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Histological and Immunohistochemical Study on the Effect of Bisphenol A on the Retina of Female Albino Rat

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ABSTRAT

Background: Bisphenol A (BPA) is a well-known endocrine disruptor used to manufacture polycarbonate plastics and epoxy resins. Exposure of rats to low doses of BPA results in histopathological effects in their retina.

Objectives: We used histological and immunohistlogical techniques for determining retina pathological changes in response to low doses of BPA and the modulating effect of both vitamin A and stem enhancer.

Methods: Twenty female albino rats orally administered with 20mg BPA /kgb.wt/day for 45 days and then divided to groups and treated with vitamin A and stem enhancer. Both eyes were examined histologically and immunohistochemicaly to determine the histopathological changes in retinal layers.

Results: A remarkable degenerative histopathological changes in the ganglion cell layer and inner nuclear layer appeared with bis phenol treated rats and a clear improvement was seen after treatment with both vitamin A and stem cell enhancer.

Conclusions: Both of vitamin A and stem enhancer have ameliorative effect on the degenerative changes in the retinal layers and damaged estrogen receptors by the action of bisphenol A.

Key Words: Estrogen - Anti estrogen receptor – BPA - Retina

INTRODUCTION

BPA was first synthesized by Dianin in 1891, and its estrogenic activity was discovered in 1936. It is therefore one of the oldest synthetic compounds known for its endocrine activity, although diethylstilbestrol (DES) has stronger estrogenic activity. In the 1950s, it was observed that BPA 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.[1]The estrogenic properties of bisphenol A (BPA) has been known since 1936 (Dodds, 1936), but recognized as an environmental endocrine disrupting compound (EDC), a xenoestrogen, in the late 1990s. Endocrine effects of BPA has been observed in both epidemiological and experimental studies [2] but yet without any solid proof of deleterious effects, and therefore more studies are required. BPA can act by the classical nuclear estrogen receptor a (ERa) and estrogen receptor b (ERb), but the estrogenicity has been regarded as weak in relation to endogenous estrogen, and thus negligible.

However, other mechanisms of the estrogen than the classic nuclear estrogen receptor a (ERa) and estrogen receptor b (ERb) suggest that there are routes where BPA can act as a rather potent estrogen, e.g. via membrane-bound estrogen receptors, mERa, mERb and G protein-coupled receptor 30 (GPR30)[3].

Vitamin A, a chemical originally used only for light sensing, is now also an essential molecule for eye development. Retinoic acid, the acid form of vitamin A, plays critical roles in retina and eye development [4].

Stem Cell Enhancers facilitates the migration of stem cells of the bone marrow to any tissue in the body needing repair.[5]

MATERIALS AND METHODS

I) Material: 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.

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II) **Experimental design:**

Experimental animals were divided into four groups (five/Each) as follows:

Group I (Control group): Normal young female rats (without any treatment) for 30 days.

Group II (Bisphinol-A group): Young female rats were orally administered with 20mg BPA /kgb.wt/day for 45 days.

Group III (Bisphinol-A group): Young female rats were orally administered with 20mg BPA /kgb.wt/day for 45 days and then orally supplied with vitamin A 4.5/100mg/day) for 15 days.

Group IV (Treated group): Rats orally received BPA daily for 45 days and orally supplied with stem enhance (4.9mg/100g/day)only for other15 days.

1- **The drugs and dosage:**

Bisphenol A:

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 **(Takahashi and Oishi 2003).**[6]

Vitamin A: Drug purchased as dietary supplements 20 gelatinous capsules of 500 mg for dosing from pharco, company.therapeutic dose calculated according to Goash table

Stem enhance:

Drug purchased as dietary supplements 60 capsules of 500mg for dosing from stem tech. health science, Inc. therapeutic dose calculated according to Goash table

II) Methods:

