Original Research Article

Effects of Food-Chain Mediated Metal Exposures on Lipid Profile in Rats

ABSTRACT

Aims: To evaluate the effect of Cd and As on lipid profile in rats by using an experimental food-chain to imitate the natural food-chain of fish to rat.

Study design: Toxicity of Cd and As was first induced in fishes through an artificial habitat; the fishes after 1 month of exposure were killed and used as source of protein in formulating rat feed. In this way, the natural food-chain of fish to rat was mimicked as the rats get the toxic metals in their fish diets.

Place and Duration of Study: Department of Biochemistry Laboratory, Faculty of Science, Delta State University, Abraka, Nigeria, from June 2016 to September 2018.

Methodology: Adult male rats weighing between 100–150 g were fed with formulated rat feed that has Cd/As (at a dose of 0.4 mg/100 ml) contaminated fish as source of protein. Control group comprises of rats that were not given metal contaminated fish as food. The rats were fed for 3 months after which they were sacrificed and the plasma and vital organs obtained for investigation of lipid metabolism function.

Results: Biochemical analysis on lipid profile status was made after 90 days of inoculation. A significant increment ($p \leq 0.05$) in plasma and organ concentrations of Cholesterol, triglyceride (TG), lipoprotein of low density (LDL), and lipoprotein of very low density (VLDL) was seen in the rats given these metals in diet in comparison to control, while plasma and organ lipoprotein of high density (HDL) concentrations declined.

Conclusion: These results infer that cadmium and arsenic produces reactive oxygen compounds that are toxic to man, hence calls for caution and further studies.

Keywords: [As; Cd; exposures; food-chain; lipid}

1. INTRODUCTION

Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms [1,2,3]. It appears that problem of heavy metals accumulation in aquatic organisms including fish needs continuous monitoring and surveillance owing to biomagnifying potential of toxic metals in human food chain [4,5,6,7,8,9]. It is important to note that Cd is a highly toxic element for all mammals and fish. Cd levels have constantly been increasing, and consequently, the research on Cd has become quite topical and urgent. Accumulation of Cd in living organisms is a major ecological concern, especially because of its ability to accumulate very quickly. The organisms developed a protective defense against the deleterious effects of essential and non-essential heavy metals and other xenobiotics that produce degenerative changes like oxidative stress in the body [10,11]. By contrast, the excretion of Cd from living organisms is a slow process. In fish, Cd can cause a number of structural and patho-morphological changes in various organs. The highest Cd levels were detected in the kidneys and liver of fish. Animals normally absorb Cd into the organism either by ingestion or inhalation. The organism does not have an effective Cd elimination pathway and as a result the biological half-life of Cd in the organism is estimated to be 15–20 years. Cd causes pleiotropic effects on organisms at both the molecular and cellular levels. It binds to cysteine residues of proteins and induces oxidative stress [12]. Long term low-level Cd intake, results in hypertensive and non- hypertensive cardiovascular diseases in humans [13]. In human, non-occupational exposure to Cd predominantly results from smoking, air pollution, contaminated food and water [14]. Cd causes a number of clinical complications including cardiovascular diseases, anemia, diabetes and disruption of endocrine system [15,16]. The toxicity of Cd consists generally, in its ability to disturb numerous cellular functions and causes damage to various cellular structures [17]. Cd^{+2} are characterized by high affinity to biological structures containing sulfhydryl (-SH), carboxyl and phosphate groups. They inhibit numerous enzymes and disturb some metabolic processes including lipid metabolism. Both experimental and epidemiological studies indicate that exposure to Cd may alter lipid metabolism and contribute to the development of cardiovascular diseases (CVD), including

UNDER PEER REVIEW

atherosclerosis, hypertension, stroke and cardiac arrest [18,19]. The main pathologies and specific biochemical changes are related to Cd toxicity and its concentration and the condition of oxidative stress in tissues [20].

