**Vitreous humor Glucose and Lipid profile levels in Rabbits Following Sodium Cyanide Exposure.**

**ABSTRACT**

This study was designed to investigate vitreous humor glucose and lipid profile levels in rabbits following sodium cyanide exposure.The rabbits were grouped into three (control, disguised and test) and the study lasted for twenty-four hours. The following parameters were investigated; fasting blood sugar (FBS), total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL). Data were expressed as mean ± Standard Deviation. Statistical differences between groups were computed using Graph pad prism 7.0 version. Results were analyzed using analysis of variance (ANOVA) and significance between groups was taken at p< 0.05. The results showed significant (p< 0.05) decrease in concentration of FBS in test group compared to control and disguised group. However, there was no significant (p>0.05) difference between control and disguised group. Also, statistical difference was not observed in TC, TG, HDL, LDL and VLDL across the groups. It can be concluded that sodium cyanide is a potential poison with high toxicity effect on glucose and lipid profile levels and the result generated could serve as a supportive tool to autopsy findings in differentiation of death involving sodium cyanide.

**Keywords**: Glucose, Lipid profile, vitreous humor, Sodium cyanide

**INTRODUCTION**

Cyanide is a fast acting, potentially and deadly chemical that can exist in various forms 1. The toxic effects of cyanide ion in humans and animals are generally similar and are believed to result from inactivation of cytochrome oxidase and inhibition of cellular respiration and consequent histotoxic anoxia. Cyanide poisoning may produce some pathologic effects on different tissues that may manifest as alterations in biochemical parameters 2. The most widespread problems arising from cyanide are from chronic /sub chronic exposures. Chronic cyanide toxicity is involved in the pathogenesis of some health problems. Moreover, chronic cyanide intoxication induces alteration in some biochemical, histological and oxidative stress parameters in experimental animal model 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 asphyxia mechanism of action. The blood is a major medium for the transportation of cyanide across the body. 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 been lipophilic access the vitreous through the eye or blood circulatory system 2. 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. Antemortem 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 3. The relative stability of vitreous biochemistry is useful in assessing the antemortem metabolic status and in predicting the antemortem serum biochemistry of an individual 4.

The suitability of rabbits as a choice animal for this study is attributed to its anatomical and physiological similarities to human in terms of histology, biochemical and heamatological parameters 5. 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 6.

There have been numerous studies of Vitreous Humor in various forensic applications relating to postmortem biochemistry for screening or confirming pre-existent pathology and determining cause of death 7. Establishing the cause of death is one of the frustrating challenges faced by a forensic pathologist, particularly in situations with limited antemortem information about the deceased individual. The postmortem analysis of the vitreous fluid has been suggested to exhibit characteristic findings in certain cases of death; post mortem vitreous humor analysis may be an important adjunct to confirm an ante mortem diagnosis. Studies have addressed the postmortem concentrations of vitreous humor glucose. A rapid decrease in vitreous glucose levels in the postmortem period is due to anaerobic degradation or glycolysis 8.

**MATERIALS AND METHOD**

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

**EXPERIMENTAL ANIMALS**: Twelve (12) rabbits were used for the experiment. The animals were purchased at Sandra Farm, Oyigbo, Rivers state, Nigeria.

**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.

**Ethical Approval**

The Animal Welfare Act of 1985 of the United State of America for research and Institutional Animal Care and Use Committee (IACUC) protocol were strictly adhered to. All experiments have been examined and approved by the appropriate ethic committee.

**STUDY DESIGN:** Twelve (12) rabbits were used for the study. The rabbits were arranged into three groups with four rabbits in each of the group and the study lasted for twenty-four hours.

**Group one**: The rabbits were sacrificed without administration of lethal dose of sodium cyanide (1 mg/kg sodium cyanide), after thirty minutes, vitreous fluid was collected from the rabbits. This represents the control group.

**Group two**: The rabbits were sacrificed and lethal dose of 1 mg/kg sodium cyanide was given to the rabbits via injection route. After thirty minutes, vitreous fluid was collected from the rabbits. This represents the disguised death group.

