IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Assessment of the Effects of Cadmium on Rat Kidney and Liver Catalase and Superoxide Dismutase Activities in the Presence of Iron

Obadoni, Isabel Ashinedu[1*], Obi, Frederick Otunuya[2]

[1]PhD Research Student

[1]Department of Biochemistry, Faculty of Life Sciences,

University of Benin, P.M.B 1154, Benin City, Edo State, Nigeria.

[2] Professor

[2]Department of Biochemistry, Faculty of Life Sciences,

University of Benin, P.M.B 1154, Benin City, Edo State, Nigeria.

ABSTRACT

The effects of iron on cadmium –induced alterations on the activities of two antioxidant enzymes and the attendant changes in lipoperoxidation status have been examined in the kidneys and livers of rats exposed singly or combined via drinking water and feed formulated with milled fish pre –exposed to the two heavy metals singly or combined also. One hundred and eight rats divided into eight experimental groups of 5 or 4, rats each were used for this study that was divided into two phases. Phase 1, in which the rats were exposed via drinking water and phase 2, in which they were exposed via feeds formulated with milled catfish pre –exposed to the heavy metals separately and concurrently. Group A rats of each phase served as the control, group B was the iron only group, group C was the cadmium only group while group D was the combined cadmium and iron group. The exposure was for 1, 2 and 3 months. At the end of each exposure period it was found that cadmium (Cd) caused significant ($p \leq 0.05$) losses in catalase (CAT) and superoxide dismutase (SOD) activities in the kidneys and livers whether in rats exposed via drinking water or diet when compared with the control group values. Percentage losses in CAT activities were in the range of 8.18 – 25.84 (kidney) and 16.50 – 51.74 (liver) while those of SOD were 30.11 – 57.71 (kidney) and 8.18 – 28.07 (liver). Correspondingly, there were significant ($p \leq 0.05$) increases in malondialdehyde (MDA) levels in the Cd only group. The percentage increases ranged from 26.24 – 254.02 (kidney) and 123.66 – 433.29 (liver). When rats were concurrently exposed to Cd and Fe, the activities of both enzymes were significantly ($p \leq 0.05$) increased relative to the Cd only group. Percentage increases in CAT activities were in the range of 17.72 – 40.00 (kidney) and 27.41 – 80.92 (liver) while those for SOD were 22.47 – 110.22 (kidney) and 5.94 – 25.48 (liver). For MDA, the levels in the Cd plus Fe treated rats were significantly ($p \leq 0.05$) reduced compared to the Cd only group. Percentage reductions were in the range of 0.58 – 43.28 (kidney) and 0.46 – 65.93 (liver). The findings in this study suggest that where Cd and Fe occur as co –contaminants at the concentrations used here, Fe would counter (i) the ability of cadmium to impair kidney and liver CAT and SOD activities and (ii) the ability of Cd to enhance MDA production.

Keywords: Cadmium, Iron, Catalase, Superoxide Dismutase

*Corresponding Author

INTRODUCTION

Products of plants consumed and water drank by humans and animals are usually contaminated with cadmium (Cd) present in the atmosphere, water and land [1]. The level of Cd in agricultural crops is not uniform. It varies according to the plant species, geographical location and also, very importantly, the season [2].

In the body, Cd is widely distributed but the major sites with fairly high loads are the kidneys and liver where it is known to accumulate and remain with extremely low rate of excretion [3]. Once Cd is taken up from the lungs or gastrointestinal tract, it is transported in the blood stream bound to albumin [4]. Cd bound to albumin preferentially ends up in the liver where it induces the synthesis of metallothionein [5], a protein that is rich in cysteine residues that have the capacity to bind and store metal ions [6]. Relatively, the Cd burden of the kidneys is higher than that of the liver but the lowest concentrations are found in the brain, bones and fat depot [7].

Cd intoxication in animals causes alteration in the status of some biochemical parameters in the blood and a good number of target organs. Among these parameters are catalase (CAT), superoxide dismutase (SOD), Na+/K+ ATPase activities as well as malondialdehyde (MDA) levels in rat liver [8] - [10]. It also increases the level of cholesterol in rat testes and prostate [11], an organ and gland known to be extremely sensitive to Cd toxicity [12] – [14].

Numerous studies on heavy metal toxicity generally focus on individual metals in isolation but the fact is that these metals do not exist alone in the environment. When a body of water is co-contaminated, as it is with many rivers, consumption of the water and/or the fish caught from the affected lake or river amounts to concurrent exposure to a cocktail of the metallic contaminants. The co-pollutants can enhance or antagonize the toxicity of each other. However, these abilities, where they exist will not be evident when toxicity studies are performed separately on individual entities like Cd. For instance, Warri River, in Niger Delta Region of Nigeria has been shown to be polluted not by a single metallic entity but by many toxic metals in various studies over the years. The concentrations of some of the metallic pollutants identified which include cadmium, chromium, copper, iron and lead were 0.229 mg/L, 0.598 mg/L, 0.1187 mg/L, 1.900 mg/L and 0.081 mg/L respectively [15]; 0.275 mg/L, 0.059 mg/L, 0.019 mg/L, 2.770 mg/L and 0.086 mg/L respectively [16]; 0.0073 mg/L, 0.0077 mg/L, 0.040 mg/L), 1.930 mg/L and 0.0001 mg/L respectively [17]; 0.0547 mg/L, 0.235 mg/L, 0.0153 mg/L, 0.400 mg/L and 0.0848 mg/L respectively [18]. The fact that the river was polluted by many toxic heavy metals and not just one gave us the opportunity to test our hypothesis which takes cognizance of environmental realities. Hence, the aim of this study was to determine whether Cd toxicity, when its concentration in water is 0.229 mg/L, will be affected by the presence of iron at a concentration of 1.900 mg/L (Warri River Cd and Fe concentrations respectively [15]). We focused on the activities of two antioxidant enzymes in the kidney and liver and the levels of malondialdeyde after rats were exposed to both agents via tainted water and food – chain based formulated heavy metals tainted diet.

