

*Full Length Research Paper*

# **Hepatotoxic and neurotoxic effects of combined lead and di-(2-ethylhexyl) phthalate exposure: Activation of total -, $\text{Ca}^{2+}$ - and $\text{Na}^+\text{K}^+$ - ATPases in the liver of male rats**

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Lead and di-(2-ethylhexyl) phthalate (DEHP) are common environmental toxicants of concern around the world. Although effects of individual exposures to both agents are well documented, there is a dearth of information on the effects of co-exposure to both agents. In this study, combined exposure to lead and DEHP was investigated for effects on ATPase activities in the liver, brain and kidney tissues of rats. Male albino rats were daily exposed to either 200 ppm lead as lead acetate in their drinking water and/or 100 mg DEHP  $\text{kg}^{-1}$  body weight in olive oil by gastric intubation for 30 days. Changes in total body weight, relative organ weights as well as brain, hepatic and renal activities of total,  $\text{Na}^+\text{K}^+$ -,  $\text{Ca}^{2+}$ - and  $\text{Mg}^{2+}$ -ATPases were used as biomarkers of toxicity. Hepatomegaly and brain atrophy heralded exposure to both agents. Individual exposure to lead and DEHP resulted in reduction in hepatic  $\text{Ca}^{2+}$ - and  $\text{Mg}^{2+}$ - ATPase activities but no significant effect on total ATPase activity, however combined exposure produced significant activation of  $\text{Ca}^{2+}$ -,  $\text{Na}^+\text{K}^+$ - and total ATPase while restoring  $\text{Mg}^{2+}$ - ATPase towards control. A potentiating effect on lead by DEHP was observed in hepatic  $\text{Na}^+\text{K}^+$ - ATPase. Lead stimulated the activities of renal  $\text{Ca}^{2+}$ - and total ATPases while DEHP on the contrary caused significant reduction in total ATPase activity and no significant effects on  $\text{Ca}^{2+}$ - ATPase activity. Co-treatment produced antagonistic effects leading to normal renal  $\text{Ca}^{2+}$ - and total ATPase activities. Brain  $\text{Na}^+\text{K}^+$ -,  $\text{Ca}^{2+}$ - and total ATPase activities were depressed in co-exposure while  $\text{Mg}^{2+}$ - ATPase was up-regulated. Lead potentiated DEHP-induced inhibition of brain total - ATPase while co-treatment produced antagonistic effects on brain  $\text{Ca}^{2+}$ - ATPase. The findings of this study highlight organ specific variations in response to combined lead and DEHP exposure in rats.

**Key words:** Hepatotoxic, neurotoxic, ATPases, DEHP, lead, co-exposure, hepatomegaly.

## **INTRODUCTION**

Despite the global move to phase out leaded-gasoline, lead has persisted as an environmental pollutant of grave concern with its deleterious effects particularly felt among children (Nriagu et al., 1996). The emission of lead by

power plants, smelters and boilers that burn used motor oil is frequently deposited in the soil, where it is absorbed by crops and leaching into underground water. Hence lead ends up in food, water and air (Allouche et al.,

2011). In the recent past, lead poisoning of significant proportions broke out in northern Nigeria and claimed the lives of over 400 children (Medecins Sans Frontieres, 2012). Lead is known to cause diseases in several systems of the body such as the hematopoietic and cardiovascular (Onunkwor et al., 2004; Adeniyi et al., 2008), immunological (Basaran and Undeger, 2000), hepatic (Sharma et al., 2011b), renal (Rastogi, 2008) and central nervous system effects (Sanders et al., 2009; Ademuyiwa et al., 2007; Sharma et al., 2011a) and it has also been implicated as a co-carcinogen and a teratogen (Pracheta and Singh, 2009; Markovac and Goldstein, 1988). Several studies have shown that lead is ubiquitous in the environment (Ademuyiwa et al., 2002; Adeniyi and Anetor, 1999) and causes oxidative stress in the body by interacting with glutathione, a known natural antioxidant in the body (Jangid et al., 2016). Glutathione is a tripeptide consisting of  $\gamma$ -glutamic acid, cysteine and glycine. It is found in several tissues (Kosnet et al., 1998) and lead's interaction with glutathione leads to replacement of the hydrogen on two sulphhydryl groups on adjacent molecules by lead. The strong bond that results effectively eliminates the two glutathione molecules from further reaction thereby eliciting all the conditions that arise as a result of oxidative stress, such as diabetes, nervous system disorders, cardiovascular diseases, aging, cancer etc (Ademuyiwa et al., 2005; 2007; Dosumu et al., 2005).

