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Original Research Article

Rescue effects of prenatal melatonin administration against bisphenol A- induced perturbations of reproductive and thyroid activities in male rat offsprings

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ABSTRACT

The current study aimed to investigate the effects of prenatal melatonin “MLT” administration against bisphenol A “BPA”- induced infertility and thyroid dysfunction in male rat offsprings (First generation “F1”). For that purpose, fifty adult albino rats (40 females and 10 males) were used and classified equally into five groups (8 females and 2 males in each group). First group (control group) in which, pregnant rats were injected with 0.3 ml of vehicle /day. The second group (low dose BPA) where rats received a daily dose of 25 µg / kg B.W. The third group (high dose BPA) where rats received a daily dose of 250 µg / kg B.W. Fourth group (low dose BPA + MLT) where rats received a daily dose of 25 µg BPA /kg B.W. plus 10 mg MLT / Kg B.W. The fifth group (high dose BPA + MLT) where the rats received a daily dose of 250 µg BPA / kg B.W. plus 10 mg /Kg B.W. All rats within each group received their specific treatment daily with subcutaneous injection starting from the fourth day of pregnancy till full term. Then, the male offsprings of each group were selected and reared until the 60th day after birth. Serum and tissue samples were collected for analyses and microscopical examination. Although prenatal administration of both BPA doses didn’t affect the body weight gain and testicular weights of male offsprings, they reduced significantly the serum levels of testosterone and triiodotyrosine when compared to the control group. Also, both BPA doses disturb significantly the oxidant/antioxidant ratio. Moreover, prenatal administration of both BPA doses affected negatively semen quality of the produced offsprings and induced marked histological alterations in their testes and prostate. Remarkably, all serological and histological alterations observed after BPA exposure were ameliorated significantly with MLT co-administration. Thus, prenatal MLT administration could be considered an optimal treatment to relieve many reproductive disorders,

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degenerative changes of testes and prostate and thyroid malfunction induced in male offsprings after gestational exposure of their dams to BPA.

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1. Introduction

The term fetal programming refers to the process by which a stimulus or insult, when occurring in the critical period of development, mainly in the intrauterine life, has permanent effects on the structure and functions of the organism in childhood, adolescence and adult life. The intrauterine phase is the stage in which the plasticity of the organism requires stable modulation of gene expression (Lucchese et al., 2017).

The current increase in the prevalence of some common reproductive and metabolic diseases may originate from unintentional exposure during development to endocrine-disrupting chemicals (EDCs), which can adversely influence the developmental trajectory of target tissue differentiation (Heindel and vom Saal, 2009). Low dose exposure during development can result in disruptions that last long after the EDC is gone from the body. (Diamanti-Kandarakis et al., 2009).

Bisphenol A (2, 2-bishydroxyphenyl propane) "BPA" is one of the most common EDCs that affect many physiological functions especially reproduction. BPA is a chemical monomer primarily used to manufacture polycarbonate plastic (e.g, water bottles), epoxy resins (e.g, inside coating in metallic food cans) and the non-polymer additive to other plastics. BPA could be released from wastewater treatment plants, river water, and surface and drinking water. Over the past three decades, the production and use of BPA have increased exponentially, and the exposure of human and animals to BPA becomes so risky and need more attention (Hoepner et al., 2013).

BPA as a potential EDC, its action mainly based on its estrogenic properties. BPA has a high affinity to bind with α - and, to a lesser

extent, β -estrogen receptors (ER) either in vitro or in vivo (Caserta et al., 2011). Moreover, BPA can also inhibit the activity of endogenous estrogens and/or disrupt estrogen nuclear hormone receptor action (Wetherill et al., 2007).

Developmental exposure to 25 μ g/kg B.W. /day bisphenol A can cause adverse effects on male fertility and semen quality (Salian et al., 2009a), alter prostate weights (Timms et al., 2005) and increase the incidence of prostatic intraepithelial neoplasia lesions in the prostate of adult rodents (Prins et al., 2011). It was reported that low-dose BPA decreased testosterone "T" levels in gestationally or neonatally exposed Holtzman rats (Salian et al., 2009a). Further, gestational exposure to low-dose BPA increased gene expression of androgen receptor, *Esr1*, aromatase, and estrogen-related receptor γ in the mouse prostate (Arase et al., 2011).

Melatonin (N-acetyl-5-methoxytryptamine) "MLT", is an endogenous compound has been identified in a wide variety of organisms including bacteria, unicellular eukaryotes, and different plants, as well as in a large number of animals (Maitra et al., 2015). In animals, it is synthesized in various organs such as the pineal gland, retina, intestine, bone marrow cells, and skin. The circulating levels of MLT vary in a daily cycle (Paredes et al., 2009). Recent studies reveal that MLT is present in different tissues and organs such as harderian gland, extraorbital lacrimal gland, retina, gastrointestinal (GI) tract and in bile in human and many vertebrates (Maitra et al., 2015).

MLT is a small soluble indoleamine in both water and lipids and hence, acts as a hydrophilic and hydrophobic antioxidant and has the effect of inhibition of the activity of a pro-oxidative enzyme, stimulation of the

activity of antioxidant enzymes, distribution in all tissues throughout the organism, and rapid diffusion through all biological membranes (Ballas et al., 2006). MLT besides its effect on the synthesis and secretion of the hypothalamic GnRH and the adenohipophyseal gonadotropins, may directly modulate testicular activity. More precisely, MLT by binding with its receptors directly regulate T secretion, increase the responsiveness of Sertoli cells to FSH during testicular development and modulates cellular growth, proliferation, and the secretory activity of several testicular cell types. It inhibits local inflammatory processes and the generation of ROS in the testis to increase the rate of spermatogenesis and fertility (Frungeri et al., 2017). Furthermore, MLT protects the testicular functions and spermatogenesis from many harmful effects of many disorders (Rocha et al., 2015). Additionally, MLT can be considered a protective reprogramming therapy to restore the nitric oxide and reactive oxygen species (NO-ROS) balance in both genetic and developmentally programmed hypertension models (Tain and Joles, 2015).

Material and methods

1.1. Chemicals and reagents

BPA (Lot No., MKBX9458V, $\geq 99\%$) and MLT (Lot No., SLBQ9501V, powder $\geq 98\%$ "TLC") were purchased from Sigma-Aldrich Co., USA and China, respectively.

