EFFECT OF DI-ETHYLHEXYLPHTHALATE ON POSTNATAL DEVELOPMENTAL CHANGES OF TESTES OF ALBINO RATS AND THE PROTECTIVE ROLE OF SELENIUM

Refaay N.E, Hegab A.S, Elmesallamy G.E.* and Lotfy H. E.
Departments of Anatomy and Embryology and Forensic Medicine and Clinical Toxicology, Faculty of medicine, Zagazig University, El-Sharkeia governorate, Egypt.*

ABSTRACT

Background: Phthalates are used as plasticizers in Polyvinyl chloride (PVC) plastics. The essential trace element selenium (Se) is the critical component of cellular antioxidant defense. It participates in the cellular antioxidant defense and helps in protection and repair of DNA and apoptosis. **Objectives:** the aim of this study was to assess the influence of di (2-ethylhexyl) phthalate **(DEHP**) on the male reproductive system in newborn and pubertal rats and to evaluate the effect of association between it and selenium on the development of male reproductive system. **Design:** forty adult pregnant female albino rats were utilized in this work (mating was performed and pregnancy was detected by daily vaginal swab). These animals were allocated randomly into four main groups. **Group I** (control group) received corn oil. **Group II:** received diphthalates (DEHP) (150 mg/kg) by gavage daily from day 6 of gestation**. Group III:** received sodium selenate (0.16mg/kg) from day 6 of gestation**. Group IV:** received (diphthalates (DEHP) 150 mg/kg + sodium selenate 0.16 mg/kg), daily from day 6 of gestation .After labour, the mother and the pups were categorized into 3 groups (a, b, c) according to the age of scarifying of the male newborn rats (postnatal day (PND) 1, PND21 "the age of weaning" and PND35 day which is the age of puberty). At the end of every stage of the experiment, 24 hours after the last administration, all animals were sacrificed and the testes were dissected out and subjected to histopathological, immunohistochemistry, and morphometrical examination. **Results:** Administration of DEHP at a dose of 150 mg/kg induced several histopathological changes in testes of albino rats as sloughed germinal epithelium and apoptosis of epithelial cells of seminiferous tubules in addition to delayed puberty. It significantly reduced tubular diameter and tubular epithelial height. The addition of selenium partially improved the state of apical processes of Sertoli cells as evidenced by vimentin immunohistochemistry. It also reduced the degree of apoptosis as evidenced by caspase-3 immunohistochemistry in addition to the improvement of tubular epithelial height. **Conclusion:** Exposure to DEHP led to pronounced testicular damage and impaired spermatogenesis in newborn and pubertal male rats. **Recommendations:** Many alternatives should be developed to improve the safety profile of the plasticizer or prevent its release from P-PVC devices especially medical ones.

Keywords: di-ethylhexylyl phthalate, germinal epithelium, testis, selenium

INTRODUCTION

Phthalates are used as plasticizers in Polyvinyl chloride (PVC) plastics. Therefore, many consumer products contain specific members of this family of chemicals, including building materials, household furnishings, clothing, cosmetics, pharmaceuticals, nutritional supplements, medical devices, dentures and children's toys. Di-(2-ethylhexyl) phthalate (DEHP) is one of the most widespread phthalate plasticizer, used in numerous consumer products, commodities, and building materials and it is still used as plasticizer in medical products **(NTP-CERHR, 2005 and 2006).** As the phthalate plasticizers are not chemically bound to PVC, they can migrate or evaporate into indoor air and atmosphere, foodstuff and other materials. Consumer products containing phthalates can result in human exposure through direct contact and use, indirectly through leaching into other products, or general environmental contamination **(Heudorf et al., 2007).** DEHP is an endocrine disrupter and a peroxisome

proliferator (PP). It is rapidly metabolized mainly to mono (2-ethylhexyl) phthalate (MEHP), which is a derivative that is even more toxic than the parent compound **(Koch et al., 2006).** Both DEHP and MEHP have been shown to induce testicular damage, decrease fertility, and decrease sperm motility in both developing and adult animals and thus are reproductive and developmental toxicants in rodents **(Erkekoglu et al., 2011a).**

Gray et al.(2000) and Moore et al.(2001) postulated that, male offspring rats display retained nipples , undescended testis, hypospadias, small accessory sex glands, epididymal and testicular abnormalities when exposed to high doses of DEHP.

Several mechanisms have been proposed for the testicular toxicity of DEHP. It was supported that oxidative stress is one of the underlying mechanisms. Oxidative stress is the imbalance between formation of reactive oxygen species (ROS) and antioxidant defense mechanisms. Oxidative stress and thus ROS play an important role in the modulation of several important physiological functions it also contribute to cellular damage, apoptosis, and cell death .Oxidative stress has also been linked to apoptosis in germ cells and to male infertility **(Erkekoglu et al., 2010a).**

The essential trace element selenium (Se) is the critical component of cellular antioxidant defense. It participates in the fundamental biological processes ranging from cellular antioxidant defense to the protection and repair of DNA and apoptosis **(Fischer et al., 2006);** also it is essential for the production of normal spermatozoa and thus plays a critical role in reproduction **(Flohe´, 2007). The aim of this work** was to elucidate the possible developmental changes that take place in the developing testes of the newborn after oral administration of diphthalates to their pregnant, lactating albino rat mothers and to their offsprings in the prepubertal period and to investigate the possible protective role of selenium during diphthalates exposure using histopathological, immunohistochemistry, morphometrical and examination

MATERIAL AND METHODS

- **Chemicals:**

1- Di-(2- ethyl hexyl) phthalates (DEHP): was obtained from Merck Schuchardt OHG, Germany, 97% pure, in the form of oily liquid. On each administration, it was freshly prepared by dissolving 1.5 ml of DEHP in 10 ml corn oil and given as 1ml /100gm/day.

2- Sodium Selenate (Na2SeO4): it was obtained from Sigma Chemical Co. (St Louis, Mo, USA). 99, 55 pure, in the form of white powder. At the time of each administration, it was freshly prepared by dissolving each 0.16 mg in 10 ml of water and gavaged as 1 ml/kg/day.

Animals

Forty healthy non pregnant adult female albino rats (100±10 days old) weighting 150-250 gm were obtained from the Laboratory Animals' Unit at the Faculty of Medicine, Zagazig University were used in the present study. All animals were kept under hygienic conditions. Standard food and water ad-libitium were allowed. All rats were handled in accordance to the standard guide for the care and use of laboratory animals.

Adult females will be housed with adult males at ratio of 2:1 respectively in each cage. After mating with males, the day of sperm detection in the vaginal smear or presence of seminal plug was considered zero day of gestation **(Sasaki, 1990**). The pregnant rats were randomly divided into four equal groups, each contains 10 rats.

Group I: (Control): this group freely received ad-libitium from the 6th day of pregnancy and during lactation.

Group II: (DEHP treated group): this group daily received Di-ethylhexyl phthalates (DEHP) in a dose 150 mg/kg /day dissolved in corn oil through gastric gavage from the 6th day of pregnancy and during lactation **(Andrade et al., 2006).** This dose is 1/20 LD_{50} **(Dobrzynska et al., 2012)**

Group III: (Selenium treated group): this group daily received 0.16 mg/kg sodium selenate dissolved in water through gastric gavage from the 6th day of pregnancy and during lactation **(Erkekoglu et al., 2011b).** It is the therapeutic dose **(Vinceti et al., 2001)**.

Group IV: (DEHP and Selenium treated group): this group daily received through gavage both Di-ethylhexyl phthalates (DEHP) (150mg/kg/day) and sodium selenate (0.16 mg/kg/day) from the 6th day of pregnancy and during Lactation (**Erkekoglu et al., 2011b).**

Then the mothers and the pups of the whole previous 4 groups were subdivided to 3 groups according to the age of the newborn males at the time of its scarification as follows:

Group A: the male pups were sacrificed at postnatal day 1

Group B: the male pups were sacrificed at the age of weaning (21st postnatal day)

Group C: the male pups were sacrificed at the age of puberty (35th postnatal day)

At the end of every stage of the experiment, 24 hours after the last administration, all animals were anesthetized by ether inhalation, their abdominal and pelvic cavities were opened and the testes were dissected out, subjected to histopathological, immunohistochemistry, and morphometrical examination.

