Original Research Article

Assessment of chronic toxicological effects of 2, 2-dichlorovinyl dimethyl phosphate (Sniper) on the kidneys of New Zealand white rabbits

ABSTRACT

Aim: the aim of this study was to assess the chronic toxicological effects of 2, 2-dichlorovinyl dimethyl phosphate (Sniper) on the kidneys of New 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, 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/kg 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 (details not included) 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 kidney function tests. Serum electrolytes (Na^+, K^+, **Cl^- and HCO_3^-)**, were determined using a chemistry auto-analyzer while urea and creatinine were estimated using the photometric methods and C-reactive protein and kidney injury marker (KIM-1) were analyzed using the Enzyme linked immune-sorbent assay method. The kidneys were also harvested and preserved in 10% formalin for histological examination. SPSS version 22.0 of windows statistical package was used to analyze the data generated and p values less than *.05* were considered significant.

Results: The results showed that the chronic oral and inhalation studies revealed significant elevation of the following biochemical indices at (*P<.05*); Na^+, K^+, Cl^-, KIM-I, urea and Creatinine when values for the rabbits that received sniper were compared with those of the control groups. Creatinine, urea and KIM-1 increased significantly as the duration of administration increased and more in the oral route when compared with the inhalation route.

Conclusion: From the results, this study has revealed that oral and Inhalation routes of sniper exposure can produce renal toxicity. Results showed that renal toxicity occurred more in the former route and as the period of administration increased.

Keywords: Chronic toxicological effects, 2, 2-dichlorovinyl dimethyl phosphate (Sniper), kidneys, Rabbits.

1. INTRODUCTION

2 2- dichlorovinyl dimethyl phosphate is an organophosphate insecticide and pesticide which is traded under names such as sniper, dichlorvos, Nuvan, vapona, DDVP and Nogos [1]. In Nigerian society, 2, 2 dichlorovinyl dimethylphosphate (sniper) is marketed by Swiss Chemical Nigerian limited [2]. Dichlorvos is a colourless to amber liquid with a boiling point of

140^0C at 2.7 Kpa. The molecular formular for dichlorvos is $C_4H_7CL_2O_3P$, its molecular weight is 220.98, vapour pressure 1.2 x 10^{-2} mmHg at 20^0C, with the density of 1.415 g/ml at 25^0C [3]. Dichlorvos is classified by the World Health Organization as a class "B" "Highly hazardous" chemical [4]. Dichlorvos has several uses. It is a household insecticide and agricultural pesticide and is the most commonly used organophosphate pesticide in the developing countries [5]. It is a contact and oral insecticide with fumigant and penetrant actions. Sniper is used for the protection of stored products and crops (mainly greenhouse crops) and for the control of internal and external parasites in livestock, for the control of insects in buildings, aircraft and outdoor areas [2]. Due to the presence of degrading enzyme in tissues and blood, dichlorvos is known for its rapid metabolism and excretion by mammals. It does not accumulate in body tissues and even at doses that could cause symptoms of poisoning, dichlorvos cannot be detected in the breast milk of mammals [6]. The major organ of dichlorvos detoxification is the liver; although, blood, lung, spleen & kidney can metabolize dichlorvos to dimethyl phosphate. Desmethyl dichlorvos, inorganic phosphate and monomethyl phosphate are other metabolites of dichlorvos [7].

Several studies have shown that the breakdown of dichlorvos is similar in all species of mammals, though there could be slight difference in quantification and rate of the metabolic pathway. One of the major routes of exposure to dichlorvos is by inhalation. Inhalation exposure occurs among individuals that reside close to harzadous waste sites that contain dichlorvos; it can also occur when it is used as a domestic insecticide and pesticide [8]. Oral exposure occurs when dichlorvos is indirectly ingested through food that is contaminated with dichlorvos [9]. Skin contact with soil that is contaminated with dichlorvos or by direct body splash is another possible means of exposure to dichlorvos [9]

