NEXUS OF MEDICINE AND LABORATORY SCIENCE JOURNAL

ISSN (ONLINE): 3027-2998

Assessment of Chronic Inhalation Exposure Effects of Dichlorvos (Dichlorvos) on Cardioprotective and Atherogenic Indices in the Blood of New Zealand White Rabbits

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Article type: Original
Cite as: Ozoemena CC. Assessment of Chronic Inhalation Exposure Effects of Dichlorvos (Dichlorvos) on Cardioprotective and Atherogenic Indices in the Blood of New Zealand White Rabbits. Nexus Med. Lab. Sci. J. 2025;2(4):11-20
Received on 23rd July, 2025; Accepted on 25th August, 2025; Published on 31th August, 2025
Publisher: ScholarlyFeed
https://doi.org/10.71462/sfpl2503011

ABSTRACT

Background: Dichlorvos, a widely used organophosphate pesticide, is frequently applied in agricultural and domestic settings. Its high volatility makes inhalation a common route of exposure, raising concerns about its chronic effects on cardiovascular health. Lipid profile–derived indices are reliable predictors of cardiovascular risk and can provide insights into pesticide-induced cardiotoxicity. **Aim:** This study evaluated the chronic effects of dichlorvos administered orally and by inhalation on cardioprotective and atherogenic indices in New Zealand White rabbits. **Methodology:** Thirty six male rabbits (1.0–1.2 kg) were divided into nine groups (n = 4 each): oral exposure, inhalation exposure, and controls for 30, 60, and 90 days. Rabbits received 10% of the LD_{50} dose of dichlorvos (0.05 mg/m^3) daily, either orally or via inhalation chambers (4 h/day). Serum lipid profiles were analyzed, and indices including Castelli's risk indices (CRI-I, CRI-II), triglyceride/HDL-C ratio (TG/HDL-C), atherogenic coefficient (AC), atherogenic index of plasma (AIP), and anti-atherogenic index (AAI) were calculated. Data were analyzed using ANOVA with Tukey's post-hoc test ($p < 0.05$). **Results:** At 30 days, only AAI showed significant reduction in exposed groups compared with controls ($p < 0.05$). By 60 and 90 days, CRI-I, CRI-II, TG/HDL-C, AC, and AIP were significantly elevated, while AAI declined progressively. Inhalation exposure produced higher elevations in atherogenic indices, whereas oral exposure caused greater reductions in AAI. **Conclusion:** Chronic dichlorvos exposure disrupts lipid homeostasis in a time-dependent manner, reducing cardioprotection and increasing atherogenic risk. Inhalation exposure appears more hazardous than oral administration, underscoring the need for stricter regulation and public health awareness.

Keywords: *Dichlorvos, Organophosphate, Atherogenic indices, Anti-atherogenic index, Cardiovascular risk, Rabbits*

1.0 INTRODUCTION

Organophosphate compounds remain one of the most extensively used groups of pesticides worldwide because of their effectiveness in controlling a broad spectrum of household, veterinary, and agricultural pests. Among these, dichlorvos (2,2-dichlorovinyl dimethyl phosphate; DDVP) is one of the most commonly applied organophosphates due to its low cost and high insecticidal potency. Despite its usefulness, concerns regarding its toxicological impact on humans and animals have continued to grow. Dichlorvos is highly volatile, making inhalational exposure a major route of entry into the body, especially in occupational and domestic settings where improper handling, lack of protective equipment, and environmental contamination are common. Studies have documented detectable residues of dichlorvos in soil, water, food crops, and even household dust, reflecting the pervasive nature of this chemical in the environment and raising questions about its long-term health consequences [1,2]

The primary mechanism of dichlorvos toxicity is inhibition of acetylcholinesterase, an essential enzyme responsible for the breakdown of acetylcholine at synaptic junctions. Inhibition of this enzyme results in the accumulation of acetylcholine in synaptic clefts, leading to overstimulation of cholinergic receptors. Clinically, this manifests as a spectrum of cholinergic symptoms ranging from headache, dizziness, miosis, muscle fasciculations, and respiratory difficulty to severe outcomes such as convulsions, paralysis, and cardiorespiratory failure [3]. Beyond these acute manifestations, evidence suggests that chronic low-dose exposure to dichlorvos may induce long-term organ-specific toxicities, particularly affecting the cardiovascular, nervous, and endocrine systems [4,5].

