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Food Chain Borne Effect of Cadmium and Cyanide on Some Biochemical Indices in the Liver of Rats

Helen Ejiro Kadiri , Ovigueroye Ekayoda
Department of Biochemistry, Faculty of Science, Delta State University, Abraka, Nigeria

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Abstract

Diverse organismsuch as mammals and fishes exposure to noxious waste in the surroundings is a continuous routine and the active absorption and propagation of contaminants in humans is through the food chain. In order to determine the level of toxicity across the food chain,this research was structured to identify some biochemical alterations in the hepatic tissue of rats fed cadmium, cyanide and a mixture of cyanide and cadmium contaminated catfish diet. Fish were assigned into four groups and were exposed to both toxicants (cadmium and cyanide) in the single and combined states. Each toxicant was administered as cadmium chloride ($CdCl_2$) and potassium cyanide (KCN) on a dose of 0.4 mg of the toxicant/100 ml water for about four weeks in a 100 Liter capacity water plastic aquarium. Fish in group A were housed in uncontaminated water (control), group B was housed in cyanide-contaminated water, group C washoused in a cadmium-contaminated water and group D was housed in cyanide + cadmium contaminated water. Subsequently the fish were sacrificed, dried and served as protein source in the prepared diet used to feed rats. The rats were also allocated into four groups and fed for 28 days.Thereafter they were sacrificed and the liver excised for biochemical assays. The end result attained specified that consumption of the contaminated diet changed the exploits of hepatic enzymes; alanine aminotransferase (ALT) and aspartate aminotransferase (AST) alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in tissue and serum of rats. A notable decrease ($P<0.05$) was detected in albumin, serum total protein and globulin level in the experimental rats. Also, a significant increase/decrease ($P<0.05$) in oxidative stress parameterswas noted in hepatic tissues of rats exposed to the contaminated diet. Thus, the discoveries of the research stipulated that interaction with cyanide and cadmium via the food-chain led to anomaly in liver function of the experimental rats so perturbing the usual metabolic actions.

Keywords: Liver, Cyanide, Cadmium, Oxidative stress, Toxicity.

Introduction

Human interaction with noxious waste in the ecosystemhas been a continuous routine and the active absorption and propagation of contaminants in humans is through the food-chain. This phenomenon which jeopardizes the populace, has developed urgent consideration from monitoring organizations and also the general public [1,2]. Ultimately there have been emergent concerns on the infinite discharge of contaminants to the food-chain with its aftermath being toxicityis a subject of crucial concern.Thus, studies as regards to the outcome of toxicity by means of the food-chain have received considerably deliberation [3,4]. Human health is endangered by pollutants which are renowned for their tenacity in the environment.

*Email: hekad@yahoo.com

Cadmium (Cd), a renowned dispensable element, is amongst numerous extremely lethal elementsdispensed into the environment. It is predominantly released by industrial processes such as painting, welding and electroplating. Cadmium is not easily transformed and has therefore turned out to be extensively accessible in the surroundings **[**5]. Consumption of cadmium-contaminated sea food consequently makes it a food-chain contaminant of severe burden **[**6]. Since Cd is not biologically transformable, exposure (chronic and acute) to it springs forth its bioaccumulation and subsequently ensures diverse adverse effects on organisms [7].

Cyanide (CN), an enormously poisonous chemical compound existing in the ecosystem, is used in several industrial processes hence making it a predominant environmental pollutant [1]. CN occurs in numerous forms in the surroundings as free cyanide, organo-cyanides and metallo-cyanides, and human contact with it effects toxicity [8]. CN primarily occurs in some food as cyanogenic glycoside and its release from industrial activity such as mining and paint industries gives rise to its availability in the environs [1]. Cadmium and cyanide which emanate from industrial activities are environmental pollutants (particularly in aquatic habitation) and their release into the aquatic habitat predominantly affects organisms leading totheir impact on terrestrial organisms through food-chain [9,10].These pollutants can occur concurrently in the ecosystem [11].

