The possible protective effect of Omega 3 fatty acids against Bisphenol A induced disruption of pituitary-testicular axis in albino rat. Biochemical, Histological and Immunohistochemical study

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ABSTRACT

Background: Infertility is a major health problem affecting human life. Multiple factors contribute to male infertility. The most important one is exposure to environmental contaminants (e.g. Bisphenol A (BPA)).

Aim of the work: To study the possible protective effect of Omega 3 against BPA induced changes in the pituitary testicular axis in rats.

Material and method: 30 adult male albino rats were used in the study. They were divided equally into 3 groups 10 animals each. Group I served as control. Group II received BPA in a dose 1.2 mg/kg orally 6 days a week. Group III received Omega 3 in a dose 0.4 g/kg subcutaneously in addition to BPA of the same previous dose and duration. At the time of sacrifice, all rats were anesthetized with ether. Blood samples were collected for estimation of testosterone, LH, FSH and prolactin. The testes and pituitary were dissected out and processed for histological and immunohistochemical study for Caspase 3, PCNA and prolactin. The number of Caspase 3 and PCNA positive cells were counted and statistically analyzed.

Results: Marked decrease in serum LH, testosterone with marked increase in serum prolactin was observed in BPA treated group. Spermatogenic cells were disorganized and degenerated. Spermatids had fragmented pyknotic nuclei. A significant increase in the rate of apoptosis and a significant decrease in the rate of proliferation of germ cells were detected on BPA exposure compared to the control. Examination of pars distalis revealed degenerative changes in acidophils and basophils in group II animals with decreased intensity of reactive mammotrophs. These changes were ameliorated in group III by administration of Omega 3 fatty acids.

Conclusion: Omega 3 may protect against BPA hazardous effects through pituitary testicular axis pathway.

INTRODUCTION

About 50% of couple's infertility is due to male factors. Male factors roughly could be classified into genetic (chromosomal abnormalities) or non genetic. Regarding non genetic factors, at least 10% are exogenous and reversible such as the impacts of lifestyle, environmental and psychological factors.

A variety of chemical compounds have been released into the environment due to marked expansion of chemical industries worldwide. Male exposure to these chemical contaminants, which are estrogen mimics and endocrine disruptors, has been claimed as one of the causative factors contributing to the increasing male infertility.

Bisphenol A (BPA) [2.2-bis (4-hydroxyphenyl) propane] is one of the endocrine disrupting chemicals. It is used in the manufacture of many products as water pipes, baby bottles, lunch boxes and toys. Humans may gain exposure to BPA through various routes like the air, absorption through the skin, fresh and marine surface waters and groundwater.

Under the effect of the hypothalamic GnRH the hypothalamic controls androgen biosynthesis; this in turn stimulates LH and FSH release from the pituitary. LH in turn stimulates Leydig cell to secrete testosterone.

Omega-3 polyunsaturated essential fatty acids are found in large amounts in fish oil. It contains eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Essential fatty acids are vital for many functions including growth, reproduction, vision, and brain development. Additionally, could affect steroidogenesis and some transcription factors controlling gene expression. Previous studies documented the antioxidant, anti apoptotic and anti inflammatory effects of omega-3 fatty acids on various tissues exposed to stress and damage.

Being a synthetic estrogen with the capability of binding to estrogen receptors in reproductive organs, BPA could disrupt the normal mechanism. So our hypothesis in this study is that BPA may affect male fertility through disruption of pituitary testicular axis and that omega-3 can protect against this harmful effect.
MATERIALS AND METHODS

Animals:
This study was carried out in accordance with the guidelines of the University Animal Ethics and approved by Research Ethics Committee considering care and use of laboratory animals. 30 adult male albino rats about (200-250) gm weight were used for this study. They were obtained from Animal facility, Faculty of Medicine, Assuit University. All rats were kept under observation for two weeks prior to the experiments to permit the animals to adjust to the environments. The animals were housed in standard suitable cages (20 x 32 x 20 cm for every 4 rats) in controlled temperature room (23°C ± 1°C) with normal light and dark cycle. They were maintained on a standard diet of commercial rat chow and tap water.

