**Renal Toxicity of Chronic Sodium Cyanide Exposure: A study of Vitreous humor in Rabbits**

**ABSTRACT**

Cyanide is a fast acting, potentially and deadly chemical that can exist in various forms. Exposure to sodium cyanide causes serious health effects and can be fatal. This study was designed to investigate renal toxicity of chronic sodium cyanide exposure using vitreous humor of rabbits. The following renal function parameters were investigated; electrolytes (Na+, K+, Cl- and Ca2-), urea, creatinine and Kidney InjuryMolecule-1(KIM-1). Renal function parameters were assessed over 24 hours in rabbits: Group 1 (Test group) received a lethal sodium cyanide dose (1mg/kg), Group 2 received it post-sacrificed (disguised death group), and Group 3 was sacrificed without administration (control group). The following were results of parameters investigated. Test group; sodium 137.90±0.17, potassium 7.74±0.21, chloride 123.20±0.52, calcium 1.26±0.05, KIM 5.95±0.58, urea 5.95±0.45 and creatinine 74.70±0.33. Disguised group; sodium 135.80±0.14, potassium 6.76±0.18, chloride 106.80±0.45, calcium 1.16±0.02, KIM 3.12±0.23, urea 3.10±0.18 and creatinine 52.89±0.66. Control group; sodium 135.60±0.16, potassium 6.30±0.12, chloride 105.60±0.48, calcium 1.15±0.04, KIM 2.91±0.24, urea 2.97±0.24 and creatinine 52.77±0.36. The results showed significant (p< 0.05) increase in concentration of electrolytes (Na+, K+, Cl-), urea, creatinine and Kidney InjuryMolecule-1(KIM-1) in test group compared to control and disguised group. However, there was no significant (p>0.05) difference in renal parameters between disguised and control group. It can be concluded that sodium cyanide is a potential poison with high toxicity effect on kidney and biochemical parameters in the vitreous humor of rabbits including, renal function test could be used in death differentiation due to sodium cyanide poisoning

 **Keywords**: Renal toxicity, vitreous humor, Sodium cyanide, Rabbits

**INTRODUCTION**

Cyanide poisoning may result from a variety of exposures, including structural fires, industrial exposures such as sodium nitroprusside and certain foods, homicide and suicide 1.

Sodium cyanide is a highly toxic substance that can have severe health effects, including potential renal damage 2. Cyanide toxicity can cause renal vasoconstriction, reducing blood flow and leading to ischemic damage 3. Severe cyanide poisoning can result in acute kidney injury, requiring medical intervention 4. Cyanide ions can also inhibit cellular respiration leading to tissue hypoxia and damage 2. Rapid onset of symptoms, include headache, dizziness, nausea and respiratory distress 5.

The severity of renal effects depends on the dose and duration of sodium cyanide exposure. Pre-existing kidney disease or other health conditions may increase susceptibility to renal damage.

Vitreous humor, the clear gel-like substance filling the eye, plays a vital role in toxicological analysis, particularly in forensic toxicology 4. It is a stable and reliable matrix for detecting xenobiotics and drugs, including alcohol 4. Since most of the xenobiotics present in the bloodstream are detected in vitreous humor after crossing the selective blood-retinal barrier, vitreous humor is an alternative matrix useful for forensic toxicology 6. Vitreous humor analysis offers particular advantages over other biological matrices; it is less prone to postmortem redistribution, easy to collect, has relatively few interfering compounds for the analytical process and shows sample stability over time after death 7. 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 6. 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.

**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.

**STUDY DESIGN:**

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 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)

**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 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.

**BIOCHEMICAL ANALYSIS**: Renal function parameters were measured using Randox Laboratories United Kingdom reagent kits and Ekrat-0201 kits. Mindray (MR-96A) was used to analyzed Kidney InjuryMolecule-1(KIM-1) using Enzyme linked immunosorbent Assay (ELISA) method.

**Statistical analysis**

 Data are expressed as mean ± Standard deviation (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 renal function parameters of vitreous humor is presented in Table 1 and 2. The result showed increase in concentration of renal function parameters in actual death compared to control and disguised death.

Table 1: Analysis of Renal Function Parameters of Vitreous Humor (Mean ± SD)

|  |  |  |
| --- | --- | --- |
| **S/N** | **Experimental Groups** |  **Parameters** |
| **Na+ (mmol/L)** | **K+ (mmol/L)** | **Cl- (mmol/L)** | **Ca2+ (mmol/L)** |
| 1 | Control  | 135.60±0.16a | 6.30±0.12a | 105.60±0.48a | 1.15±0.04a |
| 2 | Actual death | 137.90±0.17b | 7.74±0.21b | 123.20±0.52b | 1.26±0.05b |
| 3 | Disguised death | 135.80±0.14a | 6.76±0.18c | 106.80±0.45c | 1.16±0.02a |
| 4 | F –value | 255.1 | 70.09 | 1654 | 9.142 |
| 5 | P –value | <0.0001 | <0.0001 | <0.0001 | 0.0068 |

**Keys**: Na+= sodium ion, K+ = potassium ion, Cl-= chloride ion, Ca2+ = ionized calcium. SD = standard deviation. Means ± SD of experimental groups with different superscripts are significantly different from each other at p<0.05.

