NEOPLASMS OF THE DUCK WITH SPECIAL REFERENCE TO HEPATOCARCINOGENESIS

By

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THESIS

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Introduction

INTRODUCTION

Poultry farming in India has made rapid strides during the last couple of decades. Ducks constitute seven percentage of the total poultry population and contribute five percentage of egg output in the country. The ducks, therefore, enjoy second position after chicken as far as their population and egg production are concerned. The Kerala state, with 8.46 lakh ducks (1987 census) is ranked fifth in the country as far as duck population is concerned.

Duck rearing is an important occupation of farmers in Kerala and diseases are less in ducks when compared to chicken and generally they do not cause concern to the farmers. However, duck farmers in Kerala experienced severe economic loss due to unexpected outbreak of a disease in their flocks in 1976. This disease outbreak almost completely wiped out the duck population in Kerala. The disease problem was diagnosed as Duck plague (Rajan et al. 1981) and now duck farmers of Kerala are very cautious about this disease.

In recent years the change from semi-intensive to an intensive system of management and feeding has also paved the way for the incidence of diseases in ducks and they do cause concern to the farmers. The common disease problems

encountered in ducks are bacterial diseases like Salmonellosis and Pastuerellosis, viral diseases like Duck pox, Duck virus hepatitis and Duck influenza and toxicological disorders like aflatoxicosis.

Mycotoxins are secondary toxic metabolites elaborated by toxigenic fungi which could adversely affect the health and production potential of livestock and poultry, when they are ingested or administered through parenteral route. In recent years, scientists and livestock farmers have become more alert to the harmful effects of mycotoxins as it often cripple the farm economy. In poultry, mycotoxicosis commonly occur as a result of consumption of processed food grains or contaminated feed mixture.

The common mycotoxicoses encountered in ducks are aflatoxicosis, ochratoxicosis, citrinin toxicosis, oosporium toxicosis, fuzariotoxicosis and stachybotryotoxicosis. Among these, aflatoxicosis is the most common type of mycotoxicosis encountered in the country.

Aflatoxins, the toxic metabolites produced by certain species of Aspergillus are often seen contaminating the feed ingredients of poultry. These potent hepatotoxins present in the feed have emerged as one of the important factors responsible for threatening poultry production programmes in

the country. The susceptibility to this toxin varies in different species and the duck is very sensitive to the toxic effects.

The biological effects of aflatoxin depends on the quantity consumed and the duration of exposure. Aflatoxin is primarily a hepatotoxin and varying degree of hepatosis is a consistent lesion in this toxicosis. Besides this, it has immunosuppressive, mutagenic and carcinogenic effects. The latter effect is seen when aflatoxin is consumed in low doses over a long period. Inspite of taking all efforts to provide an aflatoxin free diet to the ducks, most often the diet of ducks contain low doses of this toxin and hepatic tumours are often encountered due to continuous exposure to aflatoxin at low levels. There has not been any systematically planned investigation to study the incidence and nature of aflatoxin induced carcinogenesis in ducks in this country.

Long term administration of peroxisome proliferators, which are non-genotoxic carcinogens, result in the development of preneoplastic and neoplastic lesions in the liver of rats and mice. Unlike genotoxic carcinogens, peroxisome proliferators are non-mutagenic and do not interact with and damage DNA. Peroxisome proliferators owe their biological activity to an induction of disproportionate increase in the activities of peroxisomal enzymes resulting in oxidative

stress leading to carcinogenesis. There has not been any report on the response of the hepatic tissue of the duck to the peroxisome proliferators which are hepatocarcinogens.

The present study, was therefore, undertaken to study the incidence, gross and histopathological features of spontaneous hepatic tumours in ducks and to assess and compare the carcinogenicity of genotoxic hepatocarcinogens like aflatoxins and non-genotoxic carcinogenic peroxisome proliferators like clofibrate employing duck as the experimental model system.

Review of Literature

REVIEW OF LITERATURE

2.1 Spontaneous cases of tumour in ducks

2.1.1 General incidence

Although, there are many reports of tumours occurring spontaneously in chicken, there are relatively few in the duck and very few in the white Pekin duck. The white Pekin duck is an excellent model for the experimental study of chemical carcinogens, aflatoxin and viruses.

The first spontaneously occurring tumour in the duck was reported by Semmer (1889). It was a sarcoma of the skin.

Alezais and Cotte (1908) observed a tumour in the mediastinum of a duck.

Fox (1923) reported a hypernephroma in the adrenal gland of black duck and a papillary adenoma of the kidney in a red-headed duck.

Eber and Malke (1932) recorded only a case of adenocarcinoma in the liver out of 692 ducks examined between 1899 and 1931.

Ratcliffe (1933) noted an adenocarcinoma in the ileum

in a male white Pekin duck and a fibrosarcoma in the pectoral muscle, with metastases in the kidney in a female duck.

Lombardt (1935) reported a 640 g teratoma in the abdominal cavity of a duck.

Worms and Klotz (1934) observed atypical thymoma in a duck.

Campbell (1946) during the period from 1944 to 1946 reported eleven tumours in ducks. They were either hepatocellular or cholangiocellular carcinomas and in one instance the two types occurred in the same duck.

Campbell (1949) studied 76 ducks, 22 of which had liver carcinoma and only one case of bile duct carcinoma was detected.

Trager (1953) observed, in the neck of two ducks, 12 and 14 months old, tumours located on the left side near the thorax and measuring 10 cm in diameter. One was a sarcoma with a metastatic nodule on the inner surface of the sternum, and the other was either an embryoma or a spindle cell sarcoma.

Dougherty (1953) found three fibromas in the oviduct in 4,000 Pekin ducks examined between 1950 and 1953.

Jennings (1957) reported a papilloma in the skin of the neck in a Scaup duck and a cystadenoma in the liver of a female Philippine duck and a cavernous hemangioma in a Coscoroba swan in 1957-1958.

Lombard and Witte (1959) reported a hepatic carcinoma in a male rosy-billed duck, a female red-headed duck, and a female Muscovy duck. They also observed a carcinoma in the esophagus in two ducks and a carcinoma in the testis of a rosy-billed duck.

Ratcliffe (1961) noticed six hepatomas in 427 birds of the family Anatidae.

Asplin and Carnaghan (1961) reported four intrahepatic tumours in ducks, two hepatomas and two cholangiocellular carcinomas.

Beer and Storey (1961) recorded a noncapsulated and ovoid mass, 12 x 7 cm, attached to the ovarian area by a vascular pedicle in a 3½ year old mallard duck. And this was a granulosa cell tumour of the ovary.

Snyder and Ratcliffe (1963) reported two hepatomas and two cholangiocellular carcinomas and two lung tumours in ducks.

Carnaghan (1965) observed hepatoma in three ducks and cholangioma in two drakes. Tumours of both types were seen in one duck.

Rao et al. (1967) reported a histiocytic sarcoma at the base of the neck in a drake.

Rigdon (1967) observed a teratoma of the gonads in 2 of 17 hermaphrodites resulting from the mating of a Muscovy male and white Pekin female.

Christopher et al. (1968) reported four cases of intrahepatic tumours during the period from 1964-1967 in Andhra Pradesh. This included hepatoma (2) and cholangiocellular carcinoma (2).

Rajan et al. (1989) described various hepatic disorders in ducks caused by ingestion of aflatoxin contaminated feed. This included hepatosis (418), hepatoma (19), hepatocellular carcinoma (19) and cholangiocellular carcinoma (10). Tumours in the airsac independently (2) and concomitantly with hepatomas (7) were also recorded.

Sharma and Pandey (1992) recorded six cases of tumours in ducks during the period from 1976 to 1985 in Zambia. They were classified as adenocarcinoma (3) teratoma (1) and bile duct adenoma (1).

2.1.2 Aflatoxin induced tumours in ducks

Yoshida and Kamota (1952) reported 148 hepatomas in 1, 113 ducks. The liver had periportal degeneration and inflammation, bile duct proliferation, regeneration and nodular hyperplasia of liver cells with adenomatous formation. These ducks were fed Brazilian groundnut meal.

Asplin and Carnaghan (1961) were the first to study the toxicity of aflatoxin in ducklings and observed that young ducks were the most susceptible host to the acute effects of aflatoxin. Hepatic tumours developed in 5 of 37 Khaki Campbell when aflatoxin was fed (0.01 per cent) to seven day old ducklings.

Carnaghan (1964) reported hepatomas in eleven ducks fed a ration containing 0.5 per cent Brazilian groundnut meal for 14 months.

Spontaneous hepatomas in ducks induced by aflatoxin were described by several investigators (Newberne et al. 1964; Carnaghan, 1965; Newberne, 1966 and Vles, 1967).

2.1.3 Chemical carcinogen induced tumours in ducks

Papillomas, squamous cell carcinomas, fibromas, hemangiomas, neurofibromas, ganglioneuromas, and Pacinian

corpuscle tumours were encountered by the local application of 3-methyl-cholanthrene (MCA) on the skin (Rigdon, 1952, 53, 54, 55, 56).

Many tumours were recorded in the respiratory tract of the Pekin duck given MCA intratracheally. This included papillomas, hamartomas, fibromas, fibrosarcomas, hemangiomas, hemangioendotheliomas, neurofibromas, ganglioneuromas, carcinomas, adenocarcinomas, squamous cell carcinomas, osteoid tumours and unclassified tumours (Rigdon, 1959, 61, 63, 70).

Hemangiomas and ganglioneuromas developed in the pectoral muscle of white Pekin ducks implanted with MCA (Rigdon, 1952, 55, 156).

2.2 Experimental studies

2.2.1 Aflatoxin induced hepatopathy

Butler (1964) encountered extensive biliary proliferation in the liver with fatty degeneration of the peripheral parenchymal cells in day-old Khaki Campbell ducklings when 15 µg of aflatoxin was given by mouth for 3 days. This proved the possible direct action upon biliary epithelium and it was suggested that aflatoxin may be an alkylating agent.

Armbrecht and Fitzhugh (1964) reported that aflatoxin caused liver damage in birds 1-2 days after oral administration

and the lower limit of liver damage detection after a single dose was 0.1 mg/kg.

Newberne et al. (1964) described the basic pathologic lesions associated with administration of the toxic compounds at various stages of purity. When administered in five daily doses, bile duct hyperplasia and hepatic parenchymal necrosis were noticed. Lesions of similar severity were caused by 15.6 µg aflatoxin G_1 and 50 µg aflatoxin B_2 indicating lower biological potency of the latter compounds. Madhavan and Rao (1966) administered 40 µg to 10 µg aflatoxin per day to ducklings and within 5 days they all died. Apart from the characteristic lesions, a number of them showed the presence of hepatic infarcts with or without arterial occlusion.

Butler (1969) described aflatoxicosis in rats, guinea pigs, ducklings, dogs, monkeys, hamsters, mice and ferrets. Liver was the organ primarily affected and the carcinogenicity of aflatoxin was demonstrated in rats, ducks and in trout.

Roebuck and Wogan (1977) studied the metabolism of (C^{14}) aflatoxin B_1 by 9000 xg supernatant fraction of livers of duck, rat, mouse, monkey and human. Duck, monkey and human livers were most active in total conversion but no consistent pattern of metabolism emerged which could be correlated with species differences in response to aflatoxin B_1 toxicity or carcinogenicity.

Cavalheiro (1981) conducted susceptibility studies in various avian species. The order of sensitivity from greatest to lowest was ducklings, turkey poults, goslings, young pheasants and chicks. The pathological changes in the liver, kidney and bile duct also varied in intensity and severity in the same order as above.

Hetzel et al.(1984) reported the mortality and post-mortem findings from flocks of Alabio, Bali, Tegal and Khaki Campbell ducks kept on a standard diet from one week until 27 months of age. The diets were routinely analysed for aflatoxins and contained 25-50 ppb of aflatoxin B_1 during most of the observation period. Egg production for the ducks was normal during the first laying period, but remained very low after 22 months. A group of about 40 ducks and 12 drakes of each breed was slaughtered at the end of the observation period. Livers from drakes were normal with respect to colour, had smaller lesions and less bile duct hyperplasia and considerably lower frequency of neoplasm than those from ducks. Alabio ducks had a higher frequency of bile duct hyperplasia and neoplastic change in the bile duct while the occurrence of hepatocellular carcinomas in Khaki Campbell ducks were significantly higher than for other breeds.

Balachandran and Ramakrishnan (1987) studied the pathology of aflatoxicosis in broiler chicken by feeding AFB_1

at concentrations of 1 and 3 ppm for a period of 4 weeks. Grossly the liver was the primary organ to be affected and it was enlarged, yellow coloured, mottled, soft and friable. Histologically the liver showed hepatocytomegaly, bile duct hyperplasia and focal necrosis. Regenerative changes of hepatic cells forming ductular patterns surrounded by a thin layer of fibrous tissue were prominent.

Uchida et al. (1988) in an attempt to determine the effect of aflatoxin B_1 intoxication on livers with duck hepatitis B virus (DHBV) infection domestic ducks were administered 0.1 mg of AFB_1 per kg body weight twice a week for a maximum period of 54 weeks employing various experimental designs. The ducks were infected with DHBV by intravenous inoculation of DHBV positive sera within 24 h post hatch. AFB_1 administration induced hepatocellular necrosis and marked biliary cell proliferation of the periportal areas, and finally liver cirrhosis. Long term AFB_1, administration provoked frequent nodular or cirrhotic changes. AFB, administration induced hepatocellular carcinoma in one DHBV positive and in two DHBV negative ducks.

2.2.2 Clofibrate induced hepatopathy

Farber et al. (1977) reported that several hepatocarcinogens induced in an apparently random fashion,

populations of resistant cells that grew in the presence of an environment that inhibited normal hepatocyte proliferation and clofibrate was one among them.

Reddy et al. (1979) observed that nafenopin and WY-14, 643, structurally unrelated to the clinically used drug clofibrate, induced primary liver cell proliferation and hepatocellular carcinomas. Acatalesemic mice were fed WY-14, 643 at a dietary concentration of 0.1 per cent (w/w) for 6 months and then at 0.05 per cent (w/w) until the termination of the experiment at 14.5 months. F 344 rats were fed this compound at 0.1 per cent level in the diet for 16 months. Hepatocellular carcinomas developed in 18 of 18 acatalesemic mice and 15 of 15 F 344 rats that survived chronic WY-14, 643 treatment.

Svoboda et al. (1979) stated that clofibrate, the most commonly used hypolipidemic drug in the US and Europe, since it contained a chlorinated phenoxy moiety can act as a carcinogen. This was fed at a concentration of 0.5 per cent in the diet of 25 male F 344 rats for 72-97 weeks and the animals were inspected for tumours upto a maximum of 129 weeks. Between 72 and 120 weeks there were 10 rats with a total of 16 tumours. These included 4 hepatocellular carcinoma, adenocarcinoma of glandular stomach, papillary carcinoma of urinary bladder, acinar cell carcinoma of the

pancreas, lymphosarcoma involving pancreas, acinar cell adenomas of pancreas, renal carcinoma and sarcomas of the lung and parotid gland.

Reddy and Azarnoff (1980) reported that several drugs including clofibrate caused massive hepatomegaly when administered to rats, mice or hamsters. This hepatomegaly was associated with a marked increase of peroxisomes. Liver tumours were observed in both rats and mice on long term administration.

Warren et al. (1980) demonstrated the carcinogenic activity of several hypolipidemic peroxisome proliferators in rodent species. Later these drugs were examined for their ability to induce damage to cellular DNA and it was proved that hypolipidemic drugs either in the absence or presence of liver microsomes did not interact with and damage cellular DNA.

Gupta et al. (1985) studied the basic mechanism of carcinogenesis of hepatocarcinogenic peroxisome proliferators such as clofibrate and WY-14, 643 by administering these drugs once daily by gavage to groups of 3 male F 344 rats for 3 days and the rats were killed 2 h after the last dose. Failure to detect peroxisome proliferator DNA adducts in hepatocytes under in vivo and in vitro conditions supported the contention

that peroxisome proliferator DNA adduct is not an essential step in the carcinogenesis by this novel class of carcinogens.

Furukawa et al. (1985) compared the biochemical effects in the liver of male rats of prolonged administration of the experimental hepatocarcinogen nafenopin, a hypolipidemic agent and peroxisome proliferator to those of another experimental liver carcinogen, phenobarbital, which acted as a neoplasm promoter.

Reddy et al. (1986) induced peroxisome proliferation in the hepatocytes of rodent and non-rodent species by structurally dissimilar hypolipidemic drugs and certain phthalate ester plasticizers. And this appeared to be a tissue specific response limited largely to the hepatocyte. Chronic administration of these non-DNA damaging and non-mutagenic peroxisome proliferators to rats and mice resulted in the development of hepatocellular carcinoma. Comparative morphometric and biochemical data from rats treated with varying dose levels of ciprofibrate a hypolipidemic drug and di (2-ethyl hexyl) adipate indicated that the hepato-carcinogenic potency of these agents could be correlated with their ability to induce peroxisome proliferation and peroxisomal oxidation.

Eason et al. (1988) investigated the potential of

ciprofibrate to inhibit gastric secretion and its role as a chemical carcinogen in rats.

Rao et al. (1988) studied the critical events in hepatocarcinogenesis by genotoxic carcinogens and non-genotoxic peroxisome proliferators using phenotypic markers such as γ-GGT and glutathione S-transferase P. By immunoblot method GST-P protein was found to be abundant in both primary and transplantable liver tumours induced by genotoxic carcinogens but not in those derived from peroxisome proliferator treatment. The GGT and AFP mRNAs were also not found in all the 18 tumours induced by peroxisome proliferators that were analysed and also in the ciprofibrate-derived transplantable liver tumours.

Diwan et al. (1988) did experimental studies on 6 week old male F 344/N rats using phenobarbital (PB) (500 ppm) or equimolar doses of either 5-ethyl-5-phenyl hydantoin (EPH) or 5, 5 diethyl hydantoin (EEH) in diet for 78 weeks. Animals were sacrificed at either 52 or 78 weeks. PB and EPH significantly enhanced the development of hepatocellular foci and hepatocellular adenomas at 52 week and hepatocellular carcinoma at 78 week.

Yeldandi et al. (1989) observed that preneoplastic and neoplastic lesions induced by peroxisome proliferators in the liver or rats and mice did not express GGT. Hepatic lesions

were induced in F-344 rats by feeding ciprofibrate (0.025 per cent) in diet for 60 or 84 week. These rats were then administered DAF in diet (0.02 per cent) for 5 week and the altered areas, neoplastic nodules and hepatocellular carcinomas were analysed for the expression of GGT. The result suggested that the GGT-negative property of ciprofibrate induced lesion is stable and not modulated by AAF.

2.3 Body weight

Huff et al. (1984) reported that the body weights of broilers were significantly decreased by aflatoxin, ochratoxin A and combination treatments of full term feeding regimen.

Ghosh et al. (1989) observed stunted growth, reduction in feed consumption and weight gain in chicken by feeding 0.3 ppm and 1 ppm of AFB_1 in the feed for 6 week.

2.4 Haematology

Sova et al. (1991) observed heterophilia and lymphopenia in broilers when fed a diet containing 5 per cent zeolite and 2.5 mg AFB_1.

2.5 Serum proteins

Harvey et al. (1989) investigated on the effect of

AFB_1 on serum concentration of total protein and albumin and found that 3 mg of AFB_1/kg of feed caused decrease in serum concentration of albumin and total protein.

Ghosh et al. (1990) reported significantly reduced albumin and globulin values in broilers under experimental aflatoxicosis.

2.6 Enzymology

Ideo et al. (1972) reported an increased level of serum γ-glutamyl transpeptidase in rats treated with 0.5 m/cc 14/kg.

Rosalki (1975) stressed the importance of GGT in liver disease and stated that GGT is the most sensitive of the enzymes in a variety of liver disease.

Kojima and Sakurada (1976) reported a five fold increase in the serum alkaline phosphatase level in mice bearing Ehrlich Ascites tumour.

Jalanko and Ruoslahti (1979) observed increased γ-glutamyl transpeptidase level in induced carcinogenesis but no elevation in spontaneous cases.

Brawn et al. (1987) noticed increased serum γ-glutamyl transpeptidase values when various drugs and chemicals were administered.

Borisova et al. (1987) observed increased serum concentrations of aspartate amino transferase, alanine amino transferase and alkaline phosphatase in broiler chicken in experimental aflatoxicosis.

Picoux et al. (1987) conducted field studies and found that aspartate amino transferase and γ-glutamyl transpeptidase were increased in hepatic diseases.

