PATHOLOGICAL EFFECTS OF AFLATOXICOSIS IN DUCKS

(Anas platyrhynchos domesticus)

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"PATHOLOGICAL EFFECTS OF AFLATOXICOSIS IN DUCKS"** (*Anas platyrhynchos domesticus*) is a bonafide record of research work done by me during the course of research and this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other university or society

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CERTIFICATE

Certified that this thesis entitled **"PATHOLOGICAL EFFECTS OF AFLATOXICOSIS IN DUCKS"** (*Anas platyrhynchos domesticus*) is a record of research work done independently by **Ms.LATHA.K** under my guidance and supervision and that this has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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Dedicated to
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and Guide

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INTRODUCTION

Ducks are one of the hardy birds of the Anatidae family, at a population of 24.0 millions second to chicken in the production of eggs and meat in India. They comprise 7 percent of the total poultry population and contributes 5 percent of egg output in the country . The largest number of ducks are found in West Bengal followed by Assam, Tamil Nadu, Kerala, Andhra Pradesh, Bihar and Orissa.

Kerala is ranked fourth in the duck population and has 0.85 million ducks (Anon,1994).Alleppey, Ernakulam, Kottayam and Pathanamthitta are the main duck rearing districts of Kerala. There are many farmers in Kerala with duck rearing as their main occupation and therefore duck rearing is one of the most important priority sector in Animal husbandary because of the hike in demands for duck egg and meat.

The management and feed cost of the duck is rated low as compared with poultry industry in this area, as they can thrive on forage and vegetation and does not require a pen for housing. Intensive rearing of ducks has emerged as an important occupation in modern poultry farming. Ducks under intensive system of management are exposed to many of the chemical and biological toxins, and their biological effects predisposes them to many disorders directly affecting their production potential. Though the ducks are comparatively quite hardy and resistant, few infectious diseases like duck plague, duck pox, duck virus hepatitis, duck influenza and toxicological disorders like aflatoxicosis cause considerable economic loss to duck farming.

One of the most limiting factor in poultry farming is mycotoxicosis as the ducks are highly susceptible to mycotoxins especially aflatoxins. Aflatoxicosis is caused by consumption of feeds spoiled by fungal growth. The quality of feed and feed ingredients plays an important role in determining the performance of birds and hence the whole profit margin of the industry. Presence of more than 12 per cent moisture in the feed favours fungus infection, mould growth and possible mycotoxin contamination. Mycotoxin contamination is more common in winter months and rainy seasons when feed is stored for more than two weeks in humid places. Feeds with high oil content are more prone to production of toxins.

Aflatoxins, one of the most potent mycotoxins, are the highly toxic metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*. This group of fungal toxins attracted the attention of all livestock entrepreneurs, agriculturists and scientists all over the world as a result of the toxic episodes of "Turkey -X disease " which occurred in 1960 in England causing death of about one million young turkey poults , due to the consumption of aflatoxin contaminated peanut meal (Asplin and Carnaghan, 1961). Aflatoxicosis can occur either in the acute fatal form or in the insidious form producing hepatic cirrhosis and tumours. Among poultry, ducklings are highly susceptible to aflatoxins followed by turkey poults, pheasant chick, chicken and quails. In India, heavy mortality occurred among ducklings in the Government duck farm at Niranam,Kerala state in 1965.

Aflatoxins are a group of toxic compounds produced by *Aspergillus flavus*.The various aflatoxin fractions are B_1, B_2, G_1, G_2, M_1, M_2 (Ayres, 1973). High temperature, humidity, unhygienic post harvest practices, storage conditions, unusual rains, sudden floods and cyclones etc., are conducive factors which favour invasion, contamination and proliferation of fungi and elaboration of aflatoxin.The toxic effects of aflatoxin vary depending upon the sex,age,breed and nutritional status of the birds. The biological effects of aflatoxin include suppression of immunity ,tumour formation, hepatosis,egg abnormalities,stunted growth, poor feed conversion,reduction in egg production, kidney dysfunction and mortality (Gopal *et al.* 1969 and Rao, 1986).

Hepatotoxic, carcinogenic and immunosuppressive effects of aflatoxins are also manifested in humans if they happen to consume aflatoxin contaminated milk,meat,eggs. etc., Animals and birds treated with antibiotics and other chemotherapeutic agents (e.g., Cortisones) are more susceptible to aflatoxins (Thaxton *et al.*1974,Wylliis and Morehouser, 1978 and Pier, 1980) .

Aflatoxins are harmful to the birds as they produce their biological effects both at low dietary levels ranging from 0.5 to 1.0 ppm. Very low levels cause drop in feed intake and feed efficiency. Varying levels of mycotoxins cause fatty degeneration of liver, hepatitis,nephritis and lesions of generalized toxaemia. Aflatoxins are hepatotoxic, mutagenic, carcinogenic and immunosuppressive to poultry. Depending upon the dose administered, the birds manifest peracute, acute, subacute and chronic forms of toxicity.

Ducks are more susceptible to aflatoxins than chickens due to the higher level of liver microsomal enzymes which metabolize aflatoxins into highly toxic metabolite like 2-8 epoxide,etc which ultimately lead to toxicity.

The liver is the major target organ for aflatoxins. The main site of action is the nucleolus where aflatoxin inhibits DNA dependent RNA polymerase leading to the inhibition of protein synthesis thereby enhancing susceptibility to various diseases. Aflatoxin reduces the activity of liver UDP glucose-glycogen transglycosylase resulting in the depletion of hepatic glycogen stores. It also inhibits synthesis of factor II & VII of prothrombin synthesis and clotting mechanism.

Aflatoxins constitute a major threat to the poultry industry. Ducks are extremely sensitive to the adverse effects of aflatoxin. Tolerance level of aflatoxin in the duck feed is fixed as 0.03ppm (Allocraft, 1969). Safe level prescribed vary from 0 to1000µg/kg for feed (Salunkhe *et al.*1987), but this wide margin is not suitable for the climatic condition prevailing in Kerala. Safe levels may vary with the different climatic conditions. The feed may contain various levels of aflatoxin and in tropical regions like Kerala with high temperature and humidity it may be difficult to get a feed without aflatoxin contamination. Therefore, it is imperative to recommend the permissible level of aflatoxin so that the feed manufacturers and farmers can have a guideline regarding the safe level of aflatoxin that can be permitted in the feed. So far there has not been any well documented study in this direction in this country.

Against this background, a project was drawn out with the following objectives taking ducks as a model;

1. Assessment of the pathological changes in ducks by feeding different levels of aflatoxin.
2. Determination of the permissible levels of aflatoxin in the ducks.
3. Evaluation of toxicity at various levels utilising sensitive tests such as serum enzyme estimation, hematology, histopathology and ultrastructural pathology of liver.

Review of Literature

REVIEW OF LITERATURE

Comprehensive brief review of the relevant literature on the species susceptibility, occurrence of aflatoxins in feeds, body weight , clinical signs, liver weight, haemogram, serum profiles, gross pathology, histopathology, ultrastructural pathology and permissible level studied have been gathered and presented in this chapter.

2.1 SPECIES SUSCEPTIBILITY

Among poultry, ducklings are reported to be most susceptible to aflatoxicosis followed by turkey poults, pheasant and chicken (Allocraft, 1965; Asplin and Carnaghan, 1961). Among breeds, there is a variation in the degree of susceptibility to aflatoxicosis. New Hampshire breed of chicken are more sensitive to aflatoxicosis (Brown and Abrams, 1965; and Gumbmann *et al.* 1970). The acute oral LD_{50} of aflatoxins in ducklings is 0.3mg/kg as compared with 6.3mg/kg in chicken (WHO,1979).

2.2 OCCURRENCE OF AFLATOXINS IN FEEDS

A large number of poultry feeds and feed ingredient samples like maize, groundnut cake etc., from Andhra Pradesh, Karnataka, Madhya Pradesh, Tamil Nadu, Punjab, Haryana and Uttar Pradesh have been analysed for aflatoxin. The aflatoxin content of the feeds and major feed ingredients vary from negligible to more than 1ppm (Neelakantan, 1980; Sastry,1982; Rao, 1982; Gopal *et al.* 1968; Kumar and Gowda, 1979 and Chopra, 1982). However, 30

to 35 per cent poultry feeds, 25 to 90 per cent of groundnut cake and about 20 to 38 per cent of other feed ingredients used in poultry rations are found to be contaminated, and toxin content of the majority feed samples ranges from 0.2 to 0.5 ppm of aflatoxin, and is higher in only a few samples.

Sudhakara Reddy (1982) analysed samples of groundnut cake, maize, bajra, broken rice and rice from feed godowns or factories in certain selected districts in Andhra Pradesh and found that groundnut cake contained more aflatoxin content than any other feed ingredients.

The presence of aflatoxin in poultry feed stuffs has been reported from various parts of India (Patel *et al.* 1981, Johri and Sadagopan, 1984, Reddy *et al.* 1984, Reddy *et al.* 1986, Johri *et al.* 1987). Sudharsan Singh and Singh (1987) recorded the overall incidence of aflatoxin in poultry feeds and ingredients as 30.76 per cent.

Selva Subramaniam *et al.* (1987) reported that more than 40 percent of feed samples contained moderate to high levels of aflatoxin. The levels ranged from 0.01 to 12 ppm.

Rajan *et al.* (1991) reported that 33.9 percent of the feed samples available in the Kerala market are aflatoxin contaminated and 16.2 percent feed samples had aflatoxin levels above 100 ppb.

2.3. EFFECT OF GRADED LEVELS OF AFLATOXINS ON BODY WEIGHT, FEED CONSUMPTION AND CARCASS WEIGHT

2.3.1 DUCKS

Butler (1964) observed that the loss in body weight of day old Khaki Campbell ducklings lasted for about two days by feeding 15μg aflatoxin orally. Subsequently he also observed that the birds regained their initial weight and continued to grow at the rate comparable to that of the control ducklings within three to four days

Leena Devi (1992) opined that the body weight of ducks were progressively reduced when the ducks were dosed with 0.04mg aflatoxin B_1/kg body weight for six months. Similar observation was also reported by Devegowda *et al.* (1994) in ducks when dosed with 100, 200 and 500 ppb of aflatoxin.

Vigil Anbiah (1996) reported that the ducks dosed with 10 and 15μg aflatoxin /kg body weight showed significant increase in their body weight from 15 to 120 days. Goswami and Mukit (1997) reported a significant decrease in weight gain in ducks when fed 10 and 20μg aflatoxin B_1/kg body weight for a period of three months.

Decreased body weight was recorded by George (1998) when ducks were dosed with 75μg aflatoxin /kg body weight for eight weeks.

2.3.2 OTHER AVIAN SPECIES

Smith and Hamilton (1970) stated that 2.5ppm (2500ppb) of aflatoxin could be considered as the growth inhibitory dose in chickens. Feeding broiler breeder roosters with a diet containing 20 ppm of aflatoxin for a period of four weeks (Wyatt *et al.* 1973. a), and at the rate of 2.5ppm (2500ppb) of aflatoxin and 5.0ppm (5000ppb) rubratoxin either separately or in combination resulted in a decrease in weight gain (Wyatt*et al* .1973. b)

Lanza *et al.* (1977) opined that feeding day old broiler chicks with 2.5 or 5.0 ppm aflatoxin (2500, 5000ppb) for two to three weeks and 5ppm (5000ppb) for 5 weeks led to a marked reduction in body weight.

Lanza *et al.* (1980) noticed that feeding of aflatoxin at the rate 2.5 or 5.0 mg from three to six weeks of age did not cause any significant reduction in the body weight. However, with higher concentration of aflatoxin from two to five weeks of age there was a reduction in the body weight.

Sharlin *et al.* (1980) reported a decrease in feed consumption and body weight in White Leghorn males when they were fed diet with 20 mμg aflatoxin/g of feed for a period of five weeks. Similarly decreased body weight was observed by Reddy (1981) at the dose rate of 0.75ppm (75ppb) in broiler chicken at four weeks of age.

Arafa *et al.* (1981) found that feeding gooslings with 0.07mg of aflatoxin/kg of feed (70ppb) caused some increase in body weight compared to the control birds. In quail chicks, they found that birds receiving a feed with 0.07mg of aflatoxin/kg of feed (70ppb), consumed less feed during the second

week of age. However, during the third week of age aflatoxin level of 2mg/kg(2000ppb) was found necessary to reduce the feed intake.

Campbell *et al.* (1981) noticed a decrease in the body weight in chicken at one week old by ochratoxin alone or in combination with aflatoxin. At two weeks, combined treatments depressed the growth and at third week, there was a significant interaction between aflatoxin and ochratoxin on the growth when fed a combination dose of 2.5ppm aflatoxin(2500ppb) and 2ppm ochratoxin(2000ppb).

Doerr *et al.* (1983) opined that dressed and chilled eviscerated carcass weight in broilers were decreased at all levels of aflatoxin 0.075,0.225 and 0.675μg/g of feed (75,225,675ppb) in trial1, whereas aflatoxin at a dose level of 2.7μg/g(2700ppb) significantly decreased the live and dressed weight in trial 2, while chilled eviscerated carcass weight decreased at dose levels of 0.3μg/g and 2.7μg/g (300 and2700ppb) of aflatoxin in trial 2. (Maurice *et al.* 1983) reported that aflatoxin B_1 (AFB_1) did not significantly alter body weight or feed intake when broiler chicks were dosed daily *per os* with 50 or 100 mg aflatoxin /kg body weight. Broiler chicken fed with the feed at the level of 2.5ppm(2500ppb) aflatoxin and 2.0ppm (2000ppb) ochratoxin A showed a significant decrease ($p<0.05$) in body weight (Huff *et al.* 1983).

Reddy and Yadgiri (1984) conducted experimental studies on broiler chicks by feeding diets containing 0.25,0.5,0.75 and 1.0mμg aflatoxin/g of diet for 28 days (experiment1) and 0.5, 0.75 and1.0mμg aflatoxin/g of feed for varying periods (experiment 2) and noted a significant reduction in the body

weight gain and feed consumption at and beyond 0.75mμg aflatoxin/g of diet after 28 days. Similar reductions in the body weight and feed conversion were also noted in turkeys and broiler chicken when dosed with 100,200,400 or 800mg aflatoxin /kg of feed for a period of five weeks (Giambroneet *al.* 1985).

Mashaly *et al.* (1986) opined that the body weight gain decreased in a dose dependant manner when broiler chicks were given 50 and 100μg aflatoxinB_1/kg feed for five weeks.

Huff *et al.* (1986)stated that the body weight was significantly decreased when 2.5μg aflatoxin /g of feed was given to young broiler chicken. Further they found that the body weight of broilers was significantly decreased when they were fed 5.0mμg aflatoxin /g of feed for six days and 2.5mμg aflatoxin /g of feed for 17 days.

Ghosh *et al.* (1989) recorded stunted growth, reduced feed consumption and decreased weight gain when chicken were fed with 0.3ppm(3000ppb) and1.0ppm(1000ppb) of aflatoxinB_1 for six weeks. Pearson *et al.* (1990) reported that addition of 2.5 and 5.0mg/kg of aflatoxin resulted in decreased body weight in broiler chicken.

Johri *et al.* (1990) observed that as aflatoxin intake increased, body weight gain decreased in broiler chickens when aflatoxin was given at the levels of 0.2, 0.3, 0.5 or 0.75 mg/kg of feed. A similar reduction in body weight of chicks were noted at the dose level of 0.3ppm (300ppb) and 1ppm (1000ppb) in the feed from day old to six weeks of age (Ghosh *and Chauhan*, 1991).

The growth inhibitory dose of aflatoxin in quails was estimated to be 0.5ppm (500ppb) (Kumar *et al.* 1993). Kubena *et al.* (1995) stated that the bodyweight gain was decreased when fumonisin B_1, aflatoxin or both were added. A similar reduction in the body weight was also observed by Rao *et al.* (1995).

2.4. CLINICAL SIGNS

2.4.1. DUCKS

Asplin and Carnaghan (1961) observed that the clinical signs of aflatoxicosis in ducklings were inappetance and poor growth rate with onset of mortality two weeks after commencement of the feeding of toxic groundnut meal.

Signs of aflatoxicosis in ducklings namely anorexia, poor growth rate, ataxia, diarrhoea, listlessness, unthriftiness, mucopurulent nasal discharge reduced feed intake and corneal opacity were recorded by many workers (Qin *et al.* 1983; Goswami and Mukit 1997; George 1998).

2.4.2. OTHER AVIAN SPECIES

Arshad *et al.* (1992) noted dullness, depression, anorexia, ruffled feathers, drooping of wings and variable degrees of diarrhoea when day old chicks were fed culture extracts of Aspergillus parasiticus containing aflatoxin at the levels of 637, 1274, 3720 and 5310ppb aflatoxin and similar findings were also reported by Chandrajit Singh *et al.* (1995).

2.5. WEIGHT OF LIVER

2.5.1. DUCKS

Devegowda (1994) reported that the relative weight of liver was increased in ducklings when they were fed aflatoxin at the dose rate of 100,200 and 500ppb.

Cheng Yeong Hsiang *et al.* (1995) observed that there was increase in the relative weight of liver of mule ducklings in aflatoxicosis.

2.5.2 OTHER AVIAN SPECIES

Feeding broiler breeder roosters with 20ppm (20000ppb) of aflatoxin for a period of four weeks resulted in an increase in liver weight (Wyatt *et al.* 1973.a). A similar increase in liver weight was also observed by a feed with 2.5ppm(2500ppb)aflatoxin by Doerr and Huff (1980).

In broiler chicken, liver weight and liver lipid content increased with an increase in dietary aflatoxin level and increased duration of the studies (Maurice *et al.* 1983; Reddy and Yadgiri, 1984).

Huff *et al.* (1986) described a decrease in the relative weight of the liver in chicken during the early stages of aflatoxicosis as an indication of hepatic atrophy. As aflatoxicosis progressed, hepatomegaly appeared due to lipid accumulation in the liver.

