Histological study of the possible protective effect of pomegranate juice on bisphenol-A induced changes of the caput epididymal epithelium and sperms of adult albino rats

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Objectives: The aim of our study was to throw light on the possible protective effect of pomegranate juice (PJ) after experimental oral administration of the widely used plasticizer Bisphenol A (BPA) on the epididymis by using light and electron microscopes, as well as cauda epididymis sperm count.

Methods: Forty adult male albino rats were divided into two main groups; (control and treated groups) Control group which was further subdivided into two equal subgroups group (Ia) was given corn oil (5 mg/kg) and the other group (Ib) given oral PJ 1 ml daily for 8 weeks. The other group was subdivided into two subgroups, one group (IIa) was given BPA dissolved in corn oil in a dose of 20 µg/kg, while the other group (IIb) given BPA in the same dose combined with 1 ml of PJ daily both for 8 weeks. All administrations were given by oral gavage. The rats were sacrificed. The right
1. Introduction

Bisphenol A (BPA) is an organic plasticizer used to line the majority of metal cans for food and beverages as well as for the coating of metal lids for glass jars and bottles. It is also found in some plastic food containers, all disposable plastics, toys, dental devices, dental fillings and sealants.

BPA may enter the human body primarily by penetration and mixing with food and water from plastic containers and to the saliva from dental sealants. Leach also occurs from the plastic lining of canned food that are cleaned with harsh detergents containing acidic or high temperature liquids or during autoclaving and sterilization.¹

Recently, Von et al.² had suggested that polycarbonate baby bottles that are made from BPA are the most evident route of exposure in infants, and canned food in adults and teenagers. Therefore, Toys-R-U-U decided to cease selling baby bottles made from BPA and there was an attempt to remove polycarbonate drinking products from their shelves.³

Investigators have reported concentrations of BPA and its metabolites in the saliva of patients 1 h after a sealant was applied to their teeth,⁴ and was mainly detected in the solid portion of canned fruit and vege.⁵ Therefore, attention has been drawn towards the possibility that even low doses of BPA could possibly affect human development and reproduction.⁶,⁷

Virgili et al.⁸ demonstrated the nutritive value of the pomegranate fruit and that a 250 ml glass of pomegranate juice provides approximately 50% of an adult’s recommended daily allowance (RDA) of the vitamins A, C and E. It was revealed that the antioxidant polyphenols account for about half of the fruit’s antioxidant ability to scavenge free radicals.⁹ However storage of the fruits and industrial food processing may affect the content of these antioxidants.¹⁰

Moreover, the strong free-radical scavenging potential of PJ has been proven to be useful in fighting certain cancers.¹¹,¹²

Recent research work concerned with treatment of male infertility problems has issued the existence of a link between the antioxidant-rich juice and male fertility, which could make pomegranate an important food supplement.¹³

The present experimental study is therefore designed to use a histological approach for evaluating the possible protective effect of PJ against BPA induced epididymal alterations in albino rats.

2. Methods

Forty adult male albino rats with an average body weight ranging from 150 to 200 g and an average age of 13–15 weeks were used in this study. The animals were housed in clean stainless steel cages and kept under the same environmental conditions regarding light, feeding and temperature. The handling of animals followed the rules for experimental research ethics approved by Research Ethics Committee at Alexandria Faculty of Medicine.

The animals were randomly divided into two main groups:

Group I (Control group): Included twenty adult male rats that were subdivided into:

Subgroup Ia (negative control group): It included ten rats which received a daily oral gavage of corn oil (5 ml/kg) vehicle.¹⁵

Subgroup (positive control group) Ib: It included ten adult male rats that received a daily oral gavage of pomegranate juice (PJ) in a dose of 1 ml.¹⁶

Group II (experimental group): It included twenty rats that were subdivided into two equal subgroups, ten rats each:

Subgroup IIa: received BPA dissolved in 5 ml/kg corn oil daily in a dose of 20 µg/kg body weight.¹⁷

Subgroup IIb: Rats were given an oral daily dose of 1 ml PJ combined with BPA in the same regimen as subgroup IIa. Bisphenol A (BPA) was supplied by Sigma Company USA as 97% purity in the form of white powder. The juice was extracted freshly. All administrations were given by an oral gavage for 8 weeks.

