Post-mortem analyses of *Oryctolagus cuniculus* acutely poisoned with potassium cyanide and dichlorvos (Sniper®)

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Abstract

Acute poisoning with toxic substances such as Dichlorvos (Sniper®) and Potassium Cyanide (KCN) has become a threat to human life especially in Nigeria where there have been increased cases of suicidal poisoning in recent times. This study aimed to ascertain the Post-mortem analyses of *Oryctolagus cuniculus* acutely poisoned with potassium cyanide and dichlorvos (Sniper®). Eighteen rabbits were used for histopathologic, haematologic, biochemical and molecular changes induced by acute oral KCN and Sniper® poisoning for a period of 21 days. The decomposition process was monitored and muscle tissue were sampled for DNA integrity and microbial assays. There was significant ($p<0.05$) difference in WBC, HGB, RBC, HCT, MCV, AST, ALT and ALP in test groups compared to control. DNA quality decreased overtime and agarose gel electrophoresis revealed damaged DNA bands. Five bacteria were isolated from this study; *Aeromonas hydrophila/caviae, Elizabethkingia meningoseptica, Pseudomonas, luteola, Vibrio fluvialis, Burkholderia cepacia.* KCN and Sniper® had similar effects on the organs studied.

Keywords: Forensic Pathology, Insecticide, Potassium Cyanide, Dichlorvos (Sniper®), Suicide, Forensic Toxicology

Introduction

Across all ages, unexpected deaths commonly occur and this relatively accounts for the low life expectancy in Nigeria. Sudden death is frequently caused by accidents, homicides and suicides and this account for a major clue for medicolegal autopsy in Nigeria (Amakiri *et al*. 1997, Aligbe *et al*. 2002, Obiorah and Amakiri 2013). Suicide is a global phenomenon that occurs all over the lifespan and is the second leading cause of death among 15 - 29-year olds globally (WHO 2018a). Suicide accounted for 1.4 % of all deaths worldwide, making it the 18th leading cause of death in 2016 (WHO 2018b). There are many methods for committing suicide e.g. Bleeding e.g. Cutting wrists or carotid artery, Carbon monoxide poisoning, Drowning, Drug overdosing, Electrocuting, firearm, Hanging, self-poisoning and Suffocation. The substances which are most commonly used for self-poisoning include medicines, narcotics, toxic chemical products and toxic gases (Garetier *et al*. 2016).

Toxic chemical substances have existed in the environment for many years and many of their toxic effects are well characterized (Meyer *et al*., 2010). However, available information and knowledge about many of these chemicals regarding human health risk is still limited. Several possible health risks arising from the toxic effects of well-established uses of these chemicals have been documented (Meyer *et al*., 2010). Nevertheless, new applications of existing chemicals may bring to light new risk scenarios that have not been previously described (Meyer *et al*. 2010).

Globally, Organophosphorus pesticides are the most widely used pesticides and their metabolites are widespread across different nationalities (Barr *et al*. 2004). The nervous

system is the primary target of organophosphorus pesticides consequently, the adverse effects of exposure to these chemicals have been mostly studied (Gupta *et al* 2001). However, there is growing concern about their possible toxicity in non-target tissues for instance the kidneys, liver and heart that have not been studied in much detail (Hreljac and Filipiˇc 2009). Organophosphorus pesticides are commonly used volatile pesticides that have detrimental effects on the liver in acute and chronic exposures (Karami-Mohajeri *et al*. 2017). Organophosphorus pesticides have harmful effects on human health through environmental or occupational exposure (Costa 2006, Ghazala *et al*. 2013). _Suicidal poisoning using Organophosphorus pesticides is common (Shadnia *et al* 2009). Acute poisoning with Organophosphorus pesticides is a worldwide threat to human health that causes more than 100,000 deaths a year (Gunnell *et al*. 2007, Yu *et al.* 2019, Aroniadou-Anderjaska *et al*. 2020). It has also been shown that model Organophosphorus pesticides can induce DNA damage and the expression of DNA damage inducible genes in HepG2 cells (Hreljac *et al*. 2008).

Dichlorvos, an organophosphate, is the main pesticide that is used in household insect control in developing nations. Acute and chronic exposure to dichlorvos may lead to death. Exposure to Dichlorvos can result in genotoxic, neurological, carcinogenic, hepatic, renal, respiratory, dermal and certain systemic effects (Okoroiwu *et al*. 2018). The toxicity of dichlorvos is due to the capability of the substance to impede acetyl cholinesterase at cholinergic coupling of the nervous system (Okoroiwu *et al*. 2018, Abaukaka *et al*. 2020). Sniper®, whose active ingredient is dichlorvos, is one of the leading causes of pesticide poisoning and death in Nigeria. Sniper induced death is reported to be increasing in Nigeria without a scientific basis for differentiating ante-mortem from post-mortem intoxication (Wankasi *et al.* 2020).

Cyanide is a mitochondrial poison and its toxicity is mediated through histotoxic hypoxia. Although it is regarded as a neurotoxin, its other toxic manifestations are also well documented (Hariharakrishnan *et al*. 2009). Cyanides is a very highly toxic compound that has been used as a weapon of terrorism throughout history (Hendry-Hofer *et al*. 2019). Cyanide is acutely toxic by all routes of administration; however, inhalation is the main exposure route (Hendry-Hofer *et al.* 2019, Rivera-Burgos *et al.* 2019). Its oral exposure can result in larger doses, compared with other modes of administration. Presently, there are no antidotes specific for use in the treatment of oral cyanide poisoning, and studies cannot be done in humans (Ng *et al*. 2018).

The toxicity of cyanide has been known for over 200 years (Bhattacharya and Lakshmana-Rao 1997). Cyanide toxicity is mainly attributed to inhibition of terminal oxidase of mitochondrial respiratory chain (Borowitz *et al*. 1991). In addition to its predominant neurotoxicity, the other effects of cyanide or cyanogenic compounds include cardiotoxicity, growth retardation, cardiovascular changes, gastrointestinal ulcerogenesis, nephrotoxicity, reproductive toxicity and teratogenic effects (Ballantyne and Marrs, 1987., Nyirenda 2020). Cyanide ingestion may as well cause central nervous system (CNS) syndromes and damage to thyroid in animals and humans (Soto-Blanco *et al*.2005). Cyanide is largely distributed in the ecosystem and is linked to toxic effects in animals and humans. Cyanide poisoning can occur due to exposure from dietary sources, inhalation of fumes from burning polymer products that use nitriles in their production, environmental effluence, work-related exposure, chemical warfare, deliberate ingestion, homicide, and sometimes by using drugs of plant origin like nitroprusside and laetrile (Watts 1998, McKenna and Hull, 2016). Cyanide triggers all those events which can lead to DNA damage (Bhattacharya and Lakshmana-Rao 1997). This study evaluated the post-mortem effects of cyanide and

dichlorvos (Sniper®) in acutely poisoned O*ryctolagus cuniculus.*

MATERIALS AND METHODS

Experimental animals

Eighteen (18) rabbits were housed in rabbits' cages at normal room temperature with dark and light cycle (12/12 hr). They were fed with vegetables and tap water *ad libitum*. The animals were kept for two weeks in order to ensure proper acclimatization. The average weight of all rabbits was 1.5 kg. Experiment was carried out in the animal house of the Department of Cell Biology and Genetics, University of Lagos, Nigeria. This research was performed according to the Organization of Economic Co-operation and Development (OECD) guidelines for testing Chemical, TG425 (OECD 2001). This study was conducted according to the rules and regulations of the University of Lagos Ethical Committee on the use of experimental animals.

