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Effect of a controlled food-chain mediated exposure to cadmium and arsenic on oxidative enzymes in the tissues of rats

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ABSTRACT

Objective: The present study aims to investigate the effect of cadmium and arsenic through a controlled food chain on the activities of some oxidative enzymes (Sulphite oxidase SO, Aldehyde oxidase AO, Monoamine oxidase MO and Xanthine oxidase, XO) in the liver, kidney, testes, heart and brain of rats.
Materials and methods: Fish (the first trophic level) were exposed to both metals (singly and in mixture) using cadmium chloride ($CdCl_2$) as the source of cadmium and arsenic trioxide (AS_2O_3) as the source of arsenic at a concentration of 0.4 mg of metals/100 ml of water for 1 month and then sacrificed. The contaminated fish were then used as a source of protein in compounding the experimental diet to which the rats (the second trophic level) were exposed to for a period of 1 and 3 months. The Cd- and As-load in the feed and tissues of rats as well as the activities of the oxidative enzymes were subsequently analyzed in the various tissues after both period of exposure.
Results: Metal analysis on the tissues of rats showed that the metals accumulated more in the liver than in other organs after the 1 month exposure but accumulated more in the kidney after the 3 months exposure. The activities of the oxidative enzymes in the liver were significantly ($P < 0.05$) decreased in all test groups after the 1 and 3 months exposure. However, after the 1 month exposure, the kidney, testes and heart showed an initial increase in the activities of these enzymes which were decreased after the 3 months exposure. In the brain, the activities of these enzymes were increased in both duration of study.
Conclusion: From the results obtained in the current study, it could be concluded that exposure to cadmium and arsenic through the food chain leads to accumulation of these metals in the tissues of experimental rats leading to the inhibition of oxidative enzymes, thus affecting several normal metabolic processes.

1. Introduction

Heavy metals occur as natural constituents of the earth crust. They are persistent environmental contaminants [28]. Once liberated into the environment, heavy metals can be taken into the body via inhalation, ingestion and skin absorption [7,49]. If heavy metals enter and accumulate in body tissues faster than the body's detoxification pathways can dispose of them, then a gradual build-up of these toxins occurs. As heavy metal accumulation occurs in body tissues gradually, it can reach toxic concentration levels over time, much beyond the permissible limits [77]. The agency for Toxic Substances and Disease Registry (ATSDR) lists arsenic and cadmium among the top seven of the 275 most hazardous substances in the environment [56].

Cadmium is considered one of the most toxic substances in the environment due to its wide range of organ toxicity and long elimination half-life of 10–30 years [47]. Cadmium was identified as a contaminant at 776 of the 1467 EPA National Priorities List sites [2]. Cadmium has been shown to be highly toxic to living cells and tissues even at low levels [6,7,55]. It is an underground metal and did not enter the air, water, or even food in significant amounts until it was unearthed as part of zinc deposits. It has become a widespread environmental contaminant [56,7,85]. It is said to be a global threat because it is ubiquitous in virtually all ecosystems [44]. Cadmium has no essential biological function and is extremely toxic to humans.

Arsenic (As) is a member of group V of the periodic table of elements along with nitrogen, phosphorus, antimony, and bismuth. It is found in the natural environment, being present in soil, ground-

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water and plants [12]. Arsenic appears in both inorganic and organic compounds, differing in their physical and chemical properties [17,34,35]. The inorganic and organic arsenic compounds differ significantly in their toxicity, since the organic arsenic compounds exhibit very low toxicity [34]. Consequently, the potential adverse effects of arsenic to animal and human health are determined by the amount of inorganic arsenic present in food [12]. The chemistry of arsenic is rather complex, and the compounds it forms are numerous. This is largely because arsenic possesses several different valence or oxidation states, which result in the markedly different biological behavior of its compound. Arsenic compounds are used in pigments and dyes, as a preservative of animal hides, in glass manufacture, agricultural pesticides, and various pharmaceutical substances [3].

Heavy metal bioaccumulation in the food chain can be especially highly dangerous to human health. These metals enter the human body mainly through two routes: inhalation and ingestion, with ingestion being the main route of exposure to these elements in human population [1]. Heavy metals intake by human populations through the food chain has been reported in many countries with this problem receiving increasing attention from the public as well as governmental agencies, particularly in developing countries [23,38]. The introduction of these metals into the food chain may affect human health, and thus, studies concerning heavy metal accumulation through the food chain have received increasing importance [24]. Since the dietary intake of food may constitute a major source of long-term low-level body accumulation of heavy metals, the detrimental impact might become apparent only after long period of exposure. Regular monitoring of these metals from effluents, sewage, in vegetables and in other food materials is essential for preventing excessive buildup of the metals in the food chain [11].

