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Toxicology

Effects of zinc on tissue uptake and toxicity of lead in Sprague Dawley rat

Andrew KATABA[1,2], Shouta M.M. NAKAYAMA[1]*, Yared Beyene YOHANNES[1,3], Haruya TOYOMAKI[1], Hokuto NAKATA[1], Yoshinori IKENAKA[1,4] and Mayumi ISHIZUKA[1]*

[1]Laboratory of Toxicology, Department of Environmental Veterinary Sciences, Faculty of Veterinary Medicine, Hokkaido University, Kita 18 Nishi 9, Kita-Ku, Sapporo, Hokkaido 060-0818, Japan

[2]Department of Biomedical Sciences, School of Veterinary Medicine, The University of Zambia, P.O. Box 32379, Lusaka, Zambia

[3]Department of Chemistry, College of Natural and Computational Science, University of Gondar, P.O. Box 196, Gondar, Ethiopia

[4]Water Research Group, School of Environmental Sciences and Development, North-West University, Private Bag X6001, Potchefstroom, South Africa

ABSTRACT. Lead (Pb) exposure occurs together with other metals including zinc (Zn). This study investigated the impact of Zn on Pb tissue accumulation and Pb-induced toxicities. Animals (n=6 rats per group) were exposed to lead acetate (PbAc) or a combination of PbAc and zinc acetate (ZnAc) under the following groups: control (deionized water), low PbAc [12 mg/kg PbAc (3 mg PbAc/rat/day)], low PbAc–ZnAc [12 mg/kg PbAc (3 mg PbAc/rat/day) + 0.2 mg ZnAc/rat/48 hr], high PbAc [120 mg/kg (30 mg PbAc/rat/day)], and high PbAc–ZnAc [120 mg/kg (30 mg PbAc/rat/day) + 1 mg ZnAc/rat/48 hr] for 8 weeks. A significant reduction in body weight gain was observed in the high PbAc group relative to the control group. Muscles and testes both had reduced and increased Pb uptake in low PbAc–ZnAc and high PbAc–ZnAc groups compared to PbAc only groups, respectively. Bone Pb levels in the high PbAc–ZnAc group were lower than the high PbAc group. Zinc co-administration attenuated Pb-induced inhibition of delta aminolaevulinic acid dehydratase enzyme and enhanced catalase enzyme activity at a high level of exposure. Moreover, ZnAc seems to have minimized the effects of Pb-induced mRNA dysregulation in antioxidant and antiapoptotic enzymes encoding genes. Heme oxygenase-1 was downregulated in the kidney and brain in the low PbAc group. Liver glutathione peroxidase and thioredoxin reductase-1 were downregulated in the high PbAc group. These findings suggest that zinc co-administration with lead may partially mitigate against Pb-induced toxicities.

KEY WORDS: lead, oxidative stress, toxicity, zinc co-administration

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Lead (Pb) is a widely recognized metal toxicant that persists in the environment and is known to induce several derangements in biochemical and physiological systems in humans and animals [42]. The most recognized biomarker of effect in Pb exposure is the impairment and inhibition of delta aminolaevulinic acid dehydratase (ALAD) enzyme activity that eventually affects heme synthesis with anemia as sequela [41]. Ingestion of dust or soil containing Pb through hand-to-mouth activities has been suggested as a major route of exposure in children [42]. Once taken into the body via the gastrointestinal tract, Pb is distributed throughout the body to soft tissues such as the kidney, liver, brain, and hard tissues (teeth or bone) through the circulatory system [34]. Hard tissues have been recognized as the preferred storage site for Pb owing to Pb's high affinity for calcified tissues [34].

The most common manifestations of Pb toxicity in human populations are neurological effects in children and renal and cardiovascular effects in adults [44]. Infants and children are highly sensitive to Pb exposure. Scientific evidence indicates that blood Pb levels below the minimum reference level of 5 µg/dl are associated with cognitive and learning deficits [6]. Lead has been linked to the disruption of dopaminergic functions and induction of oxidative stress, which are both hypothesized to cause neurodegenerative age-related diseases [13].

In the natural environment, Pb occurs in combination with other toxic or essential metals such as zinc (Zn), implying that children with hand-to-mouth activities are likely to ingest Pb together with other metals [7]. A unique setup has been reported in

*Correspondence to: Nakayama, S. M. M.: shouta-nakayama@vetmed.hokudai.ac.jp; shoutanakayama0219@gmail.com, Ishizuka, M.: ishizum@vetmed.hokudai.ac.jp

children with Pb blood levels above the 5 µg/dl reference value from Kabwe, Zambia [42]. The Kabwe Pb poisoning situation represents an environmental Pb exposure where both Pb and Zn occur together in high amounts and with both metals being highly bioavailable [9]. The above situation suggests that children with hand-to-mouth activities may be exposed to both metals at the same time.

Dietary zinc supplementation has been reported to be protective against Pb-induced toxicities as well as enhancing the chelation therapy outcomes in leaded rats [11, 30]. Zinc supplementation has further been associated with ameliorative effects against Pb-induced ALAD enzyme activity inhibition, besides its ability to reduce tissue Pb accumulation in rats [3, 20]. Moreover, Zn supplementation was associated with the maintenance of the oxidative stress system integrity during Pb exposure [33]. Zinc protects the antioxidant system directly as an antioxidant or indirectly by stabilizing biochemical molecules of antioxidant enzymes and modulation of molecular gene responses in antioxidant enzymes [30, 33]. Furthermore, Zn promotes normal cellular differentiation and growth, and it is required for the enzymatic activity of more than 300 enzymes in the body [38]. On the other hand, tightly controlled Zn homeostasis in the body systems naturally limits the extent to which dietary Zn supplementation offers protection against Pb exposure [32]. Notwithstanding the above, reports with systematic approaches to unravel the impact of Zn when it occurs with Pb exposure on Pb toxicity are limited.

The study was conducted to investigate the impact of zinc supplementation particularly in mitigating Pb toxicity. Specifically, we investigated tissue Pb accumulation and ensuing Pb toxicity on ALAD and catalase (CAT) enzyme activities, and the oxidative stress molecular gene responses in the liver, kidney, and brain of Sprague-Dawley rats.

MATERIALS AND METHODS

Animal husbandry and exposure of lead and zinc

Animal husbandry and chemical exposures were conducted under ethical conditions approved by Hokkaido University (approval number: 16-0017). Seven weeks-old Sprague Dawley (SD) male rats (n=36) were procured from Sankyo Labo Service Corporation, Inc. (Tokyo, Japan). The rats were kept and housed in 12 Pb-free polypropylene cages in a community of three rats (n=3 per cage) with *ad libitum* access to food (rodent chow, Labo MR Stock, Nosan Corp., Yokohama, Japan) and deionized water. The rats were acclimatized for 7 days prior to the experiment. Two cages with six (n=6) rats were randomly assigned to one of the five exposure levels. There were no significant body weight differences among all the groups prior to exposure.

Two exposure concentrations of lead acetate (PbAc): 100 and 1,000 mg/l PbAc (Wako Pure Chemical Industries, Osaka, Japan) and zinc acetate (ZnAc): 100 and 500 mg/l ZnAc (Kishida Chemical Co., Ltd., Osaka, Japan) were prepared. The PbAc in drinking water daily and 2.0 ml ZnAc/rat once every other day via gastric gavage were administered for eight weeks. The exposure duration and the levels of PbAc and ZnAc in this study were selected based on the effective doses that had been previously reported [3, 15, 39]. The Pb intake per day per rat was calculated using an estimated average daily water consumption of 30 ml [43], rat body weight, and the concentration of the Pb acetate solution. The five levels of exposure included control (deionized water), low PbAc [12 mg/kg PbAc (3 mg PbAc/rat/day)], low PbAc–ZnAc [12 mg/kg PbAc (3 mg PbAc/rat/day) + 0.2 mg ZnAc/rat/48 hr], high PbAc [120 mg/kg (30 mg PbAc/rat/day)], and high PbAc–ZnAc [120 mg/kg (30 mg PbAc/rat/day) + 1 mg ZnAc/rat/48 hr].

Body weight measurements were taken every two weeks and on the day of sacrifice to monitor body weight changes as shown in Fig. 1. After fasting overnight, rats were euthanized under carbon dioxide with sevoflurane. After sacrifice, blood, liver, kidney, brain, thyroid gland, spleen, femur (bone), muscle (*biceps femoris*), and testis were collected for metal and biochemical analyses. Some blood samples were immediately centrifuged for 10 min at 3,000 × *g* to obtain plasma for biochemical analyses. Small pieces of liver, kidney and brain tissues were preserved in RNA Later solution (SIGMA Life Science, St. Louis, MO, USA) for molecular gene analysis. The rest of the tissues were stored at −80°C until analysis.