- **Methods**

1-Histopathological study

Samples from testis were rapidly fixed in Bouin's fixative solution, dehydrated through graded alcohols and embedded in paraffin. Transverse sections of 5µm thickness were obtained from all specimens stained with hematoxylin and eosin stain **(Bancroft and Gamble, 2008).**

2-Immunohistochemical study

Immunohistochemistry was performed by following the method of **Ramos-Vara et al. (2008).** The paraffin sections were processed by Streptavidin-biotin complex (Strep ABC) paraffin sections were deparaffinized in xylene, hydrated and then placed in phosphate buffered saline (PBS; pH 7.6). Antigen retrieval was performed by boiling for 15 min in citrate buffer (0.01 M). Sections were treated with 3% hydrogen peroxide for 5 min to quench endogenous peroxidase activity, rinsed with deionized water and then washed with PBS. Sections were incubated first with 1% pre-immune rabbit serum to decrease non-specific staining and then with a monoclonal antibody against Vimentin, caspase-3 (Dako, Carpinteria CA, USA) at 23 °C in a moist chamber for 1 hour. Detection of the antibody was performed using a biotin-streptavidin detection system (Bio-Genex, San Ramon CA, USA) with 3-amino 9-ethyl carbazole (AEC) as chromogen (Dako, Carpinteria CA, USA). Sections were counterstained with Mayer's hematoxylin, sections were evaluated using a light microscope.

3-Morphometric study:

The morphometric study was done using Image analyzer software (Image analyzer, Maryland, USA). The total images per animal were 15 images. According to this method, we used an optical magnification of 400 for calculating the mean tubular diameter, epithelial height and percentage of tissue stained with vimentin/ unit area (Unit area = microscopic field). Tubular diameter and epithelial height were performed on routine H x E sections while percentage of tissue stained with vimentin was performed on sections stained with vimentin only.

4-Statistical analysis

All the grouped data were statistically evaluated with SPSS; version 10 software Testing methods included one-way analysis of variance (ANOVA) for comparisons between more than two groups followed by least significant difference (LSD) test for comparison between two groups. *P*-values of ≤ 0.05 were considered to indicate statistical significance. All the results were expressed as mean ± S.D**.**

RESULTS

1- Histopathological results:

Examination of H&E stained sections of testes of control albino rats at PND1 (IA)

revealed the normal histological pattern as the testis was surrounded by a relatively thin connective tissue band named tunica albugina with some blood vessels under beneath within tunica vasculosa. The parenchyma of testis was consisted of multiple rounded and elongated seminiferous cords with no lumina. In the peripheral part of testis, the seminiferous cords were separated by thin amount of connective tissue stroma (interstitium) while in the central part of the testis by thick amount **(Figure 1).**

The seminiferous cords were lined by spermatogonia and pre-Sertoli cells which were seen resting on the basement membrane. These two types of cells were arranged in a single row. Spermatogonia were rounded cells with rounded nuclei while pre-Sertoli cells (supporting cells) were tall cells with oval nuclei and located peripherally along the basal lamina. The most prominent finding on PND 1 was the presence of gonocytes (spermatogonia stem cells) which were fewer in number, larger in size, and spherical in shape, with large lightly stained vesicular nuclei, and a pale stained cytoplasm that occupied the central portion of the cords. The basement membrane was consisted of a single layer of myoid cells with flattened nuclei. The interstitial spaces showed clusters of fusiform cells with oval nuclei and acidophilic cytoplasm called Leydig cells **(Figure 1).**

In PND 21 (group IB) the testis parenchyma consisted of multiple closely packed ovoid shaped seminiferous tubules with central lumens. Normal seminiferous tubules were lined by stratified germinal epithelium. The germinal epithelium revealed two types of cells; spermatogenic (germ) and Sertoli cells. Sertoli cells were detected in-between spermatogenic cells as pyramidal cells with pale basal oval or triangular nuclei. The spermatogenic cells were seen in regularly arranged rows at different stages of spermatogenesis. They were arranged from the basal compartment to the lumina of the tubules starting from spermatogonia, primary spermatocytes and secondary spermatocytes. Spermatogonia appeared as small rounded cells with spherical nuclei resting on the basement membrane. Toward the inner side, primary spermatocytes appeared relatively larger in size with large rounded nuclei arranged in one or two layers. The gonocytes appeared towards the tubular lumen. The narrow interstitial spaces showed Leydig cells with oval nuclei and acidophilic cytoplasm **(Figure 1).**

In group (IC) in addition to the cells seen in (group IB) by light microscope, Spermatids were found by their two types, the small rounded or polygonal contour cells with pale nuclei and the elongated slender mature spermatids with deeply stained nuclei that are released into the lumen of the seminiferous tubule with no evidence of presence of gonocytes within the tubules **(Figure 1).**

Examination of H&E testicular sections of PND1 DEHP treated albino rats (group IIA) showed that tunica albugina was thinner than that of the control group with variable degree of laceration, splitting and degeneration. The testis consisted of multiple seminiferous cords with increased amount of connective tissue between them. Many seminiferous cords appeared smaller in diameter and dispersed by wide interstiatial space in the central as well as the peripheral part of testis when compared with the control group. In addition, some of these cords were atrophied in the center of the testis **(Figure 2).** There were also degeneration of the basement membrane of the seminiferous cords and disturbance in their epithelial lining with decrease in the number of spermatogenic cells. Abnormal gonocytes (usually binucleated cells) were also present, mostly in the centre of the cords. Apoptotic gonocytes appeared as vacuolated cells with dark nuclei surrounded by empty space. Vacuolization of pre-Sertoli cells and abnormal clusters of Leydig cells (focal leydig cell hyperplasia) were demonstrated in between the cords **(Figure 2).**

In PND 21albino rats exposed to DEHP (group II B), the seminiferous tubules showed signs of reduced germ cell differentiation and had wide central lumens. The affected tubules

exhibited small diameter and separated by wide interstitial space (**Figure 2).** The germinal epithelium of some seminiferous tubules was sloughed off with the migration of germ cells towards tubular lumen leaving wide spaces. There was also reduction of germ cell layers through adjacent small seminiferous tubules with increased homogenicity of them. The arrangement of cells lining affected seminiferous tubules was highly disrupted indicating disorganization of tubules. The different germinal epithelium cells were not determined and most of them were with darkly stained pyknotic nuclei indicating their death and necrosis. Vacuolization of Sertoli cell was also observed. Moreover, Focal Leydig cell hyperplasia was noticed between the tubules **(Figure 2).**

In PND 35 (group II C) the seminiferous tubules were smaller in their diameter as compared to the same age in the control group. The seminiferous tubules were discrete and disorganized showing wide lumina and disturbance in their epithelial lining (**Figure 2).** The affected tubules showed marked loss of spermatids leaving the lumina empty and wide. Many germ cells appeared with pyknotic nuclei indicating cellular necrosis**.** These affected tubules were lacerated and others were branching or anastmosing (**Figure 2).**

Examination of testicular sections obtained from members of selenium treated group (III A, B and C) revealed that the testes appeared more or less similar to the control group (results not shown)

As regard to the selenium and DEHP treated group at PND1(IV A) Examination of H&E testicular sections showed that the tunica was thick as compared to the control or Se treated group. The seminiferous cords were closely packed with increase in their diameter as compared to the DEHP treated group with decrease in connective tissue stroma **(Figure 3).** The cords were lined by one row of spermatogonia and pre-sertoli cells which were seen resting on the normal basement membrane. Many normal gonocytes (spermatogonia stem cells) with large single nucleus appeared near the centers of the cords. Few numbers of abnormal gonocytes were demonstrated as vacuolated or giant multinucleated cells within the cords. The number of spermatogonia increased in relation to DEHP group. Vacuolization of Sertoli cell may be seen. Some focal accumulation of Leydig cell was also present between the cords **(Figure 3).**

At PND21 (IV B) Examination of H&E testicular sections revealed that tunica of testis was normal. The testis consisted of multiple rounded or ovoid normal seminiferous tubules with normal lumina in addition to presence of distorted tubules with wide lumina. The tubules were separated by narrow interstitial spaces and lined by several layers of germinal epithelium **(Figure 3).** The seminiferous tubules appeared normal with normal epithelial lining. The germinal epithelium revealed two types of cells; spermatogenic cells and Sertoli cells. Some germ cells were still exfoliated in the center of tubules. The gonocytes were seen with large dark vesicular nuclei in the tubular lumen. The narrow interstitial spaces showed normal Leydig cells with oval nuclei and acidophilic cytoplasm **(Figure 3).**

As regard to PND 35(IV C) the testis was surrounded by relatively thick tunica albugina which was still splitted by congested blood vessels. Many normal seminiferous tubules were apparent. Few distorted tubules were still seen and showed decreased germ cell layers lining and cell differentiation **(Figure 3).** The seminiferous tubules exhibited normal lumina and were lined by relatively normal distributed germinal epithelium. The lining germinal epithelium showed no pyknosis or sloughing of their cells. There were neither multinucleated gonocytes nor spermatids were apparent in the tubules. The interstitial spaces between the tubules appeared normally narrow and contained group of normal Leydig cells **(Figure 3).**