Fish are exposed to varying concentrations of Cd and As in their natural habitats and have an ability to accumulate metal burdens exceeding aquatic levels [66,18,67,71,27]. Fish may therefore be an important vector of Cd and As transfer to higher levels of the food chain, including humans. Moreso, fish has been described as a good bio-indicator because it can be obtain easily in large quantity, easy to sample and can accumulate metals for analysis [13] of which humans are exposed to via food web. Since toxicity studies of this nature cannot be carried out directly on humans, and rats feed very well on fishes, rats were used for this purpose as the sensitivity of the test animals represents, at best, the average sensitivity of the highly heterogeneous human population of which it could be much higher for some members of the human population [54]. Various studies [73,5,42,39,15] have been carried out using fish as a feed source in terms of contamination for rats. Since fish is capable of accumulating Cd and As and thus may contribute to the intake of these metals, it is therefore important to determine their absorption and toxicity when consumed through fish. However, studies on the possible toxicity of these metals when provided in fish are scarce in literature. This forms the basis of the current study.

Xenobiotic metabolizing enzymes play central role in the biotransformation, metabolism and detoxification of xenobiotics or foreign compounds that are introduced to the human body. These enzymes protect or defend the body against the potential harmful insults from the environment [72]. Xenobiotic metabolizing enzymes enzymatically transformed foreign compounds to less harmful excretable compounds. This biotransformation process occurs mostly in the hepatic tissues and to a lesser extent, some extra hepatic tissues [82]. In the phase I reactions in xenobiotic biotransformation, oxidation reactions are probably the most common. These reactions require a group of non-specific, cytochrome P-450 dependent mixed functionoxidases (MFO). Aldehyde, xanthine, and sulphite oxidases are molybdenum and haem containing soluble enzymes that are present in the liver and other tissues and are also involved in the oxidation of xenobiotics [70,41]. Monoamine oxidase carries out the biotransformation of aromatic monoamines, including classical neurotransmitters such as serotonin, adrenalin, histamine and dopamine [7]. Despite the role played by these important oxidative enzymes in the biotransformation of xenobiotics, animal studies on the effect of cadmium and arsenic on these enzymes through the food chain are missing. Thus, the present study examines the effect of the exposure to cadmium and arsenic (singly and in mixture) on the activities of these oxidative enzymes (viz Sulphite oxidase (SO), Aldehyde oxidase (AO), Monoamine oxidase (MO) and Xanthine oxidase, (XO)) in the liver, kidney, testes, heart and brain through the food chain using rat as the animal model.

2. Materials and methods

2.1. Treatment of fish and preparation of diets

Catfish were gotten from a local fish pond in Imoje-Orogun, Delta State, Nigeria. These fish were exposed to both cadmium and arsenic in the form of cadmium chloride ($CdCl_2$) and arsenic trioxide (AS_2O_3), respectively, in plastic trough for 4 weeks and subsequently used as sources of protein in wholly compounded diets (Table 1). The fishes were divided into four (4) groups and left to acclimatize for 1 week before the commencement of the experiment. In Group A (Control), fishes were kept in fresh water. In Groups B, C and D, fishes were kept in water (100 l) contaminated with 0.4 mg cadmium/100 ml of water, 0.4 mg arsenic/100 ml of water and 0.4 mg arsenic + cadmium/100 ml of water, respectively. This concentration is equivalent to a dose of 4 ppm. For each group, the water was changed and re-contaminated every 24 h for 4 weeks. All the fishes received normal commercial feed for the duration of the 4 weeks after which they were sacrificed and dried in an oven and used as protein source in the compounded experimental diet.

Table 1
Composition of experimental diets.

Ingredients	Percentage (%) composition			
	Control	Cadmium (Cd)	Arsenic (As)	Combined (Cd + As)
Control fish	20	–	–	–
Cadmium-contaminated fish	–	20	–	–
Arsenic-contaminated fish	–	–	20	–
Cadmium + Arsenic-contaminated fish	–	–	–	20
Corn starch	45	45	45	45
Sugar	10	10	10	10
Palm oil	10	10	10	10
Fibre (dried groundnut husk)	10	10	10	10
Multi vitamin/mineral mix	5	5	5	5

The Cd and As contents of these diets were determined by atomic absorption spectrophotometry.

