academicJournals

Vol. 8(3), pp. 65-73, March 2014
DOI: 10.5897/AJBR2013.0728
Article Number: D7409EE44227
ISSN 1996-0778

http://www.academicjournals.org/AJBR

African Journal of Biochemistry Research

Full Length Research Paper

Usefulness of liver and kidney function parameters as biomarkers of 'heavy metals' exposure in a mammalian model *Mus musculus*

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Received 8 October, 2013; Accepted 5 March, 2014

The toxicological evaluations of cadmium (Cd), iron (Fe), manganese (Mn), lead (Pb) and zinc (Zn) were carried out against Albino mice model, *Mus musculus.* On the basis of LC_{50} value, Cd (0.47 mM) was found to be the most toxic followed by Zn (2.40 mM), Pb (2.42 mM), Fe (4.52 mM) and Mn (5.70 mM) as least toxic. The results of the sublethal concentration of the heavy metals ($1/10^{th}$ of 96 hrLC_{50}) on the liver function parameters and kidney markers showed that total glycerol (TG) levels increased significantly ($P < 0.05$) in Cd and Mn treated groups while Zn, Pb and Mn induced a significantly higher ($P < 0.05$) level of total protein. Mn and Fe induced significantly ($P < 0.05$) increase in the level of total bilirubin (T.BIL), urea and albumin (ALB) in exposed mice relative to the control group. The outcome of this study implied that sublethal responses of liver function parameters and kidney markers were found to have good potentials in serving as a combination of biomarkers for early detection and diagnosis of 'heavy metals' pollution in the mammalian model.

Key words: Biomarkers, heavy metals, kidney markers, liver function parameters, *Mus musculus.*

INTRODUCTION

Recently in Nigeria there was a report on lead (Pb) poisoning incident in Zamfara State (Dareta, Tunga Daji community) that claimed over 400 lives of children due to illegal mining of gold and exposure to contaminated soil and household dust in gold ore processing villages (MSF, 2012). This incidence has triggered a growing concern on the lethal effects of 'heavy metals' and also instigated greater research activities into biological effects of heavy metals pollutants in different parts of the world including Nigeria. Thus the need to acquire information on parameters such as kidney and liver function biomarkers that can be utilized in the early detection and diagnosis of heavy metals effects.

'Heavy metals' are toxic at relatively low concentrations and persist in the environment long after the source of emission has been removed. Thus could be classified as important sources of pollution (DeVagi and Arfiziah, 2009). 'Heavy metals' also bioaccumulate in one or several com-

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partments across food webs as shown by several scientific observations (Chukwu, 1991; Otitoloju and Don-Pedro, 2002; Otitoloju and Don-Pedro, 2004). The myriads of 'heavy metals' are grouped into essential and non essential. The essential elements play important roles as prosthetic groups in enzymes and key metabolic activities in living organisms, for example, iron (Fe), copper (Cu), manganese (Mn), cobalt (Co), vanadium (V), molybdenum (Mo) and zinc (Zn). The non-essential metals, such as arsenic (As), mercury (Hg), cadmium (Cd) and lead (Pb) are not needed in the physiological activities of living organisms hence they are usually toxic at relatively low concentrations (Falusi and Olanipekun, 2007; Raymond and Felix, 2011). The route of exposure of these 'heavy metals' to living forms are through air, water, soil, plants and food which can occur through dermal absorption (skin), inhalation (lungs) and ingestion (mouth). Presently, the amount of 'heavy metals' exposure is hundred times higher (Howard, 2002) than in the past thus living forms have become a “warehouse” of 'heavy metals'. Heavy metal bioaccumulation can be of public health significance especially, when it bioaccumulates in vital organs of man causing damages that can eventually lead to death. For example, calcium (Ca) when replaced by Pb in the bones can contribute to weakened bones and osteoporosis. Likewise Zn when displaced by Cd in the arteries cause inflammation and hardening of the arteries. Fe that replaces Zn and other minerals in the pancreas, adrenals and elsewhere can contribute to impaired blood sugar tolerance and diabetes. Cu that replacement by Zn in the brain is associated with migraine headaches, premenstrual syndrome, depression, anxiety, panic attacks and much more. Hg and Cu that replace selenium in various tissues impairs the conversion of thyroxine (T4) to triiodothyronine (T3), contributing to thyroid imbalances (Larissa et al., 2005). Exposure to ‘heavy metals’ result in acute and chronic toxicity. Therefore the toxic effects are multidirectional and manifested via changes in the physiological, chemical processes of the living things, impairment and dysfunction in the eliminative pathways (colon, liver, Kidney and skin). It has also been shown that the kidney and liver which are important organs of metabolism, detoxification and excretion of xenobiotics are especially vulnerable to damage when exposed to heavy metals (Alsaleh et al., 2006).

