Original Research Article

Evaluation of 2, 2-dichlorovinyl dimethyl phosphate (Sniper) induced hepatotoxicity and oxidative stress in New Zealand white rabbits

.

ABSTRACT

Aim: The aim of this study was to evaluate chronic hepatotoxicity and oxidative stress induced by 2, 2-dichlorovinyl dimethyl phosphate (Sniper) in New Zealand white rabbits.

Study design: Experimental design.

Place and Duration of Study: Department of Biological Science, Rivers State University, Port Harcourt animal house, Rivers State Teaching Hospital and Nigerian National Petroleum Corporation Hospital Laboratory, between January, 2020 and April 2020.

Methodology: Thirty six (36) male New Zealand white rabbits weighing approximately 1.0mg were used for the study. 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 divided into three (3) groups of four (4) rabbits each with four (4) matched control. For the chronic oral study, 10% of the LD50 (previously determined) 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 feed 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. At day 30, 60 and 90, 4 rabbits were sacrificed each from the chronic oral and inhalation study groups and the matched control group. Blood specimens were collected at each stage, about 5.0mls of blood was collected into lithium heparin specimen container for the investigation of liver function tests. Serum alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), alkaline phosphatase (ALP), total and conjugated bilirubin, (TB and CB) total protein (TP), albumin (ALB), lactate dehydrogenase (LDH), malondealdehide (MDA) and total antioxidant capacity (TAC) were estimated using the spectrophotometric method. SPSS version 22.0 was used for statistical analysis and p values less than *.05* were considered statistically significant.

Results: The results showed that there were significant increases in the activity of ALT, AST, ALP, MDA and LDH (at *p<.05*), when values for the controls were compared with those of rats administered with sniper. Significant decreases (at *p<.05*) were also observed in TP, ALB and TAC levels after sniper administration.

Conclusion: Chronic inhalation and oral administration of sniper led to hepatotoxicity and oxidative stress, toxic effects were more in rabbits administered through the oral route and increased with period of administration.

Keywords: 2, 2-dichlorovinyl dimethyl phosphate (Sniper), hepatotoxicity, New Zealand white rabbits

1. INTRODUCTION

Dichlorvos (2, 2-dichlorovinyl dimethyl phosphate) 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].

Dichlovos 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 lmg/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 microgram 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, Srilanka 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 incordination, 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 this study was to evaluate chronic hepatotoxicity and oxidative stress induced by 2, 2-dichlorovinyl dimethyl phosphate (Sniper) in New Zealand white rabbits.

2. MATERIALS AND METHODS

2.1 Experimental Animals

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. 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

Chart 1: 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:

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

2.4 Sample Collection, Storage and Analysis

2.4.1 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 liver function tests. The liver was harvested and preserved in 10% formalin for histological examination.

2.4.2 Laboratory investigation of parameters

Liver function tests were carried out at Nigerian National Petroleum Corporation (NNPC) Clinic, Akpajo, Port Harcourt, while the histological study was carried out at Rivers State University Teaching Hospital, Port Harcourt, Rivers State, Nigeria.

2.4.2.1 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.

2.4.2.2 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.

2.4.2.3 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.

2.4.2.4 Determination of Lactate Dehydrogenase (LDH)
Lactate dehydrogenises enzyme was determined using photometric method.

Principle
Lactate dehydrogenase (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.

2.4.2.5 Quantitative Determination of Total Antioxidant Capacity (TAC)

Method: Colorimetric. Catalog Number: E-BC-K136

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 (TAC) can be calculated by measuring the absorbance at 520 nm.

2.4.2.6 Determination of Malondialdehyde (MDA)
The estimation of malondialdehyde was carried out by photometric method.

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

2.4.2.7 Laboratory estimation of albumin

Principle
Sulphonphthalein dyes as bromocresol purple or bromocresol green yield with albumin in the presence of detergents in a blue-green complex suitable for the photometric determination.

$$\text{Albumin concentration (g/l)} = \frac{\text{A sample}}{\text{A standard}} \times \text{concentration of standard.}$$

2.4.2.8 Laboratory Analysis of Total Proteins

Principle
Proteins and peptides, similarly to biuret, react with cupric ions in alkaline solutions to form a violet complex suitable for the photometric determination.

$$\text{Total protein concentration (g/l)} = \frac{\text{A sample}}{\text{A standard}} \times \text{concentration of standard}$$