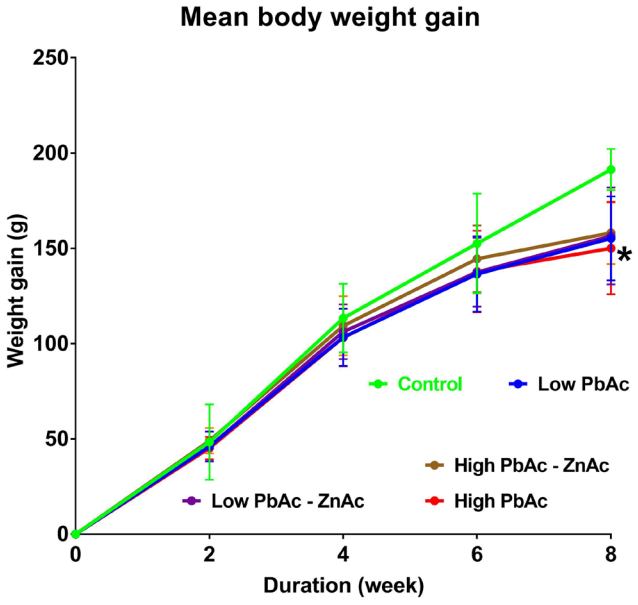

Fig. 1. Rat body weight changes in exposed and control groups during the 8-week exposure period (mean ± SD; n=6, * represent $P<0.05$). Low lead acetate (100 mg/l PbAc), Low lead acetate–zinc acetate (100 mg/l PbAc; 100 mg/l ZnAc), High lead acetate (1,000 mg/l PbAc) and High lead acetate–zinc acetate (1,000 mg/l PbAc; 500 mg/l ZnAc).

Lead tissue quantification

Blood, liver, kidney, brain, thyroid gland, spleen, femur (bone), muscle, and testis were analyzed for Pb metal analysis. Then 0.05−0.2 g soft and hard tissues were cut and oven-dried for 48 hr at 50°C before microwave digestion. The dried samples, and in the case of the blood samples, 0.1 ml blood were placed in separate prewashed digestion vessels. Then 5 ml 30% nitric acid (atomic absorption spectrometry grade, Kanto Chemical, Tokyo,

Japan), and 1 ml 30% hydrogen peroxide (Cica reagent; Kanto Chemical) were added to each vessel that contained tissues. The sample digestion process was done in a closed microwave system using a ramped temperature program (Speed Wave MWS-2 microwave digestion system; Berghof, Eningen, Germany). Following cooling, the sample solutions were transferred into 15 ml polypropylene tubes and diluted to a final 10 ml solution with Milli-Q water.

The Pb tissue quantification was performed using the ICP-MS (7700 series; Agilent Technologies, Tokyo, Japan) as previously described by Nakata *et al.* [24, 25] with some minor modifications. The quality control analysis was performed using DOLT-4 certified reference material (dogfish liver; National Research Council of Canada) and showed good recovery rates (95–105%) for replicate analysis with the instrument detection limit of 0.001 mg/l.

Plasma biochemistry analysis

Plasma samples kept at −80°C were thawed for biochemical analysis using an auto dry chemistry analyzer SPOTCHEM^{TM} SP-4410 (Arkray Inc., Kyoto, Japan). Twelve (12) parameters that included alkaline phosphatase (ALP), lactate dehydrogenase (LDH), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and blood urea nitrogen (BUN), triglycerides, albumin, uric acid, creatine, total cholesterol, total bilirubin, and total protein were measured. A total volume of 100 µl plasma per sample (n=4) was used to measure the above listed parameters using SPOTCHEM^{TM} II strips (Arkray Inc.) following the manufacturer's instructions.

ALAD enzyme, CAT enzyme, total antioxidant capacity and malonaldehyde (MDA) assays

The ALAD enzyme activity assay analysis was carried out following the whole blood ALAD assay protocol according to Espín *et al*. [8] and as previously reported by Nakata *et al.* [25]. From each whole blood sample, 60 µl was obtained and divided into 20 µl volumes that were placed into three separate 1.5 ml Eppendorf tubes for enzyme activity, reactivated enzyme activity and the blank. Then freshly prepared reagents were used to treat the samples following the procedure as was earlier detailed by Nakata *et al.* [25]. The ALAD activity was expressed in µmol porphobilinogen (PBG)/hr/l blood.

The CAT activity assay was performed using a modification of the colorimetric method described by Sinha *et al*. [36]. Briefly, 50 mg rat liver tissue was homogenized in 50 mM PBS (pH 7.0) and centrifuged at 16,000 × *g* for 45 min. Then the supernatant was obtained and used as the enzyme source. The reaction mixture of 2 ml PBS, 0.45 ml hydrogen peroxide (30% H_2O_2) (Kanto Chemical Co., Tokyo, Japan) and 0.025 ml supernatant was constituted. The absorbance was read at 570 nm against the blank using an ultraviolet spectrophotometer. The enzyme activity was expressed as micromoles (µmol) of H_2O_2 consumed per minute.

The total antioxidant capacity was performed following a ferric reduction power (FRAP) method previously described by Benzie and Stain [5]. Pure grades of freshly prepared reagents were used. First, 300 mM acetate buffer (pH 3.6) was prepared from sodium acetate (Wako Pure Chemical Industries, Osaka, Japan) and acetic acid (Wako Pure Chemical Co.). Followed by the preparation of 30 ml FRAP working reagent mixture of 25 ml 300 mM acetate buffer (pH 3.6), 2.5 ml 2,4,6- Tris (2-pyridyl)-S-Triazine)$_2$ abbreviated as TPTZ (Tokyo Chemical Industry Co., Tokyo, Japan) and 2.5 ml ferric chloride hexahydrate ($FeCl_3.6\ H_2O$) (Nacalai Tesque, Kyoto, Japan). Then the assay was carried out in a 96 well plate. To each well, 65 µl 300 mM acetate buffer was added followed by 5 µl ferrous sulphate heptahydrate ($FeSO_4.7H_2O$) (Nacalai Tesque) standard solution and 300 mM acetate buffer as a blank. Then 5 µl plasma sample was added to the sample wells, followed by 100 µl FRAP working reagent across the wells. The absorbance was measured using an enzyme-linked immunosorbent assay plate reader at 595 nm. The reaction was monitored for 8 min and readings at the 4th min were taken as optimal reaction values. A standard curve plot based on the differences in absorbance at 595 nm was used to estimate the total antioxidant capacity for each sample and the capacity was expressed as µM of ferrous iron (Fe (II)) equivalent.

The MDA concentration in liver tissue was assayed by the thiobarbituric acid (TBA) reaction as described by Ohkawa *et al.* [28] and Lakshmi *et al*. [19] with minor modifications: Briefly, 1 g rat liver tissue was homogenized in 10 ml 0.1 M phosphate buffer (pH 7.0) that contained 150 mM potassium chloride (Kanto Chemical Co.) followed by incubation for 30 min at 37°C. Then 1 ml tissue homogenate and 1 ml 10% trichloroacetic acid (Wako Pure Chemical Co.) were mixed and centrifuged at 3,500 × *g* for 10 min. The supernatant was obtained and kept as the reaction source. A final reaction mixture of 1.0 ml supernatant and 1 ml 0.65% TBA (Nacalai Tesque) was constituted and incubated in a hot water bath (65°C) for 15 min. The absorbances were then read at 535 nm against the blank using an ultraviolet spectrophotometer (Shimadzu UV-2600, Shimadzu Inc., Kyoto, Japan). The values of MDA were expressed in nmol of MDA /g sample tissue weight.

Oxidative stress and related gene analyses in liver, kidney and brain tissues

Total RNA from the liver, kidney and brain tissues was extracted using a modification of two protocols. The initial extraction steps followed the Trizol method. Briefly, about 30 mg samples were homogenized in TRI Reagent® (SIGMA Life Science, St. Louis, MO, USA) using a Qiagen TissueLyser (Qiagen RetSch, Hilde, Germany). Then 0.2 ml chloroform (Kanto Chemical Co., Tokyo, Japan) was added, and samples were vortexed followed by centrifugation at 13,000 × *g* for 20 min at 4°C. The supernatant was then mixed with 350 µl 70% ethanol (Kanto Chemical Co.) and the rest of the steps were done according to the NucleoSpin® kit (MACHEREY-NAGEL, Düren, Germany) protocol with strict adherence to the manufacturer's instruction. The cDNA (20 ng of cDNA) was synthesized with the TOYOBO cDNA kit using the ReverTra Ace® qPCR RT Master Mix with gDNA Remover (TOYOBO Co., Ltd., Life Science Department, Osaka, Japan). Most of the antioxidants and related gene primer sets used in this study were obtained from previously published works [1, 14] as shown in Table 1. The real-time quantitative PCR was done using the StepOnePlus Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). The total PCR reaction mixture of

Table 1. Antioxidant and related gene primers used for real quantitative polymerase chain reaction analyses