Experimental design:
Animals were divided into 3 groups 10 animals each as follow:

Group I: Animals were served as the control group

Group II: Animals were received BPA dissolved in distilled water and given in a dose 1.2 mg/kg orally via gastric gavage 6 days a week for 3 weeks. The dose chosen below a dose that is previously considered safe[12,13].

Group III: Rats were received omega-3 in a dose (Fresenius Company, Germany) of 0.4 g/kg subcutaneously in addition to BPA in the same previous dose and duration[14].

Materials:
BPA (purity >99%) was purchased from Cornel Lap Company, Cairo, Egypt.

Omega-3 used in this study was purchased from Corniel Lap Company, Cairo, Egypt.

3-Monoclonal antibodies for immunohistochemical study;

AntiPCNA, anti caspase 3 and antiprolactin antibodies. They were purchased from Medico company, Cairo, Egypt.

Hormonal assay
At the end of experimental period, blood samples were collected from the retro-orbital venous plexus by using heparinized capillary tubes (about 0.75 – 1.0mm internal diameter) inserted in the medial canthus. The collected blood samples were kept in dry graduated plastic centrifuge tubes until coagulated. Blood samples were centrifuged at 4000 rpm for about 10 minutes to separate the serum. The serum was sucked out into Eppendorf tubes and all specimens of sera were stored at -20°C until used for the determination of:

- Testosterone[15].
- LH, FSH and prolactin[16].

These hormones were measured by an ELISA kit (R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturer’s protocol.

Histological and immunohistochemical study
Animals were anaesthetized using ether inhalation then they were sacrificed. Testicular specimens were fixed in Bouin solution while pituitary specimens were fixed in 10% formalin and processed for histological and immunohistochemical examination as follow:

Haematoxyline and eosin for general histological examination.

PCNA and Caspase 3 antibodies for assessment of proliferation and apoptosis of spermatogenic cells respectively.

Prolactin antibody for demonstration of mammotrophs.

Immunohistochemical staining procedure: Paraffin-embedded tissue sections were prepared and mounted on coated glass slides. The sections were deparaffinized and rehydrated through descending grades of alcohol. Endogenous peroxidase activity was blocked with 0.6% hydrogen peroxide for 10 min using a peroxidase blocking reagent. Antigen retrieval was performed by boiling the slides in citrate buffer solution (pH 6.0). The slides were microwaved at high medium for 10 min. The sections were incubated with the following dilutions of primary antibodies; anti-caspase 3 (1/200) and anti-PCNA (1/400) and for antiprolactin (1/100) at 4°C overnight, washed, and incubated with biotinylated secondary antibodies; they were then incubated with the avidin–biotin complex. Finally, the sections were counterstained with hematoxylin, dehydrated, cleared, and mounted[17]. Negative control sections were prepared with omission of the primary antibody.

Morphometric study
Using Leica Qwin 500 C microscope at the Histology Department, Faculty of Medicine, Sohag University, the number of PCNA and Caspase 3 positive cells were counted in 10 non overlapping high-power fields.

Statistical analysis
Statistical analysis was done using the computer software program SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as means ± S.E. Statistical significance for data was determined using a one-way analysis of variance (ANOVA), followed by a post-hoc test to make multiple comparisons between the 3 groups. Also correlation coefficients were done. $P<0.05$ was considered statistically significant.

