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Di(2-ethylhexyl) phthalate induced hepatotoxicity in quail (*Coturnix japonica*) via modulating the mitochondrial unfolded protein response and NRF2 mediated antioxidant defense

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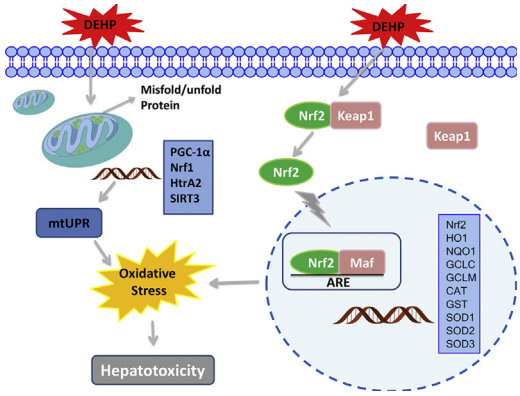
HIGHLIGHTS

- DEHP causes hepatotoxicity through oxidative stress.
- DEHP causes mitochondrial ultrastructural abnormality and dysfunctions.
- DEHP triggers the Nrf2-mediated antioxidant defense.
- MtUPR relieves DEHP-induced mitochondrial damage.

GRAPHICAL ABSTRACT



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ABSTRACT

Among ubiquitously found environmental contaminants in the ecosystem, di(2-ethylhexyl) phthalate (DEHP) is an important environmental contaminant used as plasticizer in medical and consumer goods. The bioaccumulation and environmental persistence of DEHP cause serious global health effects in wildlife animals and human, especially hepatotoxicity. Herein, to explore the mechanisms of DEHP induced hepatotoxicity, quail were exposed with 0, 250, 500 and 1000 mg/kg BW/day DEHP by gavage administration daily for 45 days. Notably, the adipose tissue degeneration was observed in the liver of DEHP-exposed quail under the histopathological analysis. DEHP exposure increased the peroxidation product (MDA), GSH and GST, but decreased antioxidant function (T-AOC, SOD and GPX). DEHP induced the oxidative stress and pulsed on NRF2 signal pathway through activating downstream genes. Furthermore, DEHP induced mitochondrial ultrastructural abnormalities and mitochondrial dysfunctions. Mitochondrial unfolded protein response (mtUPR) was activated to relieve mitochondrial dysfunctions and mitigated oxidative stress. These findings showed that mitochondrial functions and redox homeostasis were affected by DEHP and resulted in irreversible hepatic injury. In Conclusion, this study suggested that DEHP-induced hepatotoxicity in quail

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was associated with activating the NRF2 mediated antioxidant defense and mtUPR. These results provided new evidence on molecular mechanism of DEHP induced hepatotoxicity.

1. Introduction

Phthalate esters (PAEs) are generally utilized as plasticizers in industrial and consumer products (Net et al., 2015). Di(2-ethylhexyl) phthalate (DEHP), is one of the most applied PAEs, widely used as plasticizers and plastic softener production, such as food containers, children's toys, plastic products, industrial plastic and medical utensils. DEHP easily releases to the environment and enters into the organisms and produced toxic effects for its weak binding with the plastic compound through a variety of pathways like air, soil (Zeng et al., 2009) and water (Bosnir et al., 2003). In a risk evaluation report, cases of liver degeneration and hepatic carcinoma were demonstrated in chronic carcinogenicity tests in whose workplace with high level of application of DEHP (Kim, 2016). While in a chick model, DEHP has shown nonspecific toxicity and teratogenicity (Abdul-Ghani et al., 2012). Mono-(2-ethylhexyl) phthalate (MEHP), a principal metabolite of DEHP and its glucuronide were wildly metabolized within 24 h in chimeric mice with humanized liver (Adachi et al., 2015). DEHP can migrate to the surface of plastic products as it is not chemically bound to polymer matrices over time and use. It has deemed as the foremost pollution contributor in the ecosystems. As one of the most sensitive birds in the ecosystem, the toxic effects of exogenous toxicants on quail have been gradually studied (Ahmed et al., 2015; Emam et al., 2018; Yusuf et al., 2016; Li et al., 2018; Y.Z. Zhang et al., 2018). However, how DEHP causes hepatotoxicity in quail remains unclear.

