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Toxicology

The effects of di(2-ethylhexyl) phthalate and/or selenium on trace element levels in different organs of rats

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ABSTRACT

Di(2-ethylhexyl)phthalate (DEHP), a widely used plasticizer for synthetic polymers, is known to have endocrine disruptive potential, reproductive toxicity, and induces hepatic carcinogenesis in rodents. Selenium (Se) is a component of several selenoenzymes which are essential for cellular antioxidant defense and for the functions of mammalian reproductive system. The present study was designed to investigate the effects of DEHP exposure on trace element distribution in liver, testis, and kidney tissues and plasma of Se-deficient and Se-supplemented rats. Se deficiency was produced by feeding 3-week old Sprague-Dawley rats with ≤0.05 mg Se/kg diet for 5 weeks, and supplementation group were on 1 mg Se/kg diet. DEHP treated groups received 1000 mg/kg dose by gavage during the last 10 days of feeding period. Se, zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) levels were measured by inductively coupled plasma mass spectrometry (ICP-MS). Se supplementation caused significant increases in hepatic, renal, and testicular Se levels. With DEHP exposure, plasma Se and Zn, kidney Se, Cu and Mn levels were significantly decreased. Besides, liver Fe decreased markedly in all the DEHP-treated groups. Liver and kidney Mn levels decreased significantly in DEHP/SeD group compared to both DEHP and SeD groups. These results showed the potential of DEHP exposure and/or different Se status to modify the distribution pattern of essential trace elements in various tissues, the importance of which needs to be further evaluated.

Introduction

Phthalates are a family of industrial chemicals used as plasticizers that impart flexibility and durability to polyvinyl chloride (PVC) products. They are also used in solvents, fixatives, food packaging materials, and in personal care products. When incorporated into PVC, phthalates are not covalently bound and are easily released into the environment, resulting in excessive animal and human exposure [1–3].

Phthalate esters are classified as endocrine disrupting chemicals (EDCs) [4]. Exposures to high concentrations were shown to induce reproductive and developmental toxicity, fetal death, cancer, malformations, and liver and kidney injury in rodents [1,5–10].

Di-(2-ethylhexyl) phthalate (DEHP) is currently the most commonly used phthalate plasticizer in PVC materials. The primary monoester metabolites of phthalates with medium- (e.g. dibutyl phthalate [DBP]) or branched long-side chains (e.g. DEHP) were shown to induce degenerative testicular lesions [11–14]. At present, the general consensus is that DEHP, and DBP have potential to disrupt normal development and reproduction. These effects were observed in one or more animal species [15].

Despite the widely cited association of EDCs and male infertility, little is known about the mechanisms by which these agents act to cause decreased fertility [16]. Recent studies show that phthalates produce free radicals by several pathways and oxidative stress in germ cells may contribute to phthalate-induced disruption of spermatogenesis [17]. Induction of reactive oxygen species (ROS) in different cell lines and germ cells by MEHP were also reported [17–20]. Moreover, we have determined that DEHP caused an imbalance in the antioxidant/oxidant state in liver, kidney and thyroid of rats [21–23]. The exact mechanisms underlying the oxidative stress produced by DEHP in different tissues are not clear.

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One of the mechanisms underlying might be the alterations in trace element status in different organs and plasma. However, there is only limited data in literature that DEHP causes alterations in trace element distribution [24]. Changes in trace element status may also alter the expression and synthesis of several antioxidant enzymes, in particular, superoxide dismutase (SOD) and catalase (CAT). Zinc (Zn), and copper (Cu) are important components of Cu, Zn-SOD. Mn is the integral component of Mn-SOD and Fe is the component of extracellular SOD (EC-SOD, Fe-SOD) and Fe-CAT. These changes may cause alterations in the cellular redox state which may subsequently result in tissue damage [24]. In addition, Se and Zn concentrations are extremely high in seminal glands and Zn deficiencies have been associated to hypogonadism [25,26].

Se is an integral component of glutathione peroxidases (GPxs) and thioredoxin reductases (TrxRs), which are essential for cellular antioxidant defense and for the functions of mammalian reproductive system. The testes preserve high concentrations of Se and studies on SePP-knockout mice stated that Se was essential for testicular function [27]. Low sperm production and poor sperm quality have been a consistent feature in Se-deficient animals [28,29]. Besides, Se has roles in the cellular processes of liver and kidney, and may act as detoxifying component against a variety of toxic substances [30,31]. In our previous studies, Se supplementation was found to be protective against the oxidant potential of DEHP in testis, liver and kidney of rats while Se deficiency worsened the oxidant effects of this particular phthalate [21–23].