Di-(2-ethylhexyl) phthalate (DEHP) is another equally widespread toxicant in the environment because of its common use as a plasticizer in the plastic making industry. It is added to plastic to make it softer and more flexible. However, DEHP easily dislodges from the complex with the plastic and leaches into the contents of the plastic containers especially at high temperatures (Fromme et al., 2012). This is how storing foods and drinks in plastic wraps and bottles could lead to phthalate toxicity. DEHP could also leach into blood when it is used in medical devices such as intravenous tubing and blood storage bags (Padmakumaran et al., 1998). Despite the potential hazard, there is a steady rise in the use of plastic bottles as against glass in the storage of drinks and beverages. Phthalates have been associated with endocrine disruption (Ye et al., 2017; Grün, 2010; Miodovnik et al., 2011), metabolic disorders, reproductive and developmental defects (Foster et al., 2001; Jiang, 2007; Engel et al., 2010). DEHP is one of the most commonly used of the phthalates and hence the most abundant in the environment (Kamrin, 2009). Since both lead and DEHP are quite ubiquitous in the environment

and may be present in air, soil and drinking water at the same time, there is a high potential for a simultaneous exposure to both toxicants. Both agents are known to exert toxicity through the generation of oxidative stress (Onunkwor et al., 2004; Dosumu et al., 2005; Xu et al., 2008; Afolabi et al., 2015; Wójtowicz et al., 2019) and this makes the study of their co-exposure of particular interest. Lead and phthalates have attracted much research attention separately, however, there is scarce information on their combined effect on key biomarker enzymes such as the ATPases.

ATPases are membrane bound enzymes involved in the uphill transport of ions coupled with energy expenditure in the form of ATP. Previous studies on lead toxicity in humans showed that erythrocyte membrane ATPases were down-regulated by lead (Yücebilgic et al., 2003; Abam et al., 2008, Okediran et al., 2009). Specifically, erythrocyte membrane  $\text{Na}^+\text{K}^+$ -ATPase and  $\text{Ca}^{2+}$ -ATPase were shown to be inhibited by lead in occupationally exposed workers (Yücebilgic et al., 2003) while phthalates were also shown to inhibit some ATPases in the organs of rats (Afolabi et al., 2016; Dhanya et al., 2003). The focus of this study therefore was to investigate the effects of combined exposure to lead and DEHP on membrane ATPases of brain, liver and kidney of rats.

## MATERIALS AND METHODS

### Chemicals

Lead acetate and DEHP were products of Sigma-Aldrich, Munich, Germany. All other chemicals used in this study were of the purest grade available and were obtained from British Drug House (BDH) Chemicals Limited, Poole, England.

### Experimental design

Twenty-four (24) male Wistar rats were obtained from the animal house of University of Ibadan (UI), Ibadan, Oyo state, weighing an average of 140 g and housed in the animal house of Bells University of Technology, Ota, Ogun State Nigeria. The animals were acclimatized for 14 days prior to the commencement of the study. Afterwards, they were randomly distributed into four groups of six animals each. Group 1 animals served as control and were administered olive oil and lead-free distilled water. Group 2 animals received 200 ppm lead as lead acetate in their drinking water. Group 3 animals were administered a homologous mixture of 100 mg DEHP  $\text{kg}^{-1}$  body weight in olive oil by gastric intubation. Group 4 received both 200 ppm lead in drinking water and 100 mg DEHP  $\text{kg}^{-1}$  body weight in olive oil. Doses were chosen based on previous

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**Table 1.** Body and relative organ weights of rats.

Groups	Initial body wt (g)	Final body wt (g)	% change in body wt (%)	Relative brain wt (g/100 g bw)	Relative liver wt (g/100 g bw)	Relative kidney wt (g/100 g bw)
Control	155.8 ± 1.53	175 ± 1.44	12.32 ± 1.96 <sup>a</sup>	1.03 ± 0.02 <sup>a</sup>	2.83 ± 0.04 <sup>a</sup>	0.54 ± 0.02 <sup>a</sup>
200ppm Pb	155.0 ± 1.18	172 ± 1.44	10.99 ± 3.97 <sup>a</sup>	0.88 ± 0.03 <sup>b</sup>	3.18 ± 0.07 <sup>a</sup>	0.61 ± 0.03 <sup>b</sup>
DEHP	153.0 ± 0.45	134.6 ± 3.18	-12.54 ± 1.16 <sup>b</sup>	0.86 ± 0.03 <sup>b</sup>	3.47 ± 0.10 <sup>b</sup>	0.51 ± 0.01 <sup>a</sup>
Pb & DEHP	151.0 ± 2.42	179.2 ± 5.15	18.38 ± 5.37 <sup>c</sup>	0.73 ± 0.02 <sup>c</sup>	3.38 ± 0.18 <sup>b</sup>	0.54 ± 0.04 <sup>a</sup>

