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Combined Effects of Cadmium- and Cyanide-Contaminated Diet on Oxidative Stress Biomarkers in Different Tissues of Rats

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Abstract

Background. Several toxicants present simultaneously in the environment have combined toxicological effects. In addition, various xenobiotics have distinct effects on oxidative stress biomarkers in animal cells and tissues.

The aim of this study was to analyze the effect of cadmium (Cd) and cyanide (CN) through the food chain on some antioxidant indices in the tissues (lungs, testes, heart, and brain) of male Wistar rats.

Materials and Methods. The study included sixty African catfish allocated to four groups, each comprising fifteen fish, treated with potassium cyanide (KCN) and cadmium chloride ($CdCl_2$), held at a temperature of 25°C in a 100-litre fish tank aquarium with water contaminated with 0.4 mg of both cyanide and cadmium/100 ml of water. All the fish were later killed, dried, and used to prepare diet for experimental animals. Twenty male rats divided into four groups, each comprising five rats, were used for this study as well, and fed for 28 days as follows: Group A – control diet; Group B – cyanide-contaminated diet; Group C – cadmium-contaminated diet; Group D – diet contaminated with cyanide + cadmium. Subsequently, they were sacrificed. Biochemical analysis of the tissues excised from the rats was done.

Results. There was a significant ($p < 0.05$) increase in lipid peroxidation level and a significant decrease in superoxide dismutase, catalase and reduced glutathione activities in the lungs, testes, heart, and brain of rats fed a catfish diet containing both cyanide and cadmium as compared to controls. In addition, contaminated diet altered acetylcholinesterase activity in the brain, glutathione peroxidase activity, glutathione-S-transferase activity, and glutathione reductase activity in the tissues of experimental rats.

Conclusions. Cadmium and cyanide, via the food chain, induce oxidative stress in the lungs, testes, heart, and brain of rats.

Keywords
Cyanide; Cadmium; Antioxidant; Diet; ROS; Toxicity

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Introduction

The negative impact of industrial processes has led to an increase in the levels of toxins in the ecosystem; thus, the intake and spread of toxicants in humans are mediated by contaminated food. The overtime continuous release of toxicants into the food chain without strict regulations is a subject of vital consideration [1–3].

Cadmium (Cd), an extremely noxious metal contaminant, is found naturally in the earth's crust at a concentration of 0.2 ppb. Basically, its concentration increases environmentally due to negative impacts of various industrial processes such as mining, smelting, battery manufacturing, pigment manufacturing, etc. [4]. Bioaccumulation of Cd in the body is due to its inability to be biodegraded [5]. Cd prompts oxidative stress which leads to physiological abnormalities in diverse organs and tissues, such as the liver, lungs, kidneys, placenta, pancreas, testes, heart, and bones [6].

Cyanide (CN), a ubiquitous cytotoxic chemical compound, is present in nature and can be anthropogenically released into the environment. Due to industrial effluents from mines, metallurgical plants, plastics industry, tobacco industry, and vehicle exhaust gases, it is readily available in aquatic environment [7]. CN toxicity has been reported to initiate various metabolic anomalies and, consequently, to induce oxidative stress in diverse tissues through high

production of free radicals [8–10].

Over time, aquatic organisms have become prone to accumulating excessive levels of toxicants due to the simultaneous existence of contaminants in the environment, which has a negative impact on terrestrial organisms [2]. Fish and other aquatic organisms serve as good bioindicators of the levels of aquatic contaminants [9] and they stand at a strategic point in the food chain, serving mainly as a source of protein and other mineral nutrients.

Living organisms produce reactive oxygen species (ROS) via normal cellular metabolism. At mild concentrations, ROS are involved in physiological cell development; however, at high concentrations, they have harmful effects on cell components, such as lipids, proteins, and deoxyribonucleic acid (DNA) [11]. In addition, ROS are free radicals of physiological importance, and include superoxide anion (O_2^-), hydroxyl radical (-OH) and hydrogen peroxide (H_2O_2). The excess of free radicals generated in cells is modulated by antioxidant activities [6]. The antioxidant defense system includes two major groups: enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST) and non-enzymatic antioxidants which comprise low-molecular-weight compounds such as reduced glutathione (GSH), β-carotene, ascorbate, α-tocopherol, etc. [12].