Trace elements have a pivotal role in maintaining the integrity of the cell and are of particular importance in prevention of toxicity. Therefore, by taking into account the widespread availability of inadequate Se intakes, the essentiality of Se in the maintenance of the cell survival and high DEHP exposure of humans, this study was designed to investigate the potential of DEHP to alter plasma, hepatic, renal, and testicular trace element distributions and to evaluate the possible modifying effects of Se status on DEHP-induced changes.

Methods and materials

Chemicals and reagents

All chemicals were obtained from Sigma–Aldrich (St. Louis, MO, USA). All animal feed (A03/R03 base) were supplied by Scientific Animal Food and Engineering (SAFE) Laboratories (Augy, France).

Animals and treatment

Male Sprague Dawley (SD) rats, 3-weeks old, supplied from Hacettepe University Experimental Animals Laboratory, were used in the experiments. The animals were divided randomly in six groups with six animals in each group and animals were housed in plastic cages with stainless-steel grid tops. The cages were placed in a room with controlled temperature (23 °C), humidity (50%) and a 12-h light–dark cycle. Body weights (bw) were monitored weekly, including before the first dose of DEHP treatment. Feeding period was 5 weeks. The animals were treated humanely and with regard for alleviation of suffering, and the study was approved by Hacettepe University Ethical Committee.

Experimental groups

(1) Control group (Control) was fed regular diet (0.15 mg/kg Se); (2) selenium supplemented group (SeS) was fed Se supplemented diet (1 mg/kg Se); (3) selenium deficient group (SeD) was fed Se deficient diet (≤0.05 mg/kg Se); (4) DEHP treated group (DEHP) was fed regular diet (0.15 mg/kg Se) and received 1000 mg/kg DEHP during the last 10 days by intragastric gavage (i.g.); (5) selenium supplemented DEHP group (DEHP/SeS) was fed Se supplemented diet (1 mg/kg Se) and received 1000 mg/kg DEHP during the last 10 days by i.g.; (6) selenium deficient DEHP group (DEHP/SeD) was fed Se deficient diet (≤0.05 mg/kg Se) and received 1000 mg/kg DEHP during the last 10 days by i.g. Animals were allowed to access ad libitum feed and drinking water.

DEHP was dissolved in corn oil, and the animals in Control, SeS and SeD groups received equivalent amount of the vehicle by i.g. during the last 10 days. Twenty–four hours after the last dose of DEHP treatment or vehicle administration, animals were weighed, and sacrificed by decapitation under thiopental anesthesia. Testis, liver and kidney tissues were removed, frozen immediately in liquid nitrogen, and stored at −80 °C until the preparation of tissue homogenates. Besides, blood samples were collected into trace element controlled heparinized tubes, plasma was separated after centrifugation at 800 × *g* for 10 min.

Trace element determination

Sample preparation

Testis, liver and kidney tissues were weighed and mineralized in HNO_3 65% (ICP-MS grade) and then 100 fold diluted in deionized water. Plasma was diluted to 1:25 with 1% (w/v) HNO_3 prior to inductively coupled plasma mass spectrometry (ICP-MS) determination. Galium ([Ga], at 650 nmol/L) was used as internal standard.

Calibration solution preparation

Standard addition calibration solutions containing Cu, Fe, Mn, Se and Zn were prepared by dilution of a parent 100 mg/L stock using 1% (w/v) HNO_3.

Instrument configuration

The trace element measurements were performed by inductively coupled plasma mass spectrometry (ICP-MS) (ThermoScientific, Bremen, Germany). The instrument operated with a Peltier cooled impact bead spray chamber, single piece quartz torch (1.5 mm i.d. injector) together with XT interface cones and the collision cell option. A standard nebulizer was used.

The instrument was run using the following parameters: RF power: 1200W, nebulizer gas flow: 0.90 L/min, auxiliary gas flow: 0.60 L/min, and cool gas flow: 13.5 L/min, Isotopes measured were ^{71}Ga (internal standard), ^{54}Fe, ^{56}Fe, ^{55}Mn, ^{63}Cu, ^{65}Cu, ^{64}Zn, ^{66}Zn, and ^{78}Se. Dwell time per isotope were 20 ms (^{71}Ga, ^{63}Cu, ^{65}Cu, ^{56}Fe, ^{64}Zn), 40 ms (^{66}Zn), 50 ms (^{54}Fe), 100 ms (^{55}Mn), and 300 ms (^{78}Se). Sample uptake was 90 s and wash time 30 s. 2 repeats were done per sample, and 20 repeats were done for the blank and or the plasma sample used for calibration.