Organisms in aquatic habitation (aquatic plants such as water hyacinth, water lettuce and duck weed and animals) are predisposed to different concentrations of diverse toxic substance exposure [12]. Thus, fish which is an important source of food turn out to be an essential means of transfer of toxic substances in the food-chain making it a good bio-indicator [13]. There have been studies of toxicity on rats using contaminated catfish diets [14]. Contact with cadmium and cyanide via the food-chain gives rise to toxicity which in turn stimulates oxidative stress and its outcome produces free radicals initiating quite a lot of organ disarrays such as hepaticdysfunction, osteo-toxicity, testicular damage, renal dysfunction, cardiovascular disorder etc. [15,16].

Hepatocellular damage and bioaccumulation of toxic substances in liver usually occurs following acute exposure to toxins in humans and other mammals.Liver is an insightful predictor of biochemical toxicity among other tissues in the mammalian body [7]. This unique trait of the liver is attributable to its role in the biochemical modification of xenobiotic substances, thus making it a significant target organ for xenobiotic induced injuries. Reports on the influence of cyanide and cadmium on hepatic tissue via contaminated food-chain are rare in literature which articulates the basis of this study. Therefore, the current study assessed the effects of cyanide and cadmium toxin on hepatocyte via the food-chain which aids in the verification of the lethal effects of the combination of the toxicants.

Materials and Methods

Handling of fish

Sixty healthy African catfish with body weight and length ranging from 250-255 g and 30-33 cm respectively, were procured from a fish farm in Obiaruku, Delta state, Nigeria. They were accustomed to temperature ranging between 24-26^{0}Cwith humidity from 80-82%, and an alternating 12hr light and dark cycle for a week.The fish were fed with fish feed pellets*ad libitum* during accustomed and test periods[17]. The fish were grouped into four with fifteen fish in each group and were housed in a 100 L plastic aquarium with water respectively.

- Group A (control) – Fish were retained in freshwater
- Group B (cyanide contaminated) – Fish were retained in contaminated water treated with 0.4 mg of cyanide /100 ml water

- Group C (cadmium contaminated) – Fish were retained in contaminated water treated with 0.4 mg of cadmium /100 ml water
- Group D (cyanide + cadmium contaminated) – Fish were retained in contaminated water treated with 0.4 mg of both cyanide and cadmium /100 ml water.

The dosage of the contaminant (0.4 mg) used in the existing study was previously reported [17].However, varying lethal concentration of contaminant usually occurs in aquatic environment.Hence this dosage of 0.4 mgwas equal to a dose of 4ppm which is sub-lethal.In each group, changing and re-contamination of water was done every 48 hours for 28 days before the fish were slain, dried and used to prepare diet.

Diet preparation

The diet of each group was prepared using the dried catfish as protein source (25 %), corn starch as carbohydrate source (50 %), vegetable oil as fat and oil source (5 %), corn cob as fiber source (5 %), granulated refined sugar as sugar source (10 %), vitamin and mineral mix as vitaminsource (5 %). These food components mixed together formed the diet that was given to the experimental animals[17].

Handling of experimental animals

Twenty healthy Wister rats (male) weighing between 80-130g were adoptedfor the existing study.For about 14 days, they were accustomed to laboratory conditions with a temperature range of between 24-26^{0}C, humidity ranging from 80-82%, and an alternating 12hr light and dark cycle. The rats had unrestricted access to water and feed along with conducive environment in accordance with the norm guiding experimental animals [18].The groupings of the animal were into four having five rat each and were fed according to their respective groups.

- Group A given control prepared diet
- Group B given cyanide-contaminated prepared diet
- Group C given cadmium-contaminated prepared diet
- Group Dgiven cyanide + cadmium-contaminated prepared diet.

For a test period of 28 days, animalsin all groups were fed the prepared diet daily andthey were finally slain by cervical decapitation. Blood samples from respective groups were taken and placed in plain tubes to coagulate. The livers also were removed and 0.5 g of it was homogenized in 4.5 ml 0.1 M phosphate buffer (pH 7.4). The coagulated blood and liver homogenate from respective groups, were subjected to centrifugation for 15 min at 4000 rpm.The separated liver and serum gotten was then preserved in refrigerator at 4^{o}C for biochemical assay.

