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Research Article

Comparative Study of Chronic Toxicological Effects of Dichlorvos on Lactate Dehydrogenase and Oxidative Stress Markers in New Zealand White Rabbits by Oral and Inhalation Exposure

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Abstract

Background: Dichlorvos, a widely used household and public health insecticide, is known to be toxic to both humans and animals, with indiscriminate use leading to frequent accidental exposures, suicides, and homicides.
Aim: This study investigated the chronic toxicological effects of dichlorvos on lactate dehydrogenase (LDH) and oxidative stress markers in New Zealand White Rabbits following oral and inhalation exposure.
Methodology: Thirty-six two-month-old New Zealand White rabbits were divided into groups for chronic oral and inhalation studies, as well as matched controls. Ten percent of the LD50 dose of dichlorvos was administered daily for stipulated periods (0-30, 0-60, and 0-90 days). Blood samples were collected to determine LDH, malondialdehyde (MDA), and total antioxidant capacity (TAC) levels.
Results: The results showed a significant increase in LDH levels, indicating tissue damage. Furthermore, dichlorvos exposure led to a significant increase in MDA, a marker of oxidative stress, and a corresponding decrease in TAC, which is duration-dependent. These findings suggest that chronic dichlorvos exposure causes an overproduction of free radicals, overwhelming the rabbits' antioxidant defense mechanisms and leading to oxidative stress and cellular damage.
Conclusion: The study concludes that dichlorvos causes a significant elevation in LDH and oxidative stress markers, along with a decrease in total antioxidant capacity, through both oral and inhalation routes of exposure, with effects showing a duration-dependent pattern.

1. Introduction

Dichlorvos is a household and public health insecticide with fumigant action, dichlorvos has wide spread use in the form of aerosol or liquid sprays or as impregnated cellulosic, ceramic or resin strips, especially against flies and mosquitoes. For the control of fleas and ticks on livestock and domestic animals (pets), impregnated resin collars are used. A granular form of an impregnated resin strip is used as an antihelmintic in domestic animals. Dichlorovos is also available as an aerosol and soluble concentrate [1]. Commercial production of dichlorvos began since 1961, and it has been available to household since 1964. It can be produced by the dehydrochlorination of trichlorfon in aqueous alkali at 40 -500C [2]; commercial production is by a reaction of trimethyl phosphate and chloral [3].

Dichlorvos is toxic to both humans and animals and it is used indiscriminately due to its affordability and availability. This has led to its use in high frequency for suicide, homicide and other accidental exposures [3]. The United States Environmental Protection Agency

has reviewed the safety data of dichlorvos severally and in 1995 they reached a voluntary agreement which restricted the use of dichlorvos in many uses especially for all aerial application [4]. A permissible exposure limit in the Workplace has been set at a concentration of $1mg/m^3$ over an 8-hour workday by the occupational safety and Health Administration (OSHA). It was also stated that dichlorvos exposure at levels of $100g/m^3$is instantly dangerous to life and health [5].The international Agency for research on cancer (IARC) has also stated that dichlorvos is a possible carcinogen to humans [6]. Again, the U.S environmental protection Agency (EPA) has also estimated that a lifetime of drinking water which contains 0.1 microgramme of dichlorvos per liter (mg/L) would cause an extra case of cancer in every million people [6].

Dichlorvos self-poisoning has become a very serious problem in Nigerian society, and it has been estimated that about 200,000 people die every year as a result of pesticide self-poisoning [7]. It has also been assumed that estimate of 873,000 cases of suicide has occurred among the world population in the year 2002 [8]. Several cases of death by pesticides in China, Malaysia, Sri- lanka and Trinidad-Tobago have been reported, and it is estimated that 330,000 people die by intentional intake of pesticides annually. Exposure to dichlorvos could cause acute or chronic toxicity. Inhalation is usually the most common route of dichlorvos toxicity because of its volatility [9].The main exposure pathway of dichlorvos in human is the inhalation route of exposure. This is because of its current use patterns. Therefore, a chronic inhalation study could help in the assessment of potential risks of dichlorvos [9].