The cardiovascular implications of organophosphate poisoning have gained considerable attention in toxicology and clinical medicine. Several clinical reports and experimental studies indicate that exposure to dichlorvos and related compounds can induce significant alterations in cardiac electrophysiology, manifesting as conduction abnormalities, arrhythmias, and ischemic changes detectable on electrocardiography [6]. Chronic organophosphate exposure has further been associated with oxidative stress, endothelial dysfunction, and dysregulation of lipid metabolism, all of which are established contributors to the pathogenesis of cardiovascular disease. Given the global burden of cardiovascular disease as a leading cause of morbidity and mortality, elucidating the cardiotoxic mechanisms of environmental and occupational toxicants such as dichlorvos remains highly relevant.

In clinical practice and epidemiological studies, cardiovascular risk is often assessed through analysis of lipid profile and derived indices. While total cholesterol, triglycerides, and low-density lipoprotein cholesterol (LDL-C) have traditionally served as markers of cardiovascular risk, increasing evidence supports the use of lipoprotein ratios and derived atherogenic indices for better predictive accuracy. Ratios such as total cholesterol/high-density lipoprotein cholesterol (TC/HDL-C), LDL/HDL-C, triglyceride/HDL-C, Castelli's risk indices (CRI-I and CRI-II), atherogenic coefficient (AC), and atherogenic index of plasma (AIP) have been demonstrated to correlate strongly with atherosclerotic risk and cardiovascular events [7]. Conversely, the anti-atherogenic index (AAI), derived from protective fractions of lipoproteins, serves as an indicator of cardioprotective status. These indices provide more robust insights into cardiovascular health than isolated lipid values and are widely recognized as essential tools in toxicological and clinical research.

Despite the widespread use of dichlorvos in low- and middle-income countries, where regulatory enforcement is weak, relatively few studies have examined its

chronic cardiovascular effects, particularly through inhalational exposure which represents the most likely route of continuous human contact. Most prior research has focused on acute toxicity, neurological outcomes, or biochemical parameters, with limited emphasis on lipid-related cardiotoxic endpoints. This creates a gap in understanding how chronic exposure to dichlorvos may predispose individuals to cardiovascular dysfunction and atherogenesis.

Therefore, the present study was designed to assess the chronic effects of dichlorvos inhalation on cardioprotective and atherogenic indices in New Zealand White rabbits, a well-established animal model in cardiovascular research. By evaluating lipid profile alterations and derived indices over different durations of exposure (30, 60, and 90 days), this work provides mechanistic insights into the potential cardiovascular risks associated with chronic environmental or occupational exposure to dichlorvos. The findings are expected to contribute to the body of knowledge on pesticide-induced cardiotoxicity and highlight the need for stricter public health interventions, regulatory policies, and safer alternatives to organophosphate use.

2.0 MATERIALS AND METHODS

2.1 Experimental Animals

A total of twenty-four (36) male New Zealand White rabbits (*Oryctolagus cuniculus*), two months old and weighing between 1.0 and 1.2 kg, were used in this study. The animals were procured from the animal facility of Rivers State University, Port Harcourt, Nigeria. Upon arrival, the rabbits were acclimatized for two weeks under controlled environmental conditions (temperature 22 ± 2 °C; 12-h light/dark cycle) in well-ventilated standard cages. They were provided with commercial rabbit feed and clean drinking water ad libitum.

