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Histopathological Alterations in Gills of Some Poecilid Fishes after Exposure to Bisphenol A

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Abstract: *Rational*: Bisphenol A (BPA) is an industrial chemical, used to manufacture polycarbonate and numerous plastic articles and leach to salt and fresh water. Low levels of BPA have also been found to cause biological effects and its mode of action appears to mimic that of the female hormone, estrogen. BPA therefore belongs to a group of chemicals termed "hormone disruptors" or "endocrine disruptors" that are able to disrupt the chemical messenger system in the body.Objective: Gills are generally considered good indicator of water quality, being models for studies of environmental impact. So this study was designed to assess the histological changes caused to the gill tissues of both mosquito-fish (G*ambusia affinis*) and a guppy-fish (*Poecilia reticulata*) after exposure to Bisphenol.Methods: Specimens of both mosquito-fish (G*ambusia affinis*) and a guppy-fish (*Poecilia reticulata*) exposed to (50µg/l of bisphenol A) for 15 days (short term) and 30 days (long term). After 15-and fifty days experiments were terminated and both treated and control fishes were fixed in alcoholic Bouin's solution were prepared by routine histological technique. Slides were then microscopically examined, photographed and described. Results revealed that gills from BPA treated fishes showed several histopathological lesions manifested in necrosis and desquamation or degenerated of secondary lamellar epithelium, lifting up of epithelium, intraepithelial edema and fusion of adjacent secondary lamellae, disorganization and rupture in secondary lamellae as well as hyperplasia of epithelial cells. In conclusions, our study concluded that, the concentration 50ug/l of BPA has ability to induce harmful effect on the gill tissue of fishes and these effects are dose and time dependent.

Key words: BPA · Mosquito Fish · Guppy Fish · Histopathology · Gills

INTRODUCTION

Bisphenol A (BPA) is an industrial compound that has generated concerns due to its high production volume, wide spread use in many consumer products and its proven estrogenicity; furthermore BPA considered as exogenous compounds that interfere with the synthesis, storage/ release, metabolism, transport, elimination or receptor binding of endogenous hormones [1, 2].

Various substances released from modern complex human societies and entering waters may produce alterations in survivability of aquatic organisms residing within such a polluted environment, because many are high toxic. Thus fishes are in the unenviable position of being within a constantly changing ecosystem, which may at some time either physiology or biochemistry or induce changes in their bodies resulting in increased susceptibility to other disease agents [3, 4]. The occurrence of endocrine-disrupting chemicals (EDCs) in aquatic environments has become a globally growing problem of concern in recent decade. EDCs consist of a wide range of natural and man-made chemicals, most of which are released to natural waters by anthropogenic activities and whose adverse effects on normal reproductive functions of aquatic species have widely been confirmed [5-9]. For instance, widespread estrogenic chemical input has been regarded as an important factor related to the decline of fish abundance in freshwater systems [10-12].

Water pollution induces histopathological changes in fish [13, 14]. As an indicator of exposure to pollutants; histology represents a useful tool to assess the degree of pollution, particularly for sublethal and chronic effects. Gills perform numerous functions which include

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respiration, osmoregulation, excretion of nitrogenous waste products and acid-base balance because it is the first organ to be affected by pollutants [14-16].

Previous histopathological studies of fish exposed to pollutants have shown that fish gills are primary markers for aquatic pollution [17, 18]. Therefore, functional impairment of gills caused by pollutants can significantly damage the health of fish. For this reason, fish gills are considered to be the most appropriate indicators of water pollution levels [15-17].

This study was designed to assess the histological changes caused to the gill tissues of both mosquito-fish (G*ambusia affinis*) and a guppy-fish (*Poecilia reticulata*) exposed to (50µg/l of bisphenol A) for 15 days (short term) and 30 days (long term) using light microscopy.

MATERIALS & METHODS

Bisphenol A (>99% pure) was purchased from Aldrich Chemical (Milwaukee, WI, USA) and dissolved in dechlorinated tap water preparing a stock solution of 50 mg/l. The experimental solution of BPA (50µg/l) was prepared by diluting this concentrated stock solution with dechlorinated tap water.

A total of 30 specimens of Mosquito-fish, *G. affinis* and 30 specimens of guppy *P. reticulata* formed the experimental animals for the present study. Fishes were purchased from aquarium fish markets and transported to the laboratory in Zoology Department, Faculty of Science, Al-Azhar University; in a plastic package filled with oxygenated water. Then, fishes of each species were allowed to be adapted for two weeks to the laboratory conditions, in well-aerated dechlorinated tap water glass aquaria of 60-liter capacity. They were fed twice daily six days a week with commercial pelleted fish diet.

