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Research Article
Protective Effect of Nano-selenium and Ionized Selenium Against the Testicular Damage, Endocrine Disruptor and Testicular Ultrastructure of Bisphenol A in Albino Male Rats

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Abstract

Objective: The current study was conducted to investigate the protective effect of selenium preparations against the reproductive toxicity of bisphenol A. **Materials and Methods:** Sixty adult Albino male rats (aged from 2-3 months and average weight 150-180 g) were used and they were randomly divided into sex equal groups. Group I served as control, group II were orally administered BPA in a dose level of 1/10 LD_{50} (150 mg kg^{-1} b.wt.) for 8 successive weeks, group III were gavaged with BPA plus ionized selenium in a dose level of (3 mg kg^{-1} b.wt.), group IV animals in this group orally given BPA plus nano-selenium particles in a dose level of (2 mg kg^{-1} b.wt.) and groups V and VI were gavaged with both IS and NSP with previous doses. All data were analyzed using one way analysis of variance (One way ANOVA) followed by Duncan test using SPSS version 16.0 statistical software. **Results:** The obtained results revealed that exposure to BPA significantly induce testicular damage observed in testicular weight, sperm morphology and significant decrease in testosterone level and increase in percent of sperm abnormalities. Ultrastructural examination of testis revealed that both selenium preparations reduced the testicular damages induced by BPA. **Conclusion:** High concentrations of Se in testis and epididymes indicate its importance for the processes of production and maturation of spermatozoa. Nano-Se has lower toxicity and possesses equal efficacy in increasing the activities of selenoenzymes. It can serve as an antioxidant with reduced risk of Se toxicity and a potential chemopreventive agent if the induction of GST by Se is a crucial mechanism for its chemopreventive effect.

Key words: Nano- selenium, ionized selenium, antioxidant, bisphenol A, reproductive toxicity, spermatogenesis, sperm morphology, testis

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bisphenol A (BPA) is an industrial compound that has generated a great deal of concern on scientists and the part of regulatory agencies due to its widespread use. Bisphenol A, one of the environmental contaminants that used in the manufacture of plastics and other products is released largely into the environment as it is widely used in preparation of a wide range of products, including plastic bottles, medical equipment and food-storage containers[1]. Bisphenol A has been reported to have estrogen-like action[2]. The relationship between chemical pollution and reproductive performance is of major public health concern. The BPA has been reported to be weakly estrogenic activity both *in vitro* and *in vivo*[3], also bisphenol A has been found to mimic estradiol action by stimulating prolactin release *in vivo* and *in vitro*[4]. Testicular toxicity of bisphenol A was suggested in many studies in rats and mice[5,6]. Oral administration of BPA at very low-dose levels (2 and 20 μg kg^{-1}) caused reduced sperm production[7] and increased prostate weight[8]. Exposure to bisphenol A induces tissue peroxidation, ultimately leading to under development of brain, kidney and testis. Selenium has a great role in a number of biological processes for humans and animals. Deficiency of this element induces cell death and predisposing to cardiovascular diseases[9,10]. Degeneration of seminiferous tubule, poor integrity of spermatozoa, reduced numbers of spermatozoa within the seminiferous tubules and low sperm motility has been demonstrated in Se-deficient rats[11]. High levels of Se in testis and epididymes are important for the processes of production and maturation of spermatozoa[12]. Nano-Se has lower toxicity and possesses equal efficacy in increasing the activities of selenoenzymes, it can consider as an antioxidant with reduced risk of Se toxicity and a chemopreventive agent[13]. The BPA is one of many endocrine disruptors and its health risk has aroused public concern recently[14]. Many studies showed that BPA exposure is related to male reproductive disorders[15]. Moreover, the results of *in vitro* and *in vivo* studies have clearly demonstrated that exposure to BPA is a main factor of spermatogenesis impairment[16]. Although, numerous studies have been conducted on BPA, its effect on the reproductive system remains controversial. The aim of the this study was to examine the adverse effects and mechanism of action of BPA on reproductive system by observing morphological, hormonal and histopathological changes in rat testis, as well as it was demonstrated the protective role of selenium preparations against the undesirable effects of BPA.

MATERIALS AND METHODS

Animals: Sixty mature male Albino rats (average weight 150-180 g) were obtained from the Breeding Unit of the Egyptian Organization for the Biological and vaccine production. The animals were acclimatized to the laboratory for 2 weeks prior to the start of the experiments. Animals were housed individually in polycarbonate cages in a room with controlled temperature (24±1°C) and humidity (50±5%) with lights on from 07:00-19:00 daily. Rats were given access to food containing 24% protein and tap water *ad libitum*. All procedures were carried out according to Institutional Animal Care and Use Committee (IACUC) guide for the care and use of laboratory animals.

