

Diagnostic Potentials of Postmortem Vitreous Biochemical Parameters in the Autopsy of Deaths Associated with Sniper Toxicity using Animal Models

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Abstract

Literature surveys revealed two major means by which perpetrators of murder crimes survive judicial scrutiny. One is by disguising the cause of death, which ultimately misleads the jury during judicial trials. The other is the fact that blood, the principal specimen for most medical investigations, putrefies shortly after death. This has created a diagnostic gap that is being exploited to mask homicide as the court finds it difficult to accurately ascertain the cause of death where there are no physical injuries, as in cases of chemical toxicities. This study aims at exploring the discriminatory potentials of selected vitreous biochemical parameters in the autopsy of death by sniper toxicity and in death disguised as sniper toxicity using rabbits. Completely randomized block design (CRBD) was used for this study. 96 male rabbits were used for this research and were structured into four groups of twenty-four rabbits each: two treatment (test) groups and two control groups. In one test group, the death of the experimental subjects was caused by sniper toxicity. In the second test group, the death of the experimental subjects was caused by strangulation; thereafter, the dead subjects were administered lethal doses of sniper as a cover-up of the actual cause of death. The remaining two groups are the baseline controls. After a postmortem interval of twenty-four hours, vitreous samples were obtained from each group of the experimental animals by Coe and Tente's methods. The samples were analyzed for the levels of sodium, potassium, carbon dioxide, chloride, and calcium using the ion-selective electrode method, while total protein, albumin, globulin, glucose, total cholesterol, triacylglycerol, urea, creatinine, uric acid, creatine kinase, and lactate dehydrogenase were analyzed using their specific standard methods. Results obtained were



then analyzed with the Statistical Package for Social Sciences (Version 18-21) and One-way Analysis of Variance (ANOVA) (Post Hoc-LSD). The results show that postmortem vitreous levels of urea, creatinine, uric acid, creatine kinase, and lactate dehydrogenase differed significantly ($P \le 0.05$) between samples from deaths caused by sniper toxicity and deaths by strangulation but disguised as sniper toxicity. The striking differences in the levels of these notable analytes can be utilized either as primary or confirmatory tests to reveal and discriminate between death disguised as sniper toxicity and death by actual sniper toxicity.

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1.0 Introduction

Incidents of poisoning and their associated lethal toxicity scarcely reflect on the body's morphology. Thereby ruling out the prospect of depending on anatomical changes as a basis for determining the cause of death. This has turned the prism of forensic investigators in the direction of thanatobiochemistry (Rosato et al., 2021). Sniper, whose bioactive constituent is dichlorvos, is one of the most patronized poisons for both suicide and homicide. The exploitation of snipers for criminal purposes is shielded by the lack of specific bio-indicators that can reliably resolve the prevailing conundrum of distinguishing antemortem exposure from that of postmortem. In this regard, vitreous biochemistry is being explored for unambiguous chemical species that can reproducibly discern pre-mortem lethal toxicological characteristics from those of postmortem. Encountering and identifying novel molecular alarms that can guide in the deciphering and simplification of shrouded and complex medico-legal questions would lighten the existing burden of actors in the criminal identification and justice dispensing arena (Wankasi et al., 2020).

Sniper, the trade name for Dichlorvos, also known as DDVP (2, 2-dichlorovinyl dimethyl phosphate), is an organophosphate insecticide with a molecular formula of C₄H₇Cl₂O₃P and a weight of 220.98 Dalton (USEPA, 2007). Dichlorvos is used as a pesticide for households and infectious disease control to eliminate insects in stored products and crops (mainly greenhouse crops) and in buildings, aircraft, and outdoor areas (CERI, 2007). It is classified by the WHO as a class 1B, "highly hazardous" chemical (WHO, 1992). DDVP is the most commonly used organophosphate pesticide in developing countries (Binukumar and Gill, 2010). It is also used as an antihelminthic in fish farming to eradicate crustacean ectoparasites (Varo et al., 2003). Dimethyl 2,2-dichlorovinyl phosphate (dichlorvos) is a colorless to amber liquid with a boiling point of 140°C at 2.7 kPa, a vapor pressure of 1.2×10^{-2} mmHg at 20°C, and a density of 1.415 g/ml at 25°C (Budavari, 1998). Dichlorvos has a water solubility of 8 g/L at 20°C and is miscible with alcohols and toluene. Dichlorvos has an aromatic chemical odor, and the log octanol/water partition coefficient for dichlorvos is 1.16 (ATSDR, 1997). In the presence of moisture, dichlorvos breaks down to form acidic products that are eventually mineralized (WHO, 1999).