Balachandran and Ramakrishnan (1988) studied the influence of dietary aflatoxin on certain serum enzyme levels in broiler chicken and found increased aspartate amino transferase and alanine amino transferase levels.

Beer et al. (1989) observed increased serum aspartate amino transferase following a single dose of aflatoxin (2 mg AFB_1/kg body weight/day) in broilers.

2.7 Serum cholesterol

Manning and Wyatt (1984) studied the toxicity of Aspergillus ochraceus contaminated wheat and different chemical forms of ochratoxin A in broiler chicks and the serum analysis of these birds revealed significant decrease in cholesterol.

Huff et al. (1988) observed that hepatotoxicity of dietary ochratoxin A resulted in significantly reduced cholesterol level in broiler chicken.

Harvey et al. (1989) reported that aflatoxin and ochratoxin singly or in combination caused reduction in cholesterol level.

Sreemannarayana et al. (1989) conducted studies on the effect of ochratoxin on various serum components in growing chicks and they recorded decreased cholesterol values.

2.8 Histochemistry

2.8.1 Demonstration of GGT and ALP foci

Kokot et al. (1965) observed that malignant cells in the liver might show pronounced GGT activity.

Rutenburg et al. (1969) described a simultaneous coupling azo dye method for the histochemical demonstration of γ-glutamyl transpeptidase activity using the new substrate γ-glutamyl 4 methoxy - 2 naphthylamide.

Ideo et al. (1971) conducted short term studies and showed that GGT was inducible in rat liver following oral phenobarbitone or ethanol administration.

Kalengayi et al. (1975) found that expression of GGT

was a common finding in liver lesion induced by aflatoxin B_1 a genotoxic carcinogen.

Schade et al. (1977) studied the effect of clofibrate on alkaline phosphatase activity in bone and liver fractions and found that the activity was increased on long term administration.

Hirota and Williams (1979) made continuous efforts to find sensitive and reliable marker for early carcinogen induced altered hepatocellular foci and found that gamma-glutamyl transpeptidase activity can be used to assess the carcinogenesis.

Gerber and Thung (1980) studied the activities of marker enzymes by histochemical technique in 10 hepatocellular carcinomas (HCC), one liver cell adenoma and one cholangio-cellular carcinoma of liver. In nine cases the non-tumorous livers were also examined. All HCCs but not the liver cell adenoma, displayed enzyme patterns that differed from normal. GGTPase activity was markedly increased in eight HCCs and cholangiocellular carcinoma.

Erikson et al. (1983) reported that hepatocyte nodules induced by chemical carcinogen showed a large increase in GGT.

Calderson and Solt (1985) observed GGT positive cells

in pre cancerous lesions and carcinoma of oral, pharyngeal and laryngeal mucosa.

Roomi et al. (1985) studied the biochemical pattern in preneoplastic hepatocyte nodules and found increased activity of GGT and alkaline phosphatase in hepatocyte nodules.

Perera et al. (1986) reported that many hypolipidemic peroxisome proliferators induced liver tumours in rats after long term feeding but they prevented the development of γ -GGT foci, the putative preneoplastic lesions.

Beer et al. (1988) observed intensely positive γ-glutamyl transpeptidase preneoplastic foci in Diethyl-nitrosamine fed rats.

Chen et al. (1989) evaluated GGT localisation in a population of 911 individuals aged 35 or older who lived in an area of China with a high incidence of esophageal squamous cell carcinoma. The presence of GGT positive cells in normal esophageal squamous epithelium proved useful for the identification of suspect population and the presence of dysplastic cells served as a diagnostic marker to detect patients who progressed to the carcinoma stage.

Yeldandi et al. (1989) reported that preneoplastic and neoplastic lesions induced by peroxisome proliferators in

livers of rats and mice did not express GGT. Hepatic lesions were induced in F-344 rats by feeding ciprofibrate (0.025 per cent) in the diet for 60 or 84 week. These rats were then administered AAF in diet (0.02 per cent) for five weeks and the altered areas, neoplastic nodules and hepatocellular carcinomas were analysed for the expression of GGT. Ninety per cent of carcinomas, 90 to 100 per cent of neoplastic nodules and more than 60 per cent altered areas were negative for GGT following AAF treatment. The results of this study suggested that the GGT negative property of ciprofibrate induced lesions is stable and not modulated by AAF.

2.8.2 Demonstration of peroxisomes

Tsukada et al. (1977) studied the inducibility of peroxisomal proliferation in the preneoplastic hepatocytes in rats fed 2-AAF and of mice fed α-BHC. Proliferation of peroxisomes was induced after various lengths of carcinogen feeding in rats and mice, but late hyperplastic nodules showed a marked variation in the number of peroxisomes. From these findings, disturbances in the cellular regulation was suggested to take place in pre-neoplastic stages and it was assumed that peroxisomal proliferation was less inducible, as the cellular de-differentiation advanced towards cancer.

Reddy and Azarnoff (1980) reported marked increase in

peroxisomes and massive hepatomegaly when a hypolipidemic drug clofibrate was administered.

Reddy et al. (1984) investigated whether ciprofibrate can induce hepatic peroxisome proliferation in rats, chicken, pigeons and two species of monkeys. In all five species a marked but variable increase in the peroxisomes was observed.

Dwivedi et al. (1984) while studying the ultra-structural changes reported an increase in the size and number of cytoplasmic peroxisomes in ochratoxicosis in young broiler chicks.

Rao et al. (1986) studied the effect of two hypolipidemic peroxisome proliferators, ciprofibrate and and di [2-ethyl (hexyl)] phthalate (DEHP) on hepatocytes. There was nine fold and five fold increase in the volume density of peroxisomes in ciprofibrate and DEHP - fed rats respectively.

Reddy et al. (1986) used structurally dissimilar hypolipidemic drugs and certain phthalate ester plasticizers to induce peroxisome proliferation in hepatocytes of rodent and non-rodent species. Comparative morphometric and biochemical data revealed that the ability to induce marked peroxisome proliferation correlated with the hepato-carcinogenic potency of these agents.

Litwin et al. (1987) investigated the immunocytochemical localization of peroxisomal enzymes and concluded that it provided a simple and highly promising approach for further elucidation of the pathophysiology of the liver with peroxisomal disorders.

Materials and Methods

MATERIALS AND METHODS

3.1 Incidence of hepatic tumours

Autopsy register maintained at the Centre of Excellence in Pathology was examined during the period from 1989 January to 1991 December and various diseases encountered were classified and pattern of mortality in ducks was assessed.

Post-mortem examination of ducks brought from the University Duck farm and by private owners was conducted, lesions were observed and the tumours encountered were classified based on the histological appearance. Gross description of the tumour was made and representative samples of tissues collected from hepatic tumours observed were subjected to histopathological examination.

Mortality register of the Government Duck farm, Niranam was examined and different types of hepatic disorders of ducks were recorded, tabulated and categorised.

3.2 Experimental studies

Thirty six numbers of white Pekin ducks in the age group of two to three months, procured from the University Duck farm, Mannuthy were used for the study. They were randomly divided into three groups of twelve birds each and were tagged

with number. The ducks were maintained in cages in separate groups and were given duck feed tested and found free of aflatoxin. Water was given ad libitum.

Group I Pure aflatoxin B_1 was obtained from the Sigma Chemical Co., St. Louis, USA. The toxin (50 mg) was dissolved in 5 ml of rectified spirit and from that 0.05 ml was taken and reconstituted to 12 ml with distilled water. The reconstituted solution (1 ml) containing 0.04165 mg of pure toxin was given to each duck per os by oesophageal intubation every third day for 6 months.

Group II Clofibrate (Atromid-s) was obtained from ICI India Ltd., Madras. Contents of six capsules (500 mg each) were dissolved in five ml of rectified spirit and then it was reconstituted to 60 ml with distilled water. The reconstituted solution (1 ml) containing 50 mg of clofibrate was given to each duck per os by oesophageal intubation every day for 6 months.

Group III Rectified spirit (5 ml) was reconstituted to 60 ml with distilled water and one ml of the reconstituted rectified spirit was given to each duck per os by oesophageal intubation every day for 6 months.

3.3 Parameters studied

Body weight, Haemogram (Haemoglobin, ESR), Total Serum Protein, Albumin, Globulin and Serum enzymes [Aspartate amino transferase (AST), Alanine amino transferase (ALT), Gamma glutamyl transpeptidase (GGT) and Alkaline phosphatase (ALP) were estimated. These parameters were studied at fortnightly intervals in the case of group I and III. In group II these parameters were studied at 3 months interval. Serum cholesterol was estimated at 3 months interval in group II.

After an experimental period of three months six ducks from each group were sacrificed by exsanguination and after six months the remaining six ducks were also sacrificed. Gross lesions in the liver were observed and tissue samples were taken in 10 per cent buffered neutral formaldehyde for histopathological study. Fresh liver tissue pieces were also taken for preparing frozen sections for histochemical demonstration of GGT, ALP and peroxisomes.

3.3.1 Body weight

Body weight of the ducks was recorded before the commencement of the experiment and thereafter at fortnightly intervals in the case of group I and III. In group II body weight was recorded at 3 months interval.

3.3.2 Haemogram

Blood (2 ml) was collected in EDTA from the jugular vein of the duck at fortnightly intervals in the case of group I and III and at 3 months interval in case of group II.

Haemoglobin level was estimated at fortnightly invervals by the cyanmethaemoglobin method described by Miale (1967) and the final readings were taken in an Erma photometer.

ESR was estimated by Wintrobe and Landsburg method (1964).

3.3.3 Total serum protein, albumin and globulin

Blood (5 ml) was collected without anticoagulant and serum was separated out.

Total serum protein and albumin were estimated employing commercially available kits (Miles India Ltd, Baroda) in Chemetrics Analyser.

Serum globulin was estimated by finding out the difference between total serum protein and albumin levels.

3.3.4 Serum enzymes

Blood (5 ml) was collected without anticoagulant and serum was separated out.

Serum gamma glutamyl transpeptidase (GGT), serum alanine amino transferase (ALT), serum aspartate amino transferase (AST), serum alkaline phosphatase (ALP) and serum cholesterol level were estimated using commercially available kits (Miles India Ltd., Baroda) in Chemetrics Analyser.

3.3.5 Histopathology

Representative tissue samples of liver were collected in 10 per cent buffered neutral formaline and processed by routine paraffin embedding technique (Armed Forces Institute of Pathology, 1968). Sections were cut at 5 micron thickness and stained by haematoxylin and eosin method of Harris as described by Disbery and Rack (1970). For the demonstration of fat, wherever necessary, frozen sections were stained with Sudan III.

3.3.6 Histochemistry

Cryostat sections were cut at 5 μ thickness and used for histochemical demonstration of GGT, ALP and peroxisomes.

3.3.6.1 Demonstration of GGT in liver

Method described by Rutenburg et al. (1969) was followed.

Solutions

Stock solution: 5 mg of 4-glutamyl-2-methoxy naphthylamide (GMNA) in 0.1 ml of Dimethyl sulfoxide, 0.1 ml of IN NaOH and 1.8 ml of distilled water.

Working solution

GMNA (2.5 mg/ml)	-	2 ml
Tris buffer 0.1 M (pH 7.4)	-	10 ml
Normal saline	-	28 ml
Glycyl glycine	-	20 mg
Fast blue BBN	-	20 mg

Staining procedure

1. Incubated the sections in working solution for 30 minutes at 37°C.
2. Rinsed in normal saline for two minutes.
3. Rinsed in 0.1 M cupric sulphate solution for two minutes.
4. Rinsed in normal saline for two minutes.
5. Rinsed in water.
6. Dehydrated through ascending grades of alcohol.
7. Cleared in xylene and mounted with DPX mountant.

3.3.6.2 Demonstration of ALP in liver

Gomori's Alkaline phosphatase cobalt method was followed for the demonstration of alkaline phosphatase activity in liver (Pearse, 1972).

Solutions

1. Buffered solution (stock)

Sodium barbital	- 6.1 g
Calcium chloride	- 1.2 g
Mag. sulphate	- 0.5 g
Distilled water	- 1000 ml

2. Cobalt solution

Distilled water	- 100 ml
Cobalt nitrate	- 2 g

3. Substrate solution

Sodium glycerophosphate (52%)	- 3.0 g
Distilled water	- 100 ml

4. Sulphide solution

Distilled water	- 40 ml
Yellow ammonium sulphide	- 0.4 ml

Incubating solution

Substrate solution - 1 part

Buffered stock solution - 4 parts

Staining procedure

1. Incubated the sections at 37°C for 60 minutes in the incubating solution.
2. Rinsed in distilled water and transferred to cobalt solution for two minutes.
3. Rinsed in tap water followed by distilled water.
4. Placed in sulphide solution for five minutes.
5. Rinsed in distilled water.
6. Dehydrated through 70 per cent, 95 per cent alcohol and absolute alcohol.
7. Cleared in xylene and mounted with DPX mountant.

3.3.6.3 Demonstration of peroxisomes in liver

An improved Benzidine-Peroxidase reaction described by Van Duijn (1955) was followed.

Solutions

1. Saturated aqueous solution of Benzidine - Dissolved 50 mg

in 200 ml distilled water at 80°C. Cooled to room temperature and filtered.

2. Three per cent hydrogen peroxide.

3. Saturated ammonium chloride - Dissolved 40 g NH_4Cl in 100 ml of hot water and cooled.

4. Five per cent EDTA - Prepared a solution of EDTA and buffered to pH 6.0 with NaOH.

Incubating solution

Added 1 ml saturated ammonium chloride and 1 ml EDTA solution to 9 ml benzidine and added one drop of H_2O_2.

Procedure

1. Incubated the sections for 5-10 minutes in incubating solution.

2. Rinsed briefly.

3. Mounted in PVA fructose medium.

Results

RESULTS

4.1 Spontaneous cases

4.1.1 Incidence

Six hundred and eighteen ducks were brought to the Centre of Excellence in Pathology for conducting post-mortem examination during the period from 1989 January to 1991 December. After conducting detailed autopsy, the diseases encountered were classified. The incidence of hepatic disorders (hepatosis, hepatitis and hepatic tumours) was the highest (44.98 per cent) followed by enteritis (13.92 per cent) and pulmonary congestion and oedema (8.89 per cent) (Table 1). Among the various hepatic disorders, hepatosis constituted 48.92 per cent, hepatitis 45.32 per cent and hepatic tumours 5.76 per cent. Hepatic tumours included both hepatocellular carcinoma (71.42 per cent) and cholangio-cellular carcinoma (28.57 per cent). A case each of airsac tumour and skeletal tumour were also encountered.

At the Government duck farm, Niranam, 14,360 ducks died during the period from 1989 January to 1991 December and out of this 6,737 ducks (46.92 per cent) had hepatic lesions (Table 2). There was no record of a tumour.

Table 1. Incidence of diseases of ducks encountered at the Centre of Excellence in Pathology during the period from 1989-1991

Disease	Year		
	1989	1990	1991
Omphalitis	7	21	2
Pul. congestion	12	27	16
Air saculitis	5	22	12
Hepatitis	27	54	45
Hepatosis	14	29	93
Enteritis	21	39	26
Hepatoma	11	5	--
Aspergillosis	2	9	32
Duck plague	7	10	19
Coccidiosis	--	1	--
Peritonitis	--	--	8
Gout	1	--	--
Mareks disease	1	--	--
Cystadenocarcinoma	1	--	--
Airsac tumour	1	--	--
Crop impaction	--	--	4
Mutilated	5	7	22
Total	115	224	279

Table 2. Incidence of hepatic disorders of ducks at the Government Duck Farm, Niranam during the period from 1989-1991

	1989	1990	1991
Hepatitis	2265	2098	2374
Others	2591	2474	2558
Total	4856	4572	4932

4.1.2 Hepatic tumours

4.1.2.1 Gross lesions

Liver of ducks with tumour was examined in detail. The liver parenchyma was pale and friable and had tumours of varying size. Out of the fourteen birds examined, twelve birds had massive multiple nodules varying in size from 2 to 6 cm on the left lobe and few small sized (1-3 cm) solitary nodules on the right lobe. The contour of the liver was irregular and it filled up the abdominal cavity. Liver tissue not involved in the growth was greenish yellow and moderately firm (Fig.1).

In two of the ducks four to six small sized greyish white nodules of 3-4 cm diameter were seen on the left lobe. Right lobe also had small greyish white nodules varying in size and number.

In one of the ducks, in addition to the liver involvement, white nodular thickening of 2-3 cm size was seen on the airsac. Since the tissue had undergone advanced putrefaction histopathological studies could not be undertaken.

In another duck, besides the growth in the liver, a large encapsulated soft mass of 2 cm diameter was seen on the dorsal aspect of the posterior half of the head. The growth

Fig.1 Duck - Spontaneous case of hepatic tumour - Multiple tumour growth

involved the bone at this region and projected about 2-3 cm on the external surface. The cut surface was gritty and fleshy and contained white specks and streaks.

4.1.2.2 Histopathological studies

4.1.2.2.1 General features

In the ducks which had growths in the liver there were multiple well defined circumscribed nodules of hepatocytes encircled and compressed by moderately thick bands of fibro-collagenous tissue. These hepatic nodules contained hepatocytes showing fatty change and necrosis. Some of the nodules contained hepatomegalocytes with foamy cytoplasm and hypertrophic nucleus which was hyperchromatic. There was biliary hyperplasia of moderate degree and stagnation of bile in the biliary canaliculi. Biliary canaliculi were seen scattered as well as grouped particularly in the portal tracts. They were lined with hyperchromatic cuboidal epithelial cells. Some of the bile ducts were cystic. There was also focal or diffuse infiltration of lymphocytes. The histological picture resembled multinodular cirrhosis.

The histological changes of the liver of ducks which had tumours consisted of degeneration, necrosis and atrophy in focal areas. Diffuse fatty changes were present in the periportal areas. Bile duct proliferation was a consistent

feature in all the cases. Hepatocytomegaly with infiltration of few mononuclears were seen in some areas. The tumours were classified histologically as hepatocellular carcinoma (10) cholangiocellular carcinoma (4) and osteoma (1).

4.1.2.2.2 Hepatocellular carcinoma

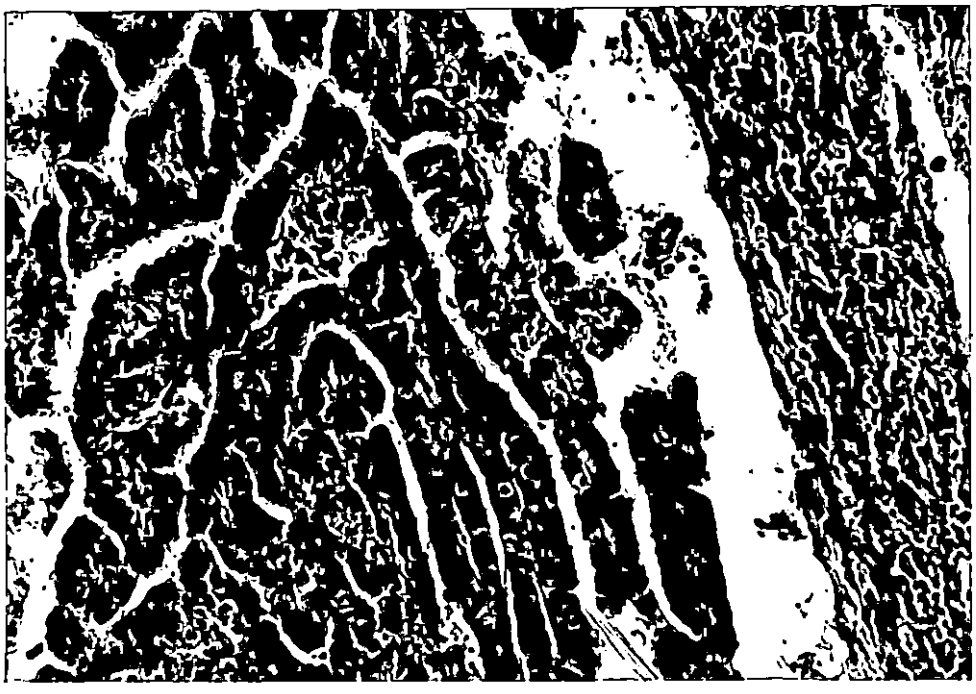
Proliferating sheets of immature hepatocytes arranged in an atypical pattern were evident. These cells were larger than the normal hepatocytes and the normal polarisation of the hepatic cords of cells was absent. These neoplastic tissue lacked a central vein or the portal structures. The hepatocytes had a central dark staining nucleus and in some of the cells the nucleus was eccentric and the cell had an oval apperance. The cell borders were indistinct. The nucleus was large hyperchromatic and nucleoli were clumped. A few of the hepatocytes were in different stages of mitosis. Some of the hepatocytes contained multiple nuclei. Groups of neoplastic cells encapsulated with a moderately thick band of fibrous tissue capsule were seen in some cases (Fig.2). In few cases proliferating biliary epithelial cells were also seen.

