



Vitamin C mitigates hematological and biochemical alterations caused by di(2-ethylhexyl) phthalate toxicity in female albino mice, *Mus musculus*

Meenakshi Soni¹ · Mohd. Zahoor ul haq Shah¹ · Vinoy Kumar Shrivastava¹

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Abstract

Di(2-ethylhexyl) phthalate (DEHP) is ubiquitous environmental contaminant and identified as endocrine-disrupting chemical (EDC), present in plastics as plasticizer. Due to its versatile use, human exposure level reaches to danger limit. The main focus of our study is to see the effect of vitamin C on hematological and biochemical alterations caused by Di(2-ethylhexyl) Phthalate toxicity in female albino mice, *Mus musculus*. It is found to cause defects of the liver, kidney, and lungs. Its anti-androgenic nature brings the main focus on its toxicity associated with reproductive and endocrine system. In this experimental study, 18 young female Swiss albino mice, *Mus musculus*, were used and divided into 3 groups of 6 animals each as control (corn oil vehicle), DEHP group (100 mg/kg body weight dissolved in corn oil), and DEHP + vitamin-C group (100 mg/kg body weight each, dissolved in corn oil and double distilled water, respectively) for 90 days. In this research, serum metabolites were evaluated to study the effect of DEHP on glucose, total protein, and lipid profile along with some hematological, enzymological, and oxidative stress parameters. Simultaneously, we compared the effectiveness of vitamin-C against DEHP toxicity to mitigate the serum homeostasis disturbance. In present study, we observed, in DEHP-treated animals, glucose, triglycerides, very-low-density lipoprotein (VLDL), total protein, alkaline phosphatase (ALP), acid phosphatase (ACP), and alanine aminotransferase (ALT) levels increased remarkably, whereas total cholesterol, high-density lipoproteins (HDL), aspartate aminotransferase (AST), total RBC count, total WBC count, and hemoglobin (Hb) level significantly decreased as compared to control group. In addition, we noticed there was a decrease in superoxide dismutase (SOD) and increase in levels of lipid peroxidation (MDA) and interleukin-6 (IL-6) in DEHP treatment group as compared to control group. The results indicated vitamin C had a better improving effect against DEHP toxicity on balancing metabolic abnormalities and inflammation-related comorbidities.

Keywords DEHP · Vitamin C · Hematology · Oxidative stress · Biochemistry

Introduction

Since its discovery in 1930, di(2-ethylhexyl) phthalate (DEHP), a member of the phthalate ester class of chemical, has been the most frequently used plasticizer. DEHP is added to plastics to make them softer and more flexible. They are ubiquitous pollutants found in toys, childcare products, food packaging, household items, personal care products, medical devices, modern electronics, detergents, vinyl flooring, air fresheners, and a variety of other consumer products as

plasticizers, solvents, adhesives, additives, binding agents, or for some other reason (Xu et al. 2010; Koniecki et al. 2011). Due to its acceptable qualities and low cost as a plasticizer in polyvinyl chloride (PVC) items, DEHP is one of the most widely used phthalates. Its extensive use causes substantially higher exposure with an average daily intake of 3–30 g/kg/day, although occupational exposure levels are even higher, reaching up to 300–600 g/kg body weight/day (Doull et al. 1999; Kavlock et al. 2002). They are easily leached out of goods by heat, agitation, and lengthy storage since they are not chemically linked to the materials (to which they are added). This can happen at any point in the product's life cycle, from conception through disposal. They infiltrate human bodies via ingestion, inhalation, or direct contact (Chen et al. 2008; Schettler 2006). The yearly global production of phthalates around us is believed to be more

✉ Mohd. Zahoor ul haq Shah
scholarendocrinology@gmail.com

¹ Laboratory of Endocrinology, Department of Bioscience,
Barkatullah University, Bhopal 462026, MP, India

than 2 million tonnes, according to reports (Latini 2005). The presence of phthalates and their metabolites in sewage and sediment was found to be greater above the European Union's safety guidelines (Gani and Kazmi 2020). DEHP and its metabolite, mono(2-ethylhexyl) phthalate (MEHP), have been found in a variety of human tissues, including urine (Silva et al. 2000; Becker et al. 2004; Genius et al. 2012; Arbuckle et al. 2016), breast milk (Hogberg et al. 2008; Arbuckle et al. 2016), blood (Hogberg et al. 2008; Genius et al. 2012), cord blood (Latini et al. 2003), and follicular fluid (Krotz et al. 2012). It has also been reported that phthalates have a greater influence on children and women. Children receive greater doses per unit body surface area due to their chewing habits and are at greater risk because of their developing endocrine and reproductive systems (Johnson et al. 2010, 2011). Adult women excrete larger amounts of phthalates metabolites in their urine, presumably as a result of their frequent use of personal care products and cosmetics (Fourth National Report on Human Exposure to Environmental Chemicals (Crinnion 2010): The Centre for Disease Control (CDC)). DEHP has been identified as a reproductive toxicant and an endocrine disruption chemical (EDC) (Kay et al. 2014; Hannon and Flaws 2015; Chiang et al. 2017; Rattan et al. 2017), and it has been linked to lung, liver, kidney, and reproductive system damage, developmental toxicity, and lowered sperm count (Rastogi et al. 2006).