MATERIALS AND METHODS

Sixty albino rats (Wistar strain) having average weight of 172.45± 6.23g were used for the water mediated cadmium and iron exposure while 48 rats, mean weight 162.45±7.52g were used for the food chain mediated exposure. Four types of waters (control and 3 tests) that differed in their metallic ion content and four diets (control and 3 tests) that differed in the same way in terms of protein source exposure that were used. The test diets contained milled Cd (0.229 mg/L), Fe (1.900 mg/L) and Cd + Fe (0.229 mg Cd/L + 1.900 mg Fe/L) exposed catfish as the source of protein. The control diet on the other hand contained milled catfish that were not exposed to any of the metallic entities as protein source. Other components of the diets were as described previously [9]. The chemicals /reagents used were: Hydrogen peroxide – 30% (Sigma- H1009, USA), $CdCl_2.2.5H_2O$ (Kermel, China), trichloroacetic acid (JHD, China), $FeSO_4.7H_2O$ (Kermel, China), 2- thiobarbituric acid (Ken Light Lab, PVT Ltd, India), epinephrine (Fluka, England) hydrochloric acid and sulphuric acid (BDH Chemicals, Poole, England).

Table 1: Composition of control and test diets.[a,a']

Ingredients	Control diet[a] (%)	Test $diet_{Fe}$ (TD_{Fe})[a] (%)	Test $diet_{Cd}$ (TD_{Cd})[a] (%)	Test $diet_{Cd+Fe}$ (TD_{Cd+Fe})[a] (%)
Milled Cd & Fe-free catfish	22.00	0.00	0.00	0.00
Milled Fe-exposed catfish	0.00	22.00	0.00	0.00
Milled Cd-exposed catfish	0.00	0.00	22.00	0.00
Milled Cd + Fe exposed catfish	0.00	0.00	0.00	22.00
Corn starch	53.00	53.00	53.00	53.00
Sugar	5.00	5.00	5.00	5.00
Palm oil	7.00	7.00	7.00	7.00
Dried peanut husk	8.00	8.00	8.00	8.00
ABC multivitamin/ minerals	5.00	5.00	5.00	5.00
Total	**100.00**	**100.00**	**100.00**	**100.00**

[a] AAS analysis showed that the Cd and Fe contents of the control and test diets were : control diet (0.03 ± 0.02 mg Cd/g and 1.30 ± 0.01mg Fe/g feed), TD_{Fe} (4.21± 0.02 mg Fe/g feed), TD_{Cd} (0.29 mg Cd/g feed) and TD_{Cd+Fe} (0.21 ± 0.01 mg Cd and 3.04 ± 0.02 mg Fe/g feed)

[a'] AAS –method of Hernandez et al. [23] was adopted for Cd and Fe analysis.

The rats used for the tainted water exposure were divided into 12 experimental groups of 5 rats each and housed in wood framed iron meshed sides and bottom cages. Cages 1A, 2A and 3A were the controls for the 1, 2 and 3 months exposure. Cages 1B, 2B and 3B were for the 1, 2 and 3 months exposure to iron tainted water (1.90 mg/L) administered by gavage. Cages 1C, 2C and 3C were for the 1, 2 and 3 months exposure to Cd tainted water (0.229 mg/L) while cages 1D, 2D and 3D were for the 1, 2 and 3 months exposure to a solution of Fe and Cd salts (1.90 mg Fe/L + 0.229 mg Cd/L). Rats in each group received the equivalent of 42.186 ml of the appropriate salt solution in water/kg body weight/day by gavage for the specified period of exposure.

The rats used for the tainted diet exposure were also divided into 12 experimental groups of 4 rats each and housed in cages as described above. Cages 1A, 2A and 3A were the controls for the 1, 2 and 3 months exposures to the control diet. Cages 1B, 2B and 3B were for the 1, 2 and 3 months exposure to iron exposed milled catfish formulated diet (TD_{Fe}). Cages 1C, 2C and 3C were for the 1, 2 and 3 months exposure to Cd exposed milled catfish formulated diet (TD_{Cd}) while cages 1D, 2D and 3D were for the 1, 2 and 3 months exposure to the cadmium plus iron exposed milled catfish formulated diet ($TD_{Cd + Fe}$). The tests and control rats were pair fed (which controlled their food consumption) but allowed free access to drinking water. At the end of each specific period of exposure, each rat was handled humanely and sacrificed as described previously [9]. A portion of the liver of known weight and one of the kidneys after weighing, were separately homogenized in ice –cold saline (1:5 w/v) to give a 20% homogenate using ice –cold motar and pestle. Each homogenate was centrifuged at 3000 rpm for 10 minutes. The supernatants were separated and stored frozen at -20^{o}C until required for biochemical assays.

CAT activity was analyzed using the method of Cohen *et al*. [19]. In this method each catalase unit specifies the relative logarithmic disappearance of hydrogen peroxide per minute and is expressed as K min^{-1}. SOD activity was analyzed by the method of Misra and Fridovich [20]. The method described by Baum and Scandalios [21] was adopted in the computation of the activity of SOD, in which the amount of enzyme required for 50% inhibition of epinephrine conversion to adrenochrome during 1 minute was regarded as 1 unit. The amounts of thiobarbituric acid reactive substances (TBARS) which are markers of lipid peroxidation were assayed for using the method described by Buege and Aust [22]. Values of TBARS were arrived at using a molar extinction coefficient of 1.56 x 10^5 $M^{-1}cm^{-1}$ and expressed in terms of malondialdehyde (MDA) units per mg tissue.

RESULTS AND DISCUSSION

The composition of the control and tests diets and their respective cadmium and iron contents are shown in Table 1. It

is obvious that the control rats were being exposed to both iron and cadmium that were environmentally introduced into their feed and/or water as AAS study revealed [23]. For Cd, this has been shown to build up progressively in their liver over time as reported earlier [9]. The presence of Cd in control experimental feed was reported by Asagba and Obi [9] and earlier by Lind et al. [24]. So, it is quite interesting to find in the present study that environmentally, iron gets incorporated in ingredients used for compounding animal feed (Table 1).