Blood enzymes are biomarkers of acute hepatic damage, thus their bioassay can serve as a diagnostic tool for assessing necrosis of the liver cells (Coppo et al., 2002). Many of these enzymes, such as a lactate dehydrogenase (LDH), aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) are released from the liver after its cellular damage and failure due to xenobiotics. The liver is the major site of intermediary metabolism and the synthesis of many important compounds, the site of conjugation and detoxification of potentially toxic substances and the site of storage of glycogen. Its role in detoxification of toxic substances makes it the organ for assessing the toxic effect of a particular substance. Literature abounds as regards the use of kidney damage and liver function parameters as indication of pathological effects of a sub-lethal concentration of heavy metals on animals such as on carp (Vinodhini and Narayanan, 2008), Wistar albino rats (Asagba and Eriyamremu, 2007; Suradkar et al., 2009; Samuel, 2010) and in humans (Orisakwe et al., 2007; Oyewole and Malomo, 2009).

The liver and kidney function markers tend to respond differently to different ‘heavy metals’ at different time therefore the response of a single or few biomarkers of the kidney and liver function cannot serve sufficiently as a tool for the diagnosis for accurate prediction of impairment in organism's health conditions. As a result the concurrent use of several biomarkers is important to minimize misinterpretation in cases of complex ‘heavy metals’ pollution situations.

In this study, the estimation of liver and kidney function parameters in *Mus musculus* was carried out to assess their importance as biological markers of environmental pollution related to heavy metals such as Cd, Fe, Mn, Pb and Zn.

MATERIALS AND METHODS

Test animals, acclimatization, selection of test animals and chemical

A total of seventy two male Albino mice, *Mus musculus* of similar sizes (19 - 24 g body weight and 10 - 15 weeks old) which served as test animals were purchased from the animal house in Nigerian Institute of Medical Research (NIMR) Yaba, Lagos, Nigeria. Mice were kept in ventilated plastic cages (length 20 cm, breath 12 cm by height 9 cm) with wood shavings under conventional conditions of natural light – dark cycle in farm house located in NIMR. The room temperature was at 30 ± 2°C and Relative Humidity - 70% ± 4%. The animals had free access to drinking fluid and a standard rodent laboratory chow (Coppens) purchased from Ladokun feeds Ltd, Ibadan. Acclimatization was for 14 days. ‘Heavy metals’ investigated in this work were obtained as metallic salts from Fisons Laboratory Reagents, Analar grades in Nigeria of the following types; zinc as $Zncl_2.3H_2O$, cadmium as $CdSO_4.8H_20$, lead as $PbSO_4$, manganese as $MnSO_4$ and ferric as $FeSO_4$. Active male mice of similar size and age (19 - 24 g body weight and 10 - 15 weeks old) were selected randomly into separate bioassay cages. This was because age and size were known to affect physiological responses of animals to chemicals.

Ethics

The experiments carried out involved the utilization of whole and live mice. However, aiming for the protection and welfare of animals, studies were conducted in accordance with University of Lagos Ethics Committee guidelines for experiments with whole animals.

Acute toxicity studies

Acute toxicity, preparation of test media for acute toxicity test, differential acute toxicity of heavy metals against mice and assessment of quantal response were determined as described by

Osuala et al. (2013).

Sublethal studies (chronic toxicity)

Mice were divided into six experimental groups (n=12 per group) for the sublethal experiment and a control group (n=3) was also included. They were treated to salts of the heavy metals [$Pb(NO_3)_2$, $FeSO_4$, $CdSO_4$, $ZnSO_4$ and $MnSO_4$] and control group was administered distilled water only. All animals were fed with their feed (Coppens).

Sub-lethal concentrations

In all the series of experiments, the mice were exposed to only sub-lethal concentrations (fraction of 96 h LC_{50} values derived from experiments carried out in acute toxicity test) (Osuala et al., 2013) of selected metal salts as specified below. A total of the sub lethal values for selected metals were as follows:

Pb 1/10 of LC_{50} = 0.24 mM, Fe 1/10 of LC_{50} = 0.45 mM
Cd 1/10 of LC_{50} = 0.05 mM, Zn 1/10 of LC_{50} = 0.24 mM
Mn 1/10 of LC_{50} = 0.57 mM

The metallic salts of the corresponding heavy metals of 1000 mg each of the salt was weighed out and diluted in 20 ml of water. Solution of 0.1 ml was administered through cannular method to the mice everyday for 28 days. The control mice were fed the same volume of distilled water. Prior to sacrificing, 3 mice from each group were separated and fasted for 24 h before dissection. This procedure was maintained every 7 days for analysis under investigation.

Collection of blood samples

At the end of the 7^{th}, 14^{th}, 2^{th} and 28^{th} day treatment periods, the animals were fasted for 24 h after the last administration for the various exposure days. The animals were anaesthetized using i.p. Ketamine (500 mg kg^{-1} i.p). Blood samples were collected from the orbital sinus.

