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EFFECTS OF DI-(2-ETHYLHEXYL) PHTHALATE ON RAT OVARIAN FUNCTION

UTICAJ DI-(2-ETILHEKSIL)-FTALATA NA FUNKCIJU JAJNIKA KOD PACOVA

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Summary: This study aimed to evaluate the effects of di-(2-ethylhexyl)phthalate (DEHP) on estrous cycle, sex hormone levels and ovary histological features. A total of 80 female SD rats were randomly divided into 8 groups (n=10 per group): short-course control group, short-course low-dose group, short-course medium-dose group, and short-course high-dose group, long-course control group, long-course low-dose group, long-course medium-dose group and long-course high-dose group. Intragastrical DEHP was administrated at 1000 mg/kg/d (low dose), 2000 mg/kg/d (medium dose) and 3000 mg/kg/d (high dose) independently for 14 days (short course) or 28 days (long course). Rats in control groups were untreated. Vaginal smearing was used to detect the estrous cycle and rats were weighed at every Monday and Thursday to evaluate the growth status. At the end of study, rats were sacrificed and bilateral ovaries were obtained for histological examination. In addition, ELISA determined levels of serum progesterone, estradiol, testosterone, follicle stimulating hormone and luteinizing hormone. DEHP treatment limited body weight gain ($P<0.05$), prolonged the estrous cycle ($P<0.05$), decreased the ovarian mass index ($P<0.05$) and ovarian weight. No evident degeneration, necrosis or other pathological features were found in the ovaries. The testosterone levels were decreased by DEHP treatment in a dose dependent manner. DEHP treatment could increase serum testosterone level, inhibit ovulation and prolong the estrous cycle of rats, exerting reproductive toxicity in a dose dependent manner. We speculate DEHP can affect the endocrine regulatory function of the ovary and limit the body weight gain, resulting in chronic toxicity.

Keywords: phthalate acid esters, di-(2-ethylhexyl) phthalate, estrous cycle, sex hormone levels, rat ovarian function

Kratak sadržaj: Cilj studije bio je da se odredi uticaj di-(2-etilheksil)-ftalata (DEHP) na polni ciklus, nivoe polnih hormona i histološke osobine jajnika. Osamdeset SD pacova ženskog roda nasumično je podeljeno u 8 grupa (n=10 po grupi): kontrolnu grupu za kratak tretman, grupu niska doza/kratak tretman, grupu srednja doza/kratak tretman i grupu visoka doza/kratak tretman, kao i kontrolnu grupu za dugačak tretman, grupu niska doza/dug tretman, grupu srednja doza/dug tretman i grupu visoka doza/dug tretman. Intragastrički DEPH primenjivan je nezavisno u dozama od 1000 mg/kg/dnevno (niska doza), 2000 mg/kg/dnevno (srednja doza) i 3000 mg/kg/dnevno (visoka doza) tokom 14 dana (kratak tretman) odnosno 28 dana (dug tretman). Pacovi u kontrolnim grupama nisu tretirani. Polni ciklus je utvrđivan pomoću vaginalnog brisa a pacovi su mereni svakog ponedeljka i četvrtka kako bi se odredio njihov rast. Na kraju studije, pacovi su žrtvovani i oba jajnika su odstranjena radi histološkog ispitivanja. Pored toga, na osnovu ELISA određeni su nivoi progesterona, estradiola, testosterona, FSH i LH u serumu. Tretman DEPH-om ograničio je rast telesne težine ($p<0,05$), produžio polni ciklus ($p<0,05$), snizio indeks mase jajnika ($p<0,05$) i težinu jajnika. U jajnicima nisu zapaženi tragovi degeneracije, nekroze ili drugi patološki nalazi. Nivoi testosterona bili su sniženi usled tretmana DEPH-om, i to u zavisnosti od doze. Tretman DEPH-om može povećati nivo testosterona u serumu, inhibirati ovulaciju i produžiti polni ciklus kod pacova, ispoljavajući reproduktivnu toksičnost u zavisnosti od doze. Naša je pretpostavka da DEPH može uticati na endokrinu regulatornu funkciju jajnika i ograničiti rast telesne težine, što za posledicu ima hroničnu toksičnost.