2- Immunohistochemical study

a- Vimentin immunohistochemical study

Vimentin immunohistochemical of the control group (group I) staining showed that, Sertoli cells are the only cells stained positive with vimentin without any extensions in PND1. Vimentin positive Sertoli cells appeared with intense basal cytoplasmic region housing the nucleus and long thin apical branched projections between germ cells in PND21.These processes reach to the tubular lumen in PND35 **(Figure 4)**

Vimentin immunohistochemical staining of group (IIA) showed a number of vimentin positive pre-Sertoli cells lining the small and atrophic seminiferous cords. Few spermatogenic cells were sloughed (detached) from thick stained basement membrane. Moreover, there were gaps between spermatogenetic cells and vimentin positive Sertoli cells which was evident in all ages of this group. The immuoreactivity was intense in the basal region of Sertoli cells with short and retracted processes in PND 21 and 35 **(Figure 4).**

Vimentin immunohistochemical staining of group (IV A) showed that, seminiferous cords were larger than those in DEHP treated group and having relatively thin stained basement membranes. No sloughing of spermatogenic cells or no gaps between them and the vimentin -positive stained pre-sertoli cells were present. The immunoreactivity was prominent at the basal part as well as apical part of Sertoli cells. The apical processes of Sertoli cells appeared longer and branched than those seen in the DEHP treated group **(Figure 4).**

b- Caspase-3 immunohistochemical study

The testes sections of group I showed negative immunoreactivity for caspase-3 in the germinal epithelium and interstitial cells especially at PND1 andPND35 while at PND21 there was minimal positive immunoreactivity in interstitial cells as shown in **figure 5.** While the testicular sections of group II stained by caspase-3 showed that, the germinal epithelial cells showed extensive positive caspase-3 immunoreactivity with many apoptotic bodies. Some of tubules showed thick lamina propria. The connective tissue stromal cells also showed extensive positive caspase-3 immunoreactivity as shown in **Figure 5.** In group (IV) Caspase-3 immunostaining showed weak positive immunoreactivity in the germinal epithelium as well as the interstitial cells of Leydig with few apoptotic bodies were still present in the tubules as shown in **Figure 5.**

3- Morphometrical study:

A. The diameter of seminiferous tubules (table 1)

The mean diameter of seminiferous cords in group I at PND1 was 80.91±5.55/ unit area. With advancement of age, the mean diameter of seminiferous tubule in the PND 21 was 160.79±11.86/ unit area while, it was 252.32±16.10/ unit area in PND 35. There was a significant increase in the mean diameter of seminiferous tubule/ unit area.

In group II, the mean diameter of seminiferous cords at PND1 was 33.71±1.84/ unit area. With advancement of age the mean diameter of seminiferous tubule in the PND 21 was 118.83±9.00/ unit area while it was 147.35±15.22 / unit area in PND35. There was no significant increase in the tubular diameter in PND1 and PND21 while the increase was significant between PND21 and PND35.

In group IV, the mean diameter of seminiferous cords at PND1 day was 80.86±6.02/ unit area. With advancement of age, the mean diameter of seminiferous tubule in PND 21 was 157.13±11.12/ unit area while it was 173.44±16.88 / unit area in PND35. So there was a significant increase in the mean values of the diameter of seminiferous tubule/ unit area.

The mean diameter of seminiferous tubules at PND1 in different studied groups showed a non significant difference between group I and group IV. However, at the same time there was a significant difference between group I, III and II groups and this was the same result obtained in PND21 however at PND35 there was a significant difference in the mean diameter between the four studied groups.

Table (1): statistical analysis of (Mean ± SD) of seminiferous cord/tubule diameter in control and treated groups

Day / Group	1d (Mean ± SD)	21d (Mean ± SD)	35d (Mean ± SD)
Control	80.91 ± 5.55^{Bc}	160.79 ± 11.86^{Cb}	252.32 ± 16.10^{Ba}
DEHP	33.71 ± 1.84^{Cb}	118.83 ± 9.00^{Bb}	147.35 ± 15.22^{Da}
SE	91.87 ± 6.23^{Ac}	217.37 ± 24.84^{Ab}	315.14 ± 19.66^{Aa}
DEHP+SE	80.86 ± 6.02^{Bc}	157.13 ± 11.12^{Cb}	173.44 ± 16.88^{Ca}

Values within the same column carrying different capital superscripts indicating significant difference at ($P < 0.05$).

Values within the same row carrying different small superscripts indicating significant difference at ($P < 0.05$).

A,a Significantly different from control group ($P < 0.05$) (LSD test).

B,b Significantly different from DEHP group ($P < 0.05$) (LSD test).

C,c Significantly different from Selenium group ($P < 0.05$) (LSD test).

D,d Significantly different from DEHP and Selenium group ($P < 0.05$) (LSD test).

Unit area= Microscopic field (n=15)

B. The epithelial height of seminiferous tubules (table 2)

The epithelial height of PND1 was not detected as there was no lumen helping to detect it

The mean epithelial height of seminiferous tubules at PND21 in Group I was 50.44±4.19/unit area. With advancement of it was 75.77±2.95 unit area on PND35. So there was a significant increase in the epithelial height of seminiferous tubule/ unit area.

In group II, the mean epithelial height of seminiferous tubules at PND 21 was 28.16±1.75unit area. With advancement of age it was 46.27±3.10/unit area at PND35 so there was a significant increase in the mean values of the epithelial height of seminiferous tubule/ unit area.

In group IV, the mean epithelial height at PND 21 was 60.74±5.16 /unit area. With advancement of age it was 59.35±6.46/ unit area at PND35 so there was no significant difference between the mean values of the epithelial height of seminiferous tubule/ unit area.

The mean epithelial height at PND21 and PND35 showed a significant difference between the different studied groups.

Table (2): Statistical analysis of (Mean ± SD) of seminiferous tubular epithelial height in control and treated groups

Day / Group	21d (Mean ± SD)	35d (Mean ± SD)
Control	50.44 ± 4.19^{Cb}	75.77 ± 2.95^{Ba}
DEHP	28.16 ± 1.75^{Db}	46.27 ± 3.10^{Da}
SE	54.37 ± 5.96^{Bb}	79.94 ± 5.20^{Aa}
DEHP+SE	60.74 ± 5.16^{Ab}	59.35 ± 6.46^{Cb}

Values within the same column carrying different capital superscripts indicating significant difference at (P < 0.05).
Values within the same row carrying different small superscripts indicating significant difference at (P < 0.05).
A,a Significantly different from control group (P < 0.05) (LSD test).
B,b Significantly different from DEHP group (P < 0.05) (LSD test).
C,c Significantly different from Selenium group (P < 0.05) (LSD test).
D,d Significantly different from DEHP and Selenium group (P < 0.05) (LSD test).
Unit area= Microscopic field (n=15)

C: The percentage of tissue stained with vimentin (table 3)

The mean percentage of tissue stained with vimentin in group I at PND1 was30.33±2.85/unit area. With advancement of age it was 37.50±1.50/ unit area at PND21 and 25.00±2.10/ unit area at PND35. So there was a significant difference between the different ages in group I.

In group II, the mean percentage of tissue stained with vimentin at PND1 was 59.00±2.10 / unit area. With advancement of age it was 49.50±1.20 / unit area at PND21 and 71.00±3.06 / unit area at PND35.so there was no significant difference between PND1 and PND21 while there was a significant difference between these previous ages and PND35.

In group IV, the mean percentage of tissue stained with vimentin at PND1 was 44.00±2.25/ unit area. With advancement of age it was 45.00±1.00 / unit area at PND21 and 67.50±5.50 / unit area at PND35 so there was a significant difference between the different ages in group IV.

The mean percentage of tissue stained with vimentin at PND1 showed no significant difference between group I and II But it showed significant difference between group I, II and IV. At PND 21 and PND 35 there was a significant difference between different studied groups

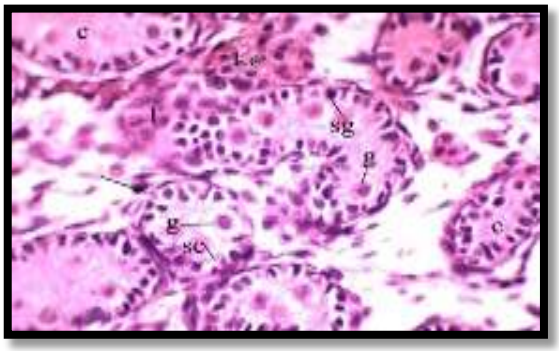
Day / Group	1d	21d	35d
Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Control	30.33±2.85Cb	37.50±1.50Ca	25.00±2.10Cc
DEHP	59.00±2.10Ab	49.50±1.20Ac	71.00±3.06Aa
SE	31.43±1.65Cb	35.50±2.57Da	20.00±1.66Dc
DHEP+SE	44.00±2.25Bb	45.00±1.00Bb	67.50±5.50Ba

Table (3): Statistical analysis of (Mean ± SD) of percentage of tissue stained with vimentin in control and treated groups

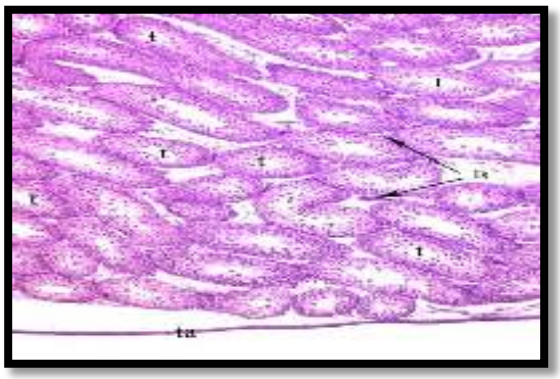
Values within the same column carrying different capital superscripts indicating significant difference at (P < 0.05).
Values within the same row carrying different small superscripts indicating significant difference at (P < 0.05).
A,a Significantly different from control group (P < 0.05) (LSD test).