A similar increase in liver weight of broiler chicken was also reported by feeding aflatoxin at the levels of 2500 ,3000, and2000ppb by many other workers (Huff *et al.* 1988; Kubena *et al.* 1990; Kubena *et al.* 1993).

Fernandez *et al.* (1994) noted that the liver and kidney weight increased in hens by 2.5 and 5 parts aflatoxin /10^6 of feed in their diet for 4,8,16 or 32 days but not in broiler chickens. Norag *et al.* (1995) found that the relative weight of the liver and kidney increased in broiler chicken when dosed with 3.5 mg aflatoxin /kg(3500ppb).

2.6 HAEMOGRAM

Anaemia was observed as a salient feature of aflatoxicosis in ducklings and chickens (Brown and Abrams, 1965).

2.6.1 PACKED CELL VOLUME

2.6.1.1 DUCKS

Maryamma *et al.* (1990) reported that packed cell volume was diminished when ducks were fed with one ppm (1000ppb) of aflatoxin. In aflatoxin dosed ducks similar observation were also reported by Vigil Anbiah (1996) and George (1998) .

2.6.1.2. OTHER AVIAN SPECIES

Wyatt *et al.* (1975) reported a low packed cell volume by both dietary aflatoxin and *Eimeria tenella*. Lanza *et al.* (1977) found lowered packed cell volume when broiler chicken were dosed with 2.5 ppm (2500ppb) and 5 ppm aflatoxin (5000ppb).

Lanza *et al.* (1980) observed that the packed cell volume values were diminished from control values in a dose related manner by graded levels of aflatoxin when the commercial broiler chicken were fed dietary aflatoxin.

Comparable reduction in packed cell volume in broiler chicken was observed by many other workers also. (Doerr and Huff, 1980; Campbell *et al.* 1981; Chang and Hamilton, 1982; Reddy and Yadgiri, 1984).

Huff *et al.* (1986) observed a decreased packed cell volume in broiler chicken maintained with a feed containing 5.0mμg and 2.5mμg aflatoxin /g of feed for 21 days and similar findings were recorded by Singh *et al.* (1992) and Mani *et al.* (1993).

2.6.2 HAEMOGLOBIN (HB)

2.6.2.1. DUCKS

Brown and Abrams (1965) observed a moderate anaemia in ducklings and New Hampshire chicks dosed with 500ppb aflatoxin for six days. Comparable reduction in haemoglobin values was also observed by Mukit and Kwatra (1978) and Maryamma *et al.* (1990) in aflatoxin treated ducks.

Leena Devi (1992) investigated the effect of aflatoxin on haemoglobin concentration and found that haemoglobin concentration was reduced at third and sixth month when ducks were dosed with 0.04mg aflatoxin /kg bodyweight. Similar observation was also recorded by Vigil Anbiah (1996) at the dose level of 10 and 15μg aflatoxin/ kg body weight.

2.6.2.2 OTHER AVIAN SPECIES

Wyatt *et al.* (1975) found that the haemoglobin values were reduced by dietary aflatoxin and *Eimeria tenella*, but a combination of these resulted in a more severe reduction in haemoglobin concentration. Lanza *et al.* (1980)

stated that the haemoglobin values were diminished from the control values in a dose related manner when broiler chicken were fed dietary aflatoxin at dose levels of 1.25, 2.5 or 5mg/g of diet from 1 to 21days of age.

Decrease in haemoglobin values were observed in broiler chicken at a dose of 2.5ppm (2500ppb) aflatoxin(Doerr and Hamilton *et al.* 1981; Campbell *et al.* 1981).

Chang and Hamilton (1982) evaluated that the haemoglobin values were lowered by graded levels of dietary aflatoxin at doses of 0.625, 1.25, 5 and 10mμg/g of diet. Similar observation was also made by Reddy and Yadgiri (1984).

Huff *et al.* (1986) reported that the haemoglobin concentration was decreased by aflatoxin levels of 5.0mμg/g at 12 days and 2.5mμg/g at 21 days. Decreased haemoglobin values were also observed by Pearson *et al.* (1990) when broiler chickens were fed with 5ppm (5000ppb) of aflatoxin. Mani *et al.*(1993) observed that haemoglobin values were lowered in chicks dosed with 75ppb and 1500ppb aflatoxin for eight weeks. Balachandran and Ramakrishnan (1987.b) found that anaemia in aflatoxicosis was due to a combination of both hemorrhagic and hemolytic type, in treated birds.

2.6.3 ERYTHROCYTE SEDIMENTATION RATE (ESR)

2.6.3.1 DUCKS

Maryamma *et al.* (1990) observed an increase in the erythrocyte sedimentation rate (ESR) in ducks with one ppm (1000ppb) aflatoxin in the

feed. Similar increase in ESR was also observed by Leena Devi(1992) in aflatoxin dosed ducks. But George (1998) reported that there was no significant variation in ESR between aflatoxin dosed and control ducks.

2.6.3.2 OTHER AVIAN SPECIES

An increase in ESR was reported by Yaman *et al.* (1989) when chicks were fed 5mμg aflatoxin daily in the feed for two months and similar observation was observed in laying hens by Fernandez *et al.* (1995).

2.6.4 TOTAL ERYTHROCYTE COUNT (TEC)

2.6.4.1.DUCKS

A reduction in the total erythrocyte count was observed in aflatoxin dosed ducks by Mukit and Kwatra (1978) and Vigil Anbiah (1996).

George (1998) reported that the total erythrocyte count decreased in proportion to the duration of aflatoxin treatment.

2.6.4.2. OTHER AVIAN SPECIES

While studying the 'X' disease of birds, Wannop (1961) observed that a significant decrease in the erythrocyte count was a prominent feature of the disease. A reduction in the erythrocyte count was also observed by Tung *et al.* (1975) in an experimental study conducted in male broiler chicks fed with 0.625μg aflatoxin/g of diet (625ppb).

Reduction in the total leucocyte count in broilers fed aflatoxin contaminated diet was observed by many research workers (Lanza *et al.* 1980; Campbell *et al.* 1981; Singh *et al.*1992). Fernandez *et al.* (1995) observed that

the total erythrocyte count was decreased when broiler chicken and laying hens of 23 days age were fed 2.5 and 5mμg aflatoxin/kg of diet for 4,8,16 and 32 days.

2.6.5 TOTAL LEUCOCYTE COUNT AND DIFFERENTIAL COUNT (TLC & DC)

2.6.5.1. DUCKS

Leucocytosis with heterophilia and lymphopenia were observed in ducks in experimental aflatoxicosis by Mukit and Kwatra (1978) and Vigil Anbiah (1996).

Maryamma *et al.* (1990) reported that the total leucocyte count was increased when ducks were fed with one ppm (1000ppb) aflatoxin. George (1998) observed that the total leucocyte count significantly decreased with the duration of aflatoxin treatment.

2.6.5.2. OTHER AVIAN SPECIES

Wannop (1961) found that a marked increase in the leucocyte count in birds affected with 'X' disease was due to an increase in the number of heterophils and monocytes. The heterophil count and monocyte count were four and eight times higher in the affected ones than the healthy birds respectively . He reported a marked reduction in the lymphocyte count.

Tung *et al.* (1975) recorded a marked increase in the leucocyte number when the male broiler chicks were fed aflatoxin at the dose of 10μg/g(10000ppb) in the feed. An increase in the heterophils and a reduction

in the basophils were also observed .Heterophilia and lymphopenia in broiler chicks were observed by Campbell *et al.* (1981) and Sova *et al.* (1991).

2.7 SERUM CHEMISTRY

2.7.1 SERUM PROTEINS

2.7.1.1DUCKS

Leena Devi (1992) reported that the total serum protein was alternatively increased and decreased when ducks were dosed with 0.04mg aflatoxin /kg bodyweight for a period of six months. Vigil Anbiah (1996) reported a significant decrease in the total serum protein when ducks were dosed with 10 and 15µg aflatoxin/kg of body weight. Similar reduction was also reported by George (1998).

2.7.1.2. OTHER AVIAN SPECIES

Doerr and Huff (1980) investigated the effect of aflatoxin and ochratoxin on the total serum protein concentration and opined that 2.5ppm aflatoxin(2500ppb) and 2ppm ochratoxin(2000ppb) caused a reduction in the total serum total protein values in chicken. A reduction in total serum protein by aflatoxin treatment was also observed by Huff *et al.* (1981).

Reddy and Yadgiri (1984) in an experimental trial observed a decrease in the total protein values when broiler chickens were fed with a diet containing aflatoxin at the levels of 0.25, 0.5, 0.75 and 1.0mµg/g of feed for 28 days and 0.5, 0.75, 1.0mµg/g feed for varying periods.

Huff *et al.* (1986) stated that the serum concentration of total protein was reduced in broilers by 2.5μg aflatoxin. They also observed that the total serum protein was reduced by a feed containing 5.0 and 2.5mg aflatoxin /g of feed at three and six days of age respectively.

Reduction in the total serum protein levels in broiler chicken was also reported by many other researchers (Harvey *et al.* 1989; Kubena *et al.*1990; Johri *et al.* 1990; Giroir *et al.* 1991).

Raina *et al.* (1991) investigated the effect of aflatoxin on total serum protein and found that the toxin caused reduction in the total serum protein. Similar observation was also recorded by Stanley *et al.* (1993).

Mani *et al.* (1993) estimated total serum protein after feeding aflatoxin and opined that the protein concentration was lowered after feeding 0.75 and 1.5mg of aflatoxinB_1/kg of feed for eight weeks. Feeding broilers with 3 or 6ppm (3000 ,6000ppb) of aflatoxin for four to six weeks resulted in decreased total serum protein values (Jassar *et al.* 1993).

2.7.2 SERUM ALBUMIN AND GLOBULIN

2.7.2.1. DUCKS

Leena Devi (1992) reported that the albumin and globulin levels increased upto third month and thereafter decreased when ducks were dosed with 0.04 mg/kg bodyweight aflatoxin B_1 for six months. Similarly reduction in serum albumin and globulin values was also noted by Vigil Anbiah (1996).

George (1998) stated that there was a relative increase in the globulin levels of experimental ducks as the albumin values decreased when ducks were dosed with 75μg aflatoxin B_1/kg body weight.

2.7.2.2. OTHER AVIAN SPECIES

A reduction in the serum albumin level was recorded by Harvey *et al.* (1989) when broiler chicken were fed with aflatoxin B_1 at the dose level of 3mg/kg of feed.(3000ppb). Reduction in the serum albumin and globulin values in broiler chicken was observed by many other workers also (Ghosh *et al.* 1990; Raina *et al.*1991; Stanley *et al.* 1993; Shukla and Pachauri, 1995; Devurkar *et al.* 1995; Sandeep *et al.* 1996).

A reduction in the serum albumin was recorded by Kubena *et al.* (1997) when male broiler chicks were fed with 3.5mg(3500ppb) aflatoxin /kg of feed upto three weeks of age.

2.7.3 SERUM ENZYMOLOGY

2.7.3.1 SERUM GAMMA GLUTAMYL TRANSPEPTIDASE (GGT)

2.7.3.1.1 DUCKS

Leena Devi(1992) reported a significant increase in the GGT levels in ducks dosed with aflatoxin at the rate of 0.04mg/kg body weight for a period of six months.

2.7.3.1.2 OTHER SPECIES

Ideo *et al.* (1972) reported an increased level of serum -glutamyl transpeptidase in rats treated with 0.5m/CCl_4/kg. Rosalki (1975) studied the importance of GGT in liver disease and stated that GGT is the most sensitive enzyme in a variety of liver diseases.

Jalanko and Ruoslahti (1979) observed an increase in gamma- glutamyl transpeptidase level in induced hepatocarcinogenesis. Similar observations were also observed in hepatic diseases by Braun *et al.* (1987) and Picoux *et al.* (1987). Feeding chicken with aflatoxin contaminated feed resulted in increased serum GGT values (Fernandez *et al.*1994).

Mammen (1994) observed an increase in the serum GGT activity in rats treated separately with CCl_4 and NMU but in combination the activity increased upto nine months after which there was a decline.

2.7.4.1. SERUM ALKALINE PHOSPHATASE

2.7.4.1.1. DUCKS

An increase in the serum alkaline phosphatase was observed in ducks dosed with high levels of aflatoxin (Leena Devi, 1992, Goswami and Mukit, 1997).

2.7.4.1.2. OTHER SPECIES

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

in the serum alkaline phosphatase activity was observed in broiler chicken after aflatoxin treatment (Jassar *et al.* 1993)

Kumar *et al.* (1993) found an increase in the serum alkaline phosphatase levels when broiler quail chicks were fed aflatoxin at the rate of 0.5, 1.0, 1.5and 2.0ppm(500,1000,1500,2000ppb).

2.8.1. GROSS PATHOLOGY

2.8.1.1. DUCKS

Butler (1964) observed that after feeding 25,50 and 100μg of aflatoxin, the liver of ducklings were putty coloured with few small areas of haemorrhage without enlargement. The author also noticed that at a dose of 8.5μg, the liver was slightly paler than normal, but was free from haemorrhages and no gross lesions of the liver could be observed at dose levels of 5 or 1.5μ g.

Carnaghan (1965) noticed that the liver was putty coloured with numerous yellowish white lesions of 1 to 2mm diameter and solid yellow nodules with a number of small irregularly shaped necrotic lesions were also noticed in ducks at the dose level of 7ppm of aflatoxinB_1(7000PPB).

The liver was brown, small with greenish discoloration and granular surface in experimental aflatoxicosis in ducks (Muller *et al.* 1970).

Rajan *et al.* (1989) recorded a 40.5 per cent incidence of hepatosis and 4.83 per cent hepatic tumours, out of 1034 ducks examined at the University poultry and duck farm, Mannuthy. Maryamma *et al.* (1990) fed aflatoxin to ducks at the dose rate of one ppm (1000ppb) and found that the liver was pale

and friable with greyish white nodules of 0.5 to 1cm diameter present over the parenchyma. The gall bladder was found to be edematous and distended with dark green bile.

Leena Devi (1992) observed moderate to severe enlargement of liver with greyish white nodules of varying sizes in aflatoxin dosed ducks. Ducks dosed with 10 and 15μg aflatoxin/kg body weight revealed enlargement of liver bearing yellowish glistening nodules, hard in consistency and with mottled appearance(Vigil Anbiah, 1996). Similar lesion was also observed by George (1998).

2.8.1.2. OTHER AVIAN SPECIES

Moorthy *et al.* (1985) described that liver was pale yellow coloured with haemorrhages and nodules in chicken in experimental aflatoxicosis. Moorthy *et al.*(1986) when fed aflatoxin to chicken at the level of 6.25ppm (6250ppb) in the feed, died within three weeks and aflatoxin at the dose level of 3.12ppm,(3120ppb) in the feed produced nodular growth of 1 to 5mm diameter in the liver.

Balachandran and Ramakrishnan (1987.a) found that the liver became enlarged pale or discoloured with moroccan leather appearance in the first two weeks and yellow coloured in the next two weeks with one ppm(1000ppb) of aflatoxin in the feed. In the broiler chicken, aflatoxin at three ppm (3000ppb) level, from the first week onwards in addition to these lesions, liver was enlarged, soft and friable with yellowish discoloration. Rama Devi *et al.* (1990) fed aflatoxin to broilers at the dose rate of 2ppm for eight weeks and found

that liver was pale yellow and enlarged with petechial haemorrhages during the initial period of the experiment and during the later period, the changes were milder but greyish foci were seen. It was found that the liver was the first organ to exhibit morphological and pathological changes in aflatoxicosis(Singh *et al.*1993).

Arshad *et al.* (1992) conducted experimental studies on day old broiler chicks and observed hepatomegaly, mottling and ecchymotic haemorrhages on the liver. Kidney was found to be enlarged and congested with urate deposits in the ureter at the dose level of 637, 1274. 3720 and 5310ppb of aflatoxin.

Kumar *et al.* (1993) noted a pale and enlarged fatty liver with a distended gall bladder, when broiler quail chicks were fed with a feed containing 0.5, 1.0, 1.5 and 2.0ppm of aflatoxin for a period of 30 days.

Bakshi *et al.* (1994) observed haemorrhagic spots along with infarction on the liver when broiler chicken were fed with aflatoxin at the dose levels of 0.38ppb (380 ppb) and 0.75 ppm (750ppb) for six weeks. At 1.5 and 3ppm levels (1500 and 3000 ppb), liver exhibited enlargement, yellowish discoloration, petechial haemorrhage and fatty change.

2.8.2. OTHER ORGANS

Splenomegaly and pale enlarged and congested kidney with a few petechial haemorrhages were also observed in broiler chicken and ducks fed with aflatoxin (Muller *et al.* 1970; Balachandran and Ramakrishnan, 1987.a; Nageswara Rao *et al.* 1988).

Thaxton *et al.* (1974) reported atrophy of bursa and thymus in chicken fed high doses of aflatoxin. Pale kidneys, petechiae on the heart, splenomegaly and atrophy of the thymus and bursa of fabricius were found in chicken fed with one ppm (1000ppb) of aflatoxin B_1 in the feed (Ghosh *et al.* 1989).

Arshad *et al* . (1992) observed enlargement and congestion of kidney with deposition of urates in the ureter when broiler chicks were fed with high doses of aflatoxin. Enlargement of kidney and initial enlargement of bursa followed by atrophy were noticed in Japanese quail at 0.5ppm (500ppb)of aflatoxin (Sadana *et al.* 1992).

Kumar *et al.* (1993) noted enlarged kidney with petechial haemorrhages and atrophy of bursa in broiler quail chicks fed with graded levels of aflatoxin.

2.8.3. HISTOPATHOLOGY

2.8.3.1. DUCKS

Butler (1964) studied the effects of aflatoxin in Khaki Campbell ducklings and observed oval cell proliferation throughout the portal system with few mitosis by 24 hours at 15μg of aflatoxin. Moreover, there was fatty change at the periphery of the lobules where many of the cells had pyknotic nuclei. The author also observed that after two days, oval cell proliferation was more marked with the first extension into the lobules between the parenchymal cells.

