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Toxicological effects of the concurrent administration of cadmium and arsenic through the food chain on the liver and kidney of rats

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Abstract

Background: Our environment is filled with a mixture of toxic elements.

Objectives: This study seeks to assess the effects of exposure to cadmium and arsenic through the food chain on the liver and kidney of rats.

Methodology: Adult male albino rats were exposed to experimental feed of which the protein composition was made of fish exposed to cadmium and arsenic for 1 month at a concentration of 0.4 mg cadmium or/and arsenic/100 ml water for 1 and 3 months. Samples of feed and tissue were assayed for cadmium and arsenic load. Tissue homogenates were used for biochemical analysis and the estimation of the expression levels of mRNA of Bax and Bcl2 genes in the liver and kidney of rats were carried out. Samples of tissues were also used for histological analysis.

Results: Results show a greater accumulation of metals in the liver than kidney of rats after the 1-month exposure of which the trend changed after the 3-month exposure. Alterations in enzymatic activities and levels of MDA and GSH were also recorded. Significant ($P < 0.05$) alterations in the level of mRNA expression of the Bax and Bcl2 genes were observed in all groups compared to the control for both duration of treatment. Treatment-related lesions were also observed for the various groups from the histopathological analysis.

Conclusion: The cadmium-contaminated diet was found to be more toxic to the kidney while the arsenic-contaminated diet was found to be toxic to the liver. In addition, the present study has shown that the toxicity of a mixture of As + Cd cannot be predicted from the toxic mechanisms of the single components.

Keywords: Bcl2, Bax, Liver, Kidney, Cadmium, Arsenic

Background

Cadmium and arsenic are well-known toxic metals capable of having adverse effects on the liver and kidney most especially on prolonged occupational or environmental exposure (Madden & Fowler, 2000). Accumulation of these metals takes place primarily in the liver and kidneys, and their toxic effects depend on the duration and dose of exposure (Eichler et al., 2006; Kim, Cho, & Kim, 2003). These two metals are usually released into our environment due to their wide industrial usage (Yuan et al., 2014). Cadmium is highly toxic to living cells and tissues. Cadmium has been found to be present in the air, food, and even water in alarming amounts. It is a widespread environmental contaminant (Hu, 1998; WHO, 1992). Cadmium has no essential biological function.

Arsenic (As) is a metalloid and a member of group V of the periodic table of elements. Being a metalloid, it is

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capable of forming alloys with metals and it also readily forms covalent bonds with carbon, hydrogen, and oxygen. Arsenic exists in several different oxidation states, and its chemistry is rather complex. Arsenic compounds are used as preservatives of animal hides, in pigments and dyes, agricultural pesticides, glass manufacture, and various pharmaceutical substances (ATSDR, 2000).

Several studies have shown high levels of these metals in water bodies (Chintaka et al., 2016; Mohankumar, Hariharan, & Rao, 2016), and fish being a major source of protein in the diet of humans and other lower animals have been shown to be a good bioaccumulator of these metals (Bradley & Morris, 1986; Dimari & Hati, 2009; Ololade & Ajayi, 2009; Ololade, Lajide, & Amoo, 2007; Roesijadi, 1996). This have raised great concerns on the safety of food and food supplies on human health (Bajpai & Upreti, 2012; Iwegbue, 2011).

Previous studies of the effect of cadmium and arsenic on animal models focus primarily on only one metal in high concentration and direct exposure. However, environmentally, humans are exposed to a mixture of toxic elements mostly through the food chain and water. Studies are therefore needed to assess the combination of these elements through the food chain. These models have been applied to numerous mixtures (Lu et al., 2014; Spurgeon et al., 2010; Yuan et al., 2014). However, limited data is available on the toxic effects of cadmium and arsenic through the food chain. In addition, available experimental evidence indicates that the absorption and toxicity of both elements are influenced by their mode of metabolism (Masami & Manabu, 2000; Obinaju, 2009). Thus, the difference in the mode of metabolism of cadmium and arsenic through the controlled food chain makes the need for a study on the comparative absorption and toxicity of both elements imperative. The present study thus investigates the effect of cadmium and arsenic in single and mixture through the food chain on mRNA expression of the liver and kidney of experimental rats.

Materials and methods

Exposure of fish to metals and diet preparation

Fish obtained from a local fish pond in Orogun, Delta State, were divided into groups and left to acclimatize for 1 week in plastic troughs. The fish were divided into group A which served as the control. Fish in groups B, C, and D were exposed to cadmium, arsenic, and cadmium + arsenic at a concentration of 0.4 mg metal/100 ml of water for 1 month. The concentration of 0.4 mg/100 ml of water was done to achieve the highest accumulation of these metals in the tissues of the fish at a sublethal dose based on previous studies (Ahmad, Qureshi, Manohar, Kaur, & Khaliq, 2011; Aruljothi & Samipillai, 2014; Chaudhari, Harshad, & Kakade and Thorat S. R., 2015; Kumar & Banerjee, 2012). The water was changed daily, and so contamination was repeated on a daily basis also. On the completion of 1-month exposure, the fish were sacrificed, strictly maintaining their groups; dried in an oven; and used as a source of protein in compounding the experimental diet. The experimental diet was made up of 20% protein (contaminated fish), 55% carbohydrate, 10% fats, 10% fiber, and 10% multivitamin/mineral mix.

Treatment of animals

Sixty-four adult male albino rats of Wistar strain were obtained from the animal house of the Faculty of Science and used for the study. The rats were divided into groups A, B, C, and D which represents the control, cadmium-contaminated, arsenic-contaminated, and cadmium + arsenic-contaminated groups, respectively. Rats were allowed free access to the experimental diet and water for a period of 1 and 3 months. After the 1-month exposure, half the number of rats in each group were sacrificed and the other half sacrificed after the 3-month exposure under chloroform anesthesia. Animal treatment is in accordance with the Nation Institute of Health guidelines (1985).