After acclimatization, fishes of each species were divided into 3 groups, each of 10 specimens in a glass aquarium of 20 liter capacity. The first group was exposed to only fresh untreated and dechlorinated water and served as a control group. The second and third groups were exposed to 50µg/l BPA for 15days and 30 days, respectively.

For histological examination, normal and treated fishes of both fish species were dissected and their gill tissues were excised out and immediately fixed in alcoholic Bouin's solution for 24 hours. These specimens were dehydrated in ascending concentrations of ethyl alcohol, cleared in xylene and embedded in paraffin wax. Transverse sections were cut at 4 to 6 microns and stained with Harri's haematoxylin and subsequently counter stained with eosin. Finally, the slides were microscopically examined and photographed using camera mounted on light microscope and described.

RESULTS

The microscopic examination of the gills of both fish species (mosquito-fish and guppy-fish) reared in a control conditions, revealed normal histological structure of fish gills. Each gill arch bears a double row of gill filaments "non-respiratory or primary filaments". Each gill filament carries two rows of gill lamellae "respiratory or secondary lamellae". The gill epithelium is composed of a multilayered filament epithelium (primary epithelium). The secondary lamellae are separated by distinct inter-lamellar spaces. Scattered mucous cells in the inter-lamellar epithelium were seen between the secondary lamellae. Each secondary lamella consists of double thin sheet of epithelial layer (secondary epithelium); separated by the centrally located pillar cell system that supports the epithelial layer and delimit blood lacunae (Fig.1_A, Fig.1_B, Fig.2_A and Fig.2_B)

Histological examination of mosquito-fish gills showed different degrees of damage after 15 days of exposure to 50µg/l BPA. The microscopic observation revealed somewhat extensive hyperplasia of the epithelial cells that causes fusion of the adjacent secondary gill lamellae (Fig.1_C). Separation and lifting of the lamellar epithelial layers, resulting in the increasing of intercellular spaces between the pillar system and epithelial lining of the secondary lamellae was recorded (Fig.1_C). Furthermore, severe congestion of blood lacunae and rupture of pillar system and capillaries that led to balloon-like lamellae packed with erythrocytes (Aneurism) or lamellar telangiectasis (Fig.1_D). In addition, edema between the primary epithelium and secondary lamellae were noticed (Fig.1_C and Fig.1_D). After 30 days of exposure to 50µg/l BPA, the microscopic observation revealed severe degeneration with fibrosis in primary filaments, hyperplasia of secondary lamellae that led to total fusion of secondary lamellae and completely disappearance of inter-lamellar spaces (Fig.1_E). Also, severe congestion led to rupture of primary filaments (Fig.1_F). Congestion was also seen in blood vessels of primary filaments, led to the ballooned-like tips packed with erythrocytes or filamentous telangiectasis; and numerous mucous cells were noticed (Fig.1_G and Fig.1_H).