Mechanism of dichlorvos toxicity involves an irreversible inhibition of neural acetyl-cholinesterase enzyme. The inhibition leads to the accumulation of acetylcholine in synapses with disruption of nerve functions [10]. Effects of the altered cholinergic neurotransmission in the parasympathetic autonomic nervous system are nausea, perspiration, lacrimation, vomiting, diarrhea, excessive bronchial secretion, coma and death [10]. On the motor nerve fibre in skeletal muscles, effects include, muscle fasciculation, muscle cramps, muscle weakness and flaccidity. Again, in the central nervous system the cholinergic effects result in fatigue, drowsiness, mental confusion, headache, convulsion, coma and death [11]. Exposure to dichlorvos could cause acute or chronic toxicity. Inhalation is usually the most common route of dichlorvos toxicity because of its volatility, [12]. 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 [12].

Several cases of suicidal and homicidal death have been reported to be associated with sniper abuse and misuse. Adequate information is not available on the actual toxicological findings in the deceased in order to differentiate between sniper intoxicated deaths and disguised or homicidal death. Measurement of biochemical markers in sniper intoxication is important in understanding the mechanism of its toxicity. A study of chronic toxicological effects of dichlorvos on the kidneys of Rabbits through oral and inhalation routes of exposure could help public health assessment of potential risks associated with dichlorvos exposure. Therefore, the aim of this study was to assess the chronic toxicological effects of 2, 2-dichlorovinyl dimethyl phosphate (Sniper) on the kidneys of 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

The rabbits were divided into three (3) groups of four (4) rabbits each with four (4) matched control.

Table 1. 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 comprehensive biochemical investigations. The kidneys were harvested and preserved in 10% formalin for histological examination.

2.4.2 Laboratory Investigation of Parameters

All the biochemical parameters investigations 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 Serum Electrolytes (Ion-Selective Electrode)

The instrument used for the analysis of (Na^+, K^+, Cl^- and HCO_3^-), was chemistry auto-analyzer

Principle

The ion selective electrode membrane for sodium and potassium respectively undergoes a specific reaction with the ion contained in the sample to be analyzed. The membrane reacts to the electric charge in the ion causing a change in the membrane potential which is built up in the film between the sample and membrane. A difference in the ion concentration between the sodium or potassium solution inside the electrode and the sample causes an electrochemical potential to form across the membrane of the active electrode. The potential is conducted by the electrode to an amplifier. This is compared with the potential of a reference electrode.

2.4.2.2 Determination of Serum Urea

Principle
The test is based on the principle that thiosemicarbazine reacts with urea in the sample to give a pink colour. The intensity of the colour formed is directly proportional to the concentration of urea in the sample. The absorbance was read at 546nm wavelength.

2.4.2.3 Determination of Serum Creatinine (Jaffe's Colorimetric Method)

Principle
The test is based on the principle that creatinine protein filtrate reacts with alkaline picrate to form a golden yellow colour, which is read at 520nm wavelength. The colour intensity is proportional to the concentration of creatinine in the sample.

2.4.2.4 Determination of Kidney Injury Molecule (ELISA Method)
This assay employs the quantitative enzyme immunoassay technique (double-antibody sandwich) to assay kidney injury molecule.

Principle
The microtitre plate provided has been pre-coated with antibody. Add standard, sample and conjugated antibody to wells. After incubation and washing to remove the uncombined enzyme, add chromogen solution and B. The colour of the liquid will change into blue. At the effect of acid, the colour finally becomes yellow. The colour change is measured spectrophotometrically at a wavelength of 450nm. The concentration of kidney injury molecule in the samples is then determined by comparing the optical density (O.D.) of the samples to the standard curve.

2.4.2.5 Determination of C - reactive protein (CRP) (ELISA Method)
Enzyme immunoassay for quantitative determination C - reactive protein.

Principle
The ELISA (enzyme-linked immunosorbent assay) is based on the principle of a solid phase enzyme-linked immunosobent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determination on the CRP molecule. This mouse monoclonal anti-CRP antibody is used for solid phase immobilization (on the microtitre wells). A goat anti-CRP antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies resulting in the CRP molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 45 minutes incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A tetramethybenzidine (TMB) reagent is added and incubated for 20 minutes, resulting in the development of blue colour. The colour development is stopped with the addition of 1N HCL changing the colour to yellow. The concentration of CRP is directly proportional to the colour intensity of the test sample. Absorbance is measured spectrophotometrically at 450nm.

