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Ovarian Histopathology In Channa Punctata Upon Exposure To Allura Red

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Abstract

Extensive and arbitrary use of food additives in restaurant dishes and food colors production in industries have caused impending hazards to aquatic organisms. Fishes occupying higher positions in trophic level of the aquatic food chain suffers the most due to the industrial discharge and hotel run off. Reproductive system is one of the most important systems which gets affected to a great extent and in turn affects the reproductive processes and fecundity of animals. Present investigation aims with potential effects of Allura red (AR), (E129) (FD&C Red Dye #40 or Red 40) is a dark-red, water-soluble, azo dye . It is used as food dye for the replacement of amaranth.. Allura red is originally derived from petroleum. It is added into soft drinks, children's medications, and cotton candy. This is the most commonly used dye in the United States for beverages, meats, cheeses, salmon, over-the-counter (OTC) medications, and liquid suspensions etc. On the histological profile of ovary of a fresh water food fish Channa punctata (Bloch) after 15, 30 and 45 days of exposure periods. Notable histopathological findings include deshaped ovum, ruptured ovarian epithelium, stromal hemorrhage, and vacuolization in the ovaries. The extent of ovarian damage was exposure dependent under the impact of individual toxicants. The results obtained will be helpful to understand the effect of Allura red on the ovary of fishes. The findings are quite suggestive of reproductive impairments leading to delayed ovarian maturity and adversely affecting process of ovulation and thus, fish production.

Keywords: *Channa fish, Allura Red, Histopathology, Oxidative stress, Ovary, Hyperplasia.*

1. INTRODUCTION

Water pollution has become inevitable owing to over-industrialization, rapid civilization, hotel kitchen run off water, and over population. Indiscriminate and extensive use of food additives and food colors in industries indirectly cause potential hazards to aquatic animals [1,2]. The hotel run-off and water from food colorant production industries that drains into the local water bodies containing disodium 6-hydroxy-5-(2-methoxy-5-methyl-4-sulfonato-phenylazo)-2-naphthalenesulfonate with subsidiary coloring matters and sodium chloride/sulfate as uncolored components are now recognized as environmental pollutants. They have considerable toxic effects on aquatic fauna including fishes. Among the chemicals this disodium naphthalenesulfonate is wide spread as a result of industrial activities [3].

Its toxicity was known as early as 16th century [4-7]. It has also been found genotoxic. It induces formation of micronuclei and also causes chromosomal aberrations infish Channa punctata [8,9]. Although food additives and colorants are chief key to industrial profit but they fall among the hazardous chemicals released by man into the environment [10] . Ideally a food colorant should not be direct lethal to the consumers , but not to non-target species, including fish. Unfortunately, this is not so, the controversy of use and abuse of food

colorants has surfaced at large scale. The rampant use of these chemicals, under the adage, "if little is good, a lot more will be better" has played havoc with humans and other life forms.

Synthetic red azo dye (25%) induced gonadal impairment in a freshwater edible fish, Channa punctatus have been reported by Srivastava et al. (2008) [11]. Multi-chemical exposure is the rule rather than an exception. Chemical mixtures in the environment can generally are complex, consisting of parent compounds, reaction or transformation products along with other residues. Exposure of biota to combinations of pollutants may sometimes result in unexpected consequences, i.e. significantly lower or greater toxic response than a simple summation of the response induced by the mixture components taken individually. Mixture of pollutants in the environment can influence the toxicity of each other which could be altogether different and more pronounced from their individual toxicological effects[12,13]. This is called synergistic effect or synergy. Ammonia which is added during this chemical production, combines with copper which is found in sediments, to form very stable complex cations of cuprammonium $[Cu(NH_3)_4]^{2+}$ and it enhances the copper toxicity.

