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Research Article

Assessment of Acute Toxicological Effects of 2,2-Dichlorovinyl Dimethyl Phosphate on Acetyl cholinesterase Enzyme and C-Reactive Protein in the Vitreous Humor of the New Zealand White Rabbits

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Abstract

Background: Dichlorvos (2,2-dichlorovinyl dimethyl phosphate), a highly hazardous organophosphate insecticide, is increasingly implicated in suicide and homicide cases globally. Forensic differentiation between true intoxication and postmortem administration remains challenging, especially in decomposed remains where conventional biomarkers degrade.
Aim: This study assessed the acute effects of dichlorvos on acetylcholinesterase (AChE) and C-reactive protein (CRP) in vitreous humor (VH) to establish reliable postmortem diagnostic markers.
Methods: Twelve New Zealand white rabbits were divided into control (A), dichlorvos-intoxicated death (DIDR; 0.5 mg/kg orally), and non-intoxicated death (NDIDR; postmortem administration) groups. VH was collected 1-hour postmortem, and AChE/CRP levels were quantified via ELISA. Data were analyzed using ANOVA and Tukey's test ($p < 0.05$).
Results:DIDR exhibited significant AChE suppression (p-value$<$0.05) and CRP significant elevation (p-value$<$0.05) between groups. Conclusion: VH AChE and CRP are specific biomarkers for antemortem dichlorvos poisoning, offering a practical tool for forensic investigations.

1. Introduction

Dichlorvos (2,2-dichlorovinyl dimethyl phosphate, DDVP) is an organophosphate insecticide marketed under trade names such as Sniper, Nuvan, and Vapona [1]. In Nigeria, it is distributed as Sniper by Swiss-Nigeria Chemical Company Limited [2]. This compound is classified by the World Health Organization (WHO) as a Class B highly hazardous chemical due to its acute toxicity [3]. Its molecular weight is 220.98 g/mol, and it exhibits a density of 1.415 g/mL at 25$^{\circ}C$ [4].

Dichlorvos toxicity primarily arises from irreversible inhibition of acetylcholinesterase (AChE), leading to acetylcholine accumulation in synaptic clefts and subsequent cholinergic overstimulation [1]. This mechanism manifests clinically as muscle weakness, paralysis, and respiratory failure, often culminating in death [5]. Chronic exposure has also been linked to oxidative stress and hepatic damage [2]. Despite these risks, dichlorvos remains widely used in agriculture and households, particularly in developing nations where regulatory oversight is limited [6].

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The global burden of dichlorvos poisoning is significant, with WHO estimating approximately 25,000 annual fatalities [7]. In forensic settings, differentiating genuine dichlorvos-induced deaths from postmortem chemical administration (e.g., disguised homicides) is challenging, especially in decomposed remains where traditional biomarkers degrade [8]. Vitreous humor (VH), however, resists putrefaction and microbial contamination, making it a reliable medium for postmortem toxicology [9]. Recent studies propose VH biomarkers—such as AChE activity and C-reactive protein (CRP)—to distinguish antemortem poisoning from postmortem interference [8]. CRP, an acute-phase protein, further reflects systemic inflammation associated with dichlorvos exposure [10].

Despite these advances, few studies have systematically evaluated VH AChE and CRP levels in dichlorvos poisoning, particularly in distinguishing acute intoxication from disguised exposure. This study aims to address this gap by analyzing the acute toxicological effects of dichlorvos on AChE and CRP in the vitreous humor of New Zealand white rabbits. We hypothesize that dichlorvos-intoxicated deaths will exhibit significant AChE suppression and CRP elevation compared to controls and postmortem-administered cases. Our findings could provide a reliable, low-cost forensic tool for dichlorvos-related death investigations, particularly in resource-constrained regions where advanced toxicology infrastructure is lacking.

