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HISTOLOGICAL CHANGES IN THE TARGET ORGANS OF *CHANNA PUNCTATUS* AFTER EXPOSURE TO ANTHRAQUINONE VAT DYES

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ABSTRACT

The present study is an attempt on histopathology of gill, liver, kidney and intestine of *Channa punctatus* after exposure to sublethal concentrations of anthraquinone vat dyes viz., vat blue 4 and vat green 1. The dyes has been found to produce several damages in the vital organs of *C. punctatus* leading to various lesions like extensive lamellar hypertrophy with some proliferation at the base of secondary lamellae and hyperplasia of intercellular epithelial cells in the gill; fat accumulation, hepatic necrosis, aggregation of polymorphonuclear leucocytes, hepatocellular degeneration and aggregation of hepatocytes in liver; while in kidney it caused hyperplastic of the haematopoietic tissue and the necrotic tubules were surrounded by interstitial haemorrhage. Moreover heavy odema and sloughing of the epidermal cells and extensive haemorrhage of the intestine were also observed.

Key words : *Channa punctatus*, Histology, Toxicity, Anthraquinone vat dyes, Pollutants, Tissues

INTRODUCTION

Anthropogenic activities do, however cause an increased discharge of the industrial effluents into the natural aquatic ecosystems. Due to this, the native aquatic organisms are exposed to extraordinarily high level of industrial pollutants. The pollutants can affect the animals directly by causing acute to chronic diseases or they could affect the animals indirectly by stressing them and thus allowing them to be vulnerable to parasites or other disease causing agents. Many attempts have been made to elucidate the relationships between pollution and disease. Prevalence of integumental lesions, skeletal anomalies and chromosomal anomalies has been found to be in good association with environmental contamination. There is an emerging relationship between particular categories of diseases and pollution. Fishes are relatively sensitive to the changes in their surrounding environment. Hence, fish health

may reflect the health status of a specific aquatic ecosystem. An early toxic effect of pollution is only evident on cellular or tissue level before significant changes can be identified in fish behaviour or external appearance. Therefore, histological analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs such as gill, liver, kidney, intestine and gonads (Dutta 1996). With this in view, the effect of two anthraquinone dyes, vat blue 4 and vat green 1 on the histology of the target organs of the native fish *Channa punctatus* was investigated.

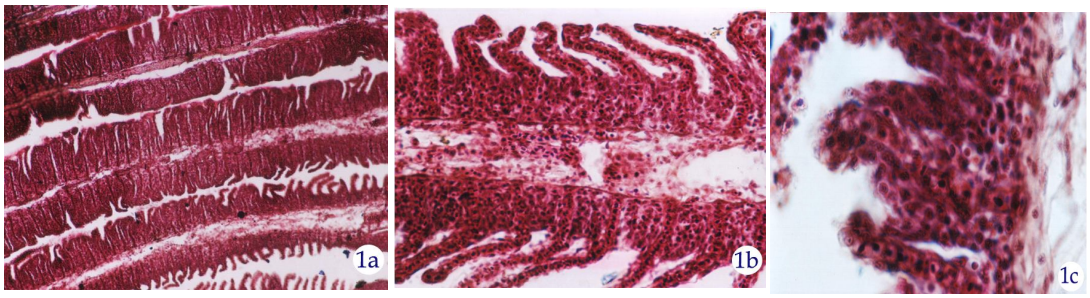
MATERIALS AND METHODS

The effluents free of Vat Blue 4 and Vat Green 1 dyes were collected from the dyeing industry. These two dyes were added to the effluent collected from the industry in varying concentrations and then LC_0, LC_{50} and LC_{100} were determined. From the values of LC_0 and LC_{100} by adopting the method of probit analysis of Finney (1971), the Safe Application Factor Equation (SAFE) was calculated. The Safe Application Range (SAR) was calculated from the figure obtained from the SAFE level and LC_{50} values adopting the formula evolved by Ramadhas (1982). Thereafter the mean values of the dyes were obtained from SAR and LC_0 for conducting laboratory bioassay. The selected mean concentration for Vat Blue 4 and Vat Green 1 are 6.15 mg/l and 6.60 mg/l, respectively. It is imperative to reiterate that only at this concentration any toxicants would slowly and adversely affect, initially physiology of the fish and eventually the other organs of the test fish (Ramadhas 1982).

