Histopathological and Immunohistochemical Changes Induced by BisphenolA in Reproductive Tissues of Female Rat

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ABSTRACT
Background: Bisphenol A (BPA) is an environmental chemical that has been widely used in the manufacture of polycarbonate plastics and epoxy resins for many years. Due to its major applications in the production of plastic food or beverage containers and the coating of food cans, people of different ages are inevitably exposed to BPA in daily life. It is a contaminant with increasing exposure to it and exerts both toxic and estrogenic effects on mammalian cells. Aim of the work: The present study was designated to evaluate the histopathological and immunohistochemical effect of BPA on the histoarchitecture of pituitary, adrenals, ovaries and uterine axis of female albino rats and the ameliorative effect of antiestrogen drug and stem enhance. Experimental model and methods: 20 female albino rats weighing 100 – 120 g, were kept under observation for about 15 days before the onset of the experiment for adaptation, then the rats were classified into 4 groups. The first group was left without any treatment for 30 days as negative control group, the second group was administered with 20 mg/kg,bw of BPA for 15 consecutive days as positive control, the third group administered with 20 mg/kg,bw of BPA for 15 consecutive days and then treated with antiestrogen drug as 0.1 mg/100gm,bw for 15 days, the fourth group administered with the same dose for the same period and the treated with stem enhance (4.5 mg/100 bw) for 15 days. All rats were sacrificed and organs were histologically examined after processing. Results: the results showed that PA has a histopathological effects on vital organs (pituitary, adrenal, ovaries and oviduct and uterus) even for a short period with minimal ameliorative effect of antiestrogen drugs and stem enhance. Keywords: Bisphenol A – xenoestrogen – antiestrogen – stem enhance.

INTRODUCTION
Bisphenol A. could be polymerized to make polycarbonate plastic, a miraculous cheap product that is lightweight, transparent, colorable, resistant to impact, heat, and chemicals, inalterable with time, and easy to mold and thermoform. BPA rapidly became one of the most produced and used chemicals worldwide, even though it was a recognized synthetic estrogen. Exposure to BPA is almost universal: most people have measurable amounts of BPA in both urine and serum. BPA is composed of two phenol groups which have structural homology with estradiol, it is suspected that it mimics estrogenic actions. BPA is capable of inducing toxic effect on nonreproductive vital organs; several studies in the literature have reported absorption of large amounts of BPA through skin which has been shown to cause extensive damage to the liver and kidney in humans. Antiestrogen drugs classified into aromatase inhibitors such as aminoglutethimide, anastrozole, exemestane, and selective estrogen receptor modulators likeraloxifene, tamoxifen.

Lonning et al., 2004. Stem Cell Enhancers facilitate and trigger the mobilization of bone marrow stem cells to the injured tissue needs for repairing. Drapeau et al., 2009. Aim of the work: The present study was designed to investigate the effect of antiestrogenic drug and stem enhancer on female rat exposed to BPA.

MATERIALS AND METHODS
Twenty female albino rats of Sprague Dawely strain, weighing around 100-120g, at the age of 6-8 weeks were purchased from Theodore Bilharz Research Institute, Giza, Egypt. They were kept under observation for about 15 days before the onset of the experiment for adaptation. Experimental design: Experimental animals were divided into four groups (five/each) as follows:
• Group 1 (Control group): Normal young female rats (without any treatment) for 30 days.
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- **Group II (Bisphenol-A group):** young female rats were orally administered with 20mg BPA /kg b.wt/day for 15 days.
- **Group III (Bisphenol-A group):** young female rats were orally administered with 20mg BPA /kg b.wt/day for 15 days and then administered orally with anti-estrogen (Nolvadex) (0.1mg/100g/day) for 15 days.
- **Group IV (Treated group):** Rats orally received BPA daily for 15 days andorally supplied with stem enhance (0.1mg/100g/day)only for other15 days.

