



Effect of Hexane Extracts of *Chromolaena odorata* (Linn.) on Hematotoxicity Induced by Cyanide in Male Albino Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate the effect of Hexane extract of *Chromolaena odorata* (HECO) on cyanide induced hematotoxicity in male Albino Wistar rats.

Methodology: Thirty-five (35) male albino rats weighing between 100g and 150g were distributed randomly into 7 groups of 5 rats each. Group 1 which comprised of normal rats received distilled water and served as the normal control, while groups 2-7 comprised of rats exposed to Potassium cyanide (KCN) (3 mg/kg). Group 2 received no treatment and served as the negative control. Groups 3, 4 and 5 received 100, 150 and 200 mg/kg of HECO respectively. Group 6 received 200 mg/kg HECO and 200 mg/kg sodium thiosulphate while group 7 was treated with a sodium thiosulphate (200 mg/kg), an established antidote, and served as the positive control. All administrations were done via the oral route and lasted for 14 days. Complete blood count was conducted after the experimental period. Data were analyzed using one-way analysis of variance, followed by Tukeys multiple comparisons test and $P < .05$ was considered significant.

Results: Results obtained indicate Red cell indices and white blood cell and differential were all significantly raised ($P < .05$) in treated rats relative to the negative control rats. Platelet value and Mean corpuscular volume were raised and lowered respectively during induction by the treatments, however, no statistical significance ($P < .05$) was observed. The results therefore suggest that *C. odorata* could be valuable in the management of the hematological changes induced by cyanide.

Conclusion: HECO reversed the adverse hematological changes in rats induced by cyanide at 100, 150 and 200 doses, with the 200 mg dose being more effective.

Keywords: Cyanide; haematotoxicity; *Chromolaena odorata*; sodium thiosulphate.

1. INTRODUCTION

Cyanide and its compounds inhibits cytochrome c oxidase irreversibly leading to inhibition of oxidative phosphorylation, reduction in ATP level, generation of reactive oxygen species (ROS) and depolarization of mitochondria membrane leading to opening of permeability transition pore. Implication of cyanide poisoning includes nausea, vomiting, diarrhea, dizziness, weakness, mental confusion, and convulsions followed by terminal coma and death [1,2]. Several factors contribute to cyanide genesis including nature of soil, fertilizers application. As plant ages, HCN concentration increases gradually [3].

Cyanide and its antecedents are abundant in the ecosystem. Man and other creatures might be presented to this poison from an assortment of sources. Cyanide ingestion happens primarily through the utilization of plants containing cyanogenic glycosides, including cassava, sorghum, linseed yams, maize, millet, locust beans and a few vegetables [4]. Cassava (*manihot esculenta*) is the most significant of the groceries in which the substance of cyanide makes wholesome issues since it shapes the staple eating regimen of individuals and animals in a few districts of Africa, Asia and South America. Cyanide carries out its toxicity effect by inhibiting cytochrome oxidase causing a cytotoxic hypoxia. It is toxic to many enzyme systems. Cyanide advertises its toxicity in several ways including; reaction with essential metal ions, production of cyanohydrins with carbonyl compounds and the removal of Sulphur in the form of thiocyanate. However, the main target enzyme is cytochrome C oxidase, the terminal oxidase of the respiratory chain and involves reaction with the ferric ion of cytochrome a₃. Cyanide can be metabolized by rhodanese. Rhodanese (EC2.8.1.1) is a ubiquitous enzyme and is widely distributed in plants [5]. It is a detoxification enzyme that involves in the conversion cyanide (a toxic compound) to

thiocyanate a less toxic compound [6]. Various studies have reported the presence of rhodanese in the cytosol and other organelles [5,7,8]. Several research reports have documented its detoxification efficacy in animal tissues [9,10]. Rhodanese has been recorded to be found at a very high concentration in the liver of mammals, though, kidney and other tissues can also contain reasonable amounts [11]. Research had made known that the enzyme also participates in other biochemical activities such as energy metabolism and iron Sulphur centres formation regardless of its widely studied detoxification role [4,12].

One of the consequences of cyanide poisoning is said to be the bright red arterializations of venous blood (because oxygen is not absorbed on passage through tissue) [13]. Blood is the main vehicle for the transport and delivery of substances to the bodys tissues and subsequent removal of their waste products [14]. Larger amount of hydrogen cyanide in blood is sequestered in the red blood cells, and a relatively small proportion is conveyed through the plasma to target organs. Red blood cells consist of high concentration of Hydrogen cyanide at a red blood cell to plasma ratio of 199:1; tissue levels of hydrogen cyanide are reflected in plasma better than levels in whole blood or erythrocytes. Plasma hydrogen cyanide levels was observed to return to normal within 4-8 h after discontinuance of exposure [15]. In rats dosed by gavage, highest concentrations of hydrogen cyanide were found in the liver, followed by the lungs and blood [4]. Hydrogen cyanide has not been shown to accumulate in the blood and tissues following oral exposure to inorganic cyanide [9], and no cumulative effect on the organism during repeated exposure has been demonstrated. Cyanide binds to the ferric form of haemoglobin (a transient physiological form of methemoglobin), which accounts for normally 1% to 2% of all haemoglobin. Binding of cyanide to the ferric form makes this type of

haemoglobin incapable of transporting oxygen [16].