Results

Plasma trace element levels

As shown in Fig. 1A, as expected Se deficiency caused a significant decrease (~90%) in plasma Se levels. DEHP also caused a significant decrease (~32%) in plasma Se while Se supplementation did not provide an increase Se levels in DEHP-treated rats vs. DEHP group. Plasma Zn levels decreased in all groups except DEHP/SeS group vs. control (Fig. 1B). Plasma Cu levels only decreased in DEHP/SeS group (~23%) (Fig. 1C). Fe (Fig. 1D) and Mn (Fig. 1E) levels did not change in any of the groups.

Fig. 1 indicates that the decrease in plasma Se levels induced by DEHP was not counteracted by Se supplementation whereas Se supplementation counteracted the decrease in plasma levels of Zn associated with DEHP. Cu is significantly decreased in DEHP/SeS

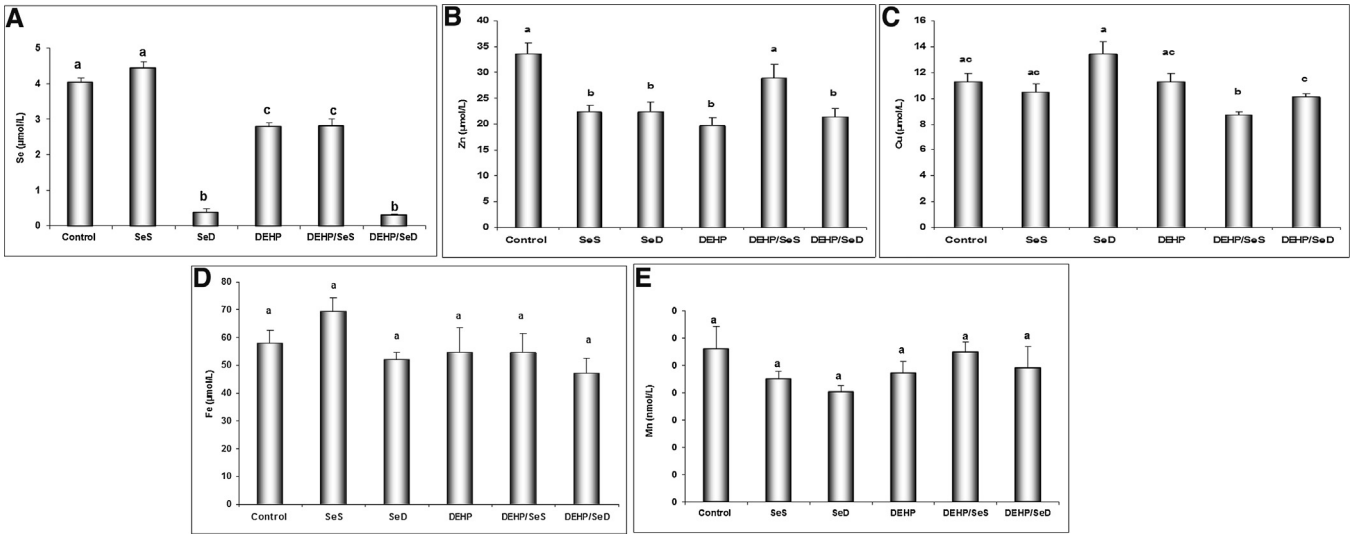

Fig. 1. Plasma trace element levels in study groups. (A) Plasma selenium levels; (B) plasma zinc levels; (C) plasma copper levels; (D) plasma iron levels; (E) plasma manganese levels. Experimental groups for 5 week were on: (Control) regular diet (0.15 mg/kg Se); (SeS) Se supplemented diet (1 mg/kg Se); (SeD) Se deficient diet (≤0.05 mg/kg Se); (DEHP) regular diet (0.15 mg/kg Se) and received 1000 mg/kg, i.g. DEHP for the last 10 d; (DEHPSeS) Se supplemented diet (1 mg/kg Se) and received 1000 mg/kg, i.g. DEHP for the last 10 d; (DEHPSeD) Se deficient diet (≤0.05 mg/kg Se) and received 1000 mg/kg, i.g. DEHP for the last 10 d All results were given as mean ± SEM of $n = 6$ animals. Bars that do not share same letters (superscripts) are significantly different from each other ($p < 0.05$).

group. In DEHP/SeD group, plasma Cu levels were significantly lower vs. SeD group.

Liver trace element levels

Liver Se levels increased significantly with Se supplementation (~113%) compared to control group while a marked decrease (~79%) was observed with Se deficiency. A statistically insignificant decrease in DEHP group (~20%) was observed; however DEHP treatment with Se deficiency did not cause any further decrease in liver Se levels compared to SeD group (Fig. 2A). Liver Zn levels decreased significantly in DEHP/SeS group (~11%) compared to control group while no other significant changes were observed in other groups (Fig. 2B). Besides, a significant increase of ~27% was observed in the liver Cu levels in DEHP/SeS group when compared to control group (Fig. 2C). Liver Fe levels decreased statistically in DEHP (~33%) and DEHP/SeS (~34%) groups when compared to control group (Fig. 2D). Only DEHP/SeS (~22%) and DEHP/SeD (~30%) groups showed significant decreases in liver Mn levels vs. control (Fig. 2E).