**The drugs and dosage:**

**Bisphenol A:**

The drugs used in this work were Bisphenol A (BPA) (2,2 Bis-4- hydroxyl phenyl propane) suspended in water and orally administered to animals. The dose of BPA was calculated according to(7)(Takahashi and Oishi 2003).

**Anti estrogen drug:**

Tamoxifen is a non-steroidal, triphenylene-based drug displays a complex spectrum of estrogen antagonist. Drug purchased as dietary supplements 60 capsules of 500mg each for dosing from Astra Zeneca Co.

**Stem enhance:** Drug purchased as dietary supplements 60 capsules of 500mg each for dosing from stem tech. health science,Inc.

**Histological and immunohistochesmcal techniques:**

Adult female rats in Diestrus cycle were sacrificed and the organs were excised for histological and immunohistochemical observations .The excised organs were fixed in Bouan’s solution for about 24 hours, washed in 70% alcohol, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and impregnated in parablast for blocking. Serial sections of 5 µm thick were prepared and stained with Hematoxylin and Eosin.

For immunohistochemical observations five-micron sections of uterus, ovary, oviduct and adrenal gland fixed in Bouan’s fixative, immunostained using anti-primary antibody (Labvision, Neomarkers, USA) for 90 minutes. This was followed by the antiestrogen secondary antibody to the excised organs using the immunoperoxidase technique (Vectastain ABC kit; Vector Laboratories, Burlingame, CA).

**Results**

**Uterus**

Control uterus showing normal endometrium with uterine glands lined with simple cuboidal epithelial cells (fig.1A). An intensive immunohistochemical reaction for antiestrogen receptors is observed in the uterine glands (fig.6A). BPA treated group showed hypertrophied glands and cells showed hyperplasia.(fig.1B). Faint immunohistochemical reaction for antiestrogen receptors is observed in the uterine glands (fig.6C).

BPA and anti estrogen drug treated rats showing uterine glands with hypertrophy and hyperplasia. (fig.1D). Strong positive immunohistochemical reaction for antiestrogen receptors is observed in the uterine glands (fig.6D).

**Ovary**

Control ovary showing normal ovarian follicles including primary and tertiary follicles ,also graffian follicle presents with centrally located oocyte .(fig.2A) and strong positive immunohistochemical reaction for antiestrogen receptor is observed in ovarian stroma (Fig 8A) BPA treated ovary showed many degenerated and atretic follicles in the ovarian stroma. (fig.2B) and weak positive immunohistochemical reaction for antiestrogen receptor is observed in ovarian stroma (Fig 8B) BPA and stem enhance treated rats showing a marked improvement in the ovarian tissue including normal follicles and well organized graffian follicle with oocyte (fig.2c) and weak positive immunohistochemical reaction for antiestrogen receptor is observed in ovarian stroma (Fig 8C) BPA and anti estrogen drug treated rats showing no improvement as many atretic and degenerated follicles were seen (fig.2D) and negative immunohistochemical reaction for antiestrogen receptor is observed in ovarian stroma (Fig 8D)

**Oviduct**

Control oviduct stained with H&E showing normal ciliated gohert columnar epithelial cells. (Fig.3A). An intensive immunohistochemical reaction for anti estrogen receptors is observed in the oviduct lined epithelium (fig.7A). BisphenolA treated rats showing no hiatopathological changes.(Fig.3B) Faint immunohistochemical reaction for anti estrogen
receptors is observed in the oviduct lined epithelium. (fig.7B) while ,Bisphenol A and stem enhance treated rats and bisphenol A and anti estrogen drug treated rats showing all normal histological picture. (Fig.3C), (Fig.3D). Also a strong immunohistochemical reaction for anti estrogen receptors is observed in the oviduct lined epithelium was observed in both bisphenol and stem enhance treated rats and bisphenol A and anti estrogen drug treated rats. (fig.7C),(fig.7D).