Chemicals: All chemicals used in the current study were obtained from Sigma Chemical Co. (St. Louis, MO). Bisphenol A (2, 2-Di (4-hydroxyphenyl) propane) of 97% purity, sodium selenite, nano-Se in the size range of 20~60 nm was prepared as described previously[17].

Treatment: Animals were divided into 6 groups of 10 rats each. Group I received the vehicle only (corn oil at 2 mL kg^{-1} b.wt.) and served as control. Group II received bisphenol A dissolved in corn oil by stomach tube in a dose of 150 mg kg^{-1} b.wt., equal[18] to 1/20 LD_{50}. Group III received bisphenol A as in group II beside sodium selenite (Na_2SeO_3) 3 mg kg^{-1} by stomach tube[19]. Group IV received bisphenol A as in group II beside nano-Se 2 mg kg^{-1} b.wt., by stomach tube[20]. Group V received sodium selenite only as in group III. Group VI received nano-Se only as in group IV. The experiment lasted for 70 days to complete time of one spermatogenesis and maturation of sperms in epididymis[21].

Reproductive organs weights: All rats were euthanized at the end of the experiment. Testis and accessory sex organs (seminal vesicles, prostate and epididymis) were dissected out and the relative weight was calculated.

Determination of serum testosterone levels: Blood samples were collected from the retro-orbital venous plexus by means of clean heparinized microcapillary tubes. The collected blood was put in clean screw-capped bottles and then incubated at 37°C until the blood is clotted and then the sample was centrifuged at 50 g for 15 min for obtaining serum and then stored at -20°C till used for the determination of serum testosterone using solid phase radioimmunoassay (RIA) in the

middle Eastern regional radioisotope center for the Arab countries, Dokki, using the specific kits of diagnostic products corporation (Los Angeles, CA, USA)[22].

Epididymal sperm count: The epididymis was minced in 5 mL of saline, placed in a rocker for 10 min and incubated at room temperature for 2 min. The supernatant fluid was diluted 1:100 with a solution containing 5 g $NaHCO_3$, 1 mL formalin (35%) and 25 mg eosin per 100 mL distilled water. About 10 µL of the diluted semen was transferred to each counting chamber of the improved Neubaur haemocytometer and was allowed to stay for 5 min on the slide to allow all sperms to be settled for counting under a light micro-scope at x200 magnification[23].

Sperm motility: Sperm-progressive motility was evaluated microscopically within 2-4 min of their isolation from the cauda-epididymis after dilution with tris buffer solution[24].

Live/dead percentage and sperm cell abnormalities: The semen was carefully mixed with eosin-nigrosin stain and thin film was spread on a clean slide. Two hundred sperms were randomly examined per slide at x400 magnifications[25]. Sperm cell total abnormalities percentage were determined using eosin-nigrosin stain and examined by binuclear research microscope (Leica, Hannover, Germany) attached with digital camera (Canon, Tokyo, Japan). The sperm abnormalities were classified according to their origin into primary abnormalities (giant or dwarf head, double head, double tail and/or coiled tail) and secondary abnormalities (detached head, bent and/or wavy tail)[26].

Ultra structural examination of testis: Specimens from testis were cut into small pieces measuring about 1 mm^3 and fixed in 2.5% glutraldehyde for 4 h and 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4). The samples were post-fixed in a buffered solution of 15 osmium tetroxide at 4°C for 1-5 h. This was followed by dehydration in ascending grades of ethyl alcohol, clearing in propylene oxide for two changes, 5 min each and embedding in epon-epoxy-resin. Semithin sections were stained with toluidine blue and investigated under a bright field microscope. Ultrathin sections were cut with a diamond knife on the ultramicrotome and mounted on formvar-coated grides, stained with uranyl acetate and lead citrate[27]. Sections were examined under ZeissEM-10 West Germany transmission electron microscope at an acceleration voltage of 60-80 kV at electron microscope unit in national research center.

Statistical analysis: All data were analyzed using one way analysis of variance (One way ANOVA) followed by Duncan test using SPSS version 16.0 statistical software[28].

RESULTS

Evaluation of reproductive organs weight: The obtained results revealed that BPA induce significant decrease in the weight of testis, epididymes, prostate and seminal vesicle of the treated male rats when compared with control group. Before administration of ISe and/or SNPs caused significant improvement in the reduced weights of male genital organs (Table 1). While administration of ISe or SNPs alone showed no changes in the weight of male genital organs of rats.

Evaluation of sperm characteristics: Epididymal sperm concentration, motility, live sperm percent and sperm abnormalities were significantly decreased ($p \leq 0.05$) in BPA and BPA+ISe treated male rats compared to the control one (Table 2). While in rats treated with BPA+SNPs, the parameters showed significant improvement than that of BPA treated groups.