At the designated time, rats were sacrificed by decapitation. Both the right and the left epididymides were dissected; the right caput epididymis was used for the light and ultrastructural study.¹⁸,¹⁹ The grids for EM were examined and photographed by Joel 100CX transmission electron microscope at the Electron Microscopy Unit, Faculty of science, Alexandria University.

The left cauda epididymides from each rat of all groups were squeezed to release spermatozoa. Sperm suspensions were collected by centrifugation and the total sperm count per rat was determined by using a counting chamber (Neubauer’s chamber).²⁰ The sperm count was done in the Clinical Pathology Department, Faculty of Medicine, Alexandria University.

The aim of studying the sperm count was to assess the effect of BPA on the count and to evaluate the potent effect of PJ intake on the sperm count.
2.1. Statistical analysis

Description of the data was done by Mean ± SD and Median. Analysis was done using Mann-Whitney test. M.W Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Histological results

Light microscopic examination of the H&E sections of the control rat caput epididymis (group Ia) showed the normal histological features of normal rat epididymis with regular outlined tubules lined with pseudo-stratified columnar epithelium. Apical cell (A) is seen in between the lining cells. Principal cells (Pr) appearing tall columnar with stereocilia (st), basal cells (arrows) lying on the basement membrane, halo cell (H), (a,b) showing little of peritubular vascular connective tissue (CT) and adequate amount of spermatozoa (S) in the lumina. (H&E stain. Mic. Mag. a x100; b x400).

Figure 1  Light photomicrographs of the caput epididymis of a control rat of group Ia. (a) Regular outlined tubules. They are lined with pseudo-stratified columnar epithelium. Apical cell (A) is seen in between the lining cells. (b) Principal cells (Pr) appearing tall columnar with stereocilia (st), basal cells (arrows) lying on the basement membrane, halo cell (H). (a,b) showing little of peritubular vascular connective tissue (CT) and adequate amount of spermatozoa (S) in the lumina. (H&E stain. Mic. Mag. a x100; b x400).

Figure 2  Light photomicrographs of the caput epididymal tubules of group IIa. (a) Wide intertubular spaces (ITS). (b) Principal cells with deeply stained nuclei (N) widening of the intercellular spaces (arrows) and the lumen contain cellular debris (D). (c) Some principal cells (Pr) show deeply stained cytoplasm. (d) Halo cells (H) are apparent at different levels. The cytoplasm of the principal cells (Pr) show vacuolation (V) (H&E stain. Mic. Mag. a x100; b–d x400).
The cellular structure of regular caput epididymal tubules. Many circular profiles of the epididymal tubules were seen representing cut sections of the supercoiled duct of the epididymis. All the tubules were lined by pseudo-stratified columnar epithelium showing minimal amount of intertubular connective tissue (arrow). (b) Principal cells are the majority (Pr), fewer apical cells (A) halo cell (H), and basal cell (B). (a, b) show good amount of spermatozoa (S) (H&E stain Mic. Mag a×100, b×400).

The epithelium lining the epididymal tubules was composed mainly of principal and basal cells. The principal cells, being the most numerous cells extended from the basement membrane to the lumen. They were closely arranged with no spaces in between. They showed rounded, pale basally located nuclei. Principal cells were topped at their apical border with numerous long stereocilia bordering the lumen (Fig. 1b).

The basal cells were scattered in a few number between the bases of the principal cells, lying on the basement membrane. They showed small amount of cytoplasm and horizontally elongated oval nuclei. Few halo cells, were depicted as...
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3.1.1. Ultrastructural study

Ultrastructural examination of the control group of rat caput epididymis (group Ia) showed the normal components of principal, basal, apical and halo cells. Clear cells were rarely seen in between the lining cells of the caput epididymis.

