Aflatoxicosis due to acute toxicity was studied through two experimental trials conducted on Indian major carp, *Catla catla* using crude as well as pure aflatoxin B₁. In the first experiment where fishes were fed crude toxin mixed with diet @ 5 mg/kg, no clinical sign as mortality could be observed, whereas in the second experiment where pure aflatoxin B₁ was fed with the diet @ 0.5 mg/kg resulted in 40% mortality with signs of lethargy, listlessness and excess mucus secretion. Histopathological alterations in the liver, kidney, spleen, heart and gastrointestinal tract have been described and discussed.

INTRODUCTION

The effect of mycotoxins on human and animal health resulting from the consumption of contaminated food and feed stuffs are of great concern worldwide. In fish, a number of problems have arisen during recent years with extensive use of feed supplements and dry pellets, which are susceptible to invasion by toxigenic fungi at some stages of their production, processing or transportation as well as storage. Among the various mycotoxins, aflatoxin produced by *Aspergillus flavus* and *A. parasiticus* are important because these compounds have been shown to be highly toxic to animals. Aflatoxin B₁ is regarded as the most potent carcinogenic substance among known aflatoxins.

Contamination of fish feed and subsequent elaboration of aflatoxins leading to the development of spontaneous and experimental hepatoma in trouts are well documented (Haddow and Blake, 1933; Nigrelli and Jakowaska, 1955; Hueper and Payne, 1961; Ashley and Halver, 1961; Halver, 1965; Scarpelli, 1967; Ashley, 1967, 1970; Sinnhuber et al., 1977; Wales et al., 1978).

Perusal of available literature reveals that substantial work have been carried out on aflatoxicosis in fish eg. brook trout, salmon, catfish (Wood and Yasutake, 1956; Ghittino, 1963; Wolf and Jackson, 1967; Halver et al., 1969; Verma et al., 1987, 1988; Jantrarotai et al., 1990). However, the work on susceptibility of Indian major carps to aflatoxin and quantum of tissue damage inflicted by this toxin has not been investigated so far in our country. The present report is based on preliminary studies on experimental acute aflatoxicosis in *Catla catla* with description of histopathological alterations of different organs.

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MATERIAL AND METHODS

Laboratory trials were conducted on Catla catla using crude and pure forms of aflatoxins, respectively. A feeding trial (No. 1) was conducted for acute toxicity test with crude toxin whereas the second experiment (No. 2) was employed using pure AFB1 toxin to observe clinical signs, survival and degree of damages inflicted in the target organs.

Experiment No. I

Twenty four fishes were reared in the laboratory in four plastic pools (100l capacity), each containing six animals of average size 150 ± 7.5 g. Control diet mixed with crude toxin of A. flavus was fed to the reared fish in pool nos. 1, 2, and 3 5 mg/kg body weight whereas, pool no. 4 served as control in which the animals were fed with diet containing rice bran, wheat bran and groundnut oil cake. Clinical symptoms were observed regularly from time to time. Fishes were sacrificed on the 10th day for necropsy and tissue pieces were collected for histopathological observations using routine histological techniques.

Experiment No. II

The experiment was conducted on 24 numbers of the same species i.e Catla catla (average weight 150 ± 7.5 g) using pure AFB1 toxin of A. flavus procured from Sigma, USA (A-6636).

Diet mixed with AFB1 was fed to the fish in pool nos. 1, 2, and 3 0.5 mg/kg body weight for a single day and then fed with control diet for ten days. Clinical symptoms and mortality if any were observed regularly. Necropsy was conducted on sacrificed animals and samples were collected and processed for histopathological examination.

RESULTS

Experiment No. I

During the ten days experiment, no external abnormality was detected. No clinical signs or mortality could be noticed. On opening the abdominal cavity, mild congestion was noticed in the kidney and spleen, however, liver was pulpy, deep chocolate in colour, mottled and congested. Heart was markedly distended due to engorgement of blood. Pericardial sac was thickened.
Histopathologically, liver tissue showed marked degenerative and vacuolar changes. Parenchymatous cells exhibited marked swelling and constriction of the sinusoids (Fig. 1). In the kidney, tubular epithelium showed hyperplastic as well as necrotic changes. Multiple granulomatous changes were discernible in the kidney parenchyma. Blood vessels were often found with disrupted endothelial lining with accumulated leucocytes in the lumen (Figs. 2 & 3). Spleen exhibited diffuse congestion and marked depletion of spleenic cells (Fig. 4). Marked changes were seen in the heart. Pericardial sac showed marked thickening with accumulation of intrapericardial fluid and connective tissue infiltrated by mononuclear cells (Fig. 5). Myocardium exhibited moderate necrosis of myocardial fibres. No abnormality could be detected in the skin, muscle and gill tissues.

Experiment No. II

In the second experiment, 40% mortality could be observed by the 10th day. Clinical signs were characterized by lethargy, listlessness and excessive mucous secretion from third day onward. Necropsy on the dead animals showed damage of various degrees in the liver, kidney, viscera and spleen. Survivors at the end of the test period showed extensive congestion of the visceral organs and marked liver damage with multiple haemorrhagic spots.

