A general survey of three types of fish feeds for the incidence of toxigenic mold infestation revealed an alarming level of aflatoxigenic *Aspergillus flavus* contamination during storage. About 89% of *A. flavus* strains were toxigenic. The toxicity of crude toxin sample was assessed on *Channa punctatus*. The experiment was conducted using pure crystalline aflatoxin B1. Four different concentrations (10, 20, 30 and 40 mg/l) of pure toxin in 0.5 ml propylene glycol were administered intraperitoneally to four groups of fish along with a control. Observations were made on day 20, 40, 60 and 80. The treated fish showed loss of appetite, followed by listlessness and increased mucus over body surface. Hemorrhagic patches and tumors were prominent on liver. Histopathologically, liver was the most prominently affected organ leading to classical trabecular hepatoma. The excretory-kidney revealed some pre-neoplastic alteration arising from tubular epithelium. The head-kidney was atrophic, but proliferation of inter-renal lobules was prominent. The muscularis of the stomach showed severe proliferation. The intestine, pyloric caeca and gills were atrophic and necrosed. Formation of atypical glandular masses in mucosal region was marked in the intestine. The splenic pulps were indistinguishable and showed a monotony of lymphocytes. The total and differential counts of leucocytes revealed a significant rise of lymphocytes in peripheral blood.

**INTRODUCTION**

Investigations since 1960's have established aflatoxins, toxic metabolites of *Aspergillus flavus* and *A. parasiticus* as model carcinogenic agents (Couch and Harshbarger, 1985). The aflatoxins (B1, B2, G1 and G2), bisfurancoumarin compounds, occur naturally in plant products and have been reported from almost all agricultural by-products. The present work was planned to study the aflatoxigenic contamination of *A. flavus* in some common fish feeds and to validate the use of an air-breathing teleost, *Channa punctatus*, as an experimental carcinogenic subject.

Scarpelli (1969) had pointed out the potential value of fish as an ideal model for research on cancer. To date, about 15 fishes (estuarine and marine) have been evaluated as experimental
carcinogenic subjects (Couch and Harshbarger, 1985). No study has been focussed on any tropical fish in general and an air-breathing fish in particular.

MATERIAL AND METHODS

Fish feed samples (mustard oil cake, mixed oil cake and ground pulses mixture) collected from different blocks of Darbhanga District (Bihar, India) were screened for mycobial infestation (Verma, 1989). Pure culture of identified A. flavus isolate was chemically assayed (Jones, 1972) and aflatoxin B₁ was detected on thin-layer chromatography plates under longwave ultraviolet-light following Reddy et al. (1970). Quantitative estimation was done by comparison of standard methods (Jones, 1972).

Culture extract of A. flavus in propylene glycol was prepared at 40 mg/l and 0.5 ml of this was administered intraperitoneally to the test fish for toxicity assessment. Studies on acute aflatoxicosis were conducted separately on the same fish using pure crystalline aflatoxin B₁ (obtained from Dr H. P. vanEgmond, Rizks Institute, The Netherlands). Four different concentrations of the toxin (10, 20, 30 and 40 mg/l) were injected in four groups of 100 fish each along with control which was given 0.5 ml of propylene glycol only. Observations were made on day 20, 40, 60 and 80 after injection. The tissues were fixed in Bouin's fluid (alcoholic) and sections were stained with hematoxylin and eosin. Routine techniques (Verma, 1989) were followed for hematological studies. Histopathological diagnoses were made with reference to Dawe (1965), Hibiya (1982) and Black (1984).