Grouping of the animals

All the rabbits were separated into three groups, with six (6) animals in each group; the control group, the KCN group and the dichlorvos (DDVP) i.e. Sniper® group. The animals in the control group were administered 10 ml kg^{-1} distilled water (p.o.) and were anesthetized using mild diethyl ether anaesthesia. The animals in the KCN and Sniper® experimental groups were orally administered 1 mg kg^{-1} per body weight of KCN and 15 mg kg^{-1} per body weight of Sniper® respectively. According to estimates, the median fatal dose (LD50) of HCN in humans is 1.52 mg/kg when taken orally, 100 mg/kg when applied topically, and 1.0 mg/kg when administered intravenously (Sidell *et al.* 1997; A. f. T. S. a. D, 2004). Acute toxicity studies with dichlorvos reported LD50 of 50 mg/kg (Durham *et al.*, 1957), 133 mg/kg and 139 mg/kg (Haley *et al.*, 1975) for female Sherman rats, female mice, and male mice, respectively. The animals in the KCN and Sniper® groups died within 5 minutes after administration.

Sample Processing

Blood samples were collected by cardiac puncture using 21-gauge (21G) needles mounted on a 10 ml syringe into Ethylene Diamine Tetra-acetic Acid (EDTA) and Lithium heparin -coated sample bottles for analysis of haematologic indices and biochemical parameters respectively. The liver, lungs, kidney and heart were also dissected and fixed in 10 % formal saline prior to the histologic studies.

Haematologic Indices

An auto Haematology Analyzer (Mindray BC3200), was used to determine Haematologic indices such as haemoglobin, white blood cell, granulocytes, monocytes et.c as described by Peng *et al*. (2008).

Assessment of Biochemical Parameters

Assessment of hepato- and nephro-toxic activity was performed by determining the activities of some biochemical parameters. These assays were carried out using Randox® reagent kits and the procedures were followed as described in the manufacturer's manual.

Histopathology.

The method of Mallory (1961) was used with a little modification as described by van-Leeuwen *et al.* (1990). The tissues were processed and sectioned in paraffin. The paraffin sections of buffered formalin- fixed tissue samples (3 µm thick) were dewaxed and rinsed in alcohol and also water. They were stained with Harris' haematoxylin (Sigma®) for 10 minutes, washed in running tap water for 1 minute, differentiated in acid alcohol for 10 seconds and washed again in running tap water for 5 minutes. The tissues were stained with eosin for 4 minutes and washed in running tap water for 10 seconds. It was then dehydrated

and mounted for photo microscopic observations of the histologic architecture.

Post-Mortem Processing and Sample collection for DNA Analysis

From each group, three animals were hanged on trees while the other three were buried. The carcasses were examined for 21 days and samples were collected for DNA analysis at the time intervals of 1, 14 and 21 days.

Deoxyribonucleic acid (DNA) extraction

Deoxyribonucleic acid samples were extracted from soft tissue samples collected from the rabbits' hind limbs (muscle) using Quick-DNA Miniprep plus Kit purchased from Zymo Research according to manufacturer's instruction.

Spectrophotometry

Spectrophotometry of the extracted DNA samples was carried out by exposing the samples to UV wavelengths 260 µm and 280 µm using a spectrophotometer (Eppendorf BioPhotometer plus). The optical density of the samples was observed and recorded.

Gel electrophoresis

One gram of the agarose powder was dissolved in 100 ml of 0.5 x TBE solution by boiling. It was then allowed to cool to approximately 60 ^{o}C and 2 drops of ethidium bromide was added. The mixture was mixed gently and a gel thickness of about 4-5 mm was obtained by pouring into a taped electrophoresis tray with the comb and allowed to solidify for 20 minutes. The rubber dam and the comb were removed carefully to avoid damaging the gel. The tray was placed in an electrophoresis tank (Bio -Rad Power-pack) and 0.5 x TBE gel buffer was poured the first well of all gels before loading the samples. Five microliters of loading dye were mixed with 5 µl of the sample. The mixture was loaded into a comb well. Electrophoresis was then initiated at 60-100V until the loading dye has migrated three quarter of the gel. Bands of the DNA on the agarose gel containing the extracted samples were observed through transillumination with the use of ultra-violet ray transilluminator (Fisher Biotech).

Microbial Assay

Identification and Characterization of Bacterial Isolates

Into each of the test tubes, 1 g of soft tissue (taken on days 1, 14 and 21) from each rabbit samples were dispensed. Ten-fold serial dilutions were performed for all the samples before culture as described by Willey *et al*. (2011). Ten-fold serial dilution was carried out before being plated at 10^{-5} in petri dishes containing nutrient agar using pour plate method in an aseptic environment. The Nutrient Agar plates were incubated for 18-24 hours at room temperature.

Colonial examination of the isolates was carried out to determine the type of shape, elevation and pigmentation pattern exhibited. Microscopic examination including Gram staining and identification of cellular morphological appearances were also carried out. Additionally, catalase, oxidase and spore tests were carried out to determine the reactions of all bacterial isolates to these chemicals and stain. Analytical Profile Index (API) using Biochemical Test Strip (API 20 NE) kits ~~to~~ carried out as further identification tests on the isolates according to the manufacturer's specification.

Identification of Insects

Identification of insects at various stages of decomposition was carried out during the insect life cycle, such as eggs, larva, and adults. The collected insects were taken to the Entomology Laboratory of Zoology Department, University of Lagos for identification.

Statistical analysis

The results were expressed as mean + SEM for six rabbits. Statistical analysis of the data was performed using ANOVA statistical SPSS package (23.0) version. The significance of differences among all groups was determined by the Tukey HSD test. P – values less than

0.05 ($p \leq 0.05$) were considered to be statistically significant.