2.2. Treatment of animals

Sixty-four adult male albino rats (Wistar strain) with average weight of 126.25 ± 3.59 g were procured from the Animal house of the Basic Medical Sciences, Delta State University, Abraka, Nigeria and used for the study. The rats were divided into four experimental groups of sixteen rats each. Rats in groups A (Control), B, C and D were maintained on the Control, Cadmium, Arsenic and Cadmium + Arsenic compounded diets, respectively. During the period of treatment, animals were allowed free access to the water and compounded feed. Animal treatments were carried out in accordance with the principles of laboratory animal care [40]. After 1 month of exposure, half the number of rats in each group were sacrificed while the other half were sacrificed after 3 months of exposure. The sacrifice of the rats were done under chloroform anesthesia. While under anesthesia, the liver, kidneys, testis, heart and brain of each rat were quickly excised, placed on ice and subsequently weighed. Portions of the tissues were homogenized to give 20% homogenates and centrifuged at 10 000*g* for 15 min to obtain clear supernatants for biochemical analysis.

2.3. Cadmium and arsenic analysis on feed and tissues

The cadmium and arsenic concentrations in the digests were measured by atomic absorption spectrophotometry (Varian AA 1475 Spectrophotometer). The test metals were dissolved in deionized water and used as standard. In all the determinations, blanks were prepared to determine the effect of reagent purity on the metal levels.

2.4. Biochemical analysis

The supernatants obtained from the tissues (liver, kidney, testes, heart and brain) were used for the determination of the activities of Sulphite oxidase (SO), Aldehyde oxidase (AO), Monoamine oxidase (MO) and Xanthine oxidase (XO). SO activity was determined by the method of Macleod et al. [57]. The principle is based on the oxidation of sulphite to sulphate by the enzyme using ferricyanide as electron acceptor. The activity of the enzyme is also expressed in units per gramme tissue and one unit represents the amount of the enzyme that reduces one micromole of ferricyanide in one minute. The activity of AO was monitored by the method of Johns [48] which is based on the oxidation of benzaldehyde to benzoate using 2,6-dichloroindolephenol (DCIP) as the electron acceptor. The activity of the enzyme is given in units per gramme tissue weight and one unit is the amount of enzyme that produces one micromole of benzoate per minute. MO activity was assayed by the method of Tabor et al. [79] based on the oxidative deamination of benzylamine to benzaldehyde. The activity of the enzyme is expressed in units per gramme tissue weight and one unit of the enzyme is defined as the amount of enzyme that is required for the production of one micromole of benzaldehyde per minute. The activity of XO was determined by the method of Stirpe and Della Corte [76] using xanthine as the substrate and oxygen as electron acceptor. The enzyme activity is expressed in XO units per gramme tissue and each XO unit is the amount of the enzyme that produces one micromole uric acid.

2.5. Statistical analysis

All the data are expressed as mean ± standard deviation (SD). Statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD). The SPSS software (version 20) was used in the statistical analysis using multiple comparison tests. A p-value of less than 0.05 ($p < 0.05$) was considered significant.

Table 2
Composition of metals in feed given to experimental animals.

Groups	Metal composition (mg/g)	
	Cadmium	Arsenic
A	BDL[a]	0.02 ± 0.56[a]
B	3.68 ± 0.62[b]	0.03 ± 0.07[a]
C	0.01 ± 0.32[a]	1.82 ± 0.18[b]
D	3.50 ± 0.14[b]	1.52 ± 0.26[b]

A, Control; B, Cadmium contaminated diet; C, Arsenic contaminated diet; D, Cadmium + Arsenic contaminated diet; BDL, below detection limit (<0.01). Results are expressed as Mean ± SD. Values not sharing same superscript in same column differs significantly at ($P < 0.05$).

Table 3
Effect of food chain mediated exposure to cadmium and arsenic on body weight gain of rats.

Group	Body weight gain (g)
One month exposure	
A	(+)3.19 ± 0.96[a]
B	(−)2.22 ± 0.38[b]
C	(−)2.91 ± 0.67[b]
D	(−)3.48 ± 0.64[c]
Three month exposure	
A	(+)9.24 ± 1.84[a]
B	(−)6.52 ± 1.34[b]
C	(−)7.81 ± 0.26[c]
D	(−)13.08 ± 0.46[d]

A, Control; B, Cadmium contaminated diet; C, Arsenic contaminated diet; D, Cadmium + Arsenic contaminated diet. Results are expressed as Mean ± SD. n = 16. Values not sharing same superscript in same column differs at ($P < 0.05$).