Dichlorvos pesticide self-poisoning is a ravaging clinical problem in the developing world and kills an estimated 200,000 people every year (Michael et al., 2008). Acute (shortterm) and chronic (long-term) exposures of humans to dichlorvos result in the irreversible inhibition of the enzyme acetylcholinesterase in the blood and nervous system, which causes neurotoxic effects, perspiration, vomiting, diarrhea, drowsiness, fatigue, headache, and, in most cases, death (USEPA, 2007). Sniper depletes the levels of AChE in the hippocampus and amygdala, and in discrete brain regions, such as the cerebellum, hippocampus, frontal cortex, medulla, spinal cord, and occipital cortex. Results from recent studies suggest that sub-chronic oral exposure to DDVP imposes hippocampal and amygdala oxidative damage and subsequent depletion of neurogenic nuclei in the hippocampus and dentate gyrus (Imam et al., 2018).

Blood, the usual analytical specimen, is unsuitable as a sample when an autopsy is conducted long after death because it decomposes shortly after death. This farreaching diagnostic gap of making a distinction between an actual cause of death and a purported cause of death is being exploited by murderers to evade justice while homicidal crimes are on the rise. Some reasons why vitreous humor would make a good sample for postmortem forensic analyses are: unlike blood, it is not degraded for a long period after death (Adam and Gail, 2013); it undergoes very slow postmortem changes (Thierauf et al., 2011); it contains several molecules and metabolites that can be assayed for or monitored (Amith, 2005); it is present in sufficient quantities that can serve as samples for multiple investigations (Garg et al., 2004); it is easy to obtain (Zilg et al., 2009).

The aim of this study is to explore the potentials of selected vitreous biochemical parameters as biomarkers in postmortem determination and discrimination of deaths by sniper toxicity using animal models. The objective of this is to measure and compare the postmortem vitreous levels of sodium, potassium, carbon dioxide, chloride, calcium, total protein, albumin, globulin, glucose, total cholesterol, triacylglycerol, urea, creatinine, uric acid, creatine kinase, and lactate dehydrogenase of dead experimental subjects in different groups based on differences in the causes of death in order to identify analytes with significant variations in quantities amongst the studied groups that can be used as biomarkers for making distinctions amongst them.

The findings of this study may reveal unexplored facts that may be useful in the development of biomarkers for distinguishing between death disguised as sniper toxicity and death by actual sniper toxicity. uncover the potentials of vitreous biochemical parameters in postmortem discrimination of death the disguised as sniper toxicity and death by actual sniper toxicity; provide useful information to aid the search for better alternatives to blood samples in the postmortem investigations of the causes of death; lead to the development of techniques/methodologies for novel corroborating existing techniques/ methodologies in the postmortem investigations of the causes of death; open up opportunities for improving the justice delivery system as it holds enormous promise of providing information that can clarify controversial cases connected with sniper toxicity; cascade into a reduction in murder crimes in the society, as it may provide novel clues for plugging one of the means by which murderers escape punishment in homicide disguised as suicidal sniper toxicity; provide data that can guide future research in the area of finding scientific solutions to postmortem legal issues.

2.0 Materials and Methods 2.1 Study area

This study was carried out at Eni-yimini Laboratories (eL) Ltd, Yenagoa, Bayelsa State. Yenagoa serves as both the capital of Yenagoa Local Government Area and the capital of



Bayelsa State. It is situated in the Niger Delta region of Nigeria, and has the following geographical coordinates: 4⁰55'29" N and $6^{0}15'51''$ E. Yenagoa is characterized by a tropical climatic variable with temperature range between 71⁰ F and 870 F, and a weather pattern of a wet rainy season and a cloudy dry season. It is characterized by four ecological zones in terms of vegetation, namely: coastal barrier island forests, mangrove forests, freshwater swamps, and lowland rain forests. The area has gradually evolved into an urban setting with petroleum oil exploration activities and other related commercial and technological development (Agoro et al., 2021).