2.4.2.6 Histological Analysis
The kidneys were 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

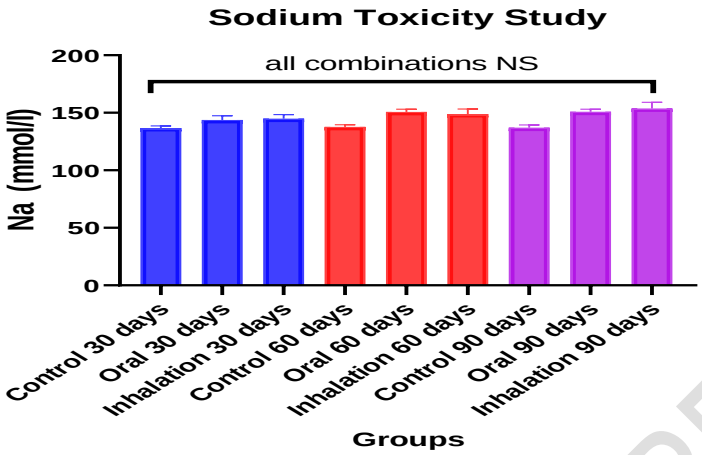

Fig. 1: Serum sodium levels comparison of the Effect of Routes of Administration of Sniper on Renal Function Parameters

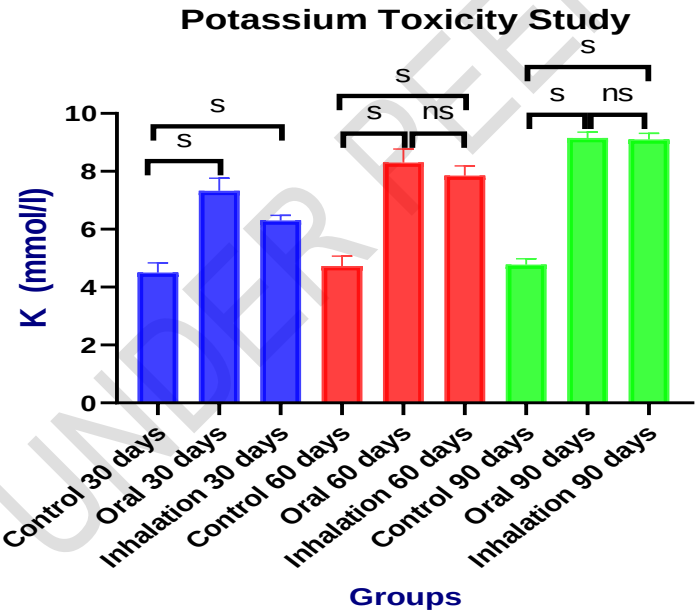

Fig. 2: Serum potassium levels comparison of the Effect of Routes of Administration of Sniper on Renal Function Parameters

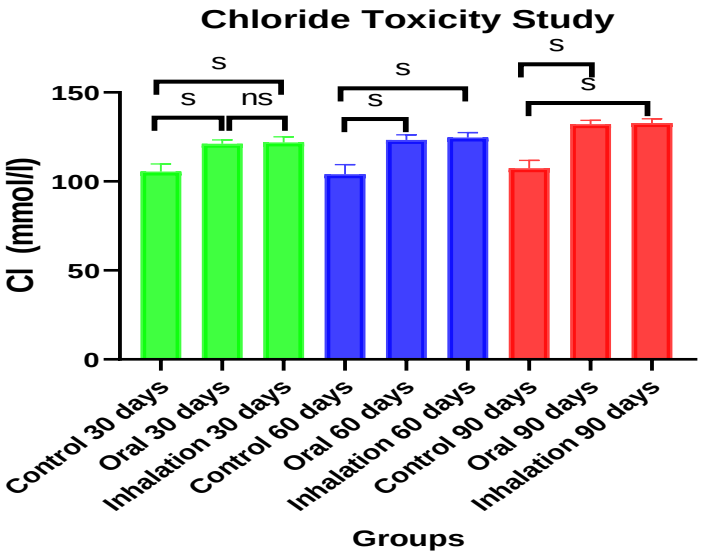

Fig. 3: Serum chloride levels comparison of the Effect of Routes of Administration of Sniper on Renal Function Parameters