Ključne reči: estri ftalatne kiseline, di-(2-etilheksil)-ftalat, polni ciklus, nivoi polnih hormona, funkcija jajnika kod pacova

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Introduction

Phthalates are the most abundant pollutants of our general environment. These substances are components of food wraps and many medical devices (e.g. tubing, blood bags, and dialysis equipment), as well as of many cosmetic products. With time, they are leached out of these plastic products, and their volatility results in pronounced human exposure to phthalates (1). Di-(2-ethylhexyl) phthalate (DEHP) is one of the important PAEs and closely related to our daily life. DEHP can enter our body through drinking, food intake, inspiration, direct contact with skin, etc. DEHP was particularly found in fatty foods including dairy products (2). Women have been exposed to DEHP in cosmetics on a daily basis (3). In recent years, with the wide application of plastic products, about 18 billion tons of PAEs are consumed annually worldwide (4) and PAEs have become one of the most common and important pollutants. The Agency for Toxic Substances and Disease Registry of America (ATSDR) has reported, generally, the maximum exposure dose is about 2 mg/kg/day/person (5) and the dose of occupational exposure is even higher. In past decades, numerous studies have demonstrated the reproductive toxicity of PAEs (6, 7, 3). However, these researches mainly focus on the reproductive toxicity of males, so the reproductive toxicity of females is not exactly definite. In addition, the incidence of reproductive endocrine disorders is increasing in women (8). Therefore, a lot of studies focus on the relationship between PAEs exposure and reproductive endocrine disorders.

The present study aimed to investigate the effects of DEHP on the rat ovarian function and to explore the reproductive toxicity of DEHP. DEHP was employed and the effects of DEHP at different doses and for different courses on the estrous cycle, sex hormone levels and ovary histological features were investigated aiming to explore the effects of DEHP on the rat ovarian function. Our study may provide evidence on the pathogenesis of reproductive endocrine disorders (polycystic ovary syndrome).

Materials and Methods

Animals

All experiments were performed in the lab of Zhongshan School of Medicine, Sun Yat-sen University from March to November 2010. Female Sprague Dawley (SD) rats (n=80) (specific pathogen free) aged 6–7 weeks weighing 170±15 g were purchased from the Anima Center of Sun Yat-Sen University. All animal experiments were approved by the Administrative Committee of Experimental Animal Care and Use of Zhongshan School of Medicine, Sun Yat-Sen University (SYU2009-0005), and conformed to the National Institute of Health guidelines on the ethical use of animals.

Reagents and instruments

DEHP (chemically pure; lot number: T20090220) was provided by the Chinese Academy of Sciences Guangzhou Branch. The ELISA kit for sex hormones was purchased from Beijing Sunbio Biotech, Co., Ltd.

Grouping and treatment

Rats were housed in an environment with layer flow ventilation in a 14:10-h dark–light cycle and the room temperature was controlled at 22–26 °C. These rats were given *ad libitum* access to food and water. Animals were divided into following groups: (1) short course low dose group: rats intragastrically received 1000 mg/kg/d DEHP for 14 days; (2) short course medium dose group: rats intragastrically received 2000 mg/kg/d DEHP for 14 days; (3) short course high dose group: rats intragastrically received 3000 mg/kg/d DEHP for 14 days; (4) short course control group: rats were untreated and sacrificed at 14 days; (5) long course low dose group: rats intragastrically received 1000 mg/kg/d DEHP for 28 days; (6) long course medium dose group: rats intragastrically received 2000 mg/kg/d DEHP for 28 days; (7) long course high dose group: rats intragastrically received 3000 mg/kg/d DEHP for 28 days; (8) long course control group: rats were untreated and sacrificed at 28 days.