Metal analysis

The feed and tissue samples were digested with 20 ml of concentrated acid mixture (98% w/v HNO_3/$HClO_4$; 4:1 v/v) at 100 °C. After digestion of samples, the Cd and As concentrations in the tissues and feeds were measured using a Varian AA 1475 spectrophotometer. An International Atomic Energy Agency (IAEA) reference biological sample was used for the evaluation of the accuracy and precision of the analysis.

Biochemical analysis

Tissue homogenates were prepared and centrifuged, and supernatant used for biochemical analysis. The activities of superoxide dismutase (SOD), catalase (CAT), and glutathione-s-transferase (GST) were assayed based on the methods of Misra and Fridovich (1972), Sinha (1972), and Habig, Pabst, and Jakoby (1974), respectively. The levels of reduced glutathione (GSH) and lipid peroxidation were determined using the method of Beutler, Duron, and Kelly (1963) and Gutteridge and Wilkins (1982), respectively.

Isolation and purification of RNA from GITC lysate

The guanidine isothiocyanate (GITC) lysate of tissue samples was prepared. Total RNA of the GITC lysate of liver and kidney were extracted using Reliaprep RNA Kit, a product of Promega Corporation (AppendixII). The integrity and purity of RNA obtained were electrophoretically verified by formaldehyde agarose gel stained with ethidium bromide based on the method of Lehrach, Diamond, Wozney, and Boedtker (1977).

cDNA synthesis protocol

In a reverse transcription reaction mixture containing 1× PCR buffer, 0.5 mMdeoxy-nucleoside triphosphates (dNTPs), one unit of RNase inhibitor, 2.5 μM of oligo d(T)16, and 2.5 units of MuLV reverse transcriptase (Perkin-Elmer, Roche Molecular Systems, Inc, NJ, USA), 1 μg of RNA was reverse transcribed into cDNA. After 10 min of incubation at room temperature to allow primer annealing, the reaction mixture was incubated at 42 °C for 15 min, heated to 95 °C for 5 min, and chilled at 4 °C for 5 min in a GeneAmp thermal cycler (Applied Biosystems). Two microliters of the resultant cDNA products was used for PCR amplification.

Real-time quantitative reverse transcription polymerase chain reaction

Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed on a Lightcycler 2.0 system (Roche Applied Systems) to analyze the expression levels of mRNA of Bax and Bcl-2 gene relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Primer sets for GAPDH, Bax, and Bcl-2 were designed using the real-time quantitative PCR probe design software (Roche Applied Systems). The primers used for the amplification of the respective genes are listed in Table 1. PCR reactions for these primers were first optimized using conventional PCR.

For the quantitative real-time PCR, 20 μl amplification mixtures (LightCyclerFaststart DNA MasterPLUS SYBR Green Reaction Mix; Roche Applied Science) were prepared per the manufacturer's instructions, containing cDNA (equivalent to 100 ng reverse transcribed RNA) and 0.5 μM of each primer. The cycling conditions were as follows: 10 min polymerase activation at 95 °C and 40 cycles at 95 °C for 15 s, 58 °C for 15 s, and 72 °C for 15 s.

Histological analysis of tissues

Tissues were stained with hematoxylin and eosin according to McManus and Mowry (1965) and viewed under the microscope. Only the right testis and kidney of each rat were used for the histological examination.

Table 1 Primers used for the amplification of the respective genes in the real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Gene	Primer
GAPDH	F: GGCTCTCTGCTCCTCCCTGTTCTA
	R: TGCCGTTGAACTTGCCGTGG
Bcl2	F: CTG GTG GAC AAC ATC GCT CTG
	R: GGT CTG CTG ACC TCA CTT GTG
Bax	F: TTCATC CAGGAT CGA GCA GA
	R: GCA AAG TAG AAG GCA ACG

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.

Results

The results of metal analyses carried out on the feed and rats' tissues for various groups are shown in Tables 2 and 3, respectively. Table 2 shows the trace amount of arsenic in the control group while the level of cadmium was below detection limit. Results shown in Table 3 indicate that after the 1-month exposure, there was greater accumulation of both metals in the liver than in the kidney, but at the end of the 3-month exposure, the trend changed with the kidney recording the highest accumulation of these metals.

The effects of the treatment on the antioxidant status of the liver and kidney are shown in Tables 4, 5, and 6. After both period of exposure, the activities of SOD, GST, and CAT were significantly ($P < 0.05$) decreased when compared to the control. The activities of AST and ALT in the liver and kidney of experiment rats were increased after both period of exposure. The levels of GSH and MDA were significantly increased when compared to the control for both duration of study.

Figures 1 and 2 show the representative photomicrographs of control and test group tissues of rats exposed to the experimental feed. Light microscopy showed that control tissues appeared normal after both duration of study, but the test groups for the liver show hepatocytes with mild visible dilation, neutrophilic infiltration into sinusoidal space, enlarged hepatocytes, and blurred nuclei. In a similar vein, the kidney shows the loss of histological details and various degenerative changes. The effect of the experimental diet on the mRNA expressions of Bax and Bcl2 in the liver and kidney of rats is as shown in Figs. 3 and 4.

Results show that after the 1-month exposure, significant increase ($P < 0.05$) in the level of mRNA expression of the Bax gene was observed for all groups except for the liver in the cadmium-contaminated group. mRNA expression of the Bcl2 gene also recorded significant decrease ($P < 0.05$) in all group except for the kidney in the arsenic-contaminated diets after the 1-month exposure. After the 3-month exposure, however, mRNA expression of the Bax gene show significant increase ($P < 0.05$) in all groups while the mRNA expression of the Bcl2 gene was significantly decreased ($P < 0.05$) in all groups.

Table 2 Concentration of metals in feed given to experimental animals

Groups	Metal concentration (mg/g)		Metal concentration (mol/g × 10^{-7})		Mole ratio (cadmium to arsenic) × 10^{-7}
	Cadmium	Arsenic	Cadmium	Arsenic	
Control	Not detected	0.02 ± 0.56^{a}	Not detected	2.67	–
Cd-contaminated diet	3.68 ± 0.62^{b}	0.03 ± 0.07^{a}	327	4.00	81.75:1
As-contaminated diet	0.01 ± 0.32^{a}	1.82 ± 0.18^{b}	0.89	243	1:273.03
Cd + As-contaminated diet	3.50 ± 0.14^{b}	1.52 ± 0.26^{b}	311	202	1.5:1

Results are expressed as mean ± SD. Values not sharing the same superscript in the same column differ significantly at $P < 0.05$

Discussion

The past few decades had witnessed an increased awareness of problems concerning food pollution of which the heavy metals are one of the most accumulative and persistent pollutants. Thus, the possible effects on the general population of long-term low-level exposure to toxic metals have been of concern in recent times. The current study was therefore designed to investigate the toxicological effects of cadmium and arsenic (singly and in combination) on the liver and kidney of rats.

