Ameliorative Effect of Vitamin E on Oxidative Stress Induced by Bisphenol A in Female Albino Rats

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ABSTRACT

Objective: Oxidative stress is induced by bisphenol A (BPA) and affects many organs. Vitamin E is an effective antioxidant which prevents the activity of free radicals. This study was aimed to clarify the effect of vitamin E on the oxidative stress induced by chronic administration of BPA.

Materials and methods: 30 female albino rats were divided into 3 groups (10/each): group 1: control rats, group 2: rats treated with bisphenol A (20 mg/kg.B.wt) for three months, and group 3: rats treated with bisphenol A (20 mg/kg.B.wt) for three months, then treated with BPA and vitamin E (0.57/100 g B.wt) for other 15 days. Results: BPA induced hormonal disrupt in liver and kidney dysfunctions and hyperlipidemia. Vitamin E ameliorated all these parameters. Conclusion: since we exposed to many harmful disruptions and oxidative stress compounds, we must take vitamin E as a protective agent.

Key words: BPA, vitamin E, sexual hormone, liver, kidney, lipids.

INTRODUCTION

Bisphenol A is one of the environmental contaminants widely used in the manufacture of polycarbonate plastic (e.g. water and baby bottles), inside coating in metallic food cans.

Hence, it becomes an integrated part of the food chain. It also found in polymers that are used in dental materials. Human exposure mainly occurs through diet as polymers containing BPA can be hydrolyze under high temperature and acidic or basic conditions. Exposure may occur through dermal contact with thermal papers used widely in cash register receipts\(^1\).

BPA is a chemical switch in endocrine process, reproduction and development. It acts like a hormone, altering cellular function. It is absorbed in the gastrointestinal tract after ingesting products packed in plastic containers. It causes liver damage, thyroid disorder, diabetes mellitus type 2 and pancreatic damage\(^2\). Vitamin E is a potent scavenger of free radicals and is able to prevent the membrane damage caused by free radicals\(^3\).

The present study was performed to evaluate the effect of vitamin E on the hazardous damage of BPA on some physiological parameters.

MATERIALS AND METHODS

Experimental animals

Thirty female albino rats of Sprague Dawley strain, weighing 100-120 g, at the age of 6-8 weeks were purchased from Theodore Bilharz Research Institute, Giza, Egypt. Animals were kept under observation at control conditions (12 hour light/dark cycle, the temperature was 23±3°C, and compressed food and water was available ad libitum) for about 15 days before the onset of the experiment for adaptation.

Drug and dose:

(a) Bisphenol-A

Bisphenol-A (2,2-Bis-(4-hydroxy phenyl propane) dissolved in sesame oil and orally administered. BPA was purchased from Sigma Chemical Co. (St Louis, MO, USA). The dose of BPA was calculated according to Takahashi and Oishi\(^4\).

Experimental design:

Experimental animals were divided into three groups (10/each) as follows:

Group I (Control group): Normal female albino rats (without any treatment) for 105 ± 2 days. Group II (Bisphenol-A group): Female albino rats were orally administered with 20 mg BPA /kg. B.wt/day for 105 ± 2 days. Group III (Stem cell Enhancer treated group): Female albino rats were administered with 20 mg BPA/kg. B.wt/day for 105 days with concomitant orally administered by Vitamin E (0.57mg /100g B.wt /day) at the last 15 ± 2 days.

Blood samples collection: At the end of the experimental periods (105±2 days, those rats that had reached the stage of diestrus), the overnight fasted animals (14 h) were anesthetized by ether, and blood samples were collected from retro-orbital sinus in a clean centrifuge tubes and left
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to incubate at 37°C and centrifuged at 3000 rpm for 15 minutes. The clear non-haemolysed supernatant sera were quickly removed and immediately stored at -20°C till been used for the biochemical analysis.