1.2. Animal exposure and experimental design

Fifty adult albino rats (40 females and 10 males) of 8-10 weeks old and average weighting 140-150 g B.w.t. were used and purchased from laboratory animal center, Beni-Suef University, Egypt.

All animals during the experiment were kept under normal environmental conditions in the lab of the physiology department, Faculty of Veterinary Medicine, Minia University. All animals were left for two weeks for acclimatization and for checking the regularity of the estrus cycle for female rats using the vaginal smear method. All the experiments in the current study were performed in accordance

with protocols and international guidelines for care and use of laboratory animals and approved by the local experimental ethics committee.

All rats were housed in polypropylene cages (BPA and estrogen-free cages) with wood bedding and glass water bottles with rubber stoppers and special rat ration with protein percentage 22% in glass containers. Food and water were available *ad libitum* throughout the study.

The rats were divided equally into five groups, each group included eight adult female albino rats and two adult male albino rats for breeding. The first group (control group; n=8); each rat in this group were injected with 0.3 ml of the vehicle (50% diluted solution of DMSO in PBS) /day. The second group (Low dose BPA; n=8); each rat received a daily dose of 25 μg / kg B.W. while in the third group (High dose BPA; n=8), each rat received a daily dose of 250 μg / kg B.W. (Acevedo et al., 2013). In the fourth group (Low dose BPA + MLT; n=8), each rat received a daily dose of 25 μg BPA /kg B.W. plus 10 mg MLT / Kg B.W. (El-Bakry et al., 2013) while in the fifth group (High dose BPA + MLT; n=8), each one received a daily dose of 250 μg BPA / kg B.W. plus 10 mg /Kg B.W. All rats within each group received their specific treatment in a 0.3 ml dose with subcutaneous injection using fibroglass needle starting from the fourth day of pregnancy till full term.

Each group was reared in 2 separated cages with 4 females in each cage plus one adult male for breeding. The day at which sperm cells within vaginal film be observed, was considered the first day of gestation (GD1).

After delivery, 10 males were selected from the litters of control and both MLT groups while in low and high BPA doses groups, because of their lower pregnancy rates, 6 males were selected. All selected rats were weighted (Initial weight) and reared under the same environmental conditions till the 60th day after birth (i.e. after the age of puberty). Then, on the sixtieth day, the adult male offsprings of each

group were weighted (Final weight), subjected to blood collection and were sacrificed and prepared for tissue samples collection.

Body weight gain

The rats were weighed after birth and then on the sixtieth day after birth using digital balance and the bodyweight gain for each rat was recorded.

1.3.Preparation of serum

At the end of the experiment, blood samples were drawn from the retro-orbital venous plexus according to the method of Sorg and Buckner (1964). Serum samples were obtained and kept at -20°C till use.

1.4. Handling of tissue samples for microscopical examination

On the sixtieth day after birth and after blood collection, the male offsprings were sacrificed and testis and prostate glands of each animal were quickly removed and rapidly weighed and then a part was taken and fixed using Bouin's fixative. Then, the organs were routinely processed and sectioned at 4–5 mm thickness. The obtained tissue sections were collected on glass slides, deparaffinized and stained with hematoxylin and eosin stain (Bancroft and Marilyn, 2008). The sections are then examined and observed under a light microscope at 100, 200, 400 and 1000 \times magnification.

Serum analyses

The collected serum samples were used to evaluate the levels of T and triiodotyrosine (T3) using competitive enzyme immunoassay (TYPE7 &5) according to Tietz (1995). Also, malondialdehyde (MDA) and total antioxidant activities (TAC) were analyzed using commercial kits (Sigma-Aldrich, USA) according to Valko et al. (2006) and Qiao et al. (2016).

1.5. Epididymal semen collection and evaluation

Semen samples were collected on clean glass slide by maceration of the tail of the epididymis using a sterile scalpel. Aliquots of sperm suspension were diluted with fresh saline,

and sperm concentration was determined using a Neubauer chamber (Aydogan and Barlas, 2006). For sperm abnormalities, semen samples were stained and percentages of abnormal sperms were calculated according to a previous protocol (Aydogan and Barlas, 2006).

Statistical analysis

The data were expressed as means \pm standard error of the mean ($M \pm SE$). All variables were tested for normal distribution and compared using the independent t-test. All analyses were performed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons using the Statistical Package for Social Science (SPSS) software in a PC-compatible computer (SPSS for Windows, version 20, USA) and the significance was set at $p < 0.05$.

2. Results

2.1. Effects of bisphenol A and melatonin administration for pregnant rats on body weight gain and testicular weight of their male offsprings (F1) on the sixtieth day after birth

Table 1 clarified that neither small nor large doses of BPA had negative effects ($P > 0.05$) on body weight gain and testicular weights of male offsprings after reaching their adult age when compared to control group. Also, Bodyweight gain and testicular weight were observed unchanged ($P > 0.05$) with MLT co-administration.

Effects of bisphenol A and melatonin administration for pregnant rats on serum testosterone and T3 levels in male offsprings

The results illustrated in Table 2 revealed that, prenatal exposure for 25 μg and 250 μg BPA reduced the serum levels of T of male neonates (F1) on the sixtieth day after birth in comparison to control group ($P < 0.05$). However, prenatal MLT co-administration was found to have positive effects against BPA exposure as it convalesced significantly the lower levels of T toward control values ($P < 0.05$).

Concerning the effect of MLT and BPA on serum level of T3 as shown in Table 2, it was clear that serum levels of T3 were decreased significantly ($P < 0.05$) with BPA 25 μg but not with BPA 250 μg administration in comparison to control group. On the other side, BPA 250 μg + MLT treatment achieved a significant increase of the activity of the thyroid gland as they increased significantly the serum levels of T3 when compared to either 250 μg BPA or control groups ($P < 0.05$). However, BPA 25 μg + MLT enhanced significantly ($P < 0.05$) the serum level of T3 greater than 25 μg BPA group and amended it to the control values.

2.2. Effects of bisphenol A and melatonin administration for pregnant rats on serum levels of total antioxidant capacity and malondialdehyde in male offsprings

It was clear from Table 3 that, serum level of TAC was reduced significantly whereas that of MDA was elevated markedly in both small and high BPA groups when compared to the control one ($P < 0.05$).