Previous study reported that DEHP can cause liver tumors in rodents (Lapinskas et al., 2005). Liver is considered as the most significant detoxification organ in vivo for large number of accumulated DEHP and its metabolites. A report showed that DEHP caused liver damage in rats (Kim et al., 2010). It has been reported that DEHP also could disrupts thyroid hormone homeostasis and caused hepatic edema through inducing hepatic enzymes (Ye et al., 2017). Reduced hepatocyte FL83B activity, increased activity of lactate dehydrogenase (LDH) and alanine aminotransferase (ALT) have been reported following DEHP exposure (Lo et al., 2014). The disadvantages of intrauterine DEHP treatment in pregnant CD-1 mice liver concluded DEHP disrupted postpartum liver growth by slowing glycogen metabolism progression (Maranghi et al., 2010). Furthermore, a high-fat diet with DEHP exposure reported various degree of nonalcoholic fatty liver disease in mice (Chen et al., 2016). Based on the above background and taking into consideration the harmful effects of DEHP exposure on live in mammalian, but till to date have very little information about the mechanisms underlying DEHP-induced hepatotoxicity in birds.

Oxidative stress is related to the harmful effects and is generated from either endogenous or exogenous substances. A report suggested that Superoxide dismutase (SOD), peroxidase (GPX) and glutathione catalase (CAT) of liver were considered as potential biomarkers for DEHP treatment (Xiang et al., 2017). Toxicity of DEHP caused abnormality in various protein indicators and antioxidant enzymes and damaged protein molecules in earthworms (Ma et al., 2017). Previous evidence elucidated that DEHP contributed to free radicals production and oxidative stress by subsequent inhibition of anti-oxidant enzymes (Culty et al., 2008). Recent study demonstrated that DEHP treatment wrecked antioxidant balance and, then enhanced oxidative stress in liver, which caused to hepatotoxicity (Erkekoglu et al., 2014). Nuclear factor erythroid 2-related factor 2 (NRF2), a nucleus transcription factor, regulated oxidative stress (Osburn and Kensler, 2008), and as an essential transcription factor that regulated the mechanism of antioxidant (He et al., 2014).

Mitochondria are the main target organelle of exogenous toxic materials and exposure to these materials cause mitochondrial damage. Exposure to DEHP obliterated the mitochondrial function and increased ROS production has led to SIRT1 attenuation (X. Li et al., 2014). MEHP increased superoxide generation and mediated perturbation in mitochondrial function in mouse Leydig cells (Savchuk et al., 2015). Furthermore, MEHP depressed the glutathione levels in HepG2 cells and destroyed mitochondrial membrane potential (Chen et al., 2012). The unfolded protein responses engendering from the mitochondrion (mtUPR) were activated to ensure correct protein handling (Mesbah Moosavi and Hood, 2017). Other study has shown that SIRT3 (NAD-dependent deacetylase sirtuin-3) is also involved in regulating mtUPR, but SIRT3 regulates mtUPR by regulating antioxidant stress and mitochondrial autophagy, and does not depend on CHOP and ER alpha (Papa and Germain, 2014).

DEHP is thought to be a chemical that interferes with detoxification and antioxidant systems in the body and leads to imbalance in the liver toxicity and antioxidant homeostasis. Mitochondria are the vital origin of ROS production (Sena and Chandel, 2012). Mitochondrial dysfunction is considered as an important contributing factor in various forms of hepatic disorders. Therefore, this study was aimed to explore the molecular mechanisms through which DEHP exposure modulats antioxidant defense and the mitochondrial unfolded protein response in the liver of quail.

2. Materials and methods

2.1. Animals and treatments

Female quail (*Coturnix japonica*) were purchased from Harbin WanJia farm. DEHP ($C_{24}H_{38}O_4$, CAS: 117-81-7, >99.0%) was bought from Aladdin Biochemical Technology Co., Ltd., (Shanghai, China). Quail were housed in cage system and maintained proper and pollution-free environment at around 50% humidity, 26 °C temperature and 12 h light/dark cycle, and regularly supplied with standard feed and drinking water ad libitum. Three hundred (300) quail were divided into five groups at random (Table S1). Quail were exposed with di(2-ethylhexyl) phthalate for gavage administration for 45 d. Quail were fasted for 12 h. Afterwards, quail were weighted and recorded, and collected heart blood for liver function tests. Finally, all quail were sacrificed and liver tissues were carefully dissected and collected, and parts of small pieces were homogenized. The supernatant was collected carefully and preserved at −80 °C for the experiments.

2.2. Light microscopic and ultrastructure analysis

For preparation of slides, parts of the liver tissues were fixed in formalin solution, embedded in paraffin, cut into thin slices and stained with hematoxylin and eosin (H & E) for microscopic observation. All the slides were observed under a light microscope for histopathological analysis at 20× magnification. Histopathological liver analysis score was estimated by a method as previously described (Puche et al., 2013; Xia et al., 2018) using a score scale from 0 to 4 for both swollen and vacuolization of hepatocyte and was as follows: 0–1, A very few or none (0–5%); 1–2, Slight (6–35%); 2–3, Medium (36–65%) and 3–4, Severe (65–100%).

Electron microscopy methodology was adopted by a method as described previously (Li et al., 2013; Lin et al., 2018; Xia et al., 2017). Fragments of liver tissues were fixed with glutaraldehyde, washed and again