1- Histological techniques

Adult female rats in Diestrus cycle were sacrificed and the eyes were immediately enucleated for histological observations. Excised organ were fixed in Bouan's solution for about 24 hours, washed in 70%alcohol, dehydrated, cleared in xylene and impregnated in parablast for blocking. Serial sections of 5 µm thick were prepared and stained with hematoxylin and Eosin

2-Immunohistoshemical techniques

For immunohistochemical observations five-micron sections of eye for retina examination fixed in Bouins fixative immunostained using anti- primary antibody (Labvision, Neomarkers, USA) for 90 minutes. This was followed by the anti estrogen receptors secondary antibody for retina using the immunoperoxidase technique (Vectastain ABC kit; Vector Laboratories, Burlingame, CA).

RESULTS

Control retina: Hematoxylin and Eosin stain showing well organized layers with normal structure for Ganglion cells with intact nucleus, inner plexiform layer, inner nuclear layer, outer nuclear layer, and photoreceptor layer**(fig.1 A)**.An intensive positive immunohistochemical reaction is observed for anti-estrogen receptors in the ganglion cell layer**(fig.2 A)**.

Bisphenol A treated group: Hematoxylin and Eosin stain showed that treatment with bisphenol A for 45 day induced retinal degeneration as GCL, EPL, PRL. RPE layers were discontinuous..Pyknosis was also seen in the ganglion cells of the ganglion layers . **(fig.1B)**. A weak immunohistochemical reaction for anti estrogen receptors in the ganglion cell layer was observed after the treatment with bisphenol A **(Fig.2B)**.

Vitamin A treated group: Hematoxylin and eosin stain showed a marked improvement in the retinal layers after the treatment of bis phenol administered rat with vitamin A**. (Fig.1 C).** The immunohistochemical reaction for anti estrogen receptor antibody showed a moderate positive reaction in the ganglion cell layer. (Fig.2 C)

Stem enhancer treated group: Hematoxylin and eosin stain showed an improvement in the retinal layers after the treatment of bis phenol administered rat with Stem enhancer**. (Fig.1 D).**The immunohistochemical reaction for anti estrogen receptor antibody showed a mild positive reaction in the ganglion cell layer. (Fig.2 D)

DISCUSSION:

Our data revealed that BPA cause many degenerative histopathological changes in the retinal layers specially OPL which may lead to disruption of vision process ,also there was immunohistochemical changes in the anti estrogen receptors in female group after 15 days of the oral administration with bisphenol-A, also a clear improvement was observed after treatment with vitamin A and stem enhancer each alone. According to (Welshons et al., 2006;[7] Alonso-Magdalena et al.,2012)[8]BPA initiates rapid responses through membrane bound estrogen receptors with about the same potency as 17bestradiol(E2).Also Takayanagi et al[9] reported that BPA estrogenic chemical able to interact with human estrogen receptors (ER).It is possible that ESR1 is more likely to bind coactivators than corepressors when

induced by BPA[10].Quesada 2002[11] found that bisphenol A is just as powerful as estradiol in binding with this estrogen receptor and alter gene expression causing serious hormonal disturbance.

Estrogen protects neurons in the retinal GCL including RGCs from both apoptosis and early changes in synaptic connections associated with ischemia and potentially preceding apoptosis.12

Current evidence indicates that estrogen receptors are found throughout the retinal thickness, concentrated prominently in the RGC and nerve fiber layers. At least in part, estrogen receptors mediate the influence of estrogens on blood viscosity and ocular vascularity13

According to Gould *et al*[14].BPA interacts with the ERa in a unique manner that differs from other known classes of ER ligands. This unique interaction with the ERa may explain, in part, differences in the effects of BPA as compared with E2 in vivo. In other wards BPA competitively bind to ERa and are potential modulators of the endocrine system, according to our study this may explain the histopathological changes in the cells of the ganglion layers.