Fig. 2 indicates that in DEHP/SeS group, liver Zn levels decreased markedly compared to DEHP group. In addition, liver Cu levels showed significant increases in DEHP/SeS group vs. control. The decrease in liver Fe levels induced by DEHP is counteracted by selenium deficiency (i.e. in DEHP/SeD group). Finally, when DEHP was applied along with SeD or SeS, we observed significantly reduced liver Mn levels compared to SeD, SeS and DEHP groups.

Kidney trace element levels

Kidney Se levels increased significantly with Se supplementation (~65%) and decreased markedly with Se deficiency (~82%) when compared to control group. Significant decreases were observed in the renal Se levels of both DEHP (37%); however DEHP treatment with Se deficiency did not cause any further decrease in kidney Se levels compared to SeD group (Fig. 3A). Significant increases in renal Zn levels in SeD (14%) and DEHP (13%), DEHP/SeS (17%) and DEHP/SeD (10%) groups were also observed (vs. control, $p < 0.05$, all) (Fig. 3B). Decrease in renal Cu levels was statistically significant in SeD (~20%), DEHP (~26%) and DEHP/SeS (~29%) groups compared to control animals (Fig. 3C). Kidney Fe levels did not show statistically significant changes in all groups (Fig. 3D). All of the groups showed significant decreases in kidney Mn levels (13% in SeS group, 30% in SeD group, 20% in DEHP group, 30% in DEHP/SeS group, 43% in DEHP/SeD group) vs. control (Fig. 3E).

Fig. 3 indicates that in DEHP/SeS group, kidney Zn increased significantly when compared to SeS group. Besides, in DEHP/SeS group, kidney Cu and Mn levels were lower when compared to DEHP group ($p < 0.05$). In contrast, in DEHP/SeD group, kidney Cu levels showed increases when compared to SeD group ($p < 0.05$). Finally, the decrease in kidney Mn levels by DEHP application was exaggerated in DEHP/SeD and DEHP/SeS groups vs. DEHP group.

Testis trace element levels

As shown in Fig. 4, significant decrease in testicular Se levels was observed in SeD (37%) and DEHP/SeD (37%) groups compared to control. Significant increases were observed in testis Zn (~15%) and Se levels in SeD group (Fig. 4A and B). Testis Cu levels showed significant increases in SeD (~46%), DEHP (~22%), DEHP/SeS (~22%) and DEHP/SeD (~24%) groups vs. control as given in Fig. 4C. There were no statistically significant changes in testicular Fe (Fig. 4D) and Mn levels (Fig. 2E).

Fig. 4 indicates that the increase in testicular Se levels in SeS group was counteracted by the association of in DEHP/SeS group. In DEHP/SeD group, the testicular Se levels were similar to the SeD group, but significantly lower than in DEHP group.

Discussion

The results of the present study showed the potential of DEHP exposure and/or Se status to modify the distribution pattern of essential trace elements in various tissues. The results can be discussed under five subtitles:

Changes in selenium levels in the study groups

Selenium is actively involved in many fundamental biological processes [32,33]. Research has shown that Se supplementation