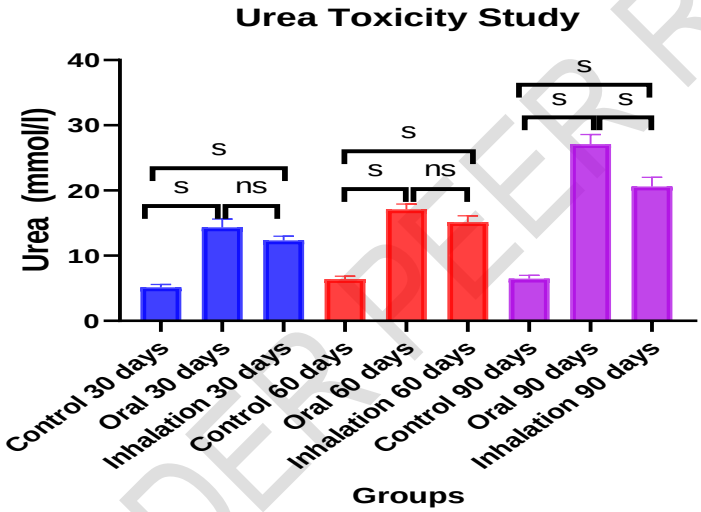

Fig. 4: Serum urea levels comparison of the Effect of Routes of Administration of Sniper on Renal Function Parameters

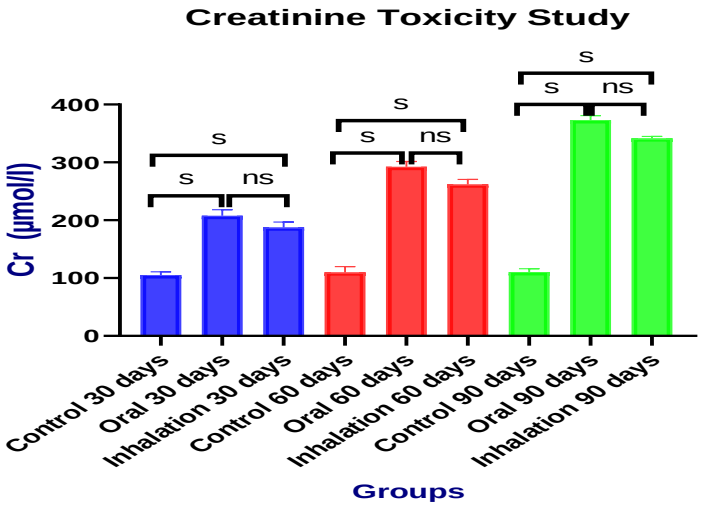

Fig. 5: Serum creatinine levels comparison of the Effect of Routes of Administration of Sniper on Renal Function Parameters

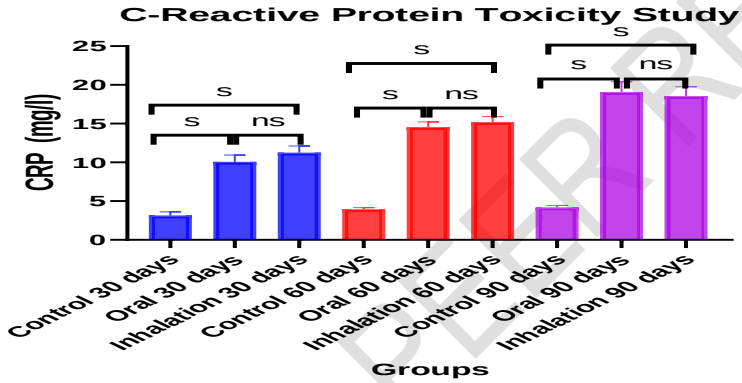

Fig. 6: Serum C-reactive protein levels comparison of the Effect of Routes of Administration of Sniper on Renal Function Parameters