EDCs exert their effects through a variety of mechanisms, including mimicking endogenous hormones, antagonizing normal hormones, and disrupting the natural cycle of hormone production or metabolism via hormone receptor activation/repression (Sonnenschein and Soto 1998; Diamanti-Kandarakis et al. 2009). Reduced conception rates, higher miscarriage rates, lower estrogen levels, and irregular ovulation are all linked to female employees' occupational exposure to DEHP, according to epidemiological research (Reddy et al. 1976; Aldyreva et al. 1975). DEHP and its bioactive metabolite MEHP have been demonstrated to cause a decrease in female sex hormone synthesis and a depletion of primordial follicles (Hannon et al. 2014; Lovekamp and Davis 2001; Hannon et al. 2015). However, the exact methods by which DEHP impacts female reproductive, however, are unknown.

DEHP is well-known for its reproductive toxicity and teratogenic consequences, which might include liver damage, neurotoxicity, nephrotoxicity, thyroid dysfunction, and immune system disruption (Carlisle et al. 2009) and cardiovascular disease (Muscogiuri and Colao 2017). Hematological analysis is also useful in determining physical health status (Parida et al. 2012, 2013, 2014; Pal et al. 2018). Changes in the blood profiles of amino acids and phosphatidylcholines identified in rats

following DEHP exposure reflect these effects on the liver (van Ravenzwaay et al. 2010). Targeted and quantitative serum metabolomics have been shown to provide functional insights, particularly in the development and progression of metabolic disorders (Suhre et al. 2011). DEHP-fed rats displayed altered glucose tolerance, which was linked to aberrant glucose intermediate metabolite levels in the liver and skeletal muscle, as well as a glucose transport deficit and a reduction in glycogen synthesis (David et al. 2000).

DEHP's negative effects have been linked to the peroxisome proliferator, hormonal instability, and free radical production (Lovekamp-Swan and Davis 2003). Choi et al. (2004) previously revealed that about 94% of the 48 EDC identified by the Centers for Disease Control and Prevention (CDC) produce free radicals, suggesting that this property is a common toxic mechanism underpinning EDC effects. As a result, antioxidant supplementation was proposed as a way to prevent a range of EDC-mediated toxicities that develop following chemical exposure. Vitamin C and vitamin E are small molecule antioxidants that have been discovered to interact directly with oxidizing radicals (Jones et al. 1995). Vitamin C decreases oxidized vitamin E and scavenges aqueous-phase ROS through fast electron transfer, whereas vitamin E stops the chain process of lipid peroxidation in biomembranes (Wang and Quinn 1999). Antioxidants may thus be beneficial in the treatment of free radical-related illnesses, such as toxins (Caxico Vieira et al. 2018; Kim and Lee 2018; Lee 2018; Roh et al. 2018). Supplementation with antioxidant vitamins (vitamins C and E) reduced DEHP-induced testicular damage (Ishihara et al. 2000; Madkour 2014; Suna et al. 2007).

All stuffs that contain ascorbic acid, not just the acid but also its isomers, can be considered to contain vitamin C (Mousavi et al. 2019). With its prominent role in electron transfer, immunological response, cell metabolism, enzymatic reactions (Ekeh et al. 2019), and oxidation–reduction equilibrium in the body, vitamin C is believed to be an efficient antioxidant. The vitamin has a broad therapeutic effect, has been studied for illness prevention, and has been observed in the treatment of health disorders as severe as cancer (Nechuta et al. 2011). Vitamin C also helps to keep other antioxidants from becoming inactive (Sharma 2013). The chemical has been studied for its ability to promote iron absorption in the small intestine (Hallberg et al. 1987) as well as its ability to reduce oxidative damage in vascular walls (Diaz et al. 1997). Because of its low cost and beneficial effects on infection therapy in critically ill or ventilated patients, vitamin C has also been investigated as a viable treatment for COVID-19 (Hemilä and Chalker 2020). The aim of the present study was to determine the detrimental effects of DEHP on young female mice and to find possible therapeutic role of vitamin C against it.

Materials and methods

Chemicals

DEHP (> 98% purity), vitamin C (L-ascorbic acid), and corn oil were purchased from TCI Chemicals (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan). The kits for glucose, cholesterol, triglycerides, and total protein were obtained from the Elab science and Meril Diagnostics. The other chemicals used in this experiment were of analytical grade.

Experimental animals

Eighteen young female Swiss albino mice, *Mus musculus*, weighing 15–20 g were purchased from the JEEVA LIFE SCIENCES, Malkajgiri, Telangana. The mice were housed at Animal House of Biosciences Department, Barkatullah University, Bhopal, and given period of 1 week for acclimatization to adjust the laboratory surroundings (temperature: $22\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$, relative humidity: 45%; light/dark cycle: 12 h). The Institutional Ethics Committee of Barkatullah University Bhopal authorized all protocols (Ethical Certificate Number 1885/GO/Re/S/16/CPCSEA/IAEC/BU/24). After acclimatization, mice were divided into three groups of 6 animals each and given different treatment for 90 days.

- Group 1: Control is fed with normal diet, water ad libitum, and corn oil (vehicle) by oral gavage.
- Group 2: DEHP-treated group is given DEHP (100 mg/kg body weight) dissolved in corn oil that acts as vehicle.
- Group 3: DEHP + vitamin C: this group received DEHP (100 mg/kg body weight) dissolved in corn oil along with vitamin C (100 mg/kg body weight) dissolved in double distilled water.

