Between day 60-90 days of dichlorvos exposure, significant elevations were observed in the levels of the treated rabbits atherogenic indices. This is an indication that chronic exposure to dichlorvos is capable of causing cardiotoxicity.

The result of this study agrees with the findings of Imam *et al.* (2018) and Meriem *et al.* (2016) who observed that sub chronic exposure to dichlorvos induced cardiotoxicity in the wistar rats; with significant elevation in the lipoprotein and atherogenic indices of the exposed rats.

The present study has shown that dichlorvos has damaging effect on the heart. The effects could be measured / assessed in the blood. From the results obtained, dichlorvos was able to induce morphological damages as well as increased lipid profiles, atherogenic indices, with decrease in antiathrogenic index (AAI). Dichlorvos can cause alteration in the lipid profiles with marked elevation of total cholesterol, VLDL, TG, LDL-C, and decrease in high density lipoproteins cholesterol level (HDL-C).

Furthermore, the toxicity of dichlorvos on the cardiovascular functions was confirmed through the outcome of the blood analysis of the anti-atherogenic index (AAI), as well as the atherogenic indices-CRI-I, CRI-II, AC and TG/HDL-C ratios. These are the essential central indicators of cardiovascular complications (Meriem *et al*., 2016). The alterations or changes in

atherogenic indices, lipid profile, and antiatherogenic index (AAI) are evidences to show that dichlorvos might cause alteration in lipid metabolism, atherogenesis, endothelial dysfunction, platelets aggregation, heart rate, blood pressure disorder and cardiotoxicity.

Conclusion: Oral exposure to dichlorvos caused a decrease in anti atherogenic index(which is a cardiac protective index) in a duration of exposure manner from day 30 to 90 days. Conversely, significant elevations were observed in the levels of Cardiac risk index 1 and 11, Atherogenic index of plasma (AIP), & Atherogenic Coefficient (AC). This is an indication that exposure to dichlorvos could lead to cardiotoxicity.

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