Oxidative stress results from a disparity between antioxidants and ROS. This outcome is either an accumulation of ROS or a depletion of antioxidants. In oxidative stress, cells try to counteract free radical effect and restore the redox equilibrium. Exposure to pollutants induces oxidative stress which leads to several pathological conditions such as cancer, atherosclerosis, neurological disorders, hypertension, dyslipidemia, ischemia/perfusion, pulmonary edema, asthma, etc. [13]. Prospective biomarkers of exposure to toxicants in diverse organisms are biochemical components such as lipid peroxidation (LPO) and antioxidant systems as they are more sensitive, less variable, extremely conserved amongst species and usually more readily available as indicators of stress [12]. Numerous studies have revealed that environmental toxicants can augment excessive intracellular ROS production resulting in oxidative damage to biological systems and this damage is neutralized by the antioxidant defense system [14–16].

Previous studies on CN and Cd effects on animal models have mostly concentrated on a single toxicant (either CN or Cd), in case of direct exposure and high concentrations. However, the food chain and water are the main pathways for human exposure to a variety of harmful environmental substances [17, 18]. Therefore, further research is needed to evaluate the interaction of these components across the food chain (from water to fish and rats). However, there is scarce information on the harmful effects of CN and Cd across the food chain. According to this study, heavy metal (Cd) and other chemical compound (CN) toxicity in aquatic habitats is caused by bioaccumulation of heavy metals in the tissues (lungs, testes, heart, and brain) above aquatic concentrations and rapid detoxification of chemical compounds that have an adverse effect on organisms. Thus, the need to assess these tissues (lungs, testes, heart, and brain) is due to several pathological conditions resulting from the adverse effects of toxicants.

In the environment, the likelihood of exposure to diverse toxicants is higher as compared to a single toxicant and, with this regard, **the objective of our study** is to establish Cd, CN, and its mixture (Cd + CN) effect on antioxidant parameters in the tissues (lungs, testes, heart, and brain) of Wistar rats.

Materials and Methods

Chemicals Used

Cd salt in the form of Cd chloride ($CdCl_2$), CN salt in the form of potassium CN (KCN) and other analytical grade chemicals used in this study were purchased from Sigma-Aldrich Co. Ltd, Poole, England.

Study Design

This study was done in two phases (fish phase and rat phase), each lasting for 28 days (Fig. 1).

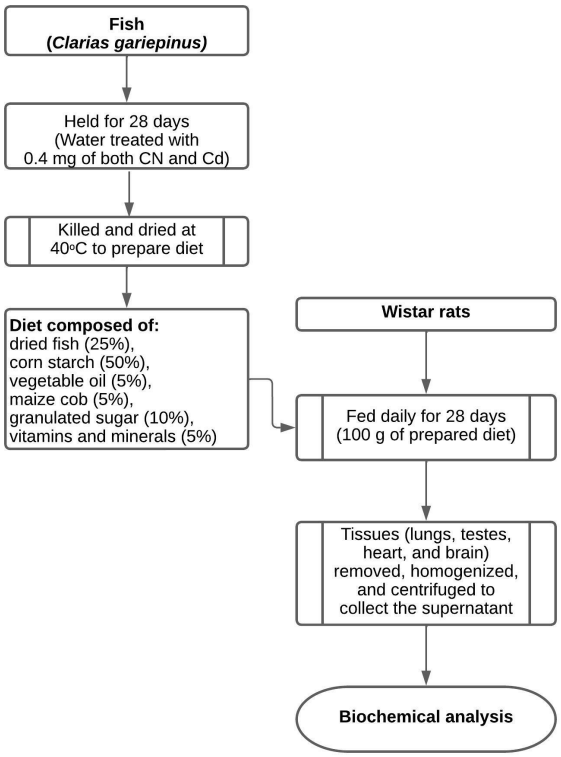

Figure 1. Study design.

