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Impact of DEHP Toxicity on Testis of Rat Leading to Disruption of Testis Histology

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Abstract

Di (2-ethyl hexyl) Phthalate (DEHP) is one of the plasticizers widely used in the manufacturing of plastics to make it flexible and durable. Present study is focussed to observe the deleterious effects of DEHP on male reproductive system of animals. For this, 1000 mg/kg body wt. of DEHP was administered to different groups of male Wistar rat for 2, 4, 6 and 8 weeks. After each interval, rats were sacrificed and histological alterations in testis of rats were observed. On hormonal assay, testosterone level decreased significantly in DEHP exposed groups. The histological structure of the testis was also observed to be disrupted significantly with increasing duration of DEHP exposure. Organisation of seminiferous tubule was found distorted and disoriented showing large gaps between them along with degenerated epithelium. Evident changes in morphology of spermatozoa were seen with gradual loss of head and tail structure. Decrease in the number of Leydig cells and sertoli cells were also found suggesting DEHP as a potent toxicant for male reproductive system.

1. Introduction

In every aspect of our lives, plastic is used in different forms for various purposes and all these forms of plastic release different kinds of chemicals causing wide range of health effects worldwide. Toxic effects caused by plastic and its components are tremendous and estimation of such damages are essential to limit its use. One such chemical is phthalate, which is added to make plastic soft, durable and elastic in various proportions depending on its application. These organic esters of alcohols and fatty acids exist in different forms like DEHP (Di(2-ethylhexyl) phthalate), BBP (Benzyl-butyl phthalate), DBP (Di-n-butyl phthalate), DIDP (Di-isodecyl phthalates), DINP (Di-isononyl phthalates) Phthalates are easily released into the surroundings from the products, as they are not covalently bonded to the polymers[1]. As a consequence, surrounding environmental factors, such as soil, water

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and air are contaminated, which eventually enter into plants, animals and humans via food, water or air directly or indirectly[2]. DEHP is also very common chemical in manufacturing industry of cosmetics, personal care products, paints, varnishes, medical equipment and many more.

The consequences of toxicity induced by phthalate esters on animals or humans range from endocrine disruption, obesity, damaged liver, type 2 diabetes, birth defects, hepatocellular carcinoma and different other types of cancers. However, prominent effects of phthalates can be observed in male reproductive system. It ranges from cryptorchidism, hypospadias TDS, effect on sperm count and quality and even to testicular cancer[3]. Phthalate exposure during prenatal condition has also been observed to result in decreased anogenital distance in infants, undescended testis and antiandrogenic properties[4], including animal[5]. Adverse effects of phthalates can be observed in the form of cryptorchidism, hypospadias, reduced testosterone production and decreased sperm count affecting male reproductive system in animals[6]. Phthalates are reported to adversely affect penile length in singleton foetus in pregnant women[7].

DEHP has been classified as a reproductive toxin (category 1B reprotoxin) by REACH (Registration Evaluation Authorization and Restriction of Chemicals) capable of damaging the fertility of the unborn child. An inverse association of serum concentration of male reproductive hormone testosterone and semen quality has been found with serum levels of DEHP metabolites in men[8]. DEHP is reported to cause long lasting epigenetic silencing of mouse sperm genes involved in the reproductive system and testicular dysgenesis syndrome in case of prenatal exposure[9]. DEHP metabolites are found to interfere with human male foetal gonadal functions causing cryptorchidism, hypospadias and other malformations[10].

Human testis steroidogenesis is also found to be inhibited by MEHP (a metabolite of DEHP) shown in a human foetal testis explant study[11]. Prenatal DEHP exposures are found to be associated with alterations in cord blood adipokine levels and birth size in human[12]. DEHP is a non-genotoxic carcinogen producing cancer through PPAR and other possible mechanism[13]. Parenteral effect of DEHP treatment in mice has been observed as increased cases of infertility, decreased size and histological damage of gonads, increased catabolic and decreased anabolic activities in gonads and decreased chances of pregnancy in both males and females[14,15] demonstrated that MEHP exposure inhibited the expression of claudin-11 and occludin mRNA in sertoli cells of rat suggesting the possible involvement of MEHP in spermatogenic function. DEHP is found to be involved in down regulations of many genes like DDx3y, Vsp9Y, RBM, EGF, FSHR, EGFR and decrease quality and quantity of spermatozoa, when treated neonatally in mice[16]. DEHP has the ability to disrupt the androgen regulated development of male reproductive tract and sexual behaviour during puberty and adulthood due to inutero and lactational exposure in Wistar rats[17]. DEHP is also found to decrease the sperm count of epididymal spermatozoa and enzymes related to sperm maturation in dose dependent manner suggesting its role in infertility[18].