The principal cells constituted the predominant cell type of the lining epithelium. They appeared as tall columnar cells with their nuclei rounded, euchromatic, regular and located in the basal half of the cells. The adluminal cytoplasm showed few endocytic vesicles. The adjacent cells were connected by intact junctional complexes with no apparent intercellular spaces. (Fig. 4a). Well developed Golgi complexes were depicted in the supranuclear region (Fig. 4b). The subnuclear cytoplasm exhibited closely packed, parallel cisternae of rough endoplasmic reticulum (Fig. 4c). The cytoplasm showed some mitochondria and few lysosomes. (Fig. 4b and c).

As regards the clear cells, they were very few and they appeared paler than the surrounding principal cells without intercellular space in between. They further exhibited basal nuclei and few lysosomes (Fig. 5a). The halo cells appeared rounded or polygonal in shape mostly located near the basement membrane. They had central rounded to oval nuclei and a clear electron lucent cytoplasm (Fig. 5a).

The basal cells appeared smaller than the principal cells, with small amount of cytoplasm. The cytoplasm surrounded a horizontally placed oval nucleus. They lie in close contact with the neighboring principal cells. Its cytoplasm showed few organelles (Fig. 5b).

The apical cells appeared in close apposition to the nearby principal cells. They exhibited a denser cytoplasm (Fig. 5c).

Ultrastructural examination of the control group of rat caput epididymis (group Ib) showed normal appearance of the principal cells without any intercellular space in between (Fig. 6a). Their cytoplasm showed prominent vesicular trafficking in the form of multiple vesicles, apically located as well as along the lateral and basal surfaces. (Fig. 6a and b).

The adluminal cytoplasm further exhibited numerous endocytic vesicles and coated pits. The apical cell membrane showed numerous microvillus projections into the lumen (Fig. 6b).

The cytoplasm of the lining principal cells showed well developed Golgi complexes, mitochondria with normal histological structure and few lysosomes (Fig. 6c), as well as the basal portion shows parallel cisternae of the rough endoplasmic reticulum (Fig. 6d). The basal cells were in normal contact with the neighboring principal cells (Fig. 6d).

Apical cell was seen in between the principal cells with no intercellular spaces, its nucleus was located above those of the neighboring principal cells (Fig. 6a).

Ultrastructural examination of the rat caput epididymis revealed widening of the intercellular spaces between the lining principal cells of most of the tubules. Also there were separations between the principal cells and the basal cells (Fig. 7a). The nuclei of some cells appeared dense

Figure 5 Electron photomicrographs of control group Ia. (a) showing a clear cell (C) in close contact with the adjacent principal cells (Pr) and a halo cell (H). (b) basal cell (B) exhibits close relation to the overlying principal cell (Pr). The latter shows parallel cisternae of rough endoplasmic reticulum (r ER). The basal cell cytoplasm shows few organelles in the form of few mitochondria (M) and few profiles of rough endoplasmic reticulum (arrow). sm; smooth muscle (sm). (c) Apical cell (A) in between the principal cells (Pr) with intact junctional complexes (J), halo cell (H). The cytoplasm of the principal cell shows Golgi complex (G) (Mic. Mag. a ×5000, b ×7500, c ×3000).
and irregular in outline (Fig. 7b). The cytoplasm of some cells showed vacuolation (Fig. 7b).

The cytoplasm of the lining principal cells showed dilated Golgi vesicles, (Fig. 8a), along with dilated profiles of rough endoplasmic reticulum were also demonstrated (Fig. 8b).

As regards the mitochondria, they showed degenerative changes in some cells in the form of swelling, focal loss of cristae and vacuolation. Moreover, the cytoplasm showed prominent lysosomes with heterogenous electron dense contents (Fig. 8c).

