Evaluation of chronic toxicological effects of 2, 2-dichlorovinyl dimethyl phosphate (Sniper) in the vitreous humorliver function parameters of New Zealand white rabbits

ABSTRACT

Aim: the aim of this study was to evaluate the chronic toxicological effects of 2, 2-dichlorovinyl dimethyl phosphate (Sniper) on the vitreous humor liver function ofNew Zealand white Rabbits.
Study design: This is an experimental study.
Place and Duration of Study: Department of Biological Science, Rivers State University, Port Harcourt animal house,RiversState Teaching Hospital and Nigerian National Petroleum Corporation Hospital Laboratory, between January 2020 and April 2020.
Methodology:A total of twelve (12) male New Zealand white rabbits, two months old, weighing between 1.0 and 1.2 kg, were used in the study. For the dichlorvos death differentiation study, 12 rabbits were assigned to 3 cages of 4 rabbits per cage for the dichlorvos death differentiation study as follows: - cage A (control) which received 0.5ml of normal saline; cage B (dichlorvos intoxicated death rabbits) which received 0.5mg/kg of dichlorvos by oral route; cage C (non-sniper intoxicated death rabbits or disguised death) which received 0.5mg/kg of sniper 30 minutes after death by intraperitoneal route. All the groups were sacrificed, exactly 1 hour after death and 3.5ml of vitreous humor was collected from both eyes. The following biochemical parameters were analyzed using the vitreous humor:vitreous liver function tests (vitreous aspartate aminotransferase, alanine amino transferase, alkaline phosphatase, bilirubin, total protein and albumin, using spectrophotometric method. Data generated were expressed as mean ± SD. ANOVA and Tukey's multiple comparison test were used for result analysis. Variation in mean of parameters were considered statistically significant at $P<0.05$.
Results: The results showed that the AST, ALT and ALP levels for the control, sniper intoxicated death and non-Sniper intoxicated death (disguised death) were respectively: AST 6.30±0.83, 171.15±6.71 and 6.78±0.97(IU/L), ALT 5.19±0.64, 125.91±6.03, 5.75±0.55 (IU/L) and ALP 20.76±0.69, 166.43±5.13, 21.66±0.48 (IU/L). There were significant differences when all the groups were compared for the three parameters. There were also significant increases in the levels of total protein, conjugated and unconjugated bilirubin and albumin levels, when values were compared for the three groups.
Conclusion: From the results, it can be concluded thatDichlorvos caused hepatoxicity in the group of intoxicated rabbits. Further studies to assay mechanism of toxicity molecularly would further elucidate the findings.

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

1. INTRODUCTION

"Dichlorvous, sometimes known as sniper, is the most widely used organophosphate pesticide in developing countries. It is also a common insecticide for use in homes and agriculture" [1]. "It works as an oral, contact, and penetrant fumigant and insecticide. Sniper is used to protect stored goods and crops, especially those grown in greenhouses, as well as to control insects in buildings, aeroplanes, and outdoor areas. It is also used to manage internal and external parasites in livestock" [2]."Although the liver is the primary organ involved in detoxifying dichlorvos, the kidney, spleen, blood, and lung can also metabolise dichlorvos to dimethyl phosphate. Other metabolites of dichlorvos include desmethyl dichlorvos, monomethyl phosphate, and inorganic phosphate" [3]. "Dichlorvos is found in tissues and blood, and it does not build up in bodily tissues or in mammal breast milk, not even at levels high enough to cause poisoning symptoms" [4].

“The liver is where diclorvors are mostly metabolised. Glutathione-dependent and esterase pathways in the liver convert dichlorvos to desmethyl-dichlorvos, dimethyl phosphate, and dichloroacetaldehyde”[5]. Numerous studies using dichlorvos have shown a significant increase in liver enzymes, suggesting that the drug is hepatotoxic. Dichlorvos-induced autoimmune hepatitis was identified in a 49-year-old woman who had been exposed to the parasite on a regular basis. Her total bilirubin level was 133.5 umol/l, alkaline phosphatase (ALP) was 182 u/L, aspartate amino transaminase (AST) was 1267 u/L, and alanine amino transaminase (ALT) was 1558 u/l at the time of her initial hospital admission, which occurred 2.5 years after the onset of exposure-related symptoms.

“Decreased activity of hepatic glucokinase was reported in rats that were acutely exposed to 20mg/kg/body weight of dichlorvos. Rats that were exposed to dichlorvos for one week were diagnosed with diffused vascular degeneration of the hepatocytes with necrotic hepatocytes as well as slight peri-portal cellular infiltration by mononuclear cells, at 2-4 weeks of exposure, severe vacuolar degeneration and necrosis of hepatocytes, loss of hepatocyte outline, Portal triad that was completely obscured, circumscribed vessels by connective tissue necrotic plaque, peri-ported cellular infiltrations and diffused necrosis were reported”[6].