2. Materials and Methods

2.1. Ethical Approval and Animal Welfare

The study protocol received full approval from the Institutional Animal Care and Use Committee, operating in strict compliance with the ethical guidelines outlined in the Guide for the Care and Use of Laboratory Animals [11]. To ensure humane treatment, predefined endpoints were established, mandating immediate euthanasia for any animal exhibiting severe distress, including prolonged convulsions or respiratory failure. Chloroform inhalation was employed for euthanasia, selected for its rapid induction of unconsciousness and minimization of suffering. These measures align with global standards for ethical animal research.

2.2. Experimental Animals and Housing Conditions

A total of twelve New Zealand white rabbits *(Oryctolagus cuniculus)*, aged two months and averaging 1.0 kg in weight, were sourced from the Port Harcourt animal shelter at Rivers State University. Following procurement, the animals underwent a 14-day acclimatization period in spacious, well-ventilated cages maintained at a controlled temperature of $25 \pm 2^{\circ}C$ with a 12-hour light/dark cycle. The rabbits were fed a standard diet of Top Feed Finisher Mash (Sapele, Nigeria) and provided water ad libitum. Environmental enrichment, such as wooden blocks and nesting materials, was incorporated to promote natural behaviors and reduce stress.

2.3. Procurement and Preparation of Dichlorvos

The dichlorvos formulation, marketed under the trade name Sniper (1000EC), was obtained from Swiss-Nigeria Chemical Company, the exclusive distributor in Nigeria. For the acute toxicity study, a lethal dose (LD50) of 0.5 mg/kg was prepared by diluting the compound in 1 mL of distilled water for oral administration.

2.4. Experimental Design and Group Allocation

For the acute toxicity assessment, twelve rabbits were allocated into three groups of four animals each. Group A served as the control and received only water ad libitum, followed by euthanasia via chloroform inhalation. Group B, the dichlorvos-intoxicated death group (DIDR), was administered 0.5 mg/kg dichlorvos orally, resulting in death within 20 minutes. Group C, the non-dichlorvos intoxicated death group (NDIDR), was euthanized with chloroform, after which 0.5 mg/kg dichlorvos was administered intraperitoneally to simulate postmortem exposure.

2.5. Sample Collection and Processing

Vitreous humor (VH) was collected systematically from both eyes of each rabbit, yielding 3.5 mL per animal. Sampling was performed precisely one hour postmortem to ensure consistency. Using a 23-gauge needle, the VH was aspirated and transferred into sterile plain bottles. The samples were then centrifuged at 8,000 rpm for 5 minutes to separate the supernatant, which was subsequently stored at $-20^{\circ}C$ to preserve biochemical integrity. Rigorous quality control measures were implemented, including processing all samples within two hours of collection to prevent analyte degradation.

2.6. Biochemical Assays

Quantification of Acetylcholinesterase (AChE) Activity

AChE activity was measured using a competitive ELISA kit, as described in the original manuscript. The assay operates on the principle of competition between AChE in the sample and an AChE-horseradish peroxidase (HRP) conjugate for binding sites on an anti-AChE antibody.

Measurement of C-Reactive Protein (CRP)

CRP levels were determined using a sandwich ELISA based on the method by Hind and Pepys [12]. The assay utilized monoclonal anti-CRP antibodies immobilized on microtiter wells to capture CRP present in the samples.

2.7. Statistical Analysis

Data analysis was performed using SPSS version 22.0. One-way analysis of variance (ANOVA) was employed to compare mean differences across groups, with Tukey's post hoc test used for pairwise comparisons. A probability value (p) of less than 0.05 was considered statistically significant. All results are presented as mean ± standard deviation (SD) of triplicate measurements to ensure reliability.

2.8. Ethical and Environmental Considerations

Throughout the study, the highest standards of animal welfare were upheld. The use of chloroform for euthanasia was chosen for its rapid action and compliance with ethical guidelines. The study also adhered to the principles of the 3Rs (Replacement, Reduction, Refinement), with the sample size (n=4 per group) carefully selected to balance statistical power and ethical responsibility.

To mitigate environmental impact, all dichlorvos-contaminated materials, including animal bedding and unused solutions, were disposed of via incineration at a licensed facility. This precautionary measure ensured that no residual toxicants entered the ecosystem, aligning with global best practices for hazardous waste management.