Gene	Accession No.	5′-Forward primer-3′	5′-Reverse primer-3	Product size	Primer efficiency (%)	Reference
ACTB	XM_039089807.1	AAGTCCCTCACCCCTCCAAAAG	AAGCAATGCTGTCACCTTCCC	98	98.4	[2]
SOD1	NM_017050.1	CTGAAGGCGAGCATGGGTTC	TCTCTTCATCCGCTGGACCG	120	104.0	NCBI
CAT	NM_012520.2	GCCCTCTTGCCTCACGTTCT	ACATCGGGTTTCTGAGGGGC	126	97.6	NCBI
GPX	NM_030826.4	TCCCGTGCAATCAGTTCGGA	GGTAAAGAGCGGGTGAGCCT	155	100.0	NCBI
HO-1	NM_012580.2	ACACGGGTGACAGAAGAGGCTAA	CTGTGAGGGACTCTGGTCTTTG	108	96.8	NCBI
TXNRD1	NM_001351984.1	GTCACACCAACTCCTCTCGG	TGTGTCCTCGAGTTTCCAGC	150	100.0	NCBI
NRF2	XM_006234398.3	GCACATCCAGACAGACACCA	CTCTCAACGTGGCTGGGAAT	189	100.0	[13]
Keap1	NM_057152.2	CAGATTGACAGCGTGGTCCG	TGAAGAACTCCTCCTCCCCGA	166	94.5	NCBI
TGFB1	NM_021578.2	AGGGCTACCATGCCAACTTC	CCACGTAGTAGACGATGGGC	168	100.0	[13]
NFKB	NM_199267.2	CATACGCTGACCCTAGCCTG	TCACTGAGCTCCCGATCAGA	79	100.0	[13]
Bax	NM_017059.2	AGGACGCATCCACCAAGAAG	CAGTTGAAGTTGCCGTCTGC	166	98.0	[13]

Key: (*ACTB*): Beta Actin, (*SOD1*): Superoxide dismutase 1, (*CAT*): catalase; (GPX): Glutathione peroxidase; (*HO-1*): Heme oxygenase-1, (*TXNRD1*): Thioredoxin reductase 1, (*NRF2*): Nuclear factor erythroid 2-related factor 2; (*Keap1*): Kelch-like ECH-associated protein,1; (*TGFB1*): Transforming growth factor beta 1; (*NFKB*): nuclear factor kappa-light-chain-enhancer of activated B cells; (*Bax*): Bcl-2 Associated X-protein.

10 µl that contained 5 µl Fast SYBR Green Master Mix (Applied Biosystems), 0.4 µl 5 µM forward and reverse primers (Thermo Fisher Scientific, Life Technologies Japan Ltd., Tokyo, Japan), 2 µl cDNA sample template, and 2.2 µl RNase-free water was used. The following qPCR conditions: 95°C for 20 sec, followed by 40 cycles of 95°C for 3 sec, 60°C for 30 sec were used. All gene expression analyses followed the relative absolute method (the $2^{-\Delta\Delta CT}$ method) and were normalized with beta-actin [23].

Statistical data analysis

The data analysis was performed using GraphPad Prism software (Prism 7 for Windows; Version 5.02, San Diego, CA, USA) and the data were summarized as means and standard deviation (mean ± SD). Since the data did not follow a normal distribution, the log-transformation of data was done for the purposes of statistical analyses and retained the original values in the results and figures for easier interpretation and comparisons with other studies. The same approach was used for gene expression data. In the present study, differences between groups were deemed to be significant at $P<0.05$ (*) using multiple Tukey's comparison test. The lower-case alphabets (a, b, c) were used to represent significant differences between or among the groups. The graphical representations were compiled using GraphPad Prism software.

RESULTS

Lead exposure effects on body weight gain

Figure 1 depicts the changes in body weight in exposed and control rats. In general, rats in both groups gained weight steadily over time. However, a general decrease in body weight gain was observed in the exposed groups compared to the control group after the sixth week. By the end of the 8th week, the high PbAc exposed group showed a significant reduction compared to the control ($P<0.05$) group. No significant differences in body weight gain were observed among the exposed groups.

Lead tissue accumulation pattern

The tissue Pb accumulation patterns are shown in Figs. 2 and 3. In general, tissue Pb accumulation followed a dose-dependent pattern. Figure 2 shows the Pb accumulation patterns in the blood, liver, kidney, and brain tissues. Compared to the control group, the exposed groups accumulated significantly much more Pb ($P<0.05$). However, no significant differences were observed between the PbAc and the PbAc–ZnAc groups. Figure 3 shows Pb distribution in muscle, spleen, thyroid gland, testis and bone. In the testes and muscles, significant tissue Pb accumulation differences were found between PbAc only and PbAc with ZnAc co-administered groups. At the low level of exposure, the testes and muscle from the low PbAc–ZnAc group had much less Pb than the corresponding PbAc only groups. At the high level of exposure, the zinc supplemented groups accumulated much more Pb than the high PbAc only group in both muscles and testes (Fig. 3). In the case of bone tissue, Pb accumulation was significantly different between the high PbAc and high PbAc–ZnAc groups, with the former accumulated much Pb (Fig. 3). No significant differences in bone Pb accumulation were observed at the lower level of exposure.

Effects of lead on plasma biochemistry

In the present study, plasma biochemistry analyses were conducted to monitor hepatic or renal damage following Pb exposure with Zn co-administration. Table 2 shows the various parameters that were considered. No significant changes were observed in most of the parameters including renal and hepatic enzymes markers among the exposed groups compared to the control group. The only significant change observed was in the levels of BUN. The BUN levels were significantly ($P<0.05$) reduced in some exposed groups relative to the control group. Quantitively, the BUN levels were lowest in the high PbAc group followed by other groups as shown in the indicated order relative to the control: high PbAc >high PbAc–ZnAc >low PbAc–ZnAc.

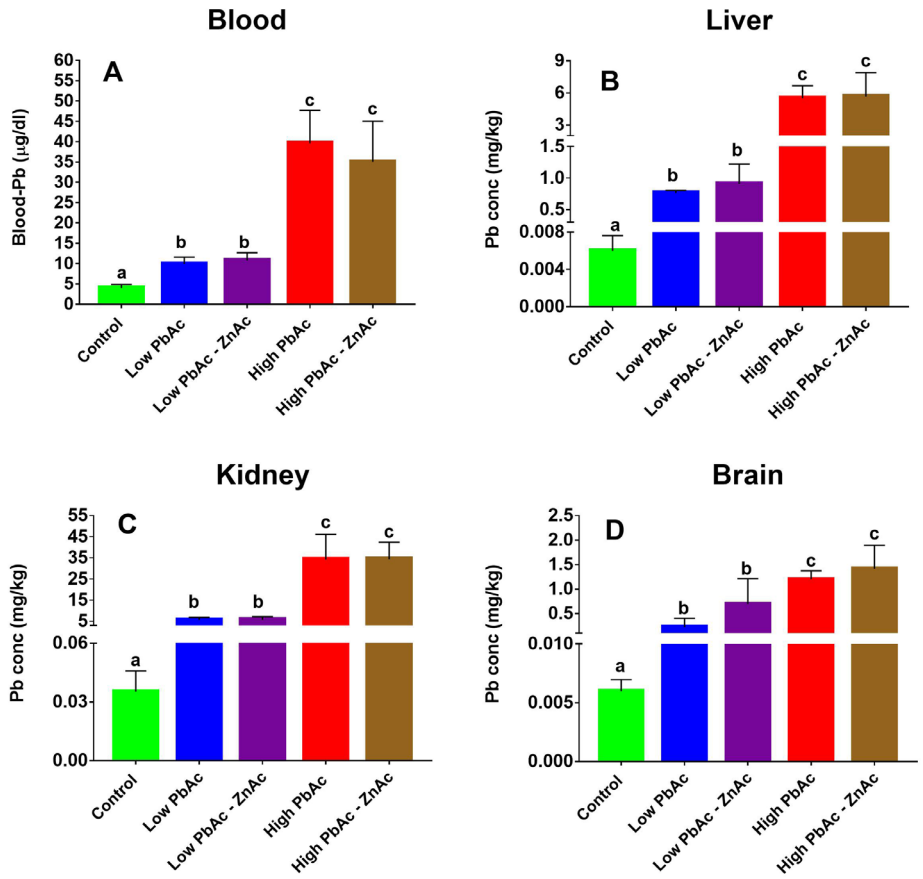

Fig. 2. Lead accumulation in blood (n=6), liver (mg/kg), kidney (n=3) and brain (n=3). (Graph: Mean ± SD, n=6, $^{a-c}$ represents $P<0.05$). Shared letter symbols among groups indicate no significant difference and different letter symbols among groups denote significant difference.

