

EVALUATION OF HEAMATOLOGICAL CHANGES IN RABBITS EXPOSED TO SODIUM CYANIDE

ABSTRACT

AIM: The aim of this study was to evaluate the haematological changes in rabbits due to sodium cyanide exposure.

Study design: An experimental 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 forty-eight (48) rabbits were used for the study. The study was divided into three groups including the control group. With the exception of the control rabbits, others were treated daily with 0.05 mg/kg sodium cyanide for 30 days, 60 days and 90 days respectively. Cardiac blood samples were extracted from the rabbits using standard procedure. Haematological parameters investigated include packed cell volume (PCV), white blood cell count (WBC), lymphocyte (L), neutrophil (N), monocyte (M), eosinophil (E) and platelet count (P). Using an automated hematology analyzer (Genrui KT 6300 automated CBC Hematology Analyzer Coulter) Data generated were expressed as mean +SD. ANOVA and Tukeys multiple comparison test were used to compare the results between means of groups. Variation in mean of parameters were considered statistically significant at $P < 0.05$.

Results: Haematological parameter results showed significant ($p < 0.05$) decrease in levels of packed cell volume, neutrophil and monocyte in 30 days, 60 days and 90 days respectively as compared to control.

Conclusion; Sodium cyanide may cause an alteration in some haematological parameters of rabbits and the severity of the changes might increase with the duration of exposure

Keywords: Haematological parameters, sodium cyanide

INTRODUCTION

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 ¹. Its harming is a type of histotoxic hypoxia in light of the fact that the cells of a living being can't make adenosine triphosphate (ATP), basically through the hindrance of the mitochondrial catalyst cytochrome oxidase ². The poisonous nature of cyanide is connected primarily to the suspension of high-impact cell digestion. It reversibly ties to the ferric particles cytochrome oxidase inside the mitochondria. This successfully stops cell breath by obstructing the decrease of oxygen to water 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. In acute intoxication, cyanide produces a rapid inhibition of cytochrome oxidase, resulting in an energy deficit within the target tissue. The enzyme cytochrome oxidase enables cells to utilize oxygen. By inhibiting this enzyme, cyanide causes cellular anoxia ⁵.

The integrity of the haematological parameters needs to be examined to determine the toxic effects of any exogenous compound, as these parameters are crucial in determining the toxicity of any compound. Any compound that accumulates excessively in erythrocytes is typically a sign of a pathological condition⁶. A drop in blood parameters during the assessment of haematotoxicity may be a sign that the production of red blood cells has been suppressed. The ability to predict changes in haematological indices accurately for human toxicity is very high.

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. Besides acute poisoning, cyanide chronic intoxication may also produce some pathologic effects on different tissues that precede alterations in biochemical parameters⁷.

Moreover, chronic cyanide intoxication induces alterations in some tissue haematological, biochemical, histological and oxidative stress parameters in experimental animal model^{8,9} reported decrease levels of erythrocyte count, haemoglobin concentration, packed cell volume and mean corpuscular haemoglobin and normal leukocyte count, leukocyte differential count and platelet count in rabbits exposed to cyanide. This study was designed to evaluate haematological parameters in rabbits exposed to 0.05mg/kg sodium cyanide.

MATERIALS AND METHODS

Study Area

The breeding and intoxication of the study was conducted at Rivers State University, Port Harcourt. The biochemical investigations were carried out at National Nigerian Petroleum Corporation (NNPC), Medical laboratory, Akpajo, Port Harcourt

Study Population

A total of forty eight (48), two-month-old New Zealand white rabbits (*Oryctolagus cuniculus*) that weighed averagely 1.0 -1.2 kg were used for this study. The rabbits were purchased from Sandre Farm Oyigbo, Rivers State, Port Harcourt. The rabbits were kept in a spacious and well-ventilated cage at room temperature, under natural circadian rhythm and were allowed to acclimatize for fourteen (14) days. They were housed in standard cages and allowed access to feed (Top Feed Finisher Mesh, Sapele, Nigeria) and water ad libitum from the animal house, Department of Biological Science Rivers State University, Port Harcourt. 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.

Ethical Approval

The Ethical clearance and experimental protocol were approved by the Ethic Committee of Rivers State Ministry of Health. 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.