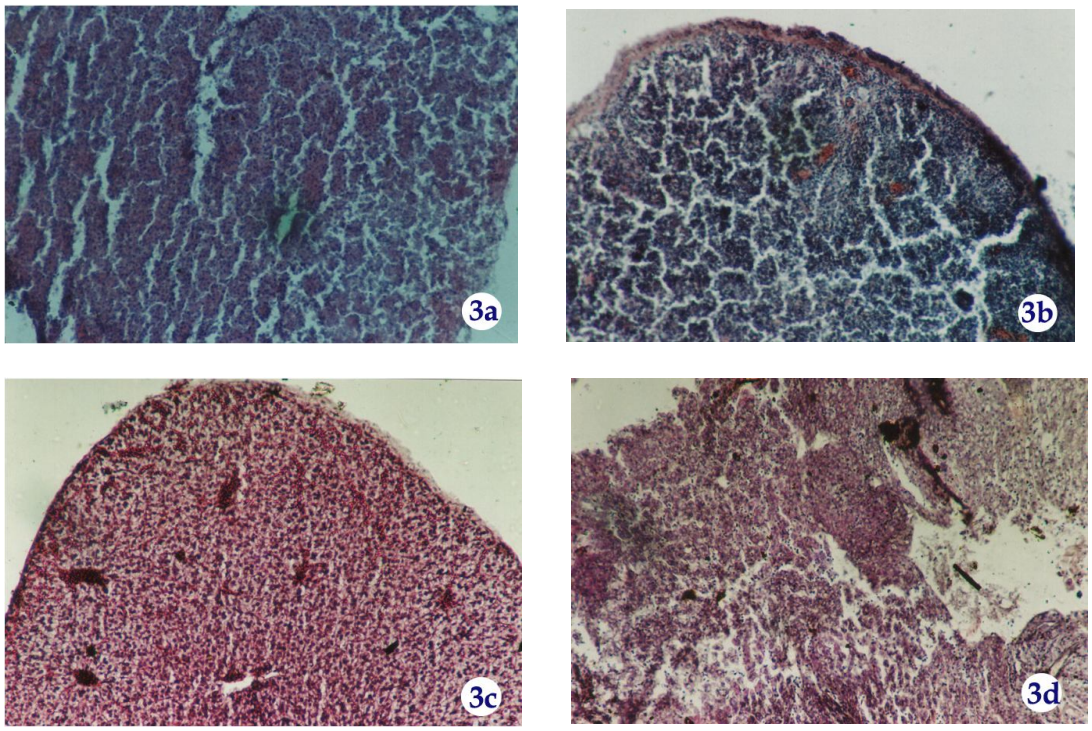
For histological investigation of the fish samples, they were treated with the dye mixture of sub-lethal concentration for a period of thirty days. The control and treated *C. punctatus* were dissected to collect the tissues of gills, liver; kidney and intestine. These tissues were fixed in Bouin's fluid for 24 to 48 hours. After fixation, the tissues were processed in up-grading alcohol of 30, 50, 70, 90 and 100 per cent. The processed tissues were then dehydrated in absolute alcohol. Since alcohol is not soluble in paraffin, it must then be removed from the tissue and replaced with xylene by 3:1, 1:1 and 1:3 ratio of alcohol and xylene and 100% xylene. For infiltration, the tissues were transferred into a solution of de-alcoholising agent, which has been saturated with paraffin at 75:25, 50:50. 25:75 and 100 % (xylene: paraffin ratio). The tissues were then embedded in paraffin wax (M.P. 58 to 60^o C). The tissues embedded in paraffin wax were then sectioned using a rotatory microtome. The thicknesses of the sections were maintained between 4 and 7 µm. These sections were then treated with xylene to remove paraffin and washed in absolute alcohol, 90, 70, 50, and 30 percent alcohol. Finally paraffin free sections were washed with distilled water and stained with haematoxylin for 10 minutes. The sections stained with haematoxylin were again washed in running tap water and distilled water and stained for one minute in eosin. Masson's triple stain and Mallory's triple stain were also used for staining the tissue sections. The sections were subsequently dehydrated in upgrading alcohol of 30, 50, 70, 90 percent and then absolute alcohol (Woods & Ellis 1994). The slides cleaned in xylene were mounted in DPX mountant. Likewise tissues of gills, liver, kidney and intestine of control and treated *C. punctatus* were also fixed and stained as per the above procedure for making comparison. In the case of control fish, visual observations made through microscope alone were recorded. In our study, the low power observations were made with X 35, X 40 and X 60, while the chosen medium powers were X 160, X 220 and X 400. The high power of X 500 and X 750 were used to take photographs as per the requirement.