After the commencement of 90 days of dosing, whole blood was taken directly from the inferior vena cava with 1 mL syringe, allowed to clot, and the serum was separated. The blood samples for hematological analysis were collected into complete blood count (CBC) bottles containing ethylenediamine tetraacetate (EDTA). For serum biochemistry analysis, blood samples were centrifuged at $2500 \times g$ for 10 min within 1 h after collection. The sera were stored at $-80\text{ }^{\circ}\text{C}$ in a freezer before analysis.

Biochemical analysis

Serum glucose and total protein levels were determined calorimetrically using commercially available kits (Elab science). Lipid-related indicators, including high-density lipoprotein (HDL), triglycerides (TG), and total cholesterol (TC), were

measured from serum samples using the corresponding kits (Meril Diagnostics) in accordance with the manufacturer's instructions. Friedewald equation is used for calculation of LDL cholesterol ($\text{LDL cholesterol} = \text{total cholesterol} - \text{HDL cholesterol} - [\text{triglycerides}/5]$).

Hematological analysis

The total red blood cell count and the total white blood cell count were done with the help of Neubauer counting chamber method (Schalm et al. 1975). The hemoglobin percentage was calculated with the help of Sahli's hemoglobinometer (Sahli's acid hematin method, Wintrobe 1975).

Enzymological analysis

King and Kings method was used for the determination of serum acid phosphatase (ACP) and alkaline phosphatase (ALP), whereas aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by using Reitman and Frankel method.

Antioxidant assay

Lipid peroxidation assay

Lipid peroxidation (LPO) was measured in ovary and expressed as malonaldehyde (MDA) content (Rehman 1984). Using a glass homogenizer, the ovaries were removed, and tissue homogenate (10%) was made in ice-cold normal saline. After that, the homogenate was centrifuged for 10 min at 3000 rpm. A milliliter of the supernatant was incubated for 2 h at $37 \pm 0.5\text{ }^{\circ}\text{C}$. Each sample received 1 mL of tris hydrochloric acid (TCA) (10%), which was correctly mixed before centrifugation at 2000 rpm for 5 min at $4\text{ }^{\circ}\text{C}$. An equivalent volume of 0.67% of 2-thiobarbituric acid (TBA) was added to 1 ml of supernatant, stirred thoroughly, and maintained in a boiling water bath for 10 min. After cooling, the samples were diluted with 1 mL of distilled water. The optical density (OD) was measured at 535 nm using a spectrophotometer. The absolute MDA levels were measured in nanomoles per gram of moist tissue.

Superoxide dismutase

The activity of superoxide dismutase (SOD) was calculated using the Marklund and Marklund method (Marklund and Marklund 1974). The absorbance was measured at 420 nm at 1-min intervals for 3 min using 2.9 ml of tissue homogenate supernatant (10%) and 100 ml of pyrogallol (0.2 mM). The final result of SOD activity was measured in units per gram of moist tissue.

Pro-inflammatory/inflammatory biomarkers (interleukin-6)

To measure the quantities of IL-6 in the blood, we used a quantitative standard sandwich ELISA technique with mouse kits from Elabscience Biotechnology Inc.

Statistical analysis

The differences among groups are presented as means \pm standard error of mean (SEM) and were analyzed using one way ANOVA followed by Tukey's multiple comparison tests with GraphPad Prism 9.3.1 software. p -value < 0.05 , < 0.001 , and < 0.0001 were treated as statistically significant, more significant, and highly significant, respectively.

Results

Effect of DEHP and DEHP + vitamin-C exposure on biochemical parameters

Table 1 illustrates the effect of DEHP and DEHP + vitamin C on glucose, total protein, and lipid profile. In DEHP-treated group, we observed significant increase in the level of blood glucose ($p < 0.0001$), total protein ($p < 0.0001$), triglycerides ($p < 0.0001$), and VLDL (0.0005), whereas levels of total cholesterol ($p < 0.0001$) and HDL ($p < 0.0001$) decreased and level of LDL ($p = 0.06$) remained unchanged as compared to control group. The results of group DEHP + vitamin C showed reduction in level of blood glucose ($p < 0.0001$), total protein ($p = 0.0001$), triglycerides ($p < 0.0001$), and VLDL ($p < 0.0001$) as compared to the DEHP group, whereas levels of total cholesterol ($p < 0.0001$) and HDL ($p = 0.0007$) increased with respect to DEHP group.

Effect of DEHP and vitamin-C exposure on hematological parameters

Figure 1A–C indicates significant decrease in total RBC count ($p < 0.0001$), total WBC count ($p < 0.0001$), and hemoglobin (Hb) level ($p < 0.0001$) of DEHP-treated group as compared to control group, whereas DEHP + vitamin-C group showed significant increases in total RBC count ($p = 0.0004$), total WBC count ($p = 0.0004$), and hemoglobin (Hb) level ($p = 0.0002$) with respect to DEHP group.

Effect of DEHP and vitamin-C exposure on enzymological parameters

Figure 2A, B indicates significant increase in alkaline phosphatase (ALP) ($p < 0.0001$), acid phosphatase (ACP) ($p < 0.0001$), alanine aminotransferase (ALT) ($p < 0.0001$), and significant decrease in aspartate aminotransferase (AST) ($p < 0.0001$) of DEHP-treated group as compared to control group. DEHP + vitamin-C group showed significant decrease in alkaline phosphatase (ALP) ($p < 0.0001$), acid phosphatase (ACP) ($p = 0.0001$), alanine aminotransferase (ALT) ($p = 0.002$), and significant increase in aspartate aminotransferase (AST) ($p = 0.0005$) with respect to DEHP group.