The current study aims to investigate the effects of DEHP on the architecture of testis and its possible role in the toxicity in male Wistar rats. Moreover, the level of testosterone in male rats has also been evaluated in order to correlate with the male reproductive health.

2. Materials and Methods

10-12 weeks old male Wistar rats were selected for the study. These rats were kept in polypropylene cages containing saw dust at the temperature 28 ± 1°C using air conditioning system in controlled light (12 hours light and 12 hours dark). Animals were maintained in ideal conditions as per the ethical guidelines approved by the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) with Registration No. 2088/PO/Re/S/19/CPCSEA, dated December 18, 2019), Government of India and Institutional Animal Ethics Committee (IAEC) of Anugrah Narayan College, Patna.

Five groups of rats were formed each containing five rats. Groups were named A, B, C, D and E, where Group A was the control group, not exposed to DEHP. Group B was treated with DEHP for two weeks, group C for 4 weeks, group D for 6 weeks and group E for 8 weeks. Rats were administered 1000 mg/kg body weight/day of liquid DEHP dissolved in corn oil orally through gavage method according to the above-mentioned periods for each group except for the control groups that were given only corn oil through gavage method. The reason for the selection of

high dose is to observe adverse effects of DEHP on male reproductive system in rats.

- **Body Weight**: Rats were measured for the body weight before and after the DEHP treatment. Testes were measured after dissection and further study was performed.
- **Assessment of Testosterone**: The blood samples from the rats were centrifuged at 3000 rpm for 10 min and the serum was transferred into fresh tubes. Testosterone estimation of these samples was done using ELISA.
- **Histological Study**: Testes were washed with normal saline and kept in 0.85% normal saline for an hour and stored in 10% formalin for 24 hours for fixation and further processing. After performing the steps of dehydration with alcohol and clearing with xylene, impregnation, embedding and blocking was done in melted wax of testis tissue. 5-6 micrometre thin section was cut with the help of microtome and dewaxing of the tissue was done. Permanent slides were prepared by double staining using Haematoxylin and Eosin and examined under the microscope.

3. Results

3.1 Body Weight and Testis Weight

DEHP exposure resulted in significant testicular weight reduction of rats, but their body weight was not affected significantly in comparison to the rats of control group.

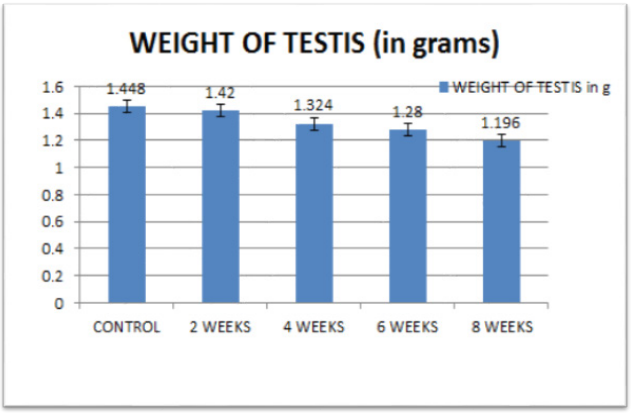


Figure 1. Weight of testis of rats of control and groups exposed with DEHP for 2, 4, 6 and 8 weeks (p value <0.001).

Significant (p<0.001) decrease in the weight of testes was observed in the rats that were exposed to DEHP for 4, 6 and 8 weeks as shown in the Figure 1.

3.2 Serum Testosterone

Value of serum testosterone in DEHP exposed rats showed clear decline with longer period of exposure. Testosterone level in control group A was found to be 2.662 ng/ml (mean value), which reduced to 2.296 ng/ml among rats of group B that were exposed to DEHP for two weeks. But the reduction of testosterone in group C, D and E were quite prominent with testosterone values as 1.626 ng/ml, 1.380 ng/ml and 1.354 ng/ml for rats exposed to DEHP for 4, 6 and 8 weeks respectively as shown in Figure 2.