Figure 6  Electron photomicrographs of parts of caput epididymal tubules of group Ib. (a) showing closely related principal cells (Pr) with multiple vesicles (V). An apical cell (A) with its denser cytoplasm is also seen. (b) shows the intact junctional complexes (J) between the cells. Their cytoplasm shows numerous small vesicles (V), together with coated pits of the apical plasma membrane (arrows) and apical microvilli (MV). (c) cytoplasm of the principal cells show well developed Golgi complexes (G), mitochondria (M) and lysosome (L). (d) well developed rough endoplasmic reticulum (rER) with normal ultrastructure. The nucleus (N) of the principal cell is euchromatic. B; basal cell, sm; smooth muscle, J; junctional complexes, M; mitochondria (Mic. Mag. a ×4000; b,c ×10,000; d ×7500).

Figure 7  Electron photomicrographs of the caput epididymal tubules of group IIa. (a) showing multiple principal cells (Pr) with evident widening of the intercellular spaces (ICS). Basal cell (B) separated from the overlying principal cells. (b) principal cells show dense irregular nuclei (N) and cytoplasmic vacuoles (V). Clear cell (C) with cytoplasmic vacuolations (V). (Mic. Mag. a ×2000; b ×4000).
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Some areas of the apical surface of principal cells exhibited exfoliation of parts of the lining cells in the lumen of some tubes (Fig. 9a). Halo cells or lymphocyte-like cells were found at different levels, some were apical and others were basal (Fig. 9a and b).

The most striking feature in this group was frequent encountering of many clear cells in between the principal cells. The clear cells showed cytoplasmic vacuoles and dilatation of the rough endoplasmic reticulum, which was marked in some cells giving the cytoplasm a cribriform appearance (Fig. 9b–d).

Ultrastructural examination of the group IIb of rat caput epididymis (BPA + PJ) showed that the lining epididymal cells were in close apposition with intact junctional complexes in between all the lining cells. The principal cells’ nuclei were located basally and appeared euchromatic, regular in outline like those of the control (Fig. 10a).

The cytoplasmic organelles of the principal cells nearly showed normal ultrastructural appearance, the cisternae of the rough endoplasmic reticulum were displayed almost in regular pattern in most of the cells and also exhibited well formed Golgi complexes (Fig. 10b).

The clear cells were found in between the principal cells without apparent spaces in between, the cytoplasm showed multiple lysosomes and numerous vesicles. The rough endoplasmic reticulum revealed some dilated profiles (Fig. 11a).

The apical cytoplasm of the principal cell showed few apical vesicles and multiple tall microvillus projections. The mitochondria depicted normal internal ultrastrucrure appearance (Fig. 11b).

Apical cells showed normal ultrastructural appearance (Fig. 11a).

Ultrastructural examination of both control groups revealed normal appearance of the sperms in the lumina. The plasma membrane is completely surrounding the whole sperm pieces. The mitochondrial sheath and the outer dense fibers appeared normal. The latter displayed the normal arrangement of nine doublets radially arranged around the two central microtubules \(9 + 2\) arrangement. The sperms’ nuclei were heterochromatic and surrounded by the cell membrane (Fig. 12a).

The mitochondrial sheath was formed of equal sizes of mitochondria completely surrounding the outer dense fibers that were also normally arranged in both cut sections and longitudinal sections (Fig. 12b). The plasma membrane was completely intact covering the different segments of the sperms (Fig. 12a and b).

As regards the effects of BPA on the sperm ultrastructure in group IIa, the sections of different parts of sperms in the caput epididymal lumen of this group showed abnormal forms; some with retained cytoplasmic droplets containing multiple heads, the nuclei showed decondensation, (Fig. 13a and b) and the outer dense fibers were disorganized in relation to the axonemal microtubules (Fig. 13c).

The mitochondrial sheath depicted some abnormalities in the form of different sizes of the mitochondria, bulging, ballooning and even loss of the mitochondrial sheath in some areas leaving gaps in between. Some sperms showed absence of the overlying plasma membrane (Fig. 14a–c).

The luminal spermatozoa illustrated similar ultrastructure to those of the control groups Ia and Ib (Fig. 15).