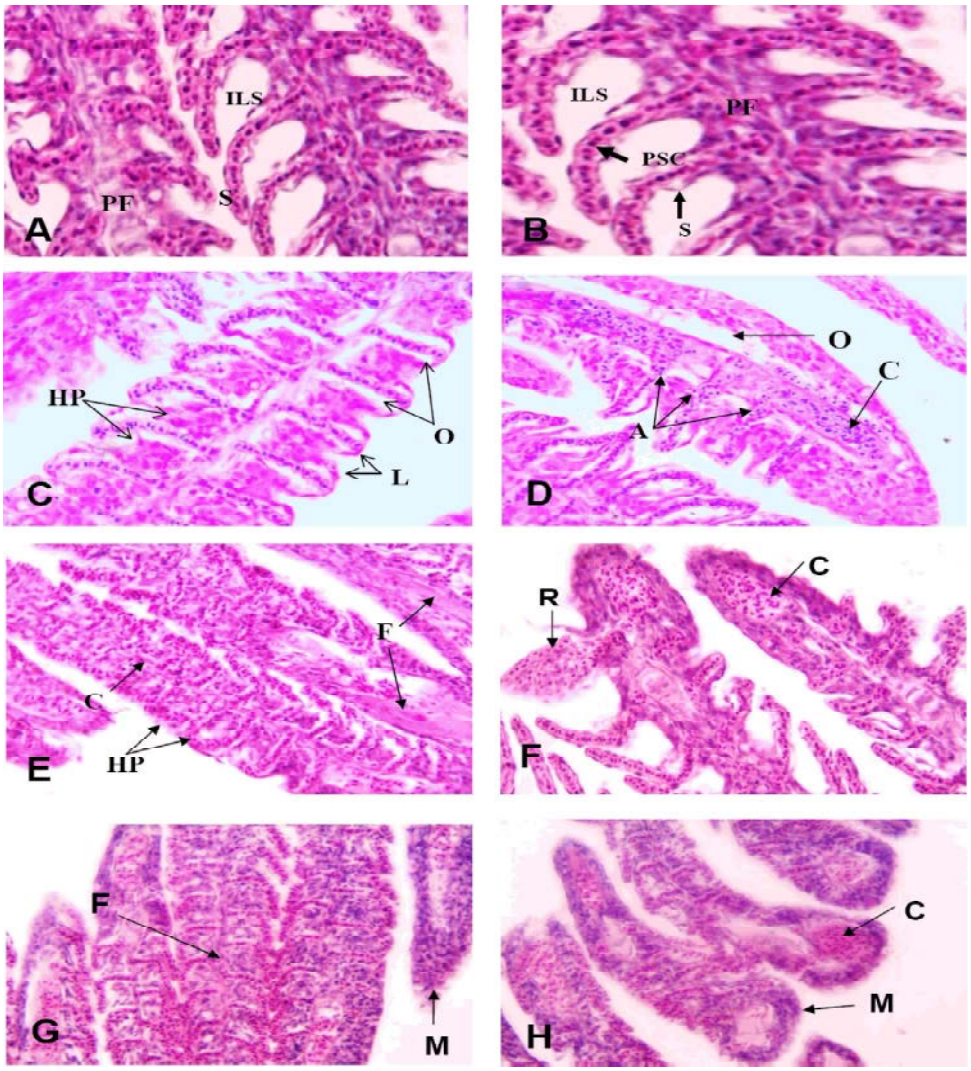

Fig. 1: A photomicrograph of gills of control mosquito fish (A and B) stained with H&E, X 100 and400 respectively demonstrating normal gill structure and normal epithelial cells of secondary lamellae

(C): Sagittal section in gills of mosquito-fish exposed to 50µg/l BPA for 15 days, showing hyperplasia of secondary gill lamellae with partial edema (H&E.X100).

(D): Sagittal section in gills of mosquito-fish exposed to 50µg/l BPA for 15 days, showing severe congestion of blood lacunae, rupture of pillar cells and capillaries that led to ballooned like lamellae packed with erythrocytes aneurism or lamellar telangiectasis. In addition to edema (H&E. X100).

(E): Sagittal section in gills of mosquito-fish exposed to 50µg/l BPA for 30 days, showing severe degeneration with fibrosis in primary filaments, hyperplasia of secondary lamellae that led to total fusion of secondary lamellae and completely disappearance of inter-lamellar spaces (H&E. X100).

(F): Sagittal section in gills of mosquito-fish exposed to 50µg/l BPA for 30 days, showing severe congestion led to rupture of primary filaments (H&E. X400).

(G and H): Sagittal section in gills of mosquito-fish exposed to 50µg/l BPA for 30 days, showing Congestion of primary filaments and numerous mucous cells were noticed (H&E. X100 & X400).

A: Anuerism **C:** Congestion, **CD:** Complete degeneration, **D:** Degeneration, **F**: Fusion, **Fi**: Fibrosis, **HP:** Hyperplasia, **ILS:** Inter lamellar space, **L:** Lifting up, **M:** Mucus cells, **N:** Necrosis, **O:** edema, **PF:** Primary gill filaments, **PCS:** pillar cell system, **R**: Rupture **S:** Secondary lamellae, **T:** Telangiectasis.

