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Di (2-Ethylhexyl) Phthalate Induced Toxicological Effects on Reproductive System of Female Mice *Mus-Musculus*

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Abstract

Di-(2-ethylhexyl) phthalate is an environmental endocrine disruptor and reproductive toxicant. It impairs the structural and functional aspects of female reproduction and also alters the molecular, endocrinological, cytological and biochemical aspects, causing ovarian cycle irregularity and infertility. DEHP was orally administered at lower dose of 10 mg/kg bwt to mice for six to twenty four weeks period which resulted in alterations of the serum levels of hormones estrogen and progesterone. The altered values of hormones were found to be statistically very significant (P<0.001). The present study indicated the adverse effects of exposure of DEHP on female reproductive health.

Keywords: Corn Oil Reproductive System, DEHP, Hormones, Infertility

1. Introduction

Phthalate esters are a class of water-insoluble organic chemicals that have been used as plasticizers for polyvinylchloride (PVC) formulations since about 1930. Exposure of the general population to DEHP is ubiquitous and occurs due to the use of various consumer products[1–7]. Among phthalates, Di-(2-ethylhexyl) phthalate (DEHP) is the most widely used[8,9] as a plasticizer commonly added to plastics to make them flexible. These plasticizers are not covalently bound to the polymer, and hence leach out into the environment, thus becoming ubiquitous environmental contaminants[10]. In a study it has been concluded that the dietary administration of DEHP (2%) to mice for 10 days resulted in liver dysfunction as evidenced from histological observations and serum analysis[11]. It has been reported that DEHP is a well known reproductive toxicant and they impair fertility by acting as endocrine disruptors, thus causing gonadal morphological or functional alterations in females[12] and also causes male reproductive organ damages in animals[13–15]. It has also been reported that exposure of adult rats to DEHP results in hypoestrogenic an ovulatory cycles and polycystic ovaries[16]. Phthalate compound induced reproductive toxicity on mammalian reproduction and fertility as well as hormonal functions have not yet been elucidated in detail at a particular dose of 10 mg/kg/b.wt in swiss albino mice. The present work is an attempt to understand the potential reproductive toxicity of DEHP in mice and its effect on level of serum concentration of estrogen and progesterone hormones.

2. Materials and Methods

2.1 Experimental Animal

Swiss albino mice procured from CDRI, Lucknow. Swiss albino mice weighing 24-30g were housed in stainless-steel wire cages at a temperature of (24 ± 1°C), humidity (55 ± 5 %), and lighting (12h light/dark cycle) randomly. Food and tap water were given *ad libitum* throughout the study. All animal experiments were carried out as per CPCSEA guidelines (Approval No.-1840 / PO /ReBi/S/15/CPCSEA).

2.2 Principal Chemical

Di(2-ethylhexyl)phthalate used as toxic chemical manufactured by Accu Sandard, America and marked by Rankem Chemical

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Pvt. Ltd. India and Loba Chem. Pvt.Ltd, India. Corn oil was used as a vehicle (Sigma Pvt.Ltd and Nieshiel Chemical Pvt. Ltd).

2.3 Experimental Design

2.3.1 Dose Administration

After acclimatization mice were administered Di(2-ethylhexyl) phthalate (DEHP) orally at a dose of 10 mg/kg/b.wt and also corn oil at a dose of 10 ml/kg/b.wt., with food and tap water *ad libitum* for 6 weeks, 12 weeks, 16 weeks, 20 weeks and 24 weeks.

2.4 Hormonal Study

It was done by the ELISA(Eliza Plate reader- Tulip diagnostic, model- lisaquant eliza plate reader) method. After DEHP administration for time period of 6 weeks, 12 weeks, 16 weeks, 20 weeks and 24 weeks, blood of treated and control mice were collected by ocular puncture. Serum was obtained for the estimation of various hormonal parameters.

3. Result

Di (2-etlylhexyl) phthalate (DEHP) administered mice groups showed alopecia (loss of fur), depressed fertility and progeny performance have been also observed. Oral administration of DEHP revealed alteration in reproductive performances in mice groups treated for 6 weeks and onward durations. Treated groups produced very less number of litter.

After DEHP administration for 6 weeks serum estrogen level decreased and it was found to be statistically significant (P<0.01) (Figure 1). In all progressive weeks after administration of DEHP decreased serum estrogen level have been recorded and the values were found to be statistically extremely significant (P<0.0001) in comparison to control group of mice (Figure 2). Decreased serum level of progesterone was recorded after 6 weeks of DEHP treatment and the observed value was found to be statistically significant (Figure 3) but decreased serum level of progesterone after 12 weeks, 16 weeks, 20 weeks and 24 weeks of DEHP treatment were statistically extremely significant (P<0.0001) in comparison to control group of mice (Figure 2).