2.2 Administration of Dichlorvos

For the chronic exposure study, a sublethal dose of dichlorvos equivalent to 10% of the median lethal dose (LD_{50}; 0.5 mg/m^3) was prepared, corresponding to 0.05 mg/m^3 of dichlorvos diluted in 1.0 mL of distilled water. The diluted solution was sprayed daily into closed exposure chambers (inhalation cages), and the rabbits were placed inside the chambers for four (4) hours before being returned to their housing cages for the inhalation exposure but for the oral exposure, same dose was adminstered orally.

Rabbit Grouping

Group	No. of Rabbit	Details
Control 30 days	4	No dichlorvos exposure for 30 days
Oral 30 days	4	Dichlorvos oral administration daily for 30 days
Inhalation 30 days	4	Dichlorvos daily inhalation for 30 days
Control 60 days	4	No dichlorvos exposure for 60 days
Oral 60 days	4	Dichlorvos oral administration daily for 60 days
Inhalation 60 days	4	Dichlorvos daily inhalation for 60 days
Control 90 days	4	No dichlorvos exposure for 90 days
Oral 90 days	4	Dichlorvos oral administration daily for 90 days
Inhalation 90 days	4	Dichlorvos daily inhalation for 90 days

Six experimental groups were established, with exposure durations of 30, 60, and 90 days, respectively. For each experimental group, a corresponding group of matched control rabbits was maintained under identical housing and feeding conditions but without dichlorvos exposure.

2.3 Biochemical Assays

2.3.1 Determination of Total Cholesterol

Serum total cholesterol was determined using the enzymatic colorimetric method. Cholesterol esters were enzymatically hydrolyzed to yield free cholesterol, which was subsequently oxidized to produce hydrogen peroxide. The hydrogen peroxide then reacted with phenol and 4-aminoantipyrine under the catalytic action of peroxidase to form a quinoneimine dye, the intensity of which was measured colorimetrically at 500 nm.

2.3.2 Determination of Triglycerides

Serum triglycerides were measured using the enzymatic glycerol-3-phosphate oxidase–peroxidase method. Triglycerides were hydrolyzed by lipase to produce glycerol, which underwent enzymatic oxidation, leading to hydrogen peroxide formation. The hydrogen peroxide reacted with 4-aminophenazone and 4-chlorophenol to yield a quinoneimine chromogen, whose absorbance was measured at 500 nm.

2.3.3 Determination of High-Density Lipoprotein Cholesterol (HDL-C)

High-density lipoprotein cholesterol (HDL-C) was determined by the precipitation method. Chylomicrons, very low-density lipoproteins (VLDL), and low-density lipoproteins (LDL) were selectively precipitated using phosphotungstic acid and magnesium chloride. The supernatant containing HDL-C was then subjected to cholesterol determination using the enzymatic colorimetric method.

2.3.4 Determination of Low-Density Lipoprotein Cholesterol (LDL-C)

Low-density lipoprotein cholesterol was calculated indirectly using the Friedewald equation:

$$LDL{-}C = TC - (HDL{-}C + TG/5)$$

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

Very low-density lipoprotein cholesterol was calculated using the following equation:

$$VLDL{-}C = TG/5$$

2.4 Derived Atherogenic and Cardioprotective Indices

From the lipid profile parameters, the following indices were computed:

Castelli Risk Index I (CRI-I):
TG/HDL-C

Castelli Risk Index II (CRI-II):
HDL-C/LDL-C

Triglyceride/HDL-C ratio:
TG/HDL-C)

Atherogenic Coefficient (AC):
(TC - HDL-C)/HDL-C

Atherogenic Index of Plasma (AIP):
Log(TG/HDL-C)

Anti-Atherogenic Index (AAI):
HDL-C/(TC- HDL-C)

2.5 Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS version 22.0. One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was applied to compare group means. A p-value < 0.05 was considered statistically significant. Graphical representations were generated using the same statistical package.

2.6 Ethical Considerations

All animal handling procedures were conducted in accordance with the "Principles of Laboratory Animal Care". The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee of Rivers State University, Port Harcourt, Nigeria.

