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Antioxidant Activity of Selenium on Bisphenol-Induced Apoptosis and Testicular Toxicity of Albino Rats

Actividad Antioxidante el Selenio sobre la Apoptosis Inducid por Bisfenol y Toxicidad Testicular en Ratas Albinas

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SUMMARY: Bisphenol A (BPA) is an industrial chemical widely used to make polycarbonate plastics for packaging and epoxy resins. This study sought to examine how selenium (Se) affects BPA toxicity in terms of albino rats' histological structure, antioxidant enzymes and reproductive organs (seminiferous tubules). Twenty-four adult male rats were divided into four experimental groups: Group 1: Control; Group 2: Orally administered BPA; Group 3: Orally administered sodium selenite; Group 4: Treated daily with BPA followed by selenium (Se). All experiment done for 4 weeks. BPA exposure caused changes in the testicular histological structure, which consists apoptosis, and led to changes in several biochemical markers: Malondialdehyde, catalase, superoxide dismutase, and glutathione peroxidase. However, these BPA side effects may be ameliorated in rats treated with BPA-plus-Se. These protective effects of Se may attributable to its ability to remove potentially damaging oxidizing agents in living organisms. The results may confirm that Se countered the oxidant effects and increased the BPA-induced stress response in rats. So, Se promotes the healthy growth and development of mammals by protecting them from oxidative stress. As human are greatly exposed to BPA and it can accumulate in tissues, there is concern about human reproductive functions particularly for occupational workers exposed usually to greater levels of BPA. Thus, the use of BPA in multiple industries must be restricted and the inaccurate usage of plastic containers should be avoided to decrease the health hazards. Administration of Se may protect against the adverse effects of BPA on reproductive functions and structures.

KEY WORDS: Bisphenol A; Selenium; Testis; Biochemical enzymes; Histology; Ultrastructure; Statistical analysis.

INTRODUCTION

BPA is considered one of the most common industrial manufactured chemicals all over the world (Bosch *et al*., 2016). Its molecule is small (228 Da), its color is white and at room temperature exists as a solid particle and has an odor of phenol (Ahmed *et al*., 2015). It is commonly used in manufacture of plastic and epoxy resin which is used a lot by consumers and acts as a monomer in the steps of polymerization reaction (Bosch *et al*.). BPA molecules remain in the environment for 2.5- 4 days which is considered a short half-life. Its pseudo persistent character comes from the constant release from plastic containers have BPA in its composition (Bosnjak *et al*., 2014).

BPA is harmful to human and animals. It is present in the environment and is commonly used in daily life. It affects normal functions of the endocrine and reproductive systems also by simulating or inhibiting the action of endogenous hormone, or modifying the synthesis of hormones (Gurmeet *et al*., 2014).

Furthermore, it was found that BPA acts as an antagonist for the androgenic receptors (AR) which considered the main regulatory component of androgen cell signaling. Androgen receptors are very important for the function of male reproductive system and its development,

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counting one of the most important processes which are spermatogenesis (Preethi *et al*., 2014). Moreover, BPA decreases the activity of aromatase enzyme via two steroidogenic enzymes. Aromatase usually appear in the brain, interstitial cells (Leydig cells) and adipose tissue and is important in the synthesis of steroid hormones. It can catalyze the irreversible alteration of androgens into estrogens (Helal *et al*., 2013).

An earlier study showed that mitochondrial enzymes in the testes diminished in mice after BPA exposure (Anjum *et al*., 2011). BPA has been described to encourage the alteration of xanthine dehydrogenase into xanthine oxidase in the rat liver cells thereby increasing the reactive oxygen species (ROS) (Sakuma *et al*., 2010). ROS generation and oxidative DNA may be responsible for the detrimental side effects of BPA on the human body (Gurmeet *et al*.).

Apoptosis is a common physiological and pathological condition which is identified as programmed cell death process. It is involved in spermatogenesis in which apoptosis of germ cell is important to remove abnormal sperm cell to provide convenient quality and quantity of sperms (Li *et al*., 2009). A lot of factors are responsible for apoptosis of germ cells such as hormone deprivation, exposure to heat and radiation and toxicants (Helal *et al*.). Occurrence of excess or unusual apoptosis of germ cells is a common cause for oligozoospermia and azoospermia.

Antioxidant molecules play a vital role in maintaining the health and defense mechanisms of biological tissues and organs. The human body has several mechanisms to counteract oxidative/nitrosative stress via the production of antioxidants (Kurutas, 2016).

In particular, selenium (Se) is an essential trace element for humans and animals (Saito *et al*., 2003) that can prevent the formation of reactive metabolites induced by many toxicants (Atif *et al*., 2008; Djeffal *et al*., 2015). Selenium is well known for its effective antioxidant influence against unfavorable residues and chemicals; furthermore, Se decreases the risk organ injury, such as intestines, heart, lungs, and kidneys (Parízek, 1990; Benstoem *et al*., 2015) and it exhibits protective effects against cadmium-induced testicular toxicity (Li *et al*.). Recently, (Dkhil *et al*. 2016) and Ullah *et al*. (2016) reported that selenium protects testicular tissue from oxidative injury and has beneficial effects on their production of experimental animals.

The effects and mechanism of BPA toxicity on reproductive system still remain unclear. The majority of the population especially in the developing countries may not be aware of the harmful effects of BPA on the human body (Srivastava & Gupta, 2016). Hence, this study aimed to observe the effects of BPA on testes of adult albino rats and explore the protective role of Se.

MATERIAL AND METHOD

Chemicals and preparation

Bisphenol A (BPA): (2,2 Bis-4- hydroxyl phenyl propane) was obtained from Sigma Chemicals Co. (Sigma, St. Louis, USA). BPA was suspended in water and orally administered to the treated animals at a dose of 10 mg/kg bw/day for 4 weeks (Takahashi & Oishi, 2003).