Selection Criteria

Only apparently healthy male and female rabbits of same age and weight were used for the study. The age range was between six to eight months. The weight brackets were 1.2 to 1.5 kg. lysed blood samples were rejected.

Procurement of materials

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

Experimental animals

Forty eight (48) rabbits were used for the experiment. The animals were purchased at Sandre Farm, Oyigbo, Rivers State, Nigeria.

Study design

The animals used for the study were divided into three groups with matched control. Four rabbits were assigned to each group and study lasted for 90 days as follows: Group one (0 – 30) days, Group two (0 – 60) days, Group three (0 – 90) days. Each rabbit in a group was given 10ml of 0.05mg/kg sodium cyanide orally daily. The matched control and treated rabbits were given water *ad-libitum* and feed daily. The blood samples were taken for analysis at day 30, 60 and 90 respectively. All animals used for the study were handled in compliance with the guide to the care and use of animals for research and teaching.

Collection of Sample

The blood samples were collected into ethylene diamine tetra acetic acid (EDTA) bottles.

2.1 Laboratory Analysis

Determination of Haematological Indices. Haematological parameters such as packed cell volume, haemoglobin as well as red cell indices were analysed with Genrui KT 6300 automated CBC Hematology Analyzer Coulter.

Statistical analysis

Graph pad prism 7.0 version of windows statistical package was used to analyze the data generated. The mean \pm standard deviation was determined. One-way analysis of variance (ANOVA) with Tukey's multiple comparison test, were also done using the same statistical package. From the values obtained statistical decision and inferential evaluation were made. A probability (p) value of less than .05 was considered statistically significant.

RESULTS

Haematological changes for rabbits given oral doses of 0.05mg/kg sodium cyanide for day 30, 60 and 90 are presented in Table 1, 2 and 3

Table 1: Mean \pm SD of Haematological Parameters of Rabbits Treated with 0.05 mg/kg Sodium Cyanide for 30 days

S/ N	Experim ental Groups	Parameters							
		PCV (%)	HB (g/dL)	WBC (x 10 ⁹ /L)	NEUT (%)	LYMP (%)	EOSI N. (%)	MON O (%)	PLT (x10 ⁹ /L)
1	Control	32.47 \pm 1.24	10.82 \pm 0.42	7.70 \pm 0.48	32.14 \pm 0.09	63.09 \pm 0.05	1.47 \pm 0.24	3.17 \pm 0.10	198.70 \pm 0.63
2	Test	31.83 \pm 1.42	10.61 \pm 0.48	7.77 \pm 0.49	31.14 \pm 0.19	63.32 \pm 0.10	1.45 \pm 0.19	4.21 \pm 0.13	198.00 \pm 0.75
3	T-value	0.6797	0.6803	0.2103	4.751	4.058	0.9765	12.7	0.8641
4	P value	0.5220	0.5217	0.8404	0.0032	0.6067	0.4145	<0.00 01	0.1787

Keys: PCV = packed cell volume, HB = haemoglobin, WBC = white blood cell, NEUT. = neutrophils, LYMP = lymphocytes, EOSIN. = eosinophil, MONO = monocytes, PLT = platelets.

Table 2: Mean \pm SD of Haematological Parameters of Rabbits Treated with 0.05 mg/kg Sodium Cyanide for 60 days

S/ N	Experim ental Groups	Parameters							
		PCV (%)	HB (g/dL)	WBC (x 10 ⁹ /L)	NEUT (%)	LYMP (%)	EOSI N. (%)	MON O (%)	PLT (x10 ⁹ /L)
1	Control	32.50 \pm 1.10	10.83 \pm 0.36	7.71 \pm 0.10	32.15 \pm 0.08	63.14 \pm 0.09	1.31 \pm 0.20	3.20 \pm 0.13	198.70 \pm 0.57
2	Test	29.04 \pm 0.29	9.68 \pm 0. 10	7.78 \pm 0.62	29.62 \pm 0.11	63.64 \pm 0.09	1.36 \pm 0.10	4.88 \pm 0.13	199.00 \pm 0.77
3	T-value	6.109	6.108	0.1684	36.78	8.082	1.353	18.93	0.5244
4	P value	0.0009	0.0009	0.8718	<0.000 1	0.6301	0.6446	<0.00 01	0.6188

Keys: PCV = packed cell volume, HB = haemoglobin, WBC = white blood cell, NEUT. = neutrophils, LYMP = lymphocytes, EOSIN. = eosinophil, MONO = monocytes, PLT = platelets.

