INTRODUCTION

Bisphenol A (BPA) is used as a monomer to manufacture polycarbonate plastic, the resin that lines most food and beverage cares, dental sealants and as an additive in other plastics and it consider one of the highest volume chemicals produced worldwide (Richter et al., 2007 and Calafat et al., 2008). Falconer et al. (2006) stated that plasticizers including bisphenol A (4,4′-isopropylidenediphenol) has become ubiquitous in the environment within the past 80 years because of its presence in a multitude of products, including polycarbonate plastics used in the manufacture of baby formula bottles, water bottles, and other clear plastic containers. Vandenberg (2007) also reported that BPA is also used in the epoxy resin that lines the inside of metal food and soda cans, medical devices, and credit card receipts.

BPA is consumed when it leaches from plastic containers into foods or drinks when they are heated or when they become broken or cracked. Other potential routes of exposure to BPA include air, dust, water, 5-gallon water coolers, printer inks, toners and thermal receipt paper (used by most gas stations and supermarkets), where BPA can rub off paper onto our hands and into our mouths. Li et al. (2009a) studied the exposure to BPA in the workplace was associated with adult male sexual dysfunction as erectile dysfunction, reduced sexual desire, ejaculation difficulties and overall dissatisfaction with their sex life than workers with no heightened BPA exposure.

Li et al. (2009b) noticed that the diameter of seminiferous tubules was significantly reduced or no lumen compared with controls, germ cells were mal-aligned, and there were a few elongated spermatids and many karyopyknotic cells. Leydig cells in the
interstitial tissue were decreased in number compared with those of the control group.

There are numerous experimental studies upon laboratory animals which showed that BPA can affect the development of gonads, accessory glands and spermatogenesis. Toyama et al. (2004) studied the effect of BPA on testes of adult mice and rats with electron and light microscopy and observed the abnormalities in spermatids (acroosomal vesicles, acrosomal caps, acrosomes and nuclei of the spermatids were severely deformed). Vom Saal et al. (2007) concluded that in animal studies, BPA has been associated with reproductive abnormalities, hormonal changes and pre-cancerous changes in the breast and prostate.

BPA has been demonstrated in vivo and in vitro experiments to act as an endocrine disrupting chemical (Welshon et al., 2006). Okada and Kai (2008) have presented a significant decrease of mature spermatids number in seminiferous tubules in 4 weeks mouse offspring after 5μg BPA administration. Nanjappa et al. (2012) reported that the testicular Leydig cells are the predominant source of the male sex steroid hormone testosterone, which supports the male phenotype and described the effects of developmental exposure of male rats to BPA. Williams et al. (2013) recorded that the California mice territorial marking behavior was reduced and presumed low dominance in the wild could have reproductive consequences for male. This study also suggests that exposure to BPA may lead to lower mate guarding and, as a consequence, increase their risk of infidelity, an atypical outcome for a monogamous species.

Manfo et al. (2014) studied the adverse effects of BPA on male rats and recorded atrophy of the testes and epididymis and alteration of adult sperm parameters (e.g. sperm count, motility and density). Rahimi et al. (2015) reported that the renal injury is one of the side effects of BPA exposure in rats by intra peri toneal injection and resulted in increase concentration of urea and creatinine. Histologically, dilation and hypercellularity of glomeruli and degeneration of epithelium of proximal tubules were found. Karnam et al. (2015) reported that reproductive study in the BPA treated rats with either 50, 200 and 600 μg/kg body weight on day 28 revealed that there was significant reduction in the epididymal sperm count, significant decrease in sperm motility percentage, dead count percentage, head and tail abnormality percentage were found to be significantly increased in rats of BPA treated groups as compared to rats of control groups. Histopathologically, testes showed necrosis of germinal layer and spermatogonial cells in the seminiferous tubules. Morgan (2014) stated that male albino rats treated orally; 3 times weekly for 50 days with 25 mg BPA/kg body weight /day showed testicular hyperemia, testicular degeneration and cellular debris in the lumen. Moreover, there were histopathological changes in liver, kidneys and brain. Paal et al. (2014) mentioned the average motility and sperm concentration of male rabbits as 55.55 ± 26.63 % and 0.55 ± 0.16 x 10^7 ml, respectively. The normal value of plasma testosterone in the male New Zealand rabbit was 7.17±0.72 ng/ml (Sanni et al., 2012).

The present study was performed to elucidate the protective role of vitamin C on testicular or reproductive toxicity and histopathological alterations induced by bisphenol A.