4.1.2.2.3 Cholangiocellular carcinoma

Large sheets of hyperchromatic low columnar and low cuboidal cells forming many acini were seen. The acini were of varying size and shape. The cells lining the acini had

Fig.2 Duck liver - Spontaneous case of Hepatocellular carcinoma - Neoplastic cells encircled by thick band of fibrous tissue capsule - H&E x 250

Fig.3 Duck liver - Spontaneous case of Cholangiocellular carcinoma - Neoplastic biliary epithelial cells seen in papillary projection - H&E x 250



enlarged nucleus with clumping of chromatin and distinct nucleoli. Besides this, there were sheets of undifferentiated hyperchromatic oval, polygonal or spheroidal cells encircled by thin bands of fibro-collagenous tissue. Numberous cells in mitotic division were also seen. In some areas these proliferating cells were thrown into papillary projections (Fig.3). Numerous proliferating ducts lined by low columnar cells were also seen.

4.1.2.2.4 Osteoma

Oval and spindle shaped cells with large hyperchromatic nucleus were seen arranged as bands. In some areas the elongated cells formed interlacing patterns. A fibrillar eosinophilic matrix showing scattered osteoid deposition and bone spicules could be observed. The elongated and polyhedral cells invaded the bony matrix and the periosteum in several locations. Moderate amount of fibrovascular connective tissue interspersed the tumour tissue. Pleomorphic cells with vesicular nuclei and multinucleated cells of irregular shape and size undergoing mitotic division were also present.

4.2 Experimental studies

Group I - Aflatoxin B_1 (0.04165 mg/kg body weight)

Group II - Clofibrate (0.05 g/kg body weight)

Group III - Control

4.2.1 Body weight

The data on the weight of ducks are shown in Fig.4. Body weight of ducks in group I and II revealed progressive reduction from three months onwards while the ducks in group III, showed gradual but appreciable weight gain.

4.2.2 Haematology

The haemogram of control and experimental ducks is presented in Fig.5 and 6. Haemoglobin concentration of ducks in both groups I and II showed reduction at three months and six months when compared to the control.

Erythrocyte sedimentation rate of ducks in group I rose to a maximum of 4 mm/h and in group II it was 3 mm/h. In the control ducks, ESR varied between 2-3 mm/h throughout the experimental period.

4.2.3 Total serum protein, albumin and globulin

Total serum protein, albumin and globulin level of ducks in groups I and II revealed a gradually increasing trend from the commencement of the experiment upto the third month and thereafter it started declining. By the end of the experimental period ducks in both group I and II had a low

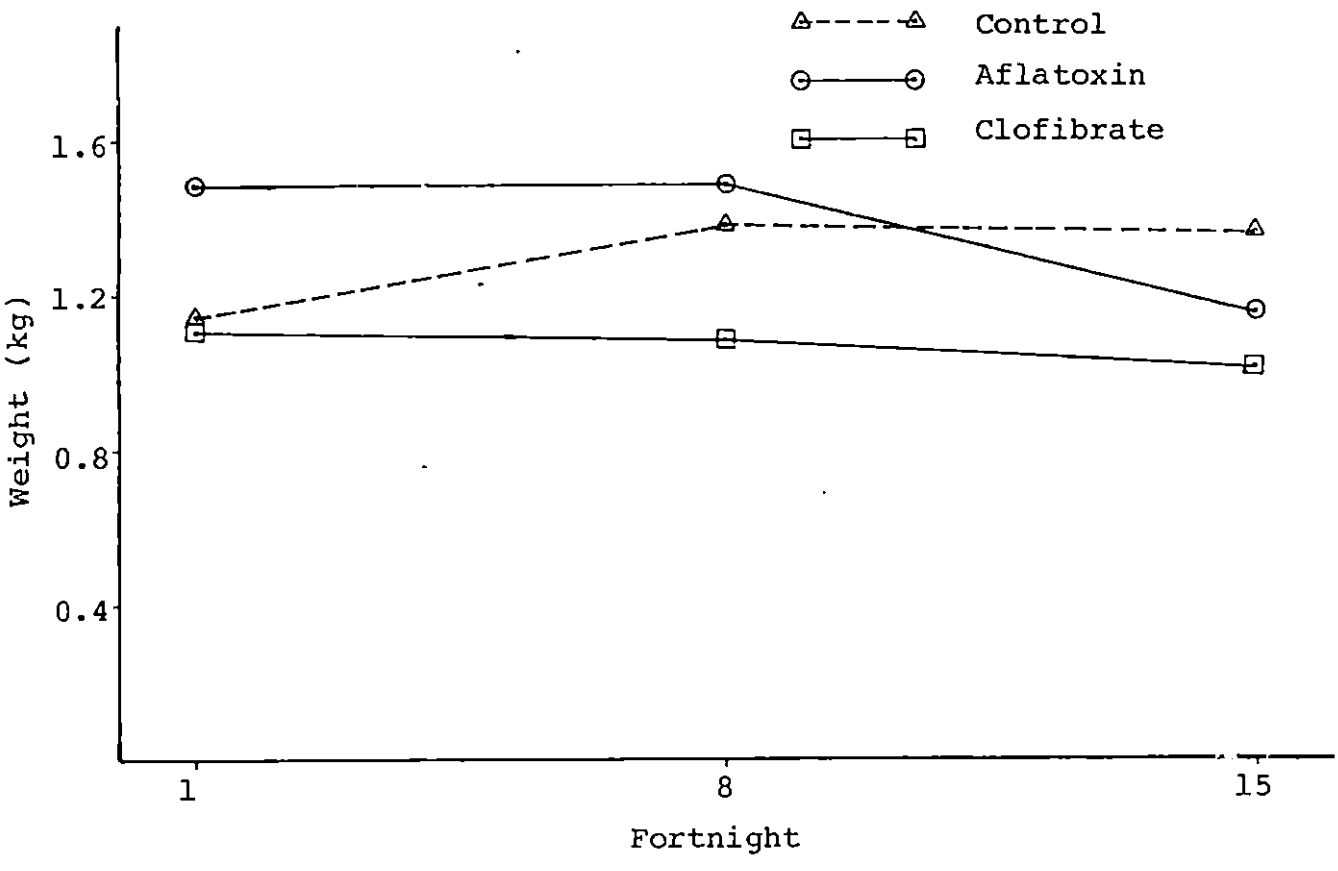


Fig.4 Body weight of ducks treated with Aflatoxin B_1 and Clofibrate

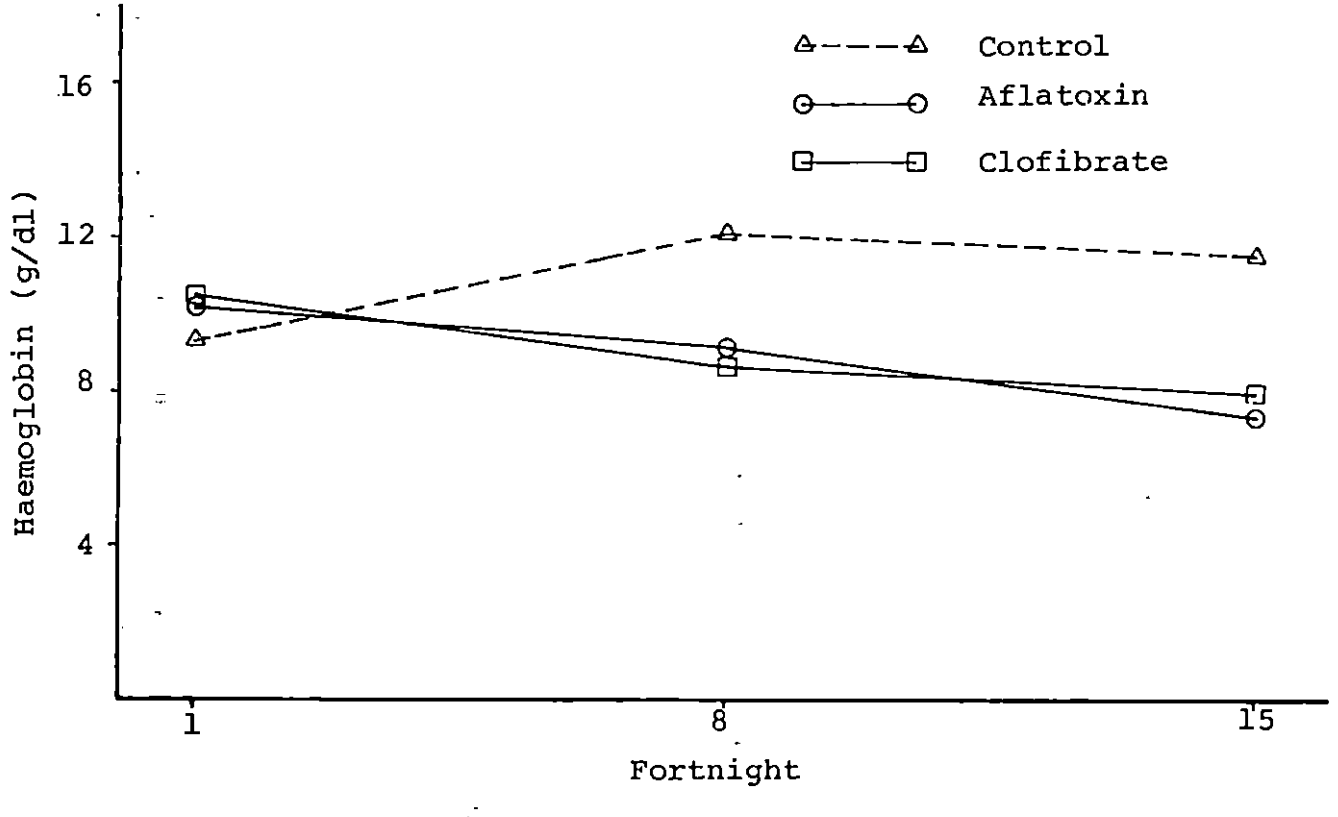


Fig.5 Haemoglobin level in ducks treated with Aflatoxin B_1 and Clofibrate

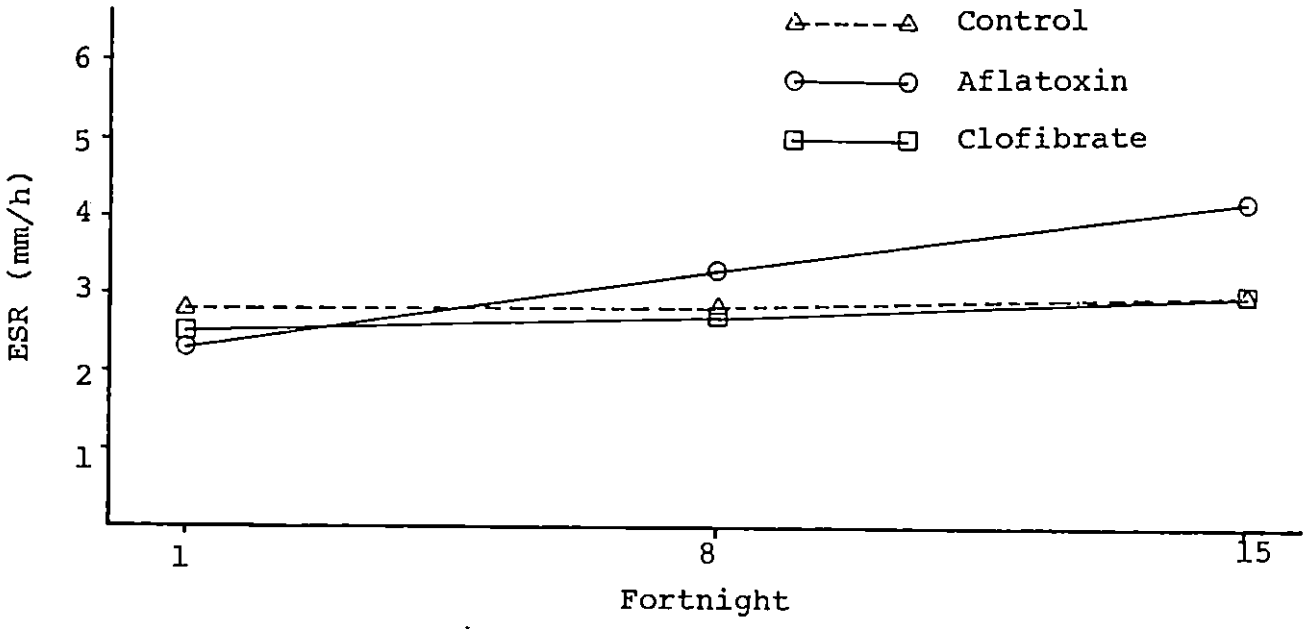


Fig.6 Erythrocyte sedimentation rate in ducks treated with Aflatoxin B_1 and Clofibrate

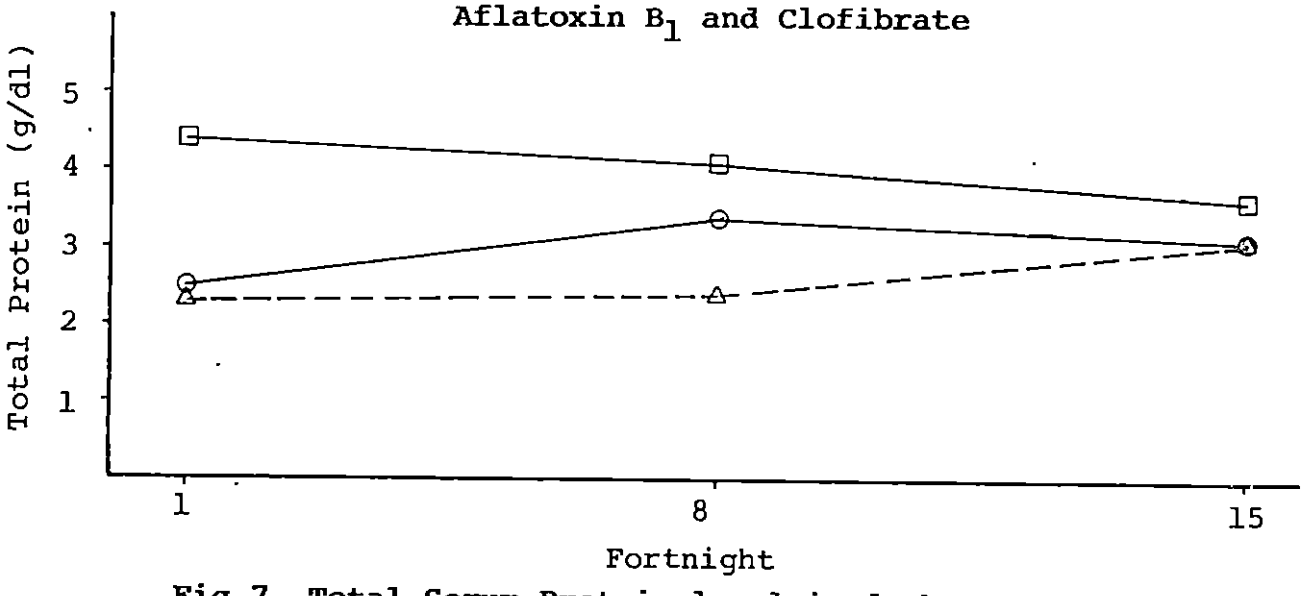


Fig.7 Total Serum Protein level in ducks treated with Aflatoxin B_1 and Clofibrate

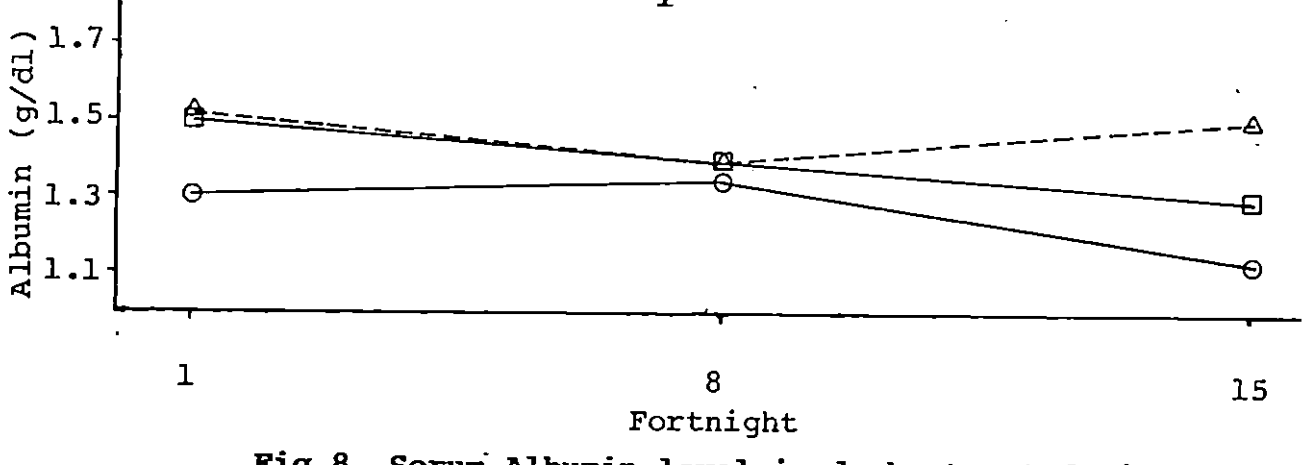


Fig.8 Serum Albumin level in ducks treated with Aflatoxin B_1 and Clofibrate

protein, albumin and globulin level when compared to the ducks in the control groups.

Ducks in group I had an initial serum protein level of 2.517 and as the experiment progressed protein level also increased gradually to 4.221 by the VIth fortnight. After this period it started declining and the mean protein level at the end of the experimental period was 3.518. In group II the mean protein level was 4.423 and it came down to 4.091 and 3.637 by the VIIIth and XVth fortnights respectively (Fig.7).

Mean albumin concentration of ducks in group I was 1.292 which later came down gradually from the VIIth fortnight to 1.133 by the XVth fortnight whereas in group II the albumin concentration came down from an initial value of 1.503 to 1.304 by the sixth month(Fig.8).

Mean globulin level in group I before the commencement of the experiment was 1.225. After a period of 6 months it came down to 2.033. In group II also the level of globulin came down from 2.920 to 2.334 by the sixth month (Fig.9).

Ducks in the control group did not reveal any change in serum protein, albumin and globulin levels.