It has also been recorded that there is reduction in incidence of pregnancy in the female mice groups treated with DEHP. Several treated groups showed complete infertility also.

4. Discussion

There has been a gradual increase in production and consumption of DEHP containing consumer products. But unconsciously during the last few decades, the use of these toxic chemicals has surpassed the tolerance level, creating imbalance in the system. Besides impairing the structural and functional aspects of female reproduction, they are altering the molecular, endocrinological, cytological and biochemical aspects as well, causing ovarian cycle irregularity and infertility.

Endocrine systems of the body play an essential and pervasive role in both the short-and long-term regulation of metabolic processes Nutritional, behavioral, and reproductive processes are intricately regulated by endocrine systems. Disorders of any of the endocrine systems, involving both overactive and under active hormone secretion, results malfunctioning of system and ultimately extend to many different associated disorders[17].

Estrogen is responsible for the development and regulation of the female reproductive system and secondary

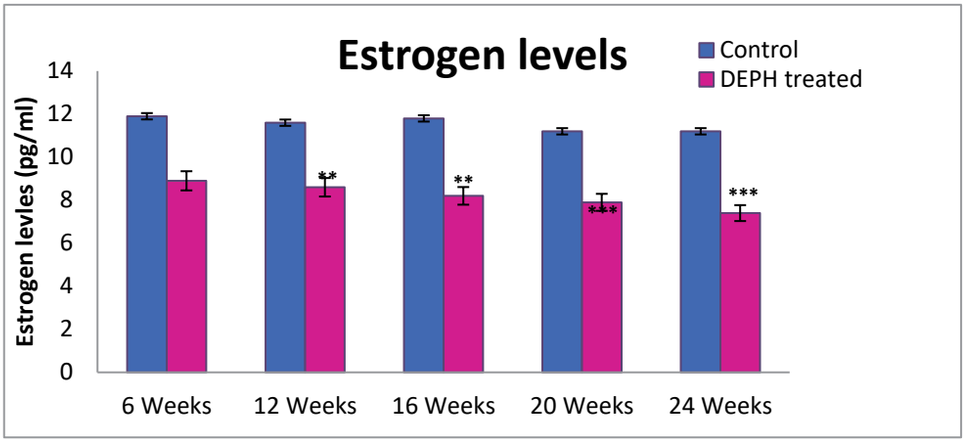

Figure 1. Showing estrogen level in control and DEHP treated mice [**considered very significant (P<0.001) and ***considered extremely significant(P<0.0001)].

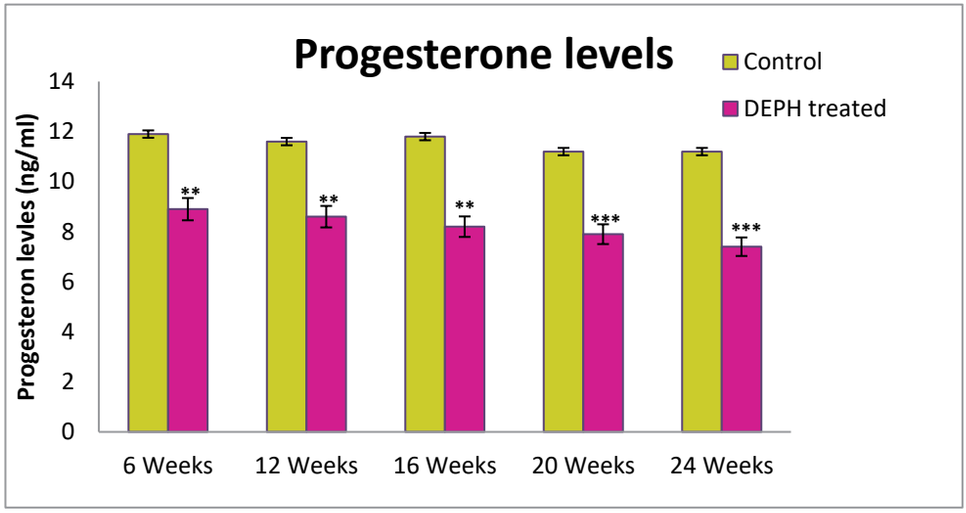

Figure 2. Showing progesterone level in control and DEHP treated mice [**considered very significant (P<0.001) and ***considered extremely significant(P<0.0001)].