Cadmium and cyanide analysis on tissue and feed

The concentrations of cadmium and cyanide in the digests were measured by atomic absorption spectrophotometry (Varian AA1475 Spectrophotometer). Deionized water was used to dissolve cadmium and cyanide, and the solvent was used as standard. Blanks were prepared for the assay in order to ascertain the outcome of the purity of the reagent on cadmium and cyanide levels [19].

Biochemical Assay

Assay for hepatic markers

Serum and tissue alkaline phosphatase (ALP),alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were evaluated with diagnostic kits (Randox Laboratories Limited, England) and lactate dehydrogenase (LDH) activity was assayed by the technique designated by Appleby and Morton (1959) [20]. Serum total protein was assessed

by the techniqueadopted by Tietz [21]. Albumin in serum was evaluated by Doumas*et al.* procedure [22], bilirubin in serum was estimated by Malloy and Evelyn method [23]. Globulin level was measured with the aid of the principle:
Globulin = Total protein – Albumin.
Albumin to Globulin (A/G) ratio was estimated using the principle:
A/G ratio = Albumin level/Globulin level

Lipid peroxidation assessment

The Niehius and Samuelson method[24] was used to assess lipid peroxidation (LPO) in hepatic tissue by evaluating thiobarbituric acid reactive substances via spectrophotometer.

Antioxidant activities analysis

Rani *et al.* procedure [25] was adopted to determine catalase activity (CAT). Superoxide dismutase activity (SOD) was analyzed by the technique of Misra and Fridovich**[**26]. Cribb *et al.* method [27] was adopted to assess glutathione reductase activity (GR). Reduced glutathione activity (GSH) was assessed by Ellman *et al.* method [28]. Rotruck*et al.* technique [29] measured glutathione peroxidase activity (GPX). Habig*et al.* procedure [30] estimated Glutathione S-transferase activity (GST).

Histological Analysis

Fixing of liver sample was done in a solution 10 % formalin for 48 hours.Thereafter the sample was dehydrated in different concentration of alcohol, cleared in xylene and infiltrated in paraffin wax. The liver was cut into sections of about 5 µ thick using a microtone and then tainted with hematoxylin and eosin (H&E) dye. The tainted tissue was mounted in light microscope and photographed. [31]

Statistical Analysis

Values were reported as Mean ± Standard error of mean (SEM). This was evaluated using analysis of variance (ANOVA) and least significance difference (LSD) with the aid of statistical software (SPSS version 22). A significant limit of $P < 0.05$ was used and values with superscript which were not same, differed significantly [32].

Results

ALT, AST, ALP and LDH Activity of RatsFed Cyanide and Cadmium Contaminated Catfish Diet.

Alteration in ALT, AST, ALP and LDH activity in liver and serum of rat fed cyanide and cadmium contaminated diet (singly and mixture) are presented in Tables 1-4 respectively. ALT, AST and ALP activity increased significantly ($p<0.05$) in liver and serum of rats in the experimental group when compared with control. However, the combined effect of the contaminated diet on the activity of these hepatic enzymes (ALT, AST, ALP and LDH) was observed to be antagonistic. Also, significant increase ($p<0.05$) was observed in LDH activity in liver and serum of rats in the experimental groups when compared with control group.

Table 1: Alteration in ALT activity inliver and serum of rat fed contaminated diet.

Groups	ALT Conc (U/L)	
	Serum	Liver
A	8.80 ± 1.80^{a}	9.20 ± 0.73^{a}
B	16.20 ± 2.06^{b}	14.00 ± 0.95^{b}
C	15.80 ± 2.03^{b}	16.60 ± 1.12^{b}
D	13.40 ± 1.12^{b}	12.00 ± 1.03^{c}

Group A-Control, Group B-cyanide polluted diet, Group C-cadmium polluted diet, Group D-cyanide + cadmium polluted diet. Values are stated in mean ± SEM (standard error of mean). Value with different superscript in same column differs significantly at $p<0.05$.