**MATERIALS AND METHODS**

**Animals:**
The present study was carried out on 15 mature male New Zealand White rabbit (6 months old, weighed about 2 kg) purchased from farm of the ministry of agriculture. All rabbits were housed in individual wire cages and received a balanced ration diet for two weeks before the start of the experiment.

**Bisphenol A and Vitamin C:**
The chemical structure of bisphenol A or hydroxylated diphenylalkanes consists of two phenolic rings joined together through a bridging carbon (CH\_2).C(C\_6.H\_12.OH\_2) (Perez et al., 1998 and Erickson, 2008). Bisphenol A was obtained from Sigma Aldrich Co. (CAS Number 80-05-7). Vitamin C (L-ascorbic acid) was purchased from El-Gomhoria Company for Chemical Products.

**Experimental design:**
Animals (15 mature male rabbits) were classified into 3 groups. Group I: Five rabbits which were daily intubated with control vehicle (olive oil). Group II: Five rabbits which were daily intubated with bisphenol A (dissolved in olive oil) at a dose level of 100 mg/kg body weight (minimal toxic dose) according to Sadek et al. (2014). Group III: Five rabbits which were daily intubated with bisphenol A (dissolved in olive oil) at a dose level of 100 mg/kg body weight and ascorbic acid (Vitamin C) (dissolved in distilled water) at a dose level of 20 mg/kg body weight according to Lebas (2000). Up to 8 weeks the whole length of spermatogenic cycle in rabbit. One rabbit was sacrificed each two weeks from three groups (i.e. 2, 4 & 6 weeks), while the other two rabbits of each group were sacrificed at the 8th week to get blood for hormonal profile and histopathological samples. Semen analysis applied weekly for each male rabbit.

**Pathological examination:**
Postmortem examination of sacrificed animals was carried out and gross findings for each organ were recorded. Tissue specimens from testes and epididymis were fixed in formal saline solution (10% formalin in 0.9% NaCl) then routinely processed in
an automated tissue processor, embedded in paraffin, sectioned at 3-5 μm, and stained with haematoxylin and eosin stain (Bancroft and Gamble, 2008), then examined by light microscopy.

**Semen collection and evaluation:**
Semen was collected every week using a teaser and an artificial vagina. The semen samples were assessed immediately after collection for individual motility, sperm abnormality, live dead ratio and sperm cell concentration according to Boiti et al. (2005).

1- Sperm individual motility using a light microscope at X100 magnification after dilution of the semen with 2.9 % sodium citrate dihydrate solution according to Woston and Martin (1972).
2- Assessment of abnormal sperms using fast green stain according to Wells and Awa (1970).
3- Live dead ratio of spermatozoa using eosin-nigrosin staining technique according to Björendahl et al. (2003).
4- Sperm concentration using the improved Neubauer hemocytometer slide after staining with eosin according to Smith and Mayer (1955).

**Testosterone hormone profile:**
Blood samples were taken from the jugular vein of rabbit bucks at the day of necropsy and kept at room temperature for complete clotting and centrifuged at 3000 rpm for the separation of serum and stored at -20ºC. Testosterone levels were assayed with ELISA technique using a commercial kit (Byk, Dietzenbach, Germany) in private lab (Olayemi, 2007).

**Statistical analysis:**
The obtained data of testosterone hormone and semen pictures was analyzed using Costat Computer Program (1986) Cottort Software.

**RESULTS**

**Histopathological examination:**
Mild testicular changes were noticed during the 2nd week consisting of thickening of basement membranes of seminiferous tubules with vacuolar degenerative changes in the lining spermatogenic cells in few seminiferous tubules and oedema in the interstitial tissues which appeared homogenous and faintly oesinophilic. In addition, degenerated spermatids and residual bodies in the lumen of some seminiferous tubules were prominent (Fig. 1).

Meanwhile on the 4th week, moderate degeneration was seen in some of the seminiferous tubules in the form of moderate thickening of basement membranes and hypospermagenesis. Interstitial tissues showed congestion and oedema. Moreover, increased number of the degenerated spermatids and residual bodies in the lumen of the seminiferous tubules were also noticed (Figs. 2& 3).

Concerning the lesions observed during the 6th week, the testicular lesions were more pronounced. Moderate thickening of the basement membranes of seminiferous tubules and depletion of spermatogenesis were observed in the most of seminiferous tubules. In addition, the tubules showed increased number of the degenerated spermatids and residual bodies in their lumen. Perivascular oedema in the interstitial tissues was also noticed (Fig. 4).