Fish Treatment / Diet Preparation

Sixty healthy African catfish (*Clarias gariepinus*) with a body weight of 250-250 g and a length of 30-33 cm were obtained from a commercial fishing pond for this experiment. They were adapted to the experiment for a week and fed fish feed. All the fish were allocated to four groups, each comprising fifteen fish, treated with KCN and $CdCl_2$. The solubility of $CdCl_2$ and KCN was 119.6 g / 100 ml (25°C) and 71.6 g / 100 ml (25°C), respectively. The fish

were held at a temperature of 25°C in a 100-litre fish tank aquarium with water treated with the contaminants as follows:

1. Group A – freshwater (controls).
2. Group B – 0.4 mg of CN/100 ml of water.
3. Group C – 0.4 mg of Cd/100 ml of water.
4. Group D – 0.4 mg of both CN and Cd/100 ml of water.

Throughout the experiment period of 28 days, the water was changed and re-contaminated every 48 hours and the dose of the pollutant used (0.4 mg) was reported [19]. The fish were killed, dried at 40°C, and used to prepare diet for experimental animals. The diet consisted of dried catfish as a protein source (25%), corn starch as a carbohydrate source (50%), vegetable oil as a source of fat and oil (5%), maize cob as a fiber source (5%), granulated sugar as a source of sugars (10%), vitamin and mineral mix as a multivitamin source (5%). These were combined to form animal feed for the experiment.

Rat Treatment / Experimental Design

Twenty animals (Wistar albino rats) weighing between 80-130 g were divided into four groups, each comprising five rats, and acclimatized for 14 days before the experiment, with free access to food and water in an acceptable environment with 12h light and 12h darkness cycle. During the experimental period, the rats were held in separate cages according to their group and were fed 100 g of the prepared diet daily for 28 days as follows:

1. Group A: control diet.
2. Group B: CN-contaminated diet.
3. Group C: Cd-contaminated diet.
4. Group D: CN- and Cd-contaminated diet.

Sample Collection

On the 28th day, the experimental rats were sacrificed by cervical decapitation; their tissues (lungs, testes, heart, and brain) were removed, and 0.5 g of each tissue were homogenized separately in 4.5 ml of 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 4,000 rpm for 15 minutes to obtain the supernatant used for biochemical analysis.

Biochemical Analysis

The supernatant obtained was used to assess the level of oxidative stress in the lungs, testes, heart, and brain of the experimental rats using standard methods and the following parameters were assessed:

1. CAT activity was evaluated using the method proposed by Rani *et al.* [20].
2. SOD activity was analyzed by the method developed by Misra and Fridovich [21].
3. GSH activity was accessed using the Ellman's method [22].
4. GPx activity was evaluated using the method reported by Rotruck *et al.* [23].
5. GST activity was analyzed by the method developed by Habig *et al.* [24].
6. GR activity was assessed by the method proposed by Cribb *et al.* [25].
7. LPO activity was evaluated by the method reported by Niehius and Samuelsson [26].
8. Acetylcholinesterase (AChE) activity was accessed using the method proposed by Ellman *et al.* [27].

Statistical Analysis

The data were processed in the IBM SPSS Statistics V.19. Descriptive statistics were stated as Mean $\pm$ Standard deviation (SD). The data obtained were compared using non-parametric analysis with the Kruskal-Wallis test.

Results

Tables 1-4 show the influence of CN- and Cd-contaminated diet on the level of antioxidant markers in the tissues (lungs, testes, heart, and brain) of the experimental rats. There was a significant ($p < 0.05$) difference in the level of all antioxidant markers in rats of the experimental groups as compared to the control group and there was no significant difference ($p > 0.05$) among the experimental groups. However, there was a significant ($p < 0.05$) difference in the level of SOD and GST activities in Group C as compared to the control group (Table 3). Moreover, there was a significant ($p < 0.05$) difference in the level of GPx activity in Group C and Group D as compared to the control group and a significant ($p < 0.05$) difference in AChE activity in Group D as compared to the experimental groups and the control group (Table 4).