Metal analysis on the compounded experimental feed and tissues of experimental rats as shown in Tables 2 and 3 shows trace contamination of the control and the test group. This is in accordance with the work of Horiguchi, Sato, Konno, and Fukushima (1996). The presence of Cd and As in the liver and kidney of the control rats and test groups which are supposed to be free of either of the metal is an indication that the water and/or feed used was tainted with Cd and As. This could be attributed to the pervasiveness of Cd and As in the general environment today. Results presented in Table 3 show that Cd and As accumulated more in the liver than kidney after 1 month of exposure. However, after the 3-month exposure, these metals were found to accumulate more in the kidney than in the liver. Experimental evidence shows that when metals are administered orally and subcutaneously, more was deposited in the liver than in the kidneys (Asagba, 2010; Pari & Murugavel, 2005). However, as the duration of exposure increases, there is a gradual mobilization of these metals from the liver to the kidney (Ercal, Gurer-Orhan, & Aykin-Burns, 2001; Smith, Klei, & Barchowsky, 2001). This may not be unconnected with the fact that the kidney being an organ whose primary function is the excretion of toxic metabolites is the final destination of these metals from various tissues and plasma. The higher uptake of Cd and As in the kidney as compared to other organs is in agreement with available reports in literature (Baykov et al., 2003; Crowe & Morgan, 1997Elsenhans, Strugala, & SchmannK, 1999; Eriyamremu, Ojimogho, Asagba, & Lolodi, 2008; Horiguchi et al., 1996; World Health Organization (WHO), 1992).

Table 3 Effect of food chain-mediated exposure to Cd and As on metal accumulation in the organs of rats

Group	Metal concentration (mg/g tissue)	
	Liver	Kidney
1-month exposure		
Control	0.02 ± 0.01^{a}	0.02 ± 0.09^{a}
Cd-contaminated diet	4.12 ± 0.34^{b}	3.01 ± 1.01^{b}
As-contaminated diet	1.53 ± 0.03^{c}	0.38 ± 0.01^{c}
Cd in Cd + As-contaminated diet	3.83 ± 0.05^{d}	2.21 ± 0.14^{d}
As in Cd + As-contaminated diet	1.38 ± 0.02^{c}	0.16 ± 0.01^{c}
3-month exposure		
Control	0.05 ± 0.03^{a}	0.04 ± 0.02^{a}
Cd-contaminated diet	7.10 ± 0.92^{b}	9.54 ± 0.13^{b}
As-contaminated diet	2.45 ± 0.21^{c}	3.21 ± 1.27^{c}
Cd in Cd + As-contaminated diet	6.24 ± 0.07^{d}	7.15 ± 1.03^{d}
As in Cd + As-contaminated diet	2.01 ± 0.12^{c}	2.79 ± 0.01^{c}

Results are expressed as mean ± SD. Values not sharing the same superscript in the same column differ significantly at $P < 0.05$

In the present study, after the 1-month exposure, the activities of the antioxidant enzymes (SOD and CAT) and GST, a phase 1 drug metabolizing enzyme, were elevated in groups B, C, and D when compared to group A in the kidney. The liver, however, recorded significant ($P < 0.05$) decrease in the activities of these enzymes after the 1-month exposure. The decrease in the activities of the enzymes in the liver even at the 1-month exposure could be attributed to the fact that the liver, being the major site of detoxification, gets a higher load of exposure to these metals due to normal metabolic activities. A decreased activity of SOD and CAT as observed after the 3-month exposure in the tissues (as well as 1-month exposure in the liver) reflects elevated production of superoxide radical anions (Yamanaka, Hasegawa, Sawamura, & Okada, 1991) and insufficient requirement of NADPH, which is required for the activation of CAT from its inactivated form (Kirkman & Gaetani, 1984). It is noteworthy that inhibition of antioxidant enzymes such as SOD by cadmium has also been linked to displacement of essential cofactor such as Zn or Cu or binding to thiol groups of the enzyme (Casalino, Calzaretti, Sblano, & Landriscina, 2002; Timbrell, 2000).

Lipid peroxidation (LPO) is a degenerative mechanism of membrane component mediated through free radical production in the cell (Veena, Josephine, Preetha, & Varalakshmi, 2007). Increased LPO implies membrane instability which correlates with decreased activity of SOD

Table 4 Effect of treatment on enzymatic activities in the liver of experimental rats

Group	Parameter				
	CAT (μmol H_2O_2/min/mg protein)	SOD (units/g testis)	GST (μmol CDNB–GSH complex formed/min/mg protein)	AST	ALT
1-month exposure					
Control	98.00 ± 4.04^a	48.50 ± 2.29^a	10.88 ± 1.69^a	39.05 ± 3.57^a	30.45 ± 1.23
Cd-contaminated diet	89.00 ± 2.82^b (– 9.18%)	28.28 ± 0.24^b (– 41.69%)	6.36 ± 0.10^b (– 41.54%)	53.31 ± 3.510^b (36.52%)	39.56 ± 3.15 (29.92%)
As-contaminated diet	44.70 ± 7.07^c (– 54.39%)	22.58 ± 0.21^b (– 53.44%)	3.80 ± 0.26^c (– 65.07%)	67.89 ± 3.134^c (73.85%)	57.57 ± 3.12 (89.06%)
Cd + As-contaminated diet	76.30 ± 7.63^d (– 22.14%)	25.75 ± 0.13^b (– 46.91%)	5.17 ± 0.46^b (– 52.48%)	59.48 ± 4.54^b (52.32%)	49.76 ± 0.27 (63.42%)
3-month exposure					
Control	205.30 ± 4.10^a	46.75 ± 1.77^a	10.12 ± 0.04^a	37.00 ± 12.12^a	33.25 ± 1.25^a
Cd-contaminated diet	189.85 ± 2.61^b (– 7.53%)	22.82 ± 0.02^b (– 51.19%)	6.12 ± 0.03^b (– 39.53%)	29.75 ± 6.80^b (– 19.59%)	21.00 ± 1.04^b (– 36.84%)
As-contaminated diet	136.95 ± 2.89^c (– 33.29%)	11.91 ± 0.46^c (– 74.52%)	2.22 ± 0.88^c (– 78.06%)	23.75 ± 7.22^c (– 35.81%)	09.25 ± 3.48^c (– 72.18%)
Cd + As-contaminated diet	157.35 ± 3.04^d (– 23.36%)	17.86 ± 0.93^c (– 61.80%)	$4.13.02^c$ (– 59.19%)	27.25 ± 5.44^b (– 26.35%)	17.75 ± 2.08^d (– 46.62%)