Values are expressed as mean ± SEM. (n = 6). Means in a column with different letters (a – d) are significantly different from each other at p < 0.05.

studies (Ademuyiwa et al., 2009; Afolabi et al., 2015, 2016). These treatments were maintained for thirty days. Animals were placed on a standard pellet diet and distilled water (or their various lead-poisoned water) *ad libitum* for the duration of the study. Weights were monitored weekly using a weighing balance with accuracy ± 0.1g. At the end of the treatment, animals were fasted overnight and blood collected by cardiac puncture under light ether anesthesia. Plasma was obtained from the blood by centrifuging at 3,000 g for 10 minutes. The brain, liver and kidney were removed and homogenized in 150 mmol L<sup>-1</sup> ice-cold KCl to obtain a 10 % homogenate which was further centrifuged at 15,000 g for 15 minutes at 4°C to obtain the post-mitochondrial fraction which was stored at -20°C until further biochemical analyses. The experiment was carried out in accordance with the Bells University of Technology, Department of Chemical and Food Sciences (Biochemistry Unit) guidelines for the care and use of laboratory animals.

### Biochemical analyses

Total ATPase (E.C.3.6.1.3) activity in tissue homogenates was assayed using the method of Evans (1969). The final assay mixture of 2 ml contained 0.1 M Tris-HCl (pH 7.4), 0.1 M NaCl, 0.1 M MgCl<sub>2</sub>, 0.01 M ATP, 0.1 M KCl and 0.1 ml of the tissue homogenate. The reaction was incubated at 37°C in a water bath for 20 min and stopped by adding 1 ml of 10% trichloroacetic acid (TCA) and then centrifuged. The inorganic phosphorus (Pi) liberated was then estimated in the supernatant by the method of Lowry and Lopez (1946) which involved the use of ammonium molybdate and vitamin C.

Assay for Na<sup>+</sup>/K<sup>+</sup>-ATPase (E.C.3.6.1.37) activity followed the procedure of Hesketh et al. (1978) as described by Afolabi et al (2016). The reaction mixture contained 0.35 M NaCl, 17.5 mM KCl, 21.0 mM MgCl<sub>2</sub>, 10 mM Tris-HCl (pH 7.4), 8.0 mM ATP and tissue homogenate. The mixture was incubated at 37°C for 1 h and the reaction terminated by the addition of 0.8 ml of ice-cold 10% (w/v) TCA. Afterwards, the mixture was allowed to stand for 20 min at 4°C and then centrifuged. Pi in the supernatant was measured as described already.

The activity of Ca<sup>2+</sup>-ATPase (E.C.3.6.1.38) was assayed according to the method of Hjerten and Pan (1983). The reaction mixture contained 125 mM Tris-HCl buffer (pH 8), 50 mM CaCl<sub>2</sub> and 10 mM ATP. The contents were incubated at 37°C for 15 min and the reaction arrested by the addition of 0.5 ml of ice-cold 10% TCA and centrifuged. Pi was then determined as described already. The activity of Mg<sup>2+</sup>-ATPase (E.C.3.6.1.39) was assayed according to the method of Ohnishi et al. (1982). The reaction mixture contained 0.1 ml each of 375 mM Tris-HCl buffer (pH 7.6), 25 mM MgCl<sub>2</sub>, 10

mM ATP and tissue homogenate. The contents were incubated at 37°C for 15 min and the reaction was then stopped with the addition of 0.5 ml of 10% TCA and centrifuged. Pi was then determined following the technique as described already.

Protein concentration was determined in the brain, liver and kidney homogenates using Bradford method (Bradford, 1976).

