**EFFECT OF SODIUM CYANIDE EXPOSURE ON RENAL PARAMETERS OF NEW ZEALAND WHITE RABBIT**

**ABSTRACT**

**Aim**: The aim of this study was to assess effect of chronic exposure of sodium cyanide on renal parameters of New Zealand White Rabbit.

**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**: Twenty-four (24) rabbits used for this study were divided into two major groups (control and experiment) with four rabbits in each group. The animals in control group were given water and feed only while others in experiment group were given 10ml of 0.05mg sodium cyanide daily for 30, 60 and 90 days respectively. Cardiac blood samples were extracted from the rabbits using standard procedure. The following renal function parameters were determined; electrolytes (Na+, K+, Cl-), urea, creatinine and Kidney InjuryMolecule-1(KIM-1).Histological examination of kidney tissue was also carried out. 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.

**Results**: Renal function parameters results showed significant (p<0.05) increase in levels of electrolytes (Na+, K+, Cl-), urea, creatinine and Kidney Injury Molecule-1 (KIM-1) in 30 days, 60 days and 90 days respectively as compared to control. Histological examination revealed significant changes on 30 days, 60 days and 90 days respectively.

**Conclusion**: The findings of this study revealed that exposure to 0.05 mg/kg sodium cyanide may cause alteration of renal function parameters due to its toxic effect on the kidney tissues as evidenced by the histological examination.

**Keywords:** Kidney function parameters, sodium cyanide and New Zealand white rabbits

**INTRODUCTION**

Cyanide is a substance ubiquitous in the environment and has been associated with many intoxication episodes in humans and animals resulting from the ingestion of foods, environmental pollution, chemical war, suicide, homicide1.

Cyanide is viewed as a compound asphyxiant on the grounds that it hinders vigorous digestion without influencing oxygen conveyance to the tissues. It has a high proclivity for iron in the ferric state, bringing about restricting to and inactivation of tissue cytochrome C oxidase 2. Its harming is a type of histotoxic hypoxia in light of the fact that the cells of a living being cannot make adenosine triphosphate (ATP), basically through the hindrance of the mitochondrial catalyst cytochrome oxidase 3. The biochemical activity of cyanide is something similar after entering the body. Once in the circulation system, cyanide frames a steady intricate with cytochrome oxidase, a chemical that advances the exchange of electrons in the mitochondria of cells during the combination of ATP 4.

Cyanide intoxication may produce some pathologic effects on different tissues that precede alterations in biochemical parameters. Consequently, certain types of cells are damaged and leak enzymes into the blood, where they can be measured as indicator of cell damage 5. 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 6.

Renal tubules and renal corpuscles also known as glomeruli and renal corpuscles, comprise the functional units of the kidney, which are represented by the nephrons from a histological standpoint. Nephrons play a critical role in osmoregulation and excretion through the ultrafiltration process. Therefore, any damage to the nephrons will cause renal failure and influence the regular excretion of metabolic waste7.

**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**: Twenty-four (24) 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:** A total of twenty-four (24) rabbits constitute the sample size. The rabbits used were divided into three groups with matched control, four rabbits were assigned to each group and the study lasted for 90 days as follows: Group one (0-30) days, Group two (0-60) days, Group three (0-90) days. With the exception of the control rabbits, others were treated daily with 0.05 mg/kg sodium cyanide for 30, 60 and 90 days respectively. The matched control and treated rabbits were given water ad-libitum and feed daily. Cardiac blood samples were extracted from the rabbits using standard procedure. The blood samples and kidney were taken for analysis and histological investigations at day 30, 60 and 90 respectively. Biochemical parameters investigated include electrolytes (Na+, K+, Cl-), urea, creatinine andKidney Injury Molecule-1 (KIM-1). All animals used for the study were handled in compliance with the guide to the care and use of animals in research and teaching.

**BIOCHEMICAL ANALYSIS**: Blood samples for biochemical analyses were collected from the rabbits and serum separated by centrifugation at 3000 rpm for 5 minutes. Serum parameters were measured using Randox Laboratories UK reagent kits and Ekrat-0201 kits. Electrolytes (Na+, K+, Cl-), urea, creatinine concentrations were measured using colorimetric method. Kidney InjuryMolecule-1(KIM-1) was analyzed using Enzyme linked immunosorbent Assay (ELISA) method.

**Histological Analysis**

The kidneys were harvested and processed for histological analysis.Sections on slide were examined and photomicrographs captured with X400 objective lens using the ScopeTek™ device and software vi.3.

**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**

Biochemical changes for kidney parameters for rabbits given the daily oral of 0.05mg/kg sodium cyanide for thirty days, sixty days and ninety days are presented in table 1, 2 and 3. The results showed that after the treatment period, the concentrations of electrolytes (Na+, K+, Cl-), urea, creatinine and Kidney Injury Molecule-1 (KIM-1) significantly (p< 0.05) increased compared to the control.