However, MLT co-administration with a small dose of BPA adjusted the oxidant / antioxidant ratio significantly ($P < 0.05$) and restored it to be near the control values. Also, the oxidative stress provoked by BPA 250 μg administration was ameliorated impressively after MLT co-administration ($P < 0.05$) but wasn't restored to control levels.

Effects of bisphenol A and melatonin administration for pregnant rats on epididymal semen quality of male offsprings

Table 4 clarified that sperm concentration was reduced significantly in 25 μg BPA -exposed group while it is reduced slightly in 250 μg BPA group when compared to control one. The improving action of MLT was obvious when co-administered with both doses of BPA as it induced a significant increase in sperm concentration in comparison to either control or both BPA groups.

By observing sperm morphology microscopically, it was noticed that prenatal exposure for low and high doses of BPA distorted the sperm cell morphology to a significant degree through increasing the percentage of second degree of abnormalities in comparison to the control group ($P < 0.05$). Also, these deformities of sperm cells were soundly recovered by MLT co-administration in relation to BPA -exposed groups ($P < 0.05$). Nevertheless, the percentages of primary abnormalities were kept unchanged among all treatments.

Effects of melatonin and bisphenol A on histopathological findings in testes and prostate glands

The microscopical findings of the current study (Fig. 1 -10) revealed that BPA at both doses showed degenerative changes in the testes as they induced degeneration of seminiferous tubules with disintegrated spermatogenic cells and Sertoli cells. Also, the sperms in the lumen of the tubules disappeared and interstitial tissue appeared with congested blood capillaries and inactive interstitial Leydig cells. Also, they induced collapse in the majority of prostatic acini and prostate glands were lined with low columnar epithelium with few secretory activities.

Interestingly, all of the degenerative changes happened with both BPA doses were recovered greatly with MLT co-administration as it showed normal seminiferous tubules lined with normal spermatogenic cells and Sertoli cells. The sperms in the lumen of the tubules increased and interstitial tissue contained uncongested blood capillaries and active interstitial Leydig cells. Also, normal prostatic acini lined with simple columnar epithelium in different stages of secretory activity were obvious with MLT co-administration.

Table 1. Effects of bisphenol A and melatonin administration for pregnant rats on body weight gain of their male offsprings (F1) on the sixtieth day after birth. (Mean \pm SE)

Groups	Body weight gain (g)	Testicular weight (g)
Control	201.40 \pm 5.82 ^a	1.20 \pm 0.09 ^a
25 μ g BPA	208.13 \pm 13.77 ^a	1.34 \pm 0.11 ^a
25 μ g BPA + MLT	210.15 \pm 11.78 ^a	1.25 \pm 0.03 ^a
250 μ g BPA	182.60 \pm 8.56 ^a	1.14 \pm 0.04 ^a
250 μ g BPA + MLT	206.93 \pm 9.18 ^a	1.10 \pm 0.07 ^a

SE: Standard error.

In each column, values with a similar small superscript letter(s) didn't show a significant difference from each other ($P > 0.05$).

Table 2. Effects of bisphenol A and melatonin administration for pregnant rats on serum levels of testosterone and triiodothyronine of their male offsprings (F1) at 60 days after birth (Mean ± SE)

Groups	Testosterone (ng/ml)	T₃ (ng/ml)
Control	2.76 ± 0.25 ^a	69.13 ± 0.64 ^a
25 µg BPA	1.28 ± 0.19 ^{bc}	60.30 ± 0.88 ^b
25 µg BPA + MLT	1.87 ± 0.34 ^{ab}	71.87 ± 0.50 ^{ac}
250 µg BPA	0.51 ± 0.21 ^c	68.73 ± 1.48 ^a
250 µg BPA + MLT	2.44 ± 0.49 ^{ab}	72.80 ± 0.44 ^c

SE: Standard error.

Values with a different superscript small superscript letter (s), show a significant difference from each other (P < 0.05).

Table 3. Effects of bisphenol A and melatonin administration for pregnant rats on serum levels of total antioxidant capacity and malondialdehyde of their male offsprings (F1) on the sixtieth day after birth. (Mean \pm SE)

Groups	Total antioxidant (mM /L)	Malonaldehyde (nmol / ml)	Oxidant antioxidant ratio
Control	3.18 \pm 0.04 ^a	1.35 \pm 0.07 ^a	0.42 \pm 0.02 ^a
25 μ g BPA	2.33 \pm 0.12 ^b	1.88 \pm 0.16 ^b	0.81 \pm 0.07 ^b
25 μ g BPA + MLT	2.61 \pm 0.09 ^c	1.68 \pm 0.13 ^{ab}	0.64 \pm 0.04 ^{ab}
250 μ g BPA	1.76 \pm 0.069 ^d	3.45 \pm 0.13 ^c	1.70 \pm 0.10 ^c
250 μ g BPA + MLT	1.80 \pm 0.06 ^d	2.62 \pm 0.06 ^d	1.47 \pm 0.04 ^d

SE: Standard error.

In the same column, values with a different small superscript letter(s), show a significant difference from each other (P < 0.05).

Table 4. Effects of bisphenol A and melatonin administration for pregnant rats on epididymal sperm quality of their male offsprings (F1) on the sixtieth day after birth (Mean \pm SE)

Groups	Sperm concentration/ $\times 10^6$ / ml	Primary abnormality %	Secondary abnormality/100 sperm
Control	85.50 \pm 1.06 ^a	2.00 \pm 0.26 ^a	24.33 \pm 3.04 ^a
25 μ g BPA	43.67 \pm 13.62 ^b	3.67 \pm 0.92 ^a	42.17 \pm 0.91 ^b
25 μ g BPA + MLT	126.50 \pm 10.44 ^c	1.67 \pm 0.42 ^a	27.33 \pm 2.04 ^a
250 μ g BPA	63.67 \pm 12.26 ^{ab}	2.50 \pm 0.22 ^a	59.50 \pm 1.75 ^c
250 μ g BPA + MLT	130.33 \pm 4.86 ^c	2.33 \pm 0.56 ^a	25.83 \pm 2.66 ^a

SE: Standard error.

In the same column, values with a different small superscript letter (s), show a significant difference from each other (P < 0.05).



Fig. 1 A photomicrograph of testis in adult male albino rat of the control group showing seminiferous tubules lined with spermatogenic cells (C) and Sertoli cells. A huge amount of sperms located in the lumen of the tubules (S). Note, an interstitial tissue containing blood capillaries (V) and interstitial cells of Leydig (arrow). (H&E) stain X200.