RESULTS AND DISCUSSION

On microscopic examination of tissues and organs of *C. punctatus* exposed to the dyeing industry effluent, following observations were made:



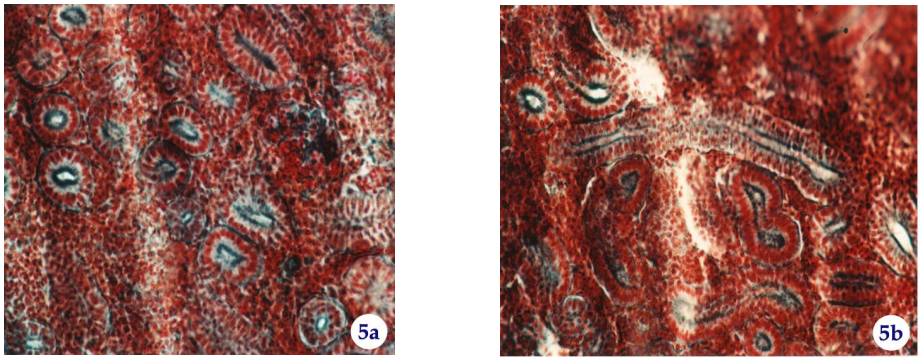
Figs. 1 a, 1b and 1 c: Gill of treated *Channa punctatus*



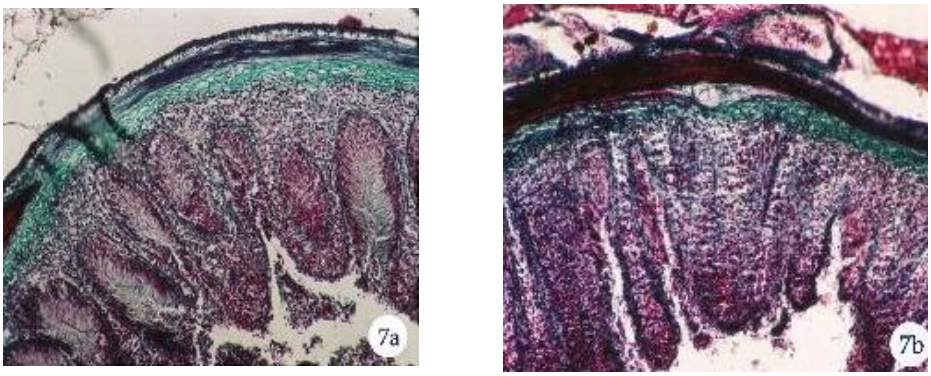
Figs. 2 a, 2 b, 2 c and 2 d: Liver of treated *Channa punctatus*

- In gills extensive lamellar hypertrophy with some proliferation at the base of secondary lamellae was observed (Fig. 1 a).

- Intercellular epithelial cells exhibited hyperplasia (Figs. 1 b).
- Secondary lamella showing lamellar hypertrophy was observed (Fig. 1 c).
- Fat accumulation and hepatic necrosis was observed in the liver (Figs. 2 a & 2 b respectively)
- An aggregation of polymorphonuclear leucocytes, hepatocellular degeneration and aggregation of hepatocytes were noticed (Figs. 2 c & 2 d respectively).



Figs. 3 a and 3 b: Kidney of treated *C. punctatus*



Figs. 4 a and 4 b: Intestine of treated *C. punctatus*

- In the case of kidney, haematopoietic tissue is hyperplastic and mild degeneration was exhibited (Fig. 3 a)
- The necrotic tubules were surrounded by interstitial haemorrhage (Fig. 3 b).
- Extensive haemorrhage of the intestine was also noted (Fig. 4 a).
- Intestine of treated *C. punctatus* exhibited heavy odema and sloughing of the epidermal cells (Fig. 4 b).