Table 2: Alteration in AST activity inliver and serum of rat fed contaminated diet.

Groups	AST Conc (U/L)	
	Serum	Liver
A	12.00 ± 1.64^{a}	15.60 ± 0.60^{a}
B	21.40 ± 2.01^{b}	23.80 ± 4.35^{b}
C	19.00 ± 2.45^{b}	21.80 ± 3.38^{b}
D	17.20 ± 1.11^{b}	19.20 ± 3.37^{b}

Group A-Control, Group B-cyanide polluted diet, Group C-cadmium polluted diet, Group D-cyanide + cadmium polluted diet. Values are stated in mean ± SEM (standard error of mean). Value with different superscript in same column differs significantly at $p<0.05$.

Table 3: Alteration in ALP activity in liver and serum of rat fed contaminated diet.

Groups	ALP Conc (U/L)	
	Serum	Liver
A	114.35 ± 23.01^{a}	136.05 ± 29.64^{a}
B	165.62 ± 12.30^{b}	192.54 ± 15.95^{b}
C	169.54 ± 8.07^{b}	180.62 ± 10.25^{b}
D	156.27 ± 11.72^{b}	169.34 ± 13.62^{b}

Group A-Control, Group B-cyanide polluted diet, Group C-cadmium polluted diet, Group D-cyanide + cadmium polluted diet. Values are stated in mean ± SEM (standard error of mean). Value with different superscript in same column differs significantly at $p<0.05$.

Table 4: Alteration in LDH activity in liver and serum of rat fed contaminated diet.

Groups	LDH Conc (U/L)	
	Serum	Liver
A	396.00 ± 48.70^{a}	333.23 ± 28.44^{a}
B	637.08 ± 53.12^{b}	509.46 ± 20.57^{b}
C	545.69 ± 95.13^{c}	531.08 ± 17.10^{b}
D	518.55 ± 54.73^{c}	554.69 ± 27.36^{b}

Group A-Control, Group B-cyanide polluted diet, Group C-cadmium polluted diet, Group D-cyanide + cadmium polluted diet. Values are stated in mean ± SEM (standard error of mean). Value with different superscript in same column differs significantly at $p<0.05$.

Total Protein, Albumin, Bilirubin (Direct and Indirect) and Globulin Level in Serum of RatsFed Cyanide and Cadmium Polluted Diet

Table 5 presents the alteration in albumin, total protein, bilirubin (direct and indirect) and globulin levels in serum of rats fed control and contaminated diet. Significant decrease ($p<0.05$) was recorded in albumin, globulin and total protein activity in serum of rats in the experimental groups compared to control. On the other hand, no significant difference

($p>0.05$) was detected in bilirubin activity (direct and indirect) in serum of rats in group C and group D compared to control. However, a decrease in bilirubin activity (direct and indirect) was detected in serum of rats in group B compared to control. Also, the albumin to globulin (A/G) ratio revealed a significant elevation ($p<0.05$) in serum of rats in the experimental groups compared to control.

Table 5: Alteration in serum proteins and bilirubin of rats fed cyanide and cadmium polluted diet

Group	A	B	C	D
Total protein (g/dl)	8.32 ± 1.41^{a}	5.57 ± 0.86^{b}	5.00 ± 0.56^{b}	4.47 ± 0.59^{b}
Albumin(g/dl)	3.65 ± 0.26^{a}	3.18 ± 0.20^{b}	3.32 ± 0.10^{b}	3.03 ± 0.16^{b}
Direct bilirubin (g/dl)	0.59 ± 0.12^{a}	0.26 ± 0.06^{b}	0.52 ± 0.08^{a}	0.57 ± 0.10^{a}
Indirect bilirubin (g/dl)	1.26 ± 0.25^{a}	0.56 ± 0.12^{b}	1.12 ± 0.17^{a}	1.22 ± 0.22^{a}
Globulin (g/dl)	5.29 ± 1.46^{a}	2.39 ± 0.79^{b}	2.35 ± 0.65^{b}	2.15 ± 0.55^{b}
A/G ratio	0.68 ± 0.17^{a}	1.33 ± 0.25^{b}	1.29 ± 0.15^{b}	1.54 ± 0.29^{b}

Group A-Control, Group B-cyanide polluted diet, Group C-cadmium polluted diet, Group D-cyanide + cadmium polluted diet. Values are stated in mean ± SEM (standard error of mean). Value with different superscript in same row differs significantly at $p<0.05$.