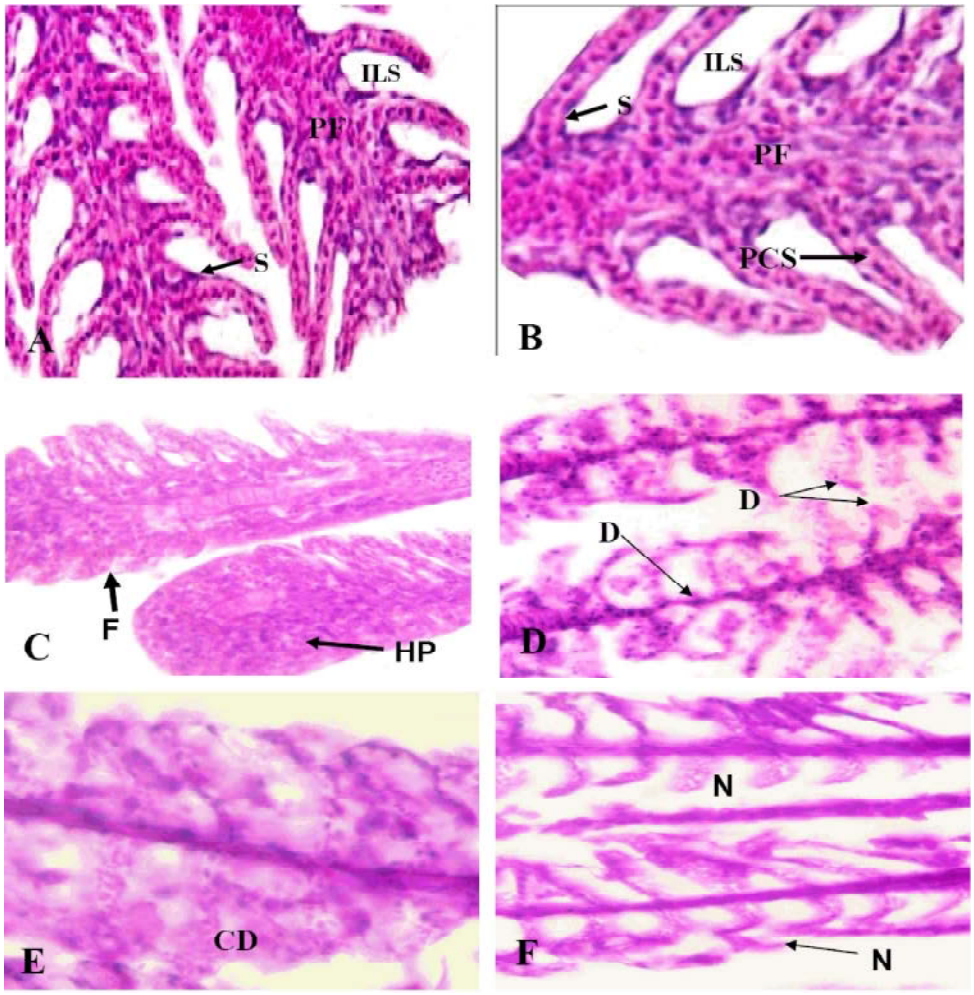

Fig 2: A photomicrograph of gills of control guppy fish (A and B) stained with H&E, X100and400 respectively demonstrating normal gill structure and normal epithelial cells of secondary lamellae.
(C): Sagittal section in gills of Guppyfish exposed to 50ug/l BPA for 2 weeks, showing hyperplasia of the lamellar epithelium lead to fusion of secondary lamellar epithelium (H&E. X100).
(D): Sagittal section in gills of Guppyfish exposed to 50ug/l BPA for 2 weeks, showing A lot of cellular necrosis and degenerated of lamellar epithelial (H&E. X400).
(E): Sagittal section in gills of Guppyfish exposed to 50ug/l BPA for 30 days, showing complete fusion of their secondary gill lamellae and numerous mucous cells (H&E. X400).
(F): Sagittal section in gills of Guppyfish exposed to 50ug/l BPA for 30 days, showing also severe gill filaments curling and necrotic of secondary lamellae (H&E. X400).

A: Anuerism **C:** Congestion, **CD:** Complete degeneration, **D:** Degeneration, **F**: Fusion, **Fi**: Fibrosis, **HP:** Hyperplasia, **ILS:** Inter lamellar space, **L:** Lifting up, **M:** Mucus cells, **N:** Necrosis, **O:** edema, **PF:** Primary gill filaments, **PCS:** pillar cell system, **R**: Rupture **S:** Secondary lamellae, **T:** Telangiectasis.

Histological examination of the guppy-fish gills after exposure to 50µg/l BPA for 15 days showed hyperplasia of the lamellar epithelium lead to fusion of secondary lamellar epithelium (Fig.2_C). A lot of cellular necrosis and degenerated of lamellar epithelial was noticed (Fig.2_D). After 30 days of exposure to BPA, the results clarified complete fusion and degeneration of secondary gill lamellae (Fig.$2_{E)}$. Furthermore, severe curling of gill lamellae, with necrotic of secondary lamellae was seen (Fig.2_F).