RESULTS

Biochemical results:
In BPA treated group (Group II), a significant decrease in both of testosterone and LH hormone levels was found ($P<0.05$). While there was a significant increase in prolactin hormone levels compared to the control group. There is a non significant increase in FSH levels when compared to control animals ($P>0.05$) (Table 1) and (Histograms 1, 2).
After Omega-3 administrations (Group III) there was a significant increase in the levels of testosterone and LH hormones \((P<0.05)\), in association with a significant decrease in prolactin level when compared to BPA treated group. Omega-3 returns levels of these hormones to normal as compared to control group \((P>0.05)\). There was a non significant increase in FSH levels when compared to control animals \((P<0.05)\) (Table 1) and (Histograms 1, 2).

**Histological examination of the testis:**

Examination of the control group revealed that, the testicular parenchyma was formed of closely packed seminiferous tubules which are surrounded with basal lamina, myoid cells and fibroblasts. The tubules are lined with many layers of spermatogenic cells namely, spermatogonia, primary spermatocytes, spermatids and sperms. Seminiferous tubules were separated by a narrow interstitium containing Leydig cells, macrophages and blood capillaries. Sertoli cells with their large pale nuclei were observed between spermatogenic cells (Fig.1&2).

BPA treated group (Group II) examination showed thickened capsule and irregular outline of the seminiferous tubules. The spermatogenic cells were disorganized and degenerated and exhibited basophilic cytoplasm with intercellular vacuoles. Apparent decrease in the number of sperms within the seminiferous tubules compared to the control group. Some tubules have dislocated germ cells within the lumen (Fig.3&4). Spermatids have fragmented pyknotic nuclei (Fig.5).

Homogenous acidophilic material with some vacuoles (transudate, interstitial edema) was observed in the interstitial space in addition to congestion of blood vessels was noticed (fig.5). Arterioles are seen under the capsule which are dilated and have thick wall (Fig. 4).

Examination of group (Group III) showed; marked improvement in testicular changes which induced by BPA was observed. Seminiferous tubules had regular basement membrane with healthy looked spermatogenic lineages, some intercellular vacuoles were still observed. Some nuclei of primary spermatocytes showed chromatinolysis. No germ cells were seen dislocated within the tubules. Some interstitial fluid exudate was still present with no vascular congestion (Figs. 6&7).

**Immunohistochemistry for PCNA:**

Seminiferous tubules of control animals had PCNA positive brown coloration in the nuclei of spermatogenic cells mainly spermatogonia (Fig. 8).

Seminiferous tubules of group II showed PCNA positive reaction in the nuclei of spermatogonia and primary spermatocytes nuclei and apparent decreased in number compared to control one (Fig. 9).While group III showed apparent increased in number of PCNA positive cells compared to BPA treated group (Fig. 10).

**Immunohistochemistry for caspase 3:**

Examination of the control group showed few apoptotic cells (fig.11), while Group II animals showed positivity in spermatogonia, primary spermatocytes and round spermatids (Fig. 12).

Examination of Group III animals, showed apparent decreased in number of positive cells compared to the previous treated group (Fig. 13).

**Morphometric study and statistical analysis**

Morphometric study and statistical analysis revealed a significant decrease in the number of PCNA positive germ in BPA treated group compared to the control group. It was noticed also a significant increase in the number of positive germ cells in group III compared to the previous group (table 2 and histogram 3). Regarding Caspase 3 results there was a significant increase in the number of positive germ in BPA treated group compared to the control group. Whereas a significant decrease in the number of positive germ cells in group III compared to the previous group was observed (table 3 and histogram 4).

**Histological examination of pars distalis:**

Pars distalis of adult control animal had chromophobes and chromophils. The latter consisted of two types, acidophils and basophils. Acidophils had rounded vesicular central nucleus and acidophilic cytoplasm. Basophils had rounded vesicular eccentric nuclei and basophilic cytoplasm. Chromophobes were seen as multiple small cells with rounded central nuclei and unstained cytoplasm. Multiple sinusoidal capillaries were noticed in between cells of pars distalis (Fig. 14).

Examination of Group II animals, Pars distalis had acidophils which have dense rounded nuclei and acidophilic cytoplasm. Numerous basophils had vesicular nuclei and marked vacuolated cytoplasm (Fig. 15).