James and Sampath (1995)[14] reported that the individual effect of copper is more toxic than ammonia and two of the combinations are more toxic than the individual effect of copper and ammonia.Trivedi and Rai (2007) [15] have reported the impact of food colorants and their mixture on histological architecture of the kidney of Channa punctata. They also investigated the effect of sub-lethal concentrations of red azo dye by exposing the fish Channa punctata for 15, 30 and 45 days, on the hepatocellular profile of the fish. They reported several histopathological changes viz. inflammation, swelling, necrosis, vacuolization and hypertrophy in hepatocytes in the liver of fish Channa punctata[16]. Potential eco-toxicological hazard of food colorants and their synergistic effect, on gills of a fresh water fish, Channa punctata as a test animal has also been reported by Rai et al. (2018)[17]. In present study, an effort has been made to evaluate the toxicity of Allura red emphasized on ovaries in the fish model, Channa punctata .

2. MATERIALS AND METHODS

2.1. Chemicals and reagents used : Allura red , as a cytotoxic agent for this study , procured from the local market.

Figure 1 : Cytotoxic chemical used Allura red.

2.2. Chemicals used for histological slides preparation :Eosin and Hematoxylin were used for staining the permanent mount slides of ovarian tissue for histological studies.

2.3. Specimen selection: In the present investigation adult and healthy specimens of Channa punctata measuring 14.90 ± 0.394 cm in length and 30.80 ± 0.789 grams in weight were collected from local fresh

water resources and brought to laboratory in wide mouthed plastic buckets, in natural water, in order to avoid stress and mechanical injuries as far as possible.

Figure 2. Model specimen used for the study : Channa punctata

2.4. Methodology :

Fishes were transferred to large glass aquaria (90×30×30cm) and thoroughly washed with tap water twice and were then given prophylactic treatment to remove external infections due to bacteria, protozoa, monogenetic, and arthropods. For this purpose, a wash of 1 mg/L solution of $KMnO_4$ for 1 hour was given to fish. Fishes were acclimatized for 10 days with continuous running aerator for proper gaseous exchange. During this period fishes were fed with minced goat liver and artificial fish food. Every effort was made to maintain optimal condition during acclimatization.

96 h LC_{50} of the test chemicals calculated by "Trimmed Spearman-Karber method"[18]. Six fishes were exposed to sub- lethal concentration of Allura red ($1/10^{th}$ 96 h. LC_{50} : 0.085 gm/L) for 15, 30 and 45 days of exposure periods respectively along with a parallel control experiment. Physico-chemical characteristics of water were analyzed at the commencement and termination of each experiment, using standard procedures[19-20].

After the termination of each experiment, fishes were dissected in Ringer's solution. Ovaries were then taken out and processed for microtomy. Permanent mount slides were stained & sectioned with the tissues of ovary of the experimental population. Significant findings were recorded by Cole Parmer MSS 200 trinocular microscope and NPK-MV TNMC 01 camera with automatic time recorder. Changes in exposed sections of ovaries were compared with that of control sections.

3. RESULTS

All of the fishes were alive up to 15 days , 2 died up to 30 days, and 2 died up to 45 days. The histo-anatomy of ovaries of one of fish exposed to sublethal concentration of Allura red ($1/10^{th}$ 96 h. LC_{50} : 0.081 mg/L) for 15, 30 and 45 days of exposure periods showed varied histopathological findings (Table 1)

Table 1 : Effect of Allura red exposure on the ovary histology of Channa punctata for 15, 30 & 60 days.

Exposure Period (Days)	Ovarian Histology Observation	
	Control Group	Exposed group
15	A) The wall of ovary consists of 3 layers, peritoneum, tunica	A) Yolk vesicles are arranged at the periphery of oocytes.

	albuginea, and germinal epithelium. B) The germ cells originate from germinal epithelium. C) A mature ovum is large in size, yellowish in color and translucent. D) It is full of yolk globules, vesicles, and nucleus. (Figure 4)	B) Stromal haemorrhage and vacuoles are more prominent. (Figure 5)
30		A) Disruption of ovarian follicle, reduction in number of mature ovum and 2 oocytes, B) Degeneration of follicles and follicular epithelial cells. C) Hypertrophy and hyperplasia. (Figure 6)
45		A) Necrotic and degenerative ovum along with stromal haemorrhage. B) Ovum swells with large sized yolk vesicles. (Figure 7)

Figure 3 : Ovaries of the Channa sp. of 15 days (Left), 30 days (Middle), and 45 days (Right) of exposure group

