

## Research Paper

# Effect of Plant Biomass Adsorbents on the Disposition of Cyanide in Wistar Rats Exposed to Sublethal Dose of Cyanide



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## ABSTRACT

**Background:** Chemical intoxication of dietary origin is one of the significant public health problems confronting the global community. Cyanide is a very toxic chemical that reacts and forms a stable complex with iron in ferric cytochrome oxidase, inhibiting its role in oxidative phosphorylation. Adsorbents are carbon materials that attract poisons to their surface, thus preventing their absorption into the gastrointestinal system. This study aims to determine the effect of adsorbents prepared from plant biomass on the disposition of cyanide in albino Wistar rats.

**Methods:** A total of 30 male Wistar rats were used for the study. The rats were randomly assigned to 5 groups of 6 rats per group. A single dose of 3 mg/kg potassium cyanide was administered orally to rats in the groups. After 15 minutes, all rats in each group were given activated charcoal from different sources. Blood samples were collected serially from the albino rats in each group for cyanide assays following standard procedures. Data were analyzed by one-way analysis of variance (ANOVA), followed by Bonferroni post hoc test using SPSS software, version 24.0.  $P < 0.05$  were considered to be statistically significant.

**Results:** In all groups, the highest blood cyanide level was reached at the 30-minute (0.5 h) mark, and from there, the blood cyanide levels declined till day 21. At the 30-minute mark, the highest blood cyanide concentration ( $34.41 \pm 0.65 \mu\text{mol/L}$ ) was seen in group 1 rats, followed by group 4 rats ( $32.54 \pm 0.40 \mu\text{mol/L}$ ). The lowest blood cyanide level ( $30.45 \pm 0.21 \mu\text{mol/L}$ ) was seen in group 5 rats.

**Conclusion:** The study demonstrated that the administration of activated charcoal samples prepared from coconut shells, castor oil seed shells, and plantain peels minimized the absorption of cyanide and the consequent toxicity in Wistar rats exposed to a sublethal dose of cyanide.

### Keywords:

Cyanide intoxication, Activated charcoal, Biomass adsorbents, Blood concentration

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## Introduction

**H**uman exposure to cyanide may occur through mining, industrial usage, smoke from a fire, propulsion motors, some food substances and tobacco [1-5]. On absorption, cyanide readily forms a reversible complex with methemoglobin and hemoglobin, which is distributed widely in the body tissues irrespective of the means of exposure to the chemical substance [6, 7].

Since these complexes are reversible, cyanide could dissociate from them under the organ system and cause the inhibition of several cellular enzymes, including cytochrome c oxidase, resulting in cellular hypoxia and cytotoxic anoxia, which is potentially fatal [8-13]. Cyanide contributes to toxicity by inhibiting other critical enzyme systems such as peroxidase, phosphatase, tyrosinase, and dehydrogenase activities [12-17].

Cyanide is a very toxic chemical that reacts and forms a stable complex with the iron in ferric cytochrome oxidase, inhibiting its role in oxidative phosphorylation. It is known to induce oxidative stress and damage in several biological systems. Protection of animals from the toxic effects of cyanide is made possible by diverting the cyanide before it reacts with the cytochrome enzyme [18-21].

The diversion is achieved by promoting the formation of additional sources of ferric ions, which will react with the cyanide, leading to the formation of less toxic cyanide product or creating a barrier to a cyanide translocation to the target cytochrome by arresting the cyanide with adsorbents [22, 23]. While the former can be achieved using such less toxic chemicals as sodium nitrite, amyl nitrite, dimethyl aminophenol, and sodium thiosulphate, the latter is usually accomplished using activated charcoal from agro-based biomass products [24-29]. Agricultural waste is usually an optimal choice when preparing commercial activated charcoal. They have a high specific surface area, high stability, efficient adsorption capacities, an easy and simple production process, and are cost-effective [24, 25].

The adsorptive capacity for cyanide by the prepared activated charcoal samples is enhanced by the increased surface area and porosity of the materials following activation by some chemical activating agents [28, 29]. This study aimed to determine the effect of some adsorbents prepared from plant biomass on the disposition of cyanide in albino Wistar rats exposed to sublethal doses of cyanide.

## Materials and Methods

### Preparation of activated charcoal from local agro-based carbon materials

The local agro-based samples were prepared from plantain peels, castor oil seed shells and coconut shells.