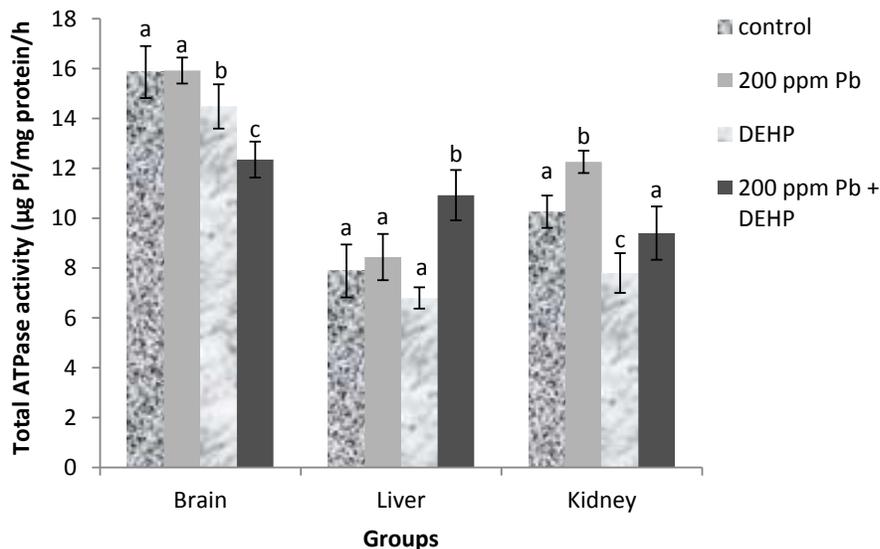
### Statistical analysis

Results are expressed as mean ± SEM. The statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by Tukey Post Hoc Multiple Comparisons using Statistical Package for the Social Sciences (SPSS) version 15.00 for Windows (SPSS Inc; CA, USA). A value of p < 0.05 was considered statistically significant between groups.

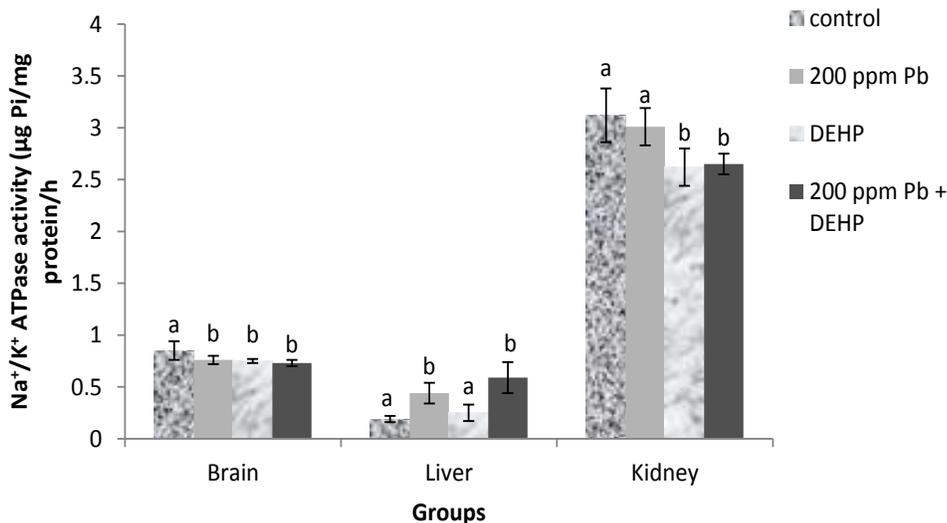
## RESULTS

Summary of percentage change in body weight and relative organ weight is depicted in Table 1. Rats treated with lead and DEHP did not show any obvious changes in behavior throughout the period of the study. Animals in all the groups increased in weight at the end of the exposure period except the animals treated with DEHP that experienced a 12.54 ± 1.16% reduction in body weight. Treatment with lead-only resulted in a weight gain of 10.99 ± 3.97% and increases in relative organ weights of the liver and kidney of the rats. Such increases were not observed in the brain in all the treatment groups (relative brain weights decreased by 15, 16 and 29% in the lead-only, DEHP and combined treatment groups respectively). DEHP and co-treatment with both toxicants resulted in increases in relative organ weights of the liver (23 and 19% respectively). Combined treatment yielded the highest gain in body weight (amounting to 18.38%) and highest decrease in relative brain weight (29%).

ATPase activities in the organs of control and experimental animals are depicted in Figures 1 to 4. Lead exposure resulted in increase in total ATPase activity of 20% in the kidney of the rats (Figure 1) while there was no significant difference in the liver and brain. Hepatic, renal and brain activities of total ATPases were significantly reduced by DEHP (14, 24 and 9%



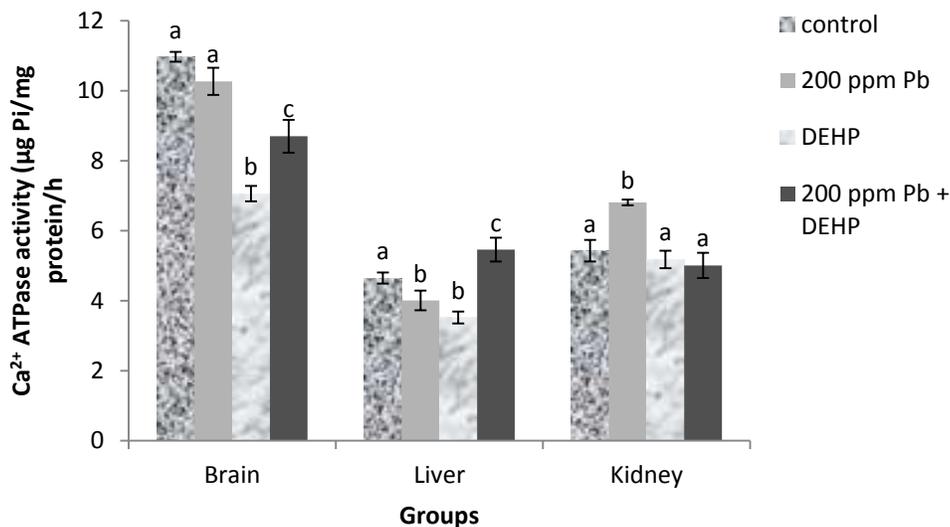
**Figure 1.** Total ATPase activities in brain, liver and kidney of rats. Bars of the same compartment carrying different letters of the alphabet are significantly different from each other ( $p < 0.05$ ).