In a previous study,the histological findings in the liver of albino rats that were treated with 11.1 to 18.5 mg/kg of dichlorvos, intraperitoneally, revealed area of necrosis, sinusoidal dilation, and infiltration of the sinuses by few mononuclear cells [7], also reported “pronounced morphological alterations in the structure and function of the liver in male albino rats that were chronically exposed to dichlorvos”. Somia & Madiha [8] also reported “abnormal size and shape of hepatic cells, massive aggregation of inflammatory cells in the portal area and hepatocytomegalocytosis in liver of mice that were orally treated with dichlorvos for 3 months”.

In a 2-year inhalation study, male carworth E rats that received 5mg of dichlorvos/m3 (0.6ppm) showed increased serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) which possibly indicated hepatic damage. Again, greyhound dog that was treated with llmg/kg of dichlorvos in capsules had a 2-fold increase in AST level, which was suggestive of liver damage. Furthermore, male Fischer 344 rats that received 4 - 8mg/kg/day of dichlorvos by oral route for 5 days weekly for 2 years were diagnosed with cytoplasmic vacuolization of liver cells, due to lipid accumulation in cells (NTP, 1989). The aim of this study was to evaluate the chronic toxicological effects of 2, 2-dichlorovinyl dimethyl phosphate (Sniper) on the vitreous humor liver function ofNew Zealand white Rabbits.

2. MATERIALS AND METHODS

2.1 Experimental Animals

A total of 12two-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. 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 Sniper

1 litre of concentrated solution of sniper (DDVP) insecticide 1000EC (which contains 1000mg of 2-2 dichloro vinyl dimethyl phosphate compound was purchased in Nigeria from Swiss–Nigeria chemical company which is the sole marketing company for sniper in Nigeria). For the chronic oral study, 10% of the LD50 dose which is 0.005mg/kg dose of sniper, 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 fed and water *ad libitum* during the study. Whilst, for the chronic inhalation study, 10% of the LD50 dose of sniper which is equivalent to 0.05mg/m^3 dose of sniper was 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 sniper to spend 4 hours daily before returning them back to their normal cages.

2.3 Experimental Design, Sample Collection, Storage and Analysis

2.3.1 Posmortem Dichlorvos Death Differentiation Study

Three (3) cages containing 4 rabbits in each cage were labelled as A, B & C.

i. Control: The control rabbits in cage A received no sniper treatment. They were sacrificed, and after 1 hour, about 3.5 ml of vitreous humor was collected from the both eyes, centrifuged at the speed of 8000 rpm for 5 minutes and the supernatant was separated and kept frozen at the temperature of -20^{o}c for biochemical investigations. The liverwas harvested and preserved in 10% formalin for histological examination.

ii.Postmortem dichlorvos intoxicateddeathrabbits: The rabbits in cage B received lethal dose of 1.0mg/kg of sniper orally, they were observed closely for the onset of signs and symptoms of toxicity. The time of death was noted. One (1) hour after death, 3.5ml of vitreous humor sample was collected from both eyes, spun at the speed of 8000 rpm for 5 minutes. The supernatant was separated and kept frozen at the temperature of - 20^{o}c prior to biochemical investigations through the vitreous humor. The liver was harvested and preserved in 10 % formalin for histological examination.

iii.Nondichlorvosintoxicateddeathrabbits (**disguiseddeath**): This group were humanely sacrificed, thirty (30) minutes after death, 1.0 mg/kg dose of dichlorvos was intraperitoneally administered, 3.5ml of vitreous humor sample was collected from both eyes, one (1) hour after dichlorvos administration, spun at 8000 rpm for 5 minutes and the supernatant was separated and preserved at the temperature of - 20^{o}c for biochemical analysis. The liver was harvested for histological examination.

2.3.2 Laboratory Investigation of Parameters

2.3.2.1 Determination of Aspartate aminotransferase (AST)

Principle

Aspartate aminotransferase (AST) catalyzes the transfer of amino group from aspartate to ketoglutarate, forming oxaloacetate and glutamate. Oxaloacetate reacts with 2,4-dinitrophenylhydrazine (DNPH) to form 2,4-Dinitrophenylhydrazone which in an alkaline medium gives a red brown colour. The absorbance of the colour is directly proportional to the concentration of the enzyme.