Examination of Group III animals, Pars distalis have some acidophils with acidophilic cytoplasm and rounded vesicular nuclei,others still had dense nuclei. Basophils had vacuolated cytoplasm and vesicular nuclei. Some basophils still had dense nuclei (Fig. 16).

**Immunohistochemistry of mammotrophs:**

Pars distalis of adult control animals had cells positive for prolactin antibody (mammotrophes). They have brown coloration of their cytoplasm (Fig. 17).

Examination of Group II animals showed apparent increase in intensity of reaction in the cytoplasm of mammotrophs compared to the control group (Fig. 18).

Examination of Group III animals showed apparent decreased in intensity of brown coloration of mammotrophs compared to the previous group (Fig. 19).
Fig. 1: A photomicrograph of control animal testis showing: seminiferous tubules (S) are surrounded by basement membrane and lined with germ cells. In between the tubules there are interstitial areas (IT) and the capsule can be seen (c). (H&E x 200)

Fig. 2: A magnified part of previous section showing; parts of five seminiferous tubules. They are surrounded by intact regular basement membrane, nuclei of myoid cells and fibroblasts. Note: Germ cells lined the tubule are spermatogonia (s), primary spermatocyte (ps), spermatid (Sp) and sertoli cell (Sr) Interstitial area has leydig cell (L), fibroblast (F) and macrophage(M). (H&E x400)

Fig. 3: A photomicrograph of testis treated with BPA showing: seminiferous tubules having irregular basement membrane (arrow head) and desquamated germ cells with wide spaces in between. Interstitial area show acidophilic material in between cells with multiple vacuoles (v) Note: desquamated cells inside the tubule (↑). (H&E X200)

Fig. 4: A photomicrograph of testis treated with PBA showing; thick testicular capsule(c), seminiferous tubules have irregular outline (arrow) and congested dilated blood vessel (arrow head) and thick walled arteriole (curved arrow). (H &E x400)

Fig. 5: A photomicrograph of testis of treated animal showing; three parts of seminiferous tubules have irregular basement membrane, spermatogonia (s) have dark condensed nuclei, primary spermatocytes (ps) with dense nuclei and basophilic cytoplasm, spermatids (sp) have fragmented and pyknotic nuclei. Sertoli cells (sr) have dense nuclei. Wide spaces and multiple vacuoles (v) in between germ cells. Note: Acidophilic vacuolated exudate is seen in interstitium with normal Leydig cells (L). (H&E x1000)

Fig. 6: A photomicrograph of testis treated with BPA and omega-3 showing: seminiferous tubules having almost regular basement membrane and germinal epithelium with few vacuoles in between (↑). Note: some exudate is still seen in interstitium (I). (H&E x200)
Fig. 7: A photomicrograph of testis treated with BPA and omega 3 showing: seminiferous tubules have regular basement membrane and spermatogenic cell. Some nuclei of primary spermatocytes show chromatolysis (P). In between germ cells few vacuoles are seen (↑). (H&E x400)

Fig. 8: A photomicrograph of control testis immunostained for PCNA showing; PCNA positive nuclei of spermatogonia (arrow head). Weak positive intensity is seen in cytoplasm of primary spermatocytes (arrow). (PCNA immunostain x400)

Fig. 9: A photomicrograph of GII testis immunostained for PCNA showing; PCNA positive nuclei of spermatogonia and primary spermatocytes (arrow head). Note: desquamated cells have positive nuclei for PCNA immunostain (arrow). (PCNA immunostain x400)

Fig. 10: A photomicrograph of GII testis immunostained for PCNA showing; increase number of PCNA positive cells, nuclei of spermatogonia (arrow head) and primary spermatocytes (arrow). Cytoplasmic positivity of Leydig cells (L). (PCNA immunostain x400)