RESULTS

Table 1 shows the effect of Potassium cyanide and Sniper® on the haematologic indices in rabbits. There was significant ($p \leq 0.05$) decrease in LYMPH, MONO, RBC, HCT and MCV levels in the experimental groups compared to control group. The GRAN value of the Sniper® group was significantly ($p \leq 0.05$) increased compared to the control and potassium cyanide groups. The RDW-CV value of the Sniper® group was significantly ($p \leq 0.05$) increased compared to the control. The PLT value of the potassium cyanide group was significantly ($p \leq 0.005$) increased compared to the control and Sniper® groups. However, there was no significant difference in the percentages of PDW and PCT in both the control and experimental groups.

In both the control and experimental groups (Table 2), Group, positively correlated with LYMPH, MONO, GRAN, HGB, RBC, HCT, MCV and MCH at 0.01 level of significance but correlated with RDWCV and MPV at 0.05 level of significance. Also, LYMPH and MONO positively correlated with HGB, RBC, HCT, MCV and MCH at 0.01 level of significance. Additionally, MCHC correlated with PLT and MPV at 0.05 level of significance. However, there was no positive correlation between PCT and other haematologic indices analysed.

Table 1: Effect of potassium cyanide and Sniper® on the hematologic indices in rabbits

Parameters	Control			Potassium cyanide			Sniper®		
	Mean ± S.E.	95% Confidence Interval for Mean		Mean ± S.E.	95% Confidence Interval for Mean		Mean ± S.E.	95% Confidence Interval for Mean	
		Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound
WBC (10^6/µl)	65.95±1.36$^{(b)}$	62.45	69.45	50.50±2.11$^{(a)}$	45.07	55.93	56.8±4.39	45.53	68.07
LYMPH(10^6/µl)	0.17±0.01$^{(b,c)}$	0.14	0.19	0.13±0.01$^{(a,c)}$	0.11	0.15	0.035±0.01$^{(a,b)}$	0.018	0.052
MONO (10^6/µl)	7.9±0.3$^{(b,c)}$	7.12	8.68	5.9±0.24$^{(a,c)}$	5.3	6.5	2.42±0.37$^{(a,b)}$	1.46	3.37
GRAN (10^6/µl)	56.3±0.5	55.03	57.57	44.55±2.1	39.16	49.94	54.55±5.98	39.18	69.92
LYMPH (%)	0.25±0.019$^{(b,c)}$	0.2	0.3	0.17±0.02$^{(a,c)}$	0.12	0.21	0.06±0.01$^{(a,b)}$	0.04	0.08
MONO (%)	14.4±0.28$^{(c)}$	13.69	15.12	11.95±0.57$^{(c)}$	1049	13.41	5.85±1.18$^{(a,b)}$	2.81	8.89
GRAN (%)	83.4±0.64$^{(c)}$	83.75	87.05	82.9±1.3$^{(c)}$	79.57	86.23	94.95±1.62$^{(a,b)}$	90.8	99.1
HGB (g/dl)	2.8±0.11$^{(c)}$	2.53	3.08	2.35±0.23$^{(c)}$	1.77	2.93	0.83±25$^{(a,b)}$	0.18	1.48
RBC (10^6/µl)	2.19±0.14$^{(b,c)}$	1.82	2.55	1.71±0.05$^{(a,c)}$	1.57	1.85	1.18±0.6$^{(a,b)}$	1.02	1.34
HCT (%)	18.65±0.7$^{(b,c)}$	16.86	20.44	13.4±0.75$^{(a,c)}$	11.48	15.32	7.75±0.15$^{(a,b)}$	7.37	813
MCV (fl)	84.65±0.35$^{(b,c)}$	83.75	85.55	76.75±0.33$^{(a,c)}$	75.9	77.6	64.9±2.69$^{(a,b)}$	58	71.8
MCH (pg)	29.25±5.39$^{(b,c)}$	15.4	43.11	13.2±0.7$^{(a)}$	11.4	15	6.13±2.13$^{(a)}$	0.65	11.61
MCHC (%)	14.95±0.25	14.3	15.6	17.15±0.72	15.31	19	11.13±3.77	1.44	20.83
RDW-CV (%)	15.2±0.52$^{(c)}$	13.85	16.55	16.1±0.49	14.84	17.36	17.3±0.61$^{(a)}$	15.74	18.87
RDW-S (fl)	42.4±0.43	41.28	43.52	40.05±0.99	37.5	42.61	38.05±3.2	29.81	46.29
PLT (%)	47±7.34	28.12	65.88	69±10.34$^{(c)}$	42.42	95.58	37±3.68$^{(b)}$	27.54	46.46
MPV (fl)	8.15±0.21	7.6	8.69	8.4±0.17	7.97	8.83	9.1±0.41	8.05	10.15
PDW (%)	17.45±0.48	16.21	1869	16.7±0.2	16.18	17.22	17.25±0.48	16	18.49
PCT (%)	0.09±0.004$^{(b)}$	0.07	0.1	0.053±0.01$^{(a)}$	0.03	0.07	0.08±0.01	0.06	0.09

Values are expressed as mean ± SE for six (6) rabbits. The mean difference is significant at the 0.05 level. (a) = $p \leq 0.05$ as compared with the normal control group. (b) = $p \leq 0.05$ as compared to Potassium cyanide group. (c) = $p \leq 0.05$ as compared with the Sniper® group. The significance of differences among all groups was determined by the Tukey HSD test.

Mean corpusclar volume (MCV), Red blood cell (RBC) Haemoglobin (HGB), Mean corpuscular haemoglobin concentration (MCHC), White blood cell (WBC)Haematocrit (HCT), Red blood cell distribution width - Coefficient of variation (RDW-CV), Mean Platelet Volume (MPV), Red blood cell distribution width - Standard deviation (RDW-SD)Lymphocytes (LYMPH), Plateletcrit (PCT), Mean corpuscular haemoglobin (MCHPlatelet count (PLT), Monocytes (MONO), Platelet Distribution Width (PDW), Granulocytes (GRAN)

Table 2: Correlations between haematologic indices in rabbits acutely poisoned with KCN and Sniper®