Changes in Antioxidant Biomarkers in Liver of RatsFed Cyanide and Cadmium Polluted Diet

Table 6 illustrates the changes in antioxidant biomarkers in livers of rats fed cyanide and cadmium polluted diet (singly and mixture). A significant reduction ($p<0.05$) was observed in CAT and SOD activity in livers of rats in the experimental groups compared to control group. Elevated activity of lipid peroxidation (LPO), reduced GSH and GR was detected in livers of rats in the experimental groups compared to control. However, no significant change was noted in the activity of GST and GPx in livers of rats fed cyanide and cadmium polluted catfish diet (singly and mixture) compared to control.

Table 6: Changes in antioxidant biomarkers in livers of rats fed cyanide and cadmium polluted diet

Group	A	B	C	D
SOD (U/mgProtein)	48.23 ± 0.90^{a}	36.44 ± 1.45^{b}	39.76 ± 0.75^{b}	39.09 ± 0.80^{b}
LPO(nmol/mgProtein)	1.66 ± 0.55^{a}	6.28 ± 0.50^{b}	4.83 ± 0.64^{b}	5.39 ± 0.23^{b}
CAT (U/mgProtein)	62.93 ± 5.78^{a}	45.76 ± 3.16^{b}	42.98 ± 2.24^{b}	45.49 ± 2.11^{b}
GST (U/mgProtein)	24.29 ± 2.11^{a}	18.16 ± 2.58^{a}	20.54 ± 0.55^{a}	21.98 ± 3.43^{a}
GPX (U/mgProtein)	58.46 ± 8.93^{a}	61.33 ± 3.93^{a}	60.78 ± 3.76^{a}	61.60 ± 8.61^{a}
GSH (U/mgProtein)	82.46 ± 7.30^{a}	107.39 ± 5.39^{b}	103.23 ± 7.48^{b}	105.60 ± 2.59^{b}
GR (U/mgProtein))	37.71 ± 3.34^{a}	49.11 ± 2.47^{b}	47.21 ± 3.42^{b}	48.30 ± 1.18^{b}

Group A-Control, Group B-cyanide polluted diet, Group C-cadmium polluted diet, Group D-cyanide + cadmium polluted diet. Values are stated in mean ± SEM (standard error of mean). Value with different superscript in same row differs significantly at $p<0.05$.

Metal/Compound Composition in Feed Administered to Experimental Animals

Table 7 shows the result of the analysis of metal/compound composition carried out on the contaminated diet. The results specifiedexistence of cadmium in control feed to be below detection limit while traces of cyanide were indicated in the control feed.

Table 7: Metal/compound composition in feed administered to experimental animal

Groups	Metal/Compound Composition (mg/g)	
	Cadmium	Cyanide
A	BDL[a]	0.12±0.28[a]
B	0.04±0.21[b]	1.95±0.36[b]
C	2.95±0.23[c]	0.13±0.09[c]
D	2.24±0.16[c]	1.46±0.11[c]

Group A-Control, Group B-cyanide polluted diet, Group C-cadmium polluted diet, Group D-cyanide + cadmium polluted diet. Values are stated in mean ± SEM (standard error of mean). Value with different superscript in same row differs significantly at $p<0.05$.BDL - Below Detection Limit (<0.01).

Metal/Compound Concentration in Tissues of Experimental Animals

The concentration of cadmium and cyanide in the tissues (liver and blood) of experimental rats fed contaminated diet is displayed in Table 8. The results showed that after exposure, the concentration of cyanide in the blood was higher than that in the liver. While the concentration of cadmium in the liver was more than that in the blood.