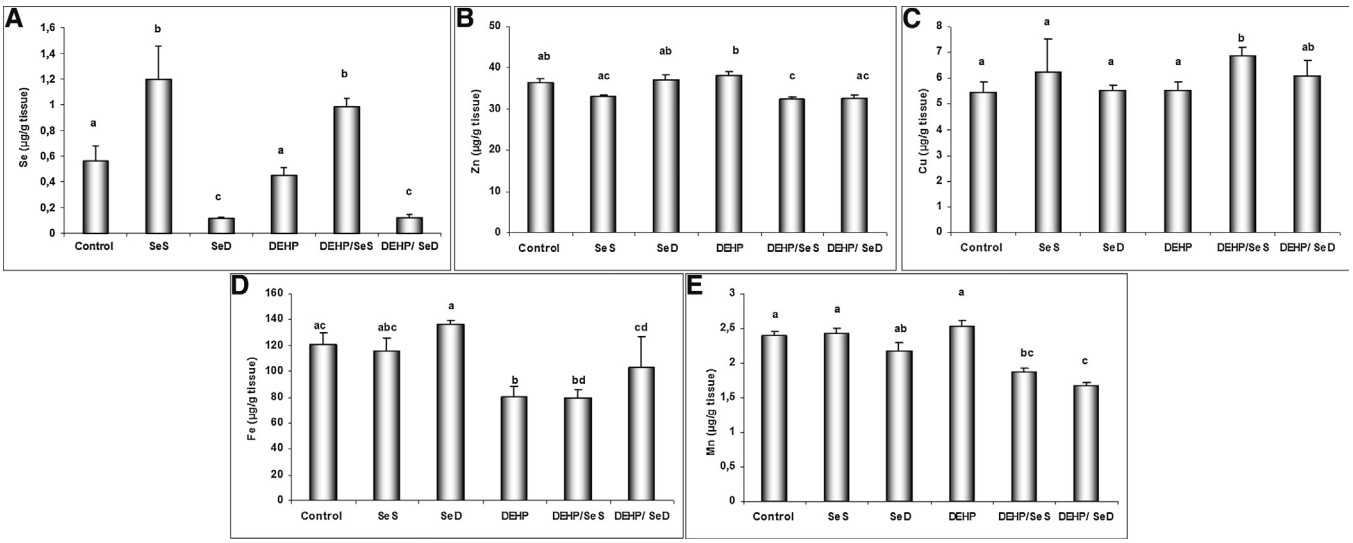

Fig. 2. Liver trace element levels in study groups. (A) Liver selenium levels; (B) liver zinc levels; (C) liver copper levels; (D) liver iron levels; liver manganese levels. Experimental groups for 5 week were on: (Control) regular diet (0.15 mg/kg Se); (SeS) Se supplemented diet (1 mg/kg Se); (SeD) Se deficient diet (≤0.05 mg/kg Se); (DEHP) regular diet (0.15 mg/kg Se) and received 1000 mg/kg, i.g. DEHP for the last 10 d; (DEHPSeS) Se supplemented diet (1 mg/kg Se) and received 1000 mg/kg, i.g. DEHP for the last 10 d; (DEHPSeD) Se deficient diet (≤0.05 mg/kg Se) and received 1000 mg/kg, i.g. DEHP for the last 10 d. All results were given as mean ± SEM of $n = 6$ animals. Bars that do not share same letters (superscripts) are significantly different from each other ($p < 0.05$).

may reduce oxidative stress and cancer incidence following exposure to a wide variety of carcinogens [19,20].

In SeD animals, Se concentrations significantly decreased in liver, kidney, testis and plasma while in SeS group, significant increase in Se levels was only observed in liver, kidney and testis, but not in plasma. Our results are also in accordance with the unchanged plasma Se levels of rats and mice, which received 0.5 ppm Se containing drinking water for 4 weeks and rats and mice fed with 0.2 mg Se/kg and 1 mg Se/kg containing diets [34–36]. In Se-deficient rats, the decreases in plasma, liver, and kidney Se were 90%, 113%, and 82%, respectively while the decrease in testis Se was only 37% when compared to control. This indicates that testis has the ability to preserve Se as indicated before [7,9,37]. Testicular Se levels were shown to reach to a plateau with 0.8 µg/g Se containing diet, providing the highest GPx1 activity in the testis of male weanling rats fed with this diet for 28 days [38]. Hepatic and testicular GPx1 activities did not increase; but renal GPx1 activity showed a moderate but significant (10%) increase by Se supplementation in these rats, suggesting that excess Se does not necessarily provide substantial increases in GPx1 activity of different organs. However, Se deficiency caused a decrease of 93% in hepatic and 80% in renal GPx1 activities, but no changes were observed in testicular GPx1 activity in SeD rats [21–23]. The ability of testis to preserve Se might be an underlying factor in the unchanged testicular activity of GPx1 as mentioned before. However, Se deficiency is able to cause an imbalance in hepatic and renal cellular redox states, possibly due to the decrease in tissue GPx1 activities. An imbalance between high oxidative stress and decreased anti-oxidative defense systems was suggested to cause a variety of liver and kidney diseases [22,39]. Therefore, antioxidants, including Se, can be