2.4.2.9 Histological Analysis

The liver was harvested for histological analysis, and were fixed in 10% formal saline solution. The organs were dissected and representative blocks were taken for histological processing each with identifying label in a tissue cassette. The fixed tissue blocks were dehydrated through ascending grades of alcohol, de-alcoholised in xylene, infiltrated and embedded in molten paraffin wax. Sections were cut at 3µm on a rotary microtome. Deparaffinised sections were then stained with the standard haematoxylin and eosin staining technique and the slides mounted in DPX. Sections on slide were examined and photomicrographs captured with X400 objective lens using the ScopeTek™ device and software v1.3.

2.5 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

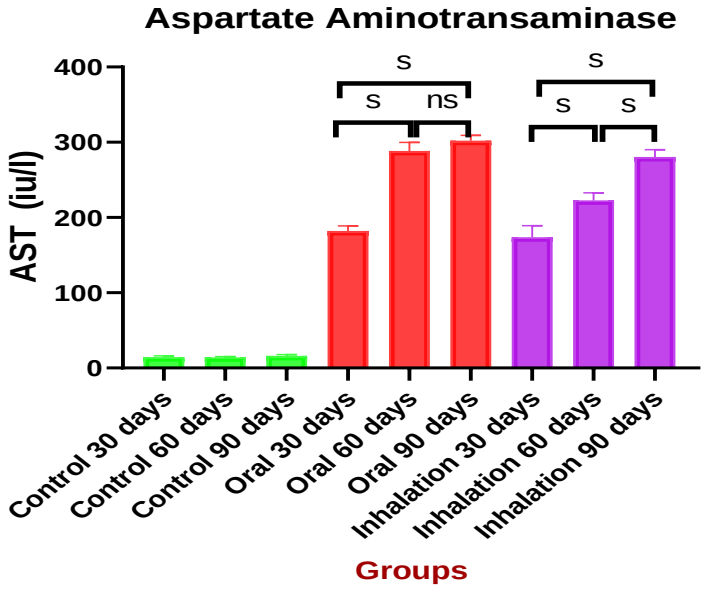

Fig. 1: Serum AST activity comparison of the effect of routes of administration of Sniper on liver function parameters *(s-significant, ns-non-significant at p<.05)*

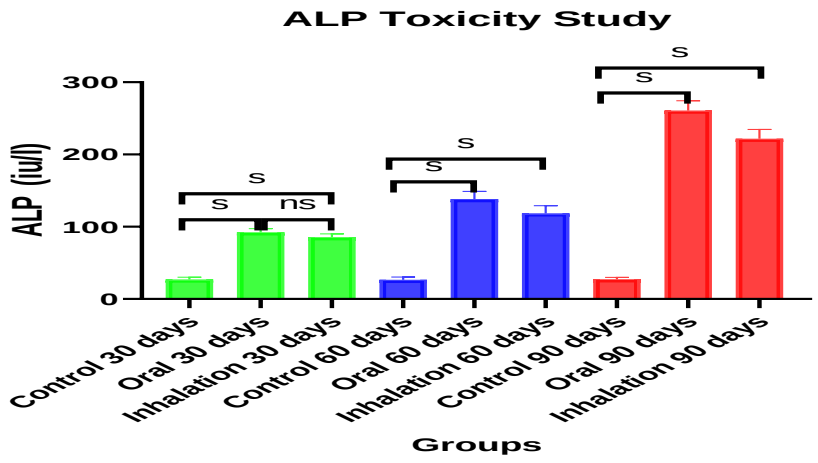

Fig. 2: Serum ALP activity comparison of the effect of routes of administration of Sniper on liver function parameters. *(S-significant, ns-non-significant at p<.05)*