	Group	WBC (10^6/µl)	LYMPH (10^6/µl)	MONO (10^6/µl)	GRAN (10^6/µl)	LYMPH (%)	MONO (%)	GRAN (%)	HGB (g/dl)	RBC (10^6/µl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDWCV (%)	RDWS (fl)	PLT (%)	MPV (fl)	PDW (%)	PCT (%)
Group	1	0.41	0.91**	0.95**	0.07	0.92**	0.88**	0.66**	0.84**	0.89**	0.96**	0.91**	0.77**	0.28	0.58*	0.38	0.19	0.52*	0.08	0.22
WBC (10^6/µl)	0.41	1	0.13	0.19	0.74**	0.31	0.04	0.11	0.12	0.38	0.47*	0.56*	0.28	0.43	0.01	0.52*	0.23	0.18	0.6**	0.46
LYMPH (10^6/µl)	0.91**	0.13	1	0.94**	0.14	0.82**	0.9**	0.77**	0.82**	0.81**	0.83**	0.81**	0.59**	0.44	0.55*	0.25	0.3	0.49*	0.07	0.02
MONO (10^6/µl)	0.95**	0.19	0.94**	1	0.12	0.84**	0.91**	0.75**	0.86**	0.82**	0.89**	0.81**	0.67**	0.48*	0.66**	0.19	0.19	0.59*	0.02	0.03
GRAN (10^6/µl)	0.07	0.74**	0.14	0.12	1	0.04	0.27	0.31	0.14	0.003	0.12	0.21	0.12	0.57*	0.24	0.14	0.19	0.24	0.56*	0.14
LYMPH (%)	0.92**	0.31	0.82**	0.84**	0.04	1	0.82**	0.56*	0.79**	0.79**	0.89**	0.86**	0.85**	0.22	0.55*	0.42	0.21	0.52*	0.05	0.37
MONO (%)	0.88**	0.04	0.9**	0.91**	0.27	0.82**	1	0.79**	0.85**	0.79**	0.8**	0.68**	0.72**	0.55*	0.64**	0.08	0.32	0.76**	0.19	0.04
GRAN (%)	0.66**	0.11	0.77**	0.75**	0.31	0.56*	0.79**	1	0.74**	0.66**	0.65**	0.62**	0.45	0.69**	0.59**	0.01	0.62**	0.58*	0.2	0.37
HGB (g/dl)	0.84**	0.12	0.82**	0.86**	0.14	0.79**	0.85**	0.74**	1	0.83**	0.89**	0.69**	0.73**	0.41	0.72**	0.1	0.5*	0.6**	0.24	0.12
RBC (10^6/µl)	0.89**	0.38	0.81**	0.82**	0.003	0.79**	0.79**	0.66**	0.83**	1	0.94**	0.8**	0.68**	0.4	0.51*	0.24	0.37	0.49*	0.07	0.2
HCT (%)	0.96**	0.47*	0.83**	0.89**	0.12	0.89**	0.8**	0.65**	0.89**	0.94**	1	0.89**	0.78**	0.26	0.59*	0.33	0.29	0.49*	0.05	0.17
MCV (fl)	0.91**	0.56*	0.81**	0.81**	0.21	0.86**	0.68**	0.62**	0.69**	0.8**	0.89**	1	0.64**	0.07	0.35	0.61**	0.2	0.31	0.24	0.22
MCH (pg)	0.77**	0.28	0.59**	0.67**	0.12	0.85**	0.72**	0.45	0.73**	0.68**	0.78**	0.64**	1	0.23	0.68**	0.17	0.32	0.55*	0.22	0.35
MCHC (%)	0.28	0.43	0.44	0.48*	0.57*	0.22	0.55*	0.69**	0.41	0.4	0.26	0.07	0.23	1	0.64**	0.52*	0.47*	0.55*	0.42	0.24
RDWCV (%)	0.58*	0.01	0.55*	0.66**	0.24	0.55*	0.64**	0.59**	0.72**	0.51*	0.59*	0.35	0.68**	0.64**	1	0.18	0.43	0.43	0.31	0.08
RDWS (fl)	0.38	0.52*	0.25	0.19	0.14	0.42	0.08	0.01	0.1	0.24	0.33	0.61**	0.17	0.52*	0.18	1	0.17	0.3	0.35	0.4
PLT (%)	0.19	0.23	0.3	0.19	0.19	0.21	0.32	0.62**	0.5*	0.37	0.29	0.2	0.32	0.47*	0.43	0.17	1	0.23	0.51*	0.46
MPV (fl)	0.52*	0.18	0.49*	0.59*	0.24	0.52*	0.76**	0.58*	0.6**	0.49*	0.49*	0.31	0.55*	0.55*	0.43	0.3	0.23	1	0.3	0.08
PDW (%)	0.08	0.6**	0.07	0.02	0.56*	0.05	0.19	0.2	0.24	0.07	0.05	0.24	0.22	0.42	0.31	0.35	0.51*	0.3	1	0.13
PCT (%)	0.22	0.46	0.03	0.03	0.14	0.37	0.04	0.37	0.12	0.2	0.17	0.2	0.35	0.24	0.08	0.4	0.46	0.08	0.13	1

** = Correlation is significant at the 0.01 level (2-tailed). * = Correlation is significant at the 0.05 level (2-tailed).

Table 3 shows the biochemical result after acute poisoning of the rabbits with potassium cyanide and Sniper®. There was significant ($p \leq 0.05$) increase in the concentrations of AST and CREA in both experimental groups compared to the control group. ALT was significantly higher ($p \leq 0.05$) in the Sniper® group compared to the control and potassium cyanide groups. There was significant decrease the concentration of CHO in both experimental groups compared to the control group. There was also significant increase in the concentrations of ALB and T BIL in the potassium cyanide group compared to the control group.

In both the control and experimental groups (Table 4), Group, positively correlated with ALT, TP, CHO, TRIG, ALP and HDL at 0.01 level of significance but correlated with AST and UREA at 0.05 level of significance. UREA

positively correlated with CHO and TRIG at 0.01 level of significance. Also, Cl positively correlated with ALB, TP and ALP at 0.01 level of significance. Additionally, CHO correlated with AST, CREA, ALT, TP, ALP, TRIG and HDL at 0.05 level of significance.

On day 1, Sniper® buried had the highest mean ± S.E value for concentration ($p \leq 0.05$) compared to other groups (Table 5). Additionally, the mean ± S.E purity values of all extracted DNA samples were within the acceptable range (1.8-2.0) in all groups except the KCN hanged group which was significantly higher ($p \leq 0.05$) than the normal range. On days 14 and 21, DNA concentration significantly ($p \leq 0.05$) decreased in Control hanged, KCN hanged and Sniper® hanged compared to their mean ± S.E values on day 1. However, purity decreased overtime in all groups.