4.2.4 Enzymology

The data on serum ALT, AST, ALP and GGT are presented

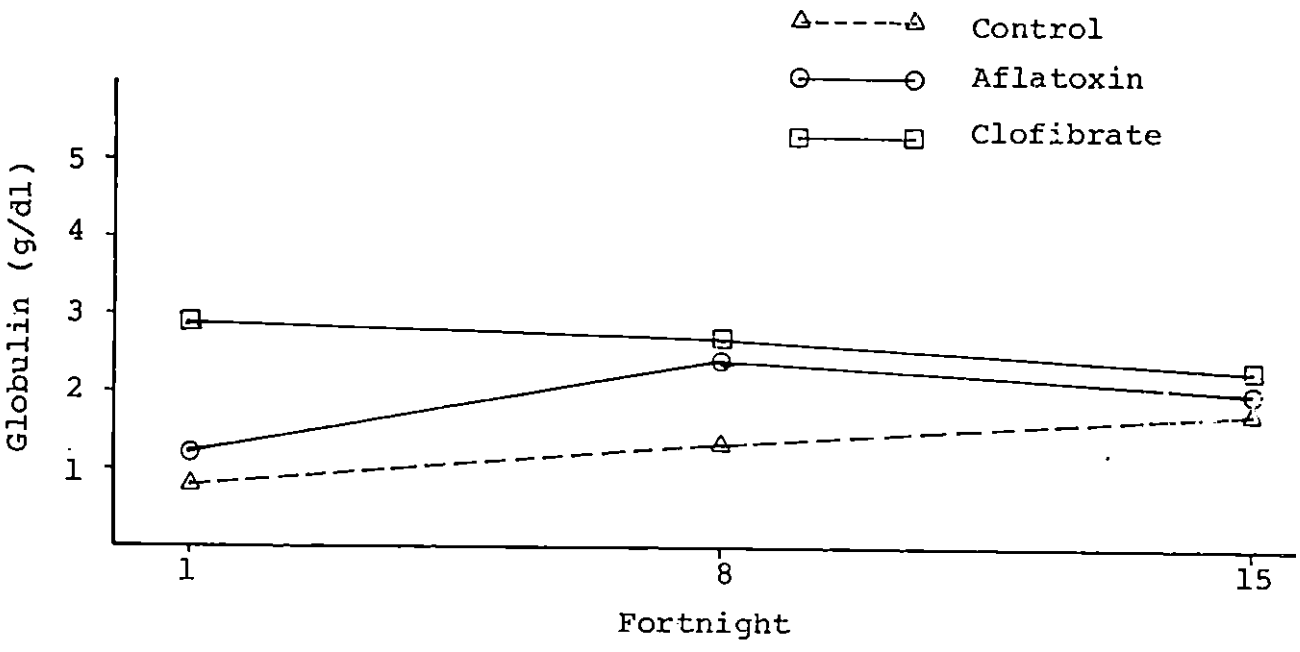


Fig.9 Serum Globulin level in ducks treated with Aflatoxin B_1 and Clofibrate

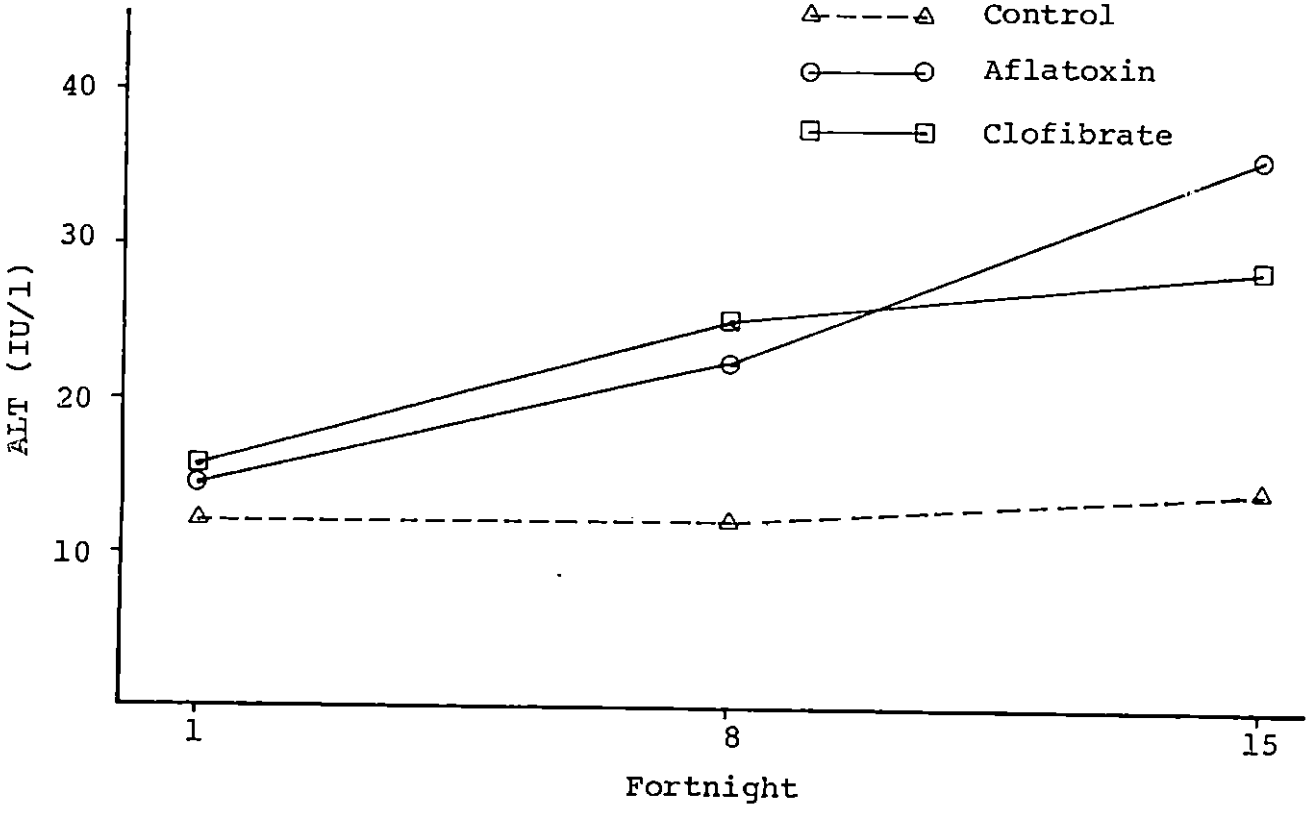


Fig.10 Serum Alanine Amino Transferase in ducks treated with Aflatoxin B_1 and Clofibrate

in (Fig.10, 11, 12 and 13). There was statistically significant increase in the level of AST from the VIIIth fortnight onwards to a maximum of 48.50 IU/l in group I and 36 IU/l in group II on the XVth fortnight when compared to the controls.

Serum ALT level showed significant increase from the VIIth fortnight onwards to 35.83 IU/l in group I and 28.5 IU/l in group II on the XVth fortnight.

Serum ALP level was increased significantly to 109.56 IU/l in group I and 76.08 IU/l in group II on the XVth fortnight.

Mean serum GGT level gradually increased from 2.16 IU/l on the 1st fortnight to 19 IU/l on the XVth fortnight in group I and from 2.5 IU/l to 6.5 IU/l in the group II.

4.2.5 Serum cholesterol

Data on the serum cholesterol level are shown in (Fig.14). Mean serum cholesterol level of 212.92 m.eq/dl at the 1st fortnight came down to 151.83 m.eg/dl on the XVth fortnight in group II.

4.2.6 Gross pathology

On the 90th day, six ducks from each group were sacrificed and gross lesions were examined in detail.

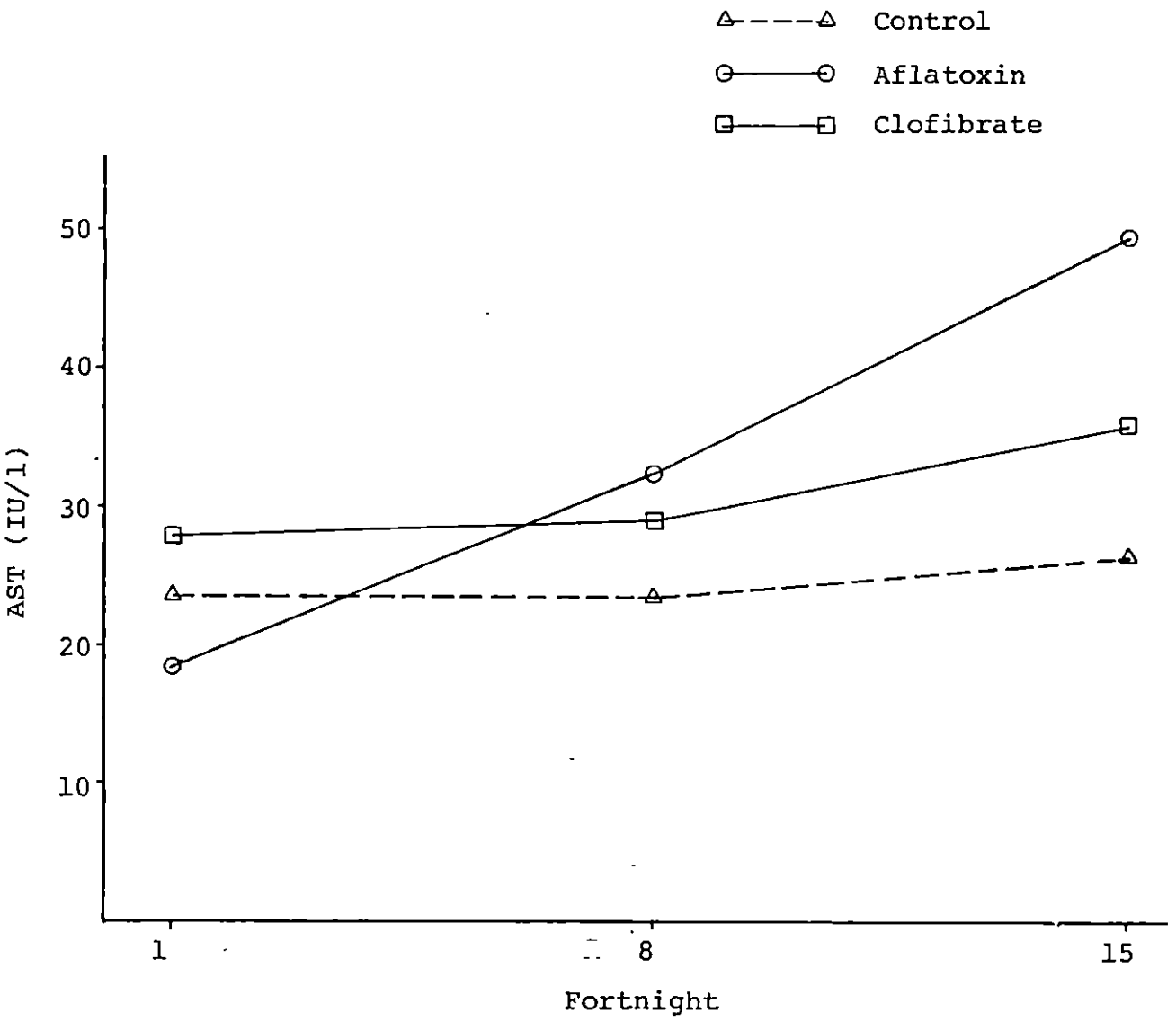


Fig.11 Serum Aspartate Amino Transferase level in ducks treated with Aflatoxin B_1 and Clofibrate

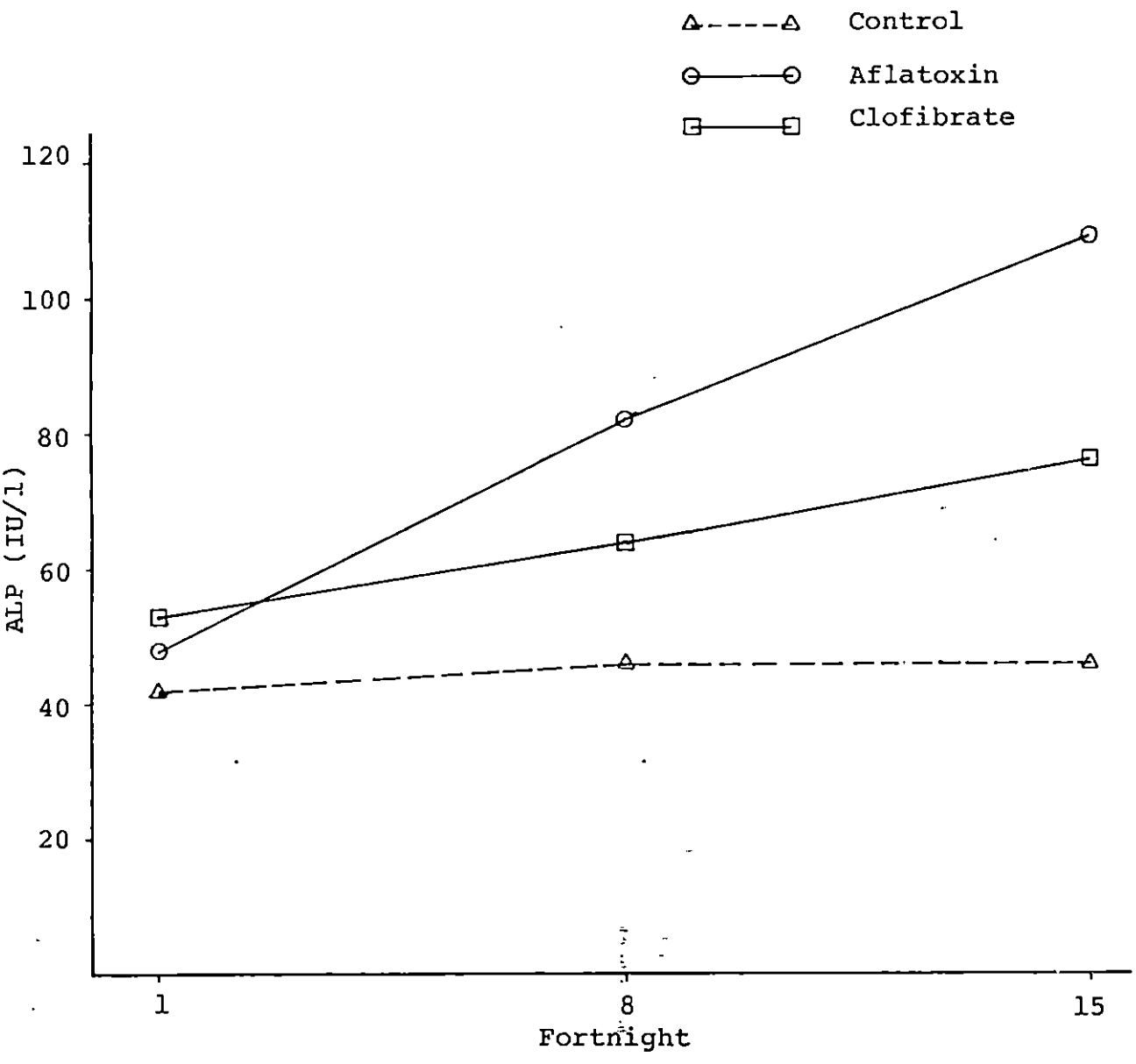


Fig.12 Serum Alkaline Phosphatase level in ducks treated with Aflatoxin B_1 and Clofibrate

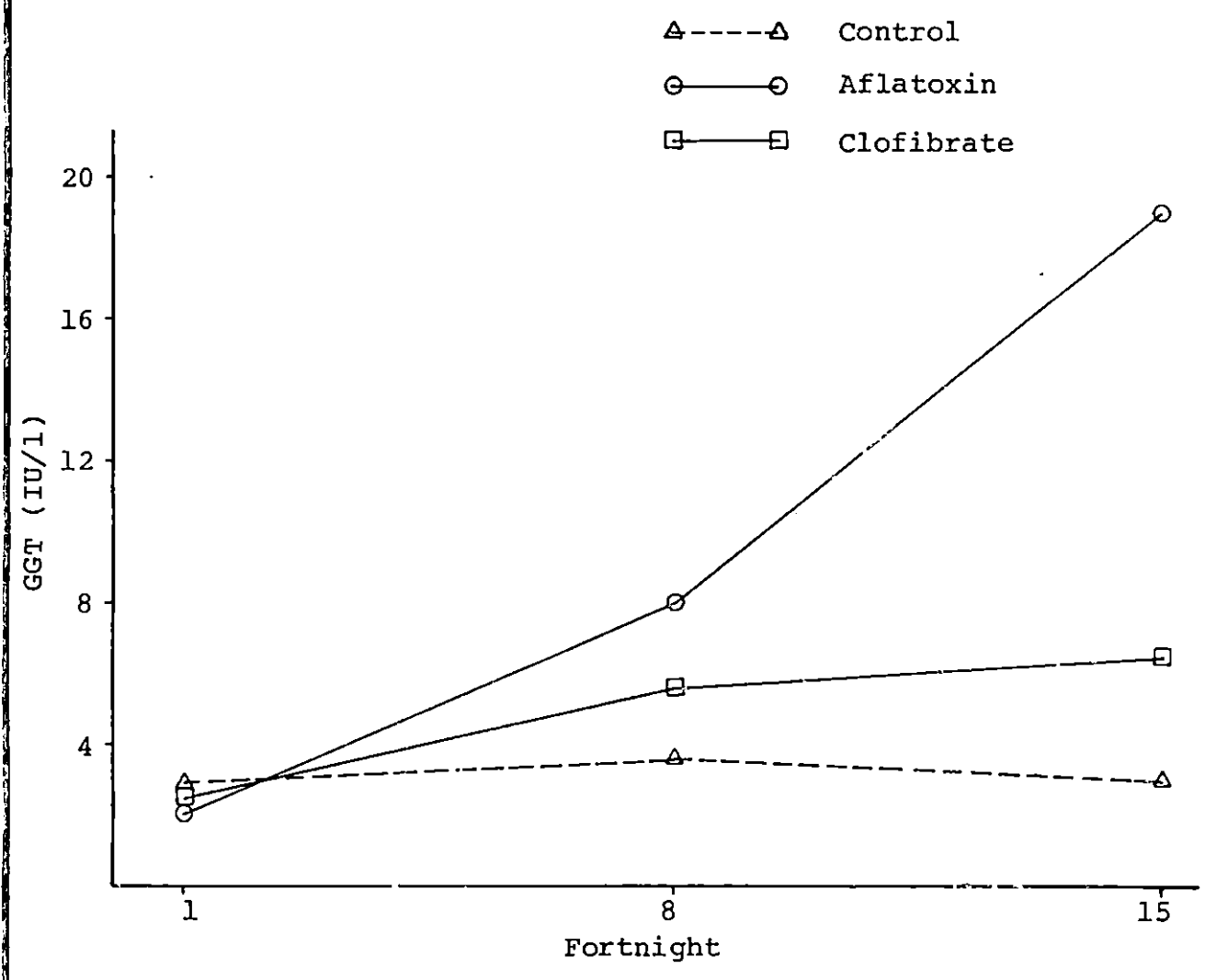


Fig.13 Serum Gamma-Glutamyl Transpeptidase level in ducks treated with Aflatoxin B_1 and Clofibrate

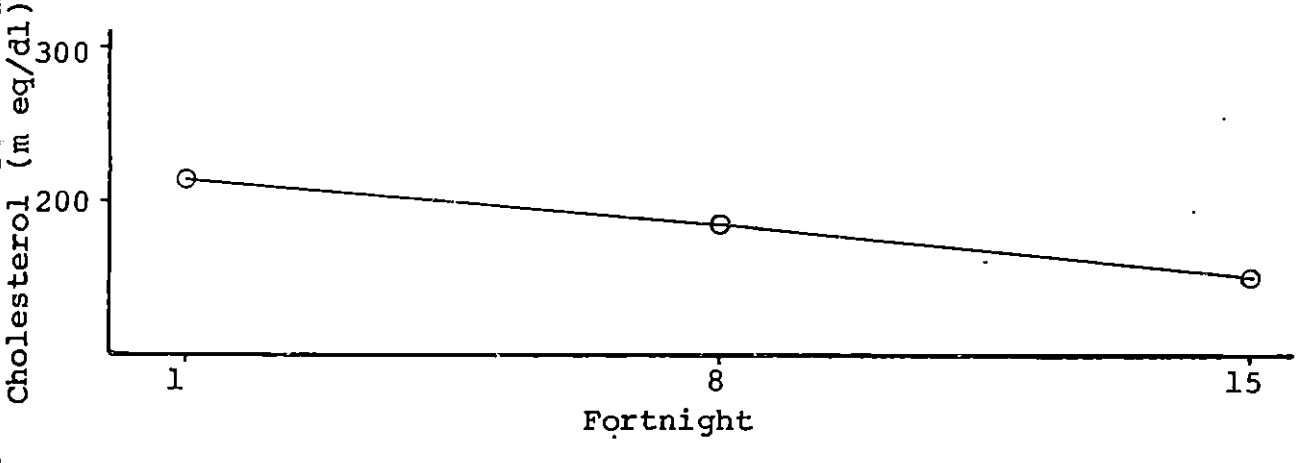


Fig.14 Serum Cholesterol level in ducks treated with Clofibrate

Group I

In all the ducks the liver was moderately and diffusely enlarged and firm in consistency. In two of the ducks the liver was moderately enlarged, congested and granular in appearance. In four ducks the liver showed scattered raised greyish white solitary well defined nodules having 1-2 cm diameter and was hard in consistency. Histologically it was classified as hepatocellular carcinoma (3) and cholangiocellular carcinoma.

Group II

The liver was moderately enlarged with greyish white foci scattered on the surface.

On the 180th day the remaining six ducks from the above groups were also sacrificed.