Exposure of rats to Cd via the Cd –only tainted water and diet caused significant reduction ($p \leq 0.05$) in kidney and liver CAT activities (Tables 2, 3, 4 and 5) at the end of 1, 2 and 3 months exposure relative to the appropriate corresponding controls. It also caused significant ($p \leq 0.05$) reduction in SOD activities in the same organs and at the same periods of exposure (Tables 6, 7, 8 and 9).

Table 2: Influence of iron on cadmium associated loss in liver catalase activity in tainted water exposed rats

		Liver Catalae Activity (K/min) Mean ± SEM (n = 5)		
Group	Treatment	Month 1	Month 2	Month 3
A	Control	2.00 ± 0.06	2.10 ± 0.06	2.33 ± 0.04
B	1.90mg Fe/L	1.96 ± 0.04	2.02 ± 0.01	2.23 ± 0.04
C	0.23mg Cd/L	1.33 ±0.07 ^{a,b,d*} (33.05%)** ↓	1.45± 0.04 ^{a,b,d} (30.95%) ↓	1.75 ± 0.01 ^{a,d} (24.89%) ↓
D	1.90mg Fe and 0.23 mg Cd/L	1.89 ± 0.02 ^{a,c*} (42.11%)** ↑	1.92±0.05 ^{a,b,c} (32.41%) ↑	2.30 ± 0.02 ^{a,c} (31.43%) ↑

*Values with superscripts a, b, c or d are statistically significantly different from the value of the group with corresponding uppercase letter A, B,C or D within the column ($p \leq 0.05$).

**Values in brackets in row C, are percentage changes relative to corresponding group A value while the values in brackets in row D, are percentage changes relative to corresponding group C values

↑% Percentage increase

↓ % Decrease

Table 3: Influence of iron on cadmium associated loss in liver catalase activity in tainted diet exposed rats

		Liver Catalae Activity (K/min) Mean ± SEM (n = 4)		
Group	Treatment	Month 1	Month 2	Month 3
A	Control	3.15 ± 0.34	3.49 ± 0.40	5.17 ± 0.46
B	1.90mg Fe/L	2.74 ± 0.38 [a*]	3.36 ± 0.44 [a,]	4.89 ± 0.97 [a,]
C	0.23 mg Cd/L	1.52 ± 0.34 [a,b,d**] (51.24%) ↓	2.59 ± 0.17 [a,b,d] (25.79%) ↓	3.53 ± 0.21 [a,b,d] (31.72%) ↓
D	1.90 mg Fe and 0.23 mg Cd/L	2.75 ± 0.09 [a,b,c] (80.92%)** ↑	3.30 ± 0.23 [a,b,c] (27.41%) ↑	4.81 ± 0.44 [a,b,c] (36.26%) ↑

Table 4: Influence of iron on cadmium associated loss in kidney catalase activity in tainted water exposed rats

		Kidney Catalae Activity (K/min) Mean ± SEM (n = 5)		
Group	Treatment	Month 1	Month 2	Month 3
A	Control	1.82 ± 0.03	1.94 ± 0.02	2.09 ± 0.05
B	1.90mg Fe/L	1.76 ± 0.04	1.91 ± 0.03	2.07 ± 0.03
C	0.23 mg Cd/L	1.42 ± 0.06 [a,b,d**] (21.98%) ↓	1.49 ± 0.04 [a,b,d] (23.08%) ↓	1.55 ± 0.05 [a,b,d] (25.85%) ↓
D	1.90 mg Fe and 0.23 mg Cd/L	1.74 ± 0.12 [c] (22.54%)** ↑	1.88 ± 0.03 [c] (26.17%) ↑	2.05 ± 0.06 [c] (32.26%) ↑

Table 5: Influence of iron on cadmium associated loss in kidney catalase activity in tainted diet exposed rats

		Kidney Catalae Activity (K/min) Mean ± SEM (n = 4)		
Group	Treatment	Month 1	Month 2	Month 3
A	Control	3.01 ± 0.08	3.58 ± 0.13	4.21 ± 0.22
B	1.90 mg Fe/L	2.89 ± 0.12	3.51 ± 0.12	3.80 ± 0.17 [a]
C	0.23 mg Cd/L	2.22 ± 0.06 [a,d**] (29.24%) ↓	2.99 ± 0.10 [a,b,d] (16.48%) ↓	3.00 ± 0.10 [a,b,d] (28.74%) ↓
D	1.90 mg Fe and 0.23 mg Cd/L	2.84 ± 0.17 [a,c] (27.93%) ↑	3.52 ± 0.03 [a,c] (17.73%) ↑	4.20 ± 0.17 [b,c] (40.00%) ↑

Table 6: Influence of iron on cadmium associated loss in SOD activity in liver of tainted water exposed rats

		Liver SOD Activity (Units/mg tissue) Mean ± SEM x 10^{-2} (n = 5)		
Group	Treatment	Month 1	Month 2	Month 3
A	Control	2.03 ± 0.13	2.05 ± 0.19	2.20 ± 0.12
B	1.90mg Fe/L	2.00 ± 0.12	2.09 ± 0.20	2.19 ± 0.03
C	0.23 mg Cd/L	1.66 ± 0.12 [a,b,d*] (18.23%) ↓	1.79 ± 0.27 [a,b,d] (12.68%) ↓	2.02 ± 0.12 [a,b,d] (8.18%) ↓
D	1.90 mg Fe and 0.23 mg Cd/L	1.83 ± 0.19 [a,b,c] (10.24%)** ↑	1.93 ± 0.21 [a,b,c] (7.82%) ↑	2.14 ± 0.11 [c] (5.94%) ↑

Table 7: Influence of iron on cadmium associated loss in SOD activity in liver of tainted diet exposed rats