Table 3: Effect of potassium cyanide and Sniper® on the biochemical indices in rabbits

Parameters	Control			Potassium cyanide			Sniper®		
	Mean ± S.E.	95% Confidence Interval for Mean		Mean ± S.E.	95% Confidence Interval for Mean		Mean ± S.E.	95% Confidence Interval for Mean	
		Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound
AST (UL)	566.03±157.94	160.03	972.03	960.27±115.59	663.13	1257.4	938.37±63.67	774.7	1102.03
ALT(UL)	60.06±10.45$^{(c)}$	33.21	86.91	76.95±0.92$^{(c)}$	74.58	79.31	157.7±3.06$^{(a,b)}$	149.84	165.56
ALP(UL)	317.08±24.95$^{(b,c)}$	252.96	381.21	131.59±6.7$^{(a)}$	114.36	148.82	153.1±12.04$^{(a)}$	122.15	184.05
TP (g L^{-1})	42.8±0.11$^{(b,c)}$	42.53	43.07	60±0.59$^{(a)}$	58.49	61.51	56.45±4.05$^{(a)}$	46.04	66.86
ALB (g L^{-1})	38.9±0.15$^{(b)}$	38.51	39.29	50.95±0.61$^{(a)}$	49.39	52.51	45.55±5.3	31.93	59.17
CHO(mmol L^{-1})	7.78±0.41$^{(b,c)}$	6.72	8.83	3.37±0.12$^{(a,c)}$	3.06	3.68	1.93±0.14$^{(a,b)}$	1.57	2.29
HDL(mmol L^{-1})	1.2±0.05$^{(c)}$	1.06	1.335	1.05±0.1$^{(c)}$	0.79	1.3	0.4±0.06$^{(a,b)}$	0.24	0.55
CREA(mmol L^{-1})	59.85±2.48$^{(b,c)}$	53.47	66.23	135.95±3.02$^{(a,c)}$	128.19	143.71	89.2±3.76$^{(a,b)}$	79.55	98.86
UREA(mmol L^{-1})	5.35±0.3$^{(c)}$	4.58	6.12	4.95±0.73$^{(c)}$	3.07	6.83	7.2±0.07$^{(a,b)}$	7.01	7.39
Cl (mmol L^{-1})	9.87±0.8	7.82	11.92	13.14±1	10.55	15.71	12.05±1.64	7.49	16.61
TRIG (mmol L^{-1})	0.99±0.18$^{(c)}$	0.52	1.46	1.33±0.15	0.94	1.72	1.83±0.09$^{(a)}$	1.59	2.07
T BIL (µmol L^{-1})	0.95±0.17$^{(b)}$	0.51	1.39	1.55±0.09$^{(a)}$	1.32	1.78	1.35±0.08	1.1537	1.5463

Values are expressed as mean ± SE for six (6) rabbits. The mean difference is significant at the 0.05 level. (a) = $p \leq 0.05$ as compared with the normal control group. (b) = $p \leq 0.05$ as compared to Potassium cyanide group. (c) = $p \leq 0.05$ as compared with the Sniper® group. The significance of differences among all groups was determined by the Tukey HSD test. Alkaline phosphatases (ALP), Creatinine (CREA), Total bilirubin (T BIL), Chloride (Cl), Alanine aminotransferase (ALT), Triglyceride (TRIG), Total protein (TP), Albumin (ALB), Aspartate aminotransferase (AST), Cholesterol (CHO), High-density lipoproteins (HDL)

Table 4: Correlations between biochemical indices in rabbits acutely poisoned with KCN and Sniper®

	GROUP	AST (UL)	CREA (µmol L^{-1})	ALT (UL)	UREA (µmol L^{-1})	Cl (µmol L^{-1})	ALB (g L^{-1})	TP (g L^{-1})	CHO (µmol L^{-1})	TRIG (µmol L^{-1})	ALP (UL)	T BIL (µmol L^{-1})	HDL (µmol L^{-1})
GROUP	1	0.47*	0.37	0.89**	0.53*	0.33	0.32	0.61**	0.93**	0.72**	0.74**	0.45	0.85**
AST (UL)	0.47*	1	0.53*	0.47*	0.3	0.33	0.06	0.26	0.66**	0.1	0.29	0.63**	0.21
CREA (µmol L^{-1})	0.37	0.53*	1	0.07	0.17	0.45	0.67**	0.79**	0.63**	0.08	0.76**	0.64**	0.001
ALT (UL)	0.89**	0.47*	0.07	1	0.7**	0.11	0.2	0.41	0.8**	0.52*	0.38	0.39	0.85**
UREA (µmol L^{-1})	0.53*	0.3	0.17	0.7**	1	0.08	0.04	0.08	0.45	0.21	0.06	0.28	0.45
Cl (µmol L^{-1})	0.33	0.33	0.45	0.11	0.08	1	0.82**	0.8**	0.34	0.44	0.63**	0.25	0.27
ALB (g L^{-1})	0.32	0.06	0.67**	0.2	0.04	0.82**	1	0.93**	0.49*	0.05	0.62**	0.36	0.09
TP (g L^{-1})	0.61**	0.26	0.79**	0.41	0.08	0.8**	0.93**	1	0.75**	0.32	0.84**	0.51*	0.33
CHO (µmol L^{-1})	0.93**	0.66**	0.63**	0.8**	0.45	0.34	0.49*	0.75**	1	0.53*	0.78**	0.65**	0.69**
TRIG (µmol L^{-1})	0.72**	0.1	0.08	0.52*	0.21	0.44	0.05	0.32	0.53*	1	0.58*	0.16	0.75**
ALP (UL)	0.74**	0.29	0.76**	0.38	0.06	0.63**	0.62**	0.84**	0.78**	0.58*	1	0.37	0.43
T BIL (µmol L^{-1})	0.45	0.63**	0.64**	0.39	0.28	0.25	0.36	0.51*	0.65**	0.16	0.37	1	0.23
HDL (µmol L^{-1})	0.85**	0.21	0.001	0.85**	0.45	0.27	0.09	0.33	0.69**	0.75**	0.43	0.23	1

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

Table 5: Effect of potassium cyanide and Sniper® on the integrity of rabbits' DNA on days 1, 14 and 21

Groups	Conc (ng/µl)			Purity		
	Day 1	Day 14	Day 21	Day 1	Day 14	Day 21
Control buried	53±1.16$^{(e)}$	35.33±11.22	50.33±8.17	1.9±0.06$^{(d)}$	1.59±0.01$^{(b,d,e)}$	1.13±0.09
Control hanged	60.33±4.84$^{(e)}$	46.67±10.65	40±8.39	1.78±0.15$^{(d)}$	1.09±0.05$^{(a,c,f)}$	1.17±0.09
KCN buried	48±2.31$^{(e)}$	47.67±8.88	40±4.36	1.83±0.1$^{(d)}$	1.54±0.08$^{(b,e)}$	1.24±0.03
KCN hanged	53.67±1.86$^{(e)}$	33.33±2.4	26±3.79	2.98±0.04$^{(a,b,c,e,f)}$	1.27±0.07$^{(a)}$	1.22±0.13
Sniper® buried	89.33±11.84$^{(a,b,c,d,f)}$	61±13.89	46±15.5	1.87±0.4$^{(d)}$	1.21±0.06$^{(a,c,f)}$	1.14±0.02
Sniper® hanged	45±4.04$^{(e)}$	29.33±8.11	18.67±1.86	1.94±0.03$^{(d)}$	1.54±0.07$^{(b,e)}$	1.48±0.05