Chromolaena odorata has been widely known among the rural population of Nigeria as ewe Akintola. It has been effectively used as a therapy against diarrhoea, malaria fever, tooth ache, diabetes, skin diseases, dysentery and colitis [17,18]. The plant has been utilized in the remediation of cyanide from contaminated sites [19,20] and this gives credence to its exploitation in this study.

2. METHODS

2.1 Collection and Preparation of *Chromolaena odorata*

Stem with Leaves of *C. odorata* were collected from an uncultivated land near a residential location at Hamama area, Ogbomoso, South-Western Nigeria and identified by Prof. A.T.J Ogunkunle at the Herbarium of Ladoké Akintola University of Technology (LAUTECH), Ogbomoso with voucher number, LHO 526. They were air dried, packed in paper bags and stored. The dried leaves were pulverized and 196 g of the pulverized sample was extracted with 500 ml of 80% hexane by maceration for 72 h. The hexane extract was concentrated in a rotary evaporator, lyophilized and thereafter preserved for further use.

2.2 Experimental Design

Thirty (35) male albino rats weighing between 100g and 150g were distributed randomly into 7 groups (Six (6) experimental groups and One (1) control group). They were transferred to the Animal House in the department of Biochemistry, Ladoké Akintola University of Technology, Ogbomoso to acclimatize for two weeks. They were fed on commercial rat pellets and water ad libitum. They were kept in cages whose dimensions are 30 cm by 15 cm by 25cm at room temperature. The cages, feed and water troughs, beddings were properly maintained in hygienic condition. The rats were subsequently distributed randomly and placed in one control and six experimental groups.

- Group 1: - this is the control group and they were maintained without the extract or cyanide.
- Group 2: - this group received 3 mg/kg body weight of potassium cyanide.

- Group 3: - this group received 3 mg/kg body weight of potassium cyanide with 100 mg/kg *Chromolaena odorata*
- Group 4: - this group received with 3 mg/kg body weight of potassium cyanide with 150 mg/Kg hexane extract of *Chromolaena odorata*
- Group 5: - this group received 3 mg/kg body weight of potassium cyanide with 200 mg/Kg hexane extract of *Chromolaena odorata*
- Group 6: this group received 3 mg/kg body weight of potassium cyanide, with 200 mg/kg of HECO and 200 mg/kg Sodium thiosulphate
- Group 7: - this group received 3 mg/kg body weight of potassium cyanide, 200 mg/kg Sodium thiosulphate

2.3 Blood Collection and Hematological Investigations

After a brief exposure of the rat to diethyl ether (anaesthetic) making the animal unconscious, the animals were placed on a dissecting board with the limbs pinned to the board. The rat is dissected starting from the central abdominal region upward to expose the internal region and organs. Using a syringe and needle, the blood was collected by cervical puncture into tube with anticoagulant ethylene di-amine tetra acetic acid (EDTA) for hematology. Hematological examination was done using the International Council for Standardization in Hematology (ICSH) standard procedures using Automated Haematology Analyzer-MC-2800 (Mindray Company, China). Packed cell volume (PCV), white blood cell count (WBC), lymphocyte count (LYM), monocyte count (MID), lymphocyte percentage (LYM%), monocyte percentage (MID%%), Granulocyte % (GRA%), Haemoglobin concentration (HB), Red blood cell count (RBC), Platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RCDW), red cell distribution width-coefficient of variation (RCDW-CV), red cell distribution width-standard deviation (RCDW-SV), were determined.

2.4 Statistical Analysis

All calculations were performed using Graph Pad Prism 5 software. All data were analyzed using one-way analysis of variance, followed by

Tukeys multiple comparisons test. The level of significance was set at $P < .05$.

3. RESULTS

3.1 Effects of HECO and Na_2SO_3 on Hemogram of Cyanide Exposed Rats

Significant ($P < 0.0001$) decrease was observed in the RBC, HB, HCT, MCH, and MCHC with exception in the PLT and MCV where no significant changes were observed. Moreover, significant increase was observed in RDW-SD, RDW-V in the cyanide group compared to the control group.

The administration of the extract alone or in combination with sodium thiosulphate caused a significant ($P < 0.0001$) increase in RBC, HB, HCT, MCH, and MCHC but significant increase ($P < 0.0001$) in RDW-SD, RDW-V and MID. Although all the treatment showed an improvement in hemogram parameters in the order; Group 6 > group 7 > group 5 > group 4 > group 3.