Carnaghan (1965) studied the effects of aflatoxin contaminated Brazilian groundnut meal on the liver of ducks and observed lymphoid foci composing mainly of mature lymphocytes surrounded by degenerating hyperplastic bile duct epithelium. Even though mitotic figures were not observed, peripheral infiltration of lymphocytes between the hepatic cells was found.

Gardiner and Oldroyd (1965) stated that the liver showed mild to moderate megalocytosis, proliferation of small ductules accompanied by mild portal tract fibrosis, fatty infiltration of liver cells, scattered necrosis and haemorrhagic foci when ducklings were fed with extracts of peanut meals.Chronic hepatic fibrosis, hepatic regenerative nodules and bile duct hyperplasia were reported in ducks and ducklings due to sub-acute aflatoxicosis by Radeleff(1970).

Jayakumar *et al.* (1988) observed fatty change, hepatic necrosis, biliary hyperplasia and hepatocytomegaly in ducks administered 25μg aflatoxin per duck daily for 3 months.

Mukit and Kwatra (1989) recorded an incidence of aflatoxicosis among ducklings in three government duck breeding farms in Assam and the histopathological changes included degeneration and necrosis of hepatocytes with mild bile duct hyperplasia.

Maryamma *et al.* (1990) observed biliary hyperplasia, periportal necrosis of hepatocytes, hepatocytomegaly, hepatokaryomegaly and several

dissociated round cells with hyperchromatic nuclei when ducks were fed one ppm (1000ppb) of aflatoxin.

Seawright *et al.* (1993) administered 25 and 50 mg/kg body weight of aflatoxin B_1 to ducks for 20 months. The ducks receiving the toxin showed almost complete regression of the early acute lesion with no evidence of neoplasia. Ducklings fed with 5ppm (5000ppb) aflatoxin B_1 for a period of 14 days showed necrosis, fatty changes and biliary hyperplasia (Soni *et al.* 1993).

2.8.3.2. OTHER AVIAN SPECIES

Ching (1981) noted fatty liver syndrome and microscopically increased fatty change in hepatic cells of broiler chicken fed with increasing amount of aflatoxin. Bile duct proliferation, cellular necrosis, vacuolisation, congestion, fatty change, mild hepatitis, lipidosis, lymphocytic infiltration in portal areas, hypertrophy of Kupffer cells, sinusoidal dilatation, phlebosclerosis and fibroblastic proliferation were observed in broiler chicken treated with high doses of aflatoxin.(Deshek *et al.* 1983; Moorthy *et al.*1985; Moorthy *et al.* 1986; Ghosh *et al.* 1989).

Rama Devi *et al.* (1990) observed sinusoidal congestion, focal haemorrhages, hydropic changes, mild fatty changes, hepatocytomegaly and Kupffer cell hypertrophy during the initial half of the experiment but during the later period, lipidosis, lymphocytic infiltration around portal tracts, sinusoidal dilatation, villous appearance of bile duct, thickened blood vessels and focal areas of necrosis were observed with 2 ppm of aflatoxin in the feed of broiler chicken for a period of eight weeks.

Arshad *et al.* (1992) noticed congestion, fatty changes, necrosis, leucocytic infiltration and haemorrhages in the liver in experimental aflatoxicosis in chicken. Similar lesions were also reported in aflatoxicosis in chicken by Singh *et al.* (1993); Fernandez *et al.* (1994) and Kubena *et al.* (1995).

2.8.4. OTHER ORGANS

Nageswara Rao *et al.* (1988) observed occasional areas of lymphoid depletion in the follicles of the spleen and bursa of fabricius when chicken was fed with 500 and 1000ppb of aflatoxin contaminated feed.

Tubular degeneration of kidney, swelling of tubular epithelium and mild fibroblastic proliferation with cellular infiltration in the interstitium were observed in chicken fed with high doses of aflatoxin (Balachandran and Ramakrishnan 1987.a; Johri *et al.* 1990)

George (1998) dosed ducks with 75μg / kg body weight of aflatoxin and studied the histopathological lesions which included congestion, mild lymphoid depletion in the spleen, shrinkage of the lymphoid follicles, focal necrosis of the lymphoid tissues, mild to moderate interfollicular edema and severe depletion of the lymphoid cells in the bursa. Similar lesions were also observed by Suresh (1999).

2.8.5. ULTRASTRUCTURAL PATHOLOGY OF LIVER

Novi (1977) conducted experimental study in female rats fed with 25g aflatoxin B_1 for a period of eight weeks and the ultrastrucural changes noted

were proliferation of smooth endoplasmic reticulum, dilatation of rough endoplasmic reticulum, increased number of microbodies, mitochondrial swelling with loss of granules and cristolysis, focal disruption and fragmentation of the nuclear membrane and the nucleolus and dilatation of secretory vesicles.

Male broiler chicks fed with 1250,2500 and5000ppb of aflatoxin for 21 days revealed hepatocellular lipidosis, enlargement of bile canaliculi and reduction in mitochondrial size.(Mollenhauer *et al.* 1989).

George (1998) conducted experimental study in the ducks and observed loss of microvilli, separation of desmosomes, appearance of lipid droplets and degranulation of rough endoplasmic reticulum (RER) in the hepatocytes. The hepatocyte nucleus became irregular with increase in the heterochromatin clumps, fragmentation of smooth endoplasmic reticulum and rough endoplasmic reticulum, dilatation of the nuclear envelop and nucleoli in many of the hepatocytes and disappearance of the nucleus. Mitochondria lost their cristae and appeared swollen.

2.9.1. TOXIC RESIDUES IN TISSUES

Blaha (1982) found residues of aflatoxin in liver, but not in muscle when broiler chickens were administered 340mμg aflatoxin for a period of 42 days.

In an experiment, with a group of chicken that received a feed containing 5510ppb of aflatoxin, the residues of toxin in the liver ranged from

1.32 to 4.95 ppb ,the highest being after eight weeks of feeding (Arshad *et al.* 1993).

Chao and Liu (1988) reported residues of aflatoxin B_1 in the liver and muscle of chicken and ducks fed with different dietary levels of aflatoxin for eight to nine weeks.

Maryamma *et al.* (1992) observed residues of aflatoxin B_1, G_1 and M_1 in stomach, liver, kidney and skeletal muscle of pigs, ducks and chicks in varying quantities.

2.9.2. PERMISSIBLE LEVEL OF AFLATOXIN

Tolerance level of aflatoxin in duck feed is fixed as 0.03 ppm (30ppb). (Allocraft,1969). Salunkhe *et al.* (1987) reported that safe level of animal feed may vary from 0 to 1000μg /kg of feed.

European economic committee (EEC) has recommended the safe limit as 20ppb (Salunkhe *et al.* 1987) in chicken. Permissible level of aflatoxin in ducks is fixed as 10 ppb (Package of practices,1994).

Materials and Methods

MATERIALS AND METHODS

3.1 EXPERMENTAL BIRDS

One hundred and twenty, four months old clinically healthy male desi ducks procured locally from Mannuthy were used for the study. These ducks were randomly divided into five groups of twenty four birds each.

3.2 EXPERIMENTAL FEED

The birds were maintained in separate compartments and control ducks were given commercial duck feed tested and found free of aflatoxin for a period of eight months.Water was provided *ad lib*.

3.3 EXPERIMENTAL DESIGN

Group I - Ducks of the control group were given commercial duck feed tested and found free of aflatoxin.

The experimental feed contaminated with aflatoxin were procured from market and the toxin levels of feed were estimated by Modified Pons method (Pons1966) and toxin levels were adjusted to the following levels with control feed :

Groups	Aflatoxin content of feed (ppb)
I	0
II	5
III	10
IV	20
V	40

3.4 EXPERIMENTAL PARAMETERS

The following parameters were studied

1. Body weight
2. Weight of the carcass
3. Weight of the liver
4. Haemogram values (erythrocyte sedimentation rate, packed cell volume,haemoglobin, total erythrocyte count, total leucocyte count and differential count).
5. Serum total protein ,albumin ,globulin and albumin /globulin ratio.
6. Serum alkaline phosphatase activity.
7. Serum glutamyl transpeptidase activity.
8. Gross and histopathology of the liver,kidney and spleen.
9. Ultrastructural pathology of liver
10. Estimation of residual aflatoxin level in the liver,kidney,muscle and blood.

3.5 TECHNIQUES

3.5.1 Clinical Symptoms

The ducks were observed daily for clinical symptoms if any, and recorded.

3.5.2 Body Weight

Initial body weight was recorded at the age of four months and thereafter at monthly intervals.

3.5.3 Collection Of Blood Samples For Laboratory Tests

Blood samples were collected during disposal of ducks from meat technology unit at two months intervals. Before bleeding, the body weight was recorded and blood smears from the peripheral blood were made. Five millilitres of blood were collected for the haematological studies using dipotassium salt of ethylene diaminetetra acetic acid (EDTA) as the anticoagulant at the rate of 1mg/ml of blood.

3.5.4. Separation of serum

The blood collected from the birds individually was left in the test tubes for one hour at room temperature, then kept at 4°C for six hours for contraction of the clot and the separated serum was collected in sterile vials and stored at -20°C until used.

3.5.5 Haemogram

Erythrocyte sedimentation rate and packed cell volume were estimated on second, fourth, sixth and eighth month of the experiment using the method described by Wintrobe (1981).

Haemoglobin level was estimated on second, fourth, sixth and eighth month employing the cyanmethoglobin method described by Miale (1967) and

the final readings were taken in an Erma photometer with Haemochek(R) solution of Agappe Diagnostics Pvt. Ltd.

Total erythrocyte count (TEC) and total leucocyte count (TLC) were determined as per the method described by Sastry (1976). The differential leucocyte count (DLC) was done with copper peroxidase method of Sato and Sekiya (1965).

3.6 SERUM CHEMISTRY

3.6.1 Total Serum Protein ,Albumin And Globulin

The values were taken at the second, fourth, sixth and eighth month of the study. The Biuret assay method of Inchiosa(1964) was adopted for the estimation of total protein. The albumin content in the serum was estimated by the Bromo cresol green (BCG) dye binding method described by Doumas *et al.* (1971). By deducting serum albumin content from total serum protein, serum globulin value was determined.

3.6.2 Serum Alkaline Phosphatase

This was estimated by employing commercially available kits (E.Merck -(India) Ltd.) and final readings were taken spectrophotometrically at the second, fourth, sixth and eighth month of the experiment.

3.6.3 Serum Gamma - Glutamyl Transpeptidase

This was estimated employing commercially available kits (Sigma Diagnostics (γ-GT) procedure NO.419) and final readings were taken using

spectrophotometer at the second, fourth, sixth and eighth month of the experiment.

3.7 LIVER WEIGHT

The weight of liver was recorded at the second, fourth, sixth and eighth month of the experiment.

3.8 GROSS PATHOLOGY

Six birds from each of the groups at the second month ,fourth month, sixth month and eighth month of experiment were disposed to the meat technology unit and subjected to detailed autopsy. Gross lesions of various organs of dead birds as well as experimental birds were noted and the liver weight were recorded. Relevant materials were procured from the unit for haematological, serological and histopathological studies. Detailed autopsy act was carried out as per the procedure advocated by **FAO/SIDA (1968)**.

3.9 HISTOPATHOLOGY

The liver, spleen, kidney and bursae were collected in 10 percent neutral buffered formalin for histopathological studies.Tissues were processed by the routine paraffin embedding technique (Armed Forces Institute Of Pathology,1968). Paraffin sections cut at five microns thickness were stained with haematoxlin and eosin (H&E) method as described by Sheehan and Hrapchack (1980).

3.10 ULTRASTRUCTURAL STUDIES

The glutraldehyde fixed liver tissues were washed three times in phosphate buffer (PH 7.4) and post fixed in one per cent buffered osmium tetroxide (Sigma, USA) at 4°C for two hours. They were then dehydrated in graded acetone and embedded in Spurr low viscosity resin (Blo, Rad, Microsciences division, USA). Sections were cut on a Reichter Jung Ultracut - R microtome. Ultrathin sections were taken on copper grids and stained with uranyl acetate and lead citrate (both Sigma products, USA) and examined in an electron microscope (Hitachl-600A) at 75KV and the electron micrographs were taken.

3.11 AFLATOXIN RESIDUES IN TISSUES

Toxin residues in muscle, liver and kidney were determined by modified Pons method (Pons *et al.* 1966) using thin layer chromatography (TLC). For quantification, the plate was scanned fluorimetrically using Hitachi - 3000 model computerized fluroscence spectrophotometer at a wavelength of excitation 365mm and emission 420mm.

Results obtained from the TLC scanning were randomly checked by analysing the samples with high performance liquid chromatography (HPLC) using pump Hitachi L-7100 model (Merck Pvt. Ltd.). For this, the extract from the Pon's method was further purified through column chromatography using chloroform as the eluting solvent. The aliquot from this purified extract were applied onto C-18 column. Methanol, acetonitrile and water (23:22:55) system

were used as the mobile phase. Aflatoxin content in the effluent was scanned by fluroscent spectrophotometer with computer attachment.

3.12 STASTICAL ANALYSIS

Stastical analysis of the data obtained during the course of the studies was carried out according to Snedecor and Cochran (1985).

Results

RESULTS

4.1 BODY WEIGHT

The mean body weights of both control and aflatoxin treated ducks at monthly intervals are furnished in Table.1 and graphically presented in Fig.1.

There was significant ($P<0.05$) growth inhibition at the first month and from the second month to eighth month ($P<0.01$) as compared with that of the controls in the aflatoxin treated groups (Groups II, III, IV and V). No significant difference could be detected in the mean body weight among groups II, III, IV and V at the first month.

At the second month of the trial, the mean body weights did not differ significantly between groups II and III and also between groups IV and V, but all the treatment groups showed significant reduction from that of the control group.

By the third and fourth month of the experiment, ducks in all the treatment groups showed significant reduction in the mean body weight from that of the control birds. Besides, significant difference could be noted between the treatment groups also. Similarly by the fifth month of the trial, birds from all the treatment groups (II, III, IV and V) showed significant depression in the mean body weight from that of the control group birds but groups II and III did not reveal any significant difference between them.

During the sixth month of the trial, birds from the control group and the experimental group II did not show any significant difference. Similarly groups

Table 1. Average (Mean ± SE) Body Weight of Ducks at Monthly Intervals

Treatment groups	Toxin (ppb) level	Observation period in months							
		1	2	3	4	5	6	7	8
Group I (control)	0	1116.00 ± 16.48^{a}	1296.667 ± 15.02^{a}	13.97.5 ± 13.66^{a}	14.88.33 ± 20.08^{a}	1654.444 ± 3153^{a}	1647.778 ± 34.83^{a}	1753.333 ± 32.95^{a}	1810 ± 22.75^{a}
Group II	5	1080.63 ± 5.48^{b}	1216.875 ± 16.88^{b}	1316.05 ± 14.76^{b}	1417.37 ± 20.05^{b}	1472.69 ± 14.75^{b}	1593.077 ± 33.94^{a}	1662.857 ± 35.66^{ab}	1732.86 ± 17.11^{b}
Group III	10	1061.4 ± 8.06^{b}	1186.4 ± 15.09^{b}	1276.58 ± 10.96^{c}	1309.74 ± 13.83^{c}	1455 ± 18.11^{b}	1457.69 ± 37.99^{b}	1555.714 ± 31.49^{bc}	1557.143 ± 31.62^{b}
Group IV	20	10.78.4 ± 11.07^{b}	1070.2 ± 6.14^{c}	1106.16 ± 11.1^{d}	1278.947 ± 1344^{d}	1375.39 ± 28.12^{c}	1442.31 ± 38.85^{b}	14.71.43 ± 57.2^{cd}	1494.29 ± 46.92^{c}
Group V	40	1085.6 ± 10.03^{b}	1044.8 ± 6.27^{c}	1067.1 ± 9.47^{e}	1170 ± 1465^{e}	1250.714 ± 24.76^{d}	1412.857 ± 14.35^{c}	1414.29 ± 44.69^{d}	1455.714 ± 22.52^{d}
F-value		3.155*	62.149**	130.271**	53.511**	35.263**	8.439**	10.379**	24.599**
CD (1,2)		30.04	37.92	36.19	49.11	70.59	101.59	125.04	90.70
CD (2,3)		25.97	32.79	32.57	44.19	62.88	90.51	120.04	87.07

CD (1,2) for comparison of control with other treatments
CD (2,3) for comparison of other treatments
Means bearing the same superscripts are not significantly different
** P<0.01 * P<0.05

Fig.1 : Average Monthly Body Weight of Ducks (g)