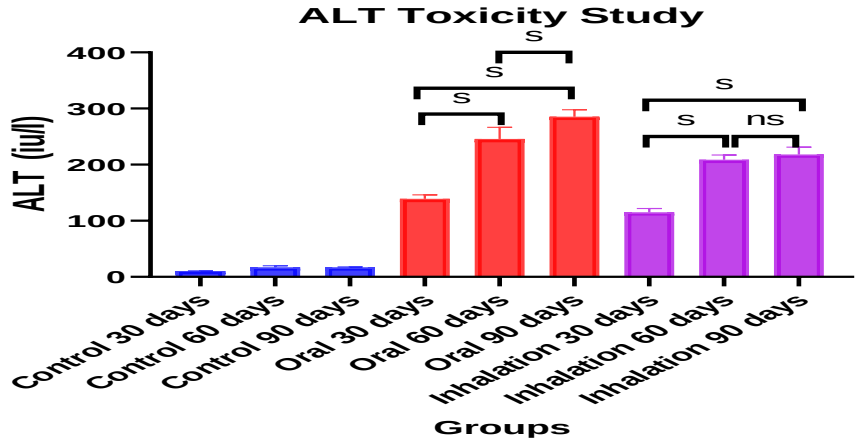

Fig. 3: Serum ALT activity comparison of the effect of routes of administration of Sniper on liver function parameters. *(S-significant, ns-non-significant at p<.05)*

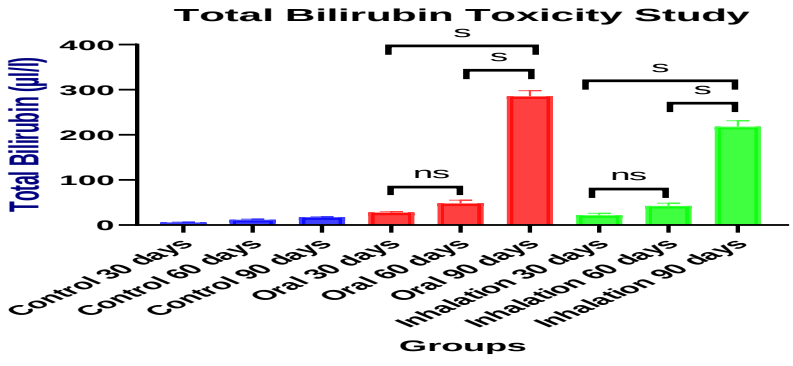

Fig. 4: Serum ALT activity comparison of the effect of routes of administration of Sniper on liver function parameters. *(S-significant, ns-non-significant at p<.05)*

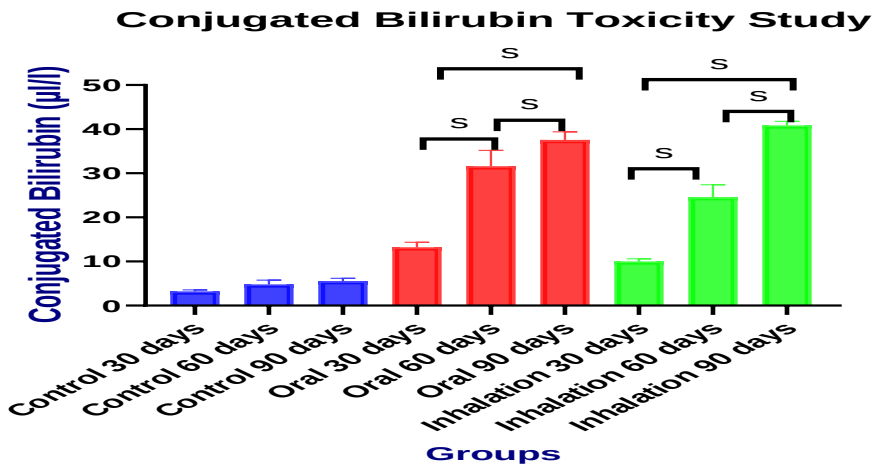

Fig. 5: Serum conjugated bilirubin comparison of the effect of routes of administration of Sniper on liver function parameters. *(S-significant, ns-non-significant at p<.05)*

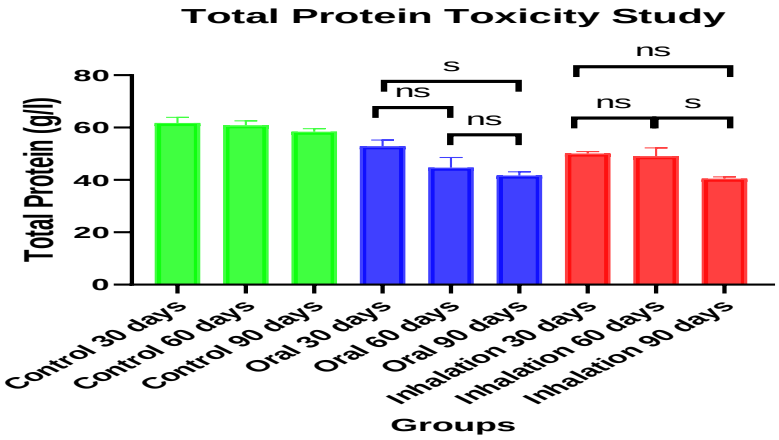

Fig. 6: Serum total protein comparison of the effect of routes of administration of Sniper on liver function parameters. *(S-significant, ns-non-significant at p<.05)*