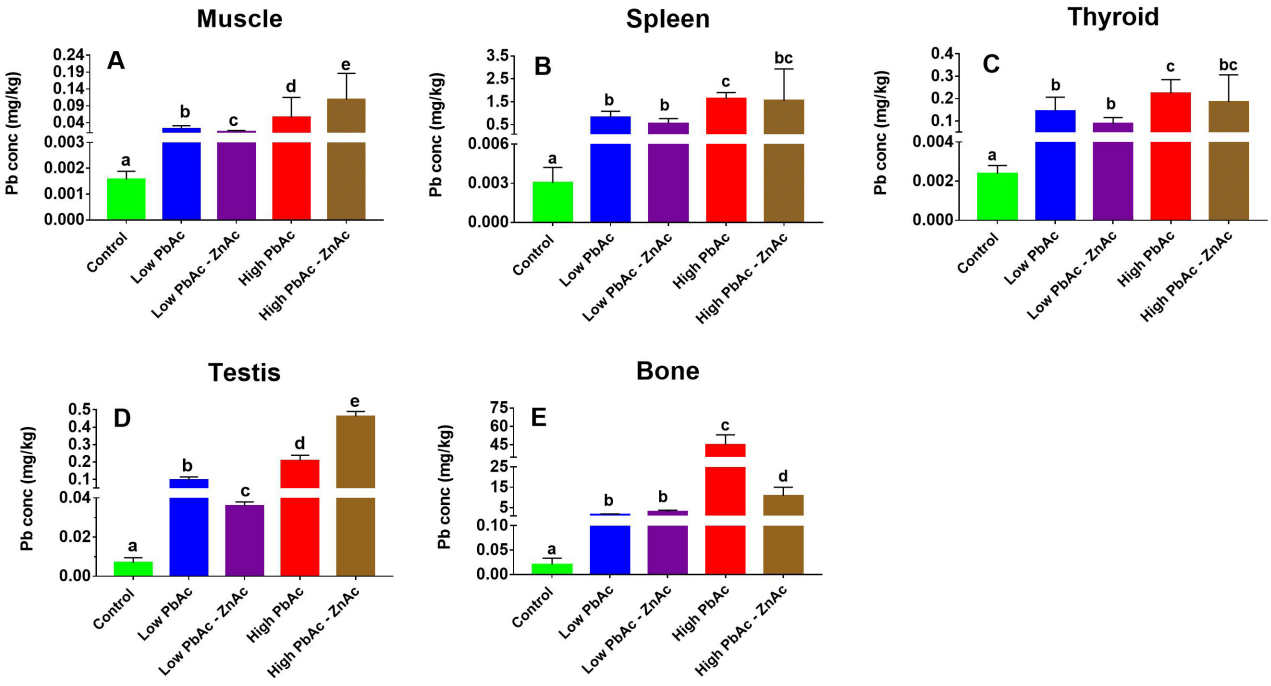

Fig. 3. Lead accumulation of in muscle (n=6), spleen (n=3), thyroid gland (n=6), testes (n=3) and bone (n=6). (Graph: Mean ± SD, n=6, $^{a-c}$ represents $P<0.05$). Shared letter symbols among groups indicate no significant difference and different letter symbols among groups denote significant difference.

Table 2. Plasma biochemistry parameters

Parameter/Group	Control	Low PbAc	Low PbAc–ZnAc	High PbAc	High PbAc–ZnAc
Total bilirubin (mg/dl)	0.4 ± 0.12	0.5 ± 0.14	0.5 ± 0.10	0.4 ± 0.15	0.5 ± 0.29
GOT (IU/l)	57 ± 11.0	67 ± 16.4	75 ± 34.4	66 ± 14.2	88 ± 33.3
GPT (IU/l)	46 ± 9.0	54 ± 12.4	52 ± 22.5	38 ± 11.2	48 ± 23.4
LDH (IU/l)	627 ± 252.3	896 ± 365.8	852 ± 539.4	1,064 ± 431.3	740 ± 234.0
ALP (IU/l)	425 ± 83.2	360 ± 162.3	435 ± 303.7	276 ± 43.6	354 ± 78.7
Triglycerides (mg/dl)	202 ± 43.60	166 ± 79.60	191 ± 68.03	214 ± 99.84	192 ± 77.80
Total cholesterol (mg/dl)	75 ± 6.5	66 ± 11.7	75 ± 11.8	51 ± 24.9	50 ± 12.6
Total protein (g/dl)	6.3 ± 0.70	6.6 ± 0.77	6.9 ± 0.75	6.8 ± 0.35	6.4 ± 0.63
Albumin (g/dl)	3.7 ± 0.13	3.6 ± 0.30	3.7 ± 0.45	3.7 ± 0.19	3.7 ± 0.57
Uric acid (mg/dl)	5.9 ± 1.5	6.7 ± 2.2	6.3 ± 2.5	5.7 ± 1.9	6.6 ± 1.6
Creatine (mg/dl)	1.1 ± 0.16	1.2 ± 0.25	1.2 ± 0.15	1.0 ± 0.22	1.0 ± 0.26
BUN (mg/dl)	23 ± 0.89^{a}	21 ± 1.83^{ab}	20 ± 0.98^{bc}	17 ± 1.47^{c}	18 ± 1.55^{cd}

[a–d] Represents $P<0.05$; Tukey's multiple comparison test. Shared letter symbols among groups indicate no significant difference and different letter symbols among groups denote the significant difference. *Key*: (ALP): alkaline phosphatase, (LDH): lactate dehydrogenase, (BUN): blood urea nitrogen, (GOT): glutamic oxaloacetic transaminase, (GPT): glutamic pyruvic transaminase.

Effects of exposure on ALAD enzyme, CAT enzyme, the total antioxidant capacity and MDA

The ALAD enzyme activity in blood is shown in Fig. 4A. Significant ALAD enzyme activity inhibition was observed in the high PbAc only group compared to the control and the low PbAc group ($P<0.05$). Among the PbAc and PbAc with ZnAc groups, the high PbAc group had significantly reduced ALAD activity compared to the high PbAc–ZnAc group only ($P<0.05$).

Figure 4B shows CAT enzyme activity. The CAT activity was significantly elevated in the low PbAc and high PbAc–ZnAc groups compared to the control ($P<0.05$). Among the exposed groups, the CAT enzyme activity in the high PbAc–ZnAc group was significantly increased than that of the high PbAc only group ($P<0.05$).

The total antioxidant capacity in plasma samples is shown in Fig. 4C. No significant differences were observed between the control and the exposed groups. Similarly, no significant differences were observed among the exposed groups.

The MDA concentrations were assayed as a measure of lipid peroxidation (Fig. 4D). In the present study, there were no significant differences in the MDA levels among all the groups.

Liver mRNA expression levels

Gene expression of some essential antioxidant liver enzymes are shown in Fig. 5. The superoxide dismutase (*SOD1*) was upregulated in the low PbAc–ZnAc and high PbAc groups. The BCL-2 associated X protein (*Bax*) gene mRNA levels were significantly upregulated in the low PbAc–ZnAc group compared to the control group. On the other hand, the following genes were downregulated namely, thioredoxin reductase 1 (*TXNRD1*) in the low PbAc–ZnAc and high PbAc groups, heme oxygenase 1 (*HO-1*) in the low PbAc–ZnAc, high PbAc and high PbAc–ZnAc groups, glutathione peroxidase (*GPX*) in the high PbAc group, Kelch Like ECH Associated Protein 1 (*Keap1*) in the high PbAc group, nuclear factor erythroid 2-related factor 2 (*NRF2*) in the high PbAc and high PbAc–ZnAc groups, *Bax* in the high PbAc group and nuclear factor kappa-light-chain-enhancer of activated B cells (*NF-KB*) in the high PbAc and high PbAc–ZnAc groups compared with the control group. Among the exposed groups, Zn co-administration restored the downregulation of the mRNA levels of *TXNRD1*, *GPX*, *Keap1*, *NF-KB* and *Bax* genes at the high level of exposure (Fig. 5).

Kidney mRNA expression levels

Figure 6 shows the mRNA expression levels of the antioxidants and related genes following exposure in the kidney. Gene expression upregulation was observed in the *SOD1* gene in the high PbAc–ZnAc groups. Both *Keap1* and *NF-KB* genes were also upregulated in the low PbAc–ZnAc group compared to the control. Lead exposure downregulated gene expression of *HO-1* (low PbAc), *Keap1* (high PbAc), *NF-KB* (high PbAc) and *NRF2* (high PbAc and high PbAc–ZnAc) in groups shown in parentheses compared to the control group.

Brain mRNA expression levels

Antioxidant and related gene expression levels in the brain tissue are shown in Fig. 7. Exposure to PbAc upregulated the mRNA levels of *HO-1* in the low PbAc–ZnAc, high PbAc and high PbAc–ZnAc groups. Furthermore, exposure downregulated *CAT* gene expression in the low PbAc, high PbAc and high PbAc–ZnAc groups. Other genes that were downregulated included *TXNRD1* (low PbAc group), *Keap1* (low PbAc and high PbAc–ZnAc groups), *TGFB1* (low PbAc and low PbAc–ZnAc groups), and *NRF2* (low PbAc group).

DISCUSSION

The present study investigated the impact of Zn supplementation with Pb on tissue Pb accumulation and the ability of Zn to

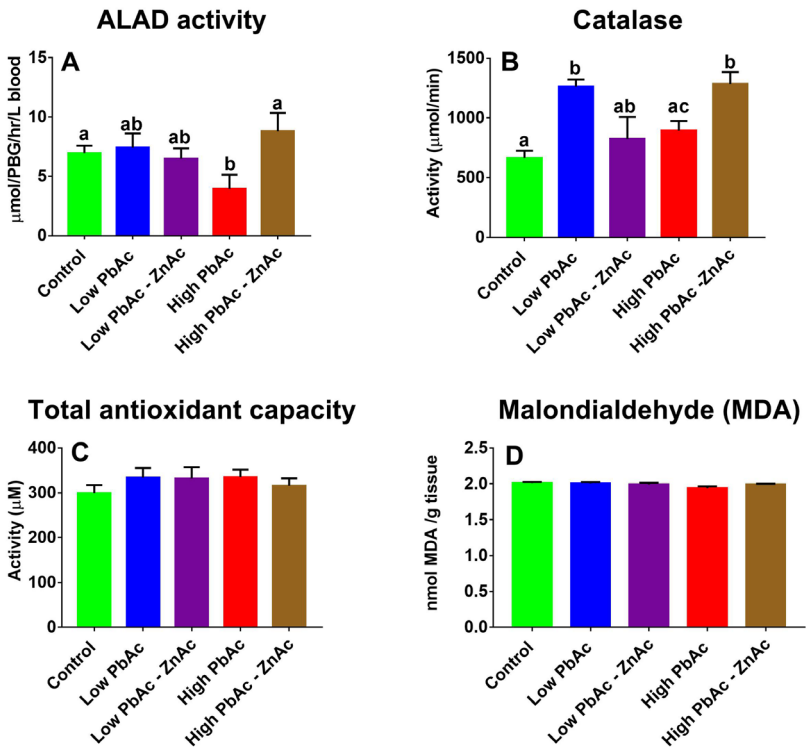

Fig. 4. Biomarkers of lead and lead/zinc exposure effects. **A.** Delta-aminolaevulinic acid dehydratase (ALAD) activity in whole blood (n=5), **B.** Catalase activity in liver tissue (n=3), **C.** Total antioxidant capacity of plasma, **D.** Malondialdehyde levels in liver tissue (n=3). (All data is presented as Mean ± SD, $^{a-c}P<0.05$, Tukey's multiple test). Shared letter symbols among groups indicate no significant difference and different letter symbols among groups denote significant difference.