**Figure 2.** Na<sup>+</sup>/K<sup>+</sup> ATPase activities in brain, liver and kidney of rats. Bars of the same compartment carrying different letters of the alphabet are significantly different from each other ( $p < 0.05$ ).

respectively), however, co-treatment produced a significant activation of total ATPase activity in the liver (39%) while lead showed a potentiating effect on DEHP in the brain. Lead and DEHP produced antagonistic effects in the kidney effectively restoring enzyme activity towards control value. There were significant decreases in Na<sup>+</sup>/K<sup>+</sup> ATPase activities between control and treatment

groups in the brain (amounting to 11, 12 and 14% in the lead, DEHP and co-treatment groups respectively) as displayed in Figure 2. On the contrary, in the liver, the activity of Na<sup>+</sup>/K<sup>+</sup> ATPase was increased by as much as 132 and 32% by lead and DEHP respectively and co-treatment produced additive effects (211%). There was no significant difference in renal Na<sup>+</sup>/K<sup>+</sup> ATPase activity



**Figure 3.** Ca<sup>2+</sup> ATPase activities in brain, kidney and liver of rats. Bars of the same compartment carrying different letters of the alphabet are significantly different from each other ( $p < 0.05$ ).

between control and lead treated groups, however DEHP and co-treatment caused approximately the same level of inhibition of the enzyme (16%).

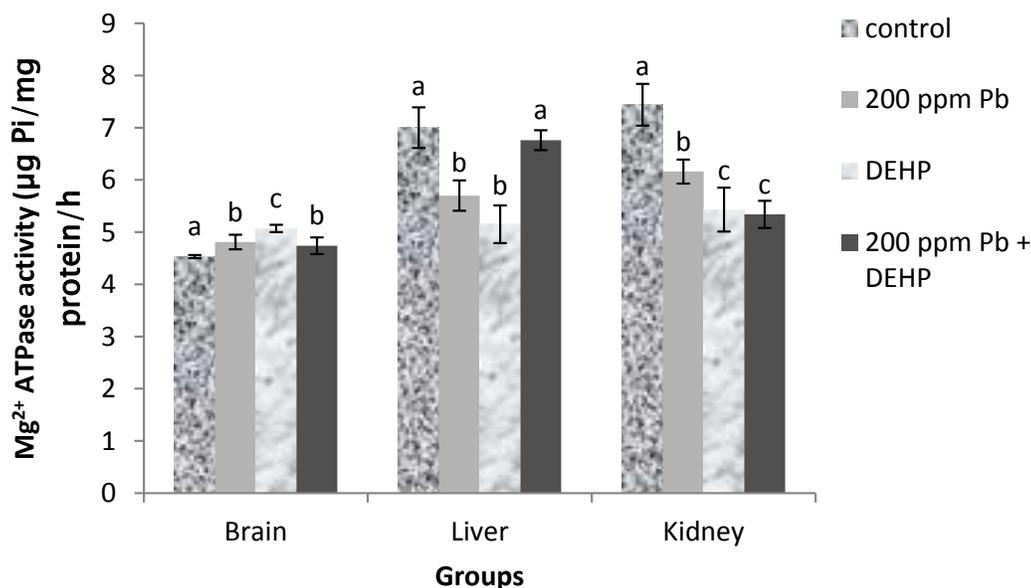
Activities of Ca<sup>2+</sup> ATPases in the organs of the rats are presented in Figure 3. After 30 days, lead and DEHP inhibited Ca<sup>2+</sup> ATPase to the tune of 6 and 36 % respectively in the brain while lead antagonized the effects of DEHP in the co-treatment group, reducing DEHP's inhibition from 36 to 21%. In the liver, there was 14% inhibition of Ca<sup>2+</sup> ATPase by lead and 24% by DEHP, while combined treatment led to the activation of the enzyme by 17 %. In the kidney, Ca<sup>2+</sup> ATPase activity was activated by 25% by lead, while there was no significant difference ( $p > 0.05$ ) in the DEHP and co-treatment groups.