**Group three**: The rabbits were given lethal dose of 1 mg/kg sodium cyanide through injection route and after thirty minutes vitreous fluid was collected. This group represents actual death from sodium cyanide (Test group)

**BIOCHEMICAL ANALYSIS**: Fasting blood sugar and lipid profile parameters were measured using Randox Laboratories United Kingdom reagent kits.

**Statistical analysis**

 Data are expressed as mean ± SD. Statistical differences between groups were computed using Graph pad prism 7.0 versions. Results were analyzed using one-way analysis of variance (ANOVA) and significance between groups was taken at p < 0.05.

**RESULTS**

The result of analysis of fasting blood sugar and lipid profile parameters of vitreous humor is presented in Table 1. The result showed decrease in concentration of fasting blood sugar in actual death compared to control and disguised death. Total cholesterol, triglyceride, high density lipoprotein low density lipoprotein and very low density lipoprotein showed no statistical difference across the groups.

**Table 1: Analysis of Lipids and Glucose of Vitreous Humor of Rabbits** (Mean ±SD)

|  |  |  |
| --- | --- | --- |
| **S/N** | **Experimental Groups** | **Parameters** |
| **FBS (mmo/L)** | **TC (mmol/L)** | **TG (mmol/L** | **HDL-C (mmol/L)** | **LDL-C (mmol/L)** | **VLDL-C (mmol/L** |
| 1 | Control  | 3.31±0.09a | 0.25±0.03a | 0.06±0.01 | 0.07±0.01 | 0.16±0.04a | 0.025±0.01 |
| 2 | Actual death | 2.10±2.10b | 0.33±0.02b | 0.07±0.02 | 0.05±0.01 | 0;25±0.02b | 0.032±0.01 |
| 3 | Disguised death | 3.25±0.09a | 0.26±0.03b | 0.06±0.02 | 0.06±0.02 | 0.17±0.03a | 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: FBS= fasting blood sugar, TC= total cholesterol, TG= triglycerides, HDL-C= high density lipoprotein cholesterol, LDL-C= low density lipoprotein cholesterol, VLDL-C= very low density lipoprotein cholesterol. Means ±SD of experimental groups with different superscripts are significantly different from each other at p<0.05.

**DISCUSSION**

This study evaluated the concentration of biochemical parameters including, glucose and lipid profile in vitreous humor of sodium cyanide poisoned rabbits. The result showed increase in concentrations of vitreous total cholesterol and triglyceride in actual death group of the study. However, the increase was not statistically significant (p>0.05) compared to control and disguised death. The explanation to this observation may not be unconnected to the duration of the study. Similarly, the concentrations of vitreous high density lipoprotein, low density lipoprotein and very low density lipoprotein was not significant (p>0.05) across the groups. Cyanide exposure can alter lipid metabolism, leading to changes in lipid profile and induce oxidative stress, which may affect lipid peroxidation and membrane integrity. Consequently, there will be massive efflux of lipid in the cell. 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 9 that reported increase in lipid profile of cassava workers that were exposed to cyanide.

This study also observed significant decrease in concentrations of vitreous glucose in the actual death group of the study. However, further comparison using tukey multiple comparison showed that there is no significant difference in vitreous glucose level of the control and disguised death. The observed decrease in vitreous glucose demonstrates that glucose utilization by the intraocular tissue continues uninterrupted after death. 10 stated that glucose concentration in postmortem fluid sample is of a little value because glycolysis continues after death leading to decrease level of glucose. Therefore, 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 11 which state that vitreous glucose level decreases within twenty-four hours after death.

Vitreous solutes are used to predict ante‐mortem serum concentrations of the same analyte and the cause of death. Postmortem analysis of the vitreous biochemistry is useful in assessing the antemortem metabolic status and in predicting the antemortem serum biochemistry of an individual 12 .Following the observation made by 11 which reported that during the first 24 h after death, both vitreous and serum glucose approach zero, with the vitreous glucose levels approximating half the serum concentration. Consequently, a vitreous glucose concentration of over 10 mmol/l at a post‐mortem interval of 1 or more days may suggest that a patient may have died during a diabetic coma or as a result of a manifestation of hyperglycaemia. Therefore, the findings generated could serve as a supportive tool to autopsy findings in differentiation of death involving sodium cyanide and predicting ante mortem analytes concentration.

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