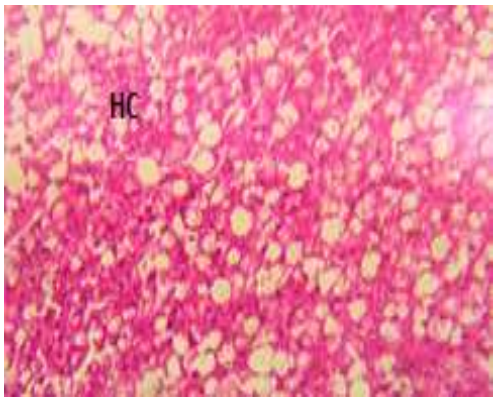
Table 8: Metal/compound concentration in tissue of experimental animals.

Groups	Liver (µg/g Tissue)	Blood (µg/g Tissue)
A	BDL[a]	BDL[a]
B	1.83±0.25[b]	2.78±0.14[b]
C	3.76±0.19[c]	1.58±0.09[c]
D (CN)	1.51±0.17[b]	2.49±0.15[b]
D (Cd)	2.86±0.05[d]	2.15±0.21[b]

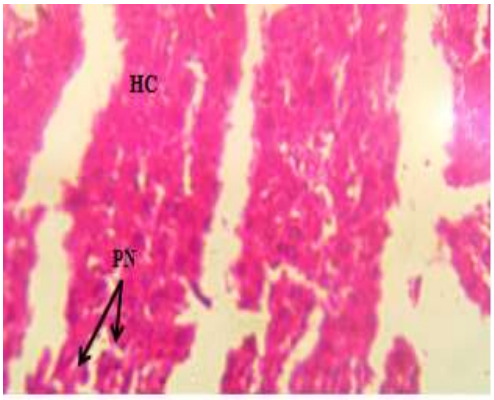
Group A-Control, Group B-cyanide polluted diet, Group C-cadmium polluted diet, Group D-cyanide + cadmium polluted diet. Values are stated in mean ± SEM (standard error of mean). Value with different superscript in same row differs significantly at $p<0.05$.BDL - Below Detection Limit (<0.01).

Liver Histological Analysis of RatsFed with Cyanide and Cadmium Contaminated Catfish Diet

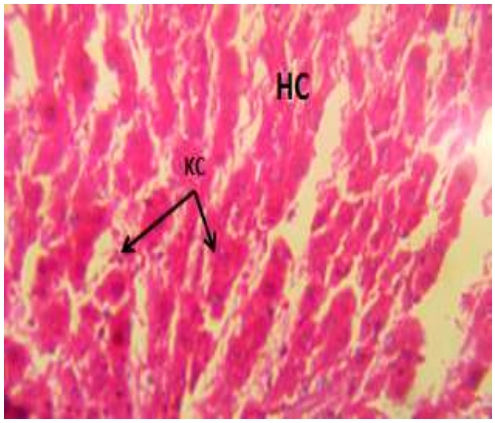
Figure 1 illustrates the liver histological structure of rats with cyanide and cadmium contaminated diet. Liver of control group indicated a normal hepatocyte. However, contact with cyanide and cadmium contaminated diet caused variations inliver structure as designated by destruction of hepatocyte, severe necrosis (N) and degeneration of kupffer cells (KC).



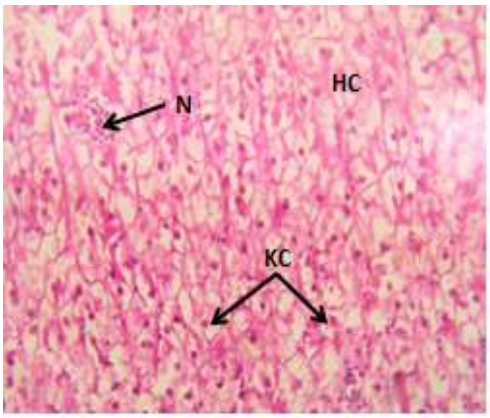
Group A - Control group photomicrograph liver section of rat showing normal hepatocytes cells (HC). H&E x400.