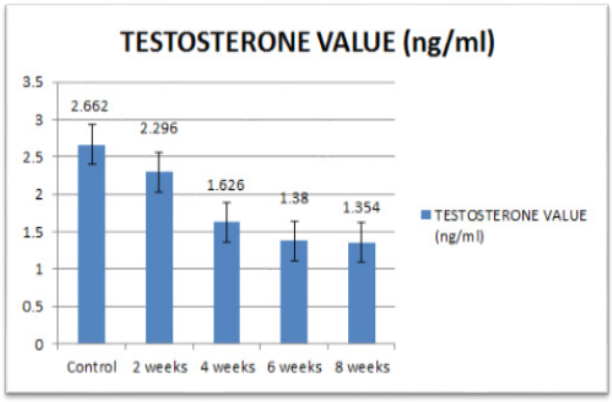
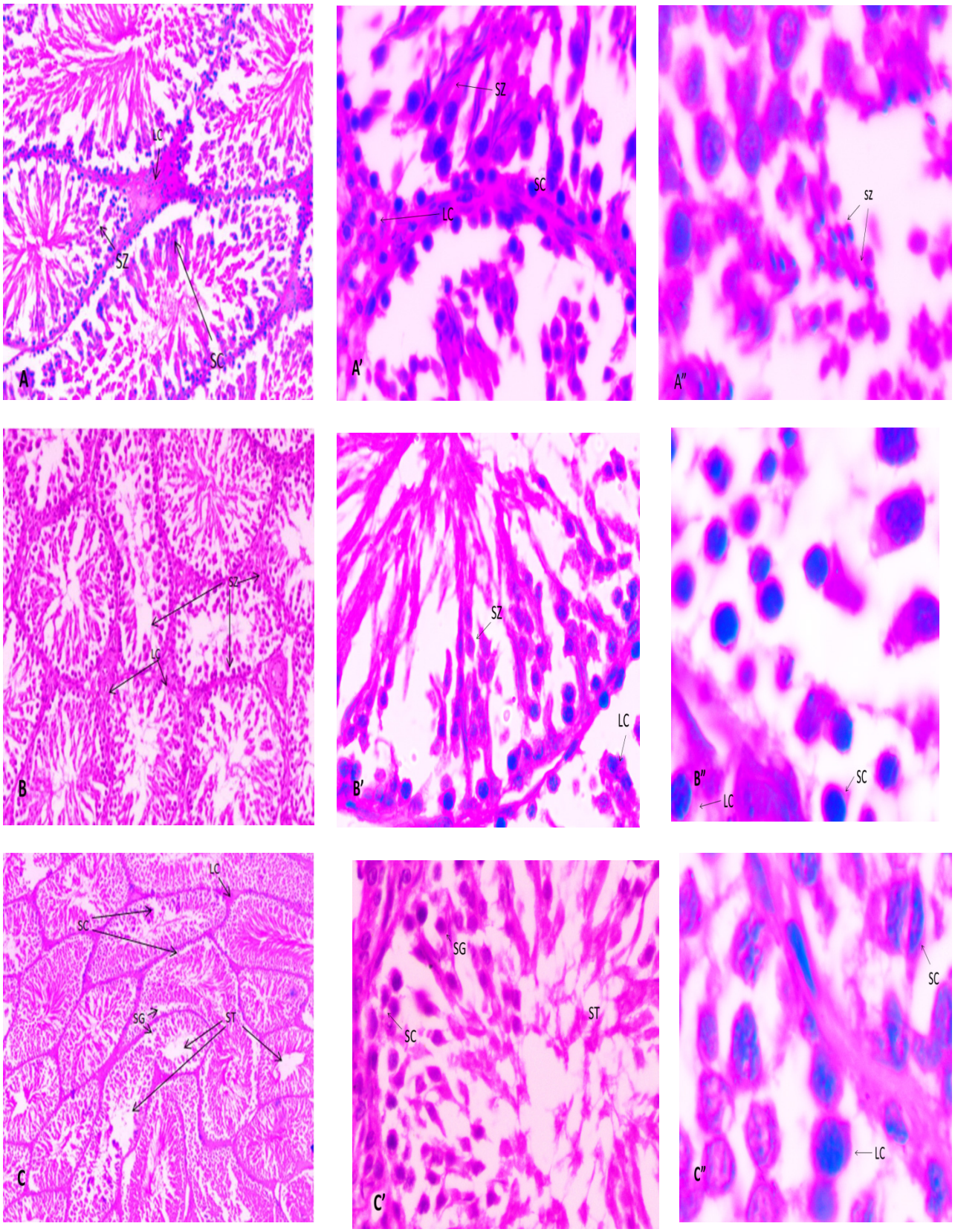


Figure 2. Representation of serum testosterone values of rats of control group and DEHP treated groups for 2, 4, 6, and 8 weeks (p value <0.001)).