Fig. 11: A photomicrograph of the testis of control group immunostained for caspase 3 showing; two adjacent seminiferous tubules with few caspase 3 positive spermatogonia (arrow head). Note, Leydig cell (arrow). (caspase 3 immunostain x400)

Fig. 12: A photomicrograph of GII testis immunostained for caspase 3 showing; two adjacent seminiferous tubules. Positive reaction appeared in spermatogonia (S), primary spermatocytes (R) and round spermatids (p). (caspase 3 immunostain x400)
Fig. 13: A photomicrograph of GIII testis immunostained for caspase 3 showing; two adjacent seminiferous tubules have apparently decreased number of positive cells compared to the previous group (Arrow head). (immunostain caspase 3 x400).

Fig. 14: A photomicrograph of adult control pituitary gland showing; Part of pars distalis have acidophils with rounded vesicular central nucleus and acidophilic cytoplasm (A). Basophils (B) have rounded vesicular eccentric nuclei and basophilic cytoplasm. Multiple small cells with rounded central nuclei and unstained cytoplasm called chromophobes (C). Note: Sinusoidal capillaries are seen between cells (arrow head). (H&EX400)

Fig. 15: A photomicrograph of GII pituitary gland showing; Part of pars distalis. Acidophils have dense rounded nuclei and acidophilic cytoplasm (A). Basophils have vesicular nuclei and marked vacuolated cytoplasm (B). (H&E x400)

Fig. 16: A photomicrograph of GIII pituitary gland showing; Part of pars distalis. Acidophils (A) have acidophilic cytoplasm and rounded vesicular nuclei. Some acidophils have dense nuclei (arrow head). Basophils (B) have vacuolated cytoplasm and vesicular nuclei. Some basophils have dense nuclei (arrow). (H &E X1000)

Fig. 17: A photomicrograph of adult control pituitary gland showing; Part of pars distalis have cells positive for prolactin antibodies (arrow head). They have brown coloration of cytoplasm. (immunostain for prolactin x1000)

Fig. 18: A photomicrograph of G II pituitary gland (Part of pars distalis) showing; apparent increased intensity of reaction in the cytoplasm of mamnotrophs compared to controls (arrow head). (immunostain for prolactin x1000)
Table 1: showing the differences in the mean levels of hormones between the three studied groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control (Group I)</th>
<th>BPA treated (Group II)</th>
<th>Treated with BPA + omega3 (Group III)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (ng/ml)</td>
<td>16.47±0.42</td>
<td>10.45±0.68</td>
<td>14.94±0.80</td>
<td>0.0001</td>
</tr>
<tr>
<td>LH (U/ml)</td>
<td>11.80±0.85</td>
<td>7.07±0.51</td>
<td>10.45±1.24</td>
<td>0.003</td>
</tr>
<tr>
<td>FSH (U/ml)</td>
<td>2.41±0.57</td>
<td>3.56±0.40</td>
<td>2.50±0.51</td>
<td>0.21</td>
</tr>
<tr>
<td>Prolactine (ng/ml)</td>
<td>5.35±0.85</td>
<td>10.01±0.85</td>
<td>6.96±0.50</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

Data represent the mean± SD of observations from 10 male rats.

* Significant compared to bisphenol group (P<0.05)
# Significant compared to control group (P<0.05).

Histogram 1: Showing the differences in the mean level of testosterone in the three studied groups

Histogram 2: Comparison of the three studied groups regarding the mean levels of LH, FSH and prolactin.

Histogram 3: Comparison of the three studied groups regarding the mean number of PCNA positive cells.

Table 2: Comparison of the three studied groups regarding the mean number of PCNA positive cells.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control group (Group I)</th>
<th>BPA treated group (Group II)</th>
<th>BPA+Omega3 treated Group III</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean± S.D</td>
<td>225.4± 54.5</td>
<td>66.4± 26.6</td>
<td>90.5± 17.9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Histogram 4: Comparison of the three studied groups regarding the mean number of Caspase 3 positive cells.