		Liver SOD Activity (Units/mg tissue) Mean ± SEM x 10^{-2} (n = 4)		
Group	Treatment	Month 1	Month 2	Month 3
A	Control	4.87 ± 0.24	5.20 ± 1.29	4.59 ± 0.05
B	1.90mg Fe/L	4.09 ± 1.41 [a]	4.54 ± 0.33 [a]	4.49 ± 0.01
C	0.23 mg Cd/L	3.62 ± 1.25 [a,b,d] (26.59%)↓	3.74 ± 0.24 [a,b,d] (28.07%)↓	3.65 ± 0.01 [a,b,d] (20.48%)↓
D	1.90 mg Fe and 0.23 mg Cd/L	3.95 ± 0.63 [a,c] (9.12%)↑	4.49 ± 0.33 [a,c] (20.00%)↑	4.58 ± 0.03 [c] (25.48%)↑

Table 8: Influence of iron on cadmium associated loss in kidney SOD activity in tainted water exposed rats

		Kidney SOD Activity (Units/mg tissue) Mean ± SEM x 10^{-2} (n = 5)		
Group	Treatment	Month 1	Month 2	Month 3
A	Control	10.99 ± 0.33	9.11 ± 0.26	9.30 ± 0.42
B	1.90mg Fe/L	8.78 ± 0.79	8.31 ± 0.19	8.99 ± 0.56
C	0.23 mg Cd/L	4.85 ±1.47 [a,b,d*] (55.87%)** ↓	5.43 ± 0.65 [a,b,d] (40.18%)↓	6.50 ± 0.25 [a,b,d] (30.11%)↓
D	1.90 mg Fe and 0.23 mg Cd/L	6.30 ± 0.91 [a,b,c] (29.89%)↑	6.65 ± 0.73 [a,b,c] (22.47%)↑	8.20 ± 0.07 [a,b,c] (26.15%)↑

Table 9: Influence of iron on cadmium associated loss in kidney SOD activity in tainted diet exposed rats

		Kidney SOD Activity (Units/mg tissue) Mean ± SEM x 10^{-2} (n = 4)		
Group	Treatment	Month 1	Month 2	Month 3
A	Control	3.24 ± 1.41	7.02 ± 2.83	8.11 ± 0.59
B	1.90mg Fe/L	4.06 ± 2.24[a]	6.56 ± 1.12	5.92 ± 1.83[a]
C	0.23 mg Cd/L	1.37 ±0.03 [a,b,d**] (57.71%)↓	4.17 ± 0.64 [a,b,d] (40.59%)↓	5.25 ± 0.10 [a,b,d] (35.27%)↓
D	1.90 mg Fe and 0.23 mg Cd/L	2.88 ± 1.67[b,c] (110.22%)↑	5.82 ± 0.43 [a,b,c] (39.57%)↑	7.02 ± 0.10 [a,b,c] (33.71%)↑

Relative to the Cd –only group of tainted water or diet exposed rats, Cd + Fe exposure via either tainted water or diet caused significant ($p \leq 0.05$) increase in CAT (Tables 2, 3, 4 and 5) and SOD (Tables 6, 7, 8 and 9) activities in the kidney and liver of rats at the end of the exposure periods. Cd intoxication has been known to be accompanied by alteration in SOD and CAT activity levels [25] – [27] and this accord with our present findings. The present study, however, shows that this is true whether Cd was ingested via a liquid or solid "vehicle". It has been reported [25],) that early effects of Cd is the inhibition of antioxidant enzymes such as SOD, although others have observed a rise in level of antioxidants enzyme activities in the heart, kidney and liver within 24 hours of intoxication of rats with Cd [26]. It would appear that longer duration of exposure can cause an inhibition in the activities of these enzymes as typified by the reduced activities of CAT and SOD in the present study.

Iron, as is evident in the findings presented here antagonizes Cd and minimizes its toxic effect particularly, in terms of its capacity to generate and sustain oxidative stress within the cell. This action of Fe would account for the relative increase in CAT and SOD activities in the kidneys and liver of rats exposed to Cd and Fe concomitantly via water or diet.

Exposure of rats to Cd via Cd –only tainted water and diet brought about significant ($p \leq 0.05$) increases in the level of MDA in the kidneys and liver (Tables 10, 11, 12 and 13) at

the end of 1, 2 and 3 months exposure when compared to the corresponding appropriate control. When compared to the Cd –only group of tainted water - or diet –exposed rats Cd + Fe exposure via either tainted water or diet brought about significant ($p \leq 0.05$) decreases in MDA levels in the kidneys and liver at the end of 1, 2 and 3 months of exposure. This was consistently so except for the liver and kidneys at the end of 1 month in the groups exposed via tainted water. Even in these there were decreases in MDA levels but not statistically significant ($p > 0.05$) (Tables 10 and 12).

Table 10: Influence of iron on cadmium associated alteration in liver malondialdehyde level in tainted water exposed rats

		Liver MDA Level (MDA Units/mg tissue) Mean ± SEM x 10^{-3} (n = 5)		
Group	Treatment	Month 1	Month 2	Month 3
A	Control	0.81 ± 0.18	0.71 ± 0.32	0.93 ± 0.10
B	1.90mg Fe/L	2.13 ± 0.37 [a*]	1.41 ± 0.04 [a]	1.55 ± 0.09 [a]
C	0.23 mg Cd/L	2.19 ± 0.05 [a**] (170.37%)↑	2.20 ± 0.23 [a,b,d] (209.86%)↑	2.08 ± 0.10 [a,b,d] (123.66%)↑
D	1.90 mg Fe and 0.23 mg Cd/L	2.18 ± 0.04 [a] (0.46%)**↓	1.47 ± 0.15 [a,c] (33.18%)↓	1.70 ± 0.11 [a,c] (18.27%)↓

*Values with superscripts a, b, c or d are statistically significantly different from the value of the group with corresponding uppercase letter A, B,C or D within the column ($p \leq 0.05$).

**Values in brackets in row C, are percentage changes relative to corresponding group A value while the values in brackets in row D, are percentage changes relative to corresponding group C value.