Vitamin A and stem enhancer treatment caused an obvious improvement in the retinal layers. This may be explained with vitamin A aldehyde which is called Retinal as it is one of the many forms of vitamin A (the number of which varies from species to species). Retinal is a polyene chromophore, bound to proteins called opsins, and is the chemical basis of animal vision4.Bone marrow adult stem cells have been shown to play an important role in tissue repair and the stem cell enhancer is an ideal mobilizer of bone marrow adult stem cells that was shown to increase the number of circulating stem.

REFERENCES

1. ***Soria Eladak, Tiphany Grisin, Delphine Moison, Marie-Justine Guerquin, Thierry N'Tumba-Byn, St_ephanie Pozzi-Gaudin, Alexandra Benachi, Gabriel Livera, Virginie Rouiller-Fabre, and Ren_e Habert.*** A new chapter in the bisphenol A story: bisphenol S and bisphenol F are not safe alternatives to this compound. J Fertility and Sterility, ***2015***; 103(1): 015-282.
2. ***Monika R♀nn a, Lars Lind b, Jan rberg c, Joel Kullberg d, Stefan S♀derberg e, Anders Larsson b,Lars Johansson d, Hkan Ahlstr♀m d, P. Monica Lind a***. Bisphenol A is related to circulating levels of adiponectin, leptin and ghrelin, but not to fat mass or fat distribution in humans. J Chemosphere, 2014; 112 : 42–48.
3. ***Watson, C.S., Alyea, R.A., Jeng, Y.J., Kochukov, M.Y.***Nongenomic actions of low concentration estrogens and xenoestrogens on multiple tissues. J Mol Cell Endocrinol, 2007; 274: 1–7.
4. ***Sun H.*** Membrane receptors and transporters involved in the function and transport of vitamin A and its derivatives. Biochim. Biophys. J Acta, 2012; 182: 99–112.
5. ***Christian Drapeau***. An increase in the number of circulating adult stem cells by Stem Enhance does not promote tumor growth 2009; Stemtech., Health Sciences, Inc.
6. ***Jensen S, Drapeau C.*** The use of in situ bone marrow stem cells for the treatment of various degenerative diseases. J Medical Hypotheses, 2002; 59:422-428.
7. ***Welshons, W.V., Nagel, S.C., vom Saal, F.S.*** Large effectsfromsmallexposures.III.Endocrinemechanisms mediating effects of bisphenol A at levels of human exposure. Endocrinology, 2006; 147, s56–s69.
8. ***Alonso-Magdalena, P., Ropero, A.B., Soriano, S., Garc¿a-Arévalo, M., Ripoll, C.,Fuentes, E., Quesada, I., Nadal.***. Bisphenol-A acts as a potent estrogen via non-classical estrogen triggered pathways. Mol. Cell. Endocrinol,2012;355: 201–207.
9. T***akayanagi S1,Tokunaga T,Liu X,Okada H, MatsushimaA, Shimohigashi Y.*** Endocrine disruptor bisphenol A strongly binds to human estrogen-related receptor gamma (ERRgamma) with high constitutive activity. J Toxicol Lett. 1 2006; 167(2):95-105.
10. ***Jason Gertz,1 Timothy E. Reddy,1,3 Katherine E. Varley,1 Michael J. Garabedian,2 and Richard M. Myers.*** Genistein and bisphenol A exposure cause estrogen receptor 1 to bind thousands of sites in a cell type-specific manner. J Genome Res, 2012; 22(11): 2153–2162.
11. ***Quesada, I, E Fuentes, MC Viso-León, B Soria, C Ripoll and A Nadal.*** Low doses of the endocrine disruptor bisphenol-A and the native hormone 17ß-estradiol rapidly activate transcription factor CREB. J FASEB, 2002; 16: 1671-1673.
12. ***Kaja S1, Yang SH, Wei J, Fujitani K, Liu R, Brun-Zinkernagel AM, Simpkins JW, Inokuchi K, Koulen PInvest***. Estrogen protects the inner retina from apoptosis and ischemia-induced loss of Vesl-1L/Homer 1c immunoreactive synaptic connections. J Ophthalmol Vis Sci, 2003; 44(7): 3155-62.
13. ***D.ManeeshKumar,1 JamesW.Simpkins,2 and Neer aj Agarwal***. Estrogens and neuroprotection in retinal diseases. J Mol Vis, 2008; 14: 1480–1486.
14. ***Janet C. Gould a , Linda S. Leonard a , Susan C. Maness a , Brandee L. Wagner b , Kevin Conner c , Tim Zacharewski d , Steve Safe C , Donald P. McDonnell b , Kevin W. Gaido,*** Bisphenol A interacts with the estrogen receptor a in a distinct manner from estradiol. J Mol. and Cell. Endo., 1998; 142: 203–214.