Collection and preparation of plasma samples for kidney and liver parameters

The blood of mice blood was collected into ethylenediaminetetra-acetic acid (EDTA) bottle immediately. Blood was then centrifuged at 4,000 g for 10 min using bench top centrifuge (MSE Minor, England) to remove red blood cells and recover plasma. Plasma samples were separated and were collected using dry Pasteur pipette, labeled and stored in the refrigerator at -20°C for analyses. All samples were analysed within 24 h of sample collection.

Biochemical measurements

Albumin was measured based on Colorimetric assay with endpoint method as described by Tietz et al. (1994). Total protein content was assayed by the Biuret method as described by Plummer, (1971). Alkaline Phosphatase (ALP) was measured as described by Wright et al. (1972).

Alanine aminotransferase (ALT) and aspartate transaminase (AST) were measured as described by Reitman and Frankel (1957). Creatinine (CREA**)** was determined by Jaffe spectrophotometric method asdescribed by Pratt (1996) and Aitken et al. (2003). Total bilirubin was measured based on Jendrassik and Grof new liquid method as described by Tietz et al., (1994). Urea concentration was determined according to the method of Veniamin and Vakirtzi-Lemonias (1970). Uric acid assayed by the method described by Fossatti et al. (1980). Total cholesterol (T.CHOL) and Triglyceride (TG) were determined by enzymatic colorimetric method of Trinder (1969). High density lipoprotein (HDL) and Low density lipoprotein (LDL) - cholesterol were assayed using Randox diagnostic kits determined following the principle described by Trinder (1969).

Statistical analysis

The biochemical data were analyzed with one way analysis of variance (ANOVA). Differences at $P < 0.05$ were considered significant. This was used to compare several treatment means in appropriately designed experiments. Further analysis was carried out only when there was a significant difference at the 5% ($P < 0.05$) level of significance (taken as minimum requirement) based on Duncan multiple range test at 0.05 levels of significance using SPSS 10.0 computer software package (SPSS Inc., Chicago, U.S.A).

RESULTS AND DISCUSSION

The results showed that there were significant ($P < 0.05$) differences in the liver function parameters and kidney function markers induced by test 'heavy metals', Cd, Mn, Zn, Pb and Fe (Tables 1 to 5). Cd significantly ($P < 0.05$) decreased plasma AST, ALT, ALP, Uric acid, Urea, ALB, CREA, T.PRO, HDL, LDL, T.BIL and T.CHOL compared to control except for TG where there was significant ($P < 0.05$) increase on the 7^{th} day of exposure but decreased with subsequent exposure days from 14^{th} -28^{th} (Table 1). The significant decrease in TG levels induced by Cd after the 7^{th} day exposure period is in agreement with Prasenjit et al. (2008) who observed alteration in TG due to Cd toxicity. It was suggested that the decrease might be related to its enhanced utilization in corticosteroidogenesis and/or a decreased *de novo* synthesis. It could also be as a result of diminished lipoprotein formation or the diminished release of complete lipoproteins, which can lead to pathogenesis of fatty liver. TG is a chain of high-energy fatty acids that provide much of the energy for body cells to function. High TG levels are strongly linked to metabolic disorders that increase the risk of heart disease. Thus the subsequent increase could probably indicate the commencement of injury induced by Cd. 'Heavy metals' toxicity has been shown to increase the risk of atherosclerosis, mainly because the toxicity increases triglyceride levels (Ballatori, 2000). The mean value of T.PRO that was significantly decreased is suggestive of the fact that Cd is known to bind to the sulfhydryl groups of enzymes containing cysteine and thus forms complexes with amino acids and protein. It is possible that Cd influenced the conversion of tissue protein into soluble fractions reaching the blood for utilization in cell repair.

Exposure to Mn showed significant ($P < 0.05$) decrease in plasma AST, Uric acid, CREA, ALT, ALP, Urea, HDL, LDL, TG and T.CHOL compared to control except for ALB and T.PRO where there was significant ($P < 0.05$)

Table 1. Liver function parameters and kidney markers induced by Cd.