Effect of DEHP and vitamin-C exposure on antioxidant assay and lipid peroxidation

Figure 3 illustrates effect of DEHP and DEHP + vitamin C on antioxidant activity and lipid peroxidation. When compared to the control group, DEHP group showed a substantial decrease in antioxidant enzyme activity SOD ($p < 0.0002$) and an increase in lipid peroxidation MDA ($p < 0.0001$). However, we discovered a substantial increase in SOD ($p < 0.0033$) activity and decrease in MDA ($p < 0.0001$) levels in DEHP + vitamin-C treatment group.

Table 1 Effect of DEHP and DEHP + vitamin-C treatment on glucose level, lipid profile, and total protein level

Parameters	Control	DEHP	DEHP + vitamin C
Glucose (mg/dl)	124.78 \pm 0.98	178.56 \pm 1.06a****	127.87 \pm 1.24b****
Cholesterol (mg/dl)	127.96 \pm 1.61	115.69 \pm 1.41a****	123.65 \pm 1.67b**
Triglycerides (mg/dl)	129.63 \pm 0.72	136.23 \pm 1.00a****	128.12 \pm 0.72b****
HDL (mg/dl)	88.92 \pm 0.94	80.84 \pm 0.67a****	86.2 \pm 0.64b***
LDL (mg/dl)	12.96 \pm 0.66	7.61 \pm 0.96a***	11.83 \pm 0.8a***
VLDL (mg/dl)	26.08 \pm 0.18	27.24 \pm 0.20a****	25.62 \pm 0.14b****
Total protein (mg/dl)	7.64 \pm 0.31	14.63 \pm 1.12a****	8.56 \pm 0.78b****

Control (corn oil); DEHP (100 mg/kg body weight in corn oil); DEHP (100 mg/kg body weight in corn oil) + vitamin C (100 mg/kg body weight in distilled water); a, control vs. DEHP; b, DEHP vs DEHP + vitamin C. Data is expressed as mean \pm SEM values

ns nonsignificant

** $p < 0.01$; *** $p < 0.001$; **** $p \leq 0.0001$ ($n = 6$)

Fig. 1 **A** Effect of different treatments on blood hemoglobin (Hb) level. Control (corn oil); DEHP (100 mg/kg body weight in corn oil); DEHP (100 mg/kg body weight in corn oil)+ vitamin C (100 mg/kg body weight in distilled water); a, control vs. DEHP; b, DEHP vs DEHP + vitamin C. Data is expressed as mean \pm SEM values. **** $p < 0.0001$, *** $p < 0.001$ ($n = 6$). **B** Effect of different treatments on blood RBC levels. Control (corn oil); DEHP (100 mg/kg body weight in corn oil); DEHP (100 mg/kg body weight in corn oil)+ vitamin C (100 mg/kg body weight in distilled water); a, control vs. DEHP; b, DEHP vs DEHP + vitamin C. Data is expressed as mean \pm SEM values. **** $p < 0.0001$, *** $p < 0.0001$ ($n = 6$). **C** Effect of different treatments on blood WBC levels. Control (corn oil); DEHP (100 mg/kg body weight in corn oil); DEHP (100 mg/kg body weight in corn oil)+ vitamin C (100 mg/kg body weight in distilled water); a, control vs. DEHP; b, DEHP vs DEHP + vitamin C. Data is expressed as mean \pm SEM values. **** $p = 0.0001$, *** $p < 0.001$ ($n = 6$)