During the 8th week of treatment, the testicular lesions turned to be severe giving the picture of testicular atrophy, the seminiferous tubules showed severe degeneration in most of seminiferous tubules and exhibited complete absence of the spermatogenesis with the presence of only one layer spermatogenic and sertoli cells (Fig. 5).

Mild vacuolar degeneration of the epididymal ductal epithelium and interstitial oedema were seen at 2nd week. These alterations gradually aggravated till the end of experiment which appeared more pronounced and clear. Moreover, hyperplasia of the lining epithelial cells of the tubules was seen more than one columnar cell layers which detected at the 6th and 8th weeks (Fig. 7& 8).

In the third group (treated with BPA and vitamin C) the testicular and epididymal lesions observed in the treated group (second group) were ameliorated gradually starting from 2nd week till the end of the experiment where the testicular structure appeared nearly normal (Fig. 6& 9).

**II- Semen evaluation:**
The following parameters of the semen analysis were statistically analyzed to compare the data of each parameter of the intoxicated group to the control value, bisphenol A plus Vit C through the spermatogenic cycle (8 weeks).

Upon the comparison of the bisphenol A group to the other two groups (control and bisphenol A plus vitamin C) there was significant decrease in the sperm count per milliliter, individual sperm motility and live dead ratio. While, there was significant increase in the total sperm abnormalities in the bisphenol A treated group rather than the other two groups (Tab. 1).

In the first and second weeks post intoxication data presented in table (1) and showed significant (P<0.05) reduction in the progressive motility in the bisphenol A treated group (63±2.99 %) compared with the control and bisphenol A plus vitamin C groups (75±1.66% and 73.5±1.83%, respectively). Similarly, there was no significant difference among the control and bisphenol A plus vitamin C groups regarding the sperm cell concentration, while there was a significant difference (P<0.05) with the bisphenol A treated group (119.5x10^6±10.46 sperm cell/ml). Also, no significant difference among the
control and bisphenol A plus vitamin C groups regarding the live dead ratio, while there was a significant difference (P<0.05) with the bisphenol A treated group (76.7±2.02%). Data also indicated that control and bisphenol A plus vitamin C groups reduced significantly (P<0.05) sperm abnormalities (14.6±1.59% and 16.3±1.23%, respectively) compared with bisphenol A treated group (28.6±2.49%).

In the third and fourth weeks post intoxication data presented in table (1) and showed significant (P<0.05) reduction in the progressive motility in the bisphenol A treated group (58.75±2.95 %) compared with the control and bisphenol A plus vitamin C groups (79.375±2.39% and 81.87±2.30%, respectively). Similarly, there was no significant difference among the control and bisphenol A plus vitamin C groups regarding the sperm cell concentration, while there was a significant difference (P<0.05) between the bisphenol A treated group (88.38±4.08 sperm cell/ml). Also, no significant difference among the control and bisphenol A plus vitamin C groups regarding the live dead ratio, while there was a significant difference (P<0.05) with the bisphenol A treated group (58.75±2.95%). Data also indicated that control and bisphenol A plus vitamin C groups reduced significantly (P<0.05) sperm abnormalities (12.3±1.28% and 15.5±1.38%, respectively) compared with bisphenol A treated group (40.13±1.69%).

In the fifth and sixth weeks post intoxication data presented in table (1) and showed significant (P<0.05) reduction in the progressive motility in the bisphenol A treated group (53.3±3.30%) compared with the control and bisphenol A plus vitamin C groups (77.5±1.71% and 75.83±2.71%, respectively).