Parameter	Control	Days of exposure			
		7	14	21	28
AST (µ/L)	79.10 ± 0.20^{d}	49.92 ± 0.01^{c}	35.17 ± 0.01^{b}	29.00 ± 0.00^{a}	35.14 ± 0.33^{b}
T.BIL (µmol/L)	0.17 ± 0.00^{a}	0.19 ± 0.04^{a}	0.47 ± 0.01^{c}	0.46 ± 0.02^{c}	0.36 ± 0.01^{b}
Uric acid (µmol/L)	82.89 ± 0.06^{d}	62.18 ± 0.91^{c}	24.42 ± 0.52^{a}	23.91 ± 0.41^{a}	28.85 ± 0.90^{b}
CREA (µmol/L)	9.07 ± 0.00^{b}	7.88 ± 0.26^{a}	7.90 ± 0.01^{a}	8.43 ± 0.04^{a}	8.33 ± 0.23^{a}
ALT (µ/L)	20.30 ± 0.00^{c}	14.52 ± 1.33^{b}	9.58 ± 0.43^{a}	11.98 ± 1.47^{ab}	14.67 ± 0.90^{b}
UREA (µmol/L)	1.98 ± 0.00^{d}	0.88 ± 0.06^{a}	1.60 ± 0.03^{c}	1.24 ± 0.00^{b}	1.30 ± 0.00^{b}
ALB (g/L)	6.51 ± 0.00^{d}	5.02 ± 0.12^{a}	6.21 ± 0.02^{cd}	5.89 ± 0.34^{bc}	5.56 ± 0.00^{b}
T.PRO (g/L)	12.29 ± 0.00^{d}	9.49 ± 0.22^{a}	10.48 ± 0.11^{bc}	11.14 ± 0.37^{c}	10.44 ± 0.16^{b}
HDL (mmol/L)	0.66 ± 0.00^{c}	0.31 ± 0.05^{a}	0.38 ± 0.00^{ab}	0.44 ± 0.03^{b}	0.42 ± 0.00^{b}
LDL (mmol/L)	0.28 ± 0.00^{d}	0.11 ± 0.00^{c}	0.07 ± 0.00^{a}	0.07 ± 0.00^{a}	0.09 ± 0.00^{b}
T.CHOL (µmol/L)	0.78 ± 0.00^{d}	0.45 ± 0.02^{bc}	0.36 ± 0.00^{a}	0.44 ± 0.02^{b}	0.48 ± 0.02^{c}
TG (µmol/L)	0.34 ± 0.00^{a}	0.53 ± 0.10^{b}	0.33 ± 0.01^{a}	0.22 ± 0.01^{a}	0.23 ± 0.00^{a}
ALP (µ/L)	20.47 ± 0.01^{c}	23.45 ± 1.47^{c}	15.63 ± 1.84^{ab}	12.73 ± 0.31^{a}	16.83 ± 0.94^{b}

Values are Mean ± SD, n=3. Values with different alphabetical superscripts along a row are significantly different at $P < 0.05$.

Table 2. Liver function parameters and kidney markers induced by Mn.

Parameter	Control	Days of exposure			
		7	14	21	28
AST (µ/L)	79.10 ± 0.20^{c}	39.71 ± 3.40^{a}	67.24±1.16^{b}	82.37±0.71^{c}	35.55±1.23^{a}
T.BIL (µmol/L)	0.17 ± 0.00^{a}	1.00 ± 0.05^{c}	0.95 ± 0.08^{c}	0.88 ± 0.055^{c}	0.52±0.02^{b}
Uric Acid (µmol/L)	82.89 ± 0.06^{d}	35.75 ± 0.42^{b}	43.65 ± 2.63^{c}	29.13 ± 1.72^{a}	26.03 ± 0.06^{a}
CREA (µmol/L)	9.07 ± 0.00^{d}	8.46 ± 0.03^{bc}	8.14 ± 0.30^{b}	8.80 ± 0.01^{c}	7.22 ± 0.03^{a}
ALT(µ/L)	20.30 ± 0.00^{d}	10.14 ± 1.58^{a}	15.30 ± 0.32^{bc}	17.47 ± 1.31^{cd}	12.83 ± 0.20^{ab}
UREA(µmol/L)	1.98 ± 0.00^{c}	1.61 ± 0.06^{b}	1.10 ± 0.00^{a}	1.57 ± 0.02^{b}	1.65 ± 0.02^{b}
ALB (g/L)	6.51 ± 0.01^{b}	6.00 ± 0.00^{a}	5.63 ± 0.17^{a}	7.15 ± 0.26^{c}	6.87 ± 0.05^{bc}
T.PRO (g/L)	12.29 ± 0.00^{b}	11.95 ± 0.25^{b}	10.42 ± 0.24^{a}	13.90 ± 0.02^{c}	23.57 ± 0.09^{d}
HDL(mmol/L)	0.66 ± 0.00^{c}	0.30 ± 0.02^{a}	0.31 ± 0.01^{a}	0.39 ± 0.02^{b}	0.34 ± 0.01^{a}
LDL(mmol/L)	0.28 ± 0.00^{c}	0.07± 0.00^{a}	0.08 ± 0.01^{b}	0.07 ± 0.00^{ab}	0.07 ± 0.00^{ab}
T.CHOL (µmol/L)	0.78 ± 0.00^{c}	0.40 ± 0.02^{a}	0.42 ± 0.01^{a}	0.50 ± 0.03^{b}	0.39 ± 0.00^{a}
TG (µmol/L)	0.34±0.00^{e}	0.26 ± 0.02^{d}	0.23 ± 0.01^{c}	0.16 ± 0.00^{b}	0.19 ± 0.01^{d}
ALP(µ/L)	20.47 ± 0.01^{b}	14.32 ± 0.11^{a}	14.95 ± 2.18^{a}	17.58 ± 2.58^{ab}	13.50 ± 0.11^{a}