Lead, DEHP and combined lead and DEHP treatment stimulated Mg<sup>2+</sup> ATPase activities by 6, 12 and 5 % respectively in the brain. On the contrary, liver Mg<sup>2+</sup> ATPase activities were down-regulated by lead and DEHP treatments (19 and 26 % respectively) while co-treatment neutralized the individual inhibitions, restoring enzyme activity towards control. Kidney Mg<sup>2+</sup> ATPase activities were inhibited by 17, 27 and 28% in the lead, DEHP and co-treatment groups respectively. Figure 4.

## DISCUSSION

In this study, animals exposed to DEHP exhibited weight loss unlike the other treatment groups. This is contrary to other studies that have shown DEHP-exposed rats to

have increased body weight and fat mass (Jia et al., 2016; Klötting et al., 2015). First and foremost, Klötting et al. (2015) worked with mice and not rats. Secondly, the mice were exposed to DEHP for 10 weeks, a much longer period than that employed in this study. Thirdly, the mice employed in the Klötting study were exposed to DEHP at a very low dose of 0.05 mg/kg bw/day. Hence summarily, the disparity in results could be attributed to the species of rodents used, duration of exposure and dose of DEHP administered factors that have been shown to influence the response of mammals to xenobiotics (Klaassen and Watkins, 2015). In this study, the DEHP-exposed rats displayed reduced appetite throughout the study compared to animals in the other groups and this might account for their weight loss. Reduced appetite could be attributed to inflammation-associated anorexia arising from the particular concentration of DEHP and the duration that it was administered (Wang et al., 2019). It was also observed that Pb antagonized DEHP - induced decrease in bodyweight in this study, and mechanisms that may be compensatory led to the highest increase in total body weight in this co-treated group. DEHP - induced hepatomegaly was observed and Pb had no effect on this observed hepatomegaly as there was no significant difference between relative liver weights of the DEHP and DEHP and Pb co-treatment groups. Other studies have also observed DEHP-induced hepatomegaly in rats (Musthag et al., 1980). The modes of action of DEHP in inducing hepatomegaly has been proposed to include: (1) activation of peroxisome proliferator-activated receptor- $\alpha$



**Figure 4.**  $Mg^{2+}$  ATPase activities in brain, kidney and liver of rats. Bars of the same compartment carrying different letters of the alphabet are significantly different from each other ( $p < 0.05$ ).

(PPAR $\alpha$ ) (2) proliferation of peroxisomes and induction of peroxisomal proteins, (3) induction of non-peroxisomal metabolism proteins, (4) induction of cell proliferation, (5) suppression of apoptosis, (6) production of reactive oxygen species, (7) oxidative DNA damage, and (8) inhibition of gap junctional intercellular communication (Blystone et al., 2010; Mitchell et al., 1985; NTP-CERHR 2006; Rusyn et al., 2006; Zang et al., 2017; Rowdhwai and Cheng, 2018; Ito et al., 2019).

Unlike in the liver, there was brain atrophy observed in the Pb and DEHP exposed groups and this effect was additive in co-treatment. Atrophy of the brain is a common feature of lead poisoning (Cecil et al., 2008) and many of the diseases that affect the brain. Brain atrophy describes a loss of neurons and the connections between them. It could be generalized, meaning that all of the brain has shrunk; or it could be focal, affecting only a limited area of the brain resulting in a decrease of the functions that area of the brain controls. The work of Mayeux and Stern (2012) estimated the global prevalence of dementia to be as high as 24 million, and predicted it to double every 20 years until at least 2040. With the global rise in prevalence of Alzheimer's and dementia correlating with a global rise in the use of phthalate – containing plastic products (Horn et al., 2004), and the result of this study that has shown an additive effect of lead and DEHP on brain atrophy, there is some scope in the hypothesis that world increase in prevalence of dementia in the aged could be linked to

accumulation of interaction of environmental pollutants such as lead and phthalates in the system (Stewart and Schwartz, 2007).