Group B - cyanide contaminated group photomicrograph liver section of rat shows destruction of hepatocytes (cells with pyknotic nuclei). H&E x400.



Group C - Cadmium contaminated group photomicrograph liver section of rat showing severe necrosis (N) and degenerationofKupffer cells(KC). H&E x400.



Group D - Cyanide and cadmium contaminated group photomicrograph liver section of rat showing destruction of hepatocyte and degeneration of Kupffer cells (KC). H&E x400.

Figure 1: Histopathology of liver.

Discussion

Living organisms exposure to xenobiotic is renowned for its association to quite a lot of detrimental consequences it has especially on humans via the food-chain [3,4]. Hepatocellular toxicity is certainly stimulated by xenobiotic materials.Reason being that the liver augments the quick clearance of poisonous substance from the blood. In this study, contact with cyanide

and cadmium polluted catfish diet prompted oxidative stress which was accompaniedwith alterations in biochemical indices in hepatic tissue and the catfish that were daily exposed to the pollutant at a concentration of 0.4mg/100ml of water within a duration of 30days in order to attain the topmost concentration of the metal/compound in the tissues of fish. The sub-lethal dose of cadmium used in this study agreed with the work ofEzedomandAsagba [17] who studied cadmium and arsenic effects on oxidative enzymes in the tissues of rats. Also, a previous study showed the average lethal concentration (LC_{50}) value of cyanide to be0.387 mg/l [33].

Liver enzymes which are sensitive pointers of hepatocyte mitochondrial damage, were assessed in this study. Consumption of Cd & CN polluted diet-initiated liver injury thatensued increased activity liver enzymes in blood. Significant increase observed in serum and tissue transaminase activities (ALT and AST) in response to Cd & CN tainted diet, can be ascribed to a loss of cellular strength and discharge of enzyme into the blood (Table 1&2). According to Kadiri [34, 35**]** injured liver tissue caused by exposure to foreign substances, prompts elevated transaminases activities in blood enormously above normal. Table 3&4 show elevated level of ALP and LDH activity in serum and liver of rats fed Cd & CN tainted diet.This further expresses the indication of cellular damage. Our resultsare coherent with prior studies done by Rufino *et al*. [36] which specified that interaction with xenobiotic brings about hepatic injury and porosity of membrane resulting in the increase activity of enzymes (ALP &LDH) in serum and tissues. Moreover, reduced effect was observed in the actions of enzymesin the combined state of the polluted diet in comparison to the single state, thus indicating that both toxicants are antagonizing one another.

Furthermore, Table 5 displays additional markers of liver dysfunction such as albumin, total protein, bilirubin and globulin which were altered by the intake of CN & Cd contaminated diet. This was pointed out by a decline in albumin, total protein, globulin and bilirubin (direct and indirect) levels in serum of rats given Cd & CN polluted diet. Our results tallied with a previous study [37] which reported that decrease in serum albumin, globulin and total protein levels may perhaps indicate liver disorder. Albumin to globulin (A/G) ratio assessesalbumin protein level compared to the globulin level present in blood. In this study, A/G ratio was observed to be significantly higher when exposed to Cd & CN polluted diet which may possibly be attributed to be an indication of disorders in the liver, kidney and intestines. Thus, these alterations in hepatic markers ensued due to hepatocellular damage.
Numerous mechanisms have been proposed for Cd-induced hepato toxicity. Hepatic injury seems to be associated with sulfhydryl groups binding, implicating membrane proteins, cytoplasmic proteins and enzymes [16]. The major mechanisms attributed to cadmium-induced liver injury include apoptosis, homeostasis of essential metals, production of inflammatory mediators and adhesion, molecules sulfhydryl groups' inactivation and oxidative stress [7]. Furthermore, studies have shown that the mechanism of cyanide toxicity is attributed to the inhibition of cytochrome oxidase which is as a result of cyanide affinity for the ferric hemeform of cytochrome, thus declining the utilization of oxygen by tissue [38]. Acute cyanide intoxication often occurs when the quantity of cyanide in tissue surpasses the minimal dose required to inhibit. Thus, the activity of several enzymes and biological systems are interrupted which ensues signs of toxicity and death [39]. The increased ROS levels in the present study propose the inhibition of antioxidant activity in liver of rats exposed to cadmium and cyanide. Therefore, accumulation of ROS in all tissues (serum and liver) leads to the impairment of antioxidant defense system.