3. Results

Figure 1 presents the comparative analysis of acetylcholinesterase enzyme activity in the vitreous humor of rabbits across the three experimental groups. The bar chart illustrates the mean values with corresponding error bars, highlighting variations in enzyme activity among the groups.

The result shows a statistically significant difference (p-value$<$0.05) in acetylcholinesterase activity among the groups, with the control group showing the highest activity, the sniper intoxicated group the lowest, and the non-sniper intoxicated group presenting an intermediate level.

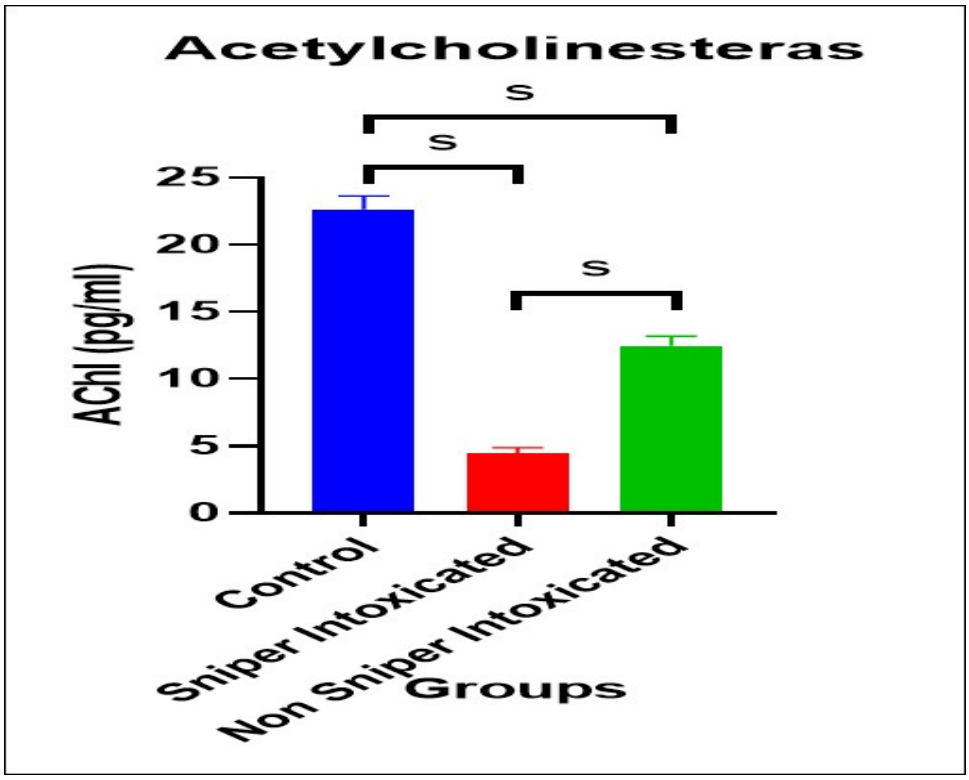

Figure 1: Analysis of Acetyl cholinesterase Enzyme in the Vitreous Humor of Rabbits

Figure 2 presents the comparative analysis of C-Reactive Protein in the vitreous humor of rabbits across the three experimental groups. The bar chart illustrates the mean values with corresponding error bars, highlighting variations in CRP among the groups.

The result shows no overall statistically significant difference (p-value$>$0.05) in C-Reactive Protein among the groups but pairwise comparison showed significant difference (p-value$<$0.05) between the group.