Adrenal gland
Control adrenal gland showing normal closely backed cells of granulosa layer with vesicular nucleus and intact cytoplasm .(fig.4A). BisphenolA treated rats showing pyknosis kareolysis in the nuclei of some granulosa cells with vacuolated cytoplasm (fig.4B). Bisphenol and stem enhancer treated rats showing normal adrenal granulosa layer (fig.4C). Bisphenol and anti estrogen drug treated rats showing granulosa layer cells with vacuolated cytoplasm and some pyknotic nuclei (fig.4D).

pituitary gland
control pituitary staining with H&E showing normal pars distalis zone (fig.5A). BPA treated group showed pyknosis in the nucleus of some cells of pars distalis zone (fig.5B). BisphenolA and stem enhancer treated rats showed no histopathological changes in pars distalis zone.(fig.5C). Bisphenol A and anti estrogen drug treated rats showing pituitary pars distalis zone with few pyknotic nuclei. (fig.5D)

DISCUSSION
BPA treated rats showed many histopathological and immunohistochemical changes represented in appearance of hypertrophied uterine glands many atretic ovarian follicles ,damaged cilia of epithelium lined oviduct and pyknosis of nucleus of some granulosa layer cells with vacuolated cytoplasm in adrenal glands. Also cells of the pituitary pars distalis zone showed some pyknotic nuclei . These findings are in agreement with90,91Boshr and Moustafa, 2011; Verma and Sangai, 2009 who reported that BPA causes cell infiltration and necrosis. Also previous studies showed that treatment with bisphenol A leads to cell rupture and membrane damage of human erythrocytes which may be due to the oxidative stress90,91Hassan et al., 2012 ; Grattagliano et al., 1999 revealed that BPA caused marked oxidative impact by decreasing the activities of antioxidant enzyme.Nitric oxide (NO) is a highly diffusible free radical. According to90Hassan et al., 2012. Levels of NO production increased after BPAXposure in the three different puberty periods. It was demonstrated that, NO reacts with superoxide (O2__) to form peroxynitrite (ONOO_) a highly reactive free radical, therefore,NO causes increased nitrosative stress .

Moreover, NO is a potent oxidant and nitrating agent is capable of attacking and modifying proteins, lipids, and DNA as well as depleting antioxidant defenses91Grattagliano et al., 1999.

The present study showed that immunohistochemical reaction for antiestrogen receptor antibody showing damaging in some estrogen receptors in uterus ovary and oviduct . BisphenolA as hormonal disruptor has a dramatic effect on the estrogen receptors in the reproductive organs92Takayanagi et al., 2006 revealed that BPA had an impact as an endocrine disruptor even at low doses and binding to ER and affecting hormonal activity in many organs.

While normal estrogen receptor distribution was seen after treatment with anti estrogen drug and stem enhances the anti estrogen drug antagonize the action of estrogen in the tissue by blocking of the estrogen receptors93Mouridsen et al., 2009 the slight improvement in the estrogen receptors after tamoxifen treatment. In the present study immunohistochemical reaction of the ovary showed strong reaction with negative control and very weak reaction with BPA & PBA and stem enhance treated rats and BPA and antiestrogen drug treated rats.These results can be explained by the results of94Brieno-Enriquez et al., 2011 who reported on a genetic bases the cytogenetic effect of BPA exposure in cultured human fetal oocyte. BPA-exposed oocytes showed lower viability in culture, a delay in meiotic progression and increased level of meiotic recombination . Also showed for the first time the gene expression of human fetal oocytes and gene expression of BPA-exposed oocytes; oocytes from ovaries exposed to PBA showed an upregulation of genes involved in DBS generation, signaling and repair as well as estrogen15 receptors. 95Seung Gee Lee et al., 2013 reported that short term BPA-exposure in adult female rats induced E2 decreases are initially provoked by reduced pituitary gonadotropin or by follicle loss via granulosa cell apoptosis at earlier time both at 1 and 2 weeks.
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In our study the weak immunohistochemical reaction of BPA treated group and BPA and stem enhance may be due to apoptotic effect of BPA and which may be modulated by regeneration stem enhance while negative immunohistochemical reaction of antiestrogen receptors with BPA and antiestrogen drug explain the destructive effect of both on ovarian tissue which the result is in aggrement with [16] Boone and Tsang 1998 who reported that Caspase-3 has been implicated in ovarian follicular atresia and leuteal regression in the rat ovary.