Table 3: Comparison of the three studied groups regarding the mean number of Caspase 3 positive cells.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control group (Group I)</th>
<th>BPA treated group (Group II)</th>
<th>BPA+Omega3 treated Group III</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean± S.D</td>
<td>1.1 ± 0.3</td>
<td>3.6 ± 2.1</td>
<td>1.5 ± 0.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
BPA is an endocrine-disrupting chemical that can induce a variety of adverse effects in mammals. Worldwide, about 50–80 million individuals are infertile, an estimate that is likely to increase considerably in the future. Several factors are known to underlie male infertility. Exposure to environmental toxicants as BPA could be one of these factors. Human body can be exposed to BPA through food containers, water bottles, thermal papers and medical equipments as dental fillings. The testis is vulnerable to damage by environmental toxicants as BPA.

Our results revealed that there was an irregular outline of the seminiferous tubules with degeneration and disorganization of spermatogenic cells with intercellular vacuolation. It is reported that a decrease in the level of testosterone in BPA-treated rats led to atrophy of seminiferous tubules, degeneration of cells, and complete absence of spermatogenesis. Also, the impairment of the basal lamina of seminiferous tubules and damaged tight junctions between sertoli cells could be attributed to BPA-induced cell injury as reported by. Additionally, the intercellular spaces represent progressive degenerative changes affecting cell membrane integrity secondary to oxidative stress induced by BPA. The free radicals oxygen species initiate oxidative phosphorylation reactions to cell membranes ending in disruption of the integrity of the intercellular junctional complexes.

An apparent decrease in the number of sperms within the lumen of some tubules was observed compared to the control. A significant increase in the rate of apoptosis and a significant decrease in the rate of proliferation of germ cells were also detected on BPA exposure compared to the control group.

Our results were in agreement with the study of Jin and his colleague who have been reported that, the number of spermatocytes, spermatogonia, and spermatids and the concentration of testosterone may be reduced in male rats on exposure to BPA at 2ug/kg. These results suggest that BPA could inhibit spermatogenesis. Furthermore, any decrease in the level of testosterone may decrease the number of sperms. While Qiu and his colleague reported that BPA dose (5mg/kg) in male rats showed a significant decline in the number of sperms.

Similar results were previously reported as well by. While Takahashi and Oishi found the delay of spermatogenesis and the disorganization of elongated spermatids of male rats when exposed to BPA only at higher levels.

BPA may act via several different mechanisms involving interaction with estrogen receptors and/or by production of a minor but potent estrogenic metabolite. A decrease in the level of androgen binding protein could be the possible mechanism for the reproductive toxicity of BPA. Others reported that a low BPA concentration can induce spermatogenesis disorders mainly through decreasing androgen receptor expression. However Previous study reported that, BPA could up-regulate mRNA expression of ERα.

A number of studies point out that the adult exposure to BPA can reduce sperm production and efficiency of spermatogenesis. Liu et al found that exposure of pregnant mice to BPA during pregnancy and lactation has some toxic effects on the testes of male offspring and these may originate from increased apoptosis.

Pengpeng and his colleagues explained germ cell apoptosis through decreasing reproductive hormones and activating the Fas/FasL signaling pathway. The Fas-signaling system is considered to be a key regulator of germ cell apoptosis during development and after testicular insults.

Furthermore BPA exposure increased the protein and mRNA levels of cytochrome C, apoptosis-inducing factor, caspase-3, 9, and Bax; caspase-3 and caspase-9 activities; and the apoptosis indices of spermatogenic cells. In addition, abnormal structure of mitochondria and decreased protein and gene levels of Bcl-2 were observed following BPA exposure.

The caspase family is a class of cysteine proteases. Active caspases play a crucial role in the transduction of apoptotic signals. Relevant caspases include the initiator caspases (caspases-8, -9, and -10) and the effector caspases (caspases-3, -6, and -7). Germ cell apoptosis can be a result of a reduced intratesticular testosterone concentration and the translocation of active caspase-3 and caspase-activated deoxyribonuclease to the nucleus which led to the induction of apoptosis.