Selenium (Se): was used in the form of sodium selenite (Na_2SeO_3). This compound was obtained from British Drug Houses Ltd. (Laboratory Chemicals Division, UK) and dissolved in distilled water for administration to the treated animals at a dose of 10 mg/kg bw/day for 4 weeks.

Corn Oil: It is used as vehicle for BPA and selenium. It was obtained from Sekem, Cairo.

Experimental Animals and Design. The study was carried out on 24 adult male albino rats with average weight of (150-200) gm; with average age of 50-60 days; they were obtained from the Animal House in King Khalid University. They were kept under hygienic conditions and fed on a balanced diet and water ad-libitum. The rats were divided into 4 groups each of 6 rats as follow:

Group 1: Served as the control and received the vehicle only (corn oil at 2 mL kg-1 b. wt).
Group 2: Received sodium selenite (10 mg/kg bw/day; administered orally).
Group 3: Received BPA (10 mg/kg bw/day; administered orally).
Group 4: Received BPA (10 mg/kg bw/day) followed by Se (10 mg/kg bw/day). All rat groups of the experiment served for 4 weeks.

Biochemical assays. The testes of all groups were rapidly isolated at two time points (2 and 4 weeks) during treatment and washed with ice-cold isotonic saline (0.9 %), then stored at -80 °C until they were homogenized in a 50 mM phosphate buffer (pH 7.4) using an electronic homogenizer to prepare the 10 % w/v homogenate. The homogenate was then divided into aliquots by volume. The level of lipid peroxidation measured as malondialdehyde (MDA) and the activity of CAT, SOD and GPx were determined using kits bought from Randox Laboratories Ltd (Antrim, UK).

Analysis of lipid peroxidation. Lipid peroxidation MDA level was measured by the content of MDA in the testis. Tissue-level MDA was determined by using the thiobarbituric acid reactive substance assay, as described by Buege & Aust (1978). The absorbance of the clear supernatant was determined spectrophotometrically (S2000 UV model) at 535 nm, and the MDA concentration calculated using 1.56×105 mol-1 cm-1 as molar absorbance coefficient. MDA results were expressed as nmol per gram of wet tissue.

Analysis of glutathione peroxidase (GPx) activity. The reduction in absorbance was measured spectrophotometrically (S2000 UV model; WPA, Cambridge, UK) against a blank at 340 nm. One unit (U) of GPx was defined as the mmol of oxidized hydrogen peroxide (NADPH) per min per mg of tissue protein. The GPx activity level was expressed as milliunits per mg of protein (that is, mU mg-1 protein).

Analysis of superoxide dismutase (SOD) activity. SOD activity was then measured by the degree of inhibition of this reaction. One unit (U) of SOD caused a 50 % inhibition of the rate of reduction of INT under the conditions of the assay. The SOD activity level was measured spectrophotometrically at 505 nm, and expressed as U mg-1 protein.

Analysis of catalase (CAT) activity. Tissue CAT activity was evaluated as described by Aebi (1984). The reaction was initiated by adding hydrogen peroxide to the reaction mixture and the enzyme's activity level was quantified spectrophotometrically as the rate at which tissue catalase decompensated the hydrogen peroxide; this was done, by monitoring the decrease in absorbance at 240 nm against a blank containing a phosphate buffer instead of the substrate (S2000 UV model). The log A1/A2 value for a measured interval was used to define a unit given the first-order reaction of the enzyme. One unit of CAT is the amount of enzyme that decomposes 1.0 nM of hydrogen peroxide per minute at pH 7.0 and 25°C.

Histopathological studies: The rats were anesthetized by ether then sacrificed. The testes were dissected and examined for the microscopic histological examination; testes specimens were fixed in Blouin's solution. Next to fixation, testes and prostate were fixed in paraffin blocks as usual and managed for the preparation of 3-5m thick sections. Sections were deparaffinized, rehydrated, and exposed to hematoxylin and eosin stain to assess variations in the morphology and examined by light microscope (Bancroft & Stevens, 1996).

Preparation of Specimens for the Semithin Sections Toluidine Blue Stain: Testes were immediately cut into cubes (1 mm in diameter) and fixed overnight in 2.5 % phosphate-buffered glutaraldehyde (pH 7.3) at 4 ºC. Fixation in 1 % buffered osmium tetroxide for 1-2 h was followed by dehydration in ascending grades of ethyl alcohol and clearance in propylene oxide and finally embedded in fresh Epon capsules. Semithin sections, 1 µm in thickness, were cut with a glass knife and stained with toluidine blue and then examined by an Olympus light microscope (Bancroft & Gamble, 2002).

Ultrastructural preparations. Specimens were cut from the testis of all animal groups into small pieces, each piece measured about 1 mm^3, then fixed immediately at 4 ºC for 18–24 h in freshly 3 % glutaraldehyde-formaldehyde. Specimens were washed in phosphate buffer (pH 7.4), and post fixed for one hour at 4 ºC in isotonic 1 % osmium tetroxide (Weakley, 1981). Semi-thin sections were stained with toluidine blue for detection the area of interest, then prepared ultrathin sections by using the ultra-microtome glass knives. Stained by uranyl acetate and lead citrate, sections were then examined by JEM-1011 transmission electron microscope, Joel Ltd., Japan, operated at 80 kv in faculty of Medicine, KKU, (Eid *et al*. 2020).

Statistical analysis. For all toxicological evaluations, results of the treatment groups were compared with those of the control group. Data collected throughout laboratory investigations and outcome measures coded, entered and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences (SPSS version 20.0) (Statistical Package for the Social Sciences) software for analysis. According to the type of data, the following tests were used to test differences for significance; differences between multiple means (quantitative variables) were compared by ANOVA test. P value was set at <0.001 for significant results.