B,b Significantly different from DEHP group (P < 0.05) (LSD test).
C,c Significantly different from Selenium group (P < 0.05) (LSD test).
D,d Significantly different from DEHP and Selenium group (P < 0.05) (LSD test).
Unit area= Microscopic field (n=15)

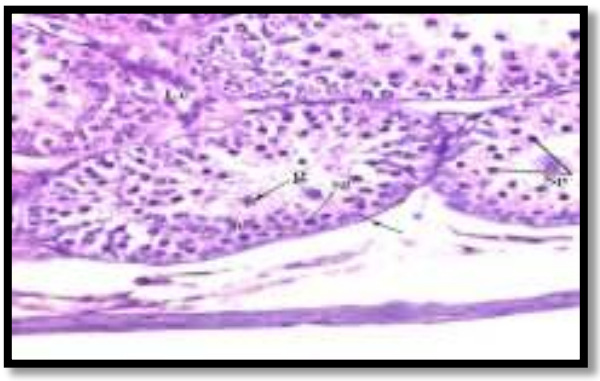
A photomicrograph of testes of subgroup I A **PND1,** showing relatively thin tunica albugina (ta) surrounding multiple seminiferous cords (c). The cords have no lumens and are separated by thin amount of interstitium (I) in the peripheral part of testis while the interstitial space (Is) was wide in the central part of the testis. Blood vessels (Bv) in tunica vasculosa are also seen beneath tunica albugina **[H&E X 100]**



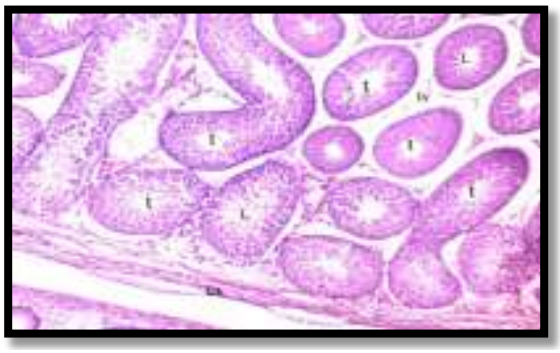
A photomicrograph of testes of subgroup I A **PND1,** showing one row of spermatogonia (sg) and pre-Sertoli (sc) cells lining the seminiferous cords(c). Large gonocytes (g) with single large nucleus are shown near the center of the cords. The basement membrane is formed of the myoid cells (arrow) with flattened nuclei. The Interstitial cells of Leydig (Lc) are seen within the interstitium (I) between the cords. **[H&E X 400]**



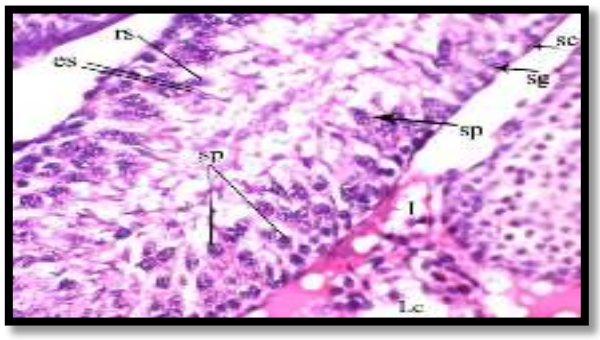
A photomicrograph of testes of subgroup I B **PND21,** showing thin tunica albugina (ta) surrounding multiple closely packed seminiferous tubules (t). These tubules have central lumens (L) and are lined by multiple layers of germinal epithelium .They are separated by narrow interstitial spaces (Is).**[H&E X 100]**



A photomicrograph of testes of subgroup I B, **PND21,** showing ovoid seminiferous tubules lined by multiple layers of germinal epithelium. The germ cells are namely Spermatogonia (sg) and spermatocytes (sp). Sertoli cells (sc) with pale basal nuclei are present between germ cells. Gonocytes (g) are seen with dark nuclei towards the tubular lumen. The Interstitial Leydig cells (Lc) are present between the tubules. Myoid cells with flattened nuclei ensheath the seminiferous tubules (arrow). **[H&E X 400]**

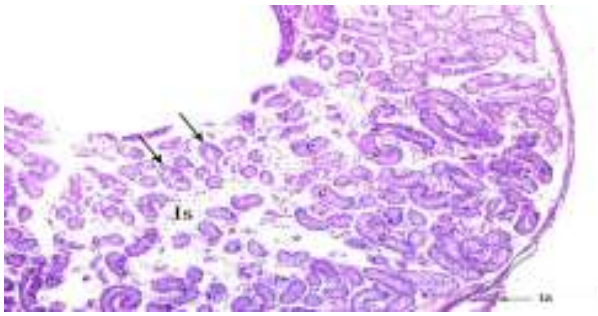


A photomicrograph of testes of subgroup I C showing thick tunica albugina (ta) surrounding multiple seminiferous tubules (t) having narrow lumina (L). These tubules are separated by interstitial spaces (Is) and are lined by germinal epithelium arranged in multiple layers.**[H&E X 100**

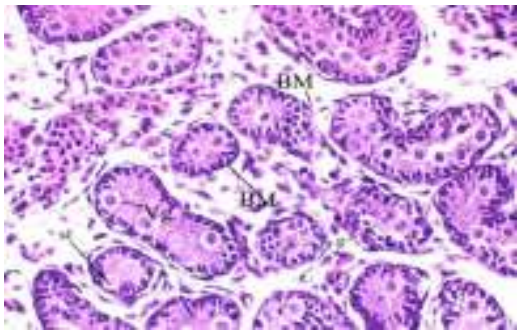


A photomicrograph of testes of subgroup I C showing different types of spermatogenic cells: spermatogonia (sg), Spermatocytes (sp), rounded spermatid (rs) and elongated spermatid (es). Sertoli cells (sc) appear with basal oval nuclei .The interstitium (I) contains group of Leydig cells (Lc).**[H&E X 400]**

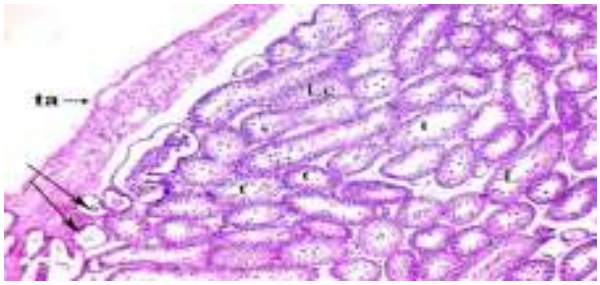
Figure (1): Photomicrographs of testes of control group (group I) at PND1, PND21 and PND35.