Wang and his colleague reported that mitochondrial pathway mediated apoptosis was observed in rat testis due to BPA exposure led to reproductive system dysfunction.

A previous in vitro study has revealed the cytotoxicity of low dose BPA through the up-regulation of Bax and the down-regulation of Bcl-2. Additionally, BPA-induced apoptotic cell death through calcium-mediated oxidative stress.
Our data in the present study revealed a statistically significant decrease in the level of LH and testosterone in BPA treated group in comparison to control group. These results come in agreement with \cite{53,54,55,56}. They reported that BPA has adverse effects on testicular function by decreasing pituitary LH secretion and reducing Leydig cells steroid genesis "primary gonadal failure" \cite{57}.

BPA suppressed testosterone production via a decreased LH secretion. There is also evidence that BPA interferes with LH receptor-legend binding \cite{58}. The decreases serum testosterone level could be primarily postulated to the decreased expression of the steroidal genes and cholesterol carrier protein "StAR" involving the testosterone synthesis as decreased LH \cite{59,60,61}. Furthermore, BPA is reported to act as anti-androgenic agent blocking the action of dihydrotestosterone \cite{62}

Our study showed disagreement with results obtained by others in rats \cite{63} and in mice \cite{64}. They found that there was no significant change in testosterone level following BPA exposure when compared with control. This variation may be due to differences in animal species, dose of BPA and time of exposure. The decreased LH level as obtained in our study could be explained by ability of BPA to interfere with LH receptor ligand binding resulting in uncoupling LH from LH receptor that potentially contributes to diminished LH stimulation of steroid genesis as reported by \cite{65} or due to increased prolactin release after BPA exposure as mentioned by \cite{66}, where hyperprolactinemia has been shown to cause reproductive dysfunction as confirmed by \cite{67}. This dysfunction is not mediated via direct action on testis but due to its effects at the level of hypothalamus-pituitary to inhibit LH-RH and LH secretion as confirmed by \cite{68}.

In contrast to the significant increase in LH levels, FSH levels showed no significant changes by BPA administration. Since FSH secretion is mainly regulated by inhibin, it could be not affected by BPA \cite{69}. The hormonal changes were associated with degenerative changes in basophils.

Our data in the present study revealed that administration of BPA increased prolactin secretion in addition to increased mammotroph immunoactivity. These results similar to those were observed by \cite{70,71,72}. The effect of BPA is probably due to direct action to anterior pituitary via calcium influx in pituitary cells \cite{73}. BPA may simulate estrogen to increase the level of serum prolactin, because estrogen has a direct role in stimulating prolactin gene expression. BPA could have hypothalamic actions and can alter levels of prostaglandin receptor expression within the medio-basal hypothalamus. This induced neural system changes that could have impact upon gonadotropin secretion \cite{74}.

Administration of omega 3 in the present study ameliorates both the hormonal changes as well as the morphological changes in both testis and pars distalis. Omega-3 (n-3) is an essential fatty acid found in large amounts in fish oil. It contains eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) \cite{75}.

We found a statistically significant increase in serum LH. Testosterone level associated with a significant decrease in serum prolactin in omega 3 treated group compared to BPA treated group. These results agree with others \cite{76,77,78,79,80}.

Omega 3 increases nitric oxide release as reported by \cite{81,82} and by previous work by \cite{83}. Nitric oxide increases the release of GnRH, which in its turn increases gonadotropin secretion in the pituitary gland \cite{84}. Nitric oxide activates Guanylate cyclase enzyme that causes the release of cyclic guanosine monophosphate and eventually by raising GnRH, LH and FSH, enhances sperm motility and induces erection in males \cite{85}. Also omega 3 is able to inhibit 5 alpha - reductase in cell cultures and cell-free systems. There by preventing the conversion of testosterone to dihydrotestosterone. In this way omega 3 prevent the reduction of plasma testosterone level \cite{86,87}.