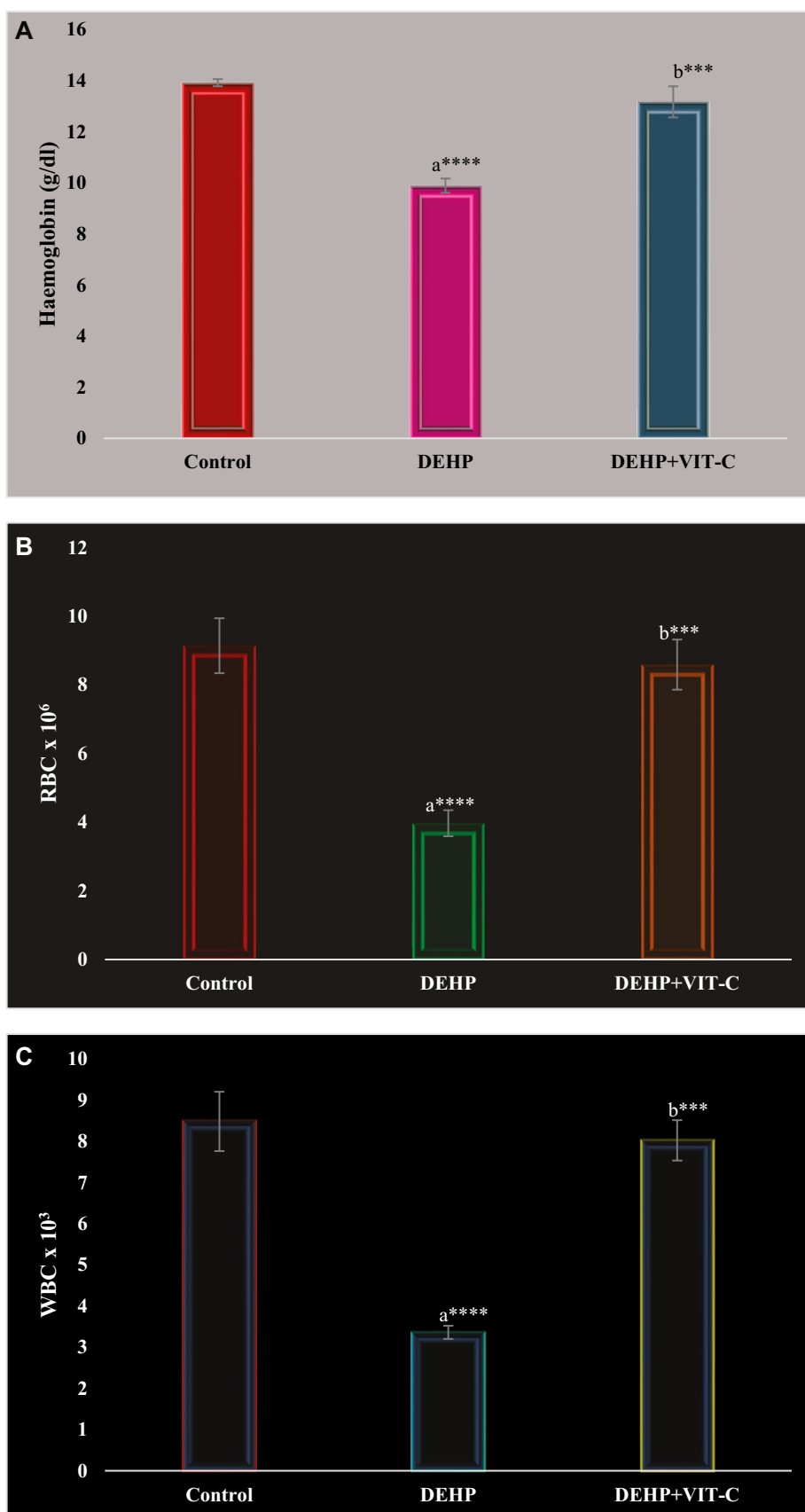
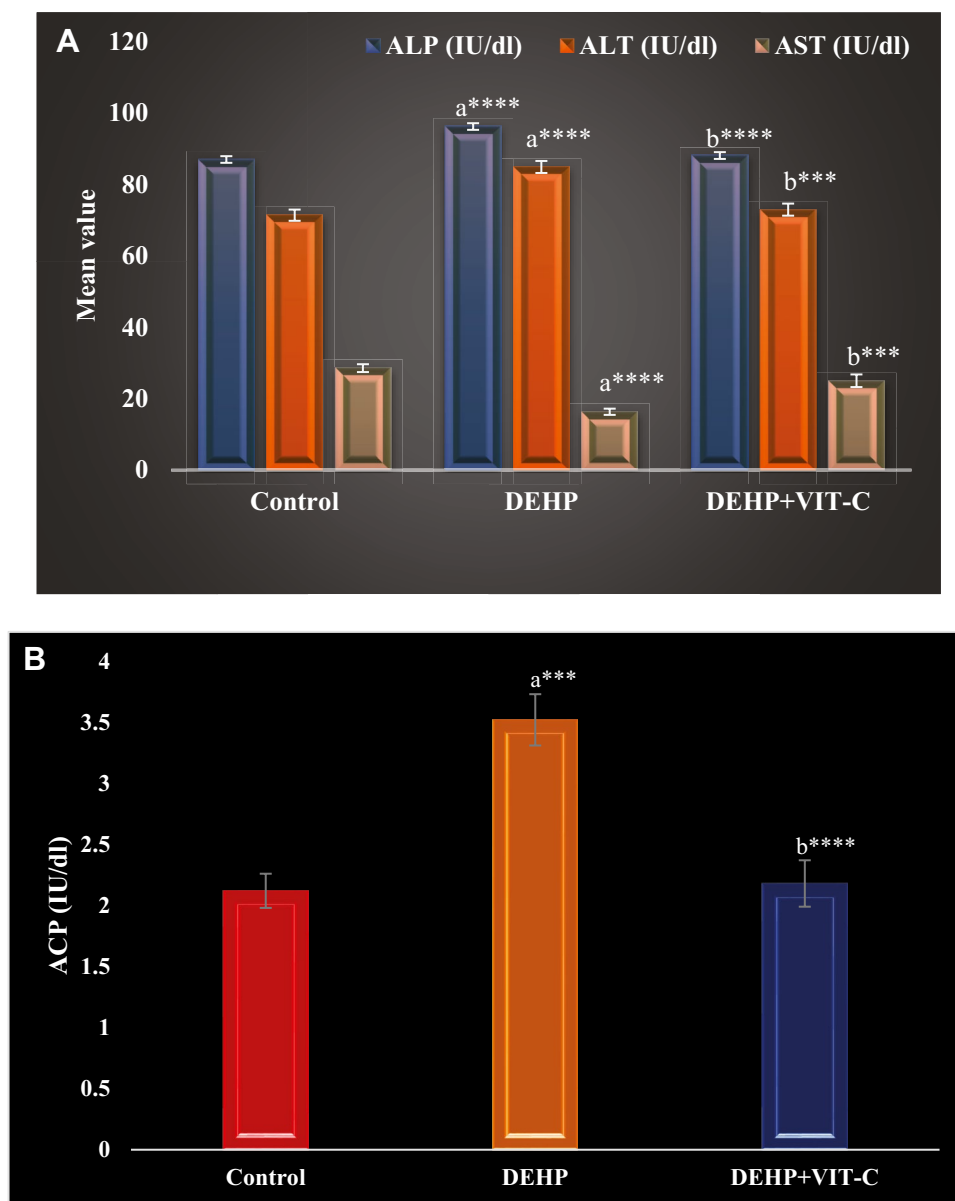