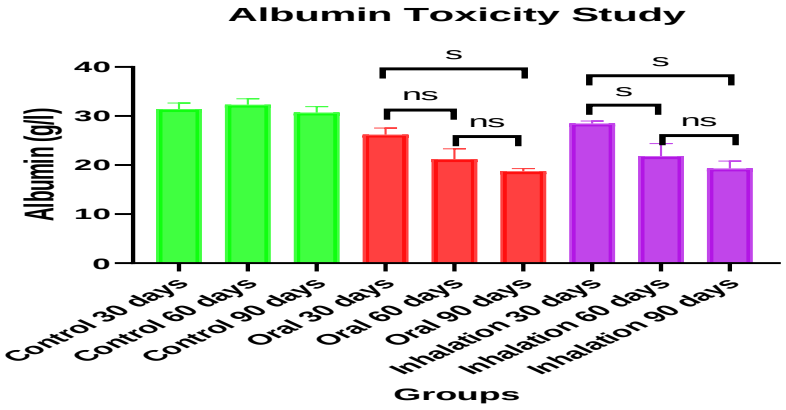

Fig.7: Serum albumin comparison of the effect of routes of administration of Sniper on liver function parameters. ***(S-significant, ns-non-significant at p<.05)***

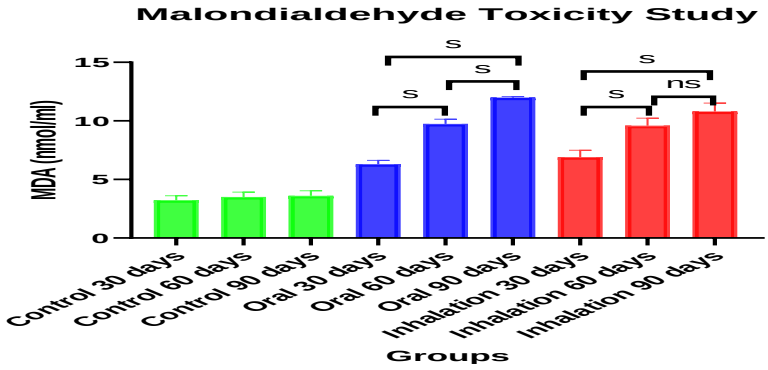

Fig.8: Serum MDA activity comparison of the effect of routes of administration of Sniper on liver function parameters. ***(S-significant, ns-non-significant at p<.05)***

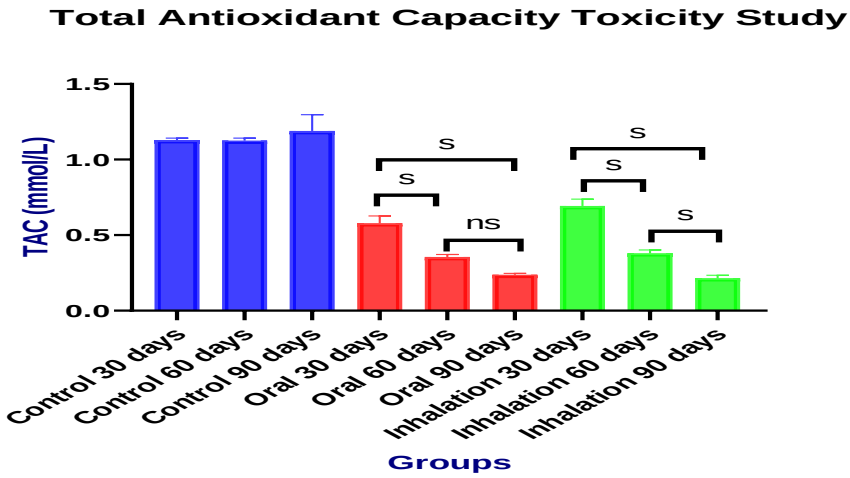

Fig.9: Serum TAC levels comparison of the effect of routes of administration of Sniper on liver function parameters. ***(S-significant, ns-non-significant at p<.05)***