RESULTS AND DISCUSSION

Aflatoxin B₁ in fish feeds

In general, 44.23% of the feed samples were contaminated with Aspergillus spp. (Verma et al., 1989). Almost 89% of A. flavus strains bore capabilities of elaborating aflatoxin B₁. The concentration of aflatoxin B₁ was relatively high in culture extracts obtained from oil cakes. The oil seeds harbour more toxigenic strains (Moreau, 1974). In the case of oil cakes, the left-out amount of oils also affect the rate of aflatoxin production (Eldridge, 1965). Congenial climatic factors increase the pace of mycobial contamination and subsequent toxic metabolite production (Verma, 1989).
Toxicity assessment

Lack of specific clinical and pathological traits that is pathognomonic for aflatoxin poisoning poses difficulties in following a particular case which may be employed for toxicity assessment in laboratory animals. Since during the present work, a wild strain of fish never employed earlier as a bioassay subject (against aflatoxins) was used, it was planned to conduct the toxicity test on the same fish. A dose of 0.5 ml of 40 mg/l crude culture extract of A. flavus induced neoplastic alterations detectable at the cytological level (Simon et al., 1967) within two months (Verma et al., 1989).

Acute aflatoxicosis

Darkening of body colour and increased mucus over body surface included early clinical manifestations, followed by lack of appetite, listlessness and loss in balance in accordance with Bauer et al. (1969). Nearly-white gill of the treated fish indicated anaemia due to variable range of hemorrhage, primarily on liver and parts of alimentary canal. Spleen and head-kidney revealed gross swelling.

Multicentric origin of tumors on liver, simulating the description laid by Halver (1969) was found on day 80 at 30 mg/l level (Fig. 1). In chronic tests on trout, such tumors were not detected earlier than 3 months (Halver, 1967). Smaller outgrowths (0.5 mm) on intestine and caecum were also marked. To date, no extra-hepatic tumours have been reported in fish exposed to aflatoxins but are common in other laboratory animals.

The general histology of the present fish in control condition was similar to that reported by Hibiya (1982). However, the variations observed have been mentioned specifically in respective cases. The histopathology has been described organwise.

LIVER: A chronological order of the histopathological alterations in toxic liver could be expressed as follows:

Parenchymal vacuolisation - sinusoidal dilatation - periportal/centrilobular necrosis - eosinophilic patches - localised dysplasia - basophilic patches/loss in normal structural plan/toxic liver cirrhosis - bile duct proliferation (minimal trace) - hyperplasia - tumor formation/carcinomatous alterations - trabecular hepatoma

Carcinomatous alterations at cytological level were marked on day 80 at 20-mg/l level in deeply basophilic cells around blood vessels (Fig. 2). The advancing lesion appeared in the form
of basophilic patches, more frequently in cirrhotic liver and developed into a classical form of trabecular hepatoma (Fig. 3). The hepatocytes contained enlarged or multiple nuclei and disturbed the nucleo-cytoplasmic (n/c) ratio. The tumors were composed of hyperplastic hepatocytes (Fig. 4). Invasive erosion of adjacent tissues (Fig. 5, 6) and trace of malignant hepatocytes in the central vein (Fig. 7) suggested local and distant metastasis of the lesion.

Impact of parenchymal cell vacuolisation on neoplastic process and cirrhosis as a necessary precursor of trout hepatoma has been an issue of debate (Halver, 1969). A high incidence of carcinomatous alterations in cirrhotic liver indicated that the condition provides increased opportunity for hepatoma development in C. punctatus. Hepatoma developing from basophilic patches has been described (Halver, 1969), but the present lesion followed a different pattern (William and Rucker, 1967) in which eosinophilic patches appeared as the preceding stage of basophilic patches. Irregular periphery of the hepatoma is indicative of the invasive nature of the lesion (Halver, 1969).

EXCRETORY-KIDNEY: Tubular nephrosis, edema of lymphoid tissue and undefined atrophy characterised acute aflatoxicosis. However, the remaining tissue, mainly lymphoid, was hyperchromatic and flooded with lymphocytes containing pleomorphic, cleaved nuclei with increased nuclearity (Fig. 8). The glomerular capillaries were dilated and epithelial cells showed albuminous degeneration characterised by cloudy swelling. A few cells containing hyperchromatic, displaced nuclei in a signet-ring stage indicated some adenocarcinomatous changes arising from tubular epithelium. The glandular cells of corpuscle of stannius were hypertrophic (Fig. 9).