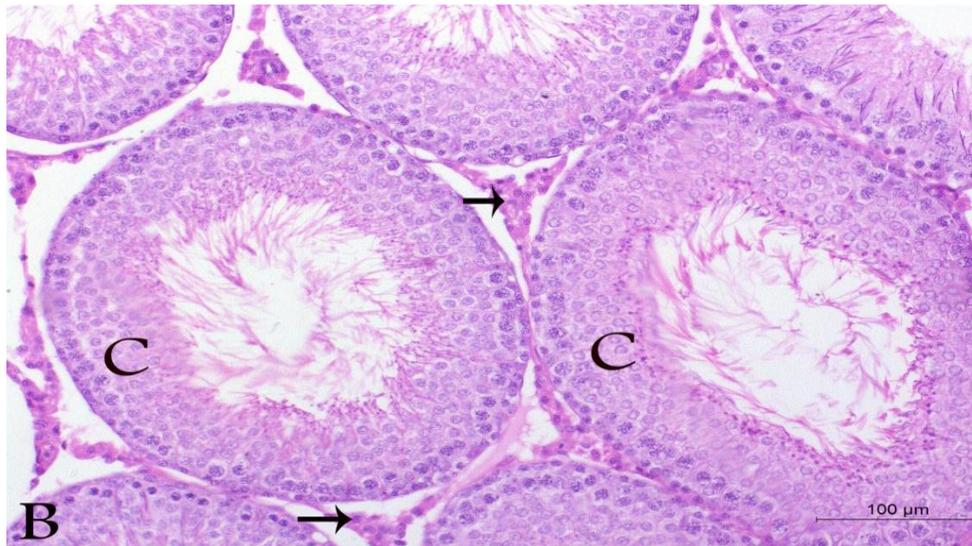


Fig. 2 A photomicrograph of testis in adult male albino rat of 25µ Bisphenol A group showing seminiferous tubules lined with spermatogenic cells (C) and Sertoli cells. The sperms in the lumen of the tubules were reduced. Note, an interstitial tissue containing blood capillaries and less active interstitial cells of Leydig (arrow). (H&E) stain X200.

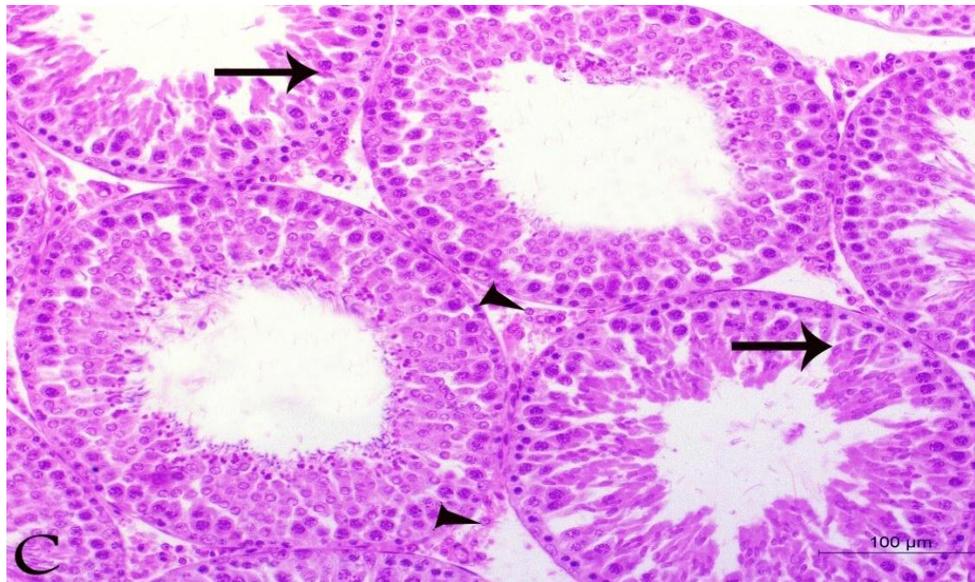


Fig. 3 A photomicrograph of testis in adult male albino rats of 250µ Bisphenol A group showing seminiferous tubules lined with spermatogenic cells and Sertoli cells suffering from degeneration (arrow). The sperms in the lumen of the tubules disappeared. Note, an interstitial tissue containing inactive interstitial cells of Leydig (arrowhead). (H&E) stain X200.

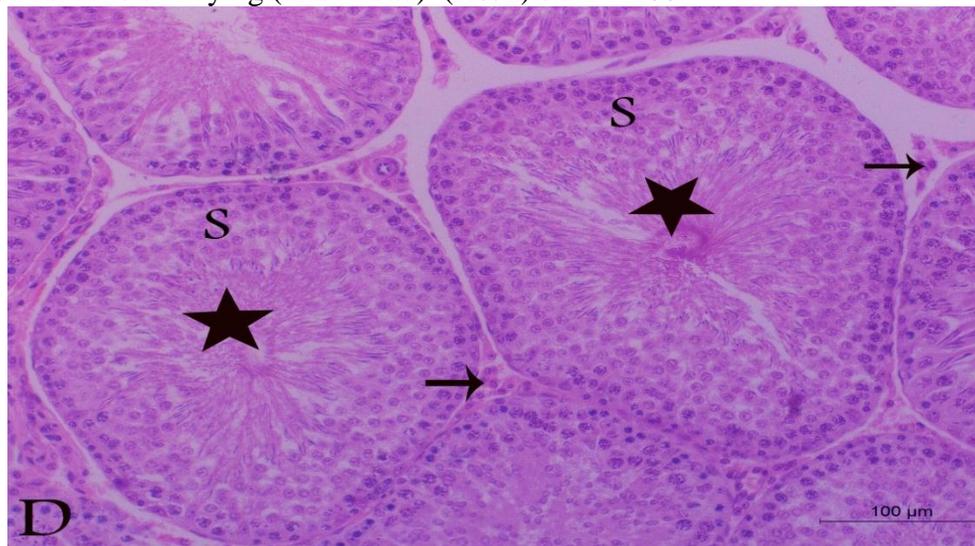


Fig. 4 A photomicrograph of testis in adult male albino rats of 25µ Bisphenol A+ Melatonin group showing normal seminiferous tubules lined with normal spermatogenic cells (C) and Sertoli cells. The sperms (Star) in the lumen of the tubules increased. Note, an interstitial tissue containing blood capillaries and active interstitial cells of Leydig (arrow). (H&E) stain X200.