Values are Mean ± SD, n=3. Values with different alphabetical superscripts along a row are significantly different at $P < 0.05$.

increase on the 21st day of exposure but decreased with subsequent exposure on the 28th day whereas T.BIL increased significantly ($P < 0.05$) from 7th - 28th day exposure (Table 2). The marked increase in plasma bilirubin could probably be as a result of fulminant hepatotoxicity, ultrastructural and functional changes in bile canaliculi leading to cholestasis and also perhaps via formation of manganese-bilirubin complexes. It is also suggestive that Mn probably induced haemolysis of the red blood cell. *In vitro* studies demonstrate that Mn ions can penetrate the erythrocyte membrane and in the presence of reduced gluthione, cause irreversible membrane damage and hence haemolysis. Therefore, elevated level of T.BIL is a probable indicator of Mn toxicity and a potential biomarker of exposure. The observed significant increase in ALB and T.PRO which was observed in this study but subsequently decreased towards the end of exposure period was probably indicative of oxidative stress induced by Mn. T.PRO level is a frequent parameter of metal poisoning in any living organism. ALB is the protein with the highest concentration in plasma. The present study showed that ALB and T.PRO were significantly decreesed but on the other hand increased the level of T.BIL in the plasma. These results may also be attributed to the

Table 3. Liver function parameters and kidney markers induced by Zn.

Parameter	Control	Days of exposure			
		7	14	21	28
AST (µ/L)	79.10 ± 0.20^{b}	52.71 ± 0.96^{a}	50.06 ± 3.53^{a}	49.52 ± 0.48^{a}	54.38 ± 4.74^{a}
T.BIL (µmol/L)	0.17 ± 0.00^{a}	0.66 ± 0.01^{c}	0.92 ± 0.04^{e}	0.82 ± 0.01^{d}	0.54 ± 0.02^{b}
Uric Acid (µmol/L)	82.89 ± 0.06^{c}	31.33 ± 0.58^{a}	30.48 ± 0.19^{a}	38.56 ± 0.24^{b}	37.89 ± 0.40^{b}
CREA (µmol/L)	9.07 ± 0.00	7.77 ± 0.19	7.95 ± 0.46	8.51 ± 0.60	8.74 ± 0.48
ALT (µ/L)	20.30 ± 0.00^{d}	14.21 ± 0.51^{ab}	13.85 ± 1.65^{a}	19.00 ± 0.59^{cd}	17.00 ± 0.90^{bc}
UREA (µmol/L)	1.98 ± 0.00^{b}	1.18 ± 0.23^{a}	1.28 ± 0.08^{a}	1.17 ± 0.07^{a}	1.23 ± 0.01^{a}
ALB (g/L)	6.51 ± 0.00^{c}	3.98 ± 0.34^{a}	5.71 ± 0.06^{b}	6.71 ± 0.04^{c}	7.00 ± 0.20^{c}
T.PRO (g/L)	12.27 ± 0.00^{bc}	7.62 ± 1.01^{a}	10.83 ± 0.32^{b}	13.31 ± 0.26^{cd}	14.43 ± 0.30^{d}
HDL (mmol/L)	0.66 ± 0.00^{b}	0.33 ± 0.07^{a}	0.33 ± 0.00^{a}	0.34 ± 0.05^{a}	0.41 ± 0.02^{a}
LDL (mmol/L)	0.28 ± 0.00^{c}	0.20 ± 0.09^{bc}	0.07 ± 0.00^{ab}	0.06 ± 0.01^{a}	0.10 ± 0.00^{ab}
TCHOL (µmol/L)	0.78 ± 0.00^{c}	0.33 ± 0.05^{a}	0.46 ± 0.01^{b}	0.4900 ± 0.01^{b}	0.52 ± 0.010^{b}
TG (µmol/L)	0.34 ± 0.03^{d}	0.18 ± 0.01^{a}	0.23 ± 0.01^{c}	0.17 ± 0.00^{a}	0.21 ± 0.00^{b}
ALP (µ/L)	20.47 ± 0.01^{a}	19.67 ± 1.68^{a}	27.0233 ± 0.68^{b}	29.56 ± 0.12^{b}	27.03 ± 1.17^{b}

Values are Mean ± SD, n=3. Values with different alphabetical superscripts along a row are significantly different at $P < 0.05$.

great demands and cellular damage that occurred in the tissues of Mn - exposed mice and manganese toxicity may probably cause protein and albumin breakdown. Bilirubin formed in the reticulo-endothelial system is transported to the liver bound albumin. In the liver, bilirubin is conjugated to glucuronic acid to form direct bilirubin. Conjugated bilirubin is excreted via the biliary system into the intestine where it is metabolised. Biliary excretion is the major elimination pathway for Mn, accounting for greater than or equal 95% of Mn excretion; consequently any existing liver damage may delay or decrease its elimination and increase the relative amount of bilirubin in plasma (Ballatori, 2000). Total bilirubin is elevated in obstructive conditions of the bile duct, hepatitis, cirrhosis, in hemolytic disorders and several inherited enzyme deficiencies. This finding is consistent with that of Veena et al. (2012). A study conducted by Goering (2003) showed that Mn increases the activity of 3-hydroxy-3-methylglutaryl coenzyme A, the rate limiting enzyme for cholesterol biosynthesis and that bilirubin decreases cholesterol 7α hydroxylase, which is important in the conversion of cholesterol into bile acids (Akoume et al., 2003).