mitigate against Pb-induced toxicity in rats. Lead exposure affected body weight gain in exposed rats. Interestingly, at the high level of exposure, significant reduction in body weight gain was observed in the high PbAc group only compared to the control group. Lead has been reported to cause reduced weight gain and stunted growth [17]. Our findings were in tandem with Kang *et al.* [17] that reported significant body weight gain reduction at a high dose (10 mg/ml PbAc), but no effect at a medium dose (3 mg/ml PbAc) and low dose (1 mg/ml PbAc) in rats administered via distilled drinking water for 8 weeks. Whereas the mechanisms behind Pb-induced body weight gain reduction without accompanying biochemical changes remain unclear, Pb-induced Zn deficiencies have been implicated in reduced body weight gain [12]. Moreover, Zn supplementation in animals has been linked to an increased appetite for food, promotion of normal growth and ability to protect gut microvilli and gut microbiota against Pb-induced toxicity [27, 39].

Zinc supplementation has been suggested to partially minimize the Pb tissue uptake and mitigate against Pb-induced toxicity. However, the specific mechanisms by which this is achieved remain unclear [3, 31, 41]. Some authors indicate that the reduced tissue Pb uptake is due to the competitive interaction of Pb and Zn divalent ions at absorption sites in the gastrointestinal tract into systemic circulation and transportation into tissues [3, 41]. In our study, zinc supplementation was associated with reduced accumulation of Pb in testes and muscles at low exposure and an increased Pb accumulation at high exposure in both tissues. The Pb accumulation pattern at low exposure in the testes agreed with what was reported by Batra *et al.* [3], in which Zn supplementation prevented testicular tissue damage. In contrast, at the high level of exposure, Zn supplementation seemed to have enhanced testicular tissue Pb uptake, a finding that was in tandem with a report in rats [31]. The possible oversaturation of the molecular binding ligands with Pb at the absorption sites at a high level of exposure could have minimized the effectiveness of the Pb/Zn interactions to reduce Pb tissue uptake [31]. In the case of bone tissue, Pb uptake was significantly reduced in Zn supplemented group at a high level of exposure only, a consistent finding with what was reported by Batra *et al.* [3]. The reduced Pb uptake by bone tissue would further reduce the total Pb body burden and minimize endogenous Pb poisoning associated with metabolic remobilization of minerals such as calcium in normal growth and physiological demands in pregnant and lactating animals [3]. In the present study, at both levels of exposure, the blood, liver, kidney and brain Pb uptake were not affected by Zn co-administration. This suggests Zn supplementation could not fully prevent the distribution of Pb to other organs or tissues through systemic circulation [31].

In the present study, Pb-induced renal and hepatic toxicities were not pronounced as evidenced by lack of changes in primary biochemical parameters of the liver or kidney and related enzymes, plasma biochemistry, MDA and the total antioxidant capacity [41]. This suggests that the Pb tissue burden may not have been severe to elicit overt tissue damage. However, low BUN levels were observed across all exposed groups, indicative of mild signs of Pb-induced hepatotoxicity [29].

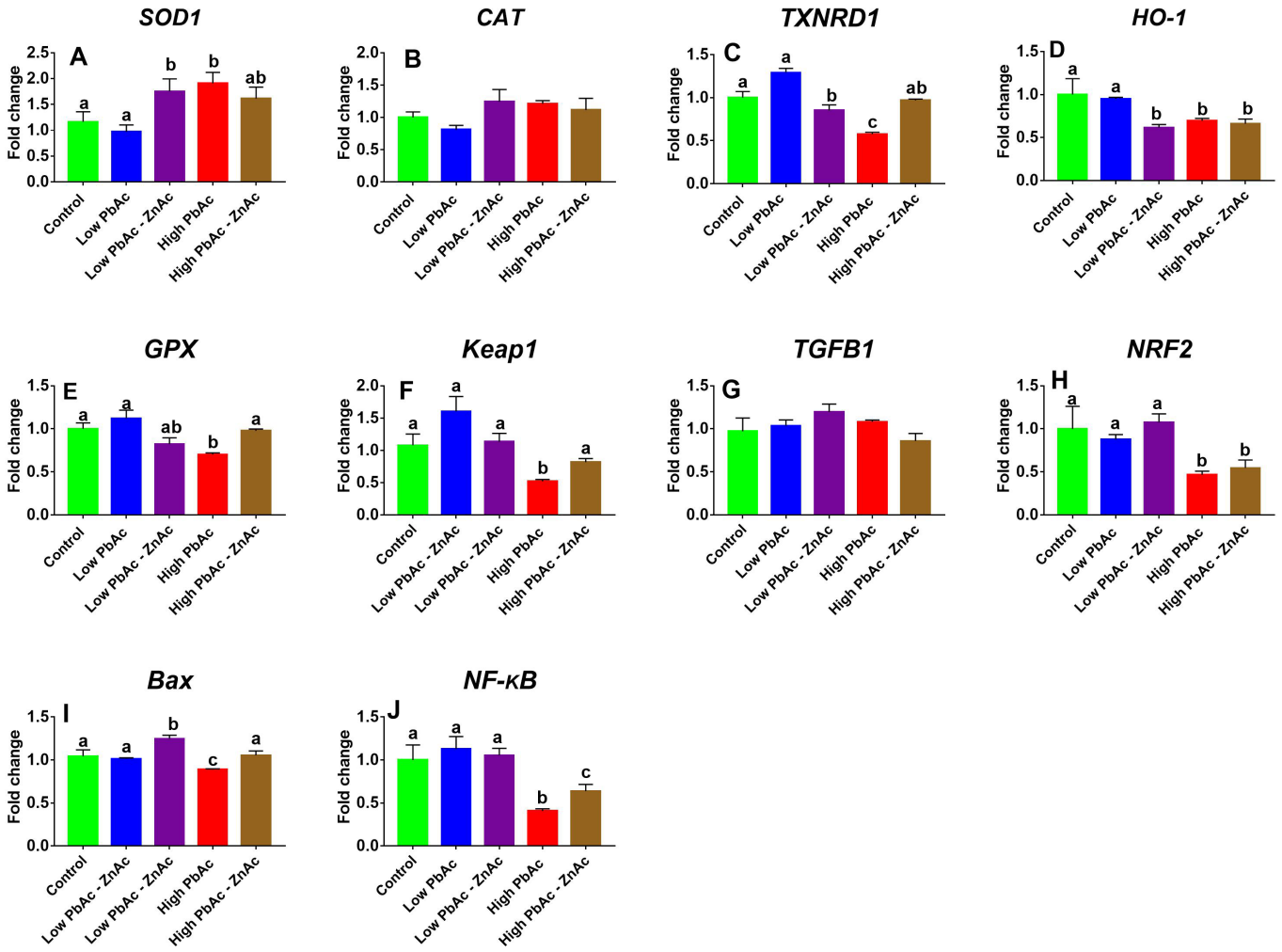

Fig. 5. Gene expression of antioxidant and related enzymes in the liver (n=3; [a–c] represents $P<0.05$). Shared letter symbols among groups indicate no significant difference and different letter symbols among groups denote significant difference. **A.** (*SOD1*): Superoxidase dismutase 1, **B.** (*CAT*): Catalase; **C.** (*TXNRD1*): Thioredoxin reductase 1, **D.** (*HO-1*): Heme oxygenase-1, **E.** (*GPX*): Glutathione peroxidase; **F.** (*Keap1*): Kelch-like ECH-associated protein,1, **G.** (*TGFB1*): Transforming growth factor beta 1; **H.** (*NRF2*): Nuclear factor erythroid 2-related factor 2, **I.** (*Bax*): Bcl-2 Associated X-protein, **J.** (*NF-KB*): Nuclear factor kappa-light-chain-enhancer of activated B cells.