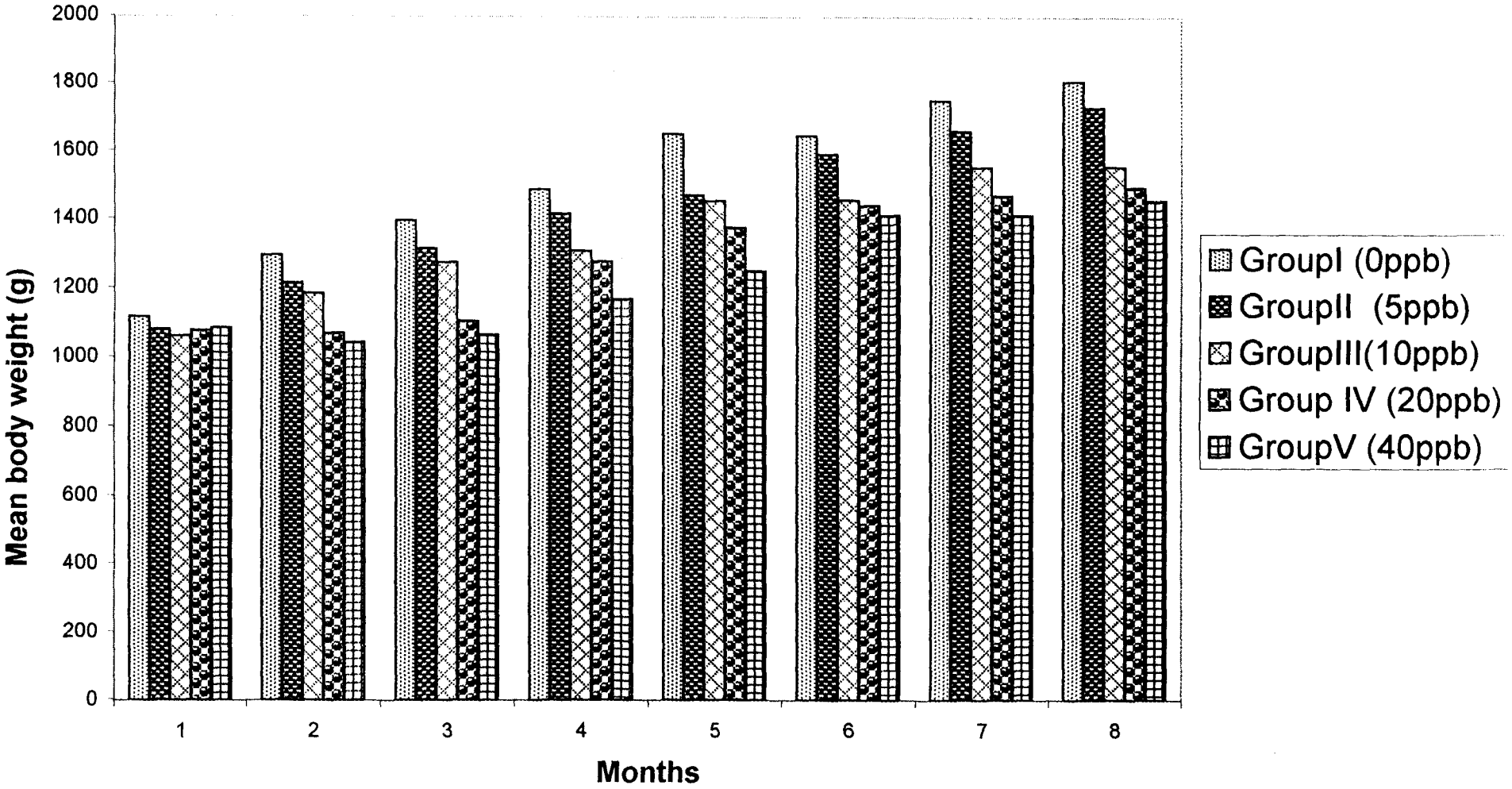
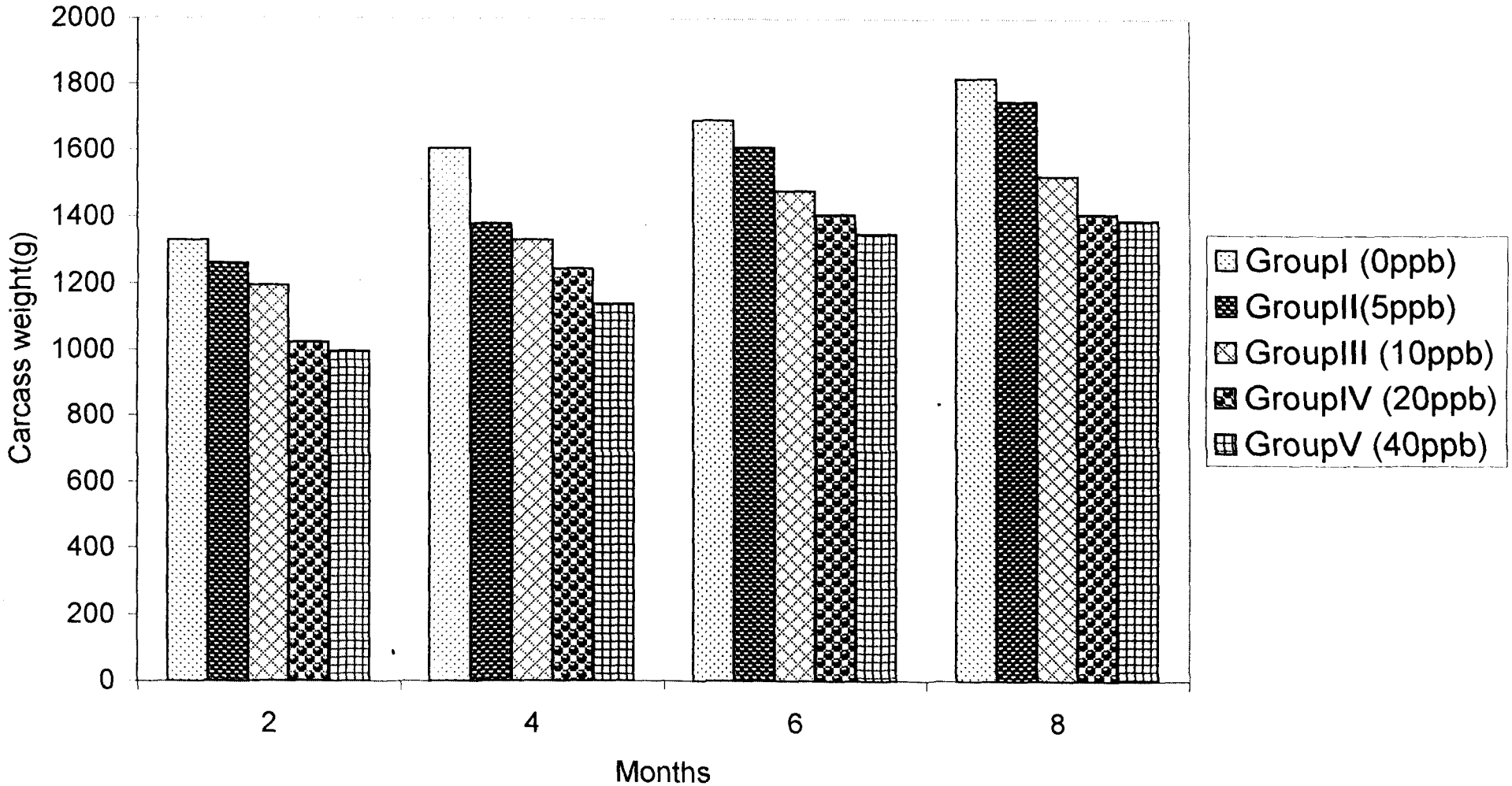


Table .2 Average (Mean ± SE) Carcass Weight (control and treated ducks)

Treatment groups	Toxin level (ppb)	Observation period in months			
		2	4	6	8
Group I (control)	0	1330.000 ± 30.03[a]	1606.667 ± 23.35[a]	1690.000±23.45	1820±19.5
Group II	5	1261.667 ± 19.11[b]	1381.667 ± 18.40[b]	1608.333±15.4	1750.333±20.53
Group III	10	1194.167 ± 16.41[c]	1333.333 ± 15.47[c]	1478.333±14.17	1523.523±11.25
Group IV	20	1024.167 ± 11.02[d]	1246.667 ± 44.94[d]	1406.667±42.95	1409.667±40.95
Group V	40	996.667 ± 18.45[d]	1140.000 ± 55.29[e]	1350.000±56.20	1390.000±49.25
F-value		60.452**	15.878**	NS	NS
CD (1,2)		60.996	129.52	-	-
CD(2,3)		49.803	105.75	-	-

CD (1,2) for comparison of control with other treatment
CD (2,3) for comparison of other treatments
Mean bearing the same superscript are not significantly different
** P < 0.01

Fig. 2. Average Carcass Weight(g)



III and IV did not show any significant difference between them but group V birds showed significant difference in the mean body weight from that of the control group and other treatment groups.

By the seventh month of the trial, the control and group II did not reveal any significant difference between them. Statistical analysis done did not reveal any difference between groups II and III, between III and IV and between IV and V.

At the eighth month of the study, the mean body weight of birds of groups I and II did not differ significantly from each other. Similarly there was also no difference in the mean body weight between groups III and IV as well as between groups IV and V, although all the treatment groups with the exception of group II showed significant reduction in the mean body weight from that of the control birds.

4.2 CARCASS WEIGHT

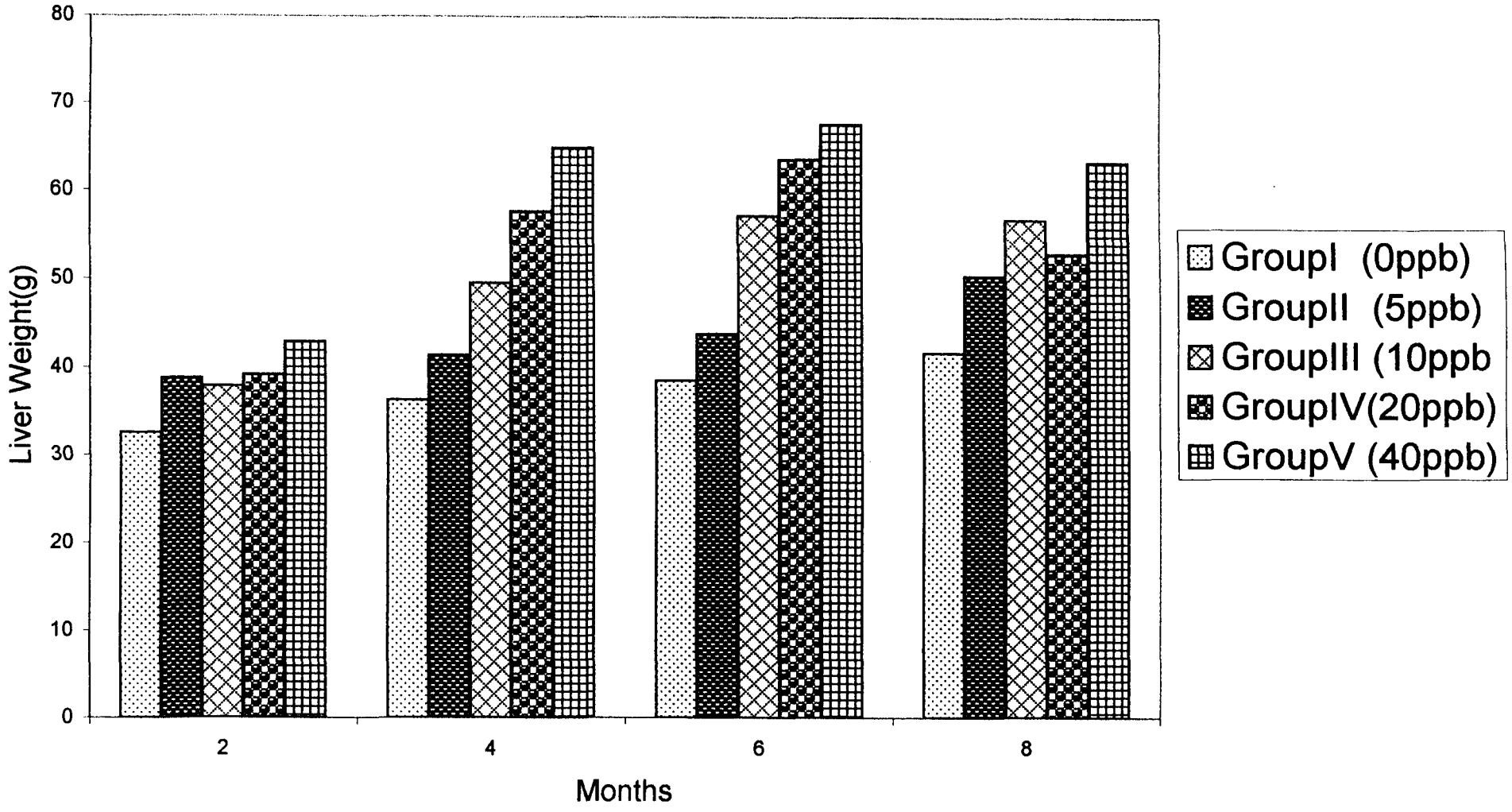
Table. 2 and Fig.2 depicts the mean carcass weight of ducks during the experimental period. The mean carcass weights of the aflatoxin treated ducks (Groups II, III, IV and V) were significantly ($p < 0.01$) reduced during the second and fourth month of the experimental trial as compared with that of the control in a dose related manner. However, there was no significant variation in the carcass weight by the sixth and eighth month. All the treatment groups showed apparent reduction in the body weight as compared with that of the control group.

Table .3 Average (Mean ± SE) of Liver Weight (control and treated ducks) observation period in months

Treatment groups	Toxin level (ppb)	Observation period in months			
		2	4	6	8
Group I (control)	0	32.571 ± 1.27 [a]	36.31 ± 0.73 [a]	38.643 ± 1.04 [a]	41.743 ± 1.97 [a]
Group II	5	38.833 ± 0.91 [b]	41.415 ± 1.55 [a]	43.904 ± 1.60 [a]	40.541 241 [b]
Group III	10	37.937 ± 2.20 [b]	49.659 ± 2.35 [b]	57.326 ± 3.33 [b]	56.920 ± 5.68 [bc]
Group IV	20	39.238 ± 2.25 [b]	57.753 ± 3.69 [c]	63.698 ± 2.91 [bc]	53.029 ± 3.34 [cd]
Group V	40	42.922 ± 1.31 [b]	65.012 ± 1.98 [d]	67.732 ± 1.68 [c]	63.429 ± 3.41 [d]
F-value		3.212*	19.080**	21.753**	4.377**
CD (1,2)		6.054	8.559	8.487	13.16
CD(2,3)		4.993	7.059	7.0001	10.74

CD (1,2) for comparison of control with other treatment
CD (2,3) for comparison of other treatments
Mean bearing the same superscript are not significantly different
* $P < 0.05$ ** $P < 0.01$

Fig. 3. Average Liver Weight (g)



4.3 WEIGHT OF THE LIVER

The mean liver weight of the aflatoxin treated ducks and control ducks are presented in Table. 3 and Fig. 3.

By the second month of the trial, ducks belonging to groups II, III, IV and V showed a significant ($p < 0.05$) increase in the liver weight from that of the control group but there was no significant difference in the mean liver weight among the experimental groups.

There was a significant ($p < 0.01$) increase in the liver weight of aflatoxin treated birds (Groups II, III, IV and V) during the fourth, sixth and eighth month of the study from that of the control birds. During the fourth month of the trial, the values of all the treatment groups were significantly different from the control and the experimental group II. However, the values of the control group (Group I) and the experimental group (Group II) did not show significant difference between them.

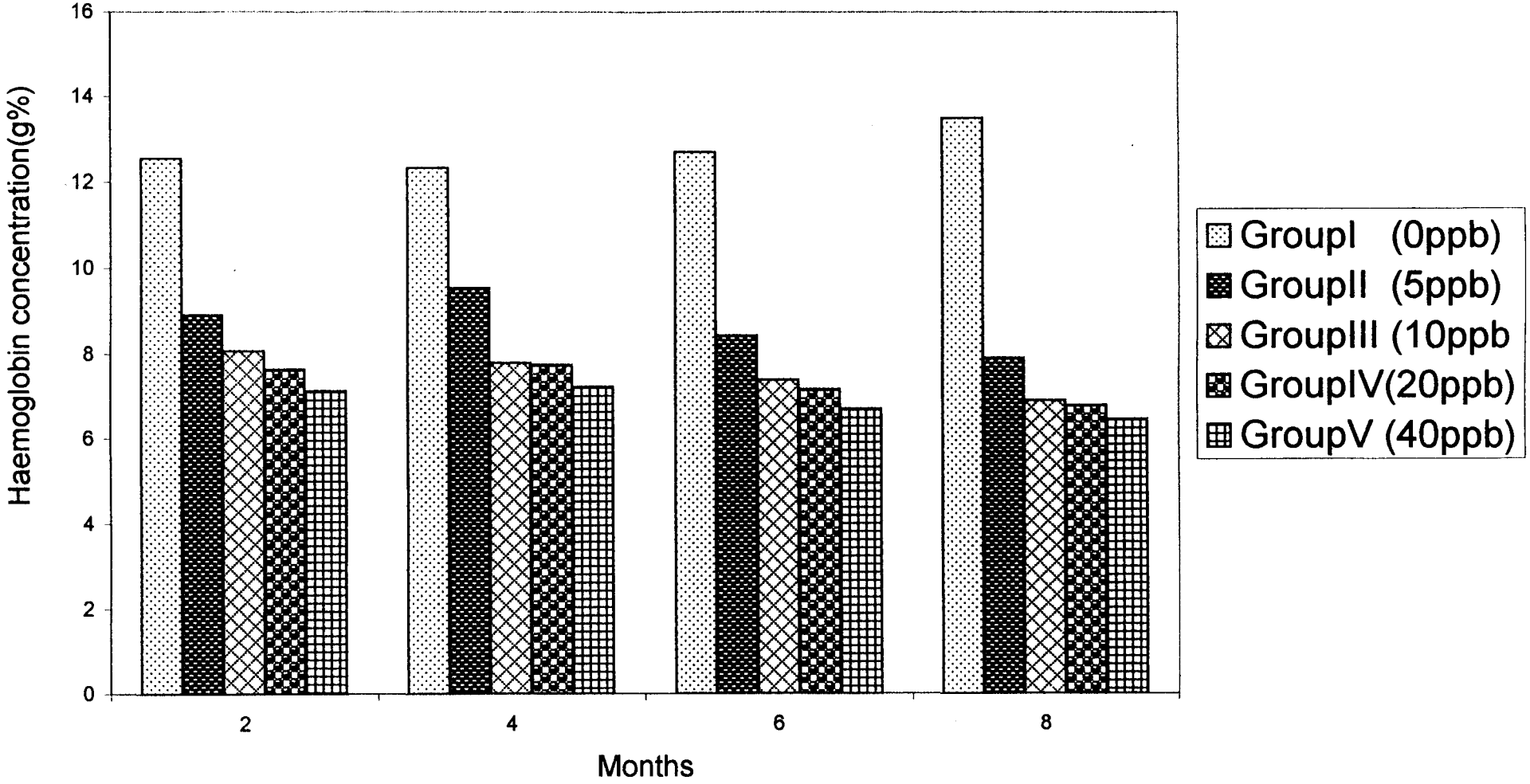
During the sixth month of the trial, the weights of the liver of groups I and II were not significantly different. There were no significant difference between the treatment groups III and IV as well as between IV and V. Similarly the mean liver weight did not differ significantly between the groups I and II, II and III, III and IV as well as IV and V by the eighth month of the trial.