Adjustment of dosage according to the body weight gain

The dosage was adjusted according to the body weight. Rats were weighed at 8 am every Monday and Thursday and the dosage of DEHP was adjusted and accurate to 1 g. The drug was administered with a 1-mL syringe and the volume was accurate to 0.01 mL.

Observation of rat estrous cycle

According to the method previously reported (9), vaginal smear was performed at 8–10 am daily to observe the estrous cycle (proestrus, estrus, metestrus and diestrus). The estrous cycle was calculated as the interval between two adjacent estruses.

Sample collection and preparation

One day before the end of experiment, rats were anesthetized and bilateral ovaries were obtained and weighed (accurate to 0.1 mg). Ovaries were fixed in 10% formaldehyde (40% formaldehyde in PBS; PH= 7.2–7.4). At the same time, blood was collected from the inferior vena cava and centrifuged at 3000 r/min for 20 min followed by collection of serum which was then stored at –20 °C. Finally, rats were sacrificed by cervical dislocation. After sample collection, conventional dehydration and transparentization,

the samples were embedded in paraffin. Tissues were then cut into 3 μm sections followed by H&E staining and mounting for use.

Detection of sex hormones with ELISA kits

According to the manufacturer's instructions, the optical densities (OD) of different concentrations of standard were determined. Standard curve was delineated with OD as the vertical coordinate and concentrations of standard as the horizontal coordinate. The concentrations of sex hormones were determined according to their OD in the standard curve.

Statistical analysis

Data were expressed as means ± standard deviation and statistical analysis was performed with SPSS version 13.0 statistic software package. A value of $P<0.05$ was considered statistically significant. Statistical analysis was performed using the One-way ANOVA, Kruskal-Wallis test, LSD-t test, Bonferroni test, and Wilcoxon rank sum test.

Results

General data and changes in body weight after DEHP treatment

No evident diseases or death were observed during the study. Rats in control groups had good mental status, body weight gain was favorable and hair had luster. After DEHP treatment, the appetite of rats was compromised and the hair had no luster. The body weight gain was not favorable. These changes were more obvious in the long course high dose group. Several rats even had decreased body weight.

After DEHP treatment, the body weight of rats in short course high/medium/low dose groups was lower than that in corresponding control groups ($P<0.05$). The body weight of rats in long course high/medium/low dose groups was lower than that in corresponding control groups ($P<0.05$). These results suggested DEHP could limit the body weight gain (*Table I*).

Changes in estrous cycle after DEHP treatment

The estrous cycle of all rats was calculated and analyzed. Results are presented in *Table II*.

The estrous cycle in the short course high dose group and long course high/medium/low dose groups was prolonged and there was a significant difference in the estrous cycle between short course high/medium/low dose groups and the corresponding control group ($P<0.05$). Bonferroni test showed a marked difference in the estrous cycle between the short course high dose group and short course medium/low dose groups and the corresponding control group ($P=0.008$, 0.019 and 0.002, respectively). These results indicated the estrous cycle in the short course high dose group was significantly longer than that in the other groups, suggesting DEHP could suppress the ovulation and estrus, normal physiological processes, in a dose dependent manner. In addition, there was a remarkable difference in the estrous cycle between long course high/medium/low dose groups and corresponding control group ($P<0.001$).

Significant difference in the estrous cycle was observed between short course medium/low dose groups and long course medium/low dose groups ($P<0.001$). But the estrous cycle in the short course high dose group was not different from that in the long course high dose group. Therefore, the effect of DEHP on the estrous cycle was not time dependent.

Table II Changes in the estrous cycle ($\bar{x}\pm s$; n=10; d).

Group	Short course	Long course
High dose	6.60±1.58★	8.25±1.78▲
Medium dose	4.95±0.90★●	8.70±1.40▲●
Low dose	5.10±0.84★◆	8.15±1.90▲◆
Control group	4.75±0.72★	4.45±0.44▲

Note: ★ $P<0.05$, short course medium dose and low dose vs. control group and high dose group
▲ $P<0.05$, long course high dose, medium dose and low dose vs. control group
◆ $P<0.05$, long course low dose vs. short course low dose
● $P<0.05$, long course medium dose vs. short course medium dose

Table I Body weight before and after treatment ($\bar{x}\pm SD$; n=10; g).