Table 2: Analysis of Renal Function Parameters of Vitreous Humor (Mean ± SD)

|  |  |  |
| --- | --- | --- |
| **S/N** | **Experimental Groups** |  **Parameters** |
| **KIM-1 (pg/L)** | **Urea(mmol/L)** | **Creatinine (mmol/L)** |
| 1 | Control  | 2.91±0.24a | 2.97±0.24a | 52.77±0.36a |
| 2 | Actual death | 5.95±0.58b | 5.95±0.45b | 74.70±0.33b |
| 3 | Disguised death | 3.12±0.23a | 3.10±0.18a | 52.89±0.66a |
| 4 | F –value | 12428 | 75.03 | 2853 |
| 5 | P –value | <0.0001 | <0.0001 | <0.0001 |

**Keys**: KIM-1 = kidney injury molecule-1. SD = standard deviation. Means ± SD of experimental groups with different superscripts are significantly different from each other at p<0.05.

**DISCUSSION**

Postmortem analysis of the vitreous biochemistry is useful in assessing the antemortem metabolic status and in predicting the antemortem serum biochemistry of an individual 9. The comparison of vitreous sodium ion concentration was statistically significant across the groups. Further comparison using tukey multiple test showed increase in sodium ion concentration of the actual death group compared to the control, whereas the difference in sodium ion concentration of the control and disguised death was not significant. The scientific basis of the difference is attributed to the cellular hypoxia effect of cyanide which causes tubular dysfunction in the kidney with consequent ureamia and electrolyte imbalance. Therefore, the cell lost the ability to maintain the concentration gradient of electrolytes between the intracellular and extracellular compartments. This result agrees with the work of 10 that reported high level of vitreous urea, creatinine, sodium ion and chloride ion in rats exposed to cyanide.

Similarly, the comparison of vitreous chloride ion concentration was statistically significant across the groups. Tukey multiple comparison test showed significant increase in chloride ion concentration of actual death group compared to the control, whereas the difference in chloride ion concentration of the control and disguised death was not significant. The scientific basis of the difference is attributed to the toxic effect of cyanide which causes tubular dysfunction in the kidney with consequent ureamia and electrolyte imbalance. The cell lost ability to maintain the concentration gradient of electrolytes between the intracellular and extracellular. Chloride ion and sodium ion are among the substances that can pass blood retina barrier into the vitreous and remain stable after death. Therefore, elevation of these electrolytes helps in predicting antemortem biochemistry status. The result agrees with the work of 11 that reported increase level of serum chloride ion in rabbits exposed to cyanide.

Vitreous potassium concentration is used for the estimation of postmortem interval (PMI), the time that elapsed after human being has died 12. The comparison of vitreous potassium ion concentration was statistically significant across the groups. Tukey multiple comparison test showed significant increase in potassium ion concentration of the actual death compared to the control, whereas the difference in potassium ion concentration of the control and disguised death was not significant. During life, intracellular potassium concentrations are maintained at high concentrations, while extracellular vitreous potassium concentrations are low because of the action of the sodium/potassium-pump. After death, postmortem vitreous potassium concentrations change because of cellular hypoxia (cause by cyanide), which induces the depletion of Adenosine Triphosphate (ATP) and loss of selective membrane permeability for ions, after which intracellular potassium diffuses with the passive diffusion into the vitreous body, leading to an increase in vitreous potassium concentrations. This finding agrees with the work of 13 which state that vitreous potassium concentration increases after death.

Calcium ion is one of the stable electrolytes in the vitreous humor. The concentration of vitreous calcium ion across the groups was statistically significant. Further comparison using tukey multiple test showed significant increase in calcium ion concentration of the actual death group compared to the control, whereas the difference in calcium ion concentration of the control and disguised death was not significant. It is of the view that the observed significant increase in the concentrations of vitreous calcium ion are dependent on the effects of cellular [hypoxia](https://www.omicsonline.org/open-access/strategies-to-combat-hypoxia-in-encapsulated-islet-transplantation-2161-1076-1000259.php?aid=70398), which lead to an increase in the cell membrane and blood vessel wall permeability, and the reduction of Adenosine Triphosphate (ATP) thereby preventing electrolyte pumps from maintaining physiological cell membrane electrical gradients. This finding is in line with the work of 14 which stated that cellular hypoxia and reduction of Adenosine Triphosphate combined with autolysis and cell disintegration leads to a considerable change in the calcium concentration in a case of cyanide poisoned.

Urea concentration has been used as a parameter to assess renal function 15. The concentration of vitreous urea across the groups was statistically significant. Tukey multiple comparison test showed significant increase in urea concentration of the actual death group compared to the control, whereas the difference in urea concentration of the control and disguised death group was not significant. Urea is relatively stable in postmortem sample and vitreous urea concentration can be used to predict antemortem serum urea concentration. High concentration of urea and creatinine indicates renal failure. The result of this study is in line with the work of 16 that reported high level of urea in rabbit exposed to cyanide.