Zinc significantly ($P < 0.05$) decreased plasma AST, ALT, Uric acid, Urea, ALB, CREA, HDL, LDL, TG and T.CHOL compared to control except for ALP from 14th - 28th day of exposure, T.PRO (21st - 28th day of exposure), T.BIL (7th - 28th day of exposure) where there was significant ($P < 0.05$) increase when compared to the control (Table 3). The consistent increase in the T.BIL concentration all through the exposure period in mice exposed to Zn is a probable indication of an adverse effect of the Zn on the haemoglobin metabolism. The implication of such an increase in bilirubin may result in jaundice. The significantly increased level of ALP observed from the 14th - 28th day in this study, is a similar finding reported by Leena et al. (2011). ALP is a 'marker' enzyme of damage for the plasma membrane and endoplasmic reticulum (Shahjahan et al., 2004). It is frequently used to assess the integrity of the plasma membrane. The increase in ALP activity following Zn exposure suggested disruption of the ordered lipid - bilayer of the membrane structure of the plasma. Rich sources of ALP are the bile canaliculi of the liver, osteoblasts in the bone, proximal tubules in the kidney and mucosal cells of the small intestine. Damage to any of these organs would lead to elevated activity of its isoform of ALP in the plasma. It seemed, therefore, that Zn induced the biosynthesis of ALP in the kidney due to the disruption of cellular integrity. This could also be attributed to the irritation of all particular liver cells by zinc or due to increased loss of intra cellular enzyme by different structures via cell membrane which appear to act as a stimulus to the synthesis of more enzymes. The initial decreased level of total protein probably reflected the compensatory processes occurring in mice after exposure to Zn. This is consistent with the findings of Leena et al. (2011). Subsequent increase in the T.PRO level from the 21st - 28th day of exposure, emphasizing the fact that T.PRO is a function of the health status which is one of the factors affecting the state of health of the animals (Igwebuike et al., 2008). Total protein level is a rough measure of protein status but reflects major functional changes in the kidney and liver functions.

Cadmium, Mn and Zn were shown in this study to have caused a decrease in plasma T.PRO which is indicative of metal toxicity. The heavy metals altered the protein concentration thus impairing the metabolism of protein. There are reports on toxicants showing decrease in protein concentration in fish (Ram and Sathyanesan, 1987; Singh et al., 1993). This also implied that the exposure

Table 4. Liver function parameters and kidney markers induced by Pb.

Parameter	Control	Days of exposure			
		7	14	21	28
AST (µ/L)	79.10 ± 0.20^{b}	24.33 ± 0.53^{a}	70.6 ± 2.52^{b}	76.93 ± 2.20^{c}	69.33 ± 2.67^{b}
T.BIL (µmol/L)	0.17 ± 0.00^{a}	0.17 ± 0.02^{e}	0.6000 ± 0.023^{d}	0.0567 ± 0.003^{a}	0.1167 ± 0.015^{b}
Uric acid(µmol/L)	82.89 ± 0.06^{c}	12.42 ± 0.12^{a}	19.1100 ± 0.779^{a}	42.3167 ± 4.893^{b}	42.1633 ± 4.400^{b}
CREA (µmol/L)	9.07 ± 0.00	7.24 ± 0.85^{ab}	6.6000 ± 0.237^{a}	8.1467 ± 0.209^{bc}	8.0100 ± 0.052^{bc}
ALT(µ/L)	20.30 ± 0.00^{d}	9.66 ± 0.20^{a}	20.06 ± 2.00^{b}	24.51 ± 1.46^{b}	20.02 ± 3.45^{b}
UREA(µmol/L)	1.98 ± 0.00^{b}	0.84 ± 0.15^{a}	1.10 ± 0.06^{b}	1.29 ± 0.01^{bc}	1.39 ± 0.02^{c}
ALB (g/L)	6.51 ± 0.00^{c}	3.6000 ± 0.615^{a}	4.6667 ± 0.220^{b}	6.7600 ± 0.012^{c}	6.4667 ± 0.081^{c}
T.PRO (g/L)	12.27 ± 0.00^{bc}	7.47 ± 0.75^{a}	9.68 ± 0.39^{b}	13.63 ± 0.16^{d}	13.46 ± 0.16^{cd}
HDL(mmol/L)	0.66 ± 0.00^{b}	0.18 ± 0.03^{a}	0.34 ± 0.02^{b}	0.42 ± 0.01^{c}	0.42 ± 0.01^{c}
LDL(mmol/L)	0.28 ± 0.00^{c}	0.05 ± 0.00^{a}	0.11 ± 0.01^{b}	0.2900 ± 0.01^{d}	0.22 ± 0.01^{c}
TCHOL (µmol/L)	0.78 ± 0.00^{c}	0.26 ± 0.04^{a}	0.46 ± 0.01^{b}	0.66 ± 0.01^{c}	0.66 ± 0.01^{c}
TG (µmol/L)	0.34 ± 0.03^{d}	0.08 ± 0.01^{a}	0.18 ± 0.01^{b}	0.30 ± 0.02^{c}	0.33 ± 0.01^{c}
ALP(µ/L)	20.47 ± 0.01^{a}	10.84 ± 0.23^{a}	14.69 ± 0.30^{b}	16.99 ± 0.81^{c}	14.35 ± 0.78^{d}

