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Impact of prenatal and postnatal exposure to bisphenol A on female rats in a two generational study: Genotoxic and immunohistochemical implications

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ABSTRACT

Environmental xenoestrogen contaminant bisphenol A (BPA), widely used as a monomer in the manufacture of epoxy, polycarbonate plastics and polystyrene resins. However, exposure to BPA has raised concerns, and the negative impacts of its exposure on reproduction have been controversial. The purpose of this work was directed to assess the potential adverse effects of BPA on dam rats and their first generation females in a comparative toxicological study. Fifteen pregnant female rats were classified into three equal groups; first group was orally administered corn oil and served as control (group1), second and third groups were orally administered BPA at dose levels of 50 and 200 mg/kg b.wt respectively (groups 2 & 3). The administration was carried out daily from zero day through the gestation period (21 days) until the last day of the lactation period (21days) and was extended after weaning for three months, in which female off springs of first generation (F1) of the three groups of dams were classified into; F1control group (group 4), F1 group treated with low dose of BPA (group 5) and F1 group treated with high dose of BPA (group 6) which continued in daily oral administration of BPA at the same previously mentioned doses for three months. The results elucidated a clear marked DNA fragmentation in the ovary of both dam and F1 female groups especially at higher examined concentration. Also, the data demonstrated a significant increase in the serum levels of GGT, ALP, glucose, total cholesterol, triglycerides, LDH and also in the serum level of estrogen hormone. Meanwhile, our study recorded a significant decrease in total protein, catalase, GST, HDL and FSH hormone in both treated dam and F1 female groups which was more significantly decreased in F1 female rats. Moreover, our experiment illustrated up-regulation in the immunoexpression of ERβ in ovary, uterus and liver of dam and F1 female groups. The histopathological investigation showed degeneration in the epithelial lining of ovarian follicles, submucosal leukocytic infiltration and increase in vaculation of hepatic cells with proliferation of kupffer cells. The lesions were more sever in groups 3 & 6 of both dam and their F1 females. Our data speculated that long- term exposure to BPA at 50 and 200 mg/kg.b.wt. depicted total genomic damage, significant alterations in liver enzymes, lipid profile, antioxidant enzymes and reproductive hormones with up-regulation in the expression of ERβ which were more significantly perturbed in group 3 and group 6 of both dam and F1 female rats.

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1. Introduction

Many environmental chemicals recently, are considered as xenoestrogens and endocrine disruptors [31,7]. Nowadays, there is growing concern regarding the impact of environmental chemicals on animal and human reproduction [19]. Such endocrine disruptors may represent a major toxicological and public health issue [35]. The xenoestrogen bisphenol A (BPA) has received much attention due to its high production volume and wide spread exposure [24].

Bisphenol A {BPA; 2,2-bis-(4-hydroxyphenyl) propane} is a plasticizer that is widely used to produce polycarbonate plastic, epoxy resin and unsaturated polystyrene. BPA can leach from linings of food cans, polycarbonate baby bottles and other beverage containers, dental sealants and composites, polyvinyl chloride plastics and recycled thermal paper [60]. This compound released to the environment both accidentally and through permitted dis-

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charges [53] and its wide spread distribution has been a major cause of concern to regulatory agencies and others [45]. Its lifetime in the environment is sufficient for it to be virtually permanently detectable and it has been described as ubiquitous in surface waters [29].

Christiansen et al. [11] examined the influence of BPA (5 and 50 mg/kg b.wt.) on early sexual development in male and female rats, they recorded significant decrease in anogenital distance of both sexes besides; the incidence of nipple retention in male appeared to increase.

Studies on BPA genotoxicity have conflicting results; both genotoxic [37] and non- genotoxic [21] effects of BPA have been reported. Where, studies of BPA genotoxicity have yielded conflicting results; BPA is considered non-genotoxic because it was negative to a set of basic genotoxicity tests [49], did not induce gene mutations [57] or chromosomal aberrations [23]. In contrast, BPA induced numerical chromosomal aberrations and morphological changes in cultured Syrian Hamster Embryo cells [57]. In addition, BPA metabolites were shown to bind to DNA in a cellular system [13].

It was reported that BPA induce oxidative stress [28], in which reactive oxygen species (ROS) are cytotoxic agents that lead to significant oxidative damage by attacking biomolecules such as membrane lipids and DNA in cells [27].

A few studies have focused on whether BPA exerts its action through nuclear receptors such as estrogen [50], androgen [56] and thyroid [44] receptors. Further, Peretz et al. [40] have shown that BPA does not exert its toxic effects via the genomic estrogenic pathway in mouse ovarian follicles.

Meanwhile, in vitro studies demonstrated that BPA binds to the estrogen receptors induces estrogen – dependant gene expression responses [30] and Caserta et al. [9] mentioned that BPA has estrogenic activity and binds to α and to a lesser extent to β-estrogen receptors. Further BPA can acts as antiestrogen, blocking these estrogen response by competing with endogenous estrogen [42].