sex characteristics. In relation to the reproductive role of estrogens, they stimulate follicular growth and maturation. Progesterone helps to maintain pregnancies and implant an egg in the uterus. The present study revealed the alteration in reproductive performances in mice groups treated with DEHP for 6 weeks and onward durations. Treated groups produced very less number of litter. In a study it has been reported that the animals that are exposed to DEHP during adulthood and pubertal periods show adverse effects in multiple reproductive parameters, such as estrous cyclicity, pubertal age, litter size, and alterations in serum hormone levels and ovarian morphology[18–20]. It has been observed that the reproductive performance of female group of DEHP treated mice were affected and the frequencies of still birth, smaller litter size, temporary sterility and high mortality among new born and were recorded during the experimental periods. These findings were not observed in control groups. It has also been recorded by other worker during the experimental period in a few DEHP treated female mice, visceral fat deposition has been observed. These female mice appeared pregnant because of the visceral fat but really these were infertile. Therefore, it can be inferred that DEHP exposure leads infertility[21].

In our investigation the decreased serum estrogen level after 6 weeks to 24 weeks of o DEHP administration has been observed. The low concentration of serum level of estrogen may be the cause of infertility among the treated groups of mice. A similar type of work reported that the transient daily oral exposures to 2 g/kg of DEHP in female rats result in prolonged estrous cycles, and delay or suppression in natural ovulation time resulting in reduced number of ovulations and hence absence of CL. Suppressed serum levels of estradiol, progesterone, and LH were also found. The primary cause of these disruptions appears to be the low levels of estradiol, insufficient to induce preovulatory LH surge[22,23]. Phthalates are well established as EDCs with estrogen disrupting properties demonstrated[24,25]. In a study it has been concluded that DEHP may stimulate peroxisome proliferator-activated receptors that suppress aromatization in the granulosa cell. This leads to the decreased serum concentration of estrogen[26].

DEHP (EDCs) can cause atresia of antral follicles or inhibit the growth of antral follicles, leading to estrogen deficiency and anovulatory cycles and ultimately infertility. Similarly, EDCs that affect the process of luteinization, the developmental transition of a follicle to a corpus luteum, or the lifespan of the corpus luteum can affect progesterone production, implantation, and pregnancy, leading to infertility. It can also directly interfere with ovarian steroidogenesis and this can cause reproductive and non-reproductive complications. Steroidogenesis can be affected either by depletion of the antral follicles and/or corpora lutea, or it can be affected by disrupting the functionality of the steroidogenic units. Specifically, the loss of antral follicles or corpora lutea from the ovary will result in a decrease in the available structures that are capable of producing steroids[27,28]. Further, EDCs can disrupt the functionality of antral follicles by decreasing ovarian mRNA, protein, and/or activity of the enzymes responsible for generating estradiol and its precursor sex steroid hormones[27,28]. The steroidogenic enzymes in the corpora lutea can also be affected in a similar manner, resulting in inadequate levels of necessary progesterone and estradiol to support a pregnancy[27,28].

In another study it has been reported that DEHP inhibits FSH-stimulated cAMP production, thereby preventing activation of the enzymes for progesterone production, and suppresses levels of *Cyp19a1via* activation of PPARs. Prolonged

exposures to a lower dose (0.05mg/kg/day) of DEHP resulted in reduced expression of *Cyp17a1*, *Cyp19a1, progesterone receptor (Pgr)*, *Lhcg*rand *Fshr* in the adult ovary (PND41) of the CD-1 mice, all which may affect ovarian steroidogenesis[29]. Besides suppressed ovarian steroid production, multiple studies have reported altered follicular dynamics as one of the major consequences of DEHP exposure. These alterations include accelerated follicular recruitment and failure in follicular maturation and ovulation. Early postnatal (PND 5-20) exposure in mice to relatively low levels of DEHP depletes primordial follicles while increasing the number secondary and antral follicles[30]. Above said reports also conclude the suppressed level of hormones which resembles with our results.

Progesterone prepares the uterus for reception of fertilized oocytes and is transported via the blood bound to plasma proteins. Progesterone also prepares the mammary tissues for milk production as well as inhibiting female reproductive behaviors associated with estrous. Investigations were made to analyze the effects of DEHP on fertility and growth of exposed mice and their offspring at a dose of DEHP [0.05 and 5 mg/kg of body weight (bwt)/day] and fertility was impaired in mice exposed to environmentally relevant doses[31]. The above mentioned report has similarity with our results also.

5. Conclusion

The present study indicated an association between the environmentally relevant concentrations of DEHP exposure and altered serum level of estrogen and progesterone hormones.

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