Table 1: Relative weight of testis, epididymis, prostate and seminal vesicle (g) in control and treated rats (Mean±SE)

Groups	Testis	Epididymis	Prostate	Seminal vesicle
Control group	6.07±1.79^{b}	2.68±2.19^{b}	1.93±7.73^{b}	3.70±2.89^{b}
Bisphenol	5.28±8.42^{a}	1.95±3.88^{a}	1.33±1.58^{a}	2.12±2.33^{a}
Bisphenol+ISe	6.11±2.52^{b}	2.48±1.64^{b}	1.81±1.29^{b}	2.80±1.84^{a}
Bisphenol+SNPs	5.90±1.05^{b}	2.09±9.88^{b}	2.09±1.06^{b}	3.04±2.65^{b}
ISe	6.45±1.49^{b}	2.50±1.45^{b}	2.16±6.81^{b}	3.27±2.69^{b}
SNPs	6.09±1.07^{b}	2.49±6.35^{b}	2.19±1.01^{b}	3.54±2.42^{b}

^{a,b}In the same column, values with different superscript letters were significantly differ ($p \leq 0.05$)

Table 2: Sperm analysis of control and treated rats (Mean±SE)

Groups	Motility (%)	Count	Live (%)	Total abnormalities (%)
Control	83.00±2.00^{b}	67.80±2.81^{b}	80.20±2.22^{b}	19.00±2.46^{b}
Bisphenol	28.00±9.16^{a}	29.77±3.08^{a}	43.40±1.07^{a}	51.20±2.15^{a}
Bisphenol+ISe	70.00±2.23^{ab}	57.80±1.39^{ab}	67.39±0.92^{ab}	29.55±1.96^{ab}
Bisphenol+SNPs	73.00±3.74^{b}	60.00±2.46^{b}	75.80±2.13^{b}	21.60±1.20^{b}
ISe	84.00±1.87^{b}	68.60±5.15^{b}	80.21±1.59^{b}	21.00±1.18^{b}
SNPs	86.00±1.87^{b}	70.40±4.00^{b}	84.44±1.20^{b}	16.22±1.59^{b}

^{a,b,ab}In the same column, values with different superscript letters were significantly differ ($p \leq 0.05$)

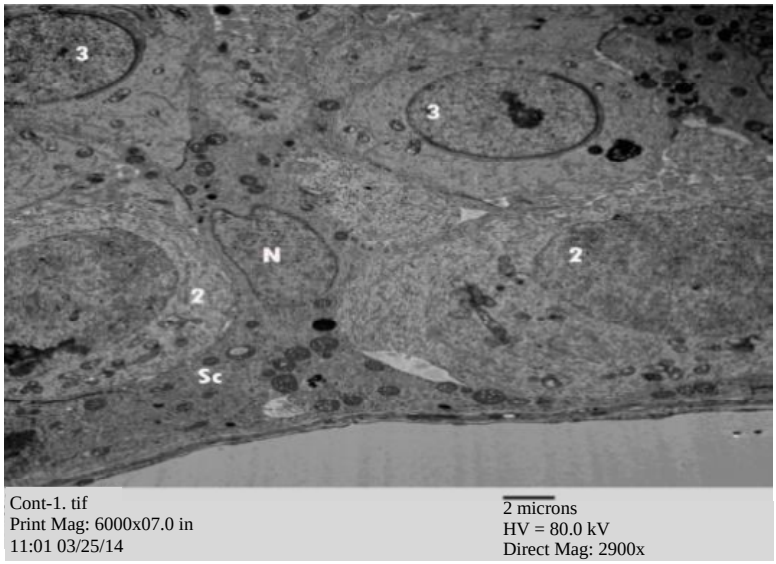

Fig. 1: T.E. micrograph of seminephrous tubule belonging to control testis (group one) showing sertoli cell (Sc) with its long and branching processes in between the spermatogenic cell, laying on the basement membrane and having dentated nucleus (N) and rich with cell organelles such as mitochondria and smooth endoplasmic reticulum. Notice presence of spermatocytes (2) and spermatid cell (3) with its acrosomal cap