Fig. 2 **A** Effect of different treatments on serum ALP, ALT, and AST levels. Control (corn oil); DEHP (100 mg/kg body weight in corn oil); DEHP (100 mg/kg body weight in corn oil) + vitamin C (100 mg/kg body weight in distilled water); a, control vs. DEHP; b, DEHP vs DEHP + vitamin C. Data is expressed as mean \pm SEM values. **** $p < 0.0001$, *** $p < 0.001$, ($n = 6$). **B** effect of different treatments on serum acid phosphatase (ACP) levels. Control (corn oil); DEHP (100 mg/kg body weight in corn oil); DEHP (100 mg/kg body weight in corn oil) + vitamin C (100 mg/kg body weight in distilled water); a, control vs. DEHP; b, DEHP vs DEHP + vitamin C. Data is expressed as mean \pm SEM values. **** $p \leq 0.0001$, *** $p < 0.001$, ($n = 6$)



Effect of DEHP and vitamin-C exposure on IL-6

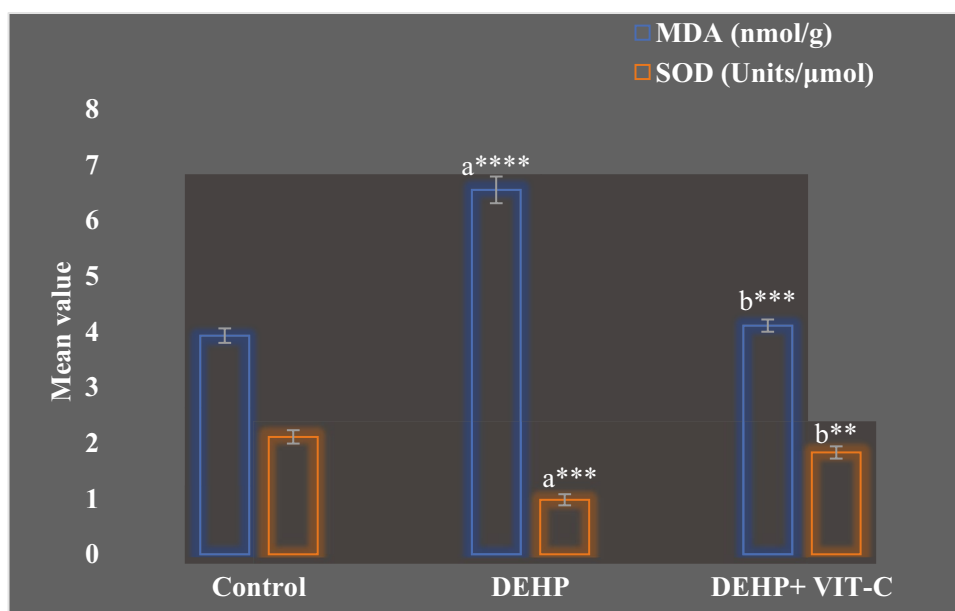
We found there was a significant increase ($p < 0.001$) in IL-6 after administration DEHP when compared to the control group. However, we observed a substantial decrease ($p < 0.0001$) in these levels in DEHP + vitamin-C treatment group (Fig. 4).

Discussion

Synthetic and natural molecules that interfere with endogenous endocrine functioning are known as “endocrine disruptors” or “endocrine-disrupting chemicals (EDCs)” (Rosenfeld et al. 2017; Yoon et al. 2014; Zawatski and Lee 2013). Phthalic

acid esters, which are major industrial compounds utilized as plasticizers in various plastic formulations, have been linked to many of the identified endocrine-modulating, deleterious reproductive, and developmental impacts (Gardner et al. 2016; Inoue et al. 2002). Because of its widespread use and hazardous effects, di(2-ethylhexyl) phthalate (DEHP) has been widely explored among phthalates (He et al. 2018; Magdouli et al. 2013). DEHP exposure comes mostly by ingestion of residues in foods, with less exposure from air and water, but occupational exposures via inhalation have the greatest potential (Fromme et al. 2007). Vitamin-C supplementation has been shown to increase the antioxidant capacity in rats indicating resistance to oxidative damage in response to DEHP treatment (Husain and Somani 1997). Recently, Wang et al. (2017a) demonstrated that vitamin E co-treatment with DEHP is protected

Fig. 3 Effect of different treatments on malondialdehyde (MDA) and SOD levels. Control (corn oil); DEHP (100 mg/kg body weight in corn oil); DEHP (100 mg/kg body weight in corn oil) + vitamin C (100 mg/kg body weight in distilled water); a, control vs. DEHP; b, DEHP vs DEHP + vitamin C. Data is expressed as mean \pm SEM values. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$ ($n = 6$)