DISCUSSION

Endocrine disrupters (EDs) reach living organisms through the air, soil, water and food, thus the major route transmission is the aquatic environment, were these substances bio accumulate through the food chain, fish ingestion is one of the main sources of human exposure to endocrine disrupters (EDs) [19]. BPA one of the most abundant endocrine disruptors in the environment, is produced by the acid-catalyzed condensation of acetone with two phenols [20]. From an economic view point, fish form the basis of a large commercial fishery and aquaculture industry, as well as having wide spread recreational value, if EDCs in the aquatic environment are affecting reproductive success of fish, thus threatening population sustainability over time, commercial and sport fisheries could be impacted [21].

Gills are generally considered good indicator of water quality [22], being models for studies of environmental impact [23-25], since the gills are the primary route for the entry of any pollutants; in fish, gills are critical organs for their respiratory, osmoregulatory and excretory functions [26,27]. In addition, gills are an important tissue because of its direct contact with water and any effect or agency has to go through it to come into the fish body [28].

In the present study, the gills of both *Gambusia affinis* and *Poecilia reticulata* exposed to 50ug/l BPA at different durations showed different signs of histopathological deformations varies in intensity according to the duration of exposure. The alternations occurred in the secondary gill lamellae were easily identified on the histological level by the microscope, like lifting or separation of the respiratory epithelium; rupture or hyperplasia of epithelial cell; lamellar thickening or lamellar fusion; congestion of blood vessels; and lamellar telangiectasis or aneurism. This result is confirmed by [16, 29, 30]. Whom concluded that, histopathological changes of gills such as cellular hyperplasia and hypertrophy, epithelial lifting have been reported after the exposure of fishes to a variety of noxious agents in the water, such as pesticides, phenols and heavy metals in their mixtures.

The interpretation of hyperplasia may in some situations represent an adaptation by the organism to protect underlying tissues from any irritant [31]. However, increased thickness of the epithelial layers including mucous cell hyperplasia and fusion of adjacent secondary lamellae as a result of hyperplasia wouldn't only decrease the surface area available for oxygen extraction but also would increase the oxygen diffusion distance between water and blood [32]. Thus, while hyperplasia may indeed be having a protective function, it may also be hindering the respiratory, secretary and excretory functions of the gills [33]. The increase of cellular layers of lamellar epithelium may be due to an increase in the number of mitotic divisions of the lamellar epithelium [34]. On another way the gill hyperplasia may increase epithelial thickness to retard or prevent the entry of toxic ions into the blood stream. [35-36].

The separation or lifting of the lamellar epithelium, which observed in the present study, can be explained on the light of [37] opinion. They stated that this separation of secondary lamellar epithelium leads to the formation of lymphoid space. Enlargement of these lymphoid spaces is associated with the presence of lymphatic fluid. Extrusion of this fluid from the central capillaries causes vascular stasis. This opinion is agreement with [38-40]. They stated that lifting, swelling and hyperplasia of the lamellar epithelium could serve a defense function against irritant. In other way alterations like epithelial lifting, hyperplasia and hypertrophy of the epithelial cells, besides partial fusion of some secondary lamellae are examples of defense mechanisms, since, in general, these result in the increase of the distance between the external environment and the blood, which serve as a barrier to the entrance of contaminants [23, 41-45]. As a consequence of the increased distance between water and blood due to epithelial lifting, the oxygen uptake is impaired. However, fishes have the capacity to increase their ventilation rate, to compensate low oxygen uptake [43, 46].

The interpretation of the lamellar telangiectasis (aneurism) may occur in association with metabolic waste or chemical pollution; histologically it is obvious that the lesion has its genesis in the rupture of the retaining pillar cells which normally join the dorsal surface of secondary lamellae to the ventral. The result is dilation of the lamellar capillary and pooling of the blood, which thrombosis and eventually fibrosis, fuses with adjacent lamellae. If there are many telangiectatic lamellae, respiratory function may be impaired especially at higher temperatures, when dissolved oxygen levels are lower and metabolic oxygen demand is high. But also if such fish are further traumatized, rupture and fatal hemorrhage may supervene [47]. Furthermore the formation of an aneurysm is related to the rupture of the pillar cells [15, 48], due to a bigger flow of blood or even because of the direct effects of contaminants on these cells.

This study shaded more light on the harmful effects of BPA one of the well-known endocrine disruptor worldwide that leach to both fresh and salt water inducing sever harms to gills of the studied fish which perform a vital function not only respiration but also osmoregulation and execration. Our study concluded that, the concentration 50ug/l of BPA has ability to induce harmful effect on the respiratory system of fishes and these effects are dose and time dependent.

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