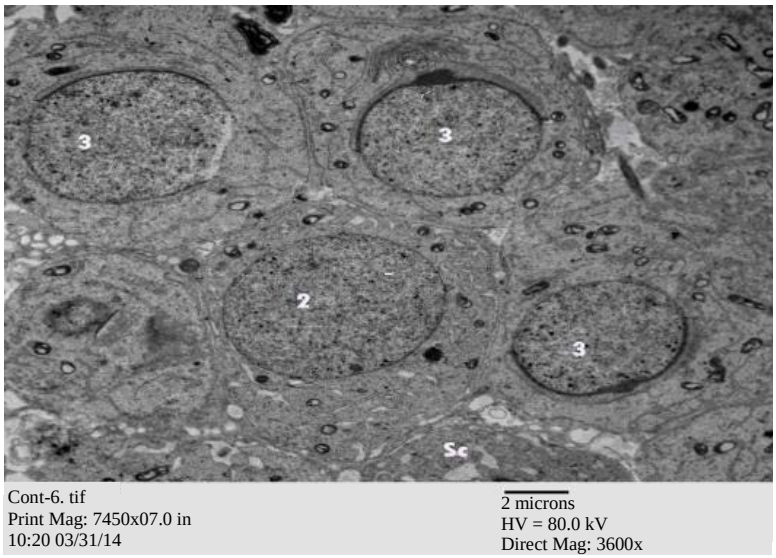

Fig. 2: T.E. micrograph of seminephrous tubule belonging to control testis (group one) showing the normal ultra structure of the spermatid cell (3), notice condensation of the nuclear chromatin forming the acrosome (arrow head) and acrosomal cap (arrow). Notice presence of spermatocyte (2) and the processes of sertoli cell (Sc)

Evaluation of serum total testosterone: The present data revealed that BPA induced significant decrease ($p \leq 0.05$) in the level of total testosterone in the male treated rats compared to the control one (Table 3). Pre-administration of ISe with BPA caused partial improvement but still significantly decreased when compared with control one. While pre-administration of SNPs with BPA induced marked protection and the level of total testosterone was significantly increased than that of BPA treated rats.

Ultra structure of the testis: Ultra structure of the testis were demonstrated in Fig. 1-11.

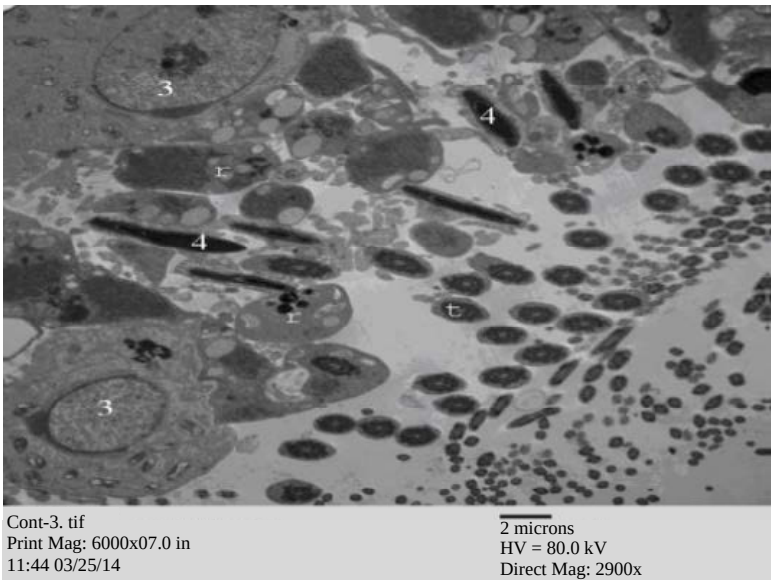

Fig. 3: T.E. micrograph of seminephrous tubule belonging to control testis (group one) showing the mature head of spermatozoa (4) near the lumen of the tubule. Notice presence of the spermatid cells (3) with its acrosomse and acrosomal cap, residual bodies (r) and large number of spermatozoal tail (t)

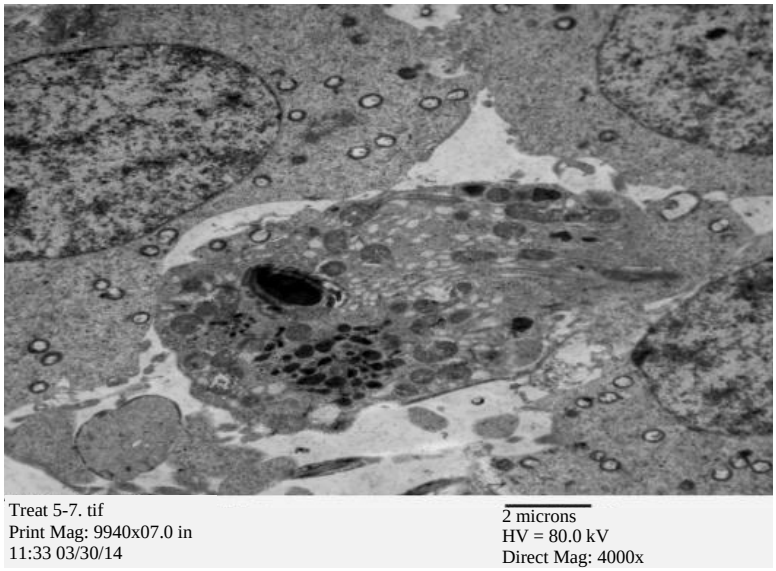

Fig. 4: T.E. micrograph of seminephrous tubule of testis belonging to (group five) showing, presence of large spermatocyte (2) containing large nucleus and vesiculated mitochondria. Notice presence of part from sertoli cell processes (Sc) contain large amount of mitochondria, dilated endoplasmic reticulum head of sperm (4) and numerous electron dens particles, also notice presence of sperm tails (t) and cytoplasmic residual bodies (r)