Results are expressed as mean ± SD. Values not sharing the same superscript in the same column differ significantly at $P < 0.05$. Percentage changes are given relative to control

and CAT as observed in the present study. The apparent increase in lipid peroxidation may be attributed to the accumulation of the heavy metals in the various organs. In the present study, significant ($P < 0.005$) increases in GSH levels in the liver and kidney were observed after the 1-month exposure; however, at the end of the 3 months of exposure, there was a significant ($P < 0.05$) decrease in the level of GSH. The increase in tissue GSH content after the 1-month exposure might be attributed to GSH rebound through enhancement of $^{\gamma}$-glutamylcysteine synthase gene expression (Yeh, Cheng, Ou, Whanger, & Chang, 2002). The depletion of GSH level however recorded after the 3-month exposure could be attributed to the oxidation of GSH by free radicals (Manna, Sinha, & Sil, 2008) or as a

Table 5 Effect of treatment on enzymatic activities in the kidney of experimental rats

Group	Parameter				
	CAT (μmol H_2O_2/min/mg protein)	SOD (units/g testis)	GST (μmol CDNB–GSH complex formed/min/mg protein)	AST	ALT
1-month exposure					
Control	88.70 ± 5.65^a	39.75 ± 0.07^a	9.01 ± 1.26^a	27.06 ± 4.319^a	29.01 ± 0.93
Cd-contaminated diet	112.05 ± 2.05^b (26.33%)	51.00 ± 2.10^b (28.30%)	17.37 ± 3.42^b (92.79%)	41.16 ± 4.26^b (52.11%)	40.67 ± 1.23 (40.19%)
As-contaminated diet	99.30 ± 2.56^c (11.95%)	42.33 ± 0.18^c (6.49%)	12.37 ± 0.15^c (37.29%)	30.63 ± 2.81^a (13.19%)	36.02 ± 0.19 (24.16%)
Cd + As-contaminated diet	107.55 ± 2.75^d (21.25%)	45.50 ± 0.03^c (14.47%)	15.62 ± 0.22^c (73.36%)	39.86 ± 3.24^b (47.30%)	37.12 ± 0.17 (27.96%)
3-month exposure					
Control	177.76 ± 5.36^a	44.67 ± 0.15^a	9.26 ± 0.25^a	32.00 ± 8.39^a	29.50 ± 10.10^a
Cd-contaminated diet	101.95 ± 0.21^b (– 42.65%)	16.77 ± 0.07^b (– 62.46%)	3.53 ± 0.22^b (– 61.88%)	22.75 ± 15.10^b (-28.91%)	13.51 ± 4.41^b (– 54.20%)
As-contaminated diet	126.70 ± 4.24^c (– 28.72%)	22.73 ± 0.59^c (– 49.12%)	5.45 ± 0.26^c (– 41.14%)	27.00 ± 8.39^b (– 15.63%)	21.01 ± 0.30^c (– 27.08%)
Cd + As-contaminated diet	103.05 ± 2.21^b (– 42.03%)	18.79 ± 0.05^b (– 57.94%)	4.53 ± 0.53^b (– 51.08%)	25.00 ± 13^b (– 21.88%)	19.25 ± 4.25^c (– 41.53%)

Results are expressed as mean ± SD. Values not sharing the same superscript in the same column differ significantly at $P < 0.05$. Percentage changes are given relative to control

Table 6 Effect of treatment on MDA and GSH in the liver and kidney of experimental rats

Group	Parameter			
	Liver		Kidney	
	MDA (units/g tissue)	GSH (mg/g tissue)	MDA (units/g tissue)	GSH (mg/g tissue)
1-month exposure				
Control	57.46 ± 3.41^{a}	22.67 ± 2.30^{a}	47.03 ± 2.46^{a}	21.33 ± 6.11^{a}
Cd-contaminated diet	62.25 ± 3.65^{b} (8.34%)	31.33 ± 1.15^{b} (38.20%)	74.13 ± 2.03^{b} (57.63%)	51.00 ± 16.97^{b} (139.10%)
As-contaminated diet	95.21 ± 2.54^{c} (65.70%)	42.00 ± 2.82^{c} (85.27%)	58.88 ± 2.52^{c} (25.20%)	48.07 ± 19.07^{c} (125.36%)
Cd + As-contaminated diet	75.60 ± 1.27^{d} (31.57%)	34.33 ± 2.30^{b} (51.43%)	69.31 ± 2.87^{d} (47.37%)	33.09 ± 1.41^{d} (55.13%)
3-month exposure				
Control	94.38 ± 1.43^{a}	32.10 ± 2.92^{a}	52.04 ± 2.33^{a}	46.03 ± 4.34^{a}
Cd-contaminated diet	115.63 ± 1.98^{b} (22.51%)	25.03 ± 2.64^{b} (– 22.02%)	110.02 ± 1.97^{b} (111.41%)	20.42 ± 2.82^{b} (– 55.64%)
As-contaminated diet	162.42 ± 2.75^{c} (72.09%)	17.00 ± 5.65^{c} (– 47.04%)	74.15 ± 0.62^{c} (42.49%)	35.01 ± 1.41^{c} (– 23.94%)
Cd + As-contaminated diet	126.65 ± 2.15^{d} (34.19%)	23.33 ± 4.04^{b} (– 27.32%)	105.34 ± 2.94^{d} (102.42%)	31.30 ± 2.48^{c} (– 32.00%)