2.2 Animal specimen and study population

A total of 96 male albino rabbits with an age range between six to eight months, and weight bracket/range between 1.5 kg and 2 kg were used for the research. The animals were obtained from the animal house of the University of Jos. Plateau State. Acclimatization of the animals were done by keeping in cages at the animal house of the in the Biochemistry laboratory of the Federal University Otuoke, Bayelsa State, for 7 days prior to the experiment. Commercial rat pellets and water were provided ad libitum. The sample size was calculated using Mead's resource equation (Kirkwood and Robert, 2010), as expressed: E = N - B - T, where N is the total number of individuals or units in the study (minus 1). B is the blocking component, representing environmental effects allowed for in the design (minus 1). T is the treatment component, corresponding to the number of treatment groups (including the control group) being used or the number of questions being asked (minus 1). E is the degrees of freedom of the error component and should be somewhere between 10 and 20. The study consisted of four groups (T = 4), with 24 animals per group, making 96 animals in total (N = 95), without any further stratification (B = 0); then E would equal 91, which is above the cutoff of 20, indicating

that the sample size is very suitable for the research.

2.3 Experimental design

The research design indicated an arrangement into four groups comprising twenty-four rabbits each: two treatment (test) groups and two control groups based on specific means of deaths. Each group was administered a unique treatment in the form of cause of death. In first treatment group, the death of the experimental subjects was caused by sniper toxicity. In the second treatment group, the death of the experimental subjects was caused by strangulation; thereafter, the dead subjects were administered lethal doses of sniper as a disguise or cover-up of the actual cause of death. The remaining two groups are the baseline controls, which were deaths by strangulation and deaths by chloroform intoxication. Acute sniper toxicity was achieved by the administration of a sniper volume of 10 ml per body weight in kg orally, while strangulation was done by hand: the thumbs of both hands were pressed directly against the neck of the experimental subject forcefully and firmly, reflecting how most human homicides are undertaken. The observed average agonal periods were 22 mins in sniper treatment, 10 mins in strangulation, and 27 mins in chloroform treatment. Ascertaining the death status of the experimental animals relied on the prescriptions of the uniform determination of death act as reported by Omelianchuk et al. (2022). Subjects in both treatment and control groups were left for 24 hours after death before their vitreous humor samples were collected for analysis, mimicking a scenario of death coverup and subsequent discovery. The selection of vitreous is based on factors such as: its long postmortem interval before deterioration, fermentation, and putrefaction as well as its similarity in biochemical concentrations to blood, coupled with insignificant age and sex influences (Agoro et al., 2018, 2019, 2020). Rabbit was selected for this research work. The suitability of rabbit for this study is attributed to



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its anatomical and physiological similarities to humans as stated by Gwon (2008). Treatments and sample collections were conducted at the Biochemistry laboratory of the Federal University Otuoke, Bayelsa State, while the sample analysis was carried out at the Eniyimini Laboratories (eL) Ltd., located in Igbogene, Yenagoa, Bayelsa State.

2.4 Selection criteria

The health status of the rabbits used for this research were ascertained and approved as healthy and active by a veterinary doctor. The following selection criteria were adopted: rabbits showing signs and symptoms of illness, form of derangements and turbid vitreous humours were excluded from the research.

2.5 Collection of samples

The method of Coe (1993) and Tente (2004) was adopted in collection of vitreous humor samples. The total extractable vitreous humor was aspirated from the eye using a 5 mL syringe and a needle, a scleral puncture was made on the lateral canthus. Extraction of eye fluid were conducted with utmost care to avoid tearing of any loose tissue fragments surrounding the vitreous chamber. A mean volume of 1.0 mL was collected from each rabbit's eye. Only crystal-clear liquid free of tissue contaminants and fragments was used in the study. The samples were collected twenty-four hours postmortem.

2.6 Preparation of samples

Samples of vitreous humor for glucose concentration measurements were transferred into fluoride oxalate tubes, while those for proteins, electrolytes, uric acid, lipid profiles, and renal and cardiac biomarkers studies were transferred into plain containers. The biochemical analyses carried were out immediately after extraction. Prior to analysis, the vitreous samples were centrifuged at 2050 rpm for 10 mins, the supernatants were separated and used for the analyses. The samples were used to determine the of sodium, potassium, chloride, calcium, bicarbonate, total protein, albumin, globulin, urea, creatinine, glucose, cholesterol, triacylglycerol, uric acid, lactate dehydrogenase (LDH), and creatine kinase (CK) using the methods of Agoro *et al.* (2021).

