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BPA induced damage to testes of *Channa punctatus*

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Abstract
Rapid industrailization has led to the introduction of a large number of xenobiotics into the environment. Bisphenol a (BPA) is a synthetic high-volume chemical that is widely used in the production of polycarbonate plastics and composites. BPA is reported to be an endocrine disrupting agent that interferes with the delicate interplay of hormones and gonadal histology. This study investigated the effect of three different BPA concentrations on testicular histology of *Channa punctatus*. Exposure to BPA resulted in degeneration of testicular tissue and proliferation of interstitial tissue. Necrosis of cells was also seen at higher concentrations of BPA after 30 days exposure. However, no ovo-testis or testicular follicles were observed.

Keywords: Bisphenol-A, BPA, *Channa punctatus*, testes, gonads

Introduction
Bisphenol A (BPA) or 2, 2-(4,4′-dihydroxydiphenyl) propane is an organic compound with two phenol moieties. It is synthesized by the condensation of acetone with two equivalents of phenol. The suffix 'An' in BPA stands for acetone and distinguishes it from other bisphenols like Bisphenol S (BPS) and Bisphenol F (BPF). BPA is a high production volume chemical that is widely used in the manufacture of polycarbonate plastics, epoxy resins, flame retardants, thermal print paper, dental sealants and other composites [1, 2]. Polycarbonate plastics are clear and tough plastics that have come to replace glass in many applications such as in bottles, lenses, windshields etc. Epoxy resins are used to coat food and beverage cans to prevent their reaction with the contents. The widespread use of plastics and epoxy resins has led to a humongous demand for BPA [3].
BPA does not occur naturally but its widespread use has made it ubiquitous in all environmental matrices [4]. However, it is the aquatic ecosystem that serves as the ultimate sink for BPA and other xenobiotics released into the environment. BPA finds its way into water bodies through effluents and discharges [1]. It has also been detected in tissues, serum and other body fluids in animals and humans, raising concern over its impact on living organisms [2, 3]. Due to the likelihood of exposure, aquatic organisms such as fish are particularly vulnerable targets to BPA.
For several years histological changes were used to evaluate the health of fish and to study the impact of xenobiotics on various organs of the fish body. Gills, kidney, spleen and liver were the organs of choice; changes in their cellular architecture being used as biomarkers of exposure to chemical stressors in the environment. Less attention was devoted to the histology of gonads. However, the presence of endocrine disrupting chemicals in the aquatic environment spiked an interest in the gonadal histopathology of fish [5].
The estrogen mimicking ability of BPA [6] is a cause of serious concern. BPA results in profound histological and reproductive changes in animals [7]. Studies on different animals have shown that BPA exposure can have adverse effects on reproductive morphology and physiology such as steroidogenesis, gonadal size and architecture and fertility [8, 9, 10]. In this study the effect of BPA exposure on the testes of *Channa punctatus*, a popular and hardy laboratory animal was investigated.

Materials and Methods
In the first week of April, adult *Channa punctatus* of length 14-16 cm and weight 25-28g were procured from Sumera reservoir (20.933°N and 77.333°E), district Aligarh, India and transported to the Department of Zoology, D. S. College, Aligarh in glass containers.

Utmost care was taken to avoid any injury to the animals during collection and transportation. As per the reproductive cycle of *C. punctatus* [11] these fish were likely to be in the preparatory phase. Fifteen days later i.e. in the third week of April another lot of fish of similar weight and length was obtained from the same source and acclimated to laboratory conditions.

Treatment and Acclimation of Fish

In the laboratory fish were first treated with 0.05% potassium permanganate solution for two minutes to avoid dermal infections [12, 13]. The fish were allowed to acclimate to laboratory conditions for at least 2 weeks [14]. Healthy, disease-free fish were chosen and used for further studies. Throughout all experimental protocols the fish were maintained in controlled laboratory conditions of pH (7.6-7.8), temperature (23-25ºC), dissolved oxygen (7.2-7.6), total hardness (175-185 mg/L) etc. The natural photoperiod was maintained and fish were fed at 12 hours intervals.

