Histological distribution and ultrastructure of exocrine pancreas in Indian major carp (*Labeo rohita* Ham.) and its alteration in aflatoxicosis

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Abstract

The distribution pattern of exocrine pancreas in *Labeo rohita* besides its general location along the course of intestinal mesentery was studied. It is evenly distributed within the liver around portal vessels and also within the spleen near a blood vessel. On ultrastructure, two cell types of different degrees of staining intensities containing abundant rough endoplasmic reticulum, mitochondria, pre-zymogen and zymogen granules were marked. During aflatoxicosis, the mesenteric pancreas and hepatic pancreas were mostly affected revealing necrotic changes to acini. The zymogen granular activities were markedly reduced. Ultrastructurally, the rough endoplasmic reticulum were fully dilated and formed whorled pattern. The damage to the exocrine pancreas might be affecting digestive enzymes' secretion which may be one of the cause of aflatoxin-induced anorexia in fish.

Key words: *Labeo rohita*, Histology, Ultrastructure, Aflatoxin, Exocrine pancreas

Introduction

The distribution of pancreatic tissue varies considerably with species and also even within a single species. The most common sites for it are in the mesentery of pyloric caeca as scattered islands of secretory tissue interspersed among the fat cells, as an external layer around the hepatic portal vein and sometimes, in the subcapsular area of the spleen. The acinar pancreatic tissue in a highly active exocrine organ, which produces digestive enzymes like lipase, amylase, trypsinogen and chymotrypsinogen. These enzymes are stored in brightly staining eosinophilic granules within the acini. The enzymes on their release after the damage to acini cause damage to surrounding tissues (Roberts 1989). In spite of these functions, this organ is yet to be studied for its distribution in many species and their alterations in various diseases. The normal fine structure of exocrine acini in Indian major carp are yet to be elucidated.

Aflatoxins are a group of extremely toxic metabolites produced by some strains of the ubiquitous fungi *Aspergillus flavus*, *A. parasiticus* and *A. nomius* grown on agricultural products under suitable conditions of temperature and moisture. Aflatoxins are best
known for their hepatotoxic, mutagenic, carcinogenic, genotoxic, teratogenic and immunosuppressive properties in animals and birds as well as few fish species viz., rainbow trout, salmon, channel catfish and chana (Ashley 1970, Smith and Mos 1985, Verma et al. 1989, Jantrarotai et al. 1990). The Indian major carp species, *Labeo rohita* (rohu) has also been proved to be a carcinogenic model animal although the aflatoxin toxicity produced changes in all most all the organs of rohu (Sahoo et al. 2000). Although, aflatoxicosis is still a major problem of tropical countries and rohu being a susceptible species (Sahoo et al. 1998) to this problem, little is known about its effect on exocrine pancreas. However, acinar necrosis and pancreatitis during acute aflatoxicosis have been reported in channel catfish and tilapia earlier, respectively (Jantrarotai et al. 1980, Chavez-Sanchez et al. 1994). Ashley and Halver (1963) have also observed metastatic liver neoplasms within pancreas due to chronic aflatoxicosis.

The main objectives of the present study were to confirm the distribution pattern of exocrine pancreas and their relevance during subchronic aflatoxicosis.

**Materials and methods**

**Laboratory conditions**

Eight 500 l plastic aquaria equipped with continuous air supply were each stocked with 10 rohu (*Labeo rohita*) fingerlings (± 50 g). The fish were fed with a nutritionally balanced commercial rohu fingerlings diet for 2 weeks before being injected with aflatoxin. The water temperature during the experiment varied between 25 to 28°C. The water was changed along with waste feed and faecal materials every 24 hour. The basic physico-chemical water parameters viz., dissolved oxygen and pH were measured systematically to maintain its optimal level throughout the experimental period. The fish were fed @ 3% of their body weight once daily. The fish were randomly divided into 4 groups with two aquaria in each group.

**Aflatoxin injection**

Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) (Sigma, USA) was dissolved in chloroform and subsequently peanut oil was added to that. The required dosings were prepared for subchronic toxicity trial as per Sahoo et al. (1998). The fish were fasted for 24 hour before administration of AFB<sub>1</sub> and anaesthetized with MS 222 (Sandoz) before injection. Two groups of fish received AFB<sub>1</sub> @ 1.25 and 2.50 mg/kg of body weight intraperitoneally (IP) once. One group was injected with oil only (control group) whereas the other one was left as such without any treatment (served as healthy normal group). The fish were observed for a period of 90 days.

**Necropsy and light microscopy**

At the end of the experiment, the fish were sacrificed by applying overdose of MS 222 and necropsied to observe the gross abnormalities in liver, spleen and pancreas. The liver, spleen and pancreatic tissues from 5 randomly selected fish of all the groups were
collected at the end of the experiment. The tissues were preserved in 10% phosphate-buffered formalin and processed for light microscopy using standard method.