RESULTS

Biochemical and statistical results. Figure 1A showed that the rats received BPS had significant elevation in the serum activities of MDA (8.4 nmol /mg, $p< 0.001$), level compared to the control group. Administration of SE to BPS group showed significant amelioration of the elevated MDA when compared with the BPS group, this indicated an antioxidant effect of SE. In addition, Figures 1B and 1C, showed a significant decrease in GPx and SOD activities in BPS group compared to control group. Administration of SE to BPS group showed significant increase in GPx and SOD activities compared to the BPS group. Moreover, Figure 1D, showed a significant decrease in catalase activities in BPS group compared to control group. Administration of SE to BPS group

showed significant increase in catalase activities compared to the BPS group.

Histological results

Groups I, II: The microscopic examination of toluidine blue stained sections of the testes of the control and Se treated rats showed seminiferous tubule of normal structure, they are lined by spermatogonia, followed by successive layers of germinal epithelium at various stages of spermatogenesis with both primary and secondary spermatocytes and normal supporting sustentacular cells (cells of Sertoli). Normal spermatids and sperms are also seen. The basement membrane of seminiferous tubule is surrounded by myoid cells. Patent blood vessels and interstitial Interstitial cells are laying in between the tubules (Figs. 2A,B).

Group III (BPA treated group): The testes showed disorganization and degenerated seminiferous tubule surrounded by myoid cells and contained damaged spermatogonia with vacuoles, sustentacular cells and degeneration in both primary and secondary spermatocytes. Pleomorphic spermatids, abnormal blood vessels and abnormal interstitial Interstitial cells are laying in the interstitium between the tubules (Fig. 2C).

Group IV (BPA and Se treated group): The testes showed marked improvement in the seminiferous tubules. Its basement membrane is surrounded by myoid cells and contained patent spermatogonia, sustentacular cells, both primary and secondary spermatocytes and spermatids and are also seen (Fig. 2D).

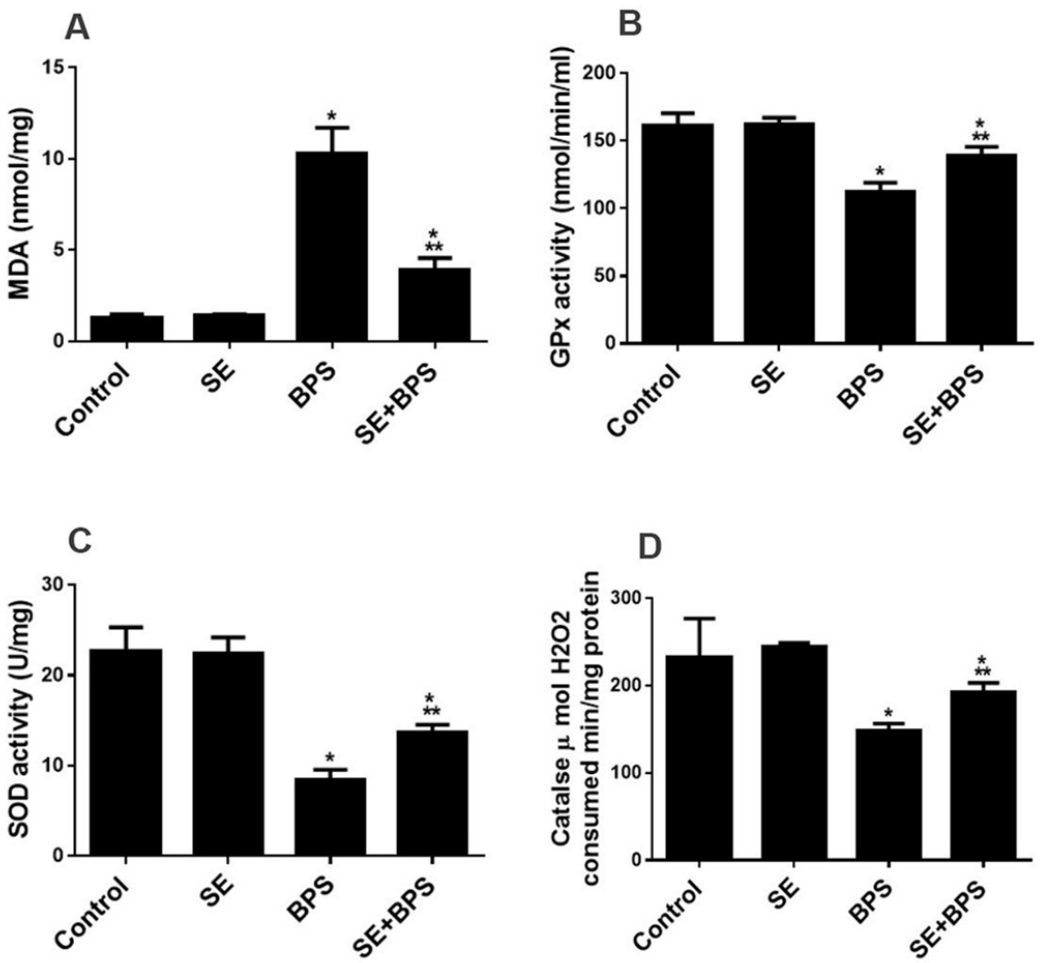

Fig. 1. A. Effect of SE on testicular MDA in BPS induced toxicity of the rats. B.&C. Influence of SE as antioxidant on glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity in BPS group. D. Influence of SE as antioxidant on testicular catalase in BPS group.