3.2. II-Caudal sperm count: (Table 1)

The mean ± SD of the caudal epididymal sperm count was significantly lower in group IIa when compared to the control groups Ia and Ib as shown in Table 1.

Comparing the mean ± SD of the caudal epididymal sperm count in group IIa and that in group IIb, it was significantly higher in group IIb as shown in Table 1.

Moreover, the statistical analysis of the mean ± SD of the caudal epididymal sperm count of group Ib was significantly higher when compared with that of the control group Ia.

4. Discussion

The epididymis secretes into its luminal environment, region specific proteins and glycoproteins, thus providing the favorable milieu for post testicular maturation of the sperms. Hence, alteration in the epididymal structure or function might contribute to male infertility.21

Bisphenol A (BPA), as one of the commonly used plasticizers nowadays, can leach and contaminate food. Its effects on testosterone levels are controversial. Some studies found that BPA reduced fertility without alteration in androgen or luteinizing hormone.22 Other studies argued that BPA exposure can cause reduction in these hormones.23 A recent study in 2011 revealed that BPA caused reduction in estradiol production.

It has been found that the principal cause of idiopathic male infertility is an underlying pathological condition known as “oxidative stress”. Some endocrine disruptors such as BPA have been shown to induce oxidative stress, and result in the onset of various diseases, including infertility.25 BPA, in previ
infertility. Dative stress which was postulated to be associated with male H2O2 generation and lipid peroxidation, thereby inducing oxidative damage, requiring a greater antioxidant defense system. In the present study, the rough endoplasmic reticulum localized family proteins is termed ERp29 which regulates epididymal Cx43 trafficking and function. Interference with ERp29 function, as a result of oxidative stress, leads to accumulation of Cx43 and reduces its transport to the plasma membrane thus inhibiting gap junction formation and hence cell communication. This could explain the widening of the intercellular spaces shown in group IIa.

Moreover, affection of the aquaporin channels (AQP) by the BPA induced free radicals could result in intercellular edema formation. At the same time, it was shown that estrogen regulates expression of the AQP in the epididymis, thus estrogen has a role in regulating the absorptive function of the epididymal epithelium, a function that is essential for fertility. The antioestrogenic effect of BPA could down regulate the expression of AQP, with failure of the absorptive function of the caput epididymal cells and accumulation of the intercellular fluid and edema formation. This also could explain the resulted wide intercellular and intertubular spaces in group IIa.

Leung et al. postulated that the basal cells not merely function as stem cells, but also as hormonal regulator for the principal cells. They are essential for the integrated function of the principal cells as they regulate principal cell electrolyte transport of ions and water by releasing prostaglandin PGE2 that act on nearby principal cell. These basal cells were seen widely separated from the overlying principal cells in group IIa that was given BPA. Thus, loss of cell–cell interactions between the both cells affects the transport of ions and water which is reflected upon the epididymal functions. As a consequence, the luminal fluid is altered and this ultimately affect sperm maturation and fertility.

In the present study, the rough endoplasmic reticulum appeared to be affected in the principal and clear cells lining the caput epididymis. In any secretory cell, proteins and motile sperms that are ready for ejaculation, therefore the caput epididymis was selected in the present study for the purpose of obtaining more reliable sperm counts compared to that in the cauda epididymis. Several light and electron microscopic histological changes were observed after the administration of BPA in group IIa for 8 weeks. The caput epididymis revealed widening of the intertubular and intercellular spaces, cytoplasmic vacuolation, degenerative changes in the mitochondria and increase lysosomes. Dilated cisternae of the rough endoplasmic reticulum and the Golgi saccules were also depicted. Some nuclei were irregular and heterochromatic. All these ultrastructural alterations represent progressive degenerative stages affecting cell membranes integrity secondary to the oxidative stress induced by BPA. The free radical oxygen species initiate oxidative phosphorylation reactions to cell membranes ending in disruption of the integrity of intercellular junctional complexes.