2.7 Analysis of samples

The method of Bolarin and Azinge (2010) was used for determination of the concentration of the selected vitreous electrolytes concentrations using the anion-selective electrode (ISE) analyzer method (analyzer ISE 4000). The Biuret method as modified by Randox Laboratories (United Kingdom) (Randox kit leaflet) was used for quantitative estimation of vitreous total protein concentration. The Biuret method is the most widely used method for protein analysis in body fluids (Peters, 1996) and by one recommended the International Federation of Clinical Chemistry (IFCC) expert panel for the determination of total protein (Randox kit leaflet). Vitreous albumin was estimated quantitatively using the Bromocresol Green Method as modified by Randox Laboratories (United Kingdom) (Randox kit leaflet). Vitreous globulin concentration was derived by subtracting vitreous albumin from vitreous total protein. The value is an estimate of vitreous globulin. Vitreous urea was estimated by the diacetyl monoxime method. The diacetyl monoxime salts are stable at room temperature and could adapt to harsh environments (Bolarin and Azinge, 2010). Determination of vitreous creatinine concentration was by Jaffe's method. It was chosen because of its stability and consistency (Bolarin and Azinge, 2010). Vitreous glucose concentration was estimated quantitatively using the glucose oxidase method as specified by Randox Laboratories (United Kingdom). The glucose kit used was a product of Randox, and the standard operating procedure as stated in the manual was strictly followed. Vitreous total cholesterol concentration was estimated quantitatively using the Agappe kit as specified by Agappe Diagnostics (Switzerland) (Agappe Kit Leaflet). Vitreous triacylglycerol



concentration was estimated quantitatively using the Agappe kit as specified by Agappe Diagnostics (Switzerland) (Agappe Kit Leaflet). Vitreous uric acid concentration was estimated quantitatively by the uricase method using the Agappe kit as specified by Agappe Diagnostics (Switzerland) (Agappe Kit Leaflet). Vitreous kinase activity creatine was estimated quantitatively using the Agappe method as modified by Agappe Diagnostics (Switzerland) Leaflet). Vitreous (Agappe Kit lactate dehydrogenase (LDH) activity was estimated quantitatively using the Agappe method as modified by Agappe diagnostics (Switzerland) (Agappe kit leaflet) (Ikimi et al., 2023; Ikimi et al., 2024).

2.8 Statistical analyses

Mean value of each parameter in the samples was determined from the triplicates values, and the data generated were analyzed with the Statistical Package for Social Sciences (SPSS) program (SPSS Inc., Chicago, IL, USA; Version 18-21) and Microsoft Excel. One-way ANOVA (Post Hoc-LSD). A student T-test was employed to compare the mean levels of the various vitreous parameters amongst the studied groups. The level of significance was assessed at P < 0.05. Pearson correlation was used to determine relationships amongst the data generated.

3.0 Results and Discussion

The results of the levels of selected studied parameters in the postmortem vitreous of rabbits in cases of death by sniper toxicity and disguised as sniper toxicity are shown in Figs. 1 to 10. The results show that Na, K, Cl, Ca, total protein, globulin, glucose, total cholesterol, and triacylglycerol levels were not statistically different in all groups ($P \le 0.05$) (Figs. 1, 2, 3, 5, and 6). Albumin level in SD (0.403 ±0 .00041 mmol/l) was significantly lower than in control 1 (3.328 ± 0.00041 mmol/l). CO₂ levels in the test groups (SD and DSD) (3.000 ± 0.40825