3. Results

The results of metal analysis carried out on the feed gotten from the different groups of fish exposed to the metals at 0.4 mg/100 ml water is shown in Table 2. Result presented indicate traces of arsenic in the control feed while the presence of cadmium in same control feed was below detection limit. Table 3 shows the changes in the body weight gain of rats used in the present study for the experimental period of 1 and 3 months. The result shows significant ($P < 0.05$) reduction in the body weights gain of rats in all test groups.

The metal load in the tissues of experimental rats exposed to the contaminated diet is presented in Table 4. Result shown in Table 4 indicates that after the 1 month exposure, cadmium and arsenic accumulated more in the liver than in the kidney and other tissues but at the end of the three months exposure, the trend changed with the kidney recording the highest accumulation of these metals followed by the liver, testes, heart and brain.

Tables 5–8 show the effect of the contaminated diet on the activities of the oxidative enzymes (sulphite, aldehyde, monoamine and xanthine oxidases) in the tissues of rats. The activities of the enzymes were significantly ($P < 0.05$) decreased in all test groups in the liver for both period of exposure. However, in the kidney, testes and heart, the activities of the oxidative enzymes were increased after one month of exposure but decreased significantly ($P < 0.05$) after 3 months of exposure. The brain however showed increase ($P < 0.05$) in the activities of these enzymes for both period of exposure.

Table 4
Metals accumulated in tissues of experimental animals after 1 and 3 months exposure.

Group	Liver (μg/g tissue)	Kidney (μg/g tissue)	Testes (μg/g tissue)	Heart (μg/g tissue)	Brain (μg/g tissue)
1 month exposure					
A	0.02 ± 0.01^{a}	0.02 ± 0.09^{a}	0.01 ± 0.11^{a}	BDL[a]	BDL[a]
B	4.12 ± 0.34^{b}	3.01 ± 1.01^{b}	2.50 ± 0.32^{b}	2.15 ± 0.41^{b}	1.19 ± 0.03^{b}
C	1.53 ± 0.03^{c}	0.38 ± 0.01^{c}	0.26 ± 0.02^{c}	0.07 ± 0.01^{c}	0.11 ± 0.08^{c}
D (Cd)	3.83 ± 0.05^{d}	2.21 ± 0.14^{d}	2.02 ± 0.12^{b}	2.86 ± 0.01^{b}	1.37 ± 0.06^{b}
D (As)	1.38 ± 0.02^{c}	0.16 ± 0.01^{c}	0.11 ± 0.02^{c}	0.08 ± 0.00^{c}	0.02 ± 0.01^{c}
3 months exposure					
A	0.05 ± 0.03^{a}	0.04 ± 0.02^{a}	0.03 ± 0.05^{a}	0.02 ± 0.31^{a}	BDL[a]
B	7.10 ± 0.92^{b}	9.54 ± 0.13^{b}	5.17 ± 0.05^{b}	4.10 ± 0.34^{b}	1.39 ± 0.09^{b}
C	2.45 ± 0.21^{c}	3.21 ± 1.27^{c}	1.36 ± 0.14^{c}	2.11 ± 0.09^{c}	1.01 ± 0.07^{b}
D (Cd)	6.24 ± 0.07^{d}	7.15 ± 1.03^{d}	5.12 ± 0.19^{b}	5.82 ± 0.12^{d}	2.57 ± 0.16^{c}
D (As)	2.01 ± 0.12^{c}	2.79 ± 0.01^{c}	1.83 ± 0.01^{c}	1.87 ± 0.00^{c}	1.86 ± 0.10^{b}

A, Control; B, Cadmium contaminated diet; C, Arsenic contaminated diet; D, Cadmium + Arsenic contaminated diet; BDL, below detection limit. Results are expressed as Mean ± SD. Values not sharing same superscript in same column differs significantly at ($P < 0.05$).

Table 5
Effect of formulated contaminated diet on sulphite oxidase activities (units/g tissue) in tissues of rats after 1 and 3 months exposure.