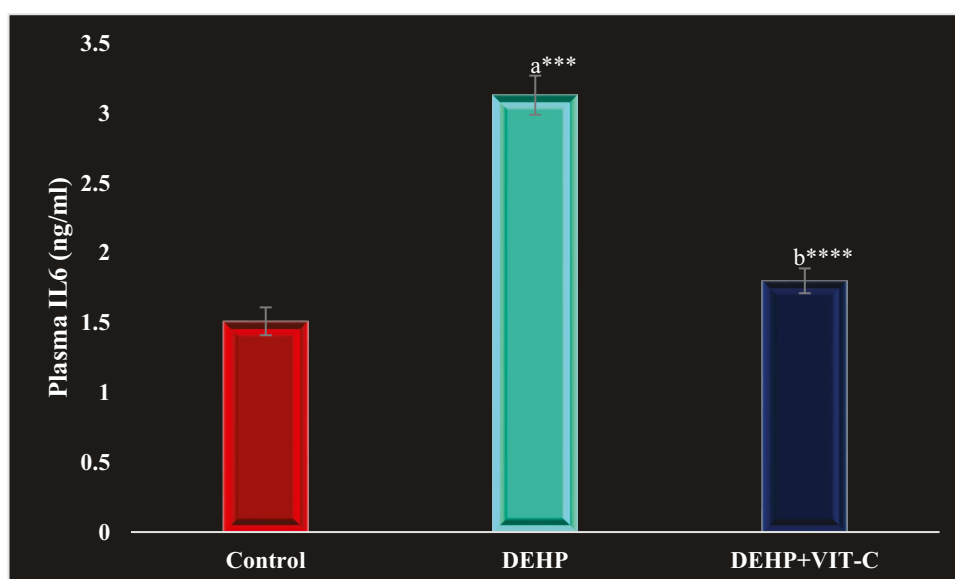


against DEHP-induced testicular toxicity via PPAR-dependent mechanism. In this study, vitamin E supplementation significantly lowered DEHP-induced PPAR upregulation especially PPAR γ (Wang et al. 2017b). A number of EDC including phthalates affect steroidogenic pathway by disturbance with LH-stimulated cAMP production, cholesterol transport and metabolism in mitochondria, and increasing oxidative stress (Wang et al. 2017a, b).

In the current study, we observed increase in the blood glucose level in mice which were treated with DEHP. Our results are in line with the previous study (Aydemir et al. 2018). In one more recent investigation, DEHP treatment was found to cause high glucose and insulin levels in rats, indicating that the rats were suffering from a glucose

metabolic disease. The disruption in the JAK2/STAT3/SOCS3 pathway is the cause of this problem (Xu et al. 2018). EDCs that interact with the endocrine (or hormonal) system, such as DEHP, have been linked to the obesity epidemic (Wassenaar and Legler 2017; Veiga-Lopez et al. 2018). Abnormal lipid levels in the blood, such as LDL-C, HDL-C, TG, and TC, are referred to as lipid metabolic disorder (Fulcher et al. 2015). In vitro and in vivo studies have shown that DEHP and its primary metabolite MEHP change serum lipids (Wang et al. 2019), EDCs that interact with the endocrine (or hormonal) system, such as DEHP, have been linked to the obesity epidemic (Wassenaar and Legler 2017; Veiga-Lopez et al. 2018). In the DEHP-treated group, we found an increase in triglycerides and VLDL but

Fig. 4 Effect of different treatments on interleukin-6 (IL-6) levels. Control (corn oil); DEHP (100 mg/kg body weight in corn oil); DEHP (100 mg/kg body weight in corn oil) + vitamin C (100 mg/kg body weight in distilled water); a, control vs. DEHP; b, DEHP vs DEHP + vitamin C. Data is expressed as mean \pm SEM values. **** $p < 0.0001$, *** $p < 0.001$ ($n = 6$)



a decrease in total cholesterol and HDL. Kwack et al. (2009) and Yu et al. (2021), on the other hand, came up with a different conclusion. They discovered that the DEHP group's blood levels of LDL-C and TC were considerably higher than the control group, implying that DEHP exposure for 23 weeks could cause lipid metabolic disorder in rats. This outcome was also consistent with earlier studies (Jia et al. 2016). Researchers included reported the raising effects of DEHP and MEHP exposure on TG in a systematic analysis of early life DEHP exposure and obesity-related outcomes in animals (Wassenaar and Legler 2017). Furthermore, our findings revealed an elevated level of total protein in serum, which is corroborated by Miura et al. (2007), who discovered an increase in total protein, ALP, and GPT in DEHP-treated mice, indicating dehydration and hepatic insufficiency. Our results experimental results indicated a significant reduction in glucose, triglycerides, and very-low-density lipoproteins (VLDL) in the DEHP + vitamin-C treatment group. This conclusion is backed up by studies that show vitamin C decreases blood cholesterol and triglycerides in humans (Sokoloff et al. 1967). Furthermore, vitamin C has been shown to affect glucose metabolism via altering glucose metabolites (Park et al. 2018). Even McRae (2008) demonstrated that taking at least 500 mg of vitamin C per day for at least 4 weeks can result in a considerable reduction in blood LDL cholesterol and triglyceride levels. However, there was no statistically significant increase in serum HDL cholesterol.

