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Acute effects of bisphenol a on testis tissue of guppy fish (*Poecilia reticulata*)

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ABSTRACT

This experiment was done for investigate the histopathological effects of bisphenol A exposure on testis tissue of guppy fish. In this study 90 guppies were examined in 2 experimental groups. In experiment groups, guppies exposed 4 mg/L and 8 mg/L BPA doses and after 96 hours fishes were sacrified. Tissues were dissected out, fixed in Bouin's fixative, stained with Hematoksilen & Eosin, and examined using light microscopy. It is observed that deterioration, atresia and decreased in the number of seminiferous tubules in experiment groups.. In addition, openings between seminiferous tubules, increase in distance between tubules and decrease in the number of spermatogonium were detected.

Introduction

Endocrine disrupting chemicals (EDCs) are compounds in the external environment that modulate the physiology of the endocrine system and often cause health disorders (Crews & McLachlan, 2006). Many studies have shown that xenoestrogens have serious damage to natural habitats (Colborn, 1995). Inspite of this, it's not clear whether xenoestrogens are caused by abnormalities in reproductive system. Because, in the nature, many pollutings contaminate ecosystem at the same time so livings are exposed to mixture of many chemicals. Therefore, it's difficult to determine the effect of each one of them.

Many of the investigations into EDCs in the aquatic environment have involved fish because of similarities in the endocrine system to higher vertebrates (Bond, 1979), a demonstrated sensitivity to EDCs in both laboratory and field investigations, ease of working with fish, and the fact that wild populations in a number of locations have exhibited effects thought to be associated with exposure to EDCs (Noaa, 2002).

Bisphenol A (2 bis(4-hydroxiphenyl) propane, BPA) is one of the most potent endocrine disrupting chemicals which mimics estrogen *in vivo* and *in vitro* (Kim et al., 2011) and therefore is classified as xenoestrogen. Chemicals that can mimic or antagonize the effects of endogenous hormones could potentially have and well serious effects not only on the development being of an individual organism, but perhaps more importantly on the ability of that organism to reproduce, and its offspring to survive and eventually reproduce (Noaa, 2002).

BPA is widely used in industry for making plastics harder. It's found in polycarbonate plastics (baby feeding bottles, carboys) (Cao & Corriveau, 2008) and inside of epoxy coated cans (Kang et al., 2006). Bisphenol A (BPA) is one of the highest production volume chemicals in the world. Nowadays, depending on the increasing the production of BPA, the rate of contamination of aquatic systems is increasing rapidly. BPA levels were reported between 0.02–21 ug/L in river water (Kang, 2007).

The guppy (*Poecilia reticulata*), is a member of the Poeciliidae family ,[1] is one of the most popular fish species in the world. They generally live in freshwater with a temperature between 25.5 and 27.8 °C Female and male individuals can be seperated easily because guppies exhibit sexual dimorphism. While wild-type females are grey in body colour, males have gonopodium and different colors of splashes, spots, or stripes.

The aim of this study is to investigate the histopathological effects of BPA on the testis tissue of male guppy fishes. Testis histology, location and shape of the cells which belong to spermatogenic serial, structure of the tissue was investigated with light microscopy with this study.

Materials and Methods

Chemical:

BPA, 2 bis(4-hydroxiphenyl), (Sigma Aldrich, CAS No: 50-05-7) was dissolved in 1 % dimethylsulfoxide (DMSO) to generate desired concentrations for treatment of the guppy fishes.