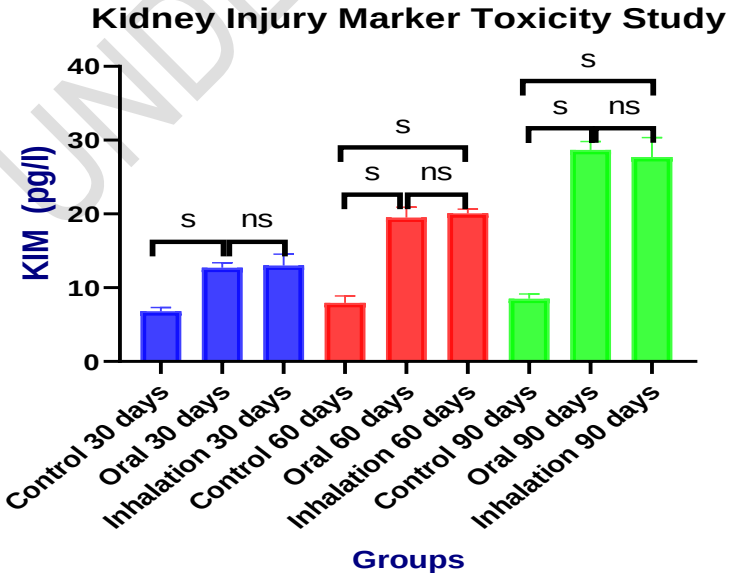

Fig. 7: Serum kidney injury marker-1 levels comparison of the Effect of Routes of Administration of Sniper on Renal Function Parameters

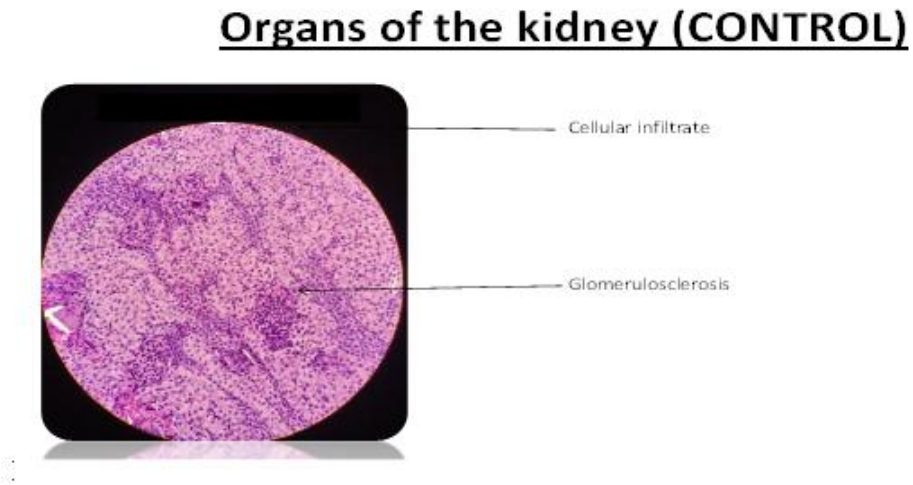

Plate 1: Micrograph of a normal kidney (from rabbit in control group- oral)