Values are Mean ± SD, n = 3. Values with different alphabetical superscripts along a row are significantly different at $P < 0.05$.

of heavy metals triggered the oxidative stress in the plasma by the generation of reactive oxygen species. The defensive surface proteins antagonise the toxic radicals resulting in elimination of protein from the liver cells. Additionally, the lowered level of T.PRO in plasma reflects the capacity of protein synthesis and denotes the osmolarity of the blood and liver impairments. Hence, would be a valuable indicator in the diagnosis of heavy metals toxicity in mammals. The observed decrease in total protein might also be due to several pathological processes induced by heavy metals including plasma dissolution, renal damage and protein elimination in the urine, a decrease in liver protein synthesis and alteration in hepatic blood flow and/or hemorrhage into the peritoneal cavity and intestine (Vinodhini and Narayanan, 2008). The present findings are in agreement with previous reports of decreased level of soluble protein, urea and creatinine reported by Al-Shinnawy (2009) and Ashok et al. (2011).

The results of Pb exposure to mice showed a signi-ficant ($P < 0.05$) decrease in AST, ALT, ALP, Uric acid, Urea, ALB, CREA, HDL, LDL, T.BIL, TG and T.CHOL compared with the control except for T.PRO where there was significant ($P < 0.05$) increase from on the 21st - 28th days of exposure (Table 4). The increase in T.PRO was also similarly observed in mice exposed to Mn which also could probably relate to the specific character of the heavy metals action. Lead may be rapidly absorbed and reach considerable amount in the blood (Haque et al., 2006). Han et al. (1996) and Moussa et al. (2001) both suggested that this element is strongly bound to macromolecules in the intracellular compartment because Pb binding protein have been observed to be deposited in kidney, liver, blood and brain. The significant decreased level of AST, ALT, ALP, uric acid, urea, ALB, CREA, HDL, LDL, T.BIL, T.PRO, TG and T.CHOL revealed in mice exposed to Pb with respect to control, are in agreement with some of the results obtained by Dhembare et al. (2011). The author reported that T.PRO and CREA contents declined in rats exposed to KH_2PO_4 but in disagreement with the findings of Veena et al. (2011), who observed that Pb caused a significant decrease in T.PRO and an increase in ALT, ALP, CREA and T.CHOL. The difference in their findings in relation to that of this study could be attributed to disparity in dosage. However, the result of this study also highlighted a significantly lowered ALB which is in agreement with the investigations of Ait et al. (2009). Albumin is the abundant protein in the human plasma representing 55 - 65% of T.PRO. It is synthesized in the liver at a rate that is dependent on protein intake subject to feed back regulation by plasma albumin level (Abd-El-Baset and Abd-El-reheem, 2009). Albumin is a useful indicator of the integrity of glomerular and other membranes. Its chief biological functions are to transport and store a wide variety of ligands to maintain the plasma oncotic pressure and to serve as a source of endogenous amino acids (Peter, 1975). Therefore from the point of view of this study, a decrease in ALB compared to the control was probably an indication of poor liver functions or impaired synthesis, either primary as in liver cells damage or secondary to diminished protein intake and reduce absorption of amino acids caused by malabsorption syndrome (Abd-El-reheem, 2008).

The results (Table 5) showed that Fe induced significant ($P < 0.05$) decrease in AST, ALP, Uric acid, CREA, HDL, LDL, TG and T. CHOL compared with the control except for T.BIL. Urea, ALB, T. PRO (7th - 28th day of exposure) and ALT increased significantly ($P < 0.05$) from 14th - 28th day of exposure where there were significant increases. The significantly increased urea level following exposure to Fe in mice is probably an indication of renal

Table 5. Liver function parameters and kidney markers induced by Fe.