Table. 4 Average (Mean ± SE) Haemoglobin Concentration (g%)

Treatment groups	Toxin level (ppb)	Observation period in months			
		2	4	6	8
Group I (control)	0	12.533 ± 0.6 [a]	12.333 ± 0.6 [a]	12.7 ± 0.47 [a]	13.47 ± 0.24 [a]
Group II	5	8.9 ± 0.54 [b]	9.533 ± 3.90 [b]	8.417 ± 0.57 [b]	7.883 ± 0.31 [b]
Group III	10	8.067 ± 0.32 [ab]	7.783 ± 3.19 [c]	7.367 ± 0.24 [c]	6.883 ± 0.14 [c]
Group IV	20	7.633 ± 0.25 [b]	7.733 ± 0.12 [c]	7.15 ± 0.09 [c]	6.767 ± 0.18 [c]
Group V	40	7.117 ± 0.31 [b]	7.217 ± 0.21 [c]	6.683 ▯1 0.18 [c]	6.417 ± 0.17 [c]
F-value		19.515**	38.630**	32.699**	109.79**
CD (1,2)		1.343	0.9553	1.668	0.7634
CD(2,3)		1.107	0.7879	0.9624	0.6233

CD (1,2) for comparison of control with other treatment
CD (2,3) for comparison of other treatments
Mean bearing the same superscript are not significantly different
** P < 0.01

Fig. 4. Average Haemoglobin Concentration(g%)



4.4 HAEMOGRAM

4.4.1 Haemoglobin

The mean haemoglobin concentration values of the different groups are shown in Table. 4 and Fig. 4.

The aflatoxin treatment, however, resulted in a significant ($P<0.01$) reduction in the haemoglobin values in all the groups (Groups II, III, IV and V) as compared with that of the control group throughout the experimental period. During the second month of the experiment, haemoglobin values of ducks belonging to groups II, III, IV and V were significantly different from that of the control. But groups II and III revealed no significant difference between them. Similarly the mean haemoglobin concentration did not differ significantly among the treatment groups III, IV and V.

By the fourth, sixth and eighth months of the trial, all the treatment groups showed significant reduction in the mean haemoglobin concentration from that of the control group. However, the haemoglobin concentration of the ducks in the treatment groups III, IV and V did not differ significantly from each other.

4.4.2 Packed Cell Volume

Data on the effect of aflatoxin on the packed cell volume are presented in Table. 5 and Fig. 5.

The aflatoxin treatment resulted in a significant ($P<0.01$) decrease in the packed cell volume of the experimental groups (Groups II, III, IV and V) in

Table .5 Average (Mean ± SE) Packed Cell Volume (%)

Treatment groups	Toxin level (ppb)	Observation period in months			
		2	4	6	8
Group I (control)	0	41.333 ± 0.66 [a]	42.000 ± 1.73 [a]	42.333 ± 1.76 [a]	45.000 ± 1.15 [a]
Group II	5	38.167 ± 1.58 [ab]	37.833 ± 1.20 [ab]	35.833 ± 1.33 [b]	33.333 ± 1.34 [b]
Group III	10	34.333 ± 1.23 [bc]	33.677 ± 2.05 [bc]	30.833 ± 1.74 [c]	28.500 ± 1.73 [c]
Group IV	20	33.500 ± 1.41 [c]	30.167 ± 3.18 [c]	28.667 ± 2.32 [cd]	26.30 ± 1.72 [cd]
Group V	40	30.833 ± 1.87 [c]	25.500 ± 2.10 [c]	25.833 ± 1.52 [d]	23.000 ± 1.31 [d]
F-value		5.762**	6.837**	9.918**	21.586**
CD (1,2)		5.245	7.7102	6.1322	5.332
CD(2,3)		4.326	6.3593	5.0577	4.353

CD (1,2) for comparison of control with other treatment
CD (2,3) for comparison of other treatments
Mean bearing the same superscript are not significantly different
** $P < 0.01$

Fig. 5. Average Packed cell volume(%)

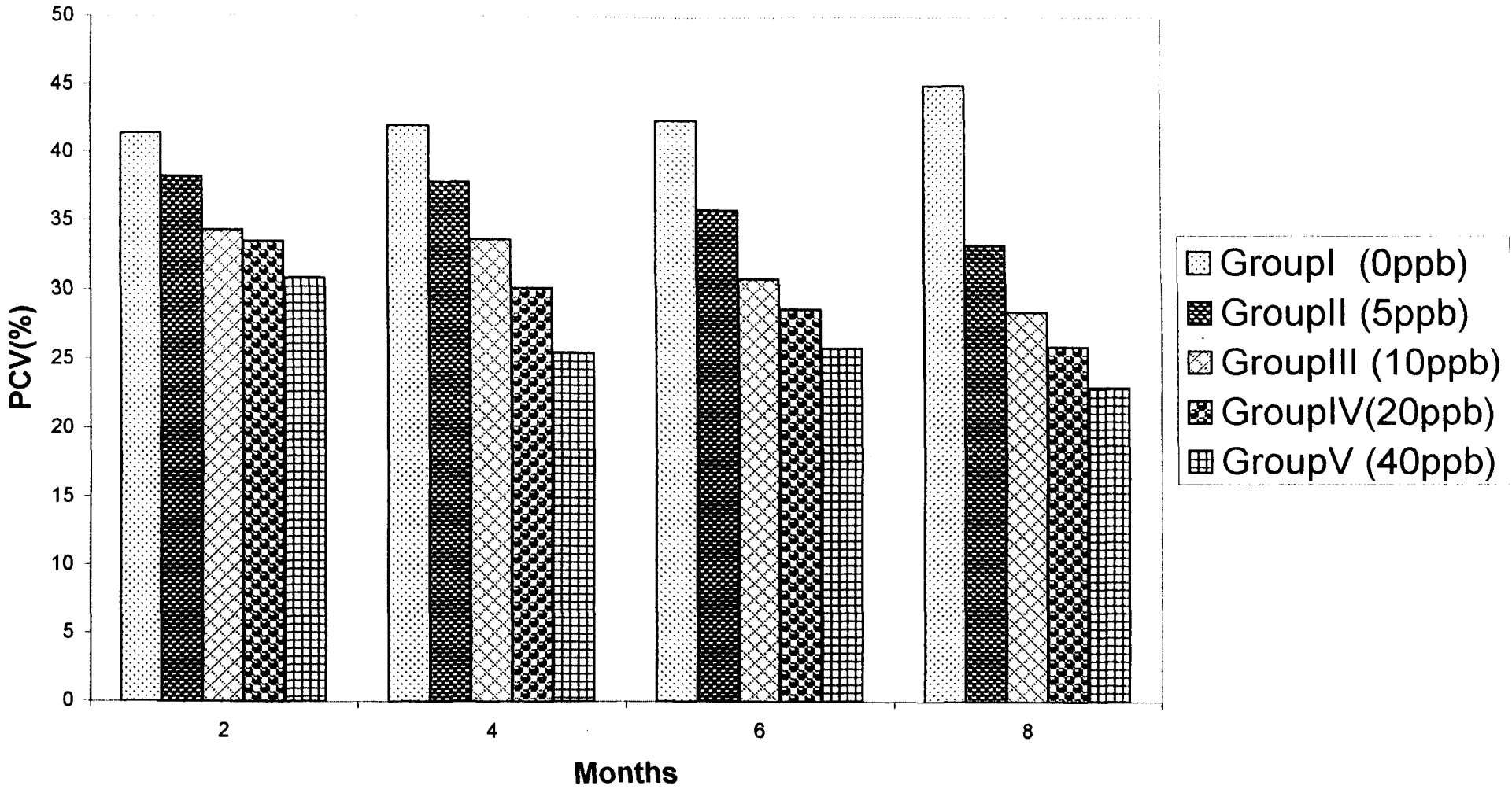
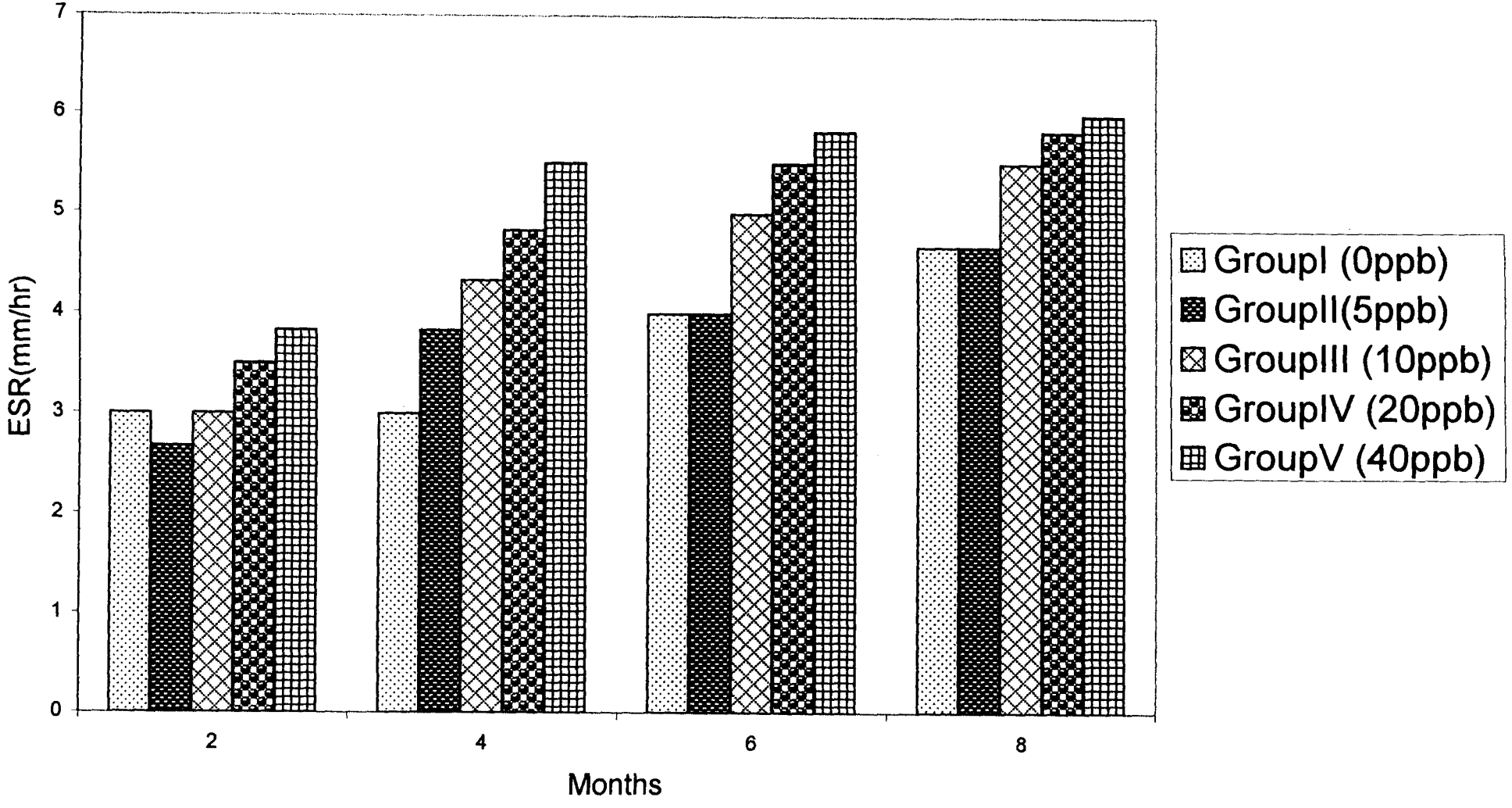


Table .6 Average (Mean ± S.E) Erythrocyte Sedimentation Rate (mm/h)

Treatment groups	Toxin level (ppb)	Observation period in months			
		2	4	6	8
Group I (control)	0	3.000 ± 0.01 [a]	3.000 ± 0.57 [a]	4.000 _ 0.02 [a]	4.667 ± 0.33 [a]
Group II	5	2.667 ± 0.21 [ab]	3.833 ± 0.16 [ab]	4.000 ± 0.25 [a]	4.667 ± 0.21 [a]
Group III	10	3.000 ± 0.25 [a]	4.333 ± 0.33 [b]	5.000 ± 0.01 [b]	5.500 ± 0.22 [b]
Group IV	20	3.500 ± 0.22 [a]	4.833 ± 0.54 [bc]	5.500 ± 0.22 [bc]	5.833 ± 0.16 [b]
Group V	40	3.833 ± 0.16 [b]	5.500 ± 0.22 [c]	5.833 ± 0.16 [c]	6.000 ± 0.25 [b]
F-value		4.853**	5.389**	18.568**	7.108**
CD (1,2)		0.7374	1.2572	0.6443	0.7870
CD(2,3)		0.1783	1.037	0.5314	0.6426

CD (1,2) for comparison of control with other treatment
CD (2,3) for comparison of other treatments
Mean bearing the same superscript are not significantly different
** P < 0.01

Fig. 6. Average Erythrocyte Sedimentation Rate(mm/hr)



a dose related manner as compared with that of the control group during the course of the experiment.

By the second and fourth month of the study, groups I and group II revealed no significant difference between them. Likewise between groups II and III and groups III, IV and V did not show any significant difference among them.

During the sixth and eighth month of the trial, group I and II revealed significant difference between them. However, treatment groups III and IV as well as groups IV and V did not differ significantly from each other.

4.4.3 Erythrocyte Sedimentation Rate (ESR)

The mean values of the erythrocyte sedimentation rate are tabulated in Table. 6 and graphically shown in Fig. 6.

A significant ($p < 0.01$) increase in the erythrocyte sedimentation rate was observed in the aflatoxin treated ducks (Groups II, III, IV and V) compared to that of the control group during the experimental period.

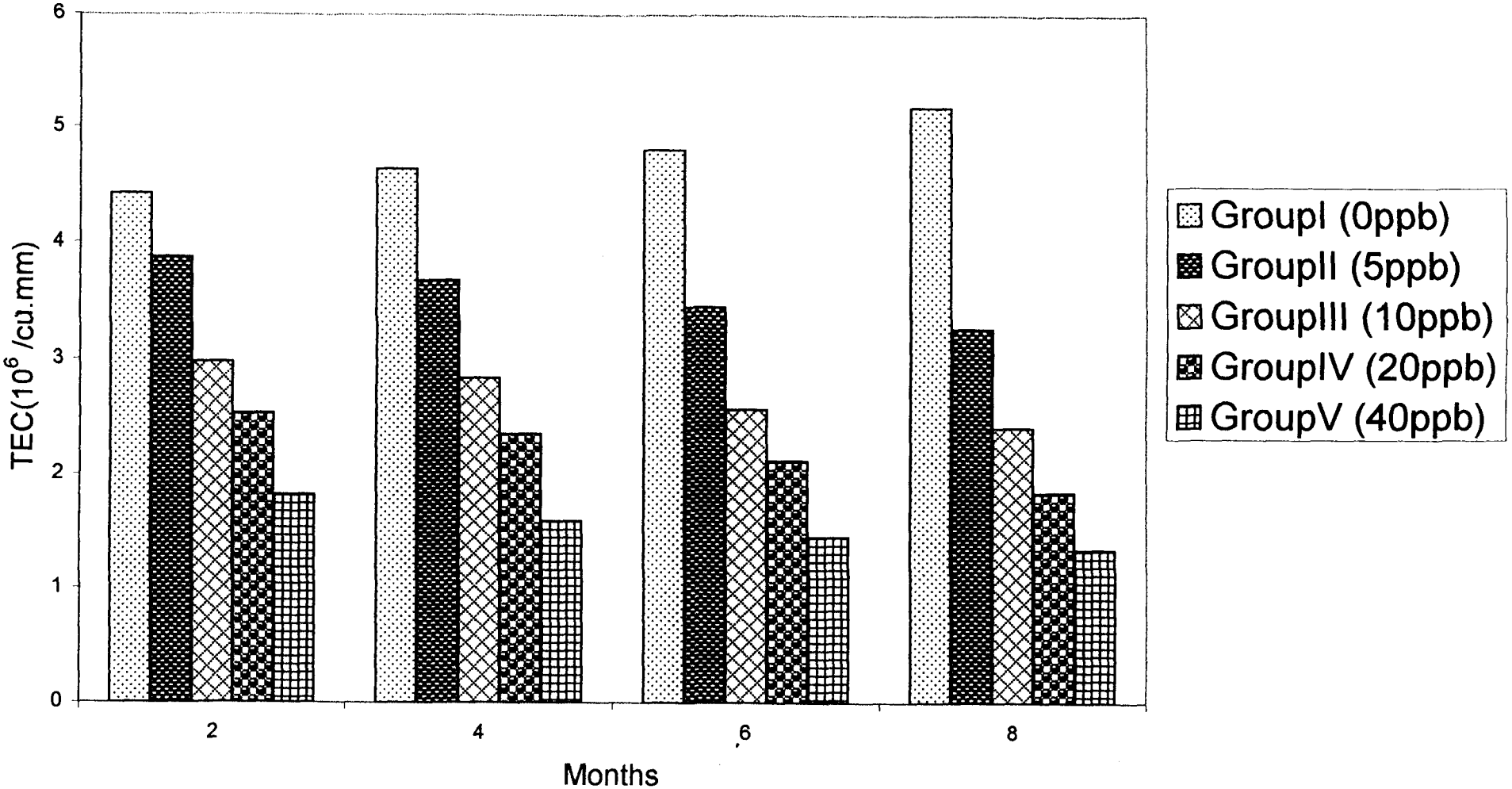
By the second month of the experiment, the ESR among the birds of groups I, II, III, IV was not significantly different. The values of the groups II and V revealed no significant difference between them. During the fourth month of the trial, ESR values of birds of groups I and II were not significantly different. Similarly, ESR of groups II, III and IV and between treatment groups IV and V did not differ from each other significantly. By the sixth month of the trial, groups I and II were not significantly different from each other. Likewise groups III and IV as well as groups IV and V did not differ significantly from

Table .7 Average (Mean ± S.E) Total Erythrocyte Count (10^6/cumm)

Treatment groups	Toxin level (ppb)	Observation period in months			
		2	4	6	8
Group I (control)	0	4.417 ± 0.28 [a]	4.637 ± 0.16 [a]	4.810 ± 0.27 [a]	5.183 ± 0.19 [a]
Group II	5	3.872 ± 0.19 [a]	3.677 ± 0.11 [b]	3.452 ± 0.15 [b]	3.263 ± 0.12 [b]
Group III	10	2.975 ± 0.15 [b]	2.833 ± 0.17 [c]	2.567 ± 0.14 [c]	2.408 ± 0.17 [c]
Group IV	20	2.530 ± 0.12 [b]	2.350 ± 0.18 [c]	2.117 ± 0.20 [c]	1.833 ± 0.16 [d]
Group V	40	1.817 ± 0.09 [c]	1.583 ± 0.10 [d]	1.442 ± 0.07 [d]	1.330 ± 0.08 [e]
F-value		35.993**	48.758**	49.005**	75.704**
CD (1,2)		0.5451	0.5173	0.5528	0.5059
CD(2,3)		0.4496	0.4267	0.46	0.4130

CD (1,2) for comparison of control with other treatment
CD (2,3) for comparison of other treatments
Mean bearing the same superscript are not significantly different
** $P < 0.01$

Fig. 7. Average Total Erythrocyte Count(10^6 /cu.mm)



each other. During the eighth month of the trial, groups I and II were significantly different from each other whereas groups III, IV and V were not significantly different from each other.