**Biochemical analysis:**
Serum total lipids (TL), triglycerides (TG), total cholesterol and high density lipoprotein cholesterol (HDL-C) content were measured using enzymatic colorimetric kits (Biodiagnostic, Egypt). Low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein (VLDL) were calculated using the Friedwald’s formula.

**Friedewald’s equation:** LDL-C (mg/dl) = TC- { HDL-C + [TG/5]}. 

VLDL = TG/5

Ratios of LDL/HDL (risk factors) and TC/HDL were also calculated. Glucose level, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, serum alkaline phosphatase (ALP), urea and creatinine concentrations, was determined by using Bio-Merieux kits (france).

Serum estradiol, progesterone, gonadotrophic (FSH-LH) and prolactin were measured by Enzyme-linked Immune Sorbent Assay (ELISA/AVISCERA BIOSCIENC-), CA-125 and CA 15-3 levels were measured using ELISA kits (BioVision, USA).

**Statistical analysis**
The data generated on various parameters were subjected to statistical analysis for reporting group means and standard error with significance between the controls and the treated groups. All the parameters characterized by continuous data were subjected to Bartlett’s test to meet the homogeneity of variance before conducting analysis of variance and Dunnett’s t test. Man Witeny (U- test was performed to calculate the significance

**RESULTS**
The data revealed that total lipids, TC, TG, LDL, LDL/HDL and TC/HDL were significantly increased in BPA treated group. This was accompanied with a significant decrease in serum HDL as compared to control rats (table 1).

Table (2) shows that the daily oral administration of BPA resulted in a significant increase in ALT,AST and ALP activities when compare to normal female rats.

Table (3) shows that chronic administration of BPA caused a significant increase in creatinine, urea and glucose (fig 1) in comparison with control female rats.

The effect of BPA on pituitary-gonadal axis is presented in table(4). According to this results, the levels of estradiol, progesterone, prolactin, FSH were significantly increased, while LH level was significantly decreased.

According to table (5), administration of BPA induced a marked increase in CA-151 and CA-125 in comparison with control group.

All the mentioned parameters were ameliorated in the vitamin treated group (in the last 15 days).

**DISCUSSION**
Bisphenol A is a chemical that ubiquitously infiltrates our environment because of continuous release. Its release can occur via effluent discharge from municipal waste water treatment plants, leaching from landfills, combustion of domestic waste and natural breakdown of plastics in the environment.

Developmental Exposure to BPA can affect adiposity, glucose or insulin regulation, lipid profiles or other read-point related to diabetes or metabolic syndrome. In the present study, hyperglycemia, hyperlipidemia and hypertriglyceridemia were observed in BPA-treated group. Insulin is known to increase lipogenesis by both post-translational protein modifications and transcriptional mechanisms. The activity and expression of sterol regulatory element binding protein 1c(SREBP-c), which regulates cholesterol metabolism, were activated by increased insulin levels. Thus, is likely to contribute to hyperlipidemia observed in BPA group. Ahmed et al. did not rule out the contribution of other mechanisms, independent of insulin and possibly involving direct effects of BPA on the liver, to the hepatic transcriptional impacts detected after BPA exposure.

AST, ALT and ALP are enzymes of the most reliable markers of hepatocellular injury or necrosis. Their levels are elevated in a variety of hepatic disorders. ALT is thought to be more specific for hepatic injury because it is presented mainly in liver cytosole and in low concentration elsewhere. When the liver hepatocytes are damaged, these enzymes are released into the blood and significantly increased. It could be suggested that the oxidative stress induced by BPA may mediate the disturbance in hepatic function which is reflected by the increase in ALT, AST and ALP.

Elevated levels of serum enzymes ALT and AST are suggestive of liver damage, while elevated value of ALP is suggestive of Cholestasis.