3.0 Results

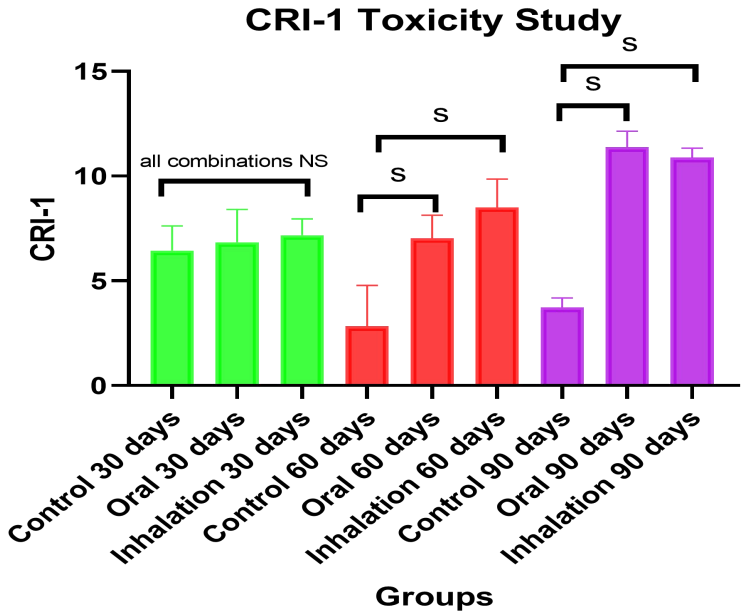

Figure 3.1: Mean ± SD Analysis of CRI-I in the serum of rabbits treated with dichlorvos by inhalation administration

In Fig 3.1, CRI-I values increased progressively with duration of exposure. At 30 days, no significant difference was observed between control and exposed groups. By 60 days, both oral and inhalation groups showed significant elevations in CRI-I compared with controls ($p < 0.05$), with inhalation exposure producing higher values. At 90 days, CRI-I was markedly elevated in both exposed groups, particularly in the oral group ($p < 0.001$).

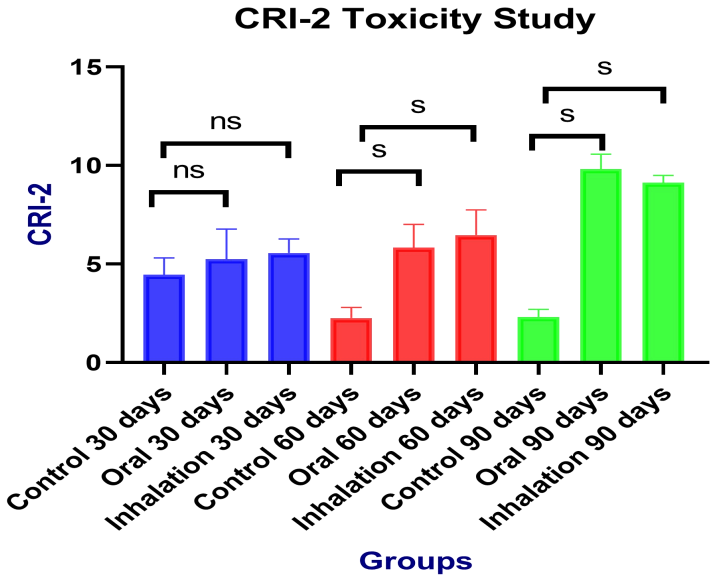

Figure 3.2: Mean ± SD Analysis of CRI-II in the serum of rabbits treated with dichlorvos by inhalation administration

Similar to CRI-I, CRI-II values in Fig 3.2 remained comparable to controls at 30 days but increased significantly in oral and inhalation groups at 60 days ($p <$

0.05). At 90 days, CRI-II was markedly higher in exposed rabbits compared with controls ($p < 0.001$), with inhalation oral exerting the strongest effect.

