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# Hepatotoxic Effects of Sodium Cyanide Exposure: Insights from Vitreous Humor Analysis in Rabbits

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**A B S T R A C T**

This study investigated the hepatotoxic effects of sodium cyanide exposure by analyzing the vitreous humor of rabbits. The rabbits were divided into three groups—control, disguised death, and test group—with the study lasting 24 hours. Liver function parameters evaluated included Total Proteins (TP), Albumin (Alb), Total Bilirubin (TB), Conjugated Bilirubin (CB), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and Alkaline Phosphatase (ALP) activities. Data were expressed as mean ± standard deviation and analyzed using one-way ANOVA via GraphPad Prism 7.0. Significant increases ($p < 0.05$) in TB, CB, AST, ALT, and ALP activities were observed in the test group compared to control and disguised death groups, while significant decreases in TP and Alb were found in the test group relative to the others. The findings indicate that sodium cyanide is a potent hepatotoxic agent, and vitreous humor analysis offers a supportive tool for forensic differentiation of sodium cyanide-related deaths.

Keywords: hepatotoxicity, sodium cyanide, vitreous humor

## INTRODUCTION

Cyanide is an extremely fast-acting and deadly chemical, existing in multiple forms (Sanchez-Verlaan et al., 2011). It causes severe health issues, including damage to liver cells (Okolie, 2002) (Duruibe et al., 2015). Cyanide intoxication may produce some pathologic effects on different tissues that precede alterations in biochemical parameters. Consequently, certain types of cells are damaged and enzymes leak into the blood, where they can be measured as indicators of cell damage (Okolie and Osagie, 1999). Aspartate aminotransferase (AST) is expressed across tissues such as heart, muscle, kidney, brain, lung, and liver; its blood level correlates with tissue damage severity. Alanine aminotransferase (ALT) is primarily produced in the liver, with small quantities from heart, muscle, and kidney, catalyzing amino group transfer between L-alanine and glutamate (Mulla et al., 2005). Changes in these enzymes reflect metabolic and functional integrity of organs (Okolie and Iroanya, 2003).

Due to its lipophilic nature, cyanide can access the circulatory system, including the vitreous humor of the eye, contributing to hypoxic and asphyxia effects. Cyanide's presence in blood can disrupt clotting and bind various components, influencing biochemical parameters. The vitreous humor, situated between the lens and retina, has a biochemical composition similar to serum and is relatively stable postmortem, making it valuable in forensic pathology to infer antemortem metabolic states and diagnoses (Lange et al., 1999, Madea and Musshoff, 2007).

Numerous studies have examined the use of vitreous humor in forensic applications, focusing on postmortem biochemistry to screen for or confirm pre-existing pathological conditions and to help determine the cause of death (Madea and Rodig, 2006).. Determining the cause of death is often a challenging task for forensic pathologists, especially when limited ante mortem information is available about the deceased. Analysis of vitreous fluid postmortem has been shown to reveal characteristic changes in specific cases, making it a valuable tool to support and confirm ante mortem diagnoses (Peclet et al., 1994).

## MATERIALS AND METHOD

### Procurement of Materials

Sodium cyanide; 98% purity, produced by Changsha Hekang Chemical Co. Ltd was purchased from Decosmiller Ventures, Ogbete, Enugu, Nigeria. Twelve rabbits used for the experiment were purchased from 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.

**Study design**

A total of twelve (12) rabbits were utilized in this study and randomly allocated into three experimental groups, each comprising four animals. The duration of the study for each group was 24 hours.

**Group 1 (Test group):** Rabbits received a lethal dose of sodium cyanide at 1 mg/kg via intraperitoneal injection. Vitreous humor samples were collected 30 minutes post-administration to represent death directly resulting from sodium cyanide toxicity.

**Group 2 (Disguised death group):** Rabbits were euthanized prior to administration of sodium cyanide. Subsequently, 1 mg/kg sodium cyanide was administered intraperitoneally, and vitreous humor was collected 30 minutes post-injection to simulate a condition of disguised cyanide-induced death.

**Group 3 (Control group):** Rabbits were euthanized without sodium cyanide exposure. Vitreous humor was collected 30 minutes after euthanasia to serve as baseline controls for comparative analysis.

**Selection Criteria**

Only apparently healthy male and female rabbits of same weight range of 1.4 to 1.8 kg were used for the study.

**Sample Collection:** Vitreous humour samples were collected using a 10 mL syringe fitted with a 21-gauge needle. A puncture was made through the sclera at the lateral canthus to access the vitreous chamber. Care was taken to aspirate the vitreous humor gently from each eye separately, avoiding disruption of surrounding ocular tissues. Approximately 2.5 mL of vitreous humour was aspirated from each eye, yielding a total volume of 5 mL per animal..

**Laboratory Analysis**

Liver function parameters were measured using Randox Laboratories United Kingdom reagent kits for ALT, AST, albumin, bilirubin and total protein

**Statistical Analysis**

The data generated were analyzed using the Windows version of GraphPad Prism 7.0 statistical software, with results reported as mean ± standard deviation. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests were conducted using the same software. Statistical interpretations and inferences were made based on these analyses, considering p-values below 0.05 as statistically significant..

## RESULTS

Table 1 presents the ANOVA analysis of the measured liver function markers, demonstrating statistically significant differences across all parameters among the three groups.