Serious poisoning with dichlorvos affects the central nervous systems which will lead to in-cordination, slurred speech, loss of reflexes, weakness, fatigue, involuntary muscle contraction, twitching, tremors of the tongue or eyelids and paralysis of the body extremities and the respiratory muscles [7]. Involuntary urination and defecation could occur in severe cases, as well as irregular heartbeats, Psychosis unconsciousness, convulsions and coma. Furthermore, respiratory failure or cardiac arrest could lead to death [7]. Chronic exposure to dichlorvos may lead to more symptoms such as impaired memory and concentration, severe depression, disorientation, irritability, insomnia and drowsiness, as well as liver toxicity and oxidative stress. An influenza- like condition has also been reported [10]. The aim of the study was to evaluate the chronic oxidative stress effect induced by 2, 2-dichlorovinyl dimethyl phosphate (Sniper) in New Zealand white rabbits.

2. Materials and methods

2.1. Experimental Design

A total of thirty six (36), 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, 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. 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.

For the chronic oral study, 10% of the LD50 dose which is 0.005mg/kg dose of dichlorvos, mixed with 1.0ml of distilled water was administered orally 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. Whilst, for the chronic inhalation study, 10% of the LD50 dose of dichlorvos which is equivalent to $0.05mg/m^3$dose of dichlorvos, mixed with 1.0ml of distilled water, sprayed in the closed cages. The rabbits were transferred into the closed cages that have been flirted with dichlorvos to spend 4 hours daily before returning them back to their normal cages.

The rabbits were divided into three (3) groups of four (4) rabbits each with four (4) matched control. A total of 20 cages were used for this experiment as shown below:

Table 1: Rabbit Grouping

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

2.2. 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.

2.3. Sample collection

At day 30, 4 rabbits were sacrificed each from the chronic oral study group, chronic inhalation study group and from the matched control group. Blood specimens were collected at each stage, about 5.0mls of blood was collected into lithium heparin specimen container for estimation of lactate dehydrogenase, oxidative stress markers and total anti-oxidant capacity. The blood sample was collected using lithium heparin anticoagulant bottle and was timely separated after collection and hemolysis avoided.

2.4. Laboratory investigation of parameters

Determination of Lactate Dehydrogenase (LDH) (ELISA Method) [11].

Principle

Lactate dehydrogenises (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.

2.5. Determination of Malondialdehyde (MDA)

The estimation of malondialdehyde was carried out using the method described by [12].

Principle of the Test

The principle is based on the quantification of a powerful light – absorbing and fluorescing adduct in a continuous reaction with thiobartituric acid (TBA).

2.6. Quantitative Determination of Total Antioxidant Capacity (TAC)

The estimation of malondialdehyde was carried out using the colorimetric method as described by [13].

Principle

A variety of antioxidant macromolecules, antioxidant molecules and enzymes in a system can eliminate all kinds of reactive oxygen species and prevent oxidant stress induced by reactive species. The total levels affect the total antioxidant capacity in the system. Many antioxidants in the body can reduce Fe^{3+} to Fe^{2+} can form stable complexes with phenanthroline substance. The antioxidant capacity (T-AOC) can be calculated by measuring the absorbance at 520 nm.

2.7. 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, bar charts were also done using the same statistical package for result presentation. From the values obtained statistical decision and inferential evaluation were made. A probability (p) value of less than 0.05 was considered statistically significant.

3. Results

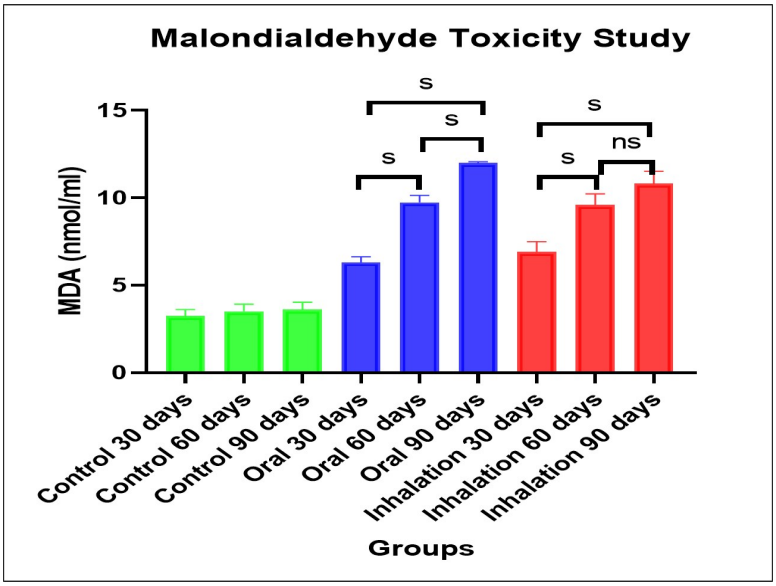

Figure 1: Serum MDA activity comparison of the effect of routes of administration of dichlorvos on oxidative stress markers.