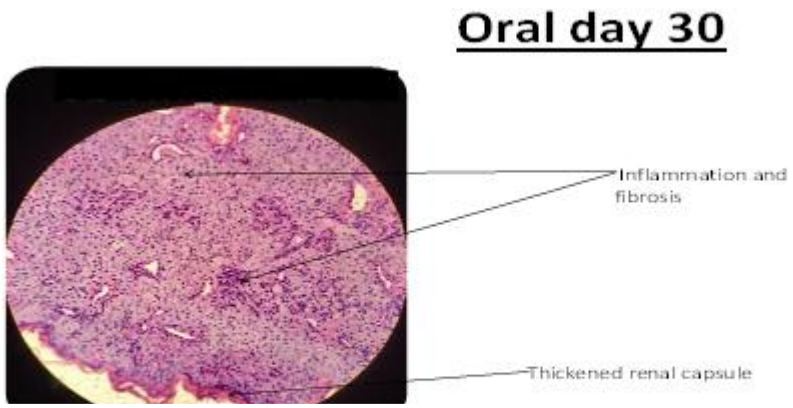

Plate 2: Micrograph of a rabbits given oral for 30 days

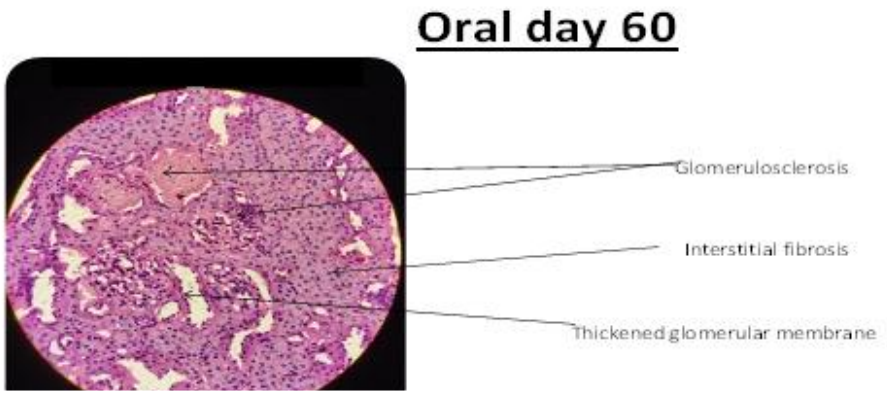

Plate 3: Micrograph of a rabbits given oral for 60 days

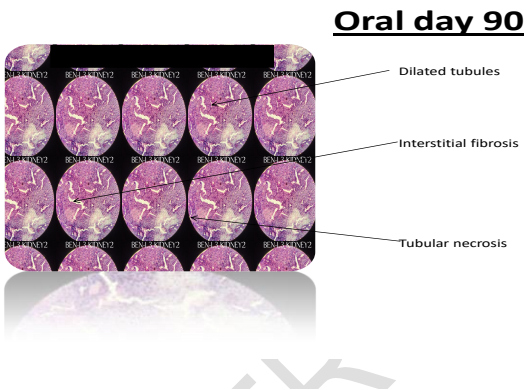

Plate 4: Micrograph of a rabbits given oral for 90 days

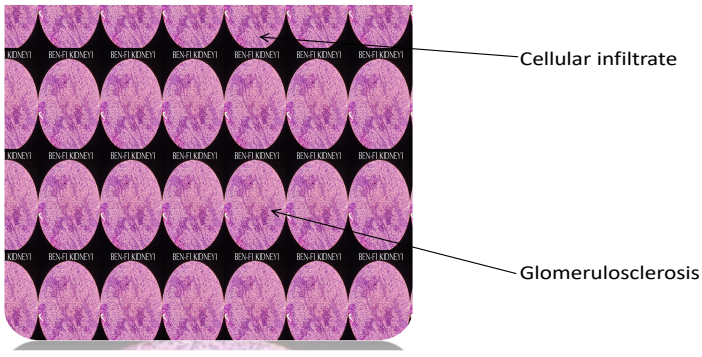

Plate 5: Micrograph of a normal rabbit kidney (from the control inhalation group)