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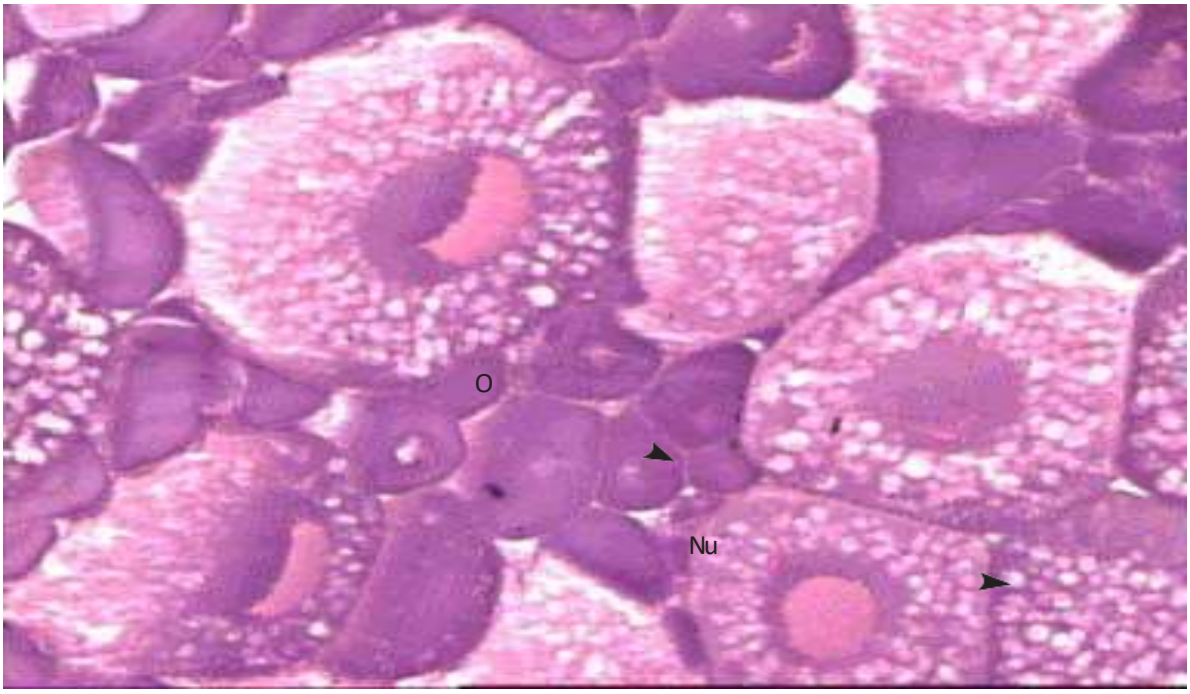

Figure 4. : Section of the ovary of C. punctata from control showing normal Oocytes (O) and nucleus (Nu), (Hematoxylin & eosin, 100X).