Fish

Adult guppies were purchased from a local breeder in İstanbul with no water pollution. The fish were separated by sex into different aquariums. Fish system water, obtained by carbon/sand filtration of municipal tap water, was maintained at 26 ± 1 ºC and pH 7.2-7.7 with a 14:10 h light:dark photoperiod. Test chambers were glass aquaria of about a 20-L capacity. The guppies were fed once daily in the morning with flake food (Tetra, Germany)

Experimental Design

Following the preliminary experiment, all determinations were repeated two times. The guppies are divided into three groups (n=30) concerning their different BPA doses. After 48 h of adaptation, the different concentrations of deltamethrin were added to the experimental aquaria. Mortality was controlled 24, 48, 72, and 96 h after the start of the tests. Dead individuals were removed immediately. Control group received 1% DMSO at its maximum volume used in dilution of the dosing concentrations (group I: 4 mg/L BPA, group II: 8 mg/L BPA, and group III: control group). After giving different doses of BPA, fishes were dissected on the fifth day of the study. The testis tissues of adults were fixed in Bouin's fixative for 18 h

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and stored in 70% methanol. The tissues were dehydrated using a series of graded ethanol solutions (70–100%), cleared in xylene, embedded in paraffin, and sectioned at 6–8 µm. The sections were then stained with hematoxylin and eosin (H&E) (hydro-soluble) and processed for histological examination by light microscopy.

Results

Control group

In transverse sections, testes contain numerous seminiferous tubules which are different in shape and size. Each tubule is at the height of spermatogenetic activity with different stages of spermatogenesis. The lumen of the seminiferous tubule contains numerous sperms. The primary spermatogonial cells, primary and secondary spermatocytes, spermatids and sperm bundles are visible. Interstitial cells and connective tissues are present in between the tubules.

Testis was covered by tunica albuginea on its outer surface. Testicular section showed many spermatogenic lobules. The compactly arranged cysts were present in the lobules. The cysts containing spermatogonia were visible in the peripheral area. Each spermatogonium was large and spherical compared to other cells, and possessed a large lightly stained spherical nucleus with distinct nucleolus. Other cysts contained primary and secondary spermatocytes. Primary spermatocytes were smaller than secondary ones, and round spermatids were also observed in some of the cysts (Fig. I).

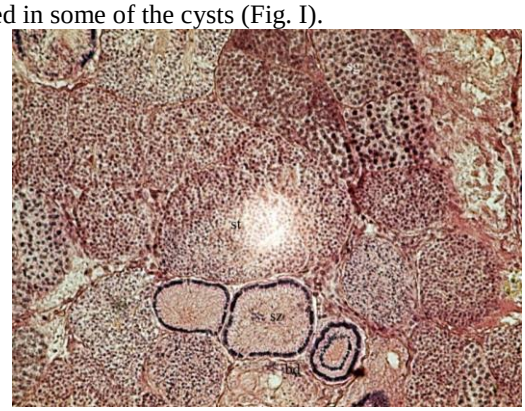

Figure 1: Control group, sg: spermatogonium, st: spermatid, sz: spermatozoa, bd: connective tissue, H&E staining, x40

Treatment Group I

Openings between seminiferous tubules following the exposure of 4mg/L BPA. Deterioration at morphology of the tubules which consists sperm and atresia at the seminiferous tubule morphology was observed (Figure 2). Increase in connective tissue were observed between tubules (Figure 3).

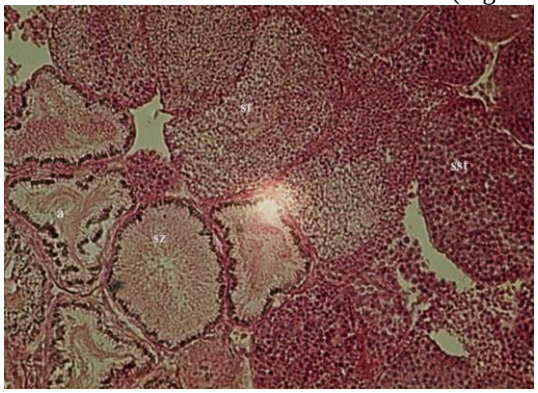

Figure 2: 4 mg/L BPA treated group, deterioration at seminiferous tubules and openings between seminiferous tubules, sz: spermatozoa, st: spermatid, sst: spermatocyte, a: atresia, H&E staining, x40.