Values are expressed as mean ± SE for three (3) rabbits. The mean difference is significant at the 0.05 level. (a) = $p \leq 0.05$ compared with the control buried group. (b) = $p \leq 0.05$ compared to control hanged group. (c) = $p \leq 0.05$ compared with the KCN buried group. (d) = $p \leq 0.05$ compared with the KCN hanged group. (e) = $p \leq 0.05$ compared to Sniper® buried group. (f) = $p \leq 0.05$ compared with the Sniper® hanged group. The significance of differences among all groups was determined by the Tukey HSD test

In Table 6, Pearson correlation analysis showed that there was a correlation between the concentration of DNA (day 1) and DNA's concentration on day 14 at 0.01 level of significance. Concentration of DNA (day 1) correlated with DNA's concentration (day 21) and DNA's purity (day 21) at 0.05 level of significance. Concentration of DNA (day 21) correlated with DNA's concentration on days 1 and 14 at 0.05 level of significance. Purity of

DNA (day 14) correlated with DNA's concentration on day 1 ($p \leq 0.05$). However, DNA's purity values on days 1 and 21 did not correlate with neither the concentration nor purity values of days 1, 14 and 21.

Table 6: Correlation between Group and spectrophometric parameters on different days

		Group	Conc (ng/µl)			Purity		
			Day 1	Day 14	Day 21	Day 1	Day 14	Day 21
Group		1	0.15	-0.004	-0.47	0.18	-0.04	0.5*
Conc. (ng/µl)	**Day 1**	0.15	1	0.68**	0.54*	-0.11	-0.5*	-0.39
	Day 14	-0.004	0.68**	1	0.58*	-0.16	-0.36	-0.44
	Day 21	-0.47	0.54*	0.58*	1	-0.22	-0.06	-0.45
Purity	**Day 1**	0.18	-0.11	-0.16	-0.22	1	-0.21	-0.01
	Day 14	-0.04	-0.5*	-0.36	-0.06	-0.21	1	0.34
	Day 21	0.5*	-0.39	-0.44	-0.45	-0.01	0.34	1

* = Correlation is significant at the 0.05 level (2-tailed). ** = Correlation is significant at the 0.01 level (2-tailed).

The genomic DNA fragmentation in tissue and bone extracts were visualized using gel electrophoresis (figures 1 and 2). Lanes A-I represent the DNA samples isolated from the animal tissues from day 1. In figure 2, L is the DNA ladder while the next 12 lanes are the DNA isolated from the animal tissue between day 14 to 21.



Figure 1: Agarose gel electrophoresis showing the DNA isolates from the tissues of the rabbits. Lanes A-F represents the DNA from the tissues gotten on day 1, the next 3 lanes (G-I) were gotten on day 7, A=Sniper1, B=Sniper2, C=Potassium cyanide1, D= Potassium cyanide2, E=Control 1, F=Control 2, G= Potassium cyanide1 hanged, H= Control 1, I=Sniper1 hanged



Figure 2: Agarose gel electrophoresis showing the DNA isolates from the tissues of the rabbits. Lanes 1-6 represents the DNA from the tissues gotten on day 14, the next 6 lanes (7-12) were gotten on day 21. L=DNA ladder, 1=Control1 buried, 2=Sniper2 hanged, 3= Potassium cyanide2, 4=Control2 hanged, 5=Sniper1 buried, 6=Potassium cyanide buried, 7=Sniper hanged, 8=control hanged, 9=Sniper buried, 10=Potassium cyanide buried, 11. Potassium cyanide hanged, 12=Control buried

Five bacteria were isolated from this study and they were identified biochemically using Analytical profiling index (API) analysis. The isolates were identified as *Aeromonas hydrophila/caviae, Elizabethkingia meningoseptica, Pseudomonas, luteola, Vibrio fluvialis, Burkholderia cepacia* as shown in table 7.

Table 7: Biochemical identification of isolates using API 20 NE kits

Groups	NO_3	TRP	GLU	ADH	URE	ESC	GEL	PNG	GLUC	ARA	MNE	MAN	NAG	MAL	GNI	CAP	ADI	MLT	CIT	PAC	OX	Organisms
Sniper® hanged	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	*Aeromonas hydrophila/ caviae*
Sniper® buried	+	-	+	-	+	+	+	+	+	-	+	+	+	-	-	-	-	-	-	-	+	*Elizabethkingia meningoseptica*
Sniper® hanged	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	*Pseudomonas luteola*
KCN hanged	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	*Vibrio fluvid*
Sniper® hanged	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	-	-	+	-	*Burkholderia cepacia*

PAC - Phenylacetic acid utilization, TRP - Tryptophan deaminase, NAG - N-acetyl glucosamine utilization, ADH- Arginine dihydrolase, ESC - Esculin ferric citrate, GEL - Gelatin liquefaction, PNPG - β-Galactosidase, URE - UreaseGNT - gluconic acid utilization ARA - Arabinose utilization MNE - Mannose utilization, MAN- Mannitol utilization, GLU - Glucose acidification, MAL - Maltose utilization, GLUC - Glucose utilization, OX - Oxidase ADI - Adipic acid utilization, MLT - Malic acid utilization, CIT - Citric acid utilization

There was observable bloating on the 3rd day in the hanged animals. On the 4th day, the bloating reduced in the hanged animals and flies perched on their carcasses. Decomposition was observed in all the hanged animals. On the 5th day, the number of flies increased. Dead flies were also noticed at the foot of the tree where the animals administered

with KCN and Sniper® were hanged. Decomposition was obvious. On the 6th day, decomposition rate was faster and more prominent in KCN intoxicated animals than in Sniper® and control group.

On the 7th day, there was little observation of skeleton from the KCN treatment, flies were still observed in the Sniper® treatment. Second set of samples were collected in sample bottles for DNA typing. On the 8th day, decomposition rate increased drastically, flies were still feeding on the carcasses. This observation continued till the 13th day. On the 14th day, skeletonization was observed in KCN treatment only, decomposition continues in Sniper® and control treatments. Samples were taken from the 3 groups for DNA processing from both the buried and hanged animals. No proper decomposition was noticed in the buried animals. However, an observed decomposition was found in buried KCN treatment. On the 15th and 16th day, numbers of flies reduced in all treatments, but drastically in KCN treatment. This observation continued till the 19th day. On the 20th day, proper skeletonization has been observed in all animals hanged. On the 21st day, skeletonization and reduced number of flies were observed in hanged animals. Buried animals were still decomposing though at a slow rate. Samples were collected from hanged and buried animals. Daily data on the temperature and humidity of the study site was obtained from the Department of Geography, University of Lagos, Nigeria.

Figure 3 shows the environmental condition over the 21 days that this study was carried out. The maximum humidity was 26 and the minimum was 15 while the minimum and maximum temperatures was 27 ºC and 31ºC, respectively.