Group	Short course		Long course	
	before	after	before	after
High dose	163.80±11.29★	187.20±29.70	166.80±17.49	208.60±11.89▲
Medium dose	170.80±15.86★	202.70±30.82	164.10±13.85	211.60±20.18▲
Low dose	169.40±14.37	181.50±25.94	169.20±17.70	220.90±25.68
Control	169.70±8.91★	215.80±25.83	168.30±11.37	233.00±13.22▲

Note: ★ $P<0.05$ short-course high dose and low dose vs. control group
▲ $P<0.05$ long course high dose and medium dose vs. control group

The estrous cycle is sensitive to external environment, especially the stimulation caused by vaginal smearing which may cause presentations of false mating. In addition, intragastrical administration can also interfere with the estrous cycle. Therefore, based on the effects of DEHP on the rat estrous cycle, the levels of hormones and histological presentations of the ovary should be taken into account to determine the effect of DEHP on the ovarian function.

Changes in the rat ovaries after DEHP treatment

Gross findings. Normal ovaries have a smooth surface and luster and the cortex and medulla of the ovary can be clearly identified. Ovary weight was calculated as the ovary mass index (10) and results are presented in *Table III*.

The ovary mass index in short course high/medium/low dose groups was lower than that in corresponding control ($P<0.05$) and the difference increased with the increase of dosage. In addition, the ovary mass index in long course high/medium/low dose groups was lower than that in the corresponding control group ($P<0.05$) and the difference also increased with the increase of dosage. These findings indicated the DEHP could affect the development of rat ovary and decrease the ovary weight, in a dose dependent manner.

Table III Changes in the ovary mass index of rats after DEHP treatment ($\bar{x}\pm SD$; n=10).

Group	Short course	Long course
High dose	0.0248±0.0039★	0.0237±0.0025▲
Medium dose	0.0257±0.0017★	0.0243±0.0123▲
Low dose	0.0273±0.0041	0.0259±0.0038
Control group	0.0297±0.0023★	0.0314±0.0043▲

Note: ★ $P<0.05$ short course high/medium dose groups vs. corresponding control group
▲ $P<0.05$ long course high/medium dose groups vs. corresponding control group

Changes in the histological features of the rat ovary

No abnormalities were found in the ovaries of short course groups and H&E staining revealed ovarian follicles at different developmental stages. Primordial follicles: round or oval, an oocyte in the center, which is surrounded by a layer of spindle-shaped granulosa cells (*Figure 1A*). Preantral follicles: oocytes are enlarged and surrounded by zona pellucida and several layers of granulosa cells (*Figure 1B*). Antral follicles: preantral follicles further develop and form a follicular cavity (*Figure 1C,D*). Mature follicles: the volume of follicles and follicular cavity are increased and follicles extrude outside the ovary (*Figure 1E*). Normal corpus luteum could be identified in sections (*Figure 1F*). In long course groups, the development of granulosa cells and oocytes in certain ovaries was not favorable, which was characterized by decreased layers or absence of granulosa cells, unclear or even absent egg structure; shrinkage of zona pellucida (*Figure 1G,H*). In long course high/medium dose groups, the mature follicles were relatively decreased. However, ovary degeneration and necrosis were not observed. According to these findings, the DEHP did not cause obvious damage to the ovary.

Changes in the sex hormone levels after DEHP treatment

ELISA determined the sex hormone levels and results are presented in *Table IV*.