Arsenic has been shown to induce atherosclerosis by increasing mRNA transcripts of growth factors including granulocyte–macrophage colony stimulating factor, transforming growth factor-α and the inflammatory cytokine-like tumor necrosis factor α [21]. Experimental studies of the effect of As on the vascular system have shown that oxidized lipids are present in all stages of atherogenesis which in turn generate several bioactive molecules (e.g. ROS, peroxides and isoprostanes), of which aldehydes are the major end products. Malondialdehyde (MDA) and 4-hydroxy-trans-2-nonenal (HNE) are the most abundant aldehydes generated from the oxidation of LDL and possess mutagenic and carcinogenic properties [22,23]. Protein adducts of MDA and HNE have been detected in atherosclerotic lesions of experimental animals and humans.

Evidence from a large number of studies indicates that inflammation plays a pivotal role in atherosclerotic plaque formation. Vascular cells generate chemokines and pro-inflammatory cytokines including monocyte chemo-attractant protein 1 (MCP 1), interleukin 6 (IL 6) and tumor necrosis factor α. This suggests that As-induced inflammation could be an important risk factor for atherosclerosis [24]. Hypertension is another disorder associated with increased As exposure [25]. As-induced hypertension has been explained by an enhanced myosin light-chain phosphorylation and an increase in calcium sensitization in blood vessels. Disruption of the antioxidant defense system leads to elevated systolic blood pressure. A number of studies revealed symptoms of hepatic injury after oral exposure of humans to inorganic As. These effects were most frequently observed after repeated exposure to doses of 0.01–0.1 mg As kg–1 per day. Clinical examination confirmed liver damage [26] and blood tests showed elevated levels of hepatic enzymes. Histological examination of the livers has revealed a consistent finding of portal tract fibrosis [27]. Individuals exposed more frequently to arsenic suffered from cirrhosis, which was considered to be a secondary effect of damage to the hepatic blood vessels.

Cd and As have been identified as the most probable causes of heavy metal-related disease observed in primary care medicine [28]. To a small extent they enter into organisms via food, drinking water and air and are bio-persistent pollutants that accumulate at the top of the food-chain. As the prevalence of heavy metal exposure is increasingly recognized and identified in individuals seen in private practice clinics, the need for effective prevention and treatment will increase.

The objective of this study is to evaluate the effect of Cd and As on lipid profile in rats by using an experimental food-chain to imitate the natural food-chain of fish to rat.

2. METHODOLOGY

2.1 Materials

2.1.1 Fishes

One hundred Catfishes were obtained from a fish pond in Obiaruku, Delta State and kept in troughs.

2.1.2 Animals

Sixteen male albino rats having average weight 129±4g were utilized for this work. The rats were obtained from the Animal House, College of Health Sciences, Delta State University, Abraka and kept in cages while copying the natural habitat.

2.1.3 Chemicals

Cadmium chloride ($CdCl_2$) and Arsenic trioxide (As_2O_3) (analytic types) were obtained from May and Baker, Dagenham, England and Qualikems fine chemicals, India respectively.

2.1.4 Preparation of diet

To induce exposure to Cd and As in the experimental food chain, four diets (1 control and 3 tests) that differed in terms of the nature of the protein were formulated. The test diets contained milled Cd (0.4 mg/100 ml), As (0.4 mg/100 ml) and Cd+As (0.4 mg/100 ml each) exposed fish as a source of protein, while the control diet contained milled non-Cd and As exposed fish [29]. Other components of the diets were cornstarch (Livestock Feed depot, Warri), multivitamin/minerals mix (Vetindia Pharmaceuticals Limited, India), vegetable oil (obtained locally in Abraka, Nigeria), cellulose (analytical grade) and granulated refined sugar (obtained from Abraka market).

2.2 Methods

2.2.1 Experimental design

The fishes were divided into four groups, the first group of fish was kept in fresh water and this served as control, the second, third and fourth groups were kept in Cd, As and Cd+As contaminated water respectively at a dose of 0.4 mg/100 ml of water. Fishes in all groups were kept for 1 month after letting them to acclimatize for 1 week and the water was changed daily and re-contaminated as the case may be.