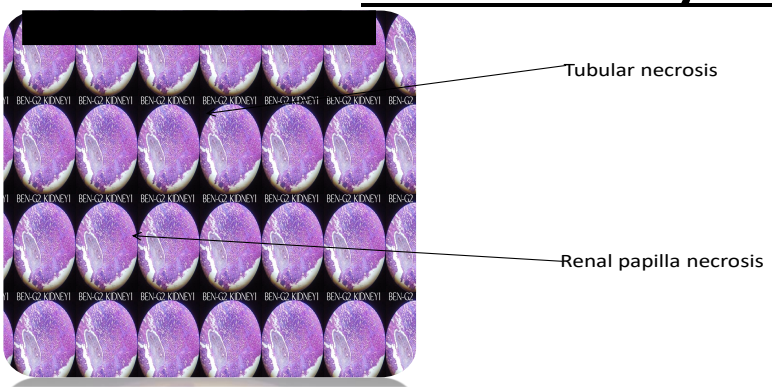

Plate 6: Micrograph of a rabbits given by inhalation for 30 days

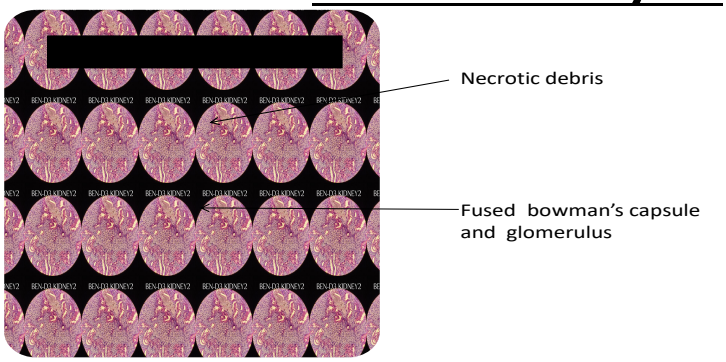

Plate 7: Micrograph of a rabbits given by inhalation for 60 days

Inhalation day 90

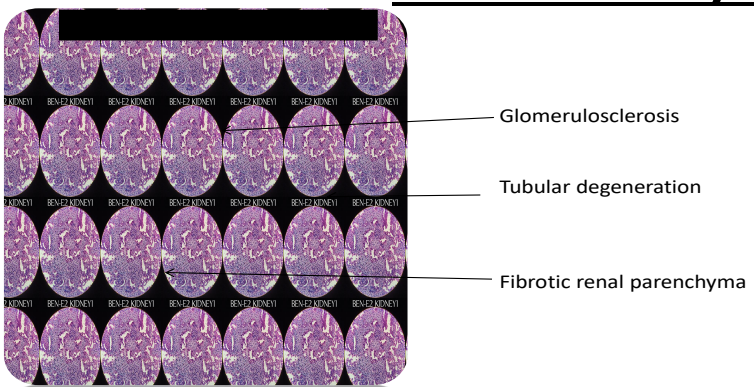

Plate 8: Micrograph of a rabbits given by inhalation for 90 days

This study investigated the chronic toxicological effects of 2 -2 – dichlovorovinyl dimethyl phosphate (sniper) in rabbits. Chronic dichlorvos exposure by oral and inhalation routes on the rabbits for the period of 30-90 days caused significant alterations in the kidney function. The kidney function parameters used for this assessment were sodium ion, potassium ion, chloride ion, calcium ion, urea, and Creatinine and kidney injury molecule (which is a biomarker of kidney function). Dichlorvos exposure caused significant increase in all the electrolytes that were measured. The increase in the level of the parameters was proportional to the duration dichlorvos exposure in the oral and inhalation studies.

Electrolyte balance is important for normal function of cells and organs. The observed elevation in the sodium, potassium and chloride ions in the studies may be due to impaired kidney function parameters. The values increased as the duration of dichlorvos treatment on the rabbits by oral and inhalation route increased. The elevation in the level of electrolyte ions may be due to the inhibitive effect of dichlorvos on the tubular cells reabsorption of the ions. Inhibition in the reabsorption of the ions may be due to serious nephrotic damage that is caused by the toxic metabolites of the dichlorvos. Again, increase in the concentration of sodium, chloride and potassium ions are known to increase glomerular filtration rate, which may contribute in kidney damage.