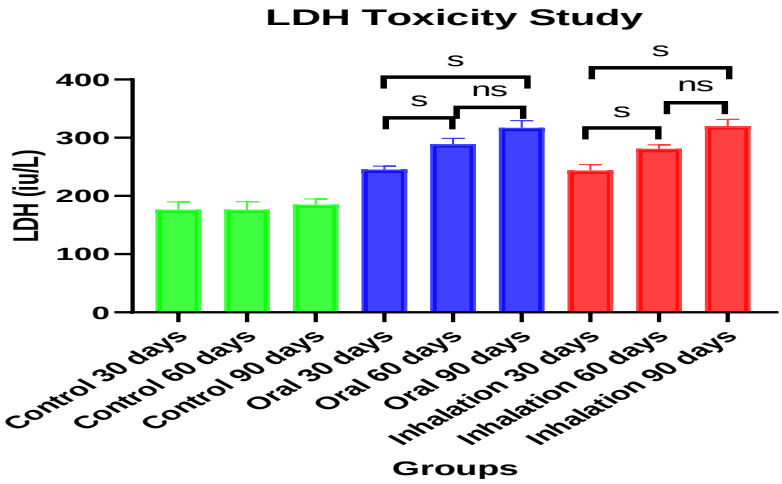

Fig.10: Serum LDH levels comparison of the effect of routes of administration of Sniper on liver function parameters. *(S-significant, ns-non-significant at p<.05)*

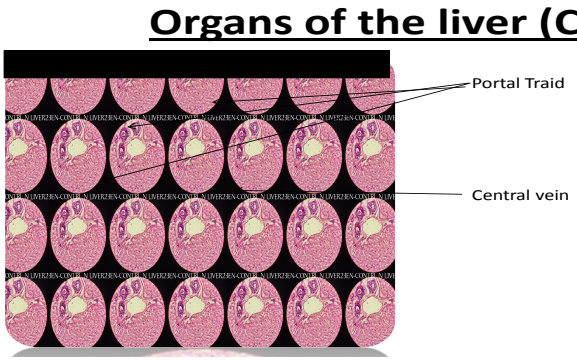

Plate 1: Micrograph of a normal liver (from rabbit in control group- oral)