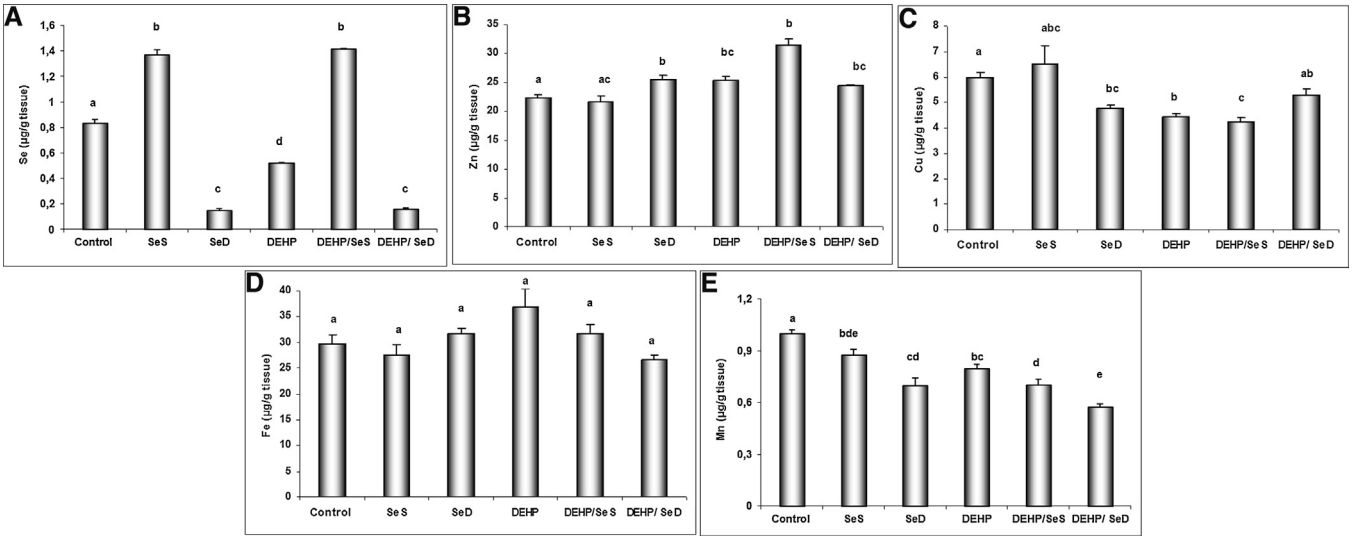

Fig. 3. Kidney trace element levels in study groups. (A) Kidney selenium levels; (B) kidney zinc levels; (C) kidney copper levels; (D) kidney iron levels; (E) kidney manganese levels. Experimental groups for 5 week were on: (Control) regular diet (0.15 mg/kg Se); (SeS) Se supplemented diet (1 mg/kg Se); (SeD) Se deficient diet (≤0.05 mg/kg Se); (DEHP) regular diet (0.15 mg/kg Se) and received 1000 mg/kg, i.g. DEHP for the last 10 d; (DEHPSeS) Se supplemented diet (1 mg/kg Se) and received 1000 mg/kg, i.g. DEHP for the last 10 d; (DEHPSeD) Se deficient diet (≤0.05 mg/kg Se) and received 1000 mg/kg, i.g. DEHP for the last 10 d All results were given as mean ± SEM of $n = 6$ animals. Bars that do not share same letters (superscripts) are significantly different from each other ($p < 0.05$).

