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Effect of Bisphenol A on the First Generation of Female Rats from Both Parents Treated with the Same Xenoestrogen

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ABSTRACT

Background: bisphenol A (BPA) is a worldwide used endocrine disruptor that is incorporated in many plastic industries. The exposure of human to such substances starts early during the fetal life, postnatal life and extends throughout the life of the individual. Many agencies raised warnings against the excessive use of such substances. The aim of the present work was to evaluate the extent to which BPA can affect the first generation (of parents treated with the same compound, during pregnancy and lactation), which treated with the same compound during their life time.

Materials and Methods: group 1: 15 control female rats. Group 2: 15 female rats of the first generation treated with BPA (20mg/kg b.wt) for one month. Sexual hormones, liver and kidney functions were measured.

Results: BPA induced increase in breast and ovarian tumor markers. It also showed significant increase in estrogen, FSH, prolactin, and progesterone. It is also increased liver function, kidney function, lipid profile. In the same time it leads to decrease in LH, HDL, and protein levels.

Conclusion: BPA induced toxicity, which is mediated by oxidative stress. This study ringing the bells of danger for using such compounds.

Key words: BPA, female, rats, liver, kidney, tumour marker, generation, lipids.

INTRODUCTION

Bisphenol A (BPA) is a high molecular polymer organic compound widely used all over the world. It used as a component of many industrial products, such as plasticizers, the epoxy resin liners of aluminium cans, and thermal receipts**,** thermal stabilizers, pesticides, paints and dental materials. In addition, it used in the production of polycarbonate and epoxy resins. Because of the use of BPA in the production of materials used for food and potable water, it has been detected in food and water consumed by humans as well as animals[1].

It is possible that humans may gain exposure to BPA through the air and by absorption through the skin. Additional studies have quantified BPA levels in various aqueous media, including fresh and marine surface waters and groundwater[2].

BPA is absorbed from gastrointestinal tract into the blood and redistributed to other tissues. It is highly conjugated in the liver to form bisphenol A glucuronide, a major metabolite, which is excreted in urine[8.] BPA has been demonstrated in both *in vivo* and *in vitro* experiments to act as an endocrine disrupting chemical[3].

Natural estrogens bind estrogens receptors and they in turn bind to estrogens responsive elements and induce the expression of genes in their target cells. These cells include those in the reproductive organs (vagina, uterus, oviduct, ovary, cervix, testis and epididymis), the mammary gland, the brain and pituitary, the thyroid gland, the skeletal and cardiovascular systems, among others. As a synthetic estrogen with the capability of binding to estrogens receptors, BPA also has the potential to alter development at various levels of organization. High doses of BPA may mediate its effects through mechanism other than those regulated by estrogens receptors (ERs)[4].

It also acts as a xenoestrogen modulating the endocrine pathways via a receptor-mediated process. Exhibit a mechanism of action similar to that of the sex hormone at the receptor. Therefore, numerous studies have investigated the effects of BPA in male and female reproductive systems. However, few studies have concern the toxic effect of BPA on other tissues and its potential to increase the risk of metabolic disorders. Indeed, the endocrine disrupting chemicals not only act as hormone-mimics or antagonists

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that act via binding to receptors, but also can interfere with hormone synthesis and clearance, as well as other aspects of tissue metabolism[5].

BPA acts as an endocrine disrupting compound (EDC). Exposure to EDC during the perinatal period can create deleterious epigenetic modifications and, in some cases, trigger carcinogenesis. It is now clear that xenoestrogens can and do negatively affect the fetus, exemplified by the multigenerational carcinogenic effects of diethylstilbestrol (DES). Mounting evidence suggests that BPA should also be classified as a carcinogen within the definitions established by the Environmental Protection Agency (EPA)[6].

However, because of its effect as a xenoestrogen, the carcinogenic effects of BPA have been studied most often with respect to reproductive organs such as the mammary glands, ovaries and testes. Perinatal exposure to BPA at environmentally relevant levels in rats has been linked to mammary carcinogenesis in the rat dams[16] and mammary gland adenocarcinoma in the female offspring as early as post natal day 90[7].