A well known relationship between the cadherin/ catenin complex and gap junctional intercellular communication is documented. Direct intercellular communication is mediated by gap junctions, which proved to be a sensitive target of oxidative stress. Parrish et al. suggested that oxidative stress selectively disrupts cadherin/catenin complexes, and disrupts cell-cell adhesion, and involve connexin 43 (Cx43) which is a major gap junction channel-forming protein. In addition, there is strong evidence for the relation between disturbed endoplasmic reticulum function and its role in gap junctional formation. One of the rough endoplasmic reticulum localized family proteins is termed ERp29 which regulates epididymal Cx43 trafficking and function. Interference with ERp29 function, as a result of oxidative stress, leads to accumulation of Cx43 and reduces its transport to the plasma membrane thus inhibiting gap junction formation and hence cell communication. This could explain the widening of the intercellular spaces shown in group IIa.

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Figure 9 Electron photomicrographs of caput epididymal tubules of group IIa. (a) showing principal cells (Pr) with exfoliation (long arrows) and blebbing (short arrow) of their apical parts. H; halo cell. (b) Clear cell with cytoplasmic vacuolation is seen in between principal cells that show irregular nuclei (N), intact junctional complexes (J) and few apical microvilli (MV). Halo cells (H) are apparent at different levels. B; basal cell. (c) Showing clear cells (C) between the principal cells (Pr), basal cell (B) and smooth muscle fiber (sm). J; junctional complexes, L; lumen. (d) A clear cell with marked dilatation of the rough endoplasmic reticulum (r ER) giving the cytoplasm a cribriform appearance, irregular nucleus (N) and the wide intercellular space (ICS) (Mic. Mag. a ×4000, b ×5000, c ×3000, d ×5000).

The present work was conducted to study the toxic effect of BPA on the caput epididymis as it is the most metabolically active region secreting about 70–80% of the total overall protein secreted in the epididymal lumen. The caput houses a vast capillary network, and it shows the highest blood flow of all epididymal regions. As such, it is particularly prone to oxidative damage, requiring a greater antioxidant defense system. Moreover, being the site for storage of fully mature sperms that are ready for ejaculation, therefore the caput epididymis was selected in the present study for the purpose of obtaining more reliable sperm counts compared to that in the cauda epididymis. Therefore, the caput epididymis was selected in the present study for the purpose of obtaining more reliable sperm counts compared to that in the cauda epididymis.

Several light and electron microscopic histological changes were observed after the administration of BPA in group IIa for 8 weeks. The caput epididymis revealed widening of the intertubular and intercellular spaces, cytoplasmic vacuolation, degenerative changes in the mitochondria and increase lysosomes. Dilated cisternae of the rough endoplasmic reticulum and the Golgi saccules were also depicted. Some nuclei were irregular and heterochromatic. All these ultrastructural alterations represent progressive degenerative stages affecting cell membranes integrity secondary to the oxidative stress induced by BPA. The free radical oxygen species initiate oxidative phosphorylation reactions to cell membranes ending in disruption of the integrity of intercellular junctional complexes.

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Moreover, affection of the aquaporin channels (AQP) by the BPA induced free radicals could result in intercellular edema formation. At the same time, it was shown that estrogen regulates expression of the AQP in the epididymis, thus estrogens have a role in regulating the absorptive function of the epididymal epithelium, a function that is essential for fertility.
glycoprotein that traverse the secretory pathway mainly depend on disulfide bonds formation for their maturation and function and any disruption of this will prevent proteins from attaining their quaternary structure conformation and lead to protein misfolding and aggregation. BPA possesses an inhibitory effect on protein disulfide bonds formation therefore it might have a potential effect in disrupting various physiological functions. The dilated endoplasmic reticulum cisterna might be due accumulation of secretory products. In addition, it could be a reaction to cell injuries and disturbance in protein synthesis. In response to accumulation of unfolded/missfolded proteins, cells adapt themselves to the stress condition via the unfolded protein response (UPR). UPR decreases the biosynthetic burden of the secretory pathway by down regulating expression of genes encoding secreted proteins. When these mechanisms fail to rescue the cell apoptosis is initiated to eliminate the diseased cell.