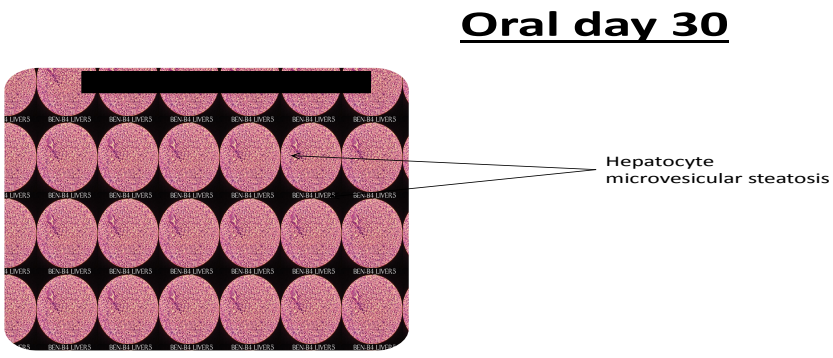

Plate 2: Micrograph of a rabbit's liver given oral for 30 days

Oral day 60

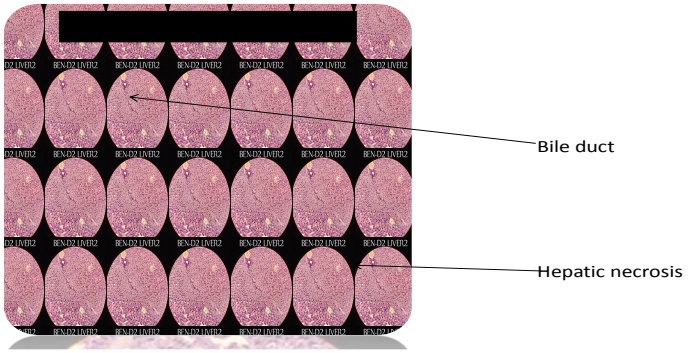

Plate 3: Micrograph of a rabbit's liver given oral for 60 days

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Oral day 90

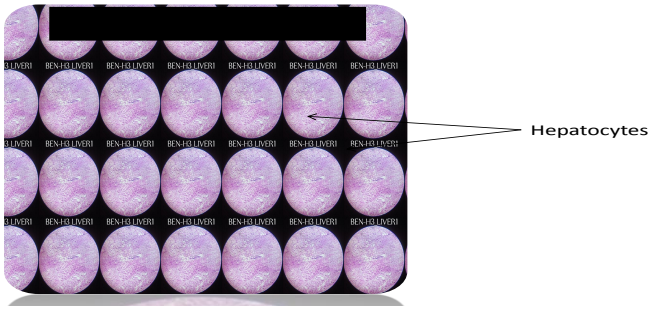

Plate 4: Micrograph of a rabbit's liver given oral for 90 days

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Inhalation administration (CONTROL)

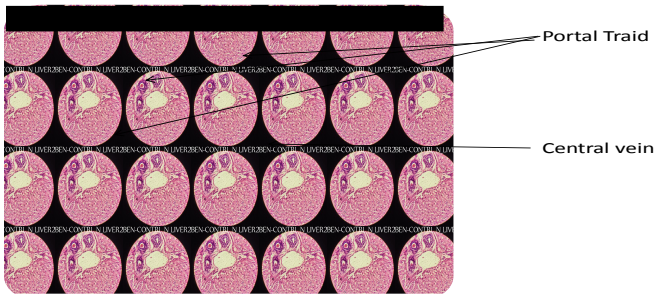

Plate 5: Micrograph of a normal rabbit's liver (control group-inhalation)

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Inhalation day 30

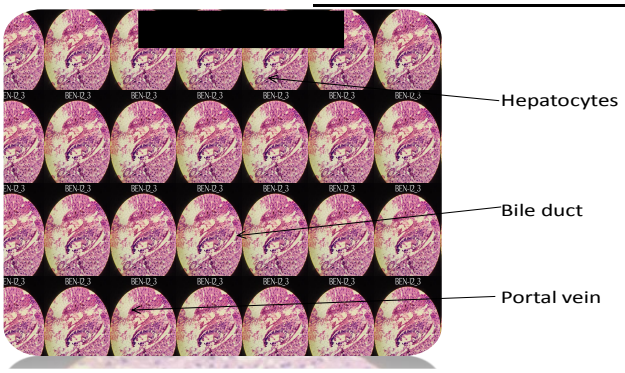

Plate 6: Micrograph of a rabbit's liver given inhalation for 30 days

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Inhalation day 60

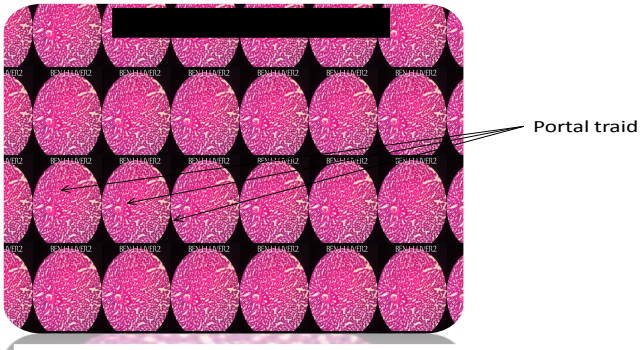

Plate 7: Micrograph of a rabbit's liver given inhalation for 60 days

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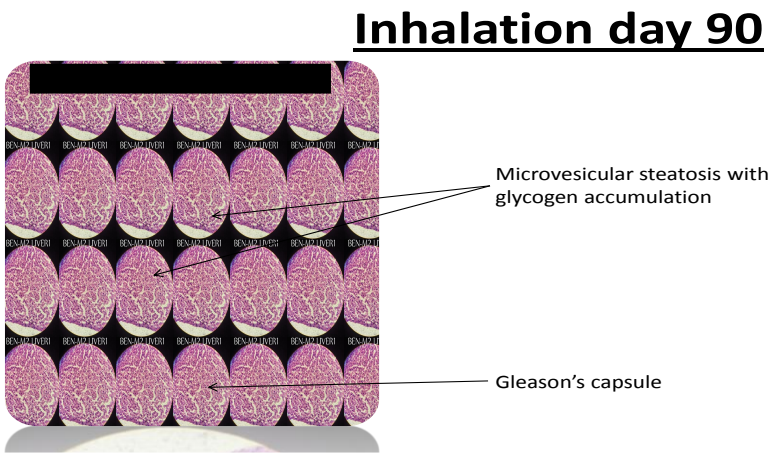

Plate 8: Micrograph of a rabbit's liver given inhalation for 90 days

This study investigated on the chronic hepatotoxic effects and oxidative stress of inhalation and oral administration of sniper in New Zealand white rats.