Grouping of the rats into four groups was made and kept in separate cages. They were fed formulated diets of control, Cd, As and Cd+As throughout the period of the study. They were supplied water which they can drink at will. Laws involving the use of animals for experimental work were kept (NIH Publication No. 85- 93, revised 1985).

2.2.2 Collection and treatment of samples

After 90 days of exposure, the animals were made to fast for three hours and weighed prior to sacrificing with chloroform anesthesia. The rats were then killed via heart puncture, using needle and syringe. Blood obtained was stored in tubes containing lithium heparin. Plasma was later obtained by centrifugation of the blood at 4000 rpm for 10 minutes. The

UNDER PEER REVIEW

heart, liver and kidney were removed and placed on ice. Portions of the liver, heart and kidney were homogenized to give 10% homogenates and centrifuged at 4000 rpm for 10 minutes to obtain clear supernatants for biochemical analysis.

2.2.3 Metal analysis on fish diet

The Cd and As concentrations in the fish diets were measured with atomic absorption spectrophotometer (AAS). Cd concentrations in the control, Cd, and Cd+As diets were <0.01, 3.45 and 3.30 mg/g respectively while As concentrations in control, As and Cd+As diets were 0.02, 1.68 and 1.42 mg/g respectively.

2.2.4 Biochemical analysis

The cholesterol contents of the samples were determined by the enzymatic endpoint method of [30] using Randox test kits. TG levels were determined by the colorimetric method of [31]. The method is based on the formation of quinoneimine (indicator) from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase. The concentrations of VLDL in the samples were determined from the relationship of [32]. The HDL contents of the samples were estimated by the precipitation method of [30]. The equation of [33] was used to estimate the LDL levels in the samples.

2.2.5 Statistical analysis

The results were expressed as mean ± SEM. Statistical analysis was done by one way analysis of variance (ANOVA) using a computer software package (SPSS version 16.0, spss inc. Cary, NC, USA). The difference between the means was tested by least significant difference (LSD) test. Statistical significance was considered at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Results

The effects of Cd, As and a combination of both metals on plasma Cholesterol, TG, VLDL, HDL and LDL levels of rats is shown in Table I. The plasma cholesterol, TG, VLDL, HDL and LDL levels of rats offered Cd, As and Cd+As contaminated diet was significantly different ($P \leq 0.05$) relative to control. There was a significant increase in plasma cholesterol levels of rats fed Cd+As diet when compared to those fed Cd and As separately, while this group comparison was also seen in the plasma TG and VLDL. Conversely, there was no significant difference in plasma TG and VLDL levels of rats fed Cd+As diet separately (Table I).

Table I. Effects of food-chain mediated metal exposure on plasma lipid profile levels in rats.

Parameter	Control	Cd	As	Cd+As
Cholesterol	176.02± 1.82[a]	228.13± 3.64[b] (29.60%)	218.27± 2.93[b] (24.00%)	230.24± 6.84[b] (30.80%)
TG	123.70± 2.21[a]	205.63± 2.15[b] (66.23%)	199.52± 2.35[b] (61.29%)	221.58± 4.38[c] (79.13%)
VLDL	24.74± 0.44[a]	41.13± 0.43[b] (66.25%)	39.90± 0.47[b] (61.28%)	44.32± 0.87[c] (79.14%)
HDL	60.18± 1.47[a]	35.77± 2.52[b] (-40.46%)	40.31± 1.43[c] (-33.02%)	32.93± 2.36[b] (-45.28%)
LDL	91.11± 1.31[a]	151.10± 5.11[b] (65.84%)	137.31± 2.85[c] (50.71%)	153.00± 5.54[b] (67.93%)

Values are given as mean ± SEM, n=4. Values not sharing a common superscript letter in the same column differ significantly at (P≤0.05). The levels of the plasma lipid profiles are in mg/dl. Bracketed figures depict percentage inhibition compared to control.