3.2 Effects of HECO and Na_2SO_3 on Leukogram of Cyanide Exposed Rats

The same trend as observed in the hemogram was observed on the Leukogram parameters after treatment with the extract and thiosulphate at all doses. Induction with KCN resulted in significant ($P < 0.0001$) reduction in White blood cell count and differentials whereas this effect was reversed by the extracts at all doses and $\text{Na}_2\text{S}_2\text{O}_3$. Combination of Treatment regimen (HECO + $\text{Na}_2\text{S}_2\text{O}_3$) however performed better than the single administrations.

4. DISCUSSION

Blood is a good predictor of health status and pathological reflector of the entire body [21]. The present study showed that cyanide induction in rats revealed alterations in hematological parameters which contributes to the life-threatening effects of cyanide compounds as speculated by Ehigie et al. [22]. The observed protection offered by *Chromolaena odorata* may be due to availability of sulphane-sulphur which

mediates the detoxification of cyanide to less toxic metabolites by the action of the sulphurtransferase, rhodanese [23]. The haemopoietic stimulating effect of the extracts is an evidence of the beneficial effects of herbs [24].

Blood indices are useful in the estimation of toxic potential of a substance. Cyanide administration caused a decrease in mean corpuscular hemoglobin level (MCH) and mean corpuscular hemoglobin count (MCHC) which agrees with the report of Ologunagba and Azubuike, [25] and Jiwuba et al. [26]. It has been reported that lysis of RBC and decrease in Haemoglobin level were responsible for the decrease in MCH, MCHC, MID, RDW [27]. Ehigie et al. [22] reported that hemaphagocytosis and bone marrow suppression are important mechanism in inducing hematological changes. The production of red blood cell (RBC) known as erythropoiesis occurs in the bone marrow and is controlled by the hormone erythropoietin (EPO) [28]. Moreover, Juxtglomerular cells in the kidney synthesize erythropoietin in response to diminished oxygen delivery (as in anemia and hypoxia). Depletion of RBC and decrease in haemoglobin may be as a result of unavailability of oxygen and nutrients to the cells and accumulation of waste products in the cells [29]. Expectedly, cyanide administration reduced the levels of the red cell indices measured in this study [22]. This is a clear indication of the hypoxic effect of cyanide. In addition to erythropoietin, red blood cell production needs adequate supplies of substrates, mainly iron, vitamin B12, folate, and heme [30]. The relief provided by the treatment agents may be by the supply of these substrates.

Administration of HECO led to reversal of the reduction in the levels of white blood cells and all related indices in the poisoned rats. This is an indication that the extract may have immune stimulating properties [31]. The increase in WBC count may have been due to improvement in the rate generation of leucocytes from hematopoietic stem cells. The development of committed stem cells responsible for the production of WBCs is regulated by haematopoietic regulatory elements including Granulocyte-macrophage colony stimulating factor, macrophage colony stimulating factor, interleukins (IL-2, IL-4 and IL-5) [32].

Table 1. Effect of HECO and Na₂SO₃ on Red cell and platelet indices in cyanide exposed rats

Paremeters	Control	KCN	KCN + 100HCO	KCN + 150HCO	KCN + 200HCO	KCN + 200HCO+ 200 Na ₂ S ₂ O ₃	KCN+ 200 Na ₂ S ₂ O ₃
RBC (mm ³)	7.35 ± 0.12	1.00 ±0.96*	2.45 ± 0.85*	3.17 ± 0.79*	4.07 ± 0.30*#	6.08 ± 0.12#	5.94±0.12#
HB (g/dL)	17.18 ± 0.29	4.41 ± 1.93*	5.88 ± 1.39*	6.80 ± 1.16*	10.02 ± 0.77*#	12.71 ± 0.96#	11.87±0.99*#
HCT (%)	45.54 ± 0.87	15.24 ± 1.98*	18.64 ± 1.38*	21.40 ± 1.29*#	30.06 ± 1.25*#	40.12 ± 0.80#	38.62±0.94*#
MCV (fL)	55.38 ± 1.560	51.38 ± 2.035	52.21 ± 1.908	52.97 ± 1.672	53.51 ± 1.611	55.11 ± 1.588	54.32 ± 1.570
MCH (pg)	5.29 ± 0.12	1.22 ± 0.69*	1.98 ± 0.59*	2.07 ± 0.41*	2.99 ± 0.36*	4.684 ± 0.28#	4.164 ± 0.26#
MCHC (g/L)	46.68 ± 1.38	17.38 ± 4.10*	23.58 ± 3.41*	26.46 ± 2.93*	31.16 ± 2.19*#	44.52 ± 1.50#	41.80 ± 1.82#
RDW-CV(fL)	16.60 ± 0.23	24.32 ± 0.49*	22.96 ± 0.81*	20.84 ± 0.70*#	18.62 ± 0.51#	17.92 ± 0.31#	17.08 ± 0.38#
RDW-SD (fL)	20.26 ± 0.31	28.84 ± 1.09*	24.48 ± 1.01*#	22.95 ± 0.99#	18.28 ± 0.88#	19.72 ± 0.77#	18.96 ± 0.66#
PLT (10 ⁶ /L)	1451 ± 284.9	2562 ± 356.2	2197 ± 332.8	1974 ± 311.1	1800 ± 307.2	1562 ± 299.7	1762 ± 302.2