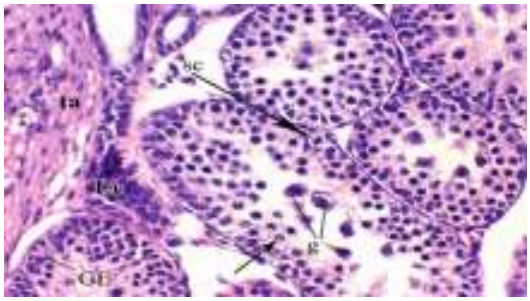
A photomicrograph of testes of subgroup II A showing lacerated and degenerated tunica albugina (ta) surrounding multiple seminiferous cords (c). Many seminiferous cords are seen small in size and dispersed by wide interstiatial space (Is) in the central and the peripheral part of testis in addition to the presence of atrophic seminiferous cords (arrows) in the center of testes. **[H&E X 100]**



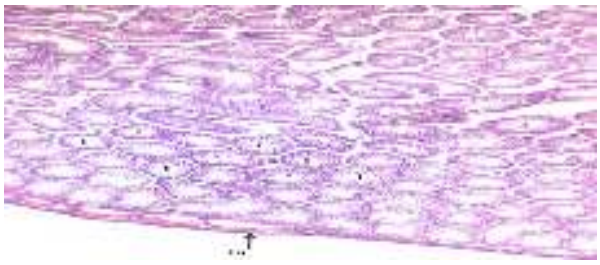
A photomicrograph of testes of subgroup II A showing multiple binucleated gonocytes (g) in the center of the cords (c). Vacuolization of both gonocytes (Vg) and Sertoli cells (sc) is also noticed. The disturbed basement membranes (BM) of some small seminiferous cords are also observed. Focal Leydig cell hyperplasia (Lc) is also seen between the cords. **[H&E X 400]**



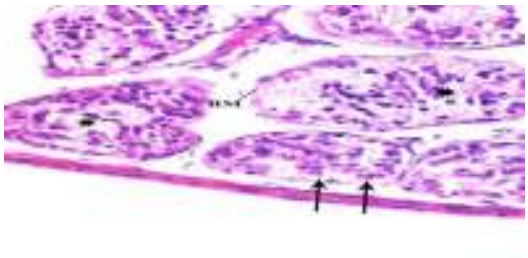
A photomicrograph of testes of subgroup II B showing very thick lacerated and degenerated tunica albugina (ta) with areas of hemorrhage surrounding multiple small seminiferous tubules (t) . Many seminiferous tubules (t) show reduced germ cell layers and have wide central lumina (L). Some degenerated seminiferous tubules (arrows) appear beneath the tunica albugina. Focal Leydig cell hyperplasia (Lc) is seen between the tubules.**[H&E X 100]**



A photomicrograph of testes of subgroup II B showing decreased germinal epithelial layers lining tubules (GE). Many germ cells appear with pyknotic nuclei (arrow). Degenerated multinucleated gonocytes (g) are shown in the lumen of tubules. Vacuolization of Sertoli cells (sc) and focal Leydig cell hyperplasia (Lc) are also seen. **[H&E X 400**

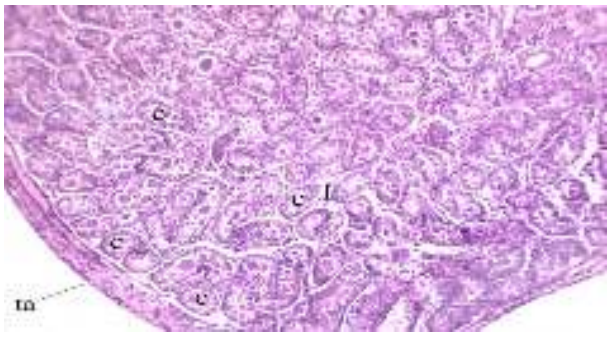


A photomicrograph of testes of subgroup II Cshowing lacerated tunica albugina with dilated blood vessels surrounding discrete seminiferous tubules (t). The seminiferous tubules (t) are disorganized with wide lumina (L) with disrupted epithelial lining. **[H&E X 100]**

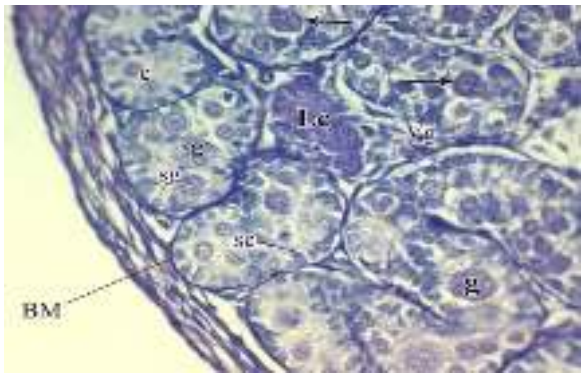


A photomicrograph of testes of subgroup II C showing distortion of many seminiferous tubules with decreased germinal epithelial layers lining (arrows). Many germ cells (arrow heads) appear with pyknotic nuclei in addition to marked loss of spermatids leaving empty wide lumina (L). Many abnormal Leydig cell (Lc) accumulate between the tubules**[H&E X 400]**

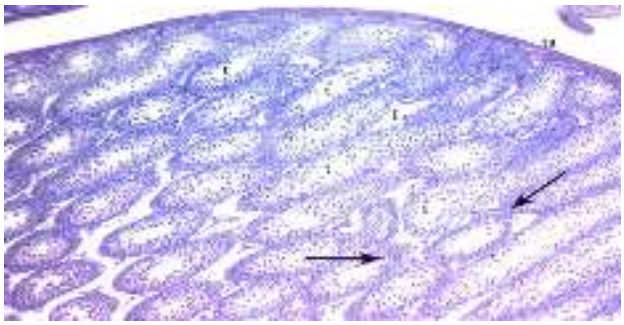
Figure (2): photomicrographs of testes of DEHP treated group (group II) at PND1 PND21 and PND35.



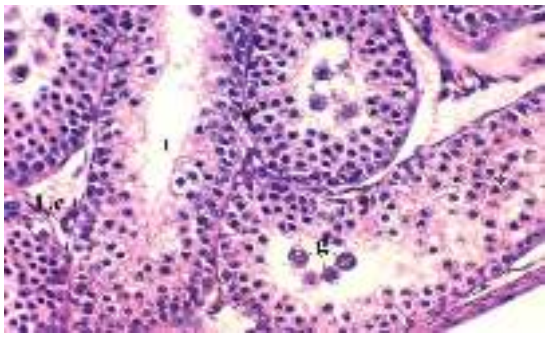
A photomicrograph of testes of subgroup IV A showing relatively thick tunica albugina (ta) surrounding closely packed seminiferous cords (c). The cords are separated by narrow interstitium (I).**[H&E X 100]**



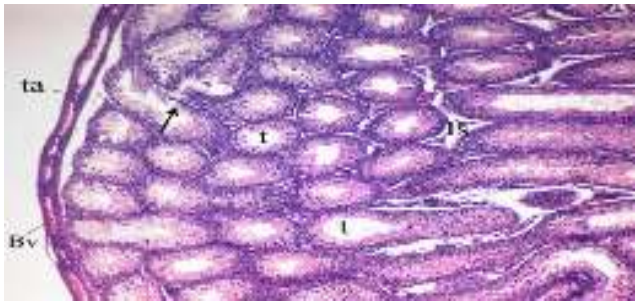
A photomicrograph of testes of subgroup IV A showing one row of normal spermatogonia (sg) and pre-Sertoli (sc) cells lining the normal basement membrane (BM) of the cords (c). Many normal gonocytes (g) appear near the centers of the cords. Few vacuolated gonocytes (vg) or multinucleated gonocytes (arrow) are seen within the cords. Some focal accumulation of Leydig cell (Lc) appears between the cords.**[H&E X 400]**



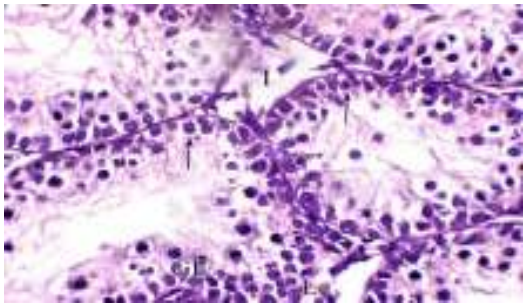
A photomicrograph of testes of subgroup IV B showing normal tunica albugina (ta). Many normal seminiferous tubules (t) with normal lumina appear separated by narrow interstitium (I). Some distorted tubules (arrow) are seen with wide lumina. **[H&E X 100]**



A photomicrograph of testes of subgroup VI B showing seminiferous tubules (t) lined by multiple layers of germinal epithelium. The gonocytes (g) are seen with dark vesicular nuclei in the tubular lumen. Few germ cells are exfoliated in the center of tubules (*). Normal Leydig cells (Lc) appear between the tubules.**[H&E X 400]**

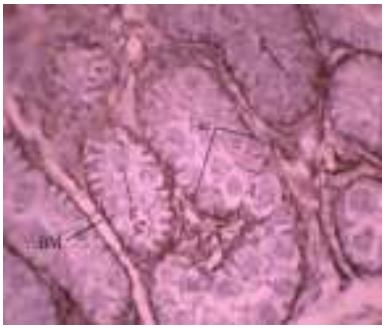


A photomicrograph of testes of subgroup VI C showing relatively thick splitted tunica albugina (ta) with congested blood vessels (Bv). Few distorted tubules (arrow) having decreased germ cell layers lining appear between many normal seminiferous tubules (t). The narrow interstitial spaces (Is) are also noticed between the seminiferous tubules. **[H&E X 100**

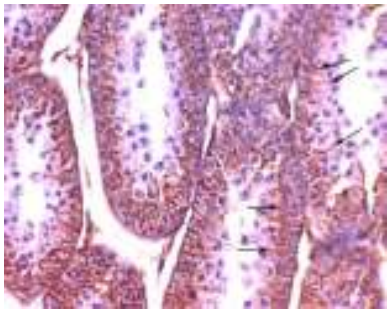


A photomicrograph of testes of subgroup VI C showing seminiferous tubules with relatively normal distribution of germinal epithelium (GE) in some parts with no pyknosis or sloughing of their cells. However decreased tubular lining in other parts (arrows) is also observed. Neither multinucleated gonocytes nor Spermatids are noticed in the tubules. The interstitium (I) contains group of Leydig cells (Lc). **[H&E X 400]**

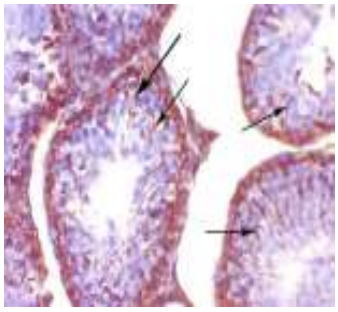
Figure (3): photomicrographs of testes of DEHP+ Se treated group (group IV) at PND1 PND21 and PND35.