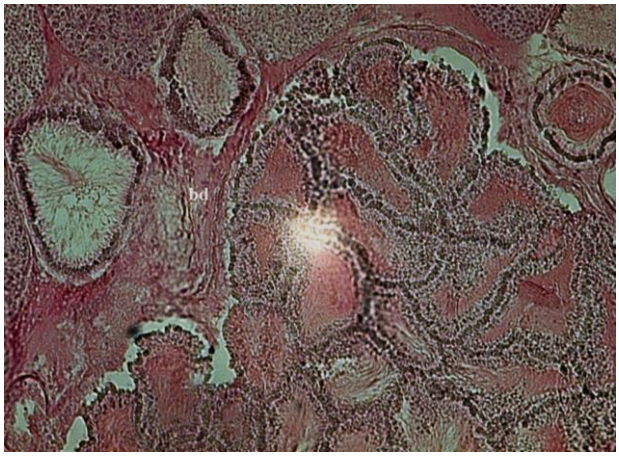

Figure 3: 4 mg/L BPA treated group, increasing in connective tissue, bd: connective tissue, H&E staining, x40

Treatment Group II

Decrease in sperm and seminiferous number was detected in 8 mg/L BPA treated group. Distortion at seminiferous tubules morphology was evident (Figure 4). In addition, atresia was observed at tubular structure. Increase in distance between tubules and connective tissue was determined. Decrease in the number of spermatogonium was detected (Figure 5).

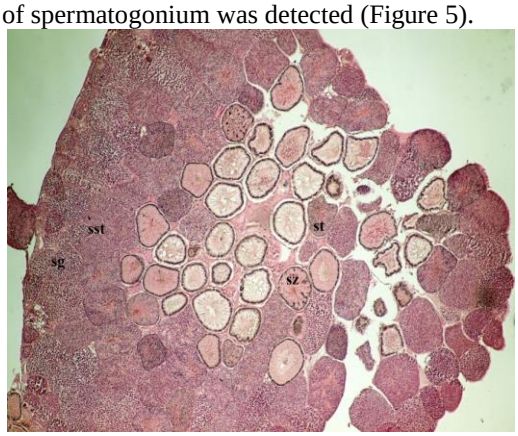

Figure 4: 8 mg/L BPA treated group, openings between seminiferous tubules, sg: spermatogonium, sst: spermatocyte, st: spermatid, sz: spermatozoa, H&E staining, x10

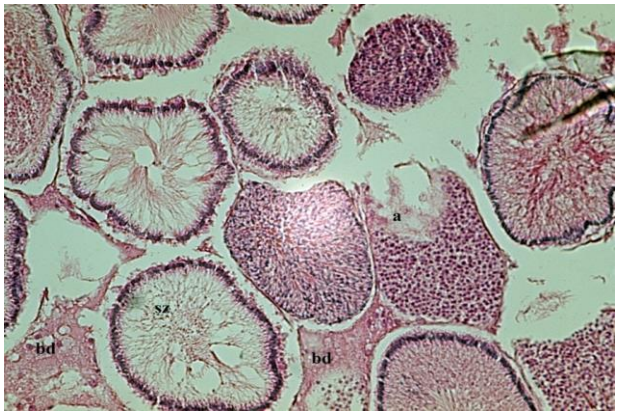

Figure 5: Increase in connective tissue and atresia at tubule structure, a: atresia, bd: connective tissue, sz: spermatozoa, H&E staining, x40

Discussion

The current study was evaluate the adverse effects of bisphenol A on testicular histology on *Poecilia reticulata*. Bisphenol A. is an estrogenic compound, capable of inducing vitellogenin in male fish. Lindholst et al. (2000) studied the estrogenic response of rainbow trout exposed to bisphenol A in a

flowthrough system. Juvenile fish were exposed to nominal concentrations of 10, 40, 70, 100, and 500 µg/ L of bisphenol A for 6 and 12 days. Plasma samples were analyzed for vitellogenin using a direct sandwich ELISA. While exposure to increasing concentrations of bisphenol A resulted in increasing levels of vitellogenin after 6 days, only the 500 µg/L concentration produced significantly higher vitellogenin levels (approximately 1 mg/ml) than controls. Interestingly, after 12 days the concentration of plasma vitellogenin in those fish exposed to 70 or 100 µg/L bisphenol A remained the same or decreased slightly.