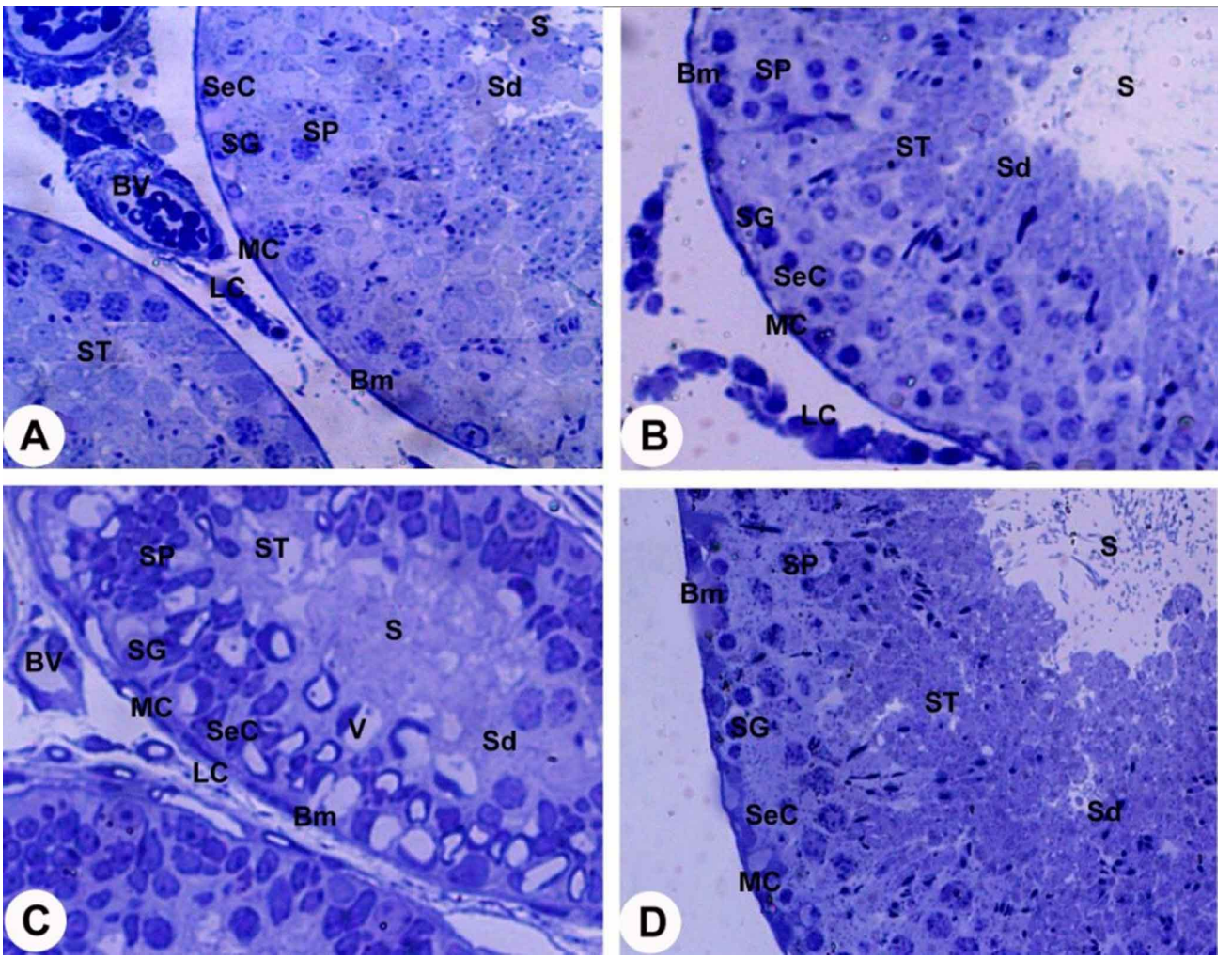

Fig. 2. Toluidine blue photomicrographs of all testis groups (X400): **A & B** Photomicrographs in the testis of control and Se-treated rats respectively, showing seminiferous tubule (ST) of normal structure, they are lined by spermatogonia (SG), followed by successive layers of germinal epithelium at various stages of spermatogenesis with both primary and secondary spermatocytes (SP) and normal supporting sustentacular cells (SeC). Normal spermatids (Sd) and sperms are also seen. The basement membrane of seminiferous tubule is surrounded by myoid cells (M). Patent blood vessels (BV) and Interstitial cells (LC) are laying in between the tubules. **C.** A photomicrograph of the testis of BPA treated rat showing degenerated seminiferous tubule (ST) surrounded by myoid cells (M) and contain damaged spermatogonia (SG) with vacuoles (V), sustentacular cells (SeC) and degeneration in both primary and secondary spermatocytes (SP). Pleomorphic spermatids (Sd) and sperms are also seen. Abnormal blood vessels (BV) and abnormal Interstitial cells (LC) are laying in the interstitium between the tubules. **D.** A photomicrograph of the testis of BPA plus Se treated rat showing marked improvement in the seminiferous tubules (ST). Its basement membrane is surrounded by myoid cells (M) and contained patent spermatogonia (SG), sustentacular cells (SeC) and both primary and secondary spermatocytes (SP). Normal spermatids (Sd) and sperms are also seen.

Ultrastructural study

Groups I & II: Electron microscopic examination of the ultrathin sections of the testes from the control and Se treated rats and showed the basal compartment of the seminiferous tubules formed of cells resting on the basement membrane formed of sustentacular cells and spermatogonia. Sertoli cell appeared as a large pyramidal cell having large indented nucleus with prominent nucleolus. Spermatogonia appeared with oval nuclei containing peripheral clumps of heterochromatin (Figs. 3A,B). The adluminal compartment showed primary spermatocytes and spermatids. The primary spermatocytes revealed rounded to oval nuclei, and secondary spermatocytes were rarely seen as they have short lifespan. The early rounded spermatids were identified by their characteristic acrosomal caps (Figs. 3C,D).