HEAD-KIDNEY: Head-kidney was diffusely necrosed. The sinusoidal spaces were dilated and foci of hypochromatic cells appeared in lymphoid tissue. The inter-renal cells, apparently, were hyperplastic (Fig. 10), but measurement of n/c ratio indicated the proliferation to be poorly differentiated anaplasia. Siperstein and Luby (1969) reported loss of cholesterol feedback synthesis in rainbow trout exposed to aflatoxins. Such loss eventually leads to overproduction of steroidal

Fig. 1. Tumors on liver-40 mg/l - 80 d; Fig. 2. Transitional stage of carcinomatous alteration around blood vessel in a cirrhotic liver-30 mg/l-80 d (x 100); BV - Blood vessel, HHP - Hyperplastic hepatocytes; Fig. 3. Classical case of trabecular hepatoma-40 mg/l - 80 d (x 267); Fig. 4. Section through a tumour on liver-40 mg/l - 60 d (x 67); Fig. 5. Invasive erosion of hepatopancreatic acinus (x 267); LYMP - Lymphocytes, H. pan. c - Hepatopancreatic cells; Fig. 6. Invasive erosion of blood vessel (x 267); CI - Carcinomatous embolus, BV - Blood vessel; Fig. 7. Malignant hepatocytes within central vein (x 400); Fig. 8. Acute lymphocytic infiltration in excretory-kidney (x 267); Lymp - Lymphocytes; Fig. 9. Hypertrophic cells of corpuscle of Stannius (x 267); Fig.
hormones and subsequent hypertrophy of the inter-renal cells (Pandey et al., 1987). Hyperplasia which may assume a pathological proportion (benign neoplasm) is closely related to hypertrophy and develop concurrently. Increased metabolic and mitotic activities provide base for carcinogenic influences.

ALIMENTARY CANAL: Oesophagus, stomach, intestine and pyloric caeca developed hyperaemia, hemorrhage, mucosal sloughing and partial atrophy in similar fashion. The submucosa of the stomach was severely hyperplastic (Fig. 11, 12). The submucosa of the intestine and caeca were also hyperplastic affecting the basic orientation of cells (Fig. 13). A defined intestinal lesion appeared in the form of a glandular mass of atypical cells replacing the native structures (Fig. 14). Preparation through tumor on caeca revealed a lesion in and around a blood vessel in the submucosa with traces of malignant hepatocytes (Fig. 15). This suggested the metastatic pathway of the hepatic lesion.

SPLEEN AND BLOOD: Spleen revealed no factor other than cellular proliferation which could be the cause of gross swelling. The distinctive appearance of splenic follicles was lost (Fig. 16, 17) and a monotony of lymphocytes containing atypically cleaved nuclei replaced the entire native cells. A total and differential count of leucocytes revealed a marked elevation of lymphocytes (mature and blast). In addition, a concomitant alteration of acute lymphocytic infiltration in different organs suggested the lesion to be a condition simulating lymphocytic leukaemia (Verma and Pandey, 1990). The toxin presumably causes mutation in hemocytoblast cells, a totipotential cell of piscine blood giving rise to all blood cells leading to uncontrolled proliferation.

GILL: The epithelial cells of the secondary lamellae were swollen, oedematous and atrophic at high doses of the toxin. The damaged epithelial cells eventually exfoliated. Consequently, haemorrhage occurred resulting in anaemia. A network of some calcareous deposition between epithelial cells was also marked (Fig. 18).

The contamination of food and feeds by molds is a recurring phenomenon. In India where 26% of the molds are toxigenic, the fungal spoilage of animal feeds deserves special attention.
particularly in geographical regions where prevalent congenial climatic factors are added by recurrent floods. Aflatoxin B₁, a toxic mold metabolite, in addition to its characteristic pathology of hepatoma, also induces some alteration at haemic level simulating lymphocytic leukemia in *C. punctatus*. The toxin is a strong mutagen (Ong, 1975) as well and presumably causes mutation in haemocytoblast cells. The problem demands elaborate investigation.

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