Oxidative stress emanates from interaction with toxicants and its effect is the excessive formation of reactive oxygen species and subsequently cell death [40]. Moreover, guarding the cells from free radicals is enhanced by antioxidant defense system. In this study, there was disparity in antioxidant activity of rats fed CN & Cd contaminated diet. A significant decline was spotted in liver CAT activity of the experimental animals. Also, there was a significant decrease in liver SOD concentration of rats fed contaminated diet. The current findings are in line with several other studies which reported a decline in SOD and CAT levels once exposed to cadmium and cyanide [37, 41]. The manifestation of SOD–CAT mutual coordination is renowned in its performance as the primary form of resistance against free radicals induced damage.Hence, they serve as indicators to pinpoint the outrageous formation of ROS [42]. Oxidative damage is enhanced by MDA which is a key ultimate product of lipid peroxidation [43]. This study showed a significant raise in LPO level in liver of rats exposed to contaminated diet. This significant raise in LPO level might be ascribed to the detriment of membrane function ensuing from the chain oxidation of poly unsaturated phospholipids by superoxide anion-radical. The outcome of this study corresponds with earlier works by Mathangi *et al.* [44] and Dabas *et al.* [45] whio revealed a significant elevation in MDA levels and a corresponding reduction in antioxidant enzymes in tissues of organisms exposed to cyanide and cadmium.

Reduced glutathione GSH, an essential antioxidant, possesses a significant feature in alleviating reactive oxygen species to a non-toxic state. GR which is also a vital antioxidant enzyme, catalyzes the restoration of GSH (reduced form) from GSSG (oxidized form), thus making it an indispensable cellular antioxidant protector. In this study, the upsurge of MDA level accompanied by a simultaneous increase in activity of GSH and GR are indicative of oxidative stress in the exposed rats. These observations coincide with the work of Abarikwu *et al.* [46] and Tabrez and Ahmad [47] who also reported elevated GSH activity in tissues of rats exposed to toxicants. In addition, Dabas *et al.* [45] also reported increased GSH and GR activity in tissues of freshwater murrel exposed to cadmium. The alterations in liver oxidative status detected in this study is in accord with earlier report [15,46].This is ascribed to the enormous production of free radicals with respect to reduced level of antioxidants needed to shield the tissues against oxidative damage. Reports have shown that the vulnerability of organisms exposed to xenobiotic together with the intensity of the stress induced along with the duration of contact to the stress is liable for repressed or elevated antioxidant activity [48]. In the existing study, the hepatic histoarchitecture of the rats treated with CN & Cd contaminated diet (singly and mixture) resulted in the destruction of hepatocyte, severe necrosis (N) and degeneration of Kupffer cells (KC). These histopathological alterations detected in the liver of rats fed CN & Cd contaminated diet may be ascribed to the formation of so many free radicals initiated by oxidative stress brought about by the polluted diet. Previous reports show that modification of liver function and hepato cellular impairment are concomitant to aberrations in hepatic markers [46, 48].

Conclusion

The outcomes of the current research designate that exposure to CN & Cd contaminated diet (single and combined state) initiated hepatotoxicity and altered hepatic biomarker, in addition to an increased and /or decreased significant changes in antioxidant indices in serum and liver of Wister rats. Exposure to CN & Cd contaminated diet tends to have deleterious effects on humans. Thus, the experimental data obtained with Wister rats can be considered as an expedient reference for evaluations with biomarker responses of organisms (animals or humans) exposed to polluted diet. The reduced effects detected in the combined state of the

contaminated diet when compared with the single state, indicates that the simultaneous occurrence of cyanide and cadmium will result in an antagonistic effect.

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