### Chemical activation and carbonization of materials

The materials were then serially activated with different chemical agents. The chemical agents used for activation were zinc chloride, phosphoric acid and nitric acid. A solution of 100 mL of 10% weight for volume of zinc chloride was mixed with 50 g of powdered carbon sample, 500 mL of 0.3 mol/dm<sup>3</sup> phosphoric acid was mixed with 50 g of prepared carbon sample, and 500 mL of 0.5 mol/dm<sup>3</sup> of nitric acid was mixed with 50 g of the carbon sample. The different mixtures were allowed to stand for 24 hours with regular stirring to enable the chemical activating agents to penetrate the carbon materials thoroughly. This procedure was adapted with minor modifications from Ekpete et al. [25] and Subramani and Rivathi [30].

Thirty male albino Wistar rats weighing 180 to 200 g were used in the study to determine their blood cyanide concentrations. They were acclimatized for one week and fed commercial poultry feed and water ad libitum. The animals were later randomly divided into five groups of six [6] rats each. The study lasted 21 days, while the cyanide and the activated charcoal administration route were oral (using an orogastric gavage tube). Each rat received potassium cyanide (KCN), 3 mg/kg stat dose at the beginning of the experiment. Single-dose activated charcoal samples prepared from different local agro-based carbon materials and standard commercial activated charcoal were administered to the rats in each group after 15 minutes of ingesting potassium cyanide at 1 g/kg body weight.

Group 1 rats received food, water, potassium cyanide (KCN) and standard activated charcoal. Group 2 rats received food, water, KCN and carbon samples prepared from plantain peels activated with zinc chloride (ZnCl<sub>2</sub>). Group 3 rats received food, water, KCN and carbon samples prepared from castor oil seed shells activated with Nitric acid-(HNO<sub>3</sub>). Group 4 rats received Food, water, KCN, water and carbon samples prepared from coconut shells activated with phosphoric acid (H<sub>3</sub>PO<sub>4</sub>).

Group 5 rats received food, KCN, water and a combination of castor shell-HNO<sub>3</sub>, coconut shell-(H<sub>3</sub>PO<sub>4</sub>)+plantain peels-(ZnCl<sub>2</sub>).

### Determination of blood cyanide levels in the wistar rats

Blood samples were collected from albino rats in all groups at the start of the experiment and different intervals until the 21<sup>st</sup> day. The blood samples were analyzed for the concentrations of cyanide. The Lundquist and Sorbo [31] method for the rapid determination of toxic cyanide concentrations in blood was used to determine the blood cyanide concentrations in the Wistar rats.

### Data analysis

Data were analyzed by one-way analysis of variance (ANOVA), followed by Bonferroni post hoc test using SPSS software, version 24.0. P<0.05 were considered to be statistically significant.

## Results

### Blood cyanide concentration

The Table 1 and Figures 1 and 2 shows the blood cyanide levels in the albino Wistar rats after ingesting potassium cyanide and treatment with the prepared activated charcoal and standard activated charcoal.

The blood cyanide levels were measured from baseline (Time 0) to day 21.

GP 1, KCN and standard commercial activated charcoal; GP 2, KCN and activated charcoal from plantain peels; GP 3, KCN and activated charcoal from castor oil seed shell; GP 4, KCN and activated charcoal from coconut shell; GP 5, KCN is a combination of activated charcoal from plantain peels, castor oil seed shells, and coconut shells.

## Discussion

The lowest blood cyanide level at 30 minutes (30.45±0.21 μmol/L) was seen in group 5 rats that received a combination of three prepared adsorbents (coconut shell, castor oil seed shell and plantain peels). The blood cyanide levels were significantly lower in the groups treated with the locally prepared activated charcoal compared with the group that received the standard commercial activated charcoal.

The maximum blood cyanide concentration in this study is relatively lower than the values obtained in other studies in which cyanide (KCN 3 mg/kg) was administered orally via gavage to the rats without any intervention [32-34]. The C<sub>max</sub> for cyanide in a study by Sousa et al. [35] in which potassium cyanide (KCN) was administered to the rats without intervention was 89 μmol/L with a T<sub>max</sub> of 15 minutes. In another study by Bhandari et al. [35], the C<sub>max</sub> of blood cyanide in rats showed 59.8