Parameter	Control	Days of exposure			
		7	14	21	28
Iron (Fe)					
AST (µ/L)	79.10 ± 0.20^{b}	54.47 ± 0.48^{a}	70.42 ± 0.06^{b}	73.15 ± 1.80^{b}	72.70 ± 1.08^{b}
T. BIL (µmol/L)	0.17 ± 0.00^{a}	0.87 ± 0.04^{c}	0.6333 ± 0.04^{b}	0.83 ± 0.06^{c}	0.61 ± 0.06^{b}
Uric Acid (µmol/L)	82.89 ± 0.06^{c}	28.27 ± 0.62^{b}	31.43 ± 1.66^{c}	20.18 ± 0.14^{a}	21.30 ± 0.59^{a}
CREA (µmol/L)	9.07 ± 0.00	8.46 ± 0.20^{ab}	7.70 ± 0.38^{a}	8.67 ± 0.27^{b}	9.75 ± 0.32^{c}
ALT	20.30 ± 0.00^{d}	17.58 ± 0.14^{a}	22.84 ± 0.93^{c}	31.75 ± 0.30^{d}	30.39 ± 0.09^{d}
UREA	1.98 ± 0.00^{b}	1.06 ± 0.00^{a}	0.9000 ± 0.07^{a}	3.30 ± 0.12^{c}	3.25 ±0.09^{c}
ALB (g/L)	6.51 ± 0.00^{c}	5.37 ± 0.04^{a}	6.09 ± 0.12^{b}	7.69 ± 0.29^{c}	7.83 ± 0.02^{c}
T.PRO (mg)	12.27 ± 0.00^{bc}	9.37 ± 0.42^{a}	9.8000 ± 0.43^{a}	13.63 ± 0.16^{c}	14.43 ± 0.13^{c}
HDL	0.66 ± 0.00^{b}	0.35 ± 0.01^{a}	0.36 ± 0.02^{a}	0.38 ± 0.00^{ab}	0.40 ± 0.00^{b}
LDL	0.28 ± 0.00^{c}	0.08 ± 0.01^{b}	0.05 ± 0.01^{a}	0.08 ± 0.01^{b}	0.09 ± 0.01^{b}
TCHOL (µmol/L)	0.78 ± 0.00^{c}	0.40 ± 0.05^{a}	0.44 ± 0.02^{a}	0.47 ± 0.01^{a}	0.48 ± 0.00^{a}
TG (µmol/L)	0.34 ± 0.03^{d}	0.21 ± 0.02^{a}	0.22 ± 0.01^{b}	0.19 ± 0.01^{a}	0.22 ± 0.01^{b}
ALP	20.47 ± 0.01^{a}	16.61 ± 1.18^{a}	19.61 ± 0.96^{a}	27.88 ± 1.26^{c}	25.72 ± 0.83^{c}

Values are Mean ± SD, n = 3. Values with different alphabetical superscripts along a row are significantly different at $P < 0.05$.

failure. The induced increase in the level of ALT following Fe exposure could also be indicative of an adaptive response to leakage into the blood stream due to the metal toxicity. ALT is a key metabolic liver enzyme (Adaramoye et al., 2008) released in damaged hepatocytes. Additionally, ALT has a part in transforming protein to glycogen, which is the major reserve fuel of the body during the stress-induced toxicity in the liver. The result indicated that under the influence of Fe inducing stress, the damage of tissues and organs may occur with concomitant elevation and liberation of ALT into the circulation. Fe is known to bind to the sulfydryl groups of enzymes which have the ability to alter the level of ALT activity in the tissues by disrupting their membrane. The 'heavy metals' enters the body by oral gavages and it is delivered to the liver through the portal blood circulation where the greatest part of it remains is stored. Only a small part of the Fe 'breaks the liver barrier' and consequently there will be a discharge of the cell content into the blood stream. The accumulated Fe in the liver can act by directly damaging the hepatocytes, primarily by destroying the permeability of the cell membrane which results in the increased release of cytosolic enzyme; ALT into the circulation. ALT is known to increase only in 'heavy metals' poisoning, toxic hepatosis and muscular dystrophy (Nduka, 1999). It has been reported that ALT is elevated significantly more than AST on heavy metals exposure, which indicates liver damage (Shalan et al., 2005). The increased liver and kidney markers such as ALT, Urea, ALB and T.PRO are suggestive of the fact that it is an indication of liver and kidney damage. This is in agreement with the findings of several researchers (Francielli et al., 2005; Asagba and Eriyamremu, 2007; Abou-Baker et al., 2011) and therefore potential tools as a biomarker of liver damage.

The observed changes in the liver and kidney parameters as early as the first week of exposure for each of the selected heavy metals is an indication that these parameters could serve as a good battery of biomarkers for early detection of pollution associated with heavy metals and their inclusion in monitoring programmes are thus recommended.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Ade-Ademilua for critical review of the manuscript.

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