mmol/l) (2.000 \pm 0.40825 mmol/l) were significantly lower than in the controls (10.000 $\pm 0.47871 \text{ mmol/l}$ (16.000 $\pm 0.40825 \text{ mmol/l}$), respectively (Fig. 4). Urea level in DSD (6.048 ± 0.00048 mcmol/l) was significantly lower than in SD (12.957 \pm 0.00041 mcmol/l) and in the controls $(10.617 \pm 0.00041 \text{ mcmol/l}) (13.275 \pm$ 0.00041 mcmol/l), respectively (Fig. 7). Creatinine level in DSD (40.081 \pm 0.0048 mcmol/l) was significantly lower than in SD $(63.865 \pm 0.00041 \text{ mcmol/l})$ and in control 1 $(83.002 \pm 0.00041 \text{ mcmol/l})$ but higher than that of control 2 ($28.663 \pm 0.0004 \text{ mcmol/l}$) (Fig. 8). Uric acid level in DSD (0.002 ± 0.00041) mmol/l) was significantly lower than in SD $(0.178 \pm 0.00041 \text{ mmol/l})$ and in control 1 (0.632 \pm 0.00041 mmol/l). Creatine kinase activity in SD (18.166 \pm 0.00041 U/L) was significantly lower than in DSD (117.627 \pm 0.00041 U/L) and in the controls $(3456.511 \pm 0.00041 \text{ mmol/L})$ $(5035.685 \pm 0.0041 \text{ U/L})$, respectively (Fig. 9). Lactate dehydrogenase activity in SD (473.055 \pm 0.00041 U/L) was significantly lower than in DSD (6834.918 \pm 0.00041 U/L) and in the controls (17491.880 ± 0.0408 U/L) (9948.605 ± 0.00041 U/L) (Fig. 10).

The observed lower level of albumin in SD $(0.403 \pm 0.00041 \text{ mmol/l})$ when compared with control 1 ($3.328 \pm 0.00041 \text{ mmol/l}$) is suggestive of renal abnormality due to sniper toxicity, particularly a dysfunction of glomerular filtration (Sembulingam and Sembulingam, 2010). The observed lower level of CO_2 in the test groups (SD and DSD) respectively (3.000±0.40825mmol/l) (2.000 ± 0.40825 mmol/l) when compared with the controls respectively $(10.000 \pm 0.47871 \text{ mmol/l})$ (16.000 \pm 0.40825 mmol/l) can be attributed to the compensatory effect in the regulation of P^H due to the metabolic acidosis introduced by dichlorvos toxicity (Vasudevan et al., 2019).





Fig. 1: Chart showing postmortem vitreous levels of Na in SD and DSD. CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); SD: Sniper administration death; SD: Disguised sniper death (strangled to death before sniper administration)



Fig. 2: Chart showing postmortem vitreous levels of K in SD and DSD. CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); SD: Sniper administration death; DSD: Disguised sniper death (strangled to death before sniper administration)

The observed lower level of urea in DSD (6.048 \pm 0.00048 mcmol/l) when compared with SD (12.957 \pm 0.00041mcmol/l) and the controls respectively (10.617 \pm 0.00041 mcmol/l) (13.275 \pm 0.00041 mcmol/l) may be due to the conversion of urea to ammonia by the

disturbance of the carcass while implementing the disguise (Agoro, 2018; D'Souza *et al.*, 2011). The observed lower level of creatinine in DSD (40.081 \pm 0.0048 mcmol/l) when compared with SD (63.865 \pm 0.00041mcmol/l) and control 1 (83.002 \pm 0.00041mcmol/l) can be



adduced to the effects of postmortem manipulations in carrying out the disguise (Zilg *et al.*, 2022). While the observed higher level of creatinine in SD ($63.865 \pm 0.00041 \text{ mcmol/l}$)

when compared with control 2 (28.663 ± 0.0004 mcmol/l) can be ascribed to the anti-putrefactive effects of chloroform on tissues (Ruchieka *et al.*, 2014; Sacco *et al.*, 2022).



Fig. 3: Chart showing postmortem vitreous levels of Cl in SD and DSD. CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); SD: Sniper administration death; DSD: Disguised sniper death (strangled to death before sniper administration)



Fig. 4: Chart showing postmortem vitreous levels of CO_2 in SD and DSD. CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); SD: Sniper administration death; DSD: Disguised sniper death (strangled to death before sniper administration)





Fig. 5: Chart showing postmortem vitreous levels of total protein in SD and DSD. CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); SD: Sniper administration death; DSD: Disguised sniper death (strangled to death before sniper administration); TP: Total protein



Fig. 6: Chart showing postmortem vitreous levels of globulin in SD and DSD. CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); SD: Sniper administration death; DSD: Disguised sniper death (strangled to death before sniper administration); GLO: Globulin