Furthermore, the significant increase in sodium (Na^+) ion level (Fig. 1) as the duration of dichlorvos exposure increased is an indication that the dichlorvos has an activation effect on the monovalent cation transport in the plasma of the rabbits. Again, the observed increases in plasma potassium, sodium and chloride ions with increase in the duration of oral and inhalation dichlorvos exposure may be due to toxic effect of the dichlorvos on the renal function thereby making is difficult for the exposed animals to regulate a proper electrolyte balance. Hyperkalaemia has been reported in conditions that cause massive destruction of blood cells with redistribution of potassium from the intracellular to the extracellular compartment as observed in severe haemolysis. The significant increase in potassium ion (Fig. 2) as the duration of dichlorvos exposure increased could be due to cation exchange of hydrogen ion (H^+) and potassium (K^+) between intracellular and extracellular spaces (erythrocyte swelling). Haemolysis would have caused mixture of the efflux of plasma potassium from intracellular compartment. Potassium is the dominant intracellular cation and plasma ionic dilution would compensate efflux into the extracellular fluid.

Hyperkalaemia in the blood occur in cases of renal failure because the kidneys lose the capacity to excrete the mineral. Severe dehydration will also cause hyperkalaemia and hypernatraemia. The resultant effects are muscle weakness and cardiac arrhythmias which could lead to heart failure [13]. The findings in this study is in corroboration with the findings of Adeoti et al. [14] which observed significant increase in the serum sodium, potassium and chloride ions of male wistar rats that were exposed via inhalation to sniper (dichlorvos). The

increased level of chloride ion (Fig. 3) may be connected with sodium retention because most sodium re-absorption is coupled with chloride ion re-absorption [15].
Dichlorvos exposure caused significant elevation in the mean urea and Creatinine from day 30-90. The significant elevation in the levels of plasma urea and Creatinine (Figs. 4 and 5) with decrease in the plasma protein and albumin levels may also signify protein catabolism and kidney dysfunction. These findings clearly reveal that dichlorvos exposure by oral and inhalation routes has serious deleterious effects on both renal and hepatic cells. Elevation in urea levels may be influenced by a number of conditions such as antidiuretic drugs use, dehydration and nature of diet; while Creatinine is more associated with the kidneys. Plasma Creatinine concentration is a good marker of glomerular filtration rate. Therefore, kidney dysfunction (damage) is the only significant factor that increases serum Creatinine levels [16]. The diagnostic accuracy of serum Creatinine increases as renal function worsens. Kidney injury molecule–I (KIM-I) is a blood sensitive biomarker that specifically reflects acute and chronic kidney injury molecule. Specifically, it is used for early detection of kidney dysfunction even when other biochemical markers of kidney dysfunction may not be elevated at the early stage. In this present study, the kidney injury molecule (KIM-I) and CRP were significantly elevated at $P<.05$ (Fig. 7) from day 30 with marked elevation as the duration of dichlorvos treatment increased up to day 90, in the oral and inhalation treatment. Kidney injury molecule-I (KIM-I) is highly up-regulated in the in proximal tubular cells following kidney injury. It is therefore the only blood marker that specifically indicates injury to the proximal tubule of the kidney. When there is injury, tubular cell polarity is lost, such that KIM-I may be released directly into the interstitium. Increased trans-epithelial permeability after tubular injury leads to back leak of tubular content into the circulation. Not only is KIM-I proven to be an early biomarker of acute kidney injury; it also has a potential role in predicting long term renal outcome [17]. Tubular epithelial cells can undergo apoptotic and necrotic cells are important in the mitigation of inflammation and to promote tissue repair. Again, after kidney injury, the proximal tubule epithelium would regenerate. This process of dedifferentiation and proliferation of viable cells around the damaged area to form an intact functional epithelial layers involves the up regulation of KIM-I expression. KIM-I is therefore an emerging biomarker for detecting acute kidney damage.
Effect of oral and inhalation routes of dichlorvos exposure on the rabbits kidney function parameters were compared as shown in table 1. The oral and inhalation routes of exposure both revealed significant increase in all the biochemical markers ($P<.05$) that were used to assess the integrity of the kidney. This study has revealed that dichlorvos is not only harmful when ingested; inhalation of subtle doses over time could as well pose serious organ/tissue damage as seen in this present study since the traditional method of observing an increase in serum Creatinine concentration and urine output may delay the detection of clinically significant kidney damage.

4. CONCLUSION

From the results, this study has revealed that oral and Inhalation routes of sniper exposure can produce renal toxicity. Results showed that renal toxicity occurred more in the former route and as the period of administration increased.

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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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