However, it appears that the increase in testosterone levels with omega-3 was due to its direct effect on Leydig cells. Omega-3 contains alpha linolenic acid, which can be converted to arachidonic acid, as a precursor to make type 2 prostaglandins like E2 \cite{88}. Arachidonic acid seems to play an important role in testicular steroid genesis. As researches indicated that, arachidonic acid increases cyclic adenylyl cyclase, thus enhancing the rate of cholesterol side-chain breakage and stimulating the production of progesterone. So, these compounds mediate the testosterone production via messaging. Previous studies on showed that all E series prostaglandins stimulated testosterone production in the testes \cite{89}. In the present study, the rate of germ proliferation was significantly increased and the rate of apoptosis was significantly decreased by administration of Omega-3. Previous studies documented the antioxidant, anti apoptotic and anti inflammatory effects of fish n-3 fatty acids on various tissues exposed to stress and damage \cite{90,91}, so it can be a promising potential cytoprotective agent against various extrinsic toxic stimuli.

Uygur and his college found that omega-3 decreased germ cell apoptosis and oxidative stress induced by doxorubicin \cite{92}. Additionally, omega-3 could protect against experimental diabetes-induced damages on testis, sperm parameters and preimplantation embryo development in rat model \cite{93}.

Also, cell membrane fluidity can be improved by increasing dietary omega-3 and changes in fatty acid contents of cell membrane reported to be achieved rapidly within weeks by altering dietary fat intake \cite{94}.

**CONCLUSION**

BPA induced disturbance in the pituitary-testicular axis in rats in low dose which is previously considered to be safe and the concomitant use of omega 3 fatty acids could protect against BPA induced changes.
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32. Chao HH, Zhang XF, Chen B, Pan B, Zhang LJ,Li L, Sun XF,Shi QH and Shen W. Bisphenol A exposure modifies methylation of imprinted genes in mouse oocytes via the estrogen receptor signaling pathway


The protective effect of omega-3 against BPA-induced disruption of the pituitary-testicular axis

Abstract

The protective effect of omega-3 against BPA-induced disruption of the pituitary-testicular axis in the rat. A chemical and histological and immunohistochemical study.


Department of Histology, Faculty of Medicine, Sohag University.

The emergence of infertility is a major health problem affecting human life. Several factors contribute to male infertility, and one of the most important is exposure to environmental pollutants such as BPSF.

Objective: The study aimed to assess the protective effect of omega-3 against the changes caused by BPSF in the pituitary-testicular axis.

Methods: We used 30 white rats in the study. They were divided into three groups, ten rats per group. The first group was the control group. The second group was treated with BPSF at a dose of 0.4 mg/kg for 3 days per week. The third group was treated with omega-3 at a dose of 1.2 mg/kg through the mouth and BPSF at the same dose and duration as the previous group. During the slaughter, all the rats were anesthetized and blood samples were collected to measure testosterone, luteinizing hormone, follicle-stimulating hormone, and prolactin levels.

Caspase 3 was tested in the testes and pituitary glands, and the tissue and immunohistochemical staining was performed to evaluate cell proliferation and programmed cell death for prolactin secreting cells. The positive PCNA was counted and statistically analyzed.

Results: There was a significant decrease in testosterone levels and a significant increase in prolactin levels in the BPSF-treated group. The spermatids were disorganized and damaged. There was also an increase in programmed cell death rate and a significant decrease in the rate of cell proliferation in the third group treated with omega-3.

Conclusion: Omega-3 may protect the pituitary-testicular axis from the harmful effects of BPSF. The results of the study suggest that omega-3 may be a potential agent to prevent the negative effects of BPSF on fertility.

Keywords: infertility, BPSF, omega-3.