Semen picture in spermatogenic cycle (1: 8 weeks) post intoxication.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Motility</th>
<th>Live Dead Ratio</th>
<th>Abnormalities</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1&amp;2 weeks) (5 rabbits)</td>
<td>75±1.66a</td>
<td>92.4±0.62a</td>
<td>14.6±1.59a</td>
<td>211±10x^2±19.06a</td>
</tr>
<tr>
<td>Bisphenol A group (1&amp;2 weeks) (5 rabbits)</td>
<td>63±2.99b</td>
<td>76.7±2.02c</td>
<td>28.6±2.49b</td>
<td>119.5±10x^2±10.46c</td>
</tr>
<tr>
<td>Bisphenol A &amp; Vit C group (1&amp;2 weeks) (5 rabbits)</td>
<td>73.5±1.83a</td>
<td>85.4±0.89b</td>
<td>16.3±1.23b</td>
<td>158.3±10x^2±5.15b</td>
</tr>
<tr>
<td>Control (3&amp;4 weeks) (4 rabbits)</td>
<td>79.375±2.39a</td>
<td>92.87±0.81a</td>
<td>12.38±1.28a</td>
<td>257.63±10x^2±26.68a</td>
</tr>
<tr>
<td>Bisphenol A group (3&amp;4 weeks) (4 rabbits)</td>
<td>58.75±2.95b</td>
<td>63.75±4.14b</td>
<td>40.13±1.69b</td>
<td>88.38±10x^2±2.30a</td>
</tr>
<tr>
<td>Bisphenol A &amp; Vit C group (3&amp;4 weeks) (4 rabbits)</td>
<td>81.87±2.30a</td>
<td>90.13±1.14a</td>
<td>15.5±1.38a</td>
<td>184.37x10^2±8.54b</td>
</tr>
<tr>
<td>Control (5&amp;6 weeks) (3 rabbits)</td>
<td>77.5±1.71a</td>
<td>94.33±0.92a</td>
<td>10.17±1.26a</td>
<td>217.67±10x^2±24.99a</td>
</tr>
<tr>
<td>Bisphenol A group (5&amp;6 weeks) (3 rabbits)</td>
<td>53.33±3.33b</td>
<td>60.16±3.06b</td>
<td>44.67±2.14b</td>
<td>78.67±10x^2±0.03b</td>
</tr>
<tr>
<td>Bisphenol A &amp; Vit C group (5&amp;6 weeks) (3 rabbits)</td>
<td>75.83±2.71a</td>
<td>88.33±1.25a</td>
<td>12.83±1.42a</td>
<td>213.67±10x^2±1.21a</td>
</tr>
<tr>
<td>Control (7&amp;8 weeks) (2 rabbits)</td>
<td>80±2.04a</td>
<td>88.75±1.31a</td>
<td>11±1.08a</td>
<td>237x10^2±24.25a</td>
</tr>
<tr>
<td>Bisphenol A group (7&amp;8 weeks) (2 rabbits)</td>
<td>50±4.08b</td>
<td>48.5±4.55b</td>
<td>46.75±3.01b</td>
<td>54±10x^2±4.24b</td>
</tr>
<tr>
<td>Bisphenol A &amp; Vit C group (7&amp;8 weeks) (2 rabbits)</td>
<td>77.5±3.22a</td>
<td>90.75±2.83a</td>
<td>12.75±2.01a</td>
<td>228.25±10x^2±24.38a</td>
</tr>
</tbody>
</table>

Values with different superscripts in the same column are significantly different at (P<0.05).
III- Blood sera testosterone hormone profile:
There was a significant (P<0.05) reduction in testosterone concentration in the bisphenol A treated group (1.72±0.13 ng/ml) compared with the bisphenol A plus vitamin C (7.19±0.47 ng/ml) and highly reduction (P<0.01) compared with the control group (7.96±0.12 ng/ml).

Table 2: Sera testosterone concentration of the treated groups 56 days post intoxication (5 rabbits for each group).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Bisphenol A</th>
<th>BPA &amp; Vit C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td>7.96±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.72±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.19±0.47&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values with different superscripts in the same column are significantly different at (P<0.05).

Fig. 1: Rabbit buck testis of BPA intoxicated group sacrificed at two weeks showing mild degeneration with thickened basement membranes of seminiferous tubules and hypospermatogenesis (H&E, X100).

Fig. 2: Rabbit buck testis of BPA intoxicated group sacrificed at four weeks showing moderate degeneration with thickened basement membranes of seminiferous tubules and hypospermatogenesis (H&E, X100).

Fig. 3: Rabbit buck testis of BPA intoxicated group sacrificed at six weeks showing moderate degeneration with thickened basement membranes of seminiferous tubules and hypospermatogenesis (H&E, X100).

Fig. 4: Rabbit buck testis of BPA intoxicated group sacrificed at eight weeks post intoxication showing severe degeneration with thickened basement membranes of seminiferous tubules and hypospermatogenesis (H&E, X40).

Fig. 5: Rabbit buck testis of BPA intoxicated group sacrificed at eight weeks post intoxication showing severe degeneration with thickened basement membranes of seminiferous tubules and aspermatogenesis (H&E, X100).

