

Di-(2-ethylhexyl) phthalate-induced reproductive toxicity and oxidative stress in male rabbits - Fayrouz A. Khaled- Omar El-Mokhtar University

Fayrouz A. Khaled

Omar El-Mokhtar University, Li

About 95% of di-ethylhexylphthalate (DEHP) produced and used as a plasticizer in polyvinyl chloride (PVC) resins for fabricating flexible vinyl products. Di(2-ethylhexyl)phthalate is a well-characterized reproductive system toxicant; it is a member of the phthalate chemical family, plasticizers that have potential endocrine-disrupting effects. DEHP and its metabolites alter proper testicular development in fetal rat models. The experiment was designed to study toxic effects of Di-(2-ethylhexyl) phthalate (DEHP) on semen characteristics, testosterone levels, testicular lipid peroxidation and testicular antioxidants in male New-Zealand white rabbits for 12 weeks. Rabbits were orally administered the doses of DEHP every day for 12 weeks. Results obtained showed that DEHP significantly ($P<0.05$) decreased libido (by increasing the reaction time), ejaculate volume, sperm concentration, total sperm output, sperm motility (%), total motile sperm per ejaculate (TMS), packed sperm volume (PSV), total functional sperm fraction (TFSF), normal and live sperm and semen initial fructose. While, initial hydrogen ion concentration (pH), and dead and abnormal sperm were increased ($P<0.05$). Also, testosterone levels, body weight (BW), relative weights of testes (RWT) and epididymis (RWE) were decreased. Thiobarbituric acid-reactive substances and lactate dehydrogenase were increased, while glutathione S-transferase, transaminases and phosphatases were decreased in seminal plasma of rabbits treated with DEHP compared to control.

Di(2-ethylhexyl)phthalate (DEHP) is a typical endocrine-disrupting chemical and reproductive toxicant. Although previous studies have attempted to describe the mechanism by which DEHP exposure results in reproductive dysfunction, few studies focused on puberty, a critical period of reproductive development, and the increased susceptibility to injury in adolescents. To elucidate the mechanism underpinning the testicular effects of DEHP in puberty,

we sought to investigate the JAZF1/TR4 pathway in the testes of pubertal rats. Specifically, we focused on the role of the JAZF1/TR4 pathway in male reproduction, including the genes JAZF1, TR4, Sperm 1, and Cyclin A1. In the present study, rats were exposed to increasing concentrations of DEHP (0, 250, 500, and 1000 mg/kg/day) by oral gavages for 30 days. Then we assayed testicular zinc and oxidative stress levels. Our results indicated that DEHP exposure could lead to oxidative stress and decrease the contents of testicular zinc. Additionally, significant morphological changes and cell apoptosis were observed in testes exposed to DEHP, as identified by hematoxylin and eosin staining and the terminal deoxynucleotidyl transferase-mediated nick and labeling assay. By measuring the expression levels of the above relevant genes by qPCR, we found the DEHP-induced increased expression of JAZF1 and decreased expression of TR4, Sperm 1, and Cyclin A1. Therefore, we have demonstrated that in vivo exposure to DEHP might induce reproductive toxicity in pubertal male rats through the JAZF1/TR4 pathway and oxidative stress.

Di-(2-ethylhexyl) phthalate (DEHP) is a widely used plasticizer with a high environmental exposure level. As a persistent organic pollutant, DEHP causes reproductive and developmental toxicity in mammals. In this paper, the reproductive toxicity of DEHP was discussed using the model organism *Caenorhabditis elegans* to determine the sensitivity indices for evaluating the ecotoxicological effects of DEHP. L4 *C. elegans* larvae to evaluate the LC50 of DEHP and the changes in brood size and generation time, we found that the LC50 of DEHP to *C. elegans* exceeded 100 mg/L. And 10 mg/L DEHP exposure significantly reduced the brood sizes but not the generation time. Results of oocyte and distal-tip cell (DTC) counting suggested that the number of oocytes were decreased and apoptotic cells that from the unilateral gonad arm were increased in the 1 mg/L and 10 mg/L DEHP exposed groups. In contrast, there was no significant difference in the fluorescence intensity of DTC. Fluorescence analysis of

HUS-1 showed that HUS-1 protein was overexpressed after DEHP exposure. The H₂O₂ level and DNA damage were measured by Bradford protein assay and AP staining respectively. The results showed that there was no significant difference in H₂O₂ level after DEHP exposure, in contrast, DNA damage was increased significantly. Moreover, 10 mg/L concentration DEHP exposure significantly increased the expression levels of apoptosis-related genes *cep-1*, *egl-1*, *ced-4*, and *ced-3* and decreased the expression levels of *ced-9*. It suggested that *cep-1*, *egl-1*, *ced-4*, and *ced-3* genes promote apoptosis and the *ced-9* gene inhibits apoptosis. Meanwhile, 10 mg/L concentration DEHP exposure decreased the expression of oxidative stress-related genes *mev-1* and *gas-1*. The *mev-1* and *gas-1* are mainly involved in the inhibition of oxidative stress in nematodes. In short, the decreased oocyte numbers and increased apoptosis oocyte numbers in *C. elegans* when exposed to DEHP, which may involve in the DNA damage induced by oxidative stress.

Di-(2-ethylhexyl) phthalate (DEHP) is the most widely used plastizer in the world and can suppress testosterone production via activation of oxidative stress. Genistein (GEN) is one of the isoflavones ingredients exhibiting weak estrogenic and potentially antioxidative effects. However, study on reproductive effects following prepubertal multiple endocrine disruptors exposure has been lacking. In this study, DEHP and GEN were administrated to prepubertal male Sprague-Dawley rats by gavage from postnatal day 22 (PND22) to PND35 with vehicle control, GEN at 50 mg/kg body weight (bw)/day (G), DEHP at 50, 150, 450 mg/kg bw/day (D50, D150, D450) and their mixture (G + D50, G + D150, G + D450). On PND90, general morphometry (body weight, AGD, organ weight, and organ coefficient), testicular redox state, and testicular histology were studied. Our results indicated that DEHP could significantly decrease sex organs weight, organ coefficient, and testicular antioxidative ability, which largely depended on the dose of DEHP. However, coadministration of GEN could partially alleviate DEHP-induced reproductive injuries via enhancement of testicular antioxidative enzymes activities, which indicates that GEN has protective effects on DEHP-induced male reproductive system damage after prepubertal exposure and GEN may have promising future in its curative antioxidative role for reproductive disorders caused by other

environmental endocrine disruptors.