3.3 Histological Results

The organisation of germ cells and Leydig cells and seminiferous tubules was absolutely normal in the rats of control group. Bundles of spermatozoa showed normal appearance with head and tail. Arrangement of seminiferous tubules appeared in healthy condition showing progressive stages of spermatogonium development that were clearly distinguishable. (Figure 3(A)).

Section of testis tissue exposed to DEHP for two weeks showed thin and broken seminiferous epithelium and a smaller number of Leydig cells. The morphology of the sperm was also observed to be abnormal. (Figure 3 (B)).


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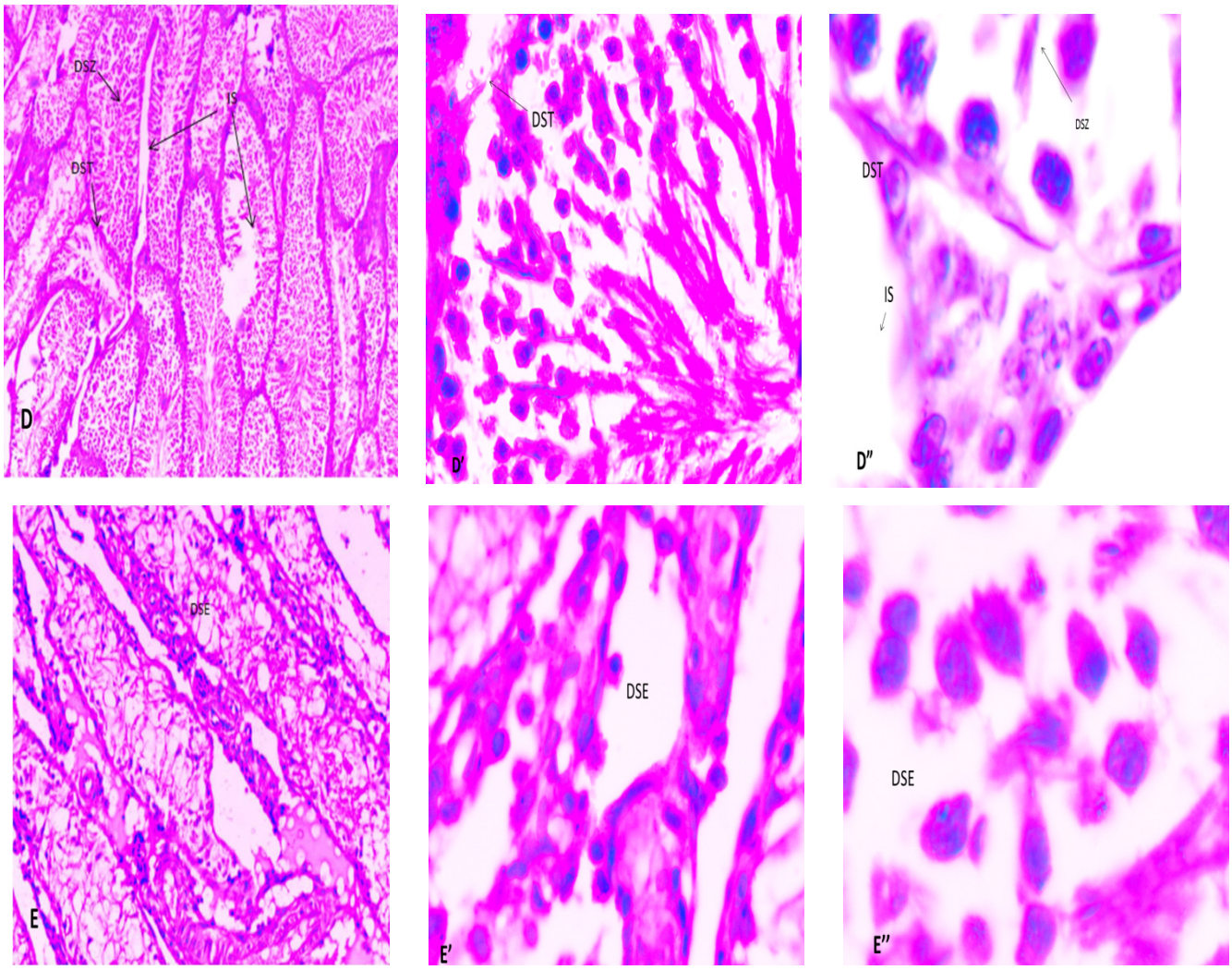