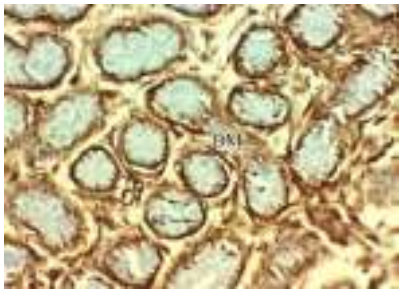
A photomicrograph of testes of subgroup I A showing spermatogonia and pre-Sertoli cells lined on thin clearly vimentin positive stained basement membrane (BM). Vimentin positive pre-Sertoli cells (sc) appear between germ cells. Large gonocytes (g) are noticed near the center of cords



A photomicrograph of testes of subgroup I B showing vimentin positive Sertoli cells with intense basal immunoreactivity and long branched apical processes between germ cells (arrows)

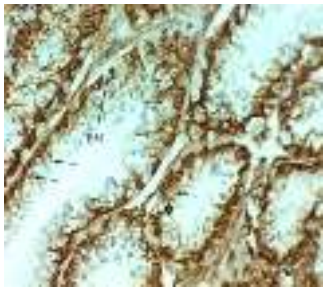


A photomicrograph of testesof subgroup I C showing vimentin positive Sertoli cells between germ cells with basal immunoreactivity and multiple thin vimentin- positive apical extensions between germ cells (arrows).

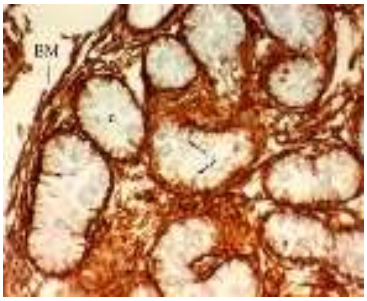


A photomicrograph of testes of subgroup II A showing sloughed germ cell (black arrow heads) from thick stained basement membrane. A number of vimentin positive pre-Sertoli cells (sc) appear lining the cords and separated by gaps (red arrow heads) from the germ cells. The degenerated basement membrane (BM) of some seminiferous cords is also noticed.

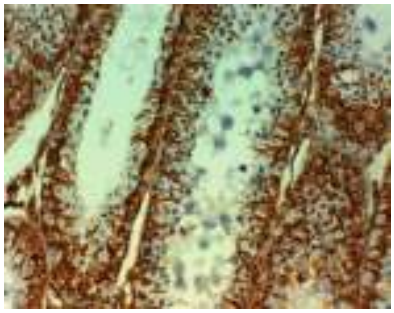
A photomicrograph of testes of subgroup II B showing vimentin positive Sertoli cells with intense basal immunoreactivity. Short non branched apical processes (arrows) with multiple multinucleated gonocytes (g) are present towards the tubular lumen.



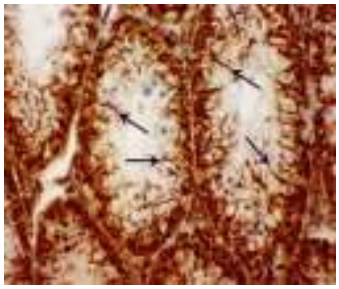
A photomicrograph of testes subgroup II C showing a number of Sertoli cells (arrow) with prominent basal immuoreactions. Gaps (*) between spermatogenic cells and Seroli cells are also observed. The distorted and less stained basement membrane of seminiferous tubules = (BM).



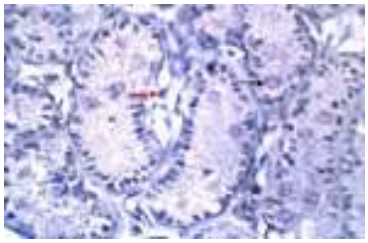
A photomicrograph of testes of subgroup IV Ashowing normal larger seminiferous cords (c) having relatively thin stained basement membranes (BM). Vimentin positive stained pre-sertoli cells (arrow) appear between normal spermatogenic cells which showed neither swelling nor vacuolization.



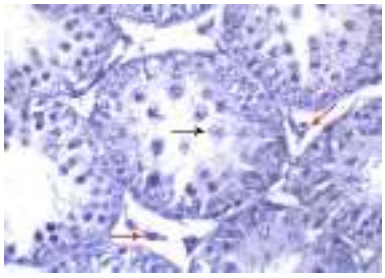
A photomicrograph of testes of subgroup VI B showing vimentin positive Sertoli cells with prominent basal immunoreactivity and long branched apical processes between germ cells (arrows).



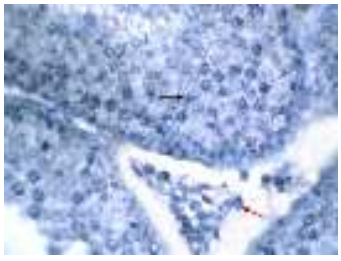
A photomicrograph of testes of subgroup VI C showing prominent immunoreactivity for both basal and apical parts of Sertoli cells. Apical Sertoli cell processes reach to tubular lumen (arrows)



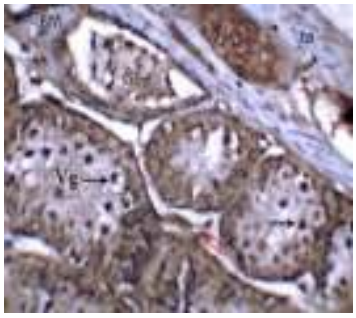
A photomicrograph of testes of subgroup I A showing negative immunoreactivity for caspase-3 in the germinal epithelium (black arrow) and interstitial cells (red arrow).



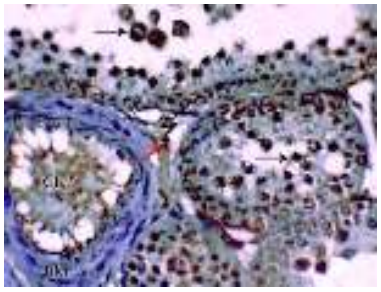
A photomicrograph of testes of subgroup I B showing negative immunoreactivity for caspase-3 in the germinal epithelium (black arrows) and weak immunoreactivity for caspase-3 in interstitial cells



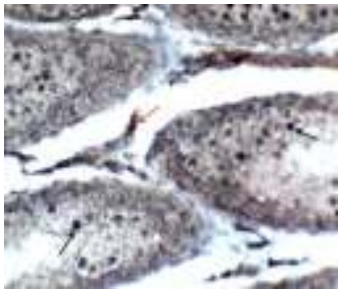
A photomicrograph of testes of subgroup I C showing negative immunoreactivity for caspase-3 in germinal epithelium (black arrows) and interstitial cells (red arrows).



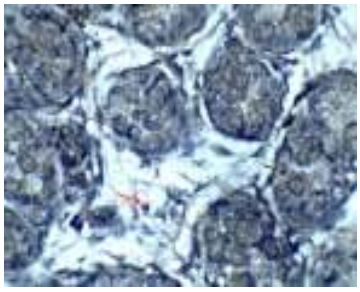
A photomicrograph of testes of subgroup II A showing excessive immunoreactivity for caspase-3 in apoptotic germ cells (black arrow) and in interstitial cells (red arrow). Caspase-3 labeling vacuolated gonocytes (Vg) with condensed nuclei are also seen. Sloughed germinal epithelium (GE) in cord centers and thickened tunica albugina with positive immunoreactivity of its cells (arrowhead) are also noticed.



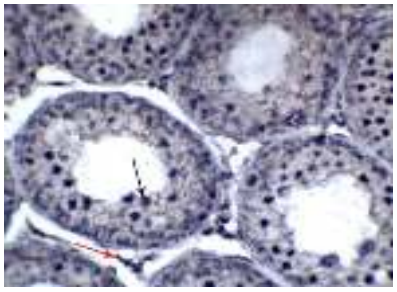
A photomicrograph of testes of subgroup II Bshowing thick lamina propria (BM), sloughed germinal epithelium (GE) with apoptotic bodies inside the tubules (black arrows) and interstiatial cells of Leydig (red arrow).