UNDER PEER REVIEW

The effects of Cd, As and a combination of both metals on liver, heart and kidney Cholesterol, TG, VLDL, HDL and LDL levels of rats is shown in Table II. The liver, heart and kidney cholesterol, TG, VLDL, HDL and LDL levels of rats offered Cd, As and Cd+As contaminated diet was significantly different ($P \leq 0.05$) relative to control. There was a significant increase in liver, heart and kidney cholesterol levels of rats fed Cd+As diet when compared to those fed Cd and As separately. Similarly, a significant increase was seen in the liver, heart and kidney TG and VLDL levels of rats offered Cd and Cd+As diet when compared to those offered As alone. Conversely, the liver, heart and kidney TG and VLDL levels of rats fed Cd and Cd+As diet showed no significant difference when compared to each other Table II.

Table II. Effects of food-chain mediated metal exposure on organ lipid profile levels in rats.

Parameter	Control	Cd	As	Cd+As
Cholesterol				
Liver	211.24±18.18[a]	267.56±18.18[b]	246.43±46.53[c]	281.64±36.36[b]
		(26.66%)	(16.66%)	(33.33%)
Heart	239.39±18.18[a]	302.76±13.48[b]	288.68±37.04[b]	337.97±11.50[c]
		(26.47%)	(20.59%)	(41.18%)
Kidney	183.06±18.18[a]	260.52±17.72[b]	232.35±17.72[c]	239.39±18.18[c]
		(42.31%)	(26.93%)	(30.77%)
TG				
Liver	189.73± 7.81[a]	259.03±15.61[b]	226.42±10.53[c]	275.33±15.60[b]
		(36.53%)	(19.34%)	(45.12%)
Heart	185.66± 6.66[a]	246.80±12.23[b]	214.19±12.23[c]	250.88±19.97[b]
		(32.93%)	(15.37%)	(35.13%)
Kidney	177.48± 4.69[a]	218.27± 6.66[b]	201.96± 6.66[c]	234.57±11.53[b]
		(22.98%)	(13.79%)	(32.17%)
VLDL				
Liver	37.95± 1.56[a]	51.81± 3.12[b]	45.28± 2.11[c]	55.07± 3.12[b]
		(36.52%)	(19.31%)	(45.11%)
Heart	37.13± 1.33[a]	49.36± 2.45[b]	42.84± 2.45[c]	50.17± 4.00[b]
		(32.94%)	(15.38%)	(35.12%)
Kidney	35.50± 0.94[a]	43.65± 1.33[b]	40.39± 1.33[c]	46.91± 2.31[b]
		(22.96%)	(13.77%)	(32.14%)
HDL				

UNDER PEER REVIEW

Liver	79.48±14.66[a]	28.39± 5.68[b]	34.07± 6.56[b]	34.07± 6.56[b]
		(-64.28%)	(-57.13%)	(-57.13%)
Heart	96.51±19.39[a]	28.39± 5.68[b]	28.39± 5.68[b]	22.71± 0.00[b]
		(-70.58%)	(-70.58%)	(-76.47%)
Kidney	56.78± 6.56[a]	34.07± 6.56[b]	34.07± 6.56[b]	22.71± 0.00[b]
		(-40.00%)	(-40.00%)	(-60.00%)
LDL				
Liver	93.81± 4.04[a]	187.36±20.41[b]	167.09±38.20[c]	193.26±38.43[b]
		(99.72%)	(78.12%)	(106.01%)
Heart	105.76±15.02[a]	225.02±12.54[b]	217.46±40.19[b]	265.08±13.93[b]
		(112.76%)	(105.62%)	(150.64%)
Kidney	90.80±12.08[a]	182.80±20.37[b]	158.71±14.14[b]	169.77±20.31[b]
		(101.32%)	(74.79%)	(86.97%)

Values are given as mean ± SEM, n=4. Values not sharing a common superscript letter in the same column differ significantly at (P≤0.05). The levels of the organ lipid profiles are in mg/dl. Bracketed figures depict percentage inhibition compared to control.