**Transmission electron microscopy (TEM)**

Similarly, the tissues were collected from 5 randomly selected fish from all the groups immediately after anaesthetization with MS 222 at the end of the experiment, sliced and minced (1 mm³) in chilled fixative (3% glutaraldehyde in phosphate buffer, pH 7.2) and fixed in fresh fixative solution for 24 hour at 4°C. They were washed in 3 changes of phosphate buffer solution for 30 min at 4°C, dehydrated in graded series of acetone and infiltrated in araldite resin (CY-212, Polysciences, Inc, Warrington). Semithin (1-2µm) sections from randomly selected blocks of each liver were cut, stained with toluidene blue for 30 seconds and examined. Ultra thin sections (60-70 nm) from not less than 3 selected blocks of each tissue were cut, mounted on copper grids and stained with aqueous uranyl acetate and lead citrate before examining under JEOL TEMSCAN-100 CX II analytical electron microscope at 60 kv.

**Results and discussion**

The distribution pattern of exocrine pancreas of healthy normal fish which had not received any treatment was studied. Like other species, the pancreas was scattered in the mesentery of pyloric caeca surrounded by fat cells. Besides, it was also abundant around periportal vein as if it follows the major portal vein tract (Fig.1). Surprisingly, it was also noticed in the subcapsular splenic tissue closely associated with splenic ellipsoids, red pulp and white pulp (Fig.2). Similar locations of splenic tissue have been recorded in few species of fish earlier (Roberts 1989). On semithin sections, intensely-stained as well as lightly-stained cell types, both packed with secretory granules were marked (Fig. 3). The two cell types were distinctly differentiated on their staining character. Abundant rough endoplasmic reticulum, glycogen, mitochondria, free ribosomes, lysosomes, rounded nucleus with nucleolus along with two types of cytoplasmic granules (one deeply osmiophilic, i.e zymogen, and the other is poorly osmiophilic, i.e prezymogen) were observed on electron microscopy (Fig. 4) in the acinar cells. The control group fish had almost similar structures on light and electron microscopy without any marked differentiation.

![Fig. 1. Liver of normal rohu showing the exocrine Pancreas (arrow) around the portal vein (H & E X197).](image1)

![Fig. 2. Cross section of normal spleen revealing exocrine pancreas (arrow) & splenic ellipsoids (arrow head) (H & E X315).](image2)
The aflatoxin-treated fish showed dose-related changes in the liver, spleen and exocrine pancreas. On necropsy, pale yellowish, enlarged and mottled liver were the most common findings in toxin-treated fish. The spleen was mildly congested. Similarly, Chavez-Sanchez et al. (1994) observed subcapsular focal congestion and hepatomegaly in tilapia fed with 3 ppm of aflatoxin for 25 days. There was no gross abnormality in the pancreatic tissue of toxin-treated fish.

On histology, the liver developed clear preneoplastic nodule to hepatocellular adenoma and there was massive lymphocytolysis in the spleen of toxin-treated fish which has been described in detail in our earlier study (Sahoo et al. 2000). The acini of pancreas located within the liver were mostly affected and found to be necrotic. The mesenteric pancreas also had necrotic acini (Fig.5) and congested blood vessels in the vicinity (Fig.6). Jantrarotai et al. (1990) also observed acinar necrosis during acute aflatoxin-toxicity in channel catfish. On the contrary, Chavez-Sanchez et al. (1994) marked pancreatitis in aflatoxin-fed Nile tilapia. The greater degree of damage to periportal pancreas might be due to its location as because liver is the main target organ of aflatoxicosis. Ashley (1965) reported that rainbow trout force-fed with high doses of aflatoxins had hypertrophic acinar cells and at times markedly desquamated and with focal hyperemia in the visceral fat. There was also marked reduction of cytoplasmic granules observed in semithin sections (Fig.6) indicating the decreased activity of pancreas which might be indicative of rendering less synthesis of pancreatic digestive enzymes thereby hampering digestion and subsequent anorexia as observed in our earlier study (Sahoo et al. 1998).
Exocrine pancreas in L. rohita

Fig. 6. Semithin section of aflatoxin-treated exocrine pancreas. Note decreased cytoplasmic granularity and engorged blood vessels in the periphery (Toluidene blue X1000).

Fig. 7. TEM section of aflatoxin-treated pancreatic acini with dilated rough endoplasmic reticulum, condensed nucleus and another type of granules with osmiophilic core (Mag. X10,800).

On electron microscopy, the rough endoplasmic reticulum were markedly dilated (Fig. 7), sometimes forming whorled-pattern. The dilated rough endoplasmic reticulum was also characteristic in the AFBl-induced hepatocellular neoplasms of rainbow trout (Nunez et al. 1991). The granules were mostly of prezymogen stage and many granules with osmiophilic core (Fig. 7) were also found. The nuclear chromatin was somewhat condensed with poorly defined nucleolus and irregular shaped nucleus was marked before death. The typical changes observed on TEM might be indicative of toxicosis and exposure to carcinogens (Ghadially 1982).

In conclusion, the present study established the distribution pattern of exocrine pancreas in rohu. This study also showed that aflatoxin-induced pancreatic damage may be one of the important factor of reduced feed intake and growth in aflatoxin exposed fish.

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