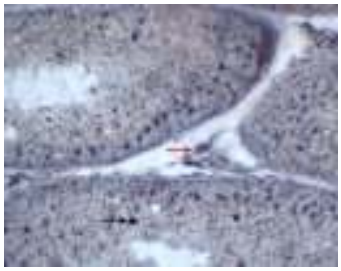
A photomicrograph of testes of subgroup II C Showing positive caspase-3 immunoreactive cells in the testes as well as apoptotic bodies in the epithelial lining (black arrows) and in the interstitium (red arrow).



A photomicrograph of testes of subgroup VI A showing mild positive caspase-3 immunoreactivity in germinal epithelium (black arrow) and in interstitial cells of Leydig (red arrow) with absence of apoptotic bodies.



A photomicrograph of testes of subgroup VI B showing weak positive immunoreactivity in the germinal epithelium with few apoptotic bodies (black arrows) and in interstitial cells of Leydig (red arrow).



A photomicrograph of testes of subgroup VI C showing mild positive immunoreactivity in the tubules and few apoptotic bodies (black arrows) and in interstitial cells of Leydig (red arrow).

Figure (4): photomicrographs of testes of Control, DEHP and DEHP and Se treated groups (group I, II and IV) at PND1 PND21 and PND35 (VimentinX 400).

Figure (5): photomicrographs of testes of Control, DEHP and DEHP and Se treated groups (group I, II and IV) at PND1 PND21 and PND35 (Caspase-3X 400).

DISCUSSION

The development of the fetal testis requires complex hormonal regulation and thus forms a highly sensitive detector for hormonal disruption (**Chauvigné et al., 2009**). Phthalates (or phthalate esters) are an important group of endocrine disruptors (EDs) which are a group of molecules capable of altering normal endocrine functions in animals and humans. These compounds are considered to mimic the

effect of estrogen and other steroid hormones, deregulating the control of several hormone-dependent developmental processes **(Li et al., 2014).**

Phthalate esters have attracted considerable public attention in the era of environmental toxicants which impair the human fertility. This is because of its adverse developmental and reproductive effects which were observed in laboratory animals, in addition to their high production volume and use as plasticizers in commercial products such as plastic food wraps, children's toys, blood transfusion and dialysis bags **(Ahbab and Barlas, 2013).**

Di ethyl-hexyl phthalates (DEHP) is a well-known peroxisome proliferator and considered a non-classic type endocrine disruptor, that is, in contrast to the classical endocrine disrupters which interfere with endocrine process at the receptor level, alters reproductive function by affecting hormone synthesis **(Akingbemi et al., 2004).**

Therefore, the present work was designed to elucidate the possible developmental changes that take place in the developing testis of the newborn after oral administration of diphthalates to their pregnant mothers and to investigate the possible protective role of selenium during diphthalates exposure.

In the present study, light microscopic examination in the day 1 postnatal albino rats, the testes showed relatively thin tunica albugina surrounding multiple solid seminiferous cords without lumen. The cords were lined by single layer of spermatogenic cells and pre-Sertoli cells resting on thin basement membrane. The most prominent finding on PND 1 control rats was the presence of gonocytes (spermatogonia stem cells) which were fewer in number, larger in size, and occupied the central portion of the cords.While in the DEHP treated group of the same age, the testes showed thick disrupted tunica albugina with variable degree of laceration and degeneration. The testis was consisted of many irregular seminiferous cords with destructed interstitial tissue between them, many of these cords were smaller in diameter and some were atrophied. Abnormal gonocytes (binucleated even multinucleated giant cells) were presen. In addition to apoptotic gonocytes which appeared as vacuolated cells with dark nuclei surrounded by empty space

The previous results were in a harmoney with the work done by **Salama et al. (2013).** They found that the tunica albugina was thickened, which might be due to an increase in type I collagen synthesis. Its blood vessels were dilated and congested, which might be due to chemical mediators released shortly after tissue damage causing dilatation of capillaries and postcapillary venules. The blood flow within them slowed and it became lodged with red blood corpuscles.

It is possible that each giant multinucleated cell represents several gonocytes whose status as individual cells has been compromised perhaps via alteration of their cell membrane properties and/ or their ability to maintain appropriate contact-mediated interactions with adjacent Sertoli cells (**Shirota et al., 2005**).

In the current study, light microscopic examination of testes in postnatal days 21and 35 showed that the cords transferred to multiple closely packed seminiferous tubules exhibiting lumina. The lining epithelium consisted of multiple arranged layers and the spermatids appeared for the first time in day 35 postnatal. In the postnatal day 21, the gonocytes were still present however, in day 35 postnatal, the gonocytes were completely absent. However, in the DEHP treated group, the distorted seminiferous tubules with disorganized spermatogenic epithelium were always obvious features. Some tubules exhibited wide lumina with reduction of germ cell layers. Abnormal gonocytes (binucleated even multinucleated giant cells) were still present on PND 21.With increased time of exposure; there was loss of stratification of the lining epithelium of the seminiferous tubules. Some germ cells exfoliated in the lumen and detached from the basement membrane, the different germinal

epithelium cells were not determined and the tubules showed arrested spermatogenesis with no spermatid in their lumens indicating delayed puberty.

This was concordat with the findings of **Barlow and Foster (2003)** who stated that, when male rats were exposed in utero to DBP at a dose of 500 mg/kg/day during the latter half of gestation, they exhibited a characteristic suite of testicular lesions with marked testicular atrophy in fetal male rats (PND31 and 42).Those effects were permanent.

In accordance with the current study, **Andrade et al. (2006)** observed delay in the pubertal age in addition to reduced germ cell differentiation lining the seminiferous tubules with reduced or absent tubular lumen in affected tubules .Also, **Christiansen et al. (2010)** reported several testicular lesions in testes of rats whose their mothers were exposed to DEHP (300 mg/kg/day) dissolved in corn oil from gestation day 7 to postnatal day 16); the testes were more immature with a delay in the development of the seminiferous epithelium and fewer germ cells were present.

On the other hand, the decrease in lining layers of the seminiferous tubules demonstrated in the days 21 and 35 postnatal DEHP-treated group of the present study, was consistent with the results of **Zhang et al. (2013).** They evaluated the effects of gestational and lactational exposure to DEHP and/ or genisteinin (GEN) in pregnant Sprague-Dawley rats gavaged from gestation day 3 to postnatal day 21 on the sexual development of male offspring rats. They stated that diameter and germ cell development of groups received DEHP 250 mg/kg and DEHP 250 + GEN50 were not significantly different from the control, but cell layers of the two groups were thinner than control layers. The diameter of the seminiferous cords of group DEHP250 +GEN400 was significantly reduced with relatively few cell layers compared to the control indicating delayed development of the testis.

Moreover, **Erkekoglu et al. (2011a)** assured the testicular injury in pubertal rats received 1000 mg/kg DEHP by intragastric gavage (i.g.) for 10 days. The previous authors found disturbed testicular histology; the lumen of the seminiferous tubules contained sloughed germ cells, apoptotic death of germ cells in addition to diminished motility in epididymal sperm.

In this study, in days 21 and 35 postnatal albino rats of DEHP treated group the affected seminiferous cords and tubules appeared smaller in diameter and dispersed by wide destructed interstitial tissue. These empty spaces between the seminiferous tubules of the DEHP treated group could be explained by phagocytosis of the apoptotic bodies by Sertoli cells, as reported by **Nishimura et al. (1995).**

On the other hand, all ages of DEHP-treated group in this study showed focal Leydig cell hyperplasia within the wide interstitial tissue between the cords and tubules which may add another explanation to the wide interstitial space. The findings in the interstitial tissues were concordant with those of **Mahood et al. (2005)** who explained that, the existence of large Leydig cell aggregates may be due to an increase in focal Leydig cell proliferation or Leydig cell migration to central regions from other points of origin in the testes after exposure to DBP.

Abnormal fetal Leydig cells (FLC) aggregation found in the current work was previously observed with DEHP exposure **as Mylchreest et al., (2000)**, reported that high dose exposures to DEHP in utero result in focal disruptions in the structure of the seminiferous epithelium as well as abnormal aggregations of FLCs. Because of their large size, the FLC aggregations were at first thought to represent Leydig cell hyperplasia or neoplasia. **Salama et al. (2013)** stated that the occurrence of large Leydig cell aggregates may be due to an increase in focal Leydig cell proliferation or Leydig cell migration to central regions from other points of origin in the testes after exposure to DBP.