Group	Liver (units per gramme tissue)	Kidney	Testes	Heart	Brain
1 month exposure					
A	46.23 ± 0.74^{a}	49.51 ± 0.70^{a}	41.15 ± 6.43^{a}	27.25 ± 0.09^{a}	27.98 ± 2.69^{a}
B	33.78 ± 1.09^{b} (−26.93%)	58.68 ± 3.55^{b} (18.52%)	44.53 ± 3.12^{a} (8.21%)	30.32 ± 1.30^{a} (11.27%)	30.40 ± 1.63^{a} (8.65%)
C	15.38 ± 3.03^{c} (−66.73%)	50.98 ± 4.20^{a} (2.97%)	46.54 ± 6.18^{a} (13.10%)	29.99 ± 0.43^{a} (10.06%)	32.24 ± 4.69^{a} (15.22%)
D	22.49 ± 1.20^{d} (−51.35%)	54.15 ± 0.78^{b} (9.37%)	68.18 ± 2.83^{b} (65.69%)	42.23 ± 1.19^{b} (54.97%)	42.05 ± 4.33^{b} (50.29%)
3 months exposure					
A	44.47 ± 1.44^{a}	45.49 ± 2.61^{a}	29.03 ± 1.27^{a}	42.39 ± 3.78^{a}	32.86 ± 0.10^{a}
B	27.58 ± 0.77^{b} (−37.98%)	16.36 ± 0.04^{b} (−64.04%)	16.16 ± 0.54^{b} (−44.33%)	31.06 ± 0.92^{b} (−26.73%)	36.81 ± 0.28^{a} (12.02%)
C	18.58 ± 1.73^{c} (−58.22%)	28.82 ± 1.11^{c} (−36.65%)	18.61 ± 1.81^{b} (−35.89%)	36.21 ± 0.53^{b} (−14.58%)	35.71 ± 0.53^{a} (8.67%)
D	22.95 ± 1.31^{b} (−48.39%)	20.21 ± 0.95^{c} (−55.57%)	8.43 ± 0.65^{c} (−70.96%)	22.28 ± 0.90^{c} (−47.44%)	42.95 ± 0.56^{b} (30.71%)

A, Control; B, Cadmium contaminated diet; C, Arsenic contaminated diet; D, Cadmium + Arsenic contaminated diet. Results are expressed as Mean ± SD. Values not sharing same superscript in same column differs significantly at ($P < 0.05$).

Table 6
Effect of formulated contaminated diet on aldehyde oxidase activities (units/g tissue) in tissues of rats after 1 and 3 months exposure.

Group	Liver (units per gramme tissue)	Kidney	Testes	Heart	Brain
1 month exposure					
A	56.90 ± 0.24^{a}	46.96 ± 4.05^{a}	29.99 ± 2.63^{a}	21.25 ± 0.09^{a}	27.98 ± 2.69^{a}
B	46.73 ± 2.03^{b} (−17.87%)	65.10 ± 2.11^{c} (38.63%)	32.22 ± 2.05^{a} (7.44%)	30.32 ± 1.30^{b} (42.68%)	31.40 ± 1.63^{a} (12.22%)
C	31.87 ± 4.00^{c} (−43.99%)	51.16 ± 3.54^{b} (8.94%)	33.29 ± 3.76^{a} (11.00%)	25.99 ± 0.43^{a} (22.31%)	32.24 ± 4.69^{a} (15.23%)
D	45.60 ± 2.03^{b} (−19.86%)	57.71 ± 1.42^{b} (22.89%)	45.83 ± 2.04^{b} (52.82%)	42.23 ± 1.19^{c} (98.73%)	42.05 ± 4.33^{b} (50.29%)
3 months exposure					
A	45.83 ± 2.62^{a}	50.80 ± 0.62^{a}	28.85 ± 0.44^{a}	33.85 ± 0.43^{a}	21.58 ± 0.99^{a}
B	28.51 ± 0.54^{b} (−37.79%)	16.22 ± 0.31^{b} (−68.07%)	15.76 ± 0.59^{b} (−45.37%)	25.92 ± 0.79^{b} (−23.43%)	34.05 ± 0.179^{b} (57.78%)
C	18.81 ± 1.13^{c} (−58.96%)	30.48 ± 1.96^{c} (−40.00%)	25.38 ± 0.26^{a} (−12.03%)	30.27 ± 0.84^{a} (−10.58%)	26.47 ± 0.56^{a} (22.66%)
D	25.29 ± 1.13^{b} (−44.82%)	23.89 ± 1.83^{d} (−52.97%)	13.96 ± 0.33^{b} (−51.61%)	23.85 ± 0.42^{b} (−29.54%)	41.43 ± 0.56^{c} (91.98%)

A, Control; B, Cadmium contaminated diet; C, Arsenic contaminated diet; D, Cadmium + Arsenic contaminated diet. Results are expressed as Mean ± SD. Values not sharing same superscript in same column differs significantly at ($P < 0.05$).