↓ % Decrease ↑ % Increase

Table 11: Influence of iron on cadmium associated alteration in liver MDA level in tainted diet exposed rats

		Liver MDA Level (MDA Units/mg tissue) Mean ± SEM x 10^{-3} (n = 4)		
Group	Treatment	Month 1	Month 2	Month 3
A	Control	0.65 ± 0.25	0.34 ± 0.11	0.83 ± 0.16
B	1.90mg Fe/L	1.13 ± 0.11 [a*]	1.74 ± 0.17 [a]	1.82 ± 0.07 [a]
C	0.23 mg Cd/L	1.53 ±0.09 [a,b,d**] (135.38%)↑	1.82 ± 0.01 [a,d] (435.29%)↑	2.43 ± 0.14 [a,b,d] (192.77%)↑
D	1.90 mg Fe and 0.23 mg Cd/L	1.18 ± 0.19 [a,c] (22.88%)↓	0.62 ± 0.34 [a,b,c] (65.93%)↓	1.00 ± 0.15 [a,b,c] (58.85%)↓

Table 12: Influence of iron on cadmium associated alteration in kidney MDA level in tainted water exposed rats

		Kidney MDA Level (MDA Units/mg tissue) Mean ± SEM x 10^{-3} (n = 5)		
Group	Treatment	Month 1	Month 2	Month 3
A	Control	6.86 ± 0.10	7.00 ± 0.54	6.93 ± 0.03
B	1.90mg Fe/L	7.98 ± 0.12 [a*]	8.53 ± 1.15 [a]	8.43 ± 0.05 [a]
C	0.23 mg Cd/L	8.66 ± 0.05 [a,b**] (26.24%)↑	9.81 ± 0.25 [a,b,d] (40.14%)↑	10.20 ± 0.12 [a,b] (47.19%)↑
D	1.90 mg Fe and 0.23 mg Cd/L	8.61 ± 0.03 [a,b] (0.58%)↓	7.79 ± 0.43 [a,b,c] (20.59%)↓	6.97 ± 0.06 [a,c] (31.67%)↓

Table 13: Influence of iron on cadmium associated alteration in kidney MDA level in tainted diet exposed rats

		Kidney MDA Level (MDA Units/mg tissue) Mean ± SEM x 10^{-3} (n = 4)		
Group	Treatment	Month 1	Month 2	Month 3
A	Control	0.87 ± 0.12	1.54 ± 0.09	1.96 ± 0.16
B	1.90mg Fe/L	2.71 ± 0.84 [a*]	3.15 ± 0.84 [a]	3.22 ± 1.10 [a]
C	0.23 mg Cd/L	3.08 ±0.12 [a,b,d**] (254.02%)↑	5.36 ± 0.35 [a,b,d] (248.05%)↑	4.53 ± 0.11 [a,b,d] (131.12%)↑
D	1.90 mg Fe and 0.23 mg Cd/L	2.43 ± 0.71 [a,c] (21.10%)↓	3.04 ± 1.78 [a,b,c] (43.28%)↓	2.87 ± 0.52 [a,b,c] (36.64%)↓

It has been demonstrated that acute exposure of rats to Cd administration increases renal MDA concentration [27]. Also, it has been found that exposure of experimental animals to Cd, irrespective of the route of administration causes lipid peroxidation in a number of tissues which include liver and kidneys as evidenced by elevated MDA production [10]. These observations are in harmony with the findings in this study vis – a – vis Cd –induced increase in tissue MDA levels. Furthermore, the present findings also show that lipid peroxidation do occur even when the exposure is chronic.

As indicated above, exposure to Cd for 1, 2 and 3 months caused profound decreases in kidney and liver CAT (Tables 2, 3, 4 and 5) and SOD (Tables 6, 7, 8 and 9) activities. Correspondingly, the Cd only group of rats had significantly elevated kidney and liver MDA levels (Tables 10, 11, 12 and 13). Cd associated lipid peroxidation as indicated by increased MDA production has been attributed to the ability of Cd ions to stimulate reactive oxygen species (ROS) production by inhibiting the electron transport process in the mitochondria [10], [28]. Increased ROS production without adequate activity of the antioxidant enzymes such as CAT and SOD culminates in lipid peroxidation, which is likely why the Cd only group had elevated MDA levels. Low CAT and SOD activities are, however, associated with acute Cd exposure as reported by others [26] whereas chronic exposure caused increases in the activities of both enzymes in red blood cells [29], [30]. However, it is pertinent to state that in the present study, chronic exposure to Cd produced reduced SOD and CAT activities when compared to the Cd – and Fe – free control group values and not an increase. The reason why we observed a decrease in the activities of both enzymes following chronic exposure while others reported an increase remains unclear at the moment apart from the fact that the authors in question [29] examined the red blood cells while we examined the liver and kidney. This however, is not entirely surprising because fluctuations in the activity of both enzymes are known and inconsistent reports on this are available in the literature [31], [32].

Like Cd, exposure of rays to Fe via Fe only tainted water and di*et al*so brought about significant ($p \leq 0.05$) increases in the level of MDA in the kidneys and liver at the end of 1, 2 and 3 months exposure relative to the corresponding control rat value (Tables, 10, 11, 12 and 13). This is in agreement with the report of Fletcher *et al*. [33] who found a significant increase in liver lipid peroxidation as measured by increased MDA level when rats were exposed to iron via dietary carbamyl iron treatment. Similarly, iron oxide nanoparticle administration to rats orally produced significant increase in lipid peroxidation in the kidney, liver and brain [34] evidently, exposure to iron via oral route is capable of causing tissue lipid peroxidation as demonstrated in the present study. Mechanistically, Cd causes lipid peroxidation indirectly since it is a redox inactive element. It does so by using an indirect route to produce reactive oxygen species (ROS), which are the lipid peroxidation causative agents [10], [28], [35] – [37].