Results are expressed as mean ± SD. Values not sharing the same superscript in the same column differ significantly at $P < 0.05$. Percentage changes are given relative to control

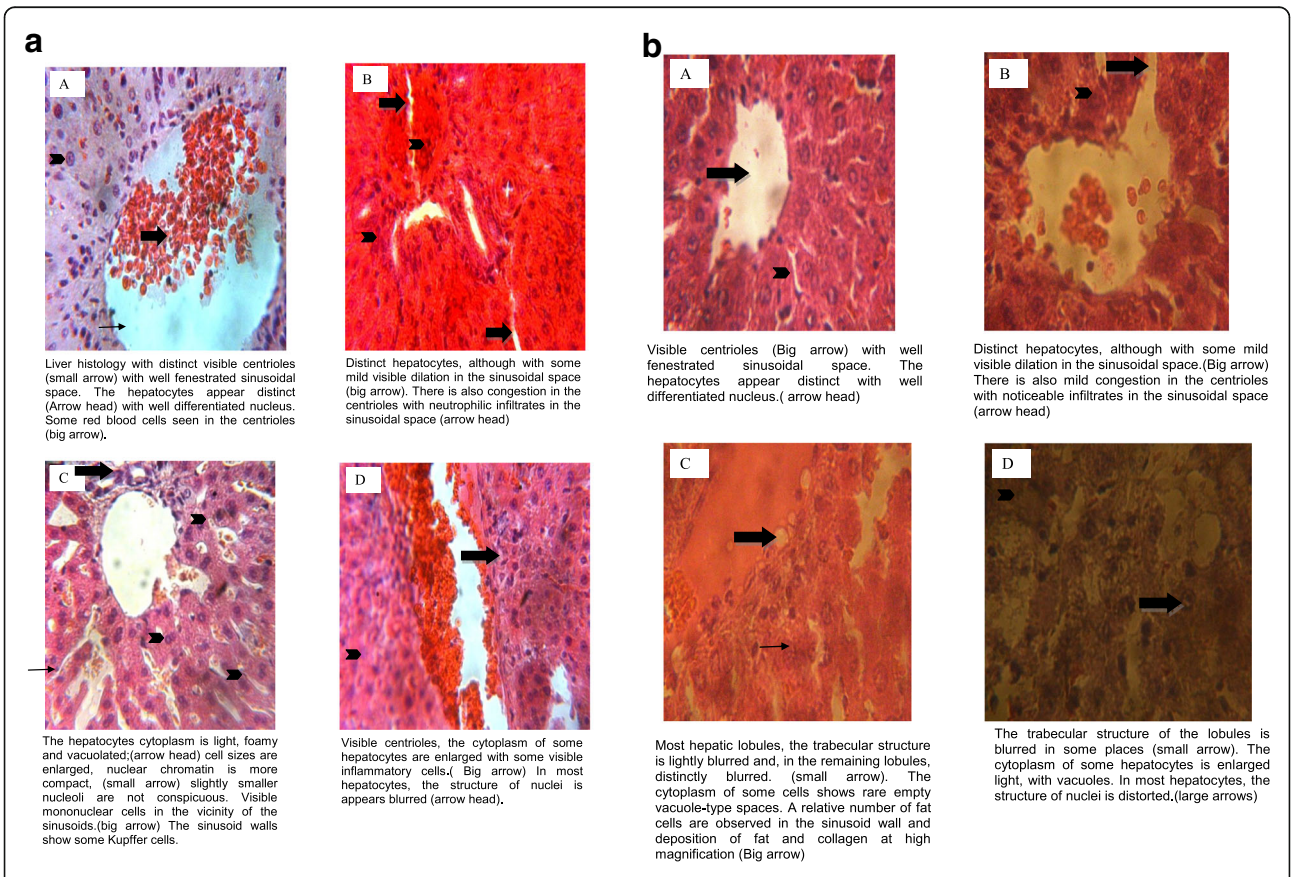

Fig. 1 a Morphological changes in the liver of rats exposed to contaminated diet after 1-month exposure. (A) Control. (B) Cd-contaminated diet. (C) As-contaminated diet. (D) Cd + As-contaminated diet. **b** Morphological changes in the liver of rats exposed to contaminated diet after 3-month exposure. (A) Control. (B) Cd-contaminated diet. (C) As-contaminated diet. (D) Cd + As-contaminated diet