Figure 3. Testis micrograph of control group rat showing normal healthy organisation of seminiferous tubule and placement of germ cells, Leydig Cells (LC), Sertoli Cells (SC), Spermatozoa (SZ) inside, 40X (A), 100X (A') and 400X (A"). Pictomicrograph of testis of 2 weeks treated DEHP administered male rat showing fewer decreased number of Leydig Cells (LC) and abnormal Spermatozoa (SZ), 40X (B), 100X (B') and (B"). Pictomicrograph of testis of 4 weeks treated DEHP administered male rat showing large gaps in between Seminiferous Tubule (ST) their degeneration and loss of Spermatogonial Cells (SG), Sertoli Cells (SC) and Leydig Cells (LS), 40X (C), 100X (C') and 400X (C"). Pictomicrograph of testis of 6 weeks treated DEHP administered male rat showing Damaged Seminiferous Tubule (DST), increased Interstitial Space (IS) and Damaged Spermatozoa (DSZ), 40X (D), 100X (D') and 400X (D"). Pictomicrograph of testis of 8 weeks treated DEHP administered male rat showing disarranged and Degenerated Spermatogenic Epithelium (DSE) layers and cells, 40X (E), 100X (E') and 400X (E").

Rats exposed to DEHP for four weeks showed significant changes in testes histology. Wide gaps were observed in between seminiferous tubules showing gradual degeneration of the epithelium layers. Spermatozoa appearance was also found to be deteriorated and number of Leydig cells decreased. Number of spermatogonia was also found to be reduced in comparison to the control group. Sertoli cells were also reduced and retracted. (Figure 3(C)).

Testis micrograph of rats that were exposed to DEHP for six weeks presented degenerated histology with abnormal spermatozoa without tails. Lesser Leydig cells

were observed in the section. Number of spermatogonia was found very less. Large gaps appeared between the seminiferous tubules. (Figure 3 (D)).

Rats that were exposed to DEHP for 8 weeks, showed complete disruption of seminiferous tubule histology and prominent gaps appeared between these in section of the testis. Disarrangement of testicular spermatogenic epithelium was evident in the micrograph. Complete degeneration of sperm tail was observed, a few sperms with head only were visible. Spermatogonia and sertoli cells were reduced to only a few (Figure 3 (E)).

4. Discussion

DEHP is a widely used plasticizer around the world acting as a major disruptor of male reproductive system. DEHP is a form of phthalate that leaches out from the plastic and enters the living world through air, water or food chain and produce several damaging effects. In this study DEHP exposure showed quite evident effect on the weight of testes of rats.Weight of testes decreased with the increase in duration of DEHP exposure. Reduction of testes weight was observed to be significant in group C, D and E rats that were exposed to DEHP for 4, 6 and 8 weeks respectively (Figure 1).

Weight of testes might be reduced due to disruption of internal structure and function of testes, i.e., disruption of the testes epithelium, shortening of seminiferous tubules, loss in number of Leydig cells, reduction of steroid synthesis in Leydig cells and spermatogenic and cell proliferation arrest inside the testes[14]. These changes altogether could result in deformity and weight reduction of testes, an evident effect of DEHP exposure, which gradually became more significant with the increase of duration of exposure[9].

Gradual reduction in level of serum testosterone is also evident with the increase in duration of DEHP exposure. Reductionof testosterone is a prominent indicator of male infertility, which could be linked with the reduction in the number of Leydig cells, shown in the histopathological micrograph. Leydig cells are involved in the synthesis of male steroid hormone, i.e., testosterone. These results support the cumulative damaging effect of DEHP on male reproductive system causing infertility[19].

Disruption of testis histology due to DEHP exposure is well exhibited in histopathological micrograph of the testes tissue of rats. Degeneration of the shape of spermatozoa, significant reduction of Leydig cells, loss of stages of spermatogonial division, degeneration of seminiferous tubules showing wide gaps in between could be the result of DEHP exposure[20]. The micrograph of testes of rats exposed to DEHP for 6 and 8 weeks showed fusion of some of the seminiferous tubules, a clear indicator that could lead to carcinogenesis[21]. Deformed spermatozoa shown in testes micrograph of DEHP exposed groups could also be linked to infertility as a result of DEHP exposure.

5. Conclusion

As evident from the results, daily exposure of 1000 mg/kg body wt. resulted in clear damage on testicular histology, viz., disorientation and degeneration of testicular epithelium, loss of germ cells, Leydig cells and population and morphology of spermatozoa as well as decreased weight of the testes. Blood level of testosterone was also found to be significantly decreased in all exposed groups. Hence, it can be concluded that DEHP acts as potent male reprotoxin targeting testis and its hormone testosterone. This study could be further elaborated for determining its potency on human population and addressing infertility problems.

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