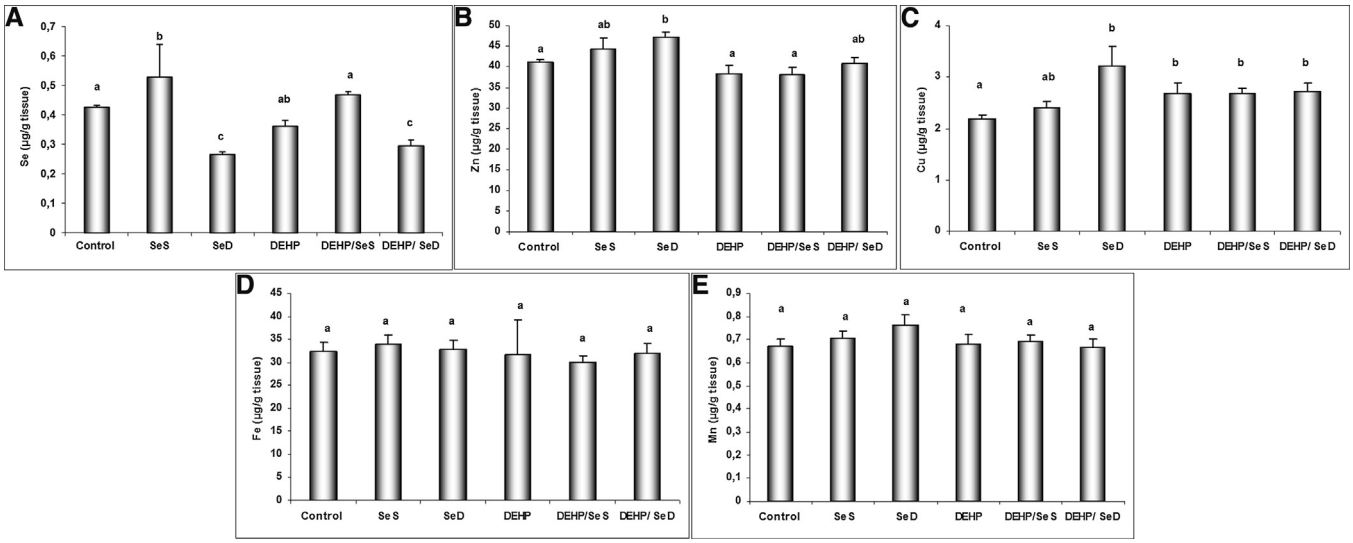

Fig. 4. Testis trace element levels in study groups. (A) Testis selenium levels; (B) testis zinc levels; (C) testis copper levels; (D) testis iron levels; (E) testis manganese levels. Experimental groups for 5 week were on: (Control) regular diet (0.15 mg/kg Se); (SeS) Se supplemented diet (1 mg/kg Se); (SeD) Se deficient diet (≤0.05 mg/kg Se); (DEHP) regular diet (0.15 mg/kg Se) and received 1000 mg/kg, i.g. DEHP for the last 10 d; (DEHPSeS) Se supplemented diet (1 mg/kg Se) and received 1000 mg/kg, i.g. DEHP for the last 10 d; (DEHPSeD) Se deficient diet (≤0.05 mg/kg Se) and received 1000 mg/kg, i.g. DEHP for the last 10 d All results were given as mean ± SEM of $n = 6$ animals. Bars that do not share same letters (superscripts) are significantly different from each other ($p < 0.05$).

beneficial to minimize the detrimental effects of oxidative stress in both liver and kidney.

DEHP caused marked decreases in plasma and, kidney Se. Kidney GPx1 activity also showed marked decreases after rats were exposed to DEHP (1000 mg/kg, 10 days, oral) as mentioned in our previous study [22]. Although hepatic GPx1 activity markedly decreased, insignificant alterations were observed in hepatic Se levels (20%) in these rats [21]. Se supplementation in DEHP-treated groups increased liver and kidney Se levels significantly when compared to DEHP group while in DEHP/SeD group plasma, hepatic, renal and testicular levels of Se decreased when compared to DEHP-treated rats.

Changes in zinc levels in the study groups

Zinc is an essential component or a cofactor for 300 specific enzymes. Zinc is necessary for normal reproduction, development and mental functions [40,41].

Both Se deficiency and Se supplementation caused decreases in plasma Zn levels. In Se deficiency, Zn release by metallothioneins (MT) might have diminished as plasma Zn levels decreased (33%) vs. control. In Se supplementation, Se may provoke Zn release by MT; however the excretion rate of Zn might have increased due to high levels of Zn in plasma. However, the mechanism underlying this phenomenon needs to be elucidated with mechanistic studies.

DEHP caused significant decreases in plasma Zn levels while a marked increase was observed in kidney but has no effect on Zn levels in liver and testis. The elevations in kidney zinc levels by DEHP exposure might be the result of the inflammation process induced by this particular phthalate. However, the increase in Zn levels in DEHP/SeS (19% vs DEHP group, $p > 0.05$) was an unexpected phenomenon as it was expected that Se supplementation would decrease, rather than increase kidney Zn levels. Miura et al. [24] found insignificant decreases of Zn in testes of mice exposed to DEHP. Other studies indicated significant changes in tissue Zn levels by DEHP administration. This discrepancy might be because of the differences in dose, duration and the route of DEHP administration as well as the age and strain of the animals. Oishi observed that the Zn concentration significantly decreased, whereas the activities of Zn-containing enzymes were increased in the testes of DEHP-treated rats [42]. Previous studies demonstrated that testicular atrophy caused by DEHP and other phthalates was accompanied by depletion of Zn content in testis [43,44]. Foster et al. [45] demonstrated by a histochemical reaction technique that the loss of Zn was the earliest finding in phthalate-treated rats. They also suggested that the loss of Zn might be a trigger factor for the induction of testicular atrophy by phthalates. However, as we did not also observe any testicular atrophy in the DEHP-treated rats in a previous study [7], we might suggest that DEHP might have different mode of action than di-*n*-phenyl phthalate and the possible depletion of Zn might start later than 10 days which was the DEHP administration period in our study. Besides, it is questionable whether the Zn depletion in testis caused by some phthalates is a causal factor in inducing testicular atrophy, because: (1) in contrast to antioxidant vitamins, the administration of Zn had failed to protect the testes from DEHP-induced injury [46–48]; (2) a single dose of MEHP consistently induces testicular atrophy but has varying effects on the testicular Zn concentration [49,50]; and (3) in a time course study by Oishi, significant morphological and biochemical alterations by DEHP occurred prior to the loss of Zn in testis [46].