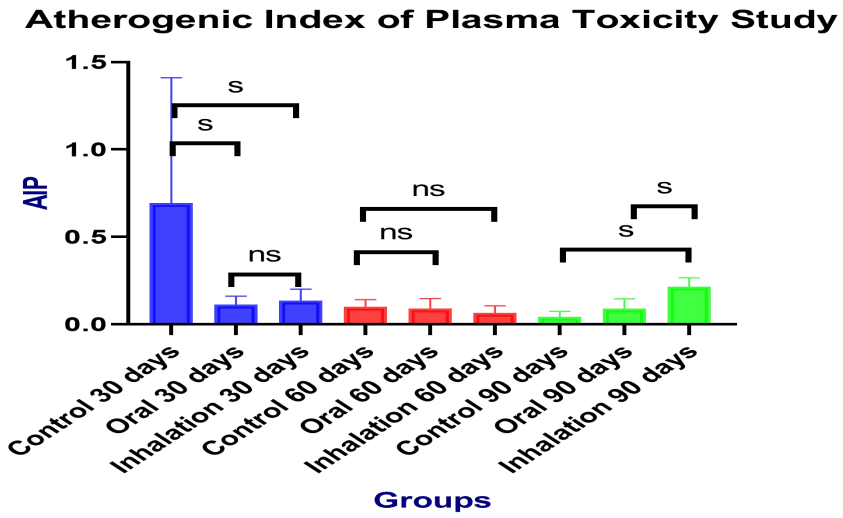

Figure 3.3: Mean ± SD Analysis of Atherogenic index of plasma in the serum of rabbits treated with dichlorvos by inhalation administration

AIP values did not differ significantly at 30 days of exposure. Similarly, by 60 days, no significant difference in AIP levels was seen in all the groups. At 90 days, AIP values were significantly elevated in both exposed groups relative to controls ($p < 0.05$), with inhalation exposure producing higher values.

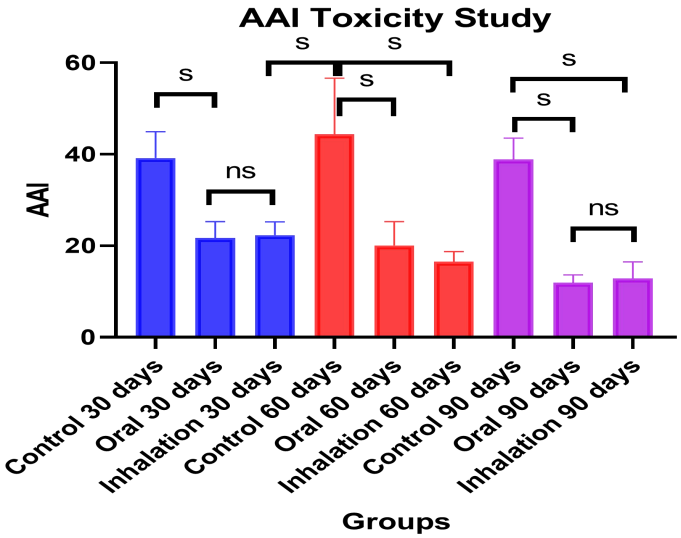

Figure 3.4: Mean ± SD Analysis of AAI in the serum of rabbits treated with dichlorvos by inhalation administration

AAI values in Fig 3.4, a marker of cardioprotective capacity, showed a consistent and significant decline from 30 days onward in both oral and inhalation groups. The reduction was progressive, reaching the lowest values at 90 days ($p < 0.001$). oral exposure caused greater reductions than inhalation exposure.