Group I

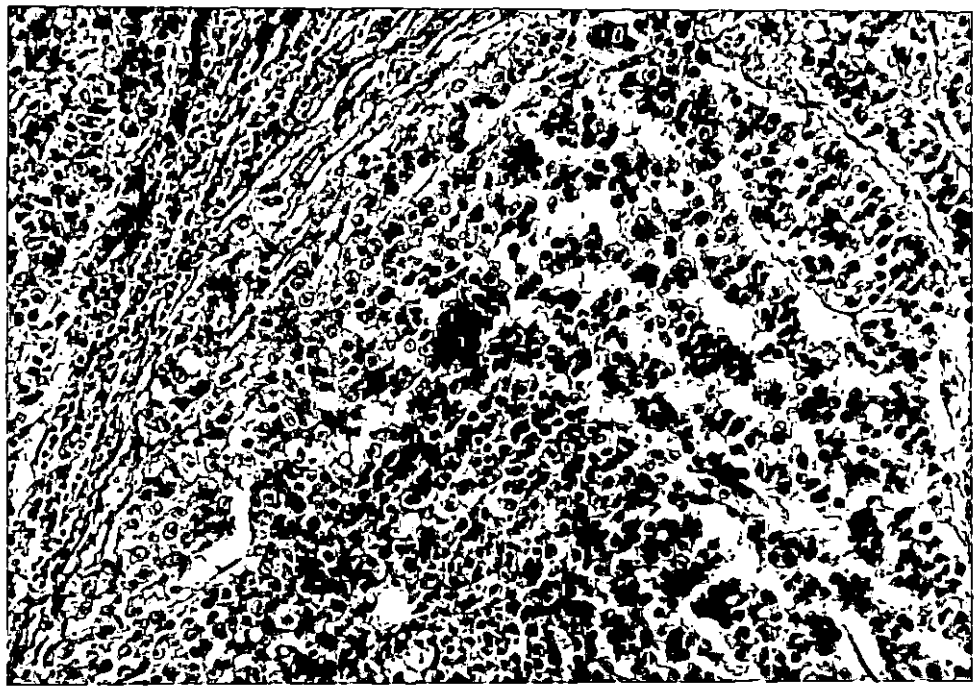
The liver of all the birds was very much enlarged and had rounded borders and almost filled the abdominal cavity. Greyish white nodules of 3-4 mm in diameter were seen bulging out on the parenchyma (Fig.15). The tissue around these nodules was highly congested and moderately firm in consistency. Microscopically they were categorised into hepatocellular carcinoma (3) and cholangiocellular carcinoma (3).

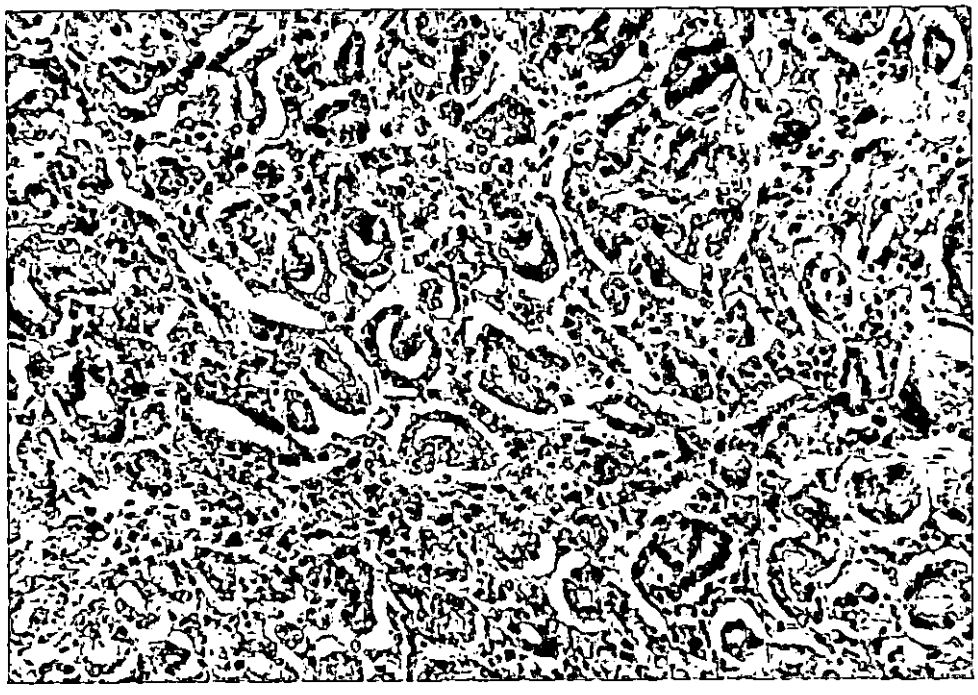
Fig.15 Hepatocellular carcinoma - Duck - Dosed with Aflatoxin for 180 days

Fig.16 Liver of ducks treated with Clofibrate - 180 days - Focal greyish white patches

Fig.17 Hepatocellular carcinoma - Aflatoxin treated duck - Sheets of neoplastic cells - H&E x 250

Fig.18 Cholangiocellular carcinoma - Aflatoxin treated duck - Neoplastic biliary epithelial cells forming acinar pattern - H&E x 250





Group II

The liver was not enlarged and it had a cooked appearance and a few focal greyish white patches of 0.5-1 cm diameter were seen on the surface (Fig.16).

Group III

The liver of ducks in this group did not reveal any gross abnormality.

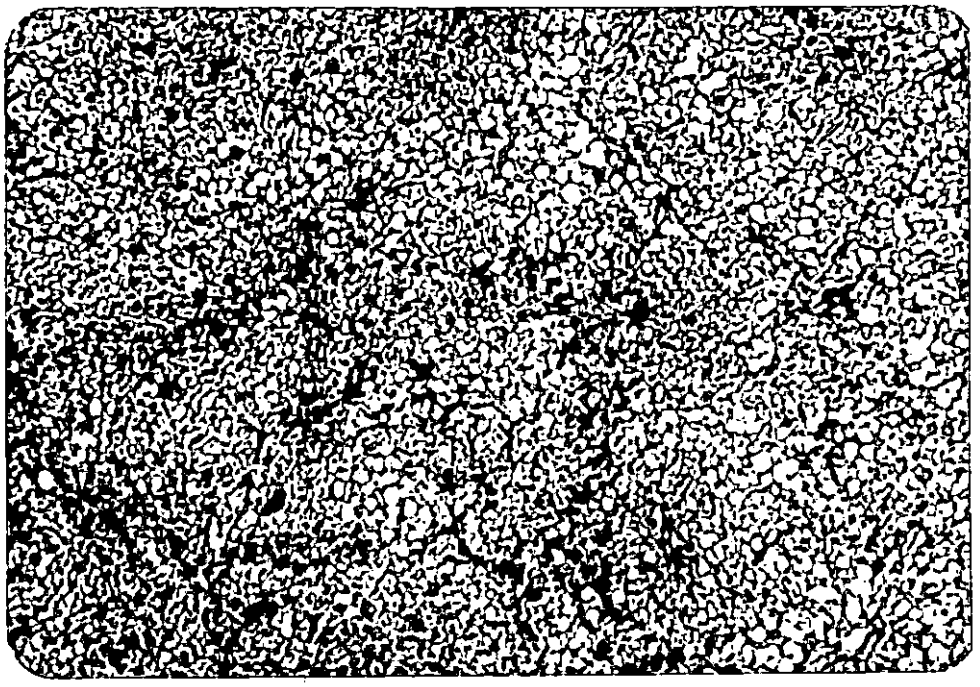
4.2.7 Histopathology

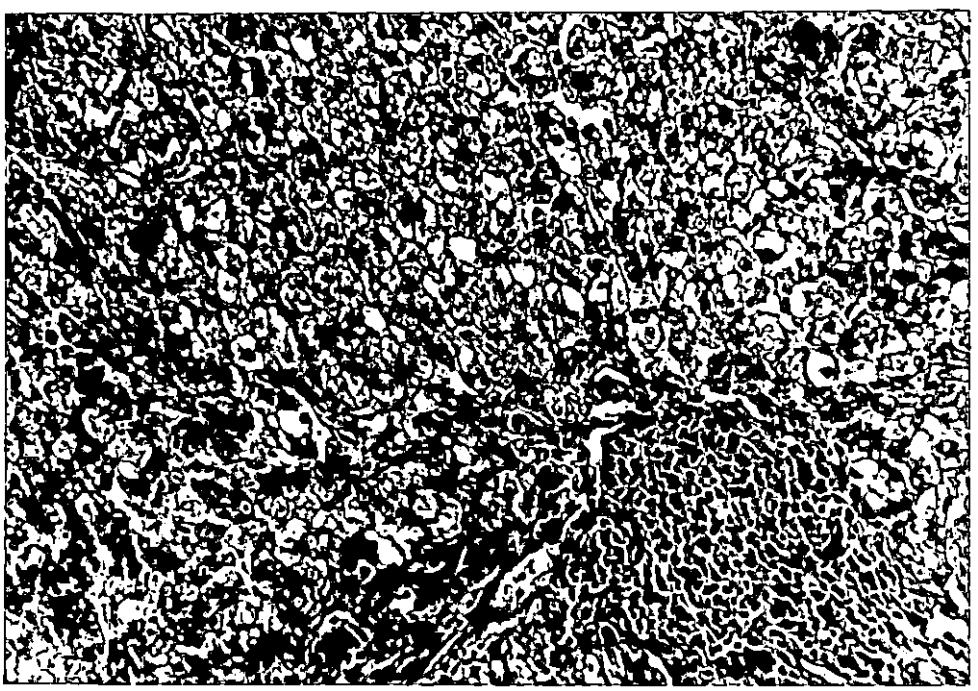
4.2.7.1 Hepatocellular carcinoma

Six ducks revealed sheets of proliferating hepatic cells without a central vein. The neoplastic cells were oval, polygonal or irregular in shape. Hepatocytomegaly and hepatokaryomegaly were consistent features. They had hypertrophic hyperchromatic nucleus and basophilic cytoplasm. The cellular borders were indistinct. Some of the cells were in different stages of mitosis. The cytoplasm of some of the cells were granular and vacuolated (Fig.17). Bile ducts did not have definite shape or pattern of arrangement and the basement membrane was lined by tall columnar to polygonal cells. Bands of fibrous tissue were seen dividing the hepatic cell cords into small lobules. This was a consistent feature. Blood vessels and sinusoids were engorged and some of them contained neoplastic cells.

Fig.19 Chronic hepatitis - Aflatoxin treated duck - Fatty change and bile duct proliferation - H&E x 250

Fig.20 Liver - Clofibrate treated duck - Fatty change - Proliferating biliary epithelial cells - H&E x 250





4.2.7.2 Cholangiocellular carcinoma

In four ducks multiple acini of varying sizes and shapes were seen. The acini were lined with columnar to low cuboidal cells. These cells had large nuclei, vacuolated cytoplasm, distinct nucleoli and clumping of chromatin. Some of the acini had larger cells and almost filled the lumen (Fig.18). In certain areas there was no acini formation but cords of columnar or cuboidal cells were seen borne on the basement membrane. In some areas these low cuboidal cells lining the lumen of the acini were thrown into small papillary projections with supporting fibrous stroma in between. Sinusoids were engorged and in some areas it contained neoplastic cells.

4.2.7.3 Chronic hepatitis

Granular livers showed varying degree of fatty change. The hepatocytes were large and had foamy cytoplasm. Large number of fat vacuoles, single and multiple, were seen in the cytoplasm of hepatocytes and there was displacement of the nucleus towards the periphery. The vacuoles stained orange red with Sudan III (Fig.19). Various stages of degeneration such as pyknosis, karyorrhexis and karyolysis were seen in many of the hepatocytes. Granularity of the cytoplasm was evident in some regions. Paracentral and centrilobular necrosis were

evident. There was marked proliferation of the bile ducts and ductules. Focal fibrosis was also observed in some of the cases, wherein groups of necrosed hepatocytes were seen encircled by fibrous tissue bands.

Group II

There was moderate to severe degree of fatty change with focal disassociated rounded up hepatocytes at the portal areas. Biliary epithelial hyperplasia was of exaggerated type and groups of proliferating bile duct epithelial cells were seen in some areas. Periportal hepatocytes revealed extensive necrosis. Focal areas revealed fibrosis of moderate degree. (Fig.20).

4.2.8 Histochemistry

On the 90th and 180th day cryostat sections of fresh liver pieces from the three groups of birds were taken and histochemically GGT, ALP and peroxisomes were demonstrated.

4.2.8.1 Histochemical demonstration of GGT

Group I

On the 90th day when the sections of liver were examined for the activity of GGT, moderate intense positive reaction was seen. The reaction was very much intense and

severe on 180th day. The activity was mainly concentrated in the cytoplasm of proliferating bile duct epithelial cells (Fig.21).

Group II

On the 90th day and 180th day when sections were examined there was mild GGT activity.

Group III

The liver sections did not reveal any GGT activity.

4.2.3.2 Histochemical demonstration of ALP

Group I

The sections of liver showed moderate positive reaction for the activity of alkaline phosphatase by 90 days (Fig.22) and it became more intense by 180 days (Fig.23). Only the bile duct epithelial cells revealed the activity.

Group II

On the 90th day and 180th day when sections of liver were examined there was moderate activity of ALP. However, degree of reaction was more intense on the 180th day. When compared to the other group, the activity was less in this case (Fig.24, 25).

Fig.21 Liver - Gamma-Glutamyl Transpeptidase activity - Aflatoxin treated duck - GGTPase x 250

Fig.22 Liver - Alkaline Phosphatase activity biliary epithelium - Aflatoxin treated duck - 90 days - ALPase x 250

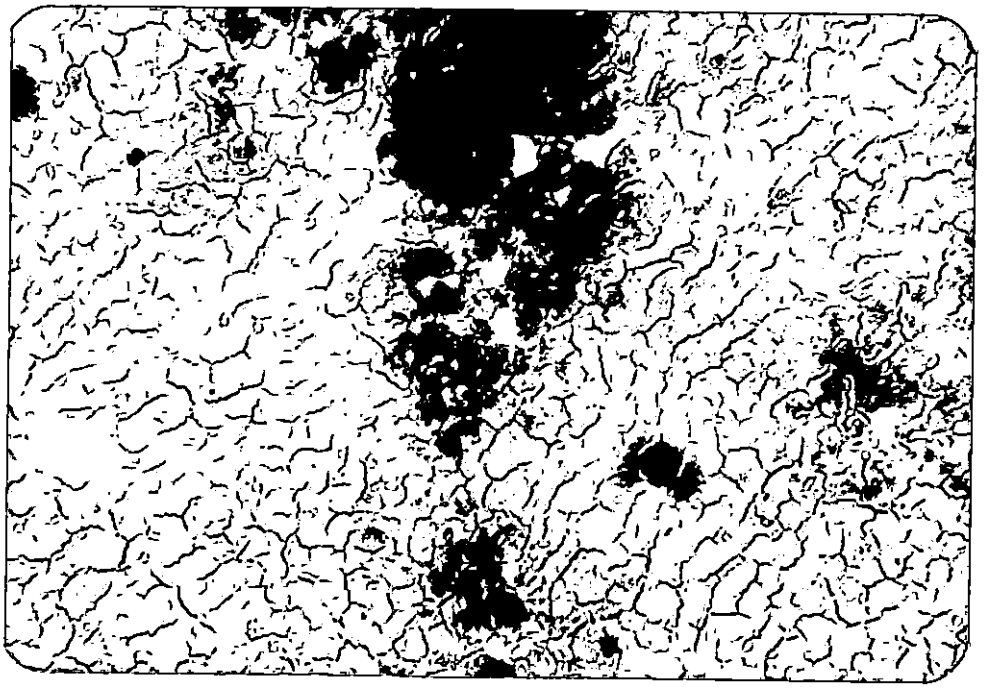
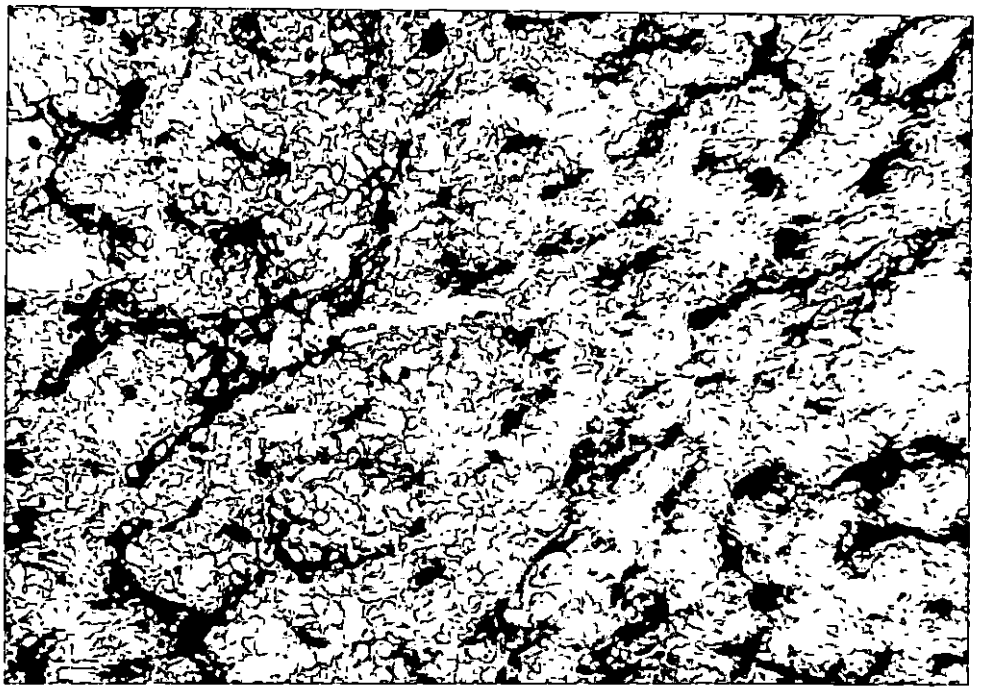


Fig.23 Liver - Alkaline phosphatase activity - Aflatoxin treated duck - 180 days - ALPase x 250

Fig.24 Liver - Alkaline phosphatase activity - Clofibrate treated duck - 90 days - ALPase x 250



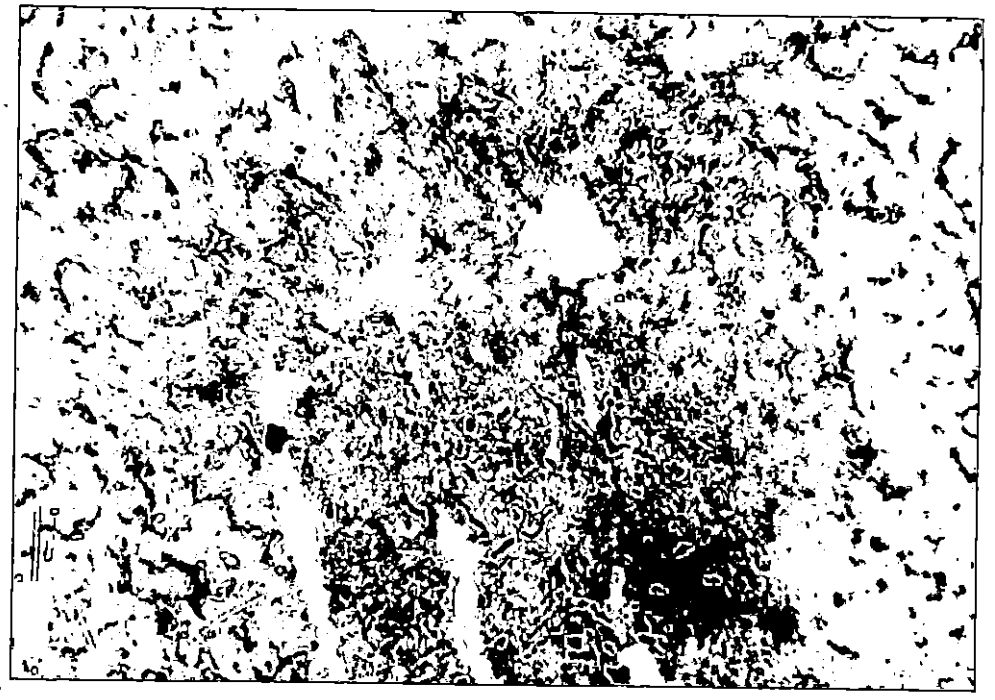
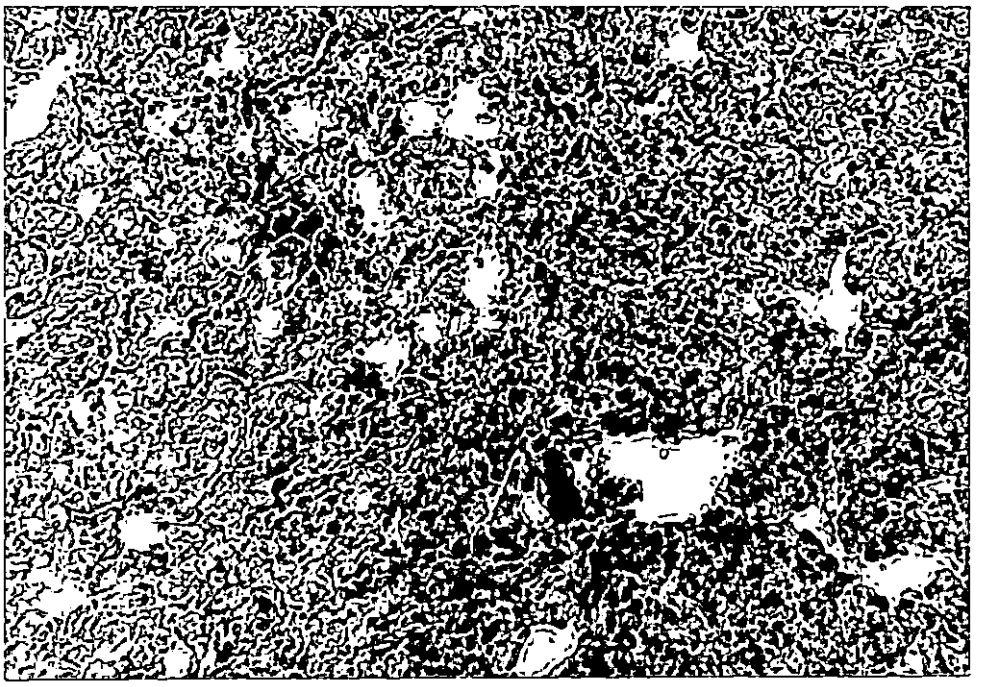


Fig.25 Liver - Alkaline phosphatase activity - Clofibrate treated duck - 180 days - ALPase x 250

Fig.26 Liver - Proliferating peroxisomes - Clofibrate treated duck - Peroxisome staining x 250



Group III

The liver sections were negative for the activity of ALP.