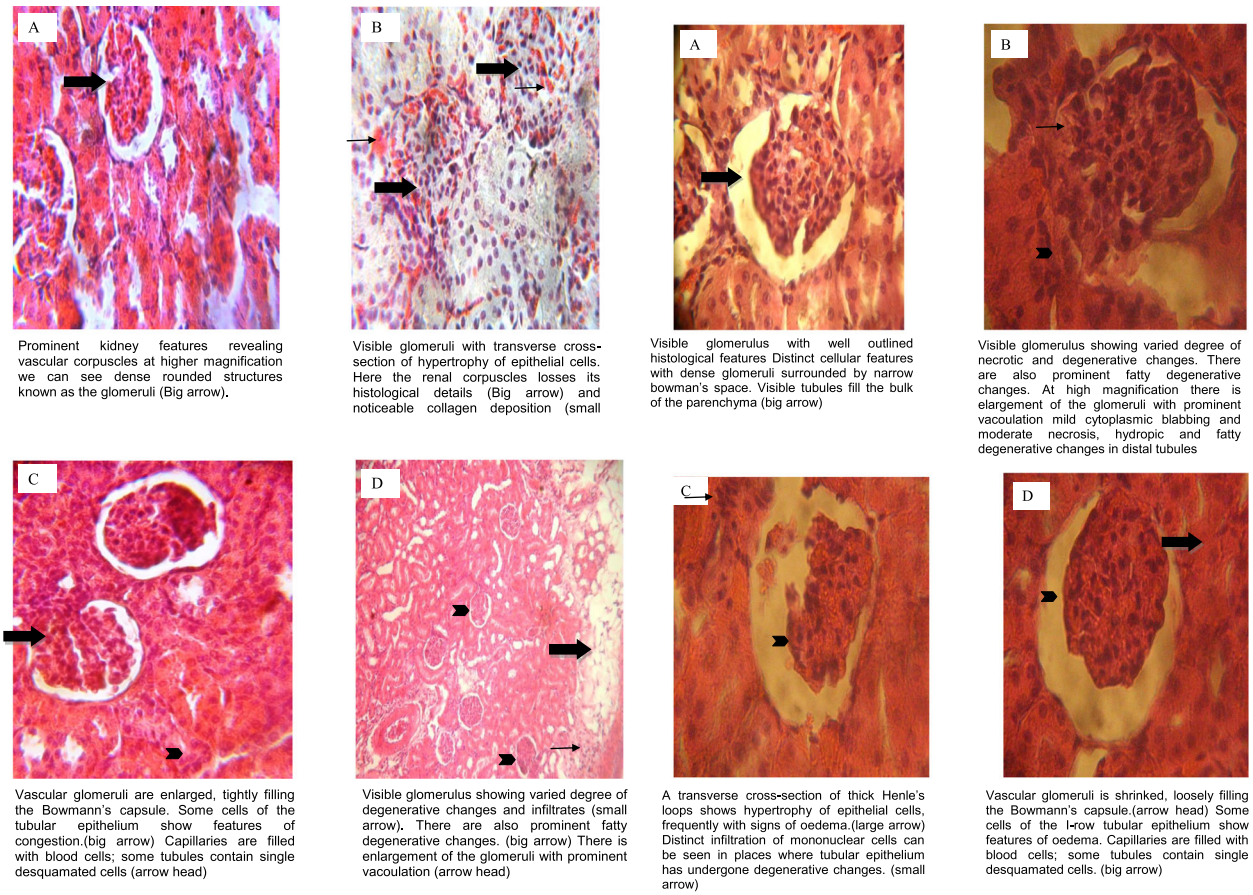

Fig. 2 a Morphological changes in the kidney of rats exposed to contaminated diet after 1-month exposure. (A) Control. (B) Cd-contaminated diet. (C) As-contaminated diet. (D) Cd + As-contaminated diet. **b** Morphological changes in the kidney of rats exposed to contaminated diet after 3-month exposure. (A) Control. (B) Cd-contaminated diet. (C) As-contaminated diet. (D) Cd + As-contaminated diet

result of depletion of the sulfhydryl group of cysteine moiety in GSH due to its high affinity for cadmium and arsenic forming Cd/As-GSH complex (Aposhian, Zakharyan, Wildfang, et al., 1999a) or its electron donor ability (Radabaugh & Aposhian, 2000).

Histopathological analysis shows treatment-related lesions in the various tissues/groups studied. Photomicrographs of the liver for both duration of study showed that arsenic had more deleterious effect on the liver than cadmium or the mixture of metals. After the 1-month exposure, arsenic was able to induce a light, foamy, and vacuolated cytoplasm while at the end of the 3-month exposure, the cytoplasm of cells shows rare empty vacuole-type spaces and a number of Kupffer cells are observed in the sinusoid wall. In the kidney, however, cadmium exerted more deleterious effects than the mixture or arsenic alone. In the kidney, after the 1-month treatment, cadmium was able to induce visible dilation in the sinusoidal space, mild congestion in the centrioles, and noticeable infiltrates in the sinusoidal space. Also, the renal corpuscles lost their histological details and there was a noticeable collagen deposition.

After the 3-month exposure, examination of the photomicrograph of the organs indicates that the glomerulus shows varied degree of necrotic and degenerative changes. Elsenhans et al. (1999) had reported that the kidneys are the critical target following long-term exposure to cadmium. Cadmium-induced hepatic and renal injury in chronically exposed rats shows the likely role of hepatic cadmium-metallothionein in nephrotoxicity (Dudley, Gammal, & Klaassen, 1985). Jadhav et al. (2007, b) investigated the effect of subchronic exposure of rats to drinking water containing arsenic, cadmium, lead, mercury, chromium, nickel, manganese, and iron. The results showed that the 3-month exposure of rats to the mixture of elements led to substantial changes in humoral and cell-mediated immune responses, in biochemical parameters, and even in vascular, degenerative, and necrotic changes in tested tissues of rats.

The effect of the experimental diet on the mRNA expressions of Bax and Bcl2 was also analyzed in the current