Creatinine concentration is used as an approximation of glomerular filtration rate. The concentration of vitreous creatinine across the groups was statistically significant. Tukey multiple comparison test showed significant increase in creatinine concentration of the actual death group compared to the control, whereas the difference in creatinine concentration of the control and disguised death group was not significant. High vitreous urea and creatinine indicates kidney malfunction that might be due to the histotoxic effect of cyanide on the renal tubules. This finding agrees with the work of 8 that reported high level of vitreous urea, creatinine, sodium ion and chloride ion in rabbits exposed to cyanide.

The concentration of vitreous kidney injury molecule 1 across the groups was statistically significant. Tukey multiple test showed significant (p<0.05) increase in kidney injury molecule 1 concentration of the actual death group compared to the control, whereas the difference in kidney injury molecule 1 concentration of the control and disguised death group was not significant.

The result of this study agrees with the report of 17 that reported increased Kidney Injury Molecule 1(KIM-1) level in rats exposed to cyanide.

This study revealed that biochemical parameters in the vitreous humor of rabbits including, renal function test could be used in death differentiation due to sodium cyanide poisoning. It also revealed that sodium cyanide is a potential poison with high toxicity effect on kidney.

**CONCLUSION**

It can be concluded that sodium cyanide is a potential poison with high toxicity effect on kidney and biochemical parameters in the vitreous humor of rabbits including, renal function test could be used in death differentiation due to sodium cyanide poisoning

**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.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

**COMPETING INTERESTS**

Authors have declared that no competing interest exist.

**REFERENCES**

1 Parker-Cote, J. L., Rizer, J., Vakkalanka, J. P., Rege, S. V. and Holstege C. P. (2018). Challenges in the diagnosis of acute cyanide poisoning*. Chemical Toxicology,* 56, 609 - 617.

2 Koschel, M. J. (2006). Management of the Cyanide-poisoned Patient. *Journal of Emergency Medicine,* 32, 19 - 26.

3 Madea, B. and Musshoff, F. (2007). Postmortem biochemistry. *Forensic Science International.* 165, 165 - 171.

4 Mulla, A., Massey, K. L. and Kalra, J. (2005). Vitreous humor biochemical constituents: evaluation of between-eye differences. *American Journal of Forensic Medicine and Pathology*, 26, 146 - 159.

5 Agency for Toxic Substances and Disease Registry (ATSDR) (2006). Toxicological profile for cyanide. Atlanta, GA. USA. 56 - 67.

6 Madea B, Rodig A. (2006). Time of death dependent criteria in vitreous humor: accuracy of estimating the time since death. *Forensic Science International*, 164: 87 - 92.

7 Lange, N. Swearer, S. and Sturner, W. (1994). Human Postmortem Interval Estimation from Vitreous Potassium an analysis of original Data from Six Different Studies.  *Forensic Science International,* 66, 159 - 174.

8 Kadiri, H. E. and Asagba, S. O. (2019). The chronic effects of cyanide on oxidative stress indices in the rabbits. *The Journal of Basic and Applied Zoology,* 80, 40 - 45.

9 Zilg, B., Alkass, K, Berg, S. and Druid, H. (2009). Postmortem identification of hyperglycermia. *Forensic Science International*,185, 89 - 95.

10 Nicholas, T. L. and Courtney, M. L. (2016). Analytical samples. *Forensic Toxicology*, 56, 463 - 472.

11 Manzano, H., Benedito, S., Soto-Blanco, B., Guerra, J. L., Maioka, P. C. and Gomiak, S. L. (2007). Effect of long-term cyanide ingestion by rabbits. *Veterinary Reseach Communications*, 31, 93 - 104.

12 Stephen, D. W., Rork, P. and Coral, B. L. (2006). Use of Vitreous Humor to Predict Postmortem Blood Morphine Concentration. *Clinical Chemistry,* 52, 70 - 73.

13 Moriya, F. and Hashimoto, Y. (2003). Chemical factors affecting the interpretation of blood cyanide concentration in fire victim*. Legal Medicine*, 5, 113 - 117.

14 Andrew, K. M. (2003). Calcium and heart a question of life and death. *Journal of Clinical Investigation,* 111, 597 - 600.

15 Sousa, A. B., Soto-Blanco, B. Guerra, J. L., Kimura, E. T. and Gorniak, S. L. (2002). Does Prolonged Oral Exposure to Cyanide Promote Hepatotoxicity and Nephrotoxicity? *Toxicology,* 174, 87 - 95.

16 Kamalu, B. P. (1993). Pathological changes in growing rabbits fed on a balanced cassava (Manihot esculenta) diet. *British Journal of Nutrition*, 65, 921 - 934.

17 Bailly, V., Zhang, Z., Meier, W., Cate, R., Sanicola, M. and Bonventre, J. V. (2002). Shedding of kidney injury molecule 1, a putative adhesion protein in renal regeneration. *Journal of Biology and Chemistry*, 277, 39739 - 39748.