Discussion

Simultaneous occurrence of several toxicants in the environment has led to combined toxicological effect associated with such toxicants [12, 16]. Cd and CN are crucial occupational and environmental pollutants whose toxic effects are linked to multiple organ damage. Therefore, the current study examined the effect of Cd- and CN-containing diet on the parameters of oxidative stress in the lungs, testes, brain, and heart of male Wistar rats. According to Kadiri (2018) and Genchi *et al.* (2020), the lung is a target organ of CN and Cd toxicity, mainly through inhalation, resulting in alveolar congestion, pulmonary edema, and respiratory stress [28, 29]. CN toxicity in the lungs is primarily associated with the cessation of aerobic cell metabolism and the resulting effect is inhibition of oxidative phosphorylation. Cd has a deleterious effect on the lung by modifying individual lung cells or reducing their viability; its toxicity causes lung damage, pulmonary fibrosis, emphysema, and inflammation in exposed organisms. This study indicated that diet containing Cd and CN altered antioxidant activity in the lungs, which could induce lung cell proliferation that might be independent of lung inflammation; that is consistent with previous studies [29, 30].

Chronic oral exposure to pollutants causes cardiovascular effects (atherosclerosis and its complications) in animals and alters nitric oxide (NO) levels in endothelial cells which enhances ROS production leading to LPO; pollutants have been reported to play a significant role in the development of cardiovascular diseases [30, 31]. Notably, a small dose of KCN, probably, decreases myocardial dysfunction

Table 1. Antioxidant level in the lungs of rats fed a diet containing cyanide and cadmium.

Group	SOD	CAT	GSH	GPx	GST	GR	LPO
A	43.63 ± 2.06^{a}	68.26 ± 1.42^{a}	56.88 ± 1.17^{a}	46.09 ± 2.34^{a}	16.68 ± 0.40^{a}	53.46 ± 2.37^{a}	1.16 ± 0.29^{a}
B	22.50 ± 1.21^{b}	44.25 ± 1.90^{b}	32.90 ± 1.51^{b}	35.36 ± 1.95^{b}	34.10 ± 1.30^{b}	40.78 ± 0.69^{b}	7.46 ± 1.43^{b}
C	25.71 ± 1.72^{b}	46.37 ± 1.78^{b}	34.86 ± 1.06^{b}	37.63 ± 1.85^{b}	27.29 ± 1.50^{b}	47.83 ± 1.35^{b}	4.00 ± 0.56^{b}
D	23.57 ± 1.12^{b}	43.88 ± 0.96^{b}	35.96 ± 1.15^{b}	31.97 ± 0.54^{b}	28.89 ± 1.22^{b}	46.76 ± 1.81^{b}	6.44 ± 1.05^{b}

Notes: Group A – control diet, Group B – CN-containing diet, Group C – Cd-containing diet, Group D – diet containing CN + Cd. Values shown are mean ± SD (standard deviation). The value with different superscripts (a, b, c) in the same column differs significantly at $p < 0.05$.

Table 2. Antioxidant level in the testes of rats fed a diet containing cyanide and cadmium.

Group	SOD	CAT	GSH	GPx	GST	GR	LPO
A	45.46 ± 1.00^{a}	63.81 ± 2.43^{a}	47.92 ± 2.78^{a}	35.27 ± 1.07^{a}	18.30 ± 0.65^{a}	56.80 ± 3.47^{a}	1.53 ± 0.52^{a}
B	25.49 ± 1.09^{b}	30.03 ± 1.18^{b}	35.37 ± 1.30^{b}	25.54 ± 1.22^{b}	31.53 ± 0.43^{b}	36.48 ± 1.51^{b}	6.74 ± 0.44^{b}
C	29.78 ± 0.86^{b}	36.61 ± 1.16^{b}	33.11 ± 1.15^{b}	20.16 ± 0.88^{b}	32.31 ± 1.03^{b}	38.66 ± 1.90^{b}	5.08 ± 0.26^{b}
D	22.51 ± 0.27^{b}	32.15 ± 1.64^{b}	30.20 ± 0.43^{b}	23.29 ± 1.18^{b}	33.85 ± 1.24^{b}	33.27 ± 1.57^{b}	5.10 ± 0.32^{b}

Notes: Group A – control diet, Group B – CN-containing diet, Group C – Cd-containing diet, Group D – diet containing CN + Cd. Values shown are mean ± SD (standard deviation). The value with different superscripts (a, b, c) in the same column differs significantly at $p < 0.05$.