Increased MDA production in the kidney (Tables 12 and 13) and liver (Tables 10 and 11) of rats exposed to iron as described here is an indication of oxidative stress in these organs. Ordinarily, this implies that the antioxidant defense system, be it molecular or enzymatic did not act efficiently to counter the stress, hence the peroxidative tissue damage. The current study examined the antioxidant enzyme status. Their activities influenced the action of iron in the induction of lipid peroxidation as evidenced by MDA generation. The results presented in this report show that iron caused significant ($p \leq 0.05$) reduction in catalase activity in the liver of tainted diet exposed rats relative to the control at the end of 1, 2 and 3 months exposure (Table 3). Same goes for the kidney of rats exposed via tainted diet but at the end of 3 months exposure only (Table 5). Liver SOD activity was significantly ($p \leq 0.05$) reduced in rats exposed to iron via tainted diet at the end of 1

and 2 months exposure but without significant ($p \leq 0.05$) reduction at the end of 3 months (Table 7). Kidney SOD activity was significantly ($p \leq 0.05$) reduced in rats exposed to Fe via tainted diet at the end of 3 months but a significant v increase was demonstrated at the end of 1 month (Table 9). It appears, therefore, that iron –induced lipid peroxidation observed in this investigation is to some extent attributable to compromised antioxidant catalase and SOD activities. The findings in this study most cases that dietary iron caused reduction in antioxidant enzyme activities in the kidney and liver of exposed rats agrees with the observation of others. For instance Fletcher *et al*. [33] showed that there was significant decrease in SOD activity in the liver of rats exposed to carbamly iron orally. Reddy *et al*. [33] also found that rats exposed to iron oxide nanoparticle orally had significantly decreased SOD activity in the brain, kidney and liver. As pointed out above, these investigators [34], [35] also found that increased MDA levels accompanied significantly reduced antioxidant enzyme activities.

Relative to the control, treatment of rats with iron or cadmium tainted water or diet in isolation triggered increased production of malondialdehyde in the kidney and liver (Tables 10, 11, 12 and 13) though Cd was more potent. When rats were exposed to both chemicals simultaneously, the levels of MDA found in the kidney and liver were significantly reduced compared to the value of the Cd –only group of rats. In this situation, the combined effect of Cd and Fe on MDA production in the kidney and liver of rats to which they were exposed simultaneously turned out to be less than the sum of their effects when rats were exposed to them independently of each other. Evidently both elements were antagonistic of each other's action.

Cadmium – induced reductions in kidney and liver CAT and SOD activities and increase in MDA levels caused by both metallic ions were reversed when the duo, Cd plus Fe were concurrently administered to rats via tainted water or diet (Tables 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13, group D). Evidently, Fe has the capacity to impair these toxic actions of Cd. The mechanism by which Fe does this is largely undefined but a few facts about the chemistry of Cd and Fe may shed light on how the latter could prevent the former from exhibiting its full toxic potential. Firstly, iron is a redox active metal while cadmium is redox inactive [27], [38]. The possibility exists that the processes by which the redox inactive Cd exerts toxicity could be undermined by iron through its redox capacity by stimulating reversed processes influenced by reduction or oxidation. Again, iron is both an essential nutrient and a potential toxicant to cells whereas cadmium is completely a toxicant bereft of evidence of nutritional value or essentiality to the mammalian cell [39].Consequently, adequate supply of iron is essential for the sustainable maintenance of a number of biochemical processes which include electron transfer reactions in the cytoplasm or organelles like the mitochondria. This capacity may somehow impact negatively on the toxic capacity of cadmium.

Secondly, since Cd can reach the mitochondria [40], [41] it is conceivable that without sufficient extra – mitochondrial iron levels, the level of iron in the mitochondria is not likely to be sufficient to antagonize Cd toxicity in Cd – exposed rats. This is particularly of interest in view of the fact that dietary Cd loading potentiates dramatic reduction in plasma iron and non – haem iron levels in the kidney and liver which in effect creates an induced iron – deficiency state [42]. This likely explains the ability of Cd to impair the activities of CAT and SOD whose activities are strongly influenced by tissue iron status [43], [44].

In the group of rats exposed to iron and cadmium concurrently, the overwhelming presence of iron (Cd:Fe ratio = 1:8.3), may give it a competitive advantage over Cd in terms of capacity for chemical activity that will ordinarily enhance or antagonize the toxic potential of Cd. However, in this context, it appears to be antagonism. If the equilibrium is competitively shifted in favour of iron, the level of ROS ordinarily generated by Cd will be reduced. Consequently, the ROS associated effects on liver and kidney levels of CAT and SOD activities and MDA level will be reduced.

In this study, rats were exposed to Cd and Fe as separate metals or combined via water or diet. Either way the exposure route was oral. The tissue levels of Cd in Cd alone – or Fe in Fe alone – treated rats were respectively greater than their tissue levels when combined. The 15.65% to 52.62% reduction in tissue Cd and Fe levels (Table 14) in the rats exposed to the duo, appears to suggest that there was relative competition between the two for uptake from the gastrointestinal tract (GIT). Also, there is evidence that Cd ions were more biologically available when rats were exposed via water than via diet but the opposite is true of Fe ions (Table 14, 15). It does appear then that the findings that CAT and SOD activities

as well as MDA level were significantly reduced in rats exposed to both metals concurrently , can be attributed to the ability of iron loading to reverse the biochemical consequences of cadmium loading [42]. The dietary route that produced more Fe ions *in vivo* was accompanied by higher reversal effect in CAT and SOD activities and MDA levels. This gives support to our belief that Fe ions are indeed impairing Cd toxicity.