2.3.2.2 Determination of Alanine Aminotransferase

Principle

The principle involved in the determination of alanine aminotransferases is such that alanine aminotransferase transfers an amino group from alanine to α-ketoglutarate producing glutamate and pyruvate. The reaction that produces pyruvic acid is established by coupling the alanine aminotransferase catalyzed reaction. Oxoacid generated is quantified by coupling oxo-derivatives formed with 2,4-dinitrophenihydrazine to form an oxo-acid hydrazine that is viewed as a reddish color in the presence of an alkaline medium.

2.3.2.3 Determination of Alkaline Phosphatase (ALP)

Principle

Alkaline phosphatase (ALP) catalyzes the hydrolysis of the colourless organic phosphate ester substrate, p-Nitrophenylphosphate, to the yellow-coloured product p-Nitrophenol and phosphate. The absorbance of the coloured product is directly proportional to the concentration of the enzyme.

2.3.2.4Determination of bilirubin using Diazo Colorimetric Method as described by (Jendrassik & Grof method.

Principle

Bilirubin reacts with the diazotized sulphanillic acid (diazo reagent) to form azobilirubin. Caffeine is an accelerator and gives a rapid and complete conversion to azobilirubin. The pink acid azobilirubin is converted to blue azobilirubin by an alkaline tartrate reagent and the absorbance of the blue-green solution is read using 600 nm wavelength. Conjugated bilirubin is measured in the absence of the caffeine-benzoate catalyst and at acid ph. Under these conditions only the conjugated bilirubin will react.

2.3.2.5 Determination of Total Protein using Biuret colorimetric method as described by Fehling Benedicts

Principle

Cupric concentration in alkaline medium interact with protein peptide bonds resulting in the formation of a blue colour complex measured at 560 nm wavelength.

2.3.2.6Determination of Albumin using Bromocresol Green Method as described by Gosslin, Can

Principle

Bromocresol green is an indicator which is yellow between ph 3.5-4.2. When it binds to albumin the colour of the indicator changes from yellow to blue green. The absorbance of the colour produced is measured in colorimeter using 600 nm wavelength.

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, 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 *.05* was considered statistically significant.

3. RESULTS AND DISCUSSION

Table 1: Analysis of Liver Function Parameters, C-Reactive Protein and ACH of Vitreous Humor (Mean ±SD)

Experimental Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control	6.30±0.83[a]	5.19±0.64[a]	20.76±0.69[a]
Sniper intoxicated death	171.15±6.71[b]	125.91±6.03[b]	166.43±5.13[b]
Non-Sniper inoxicated death (Disguised death)	6.78±0.97[a]	5.75±0.55[a]	21.66±0.48[a]
F –value	1378.74	927.70	1037.35
P –value	<0. 001	<0.001	<0.001

Keys: Sniper = 2-2 dichlorovinyl dimethyl phosphate, AST = aspartate aminotransferase, ALT = alanine aminotransferase, ALP = alkaline aminotransferase, Means ±SD of experimental groups with different superscripts are significantly different from each other at p<0.05.a,b – significant difference when compared to the next group.

Table 2: Analysis of Liver Enzymes, of Vitreous Humor

Experimental Groups	Total Bilirubin (µmol/L)	Conjugated Bilirubin. (µmol/L)	Total Protein (g/dl)	Albumin (g/dL)
Control	8.03±0.33[a]	6.25±0.59[a]	6.11±0.18[a]	0.65±0.04[a]
Sniper intoxicated death	66.14±4.04[b]	30.45±3.41[b]	3.56±0.45[b]	0.13±0.05[b]
Non-Sniper intoxicated death (Disguised death)	8.78±0.28[a]	6.89±0.56[a]	5.75±0.19[a]	0.59±0.03[a]
F –value	485.04	108.54	55.66	53.33
P –value	<0.001	<0.001	<0.001	<0.001

Keys: Sniper = 2-2 dichlorovinyl dimethyl phosphate, CRP = C-reactive protein and ACH = acetyl cholinesterase. Means ±SD of experimental groups with different superscripts are significantly different from each other at p<0.05.a,b – significant difference when compared to the next group.

This study also found that vitreous total bilirubin concentration was significant across the groups. Further comparison using Tukey multiple tests showed significant increase in total bilirubin concentration of the actual death group compared with the control, whereas the difference in total bilirubin concentration of the control and disguised death group was not significant. The observed increased vitreous concentration of total bilirubin resulted from the damage caused by dichlorvos in the hepatocytes with consequent impaired hepatic uptake and conjugation of bilirubin.