Table 3. Antioxidant level in the heart of rats fed a diet containing cyanide and cadmium.

Group	SOD	CAT	GSH	GPx	GST	GR	LPO
A	81.18 ± 2.16^{a}	62.13 ± 1.24^{a}	48.28 ± 1.22^{a}	59.43 ± 3.96^{a}	15.97 ± 1.60^{a}	86.57 ± 2.29^{a}	1.04 ± 0.11^{a}
B	52.38 ± 1.23^{b}	50.27 ± 0.42^{b}	23.82 ± 1.35^{b}	35.71 ± 1.01^{b}	29.86 ± 1.45^{b}	75.51 ± 1.73^{b}	5.68 ± 0.17^{b}
C	69.50 ± 1.54^{c}	53.33 ± 1.40^{b}	29.78 ± 1.81^{b}	30.05 ± 1.08^{b}	20.26 ± 0.58^{c}	79.91 ± 1.66^{b}	4.81 ± 0.21^{b}
D	49.10 ± 1.38^{b}	54.86 ± 1.51^{b}	25.43 ± 0.65^{b}	29.47 ± 1.18^{b}	27.86 ± 2.48^{b}	77.15 ± 1.24^{b}	5.16 ± 0.124^{b}

Notes: Group A – control diet, Group B – CN-containing diet, Group C – Cd-containing diet, Group D – diet containing CN + Cd. Values shown are mean ± SD (standard deviation). The value with different superscripts (a, b, c) in the same column differs significantly at $p < 0.05$.

Table 4. Antioxidant level in the brain of rats fed a diet containing cyanide and cadmium.

Group	SOD	CAT	GSH	GPx	GST	GR	LPO	AChE
A	89.75 ± 4.36^{a}	73.85 ± 2.43^{a}	71.13 ± 2.13^{a}	69.38 ± 1.04^{a}	18.85 ± 0.89^{a}	81.56 ± 2.21^{a}	0.50 ± 0.17^{a}	78.06 ± 2.78^{a}
B	48.68 ± 2.47^{b}	37.26 ± 3.88^{b}	35.35 ± 1.90^{b}	39.74 ± 1.19^{b}	35.65 ± 2.85^{b}	62.69 ± 1.03^{b}	8.99 ± 0.26^{b}	75.12 ± 2.68^{b}
C	55.14 ± 2.04^{b}	36.51 ± 1.60^{b}	34.12 ± 0.45^{b}	32.18 ± 0.16^{c}	30.64 ± 0.33^{b}	63.74 ± 1.06^{b}	6.85 ± 0.23^{b}	74.62 ± 1.57^{b}
D	48.95 ± 1.36^{b}	33.98 ± 0.70^{b}	36.28 ± 1.04^{b}	30.43 ± 1.70^{c}	29.68 ± 0.15^{b}	65.64 ± 1.38^{b}	5.64 ± 0.14^{b}	60.96 ± 1.06^{c}

Notes: Group A – control diet, Group B – CN-containing diet, Group C – Cd-containing diet, Group D – diet containing CN + Cd. Values shown are mean ± SD (standard deviation). The value with different superscripts (a, b, c) in the same column differs significantly at $p < 0.05$.

and ROS production in characteristic cardiac preconditioning; hence, CN significantly induces cardiac failure [32]. Cd predominantly accumulates in the kidneys and liver; the heart usually has small Cd concentrations [30]. Thus, even at low concentrations, Cd induces molecular and biochemical alterations in the heart which are characteristic features of oxidative damage that can possibly result in hypertension and early cardiac failure. Among other toxicants, Cd is responsible for the highest mitochondrial damage [33].