Table 14: Kidney Metal Burdens in 3 Months Exposed Rats

Kidney Mean ± SEM x 10^{-2} (n=5) (mg/g tissue)					
		Cadmium		Iron	
Group	Treatment	Via Water	Via Diet	Via Water	Via Diet
A	Control	1.90 ±0.02	1.80 ± 0.03	84.29 ± 2.00	127.32 ± 2.00
B	Cd - only	5.78 ±0.08	3.77 ±0.60	_	_
C	Fe - only	_	_	216.37 ± 0.05	456.23 ± 5.00
D	Cd + Fe	3.78 ±0.33	3.18 ±0.02	102.51 ± 0.05	365.87 ± 1.58
	% Decrease	34.60*	15.60*	52.62**	20.03**

*Percentage decrease of group D value relative to group B value

**Percentage decrease of group D value relative to group C value

Table 15: Liver Metal Burdens in 3 Months Exposed Rats

Liver Mean ± SEM x 10^{-2} (n=5) (mg/g tissue)					
		Cadmium		Iron	
Group	Treatment	Via Water	Via Diet	Via Water	Via Diet
A	Control	1.92 ± 0.27	1.00 ± 0.30	200.36 ± 0.01	158.00 ± 1.00
B	Cd - only	5.78 ± 0.15	5.00 ± 0.40	_	_
C	Fe - only	_	_	468.39 ± 0.32	660.00 ± 4.58
D	Cd + Fe	3.78 ± 0.15	3.83 ± 0.30	347.46 ± 0.10	456.00 ± 3.98
	% Decrease	34.60*	23.40*	25.82**	30.91**

*Percentage decrease of group D value relative to group B value

**Percentage decrease of group D value relative to group C value

The findings presented in these reports which show that Cd toxicity can be mellowed by Fe, at least as far as kidney and liver CAT and SOD activities are concerned gives credence to the hypothesis on which this study was predicated. Our hypothesis is that in the presence of other metals, a toxic heavy metal may be more or less toxic than when examined in isolation. Evidently, the fear entertained by investigators that the consumption of food or water contaminated by Cd or indeed any other heavy metal is hazardous may be unduly exaggerated since none of the metals exists in the environment in isolation.

REFERENCES

[1] K, Haki, K. Fikretand C. Halit, 2005 "Effect of Single Dose Cadmium Chloride Administration on Oxidative Stress in Male and Female Rats," Turkish Journal of Veterinary and Animal Sciences, 29: 37-42.

[2] M. Ursínyová, V. Hladíková, 2000 "Cadmium in the environment of Central Europe" Trace Metals in the Environment, 4: 87-107.

[3] A. Cuypers, M. Plusquin, T. Remans, M. Jozefczak, A. Keunen, H. Gielen, 2010 "Cadmium stress: an oxidative challenge," Biometals, 23 (5): 927-940.

[4] S. Tim, E. Nawrot, H. Van, T. Lutgarde, R.. Tom, K. Tatiana, J. Yu, V.,Jaco, A.R. Harry, A. S. Jan, 2008

"Cadmium- Related mortality and long-term secular trends in the cadmium body burden of an environmentaly exposed Population," Environmental Health Perspectives. 116 (12): 1620-1628.

[5] J. Liu, M.B. Kadiiska, J.C. Corton, W. Qu, M.P. Waalkes, R.P. Mason, 2002 "Acute cadmium exposure induces stress-related gene expression in wild-type and metallothionein-I/II null mice," Free Radial Biology and Medicine, 32: 525–535.

[6] R. N. V. D. Krishna, C. Brijesh, S. Ramasubbu, 2017 "Comparison of metal-binding strength between methionine and cysteine residues: Implications for the design of metal-binding motifs in proteins," Biophysical chemistry, 224: 32-39.

[7] H.C. Gonick 2008 "Nephrotoxicity of cadmium and lead," Indian Journal of Medical Research, 128: 335-352.

[8] M.A. Adaikpoh, F.O Obi, 2010 "Protective effect of vitamin E on cadmium – induced testicular toxicity in rats," Journal of Science, Engineering and Technology, 12 (2): 9352 – 9371.

[9] S.O. Asagba, F.O. Obi, 2005 "Comparative evaluation of the biological effects of environmental cadmium – contaminated control diet and laboratory –cadmium supplemented test diet," Biometals, 18:155-161.

[10] S.J. Stohs, D. Bagchi, E. Hassoun, M Bagchi,. 2001 "Oxidative mechanisms in the toxicity of chromium and cadmium ions," Journal of Environmental Pathology, Toxicology and Oncology, 20:77–88.

[11] M.A. Adaikpoh, F.O Obi, 2009 "Prevention of cadmium – induced alteration in rat testes and prostate lipid patterns by alpha –tocopherol," African Journal of Biochemistry Research, 3 (10): 321 – 325.

[12] W,C. Prozialock, J.R.. Edwards, D,W. Nerbert, J.M. Woods, A. Barchowsky, W.D. 2008Atchison, "The vascular system as target of mental toxicity," Toxicological Science, 102: 207-218.

[13] S. Benoff, A. Jacob, I. Hurly, 2000 "Male infertility and environmental exposure to lead and cadmium," Human Reproduction, 6: 107-121.

[14] E.R. Siu D.D. Mruk, C.S. Porto, C.V. Cheng, 2009 "Cadmium induced testicular injury," Toxicology and Applied Pharmacology 238(3): 240-249.

[15] A.B.M. Egborge, 1994 "Water Pollution in Nigeria, Vol, 1: Biodiversity and chemistry of Warri River. Ben Miller Books, Nigeria Limited, Warri. pp 34-77.

[16] L.I.N. Ezemonye, "Heavy metals concentration in water, sediment and selected fish of Warri river and its tributaries," Ph.D Thesis, University of Benin, Benin city, Nigeria, 1992.

[17] M.D. Wogu, C.E. Okaka, 2011 "Pollution studies on Nigerian rivers: heavy metals in surface water of Warri river, Delta state," Journal of Biodiversity and Environmental Sciences, 1 (3): 7-12.

[18] A. A. Ogaga, A. O. Olusegun, I.O. Elijah, 2015 "Heavy metal levels in water and sediment of Warri River, Niger Delta, Nigeria," International Journal of Geology, Agriculture and Environmental Sciences, 3 (1): 235-247.

[19] G. Cohen, D. Denbliec, S. Marcus, 1970 "Measurement of catalase activity in tissue extracts," Analytical Biochemistry, 34: 319-329,.

[20] H.P. Misra, I. Fridovich, 1972 "The role of superoxide anion in the auto oxidation of epinephrine and a simple assay for superoxide dismutase," Journal of Biological Chemistry, 247: 3170- 317.

[21] J.A. Baum, J.G. Scandalios, 1981 "Isolation and characterization of cytosolic and mitochondrial superoxide dismutase of maize," Archives of Biochemistry and Biophysics, 206: 249-264.