AST, ALT, ALP, total bilirubin, conjugated bilirubin, LDH, TAC, MDA, total protein and albumin.

The liver enzymes are markers of hepatocyte organelles or biliary ductile that leaks out into the circulation in response to injury. The injury could be caused by reactive metabolites, resulting from xenobiotic metabolism in the liver. The serum level of these enzymes therefore reflects physiological state of the liver.

Bilirubin, ALT, AST and ALP are present in the blood usually in low levels. When liver cells are diseased or its integrity disturbed by pathologic conditions, the activity of the enzymes increases in the blood. The level of these enzymes released into the blood depends on the severity of the cellular damage; hence the need to monitor these enzymes to ascertain the state of the liver of the dichlorvos treated rabbits.

As shown in Figs. 1 to10. In the 90 days oral and inhalation dichlorvos treatment of the rabbits, corresponding increase in the activity of the liver parameters AST (Fig. 1), ALP (Fig. 2), ALT (Fig. 3) and bilirubin (Figs. 4 & 5) was observed as the duration of dichlorvos treatment on the rabbits increased. The mean value of the liver enzymes was highest in rabbits that were treated orally for 90 days, followed by the inhalation exposure mean value of day 90. Generally, comparison of the effect on routes of administration of the dichlorvos on liver function parameters showed that in the orally treated rabbits, the liver enzymes activities were slightly higher when compared with the mean values of the inhalation route exposure.

As shown in (Figs. 1 to 10), the day 90 oral and inhalation dichlorvos study produced significant or remarkable elevation in all the liver function parameters at $P<.05$, as the duration of dichlorvos exposure on the rabbits increased from 30-90 days when compared with the control.

Dichlorvos primarily affects the nervous system of the exposed organisms by inhibiting acetyl cholinesterase (AChE) and increasing acetylcholine levels in the cholinergic synapse. Again, dichlorvos induces oxidative stress, causes disturbance of metabolic pathways, and results in multiple organ dysfunctions such as hypoxia and inadequate tissue perfusion of the liver and heart [11].

In the liver, dichlorvos causes ultrastructural, biochemical, metabolic and mitochondrial damage which results to changes in the hepatic biomarkers such as serum ALT, AST, ALP and bilirubin levels as observed in this present study agrees with the findings of [12] who

observed increase in the liver function parameters of rats that were exposed to repeated doses of organophosphorus pesticides.

AST and ALT are usually released from the liver when parenchymal cells of the liver become necrotic due to viral infection or cell destruction due to exposure to toxic substsnces. ALT enzyme is more specific for the liver than the AST due to their biological location. AST may be elevated in other pathological conditions. AST may be elevated also in extra hepatic disease.

The activities of the liver enzymes were markedly raised *(P<.05)* from day 30-90. The enzymes were greater than three folds increase when compared with the control. The marked increases in the plasma level of the enzymes was an indication of hepatotoxicity, which may also indicate degenerative changes and mal-functioning of the liver. The liver cell membrane and cytolic dysfunction by dichlorvos could also be the reason for enzyme leakages into the plasma as revealed by the elevated activities. This present study has shown that dichlorvos has a harmful effect on the hepatic tissue.

Furthermore, the marked increase in the liver enzyme activities in the oral and inhalation dichlorvos treated rabbits may be attributed to oxidative stress effect of dichlorvos on the liver cells. The elevation of AST and ALT along with ALP may reflect some inflammatory conditions or injury to the liver (hepatocellular disease). In the present study, more than 3-folds of increase in the liver enzyme levels of ALT, AST and ALP in the oral and inhalation study at day 90; indicating hepatocellular damage. The result of this study agrees with the finding of [13] who observed marked increase in AST, ALT and ALP in a 49 year old woman who was chronically exposed to dichlorvos, who was diagnosed of dichlorvos induced immune hepatitis on initial hospital admission.