Table 3: Serum total testosterone (ng mL^{-1}) of control and treated rats (Mean±SE)

Groups	Control	BSA	BSA+ISe	BSA+SNPs	ISe	SNPs
Total testosterone	12.42 ± 1.10^{b}	3.27 ± 0.20^{a}	6.83 ± 0.12^{ab}	11.15 ± 1.26^{b}	10.31 ± 0.55^{b}	11.26 ± 0.25^{b}

^{a,b,ab}In the same row, values with different superscript letters were significantly differ ($p \leq 0.05$)

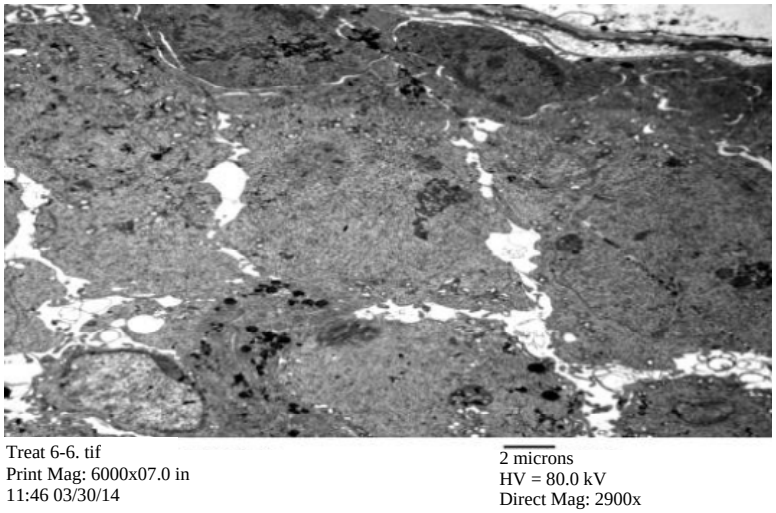

Fig. 5: T.E. micrograph of seminuphrous tubule of testis belonging to group six showing, spematogonia (1), sertoli cell (Sc) laying on basement membrane (arrow). Notice presence of intercellular spaces (x) between spermatocyte (2) and deformity of spermatid cell (3)

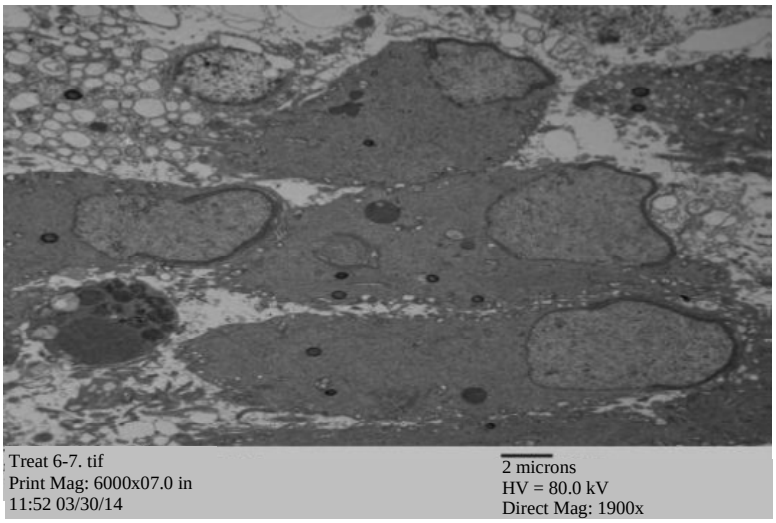

Fig. 6: T.E. micrograph of seminephrous tubule of testis belonging to (group six) showing, abnormal formation of spermatid cells (3) which lead to deformity in the nucleus and acrosomal cap and its cell membrane having microvilli variable in length and the cytoplasm contain fat globule (f) and presence of intercellular spaces. The sertoli cell (Sc) processes contain numerous dilated endoplasmic reticulum

DISCUSSION

The BPA was ultimately becoming a part of the food chain as an environmental estrogenic pollutant[29]. Daily sperm production is reduced significantly and the structure of sperm cells undergoes marked changes in adult mice fed BPA[30]. The BPA induced alterations in hypothalamic pituitary-gonadal axis and reduction in sertoli cell phagocytic function. Weights of the reproductive organs are the parameters used for evaluation of reproductive toxicity[31]. In the current study, BPA caused significant reduction in the reproductive organ weights which might be due to the decrease in serum testosterone levels. Other studies showed that, the weight of the testis is basically dependent on the mass of the differentiated spermatogenic cells[32,33]. The reduction on the weight of the testis may be due to decreased number of germ cells, inhibition of spermatogenesis and steroidogenic enzyme activity[6]. The epididymes and seminal vesicles both