Testicular tissues are rich in polyunsaturated fatty acids and, therefore, subject to oxidative stress, which causes changes in biochemical function of the testes and testicular damage when exposed to xenobiotics. The variation in the function of male reproductive system by its response to toxic substances serves as a subtle sign of the lethal effect of an environmental pollutant on animal and human health, which clearly indicates the damage to the reproductive function [34]. Zhu et al. [35] reported that exposure of male rats to Cd caused damage to the blood-testicular barrier, decreased germ cell adhesion resulting in germ cell loss, reduced sperm count, and led to subfertility or infertility. Cd prompts ROS production which leads to a decrease in the activity of antioxidative enzymes, which initiates oxidative damage to the testes. Additionally, CN exposure has been reported to initiate anomalies in the testicular structure along with alteration of the seminiferous tubules attributable to the reduction in the number of germ cells [36].

Exposure to toxicants induces neuronal impairment,

that is facilitated by numerous processes resulting in oxidative stress in the brain. CN toxicity blocks cellular respiration by obstructing cytochrome c oxidase, leading to some defects such as hypoxic brain injury and, eventually, death [37]. Neuronal damage caused by CN toxicity is claimed to be triggered by numerous mechanisms. Furthermore, LPO which is toxic to neurons, occurs by increasing ROS production as a response to CN poisoning. In addition, blocking mitochondrial respiration by CN poisoning causes an increase in ROS in the brain following severe oxidative damage in neurons [38]. Neurotoxicity is induced by Cd via diverse mechanisms and Cd accumulate in brain. Cd enters the brain parenchyma and neurons, causing damage via complex processes, and has a direct lethal effect on the brain resulting in some neurological alterations such as learning difficulty, memory loss, and hyperactivity [39].

LPO, a process of oxidative breakdown of polyunsaturated fatty acids with malondialdehyde (MDA) as its foremost end-product, affects cellular components via oxidative stress and ultimately results in cell damage. MDA assessment aids in verifying the degree of xenobiotic-induced damage to biological membranes [40]. The present study showed significant elevation in LPO levels in the tissues of rats fed a contaminated diet as compared to the control group. This significant elevation in LPO levels might be attributed to the impaired membrane function resulting from chain oxidation of polyunsaturated phospholipids by superoxide anion radical; this caused inhibition of antioxidant activities that led to oxidative tissue damage. Oxidative damage explains the significant increase observed in LPO levels in the lungs, brain, testes, and heart of exposed rats as compared to the control group. In agreement with our findings, elevated LPO levels were reported in several tissues exposed to CN and Cd [37, 41].

The detrimental effect of exposure to xenobiotics is associated with the initiation and proliferation of free radicals, which causes oxidative damage to all biological macromolecules (proteins, lipids, sugars, and DNA) and, eventually, cell apoptosis [42]. Antioxidants are molecules capable of preventing the oxidation of other molecules. This process generates free radicals which initiate chain reactions leading to cell/tissue injury, and these chain reactions are terminated by antioxidants which destroy free radical intermediates [43]. SOD is a universal enzyme which protects cells against oxidative stress. In this paper, a significant decrease in SOD activity in rat tissues was observed, which was consistent with an earlier report [28]. Superoxide anions are made in cells through toxicants, and it initiates the vital role of SOD which destroys the harmful radicals in cells leading to the decline in SOD activity [44]. CAT is a tetrameric enzyme liable to remove intracellular H_2O_2 by converting H_2O_2 into H_2O and O_2. In this study, CAT activity showed a significant decrease in the lungs, testes, brain, and heart of exposed rats and this decrease could be attributable to the inhibition of CAT activity by the excess free radical generation. The activities of SOD-CAT are recognized as a first line antioxidant defense system and are used as a biomarker to elucidate extreme production of ROS [45]. The reports have also demonstrated similar findings in increased MDA concentration complemented by a simultaneous decrease in SOD and CAT activities in tissues of organism [18, 28].