BPA is an estrogenic endocrine disruptor molecule of phenolic structure used in plastics, which has renal elimination and builds up when the glomerular filtration rate decreases. The renal tubules are particularly sensitive to toxic influences, in part because they have high oxygen consumption and vulnerable enzymes systems, and because they have complicated transport mechanism that may be used for transport of toxins and may be damaged by such
toxins. Also the tubules come in contact with toxic chemicals during their excretion and elimination by the kidneys\(^9\).

Data presented in this study revealed that BPA increased urea, creatinin and BUN levels in BPA treated group than control group. BPA is eliminated by the kidney and increased blood levels has been observed in chronic kidney disease (CKD)\(^10\).

The present study examined the relationship between BPA and several serum productive hormones, there was highly significant increase in FSH, prolactin, progesterone and estrogen levels accompanied with a decrease in LH level. In vitro studies showed that BPA can bind to estrogen receptors which are capable of nongenomic steroid action. GH3/B6 pituitary cells, which express membrane estrogen receptors (mRE), respond to BPA exposure by producing calcium flux which leads to prolactin release. BPA can also induce prolactin gene expression and cell proliferation in both primary cells and GH3 cells. The increased progesterone in the fining of an alteration of preexpression following BPA exposure\(^11\).

In the present study, it was found that BPA induced a significant elevation in FSH and estrogen with concomitant reduction in LH level. Chronic exposure to BPA lead to significant increase in serum E\(_2\) level in comparison with control group. The altered expression levels of hormones at the hypothalamus and pituitary levels may be the cause of or the sequence of the changes in gonads steroidogenesis and sex hormone production. There are possible mechanistic effects of BPA on the local regulatory circuits of hypothalamus and pituitary. BPA produces its effect by interfering with one or both of the primary forms of the estrogen receptors within the hypothalamic-pituitary-gonadal axis. The decreased of serum LH level in female rats could be resulted from BPA induced reduction in luteinizing hormone releasing hormone biosynthesis in the hypothalamus and/or from direct effect of BPA on LH secretion from pituitary due to decrease stimulation of gonadotrops by GnRH as a result of impairing Lp/inositol system. The decreased serum LH level by BPA may be due to the consequence of the increase in GnRH frequency, leading to desensitization of the pituitary\(^11\). BPA can alter gene expression in mammary tissues from mature adults. In the present study chronic exposure to BPA increase breast and ovarian marker levels in the female rats. Chronic exposure of adult mice to BPA decreased the latency of tumor appearance and increased the number of mammary tumors as well as their rate of metastasis; it also enhanced the rate of mammary cell proliferation\(^12\).

BPA affects cellular functions through interaction with the membrane estrogen-receptor. BPA reduces the common chemotherapy agents in their blocking the proliferation of cancer cells. It also lead to an increased risk for development of breast tumors, and exposure to it during chemotherapy treatment make the treatment less effective\(^13\). It has been no documented that vitamin E is a potent scavenger of free radicals and is able to prevent the membrane damage mediated by free radicals. In the present study\(^14\), Vit. E ameliorated all the damages happened in all the tested parameters.

In the light of these results, it could be concluded that chronic administration of BPA induce serious problems in hypothalamus-pituitary-gonadal axis hormones, liver and kidney functions, lipid profile and breast and ovarian tumor markers. These effects are mediated by the oxidative stress induced by BPA and could be ameliorated by the antioxidant effects of vitamin E. Thus, the use of BPA in different plasticizers and other industries should be limited and the erroneous handling of plastic containers should be avoided to reduce the health risks resulting from exposure to these endocrine disruptors including BPA.