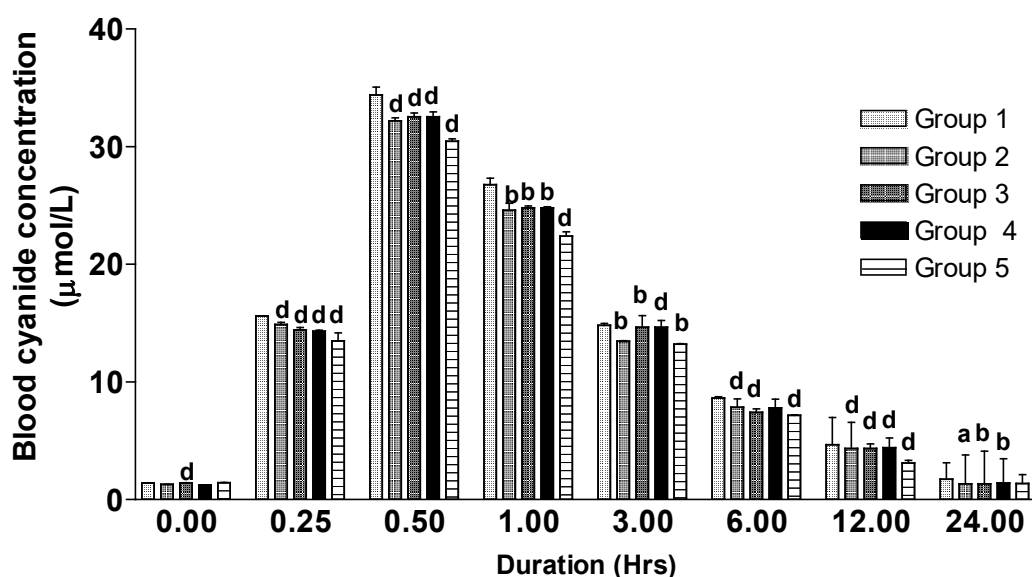


Figure 1. Blood cyanide concentrations (μmol/L) over time (hours) from baseline to 24 hours in the wistar rats

**Table 1.** The blood cyanide levels ( $\mu\text{mol/L}$ ) after ingestion of KCN and treatment with the prepared activated charcoal samples and standard activated charcoal

Time (hours)	Group 1	Group 2	Group 3	Group 4	Group 5
0	1.40±0.01	1.30±0.003 <sup>d</sup>	1.42±0.001 <sup>d</sup>	1.24±0.003 <sup>d</sup>	1.44±0.011 <sup>d</sup>
0.25	15.62±0.001	14.87±0.20 <sup>d</sup>	14.42±0.20 <sup>d</sup>	14.30±0.16 <sup>b</sup>	13.49±0.69 <sup>d</sup>
0.5	34.41±0.65	32.21±0.25 <sup>d</sup>	32.55±0.32 <sup>d</sup>	32.54±0.40 <sup>d</sup>	30.45±0.21 <sup>d</sup>
1	26.77±0.56	24.60±0.60 <sup>b</sup>	24.80±0.15 <sup>b</sup>	25.40±0.10 <sup>b</sup>	22.39±0.38 <sup>d</sup>
3	14.80±0.17	13.45±0.05 <sup>b</sup>	14.64±1.00 <sup>b</sup>	14.64±0.58 <sup>d</sup>	13.21±0.05 <sup>b</sup>
6	8.62±0.11	7.85±0.70 <sup>d</sup>	7.40±0.31 <sup>d</sup>	7.79±0.75	7.17±0.03 <sup>b</sup>
12	4.66±2.31	4.35±2.21 <sup>d</sup>	4.34±0.40 <sup>d</sup>	4.42±0.81 <sup>d</sup>	3.11±0.22 <sup>d</sup>
24 (1 day)	1.73±1.40	1.35±2.45 <sup>a</sup>	1.34±2.77 <sup>b</sup>	1.40±2.06 <sup>b</sup>	1.37±0.76
48(2 days)	1.31±0.22	1.25±0.34	1.31±0.95	1.27±2.55 <sup>a</sup>	1.18±0.12 <sup>b</sup>
168 (7 days)	1.22±0.25	1.13±1.01	1.20±0.43 <sup>b</sup>	1.20±2.99	1.16±1.01
336 (14 days)	1.20±0.23	1.12±0.22 <sup>b</sup>	1.10±2.97 <sup>b</sup>	1.15±0.74 <sup>b</sup>	1.13±0.55 <sup>a</sup>
504 (21days)	1.19±0.90	1.12±1.91 <sup>a</sup>	1.10±3.80 <sup>a</sup>	1.15±2.23	1.10±1.91
F	118.56	99.01	82.47	96.57	816.07
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