4.2.8.3 Histochemical demonstration of peroxisomes

Group I and III

Peroxisomes could not be demonstrated in liver sections of group I and III.

Group II

On the 90th day when liver sections were examined it was very difficult to localise the peroxisomes. On the 180th day peroxisomes appeared as blue crystals and there was increase in the number of peroxisomes. Proliferating peroxisomes were seen in the cytoplasm of hepatic cells (Fig.26).

The data on the Body weight, Hemogram (hemoglobin and ESR), total serum protein, albumin and globulin, serum enzymology (AST, ALT, GGT and ALP) and serum cholesterol are shown in Appendix.

Discussion

DISCUSSION

Duck rearing is an important occupation of farmers in Kerala and generally diseases are less in ducks when compared to chicken. The ducks received for autopsy at the Centre of Excellence in Pathology was mainly from the University duck farm and in the farm regular culling is practised and ailing ducks are disposed off. Therefore, only few ducks which died spontaneously before culling are brought for autopsy. In the present study out of the 618 ducks examined during the period from 1989 to 1991, only 14 ducks had hepatic tumours. It would appear that, the incidence of neoplastic conditions recorded is very low. But in reality the incidence may be high. Apart from the neoplastic conditions observed there were also hepatosis (22 per cent) and hepatitis (20.39 per cent).

At the Government duck farm, Niranam 14,360 ducks were examined during the period from 1989 to 1991. Out of these ducks examined 46.92 per cent had hepatic lesions like hepatosis and hepatitis. The hepatosis and hepatitis may be a manifestation of aflatoxicosis. No data are available in the farm regarding aflatoxin level in the feed. Since in the farm no systematic post-mortem examinations are done and detailed classification of diseases are made, the incidence of hepatic tumours might have been overlooked and hepatic tumours were

not recorded. Moreover, in the farm systematic culling of unproductive birds is done and ducks are not allowed to have a natural death. This might also be a reason for not recording cases of hepatic tumours at the Niranam Duck Farm. In reality there might have been many cases of hepatic tumours.

Rajan et al. (1989) recorded 40.5 per cent incidence of hepatosis and 4.83 per cent of hepatic tumours, out of the 1034 ducks examined during the period from 1986 to 1989. The source of material for the study was again the University duck farm. They also reported that, out of the twenty feed samples analysed during 1987-88, 40 per cent contained Aflatoxin B_1 ranging from 20-100 ppb and 20 per cent contained Aflatoxin B_1 ranging from 101-600 ppb and attributed the incidence of hepatic lesions to consumption of aflatoxin contaminated feed. Maryamma et al. (1982) reported that 35 per cent of the feed sample available in the market of Kerala are contaminated with aflatoxin. Rigdon (1972) suggested, that aflatoxin is a significant factor in the etiology of many of the spontaneously occurring hepatomas in ducks. Hence, the etiology of hepatic tumours reported in this investigation can be ascribed to aflatoxin contaminated feed.

In spontaneous cases of tumour, the lesions were striking. Grossly there was diffuse enlargement of the liver and it almost filled the abdominal cavity. The enlarged liver

contained many well defined slightly raised greyish white or pale brown nodules and slightly raised whitish patches. There was no indication of ascites in any of the cases. No metastatic foci were also seen. This is a significant observation. The poor development of lymphatic system and the absence of lymphnodes may be factors which do not favour metastatic growth. Histopathologically the hepatic tumours were classified into hepatocellular carcinoma (10) and cholangiocellular carcinoma (4). In the hepatocellular carcinoma, the striking histological feature was intense stromal reaction and pseudolobulation. This stromal response may be an inherent mechanism of the body to contain the neoplastic foci to the site of origin of the neoplasm. This feature can also be considered as a factor responsible for the absence of metastasis in these cases. The histological features observed and the nature of distribution of gross lesions pointed out that neoplastic transformation was of multicentric origin. Among the malignant liver neoplasms observed, hepatocellular carcinoma was more common than cholangiocellular carcinoma. It would, therefore, appear that malignant transformation of hepatocytes by aflatoxin occurred much more commonly than bile duct epithelium. The histological features of the tumour encountered were similiar to that observed by other workers (Christopher et al. 1968; Carnaghan, 1965 and Rajan et al. 1989). The cholangiocellular carcinoma cases showed papillary pattern other than the usual acinar

pattern observed by others (Carnaghan, 1965 and Rajan et al. 1989). In the case of hepatitis, altered foci were also seen. Altered foci were considered as preneoplastic foci (Rogers and Newberne, 1969 and Maronpot et al. 1986). This observation would suggest that altered foci associated with hepatosis are sites of cellular transformation. In some of the hepatocellular carcinoma cases also altered foci were seen.

A case of airsac tumour was observed along with hepatocellular carcinoma. Rajan et al. (1989) recorded 7 cases of airsac tumour associated with hepatocellular carcinoma in ducks. They suggested that aflatoxin might have a remote carcinogenic effect on the respiratory epithelium of the airsac. They also stressed the need for further experimental studies to confirm this. A case of osteoma was also recorded along with hepatocellular carcinoma. This also might be a manifestation of the remote carcinogenic action of aflatoxin. The histologic, features of the tumour in the airsac and in the bone indicated that, they are not the secondaries of the tumour developed elsewhere. Rajan et al. (1989) while making observations on the incidence of hepatosis and hepatic tumour in the University Duck Farm, observed that the histological changes observed in the ducks and the presence of aflatoxin in the feed of ducks indicated the possibility of aflatoxin induced carcinogenesis.

In the experimental study, out of twelve ducks administered aflatoxin, ten ducks developed hepatic tumours. The six ducks which were sacrificed on the 90th day of administration, three ducks had hepatocellular carcinoma and one had cholangiocellular carcinoma. Two of the ducks had hepatosis. By the 180th day all the 6 ducks developed tumours and they were classified as hepatocellular carcinoma (3) and cholangiocellular carcinoma (3). The experimental investigation undertaken have indicated that at this dose level hepatic tumour could be induced in 66.6 per cent cases within 90 days. The 1/10th LD_{50} level, was therefore, demonstrated to be carcinogenic in ducks. Aflatoxin was found to be carcinogenic in rats (Barnes and Butler, 1964; Carnaghan, 1967 and Newberne et al. 1968) in mice (Wogan, 1969) in hamsters (Chesterman and Pomerance, 1965) in Rhesus monkeys (Gopalan et al. 1972 and Adamson et al. 1973) and also in several species of other laboratory animals (Newberne and Butler, 1969; Enomoto and Saito, 1972).

It is very significant to observe that at the same dose level by the 180th day all the six ducks developed tumour. This observation clarified that the cancer induction is dose and time dependent. The development of tumour in all the ducks maintained for six months highlights the carcinogenic potential of aflatoxin and the sensitivity of the

duck to aflatoxin. The variation in size, distribution and histological appearance of hepatic nodules will also point out that the neoplastic transformation of hepatocytes was initiated early.

The progressive nature of hepatocarcinogenesis was self evident by the development of tumours in all the ducks after 180 days of aflatoxin administration. This observation highlights the practical importance of aflatoxin contaminated feed and its deleterious effects on the productive performance of the duck.

In the field conditions there is every chance of exposure of ducks to such a low level of aflatoxin, through contaminated feed. According to Rigdon (1972), white Pekin duck is regarded as a breed in which spontaneous neoplasms are relatively less. The experimental observation of high incidence of hepatic tumours in this breed, which is regarded as a breed in which spontaneous neoplasms are relatively low is significant. Therefore, the other breeds of ducks like Khaki Campbell and Desi ducks which are common in the state of Kerala will have a great risk of development of hepatic neoplasms.

The climatic conditions of Kerala is favourable for the growth of toxigenic fungi and production of toxin. So this study points to the fact that, farmers of Kerala should

take adequate precautions to avoid toxin contaminated feed to their ducks. The high tumour induction rate in the experimental groups of ducks suggest that, the duck could be used as an experimental model for carcinogenic studies.

From the histopathological observations, it appears that both hepatocyte and bile duct epithelium are amenable to the carcinogenic action of aflatoxin. But it is of interest that, though both the hepatocyte and bile duct epithelium are responsive to the carcinogenic action of aflatoxin, no concomitant neoplasia of hepatocytes and bile duct epithelium was seen in any of the cases. In all the cases the neoplastic nodules were either from the hepatocyte or from the bile duct epithelium. The reason for the abscence of a concomitant neoplasia of both hepatocytes and bile duct epithelium is yet to be elucidated.

As far as duck is concerned biliary epithelium is very sensitive to the effect of aflatoxin, and biliary hyperplasia is considered as a marker to quantify the aflatoxin level. In spite of this cholangiocellular carcinoma was not seen in all the ducks. This will indicate that, the development of tumour and the involvement of the type cell in carcinogenesis are controlled by certain other factors. Perhaps, the age may play an important role as biliary hyperplasia is a marker index in day old ducklings. As they mature, the sensitivity may vary. This aspect require further work and elucidation.

Armbrecht et al (1971) indicated that the effect of aflatoxin on weight gain and feed conversion was among the most sensitive indicators of aflatoxicosis. There was significant decrease in the weight gain of ducks dosed with aflatoxin in this investigation and this is an observation which will support the observation made by earlier investigators (Huff et al. 1984 and Ghosh et al. 1989). The observation made have great practical relevance in the field situation, as the diet may contain low levels of aflatoxin and this will lead to decrease in growth rate and feed consumption. This is going to seriously affect the productivity of ducks and the farmer may not get the anticipated weight gain. This, would perforce lead to great economic loss to the farmer. This study, therefore, points out the need for regular screening of the feed fed to ducks for aflatoxin. This observation has great practical relevance since it has been reported that 35 per cent of the feed samples available in the market of Kerala are contaminated with aflatoxin (Maryamma et al. 1982).

There was significant increase in the ESR of ducks dosed with aflatoxin. This can be ascribed to hepatic damage leading to variation in the total protein and albumin globulin ratio. Anaemia was observed in these ducks as reflected by reduced level of haemoglobin. Reduction in the haemoglobin in the toxin fed ducks reflects anaemia and this again can be a factor which could contribute to the increase in ESR.

The enzyme γ-glutamyl transpeptidase (GGT) catalyses the transfer of γ-glutamyl groups from γ-glutamyl peptides to other peptides, to amino acids and to water. In the liver GGT was demonstrated histochemically in the parenchyma and especially in the luminal border of the epithelial cells lining the fine biliary ductules. Slight histochemical activity was observed within the periportal hepatic cells and such activity may be increased by inflammation or cirrhosis. Serum GGT estimation is a sensitive test for liver diseases (Szczeklik et al. 1961). The highest values were observed in biliary obstruction (Rutenburg et al. 1963 and Szczeklik et al. 1961) and in malignant hepatic involvement (Rutenburg et al. 1963 and Szczeklik et al. 1961). The elevation may result from cholestasis or originate from the reactive normal liver cells. Kalangayi et al. (1975) observed that expression of GGT was a common finding in liver lesions induced by aflatoxin B_1 a genotoxic carcinogen. In the present study serum GGT levels were seen increasing from the VIIth fortnight. From the VIIth to VIIIth fortnight the elevation in GGT was very prominent and continued to increase till the day of sacrifice on the 180th day. This may be explained as the effect of regenerative proliferation of hepatocytes consequent to liver damage induced by aflatoxin administration.

The post-mortem examination of the experimental ducks showed lesions in the liver ranging from hepatitis, hepatic

degeneration and neoplastic nodules. These histopathological changes in the liver confirm the increase in the serum GGT levels. This was confirmed further by the histochemical demonstration of GGT in the liver sections. The intensity of GGT activity in the liver section of the ducks slaughtered on the 180th day was much more when compared to the liver of ducks slaughtered on the 90th day. The increase in the intensity of GGT activity in the long term experimental group is due to the increased liver damage in this group. Gangadharan (1992) showed that in ethmoid carcinoma sections, there was expression of GGT activity and he opined that expression of GGT was an indication of involvement of a genotoxic carcinogen like aflatoxin. In the case of ducks, hepatocytes contained GGT and in neoplastic transformation the activity was increased.

The use of serum enzymes for the diagnosis of hepatic diseases was introduced four decades ago with the demonstration of usefulness of alkaline phosphatase assay in the differential diagnosis of jaundice. Of the more than 100 tests of hepatic function that have been deviced, none by itself has been reliable in the differentiation of hepatic and obstructive jaundice, in the separation of hepatic from non-hepatic disease, or in the identification of specific hepatic diseases (Zimmerman and Seeff, 1970). The obstruction of the biliary tree and intra-hepatic cholestasis as well as

carcinoma of the liver and other infiltrating lesions lead to high level of alkaline phosphatase. These conditions, however, lead to only moderately elevated levels of AST and ALT. The usefulness of serum levels of ALP in the differential diagnosis of jaundice and in the recognition of infiltrating space occupying lesions of the liver is well known. Nodular or infiltrating lesions despite the usual absence of jaundice regularly lead to elevated ALP values that may be as high as those seen in obstructive jaundice (Zimmerman and Seeff, 1970).

In the present study serum values of ALP were found increasing from the VIth fortnight onwards, which was quite perceptible after VIIIth fortnight and became almost double by the XVth fortnight. This increase in the serum ALP value was related to the pathological changes observed in the liver of ducks. The space occupying lesions in the form of primary neoplastic nodules will cause biliary obstruction and it will lead to the release of ALP enzyme into the circulation (Zimmerman and Seeff, 1970). This was also clarified by demonstrating the activity of ALP histochemically in the liver sections. Liver sections of the duck sacrificed after 3 months of aflatoxin administration showed mild activity of ALP in the biliary epithelium. The activity of ALP was intense in the liver sections of ducks sacrificed after 6 months.

The enzymes like AST and ALT reflect hepatobiliary disease in a manner which is essentially reciprocal to that of ALP. Serum levels of AST and ALT are usually much higher in patients with acute hepatitis than patients with biliary obstruction (Zimmerman and Seeff, 1970). Elevated values of AST may also be found in patients with myocardial or muscle disease. The enzymes ALT is uniquely or particularly richly concentrated in the liver. Their blood levels are usually markedly elevated in acute hepatic injury or moderately or slightly elevated in biliary obstruction and usually normal or only slightly increased in diseases that do not involve the liver and biliary tree (Zimmerman and Seeff, 1970).

The ducks dosed with aflatoxin in this investigation showed a gradual increase in the serum levels of AST and ALT throughout the course of the experiment. From the analysis of serum enzyme profile of ducks in this experimental protocol showed that there was a steady increase in the levels of ALP, AST and ALT. From this observation it is evident that, there was both hepatocellular damage and biliary obstruction. The changes in the enzyme level of AST indicated that aflatoxin had no direct pathological effect on organs like heart and skeletal muscles. If it had an effect on the heart and muscle, it should have shown a much more increase in AST level. The increase in serum levels of AST

and ALT throughout the experimental period was similar indicating that the pathological effects of aflatoxin was mainly confined to the liver.

The serum protein level showed a gradual decrease in the levels of total protein, albumin and globulin. The gross and histopathological changes observed in the liver of these ducks will serve to explain the reduced level of protein fractions in the serum of these ducks. The pathological effects of aflatoxin on the B-lymphocytes and the consequent immunosuppressive effect has been well documented (Ho, 1982 and Osuna and Edds, 1982b; Southern and Clawson, 1979). The effect of aflatoxin on B-lymphocytes may be responsible for the lower level of globulin in these ducks. Reduction in the level of serum albumin and globulin must be the reason for the reduced total protein level in the serum of ducks.

Hyperlipidemia is an important emerging disease condition of human beings. Several hypolipidemic drugs like Clofibrate, Ciprofibrate, Nafenopin, BR-931, and Tibric acid are used to treat hyperlipidemia. Peroxisome proliferation was induced in hepatocytes of rodent and non-rodent species by these hypolipidemic agents. Reddy et al. (1986) observed that the induction of peroxisome proliferation is tissue specific towards hepatocytes. Chronic administration of these non-DNA damaging and non-mutagenic peroxisome proliferators to

rats and mice resulted in the development of hepatocellular carcinoma. Svoboda et al. (1979) observed the development of hepatic tumours in rats fed clobibrate (Atromid-S) at the level of 0.5 per cent in the diet for 72-97 weeks. Reddy et al. (1976) reported hepatocellular carcinoma in mice treated with nafenopin, a hypolipidemic peroxisome proliferator. Reddy et al. (1979) also reported the carcinogenic effect of W_4-14, 643, another hypolipidemic agent on rat and mouse liver. While reporting the carcinogenic effects of hypolipidemic agents like BR-931 and Tibric acid in rats (Reddy et al. 1980) confirmed that the hypolipidemic peroxisome proliferator group of chemicals form a novel class of chemical hepatocarcinogens. The potent peroxisome proliferator, ciprofibrate could proliferate peroxisomes in the liver of non-rodent species like cats, chickens, pigeons, Rhesus monkey and Cynomolgus monkeys, in a dose dependent manner (Reddy et al. 1984).

Clofibrate is the common drug in the market to treat hyperlipidemic patients. Though clofibrate is less potent compared to ciprofibrate and nafenopin in inducing peroxisome proliferation, its potential as a carcinogen in rodents, on long term use should be taken into account (Reddy and Krishnakantha, 1975).

White Pekin duck is considered as a good experimental model for chemical hepatocarcinogenicity studies (Rigdon,

1972). This experiment was designed to assess the effect of clofibrate feeding in white Pekin ducks for a period of 6 months with the dose rate of 50 mg/bird/day orally. Grossly the liver of ducks dosed with clofibrate showed perceptible enlargement, even after 3 months of treatment and became prominent after 6 months of treatment. Histopathologically there was fatty change and biliary proliferation in a time dependent manner. These changes were reflected in the serum enzymes like ALP, AST and ALT haemogram and serum proteins. The effect of hypolipidemic drug treatment was monitored by analysing the serum cholesterol level which showed a gradual reduction. Peroxisomes were seen proliferated in the hepatocytes. The induction of peroxisome proliferation and decrease in cholesterol level are changes similar to that described in mice and rats following clofibrate administration (Svoboda et al. 1979 and Reddy et al. 1980). Peroxisome proliferators as a class have been classified as carcinogens and the carcinogenic potential has been evaluated in rodents like rats and mice by various workers. However, in the experiment undertaken in this investigation taking duck as a model it did not induce cancer development. However, the histological changes of biliary hyperplasia in portal areas in particular suggested a preneoplastic change.

It would appear that the dose adopted and the duration of experiment were not sufficient enough to induce neoplasms.

There was mild expression of GGT in the liver of ducks dosed with clofibrate. The expression of GGT is considered as a marker for the involvement of a genotoxic carcinogen like aflatoxin and 2-acetyl aminofluorene but not considered as a marker for peroxisome prolifertors like ciprofibrate (Rao et al. 1988 and Yeldandi et al. 1989). In the duck, there was expression of GGT, which is normally present in the liver of ducks and there was no increase in the activity of GGT compared to the control. The peroxisome proliferator, therefore, was not found to influence GGT expression.