Accordingly, the main mechanism for the BPA –induced degenerative lesions in cells lining the epididymis is attributed to oxidative stress which triggers the chain reactions of lipid peroxidation. Lipid peroxidation reactions involve the activation of a series of hydrolytic enzymes, which explain the increased observation of lysosomes in the epididymal cells in group IIa of the present study. The lipid peroxidation reactions also contribute to activation of endonuclease enzymes, which end up with break of nuclear DNA. This fact clarifies the present finding of cells with dense deformed nuclei.

The electron microscopic examination of the caput epididymal cells of group IIa revealed that there was extensive cytoplasmic vacuolation of some of the lining cells. These were most probably caused by ionic and osmotic imbalance leading to imbibition of water causing cellular vacuolation, which is known to be a sort of cell degeneration. The swollen mitochondria with disintegrated cristae was the result of increased reactive oxygen species production and alteration of the mitochondrial membrane permeability, leading to membrane depolarization. This finally ends with decreases in the generation of adenosine triphosphate (ATP).

The histological results of this study in lumen of group IIa revealed apical exfoliation of cells of the epididymal epithelium complying with the degenerative changes in the epididymis. It has been postulated that clear cell hypertrophy and an increase in their number and activity coincide with excessive phagocytosis of the abnormal structures in the lumina as cellular debris and abnormal sperms. Clear cells are also responsible for removing the immature damaged sperms, and the absorption of the cytoplasmic droplets of sperms.

The finding that intraepithelial halo cells were more frequently encountered and acquired a higher position towards the lumen is attributed to increased spermophagy along the excurrent ducts as a result of impaired spermatogenesis and increased number of abnormal cells, cell debris and foreign cells leading to more pronounced macrophagic activity.

Examination of the protection group of rats (group II) (BPA + PJ) showed that the lining epididymal cells were in close apposition. The cytoplasmic organelles of the cells showed nearly normal ultrastructural appearance.
Cisternae of the rough endoplasmic reticulum were displayed almost in regular pattern in most of the cells and also exhibited well formed Golgi complexes. Several studies proved the protective effect of PJ, with its powerful antioxidant effect. In this study, group IIb showed no apparent intercellular spaces between cells suggesting the antioxidative role of PJ in improving such changes seen in group IIa. Increasing evidence also suggests that retinoic acid receptors (RAR) were more in the principal cells of the caput, than in other regions of the epididymis. As PJ provide the daily recommended dose of vitamin A and it has an inhibitory effect on the estrogenic activity of BPA. This might explain the close apposition between the lining cells as well as between the adjacent tubules. Similarly, a previous electron microscopic study has revealed the protective effect of vitamin-E which led to lesser histological changes, and regaining of the normal intact junctional complexes between adjacent cells.

This was explained to be a result of the antioxidant effect of vitamin-E that could cancel out the cell-damaging effects of free radicals. The antioxidant enzymatic activities, after PJ intake, were found to be increased and it also protects the DNA from free radical damage. Cárcamo et al. showed that vitamin C could protect mtDNA against oxidative damage thus preventing mitochondrial degenerative changes.
Previous studies showed that similar changes, seen in group IIa (BPA), were reversed after the addition of vitamin C to diet. This is because vitamin C has a role in protein maturation, oxidative protein folding, rapid reaction with many unfolded or malfolded proteins, eliminating ER stress, and promoting protein disulfide formation, which at the same time, requires the participation of vitamin E. Treatment with both vitamin C and E produce improvement in protein content, they act to decrease damaged protein in tissues and play an important role in stimulating intercellular signals for activation of the gene responsible for protein synthesis. Polyphenolic compounds as those found in PJ can rescue the rough endoplasmic reticulum oxido-reductase function and maintain its function.

Furthermore, polyphenols can prevent the formation of misfolded protein forms that accumulate upon exposure to oxidative stress. PJ intake, in the current study, with its content of polyphenolic compounds and vitamin C showed improvement of the rough endoplasmic reticulum cisternae which mostly resumed its normal ultrastructural appearance and probably regained its secretory role in protein synthesis, thereby increasing the secretory function of the principal cells and improving the microenvironment through which sperms are traversing and hence this could improve fertility.