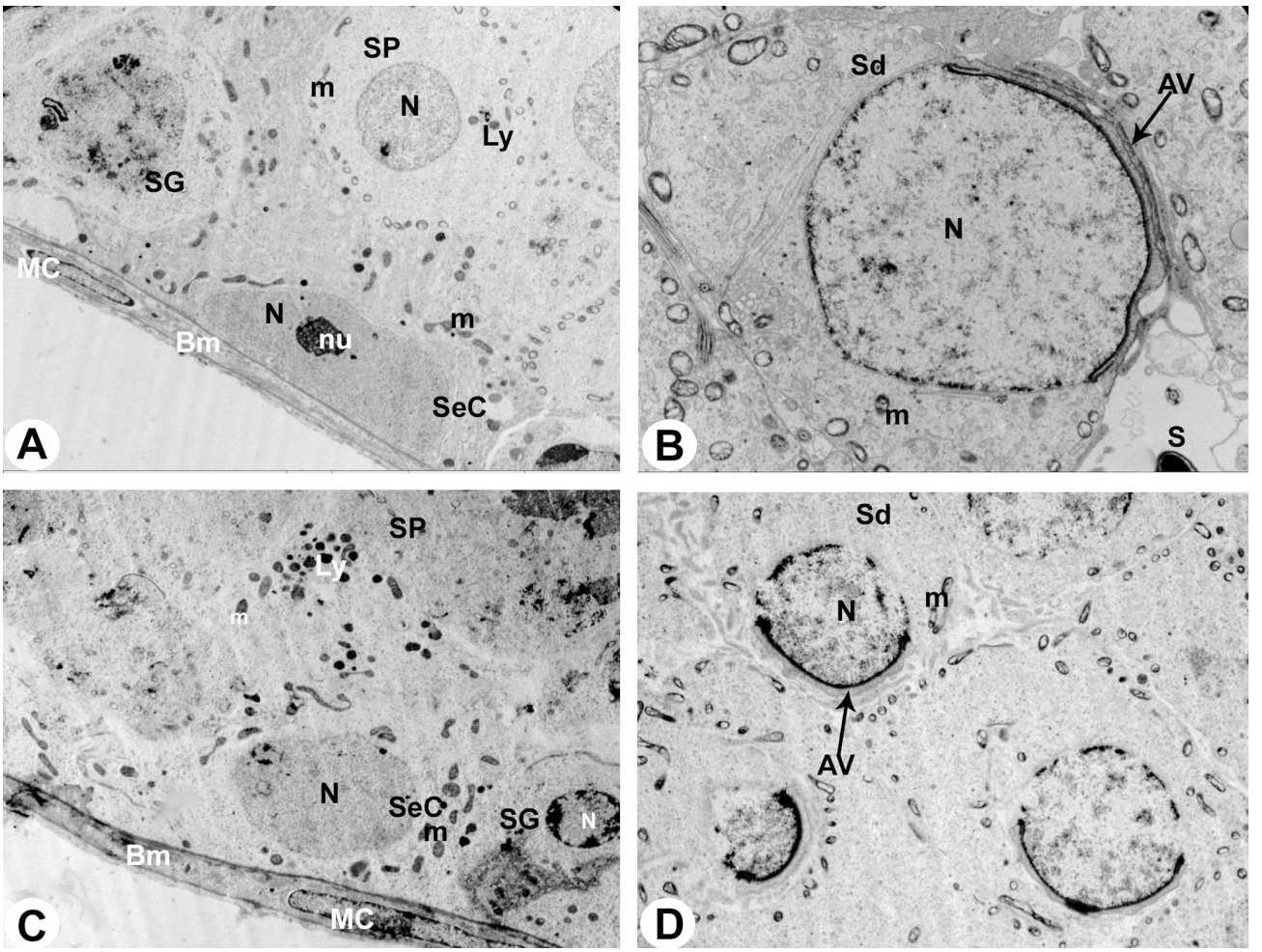

Fig. 3. Transmission electron micrographs (TEM) of control and selenium - treated rat testis: **A.** An electron micrograph of the testis of the control rat's showing spermatogonial cells (SG) are found regularly arranged and with nuclei lying parallel to the tubular membrane with no signs of abnormality. Sertoli (SeC) shows its nucleus appears triangular and euchromatic (N) with well-developed nucleolus (nu) and its cytoplasm contains many mitochondria. Notice the basement membrane (Bm) enclosing myoid cell (MC) with central flat nucleus. The primary spermatocytes appear as large rounded cells, with rounded nucleus (N) and dispersed granular euchromatin. The cytoplasm shows mitochondria (m) and lysosomes (Ly). X5000. **B.** An electron micrograph of the testis of the control rats showing intact rounded spermatids (Sd) with rounded nucleus (N) with fine granular chromatin, mitochondria (m) and acrosomal vesicles (AV). X15000. **C.** An electron micrograph of the testis of the Se treated rats showing intact spermatogonial cells (SG), Sertoli cell (SeC) and primary spermatocytes (SP). N, nucleus; m, mitochondria; Ly, lysosomes. X5000. **D.** An electron micrograph of the testis of the Se treated rats showing intact rounded spermatids (Sd) with rounded nucleus (N) and acrosomal vesicles (AV). X15000

Group III (BPA treated group): Ultrathin sections of this group revealed damaging effects of BPA on the cells. The cytoplasm of these spermatogonial cells along with surrounding Sertoli cell was relatively damaged, its cytoplasm appears degenerated with enlarged intracellular vacuoles and electron dense bodies, swollen mitochondria, cell debris and cytoplasmic vacuoles. A few spermatogonial cells were shrunken and separated from the thickened basement membrane and neighboring cells by remarkable spaces (Figs. 4A,B). Some degenerated spermatids and primary spermatocytes with mitotic division nuclei, cytoplasmic damaged and vacuolation were noticed. The primary spermatocytes showed cytoplasmic vacuoles, excessive lipid droplets, corrugated nuclear envelope. The cytoplasm contained numerous mitochondria and vacuoles in neighboring cells (Fig. 4C). Distorted spermatids with shrunken nucleus and marked accumulation of cytoplasmic vesicles and shrunken pyknotic nucleus were shown (Fig. 4D).