The experimental investigation undertaken with two different classes of carcinogens, aflatoxin and clofibrate revealed the high carcinogenic potential of aflatoxin and low degree of carcinogenic potential of clofibrate. It would appear that the dose and the duration are critical factors in carcinogenesis. The induction of proliferation of bile ducts indicated the possible carcinogenic effect of clofibrate in the duck also. Clofibrate was a drug marketed to reduce cholesterol level in hyperlipidemic patients and since the identification of its carcinogenic potential, it is not being marketed now. By this investigation taking duck as a model, carcinogenic effect of genotoxic carcinogen like aflatoxin was clearly established and its significance was brought to light, and the possible carcinogenic effect of non-genotoxic carcinogen like clofibrate was indicated.

Summary

SUMMARY

1. Analysis of mortality pattern of ducks brought to the Centre of Excellence in Pathology for post-mortem diagnosis from the University Poultry farm and private owners during the period from 1989-1991 showed that out of the six hundred and eighteen ducks examined, two hundred and seventy eight ducks had hepatic lesions like hepatosis (136), hepatitis (126) and hepatic tumours (16).

2. Survey studies conducted at the Government duck farm, Niranam showed that out of 14,360 ducks examined during the period from 1989 to 1991, 6,737 ducks had hepatic lesions. No tumours were seen recorded.

3. An experiment was designed to assess the response of duck hepatic tissue to carcinogens like aflatoxin and clofibrate. Aflatoxin was administered at the rate of 0.04165 mg/kg body weight every third day for a period of six months. Clofibrate was administered at the rate of 0.05 g/kg body weight per day for a period of six months.

4. In aflatoxin fed group out of the six ducks sacrificed on the 90th day, four of them had hepatic tumours and the rest two ducks revealed chronic hepatitis. Histologically

the tumours were classified into hepatocellular carcinoma (3) and cholangiocellular carcinoma (1). On the 180th day when the remaining six ducks were sacrificed all of them had hepatic tumour. They were classified histologically as hepatocellular carcinoma (3) and cholangiocellular carcinoma (3).

The sequence of pathological changes in the neoplastic process was identified as hepatic degeneration, hepatic necrosis and hepatocellular carcinoma. A detailed descriptive account of the pathological features of the tumours encountered was given.

5. During the experimental observation period the body weight, haemoglobin, total serum protein, albumin and globulin levels showed gradual and progressive reduction from the third month onwards. It was therefore clarified that the aflatoxin had induced hepatosis. The induction of hepatosis was associated with increase in serum enzymes like AST, ALT, GGT and ALP from the third month onwards.

6. Histochemically the activity of GGT and ALP were demonstrated in the hepatic tumours. GGT expression was considered as a manifestation of neoplastic transformation of hepatocytes.

7. In clofibrate fed group all the six ducks sacrificed on the 90th day showed fatty change and biliary hyperplasia. On the 180th day these changes were more severe. It was demonstrated that the clofibrate caused progressive hepatosis and preneoplastic change.

8. Body weight, haemoglobin, total serum protein, albumin and globulin levels and serum cholesterol showed mild and gradual reduction from the third month onwards. Serum enzymes such as AST , ALT , GGT and ALP revealed progressive increase from the third month onwards. All these parameters pointed out to hepatosis.

9. Although there was no well defined cancer development there was histological evidence of preneoplastic changes in the liver of ducks dosed with clofibrate.

10. By this investigation the carcinogenic response of the duck hepatic tissue to aflatoxin was evaluated and the nature of the hepatic tumours induced was clarified. The histogenesis of neoplastic transformation was delineated.

11. Clofibrate was demonstrated to induce hepatosis and pre-neoplastic changes in the hepatic tissue and the possible carcinogenic potential of clofibrate was elucidated.

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NEOPLASMS OF THE DUCK WITH SPECIAL REFERENCE TO HEPATOCARCINOGENESIS

By

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ABSTRACT OF A THESIS

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ABSTRACT

At the Centre of Excellence in Pathology during the period from 1989-1991 six hundred and eighteen ducks were subjected to detailed post-mortem examination and out of this one hundred and thirty six ducks showed hepatosis, one hundred and twenty six ducks had hepatitis and sixteen ducks had hepatic tumours.

At the Government duck farm, Niranam, 14,360 ducks were examined and 46.92 per cent of ducks had hepatic lesions. However, tumours were not recorded. The possible aetiological role of aflatoxin for hepatosis and hepatic tumour was indicated.

An experiment was designed taking duck as a model to assess the carcinogenic effect of genotoxic carcinogen aflatoxin and non-genotoxic carcinogen clofibrate. Aflatoxin was administered to twelve ducks at the dose rate of 0.04165 mg/kg body weight every third day for a period of six months. Hepatic tumours were recorded in four ducks out of the six ducks sacrificed on the 90th day and in all the six ducks sacrificed on the 180th day.

The body weight, haemoglobin, ESR, total serum protein, albumin, globulin, serum enzymes such as AST, ALT, GGT and ALP were estimated at fortnightly intervals.

Clinically there was reduction in the body weight, haemoglobin, total serum protein, albumin and globulin levels by the third month. There was significant increase in ESR, serum AST, ALT, GGT and ALP levels when compared to the control ducks. These clinical changes were attributed to hepatosis and hepatic tumours.

Hepatosis characterised by moderate to severe enlargement of the liver with scattered greyish white nodules of varying sizes were the chief lesions encountered in aflatoxin fed ducks at autopsy.

The tumours encountered were classified as hepatocellular carcinoma (6) and cholangiocellular carcinoma (4). The gross and histopathological features of these lesions were described in detail. Histochemically the activity of GGT and ALP was moderate to severe in the liver tumours. The sequence of histological changes seen was hepatic degeneration necrosis and tumour formation.

Clofibrate was given at the dose rate of 0.05 g/kg body weight per day for six months. In these ducks the liver had a cooked appearance with few focal greyish white patches when sacrificed at the sixth month.

There was significant reduction in the body weight, haemoglobin, total serum protein, albumin and globulin levels

and serum cholesterol level at the third and sixth month. However, ESR, serum SGOT, SGPT, GGT and ALP showed significant increase as compared to the control ducks. These clinical parameters suggested hepatosis.

Histologically there was moderate fatty change, focal disassociation of hepatocytes and biliary hyperplasia. Histochemical expression of ALP was moderate to intense in the liver and there was moderate to severe proliferation of peroxisomes. There was histological evidence of preneoplastic changes although no tumours were seen.

By this investigation the high sensitivity of ducks to aflatoxin was clarified and the tumour induction potential of aflatoxin in ducks was evaluated and the nature of hepatic tumours induced was delineated.

Clofibrate was demonstrated to induce hepatosis and preneoplastic changes in the hepatic tissue and the possible carcinogenic potential of clofibrate was indicated.

Appendix

Table 1. Body weight (kg) of ducks fed aflatoxin (Group I)

Sl. No.	Fortnight														
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
1.	1.160	1.260	1.720	1.580	1.560	1.580	1.520	1.240							
2.	1.680	1.800	1.900	1.960	1.900	1.860	1.800	1.740							
3.	0.920	1.060	1.420	1.340	1.520	1.620	1.520	1.320							
4.	1.700	1.620	1.780	1.580	1.780	1.600	1.800	1.620							
5.	1.600	1.300	1.440	1.420	1.400	1.360	1.520	1.380							
6.	1.620	1.560	1.700	1.780	1.750	1.760	1.800	1.540							
7.	1.340	1.560	1.600	1.620	1.600	1.520	1.620	1.280	1.200	1.180	1.140	1.120	1.140	1.120	1.100
8.	0.940	1.180	1.600	1.660	1.680	1.740	1.600	1.380	1.360	1.320	1.300	1.280	1.200	1.180	1.140
9.	1.130	1.180	1.460	1.660	1.640	1.540	1.500	1.360	1.340	1.300	1.280	1.200	1.180	1.080	1.100
10.	1.160	1.160	1.580	1.800	1.720	1.760	1.740	1.560	1.480	1.280	1.260	1.180	1.160	1.140	1.180
11.	1.120	1.240	1.740	1.920	1.900	1.720	1.860	1.620	1.420	1.260	1.240	1.220	1.200	1.220	1.200
12.	1.000	1.000	1.400	1.500	1.680	1.680	1.620	1.380	1.380	1.360	1.300	1.320	1.300	1.280	1.220
Mean ± SE	1.447 ± 0.852								1.470 ± 0.456						1.157 ± 0.206

Table 2. Bodyweight (kg) of ducks fed clofibrate (Group II)

Sl. No.	Fortnight		
	I	VIII	XV
1.	1.020	1.000	--
2.	0.820	0.680	--
3.	0.700	0.600	--
4.	0.780	0.580	--
5.	0.880	0.800	--
6.	0.900	0.820	--
7.	1.020	1.000	0.880
8.	1.120	1.020	1.000
9.	1.240	1.160	1.080
10.	1.180	1.080	1.020
11.	1.120	1.100	1.000
12.	1.160	1.140	1.080
Mean ± SE	1.140 ± 0.505	1.083 ± 0.607	0.010 ± 0.295

Table 3. Body weight (kg) of control group of ducks (Group III)

Sl. No.	Fortnight														
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
1.	1.040	1.060	1.080	1.080	1.060	1.140	1.200	1.220							
2.	1.120	1.120	1.160	1.200	1.240	1.280	1.320	1.360							
3.	1.200	1.240	1.320	1.380	1.400	1.420	1.460	1.460							
4.	1.210	1.200	1.260	1.300	1.360	1.400	1.440	1.480							
5.	1.180	1.160	1.200	1.260	1.340	1.380	1.360	1.400							
6.	1.200	1.180	1.220	1.240	1.280	1.300	1.320	1.400							
7.	0.980	1.020	1.060	1.080	1.120	1.240	1.360	1.360	1.380	1.400	1.500	1.510	1.540	1.560	1.480
8.	0.960	0.940	0.980	1.020	1.000	1.080	1.100	1.140	1.160	1.200	1.180	1.160	1.220	1.240	1.260
9.	1.000	1.040	1.020	1.100	1.120	1.140	1.180	1.220	1.280	1.240	1.220	1.260	1.280	1.300	1.340
10.	1.020	1.000	0.980	1.100	1.130	1.140	1.180	1.210	1.240	1.240	1.300	1.280	1.320	1.280	1.300
11.	1.040	1.040	1.020	1.060	1.080	1.100	1.140	1.160	1.200	1.200	1.220	1.180	1.240	1.200	1.260
12.	1.020	1.000	1.040	1.060	1.100	1.120	1.160	1.200	1.240	1.240	1.280	1.300	1.280	1.320	1.340
Mean ± SE	1.158 ± 0.273							1.380 ± 0.349							1.330 ± 0.327

Table 4. Haemoglobin level (g/hl) in ducks fed aflatoxin (Group I)

Sl. No.	Fortnight														
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
1.	8.8	9.2	9.0	9.2	10.2	9.8	9.6	9.6	8.6	8.2	8.0	7.8	8.0	7.8	8.4
2.	10.0	10.0	9.8	10.0	9.8	9.4	9.4	9.0	8.4	8.2	8.2	7.8	8.0	6.0	7.2
3.	9.6	9.8	9.6	9.8	10.0	9.8	9.8	9.2	8.2	8.0	7.6	8.0	7.8	7.4	7.4
4.	10.2	10.0	10.0	9.8	9.2	9.0	8.8	8.8	9.4	9.0	8.4	8.0	7.8	7.8	7.2
5.	10.6	10.4	10.4	10.0	10.2	10.0	9.8	9.6	8.6	8.4	8.6	8.2	8.0	8.2	7.8
6.	9.8	10.2	9.8	10.2	10.4	10.2	10.0	10.0	8.8	8.8	8.6	8.4	8.2	8.0	7.8
7.	8.2	8.6	8.4	9.2	9.8	9.6	9.4	9.2							
8.	11.2	10.8	10.8	10.4	10.2	10.0	9.8	9.0							
9.	10.8	10.6	10.4	10.2	10.4	10.0	9.6	8.8							
10.	10.4	10.2	10.0	10.4	10.2	10.0	10.0	9.8							
11.	9.8	10.0	9.8	10.2	10.0	9.6	9.2	8.8							
12.	10.2	10.0	10.2	10.4	10.2	10.0	9.4	9.0							
Mean ± SE	10.10 ± 0.239							9.10 ± 0.120							7.63 ± 0.189

Table 5. Haemoglobin level (g/dl) in ducks fed clofibrate (Group II)

Sl. No.	Fortnight		
	I	VIII	XV
1.	10.8	10.6	
2.	10.6	9.8	
3.	10.2	8.4	
4.	8.8	9.0	
5.	10.2	10.0	
6.	10.4	8.8	
7.	9.8	8.4	8.6
8.	10.4	9.2	8.4
9.	11.2	8.8	8.0
10.	9.8	9.2	7.8
11.	10.0	8.2	8.0
12.	10.2	8.4	7.6
Mean ± SE	10.23 ± 0.172	8.70 ± 0.214	8.07 ± 0.152

Table 6. Haemoglobin level (g/dl) in the control group of ducks (Group III)

Sl. No.	Fortnight														
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
1.	9.2	8.6	9.0	9.2	9.8	10.0	9.8	10.2							
2.	9.0	9.2	9.4	9.8	10.0	10.2	10.4	9.8							
3.	9.8	10.0	10.0	8.8	9.2	7.8	8.8	11.2							
4.	8.6	8.8	9.2	10.4	10.6	10.8	14.6	10.8							
5.	9.6	10.2	10.4	11.0	11.2	11.4	11.2	12.4							
6.	9.4	9.8	9.8	10.2	10.8	11.2	12.0	13.2							
7.	10.0	11.0	11.4	12.2	12.6	12.8	13.2	14.6	14.2	14.4	15.2	14.8	15.0	14.6	15.0
8.	10.2	10.2	7.8	10.6	13.2	13.2	12.6	16.8	12.8	12.6	12.8	13.0	13.2	13.4	12.8
9.	9.8	9.8	10.2	10.4	10.8	10.6	11.8	10.2	11.2	11.4	12.2	12.4	12.6	12.8	11.6
10.	9.4	9.6	11.2	11.0	11.2	11.8	10.6	7.8	10.8	11.0	11.2	10.8	11.2	11.4	10.2
11.	8.8	9.0	10.0	12.6	12.8	12.6	12.4	10.2	11.4	11.6	12.0	12.4	12.6	128	9.8
12.	8.2	9.0	10.2	11.2	14.4	13.2	12.8	13.0	12.8	12.8	13.0	14.2	13.2	13.6	10.2
Mean ± SE	9.40 ± 0.173							12.10 ± 0.704							11.60 ± 0.819

Table 7. ESR (mm/h) in ducks fed aflatoxin (Group I)

Sl. No.	Fortnight														
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
1.	3	2	2	3	3	4	4	3	3	3	3	3	3	3	5
2.	4	3	3	3	4	3	3	2	3	4	4	4	4	4	4
3.	3	4	3	4	3	4	4	4	4	4	4	4	4	3	4
4.	2	3	4	4	2	3	3	3	3	3	4	5	4	4	3
5.	3	2	3	3	1	3	2	3	4	5	5	3	5	7	6
6.	4	3	2	4	3	4	3	2	6	4	4	4	3	5	3
7.	3	4	1	3	4	3	3	4							
8.	2	3	3	2	2	4	3	3							
9.	1	2	0	2	3	2	4	2							
10.	2	1	2	3	3	3	3	5							
11.	3	3	1	4	4	3	3	2							
12.	3	4	2	3	3	4	3	6							
Mean ± SE	2.33 ± 0.342							3.25 ± 0.313							4.16 ± 0.285

Table 8. ESR (mm/h) in ducksfed clofibrate (Group II)

Sl. No.	Fortnight		
	I	VIII	XV
1.	1	2	
2.	2	2	
3.	2	3	
4.	1	2	
5.	1	3	
6.	3	2	
7.	4	3	2
8.	3	3	2
9.	2	4	4
10.	1	2	4
11.	2	3	3
12.	3	3	3
Mean ± SE	2.50 ± 0.281	2.66 ± 0.302	3.00 ± 0.216

Table 9. ESR (mm/h) in control group of ducks (Group III)

Sl. No.	Fortnight														
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
1.	3	4	4	4	3	3	2	3							
2.	4	3	3	3	2	4	3	4							
3.	4	2	2	2	4	3	4	2							
4.	3	3	3	3	3	2	3	3							
5.	2	4	4	4	2	1	2	3							
6.	1	2	3	3	3	3	3	4							
7.	0	1	2	2	4	4	4	2	3	3	4	3	3	3	3
8.	3	3	1	2	3	2	3	3	4	2	3	2	4	2	2
9.	4	2	2	3	3	3	2	3	3	3	2	3	2	3	4
10.	4	3	3	3	2	1	3	4	2	4	1	0	3	1	3
11.	3	3	4	3	0	4	3	3	4	3	3	4	2	2	4
12.	3	4	3	1	3	3	3	2	3	2	2	3	4	0	2
Mean ± SE	2.83 ± 0.365							2.83 ± 0.213							3.00 ± 0.365

Table 10. Total serum protein level (g/dl) in ducks fed aflatoxin (Group I)

Sl. No.	Fortnight														
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
1.	1.827	5.635	3.756	4.711	4.151	3.551	3.413	3.042							
2.	7.675	3.010	4.689	3.900	2.965	1.490	1.289	1.475							
3.	2.746	2.802	3.605	3.347	5.512	3.184	3.021	2.985							
4.	3.076	3.015	3.857	4.011	2.407	1.592	1.337	1.546							
5.	2.041	5.665	3.706	6.284	4.151	3.806	3.651	3.743							
6.	2.376	6.426	6.107	4.926	5.163	4.969	4.901	4.872							
7.	3.289	3.898	3.908	1.699	4.326	4.071	3.929	4.006	3.878	3.692	3.580	3.460	3.360	3.320	3.260
8.	2.680	5.149	4.185	3.240	3.733	3.337	3.011	3.124	3.004	3.010	3.000	3.000	3.004	3.989	2.900
9.	2.071	3.442	4.109	4.074	5.198	5.265	4.943	4.990	4.423	4.368	4.352	4.250	4.180	4.120	4.110
10.	1.748	2.146	3.563	3.284	4.128	3.468	3.793	3.895	3.454	3.342	3.125	3.110	3.100	3.000	3.000
11.	3.071	2.904	4.217	4.151	2.979	4.087	2.315	2.476	2.328	2.310	2.240	2.238	2.224	2.200	2.208
12.	2.241	3.013	3.386	3.970	3.641	4.221	4.006	4.023	4.006	4.000	3.978	3.871	3.764	3.521	3.578
Mean ± SE	2.517 ± 0.458							3.348 ± 0.328							3.166 ± 0.114

Table 11. Total serum protein level (g/dl) in ducks fed clofibrate (Group II)

Sl. No.	Fortnight		
	I	VIII	XV
1.	3.820	3.760	--
2.	4.450	4.420	--
3.	4.620	4.600	--
4.	4.580	4.280	--
5.	3.726	3.580	--
6.	4.600	4.162	--
7.	4.200	3.892	3.518
8.	4.560	4.146	3.210
9.	4.720	4.510	4.080
10.	4.262	4.080	3.910
11.	4.341	4.100	3.752
12.	4.454	3.820	3.354
Mean ± SE	4.423 ± 0.091	4.091 ± 0.089	3.637 ± 0.136

Table 12. Total serum protein level (g/dl) in control group of ducks (Group III)

Sl. No.	Fortnight														
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
1.	4.003	4.421	4.438	4.459	4.447	4.456	4.448	4.512							
2.	5.641	3.089	3.141	3.167	3.201	3.208	3.231	3.242							
3.	2.151	2.160	2.158	2.118	2.165	2.164	2.204	2.201							
4.	2.089	2.242	2.255	2.257	2.277	2.282	2.300	2.310							
5.	3.033	3.121	3.023	3.031	3.042	3.061	3.127	3.161							
6.	2.046	2.061	2.174	2.197	3.008	3.144	3.144	3.108							
7.	2.121	2.121	2.127	2.260	2.251	2.310	2.309	2.318	2.362	2.374	2.460	2.480	2.580	2.420	2.660
8.	3.147	3.153	3.155	3.138	3.106	3.204	3.212	3.220	3.260	3.280	3.420	3.460	3.470	3.540	3.580
9.	1.923	1.919	1.920	2.004	2.001	2.115	2.123	2.174	2.282	2.300	2.360	2.400	2.280	2.360	2.440
10.	1.211	1. 220	1.232	1.241	1.253	1.277	1.284	1.305	1.840	1.860	1.940	1.980	2.400	2.440	2.440
11.	3.017	3.020	3.631	3.076	3.085	3.116	3.174	3.191	3.220	3.280	3.380	3.400	3.580	3.660	3.760
12.	2.212	2.220	2.218	2.241	2.241	2.253	2.269	2.285	2.320	2.360	2.400	2.500	2.620	2.720	2.820
Mean ± SE	2.272 ± 0.339							2.412 ± 0.236							2.950 ± 0.299