4.4.4 Total Erythrocyte Count (TEC)

Total erythrocyte count of ducks are furnished in Table. 7 and Fig. 7. A significant ($p<0.01$) decrease in the erythrocyte count of aflatoxin treated birds (Groups II, III, IV and V) was observed by the second, fourth, sixth and eighth month of the trial in a dose related manner. By the second month of the trial, only birds of the group V showed significant decrease.The values of the treatment group II did not show significant decrease from the control group and similarly the total erythrocyte count of groups II and IV did not differ significantly. During the fourth and sixth month, the total erythrocyte count of groups III and IV were not statistically different from each other, but on the contrary the erythrocyte counts of groups I, II and V were significantly different. During the eighth month of the trial, all the treatment groups showed significant reduction in the erythrocyte count. The treatment groups differed significantly from each other.

4.5.5 Total Leucocyte Counts (TLC)

The mean values of the total leucocyte count are furnished in Table. 8 and Fig.8.

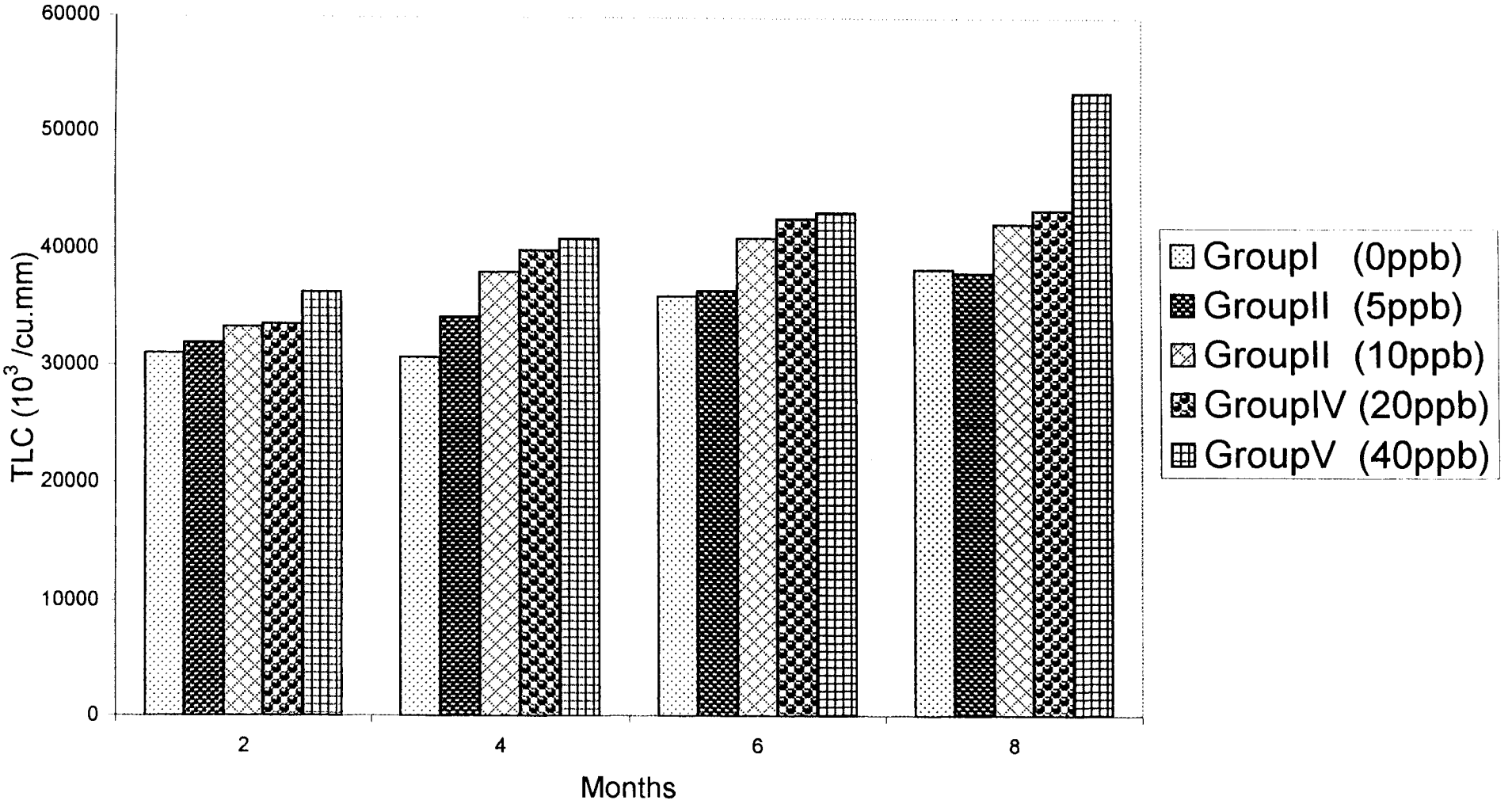
There was no significant variation in the total leucocyte count in treatment groups at the second and eighth month but aflatoxin treated ducks

Table.8 Average (Mean ± S.E) Total Leucocyte Count (10^3/cumm)

Treatment groups	Toxin level (ppb)	Observation period in months			
		2	4	6	8
Group I (control)	0	31000.000 ± 2648.89	30666.667 ± 2966.24 [a]	36000 ± 2084.13 [a]	38333.333 ± 2029.96
Group II	5	31916.667 ± 1218.64	34200.000 ± 1512.51 [a]	36458.33 ± 1092.84 [b]	38041.667 ± 1700.22
Group III	10	33333.333 ± 1627.34	38041.667 ± 469.15 [b]	41000 ± 849.85 [c]	42250.000 ± 827.31
Group IV	20	33583.333 ± 1524.77	39916.667 ± 813.67 [c]	42666.667 ± 630.49 [d]	43416.667 ± 540.86
Group V	40	36333.333 ± 856.41	40916.667 ± 487.80 [d]	43208.333 ± 716.96 [e]	53517.88 ± 690.46
F-value		NS	11.139**	11.546**	NS
CD (1,2)		-	3842.47	3249.08	-
CD(2,3)		-	3162.22	2679.80	-

CD (1,2) for comparison of control with other treatment
CD (2,3) for comparison of other treatments
** $P < 0.01$

Fig. 8. Average Total Leucocyte Count (10^3 /cu.mm)



(Groups II, III, IV and V) showed a relative increase in the total leucocyte count when compared to that of the control group. During the fourth month of the trial groups III, IV and V revealed significant ($P<0.01$) difference from the group II and the control group, but the values of groups I(control) and II were not significantly different from each other. By the sixth month of the trial, all the treatment groups including the control revealed significant ($P<0.01$) difference from each other.

4.4.6 Differential Leucocyte Count (DLC)

The mean values of the differential leucocyte count are shown in Table. 9.a,b,c,d and e. Aflatoxin treated ducks (Groups II, III, IV and V) showed a significant ($P<0.01$) increase in the heterophil count depending on the dose and duration of treatment as compared to that of the control. During the second, sixth and eighth month of the study, the values of all the treatment groups including the control were significantly different from each other but groups I and II were not significantly different from each other. At the fourth month of the trial, groups III, IV and V revealed no significant difference among them.

There was no significant variation in the eosinophil count at the second and eighth month of the study. There was significant ($P< 0.01$) decrease in the eosinophil count in the treatment groups during the fourth month of the trial when compared to the control.

The eosinophil count of groups I, IV and V were not statistically different from each other, but that of groups II, and III were significantly

Table. 9.a Average Heterophil Count (%)

Treatment groups	Toxin level (ppb)	Observation period in months			
		2	4	6	8
Group I (control)	0	18.333 ± 0.88[a]	25.667 ± 2.02[a]	34.333 ± 2.33[a]	37.333 ± 2.67[a]
Group II	5	25.500 ± 0.96[b]	36.500 ± 1.88[b]	39.833 ± 0.075[b]	43.667 ± 1.11[b]
Group III	10	30.333 ± 1.05[c]	42.333 ± 1.77[c]	42.167 ± 1.30[c]	47.000 ± 1.46[c]
Group IV	20	30.833 ± 1.93[d]	41.667 ± 1.80[c]	46.500 ± 0.93[d]	49.667 ± 1.73[d]
Group V	40	40.500 ± 0.67[e]	42.667 ± 1.02[c]	45.167 ± 0.98[e]	50.500 ± 2.02[e]
F-value		34.588**	11.877**	13.579**	7.097**
CD (1,2)		4.2646	5.808	3.849	5.889
CD(2,3)		3.5174	4.790	3.175	4.808

CD (1,2) for comparison of control with other treatment
CD (2,3) for comparison of other treatments
Mean bearing the same superscript are not significantly different
** $P < 0.01$

Table .9.b Average Eosinophil Count (%)

Treatment groups	Toxin level (ppb)	Observation period in months			
		2	4	6	8
Group I (control)	O	1.33 ± 0.12	2.66 ± 0.09 [a]	1.33 ± 0.12 [a]	2.0 ± 0.18
Group II	5	2.0 ± 0.08	1.66 ± 0.18 [b]	0.83 ± 0.08 [a]	1.67 ± 0.16
Group III	10	1.66 ± 0.07	1.66 ± 0.06 [b]	1.83 ± 0.11 [ab]	1.0 ± 0.15
Group IV	20	1.33 ± 0.20	2.0 ± 0.08 [a]	1.83 ± 0.13 [a]	1.0 ± 0.18
Group V	40	1.66 ± 0.16	2.33 ± 0.06 [a]	2.83 ± 0.08 [b]	1.33 ± 0.28
F-value		N.S.	4.510**	5.590**	N.S.
CD (1,2)		-	0.3672	0.3729	-
CD(2,3)		-	0.3029	0.3076	-

CD (1,2) for comparison of control with other treatment
CD (2,3) for comparison of other treatments
Mean bearing the same superscript are not significantly different
** P < 0.01

Table .9c Average Basophil Count (%)

Treatment groups	Toxin level (ppb)	Observation period in months			
		2	4	6	8
Group I (control)	O	0.67 ± 0.17	0.67 ± 0.17	0.67 ± 0.17	0.67 ± 0.17
Group II	5	0.83 ± 0.14	0.67 ± 0.11	0.83 ± 0.11	0.67 ± 0.15
Group III	10	0.50 ± 0.15	1.0 ± 0.15	0.67 ± 0.15	0.83 ± 0.14
Group IV	20	1.0 ± 0.18	1.83 ± 0.16	1.16 ± 0.13	0.83 ± 0.17
Group V	40	1.66 ± 0.18	1.50 ± 0.16	1.50 ± 0.18	0.67 ± 0.15
F-value		N.S.	N.S.	N.S.	N.S.
CD (1,2)		-	-	-	-
CD(2,3)		-	-	-	-

CD (1,2) for comparison of control with other treatment
CD (2,3) for comparison of other treatments
Mean bearing the same superscript are not significantly different

Table .9.d Average Monocyte Count (%)

Treatment groups	Toxin level (ppb)	Observation period in months			
		2	4	6	8
Group I (control)	O	1.0 ± 0.01[a]	1.0 ± 0.02[a]	2.0 ± 0.11[a]	1.0 ± 0.25
Group II	5	2.3 ± 0.08[b]	1.83 ± 0.10[ab]	3.0 ± 0.12[ab]	1.5 ± 0.15
Group III	10	4.0 ± 0.11[b]	2.1 ± 0.21[a]	2.33 ± 0.09[a]	0.83 ± 0.17
Group IV	20	4.0 ± 0.06[b]	2.66 ± 0.06[b]	2.5 ± 0.06[a]	1.17 ± 0.17
Group V	40	3.0 ± 0.08[b]	3.5 ± 0.09[c]	4.0 ± 0.11[b]	0.67 ± 0.15
F-value		12.038**	7.191**	3.993*	N.S.
CD (1,2)		0.2939	0.4451	0.3526	-
CD(2,3)		0.2424	0.3671	0.2908	-

CD (1,2) for comparison of control with other treatment
CD (2,3) for comparison of other treatments
Mean bearing the same superscript are not significantly different
** P < 0.01

Table .9.e Average Lymphocyte Count (%)

Treatment groups	Toxin level (ppb)	Observation period in months			
		2	4	6	8
Group I (control)	O	78.667 ± 0.88 [a]	70.000 ± 1.73 [a]	62.333 ± 2.40 [a]	45.000 ± 1.75
Group II	5	69.00 ± 1.53 [b]	59.833 ± 1.35 [b]	55.833 ± 1.43 [b]	54.667 ± 0.61
Group III	10	68.833 ± 1.08 [c]	53.333 ± 1.89 [c]	52.500 ± 1.31 [b]	50.333 ± 1.52
Group IV	20	62.500 ± 1.18 [c]	51.833 ± 1.82 [c]	47.333 ± 1.38 [c]	47.333 ± 1.43
Group V	40	52.167 ± 0.65 [d]	48.833 ± 1.20 [c]	46.833 ± 0.87 [c]	47.667 ± 1.84
F-value		55.303**	19.103**	16.964**	N.S.
CD (1,2)		3.9618	5.552	4.665	-
CD(2,3)		3.2676	4.579	3.847	-

CD (1,2) for comparison of control with other treatment
CD (2,3) for comparison of other treatments
Mean bearing the same superscript are not significantly different
** P < 0.01

different from the control group (group I) although they did not differ significantly between them.

At the sixth month of experiment there was anincrease ($P<0.01$) in eosinophil count in groups III, IV and V and decrease in the count in group II when compared to the control. Groups I, II, II and IV as well as groups II and V did not differ significantly among them.

There was no significant variation in the basophil count throughout the experimental trial. A significant ($P<0.01$) increase in the monocyte count was noticed in the aflatoxin treated birds (Groups II, III, IV and V) at the second, fourth and sixth month as compared with that of the control. There was no significant variation in the monocyte count between the treatment and control ducks at the eighth month.

At the second month of the trial, all the experimental groups showed significant ($P<0.01$) increase in the monocyte count but the treatment groups did not differ significantly among them. By the fourth month of the trial only group V showed significant ($P<0.01$) increase when compared to the other treatment groups, whereas groups I, II and III revealed no significant difference among them and groups II and IV were also found to be not statistically different. At the sixth month of the trial, values of the groups I, II, III and IV were not significantly different.