CONCLUSION

BPA has dramatic effect in the reproductive organs as a hormonal disruptor mimic the hormonal effect of external estradiol while the stem enhance improve the histopathological changes occurred by the action of BPA ,on contrast the anti estrogen drug increase the damaging effect on the reproductive organs including uterus ,ovary and not the oviduct which showed no histopathological changes.

REFERENCES


Fig. 1 Photomicrographs of female rats uterus showing A: Control normal uterine glands. B: bisphenol A treated rats with uterine glands showing hyper trophy and hyperplasia. C: bisphenol and stem enhancer treated rats showing hypertrophied cells of uterine gland. D: bisphenol and anti estrogen drug treated rats showing uterine glands with hyperplasia (black arrow).

H&E X400

Fig. 2 Photomicrographs of female rats ovaries showing A: Control normal ovarian follicles (f). B: bisphenol treated rats with ovarian artistic follicle (AF). C: bisphenol A and stem enhancer treated rats showing normal ovarian follicles (f). D: bisphenol and anti estrogen drug treated rats showing ovarian follicles with atresia (AF). H&E X400
Fig. 3 Photomicrograph of rat oviducts A: Control normal oviduct with ciliated cuboidal lined epithelial cells. Also B: bisphenol A treated rats showing no histopathological changes. C: bisphenol A and stem enhancer treated rats and D: bisphenol A and anti estrogen drug treated rats showing all normal histological picture. H&E X400

Fig. 4 Photomicrograph of rat adrenal glands showing A: Control normal adrenal granulosa layer. B: bisphenol A treated rats showing pyknosis, karyolysis in the nucleus of some granulose cells with vacuolated cytoplasm. C: bisphenol and stem enhancer treated rats showing normal adrenal granulosa layer. D: bisphenol and anti estrogen drug treated rats showing granulosa layer cells with vacuolated cytoplasm and some pyknotic nuclei. H&E X400
Fig. 5 Photomicrographs of rats pituitary gland showing A: Control normal pituitary pars distalis zone. B: bisphenol A treated rats showing pituitary pars distalis zone with some pyknotic nuclei. C: bisphenol A and stem enhancer treated rats showing normal pituitary pars distalis zone. D: bisphenol A and anti estrogen drug treated rats showing pituitary pars distalis zone with few pyknotic nuclei (black arrow) H&E X400

Fig. 6 Photomicrographs of rats uterus showing A: Strong positive immunohistochemical reaction for control uterus. B: bisphenol A treated rats showing weak immunohistochemical reaction. C: bisphenol and stem enhancer and D: bisphenol A and antiestrogen drug treated rats showing strong positive immunohistochemical reaction. Anti Est. receptor reaction x400
Fig. 7. Photomicrographs of rat oviducts showing A: Strong positive immunohistochemical reaction for control oviduct. B: Bisphenol A treated rats showing weak immunohistochemical reaction. C: Bisphenol A and stem enhancer and D: Bisphenol A and anti estrogen drug treated rats showing strong positive immunohistochemical reaction.

Anti Est. receptor reaction x400

Fig 8A. A photomicrograph of female rat ovary showing A: Strong positive immunohistochemical reaction for control ovary (black arrow).

Anti Est. receptor reaction x100
Fig. 8B. Photomicrographe of rat ovary showing weak positive immunohistochemical reaction for BisphenolA treated rats. *Anti Est. receptor reaction x100*

Fig. 8C. Photomicrograph of female rat ovary showing *weak* positive immunohistochemical reaction for BisphenolA and stem enhancer. *Anti Est. receptor reaction x100*

Fig 8D. Photomicrograph of female rat ovary showing *negative* immunohistochemical reaction for BisphenolA and anti estrogen drug treated rats. *Anti Est. receptor reaction x100*