[22] J. A. Buege, S.D. Aust, 1978 "Microsomal lipid peroxidation. Methods in enzymology. Vol LII – Biomembranes," Fleischer and Parker (eds.), Academic Press, New York, pp. 302- 310.

[23] O. M. Hernandez, J.M.G. Fraga, A.I. Jimenez, F. Jimenez, J.J. Arias, 2004 "Determination of the mineral content by atomic absorption spectrophotometer," Journal of Food Chemistry. 93: 449 – 458.

[24] V. Lind, A. Wicklund-Glynn, J. Engman, L. Jorhem, 1995 "Bioavailabilty of cadmium from crab hepatopancrease and mushroom in relation to inorganic cadmium: a nine week feeding study in mice," Food Chemistry and Toxicology, 33: 667-673.

[25] S. Gupta, M. Athar, J.R. Behari, R.C. Srivastava, 1991"Cadmium mediated induction of cellular defence mechanism: a novel example of the development of adaptive response against a toxicant," Industrial Health, 29: 1-9.

[26] S. Sakar, P. Yadev, , R. Triveda, A.K. Bansal, D. Bhatnagar,. 1995 "Cadmium-induced lipid peroxidation and status of the antioxidant system in rat tissues," Journal of Trace Element Biology, 9: 144-147.

[27] A.M. Abdel-Moneim,K .M. Said, 2007"Acute effect of cadmium treatment on the kidney of rats: Biochemical and ultra-structural studies," Parkistan Journal of Biological Sciences, 10 (4): 3497-350.

[28] E. Tatrai, Z. Kovacikova, A. Hudak, Z. Adamas, 2001"Comparative in vitro toxicology of cadmium and lead on redox cycling in type II pneumocytes," Journal of Applied Toxicology, 11 (6): 479-483..

[29] M.M. Kostic, B. Ognjanovic, S. Dimitrijevic, R.V. Zikic, G.L. Rosic, R.V. Zivkovic,1993 " Cadmium –induced changes in antioxidant and metabolic stutus in red blood cells of rats: in vivo effects," European Journal of Haematology, 51 (2):63 – 124.

[30] V. Matovic, A. Buha, D. Dukic-Cosic, Z. Bulat, 2015"Insight into oxidative stress induced by lead and/or cadmium in blood, liver and kidneys," Food and chemical Toxicology, 78: 130-140.

[31] B.I. Ognjanovic, S.D. Markovic, S.Z. Pavlovic, R.V. Zikic, A.S. Stajn, Z.S. Saicic, V. M. Petrovic, 2003 "Protective influence of vitamin E on antioxidant defense system in the blood of rats treated with cadmium," Physiological Research, 52 (5): 563 -570.

[32] S. Yalin, U. Comelekoglu, S. Bagis, N.O. Sahin, O. Ogenler, R. Hatungil, 2006 "Acute effect of single dose cadmium treatment on lipid peroxidation and antioxidant enzymes in ovariectomized rats," Ecotoxicology and Environmental Safety, 65 (1): 140-144.

[33] L.M. Fletcher, F.D. Roberts, M.G. Irving, L.W. Powell, J.W. Halliday, 1989 Effects of iron loading on free radical scavenging enzymes and lipid peroxidation in rat liver," Gastroenterogy, 48: 1011 – 1018.

[34] U.A. Reddy, P.V. Prabhakar, M. Mahboob, 2017 Biomarkers of oxidative stress for in vivo assessment of toxicological effects of iron oxide nanoparticles," Saudi Journal of Biological Sciences, 24 (6): 1172 – 1180.

[35] E. Birben, U.M. Sahiner, C. Sackesen, S. Erzurum, O. Kalayci, 2012 "Oxidative stress and antioxidant defense," The World Allergy Organization Journal, 5 (1): 9-19.

[36] H.J.H. Fenton, 1984 "Oxidation of tartaric acid in the presence of iron," Journal of Chemical Society, 65: 899 – 910.

[37] F. Haber, J.J. Weiss, 1934 "The catalytic decomposition of hydrogen peroxide by iron salts," Proceeding of Royal Society of London 248: 332- 351.

[38] A.L. Luz, X. Wu, and E.J. Tokar, 2018 "Toxicology of inorganic carcinogens. Advances in Molecular Toxiciology," Vol 12. Elsevier, pp 1-46.

[39] K. Yokoi. 2014 "Nutritional aspects ofmetals and trace elements. Encyclopedia of inorganic and bio-inorganic chemistry," John Willy and Sons, Ltd, Online Library https://doi.org/10.1002/9781119951438. eibc 0153.pub2.

[40] R, Aziz, M.T, Rafiq, T, Yang, D. LiuLu, Z. He, M.K. Daud, T. Li, and T. Yang, 2014 "Impact of assessment of cadmium toxicity and its bioavailability in human cell lines (Caco-Z and HL-7702)," BioMed Research International, http://doi.org/10-1155/2014/839538

[41] M. Sokolova, 2004 "Cadmium effects on mitochondrial function are enhances by elevated temperatures in marine poikilotherm, Crassostrea virginica Gomelin (Bivalvia: Ostreidae)," Journal of Experimental Biology 208: 2639-2648.

[42] A. Crowe, E.H. Morgan, 1997 "Effect of dietary cadmium on iron metabolism in growing rats," Toxicology and Applied Pharmacology, 145 (1):136 – 146.

[43] Z. Zaka-Ur-Rab, M. Adnam, S.M. Ahmad, N. Islam, 2006 "Effect of oral iron on markers of oxidative stress and antioxidants status in children with iron deficiency anaemia," Journal of Clinical and Diagnostic Research, 10 (10): 13 - 19.

[44] M. Isler, N. Delibas, M. Guclu, F. Gultekin, 2002 Superoxide dismutase and glutathione peroxidase in erythrocytes of patients with iron deficiency anaemia: effects of deficient treatment modalities," Croatian Medical Journal, 43 (1): 16-19.

IJCRT