Values are given as Mean ± SEM (Standard Error of Mean), n =5.

*represents significant difference with respect to the control group, # represents significant difference with respect to the KCN group.

Table 2. Effect of HECO and Na₂SO₃ on White blood cell and differentials in cyanide exposed rats

Paremeters	Control	KCN	KCN + 100HCO	KCN + 150HCO	KCN + 200HCO	KCN + 200HCO+ 200 Na ₂ S ₂ O ₃	KCN+ 200 Na ₂ S ₂ O ₃
WBC (10 ⁶ /L)	15.16 ± 0.89	1.25 ± 1.09*	4.55 ± 1.00*	6.01 ± 0.99*#	8.95 ± 0.91*#	13.22 ± 0.89#	11.89±0.89#
LYM# (10 ⁹ /L)	23.76 ± 0.75	8.65±1.59*	12.55±1.32*#	15.98±1.12*#	18.99±1.02*#	22.00 ± 0.81#	20.99± 0.98#
LYM (%)	80.96 ± 1.02	52.65±2.08*	61.28 ± 2.02*	67.76±1.65*#	70.05 ± 1.36#	ab78.54 ± 1.12#	75.35 ± 1.25#
GRAN# (ml)	11.54 ± 0.34	3.22 ± 1.03*	5.67 ± 0.96*	7.29 ± 0.89*#	8.05 ± 0.54*#	ab10.72 ± 0.39#	8.87± 0.42#
GRAN (%)	12.70 ± 0.79	2.55 ± 1.75*	3.90 ± 1.53*	5.00 ± 1.32*	7.21 ± 1.18	11.00 ± 0.82#	9.98 ± 0.89#
MID# (ml)	0.22 ± 0.04	1.53 ± 0.19*	1.28 ± 0.18*	0.95 ± 0.13*#	0.73 ± 0.09#	0.33 ± 0.05#	0.44 ± 0.08#
MID (%)	5.34 ± 1.23	15.21 ± 1.98*	12.22 ± 1.76	9.55 ± 1.64	7.66 ± 1.52#	5.78 ± 1.32#	6.01 ± 1.43#

Values are given as Mean ± SEM (Standard Error of Mean), n =5.

*represents significant difference with respect to the control group, # represents significant difference with respect to the KCN group.

Differential white blood cell counts have been used in the assessment of stress effects [33]. There exists a positive correlation between stress conditions and white blood cell differentials. The cyanide-induced leukocytosis may be an indication of cyanide toxicity on the immune system. White cell differential counts important indices of the ability of an organism to eliminate infection. An increase in the number of circulating leukocytes is rarely due to an increase in all the types of leukocytes. Decrease in total white blood cell count (WBC) and differentials (lymphocytes and granulocytes) levels was also observed which correspond to the findings of Kadiri [34]. The leucopenia and neutropenia may be through the defects in the hematopoietic organs such as the spleen and bone marrow which is said to slow down leucopoiesis [22], while the lymphocytosis and monocytosis may be due to an increased release of cells from lymphoid/myeloid tissues [35].

Neutrophils attack and destroy pathogens in the blood [36]. Improved neutrophil counts increased the phagocytic activity in the animals. Lymphocytes are the major player in the immune system. The increase in their level observed in this work may improve the immune system. In a similar way, the increased levels of eosinophils and basophils observed in the present study indicate potent immune system. The defect in the white blood cell subsets and differentials suggests that not only does the immune system reacts to pathogens or allergies but to any form of stressors.

Sodium thiosulfate is considered an ineffective antidote for acute cyanide toxicity because of poor intracellular penetration, slow onset of effect, a short half-life, and limited distribution volume, however, it is often used in combination with antidotes with rapid action. Its single use as an antidote for cyanide poisoning is no longer approved of Alzhrani [37].

5. CONCLUSION

HECO reversed the adverse hematological changes in rats induced by cyanide at 100, 150 and 200 doses, with the 200 mg dose being more effective. However, by comparison with sodium thiosulphate alone, HECO combined with sodium thiosulphate did not have a better protective effect. Further studies are needed to explore possible ways through which HECO could potentiate the action of Sodium thiosulphate and help overcome its limitations.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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