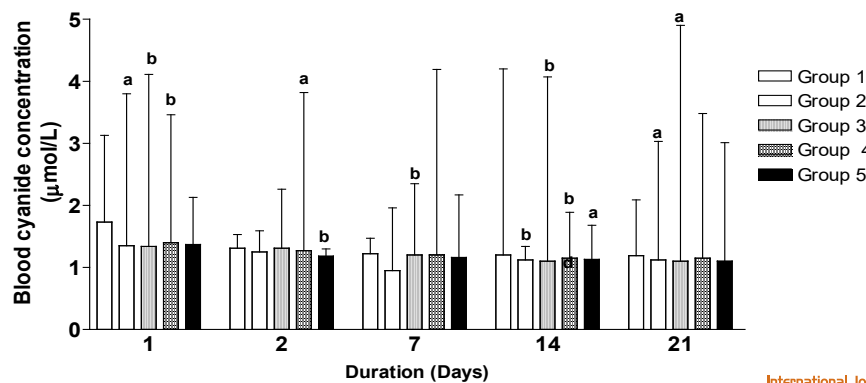
<sup>a, b, d</sup>Comparison of control with group 1 represents P<0.05, P<0.01, P<0.0001 respectively.

$\mu\text{mol/L}$ . The  $C_{\text{max}}$  for the blood cyanide in these studies can be seen to be significantly higher than the  $C_{\text{max}}$  for blood cyanide in this present study, in which biomass adsorbents were administered to the rats after exposure to cyanide.

In another study by Abraham et al. [18], in which blood cyanide levels were measured after consumption of cyanogenic food (cassava and bitter apricot) consumption, the  $C_{\text{max}}$  was 19.5  $\mu\text{mol/L}$  and the  $T_{\text{max}}$  was 30 minutes, after bitter apricot consumption,  $C_{\text{max}}$  was 15.4  $\mu\text{mol/L}$ ,

and  $T_{\text{max}}$  was 15 minutes. The lower  $C_{\text{max}}$  in this study by Abraham may be due to some extraneous factors and the fact that the level of cyanide in the food items does not compare with the direct administration of potassium cyanide to the Wistar rats as used in this present study.

One of the main factors that affect absorption is the relationship between the pKa of the substance and the pH of the gastrointestinal environment. Because cyanide is a weak acid with a pKa of 9.22 [36-38] and considering that the pH in the monogastric stomach is between 1.0



**Figure 2.** In the wistar rats, blood cyanide concentrations ( $\mu\text{mol/L}$ ) over time (days) from day 1 to day 2

and 2.0 [39], it may be concluded that cyanide should be more efficiently absorbed in the monogastric stomach because more of the non-ionized form of the molecules will be generated.

Studies done by Akyildiz et al. [40] and Arabizadeh et al. [41] showed improvement in cyanide-intoxicated patients treated with activated charcoal after some hours. Lambert et al. [38] made the same findings in rats that received granular potassium cyanide and were immediately treated with super-activated charcoal. All the rats exposed to cyanide in the various groups in this study survived and showed little signs of cyanide intoxication owing to the immediate administration of activated charcoal samples from local agro-based sources [40, 41]. Chijioke et al. [42] also showed an improved biochemical profile in cyanide-exposed Wistar rats treated with activated charcoal prepared from plant biomass. Some adsorbents in this study are believed to have better adsorptive capacities owing to their enhanced porosity and surface morphology.

## Conclusion

The study demonstrated that administering activated charcoal samples prepared from coconut shells, castor oil seed shells and plantain peels minimized the absorption of cyanide and the consequent cyanide toxicity in Wistar rats exposed to a sublethal dose of cyanide. The adsorption capacities of these biomass adsorbents in this experiment were better than those of conventional commercial activated charcoal in clinical use. Because of the findings from this study, adsorbents prepared from these plant materials may be utilized in clinical practice and for purifications in industrial settings. These materials can also be used in clinical trials in the future to facilitate the treatment of chemical intoxication in patients. Limitations of this study include low sample size, variability in the properties of the plant biomass, and lack of clinical trials in humans

## Ethical Considerations

### Compliance with ethical guidelines

This study was approved by the Ethics Committee of the [University of Nigeria Teaching Hospital](#), Enugu State, Nigeria (Code: NHREC/05/01/2008B-FWA00002458-IRB00002323).

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## Authors' contributions

Conceptualization and study design: Casimir C Ofor, Uchenna D Mbah and Godwin C Akuodor; Data collection, data analyzed and data interpretation: C Ofor, Mansur A. Ramalan, Donatus O Anele, Eugene O Ohanme, Daniel O Aja, and Uzochukwu Ofonakara; drafting the manuscript and critically reviewing: Casimir C Ofor, Godwin C Akuodor, Benjamin N Nwakelu and Anthony U Megwas; Final approval: All authors.

## Conflict of interest

The authors declared no conflict of interest.

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