Lead possesses a very high affinity for sulfhydryl (SH) functional groups [16]. Thus, the inhibition of several essential antioxidant enzymes such as ALAD enzyme, CAT, GPX, superoxide dismutase (SOD) by Pb via the SH group attachment is postulated to be one of the mechanisms of Pb toxicity [44]. In the present study, Zn co-administration partially mitigated against blood ALAD enzyme activity inhibition at a high level of exposure. The lack of Pb-induced ALAD enzyme activity inhibition effects at a low level of exposure in the current study agreed with Flora *et al.* [11] who reported lack of ALAD enzyme activity inhibition effects in rat blood with Pb levels below 15 µg/dl. On the other hand, the observed ALAD enzyme activity inhibition effects at the high level of exposure were consistent with a report that showed more pronounced inhibition of ALAD enzyme activity in subjects with blood Pb levels above 25 µg/dl [30]. The protective effects of Zn against Pb-induced inhibition of ALAD enzyme activity are linked to Zn's ability to displace Pb ions from sulfhydryl groups and it being a component of the functional unit of the ALAD enzyme [20].

The CAT is a tetrameric enzyme that is involved in combating oxidative stress tissue damage by catalytically converting hydrogen peroxide into water and an oxygen molecule [26]. Presently, no significant inhibition of the CAT enzyme activity in the presence of Pb ions was observed. However, an increase in the CAT enzyme activity was observed in the low PbAc and high PbAc-ZnAc groups. The increased CAT enzyme activity in the low PbAc exposed group may have been a protective hormetic response [10]. On the other hand, the enhanced CAT enzyme activity response in the high PbAc–ZnAc group could be linked to Zn supplementation [26]. This finding agreed with Barman *et al.* [4] who demonstrated that zinc supplementation was accompanied by an increased CAT enzyme activity in rats [26].

The evidence of zinc involvement in cellular protection and its role in several nuclear enzymatic activities as a transcription factor is beyond contestation [22]. In the present study, we investigated antioxidant and related genes' molecular responses to Pb

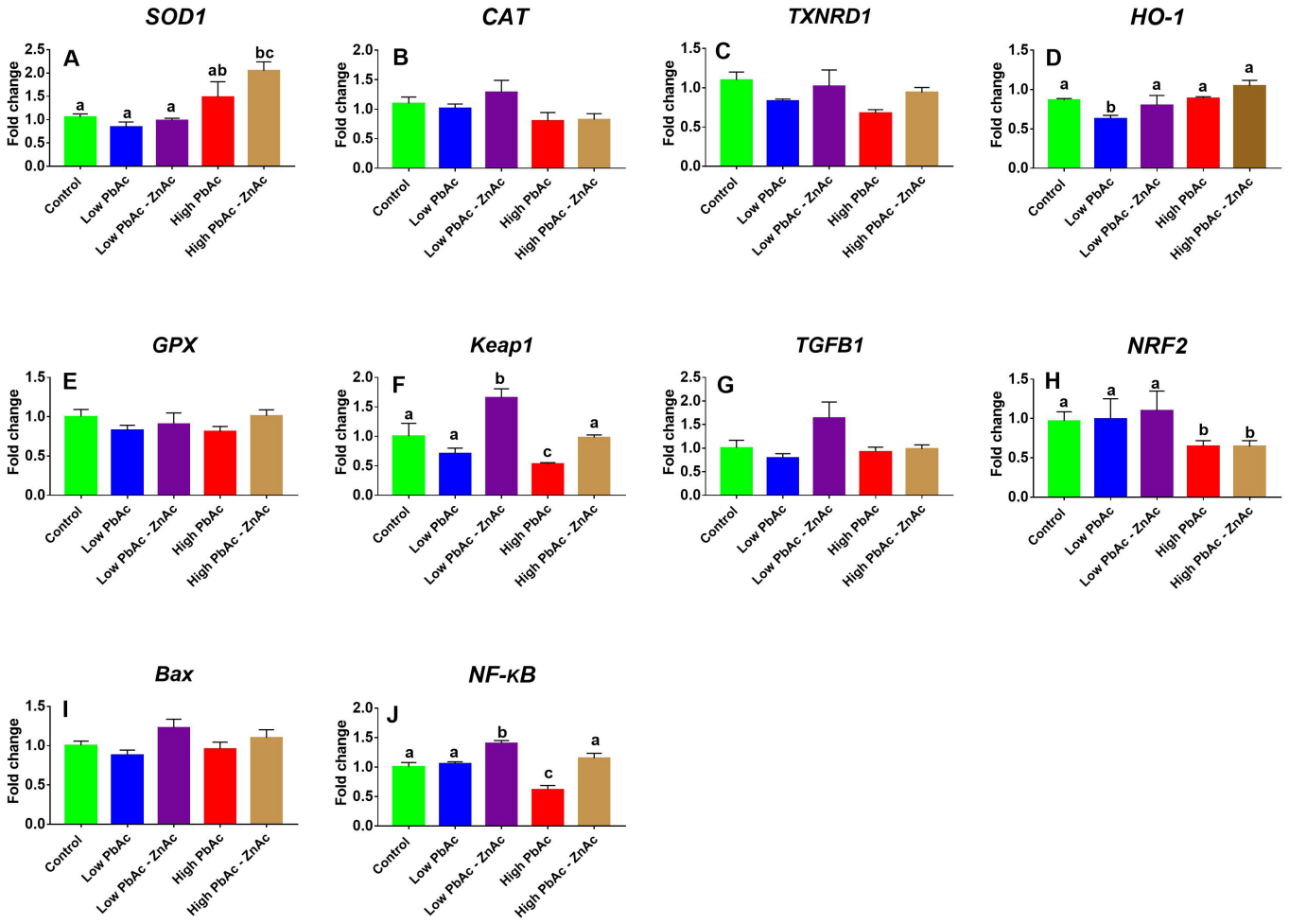

Fig. 6. Gene expression of antioxidant and related gene in the kidney (n=3; $^{a-c}$ represents $P<0.05$). Shared letter symbols among groups indicate no significant difference and different letter symbols among groups denote significant difference. **A.** (*SOD1*): Superoxidase dismutase 1, **B.** (*CAT*): Catalase; **C.** (*TXNRD1*): Thioredoxin reductase 1, **D.** (*HO-1*): Heme oxygenase-1, **E.** (*GPX*): Glutathione peroxidase; **F.** (*Keap1*): Kelch-like ECH-associated protein,1, **G.** (*TGFB1*): Transforming growth factor beta 1; **H.** (*NRF2*): Nuclear factor erythroid 2-related factor 2, **I.** (*Bax*): Bcl-2 Associated X-protein, **J.** (*NF-KB*): Nuclear factor kappa-light-chain-enhancer of activated B cells.

exposure with or without Zn co-administration in the liver, kidney and brain of rats. The liver and kidney were chosen due to their prominent metabolic and excretory functions, respectively [26]. The brain was chosen owing to its reported high sensitivity to Pb-induced oxidative stress toxicity [40]. The protective effect of Zn co-administration against Pb-induced oxidative stress toxicity through the induction of encoding genes of primary antioxidant enzymes such as SOD-1, CAT, HO-1, GPX and TXNRD1 in the liver, kidney and brain was observed. The TXNRD1 enzyme is involved in the protection of the brain tissue against oxidative stress damage [37], hence the observed induction suggests that Zn could play a protective role for the brain tissue under Pb toxicity. Moreover, Zn protection against oxidative stress tissue damage occurs in various ways. Zinc plays a role as a co-factor and as a component of essential antioxidant metalloenzymes such as GPX and SOD enzymes. Furthermore, Zn acts to stabilize cell membranes against oxidative stress and induces the expression of metallothionein, a small protein that sequesters Pb from tissues and acts as an antioxidant [38]. In our study, zinc co-administration was protective against the oxidative stress response regulating gene, Kelch-like ECH-associated protein 1 (Keap1). Keap1 is a cysteine-rich component of the Keap1-NRF2 complex that regulates cytoprotection against reactive oxygen species through the activation of an ameliorative antioxidant response [18]. This induction of *keap1* could protect against oxidative stress tissue damage by preserving the transcription and translation of antioxidant enzymes, boosting the intracellular glutathione levels and prevent protein oxidation through the Keap1-NRF2 signalling pathway [35].

Lead-induced apoptosis is the major mechanism accounting for cellular and tissue damage [12]. In our study, the apoptosis inhibitor gene, the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-KB) mRNA expression levels were induced in the Pb with Zn co-administered groups in the liver and kidney suggestive of antiapoptotic protective response [2]. This finding agrees with Leccia *et al.* [21] that reported Zn had a significant contribution towards stabilizing the factors that control apoptosis including *NF-KB* a complex protein that regulates the deoxyribonucleic acid transcription. This implies that the dysregulation in the