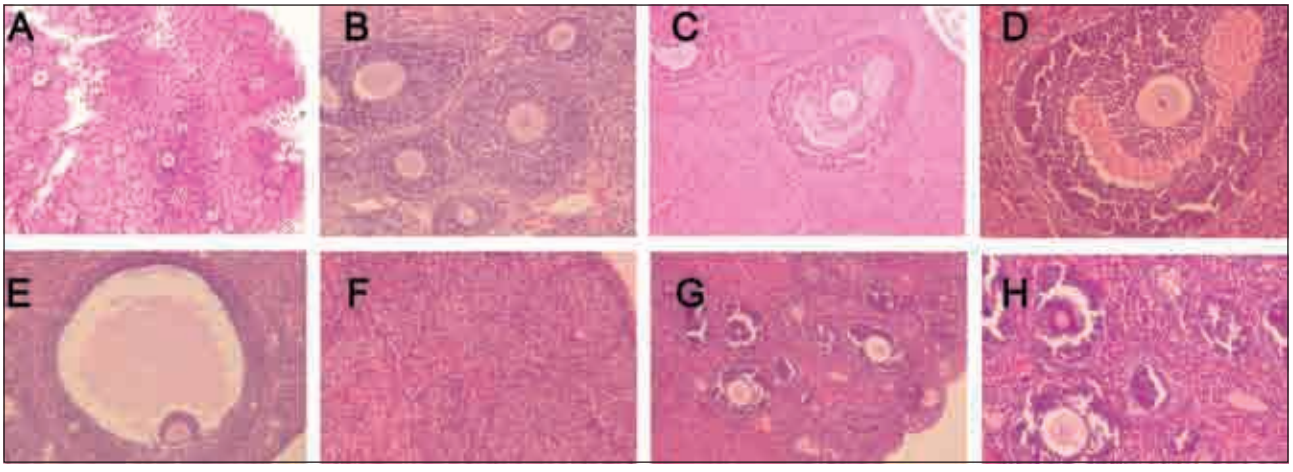

Figure 1 Changes in the histological features of rat ovary. A, primordial follicle (200×); B, preantral follicle (200×); C, antral follicles (100×); D, antral follicles (200×); E, mature follicle (100×); F, corpus luteum (100×); G, abnormal follicles (100×); H, abnormal follicles (200×).

Table IV Sex hormone levels in different groups ($\bar{x}$±SD; n=10).

Group		FSH (mIU/mL)	LH (mIU/mL)	P (ng/mL)	E2 (pg/mL)	T (ng/mL)
Short course	High dose	0.570±0.508	2.309±0.285	18.33±8.38	71.96±7.18	2.966±0.525★
	Medium dose	0.913±1.091	2.442±0.429	17.42±13.57	72.19±9.81	2.621±0.320
	Low dose	0.536±0.360	2.201±0.178	20.97±14.92	72.56±6.07	2.484±0.310★
	Control	0.585±0.522	2.265±0.259	16.39±9.88	74.54±14.36	2.210±0.171★
Long course	High dose	0.696±0.624	2.539±0.527	13.24±7.55	82.67±17.33	2.799±0.674▲
	Medium dose	0.623±0.316	2.425±0.339	14.48±10.18	81.31±12.98	2.431±0.339
	Low dose	0.674±0.320	2.874±0.892	11.21±5.00	81.21±15.17	2.357±0.197
	Control	0.934±1.130	2.758±0.826	15.29±9.91	87.13±13.93	2.127±0.196▲

Note: ★ $P<0.05$ short course low dose group and control group vs. short course high dose group
▲ $P<0.05$ long course high dose group vs. control group

Significant difference was observed in the testosterone level between the short course high/medium/low dose groups and corresponding control group ($P<0.05$) and in the short course groups, the testosterone level had a tendency to increase with the increase of DEHP dosage, suggesting a dose dependent manner. A marked difference was observed in the testosterone level between the long course high/medium/low dose groups and the corresponding control group ($P<0.05$) and in the long course groups, the testosterone level had a tendency to increase with the increase of DEHP dosage, suggesting a dose dependent manner. However, there was no significant difference between the short course groups and long course groups at each dose level, suggesting the effects of DEHP on testosterone level were not time dependent.