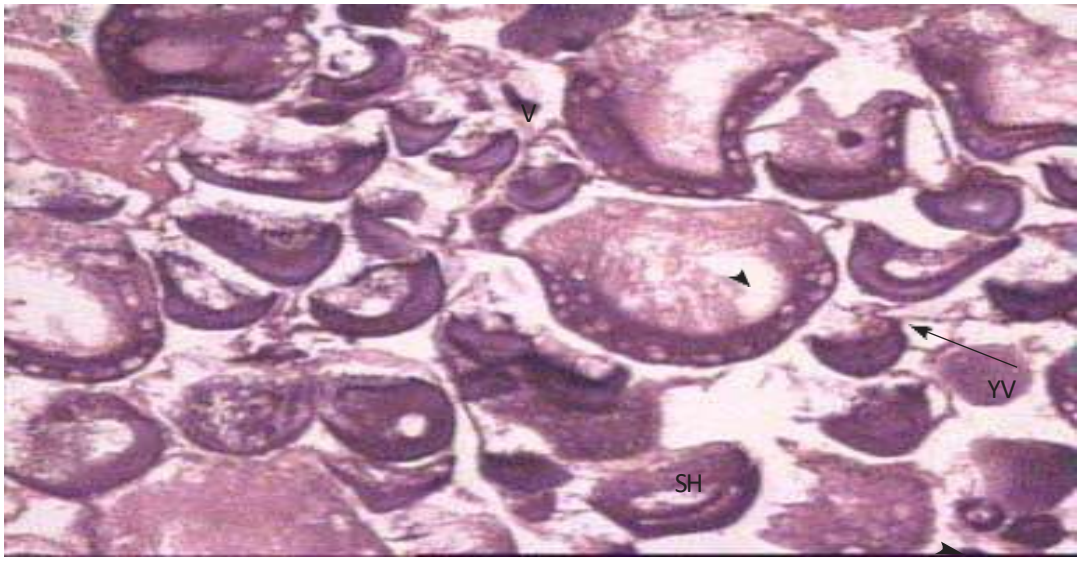

Figure 5. Section of ovary of C. punctata exposed to 96 h LC_{50} 1/10 of Allura red for 15 days showing Yolk Vesicles (YV) at the periphery of Oocytes, Vacuolization (V) and Stromal Haemorrhage (SH), (Hematoxylin & eosin, 100X).

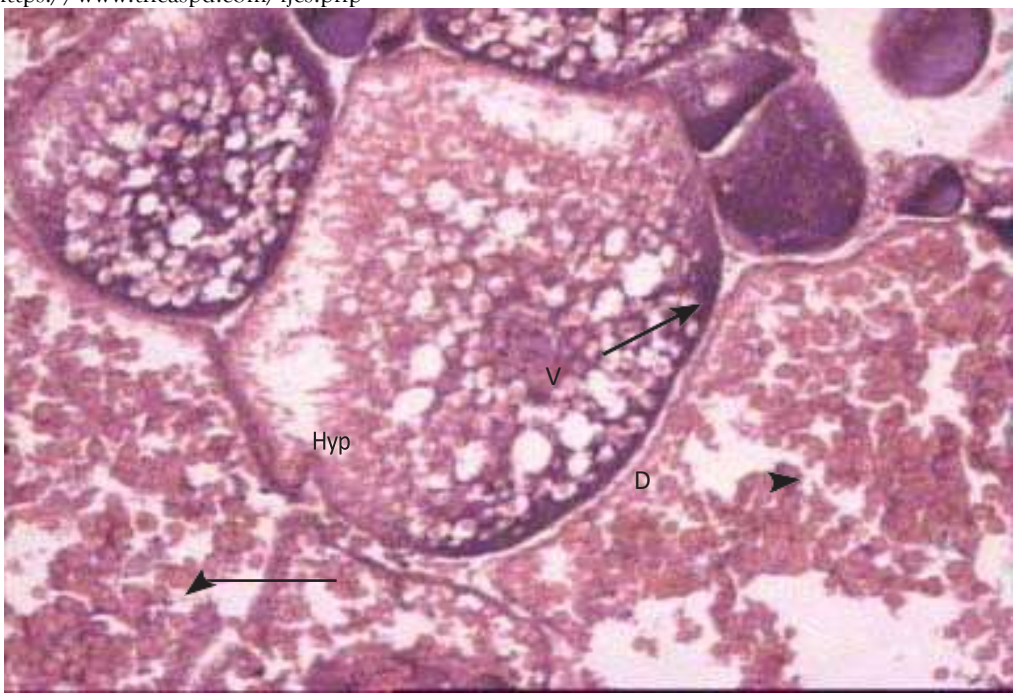

Figure 6. Section of ovary of C. punctata exposed to 96 h LC_{50} 1/10 of Allura red for 30 days showing Oocytes of abnormal size having large number of Vacuoles (V), degeneration of follicles (D) and hyperplasia (Hyp), (Hematoxylin & eosin, 100X).