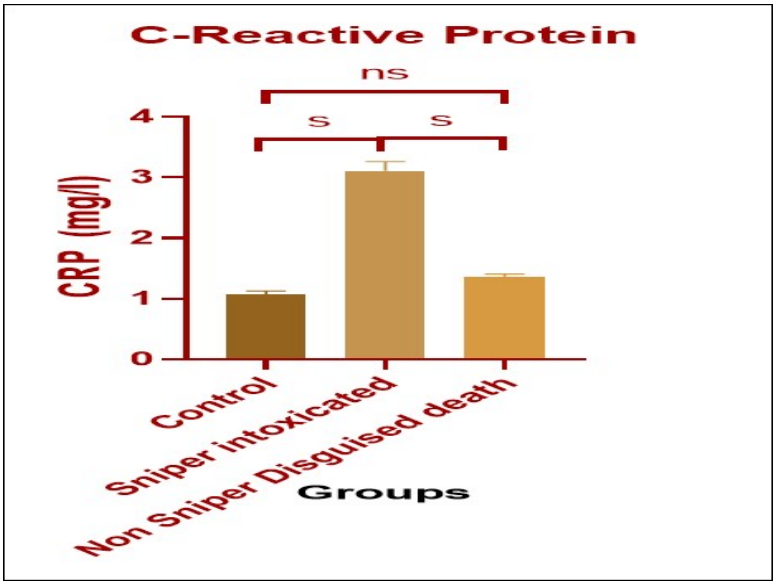

Figure 2: Analysis of C-Reactive Protein in the Vitreous Humor of Rabbits

4. Discussion

The present study demonstrates that acute dichlorvos intoxication in New Zealand white rabbits produces significant biochemical alterations in vitreous humor (VH), characterized by marked suppression of acetylcholinesterase (AChE) activity and elevated C-reactive protein (CRP) levels. These findings corroborate the neurotoxic and inflammatory effects of dichlorvos and highlight the forensic utility of VH analysis in differentiating true poisoning from postmortem chemical interference

4.1. Acetylcholinesterase Inhibition and Neurotoxicity

The observed decrease in VH AChE activity in the DIDR group aligns with dichlorvos's known mechanism as an irreversible AChE inhibitor [1]. This inhibition leads to acetylcholine accumulation at synaptic junctions, causing cholinergic hyperstimulation and the clinical symptoms documented in our study (e.g., convulsions, respiratory paralysis). The absence of AChE suppression in the NDIDR group confirms that postmortem dichlorvos administration does not replicate antemortem enzymatic inhibition, as AChE synthesis ceases after death. These results mirror findings by Bui-Nguyen et al. [5], who reported similar AChE inhibition in zebrafish liver following dichlorvos exposure.

4.2. CRP Elevation and Systemic Inflammation

The significant rise in VH CRP levels in DIDR rabbits suggests dichlorvos-induced systemic inflammation. CRP, an acute-phase protein, is synthesized in response to tissue damage or oxidative stress [10]. The lack of CRP elevation in the NDIDR group implies that postmortem dichlorvos exposure does not trigger inflammatory pathways, supporting CRP's specificity as an antemortem biomarker. This finding parallels studies linking organophosphate exposure to oxidative stress in mammalian models [2].

4.3. Forensic Implications of Vitreous Humor Analysis

The stability of VH in postmortem settings makes it uniquely valuable for forensic toxicology, particularly in decomposed remains where blood and soft tissues are compromised [9]. Our study establishes that VH AChE and CRP can reliably distinguish dichlorvos intoxication from disguised homicides, addressing a critical gap in forensic investigations in resource-limited regions. Notably, the biochemical profile of NDIDR rabbits mirrored controls, shows that postmortem chemical administration does not replicate antemortem metabolic responses.

4.4. Limitations and Future Directions

While this study focused on acute toxicity, chronic dichlorvos exposure may elicit different biomarker patterns, warranting further investigation. Additionally, interspecies variability necessitates caution when extrapolating results to humans. Future studies could explore correlations between VH biomarkers and histopathological changes in target organs (e.g., brain, liver) to strengthen diagnostic specificity.

5. Conclusion

This study confirms that acute dichlorvos poisoning significantly reduces acetylcholinesterase activity and increases C-reactive protein levels in vitreous humor, providing reliable biomarkers to distinguish true intoxication from postmortem exposure. These findings offer valuable tools for forensic investigations, particularly in cases where decomposition limits traditional toxicological analysis.

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