4. Discussion

Cadmium and arsenic are well known to induce a variety of toxicity symptoms in both experimental and exposed populations. The present study aims to assess the effect, if any, of cadmium and arsenic (singly and in mixture) on oxidative enzymes in the tissues of rats through the food chain using fish as the first trophic level. The exposure of the fish to the contaminants at a concentration of 0.4 mg/100 ml of water daily was done to achieve the highest concentration of these metals in the tissues of fish within a period of 30 days. The use of this sub-lethal dose of cadmium is in consonance with the work of Chaudhari et al. [19] who studied the effect of cad-

Table 7
Effect of formulated contaminated diet on monoamine oxidase activities (units/g tissue) in tissues of rats after 1 and 3 months exposure.

Group	Liver (units per gramme tissue)	Kidney	Testes	Heart	Brain
1 month exposure					
A	46.23 ± 0.74^{a}	36.51 ± 0.70^{a}	41.15 ± 6.43^{a}	37.87 ± 1.03^{a}	36.63 ± 3.01^{a}
B	33.78 ± 1.09^{b} (−26.93%)	54.15 ± 0.78^{b} (48.32%)	47.53 ± 3.12^{a} (15.50%)	40.70 ± 3.03^{a} (7.473%)	45.87 ± 4.01^{b} (25.23%)
C	15.38 ± 3.03^{c} (−66.73%)	45.98 ± 4.20^{c} (25.94%)	44.54 ± 6.18^{a} (8.23%)	39.20 ± 1.06^{a} (3.51%)	40.50 ± 3.01^{a} (10.57%)
D	22.49 ± 1.20^{d} (−51.35%)	49.68 ± 3.55^{c} (36.07%)	52.18 ± 2.83^{b} (26.80%)	47.54 ± 2.01^{b} (25.53%)	49.25 ± 2.01^{c} (34.45%)
3 months exposure					
A	61.70 ± 1.05^{a}	35.47 ± 0.25^{a}	46.32 ± 2.72^{a}	38.91 ± 2.046^{a}	31.87 ± 1.07^{a}
B	49.20 ± 0.83^{b} (−20.26%)	14.19 ± 0.31^{b} (−59.99%)	34.71 ± 3.54^{b} (−25.06%)	20.96 ± 0.85^{b} (−46.13%)	47.67 ± 0.79^{b} (49.58%)
C	23.66 ± 1.10^{c} (−61.66%)	20.99 ± 0.11^{c} (−40.82%)	52.24 ± 3.50^{c} (12.78%)	29.70 ± 1.68^{b} (−23.67%)	38.02 ± 0.31^{a} (19.30%)
D	33.37 ± 1.44^{d} (−45.92%)	19.83 ± 1.31^{b} (−44.09%)	24.50 ± 1.26^{d} (−47.12%)	13.45 ± 0.36^{c} (−65.43%)	50.57 ± 0.59^{b} (58.68%)

A, Control; B, Cadmium contaminated diet; C, Arsenic contaminated diet; D, Cadmium + Arsenic contaminated diet. Results are expressed as Mean ± SD. Values not sharing same superscript in same column differs significantly at ($P < 0.05$).

Table 8
Effect of formulated contaminated diet on xanthine oxidase activities (units/g tissue) in tissues of rats after 1 and 3 months exposure.

Group	Liver (units per gramme tissue)	Kidney	Testes	Heart	Brain
1 month exposure					
A	60.75 ± 3.09^{a}	50.01 ± 2.03^{a}	50.32 ± 1.55^{a}	32.53 ± 0.08^{a}	28.73 ± 6.99^{a}
B	48.28 ± 4.54^{b} (−20.53%)	64.53 ± 2.02^{b} (29.03%)	45.55 ± 1.45^{b} (−9.48%)	41.70 ± 2.26^{b} (28.19%)	41.55 ± 2.35^{b} (44.62%)
C	41.54 ± 2.91^{b} (−31.62%)	57.54 ± 1.01^{c} (15.05%)	58.04 ± 2.01^{c} (15.34%)	40.57 ± 0.05^{b} (24.72%)	37.00 ± 1.85^{c} (28.79%)
D	44.69 ± 1.01^{b} (−26.44%)	60.02 ± 1.05^{b} (20.02%)	64.03 ± 0.35^{c} (27.25%)	45.37 ± 0.66^{b} (39.47%)	45.54 ± 1.78^{b} (58.51%)
3 months exposure					
A	75.51 ± 1.49^{a}	86.43 ± 1.05^{a}	58.96 ± 0.49^{a}	39.30 ± 1.01^{a}	32.70 ± 3.02^{a}
B	62.43 ± 0.43^{b} (−17.32%)	58.05 ± 1.01^{b} (−32.84%)	37.05 ± 0.178^{b} (−37.16%)	45.03 ± 0.89^{b} (14.58%)	43.01 ± 1.50^{b} (31.53%)
C	49.15 ± 0.63^{c} (−34.91%)	71.17 ± 0.02^{c} (−17.66%)	43.60 ± 0.65^{c} (−26.05%)	46.20 ± 2.46^{b} (17.56%)	37.02 ± 0.16^{a} (13.21%)
D	53.01 ± 2.83^{d} (−29.80%)	65.81 ± 2.64^{d} (−23.86%)	26.17 ± 0.48^{d} (−55.61%)	41.25 ± 0.71^{b} (4.96%)	46.81 ± 1.68^{b} (43.15%)