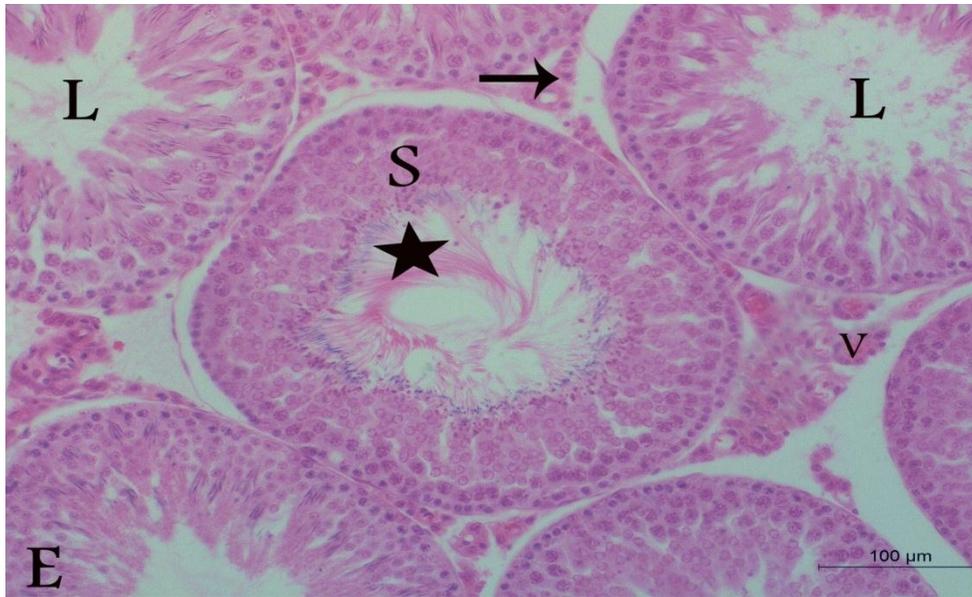


Fig. 5 A photomicrograph of testis in adult male albino rats of 250µ Bisphenol A+ Melatonin group showing normal seminiferous tubules (S) lined with spermatogenic cells and Sertoli cells with sperms (star) in the lumen of the tubules. The other tubules (L) lined with degenerated spermatogenic cells and free from any sperms. Note, an interstitial tissue containing blood capillaries (v) and less active interstitial cells of Leydig (arrow). (H&E) stain X200.

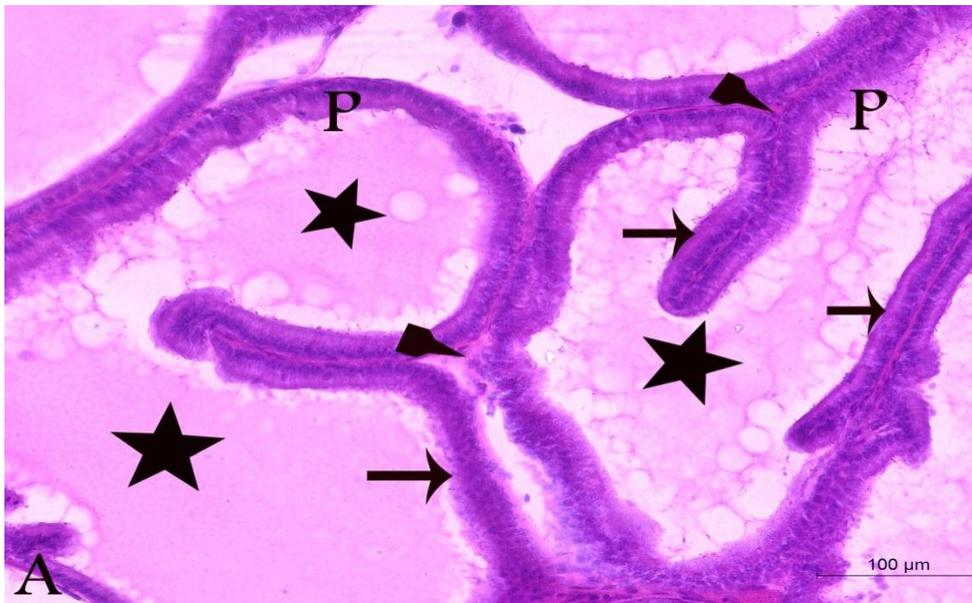


Fig. 6 A photomicrograph of the prostate gland in the adult male albino rat of the control group showing normal prostatic acini (P) lined with simple columnar epithelium (arrow) in different stages of secretory activity. The acini were separated by very thin connective tissue stroma (arrowhead). Note, the lumen of acini expanded containing huge amount of secretory materials (star). (H&E) stain X200.