Previous studies have proved that the Golgi apparatus in cells receiving vitamin A showed significant increase both in the number of cisternae per Golgi apparatus stack and in the number of transition vesicles of the cis Golgi apparatus face. These vesicles are involved in the transfer of membrane material to the Golgi apparatus from endoplasmic reticulum, increasing the secretory function. Therefore, the size of the associated Golgi vesicles seen in group IIb and Ib was an indication for the increased cellular secretory activity. The most striking sperm changes were observed in group IIa rat epididymis. They revealed chromatin decondensation of sperm heads, complete absence of the plasma membrane over the entire tail as well variable alterations in the mitochondrial sheath.

Studies proved that the male specific steroidogenic cytochrome p450 isoform (cytochrome p45017), that catalyze the oxidation of androgen, is necessary for the structure and organization of the sperm mitochondria and that BPA inhibited CYP17 activities. This might explain the changes depicted in sperm mitochondria. BPA also induces overproduction of hydrogen peroxide which is a powerful membrane permeant oxidant that has to be rapidly eliminated from the cell.

The elimination of H2O2 is done by glutathione peroxidase isofrom (GPX5), which is expressed exclusively in the caput epididymis and constitutes 6% of the secretory epididymal proteins. Thus, the protection of the sperm membrane against peroxidation is a function of this epididymis-specific isofrom which if failed causes infertility. In addition it was found that sperms that lack an intact plasma membrane are unlikely to be motile. Sperm membranes harbor a higher concentration of polyunsaturated fatty acids (PUFA) than other human cells. These fatty acids are extremely sensitive to oxidative stress causing instability in membrane permeability and ultimate damage that might cause loss of sperm motility, abnormal sperm morphology, reduced capacity for oocyte penetration and infertility.

Statistical analysis of the caudal sperm count in group IIa given BPA revealed marked reduction in sperm counts compared to control groups. A dramatic decrease in caudal epididymal sperm numbers was previously considered to be the result of a lower sperm output by the testis and to increase sperm resorption (phagocytosis) in the rat testis and epididymis as a result of increase the number of mal-formed sperms.

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<th>Table 1</th>
<th>Comparison between the different studied groups according to number of sperms.</th>
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<td>Group Ia</td>
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<tr>
<td>Number of sperms</td>
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<tr>
<td>Range</td>
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<tr>
<td>Z2 (p)</td>
<td>3.790* (&lt; 0.001)</td>
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<td>Z3 (p)</td>
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Z1: Z for Mann Whitney test between group Ia and other groups.
Z2: Z for Mann Whitney test between group Ib and other groups.
Z3: Z for Mann Whitney test between group IIa and group IIb.
Statistically significant at p < 0.05.
In group IIb (BPA + PJ) well organized mature sperms were seen in the caput epididymal lumina. Moreover, the present work revealed a significant high caudal sperm count in animals given PJ. Vitamin A, provided by PJ, is required for the maturation process of epididymal spermatozoa, together with the potent antioxidant role of PJ\(^13\) might explain the protective effect of this juice on sperms. A study also showed that the rats which drank concentrated PJ revealed increase in spermatogenic cell density, sperm motility, epididymal sperm concentration as well as decrease in abnormal sperm rate.\(^{14,54}\)

In conclusion, the present study showed that BPA produced remarkable histological degenerative changes in the epithelium and the sperms of the caput epididymis, as well as causing marked decrease in the number of caudal sperm count. It is assumed that these changes would interfere with the proper function of the epididymis and accordingly contribute to infertility. On the other hand, the results obtained proved the PJ potentials to counteract all BPA induced degenerative changes thus allowing recovery of the rat epididymis from oxidative stress and hence contributing in improving male fertility.

Therefore, it is recommended to supplement PJ in male individuals suffering from infertility.

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References