Histopathologically, liver tissue showed marked necrotic changes of the hepatic cells with severe damage to the blood vessel (Fig. 6). Accumulation of lymphocytes, sometimes in bunches were discernible in the vicinity of the blood vessels. Disruption of hepatic cords and severe generalised hepatocellular degeneration were evident in most of the cases with darkly stained hepatocytes scattered throughout the parenchyma (Fig. 7). The hepatocyte appeared granular with either pyknotic or karyorrhectic nuclei. In the kidney, marked changes were noticed in the tubular epithelial cells with extensive degeneration and necrosis. Multiple granulomatous lesions with central necrotic tissue were seen in the parenchyma. Focal accumulation of lymphocytes was seen in the interstitium (Fig. 8). Spleen exhibited extensive loss of stroma and depletion of the spleenocytes. Dark eosinophilic grape like material was seen accumulated in the parenchyma (Fig. 9). At some places spleenic artery contained emboli with caseation. Perivascular accumulation of lymphocytes were evident at many places (Fig. 10).

DISCUSSION

The effect of aflatoxin in rainbow trout and salmon has been well documented and reviewed by several workers (Ashley, 1967; Halver, 1967; Halver et al., 1969). Although toxic effect of aflatoxin B₁ has been reported from India in airbreathing teleost,
Fig. 1. Hepatocytes showing marked swelling and constriction of sinusoids. Note the distortion of hepatocellular arrangement (H&E x 200); Fig. 2. Kidney showing multiple granulomatous lesions and tubular necrosis (H&E x 200); Fig. 3. Higher magnification of Fig. 2. Note the disrupted endothelial lining of the blood vessel and accumulation of leucocytes in the lumen (H&E x 400); Fig. 4. Marked depletion of splenic cells (H&E x 400); Fig. 5. Microphotograph showing marked thickening of the pericardium with cellular infiltration. Note myocardial necrosis (H&E x 200); Fig. 6. Hepatic cells showing swelling and necrosis and central vein endotheliosis. Note extensive hepatocellular necrosis (H&E x 400); Fig. 7. Darkly stained hepatocytes are scattered throughout the parenchyma (H&E x 400); Fig. 8. Kidney showing degenerative and necrotic changes with extensive accumulation of lymphocytes in the interstitium (H&E x 400); Fig. 9. Darkly stained materials in the stroma of cell-depleted spleen (H&E x 200); Fig. 10. Photograph showing perivascular accumulation of lymphocytes in a splenic artery. Note another blood vessel with caseated embolus in the lumen (H&E x 200).
Channa punctatus by Verma and Pandey (1987) and Pandey et al. (1985), available literature failed to reveal any report of the effect of aflatoxin on Indian major carps. The present study is probably the first to highlight the effect of crude aflatoxin as well as aflatoxin B₁ on tissues of Catla catla.

In the first experiment, no clinical signs or external lesions could be observed in the test animals where crude aflatoxin was used at a higher dose than the AFB₁ used in the second experiment. Clinical signs were pronounced in the fish which received pure aflatoxin B₁ through feed. In both the cases, of course, liver tissue showed appreciable changes on gross examination. Histopathologically, the changes in the hepatocytes were milder in case of crude aflatoxin in comparison to AFB₁ where the hepatic cells showed severe damage with necrotic changes. Halver (1969) while doing acute aflatoxicosis trials in rainbow trout with single dose of 5 mg/kg body weight, found moribund fish with gross multiple hyperaemic areas throughout the liver and other viscera within 7 days. He has recorded multiple hyperaemic areas interspersed with hard, firm yellow tissue in liver within 10 days. The changes were by far minimum in our case where the liver tissue were only mottled and congested. Halver (1969) recorded necrotic liver cells with pyknotic to karyolytic nuclei with more extensive hyperaemic areas in rainbow trout whereas the changes in the liver tissue were limited to degeneration and vacuolation only in our experiment. This showed that Catla catla may be comparatively more resistant to aflatoxicosis than the rainbow trout.

The single dose LD₅₀ for crystalline aflatoxin B₁ was determined as 0.5 mg/kg body weight for rainbow trout (Halver, 1969; Halver et al., 1969) and thus the second experiment was designed with a single dose of 0.5 mg/kg body weight through feed. In this case also, the mortality in Catla catla reached up to 40% by the end of 10th day as against 50% in case of rainbow trout recorded by earlier workers. Halver et al. (1969) agreed that coho salmon were 10 to 30 times more resistant to aflatoxicosis than rainbow trout and were generally refractory to chronic dietary treatment.

Gross observation of samples of fish showed characteristically less specific damage in liver and other visceral organs than observed in rainbow trout and Channa punctatus. More time may be necessary for acute effects in Catla catla to become evident and repetitive feeding of the toxin is necessary before frank toxicity to be observed.

A very prominent histopathological change that noticed was in the cardiovascular system in case of crude aflatoxicosis. The myocardial necrosis with infiltrating cells (Fig. 6) and blood vessels of kidney and spleen indicated damaging effect of the toxin on blood vascular system. This type of lesions have never been recorded in any earlier reports. Histopathology of tissues disclosed and confirmed typical extensive liver damage showing marked disruption of hepatic cords and darkly stained
hepatocytes scattered throughout parenchyma due to acute AFB1 toxicity. Aflatoxins are supposed to be immunosuppressive in nature. This was evident from the histological changes in the spleen which showed marked depletion of spleenocytes. Changes like sloughing of the gastro-intestinal epithelium are also indicative of the cytotoxic effect of the toxin. It will be important to note the long term effect of aflatoxin in Indian major carps by subjecting the fish to frequent low dose exposure for a longer period of time.

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REFERENCES