Table 3: Mean \pm SD of Haematological Parameters of Rabbits Treated with 0.05 mg/kg Sodium Cyanide for 90 days

S/ N	Experim ental Groups	Parameters							
		PCV (%)	HB (g/dL)	WBC (x 10 ⁹ /L)	NEUT (%)	LYMP (%)	EOSI N. (%)	MON O (%)	PLT (x10 ⁹ /L)
1	Control	32.78 \pm 1.02	10.92 \pm 0.34	7.70 \pm 0.65	32.25 \pm 0.12	63.09 \pm 0.05	1.44 \pm 0.11	3.14 \pm 0.12	198.70 \pm 0.52
2	Test	25.30 \pm 0.21	8.43 \pm 0. 07	7.86 \pm 0.57	27.67 \pm 0.15	63.78 \pm 0.10	1.49 \pm 0.03	5.14 \pm 0.10	199.10 \pm 0.63
3	T-value	14.43	14.52	0.3524	46.91	31.14	2.531	28.91	1.008
4	P value	<0.000 1	<0.000 1	0.7366	<0.000 1	0.4201	0.0646	<0.00 01	0.3524

Keys: PCV = packed cell volume, HB = haemoglobin, WBC = white blood cell, NEUT. = neutrophils, LYMP = lymphocytes, EOSIN. = eosinophil, MONO = monocytes, PLT = platelets.

DISCUSSION

The result of this study showed that haematological parameters of rabbits fed with topfeed mixed with sodium cyanide solution for 30, 60 and 90 days respectively caused a significant reduction in haemoglobin concentration at $p < 0.05$ when compared with the match control level. Sodium cyanide can affect the haematopoietic system through restraining synthesis of haemoglobin by hindering key enzymes involved in haeme synthesis pathway such as aminolevulinic acid dehydratase (ALAD). Cyanide is found to be highly poisonous to living organism, primarily due to the formation of complexes with metal ions that present as

enzyme cofactors¹⁰. The notable effect of cyanide occurs with Fe³⁺ ion in cytochrome, thereby obstructing cellular respiration and hence oxidative phosphorylation¹¹.

Haematological parameters (Tables 1 and 2) showed that there was an increase (p<0.05) in monocyte count with a decrease (p<0.05) in packed cell volume, haemoglobin concentration and neutrophil count. This result agrees with the work of¹² that observed increase levels of lymphocyte, monocyte and a decrease levels of neutrophile, eosinophile and platelets in rabbits fed with *Manihot utilissima*. The observed reduced packed cell volume could be attributed to histotoxic hypoxia effect of cyanide on cell which is capable of hemolysis.

The haematological parameters (Table 3) showed that chronic exposure could cause anaemia as showed in decreased hemoglobin and packed cell volume. This result is in line with the work of¹³ that observed sodium cyanide induced toxicity which caused anaemic conditions in rabbits. The Toxicity effects observed might be the effect of cyanide on the red blood cells.

Haematological indices are of diagnostic significance in routine clinical evaluation of health. The reduction in the hematocrit values observed in this study was an indication of an anaemic condition and this condition could be attributed to hemolysis which was beyond the capacity of the bone marrow to compensate for the loss. Also, the toxic effect of sodium cyanide on the hematopoietic cells in the bone marrow could be associated with the metabolites of sodium cyanide that were released in high concentration which hindered the normal mechanism that regulate blood cell formation.

The total white blood cell of rabbits in control group differed significantly (p<0.05) from the rabbits at 60 and 90 days groups, this observation could be associated with the toxic effect of

sodium cyanide on the immune system. This effect could cause an immunosuppressive effect, which is suggestive of an impairment of the ability of the leukocyte to respond to antigenic mutagenic stimuli due to rapid proliferation.

CONCLUSION

It can be concluded that long term exposure to 0.05mg/kg sodium cyanide may cause an alteration in some haematological parameters and the severity would depend on the duration of exposure.

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