The outcome of the concentration of both electrolytes and renal function indices in this study varies with the findings reported by Wankasi *et al.*, (2020). This disparity can be explained by the large difference in postmortem intervals before sample collection and



subsequent analysis in these studies. This further underscores the fact that postmortem interval is a critical factor in determining postmortem chemical compositions. The observed lower level of uric acid in DSD ($0.002 \pm 0.00041 \text{ mmol/l}$) when compared with SD ($0.178 \pm 0.00041 \text{ mmol/L}$) and control 1 ($0.632 \pm$

0.00041 mmol/l) could be related to the alterations in chemical and physiological processes by reasons other than the causa mortis such as the effects of staging a disguise to mask the actual means of death (Gill and Landi, 2011). This argument is in tandem with the position of Rosato *et al.*, (2021).



Fig. 7: Chart showing postmortem vitreous levels of urea in SD and DSD. CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); SD: Sniper administration death; DSD: Disguised sniper death (strangled to death before sniper administration); UR: Urea





Fig. 8: Chart showing postmortem vitreous levels of creatinine in SD and DSD. CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); SD: Sniper administration death; DSD: Disguised sniper death (strangled to death before sniper administration); CR: Creatinine



Fig. 9: Chart showing postmortem vitreous levels of creatine kinase in SD and DSD. CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); SD: Sniper administration death; DSD: Disguised sniper death (strangled to death before sniper administration); CK: Creatine kinase



Fig. 10: Chart showing postmortem vitreous levels of lactate dehydrogenase in SD and DSD. CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); SD: Sniper administration death; DSD: Disguised sniper death (strangled to death before sniper administration); LDH: Lactate dehydro genase



The observed lower level of Creatine kinase activity in SD (18.166 \pm 0.00041 U/L) when compared with DSD (117.627 \pm 0.00041 U/L) and the respective controls (3456.51 \pm 0.00041 U/L) (5035.685 \pm 0.00041 U/L) signals a lesser extent of muscle tissue damage in death by means of SD than that of the controls. This revelation reinforces the prevailing position that the mechanism of action of sniper exerted toxicity is through nervous transmission interferences as it inhibits acetylcholinesterase, which is crucial in the conduction of nerve impulse across synaptic gaps (USEPA, 2000; Zwirner *et al.*, 2021).

The observed lower level of lactate dehydrogenase activity in SD (473.055 ± 0.00041 U/L) when compared with DSD $(6834.918 \pm 0.00041 \text{ U/L})$ and the respective controls (17491.880 ± 0.000408 U/L) (9948.605 \pm 0.00041 U/L) hints on the fact that the magnitude of hypoxic trauma in deaths occasioned by dichlorvos insult is lesser than those orchestrated in deaths by means of the controls. This may explain why death by means of the controls occurs faster than by sniper intoxication (Farhana and Lappin, 2023; Spriet et al., 2000).

4.0 Conclusion

Postmortem vitreous levels of urea, creatinine, uric acid, creatine kinase and lactate dehydrogenase differed significantly ($P \le 0.05$) between samples from death by means of sniper toxicity and deaths by means of strangulation but disguised as sniper toxicity. Postmortem vitreous levels of albumin and CO2 differed significantly ($P \le 0.05$) between samples from deaths by means sniper toxicity and deaths by means of strangulation. These identified analytical metabolites with discriminatory potentials between death by sniper toxicity and death by other means but disguised as sniper toxicity can be exploited as indicators in forensic investigations for the resolution cases where the cause of death is unclear and in contention of

being either truly caused by sniper toxicity or suspected to be by other means but purported to be by sniper toxicity.

Authors contributions

Dr. C.G. Ikimi conceptualized the work, did the laboratory analysis and wrote the manuscript. Dr. M. T. Bekinbo conducted the sample collection. Dr. E. C. Onuoha did the statistical analysis. All authors read and approved the manuscript.

Ethical Approval

Ethical clearance was obtained from the animal research ethics committee of the Nnamdi Azikiwe University, Awka. The Animal Welfare Act of 1985 of the United States of America for research and Institutional Animal Care and Use Committee (IACUC) protocols were stringently adhered to (Benjamin and Jean, 2016).

Conflict of interest

The authors declared no conflict of interests.

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Availability of data and materials

Data would be made available on request.

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