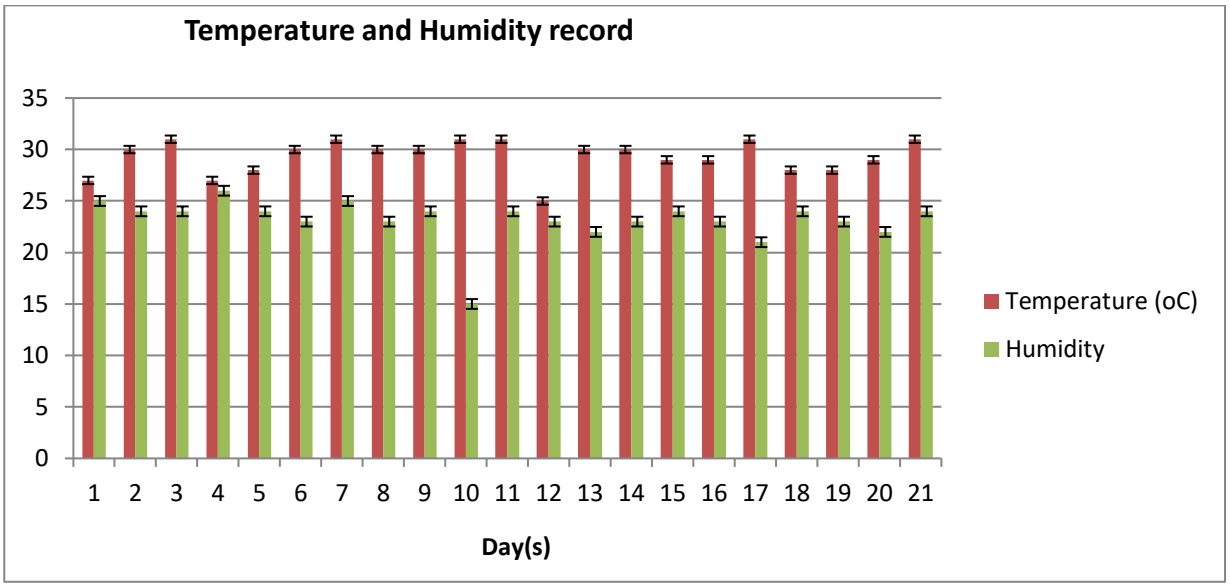


Figure 3: Temperature and Humidity reading from day 1 to day 2

The insects captured on the site of the experiment were identified. For the hanged animals, *Sarcophage Exuberans* (Fleshfly), Phidole, *Chrysomya Chlorpyga* (Blowfly), *Camponostus perrissi* (Black carpenter ant) and *Musca domestic* (Housefly) were identified while only Maggots and *Camponostus perrissi* (Black carpenter ant) were identified in the buried animals. *Camponostus perrissi* (Black carpenter ant) were collected from both the hang and burial sites.

The liver, kidney, lungs and heart were collected from each rabbit. Histological sections of kidney tissues (Figures 4 - 6) of

both the control and experimental groups showed normocellular glomerular tufts disposed on a background containing viable tubules. Congested blood vessels were seen in the experimental groups. No abnormalities were seen in the control group.

Histological sections of heart muscles both the control and experimental groups (Figures 7 - 9) showed interlacing fascicles of cardiac myocytes/ myocardial cells. Congested blood vessels were also seen in the experimental groups. No abnormalities were seen in the control group.

Histological sections of liver of the animals revealed their general structures, Central vein, portal vein and the basophilic portion with nucleus and the acidophilic cytoplasm of the acinar cells (figures 10 -12). Congested blood vessels were also seen in the experimental groups while the histologic section of liver of the control group revealed general structure, Central vein, portal vein and the basophilic portion with nucleus and the acidophilic cytoplasm of the acinar cells. No abnormalities are seen in the control group.

Histologic sections of lung tissues of the experimental groups (Figures 13 -15) showed alveolar air spaces and bronchioles on a background interstitium containing blood vessels and few inflammatory cells. Some congested blood vessels were also seen in them. However, no abnormalities were seen in the control group.

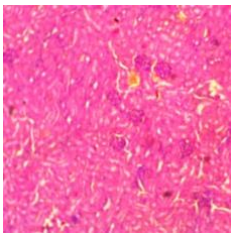


Figure 4: Histological sections of kidney tissues (Control group)

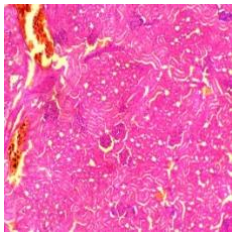


Figure 5: Histological sections of kidney tissues (Potassium cyanide group)

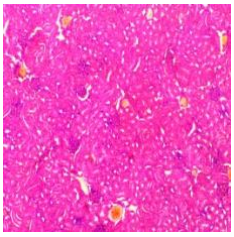


Figure 6: Histological sections of kidney tissues (Sniper® group)

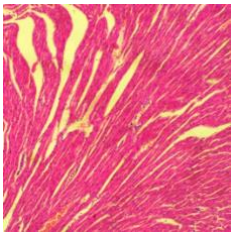


Figure 7: histological sections of the heart tissue (Control group)

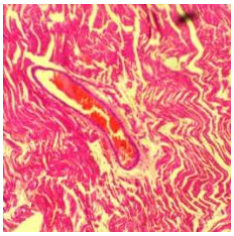


Figure 8: histological sections of the heart tissue (Potassium cyanide group)

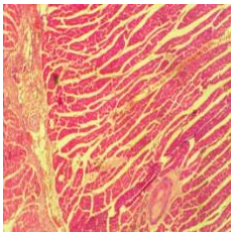


Figure 9: histological sections of the heart tissue (Sniper® group)

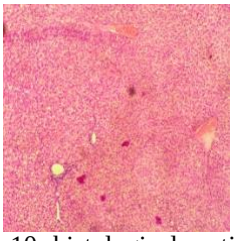
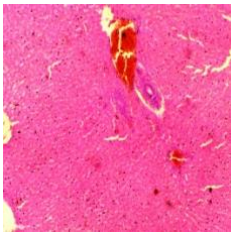


Figure 10: histological sections of the liver tissues (Control group)



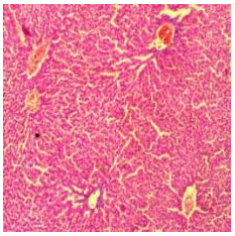


Figure 12: histological sections of the liver tissues (Sniper® group)

Figure 11: histological sections of the liver tissues (Potassium cyanide group)

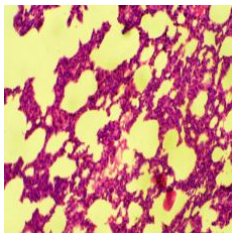


Figure 13: histological sections of the lung tissues (Control group)