**REFERENCES**


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Table 1: The effect of bisphenol-A and vit. E on serum lipid profile (mg/dl) in female albino rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Bisphenol</th>
<th>Vitamin E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1001.23±20.72</td>
<td>1178.52±26.48**</td>
</tr>
<tr>
<td>Total lipids (mg/dl)</td>
<td></td>
<td>154.87±1.54</td>
<td>178.85±2.38***</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td></td>
<td>50.83±3.95</td>
<td>69.37±3.14***</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td></td>
<td>99.0±13.0</td>
<td>91.85±6.87</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td></td>
<td>55.7±1.50</td>
<td>80.13±2.06***</td>
</tr>
<tr>
<td>LDL</td>
<td></td>
<td>0.56±0.17</td>
<td>0.87±0.02</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td></td>
<td>1.56±0.10</td>
<td>1.95±0.03***</td>
</tr>
<tr>
<td>HDL</td>
<td></td>
<td>25.97±0.14</td>
<td>69.39±2.47***</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td></td>
<td>36.85±0.79</td>
<td>66.43±0.66**</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td></td>
<td>46.80±0.64</td>
<td>88.27±0.83***</td>
</tr>
</tbody>
</table>

***p<0.001 significant increase than control; **p<0.01 significant increase than control; *p<0.05 significant increase than control; a p<0.001 significant decrease than Bisphenol; b p<0.05 significant decrease than Bisphenol.

Table 2: Effect of BPA and vitamin E on liver function enzymes.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Bisphenol</th>
<th>Vitamin E</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (IU/L)</td>
<td>25.97±0.14</td>
<td>69.39±2.47***</td>
<td>26.10±0.81a</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>36.85±0.79</td>
<td>66.43±0.66**</td>
<td>35.5±0.88a</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>46.80±0.64</td>
<td>88.27±0.83***</td>
<td>48.37±1.18a</td>
</tr>
</tbody>
</table>

***p<0.001 significant increase than control; *p<0.001 significant decrease than Bisphenol.

Table 3: Effect of BPA and vit. E on kidney function.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Bisphenol</th>
<th>Vitamin E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.87±0.02</td>
<td>2.17±0.06***</td>
<td>0.84±0.03a</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>16.03±0.12</td>
<td>37.33±1.45***</td>
<td>15.97±0.19a</td>
</tr>
</tbody>
</table>

***p<0.001 significant increase than control; *p<0.001 significant decrease than Bisphenol.
Fig. (1): Effect of bisphenol A and vit.E on serum glucose level

Table. 4: Estradiol and Progestron levels in control and treated group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Control</th>
<th>Bisphenol</th>
<th>Vitamin E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol (Units)</td>
<td>75.37±0.54</td>
<td>88.70±1.97***</td>
<td>71.66±1.45a</td>
<td></td>
</tr>
<tr>
<td>Progesterone (Units)</td>
<td>0.47±0.003</td>
<td>0.71±0.02**</td>
<td>0.47±0.04a</td>
<td></td>
</tr>
<tr>
<td>Prolactin (Units)</td>
<td>4.37±0.13</td>
<td>6.10±0.36***</td>
<td>4.23±0.14a</td>
<td></td>
</tr>
<tr>
<td>FSH (Units)</td>
<td>4.07±0.29</td>
<td>5.19±0.19***</td>
<td>3.93±0.18a</td>
<td></td>
</tr>
<tr>
<td>LH (Units)</td>
<td>1.80±0.06</td>
<td>0.90±0.15***</td>
<td>1.62±0.05a</td>
<td></td>
</tr>
</tbody>
</table>

***p<0.001 highly significant increase than control; *p<0.05 significant decrease than Control

Table. 5: CA-153 and CA 125 levels in control and treated groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>Control</th>
<th>Bisphenol</th>
<th>Vitamin E</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA 15-3 (mg/dL)</td>
<td>0.02±0.003</td>
<td>0.23±0.06**</td>
<td>0.02±0.006</td>
<td></td>
</tr>
<tr>
<td>CA 125 (mg/dL)</td>
<td>0.02±0.008</td>
<td>0.17±0.03**</td>
<td>0.02±0.007</td>
<td></td>
</tr>
</tbody>
</table>

***p<0.001 highly significant increase than control; **p<0.01 significant increase than control;
bp<0.01 significant decrease than bisphenol ;<p<0.001significant decrease than bisphenol