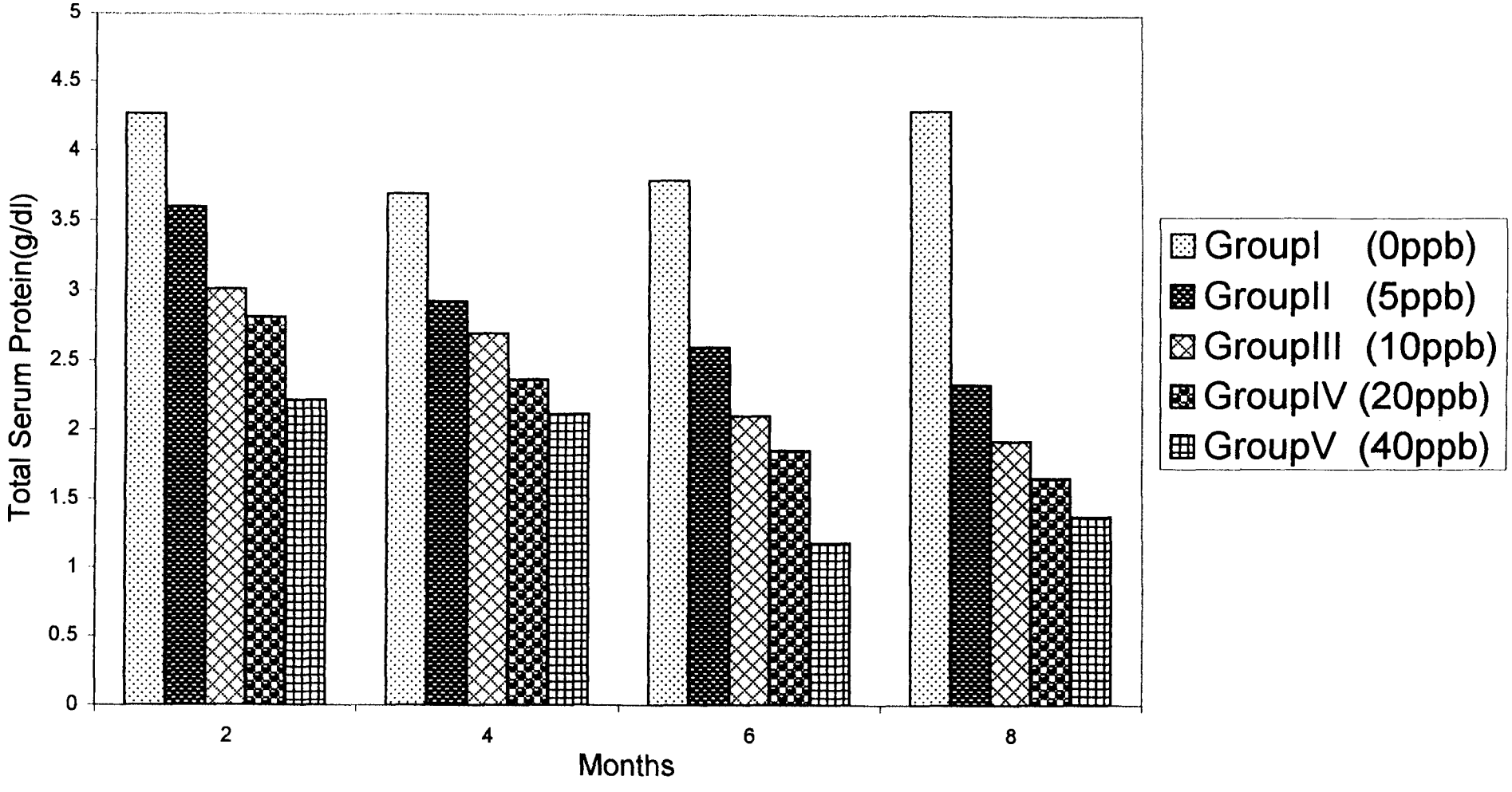
During the second, fourth and sixth months, aflatoxin fed ducks (Groups II, III, IV and V) showed a significant ($P< 0.01$) reduction in the lymphocyte count in a dose dependant manner, but at the eighth month there

Table .10 Average (Mean ± S.E) Serum Protein (g/dl)

Treatment groups	Toxin level (ppb)	Observation period in months			
		2	4	6	8
Group I (control)	0	4.267 ± 0.14 [a]	3.700 ± 0.25 [a]	3.800 ± 0.15 [a]	4.300 ± 0.30 [a]
Group II	5	3.600 ± 0.15 [b]	2.933 ± 0.05 [b]	2.600 ± 0.13 [b]	2.333 ± 0.14 [b]
Group III	10	3.017 ± 0.13 [c]	2.700 ± 0.3 [b]	2.100 ± 0.14 [c]	1.917 ± 0.14 [bc]
Group IV	20	2.817 ± 0.10 [d]	2.367 ± 0.09 [c]	1.850 ± 0.12 [d]	1.650 ± 0.15 [cd]
Group V	40	2.217 ± 0.13 [e]	2.117 ± 0.05 [d]	1.175 ± 0.19 [e]	1.367 ± 0.18 [d]
F-value		27.23**	36.811**	30.786**	30.713**
CD (1,2)		0.4567	0.2939	0.5173	0.5847
CD(2,3)		0.3767	0.2424	0.4267	0.4774

CD (1,2) for comparison of control with other treatment
CD (2,3) for comparison of other treatments
Mean bearing the same superscript are not significantly different
** P < 0.01

Fig. 9. Average Total Serum Protein(g/dl)



was no significant difference among the treatment groups and the control groups.

During second month of the trial, groups III and IV revealed no significant difference, but were significantly different from II and IV.

During the fourth month of the trial all the treatment groups showed significant ($P<0.01$) reduction in lymphocyte count from the control groups, but the count among the groups III, IV and V were not significantly different. Similarly by the sixth month all the treatment groups showed significant ($P<0.01$) reduction in the lymphocyte count from the control groups, however treatment groups II and III as well as groups IV and V did not differ significantly between them. By the eighth month, there was no significant difference among the treatment groups and control.

4.5 SEROLOGY

4.5.1 Serum Protein

The values are tabulated in Table. 10 and shown in Fig. 9. There was a progressive and significant ($p< 0.01$) dose and time dependent reduction in the levels of serum protein in the ducks of the treatment groups. At the fourth month, ducks in groups II and III revealed no significant difference between them though they differed significantly from that of the control group.

At the second and sixth month of the trial, all the aflatoxin treated groups showed significant reduction in the serum protein values from that of the control groups besides showing significant difference among them.

Table .11 Average (Mean ± S.E) Serum Albumin (g/dl)

Treatment groups	Toxin level (ppb)	Observation period in months			
		2	4	6	8
Group I (control)	0	2.867 ± 0.09 [a]	2.400 ± 0.25 [a]	2.900 ± 0.05 [a]	3.233 ± 0.28 [a]
Group II	5	1.817 ± 0.14 [b]	1.288 ± 0.12 [b]	0.948 ± 0.045 [b]	0.820 ± 0.04 [b]
Group III	10	1.567 ± 0.14 [c]	0.960 ± 0.04 [c]	0.783 ± 0.060 [c]	0.702 ± 0.05 [c]
Group IV	20	1.228 ± 0.17 [d]	0.478 ± 0.09 [d]	0.463 ± 0.070 [d]	0.302 ± 0.03 [d]
Group V	40	0.613 ± 0.14 [e]	0.213 ± 0.02 [e]	0.198 ± 0.050 [e]	0.110 ± 0.02 [e]
F-value		23.082 **	52.801**	216.346**	173.321**
CD (1,2)		0.5091	0.3404	0.2001	0.2623
CD(2,3)		0.4199	0.2808	0.1650	0.2142

CD (1,2) for comparison of control with other treatment
CD (2,3) for comparison of other treatments
Mean bearing the same superscript are not significantly different
** $P < 0.01$

Fig. 10. Average Serum Albumin(g/dl)

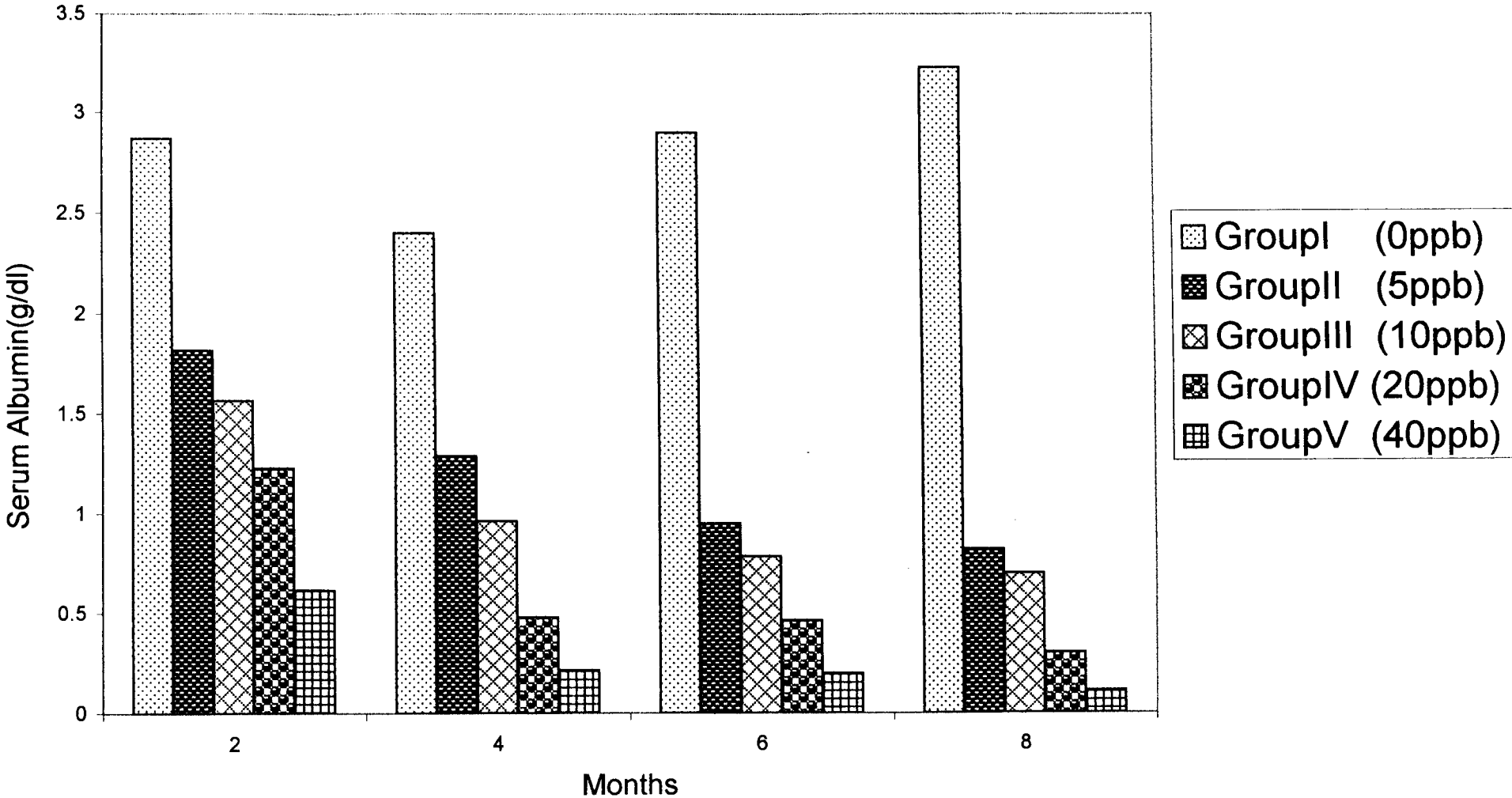
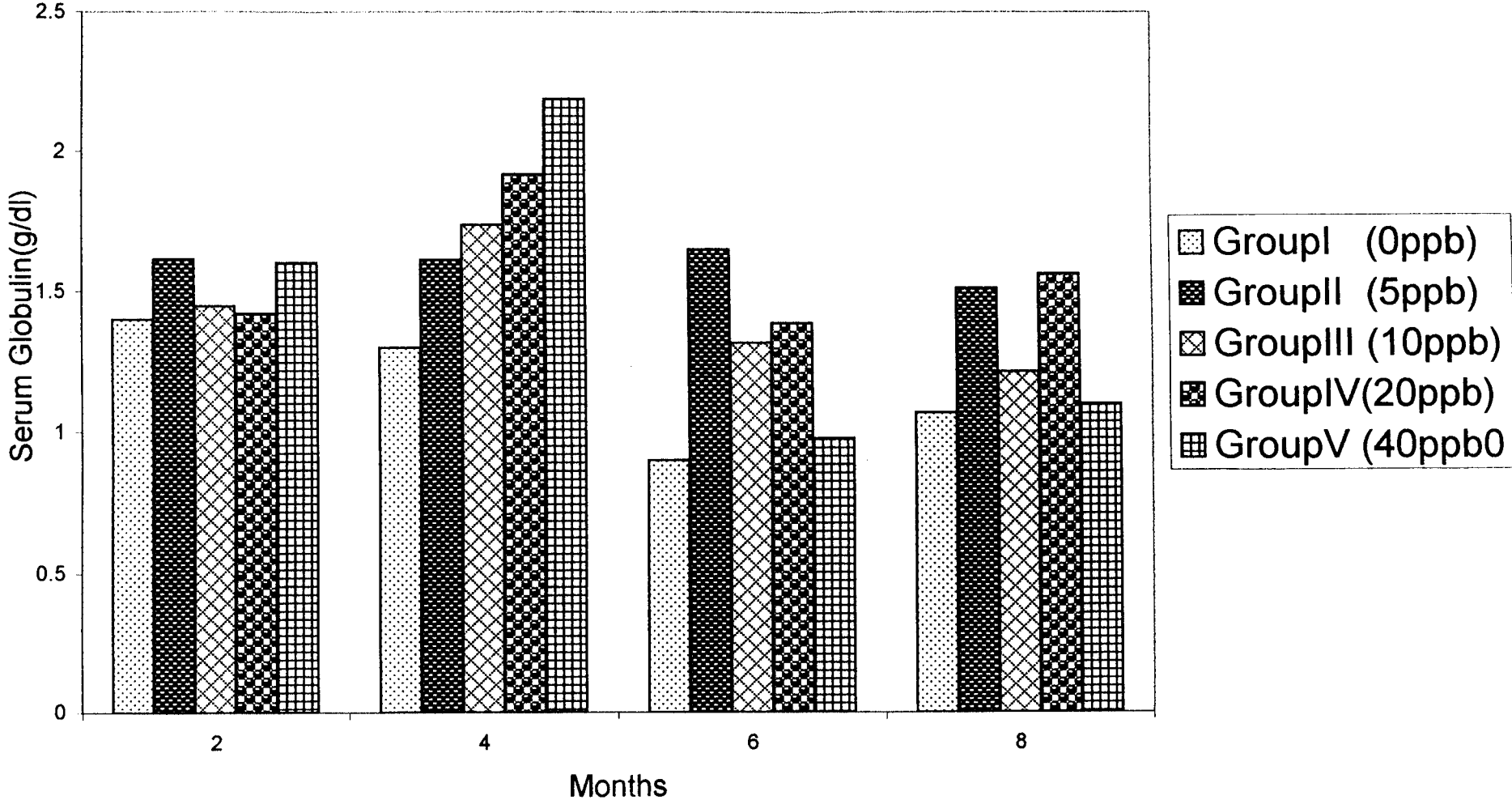


Table.12 Average (Mean ± S.E) Serum Globulin (g/dl)

Treatment groups	Toxin level (ppb)	Observation period in months			
		2	4	6	8
Group I (control)	0	1.400 ± 0.15	1.300 ± 0.21 [a]	0.900 ± 0.15 [a]	1.067 ± 0.034
Group II	5	1.617 ± 0.13	1.645 ± 0.11 [ab]	1.652 ± 0.13 [b]	1.513 ± 0.16
Group III	10	1.450 ± 0.15	1.740 ± 0.04 [b]	1.317 ± 0.12 [b]	1.215 ± 0.15
Group IV	20	1.422 ± 0.12	1.922 ± 0.13 [bc]	1.387 ± 0.11 [b]	1.565 ± 0.13
Group V	40	1.603 ± 0.12	2.187 ± 0.08 [c]	0.977 ± 0.17 [a]	1.098 ± 0.20
F-value		NS	7.340**	4.287**	NS
CD (1,2)		-	0.3701	0.4815	-
CD(2,3)		-	0.3052	0.3917	-

CD (1,2) for comparison of control with other treatment
CD (2,3) for comparison of other treatments
Mean bearing the same superscript are not significantly different
** P < 0.01

Fig. 11. Average Serum Globulin(g/dl)



But no significant variation could be observed between groups II and III, groups II and IV and groups IV and V, but all the treatment groups showed significant reduction from that of the control group at the eighth month of the trial.

4.5.2 Serum Albumin

The values are tabulated in Table. 11 and represented graphically in Fig.10. A significant ($P< 0.01$) decrease in the albumin values of aflatoxin treated ducks (Groups II, III, IV and V) was noticed as compared with that of the control group at the second, fourth, sixth and eighth month of the study. At the second, fourth and sixth month of the trial, control and aflatoxin fed groups showed significant difference from each other. At the eighth month of the study, groups II and IV as well as groups IV and V revealed no significant difference between them.

4.5.3 Serum Globulin

The serum globulin values are presented in Table. 12 and shown in Fig. 11. At the second and eighth month, there was no significant increase in the globulin levels of the ducks of groups II, III, IV and V from that of the control group.

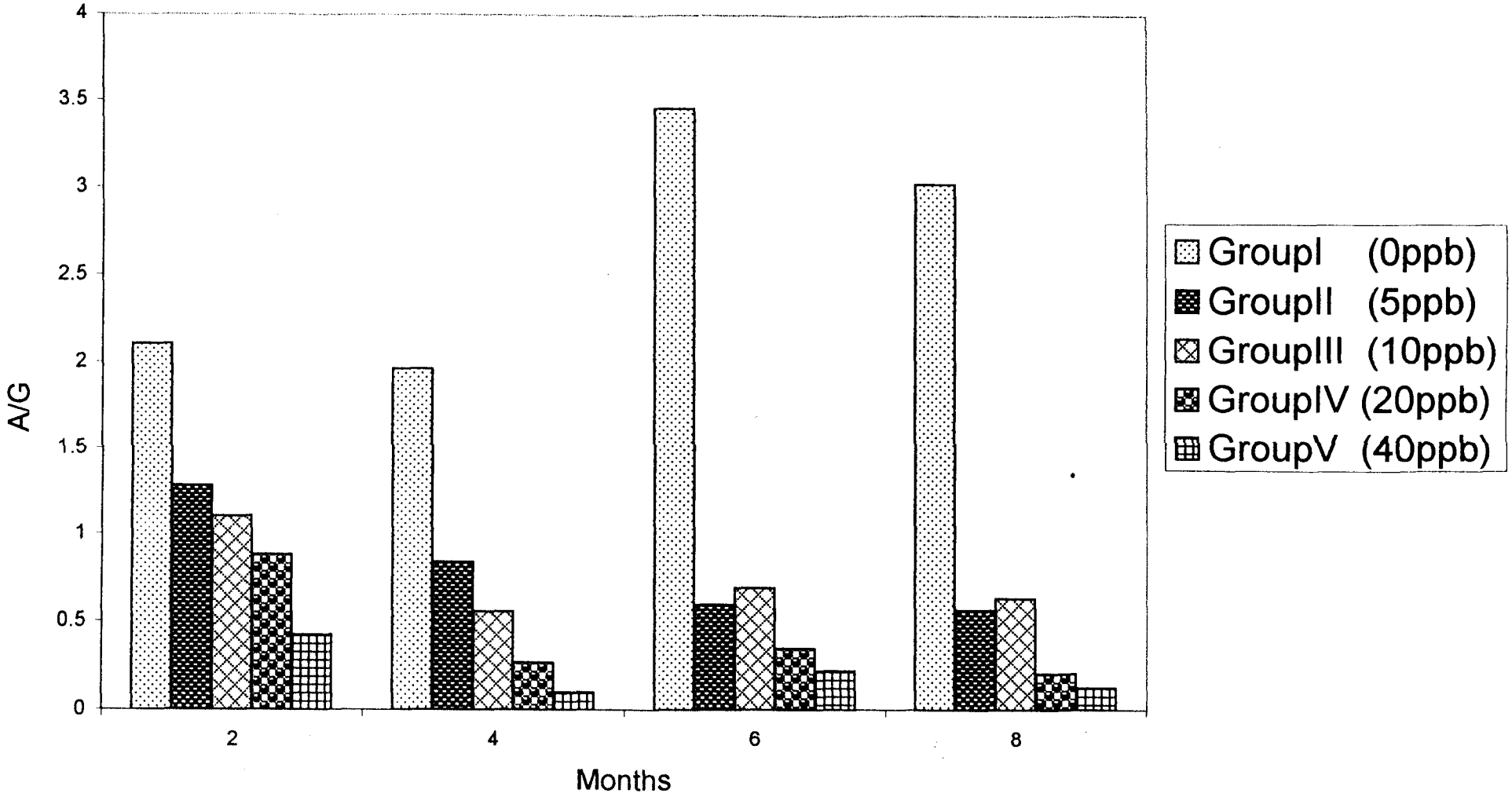
At the fourth month of the study, ducks of the group II did not show any significant increase from that of the control group. Similarly, groups, II, III and IV as well as groups IV and V revealed no significant difference among them although they all showed significant reduction from that of the control group.