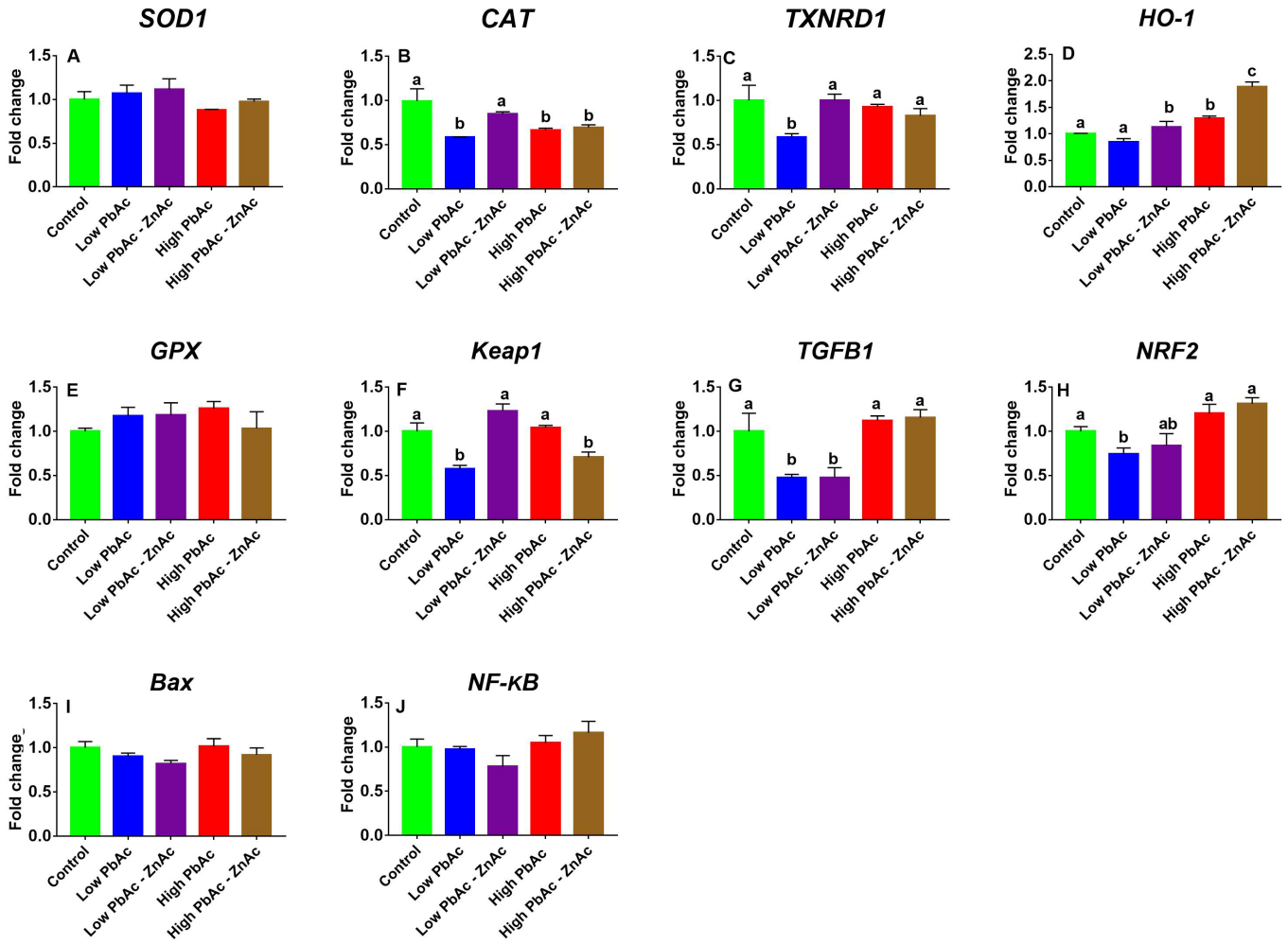

Fig. 7. Gene expression of antioxidant and related gene in the brain (n=3; [a–c] represents $P<0.05$). Shared letter symbols among groups indicate no significant difference and different letter symbols among groups denote the significant difference. **A.** (*SOD1*): Superoxidase dismutase 1, **B.** (*CAT*): Catalase; **C.** (*TXNRD1*): Thioredoxin reductase 1, **D.** (*HO-1*): Heme oxygenase-1, **E.** (*GPX*): Glutathione peroxidase; **F.** (*Keap1*): Kelch-like ECH-associated protein,1, **G.** (*TGFB1*): Transforming growth factor beta 1; **H.** (*NRF2*): Nuclear factor erythroid 2-related factor 2, **I.** (*Bax*): Bcl-2 Associated X-protein, **J.** (*NF-KB*): Nuclear factor kappa-light-chain-enhancer of activated B cells.

expression of *NF-KB* encoding genes could eventually promote enhanced cellular apoptosis [2].

Taken together, our study has demonstrated the impact of Zn co-administration with Pb in ameliorating Pb-induced toxicities. While the impact of Zn co-administration on reducing tissue Pb uptake was lacking in the blood, liver, kidney and brain, Zn conferred ameliorative benefits in the supplemented groups. For instance, despite the non-significant differences in the blood and liver tissue Pb accumulation at the high level of exposure, the ALAD enzyme activity was significantly inhibited in the high PbAc only groups and not in the high PbAc–ZnAc groups. Zinc's versatile properties in preserving enzymatic sulfhydryl groups through either direct binding to the thiol group, steric hindrance (binding near the thiol group) or a conformational change of the thiol structures seem to have preserved the ALAD enzyme sulfhydryl groups and their activity in the high PbAc–ZnAc group [32]. Moreover, Zn increased the catalase enzyme activity in the liver of high PbAc-ZnAc exposed rats as an indirect protective response against Pb-induced oxidative stress [31]. Furthermore, the restorative inductions of antioxidant and antiapoptotic molecular gene responses were observed in zinc co-administered groups in our study.

In conclusion, the current study revealed that the protective effects of Zn on Pb tissue accumulation and Pb toxicity might depend on the quantities of the metals ingested. Whereas Zn supplementation may reduce tissue Pb accumulation in some tissues at low levels, the reverse may happen with an increased level of exposure. Moreover, Zn co-administration partially mitigated against Pb-induced inhibition of ALAD enzyme activity and enhanced catalase enzyme activity at a high level of exposure. Zinc's restorative effects against Pb-induced mRNA dysregulation in antioxidant enzymes encoding genes were observed. Further studies are required to investigate the Zn dose effect that seems to undo the beneficial effects of Zn supplementation in Pb exposure. Furthermore, it must be noted that Zn in Pb and Zn co-exposure scenarios may partially attenuate Pb-induced toxicities and not fully protective.

CONFLICT OF INTEREST. The authors declare no conflict of interest in the current study.