The E2 level in the short course and long course high/medium/low dose groups was slightly higher than that in the corresponding control group ($P>0.05$). Furthermore, there was no marked difference in the E2 level between the short course groups and long course groups at each dose level. In addition, no significant differences were observed in the levels of P, FSH and LH between the short course groups and long course groups at each dose level, because animals were sacrificed at distinct stages of the estrous cycle and the levels of E2, P, FSH and LH are closely related to the stages of the estrous cycle and vary across the cycle. Several parameters were even at the maximal level before ovulation. Therefore, the reference value of the levels of these sex hormones was limited.

Discussion

Effect of DEHP on the body weight gain

Body weight is an important non-specific indicator comprehensively reflecting the toxicity of substances (11), and can be used to evaluate the effect of DEHP on the growth status of rats. Study has shown DEHP can limit the body weight gain through interfering with the fat metabolism and fat synthesis (12). Our study showed DEHP treatment could decrease the appetite and cause loss of hair luster. Body weight gain was also not favorable. These changes were more obvious in the long course high dose group in which several rats even had decreased body weight. Under similar conditions (intragastrical medication and stimulation by vaginal smearing), the body weight of rats with DEHP treatment was lower than that of rats in control groups. The decrease in body weight was more evident in high dose groups. According to these findings, we speculated that DEHP could limit the body weight gain. Raymond et al. also investigated the long term effect of DEHP on the body weight and their results were consistent with ours. These results suggest DEHP has chronic toxicity in a rat model (13).

Effect of DEHP on the ovarian function

Since the 1980s, studies have found an abnormal development of the reproductive system in wild animals. Numerous studies have demonstrated some chemicals can interact with distinct hormone receptors interfering with normal endocrine function, which may be the main cause of abnormal development of the reproductive system in wild animals (14). Thereafter, these chemicals are named Environmental Endocrine Disrupters (15). The endocrine receptor system in humans is similar to that in animals. The detrimental effects of these chemicals elicit the attention of researchers and the dangers of these chemicals to humans and their toxicities have been the hot topics in researches worldwide. There are a lot of Environmental Endocrine Disrupters widely distributed in the living and production environment threatening the health of organisms. In recent years, a variety of *in vivo* and *in vitro* studies have been

conducted to extensively investigate the reproductive toxicity, developmental toxicity, and genetic toxicity of Environmental Endocrine Disrupters at different levels (16–18).

PAEs are one of the important Environmental Endocrine Disrupters and have been widely used in our daily life rendering them widely distributed in the environment. PAEs have been the main environmental pollutants worldwide. DEHP is an important PAE and its toxicity represents the toxicity of PAEs. Furthermore, DEHP is the most widely used, resulting in the most serious pollution. Numerous studies have explored the reproductive toxicity of DEHP in men. In the past decade, the incidence of reproductive endocrine disorders (such as polycystic ovary syndrome) with unknown cause is increasing in women and their relation to Environmental Endocrine Disrupters is still unclear. In the present study, DEHP was employed and the effects of DEHP at different doses and for different courses on the estrous cycle, sex hormone levels and ovary histological features were investigated, aiming to explore the effects of DEHP on the rat ovarian function. Our results may provide evidence on the pathogenesis of reproductive endocrine disorders (such as the polycystic ovary syndrome).

The ovary has an endocrine function and a reproductive function characterized by periodic ovulation and periodic secretion of sex hormones accompanied by periodic alteration of vaginal epithelial cells. Under normal conditions, maintenance of the estrogen level assures a stable estrous cycle (19), and the estrogen level is regulated by negative feedback and positive feedback (20, 21). In addition, excessive androgen may counteract with the estrogen and affect the estrous cycle. Some studies report the decreased secretion of estradiol or increased secretion of testosterone can lead to prolongation of the estrous cycle, and the estrous cycle of rats is closely related to the hormone levels (22, 23). Our results showed, under same conditions (intragastrical medication and stimulation by vaginal smear), DEHP treatment could prolong the estrous cycle and interfere with the endocrine function. The estradiol level was decreased but without significant difference, which was inconsistent with the studies of Davis et al. (24) and Lovekamp et al. (25). Because the estradiol level is closely related to the stages of the estrous cycle, the contribution of time of blood collection could not be excluded.