Preparation of BPA Stock Solution

As BPA is only moderately soluble in water [15], ethanol was chosen as the carrier solvent. 20% stock solution [16] of BPA (HiMedia Laboratories Pvt. Ltd, Mumbai, India) was prepared and different dilutions of the stock solution were used for treatment.

Sublethal Concentrations of BPA

In a separate study the 96 hours LC50 of BPA to *C. punctatus* was estimated to be 13.1 mg/L. Three sublethal concentrations of BPA, namely, 10%, 20% and 30% of 96 hours LC50 were chosen for the study.

Treatment Groups

Following acclimation healthy fish were randomly divided into 5 treatment groups which have been summarized in Table 1.

Table 1: Treatment Groups

Group I	Negative Control
Group II	Solvent Control
Group III	10% of LC50 – 1.31mg/L
Group IV	20% of LC50 – 2.62mg/L
Group V	30% of LC50 – 3.92 mg/L

Histopathology of testes

At the end of treatment period (15 days for second lot & 30 days for first lot) the fish which were now in the pre-spawning phase were taken out, anaesthetized by immersing in 50 mg/L benzocaine solution [17, 18] and dissected. The testes were removed from male fish, fixed and processed for histopathology. Using a rotary microtome 6 microns thick sections were cut from testes embedded in paraffin blocks. Double staining technique with Haematoxylin and Eosin was used. The slides were first viewed at low power and then at high power using Metzer Magnus MLX microscope. The images were captured using Capture Pro 4.6 software.

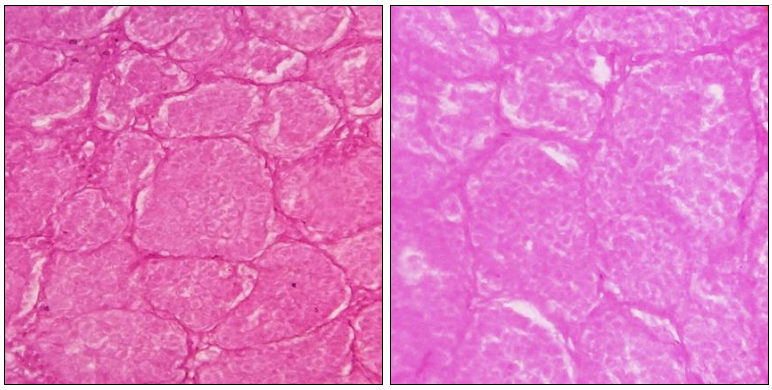
Results and Discussion

Sections of testes of the fish in the control and solvent control groups had numerous seminiferous lobules with intact and distinct lobular walls and interstitial tissue. The lobules contained germ cells, primarily spermatogonia, although few other stages were also seen. These observations are in accordance with normal testicular histology of *Channa punctatus* as reported in earlier studies [19, 20].

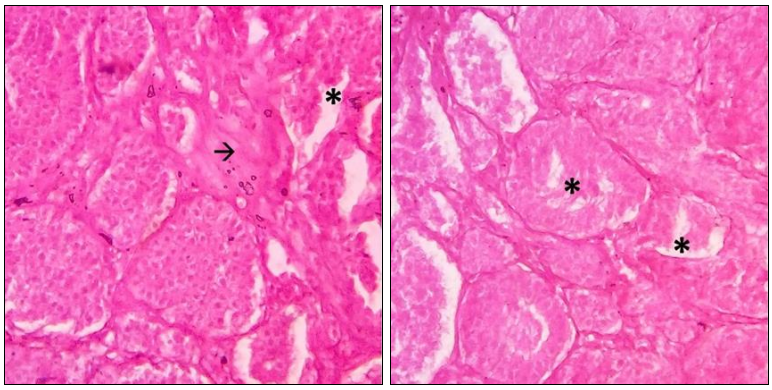
In BPA treated groups, the lobules tended to be smaller than those of the control groups. The lobular walls were not distinct. The lobules showed atrophy and there was a marked proliferation of interstitial tissue in the testes of treated groups. At higher concentrations, after 30 days exposure necrosis of cells was also seen. Large acellular areas and patches of necrotic tissue were visible in the testes of BPA treated fish. These observations are in agreement with previously reported data on effect of BPA on male *Cyprinus carpio* [8] and *Cyprinus auratus* [21].

