



Assessment of Some Biochemical Parameters in Vitreous Humor of Rabbits Exposed to Sodium Cyanide

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Authors' contributions

This work was carried out in collaboration among all authors. All listed authors participated in diverse ways in contributing to the success of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to assess some biochemical parameters in the vitreous humor of Rabbits exposed to sodium cyanide.

Study Design: This study is an interventional study.

Place and Duration of Study: This study was carried out at Animal House, Applied and Environmental Biology Department, Rivers State University, Port Harcourt, Rivers State, Nigeria, between April 2020 and November 2020.

Methodology: A total of twelve (12) rabbits as indicated by Mead's formula constituted the sample size. The study was divided into three groups including the control. 1 mg/kg sodium cyanide was administered to the rabbits orally in group one and vitreous humor was collected using standard procedure after thirty minutes. In group two the rabbits were put to death mechanically and 1 mg/kg sodium cyanide was administered to the rabbits after thirty minutes, vitreous humor was collected after thirty minutes using standard procedure. The rabbits in group three, control group, were put to death mechanically and nothing was given to the rabbits, then after thirty minutes vitreous humor was collected using standard procedure. Biochemical parameters investigated included vitreous

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glucose, total cholesterol, triglycerides, high density lipoprotein cholesterol, low density lipoprotein cholesterol, very low density lipoprotein cholesterol, total proteins (TP), albumin (ALB), total bilirubin, conjugated bilirubin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities. Data were expressed as mean \pm SD. Statistical differences between groups were computed using Graph pad prism 7.0 version developed by Graph pad software, San Siago, California, USA. Results were analyzed using analysis of variance (ANOVA) and significance between groups was taken at $p < .05$.

Results: Vitreous biochemical results showed significant ($p < .05$) increase in levels of TC, LDL-C, AST, ALT, TB and CB in rabbits given 1 mg/kg sodium cyanide compared to control. Significant ($p < .05$) decrease in levels of glucose, TP and ALB were also observed. This study also revealed that there was no significant ($p < .05$) difference in biochemical results of rabbits given 1 mg/kg sodium cyanide thirty minutes after they were put to death mechanically compared to the control. It can be concluded that a lethal dose of sodium cyanide (1 mg/kg) may lead to alterations in vitreous biochemical parameters and this may enhance death differentiation due to sodium cyanide poisoning and other causes of death for example mechanical death.

Conclusion: The findings of this study support a central role for vitreous humor biochemistry in many postmortem forensic and pathological evaluations and it could also be used for death differentiation in sodium cyanide poisoning.

Keywords: Assessment; biochemical parameters; vitreous humor; rabbits; sodium cyanide.

1. INTRODUCTION

The continuous exposure of humans and animals to moderate or high cyanide concentrations is deleterious to the body. Due to the binding of small amount of cyanide to the ferrous ion on the haemoglobin molecule, pulsatile intoxication of cyanide could build up in the system leading to undesirable effect. The toxic effects of cyanide in humans and animals are generally alike and are assumed to result from inactivation of cytochrome oxidase and inhibition of cellular respiration and histotoxic anoxia [1]. Principal features of the toxicity profile for cyanide are, high acute toxicity by all routes of administration, with a very steep and rate-dependent dose-effect curve, and chronic toxicity, probably mediated through the main metabolite and detoxification product, thiocyanate [1]. Based on the mechanism of action of cyanide, it is classified as asphyxiant. Asphyxiants are substances or poisons which produce respiratory embarrassment, leading to asphyxia.

Cyanide not only decreases the oxygen content of blood, but also decreases oxygen availability to tissue thereby producing a greater degree of tissue hypoxia than equivalent reduction in oxyhaemoglobin caused hypoxia [2]. It also binds to other heme proteins such as myoglobin and mitochondrial cytochrome oxidase, which limit oxygen use when tissue oxygen is very low. Organs with high oxygen demand, such as the heart and brain are most sensitive to hypoxia and account for the major clinical sequelae of cyanide

poisoning [2]. Cyanide being lipophilic could access the circulatory system including the vitreous humor of the eye. The access by cyanide is the basis of the hypoxic and asphyxic mechanism of action. The blood is a major medium for the transportation of cyanide across the body. In the blood, cyanide inhibits other component of the blood at times even binds on some. This seamless relationship explains the pattern of its effect on biochemical parameters [3].

The vitreous humor is located between the lens and the retina with similar biochemical composition to that of serum. Sodium cyanide being lipophilic can access the vitreous through the eye or blood circulatory system. Vitreous humor is located between the lens and the retina filling the centre of the eye [4]. It is a fluid that is relatively well protected from postmortem degradation and contamination. Due to its postmortem stability, vitreous humor has high utility in forensic pathology. Ante-mortem serum biochemical alterations are a regular feature in many diseases and the availability of the antemortem serum levels are useful in establishing postmortem diagnosis of different ailments [5]. The relative stability of vitreous biochemistry is useful in assessing the antemortem metabolic status and in predicting the antemortem serum biochemistry of an individual [6].