Figure 1: Bar chart showing the mean ± SD values for three groups across multiple time points. Green bars represent the Control group, blue bars represent the Oral treatment group, and red bars represent the Inhalation treatment group. Error bars indicate the standard deviation of the mean (SD). Asterisks above the bars indicate statistically significant differences between groups ($p < 0.05$).

The chart above shows that the control group (green) maintains relatively stable, low mean values across all observed time points. Oral treatment (blue) exhibits a significant increase in MDA levels reaching a peak at the 90 days of administration. Similarly, the Inhalation treatment (red) continues to increase over time, maintaining significant exposure-dependent elevated levels of MDA.

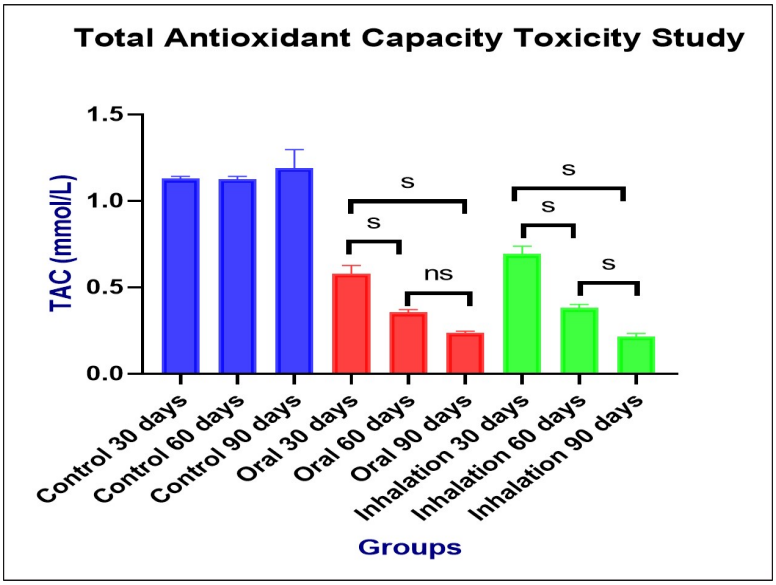

Figure 2: Serum TAC levels comparison of the effect of routes of administration of dichlorvos on oxidative stress markers.

Figure 2: Bar chart showing the mean ± SD values for three groups across multiple time points. Blue bars represent the Control group, red bars represent the Oral treatment group, and green bars represent the Inhalation treatment group. Error bars indicate the standard deviation of the mean (SD). Asterisks above the bars indicate statistically significant differences between groups ($p<0.05$).

The chart above shows that the control group (green) maintains relatively stable, low mean values across all observed time points. Oral treatment (red) exhibits a significant decrease in TAC levels reaching the lowest level at the 90 days of administration. Similarly, the Inhalation treatment (green) continues to decrease over time, maintaining significant exposure-dependent decline of TAC.