In our experiment, we detected a drop in total RBC count, total WBC count, and hemoglobin levels when focusing on the hematological modifications induced by DEHP exposure. David et al. (2000) observed hematological changes such as decreased erythrocyte count, hemoglobin, and hematocrit level, which were similar to our findings. Kwack et al. (2009) also discovered that DEHP-treated rats had significantly lower red blood cell (RBC) and hematocrit (Ht) levels while having significantly higher mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet (PLT) levels. Erythrocytes are particularly vulnerable to DEHP-induced ROS and oxidative damage due to their high iron and PUFA content (Bulle et al. 2017). By lipid peroxidation of membrane PUFAs, free radicals such as ROS can directly damage erythrocyte membranes (Bulle et al. 2017). Vitamin C is a water-soluble vitamin and coenzyme whose insufficiency has been linked to the aging of several cells and tissues (Camarena and Wang 2016). In both in vivo and in vitro experiments, it has been shown to reduce vascular dysfunction in a variety of illnesses (Oudemans-van Straaten et al. 2014). Vitamin-C supplementation improves mesenchymal stem cell (MSC) proliferation and metabolism through activating mitochondria (Fujisawa et al. 2018). The delivery of vitamin C to the mice had a good overall effect

on the numerous parameters studied, indicating that it can be useful for drug toxicity studies and studying the long-term effects of pharmaceuticals. In addition, the current study suggests that increasing vitamin-C intake may have hematological benefits. The outcomes of DEHP + vitamin C in our study showed that total RBC count, total WBC count, and hemoglobin levels were successfully restored.

According to our blood serum results, DEHP administration resulted in a considerable increase in the levels of serum ALP, ACP, and ALT and a drop in the level of AST, implying that DEHP may induce liver injury. In addition, one study confirmed that DEHP-treated mice showed signs of liver disease, including higher ALT and ALP levels. These findings were in line with the prior research, in comparison with the control group. Aydemir et al. (2018) found a significant increase in ALT and AST levels in DEHP treatment group. ALT and AST activities were measured in serum samples as a biochemical marker that could indicate liver injury at the conclusion of the experiment. Serum aminotransferases are sensitive markers of hepatocellular injury, and elevated levels of AST and ALT enzymes in the serum can detect acute and chronic liver disease before symptoms appear (Fraga 2005). DEHP and its metabolites are biochemically processed mostly in the kidney and liver. As a result, DEHP metabolism may have chemically induced toxicological effects on the liver and kidney at a higher degree. AST is present in numerous organs, including the liver, cardiac muscle, skeletal muscle, and erythrocytes, whereas ALT is found mostly in the liver. These two enzymes' enzymatic activity are markers of parenchymal hepatocyte proliferative damage (Sekas and Cook 1979). Vitamin-C supplementation reduces the damage of hepatocytes and erythrocytes and aids in the rehabilitation of postoperative liver function. Vitamin C, we believe, may play an indirect effect in the reduction of hepatic markers of parenchymal hepatocyte proliferation (Hamden et al. 2009; Zaldi et al. 2005). Vitamin C inhibits lipid peroxidation and suppresses the activation of serum AST and ALT. Our findings in the DEHP + vitamin-C group indicated a promising reversal of ACP, ALP, ALT, and AST levels that were close to control.

In tissues, oxidative stress is defined as the production of reactive oxygen species (ROS) and/or a decrease in the quantity of endogenous antioxidants. As a result, successful techniques to boost intracellular antioxidant defenses in tissues may aid in liver injury prevention. Antioxidants are necessary for preventing free radical damage to cells. Vitamins E and C are antioxidants that protect the body from the oxidative damage produced by free radicals. Oxidative stress has been proposed as a coexisting pathogenic process that has a role in the onset and progression of liver injury. Increased peroxidation of membrane lipids is reflected by a rise in testicular MDA levels accompanied by a decrease in antioxidant defense mechanisms. DEHP-induced increases

in testicular MDA show that this EDC compromises testicular function by causing oxidative stress. Important testicular activities like steroidogenesis and gametogenesis were harmed as a result of the oxidative stress (Choi et al. 2018). In the rat testes, Kasahara et al. (2002) found that oral administration of DEHP increased the formation of reactive oxygen species (ROS) (O_2^{2-} and H_2O_2) with simultaneous reductions in glutathione and ascorbic acid. According to one study, DEHP additionally causes oxidative stress in granulosa cells by boosting ROS production and inhibiting steroid synthesis by altering the expression of steroidogenic response genes; it also causes apoptosis by activating the Bax/Bcl-2 and caspase-3-mediated mitochondrial apoptotic pathway (Tripathi et al. 2019). DEHP causes oxidative stress in the ovaries and changes ovarian function, resulting in female reproductive damage. The particular mechanism underlying DEHP's harmful effects, on the other hand, is mostly unknown. To evaluate the damage produced by ROS, the MDA, SOD, and IL-6 concentration were determined in mice treated with DEHP and DEHP + vitamin C. After DEHP exposure in our study, oxidative stress and inflammation increased, as expected. The levels of MDA and IL-6 were found to be increased, and levels of SOD were detected to be reduced in DEHP-treated group as compared to control group. As a result, it can be concluded that oxidative stress in the ovary and systemic inflammation could be the primary causes of female reproductive toxicity. Our results are supported by Fu et al. (2021). Moreover, the results of DEHP + vitamin-C treatment group showed improving effects on levels of MDA, SOD, and IL-6 which touched nearly the level of control group.

The return of all biochemical, hematological, enzymological, oxidative stress and inflammatory parameters taken into consideration, to approximate control levels, appeared to be related to vitamin C, antioxidant supplementation in DEHP-treated animals, although the precise mechanisms remained to be determined.

Conclusion

The treatment of the mice with vitamin C indicates an overall positive effect on the various parameters taken into consideration, so it is evident that the administration of vitamin C can be helpful for reversal of DEHP toxicity.

Declarations

Ethics approval This research work was approved by Institutional Ethics Committee of the Barkatullah University Bhopal (Ethical Certificate Number 1885/GO/Re/S/16/CPCSEA/IAEC/BU/24).

Consent to participate None.

Conflict of interest The authors declare no competing interests.

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