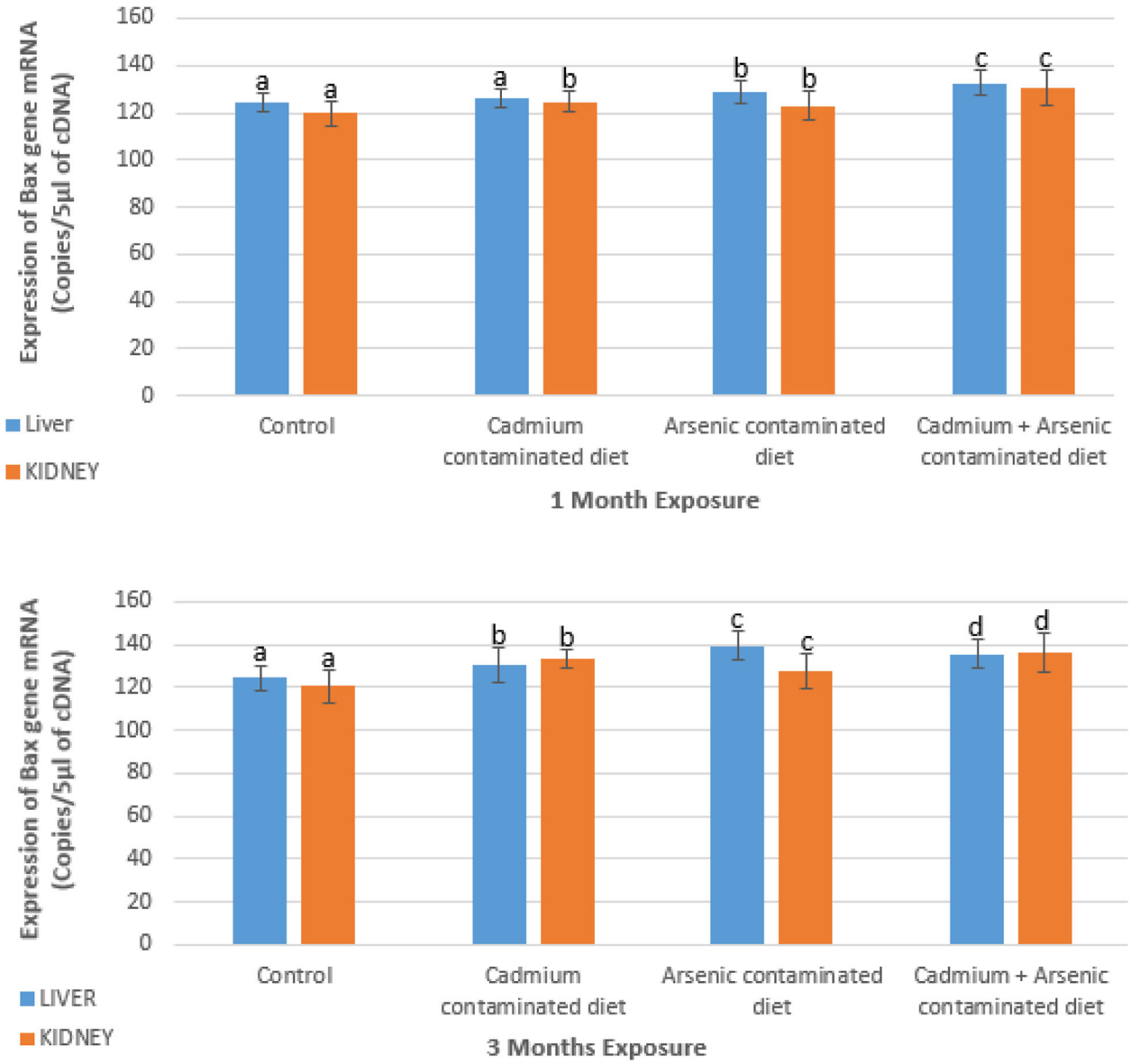

Fig. 3 Changes in the expression of Bax gene mRNA in the liver and kidney of rats after 1- and 3-month exposures to experimental diet. Values not sharing the same superscript within a tissue differ significantly at $P < 0.05$

study (Figs. 3 and 4). One month after exposure to these metals through the food chain, there was a significant ($P < 0.005$) increase in the level of Bax mRNA in the kidney and liver of rats except for the liver which was exposed to cadmium-contaminated diet only. The levels of Bcl-2 were, however, non-significantly ($P < 0.005$) downregulated in all groups except for the kidney in the arsenic-contaminated diet. After the 3-month exposure, Bcl-2 was significantly ($P < 0.05$) downregulated while Bax was significantly ($P < 0.05$) increased in all test groups in the liver and kidney. Interactions between Bcl-2 and Bax regulate cytochrome c release from mitochondria and establish baseline sensitivity to apoptotic stimuli. El-Sayed, Salem, El-Garhy, Rahman, and Kandil (2013) reported that cadmium administrated to pregnant mice increased primary DNA damage and activated the apoptotic pathway. Bcl-2 and Bax produce mitochondrial-related proteins with antagonistic effects; the former having an anti-apoptotic activity and the latter a pro-apoptotic activity.

Very little information is available on the interactive effects of As and Cd upon ingestion. Fowler and Mahaffey (1978), Mahaffey and Fowler (1977), Schmolke, Elsenhans, Ehtechami, and Forth (1992), and Mahaffey, Capar, Gladen, and Fowler (1981) have shown using subchronic dietary studies in rats that neither metal significantly affected accumulation of the other in the