A, Control; B, Cadmium contaminated diet; C, Arsenic contaminated diet; D, Cadmium + Arsenic contaminated diet. Results are expressed as Mean ± SD. Values not sharing same superscript in same column differs significantly at ($P < 0.05$).

mium chloride (0.4 mg/100 ml) on the biochemical characteristics of fresh water fish, *Cyprinus carpio*. Also, Siddiqui and Chang [74] used a sub-lethal dose of 4.0 μg/ml to analyse cadmium chloride intoxication in *Clarias batrachus* (Linn). Ahmad et al. [4] also studied the effect of cadmium chloride on the histoarchitecture of liver and kidney of a freshwater catfish, *Clarias batrachus* using 4 and 8 ppm. Exposure of cat fish to sub-lethal levels of arsenic has also been established by several authors [6,53]. Environmentally, in contaminated areas, the concentration of arsenic ground water could range from 0.25 to 2.1 ppm and even reaches >4.0 ppm in severely contaminated areas [20,22,69,80]. While for cadmium, river water contains dissolved cadmium at concentrations of between <1 and 13.5 ng/l [45]. McCready et al. [60], Fatoki and Mathabatha [36] and Ololade et al. [67] showed that ecosystems could be affected by sediments that serve as a metal pool which releases metals to the overlying water via natural or anthropogenic processes, causing potential adverse health effects to the ecosystems. Moreso, marine organisms can take up metals, further enhancing the potential of some metals entering into food chain.

The results of the present study revealed that the general metabolic condition of the animals exposed to either cadmium or arsenic contaminated diet (Groups B and C) for 1 and 3 months were not within normal range as manifested in the body weight loss observed when compared with control rats (Table 2). These could be attributed to the reduction in food intake as observed at commencement of study. In Group D of the present study where both metals were combined, there was also a significant reduction in body weights of rats as compared to the control. This supports the observations reported by Mahaffey et al. [59] who showed that both As and Cd decreased body weight gain and food utilization and that these effects were more pronounced for the mixture. Available reports [63,62,78] also indicate that long term exposure to arsenic and cadmium could lead to significant reduction in body weights.

Weight gain has been shown to be influenced by the availability and absorption of nutrients [8]. Elsenhans et al. [29] and Eriyamremu et al. [31] have also shown that cadmium decreases nutrient digestion and absorption. Other authors [62,59,8,43] have also shown the effect of cadmium and arsenic (singly and in mixture) on body weight gain and results obtained are consistent with the observations of the current study. Gaskill et al. [37], Yousef et al. [86] and Bhatia et al. [16] had shown that reduction of body weight can be used as an important indicator for the deterioration

of rat general health status. Cd and As inhibitory effect on digestive and absorption enzymes [7], may also account for the decreased in weight of rats as observed in this study.

Results presented in Table 4 shows that Cd and As accumulated more in the liver than other tissues after one month of exposure. However, after 3 months of exposure, these metals were found to accumulate more in the kidney than in the liver or other tissues. Studies have shown increased accumulation of Cd and As in the tissues of exposed rats [7,64,21,43]. Experimental evidence has shown that more metals are deposited in the liver than in the kidneys when administered orally and subcutaneously [68,7]. However, as the duration of exposure increases, there is a gradual mobilization of these metals from the liver to the kidney [30,75]. This may not be unconnected with the fact that the kidney as an excretory organ is the final destination of these metals from various tissues and plasma. The present finding on the higher uptake of Cd and As in the kidney as compared to other organs is in harmony with available reports in literature [43,29,14,32].