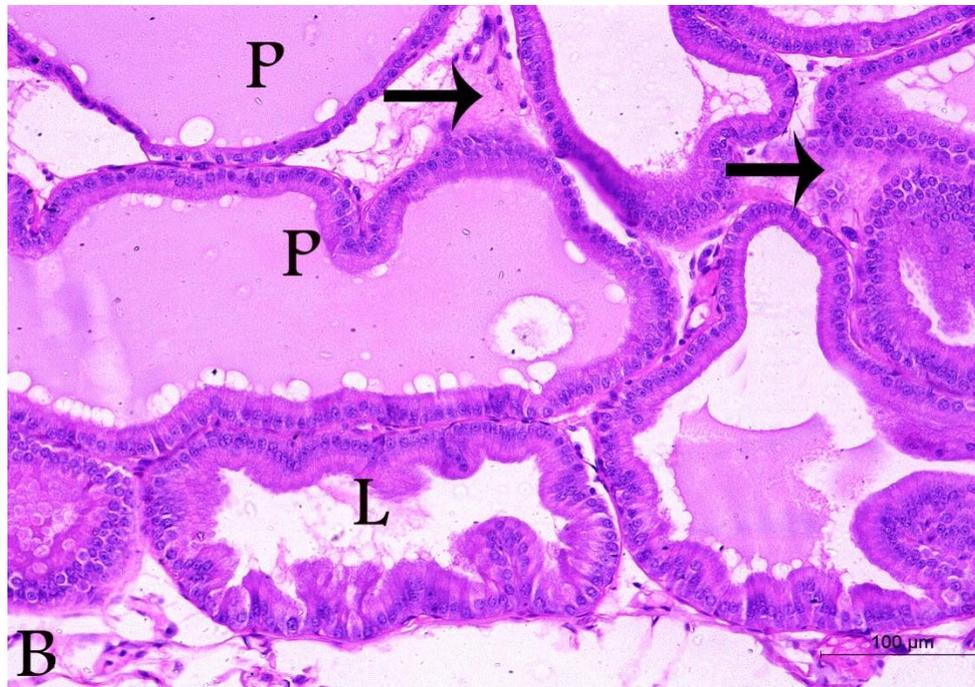


Fig. 7 A photomicrograph of the prostate gland in adult male albino rats of 25μ Bisphenol A group showing some prostatic acini (P) lined with simple columnar epithelium in different stages of secretory activity containing secretory materials. The others (L) appeared collapsed and inactive separated by thick connective tissue stroma (arrow). (H&E) stain X200.

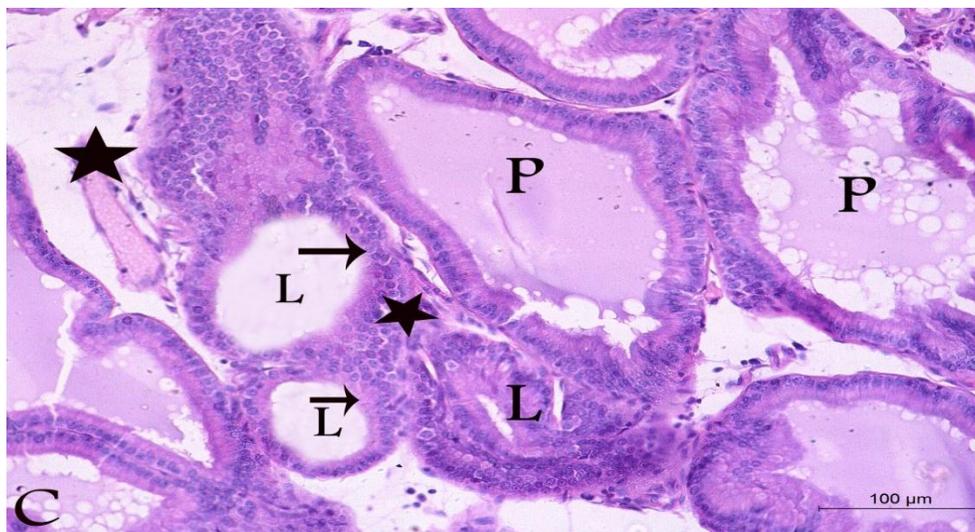


Fig. 8 A photomicrograph of the prostate gland in adult male albino rats of 250μ Bisphenol A group showing the majority of prostatic acini (L) appeared collapsed and inactive separated by thick connective tissue stroma (star). It was lined with low columnar epithelium with few secretory activity (arrow). Note, few prostatic acini (P) appeared active and secretory (H&E) stain X200.

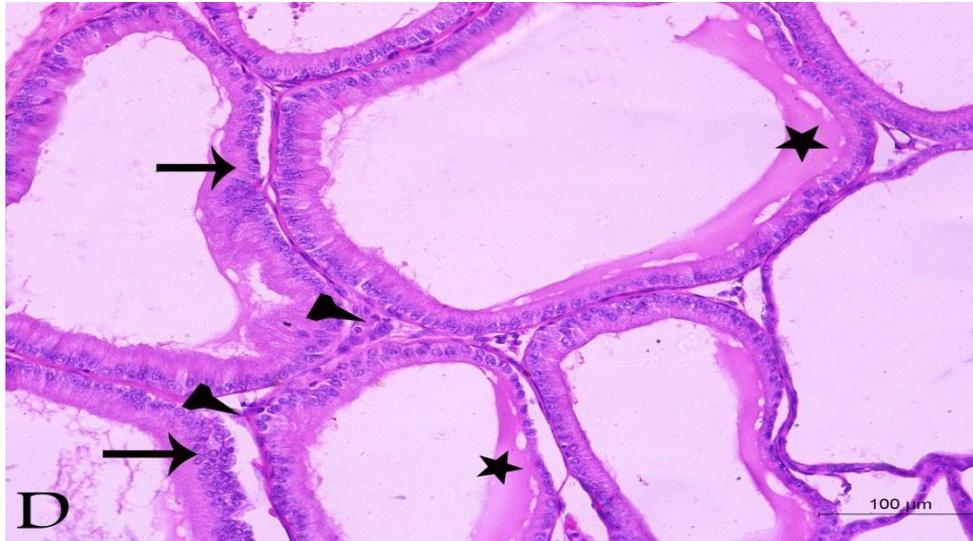


Fig. 9 A photomicrograph of the prostate gland in adult male albino rats of 25 μ Bisphenol A+ Melatonin group showing normal prostatic acini lined with simple columnar epithelium in different stages of secretory activity (arrow). The acini were separated by very thin connective tissue stroma (arrowhead). Note, the lumen of acini contain few secretory materials (star). (H&E) stain X200.