Group IV (BPA and Se treated group): The rat testis of this group resulted in marked improvement in the

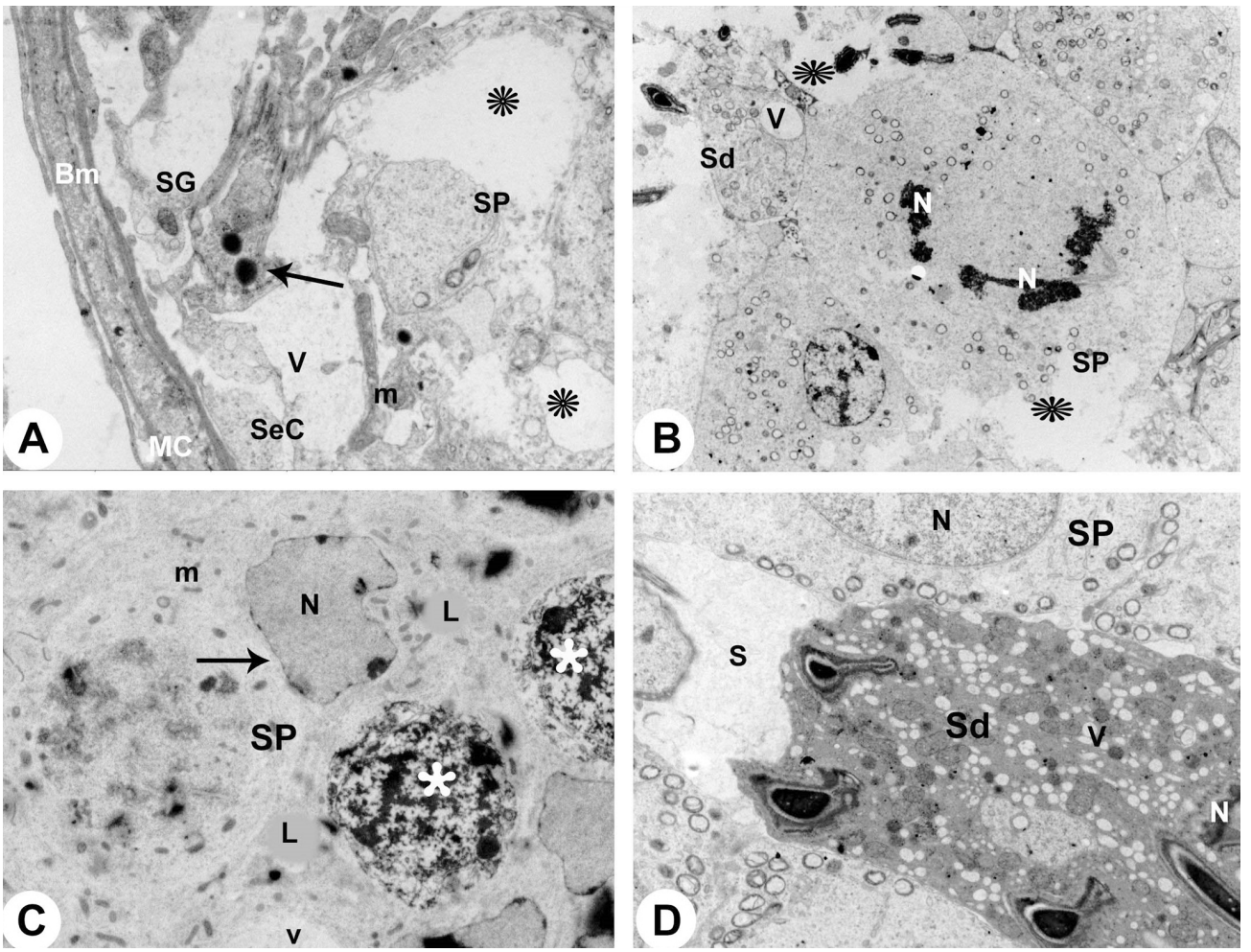

Fig. 4. Transmission electron micrographs (TEM) of bisphenol A –treated rat testis: **A.** An electron micrograph of the testis of BPA treated rats showing cellular alterations in some spermatogonial cells (SG). The cytoplasm of these spermatogonial cells along with surrounding Sertoli cell (SeC) is relatively damaged, its cytoplasm appears degenerated with enlarged intracellular vacuoles (V) and electron dense bodies (arrow), swollen mitochondria (m), cell debris and cytoplasmic vacuoles (asterisks). A few spermatogonial cells are shrunken and separated from the thickened basement membrane (Bm) and neighboring cells by remarkable spaces. X5000. **B.** An electron micrograph of the testis of BPA treated rats showing some degenerated spermatids (Sd) and primary spermatocytes (SP) with mitotic division nuclei (N) and cytoplasmic damaged (asterisks) and vacuolation (V). X 15000. **C.** An electron micrograph of the testis of BPA treated rat's showing primary spermatocytes (SP) with cytoplasmic vacuoles (stars), excessive lipid droplets (L), corrugated nuclear envelope (arrow). The cytoplasm contains numerous mitochondria (m). Notice vacuoles (v) in neighboring cells. X 5000. **D.**An electron micrograph of the testis of BPA treated rats showing distorted spermatids (Sd) with shrunken nucleus (N) and marked accumulation of cytoplasmic vesicles (V) and shrunken pyknotic nucleus (N). The primary spermatocytes (SP) are also seen. Notice wide separation (s) between neighboring cells. X 15000