The increased accumulation of Cd in the kidney and liver is consistent with previous reports which indicate that these two organs account for most of the body burden of the metals [85,25]. The increased accumulation of Cd in the liver and kidney has been attributed to their ability to induce the synthesis of metallothionien (MT) [52,82]. MT is a heavy metal binding protein which is believed to influence the uptake, distribution and toxicity of Cd [84,51]. In a similar vein, the metabolism of arsenic for elimination through the oxidation and reduction reactions, methylation reactions as well as arsenic binding to unidentified proteins takes place in the liver while the kidney is responsible for the excretion of the metabolites [56]. Thanh and Stillman [81] has shown that arsenic is capable of binding to biological thiols including mammalian metallothionein. The liver and kidney are thus, more exposed to foreign substances than other organs because they are the sites of metabolic activities and this may have also contributed to the increased concentration of these metals in these organs. The relatively low level of Cd and As in the brain of the 1 and 3 months exposed rats is consistent with the findings of a Eriyamremu et al. [33]. This underscores the importance of the blood-brain barrier which has been reported to limit the uptake of toxicants into the brain [83] unlike the liver and kidney which do not possess such a barrier mechanism.

In the present study, it was observed that the control also has some very minimal level of contamination of cadmium and arsenic. The presence of Cd and As in the liver and kidney of the control rats and the progressive manner in which it increased are indications that the water and/or feed used for the controls were tainted with Cd and As. This could be attributed to the broad distribution of Cd and As in the general environment. Reproducible results were obtained by Horiguchi et al. [43] who detected trace quantity of cadmium in the kidney at the end of the 6 and 9 months exposure period after administering supposedly Cd-free saline solution to control rats. Asagba et al. [9,10], Ognjanovic et al. [65] and Mafulul and Okoye [58] have also reported trace quantities of cadmium in the liver and kidney of rats on the control diet. This is a further proof of the wide distribution these metals in the food chain and the environment.

The elevation of the activities of the oxidases (sulphite, aldehyde, monoamine and xanthine oxidases) in the tissues analysed (Liver excluded) after 1 month of exposure shows that the metals were capable of elevating enzyme activities for the duration of exposure. This apparently may be due to the level of these metals still at a low accumulated state after the 1 month exposure. However, the decrease in the activities of the various enzymes in the liver even at the 1 month exposure could be attributed to that fact that the liver, being the major site of xenobiotic activities, gets a higher load of exposure to these metals due to normal metabolic activities and thus led to the inhibition of the activities of these enzymes even at low concentration.

Similarly, the decreased activities of the oxidative enzymes in the tissues of rats after the 3 months exposure could be attributed to increased pathological condition of tissues as the metals (singly and in mixture) enter and accumulate in body tissues faster than the body's detoxification pathways could dispose of them. The inhibition of these enzymes by the metals may be linked to the displacement of essential metal cofactors from the enzyme active site, or the formation of covalent bonds by the metals with sulphydryl and other groups essential for the enzyme action [82,46,87]. Asagba [7] also reported that the inhibiting activity towards the molybdenum hydroxylases (Aldehyde oxidase (AO) and xanthine oxidase (XO)) and the molybdenum-dependent sulphite oxidase (SO) may be associated with metal-metal interactions, while the reduction of FAD-activated monoamine oxidase (MO) flavoprotein activity may be due to the prooxidative activity of these metals.

The metabolic functions of the various oxidative enzymes have been listed in literature [7,50]. Kitamura et al. [50] reported that AO and XO both play important roles in the metabolism of many exogenous and endogenous compounds as they exhibit oxidative activity towards various heteroxcyclic compounds and aldehydes and the liver cytosol of various mammals also exhibits a significant reductive activity toward nitro, sulfoxide, N-oxide and other moieties catalyzed by Kitamura et al. [50] also reported that XO catalyzes the oxidation of hypoxanthine to xanthine, then to uric acid, an important process in purine catabolism. Asagba [7] reported that SO is another molybdoprotein that is involved in the oxidation of endogenous sulphite arising from the degradation of sulphur amino acids. While, Murphy and Kalin [61] reported that MO inhibition is accompanied by marked changes in the sensitivity of the organism to some dietary constituents such as tryptophan,tyramine and other amines and their precursors as well as many drugs (e.g. opiates, reserpine and caffeine).

From the above findings, it can be concluded that cadmium and arsenic, at low concentrations through the food chain are potential inducers/inhibitors of the activities of the oxidative enzymes in the tissues studied depending on the duration of exposure. The decreases observed in the activities of these enzymes with increase in the duration of study, would not only affect their contribution towards the detoxification of xenobiotics but may also affect other aspects of metabolism thus aggravating the toxicity of these metals.

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