Lovekamp et al. (25) speculated that the toxicity of DEHP to the ovary was conferred by suppressing the synthesis of aromatase in granulosa cells. Aromatase is a rate-limiting enzyme in the conversion of androgen to estrogen and the decrease of aromatase leads to the suppression of conversion of androgen to estrogen, resulting in the decrease of estrogen and increase of androgen, which was consistent with our results. Whether the changes in rat estrous cycle were affected by serum testosterone level is still unclear. We speculate that the increase of serum testosterone level can counteract with estrogen and inhibit the maturation of follicles and subsequent ovulation leading to the prolongation of estrous cycle. Under the same conditions, the serum testosterone level increased with the increase of DEHP dose which suggests the effect of DEHP on rat testosterone level was dose dependent. However, there was no significant difference in testosterone level between long course groups and short course groups at each dose level, suggesting the absence of time dependence.

Davis et al. (24) showed the ovaries of rats had polycystic-like changes. In the present study, DEHP affected the weight of ovaries, unfavorable development was only found in certain granulosa cells and oocytes, and the number of mature follicles was relatively low in the long course high/medium dose groups. But substantial pathological changes were not found in the ovaries of these rats. Whether the medication was not long enough or whether other factors also confounded our results should be further studied. Distinct compounds can exert reproductive toxicity at different sites, and interfering with endocrine function is the main cause of reproductive toxicity of these compounds. According to the results of our study, the reproductive toxicity of DEHP was less likely to be related to direct damage to reproductive organs. Interfering with the endocrine regulatory function of the ovary leading to endocrine dysfunction may play a critical role in the reproductive toxicity of DEHP. Furthermore, DEHP can affect the serum testosterone level and inhibit the estrous cycle and ovulation, influencing normal reproductive function. The serious pollution caused by these compounds may be one of the main contributions to increased incidence of endocrine disorders in women.

There is a variety of endocrine disrupters, and the mechanisms underlying the toxicities of these disrupters are distinct and synergism in toxicities may even be found in some disrupters (26). Study has shown the toxicities were significantly increased when exposed to two or more endocrine disrupters. These findings render the researches on endocrine disrupters difficult and complex. In the present study, we investigated the effects of DEHP on the rat ovarian functions. Our results added evidence for the toxicity of DEHP to ovarian function and provided a scientific basis for the evaluation of toxicity of PEAs. Due to limitations, we investigated the toxicity of only one compound of PEAs and the combination effects of several PEAs were not explored. In the future studies, the synergistic effects of different PEAs will be investigated, which may provide evidence for the scientific evaluation of the toxicity of PEAs to humans and offer a theoretical basis for the prevention of endocrine disrupter induced ecological imbalance and human diseases.

Conclusion

In our study, DEHP was employed and the effects of DEHP at different doses and for different courses on the estrous cycle, sex hormone levels and ovary histological features were investigated aiming to explore the effects of DEHP on the rat ovarian function. We speculate DEHP can affect the endocrine regulatory function of the ovary and limit the body weight gain, resulting in chronic toxicity. DEHP treatment could prolong the estrous cycle and interfere with the endocrine function. The serum testosterone level increased with the increase of DEHP dose, which suggests the effect of DEHP on the rat testosterone level was dose dependent. However, there was no significant difference in the testosterone level between the long course groups and short course groups at each dose level, suggesting the absence of time dependence. Interference with the endocrine regulatory function of the ovary leading to endocrine dysfunction may play a critical role in the reproductive toxicity of DEHP. Furthermore, DEHP can affect the serum testosterone level and inhibit the estrous cycle and ovulation influencing the normal reproductive function. The serious pollution caused by these compounds may be one of the main contributions to the increased incidence of endocrine disorders in women. Our results added evidence for the toxicity of DEHP to ovarian function and provided a scientific basis for the evaluation of PEAs toxicity. It might also provide evidence on the pathogenesis of reproductive endocrine disorders (polycystic ovary syndrome).

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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