constituents of the testis. The basal lamina restored its normal structure and sustentacular cells retained their normal organelles except for the presence of a few degenerated mitochondria. The basement membrane and spermatogonia appeared normal (Fig. 5A). The spermatids retained their normal structure except for the presence of a few vacuoles. They had rounded nuclei and numerous mitochondria located in one layer subjacent to the cell membrane. The primary spermatocytes and sperms appeared nearly normal (Fig. 5B).

DISCUSSION

Exposure to BPA resulting from consuming food in containers which have BPA in their component, for example baby bottles, table ware and cans for food preservation which are coated with epoxy resins producing a direct absorption as an exposure pathway (Heindela *et al*., 2015). Recently, it has been revealed that BPA can be transmitted directly

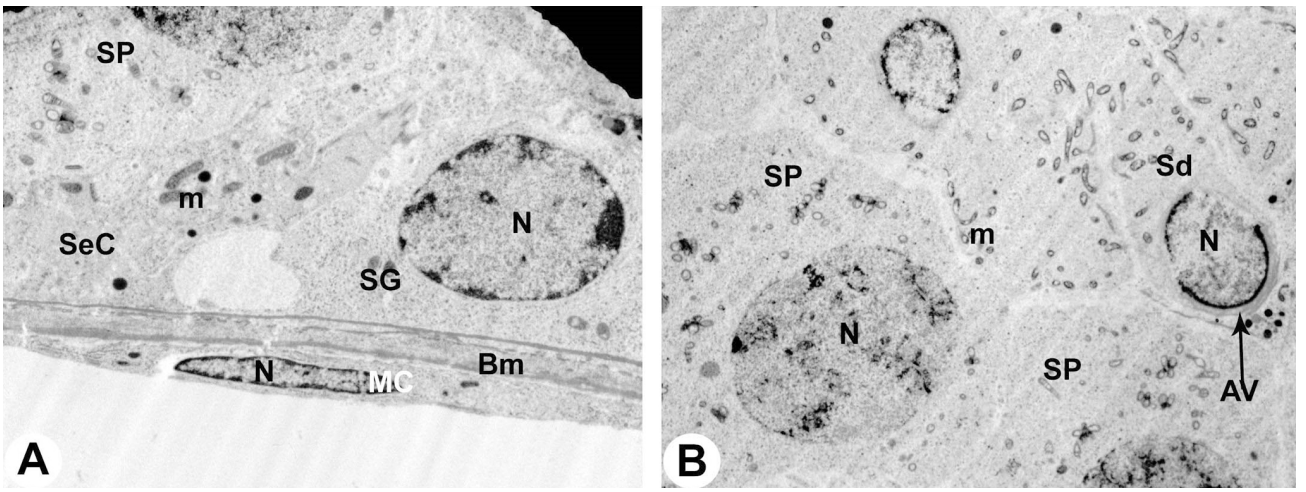

Fig. 5. Transmission electron micrographs (TEM) of BPA plus Se treated rat testis: **A**. An electron micrograph of a section of testis of a rat treated with BPA simultaneously with Se showing thin and regular basement membrane (Bm). The spermatogonia (SG) with rounded nuclei (N) and sustentacular cells (Sec) retained their normal structure and contained normal mitochondria (m). Note normal enclosing myoid cell (MC) with central flat nucleus (N). X 5000. **B.** An electron micrograph of a section of testis of a rat treated with BPA simultaneously with Se showing spermatids (Sd) with rounded nuclei (N), numerous mitochondria (m) and acrosomal vesicles (AV), located in one layer subjacent to the cell membrane. X 15000.

through the skin from some types of thermal printing paper, for example: cashier's receipts (Helal *et al*.). Subsequently, BPA is a universal synthetic material in the environment found nearly in all examined serum samples taken from people in developed countries; it can be found in human serum, urine and placental tissue samples, amniotic fluid, and blood taken from umbilical cord (Bosch *et al*.).

Reproductive function eventually determined by the suitable organization of the hypothalamic-pituitary-gonadal (HPG) axis, proper harmony of the neurological and endocrinal systems including the hypothalamus; the pituitary gland, found underneath the brain; and the gonads (Heindela *et al*.).

The light microscopic observations of our study in the treated group by BPA showed disorganization and degenerated seminiferous tubule surrounded by myoid cells and contained damaged spermatogonia with vacuoles, sustentacular cells and degeneration in both primary and secondary spermatocytes. Pleomorphic spermatids, abnormal blood vessels and abnormal interstitial Interstitial cells are laying in the interstitium between the tubules. The increased level of FSH can affect the sustentacular cells which found in the basement membrane of the somniferous tubules and provide the environment necessary for germ cell differentiation and maturation of testis tissues (Sweeney *et al*., 2000). Decreased level in LH hormone can reduce Leydig cell steroidogenesis. Interstitial cells are scattered in the connective tissue between the coiled somniferous tubules which produce testosterone in response to LH, so, testosterone hormone level can be lowered (Akingbemi *et al*., 2004).