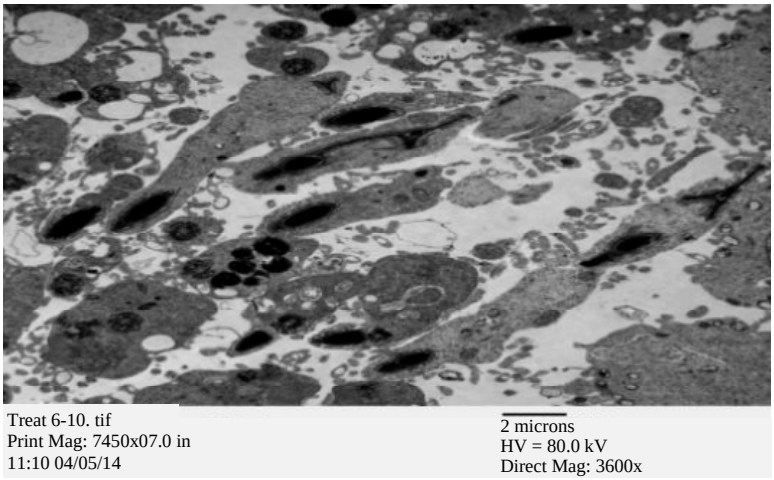

Fig. 7: T.E. micrograph of seminephrous tubule of testis belonging to (group six) showing numerous spermatozoa (4) near the lumen some of them showing miss shaped in its morphology. Notice presence of numerous residual bodies (r) containing vacuoles (v) or electron dens particles similar to lysosomes (arrow)

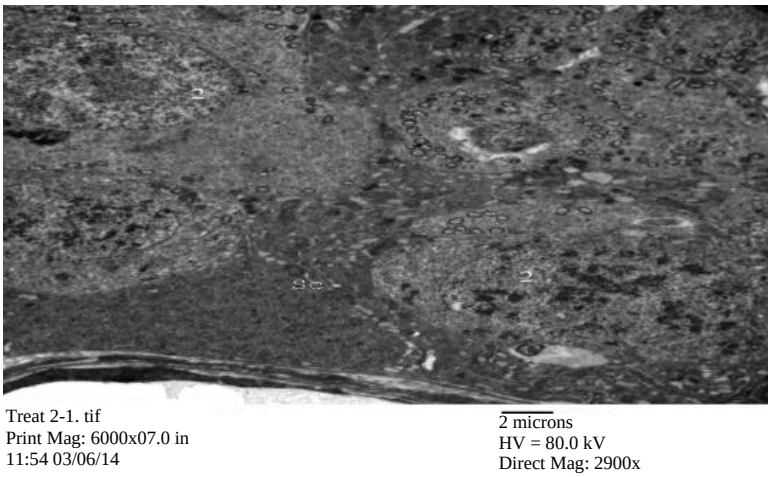

Fig. 8: T.E. micrograph of seminephrous tubule of testis belonging to (group two) showing, presence of sertoli cells (Sc) with its long branching processes laying on the basement membrane and contain large amount of dilated smooth endoplasmic reticulum forming vesicle, mitochondria and increase the electron density of the nucleus and the cytoplasm. Notice presence of spermatocyte (2) contain large nucleus, free ribosomes and vesiculated mitochondria

are androgen dependent organ. Testosterone is more essential for their growth and function so a reduction in their weights may reflect a decline in the level and production of testosterone. The sperm number is one of the most sensitive parameter for spermatogenesis and is in high correlation with fertility[34]. The results of the present study indicated that BPA administration at the dose of 150 mg kg^{-1}, resulted in a significantly decrease in sperm motility, count, alive sperm percent and significantly increased sperm abnormalities. Several investigators have suggested that BPA treatment may adversely affect the quality and quantity of sperm, which is responsible for male fertility[6]. The reduction in sperm count may be due to an adverse effect of BPA on spermatogenesis. The harmful effect of BPA on spermatogenesis could be ascribed to either the reduction in serum testosterone level or BPA induced LPO[35]. Increased oxidative stress due to BPA action, damages the sperm membranes, proteins and DNA associated with male fertility[36]. The obtained results revealed that rats treated with BPA significantly decreased alive sperm percent and increased sperm cell abnormalities[33,35,37,38]. These abnormalities may be attributed to oxidative stress and damage of DNA by BPA during the spermatogenesis. One of