This study has also shown that combined exposure to Pb and DEHP displayed different effects on organ ATPases from individual exposures to these toxicants. Response of the liver ATPases were especially significant and different in combined exposure.  $Ca^{2+}$  ATPase functions in the transfer of  $Ca^{2+}$  ions across cell membranes, mediating diverse physiological processes such as gene expression, contraction, secretion, fertilization, proliferation and apoptosis (Jiang and Zi-jiang, 2008; Mata and Sepulveda, 2010). One of the mechanisms through which  $Pb^{2+}$  exerts its toxic effects is by mimicking the action of  $Ca^{2+}$  in many physiological processes hence the supplementation of  $Ca^{2+}$  and  $Zn^{2+}$  has been shown to ameliorate  $Pb^{2+}$  toxicity (Prasanthi et al., 2006). This is because these divalent cations compete for the same binding sites as  $Pb^{2+}$  in the intestinal mucosa. In this study, individual exposure to  $Pb^{2+}$  and DEHP was found to inhibit hepatic  $Ca^{2+}$ -ATPase - which is in consonance with the work of Abam et al. (2008) and Afolabi et al. (2016), however, co-treatment with both agents resulted in significant induction of the protein. An explanation for this increase in hepatic activity of  $Ca^{2+}$  ATPase could be that the interaction of  $Pb^{2+}$  and DEHP in co-treatment resulted in a greater bioavailability of  $Ca^{2+}$  ions leading to an increase in the activity of  $Ca^{2+}$ -ATPase in order to

maintain low intracellular levels of calcium. Calcium is tightly regulated because of its significance as a powerful second messenger crucial in proliferation, differentiation, mitosis, and motility (Capiod et al., 2011) hence calcium is removed to the extracellular environment and/or sequestered in the endoplasmic reticulum. Secondly, intracellular calcium is also known to be regulated by  $\text{Na}^+\text{K}^+\text{-ATPase}$  (Tian and Xie, 2008). For example, binding of ouabain (a well-known physiological inhibitor of  $\text{Na}^+\text{K}^+\text{-ATPase}$ ) to the enzyme leads to increase in intracellular calcium in cardiac myocytes, resulting in increases in myocardial contraction. This is the basis for the administration of digitalis in treating congestive heart failure. Mechanistically, the  $\text{Na}^+\text{K}^+\text{-ATPase}$  has been shown to regulate calcium entry through the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (NCX) by altering intracellular  $\text{Na}^+$  concentration in cardiac myocytes (Blaustein et al., 1998). Physical coupling among the  $\text{Na}^+\text{K}^+\text{-ATPase}$ , NCX, and sarcoplasmic reticulum calcium store has been demonstrated in smooth muscle cells (Moore et al., 1993). Other workers (Juhaszova and Blaustein, 1997; Song et al., 2006) have provided evidence that the  $\text{Na}^+\text{K}^+\text{-ATPase}$  interacts with NCX to form a specific calcium-signaling microdomain in many different cell types. The greater bioavailability of  $\text{Ca}^{2+}$  due to  $\text{Pb}^{2+}$  and DEHP co-treatment in the liver may have triggered an increase in NCX activity (Matsuoka, 1995) which in turn may have led to the observed increase in activity of  $\text{Na}^+\text{K}^+\text{-ATPase}$  activity. Renal  $\text{Ca}^{2+}$  ATPase activity was also observed to be upregulated by  $\text{Pb}^{2+}$  in this study, which could be explained as hormetic. Hormesis is the concept whereby non-nutritional toxic substances impart beneficial or stimulatory effects at low to moderate concentrations but adverse effects at high concentrations (Klaassen and Watkins, 2015). DEHP imparted no effects on the enzyme while co-treatment with  $\text{Pb}^{2+}$  and DEHP produced antagonistic effects. A similar antagonistic association between  $\text{Pb}^{2+}$  and DEHP combined treatment was also observed in brain  $\text{Ca}^{2+}$  ATPase activity suggesting that the binding of one toxicant may decrease the binding of the other toxicant through a conformational change in the enzyme.

Unlike  $\text{Ca}^{2+}$  ATPase, individual exposures to  $\text{Pb}^{2+}$  and DEHP upregulated hepatic  $\text{Na}^+\text{K}^+\text{-ATPase}$  activities. This is contrary to various works that have recorded an inhibitory effect on the hepatic enzyme by both agents (Yücebilgiç et al., 2003; Dhanya et al., 2003; Krstić et al., 2008; Afolabi et al., 2016). This disparity in results may be explained by differences in conditions, concentration of toxicant administered, ages and species of animals and subjects employed in the study – well established indices that have been shown to affect response to toxicants. Yücebilgiç et al. (2003) employed human subjects in their study and they observed the enzyme inhibition in the blood of the lead-exposed workers.