Fig. 6: Rabbit buck testis of BPA plus vitamin C group showing more or less the normal appearance of seminiferous tubules and interstitial tissues (H&E, X100).
DISCUSSION

Exposure to several chemicals and environmental contaminants has been reported to increase oxidative stress in body by disturbing the prooxidant/antioxidant balance of cells (Ho et al., 1998 and Aydogan et al., 2008). BPA was previously reported to induce oxidative damage in several tissues (Aydogan et al., 2010 and Korkmaz et al., 2010 and 2011). It is widely accepted that antioxidants may delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation, or propagation of oxidizing chain reactions (Velioglu et al., 1998 and Aitken and Roman, 2008). These involve absorption and neutralization of free radicals, quenching singlet and triplet oxygen, and decomposing peroxides (Osawa, 1994). Considerable attention has recently been focused on some nutritional factors such as polyphenols that could counteract oxidative damages and therefore be beneficial through their antioxidant properties (Rice-Evans et al., 1997 and Luczaj et al., 2009). Also, Chitra et al. (2003b) reported that bisphenol A induced oxidative stress in epididymis and caused degeneration of the epididymal epithelium of rats. In consistent with our histopathological findings, the testis and epididymis showed degeneration, congestion, atrophy, and germinal cell debris that come in contact with Aydogan et al. (2010) of rats administrated BPA. Chitra et al. (2003b) mentioned that degeneration of epididymal epithelium with reduction in the number of sperms was observed at all doses of bisphenol A treated rats. BPA has been shown to be decomposed to many kinds of metabolites including BPA radical by a reaction with radical oxygen (Sajiki, 2001). Several evidences indicated that BPA exerts a potent oxidant activity in various tissues via reactive oxygen species (ROS) production which results in inhibition of antioxidant enzymes; increased \( \text{H}_2\text{O}_2 \) and lipid peroxidation products as recorded by (Bindhumol, 2003; Chitra et al., 2003a&b; Kabuto, 2003; Obata and Kubota, 2000 and Kourouma et al., 2015). Moreover, polyphenols act as reactive oxygen and nitrogen species scavengers, redoxactive transition metal chelators and enzyme modulators as recorded by (Rice-Evans et al., 1997; Jian, 2010; El Tohamy and

Fig. 7: Rabbit buck epididymis of BPA intoxicated group sacrificed at two weeks showing mild vacuolar degenerated tubules with few sperms in lumen (H&E, X100).

Fig. 8: Rabbit buck epididymis of BPA intoxicated group sacrificed at four weeks showing moderate vacuolar degenerated tubules with cellular debris and few sperms in lumen and epithelial cells hyperplasia in many tubules (H&E, X100)

Fig. 9: Rabbit buck epididymis of BPA plus vitamin C group showing more or less the normal appearance of tubules and sperms in lumen (H&E, X100).
Semen evaluation of this study among the whole spermatogenic cycle reach similar to the results by Paal et al. (2014) in the average motility and sperm concentration of male rabbits as 55.55±26.63 % and 0.55±0.16 x 10⁹ ml, respectively. Co-administration with vitamin C reversed the effect of bisphenol A induced reduction in semen motility, live dead ratio and concentration with increase of sperm abnormalities so vitamin C considered as antidote for bisphenol A toxicity (Chitra et al., 2003b).

Testosterone level in the present study showed reduction in bisphenol A intoxicated group in comparison with control and bisphenol A plus vitamin C groups and that get in accordance of results of (Gunnarsson et al., 2003 and Sanni et al., 2012). It was an expected event because the corresponding cytotoxic effect of bisphenol A on Leydig cells responsible for reduction of testosterone production.

Vandenberg et al. (2007) and Li et al. (2009b) found that BPA may disrupt spermatogenesis by impacting various testicular cell types and given that human exposure to BPA is continuous and BPA can bioaccumulation in some conditions, there is concern about human reproductive health especially for occupational workers exposed to higher levels of BPA.

CONCLUSION

The results of this study revealed that oral BPA administration induced adverse oxidative stresses on the exposed animals as evidenced by the recorded abnormalities and histopathological alterations in the genital organs. Co-treatment with vitamin C provided a protective antioxidant role against such adverse effects. So, it is recommended to use vitamin C as a protector for all living organisms intensively exposed to BPA especially in occupational settings.

REFERENCES


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and Biotechnological Engineering, 8, 11; 1206: 1212.