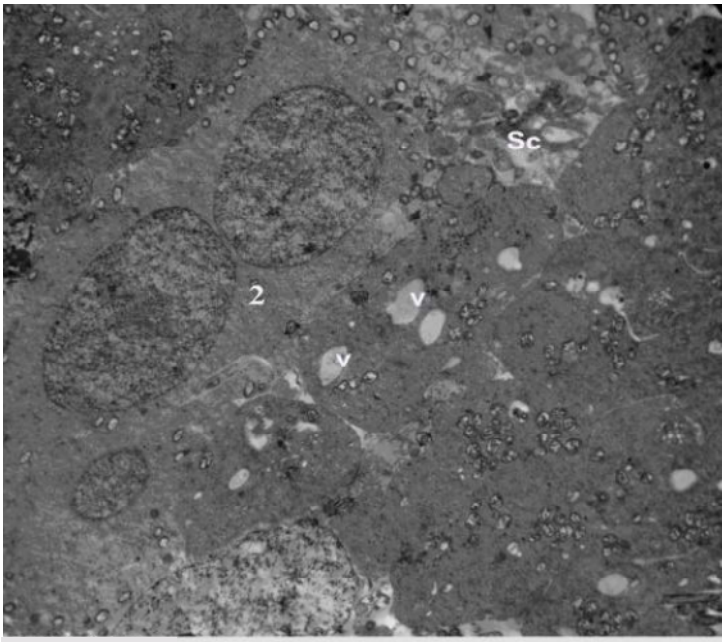

Fig. 9: T.E. micrograph of seminephrous tubule of testis belonging to (group two) showing contain bi-nucleated spermatocyte (2) and large amount of residual bodies containing vesiculated mitochondria and numerous vacuoles (v). Notice presence of sertoli cell processes (Sc)

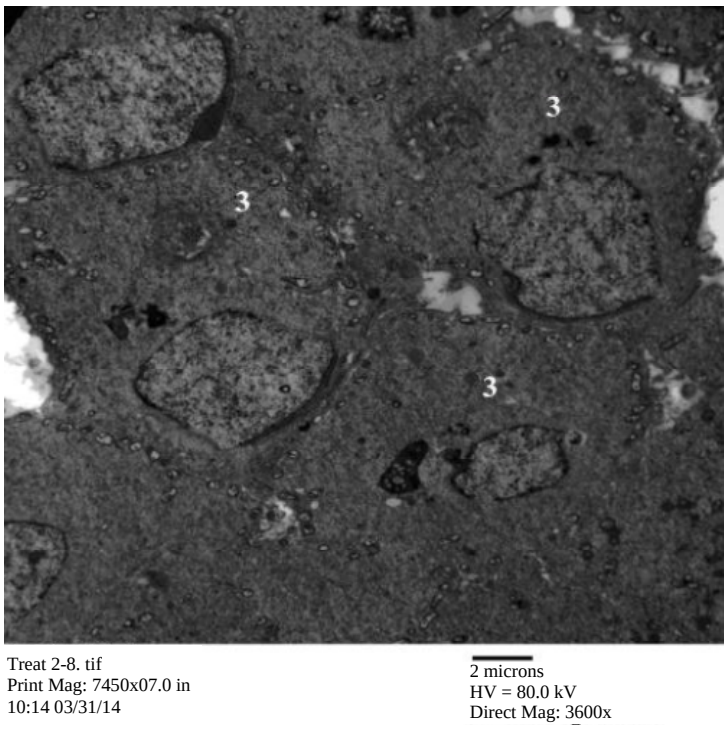

Fig. 10: T.E. micrograph of the seminephrous tubules of testis belonging to (group two) showing the abnormal development of the spermatid (3). Notice the deformity in the nucleus and the acrosomal cap (arrow) and increase electron density of the cytoplasm with vsiculation of the mitochondria