3.2 Discussion

This research was done to provide biochemical information on Cd and As toxicity when inoculated in fish used in rat feed formulation as fish may be a vital agent of Cd and As passage into humans. In vast studies relating to metal toxicity, contamination by metals is not usually through an agent but directly added to the meal provided for the rats.

The changes in values of plasma (Table I) as well as organ (Table II) TG in rats after ingesting Cd inoculated meal along the experimental food chain, likewise the decrement in HDL-Cholesterol, conspicuously insinuates lipoprotein metabolic problems. This arises from the fact that TG build up supersedes its breakdown. Increments in both plasma TG, VLDL and LDL-Cholesterol concentrations after Cd inoculums in rats have been reported earlier by [19]. They ascribed the increments to lipoprotein lipase (LPL) activity decrement in the animals that yielded an increment in the TG-rich VLDL in circulation. Available literatures have shown an unparallel relation between TG concentrations and the chance of cardiovascular disease [34]. TG-rich lipoprotein a, and their metabolites which are known to be atherogenic, may directly lead to the formation of arterial wall foam cells.

Plasma and organ Total Cholesterol contents were significantly elevated in the rats fed Cd inoculated diet (Table I and II). The observed increment in plasma and organ Cholesterol was linked to an increment in VLDL and LDL-Cholesterol portion in the Cd inoculated rats (Table I and II). The changes in the cholesterol profile and other lipid compounds noted in Cd inoculated rats may be explained by the potential of the metal to elevate the activity of hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase via releasing inflammatory cytokines and interleukins [35]. The increment in plasma cholesterol may likewise result from the potential of Cd to decrease its absorption by macrophages that are involved in processing of cholesterol [36]. Studies reveals that increased TG and VLDL levels are dependent on cytokines, thereby promoting lipid synthesis while suppressing its oxidation [37,38,18,39].

Like Cd-inoculated rats in diet, exposure to As in diet also caused significant elevation of lipid markers of cardiac damage indicated by increment in level of Cholesterol, TG, LDL, VLDL with a decreased HDL level in plasma and organs (found in Table I and II). This is not surprising as experimental evidence showed incidence of cardiovascular disease in As exposed rats [40]. Moreover, report has it that As causes overt injury to the endothelium, cellular proliferation and changes in monolayer binding of labelled lipoprotein of low density (LDL) and permeability of albumin. The general trend of the gotten results depicts that the separate effect of the two metals was lesser than the effect put together. This is evident in the data

UNDER PEER REVIEW

gotten for plasma alongside organ Cholesterol concentration (Table I and II) and TG in plasma, kidney and liver. The combination effect of the duo may probably have increased arising from the additive and/or synergism of two metals on the above parameters.

The findings in the study at present are in agreement with the study of [41] which showed that the change in enzymes of hepatocyte like plasma transaminase activity and total bilirubin level after combined exposure of rats to Mn and Pb was because of synergism of the duo. These results are in conformity with the conclusion of a previous study about theoretical assessment of Pb, As and Cd [42] which demonstrated that chemicals with common way of action will wax together to give combination effect higher than if it was singly given.

4. CONCLUSION

The results gotten depicts that rats inoculated with Cd and As along the experimental food-chain alter plasma and tissue lipid panel levels. Also, the joint inoculation of rats with Cd and As (via the experimental food-chain) shows the possibility for additive chemical effects on some biochemical marker for cardiovascular disease as per the nature of the parameter.

COMPETING INTERESTS

The authors declare that they have no conflict of interests.

ETHICAL APPROVAL

All applicable institutional guidelines for the care and use of animals were followed with the approval of the Ethics committee of the College of Health Sciences of the Delta State University, Abraka.

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UNDER PEER REVIEW

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UNDER PEER REVIEW