Another significant mechanism by which xenobiotic brings about oxidative stress is intracellular GSH depletion. The tripeptide GSH has been shown to be the foremost thiol-disulfide redox buffer of the mammalian cell that provides the first line of defense against oxidative damage [46]. GSH is capable of decreasing toxicant-induced LPO, acting as a reducing substrate in oxidative processes, and eliminating extricating ability of toxicant. GSH is known for its ability to demonstrate a crucial role in combating unregulated oxidative stress in a cellular defense of mammalian cells [47]. GR is accountable for the existence of reduced GSH and is an indispensable enzyme which catalyzes the regeneration of GSH (its reduced form) from oxidized glutathione disulfide (GSSG), thus making it an essential cellular antioxidant protector. In most cells, GR is part of most sufficient reducing thiols [48]. GPx is a significant intracellular enzyme found primarily in the mitochondria and occasionally in the cytosol; it degrades hydrogen peroxides (H_2O_2) to water and lipid peroxides to their corresponding alcohols. Most times, GPx activity depends on selenium, a micronutrient cofactor. Thus, GPx is typically referred to as selenocysteine peroxidase. In the cell, GPx catalyzes the reduction of peroxides and converts reduced GSH to GSSG. It possesses a vital function of inhibiting LPO processes, thereby protecting cells from oxidative stress. In addition, GPx catalyzes hydroperoxide reaction with reduced GSH to form GSSG and the reduction product of the hydroperoxide [4, 45]. GST is a multifunctional enzyme involved in the detoxification of cells from endogenous toxic metabolites, exogenous toxic chemicals, and superoxide radicals, which is presumed to be a xenobiotic metabolizing enzyme that prevents cells from toxic substances. In addition to its detoxification mechanisms, GST inactivates hydroperoxides formed during oxidative stress. Thus, GSTs represent different category of enzymatic antioxidants catalyzing the breakdown of lipid peroxides. Moreover, GST critically regulates the maintenance of the cellular antioxidant GSH in different cellular compartments [49]. Hence, with the aid of reduced GSH, GST catalyzes the conjugation reaction of xenobiotics, and this study showed a significant increase in GST activity in the tissue of rats fed a contaminated diet. Furthermore, a significant decrease in GR, GPx, and GSH activity in the tissues of experimental animals was noted, and this decline was attributed to an increase in the production of ROS induced during oxidative stress as the antioxidant tends to protect injured cells. The findings of the current study correspond with another study, which recorded a similar trend in decreasing GSH activities when exposed to a xenobiotic as well [50].

The principal neurotransmitter in the neuromuscular and sensory systems in many species is acetylcholine with its activity being vital and characterizing a major target wherein the detrimental effect of several toxicant can be exerted [51]. AChE is a significant enzyme in the brain which spots the neurotoxic effect of some xenobiotic substance.

The neurotransmitter acetylcholine, which plays a significant role in the nervous system function, is hydrolyzed by AChE in the synaptic cleft of the cholinergic synapses and the neuromuscular junctions [52]. AChE may probably be a target for toxins, which leads to the inhibition of its activity, failure of synaptic transmission, and muscle overstimulation. Thus, changes in AChE activity suggested that this enzyme is a significant pathological and physiological biomarker in assessing the neurotoxicity of pollutants [53]. Previous reports have demonstrated that the production of free radicals is, to some extent, associated with changes in AChE activity in the brain [54, 55]. The findings of the current study showing the inhibition of AChE activity in the brain exerted by Cd and CN confirm the observations of previous studies suggesting that the inactivation of AChE enzymes is due to the occupation of its active sites by pollutants [56, 57].

Conclusions

This study has shown that low concentrations of CN and Cd, through a controlled food chain, accumulate in fish in their natural habitat and are transferred to the next trophic level through the food chain as demonstrated by the gradual increase/decrease in the levels of most biomarkers found in the lungs, testes, heart, and brain of rats. The study demonstrated that Cd, CN, and their combination, via the food chain, induce oxidative stress in the lungs, testes, heart, and brain in rats.

Ethical Statement

The Faculty of Science Ethics Committee approved the current study (Approval Number ETH/17/18/PG245575). The ethics for the study is in line with the Statement for the Use of Animals in Research by the National Institutes of Health and World Medical Association Statement on Animal Use in Biomedical Research.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare that no conflicts exist.

Financial Disclosure

The authors declared no financial support.

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