One of the primary targets of phthalates in testis was Sertoli cells. These are the supportive cells of the seminiferous epithelium that provide an appropriate hormonal and nutritional environment necessary for the differentiation of immature spermatogenic cells to spermatozoa Many reproductive toxicants have been shown to disturb Sertoli cell junctional complexes, resulting in loss of intercellular contacts between Sertoli cells and germ cells, which may contribute to alteration of Sertoli-cell functions and germ cell losses through apoptosis **(Fiorini et al .,2004).**

Vacuolization of Sertoli cells was also seen in the current study in postnatal days 1 and 21 in the DEHP treated group. This finding was in a harmoney with the results of **Kasahara et al. (2002)** who postulated that administration of DEHP induces vacuolation in Sertoli cells. Also, autoradiographic experiments showed that radioactive marked DEHP can pass the blood – testis barrier into Sertoli cells. Hence Sertoli cells can only support a finite number of germ cells, so that the number of spermatids in adults can decrease when there has been Sertoli cell reduction in neonatal rats **(Okdah, 2013).**

The presence of sloughed germ cells and vacuoles in Sertoli cells in the present work was in accordance with **Kai et al. (2005**) who suggested that, the primary target of the phthalate esters is the Sertoli cells in adult rat testes.

In the current study, by light microscopic examination, many germ cells appeared with pyknotic nuclei indicating cellular necrosis and death. Also in this study, there was negative (in germinal epithelium) to weak immunoreactivity (in interstitial cells) for caspase 3 in the developing rats of the control group. However, in the DEHP treated group there was moderate to intense immunoreactivity for caspase 3 with the appearance of apoptotic bodies. This was explained by **Yan et al. (2000)** who reported that, apoptosis normally occurs during development and aging and as a homeostatic mechanism to maintain the cell population in tissues. **Norbury and Hickson (2001)** supported the previous hypothis. They stated that during the first wave of spermatogenesis (which occurs from the first to the sixth/seventh week of postnatal life), a significant number of spermatogenic cells undergo apoptosis, and the highest rate of apoptosis occurs at 18–26 days of age to maintain an optimal Sertoli and spermatogenic cell ratio.

DBP-treated rats exhibited strong immunoreactivity for caspase 3 in this study could be explained by the work of **Alam et al. (2010)** who stated that, vacuolation is the earliest morphological sign of testicular injury, as observed in this study, and believed that vacuolation, followed by ultrastructural alterations in Sertoli cells induced by phthalate administration, causes detachment of spermatogenic cells from Sertoli cells, which is the first step towards cell death or apoptosis.

Statistical analysis of the morphometric study in the present work showed a significant decrease ($P < 0.05$) in tubular diameter and epithelial height of all ages in the DEHP-treated group, when compared with the control and selenium treated groups. **Andrade et al. (2006)** exposed the rats in utero and lactational period to DEHP and observed a reduction in the diameter of seminiferous tubules that was only seen at 15 mg/kg/day. **Ahbab and Barlas (2013)** observed decreased seminiferous tubule diameter only in high dose DHP and all DCHP and referred the controversy in those findings to the exposure period which was only in utero in the former study. Also, **Zare et al. (2011)** found a significant decrease in tubular diameter and epithelial height in 4 weeks old male mice, when exposed to different doses of DEHP by gavaging for 2 weeks.

On the other hand, the group of rats simultaneously treated with selenium in accompany with DEHP in the current study revealed minimal preserved histological structure with the ordinary light microscope. However, some seminiferous tubules showed disrupted germinal epithelium. Selenium was

effective in preventing some of the toxic effects induced by DEHP and this was evident by the immunohistochemical examination. These findings were consistent with **Erkekoglu et al. (2011b),** who found small vimentin-positive extensions of Sertoli cells in rats received Se and DEHP with some recovery in vimentin assembly of Sertoli cells, especially in unaffected tubules, indicating a protective effect of excess Se against DEHP toxicity.

The essential trace element Se with its several forms of cellular selenoproteins is primarily involved in the modulation of intracellular redox equilibrium and plays a vital role in cellular antioxidant defense. Se is remarkably and preferentially maintained in testis and is essential for normal spermatogenesis. Glutathione peroxidase 4 (GPx4), which is necessary for the integrity of mature sperm and required for proper sperm stability, is the most abundantly present selenoprotein in rat testis thus, it is vital for male fertility **(Flohe´ 2007).**

Seiler et al. (2008) demonstrated that GPx4 is also a sensor of oxidative stress and controls a distinctive cell death–signaling pathway involving 12, 15-lipoxygenase-derived lipid peroxides and apoptosis inducing factor–mediated cell death. Decrease of GPx4 expression was shown to cause termination of spermatogenesis. So, decreased GPx4 activity may be used as a marker for germ-cell apoptosis in rats. Also, **Erkekoglu et al. (2011)** found significant decrease in GPx4 activity along with a significantly high redox ratio that in selenium deficient rat exposed to DEHP.

CONCLUSIONS

From the previously mentioned results, it can be concluded that intrauterine exposure to DEHP at a dose of 150 mg/kg/day produced toxic effects in the reproductive organs of male albino rat offsprings. These effects were evidenced by the histopathological and immunohistopchemical changes of reproductive organs. Contuning exposure to DEHP through lactation worsens these hazardous effects. Selenium could provide partial protection against those toxic effects.

RECOMMONDATIONS

Much more attention should be paid for limiting the occupational and environmental exposure to DEHP. Special precautions must be taken to limit the level of the environmental, water and food contamination. Many alternatives should be developed to improve the safety profile of the plasticizer and or prevent its release from P-PVC devices especially medical ones. Moreover, people especially pregnant mothers should be advised to take Se supplementations in the recommended dose.

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تأثيرثنائي ايثيل هكسيل الفيثالات على التغيرات التكوينية بعد الولادة في خصي الجرذان البيضاء والدور الوقائي للسيلينيوم

نهال السيد رفاعي ؛ أشرف صابر حجاب؛ غادة السيد احمد المسلمي* ؛وحنان السيد لطفي

أقسام التشريح الآدمي وعلم الأجنة؛ الطب الشرعي والسموم الاكلينكية*- كلية الطب البشري - جامعة الزقازيق- محافظة الشرقية- جمهورية مصر العربيه.

تستخدم الفيثالات كملدنات لبلاستيك البولي فينيل كلوريد و من ثم فإن العديد من المنتجات الاستهلاكية تحتوي على واحدا أو أكثر من هذه العائلة الكيميائية مثل مواد البناء ، أثاث المنزل ، الملابس، مواد التجميل،الدوائيات ، مكملات الغذاء ، الاجهزة الطبية ، وقد ثبت أن ثنائي الفيثالات يعتبر من أكثر الملدنات . استخدمت في هذه الدراسة أربعون من اناث الجرذان البيضاء البالغة العذراء التي لم يسبق لها الحمل وتم استبعاد الجرذان المريضة (تم تزاوج الحيوانات في بيت الحيوان بكلية الطب البشري –جامعة الزقازيق، وتم التأكد من حدوث الحمل بمسحه مهبليه يوميه). قسمت هذه االاناث على ٤ مجموعات رئيسية (٢،١ ، ٣، ٤) .تم تقسيم الأجنة بعد الولادة الي ٣ مجموعات (ا، ب، ج) حسب يوم ذبح المولود الذكر (اليوم الأول بعد الولادة واليوم ٢١ بعد الولادة وهو عمر الفطام وعند عمر٣٥ يوم وهو سن البلوغ). كما ظهر أن تعاطي الفيثالات قد تسبب في إحداث تغيرات عدة في التركيب النسيجي للخصية وتتمثل هذه التغيرات في التالي: أظهر الغلاف الخارجي للخصية تعرجات كثيرة مع زيادة أليـاف الكولاجين مصحوبا زيادة اعداد الخلايا الجنسية ثنائية النواة وضمور الحبال والأنابيب المنويه مع زيادة اعداد خلايا ليديج الموجودة بالأنسجة البينية بصورة مرضية. بالاضافة الي تقشر الخلايا المبطنة للأنابيب المنوية وتمركزها في التجاويف مع موت بعضها .وكذا تأخر البلوغ المصاحب باختفاء النطفة الأرومية عند عمر ٣٥ يوم. كما وجد أن تناول السيلينيوم متزامنًا مع الفيثالات قد أدى إلى تحسن في التركيب النسيجي للخصية حيث زادت أحجام الأنابيب والحبال المنوية وانتظمت الطبقات المبطنة بها الي حد ما ، مع ظهور الزوائد القمية للخلايا الدعامية ، وتقلص رد الفعل الايجابي للكاسبس ٣. لذا ينصح بتقليل التعرض للفيثالات والحرص علي وجود بدائل لهذه المادة وعدم استخدامها في صناعة لعب الأطفال والأجهزة الطبية .إضافة إلى ذلك ، يوصى بتناول عنصر السيلينيوم لما له من دور وقائي فعال.