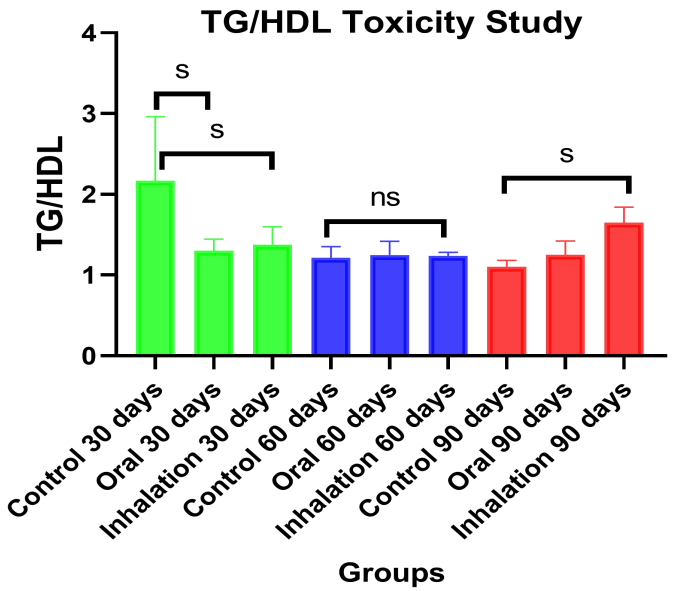

Figure 3.5: Mean ± SD Analysis of TG/HDL in the serum of rabbits treated with dichlorvos by inhalation administration

In Fig 3.5, at 30 days, TG/HDL-C ratios in exposed rabbits were not significantly different from controls. However, by 60 days, a stable trend was observed in all groups. At 90 days, TG/HDL-C was significantly elevated in exposed groups ($p < 0.05$), with the inhalation route showing the most pronounced increase.

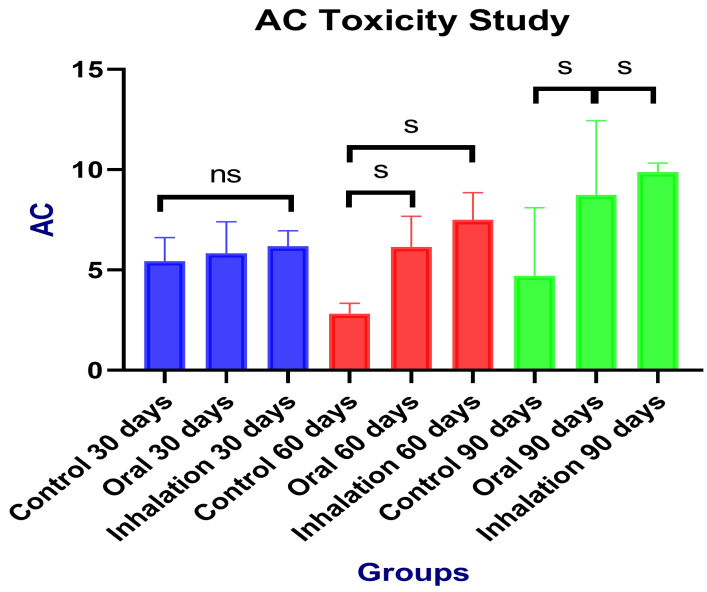

Figure 3.6: Mean ± SD Analysis of AC in the serum of rabbits treated with dichlorvos by inhalation administration

Fig 3.6 shows that AC values remained unchanged at 30 days but rose progressively with longer exposure. At 60 days, oral and inhalation groups showed significantly higher AC compared with controls. By 90 days, AC was significantly elevated in both oral and inhalation groups ($p < 0.01$), with inhalation exposure producing the highest values.

4.0 DISCUSSION

The present study investigated the chronic effects of dichlorvos exposure, administered either orally or through inhalation, on cardioprotective and atherogenic indices in New Zealand White rabbits over 30, 60, and 90 days. The findings demonstrate a clear time-dependent alteration in lipid-derived cardiovascular risk indices, with inhalation exposure exerting more pronounced effects than oral administration in most parameters. These results provide important toxicological insights into the potential cardiovascular risks associated with chronic exposure to dichlorvos, a widely used organophosphate pesticide.

4.1 Alterations in Atherogenic Indices

The progressive elevation of Castelli's Risk Index I (CRI-I) and Castelli's Risk Index II (CRI-II) observed from 60 to 90 days in both oral and inhalation groups reflects an increased burden of atherogenic lipoproteins relative to protective HDL-C. These indices are widely recognized predictors of cardiovascular disease risk, and their elevations suggest a shift toward a more atherogenic lipid profile in dichlorvos-exposed rabbits. This aligns with earlier reports by Imam et al. [6], who documented significant increases in CRI values in Wistar rats following sub-chronic dichlorvos exposure, supporting the cardiotoxic potential of this compound.