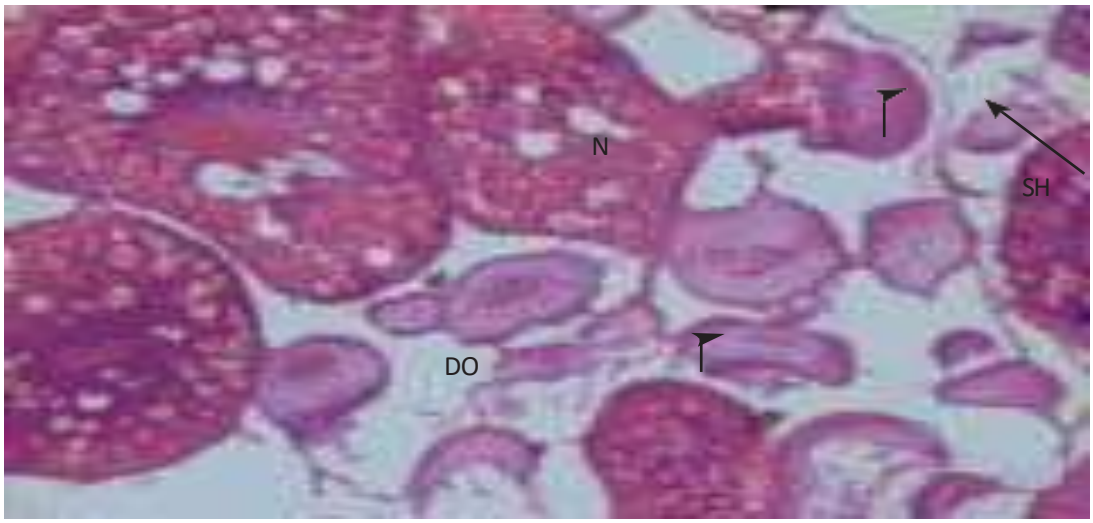

Figure 7 : Section of ovary of C. punctata exposed to 96 h LC_{50} 1/10 of Allura red for 45 days showing Necrosis (N), degenerative ovum (DO) along with Stromal Hemorrhage(SH).(Hematoxylin & eosin, 100X)

4. DISCUSSION

Fishes occupy the utmost position in the trophic level of aquatic ecosystem, thus suffer most due to the industrial discharge and pesticides which impinge in different systems. Reproductive system is one of the most important systems which to a great extent is affected by these toxicants. It is now evident that toxic substances such as heavy metals and pesticides introduced in the environment affect the reproductive processes and fecundity of animals[21]. In the present

investigation, fish C. punctata, exposed to sub lethal concentration of Allura red registered gross histopathological changes in their ovaries and the normal configuration of the ovary was almost lost. Potential ecotoxicological hazard of Allura red and its synergistic effect, on gills of a fresh water fish, Channa punctata as a test animal has also been reported by Rai et al.,2018 [22] . Inadequate literature is available supporting such histopathological changes in other visceral organs due to Allura red exposure to the fish and other animals. But it is quite suggestive that these alterations may lead to the suppression of ovarian activity, resulting in the impairment of reproductive potential of the fish due to this chemical which is now prominent after this research.

Present study concludes that food colorant Allura red exposure alters the normal architecture of ovarian tissue of C. punctata. The toxicity of Allura red is solely dose and time dependent. It may be assumed that reactive oxygen species of phagocytes of leucocytes i.e. oxidative stress is one of the major cause for such aforementioned histo-pathological alternations in the fishes. Definitely, there is an urgent need for proper monitoring and checking of food colorant release into water channels and their residues in aquatic habitats not only to safeguard fish biodiversity, but consumer's health as well. This research gives alarming message to us about its gonado-toxic role on aquatic animals especially edible fishes.

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6. ETHICAL APPROVAL

This study was approved by the ethical research committee board of Studies, GMRD College, Samastipur, Bihar, India. Since , the entire experiment was done there on edible fish available to market so no additional approval was required from Animal Rights Board of India.

7. ACKNOWLEDGEMENT

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8. CONFLICT OF INTEREST

The authors declare that no conflict of interest among the contributing authors of this paper is there.

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The research object chemical Allura red was procured from the local market, Kermy synthetic food sellers & all the required reagents were provided by the Department of Zoology, GMRD college Samastipur, Bihar.

10. AUTHORS' CONTRIBUTION

The entire research was conducted by the corresponding author , Supriyo Acharya with the supervision of Dr. Paramesh Chaudhari and Dr. M.S. Gaikwad.

11. DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.