DISCUSSION

Fishes like any other living organisms require energy for locomotion, respiration, digestion, reproduction and blood chemistry. Energy is also required for their internal metabolic functions. The requirement of energy is met through the metabolic activities of the body with the help of oxygen. Therefore the oxygen is the dominant constituent of metabolic function of any animal and also plants. It is known fact that atmosphere contains oxygen and it is also present in large quantities in water. Fishes obtain the required amount of oxygen from the water in which they live by utilizing a slightly different mechanism i.e., they utilize the unidirectional passive movement of water through the external gills. There are a number of mechanisms that fish can utilize to extract oxygen from the water but the most common mechanism involves the gills. If the water in which they live is polluted with toxicants, gills are prone to get damaged. On long run, pollutants are slowly transmitted to other vital organs of fish responsible for physiological functions and these toxicants may also trigger catabolism due to unremitting physiological stresses. To sum up, gills rank first among the other organs, with respect to the adverse effect of pollutants present in the water. Gills are lined with single layer of delicate sensitive tissues and these tissues are susceptible to be damage by any irritant dissolved or suspended in the media in which they live (Lemke & Mount 1963). Grevan (1953) and Waluga (1966) recorded gill oedma and detachment of the cells of the respiratory epithelium when gold fishes were exposed to phenol. Kuhn and Koeeka (1956) considered that under damaged conditions, gills could continue its respiratory function but poisonous materials of the medium in which they live would penetrate along the damaged portion of the gills into the blood vessels. According to them, the gills owing to their structure and function are more vulnerable to allow the penetration of the pollutants present in the habitat. In the present study of both the control and treated *Channa punctatus*, gill damage took place, predominantly due to the dyeing industry effluent. The results of the present investigation are in conformity with the previous reports of Grevan (1953) and Waluga (1966). The observations made in the present study establish that the anthraquinone dyes damaged the organ directly as a pollutant and indirectly as an agent triggering the proliferation of microbes, which in turn would progressively degenerate different organs responsible for many vital physiological functions.

A close observation of photomicrographs of liver tissues disclosed the fact that hepatocytes were under different patterns of degeneration with swollen cells. The swelling of these cells might be due to the cumulative affect of toxicity pollutants. Similar type of changes was reported by Ram and Sathyanesan (1987) who investigated the impact of mercurial fungicide on *C. punctatus*. Narayanan *et al* (1987) and Othuman (1994) established that the quantity of fat and glycogen deposited was in consonance with increase in the concentration of the toxicant under investigation. Mandal and Kulshrestha (1980) observed that blood vessels

traversing the liver of *Clarias batrachus* exposed to sub-lethal dose of malathion for 90 days were found to be more or less empty. Similarly, lack of sufficient blood supply could be one of the major reasons for the damage of the liver in the present study and thus it also confirmed the above findings.

The primary function of kidney is to maintain the homeostatic balance of bodily fluids by filtering and secreting metabolites such as urea and minerals from the blood and excreting them, along with water when liver is detoxified. Thus, it is important to note the structure and function of any organ subjected to physiological stress because it may inevitably leave its effect on the organ. The structure of kidney undergo modification and changes abnormally as it struggles to eliminate the ions and molecules present in the effluent. The extent of these changes has been known to be effected by the total exposure to the effluent. In the present study, increased level of damage confirmed the toxic nature of the effluent. Mandal and Kulshrestha (1980) recorded striking abnormalities in *C. batrachus* exposed to sub-lethal concentration of malathion. Similarly, Wobeser (1975) observed slight swelling of the epithelial cells lining Bowman's capsule of kidneys of rainbow trout fingerlings orally administrated with methyl mercury chloride.

Being the site of absorption, the gut and intestine are naturally susceptible to the toxic effects of any pollutants entering through either the food or water. The pollutants, primarily affect the tissue leading to dysfunction. In the present study extensive hypertrophy and necrosis was observed in *C. punctatus* exposed to the effluent and similar changes were also reported by Murugesan (1988) in *Heteropneuolus fossilis* exposed to textile mill effluents. Othuman (1994) observed similar effects in *Oreochromis mossambicus* exposed to distillery effluents. The hypertrophy of globet cells usually results in copious mucus secretion that is a common response of the epithelium to many irritants (Roy 1988). From the above discussions, it is apparent that the test animal of the present study has revealed that the vital organs were damaged at cellular level due to the synergetic effect of pathogenic microbes and disease causing dyes. Thus the present observation strongly recommend for increased scope for further investigations by including viable pathogens.

The industrial growth and the pollution go hand in hand. The industrial growth is directly proportionate to the pollution. The toxic effluents drained into the water bodies located in and around the human habitats threaten the aquatic environment. The continuous draining of toxic pollutants into the aquatic environment would increase the level of concentration of toxic pollutants to alarming level, threatening seriously, the aquatic organisms including fish population. The concentration of these toxic substances and its effect on the aquatic organisms are to be monitored and regulated regularly to ensure sustainable living and conservation of aquatic biodiversity. The present study amply affirms this fact. Any negligence in monitoring the level of pollutants in the aquatic environment would lead to the outbreak of diseases in the fish population by dyes

through the process of biomagnifications through food tier. To conclude, it is strongly recommended that wastewaters containing toxicants be properly processed prior to releasing it into aquatic ecosystems harbouring rich floral and faunal diversity. Investigations carried out in this line on the species of native fish confirm this phenomenon to be true.

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