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REFERENCES

1. Albertini, R., Aimbire, F., Villaverde, A. B., Silva, J. A. Jr. and Costa, M. S. 2007. COX-2 mRNA expression decreases in the subplantar muscle of rat paw subjected to carrageenan-induced inflammation after low level laser therapy. *Inflamm. Res.* **56**: 228–229. [Medline] [CrossRef]
2. Babaei, M. A., Huri, H. Z., Kamalidehghan, B., Yeap, S. K. and Ahmadipour, F. 2017. Apoptotic induction and inhibition of NF-KB signaling pathway in human prostatic cancer PC3 cells by natural compound 2,2′-oxybis (4-Allyl-1-Methoxybenzene), Biseugenol B, from litsea costalis: an *in vitro* study. *OncoTargets Ther.* **10**: 277–294. [CrossRef]
3. Batra, N., Nehru, B. and Bansal, M. P. 1998. The effect of zinc supplementation on the effects of lead on the rat testis. *Reprod. Toxicol.* **12**: 535–540. [Medline] [CrossRef]
4. Barman, S. and Srinivasan, K. 2017. Attenuation of oxidative stress and cardioprotective effects of zinc supplementation in experimental diabetic rats. *Br. J. Nutr.* **117**: 335–350. [Medline] [CrossRef]
5. Benzie, I. F. F. and Strain, J. J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* **239**: 70–76. [Medline] [CrossRef]
6. Dapul, H. and Laraque, D. 2014. Lead poisoning in children. *Adv. Pediatr.* **61**: 313–333. [Medline] [CrossRef]
7. de Queiroz, C. A. A., Fonseca, S. G. C., Frota, P. B., Figueiredo, Í. L., Aragão, K. S., Magalhães, C. E. C., de Carvalho, C. B. M., Lima, A. Â. M., Ribeiro, R. A., Guerrant, R. L., Moore, S. R. and Oriá, R. B. 2014. Zinc treatment ameliorates diarrhea and intestinal inflammation in undernourished rats. *BMC Gastroenterol.* **14**: 136. [Medline] [CrossRef]
8. Espín, S., Martínez-López, E., Jiménez, P., María-Mojica, P. and García-Fernández, A. J. 2015. Delta-aminolevulinic acid dehydratase (δALAD) activity in four free-living bird species exposed to different levels of lead under natural conditions. *Environ. Res.* **137**: 185–198. [Medline] [CrossRef]
9. Ettler, V., Štěpánek, D., Mihaljevič, M., Drahota, P., Jedlicka, R., Kříbek, B., Vaněk, A., Penížek, V., Sracek, O. and Nyambe, I. 2020. Slag dusts from Kabwe (Zambia): Contaminant mineralogy and oral bioaccessibility. *Chemosphere* **260**: 127642. [Medline] [CrossRef]
10. Farmand, F., Ehdaie, A., Roberts, C. K. and Sindhu, R. K. 2005. Lead-induced dysregulation of superoxide dismutases, catalase, glutathione peroxidase, and guanylate cyclase. *Environ. Res.* **98**: 33–39. [Medline] [CrossRef]
11. Flora, S. J. S., Mittal, M. and Mehta, A. 2008. Heavy metal induced oxidative stress & its possible reversal by chelation therapy. *Indian J. Med. Res.* **128**: 501–523. [Medline]
12. Flora, S. J. S. and Tandon, S. K. 1990. Beneficial effects of zinc supplementation during chelation treatment of lead intoxication in rats. *Toxicology* **64**: 129–139. [Medline] [CrossRef]
13. Gurer, H. and Ercal, N. 2000. Can antioxidants be beneficial in the treatment of lead poisoning? *Free Radic. Biol. Med.* **29**: 927–945. [Medline] [CrossRef]
14. Hamid, M., Liu, D., Abdulrahim, Y., Liu, Y., Qian, G., Khan, A., Gan, F. and Huang, K. 2017. Amelioration of CCl_4-induced liver injury in rats by selenizing Astragalus polysaccharides: Role of proinflammatory cytokines, oxidative stress and hepatic stellate cells. *Res. Vet. Sci.* **114**: 202–211. [Medline] [CrossRef]
15. Highab, S. M., Raji, I., Aliyu, M. and Danjuma, N. 2020. Amelioration of lead toxicity in selected organs and tissue of wistar rats using resveratrol. *Dutse J. Pure Appl. Sci.* **6**: 87–97.
16. Hsu, P. C. and Guo, Y. L. 2002. Antioxidant nutrients and lead toxicity. *Toxicology* **180**: 33–44. [Medline] [CrossRef]
17. Kang, J. K., Sul, D., Kang, J. K., Nam, S. Y., Kim, H. J. and Lee, E. 2004. Effects of lead exposure on the expression of phospholipid hydroperoxidase glutathione peroxidase mRNA in the rat brain. *Toxicol. Sci.* **82**: 228–236. [Medline] [CrossRef]
18. Kansanen, E., Kuosmanen, S. M., Leinonen, H. and Levonen, A. L. 2013. The Keap1-Nrf2 pathway: Mechanisms of activation and dysregulation in cancer. *Redox Biol.* **1**: 45–49. [Medline] [CrossRef]
19. Lakshmi, B. V. S., Sudhakar, M. and Aparna, M. 2013. Protective potential of Black grapes against lead induced oxidative stress in rats. *Environ. Toxicol. Pharmacol.* **35**: 361–368. [Medline] [CrossRef]
20. Lamidi, I. Y. and Akefe, I. O. 2017. Mitigate effects of antioxidants in lead toxicity. *Clinical Pharmacol. Toxicol. J.* **1**: 1–9.
21. Leccia, M. T., Richard, M. J., Favier, A. and Béani, J. C. 1999. Zinc protects against ultraviolet A1-induced DNA damage and apoptosis in cultured human fibroblasts. *Biol. Trace Elem. Res.* **69**: 177–190. [Medline] [CrossRef]
22. Li, B., Cui, W., Tan, Y., Luo, P., Chen, Q., Zhang, C., Qu, W., Miao, L. and Cai, L. 2014. Zinc is essential for the transcription function of Nrf2 in human renal tubule cells *in vitro* and mouse kidney in vivo under the diabetic condition. *J. Cell. Mol. Med.* **18**: 895–906. [Medline] [CrossRef]
23. Livak, K. J. and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Δ Δ C(T)) Method. *Methods* **25**: 402–408. [Medline] [CrossRef]
24. Nakata, H., Nakayama, S. M. M., Yabe, J., Liazambi, A., Mizukawa, H., Darwish, W. S., Ikenaka, Y. and Ishizuka, M. 2016. Reliability of stable Pb isotopes to identify Pb sources and verifying biological fractionation of Pb isotopes in goats and chickens. *Environ. Pollut.* **208** Pt B: 395–403. [Medline] [CrossRef]
25. Nakata, H., Nakayama, S. M. M., Yabe, J., Muzandu, K., Toyomaki, H., Yohannes, Y. B., Kataba, A., Zyambo, G., Ikenaka, Y., Choongo, K. and Ishizuka, M. 2021. Clinical biochemical parameters associated with the exposure to multiple environmental metals in residents from Kabwe, Zambia. *Chemosphere* **262**: 127788. [Medline] [CrossRef]
26. Nandi, A., Yan, L. J., Jana, C. K. and Das, N. 2019. Role of catalase in oxidative stress- and age-associated degenerative diseases. *Oxid. Med. Cell.*

Longev. **2019**: 9613090. [Medline] [CrossRef]
27. Ninh, N. X., Thissen, J. P., Collette, L., Gerard, G., Khoi, H. H. and Ketelslegers, J. M. 1996. Zinc supplementation increases growth and circulating insulin-like growth factor I (IGF-I) in growth-retarded Vietnamese children. *Am. J. Clin. Nutr.* **63**: 514–519. [Medline] [CrossRef]
28. Ohkawa, H., Ohishi, N. and Yagi, K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95**: 351–358. [Medline] [CrossRef]
29. Olayinka, E. T. and Olukowade, I. L. 2010. Effect of amoxycillin / clavulanic acid (Augmentin 625®) on antioxidant indices and markers of renal and hepatic damage in rats. *J. Toxicol. Environ. Heal. Sci* **2**: 85–92.
30. Patil, A. J., Bhagwat, V. R., Patil, J. A., Dongre, N. N., Ambekar, J. G., Jailkhani, R. and Das, K. K. 2006. Effect of lead (Pb) exposure on the activity of superoxide dismutase and catalase in battery manufacturing workers (BMW) of Western Maharashtra (India) with reference to heme biosynthesis. *Int. J. Environ. Res. Public Health* **3**: 329–337. [Medline] [CrossRef]
31. Piao, F., Cheng, F., Chen, H., Li, G., Sun, X., Liu, S., Yamauchi, T. and Yokoyama, K. 2007. Effects of zinc coadministration on lead toxicities in rats. *Ind. Health* **45**: 546–551. [Medline] [CrossRef]
32. Powell, S. R. 2000. The antioxidant properties of zinc. *J. Nutr.* **130** Suppl: 1447S–1454S. [Medline] [CrossRef]
33. Prasanthi, R. P. J., Devi, C. B., Basha, D. C., Reddy, N. S. and Reddy, G. R. 2010. Calcium and zinc supplementation protects lead (Pb)-induced perturbations in antioxidant enzymes and lipid peroxidation in developing mouse brain. *Int. J. Dev. Neurosci.* **28**: 161–167. [Medline] [CrossRef]
34. Rabinowitz, M. B. 1991. Toxicokinetics of bone lead. *Environ. Health Perspect.* **91**: 33–37. [Medline] [CrossRef]
35. Shanmugam, G., Challa, A. K., Litovsky, S. H., Devarajan, A., Wang, D., Jones, D. P., Darley-Usmar, V. M. and Rajasekaran, N. S. 2019. Enhanced Keap1-Nrf2 signaling protects the myocardium from isoproterenol-induced pathological remodeling in mice. *Redox Biol.* **27**: 101212. [Medline] [CrossRef]
36. Sinha, A. K. 1972. Colorimetric assay of catalase. *Anal. Biochem.* **47**: 389–394. [Medline] [CrossRef]
37. Soerensen, J., Jakupoglu, C., Beck, H., Förster, H., Schmidt, J., Schmahl, W., Schweizer, U., Conrad, M. and Brielmeier, M. 2008. The role of thioredoxin reductases in brain development. *PLoS One* **3**: e1813. [Medline] [CrossRef]
38. Soussi, A., Gargouri, M. and El Feki, A. 2018. Effects of co-exposure to lead and zinc on redox status, kidney variables, and histopathology in adult albino rats. *Toxicol. Ind. Health* **34**: 469–480. [Medline] [CrossRef]
39. Talpur, S., Afridi, H. I., Kazi, T. G. and Talpur, F. N. 2018. Interaction of lead with calcium, iron, and zinc in the biological samples of malnourished children. *Biol. Trace Elem. Res.* **183**: 209–217. [Medline] [CrossRef]
40. Togao, M., Nakayama, S. M. M., Ikenaka, Y., Mizukawa, H., Makino, Y., Kubota, A., Matsukawa, T., Yokoyama, K., Hirata, T. and Ishizuka, M. 2020. Bioimaging of Pb and STIM1 in mice liver, kidney and brain using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) and immunohistochemistry. *Chemosphere* **238**: 124581. [Medline] [CrossRef]
41. Ugwuja, E. I., Vincent, N., Ikaraoha, I. C. and Ohayi, S. R. 2020. Zinc ameliorates lead toxicity by reducing body Pb burden and restoring Pb-induced haematological and biochemical derangements. *Toxicol. Res. Appl* **4**: 1–11.
42. Yabe, J., Nakayama, S. M. M., Ikenaka, Y., Yohannes, Y. B., Bortey-Sam, N., Oroszlany, B., Muzandu, K., Choongo, K., Kabalo, A. N., Ntapisha, J., Mweene, A., Umemura, T. and Ishizuka, M. 2015. Lead poisoning in children from townships in the vicinity of a lead-zinc mine in Kabwe, Zambia. *Chemosphere* **119**: 941–947. [Medline] [CrossRef]
43. Yousaf, S., Butt, M. S., Suleria, H. A. R. and Iqbal, M. J. 2014. The role of green tea extract and powder in mitigating metabolic syndromes with special reference to hyperglycemia and hypercholesterolemia. *Food Funct.* **5**: 545–556. [Medline] [CrossRef]
44. Zhai, Q., Narbad, A. and Chen, W. 2015. Dietary strategies for the treatment of cadmium and lead toxicity. *Nutrients* **7**: 552–571. [Medline] [CrossRef]