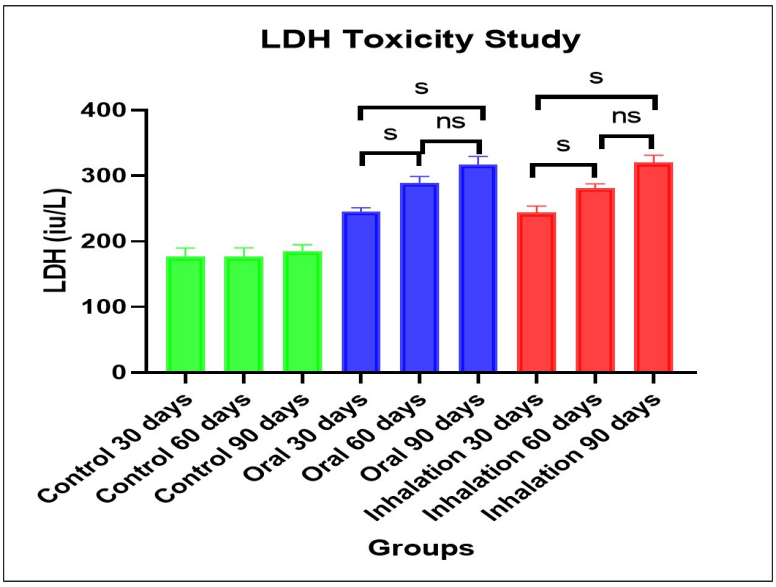

Figure 3: Serum LDH levels comparison of the effect of routes of administration of dichlorvos to assess the extent of tissue damage.

Figure 3: Bar chart showing the mean ± SD values for three groups across multiple time points. Green bars represent the Control group, blue bars represent the Oral treatment group, and red bars represent the Inhalation treatment group. Error bars indicate the standard deviation of the mean (SD). Asterisks above the bars indicate statistically significant differences between groups ($p < 0.05$).

The chart above shows that the control group (green) maintains relatively stable, low mean values across all observed time points. Oral treatment (blue) exhibits a significant increase in LDH levels reaching the peak level at the 90 days of administration. Similarly, the Inhalation treatment (red) continues to increase over time, maintaining significant exposure-dependent rise in LDH level.

4. Discussion

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 sof LDH enzyme from the affected or damaged cells of the liver, kidney, heart or 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. This result is consistent with the report of [14] who observed significant increase in AST, ALT, LDH and total cholesterol at the end of 4th and 7th weeks in the dichlorvos and vitamins C & E treated rabbits.

Oxidative stress is a state of imbalance between the cellular oxidant species production and antioxidant capability in favour of the oxidants resulting in tissue or organ damage [15]. Reactive oxygen species (ROS) are products of normal cellular metabolism. Most of the bodies' energy is produced by the enzymatically controlled reaction of oxygen with hydrogen in oxidative phosphorylation which takes place within the mitochondria during oxidative metabolism. During the process of enzymatic reduction of oxygen to produce energy, free radicals are produced (reactive oxygen species and or reactive nitrogen species). Excessive generation of these free radicals results in decrease in antioxidant defense mechanism leading to oxidative stress. Based on the results obtained in this study, dichlorvos exposure caused significant increase in the mean levels of malondialdehyde with a corresponding decrease in the mean total antioxidant capacity which were all duration of exposure-dependent.

From the above findings, chronic exposure of the rabbits to dichlorvos led to overproduction of free radicals which overwhelmed the ability of the defense mechanism of the rabbits to detoxify and remove the harmful toxic intermediates produced by oxidation. This caused an imbalance in the oxidant - antioxidant ratio. The toxicity of dichlorvos on the rabbits under chronic exposure could cause a continual formation of free radicals, and in the absence of proper antioxidant balance; this resulted in oxidative stress[16]. Generation of reactive oxygen species or free radicals have been implicated in the progression of diverse human pathologies such as tumorgenesis, atherosclerosis, neurodegenerative illness (Alzheimer's & Parkinson's diseases), liver and kidney diseases. This could be the reason for alterations observed in this present study. Similar results were also obtained by [17]. who reported significant increase in malondialdehyde level in male mice that were sub chronically orally treated with dichlorvos. Again, [18], observed significant decrease in total antioxidant capacity and significant elevation in malondialdehyde (MDA) levels in 55 adult males who were exposed to organophosphate pesticide and carbamates in pesticide-sprayers.

5. Conclusion

In conclusion, dichlorvos caused significant elevation in the levels of lactate dehydrogenase enzyme, malondialdehyde and lipid peroxidase index with significant decrease in total anti-oxidant capacity in the oral and inhalation routes of exposure. Comparison of the effect in the oral and inhalation routes of exposure showed duration-dependent pattern in both routes of administration.

Competing interests

Author has declared that no competing interests exist.

Authors Contributions

Author C.C.O designed the study, performed the statistical analysis, wrote the protocol, wrote the draft of the manuscript and managed the literature searches.

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