Significant degeneration of testes after 30 days exposure to BPA was observed. It is probable that chronic exposure to high concentrations of BPA affects the hypothalamic pituitary gonadal axis [10, 21], alters the interplay of estrogens and androgens [22], targets Leydig and Sertoli in males [23, 24] and induces oxidative stress in gonads [25]. The resulting perturbations of the delicate homeostasis are manifested in the gross morphological changes in gonadal architecture in both male fish. Alterations in gonadal histopathology and reproductive physiology following exposure to BPA have also been observed and reported in mice [26], oysters [27], painted turtles [28] etc.

In this study no ovo-testis or testicular follicles were seen. A large number of studies have reported the presence of oocytes in testicular tissue, [29, 30], intersexes [8, 31] increased feminization [32] and subsequent changes in sex ratios [33] in fish populations after exposure to endocrine disrupting agents. However, in this study we did not observe any hermaphroditic histological structure. This difference in results could be attributed to differences in species sensitivity, administered BPA doses, duration of exposure and stage at which the organism was exposed to the toxicant. A better evaluation of endocrine disruptors is possible through long term studies of natural fish populations spanning various developmental stages [34].



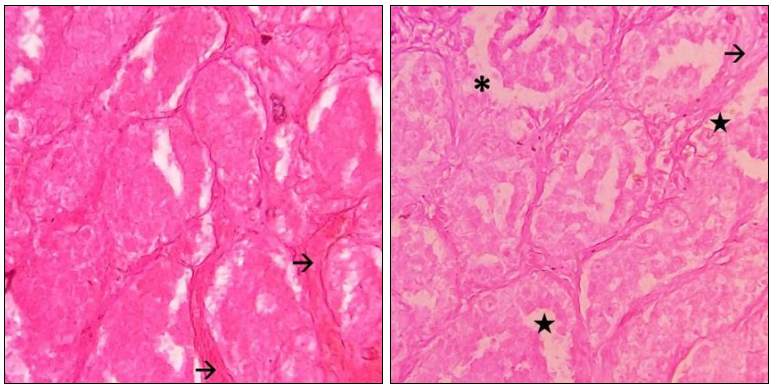
Control Solvent control



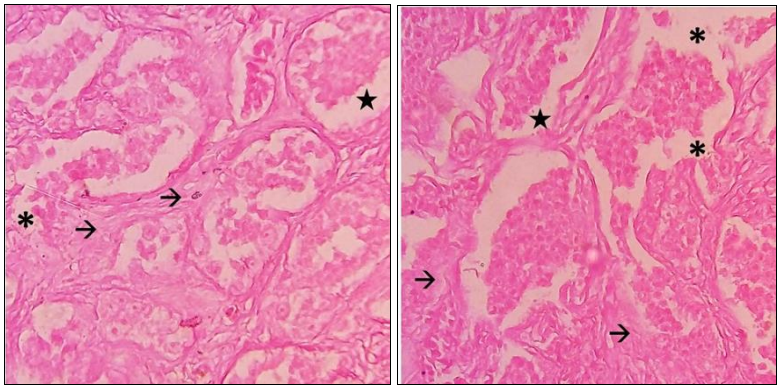
BPA 1.31 mg/L - 15 Days BPA 2.62 mg/L - 15 Days

> Proliferation of interstitial tissue * Necrosis (H & E, X 1200).

Fig 1: Sections of Testis of *C. punctatus*



BPA 3.92 mg/L – 15 Days BPA 1.31 mg/L – 30 Days



BPA 2.62 mg/L – 30 Days BPA 3.92 mg/L – 30 Days

> Proliferation of interstitial tissue * Necrosis ★ Acellular area (H & E, X 1200)

Fig 2: Section of Testis of *C. punctatus*

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