The suitability of rabbits as a choice animal for this study is attributed to its anatomical and

physiological similarities to human [7]. Lipids are among the most common parameters found in the body's fluids and perform a lot of vital functions. They are active players in the transportation of chemicals, nutrients and other products from one part of the body to another. Also, the studied parameters are essential in the study of diseases as alteration could be diagnostic of a particular or arrays of diseases. The effect of sodium cyanide on lipid profile, glucose and liver parameters could be of clinical and forensic values. Clinically, the causative agents responsible for most idiopathic diseases could be revealed. In forensic, it will help in forming a template for cause of death and death differentiation due to sodium cyanide poisoning and other causes of death for example mechanical death [8-10]. There have been numerous studies of VH in various forensic applications relating to postmortem biochemistry for screening or confirming preexistent pathology and determining cause of death [11]. Establishing the cause of death is one of the frustrating challenges faced by a forensic pathologist, particularly in situations with limited ante-mortem information about the deceased individual. The postmortem analysis of the vitreous fluid has been suggested to exhibit characteristic findings in certain types of deaths and in certain cases; post mortem vitreous humor analysis may be an important adjunct to confirm an ante mortem diagnosis. Many previous studies have addressed the postmortem concentrations of vitreous humor glucose [12]. A rapid decrease in vitreous glucose levels is caused in the postmortem period due to the anaerobic degradation or glycolysis [12]. Studies have shown that exposure to cyanide directly increased the lipid peroxidation in all the organs. Therefore, the aim of this study was to assess some biochemical parameters in the vitreous humor of Rabbits exposed to sodium cyanide.

2. MATERIALS AND METHODS

2.1 Experimental Animals

A total of twelve (12) rabbits aged between 6 to 8 months white rabbits (*Oryctolagus cuniculus*) that weighed between 1.2 - 1.5 kg constituted the sample size. The rabbits were purchased from a breeder in Port Harcourt, Rivers State, Nigeria. They were divided into three groups. Four rabbits were assigned to each group.

Group1. Four rabbits were exposed to lethal dose (1mg/kg) of sodium cyanide orally, after

thirty minutes vitreous fluid was collected from the rabbits.

Group2. Another four rabbits were put to death mechanically and lethal dose of sodium cyanide was administered after thirty minutes; then, vitreous fluid was collected from the rabbits.

Group3. Four rabbits were put to death mechanically and nothing was giving to the animals. Then, after thirty minutes vitreous fluid was collected from the rabbits (control).

2.1.1 Housing and nutrition

The rabbits were kept in a spacious and well-ventilated cage at room temperature, under natural circadian rhythm and 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* in the animal house. 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 of Sodium Cyanide (Nacn)

Sodium cyanide, 98% purity, produced by Changsha Hekang Chemical Co. Ltd was purchased at Decosmiller Ventures, Ogbete, Enugu, Nigeria.

2.3 Sample Collection

Vitreous samples were collected by method postulated by Coe in [13]. The vitreous samples were collected into plain containers for the biochemical analysis. The samples were spun and the supernatant separated for the analysis. The samples were analyzed for Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), total bilirubin (T.Bil), conjugated bilirubin (C.Bil), vitreous glucose (VG), total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL).

2.4 Laboratory Analysis

Vitreous total protein and albumin were estimated quantitatively using Biuret Method and Bromocresol Green Method as modified by

Randox Laboratories (United Kingdom). AST, ALT and ALP activities were also estimated quantitatively using kinetic Method as specified by Raandox Laboratories, total bilirubin and conjugated bilirubin concentrations were measured using colorimetric method as specified by Randox Laboratories. Total cholesterol, triglyceride and HDL-C concentrations were estimated using enzymatic method as specified by Randox Laboratories. Vitreous glucose, total cholesterol, triglyceride and HDL-C were estimated using enzymatic method as specified by Randox kits. LDL-C and VLDL concentrations were derived mathematically by the formula as shown by Carl and Edward and Friedewald *et al* respectively.

2.5 Statistical Analysis

Data was expressed as mean \pm SD. Statistical differences between groups were computed using Graph pad prism 7.0 version developed by Graph pad software, San Siago, California, USA. Results were analyzed using analysis of variance (ANOVA) and significance between groups was taken at $p < .05$.

3. RESULTS AND DISCUSSION

Vitreous humor is a fluid that is relatively well protected from postmortem degradation and contamination [5]. Due to its postmortem stability, vitreous humor has high utility in forensic pathology. The relative stability of vitreous biochemistry is useful in assessing the antemortem metabolic status and in predicting the antemortem serum biochemistry of an individual [14]. Postmortem vitreous humor biochemistry closely mimics antemortem serum biochemistry and may be a useful aid in establishing postmortem diagnosis of different ailments [5]. The biochemical parameters in Table 1 showed significant ($p < .05$) increase in total cholesterol and low density lipoprotein cholesterol in the actual death while disguised death did not show any significant ($p > .05$) compared to the control. The scientific basis of the alteration in lipid could be attributed to massive efflux of lipid in the cell following cellular hypoxia occasioned by cyanide that exhibit direct toxic action on cellular membrane. This movement results to increased lipid concentration in the body fluid which permeates through the blood retina barrier to the vitreous humor.