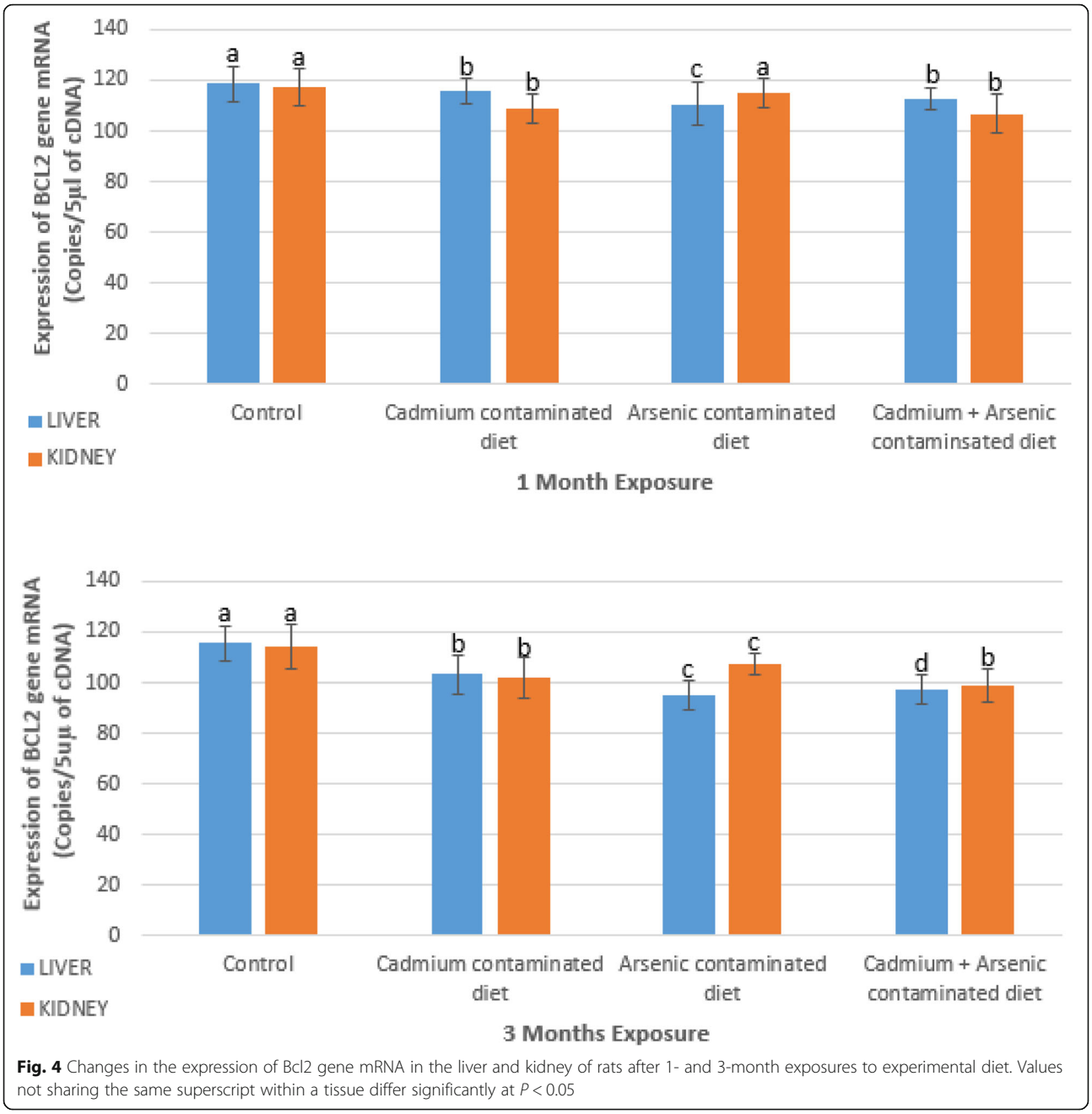

Fig. 4 Changes in the expression of Bcl2 gene mRNA in the liver and kidney of rats after 1- and 3-month exposures to experimental diet. Values not sharing the same superscript within a tissue differ significantly at $P < 0.05$

kidney, liver, or brain tissue. Contrary to the observation of Diaz-Barriga et al. (1990) and Yanez, Carrizales, Zanatta, Mejia, and BatRes.L. and Diaz-Barriga F. (1991) that showed that As (as arsenite) and Cd were more lethal to rats when administered as a mixture than when injected alone, the present study showed that arsenic was more toxic to the liver than the mixture while cadmium showed more deleterious effects on the kidneys than the mixture. Thus, both metals appear to be antagonistic to each other when consumed together. This could be attributed to the fact that in the mixture, arsenic together with cadmium was able to induce the synthesis of metallothioneins. Thus, while all of the arsenics absorbed in the intestines of rats in group C are available to exert their toxic effect, in group D, where the mixture of the metals was included in the experimental diet, some of the arsenic might have been bounded by metallothionein induced in the liver due to the presence of cadmium. Falnoga et al. (2000) have shown that metallothionein is also capable of binding to arsenic, and so reduce the amount of arsenic available for toxicity. Metallothionein has thus been shown to prevent acute cadmium-induced hepatotoxicity and cell death in animal studies (Klaassen, Liu, & Choudhuri, 1999). Studies (Diaz-

Barriga et al., 1990; Yanez et al., 1991) have also shown that Cd-induced toxicity could be inhibited by As-induced glutathione. In addition, studies have shown that MMA and DMA are toxic forms of arsenic due to the ability to induce enzyme inhibition, oxidative stress, DNA damage, and complex with glutathione and other sulfhydryl proteins in enzymes (Ercal et al., 2001; Goering, Aposhian, Mass, et al., 1999; NAS, 2001).

The results of the present study are in consonance with the report of Yanez et al. (1991) which showed that the toxicity of a mixture of As + Cd cannot be predicted from the toxic mechanisms of single components. They also demonstrated differences between the mechanisms of toxic behavior of both elements. In their experiment, the mixture of As + Cd behaved as arsenic in the induction of lipid peroxidation and glutathione and as cadmium in the metallothionein induction.

Conclusion

This study has provided evidence that cadmium and arsenic, at low concentrations through a controlled food chain, can potentially be accumulated in fish in their natural habitat which can be passed on to the next trophic level through the food chain as evidenced by the metal burdens recorded in the tissues of experimental rats. The cadmium-contaminated diet was found to be more toxic to the kidney than the liver while the arsenic-contaminated diet was found to be toxic to the liver. These toxicities altered the activities of the examined enzymes as well as the levels of GSH and MDA and expression levels of mRNA of Bax and Bcl genes of exposed rats. Treatment-related morphological changes were also observed in the tissues of experimental rats. In addition, the present study has shown that the toxicity of a mixture of As + Cd cannot be predicted from the toxic mechanisms of the single components.

Abbreviations

As: Arsenic; CAT: Catalase; Cd: Cadmium; cDNA: Complementary DNA; dNTPs: Deoxnucleoside triphosphates; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; GITC: Guanidine isothiocyanate; GSH: Reduced glutathione; GST: Glutathione-s-transferase; LPO: Lipid peroxidation; mRNA: Messenger ribonucleic acid; NADPH: Reduced form of nicotinamide adenine dinucleotide phosphate; PCR: Polymerase chain reaction; qRT-PCR: Real-time quantitative reverse transcription polymerase chain reaction; RNA: Ribonucleic acid; SD: Standard deviation; SOD: Superoxide dismutase

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Authors' contributions

ET contributed to the conception and design of the study, acquisition of materials required for the study, and analysis and interpretation of data; drafted the manuscript; critically revised the manuscript; and agreed to be accountable for all aspects of work ensuring integrity and accuracy. AOS also contributed to the conception and design of the study and acquisition of materials required for the study, gave the final approval, and agreed to be accountable for all aspects of work ensuring integrity and accuracy. TJN also contributed to the conception and design of the study and analysis and interpretation of data. The authors read and approved the final manuscript.

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Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The research was carried out in accordance with the National Institute of Health (NIH), and approval was given by the Committee on Animal Research and Ethics, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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