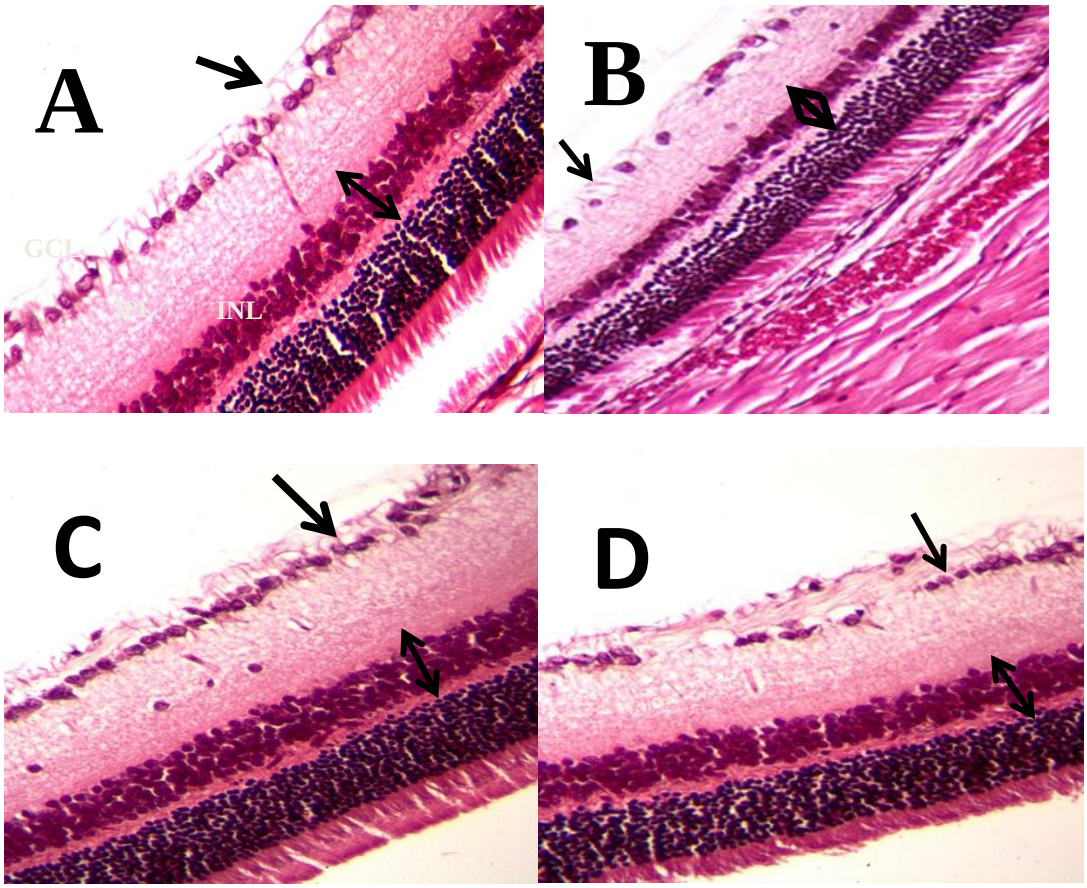

Fig.1 A) Photomicrograph of control group showing Ganglion cells **(↓)**, Inner plexiform layer, inner nuclear layer, outer nuclear layer, and photoreceptor layer exhibited normal structure. **B**) Bisphenol treated group showing severe retinal degeneration IPL,INL**(↕)** and OPL. Also sever pyknosis in the ganglion cells (↓). **C)** Vitamin A treated group showing a marked improvement in the retinal layers. **D)** Stem enhancer treated group showing a mild improvement in the retinal layers.