Lindholst et al. (2000) attributed this to a possible increase in the degradation rate of bisphenol A as a result of cytochrome P450 induction. After 12 days, vitellogenin levels in fish exposed to 500 µg/L continued to increase, which the authors suggested was the result of detoxification processes not being able to keep pace with the higher concentration of bisphenol A. Sohoni et al. (2001) investigated the long term reproductive effects of bisphenol A in fathead minnows (*Pimphales promelas*). Mature male and female fish were exposed to nominal bisphenol A concentrations of 1, 16, 160, 640 or 1,280 µg/L in flow-through chambers. At days 43, 71, and 164, selected fish were sacrificed for measurements of somatic growth, GSI, and plasma vitellogenin (ELISA). The testes were processed for histologic analysis. At concentrations of 640 and 1.280 µg/L, bisphenol A had a significant inhibitory effect on weight and length in males by Day 71. In males, exposure to bisphenol at concentrations of 160 µg/L and higher for at least 71 days resulted in vitellogenin concentrations between 120 and 144 µg/L. The GSIs were also significantly decreased in both males and females exposed to bisphenol A at concentrations of 640 µg/L and higher for 164 days. Histological examination revealed a decrease in spermatozoa in fish exposed to nominal bisphenol A concentrations of 16 µg/L and greater for 164 days. Results from breeding pair experiments begun on Day 42 indicated that egg production was inhibited at 1,280 µg/L, and that hatchability was reduced at a nominal bisphenol A concentration of 640 µg/L. for a number of xenoestrogens, including bisphenol A, to induce vitellogenesis in juvenile rainbow trout.

Koç et al. (2012) investigated the histopathological changes in testis of the swordtail fish *Xiphophorus helleri* exposed to deltamethrin. Depending on increasing doses, numbers of mature sperm were significantly reduced, and degenerative changes in seminiferous tubules were observed.

Kalsoom et al. (2005) investigated the effetcts of endosulfan on histomorphology of fresh water Cyprinid fish *Cyprinion watsoni*. The fish was exposed to 0.75 and 1 ppb endosulfan on alternate days for 30 days during early spawning season (March). The testicular weight, increased significantly ($p<0.05$) with 0.75 ppb, whereas a dose of 1 ppb did not cause any significant change in testicular weight and breadth. The testicular length, however, showed an increase. The mean diameter of spermatogonia decreased significantly in 1 ppb group. Histomorphological studies showed loosely arranged lobules, irregular nuclear and cell membrane of spermatogonia, clumping of spermatocytes and spermatids and reduction in sperm count. Similar results (disintegration of spermatogenic cells) were observed by Sangalang et al. (while studying the effect of 2.5 µg/g PCB diet on Gadus morhua. In this study, spermatogonia numbers decreased and showed clumping and irregular cell membranes. Clumping of early spermatocytes was also observed by Sangalang et al (1981).

According to Kinnberg et al. (2000), nonylphenol exposure dramatically altered testis structure. Testis from the control fish contained regularly organized cysts with the different spermatogenetic stages. Squamous Sertoli cells surrounded the developing spermatozeugmata, and mature spermatozeugmata were observed in the efferent ducts.

It is concluded from this study that exposure of endocrine disruptors cause decrease in number of spermatogonia, spermatid and sperm. In the present study, changes associated with testicular atresia and damage of the seminiferous tubules and spermatogenic cells have been observed in BPA exposed fish. With this study, it is proved that BPA exposure inhibits spermatogenesis.

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