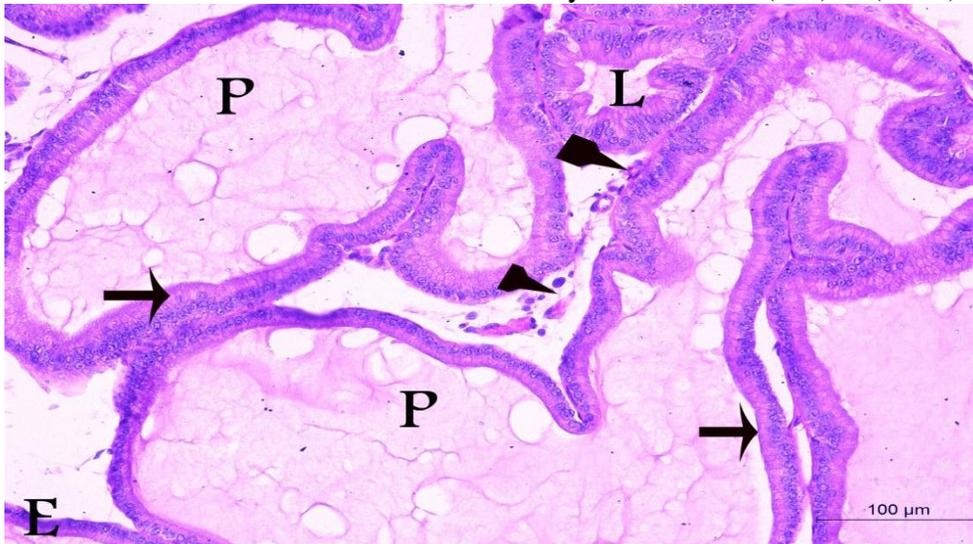


Fig. 10 A photomicrograph of the prostate gland in adult male albino rat of 250 μ Bisphenol A+ Melatonin group showing that the most of prostatic (P) acini were lined with columnar epithelium with secretory activity (arrow). The others remained collapsed and inactive (L). The acini were separated by thin connective tissue stroma (arrowhead). (H&E) stain X200.

3. Discussion

Bisphenol A is a potent EDC and induced many complications in the fertility of animals and human especially if it is exposed during gestation. Thus, this study was conducted to investigate the effects of prenatal MLT

administration against BPA- induced impairment of fertility as well as thyroid activities of male rat offsprings (F1).

It was clear that neither small nor large doses of BPA had negative effects ($P>0.05$) on body weight gain of male offsprings (Table 1)

after reaching their adult age when compared to control group. Also, bodyweight gain was observed unchanged ($P>0.05$) with MLT co-administration. This met agreement with the previous study of Kobayashi et al. (2002) who reported that in utero and lactational exposure of rats to various doses of BPA didn't induce any significant changes in body weight gain compared to the vehicle-exposed control. A study of Kwon et al. (2000) clarified that oral administration of BPA at high doses had no effects on the bodyweight of rats. In fact, the discrepancy in the findings of body weight changes remain enigmatic and could be due to many factors such as differences in the sensitivity of the strain used, dose, route of exposure, window of exposure (age) and duration of exposure (Mendoza-Rodriguez et al., 2011).

Also, the findings in Table 1 displayed that neither small nor high doses of BPA had significant effects on the testicular weights when compared with control values. Additionally, MLT co-administration didn't induce any significant effects on testicular weights. This met agreement with the previous study of Kobayashi et al. (2002) who reported that in utero exposure of rats to various doses of BPA didn't induce any significant changes in testes weight compared to the control group. Also, rats when were given BPA at 0.2, 2, 20 or 200 $\mu\text{g}/\text{kg}/\text{day}$ by gastric intubation throughout the study beginning at the onset of a 10- and 2-week pre-mating period, in F0 males and females, respectively, and continuing through the mating, gestation, and lactation periods, for two generations. They observed no changes in main organ weights including testes under their conditions (Ema et al., 2001).

Prenatal exposure for both BPA doses reduced the serum levels of T of male offspring (F1) in comparison to control group ($P<0.05$). This met agreement with a study of Quan et al. (2016) who reported that gestational exposure of rats to different doses of BPA induced a significant reduction in serum T level of male

offspring compared with the control group and the reduction level run parallel with increasing the doses. Besides, low-dose BPA decreased T levels in gestationally or neonatally exposed Holtzman rats (Salian et al. 2009a, 2009b), adult-exposed albino rats (El Beshbishy et al., 2012) and adult exposed Wistar rats (D'Cruz et al., 2012). These adverse effects could be explained by studies of Horstman et al. (2012) and Qiu et al. (2013) who reported that BPA decreases the expression of steroidogenic enzymes. Also, previous reports proved that perinatal and postnatal BPA exposure disrupting the hypothalamic-pituitary-testicular axis of male animals and consequently affecting the hormone secretion (Wisniewski et al., 2015). Moreover, the reduced level of T has been suggested as being caused by the oxidative damage of BPA to the Leydig cell population (Nakamura et al., 2010). Furthermore, the exposure to BPA or its fluorinated derivative resulted in a dramatic decline in genes and protein involved in cholesterol biosynthesis, transport, and steroid biosynthesis (Feng et al., 2012) and caused a failure of spermatogenesis (Qiu et al., 2013).

However, prenatal MLT co-administration was found to have positive effects against BPA exposure as it convalesced significantly the lower levels of T toward control values ($P<0.05$). This coincided with the study of Othman et al. (2016) who documented that MLT treatment along with BPA significantly maintained T levels near the control values compared with the BPA-treated group. The protective effects of MLT could be referred to its known action on the hypothalamic-pituitary-gonadal (HPG) axis, resulting in modification of sex hormone production, including estrogen, T, FSH, and LH (Didolkar et al., 1980). Moreover, keeping the T level by MLT against BPA-induced oxidative stress is due to the strong antioxidant properties of MLT as it activates antioxidant enzymes including superoxide dismutase, glutathione

reductase and glutathione peroxidase (Reiter et al., 2000).

In the current study, it was clear that serum levels of T3 were decreased significantly ($P < 0.05$) with BPA 25 μg but not with BPA 250 μg administration in comparison to the control group (Table 2). In this respect, BPA may act as an agonist or antagonist of the thyroid hormone receptor because of its structural similarity to thyroid hormone. Furthermore, BPA was observed to distribute rapidly in fetuses via placental transfer after a single BPA administration to pregnant female rats (Takahashi et al., 2000) as the placental barrier cannot block BPA transfer. Furthermore, BPA was shown to suppress T3-stimulated transcriptional activity (Moriyama et al., 2002). All of the previous studies confirmed and explained why serum T3 levels were dramatically decreased with gestational BPA exposure.

On the other side, BPA 250 μg + MLT treatment achieved marked improvement of the activity of the thyroid gland as they increased significantly the serum levels of T3 when compared to either 250 μg BPA or control groups ($P < 0.05$). However, BPA 25 μg + MLT enhanced significantly the serum levels of T3 greater than 25 μg BPA group and amended it to the control values. In this regard, Garcia –Marin et al. (2015) showed evidence for the involvement of MLT in thyroid function by directly-regulating thyroglobulin gene expression in follicular cells. In juvenile and adult mammals, MLT controls reproductive endocrine function through effects on the pars tuberalis (PT) of the anterior pituitary gland. This tissue contains MLT1 receptor-expressing thyrotrophic endocrine cells (Garcia-Marín et al., 2012), which produce thyroid-stimulating hormone (TSH) (Chiamolera et al., 2009). TSH produced by the PT acts on ependymal cells in the neighboring basal hypothalamus, known as tanycytes, which express TSH receptors (TSHRs), and in turn regulate local thyroid hormone (TH) levels (Chiamolera et al., 2009).