**Table 1.Mean ±SD of Renal Parameters of Rabbits Treated with 0.05 mg/kg sodium cyanide for 30 Days**

|  |  |
| --- | --- |
| S/N | Experimental Groups |
| Na+ (mmol/L) | K+ (mmol/L) | Cl- (mmol/L) | KIM-1 (pg/L) | Urea (mmol/L) | Creatinine (µmol/L) |
| 1 | Control  | 138.00±1.05 | 4.98±0.13 | 104.00±2.16 | 7.89±0.63 | 6.34±0.32 | 124.80±1.36 |
| 2 | Treated Group | 149.60±1.62 | 6.77±0.17 | 116.20±2.91 | 10.36±0.78 | 7.86±0.07 | 137.00±1.85 |
| 3 | T value | 11.98 | 16.88 | 6.70 | 4.934 | 9.24 | 10.65 |
| 4 | P value | <0.0001 | <0.0001 | 0.0005 | 0.0026 | <0.0001 | <0.0001 |

**Keys:** Na+= sodium ion, K+ = potassium ion, Cl-= chloride ion, Ca2+ = ionized calcium, KIM-1 = kidney injury molecule-1.

**Table 2. Mean ±SD of Renal Parameters of Rabbits treated with 0.05 mg/kg sodium cyanide for 60 Days**

|  |  |
| --- | --- |
| S/N | Experimental Groups |
| Na+ (mmol/L) | K+ (mmol/L) | Cl- (mmol/L) | KIM-1 (pg/L) | Urea (mmol/L) | Creatinine (µmol/L) |
| 1 | Control  | 137.90±1.03 | 5.04±0.29 | 104.80±1.83 | 7.84±0.64 | 6.39±0.32 | 126.00±0.97 |
| 2 | Treated Group | 159.80±1.17 | 7.70±0.18 | 126.40±2.92 | 12.46±0.62 | 8.92±0.52 | 147.40±1.67 |
| 3 | T value | 28.13 | 15.45 | 12.55 | 10.13 | 8.194 | 22.14 |
| 4 | P value | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0002 | <0.0001 |

**Keys**: Na+= sodium ion, K+ = potassium ion, Cl-= chloride ion, Ca2+ = ionized calcium, KIM-1 = kidney injury molecule-1.

**Table 3. Mean ±SD of Renal Parameters of Rabbits treated with 0.05 mg/kg sodium cyanide for 90 Days**

|  |  |
| --- | --- |
| S/N | Experimental Groups |
| Na+ (mmol/L) | K+ (mmol/L) | Cl- (mmol/L) | KIM-1 (pg/L) | Urea (mmol/L) | Creatinine (µmol/L) |
| 1 | Control  | 137.90±1.02 | 5.03±0.18 | 104.00±1.59 | 8.04±0.66 | 6.63±0.33 | 125.80±1.40 |
| 2 | Treated Group | 165.70±1.45 | 8.41±0.38 | 130.30±0.60 | 14.86±0.24 | 11.22±0.51 | 155.00±2.56 |
| 3 | T value | 31.44 | 16.1 | 31.03 | 19.36 | 15.04 | 19.95 |
| 4 | P value | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

**Keys**: Na+= sodium ion, K+ = potassium ion, Cl-= chloride ion, Ca2+ = ionized calcium, KIM-1 = kidney injury molecule-1.

Pathological changes in the kidney section were observed following treatment of the rabbits with 0.05 mg/kg sodium cyanide for the period of 30, 60 and 90 days respectively. Figure 1 showed normal kidney section of rabbits with no lesion while figure 2 (treated with 0.05 mg/kg sodium cyanide for 30 days) showed mononuclear cell infiltrate and tubular degeneration. Figure 3 (treated with 0.05 mg/kg sodium cyanide for 60 days) also showed infiltration areas and thickened renal capsule, while figure 4 (treated with 0.05 mg/kg sodium cyanide for 90 days) showed tubular necrosis and renal papilla necrosis.







**DISCUSSION**

Cyanide poisoning may result from a variety of exposures, including structural fires, industrial exposures such as sodium nitroprusside and certain foods 8. Cyanide intoxication may produce some pathologic effects on different tissues that precede alterations in biochemical parameters. Consequently, certain types of cells are damaged and leak enzymes into the blood, where they can be measured as indicator of cell damage 5. Biochemical alterations are considered as sensitive indicators of toxicity before the expression of visible hazardous effects5.

The result of this study showed significant increase in serum concentration of renal function parameters in thirty, sixty ninety days respectively. The observed increase in serum concentration of the renal function parameters indicates long-term effect of cyanide toxicity on kidney.