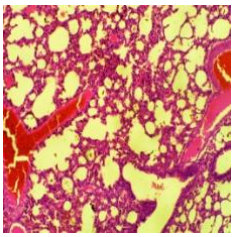


Figure 14: histological sections of the lung tissues (Potassium cyanide group)

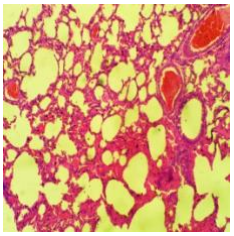


Figure 15: histological sections of the lung tissues (Sniper® group)

DISCUSSION

Forensic pathology is a subspecialty of forensic medicine and medico-legal practice that deals mainly with the dead (Balachandra *et al*. 2011). Forensic toxicology is the science which applies the principles of analytical chemistry and clinical toxicology for medico-legal purpose where the results are probably used in court (Wyman, 2012). The overall results of this study indicated that exposure to KCN and Sniper® could induce oxidative stress in plasma and changes in certain haemorheological parameters. There was a significant general decrease in WBC, HGB, RBC, HCT and MCV in both experimental groups compared to the control group. This agrees with the report of Zhang *et al*., (2010) who also documented certain alterations in the haematological parameters of their animals.

This study also evaluated the deleterious effects of oral potassium cyanide and Sniper® insult on various organs and tissues in rabbits. Histologic sections of kidney tissues of both the control and experimental groups showed normocellular glomerular tufts disposed on a background containing viable tubules. Congested blood vessels were seen in the experimental groups. However, no abnormalities were seen in the control group. This agrees with the findings of Avais *et al*., (2018) who reported that the kidneys of rabbits in experimental group of their study demonstrated severe glomerular and tubular necrosis and congestion. Sousa *et al*., (2003) found that the histological kidney sections of rats fed with KCN had alterations in kidney tissue, characterized by congestion and cytoplasmic vacuolation of epithelial cells of proximal tubules. Organophosphorus insecticides poisoning damages the capillary wall of the kidney during poisoning and aggravates necrosis of the renal tubule. It also destroys erythrocytes after entering the body, which causes acute intravascular haemolysis. The kidneys are impaired by a large amount of haemoglobin accumulating in renal tubules (Cao *et al*. 2006).

Congested blood vessels were also seen in both experimental groups while the histologic section of liver of the control group revealed general structure, Central vein, portal vein and the basophilic portion with nucleus and the acidophilic cytoplasm of the acinar cells. This is consistent with the findings of Avais *et al*., (2018) who stated that the histopathological examination of the animals in experimental group revealed severe hepatocyte vacuolation and degeneration in liver. They also reported excessive congestion in liver and bile duct of rabbits in experimental group. Hence, no abnormalities were seen in the control group. The study revealed elevated serum levels of urea and creatinine in both experimental groups compared to control group. These findings are agreement with those of Okolie and Osagie (1999) who also documented that serum levels of urea and creatinine were significantly higher in cyanide treated rabbits compared to control values. Exposure to cyanide has been documented to produce pathology in the liver and kidney of

experimental animals without any significant change in the levels of urea and creatinine (Iyayi 1991, Soto-Blanco and Gorniak 2003). Okolie and Osagie, (1999) also observed focal areas of congestion and necrosis in the liver of cyanide fed rabbits. Alterations in AST and ALT activities were observed in both experimental groups, which could be the consequence of hepatic lesion (Sousa *et al.* 2003) and indication of damage to hepatocytes (Watson *et al.* 2014) respectively.

Histologic sections of heart muscles of both experimental groups showed interlacing fascicles of cardiac myocytes/myocardial cells with congested blood vessels. There were no abnormalities in the histological heart tissue sections of the control group. Prolonged exposure to cyanide has been linked to disturbance in thyroid, lesions in liver, kidneys, lungs (Okolie and Osagie 1999) and also CNS pathology (Soto-Blanco *et al* 2002a, 2002b). Organophosphorus insecticides poisoning has been implicated in the destruction erythrocytes after entering the body, which causes acute intravascular haemolysis (Cao *et al.* 2006). Cyanide or cyanogenic compounds have been documented to produce deleterious effects such as neurotoxicity, cardiotoxicity, cardiovascular changes, gastrointestinal ulcerogenesis, nephrotoxicity, reproductive toxicity and teratogenic effects (Ballantyne and Marrs 1987).

DNA was extracted from the muscle tissues of the all rabbits in the three groups on days 1, 14 and 21. On day 1, the animal which was poisoned with Sniper® and buried yielded more DNA compared to other groups. Overtime, DNA purity decreased in all groups from day 14 to day 21 and thus, the DNA quality was below the acceptable range of 1.8 - 2.0 at 260/280 absorbance. Decomposition degrades DNA and therefore decrease DNA profiling success (Tozzo *et al.* 2015). Agarose gel electrophoresis revealed general degradation of all DNA samples which were extracted from all experimental groups as the visible bands appeared smeared. Hreljac *et al*., (2008) also reported that organophosphorus pesticides are genotoxic and mitogenic.

Decomposition rate was higher and prominent in KCN intoxicated animals than in the Sniper® and control groups. The limited insect activity caused by Sniper® explains the delay in decomposition of Sniper® treated animals. This prevents colonization by Diptera which are usually the first set of insects to colonize dead bodies Paula *et al.* (2016). The following bacteria were isolated from this study *Aeromonas hydrophilia/caviae, Elizabethkingia meningoseptica, Pseudomonas luteola, Vibrio fluidis, Burkholderia cepacia*. Microbial forensics, also known as the microbiology of death, is an emerging branch of science that is still underused in criminal investigations (Speruda *et al.* 2022). Thanatomicrobiome and epinecrotic communities are used in forensic sciences especially in the prediction of post-mortem interval. The following insects were also collected and identified from the experimental sites; *Sarcophage Exuberans* (Fleshfly), *Chrysomya Chlorpyga* (Blowfly), *Camponostus perrissi* (Black carpenter ant) and *Musca domestic* (Housefly). In forensic entomology, accurate identification of insects that colonize a decomposing body is very crucial in estimation post-mortem interval. Right from the early stages of putrefaction, insects are attracted to the decomposing body and may lay eggs in it. Therefore, studying the insect population and the developing larval stages, forensic scientists can estimate the postmortem index, any change in position of a decomposing body (Joseph *et al*. 2011).

CONCLUSION

There have been increased cases of suicide in Nigeria where the victims ingested Sniper® and these incidences of death by ingestion of Sniper® in Nigeria have become worrisome. Oral potassium cyanide and Sniper® administration have harmful effects on liver, kidney and hematologic parameters. Potassium cyanide and Sniper® had similar effects on the organs studied. There is a great need for the Nigerian government to come up with more stringent measures that will limit the access of individuals to these toxic chemicals especially Sniper®.

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