As shown in (Figs. 4 & 5), there was a significant increase in the mean values of the conjugated and total biliubin levels in the oral and inhalation study as the duration of dichlorvos exposure prolonged. More than 3-fold increases in the conjugated and total bilirubin was observed at day 90 dichlorvos exposure. The hyperbilirubinaemia could suggest an over production of bilirubin due to excessive breakdown of red cells. Excessive red cells breakdown could be due to antigen antibody reaction caused by several toxins and metabolites that were released from the dichlorvos. The elevated levels of conjugated and total bilirubin, alongside with the liver enzymes also suggest hepatocellular damage. This result corroborates with the findings of Owoeye, [12] who also indicated increased levels of conjugated and total bilirubin in rats that were exposed to dichlorvos.

Serum total protein and albumin were also used in this study to assess the effect of dichlorvos on the liver function. The oral and inhalation chronic dichlorvos exposure on the rabbits caused an exposure duration dependent decrease in total protein and albumin levels at *(P<.05)* from the period of 30-90 days (Figs. 6 & 7). Significant reduction in the levels of serum protein and albumin were observed from day 30-90. The both routes of exposure produced similar effects. Albumin concentration reflects the balance between synthesis and degradation and may be influenced by factors other than the functional state of the liver. It can be suggested that the depression of serum total protein and albumin levels which were observed in this study may be due to the suppressive action of Metabolites of dichlorvos on the synthetic capacity of the liver and or the increase of the renal tubular excretion of protein which is lost in the urine. The synthesis of albumin occurs exclusively in the liver and the synthetic rate is influenced by thyroid hormones, glucocorticoids, plasma colloidal osmotic pressure, hepatic function and toxins.

The parameters used in the assessment of the effects of DDVP on the oxidative stress markers were total antioxidant capacity and lipid peroxides index. They were used in the male rabbits at the various months of exposure. Total antioxidant capacity and lipid peroxides index can be used to assess cellular injury which could result in the generation of reactive oxygen species. Oxidative stress usually occur due to the production of damaging reactive oxygen species (ROS) which is beyond the capacity of the body's natural antioxidant defenses, resulting in cellular destruction [12]. Furthermore, oxidative stress is a condition which is characterized by elevated levels of intracellular reactive oxygen species

(ROS) which are the progenitors of free radicals. Free radicals are highly reactive, and are capable of damaging almost all types of biomolecules (proteins, lipids, carbohydrates and nucleic acids) [14]. Oxidative stress effect on protein results in site-specific amino acid modifications, fragmentation of the peptide chain, aggregation of the cross-linked reaction products, altered electrical change and increased susceptibility to proteolysis. Oxidative attack on iron-sulphur centers in biomolecules by superoxides will lead to the destruction of enzymatic functions [15].

Total antioxidant capacity is the estimate of antioxidant capacity which includes the antioxidants that are not yet recognized or not easily measured. It represents the overall free radical scavenging ability of various antioxidants. It is an important measurement for investigating oxidative stress, which has been implicated in the pathological mechanisms of many diseases. It measures both the antioxidant capacity of a single compound, and the antioxidant capacity of all antioxidants in a biological sample. The administration of DDVP on the male rabbits caused a significant reduction in the level of total antioxidant capacity (TAC) when the results obtained from the experimental animals were compared with the matched control values at *(P<.05)* (Figs 8 & 9). Sniper causes damage to the tissues by inducing oxidative stress, which leads to an increase in the reactive oxygen species in the body, thereby, depleting the antioxidant enzymes. It has the ability of increasing the production of reactive oxygen species (ROS) due to its abilities to increase lipid peroxidation, thereby inhibiting the activity of antioxidant production such as gluthathione peroxidase, (GPX), superoxide dismutase and catalase.

4. CONCLUSION

Chronic inhalation and oral administration of sniper led to hepatotoxicity and oxidative stress, toxic effects were more in rabbits administered through the oral route and increased with period of administration.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

Authors ESB, EON and FUI designed the study, author ESB performed the statistical analysis, Author CCO wrote the protocol, and wrote the first draft of the manuscript and managed the literature searches. All authors read and approved the final manuscript.

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.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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