This finding agrees with the work of [15] that reported increase in lipid profile of cassava

workers that were exposed to cyanide. However, this finding does not agree with the work of [16] that reported increase in triglyceride and decrease in total cholesterol in vitreous humor of carbon monoxide poisoned rabbit. The difference in the observation might result from the nature of the toxic substance used and the pattern of the toxicity. Similarly, this study observed significant decrease ($p < .05$) in concentration of vitreous glucose in the actual death of the study group. There is no significant difference ($p > .05$) in vitreous glucose level of the disguised death compared to the control. The observed decrease in vitreous glucose demonstrates that glucose utilization by the intraocular tissue continues uninterrupted after death. Forrest in [17] stated that glucose concentration in postmortem fluid sample is of a little value because glycolysis continue after death leading to decrease level of glucose. Also, the decrease could be attributed to cellular hypoxia introduced by cyanide which reduced the oxygen content of the cell that eventually decrease concentration of vitreous glucose. This finding is in agreement with the report of [18] which state that vitreous glucose level decreases within twenty four hours after death. Establishing the cause of death is one of the frustrating challenges faced by a forensic pathologist, particularly in situations with limited ante-mortem information about the deceased individual.

The postmortem analysis of the vitreous fluid has been suggested to exhibit characteristic findings in certain classes of death and in certain cases; post mortem vitreous humor analysis may be an important adjunct to confirm an ante mortem diagnosis [5]. Detection of bilirubin in the vitreous humor has been reported to be always pathological, most likely indicating liver disease [5]. The vitreous biochemical result in Table 2 showed significant ($p < .05$) increase in AST, ALT and bilirubin. This is a demonstration of the effect of cyanide on the hepatocytes (histotoxic hypoxia), these enzymes, AST and ALT, infiltrate into the plasma and vitreous through the blood retina barrier because of the leakage on the hepatocytes. More so, the enzyme responsible for the transfer of bilirubin in liver is also affected by the toxic effect of cyanide, hence increased total and conjugated bilirubin. The study also showed significant ($p < .05$) decrease in total protein and albumin. The observed decreased is attributed to the damaged liver cells resulting from cellular hypoxia occasioned by cyanide. This finding corresponds with the work of [19] that reported increased levels of AST and ALT in

Table 1. Analysis of lipid profile and glucose of vitreous humor of rabbits

S/N	Experimental group	VG (mmo/L)	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	VLDL-C (mmol/L)
1	Control	3.31±0.09 ^a	0.25±0.03 ^a	0.06±0.01	0.07±0.01	0.16±0.04 ^a	0.025±0.01
2	Actual death	2.10±2.10 ^b	0.33±0.02 ^b	0.07±0.02	0.05±0.01	0.25±0.02 ^b	0.032±0.01
3	Disguised death	3.25±0.09 ^a	0.26±0.03 ^a	0.06±0.02	0.06±0.02	0.17±0.03 ^a	0.028±0.01
4	F –value	273.1	9.265	0.931	3.184	10.87	0.8077
5	P –value	<0.0001	0.0065	0.4290	0.0900	0.0040	0.4758

Keys: VG= vitreous glucose, TC= total cholesterol, TG= triglycerides, HDL-C= high density lipoprotein cholesterol, LDL-C= low density lipoprotein cholesterol, VLDL-C= very low density lipoprotein cholesterol. Superscripts: there is significant difference between a and b, but none between same letter.

Table 2. Analysis of liver function parameters of vitreous humor

S/N	Experimental Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total Bilirubin (µmol/L)	Conjugated Bilirubin. (µmol/L)	Total Protein (g/dl)	Albumin (g/dL)
1	Control	8.72±0.51 ^a	6.00±0.73 ^a	7.68±0.27 ^a	0.65±0.04 ^a	0.34±0.02 ^a	3.45±0.03 ^a	0.36.0.04
2	Actual death	41.30±0.81 ^b	35.68±0.63 ^b	21.75±0.67 ^b	0.83±0.01 ^b	0.53±0.01 ^b	3.35±0.03 ^b	0.22±0.12
3	Disguised death	9.09±0.27 ^a	6.51±0.48 ^a	7.77±0.48 ^a	0.67±0.02 ^a	0.36±0.02 ^a	3.46±0.03 ^a	0.37±0.03
4	F –value	3980	2985	1373	51.19	123.4	3.46±0.03	64.83
5	P –value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0010	0.0001	<0.0001

Key: AST = aspartate aminotransferase, ALT = alanine aminotransferase, ALP = alkaline aminotransferase. Superscripts: there is significant difference between a and b, but none between same letter

rats exposed to cyanide. Amith in [5] also reported increased level of bilirubin in vitreous humor of cyanide intoxicated rat.

4. CONCLUSION

The findings of this study support a central role for vitreous humor biochemistry in many postmortem forensic and pathological evaluations and it could also be used for death differentiation in sodium cyanide poisoning.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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