Serum urea concentration has been used for many years as a parameter to assess renal function and this study observed significant increase in serum urea concentration in thirty, sixty and ninety days. This result agrees with the work of 9 which reported that cyanide poisoning is closely associated with ureamic complication, also 10 found significant increase in urea and creatinine levels from patients following ingestion of sodium cyanide. The increased serum urea concentration could result from the histotoxic hypoxia effect of cyanide on the renal proximal tubular epithelium and glomeruli. Although kidney converts cyanide to thiocyanate, a less toxic substance, its long-term exposure could cause tubular necrosis as shown by the histology report in Fig 2, which could eventually lead to retention of nitrogenous waste (urea, creatinine).

Serum creatinine concentration has been used as markers of glomerular filtration rate, because its concentration in plasma is maintained within narrow limits by glomerular filtration 1. We observed that serum creatinine concentration was significantly increased in ascending order from thirty, sixty and ninety days respectively. The observed increased serum creatinine concentration indicates renal abnormalities, thus reflecting altered glomerular filtration function as shown by the histology report in Fig 3. The production of creatine and subsequently creatinine depends on muscle mass which fluctuates very little. Creatinine is excreted entirely by the kidneys and therefore is directly proportional to renal excretory function. With abnormal excretory function, the serum creatinine concentrations should be abnormal. The increase in concentration of creatinine and urea is a pointer to the fact that renal dysfunction is a long term outcome of chronic exposure to cyanide. The result of this study agrees with the work of 11 that showed increase level of creatinine in rabbit following cyanide exposure.

Sodium (Na+) ion is the major cation of extracellular fluid. This study found that serum concentration of sodium ion was significantly increased. The kidney regulates the amount of sodium ion in the body. It is therefore thought that kidney dysfunction would generally present with abnormality in sodium ion concentration and this could be due to the increasing periods of exposure to cyanide. The biochemical bases for this induced hypernatremia could be three-fold, that is, neurological, hypovolemia and normovolemia12. The path utilized by sodium cyanide could be neurological as cyanide is associated with neurons. Also, cyanide could utilize the path of normovolemia by altering the capacity of anti-diuretic hormone (ADH) responsible for the regulation of extracellular water composition. It is an established fact that cyanide affects the brain, ADH being a product of the brain could be affected 12(Andrew 2003). This result agrees with the report of 13that showed increase sodium ion level in rabbits exposed to cyanide

Chloride (Cl+) is the major anion in the extracellular fluid. In this study, the chloride ion concentration was significantly increased. Like sodium ion, chloride ion is involved in the maintenance of water distribution, osmotic pressure and anion-cation homeostasis in the extracellular fluid. Chloride ion is an important driver of numerous homeostatic mechanisms including regulation of rennin secretion, tubuloglomerular feedback and renal sodium handling; also, chloride is excreted by the kidneys 14. The possible explanation for the observed increased serum chloride ion concentration is link to metabolic acidosis occasioned by long term cyanide exposure. Since higher serum chloride ion concentrations are often seen concurrently with metabolic acidosis, which is associated with increased risk of chronic kidney disease and metabolic acidosis results from cellular hypoxia occasioned by cyanide exposure 15. The finding is in line with the work of 14who reported that higher serum chloride ion is associated with an increased glomerular filtration rate.

Kidney Injury Molecule 1(KIM-1) is a useful biomarker for renal proximal tubule injury 16. KIM-1 is not only an early biomarker of acute kidney injury, but also has a potential role in predicting the long term renal outcome 16. This study found serum concentration of KIM-1 to be statistically significant. Based on this finding, the increased serum concentration of KIM-1 correlates highly with other renal biochemical parameters; therefore, KIM-1 is a sensitive and specific marker of kidney injury. The observed increased serum concentration of KIM-1 in thirty, sixty and ninety days is attributed to the loss of tubular cell polarity, increase in transepithelial permeability and the disruption of actin cytoskeletal architecture in renal microvascular cells. This result agrees with the work of 17 which observed that serum concentration of Kidney Injury Molecule 1 correlates highly with the incidence of progression of chronic kidney disease.

This study revealed that the chronic exposure to sodium cyanide could cause renal impairment; consequently, the biochemical parameters for renal function test, the regulatory and homeostatic functions of the kidneys would be altered.

The histological examination of kidney tissues revealed the following features; area of inflammation, glomerulosclerosis, tubular degeneration and tubular necrosis. This result showed the scientific basis for the observed renal function parameters alteration and confirmed the impairment of kidney function detected by the biochemical evaluation. This result also shows that the kidney tissues were severely impacted by the chronic effect of cyanide (30, 60 and 90 days respectively). This result is consistent with the work of 18 that reported severe glomerular and tubular necrosis in kidney tissue of rabbit exposed to cyanide.

**CONCLUSION**

The result of this study revealed that the chronic exposure to sodium cyanide could cause renal impairment; consequently, the biochemical parameters for renal function test would be abnormal as evidence in the histological examination.

**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):**

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

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