The mechanism by which BPA might influence breast cancer in humans is so far unknown[7]; however, Acevedo *et al.*[8] showed that in human breast cancer cell lines, BPA increases DNA repair gene expression including BRCA1 and BRCA2. They concluded that women who have mutations in these two genes may be particularly susceptible to the negative effects of environmental BPA exposure. While other mechanisms may exist, there is no doubt that the epigenetic effects of BPA on the binding, synthesis, and metabolism of natural estrogens plays a large role[8].

This study aims to throw light on the danger of use BPA on the female rats (first generation) of parents treated with BPA.

MATERIALS AND METHODS

Chemicals:

Bisphenol A (BPA, Purity: 99%) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Appropriate amounts of BPA were mixed with corn oil to achieve the desired concentrations were prepared each two days.

Animals of the study:

The present study included 40 mature female and 20 mature male albino rats. Rats were obtained from the Laboratory Animal Unit, College of Science, Al-Azhar University, Egypt. They were 14 to 16 weeks old with an average body weight (150-170gm). The animals were clinically healthy, kept under hygienic conditions, housed in metal cages to avoid bisphenol exposure from old polycarbonate cages. Tab water were provided via glass bottles, and feed were giving *ad – libitum* throughout the experimental period. The animals were accommodating to the laboratory conditions for 30 days before beginning of experiment. The light system was 12/12 hrs light/dark cycle. All rats were treated with bisphenol A (20mg/kg B.wt) before and during pregnancy and lactation. Kids of these treated rats were considering the first generation.

Experimental Design:

Group1: 15 control female rats.

Group 2: 15 female rats of the first generation, 45 days old treated with bisphenol A (20 mg/Kg B.wt) for 30 days ±2 days, female must be in the diestrus phase.

At the end of the experiment, rats from treated as well as control groups were fasted overnight, weighed and anaesthetized with ether. Blood samples were collected without anticoagulant to obtain serum. Serum was separated from blood by centrifugation at 5000 rpm for 10 min and was stored at-20ºC for analysis.

Parameters of the study:

1. **Hormonal assay**: Estimation of serum prolactine, estrogen, progesterone, luteinizing hormone (LH) and follicles-stimulating hormone (FSH) levels by follow manufacture instructions of kit. All kits used for hormone assay were (Monobind Inc. lake forest CA 92630, USA).
2. **Biochemical analysis**: Serum total lipids (TL), triglycerides (TG), total cholesterol (TC) 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[9]. Friedewald's equation: LDL (mg/dl) = TC-{HDL + [TG/5]}. Ratios of LDL/HDL (risk factors) and TC/HDL were also calculated. Glucose level was estimated according to Trinder. Also, glucose, total proteins (TP), gamma glutamyl transferase (GGT), aspartate

aminotransferase (AST) and alanine aminotransferase (ALT) activities , serum alkaline phosphatase (ALP), urea, blood urea nitrogen (BUN) and creatinine concentrations were estimated by using Bio-Merieux kits (France).

3. **Tumor markers**: Levels of the tumor markers (breast cancer) CA-15.3 (ES 700; Enzymun, Roche Diagnostics, Germany) and CA-125 (ovarian cancer) (R&D Systems Inc.) in sera were determined by automated test systems using ELISA assay kits according to the protocols provided by the manufacturers.

STATISTICAL ANALYSIS

The results were expressed as mean ±SE. The comparisons between groups were performed t-test by using computerized SPSS program (Statistical Program for Social Sciences). $P<0.05$ was considered to be least limit of significance. Least significant different test (LSD) was calculated to test difference between means (groups) for t-test.

RESULT

Effect of BPA on the biochemical assays:

In Table 1, the activities of liver function; γGT, AST, ALT and ALP levels in BPA group exhibited highly significantly increased when compared to the corresponding control values. Also kidney function parameters as creatinine, urea, BUN,serum glucose were also highly significant in the BPA-treated group. On the other hand, significant decrease in and total protein levels were observed in the BPA group compared to the corresponding control value.

Effect of BPA on lipid profile:

In Table 2, total lipids, cholesterol, triglyceride and LDL values significantly increased in BPA-treated group, while a significant decrease in HDL value was recorded between the control group and the BPA- treated group.

Effect of BPA on hormones:

In Table 3, LH values significantly decrease in BPA-treated group, while significant increase in prolactin,FSH, estrogen and progesterone levels between the control group and the BPA- treated group was showed.

Table 4 shows very high significant increase in both breast and ovarian tumor markers in BPA- treated rats when compared with control rats.