In this spirit and because there is paucity of information concerning studying the comparative toxic impacts from long-term exposure to BPA on dam rats and their female generations. We show in this study for the first time the effects of BPA in a comparative manner on dam rats and their first generation females concerning DNA damage, expression of estrogen receptor β, with some biochemical parameters and histopathological examination of liver and reproductive organs.

2. Materials and methods

2.1. Chemical compound

Bisphenol A (purity 97% of CAS number 80-50-7) was obtained from sigma- Aldrich Company and dissolved in corn oil [32].

2.2. Animals and dosing

Eighty mature albino rats (sixty of females weighing 150–200 gm b.wt. and twenty of males weighing 220–260 gm b.wt., used for mating) were obtained from experimental Animal Unit of Faculty of Veterinary Medicine, Zagazig University, Egypt. Animals were kept in metal cages under hygienic conditions, fed on well balanced ration and provided with water *ad-libitum* throughout the experiment.

Experiments were conducted in accordance with the guidelines set by Animals Health Research Ethics Training Initiative, Egypt, and experimental protocols were approved by the institutional animal ethics committee.

Female dam rats were daily examined to ensure estrous phase using vaginal smear technique, females in estrous were paired with mature males, the presence of sperms in vaginal smear indicating zero day of gestation [4]. Fifteen pregnant female rats were classified into three equal groups; first dam group orally administered corn oil and served as control (group1), second dam group orally administered BPA at dose level of 50 mg/kg b.wt. [61] (group2) and third dam group orally administered BPA at dose level of 200 mg/kg b.wt. [48] (group3). The administration was carried out daily from zero day throughout the gestation period (21days) until the last day of lactation period (21days) and extended after weaning for three months.

After weaning, female off springs of first generation (F1) of the three groups of dams; each group contain seven females of F1 were classified into; F1control group (group 4), F1 group treated with low dose of BPA (group 5) and F1 group treated with high dose of BPA (group 6) which continued in oral daily administration of BPA at the same previously mentioned doses for three months.

At the end of the experiment, blood samples were collected from the retro-orbital sinus without anticoagulant in sterile test tubes for separation of serum which kept at −20 °C till biochemical analysis and then dam rats of the three groups and their F1 females that examined for pro-estrus phase were anesthetized and euthanized by decapitation. Tissue samples from ovaries of all groups were taken and kept at −20 °C for applying DNA fragmentation assay. For immunohistochemical and histopathological studies, specimens from ovary, uterus and liver were collected and fixed in 10% buffered neutral formalin solution.

2.3. DNA fragmentation assay

DNA damage determined by DNA fragmentation assay according to Bortner et al. [8] that could be summarized as following:

Small pieces of ovarian tissues were put in 1.5 ml microfuge tube. Extraction buffer was added to 0.3 ml mark. Tissues were crushed and then extraction buffer was added till 0.5 ml mark. 50.0 μl of proteinase-K solution (10.0 mg/ml) was added then the tubes were closed and inverted to mix. Tubes were incubated at 50 °C for 12 h: 3 days with occasional vigorous mixing. DNA was extracted with a mixture of phenol, chloroform and isoamyl alcohol (25: 24: 1) and vortex samples 5.0 s. Samples were centrifuged at 12000 rpm for 5.0 min. Then 500 μl of aqueous layer for each sample was removed carefully into a new tube and 50.0 μl of 3.0 M sodium acetate (pH = 5.3) was added to each tube. Pure ethanol (100%) was added till mark 1.5 ml. The tubes were inverted for mixing and DNA precipitation then let to be set at −20 °C overnight and centrifuged at 12000 rpm for 20 min. The supernatant was removed and 50.0 μl of Tris EDTA buffer was added overnight till complete dissolving. Samples were run on electrophoresis using 1.2% agarose gel at 50.0 voltages, gel was stained using ethidium bromide. Samples were analyzed using image analyses software.

2.4. Immunohistochemical examination for determination of estrogen receptor–β (ERβ)

The paraffin embedded ovaries, uteri and livers were fixed in 10% formalin, sectioned into 5 μm sections, and mounted on positively charged slides for immunostaining of ERβ. Sections were deparaffinised, rehydrated and autoclaved at 120 °C for 10 min in 10 Mm citrate buffer (pH 6) for ERβ. After washing with PBS endogenous peroxidase was blocked using 0.3% hydrogen peroxide in methanol (15 min). Thereafter, slides were washed in PBS again and blocking was performed by adding blocking buffer and incubated for 30 min at room temperature. Primary antibody for ERβ (Cat. No. RB- 10658-R7, Thermo Scientific Co., UK). It was diluted by PBS (1:100) then added to the slides and incubated for 30 min.

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