Significant decrease was observed in the mean concentration of vitreous humor total protein level across the groups. Further comparison using Tukey multiple test showed significant decrease in vitreous total protein concentration of the actual death group compared with the control, whereas the vitreous total protein concentration of the control and disguised death group was not significant. Therefore, liver impairment that was observed in this study would affect serum total protein concentration with a resultant decrease in its concentration. This result is in line with the work of Agoro et al. [9] that observed significant decrease in concentration of serum and vitreous total protein in rabbit exposed to carbon monoxide.

Liver enzymes are released when there is liver impairment. This study observed significant increase in vitreous humor concentration of the liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) of the dichlorvos intoxicated death rabbits when compared with the control and the disguised death group. Further comparison using tukey simultaneous test showed significant increase in vitreous AST, ALT and ALP concentrations of the actual dichlorvos intoxicated death rabbits when compared with the control, whereas the vitreous AST, ALT and ALP concentrations of the control and disguised death rabbits showed no significant difference. ALT is a cytoplasmic enzyme, and its elevation is an indication that dichlorvos exposure can cause injury to the liver cells. Vermeulen et al. [10] also reported that subtle membrane changes are sufficient to allow passage of intracellular enzyme to extracellular space. Therefore, dichlorvos exposure caused increase in serum AST and ALT concentrations in this study which could be attributed to the damaged structural integrity of the liver, because these enzymes are normally located in the cytoplasm of hepatocytes and are released into circulation after cellular damage. ROS have been implicated in dichlorvos toxicity leading to liver damage, which is an organ of metabolism and detoxification and therefore a target organ for toxic agents' accumulation, resulting in oxidative stress and tissue damage. Adedeji et al. [2] observed increased activities of AST and ALT in dichlorvos exposed rats.

4. CONCLUSION

From the results, it can be concluded that Dichlorvos caused hepatoxicity in the group of intoxicated rabbits. Further studies to assay mechanism of toxicity molecularly would further elucidate the findings.

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.

REFERENCES

1. Wankasi, M. M., Agaro, E. S. & Ikimi, C. G. Vitreous Humor, Biochemical Parameters as Indicators corroborating Acute Sniper (Dichlorvos) Induced Death. *JournalofForensicTechnologyandPharmacology*, 2020; 9 (2): 168-71.
2. Adedeji, A. L., Ogunniyi, A., Wusu, D. & Afolabi, O.K. Dichlorvos Exposure Aggravates Complications Associated with Diabetes in Wistar Rats. *InternationalJournalofResearchStudiesinBiosciences* (*IJRSB*), 2016; 4(7): 22 - 31.
3. Beydilli, H., Yilmas, N., Cetin, E. S., Topal, Y., Celik, O. I. & Sabin, C. Evaluation of the Protective Effect of Silibinin against Diazinon Induced Hepatotoxicity & Free Radical Damage in Rats' Liver. *Iran Red Crescent Medical Journal,* (2015; 17(4): 134 - 9.
4. Ender, Y. & Onder, G. Effects of Dichlorvos on Lipid Peroxidation in Mice on Subacute and Subchronic Periods. *Pesticide Biochemistry and Physiology*, 2006; 86(2): 106 -9.
5. Wang, Q., Zhang, Y., Zhou, C., Zhang, J., Dou. Y. & Li, Q. Risk Assessment of Mouse Gastric Tissue Cancer induced by Dichlorvos & Dimethoate. *Oncology letters*, 2013; 5: 1385-9.
6. Gbadegesin, M. A. Hepatotoxicity and Clastogenicity of Dichlorvos at High Doses in Male Wister Rat. *AfricanJournalofMedicineandMedicalsciences*; 2018; 47(2): 47-52.
7. Nwachuku, E. O., Beega, F., Ben-Chioma, A. & Brisibe, N. Evaluation of Protective Properties of Elaeis Oleifera Fruit Extract on Renal Parameters of Dichlorvos – Induced Nephrotoxicity in Albino Rats. *Journal of Advances in Medical and Pharmaceutical Sciences*, 2020; 22(6): 1 - 13.

8. Somia, E. M. & Madiha, F. Pathological Effects of Dichlorvos & Fenitrothion in Mice. *Journal of Research Practice,* 2012; 208: 286 - 91.
9. Agoro, E. S., Akubugwo, E. I., Chinyere, G. C. & Samul, R. Comparison of Vitreous Protein Profiles of Rabbits subjected to Acute Carbon Monoxide Poisoning & Normal Animal after death. *Journal of forensic science Resource,* 2018;22:40 - 5.
10. Vermeulen, N., Bessems R. & Vande, S. Molecular Aspects of Paracetamol-Induced Hepatotoxicity and its Mechanism of Prevention. *DrugMetabolismRevolution*, 1992; 24: 367-407.