Similarly, the atherogenic coefficient (AC) and triglyceride/HDL-C (TG/HDL-C) ratio showed significant increases by 90 days, further confirming the development of an unfavorable lipid profile. Elevated AC and TG/HDL-C ratios have been associated with endothelial dysfunction, oxidative stress, and early onset of atherosclerosis (Rifai et al., [7]. The more pronounced changes in the inhalation group suggest that inhalation may facilitate greater systemic absorption and toxicity compared to the oral route, possibly due to the high volatility of dichlorvos and the efficient uptake of vapors through the respiratory system.

4.2 Reduction in Cardioprotective Index

A consistent and significant decline in the anti-atherogenic index (AAI) was observed from 30 to 90 days, with the steepest reductions recorded in orally exposed rabbits. AAI is a strong indicator of cardioprotective capacity, with higher values reflecting a favorable balance of HDL-C relative to total cholesterol. The persistent decline across all exposure groups underscores the potential of dichlorvos to deplete cardioprotective lipoproteins, thereby reducing resistance to lipid peroxidation and atherogenesis. Similar reductions in cardioprotective indices following pesticide exposure have been documented in studies linking organophosphate toxicity to oxidative stress and lipid metabolism dysregulation [8].

Interestingly, oral exposure produced greater reductions in AAI than inhalation, contrasting with the trend seen in the atherogenic indices. This may indicate route-specific variations in how dichlorvos alters lipid metabolism, with oral administration potentially exerting a stronger effect on hepatic processing of lipids, while inhalation favors systemic atherogenic shifts.

4.3 Mechanistic Considerations

The alterations observed in this study may be attributed to the established mechanism of dichlorvos toxicity—irreversible inhibition of acetylcholinesterase, leading to acetylcholine accumulation and overstimulation of cholinergic receptors. Chronic cholinergic overstimulation has been linked to autonomic imbalance, oxidative stress, and metabolic disturbances that adversely affect cardiovascular function [3,4]. Furthermore, organophosphate-induced oxidative stress may impair lipid metabolism, reduce HDL synthesis, and promote LDL oxidation, thereby driving

the observed changes in cardioprotective and atherogenic indices.

The route-dependent variations in toxicity observed here emphasize the importance of inhalation as a particularly hazardous mode of exposure. This is consistent with environmental and occupational health studies showing significant health risks associated with pesticide inhalation, particularly in poorly ventilated agricultural and household settings [2].

4.4 Public Health Implications

The findings of this study shows the cardiovascular risks associated with chronic dichlorvos exposure, particularly through inhalation. In regions where pesticide regulation and protective equipment use are inadequate, individuals may be at heightened risk of developing cardiovascular complications over time. Considering the already high global burden of cardiovascular disease, exposure to cardiotoxic pesticides such as dichlorvos may contribute significantly to disease progression, particularly in low- and middle-income countries where such chemicals are widely used.

4.5 Limitations and Future Directions

While this study provides valuable insights, certain limitations should be acknowledged. The sample size, though adequate for preliminary toxicological assessment, limits broader generalization of the findings. Moreover, only lipid-derived cardiovascular indices were evaluated; future studies should include histopathological analysis of cardiac tissues, oxidative stress biomarkers, and electrophysiological assessments to provide a more comprehensive understanding of dichlorvos-induced cardiotoxicity. Long-term epidemiological studies in exposed human populations would also be valuable in confirming the translational relevance of these findings.

Conclusion

Chronic exposure to dichlorvos, whether by oral or inhalation routes, significantly alters lipid profile–derived cardiovascular indices in rabbits. Inhalation exposure generally produced more severe elevations in atherogenic indices, while oral exposure was associated with greater reductions in cardioprotective indices. Collectively, these findings highlight the cardiotoxic potential of dichlorvos and emphasize the need for stricter control of its use, as well as public health interventions aimed at minimizing exposure risks.

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