Changes in copper levels in the study groups

Copper proteins have diverse roles in biological electron transport and oxygen processing and transportation [51]. Copper is found in Cu, Zn-SOD protein that catalyzes the dismutation of superoxide [52].

Plasma Cu levels were only decreased in DEHP/SeS and DEHP/SeD groups while liver Cu levels only increased in DEHP/SeD group. Kidney Cu levels decreased in SeD, DEHP and DEHP/SeS groups. Testis Cu levels increased in SeD and in all DEHP-treated groups. However, Miura et al. [24] found insignificant decreases in Cu in testes of mice in contrary with our findings.

In our recent studies, DEHP caused decreases in testis and kidney Cu, Zn-SOD activity [21,22] and significant increases in liver Cu, Zn-SOD activity [21]. It is likely that Cu- and Zn-related proteins, including SOD, are down-regulated by DEHP exposure transcriptionally or translationally, to a certain degree in the testis and kidney, whereas DEHP exposure caused an accumulation of Cu in testis. Wang et al. [53] showed that DEHP induced oxidative

stress in isolated antral follicles by suppressing the expression and activity of Cu, Zn-SOD in rats. Total SOD activity showed different patterns as it showed decreases in kidney and increases in liver [21,22]. Moreover, the hepatic Cu, Zn-SOD activity increased 60% by DEHP exposure [21]. The decrease in kidney Cu, Zn-SOD activity is in line with the decrease in Cu levels in DEHP exposure.

Changes in iron levels in the study groups

Iron is an element found in nearly all living organisms. Iron-containing proteins often contain heme prosthetic groups and participate in many biological oxidations [54,55]. Human and rodent CATs have Fe in the center of heme group attached to the enzyme [56]. In the current study, plasma, testis and kidney iron levels did not change by DEHP administration. However, we have shown that liver iron levels significantly decreased although hepatic CAT activity showed significant increases by DEHP exposure. The increase in CAT activity might be independent of hepatic Fe concentration and may be related to the peroxisome proliferating effects of phthalates as these substances enhance activity of peroxisomal enzymes, such as CAT [57,58]. Selenium status did not modify the effect of DEHP on plasma Fe levels whereas Se deficiency counteracts the effect of DEHP on hepatic Fe levels.

Changes in manganese levels in the study groups

Although no changes were observed in plasma and testis Mn levels, kidney Mn levels decreased significantly in all of the study groups whereas hepatic Mn levels were only lower in DEHP/SeS and DEHP/SeD groups. It is likely that Mn levels were regulated differently in various organs by Se and/or DEHP exposure. However, in SeD animals, both hepatic ($p < 0.05$) and testicular Mn-SOD activity increased vs. control [21,22]. In DEHP-exposed rats, only hepatic Mn-SOD activity showed marked increases [21]. As Mn-SOD is a good indicator of mitochondrial oxidative stress, we can postulate that DEHP might cause an imbalance in mitochondrial redox state and this phenomenon induces an increase in Mn-SOD in liver which is independent of tissue Mn concentration.

The cellular homeostases of Mn, Zn, Cu and Fe are closely interlinked. The physical and chemical properties of these elements are very similar. Their metabolic pathways have several synergistic and antagonistic interactions. If one of these elements becomes deficient, another element may accumulate. A positive interaction is suggested to exist between Cu and Fe homeostasis. Cu is necessary for the activity of ceruloplasmin and hephaestin and facilitates iron excretion by ferroportin, which act as ferroxidases. These proteins convert Fe(II) to Fe(III), generate an iron gradient, and facilitate export of Fe and uptake by proteins. Cu is also necessary for the activity of tyrosinase and synthesis of melanin, a storage protein for Fe, Zn, and Cu in different tissues. Zn-deficient animals accumulate Cu and Fe in several organs. Numerous genes respond to alterations in cellular Zn levels with changes in mRNA levels [59]. Se provokes Zn release by MTs, via reduction of GPx1 [60]. However, when the organism is subject to an EDC such as DEHP, these interrelationships seem to become more complicated. Mechanistical studies on the levels of Se, Zn, Cu, Fe and Mn in different cellular compartments need to be performed in order to understand these interactions.

In conclusion, we can suggest that DEHP exposure as well as changes in Se status could alter the plasma and tissue distributions of currently measured elements. Such changes might subsequently bring a reduction in reproduction and development and might cause tissue damage, the importance of which needs to be further evaluated. Therefore, plastic materials containing DEHP should be avoided particularly in growing children because they might lead to significant alterations in trace elements of tissues and organs.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgements

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