Table 1: Liver Function Parameters in the

| **Experimental Group** | **AST (IU/L)** | **ALT (IU/L)** | **ALP (IU/L)** | **Total Bilirubin (µmol/L)** | **Conjugated Bilirubin (µmol/L)** | **Total Protein (g/dL)** | **Albumin (g/dL)** |
|---|---|---|---|---|---|---|---|
| **Control** | 8.7 ± 0.5 | 6.0 ± 0.7 | 7.6 ± 0.3 | 0.65 ± 0.04 | 0.34 ± 0.02 | 3.5 ± 0.03 | 0.36 ± 0.04 |
| **Actual Death** | 41.3 ± 0.8 | 35.68 ± 0.6 | 21.8 ± 0.7 | 0.83 ± 0.01 | 0.53 ± 0.01 | 3.4 ± 0.03 | 0.22 ± 0.12 |
| **Disguised Death** | 9.09 ± 0.3 | 6.51 ± 0.5 | 7.8 ± 0.5 | 0.67 ± 0.02[a] | 0.36 ± 0.02 | 3.46 ± 0.04 | 0.46 ± 0.03 |
| **F-value** | 3902.34 | 4456.26 | 1025.83 | 77.22 | 83.61 | 9.83 | 10.80 |
| **p-value** | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | 0.0055 | 0.0 |

Table 2 presents the Tukey's HSD post hoc analysis for pair wise group comparisons of the liver function parameters. Significant differences consistently existed between the Control and Actual Death groups, as well as between the Actual Death and Disguised Death groups for all parameters except albumin

which showed significance only in Actual Death vs Disguised Death. There were no statistically significant differences between the Control and Disguised Death groups for all the parameters, indicating similar biochemical profiles in these two groups.

Table 2: Tukey's HSD Post Hoc Analysis of the Liver Function Parameters.

| **Parameter** | **Control vs Actual Death (p- value)** | **Control vs Disguised Death (p- value)** | **Actual Death vs Disguised Death (p- value)** |
|---|---|---|---|
| **AST (IU/L)** | < 0.001 | 0.317 | 0.001 |
| **ALT (IU/L)** | < 0.001 | 0.269 | 0.001 |
| **ALP (IU/L)** | < 0.001 | 0.783 | 0.001 |
| **Total Bilirubin (µmol/L)** | 0.001 | 0.505 | 0.002 |
| **Conjugated Bilirubin (µmol/L)** | 0.001 | 0.439 | 0.002 |
| **Total Protein (g/dL)** | 0.029 | 0.999 | 0.044 |
| **Albumin (g/dL)** | 0.094 | 0.567 | 0.038 |

## Discussion

The results of this study unequivocally demonstrate the hepatotoxic effects of sodium cyanide exposure as evidenced by significant alterations in vitreous humor biochemical parameters in rabbits. One-way ANOVA analysis revealed statistically significant differences ($p < 0.05$) among the control, actual death, and disguised death groups across all measured liver function parameters, indicating pronounced hepatic dysfunction in the actual death group attributable to cyanide toxicity.

Vitreous concentrations of hepatic enzymes—AST, ALT, and ALP—were markedly elevated in the actual death group relative to both control and disguised death groups. These enzymes serve as critical biomarkers of hepatocellular injury; ALT elevation predominantly reflects cytoplasmic hepatic damage, while increased AST levels indicate more severe mitochondrial involvement. The elevated alkaline phosphatase levels further underscore cholestatic or hepatic membrane compromise. These findings are consistent with previous studies demonstrating cyanide-induced hepatocellular damage leading to enzyme leakage into systemic circulation (Amodu et al., 2016, Vermeulen et al., 1992). The lack of significant enzyme elevation in the disguised death group relative to controls highlights the necessity of in vivo metabolic activity for cyanide's hepatotoxic manifestations.

Bilirubin analysis revealed significant increases in total and conjugated bilirubin levels in the actual death group compared to controls and disguised death animals. This hyperbilirubinemia likely stems from hepatocellular impairment, disrupting bilirubin conjugation and clearance mechanisms. The findings align with established literature indicating hepatotoxic substances inhibit hepatic uptake and conjugation of bilirubin, resulting in elevated serum and vitreous bilirubin levels (Amodu et al., 2016, Zilg et al., 2009). The absence of significant bilirubin changes in the disguised death group further supports the concept that postmortem cyanide administration does not replicate antemortem hepatotoxic biochemical changes.

Conversely, total protein and albumin concentrations in the vitreous humor were significantly reduced in the actual death group compared to controls, suggestive of impaired hepatic protein synthesis attributable to cyanide-mediated hepatocyte dysfunction. This finding contrasts with reports indicating serum protein elevation following acute cyanide exposure ((Amodu et al., 2016), but parallels observations of decreased protein levels in carbon monoxide toxicity, another asphyxiant with comparable cellular effects (Agoro et al., 2018). The unchanged protein and albumin levels between control and disguised death groups reaffirm the specificity of these alterations to the toxic metabolic state preceding death.

Collectively, these biochemical aberrations observed in vitreous humor authentically reflect underlying pathophysiological hepatic injury induced by sodium cyanide, demonstrating that vitreous humor analysis is a reliable postmortem surrogate for serum biochemistry. The differentiation of actual cyanide toxicity from simulated postmortem administration, based on distinctive biochemical profiles, underscores the forensic utility of vitreous analysis in elucidating sodium cyanide-related causes of death. These results substantiate previous assertions that postmortem vitreous biochemistry can serve as a vital marker in forensic investigations where antemortem data are unavailable (Madea, 2004).

The use of vitreous humor for forensic toxicology is particularly advantageous due to its biochemical stability and protection from postmortem artifact, making it an indispensable matrix for confirming cyanide-induced hepatocellular injuries and differentiating between genuine toxic deaths and artifactual or disguised scenarios.

## CONCLUSION

The result of this study corroborates the hepatotoxic potential of sodium cyanide and validates vitreous humor biochemical analysis as an effective, minimally invasive forensic tool for distinguishing sodium cyanide poisoning, contributing valuable evidence for medico-legal investigations.

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