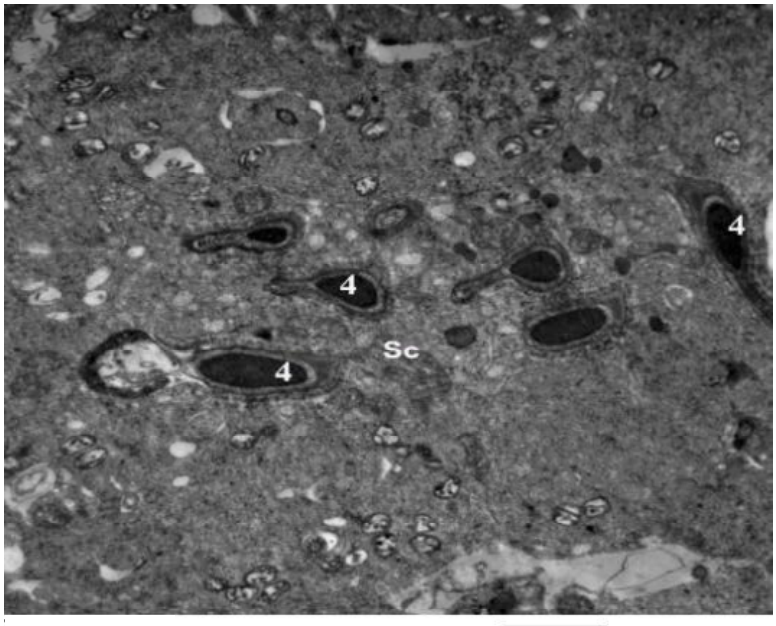

Fig. 11: T.E. micrograph of the seminephrous tubule of testis belonging to (group two) showing deformity of the spermatozoa (4) and phagocytozed by sertoli cell (Sc) with vesiculation of the mitochondria of the spermatocytic cells

the indicators of the chemical toxicity on reproductive system is the decreased level of testosterone[39]. The BPA treated rats had a significant reduction of serum testosterone level, in the same way it has been found that BPA induces a significant decrease in testosterone in male rats[40,41]. It is postulated that the low testosterone level may have caused failure of spermatogenesis and distruction of seminiferous epithelium. The low level of plasma testosterone in BPA-treated animals was probably due to proliferative activity and development of leydig cell in rats[42]. The endocrine disruptors and impairment of the hypothalamus-pituitary-gonadal axis were also evident at the testicular level[6].The ultrastructural examination, BPA induced some histopathological changes in the testis such as sertoli cell with its branching processes laying on the basement membrane and contain large amount of dilated smooth endoplasmic reticulum forming vesicle, mitochondria and increase the electron density of the nucleus and the cytoplasm[33,41]. The present study revealed that BPA caused marked decreases of spermatozoa which in the form of numerous deeply stained bodies or phagocytosed by sertoli cell processes, abnormal development of the spermatid was also evident and deformity of the spermatozoa. The electron microscopic examination of seminiferous tubules of BPA treated male rats revealed that there was extensive cytoplasmic vaculation of some of the lining cells[40,41]. These may be attributed to the ionic and osmotic imbalance leading to inhibition of water causing cellular vaculation which is known to be a sort of cell degeneration[43]. Also the vacuolization of the germinal cells and sertoli cells may be due to the dilation of smooth endoplasmic reticulum that possibly represents cellular permeability changes[44]. Also the ultrastructural observations suggested that all animals exposed to BPA had apoptosis in different germinal cells. The BPA induced degenerative changes in basement membrane which maintains the structural and functional integrity of testicular tissues[45]. The ultrastructural changes in testis of BPA treated male rats may be due to BPA-induced LPO and decrease in testosterone level. Where, testosterone is required for the attachment of different generations of germ cells in seminiferous tubules. Therefore, low level of intra testicular testosterone may lead to detachment of germ cells from seminiferous epithelium and initiate germ cell apoptosis and consequently male infertility[46]. Several lines of evidences investigate the beneficial effect of natural and synthetic antioxidant in protecting male reproductive system from toxic effect of xenobiotic substances[20,43,47]. In the current study, ionized selenium (ISe) and selenium nanoparticles were used against the reproductive toxicity of BPA. Co-administration of ISe and/or SNPs improved the evaluated parameters albeit not all were identical to control levels especially in case of Ise

while SNPs induced marked protective effect and the examined parameters were nearly similar to the control. The ISe and/or SNPs ameliorated the reduction in the reproductive organ weights, sperm characteristics and the ultrastructural alterations of the testis. This observation was correlated with several studies which mentioned that nano-Se appears to be more effective than that of other Se sources like sodium selenite, sodium selenosulfate, selenomethionine and Se-methyl selenocysteine[13,48-50] at inducing selenoenzymes like GSH-PX and thioredoxin reductase, scavenging of free radicals, prevention of oxidative DNA damage through potent antioxidant activity and antiapoptic properties[13,51]. The protective mechanism of nano-Se may be related to its androgenic stimulatory affect. Nano-Se treatment reversed toxic effect of BPA on testosterone level and increased it when given together with BPA. Selenium has been proved to be essential for testosterone synthesis and the normal development of spermatozoa[52]. Selenium was found to play a dominant role in sperm motility and male fertility, through up regulation of the catsper genes expression in sperm[53]. So, that Se deficient cases exhibit low sperm production, as well as decreased testosterone concentration[54]. The improvement in the ultrastructure of the testis was correlated to powerful antioxidant effect of selenium and the potential role of Se in scavenging ROS generated by BPA.

CONCLUSION

The study demonstrated the detrimental effect of BPA on male reproductive organs, semen quality, testosterone level and ultra structure of testis. Moreover, the co-administration of ISe and/or SNPs partially or completely allivated the toxic effect of BPA on male fertility.

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