Thus, MLT could be considered a useful treatment to maintain the secretory activity of the thyroid gland against the undesirable effects of BPA.

Serum levels of TAC were reduced significantly whereas that of MDA was elevated markedly in both BPA groups in male offsprings (Table 3) when compared to the control group ($P < 0.05$). In this concern, many studies have shown that BPA can induce oxidative stress and reproductive toxicity in testes (Anjum et al., 2011). Kabuto et al. (2004) also reported that perinatal BPA exposure increased oxidative injury and caused underdevelopment of the testes in mice. These results indicated that although partly metabolized and eliminated by the mothers, BPA still can induce oxidative stress in offspring, because it can cross the placental barrier readily and accumulate both in the placenta and the fetus (Avissar-Whiting et al., 2010). Quan et al. (2016) proved that feeding pregnant rats BPA at doses ranging from 1 to 100 mg/kg b.w.t. during gestation days 14–21 resulted in significant elevation of superoxide production and MDA contents in the medium- and high-dose groups ($P < 0.05$).

However, MLT co-administration with both BPA doses adjusted the oxidant / antioxidant ratio significantly ($P < 0.05$). This comes in accordance with a study of Othman et al. (2016) who found that treatment with MLT and BPA concurrently resulted in a significant decrease in the MDA and H₂O₂ levels in the testes and sperm compared with the BPA-treated rats. MLT is attracting increased attention in recent years due to its known ability to reduce oxidative stress (Othman et al., 2008), with negligible toxicity even in very high doses (Reiter et al., 2013). MLT is not only an effective hydroxyl radical scavenger (Reiter et al., 2004), but also can detoxify other ROS and reactive nitrogen species as well as their metabolites (peroxynitrous acid and intermediates H₂O₂) (Tan et al., 2002). Moreover, MLT enhanced the antioxidant

potential of the cell by the upregulation of several antioxidant enzymes (Pandi-Perumal et al., 2013).

Epididymal semen quality was reduced significantly in BPA - exposed groups whereas MLT co-administration improved them greatly. In this regard, Quan et al. (2016) found that prenatal BPA exposure can cause endocrine disruption and oxidative stress in male offspring, leading to inhibition of spermatogenesis by suppressing the Akt/mTOR pathway and activating the mitochondrial apoptosis pathway. Moreover, BPA was suggested to have adverse effects on spermatogenesis in the adult following either prenatal or early postnatal exposure (Richter et al. 2007). Gestational exposure to low-dose BPA resulted in a decreased number of elongated spermatids present in seminiferous tubules in pubertal ICR mice (Okada and Kai, 2008) and decreased sperm counts in Holtzman rats (Salian et al. 2009a). Similarly, both low- and high-dose BPA exposure during early postnatal development or around the time of puberty increased apoptosis and/or decreased spermatogenesis in male mice and rats (Liu et al. 2013; Qiu et al. 2013). Besides, many studies using various routes of exposure (oral and subcutaneous) and different exposure times (embryonic, fetal, perinatal, and adult) have reported that low dose BPA impairs sperm motility in rats and mice (Tiwari and Vanage, 2013). These harmful effects on semen quality could be attributed to the oxidative stress induced by gestational exposure of BPA (Quan et al., 2016).

In contrast, when MLT was administered concurrently with BPA, it significantly ameliorated the effects of BPA on the percentages of ploidy levels in the sperm (Othman et al., 2016). Recently, Zhang et al. (2018) reported that in vitro exposure of prepuberal mouse testes to two well-known endocrine disruptors (EDs), BPA or diethylhexyl phthalate (DEHP), impairs spermatogenesis with perturbing self-renewal,

spermatogonia activity and meiosis. On the other side, MLT was found to protect the testis from the negative ED impacts with preserving spermatogonia stem and meiotic cells, along with maintaining normal H3K9 di-methylation in these cells. Evidence showed that MLT can pass through blood-testis barrier (Guneli et al., 2008) and is present in the testis which also expresses MLT receptors (Frungeri et al., 2017). Several publications indicated that MLT is potentially a key local player in the regulation of testicular steroidogenesis and spermatogenesis (Yang et al., 2014; Moayeri et al., 2018). Interestingly, recent findings show that the action of MLT converges on epigenetic modification of DNA and histones (Mayo et al., 2017). Further, MLT is a potent antioxidant and can protect testis and enhance spermatogenesis against BPA-induced oxidative stress (Othman et al., 2016). Taken together, the previous studies established that BPA could adversely affect spermatogenesis and perturb crucial epigenetic activities in male germ cells and proved the useful roles of MLT to protect spermatogenesis and accordingly to improve semen quality.

The microscopical findings in the current study were parallel with the serological findings and revealed that BPA at both doses showed degenerative changes in the testes and prostate glands which recovered markedly with MLT co-administration. In this regard, gestational exposure to low-dose BPA resulted in a decreased number of elongated spermatids present in seminiferous tubules in pubertal ICR mice (Okada and Kai, 2008). The testes of rats treated with BPA for 3weeks displayed vacuolization, sloughing, and reduction of spermatogenic cells. Also, the testes of 6-week BPA-treated rats displayed extensive histopathological alterations including atrophy with significant loss of spermatogenesis in most of the seminiferous tubules, marked vacuolization degeneration, sloughing, and reduction of spermatogenic cells. Moreover, interstitial hemorrhage, vacuolated, degenerated,

and poorly developed Leydig cells were noticed. The administration of MLT concurrently with BPA for 3 and 6 weeks markedly ameliorated BPA-induced histopathological effects (Othman et al., 2016). Also, prenatal exposure to BPA may affect the development of prostate cancer in later life Timms et al. (2005). The degenerative effects of BPA on testes and prostate glands could be attributed to the oxidative stress induced by BPA and the ameliorative effects of MLT might be due to its potent antioxidant properties.

5- Conclusion

Thus, MLT might be considered an optimal treatment that can relieve degenerative changes of testes and many reproductive disorders as well as thyroid malfunction induced in male offsprings after gestational exposure of their dams to BPA.

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