DISCUSSION

Exposure to several chemicals and environmental contaminants has been reported to increase oxidative stress in body by disturbing the pro-oxidant/antioxidant balance of cells. BPA was reported to induce oxidative damage in several tissues [9].

In this study, the increase in blood glucose level may be due to the over stimulation of the estrogen receptors alpha (ERα) in pancreatic B-cells by BPA which produced an excessive insulin signaling in the liver, endothelium and in fats, thus leading to obesity, glucose intolerance and dislipedimia[10].

The elevated creatinine levels recorded in our results may be due to BPA induced peroxidative effects. BPA also induced hyperglycemia that may result in kidney damage and renal dysfunction and consequently greatly increased serum creatiin[11].

The significant change in the present activities of γGT, ALT and AST showed the toxic effect of BPA. Elevated levels of serum enzymes are indicators of cellular leakage and loss of functional integrity of the cell membrane in liver. These are of major importance in assessing and monitoring functional status of liver. Thus, their increase presence in serum may give information on organ dysfunction. It has been reported that ALT activity is an important index to measure the degree of cell membrane damage, while AST is an indicator of mitochondrial damage since it contains 80% of this enzyme. γGT activity suggests that something is damaging the liver, the higher the level, the greater the damage[12].

Alkaline phosphatase (ALP), a marker enzyme, for plasma and endoplasmic reticulum is often employed to assess the integrity of plasma membrane. The increase in serum ALP activity may be attributed to either *de novo* synthesis of the enzyme molecules or loss of other proteins from tissues. The increase in enzyme activity may have resulted from excess leakage of enzymes from hepatocytes in serum due to BPA treatment. Such increase in ALP activity can constitute a threat to the life of cells that are dependent on a variety of phosphate esters for their vital process since there may be indiscriminate hydrolysis of phosphate estrus of the tissue.

This suggests that BPA may act as a plasma membrane labilizer[12].

A significant decrease was observed in the serum protein in BPA-treated group. All the serum proteins are invariably secreted by liver. Decreased biosynthesis and secretion of protein might be due to formation of BPA adducts with DNA, RNA and protein. The ability of BPA to form DNA adducts both *in vivo* and *in vitro* has been confirmed. BPA is converted to bisphenol O-quinon, which might be the ultimate DNA and can inhibit the formation of mRNA. A failure in mRNA formation can result in an inhibition of protein synthesis, which may be considered to be the live cell necrosis[13].

Liver is the principle organ to maintain the body's homeostasis .It plays a key role in the control of results of the present study revealed a disturbance in lipid profile as reflected by the significant increase in the level of total lipids, total cholesterol, triglycerides, LDL, LDL/HDL and Tc/HDL accumulation and/or secretion accompanied with significant decrease in HDL[14].

Bisphenol A has the potential to affect lipid and glucose homeostasis in various tissues by interfering with different nuclear receptors involved in regulation of metabolism lipid metabolism. Bisphenol A has been found to stimulate lipid accumulation and up-regulate genes involved in lipid metabolism in adipocytes[14].

The present study examined the relationship between BPA exposure and several serum reproductive hormones, in the first generation (G1) female rats of parents treated with the same compound throughout pregnancy and lactation periods. There was highly significant increase in FSH, progesterone, prolactin and estradiol levels accompanied with a significant decrease in in LH level in the female treated group (G1) in compare with control group. In vitro studies showed that BPA can bind to estrogen receptors which are capable of nongenomic steroids action. GH3/B6 pituitary cells, which express membrane estrogen receptors (mER), respond to BPA exposure by producing calcium flux which leads to prolactin release[15]. BPA can also induce prolactin gene expression and cell proliferation in both primary anterior pituitary cells and GH3 cells. The increased progesterone in the present study is supported by the finding of an alteration of PR expression following BPA exposure [15].