Table 13. Albumin level (g/dl) in ducks fed aflatoxin (Group I)

Sl. No.	Fortnight														
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
1.	0.675	1.762	1.811	1.813	1.804	1.421	1.265	1.137							
2.	2.143	1.423	1.912	1.805	1.072	0.676	0.763	0.572							
3.	1.823	1.232	1.672	1.563	2.212	1.413	1.154	1.213							
4.	1.675	1.465	1.684	1.726	1.086	0.872	0.672	0.571							
5.	1.510	1.572	1.573	1.585	1.567	1.414	1.154	1.143							
6.	1.312	2.132	2.108	2.113	2.131	1.398	2.013	2.001							
7.	1.576	2.224	2.100	0.923	1.721	1.265	1.487	1.482	1.463	1.454	1.432	1.420	1.420	1.414	1.410
8.	1.080	2.100	2.136	1.571	1.489	1.203	1.012	1.008	0.892	0.873	0.781	0.663	0.651	0.631	0.617
9.	1.362	1.487	2.209	2.116	2.236	2.211	2.103	2.098	2.002	2.000	1.987	1.931	1.927	1.916	1.910
10.	1.211	1.062	1.914	1.751	2.007	1.614	1.113	1.109	1.008	1.000	0.993	0.987	0.972	0.961	0.934
11.	1.423	1.571	2.089	2.091	0.993	1.539	1.101	0.991	0.872	0.858	0.844	0.834	0.825	0.812	0.809
12.	1.121	1.672	1.743	1.913	1.116	1.672	1.423	1.421	1.300	1.210	1.192	1.190	1.187	1.160	1.120
Mean ± SE	1.292 ± 0.110							1.352 ± 0.1356							1.133 ± 0.170

Table 14. Albumin level (g/dl) in ducks fed clofibrate (Group II)

Sl.No.	Fortnight		
	I	II	III
1.	1.680	1.660	
2.	1.720	1.560	
3.	1.500	1.372	
4.	1.480	1.480	
5.	1.380	1.500	
6.	1.520	1.380	
7.	1.392	1.262	1.220
8.	1.560	1.400	1.354
9.	1.620	1.530	1.428
10.	1.480	1.410	1.280
11.	1.442	1.360	1.300
12.	1.522	1.360	1.240
Mean ± SE	1.503 ± 0.03	1.387 ± 0.032	1.304 ± 0.03

Table 15. Albumin level (g/dl) in control group of ducks (Group III)

Sl. No.	Fortnight														
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
1.	2.462	2.521	2.678	2.434	2.126	2.080	2.078	2.052							
2.	1.898	1.919	1.854	2.123	1.679	1.700	1.710	1.720							
3.	2.100	2.002	1.762	1.926	1.828	1.628	1.640	1.652							
4.	2.020	1.876	1.654	1.672	1.674	1.654	1.648	1.582							
5.	1.926	1.745	1.542	1.554	1.556	1.621	1.580	1.591							
6.	1.676	2.008	1.673	1.678	1.711	1.673	1.592	1.613							
7.	1.542	1.976	1.782	1.811	1.830	1.911	1.878	1.808	2.100	2.112	2.118	2.201	2.212	2.221	2.234
8.	1.897	2.002	2.100	1.200	1.626	1.631	1.721	1.692	1.546	1.554	1.562	1.563	1.573	1.581	1.620
9.	1.298	1.763	0.920	1.080	1.521	1.511	1.616	1.426	1.436	1.444	1.483	1.511	1.514	1.519	1.520
10.	1.043	1.092	0.581	1.008	1.010	1.012	1.008	0.989	0.980	0.991	0.997	1.000	1.008	1.010	1.008
11.	1.677	2.143	1.008	1.020	1.026	1.030	1.020	1.000	1.004	1.016	1.020	1.040	1.043	1.045	1.076
12.	1.523	1.914	1.143	1.157	1.342	1.421	1.820	1.420	1.422	1.431	1.473	1.464	1.472	1.473	1.475
Mean ± SE	1.497 ± 0.090							1.389 ± 0.350							1.489 ± 0.180

Table 16. Globulin level (g/dl) in ducks fed aflatoxin (Group I)

Sl. No.	Fortnight														
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
1.	1.152	3.873	1.945	2.898	2.347	2.130	2.148	1.905							
2.	5.532	1.587	2.777	2.095	1.893	0.814	0.576	0.903							
3.	0.923	1.570	1.933	1.784	3.300	1.771	1.867	1.767							
4.	1.401	1.550	2.173	2.285	1.401	0.720	0.665	0.955							
5.	0.531	4.153	2.133	4.699	2.584	2.392	2.497	2.606							
6.	1.064	4.294	3.999	2.813	3.032	3.571	2.888	2.871							
7.	1.713	1.674	1.808	0.776	2.605	2.806	2.442	2.524	2.415	2.238	2.148	2.040	1.940	1.906	1.850
8.	1.600	3.049	2.049	1.669	2.244	2.134	1.999	2.116	2.112	2.137	2.219	2.337	2.353	2.358	2.283
9.	0.729	1.955	1.900	1.958	2.962	3.054	2.840	2.892	2.421	2.368	2.365	2.319	2.253	2.204	2.200
10.	0.537	1.084	1.649	1.633	2.121	1.854	2.680	2.786	2.446	2.342	2.132	2.123	2.128	2.039	2.066
11.	1.648	1.333	2.128	2.060	1.986	2.548	1.214	1.485	1.456	1.452	1.396	1.404	1.399	1.388	1.399
12.	1.120	1.341	1.643	2.220	2.525	2.549	2.583	2.602	2.706	2.790	2.786	2.681	2.577	2.561	2.398
Mean ± SE	1.225 ± 0.385							2.401 ± 0.206							2.033 ± 0.155

Table 17. Globulin level (g/dl) in ducks fed clofibrate (Group II)

Sl. No.	Fortnight		
	I	VIII	XV
1.	2.140	2.100	
2.	2.730	2.860	
3.	3.120	3.228	
4.	3.100	2.800	
5.	2.346	2.080	
6.	3.080	2.782	
7.	2.808	2.630	2.298
8.	3.000	2.746	1.856
9.	3.100	2.980	2.652
10.	2.780	2.670	2.630
11.	2.899	2.740	2.452
12.	2.932	2.46	2.114
Mean ± SE	2.920 ± 0.089	2.704 ± 0.095	2.334 ± 0.126

Table 18. Globulin level (g/dl) in the control group of ducks (Group III)

Sl. No.	Fortnight														
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
1.	1.541	1.900	1.760	2.025	2.321	2.376	2.370	2.460							
2.	3.743	1.170	1.287	1.044	1.522	1.508	1.521	1.522							
3.	0.051	0.158	0.398	0.192	0.337	0.536	0.564	0.549							
4.	0.069	0.366	0.601	0.585	0.603	0.628	0.652	0.728							
5.	1.104	1.376	1.579	1.477	1.486	1.440	1.547	1.570							
6.	0.370	0.053	0.501	0.519	1.297	1.441	1.522	1.495							
7.	0.579	0.145	0.345	0.449	0.421	0.399	0.431	0.510	0.262	0.262	0.342	0.279	0.368	0.399	0.426
8.	1.250	1.145	1.055	1.938	1.480	1.573	1.491	1.528	1.714	1.726	1.858	1.897	1.897	1.959	1.960
9.	0.625	0.160	1.000	0.924	0.480	0.604	0.507	0.748	0.664	0.756	0.777	0.889	0.766	0.841	0.920
10.	0.168	0.128	0.651	0.233	0.243	0.265	0.276	0.316	0.880	0.869	0.943	0.980	1.392	0.430	1.432
11.	1.340	0.877	2.623	2.056	2.059	2.086	2.154	2.191	2.216	2.264	2.360	2.360	2.537	2.615	2.684
12.	0.689	0.306	1.037	1.084	0.899	0.832	0.449	0.865	0.898	0.924	0.927	2.036	1.148	1.247	1.345
Mean ± SE	0.775 ± 0.292							1.026 ± 0.198							1.461 ± 0.332

Table 19. Serum alanine aminotransferase level (IU/l) in aflatoxin fed ducks (Group I)

Sl. No.	Fortnight														
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
1.	10	15	14	12	13	16	18	20							
2.	12	14	12	14	14	15	20	16							
3.	19	13	13	16	12	17	23	22							
4.	13	16	12	13	11	10	20	13							
5.	16	18	14	10	10	16	32	20							
6.	17	17	15	9	13	18	28	26							
7.	14	13	13	8	12	20	24	24	26	28	30	28	30	31	32
8.	18	14	10	10	15	19	20	22	24	30	34	32	33	28	34
9.	13	12	9	9	14	17	22	20	28	36	36	36	38	36	32
10.	12	10	10	10	13	16	18	22	30	34	28	34	35	40	44
11.	15	11	14	11	12	18	22	24	32	28	30	33	34	32	33
12.	16	12	10	12	10	14	24	22	34	32	32	31	32	42	40
Mean ± SE	14.67 ± 0.782							22.33 ± 1.026							35.83 ± 2.039

Table 20. Serum alanine aminotransferase level (IU/l) in ducks fed clofibrate (Group II)

Sl. No.	Fortnight		
	I	II	III
1.	12	18	--
2.	13	17	--
3.	15	20	--
4.	16	21	--
5.	18	22	--
6.	16	26	--
7.	15	24	28
8.	17	26	30
9.	16	23	26
10.	17	25	27
11.	14	28	32
12.	15	26	28
Mean ± SE	15.67 ± 0.580	25.33 ± 1.00	28.50 ± 0.885

Table 21. Serum alanine aminotransferase level (IU/l) of ducks in the control group (Group III)

Sl. No.	Fortnight														
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
1.	12	15	12	13	15	14	15	14							
2.	13	16	10	16	13	13	16	13							
3.	8	13	11	14	14	16	13	12							
4.	10	18	16	16	12	15	14	11							
5.	10	19	18	18	13	13	13	10							
6.	19	10	17	13	11	14	12	16							
7.	15	11	14	12	12	12	10	14	10	11	12	16	13	14	15
8.	12	12	13	11	13	11	11	13	12	13	13	17	14	16	12
9.	13	13	12	12	16	12	17	12	13	14	14	10	16	17	18
10.	12	14	15	13	14	13	14	11	14	16	12	11	10	12	16*
11.	10	16	12	12	15	14	13	10	16	15	13	12	12	13	13
12.	11	12	14	14	12	10	12	13	17	18	15	14	13	10	12
Mean ± SE	12.17 ± 0.82							12.16 ± 0.514							14.33 ± 0.988

Table 22. Serum aspartate aminotransferase level (IU/l) in ducks fed aflatoxin (Group I)

Sl. No.	Fortnight														
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
1.	20	20	21	19	20	20	21	26							
2.	22	23	24	25	25	21	26	27							
3.	18	19	26	27	28	26	27	28							
4.	19	21	22	23	24	27	30	29							
5.	19	23	27	26	25	28	34	32							
6.	15	16	22	23	24	26	32	36							
7.	12	14	19	20	21	24	28	32	40	42	46	54	56	55	53
8.	13	17	20	21	22	23	27	34	33	38	48	45	46	44	42
9.	17	22	21	22	23	26	29	28	36	41	42	42	44	38	40
10.	21	21	22	23	22	23	30	36	40	44	42	44	48	44	45
11.	22	23	20	21	18	21	28	32	39	43	43	38	42	40	56
12.	26	25	23	24	17	20	29	34	40	52	50	52	51	52	55
Mean ± SE	18.50 ± 1.1569							32.67 ± 1.006							48.50 ± 2.86

Table 23. Serum aspartate aminotransferase level (IU/l) in ducks fed clofibrate (Group II)

Sl. No.	Fortnight		
	I	VIII	XV
1.	22	26	--
2.	24	28	--
3.	33	32	--
4.	28	34	--
5.	26	36	--
6.	25	31	--
7.	24	30	30
8.	30	32	36
9.	28	30	42
10.	32	36	40
11.	29	25	32
12.	26	22	36
Mean ± SE	28.17 ± 0.98	29.17 ± 1.248	36.0 ± 1.8629

Table 24. Serum aspartate aminotransferase level (IU/l) in control group of ducks (Group III)

	Fortnight														
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
1.	16	17	18	10	19	20	21	22							
2.	17	18	19	16	17	18	20	18							
3.	20	21	22	14	15	20	22	23							
4.	22	16	17	18	16	21	23	25							
5.	23	14	15	19	21	22	25	27							
6.	24	22	21	20	20	23	24	22							
7.	26	23	23	21	23	21	19	23	24	25	23	22	20	21	22
8.	28	18	24	22	29	20	21	20	21	22	19	22	23	24	23
9.	30	19	25	24	27	19	22	26	27	28	23	23	25	26	25
10.	20	24	21	20	28	29	23	25	26	18	24	26	27	28	29
11.	19	22	22	23	26	16	22	24	28	19	28	29	30	30	22
12.	18	23	19	22	25	14	19	23	24	27	26	27	28	29	30
Mean ± SE	23.50 ± 1.276							23.50 ± 0.742							25.17 ± 1.02°

Table 25. Serum alkaline phosphatase level (IU/l) in ducks fed aflatoxin (Group I)

Sl	Fortnight														
	I	II	III	VI	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
1.	36.52	45.65	45.65	45.65	45.65	54.78	54.78	73.04							
2.	45.65	36.52	45.65	54.78	54.78	54.78	63.91	73.04							
3.	36.52	45.65	36.52	63.91	63.91	54.78	73.04	82.17							
4.	45.65	36.52	45.65	54.78	54.78	63.91	82.17	91.30							
5.	36.52	45.65	54.78	45.65	54.78	73.04	91.30	100.40							
6.	54.78	54.78	45.65	36.52	45.65	63.91	73.04	73.04							
7.	45.65	63.91	54.78	45.65	54.78	73.04	82.17	82.17	91.30	82.17	100.40	91.30	100.43	109.56	109.56
8.	36.52	36.52	36.52	54.78	63.91	82.17	91.30	100.40	109.56	118.69	118.69	100.40	109.56	118.69	118.69
9.	54.78	54.78	36.52	54.78	63.91	73.04	63.91	73.04	91.30	109.56	100.40	109.56	118.69	109.56	109.56
10.	63.91	45.65	45.65	45.65	54.78	82.17	73.04	82.17	100.40	109.56	100.40	91.30	100.43	109.56	91.30
11.	36.52	45.65	36.52	36.52	54.78	63.91	73.04	82.17	109.56	109.56	109.56	100.40	109.56	118.69	109.56
12.	45.65	36.52	45.65	36.52	45.65	54.78	63.91	73.04	100.40	91.30	100.40	109.56	118.69	118.69	118.69
Mean ± SE	47.17 ± 2.626							82.17 ± 2.971							109.56 ± 4.083

Table 26. Serum alkaline phosphatase level (IU/l) in ducks fed clofibrate (Group II)

Sl. No.	Fortnight		
	I	II	III
1.	36.52	63.91	--
2.	45.65	54.78	--
3.	36.52	36.52	--
4.	54.78	63.91	--
5.	45.65	45.65	--
6.	54.78	63.91	--
7.	63.91	73.04	82.17
8.	54.78	63.91	82.17
9.	63.91	82.17	91.30
10.	45.65	54.78	63.91
11.	36.52	45.65	63.91
12.	54.78	63.91	73.04
Mean ± SE	53.26 ± 2.85	63.91 ± 3.64	76.08 ± 4.514

Table 27. Serum alkaline phosphatase level (IU/l) in the control group of ducks (Group III)

Sl.No.	Fortnight														
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
1.	36.52	45.65	36.52	45.65	36.52	45.65	36.52	45.65							
2.	36.52	36.52	45.65	54.78	45.65	54.78	45.65	36.52							
3.	45.65	45.65	36.52	45.65	54.78	36.52	45.65	54.78							
4.	36.52	36.52	45.65	36.52	45.65	36.52	54.78	54.78							
5.	45.65	45.65	36.52	54.78	45.65	45.65	54.78	36.52							
6.	36.52	36.52	45.65	36.52	45.65	54.78	45.65	63.91							
7.	45.65	45.65	36.52	45.65	54.78	45.65	36.52	45.65	45.65	36.52	45.65	36.52	36.52	36.52	36.52
8.	36.52	36.52	45.65	54.78	45.65	54.78	45.65	54.78	45.65	54.78	63.91	54.78	54.78	45.65	45.65
9.	45.65	45.65	36.52	36.52	45.65	54.78	54.78	45.65	54.78	45.65	63.91	63.91	54.78	54.78	54.78
10.	36.52	45.65	54.78	45.65	54.78	45.65	63.91	54.78	36.52	45.65	54.78	54.78	45.65	45.65	45.65
11.	45.65	36.52	36.52	54.78	45.65	36.52	45.65	36.52	45.65	54.78	63.91	45.65	54.78	36.52	36.52
12.	36.52	36.52	45.65	36.52	45.65	54.78	45.65	36.52	36.52	45.65	54.78	36.52	45.65	54.78	54.78
Mean ± SE	41.085 ± 1.357							45.65 ± 2.714							45.65 ± 3.334

Table 28. γ -glutamyl transpeptidase level (IU/l) in the ducks fed aflatoxin (Group I)

Sl. No.	Fortnight														
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
1.	3	3	2	3	4	5	6	8	10	11	8	10	12	13	14
2.	2	1	2	3	3	7	7	9	8	10	9	12	14	15	18
3.	3	7	3	1	4	6	8	6	7	12	13	17	18	19	20
4.	1	4	2	2	5	4	5	7	9	10	16	16	16	17	19
5.	2	3	1	3	3	5	6	6	8	6	15	14	15	16	21
6.	0	2	1	2	4	6	9	10	10	8	12	13	13	14	22
7.	3	0	3	2	3	7	8	8							
8.	2	3	2	1	5	8	9	9							
9.	3	2	3	3	3	5	10	10							
10.	1	1	2	2	4	6	6	6							
11.	4	2	3	1	2	4	7	8							
12.	2	3	2	2	5	5	8	9							
Mean $\pm$ SE	2.16 $\pm$ 1.02							8 $\pm$ 0.972							19 $\pm$ 1.32

Table 29. γ-glutamyl transpeptidase level (IU/l) in ducks fed clofibrate (Group II)

Sl. No.	Fortnight		
	I	VIII	XV
1.	3	4	8
2.	2	5	19
3.	4	6	12
4.	5	7	10
5.	6	8	15
6.	2	9	14
7.	1	4	
8.	0	6	
9.	2	2	
10.	3	4	
11.	0	5	
12.	2	7	
Mean $\pm$ SE	2.5 $\pm$ 0.761	5.58 $\pm$ 0.892	6.5 $\pm$ 1.02

Table 30. γ -glutamyl transpeptidase level (IU/l) in control group of ducks (Group III)

	Fortnight														
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
1.	2	1	2	1	3	1	2	3	5	2	2	3	2	3	4
2.	0	0	3	3	1	2	3	2	6	3	4	2	3	2	2
3.	4	3	4	0	7	3	2	1	3	4	3	4	2	1	0
4.	0	0	0	2	6	1	6	4	2	2	2	3	4	4	3
5.	2	3	6	3	4	0	3	2	4	1	3	2	2	6	4
6.	3	1	1	1	2	3	2	1	1	2	6	3	3	2	2
7.	4	0	0	2	3	2	1	2							
8.	0	0	0	2	2	1	2	3							
9.	0	4	2	3	4	3	3	2							
10.	9	1	3	3	5	1	0	1							
11.	9	4	1	1	4	2	2	0							
12.	3	2	2	3	3	3	3	2							
Mean $\pm$ SE	3 $\pm$ 0.981							1.91 $\pm$ 0.872						2.5 $\pm$ 0.864	

Table 31. Cholesterol level (m eq/dl) in ducks fed clofibrate (Group II)

Sl.No.	Fortnight		
	I	II	III
1.	188	150	--
2.	212	200	--
3.	230	180	--
4.	220	200	--
5.	250	195	--
6.	200	180	--
7.	210	160	140
8.	198	190	156
9.	187	178	145
10.	220	200	170
11.	210	190	160
12.	230	200	140
Mean ± SE	212.92 ± 1.12	185.25 ± 0.98	151.83 ± 1.20