These changes are confirmed by ultrastructural examination such as Sertoli cell exhibited enlarged intracellular vacuoles and electron dense bodies, swollen mitochondria, cell debris and cytoplasmic vacuoles (Gurmeet *et al*.). The electron microscopic examination of seminiferous tubules of BPA treated male rats revealed that there was extensive cytoplasmic vacuolation of some of the lining cells (Gurmeet *et al*.). These may be attributed to the ionic and osmotic imbalance leading to inhibition of water causing cellular vacuolation which is known to be a sort of cell degeneration (El-Gerbed, 2013). Also, the vacuolization of the germinal cells and sustentacular cells may be due to the dilation of smooth endoplasmic reticulum that possibly represents cellular permeability changes (Creasy & Foster, 2002). Moreover, 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 (Liu *et al*., 2013).

The ultrastructural changes in testis of BPA treated male rats may be due to BPA-induced lipid peroxidation 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 (Blanco-Rodríguez & Martínez-García, 1998). Several lines of evidences investigate the beneficial effect of natural and synthetic antioxidant in protecting male reproductive system from toxic effect of xenobiotic substances (Rezvanfar *et al*., 2013; El-Gerbed). Since the tested compound is endocrine disruptors, it induced oxidative stress in testis, liver and kidney (Bindhumol *et al*., 2003). Oxidative stress influenced by excess reactive oxygen species (ROS) produced in mitochondria and microcosms is known to damage nucleic acid, lipid and proteins (Chitra *et al*., 2003). This can lead to various diseases, including cancer, infertility and neuro-degenerative diseases (Kabuto *et al*., 2004; Zhang *et al*., 2005).

The obtained results revealed a marked improvement in the testicular structure in the group given BPA with Se when compared with group given only BPA. It has been reported that sodium selenite protects testicular gametogenic and spermatogenic disorders against carbimazole and prevents testicular oxidative stress by increasing the antioxidant status (Long *et al*., 2016). Recently, Erkekoglu *et al*. (2012) reported that supplementation of Se to di (2-ethylhexyl) phthalate-treated rats lowered the activity of testicular germ cell apoptosis. In another study, a notable decrease in the apoptotic index of spermatogenic cells was recorded after scrotal hyperthermia in Se-supplemented mice (Kaur & Bansal, 2015).

Concerning the biochemical results, exposure to BPA led to significant changes in the activity of antioxidant enzymes in rats. Specifically, BPA caused an elevation in the lipid peroxidation marker, testicular malondialdehyde (MDA) and a reduction of the CAT, SOD, and GPx antioxidant enzymes in rat testes. These results confirmed that BPA induced oxidative stress and changes in SOD and CAT levels in testes, reduce the epidydimal sperm count, and cause abnormal reactions with radical oxygen in several biological systems (Aitken & Roman, 2008). As such, antioxidant enzymes can become inhibited with an increase in the lipid peroxidation products such as MDA (Obata & Kubota, 2000).

The results also showed that co-administration of Se led to a decrease in MDA and an increase in the activities of CAT, SOD, and GPx. These effects are likely linked to a suppression of GPx1 gene expression, and GPx4, leading to a disruption in protein oxidation (Fátima *et al*., 2013). Ahmad *et al*. (2012) reported that changes in antioxidant enzyme activities (CAT, SOD) are related to the overexpression of the selenoprotein iodothyronine deiodinase, thus leading to the high-level production of reactive oxygen species (ROS). Some studies also suggest that the stimulation of antioxidant activity by Se may be related to the effects of GH and IGF in vitro (Aksu *et al*., 2013), which would promote oxidation and diminish GPx activity when they act as somatic growth inductors. Therefore, Se and GPx, among other selenoproteins, may thus play a key role in biological development, namely by preventing concomitant oxidation.

CONCLUSION

The results may confirm that Se countered the oxidant effects and increased the BPA-induced stress response in rats. Hence, Se promotes the healthy growth and development of mammals by protecting them from oxidative stress.

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RESUMEN: El bisfenol A (BPA) es un químico industrial ampliamente utilizado para fabricar plásticos de policarbonato para envases y resinas epoxi. Este estudio examinó el efecto de selenio (Se) en la toxicidad del BPA en términos de la estructura histológica, enzimas antioxidantes y los órganos reproductivos (túbulos seminíferos) de ratas albinas. Se dividieron veinticuatro ratas macho adultas en cuatro grupos experimentales: Grupo 1: control; Grupo 2: BPA administrado por vía oral; Grupo 3: BPA administrado por vía oral para; Grupo 4: tratado diariamente con BPA seguido de selenio (Se). El experimento se realizó durante cuatro semanas y se observó que la exposición al BPA provocó cambios en la estructura histológica testicular, incluyendo apoptosis, y alteraciones en varios marcadores bioquímicos:malondialdehído, catalasa, superóxido dismutasa y glutatión peroxidasa. Sin embargo, estos efectos secundarios del BPA pueden mejorar en ratas tratadas con BPA-plus-Se. Estos efectos protectores del Se pueden ser atribuidos a la capacidad de eliminar agentes oxidantes potencialmente dañinos en organismos vivos. Los resultados indicaron que se contrarrestaron los efectos oxidantes y aumentó la respuesta al estrés inducido por BPA en ratas, y favorece el crecimiento y desarrollo en los mamíferos al protegerlos del estrés oxidativo. Debido a la exposición al BPA en el ser humano, se puede acumular en los tejidos, por lo que existe una preocupación por el daño a las funciones reproductivas en particular de los trabajadores que generalmente están expuestos a nive-

les más altos de BPA. Por lo tanto, se debe restringir el uso de BPA en las industrias y evitar el uso incorrecto de envases de plástico para así disminuir los riesgos para la salud. La administración correcta de Se puede proteger contra los efectos adversos del BPA en las funciones y estructuras reproductivas.

PALABRAS CLAVE: Bisfenol A; Selenio; Testículo; Enzimas bioquímicas; Histología; Ultraestructura; Análisis estadístico.

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