In the present study, it was found that BPA induced a significant elevation in FSH and estrogen levels with concomitant reduction in LH level. Exposure to BPA during, in utero stage in the present study led to significant increase in serum E level in compare with control rats. During critical periods of embryonic and post natal development, the hormonal milien is crucial for the correct organization of neuroendocrine circuits that coordinate sex-specific physiology, so, the altered expression levels of hormones at the hypothalamus and pituitary levels may be the cause and/or the consequence of the changes in gonadal steroid genesis and sex hormone production[15]. 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. These finding suggest that the increase of serum E level in the present study may be resulted from interference of BPA with developmental mechanisms of local regulatory circuits of hypothalamus and pituitary which occur during gestation and neonatal period and seems to be a critical for BPA to affect reproductive neural circuit in hypothalamus of female rats. The decreased of serum LH level in females could be resulted from BPA-induced reduction in luteilizing hormone releasing hormone biosynthesis in the hypothalamus and/or from direct effect of BPA on LH secretion from pituitary due to decrease stimulation of gonadotrophs by GnRH as a result of impairing IP3/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[15].

The present results revealed that BPA induce breast and ovarian cancer in the first generation treated with BPA and their parents were treated with the same compound through their life time. BPA is associated with increased breast and prostate cancer, reproductive and sexual dysfunctions, metabolic problems and diabetes[16]. Exposure to BPA during gestation or around the time of birth lead to changes in mammary tissue structure predictive of later development of

tumors. Also increased sensitivity to estrogen puberty and abnormalities in mammary tissue development during gestation and maintained into adulthood. Prenatal exposure of rats to BPA increased the number of pre-cancerous lesions and situ tumors (carcinoma), and increased mammary tumors following adulthood exposure to BPA. Another mechanism by which prenatal exposures to BPA may affect mammary tissue development at puberty and into adulthood is through increased synthesis of the progesterone receptors and activation of progesterone-regulated mammary-cell proliferation. Neonatal exposures to BPA resulting in different timing and profiles of changes in gene expression in cells of the mammary gland[17].

CONCLUSION

The results of the present study suggest that exposure to BPA in the first generation of parents treated with the same compound, increased the hazardous effect on different body organs. So, people must use BPA-free plastic containers and minimize the use of other xenoestrogen substances.

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Tables

Table (1): The Effect of Exposure to BPA on Biochemical Analysis in G1 Mature Female Rats (Means ± SE)

	Control	Bisphenol A	P. value
Blood glucose mg/dl	97.06±1.96	191.0±1.69	<0.01**
BUN (mg/dl)	10.67±0.33	33.63±1.00	<0.01**
Urea (mg/dl)	16.03±0.12	57.32±1.45	<0.01**
Creatinine mg/dl	0.15±0.01	0.87±0.02	<0.01**
γGT U/L	9.92±0.52	14.8±1.7	<0.01**
AST (IU/L)	26.85±0.14	86±2.47	<0.01**
ALT (IU/L)	16±0.79	80±0.66	<0.01**
ALP (IU/L)	36.80±0.64	98.27±0.83	<0.01**
Total protein g/dl	6.16±0.16	4.24±0.04	<0.01**

Table (2): The Effect of Exposure to BPA on Lipid Profile in G1 Mature Female Rats (Means ± SE)

	Control	Bis-phenol A	P. value
Total lipids (mg/dl)	1101.23±20.72	1378.52±26.4	<0.01**
Cholesterol mg/dl	83.02±2.15	133.47±1.54	<0.01**
Triglyceride mg/dl	22.98±1.8	41.93±2.89	<0.01**
HDL mg/dl	59.5±2.57	49.0±13.0	<0.01**
LDL mg/dl	11.94±0.26	65.87±1.5	<0.01**
Cholesterol/HDL	1.22±0.39	2.72±0.11	<0.01**
LDL/HDL	0.20±0.10	1.34±0.11	<0.01**

Table (3): The Effect of Exposure to BPA on Reproductive Serum Hormones Levels in G1 Mature Female Rats (Means ± SE)

	Control	Bis-phenol A	P. value
FSH mIU/ml	3.06±0.88	4.24±0.01	<0.01**
LH mIU/ml	2.20±0.15	1.17±0	<0.01**
Prolactin ng/ml	2.4±0	6.80±0.06	<0.01**
Estrogen Pg/ml	26.27±3.78	92.83±4.28	<0.01**
Progesterone ng/ml	0.44±0.03	2.87±0.28	<0.01**

Table (4): CA-15.3 and CA-125 Levels in the Control and Treated Groups (Means ± SE)

Groups / Parameters	Control	*BPA-treated group*	*P*-value
CA-15.3 (kU/L)	0.02 ± 0.002	0.33 ± 0.002	<0.001***
CA-125 (kU/L)	0.02 ± 0.004	0.27 ± 0.004	*<0.01***