Although Dhanya et al. (2003) worked on rats, DEHP was administered intraperitoneally and at a much lower dose of 750  $\mu\text{g}/\text{kg}$  body weight (compared to 100  $\text{mg}/\text{Kg}$  body weight employed in this study). Krstić et al. (2008) employed very large adult rats (average of 330 – 400 g) in their study unlike the present study that used rats with average weights of 140 g. Afolabi et al. (2016) employed rats with an average weight of 80 – 100 g (7 – 8 weeks old) unlike the present study. Co-treatment produced potentiating effects on  $\text{Na}^+\text{K}^+\text{-ATPase}$  activity. Three mechanisms proposed for the potentiation of  $\text{Pb}^{2+}$ -induced activation of hepatic  $\text{Na}^+\text{K}^+\text{-ATPase}$  by DEHP in co-treatment are: (1) an increase in concentration of  $\text{Na}^+$  or  $\text{K}^+$  or  $\text{Ca}^{2+}$ , (2) some natural physiologic inhibitor of  $\text{Na}^+\text{K}^+\text{-ATPase}$  such as ouabain may have been downregulated as a result of  $\text{Pb}^{2+}$  and DEHP interaction in co-treatment leading to an up-regulation in the activity of the enzymes, (3) Some compensatory mechanisms. Increase in  $\text{Na}^+\text{K}^+\text{-ATPase}$  activity as a result of higher bioavailability of  $\text{Ca}^{2+}$  has already been discussed in the preceding paragraph. In the brain,  $\text{Na}^+\text{K}^+\text{-ATPase}$  activities were generally downregulated by  $\text{Pb}^{2+}$  and DEHP (in accordance with the work of Okediran et al., 2009; Dhanya et al., 2003) while combined treatment produced no significantly different effects from the individual single exposures.  $\text{Na}^+\text{K}^+\text{-ATPase}$  plays a crucial role in the maintenance of the  $\text{Na}^+$  and  $\text{K}^+$  gradients across the plasma membrane of neuronal cells hence the function of these cells are hinged on the efficient regulation of the transport function of this ion pump. Research on human genetic diseases and various animal models have confirmed that neuronal activity and survival are dependent on a normal functioning of the  $\text{Na}^+$  pump. Adverse effects on the  $\text{Na}^+\text{K}^+\text{-ATPase}$  pump may lead to neuronal death (Horisberger and Geering, 2009).  $\text{Pb}^{2+}$  had no effects on renal  $\text{Na}^+\text{K}^+$  ATPase activity at the dose and duration administered, however DEHP and co-treatment caused approximately the same level of inhibition of the enzyme showing the absence of any  $\text{Pb}^{2+}$  effects on DEHP induced inhibition of the enzyme in the kidney.

$\text{Mg}^{2+}$  ATPase activities showed organ-specific responses to  $\text{Pb}^{2+}$ , DEHP and combined treatments with both toxicants just as already observed with the other ATPases. In the brain,  $\text{Mg}^{2+}$  ATPase activity was stimulated by individual exposure to  $\text{Pb}^{2+}$  and DEHP while co-exposure to both toxicants produced an antagonistic effect of  $\text{Pb}^{2+}$  on DEHP. On the contrary, liver and kidney  $\text{Mg}^{2+}$  ATPase activities were down-regulated by lead and DEHP treatments while combined treatments with both toxicants restored enzyme activity towards control value in the liver but sustained DEHP induced inhibition in the kidney. Compensatory and adaptive mechanisms may explain the restoration of enzyme activities to control values in the liver. Such mechanisms are popular in organ

responses to toxicants (Klaassen and Watkins, 2015). Total ATPase enzyme responses to  $Pb^{2+}$  and DEHP treatment were also different from one organ to the other.  $Pb^{2+}$  showed a potentiating effect on DEHP induced inhibition of total ATPase in the brain, while an antagonistic effect was observed between both toxicants in the kidney. On the contrary in the liver, a significant activation of the enzyme was observed, which was in consonant with liver  $Na^+K^+$  ATPase and  $Ca^{2+}$  ATPase. Only in  $Mg^{2+}$  ATPase liver activity was the compensatory mechanism not complete in co-treatment, rather, the individually induced inhibitions were reversed to control value.

In this study, it is observed that DEHP acted somewhat like a chelator for  $Pb^{2+}$  in the liver. The association between DEHP and  $Pb^{2+}$  seemed to relieve  $Pb^{2+}$  and DEHP induced inhibitions of the ATPases in the liver leading to neutralization of  $Pb^{2+}$  and DEHP individual inhibitions of  $Mg^{2+}$  ATPase, conversion of individual inhibitions of  $Ca^{2+}$  ATPase to a 17 % activation of the enzyme, additive increase in  $Na^+K^+$  ATPase activity over and above the increase in activity of the enzyme observed in individual exposures to both toxicants. This shows that at some concentrations, combined exposures to toxicants may actually neutralize their individual toxic effects in some organs while magnifying the toxic effects in other organs.

## Conclusion

The findings of this study demonstrate tissue specific responses of ATPases to lead and DEHP co-exposure in rat, with hepatic effects deviating significantly from the brain and renal compartments. More work is needed to evaluate the compensatory and adaptive mechanisms that could have accounted for these deviations and how this contributes to or protects from hepatic degeneration.

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## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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