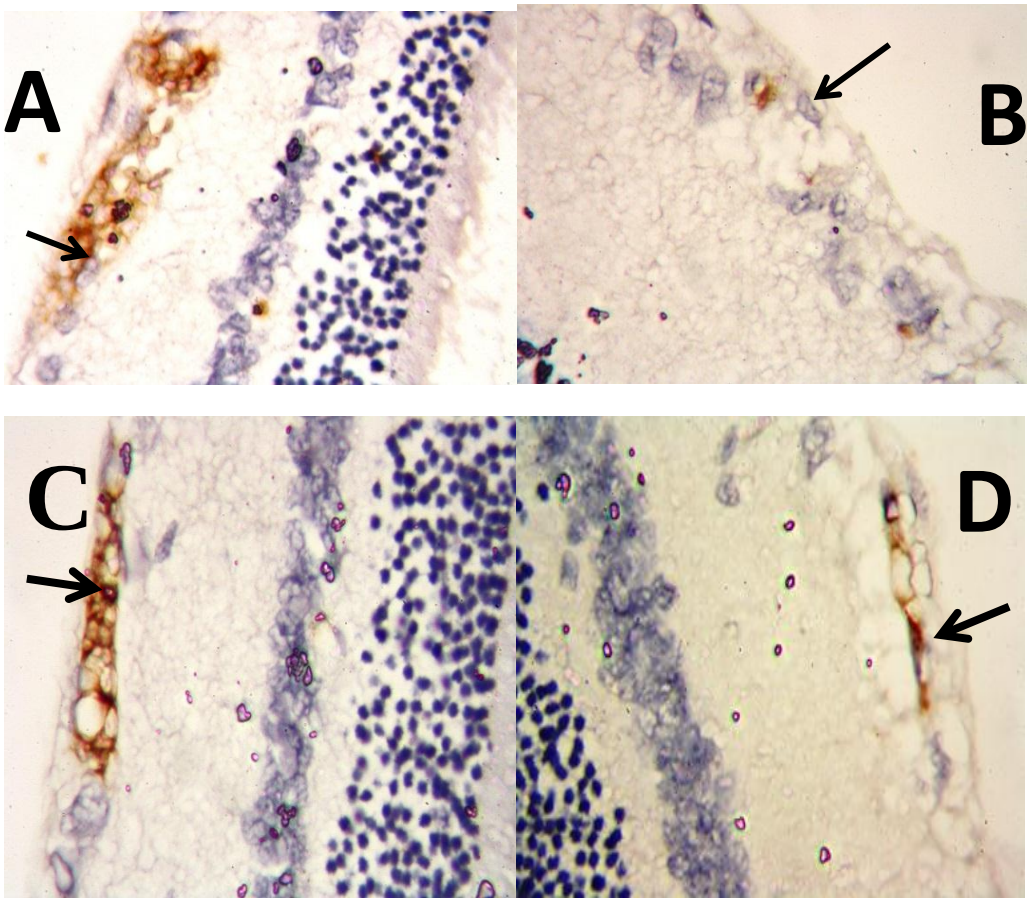

Fig.2 **A)** Photomicrograph of control group showing +ve reaction for Immunolocalization of Era in Ganglion cells **(↓). B**) Bisphenol treated group showing weak immunohistochemical reaction for Era in the ganglion cells (↓). **C)** Vitamin A treated group showing a marked improvement in immunolocalization of Era in Ganglion cells. **D)** Stem enhancer treated group showing a mild improvement in the immune histochemical reaction in the ganglion layers.