Table .13 Average (Mean ± S.E) Albumin-Globulin Ratio

Treatment groups	Toxin level (ppb)	Observation period in months			
		2	4	6	8
Group I (control)	0	2.106 ± 0.28 [a]	1.960 ± 0.39 [a]	3.460 ± 0.69 [a]	3.027 ± 0.24 [a]
Group II	5	1.279 ± 0.14 [b]	0.835 ± 0.16 [b]	0.595 ± 0.06 [b]	0.565 ± 0.06 [b]
Group III	10	1.101 ± 0.20 [c]	0.555 ± 0.03 [c]	0.689 ± 0.10 [b]	0.632 ± 0.10 [b]
Group IV	20	0.877 ± 0.19 [d]	0.267 ± 0.06 [cd]	0.347 ± 0.06 [b]	0.205 ± 0.04 [c]
Group V	40	0.421 ± 0.13 [e]	0.099 ± 0.02 [d]	0.220 ± 0.049 [b]	0.124 ± 0.04 [c]
F-value		9.192 **	23.711**	38.789**	128.38**
CD (1,2)		0.5968	0.4232	0.5807	0.2896
CD(2,3)		0.4922	0.3491	0.4789	0.2364

CD (1,2) for comparison of control with other treatment
CD (2,3) for comparison of other treatments
Mean bearing the same superscript are not significantly different
** $P < 0.01$

Fig. 12. Average Albumin/Globulin Ratio



Groups I and V revealed no significant difference and similarly the values in groups II, III and IV were not statistically different from each other at the sixth month of the study though they showed significant increase from that of the control groups.

4.5.4 Albumin/Globulin Ratio (A/G)

The values of A/G are furnished in Table. 13 and Fig. 12. Ducks of the aflatoxin treated groups (Groups II, III, IV and V) showed significant ($p < 0.05$) reduction in the A/G ratio.

At the second month of the experiment all the groups showed significant ($P< 0.01$) difference among them. On the other hand, by the fourth month groups II and III, and groups III and IV as well as group IV and V did not differ significantly ($P<0.01$) among them.

At the sixth month of the study, all the treatment groups showed significant ($P<0.01$) reduction in the albumin globulin ratio. However, the values of groups II, III, IV and V were not statistically different from each other. By the eighth month of the trial, groups II and III as well as groups IV and V did not reveal any significant difference between them, though all the treatment groups showed significant reduction when compared to that of the control group.

4.5.5 Serum Alkaline Phosphatase Activity

The data on the serum alkaline phosphatase level after feeding of graded levels of aflatoxin are detailed in Table. 14 and shown in Fig. 13.

Table .14 Average (Mean ± S.E) Serum Alkaline Phosphatase (IU/L)

Treatment groups	Toxin level (ppb)	Observation period in months			
		2	4	6	8
Group I (control)	0	45.65 ± 1.35 [a]	45.65 ± 2.5 [a]	54.78 ± 2.71 [a]	63.91 ± 3.03 [a]
Group II	5	50.78 ± 2.60 [b]	56.00 ± 3.0 [b]	60.02 ± 2.81 [b]	69.05 ± 2.93 [b]
Group III	10	55.78 ± 2.92 [c]	63.91 ± 1.92 [c]	73.04 ± 2.72 [c]	82.17 ± 3.02 [c]
Group IV	20	63.91 ± 2.02 [d]	82.17 ± 2.9 [d]	100.40 ± 2.05 [d]	110.56 ± 4.08 [d]
Group V	40	73.04 ± 2.50 [e]	91.30 ± 2.79 [e]	109.56 ± 2.5 [e]	118.69 ± 4.93 [e]
F-value		3.93**	3.953**	4.43**	4.45**
CD (1,2)		4.932	5.032	6.35	5.95
CD(2,3)		3.235	4.959	5.495	4.039

CD (1,2) for comparison of control with other treatment
CD (2,3) for comparison of other treatments
Mean bearing the same superscript are not significantly different
** $P < 0.01$

Fig. 13. Average Serum Alkaline Phosphatase(IU/L)

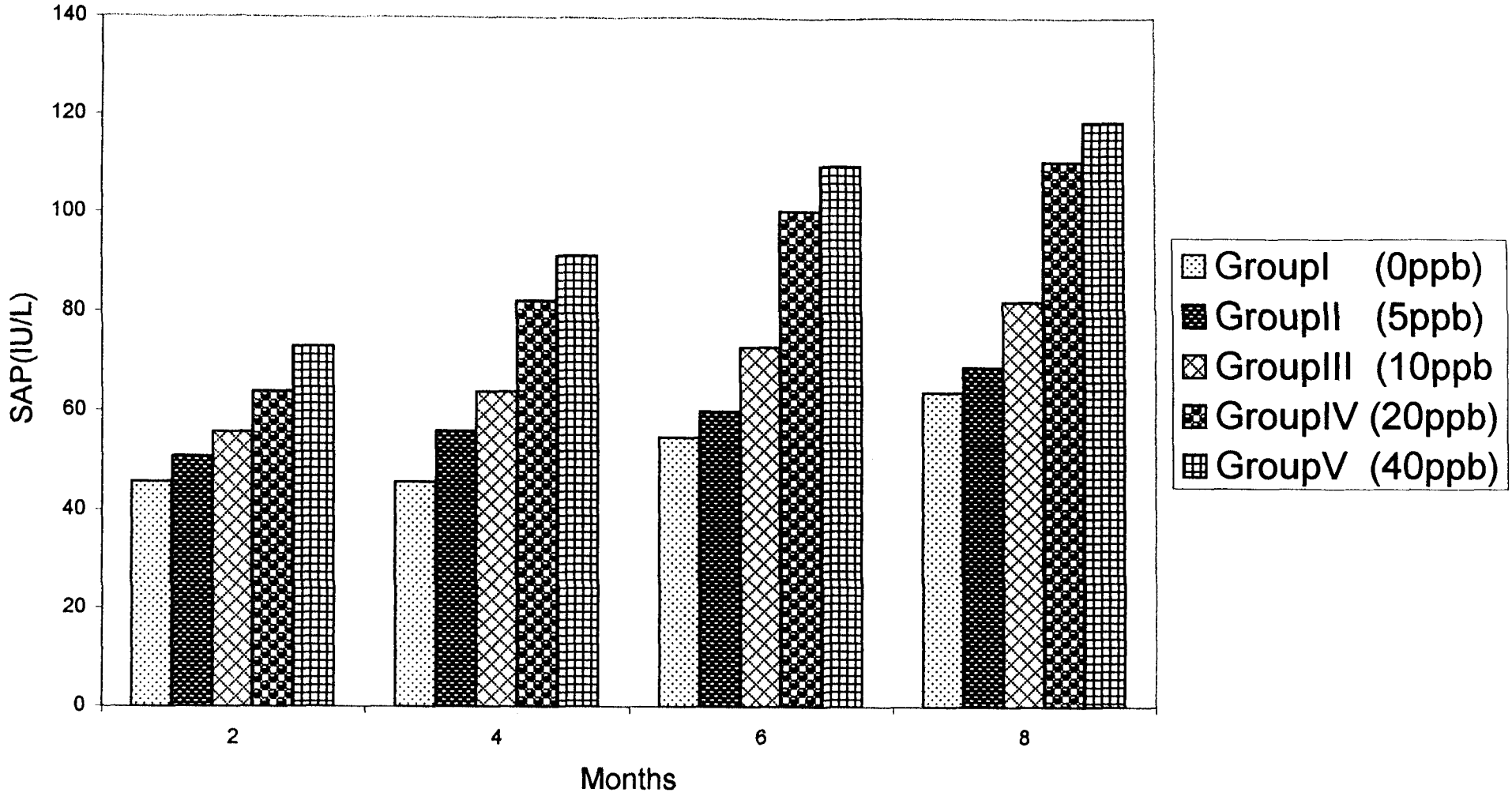
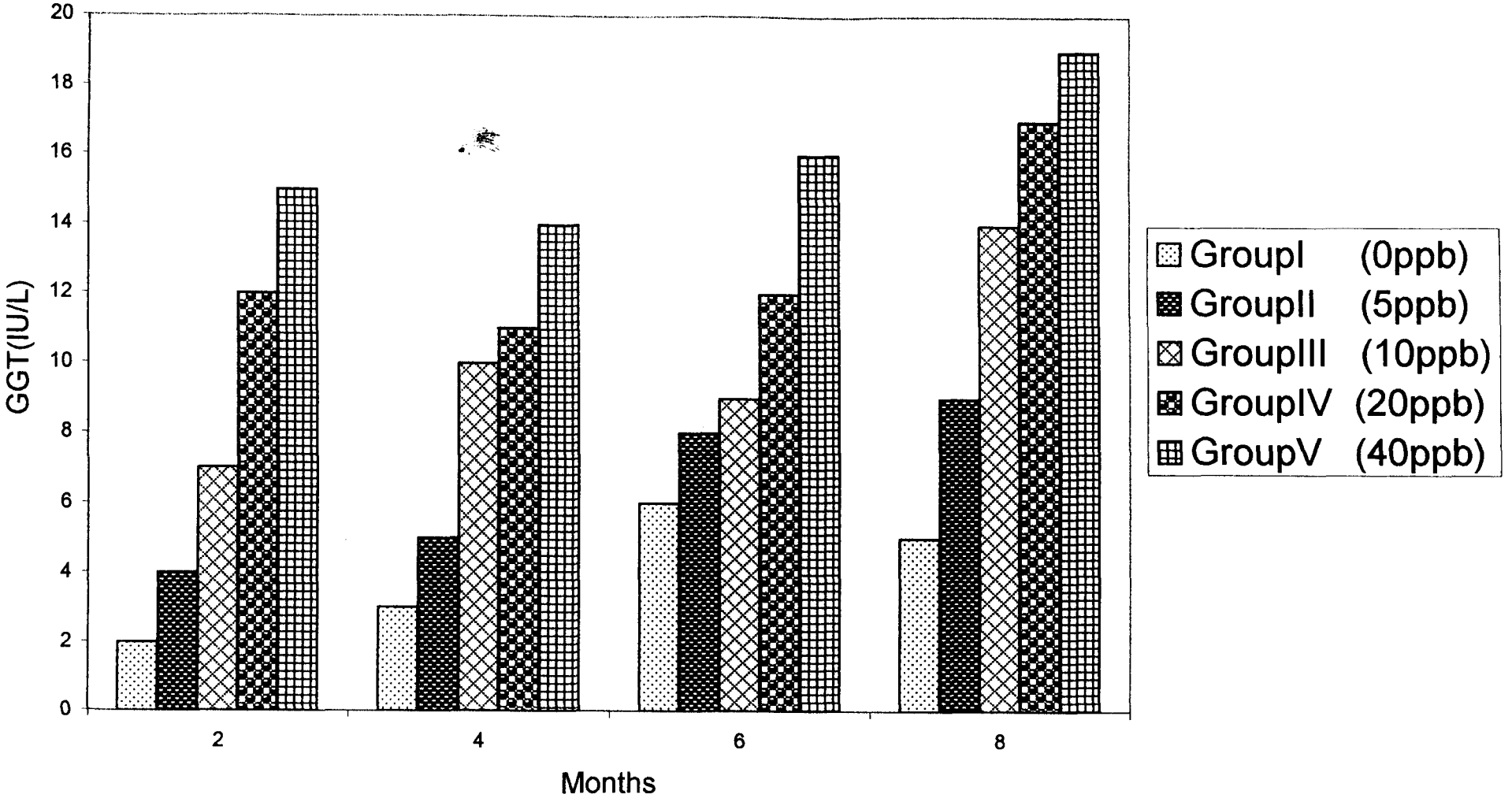


Table .15 Average (Mean ± S.E) Gamma Glutamyl Transpeptidase (IU/L)

Treatment groups	Toxin level (ppb)	Observation period in months			
		2	4	6	8
Group I (control)	0	2 ± 0.982 [a]	3 ± 0.872 [a]	6 ± 0.864 [a]	5 ± 0.756
Group II	5	4 ± 0.761 [b]	5 ± 0.02 [b]	8 ± 0.956 [b]	9 ± 0.782
Group III	10	7 ± 1.03 [c]	10 ± 0.982 [c]	9 ± 0.56 [c]	14 ± 0.762
Group IV	20	12 ± 0.56 [d]	11 ± 0.653 [d]	12 ± 0.465 [d]	17 ± 0.32
Group V	40	15 ± 0.632 [e]	14 ± 0.758 [e]	16 ± 0.26 [e]	19 ± 0.45
F-value		65.07**	694.11**	360.92**	195.54**
CD (1,2)		0.7309	0.4904	0.3975	0.6576
CD(2,3)					

CD (1,2) for comparison of control with other treatment
CD (2,3) for comparison of other treatments
Mean bearing the same superscript are not significantly different
** P < 0.01

Fig. 14. Average Serum Gamma Glutamyl Transpeptidase(IU/L)



At the second, fourth, sixth and eighth month of the trial, ducks of the groups II, III, IV and V showed significant ($p < 0.01$) increase in the serum alkaline phosphatase level when compared to that of the control. All the groups including the control showed significant difference from each other throughout the study.

4.5.6 Serum Gamma Glutamyl Transpeptidase Activity

The mean values are presented in Table. 15 and shown in Fig. 14. Ducks belonging to the groups II, III, IV and V showed significant ($p < 0.01$) increase in Serum GGT in a dose dependant manner as compared with that of the control group by the second, fourth, sixth and eighth month of the study.

4.6 CLINICAL SYMPTOMS

In general, reduced feed consumption was noticed in the case of the aflatoxin treated ducks. Besides this, ducks belonging to the group IV and V showed signs of diarrhoea. The control birds were healthy and active throughout the experimental period.

4.7 GROSS PATHOLOGY

Group I (Control)

The liver, kidney and spleen showed no changes throughout the experiment.

Group II (5 ppb)

By the second month of the experiment, grossly the liver of ducks fed with 5 ppb aflatoxin was congested, pale and soft. At the fourth, sixth and eighth month of study, the liver was pale to tan in color and enlarged (Fig.16.A)

Spleen and the kidneys did not reveal any changes except congestion but the bursa was apparently normal.

Group III (10ppb)

The liver of ducks fed with 10 ppb aflatoxin was pale, moderately enlarged and soft. Besides this, a few small areas of haemorrhage were seen by the second month of the experimental trial. By the fourth month of the experiment, liver was severely enlarged, and the surface showed scattered pin head sized necrotic areas, yellow discoloration and subcapsular haemorrhages (Fig.16.B). Hepatomegaly, congestion, mottling, petechial to ecchymotic haemorrhages and pale yellow discoloration with soft consistency were evident by the sixth month of the trial. Haemorrhagic spots along with pale infarction, enlargement, yellowish discoloration, petechial haemorrhage and fatty change were the characteristic lesions in the liver by the eighth month of the study.

The spleen, kidney and bursa showed congestion and haemorrhage of variable intensity depending on the duration of the treatment.

Group IV (20 ppb)

At the second month of the treatment, pale and enlarged fatty liver was noticed with a few pin point haemorrhages in focal areas in different lobes. By the fourth month of the trial, the liver of ducks showed ecchymotic haemorrhages, subcapsular haemorrhages, pin point necrotic areas and pale yellow discoloration. Diffuse enlargement with rounded edges, pale discoloration and congestion along with a few necrotic areas were noticed at the sixth month of the experiment (Fig. 17). By the eighth month of the trial, the liver was pale yellow and enlarged (Fig. 18.A) compared to the control, (Fig.18.B) along with petechial haemorrhages (Fig.19). Varying degrees of splenomegaly, pallor of kidney and atrophic bursa were also observed depending upon the duration of the exposure of aflatoxin in this group.

Group V (40 ppb)

At the second month of the trial, that the liver revealed severe enlargement, with numerous small irregularly shaped necrotic areas, subcapsular and ecchymotic haemorrhages. At the fourth month of the experiment, necrotic areas, severe enlargement, ecchymotic haemorrhages and rounded edges were noticed in the liver. By the sixth month of the study, liver appeared grossly enlarged, yellow in colour (Fig. 20) and was hard in consistency. The surface of the liver was granular and bearing irregularly shaped necrotic areas (Fig.21). By the eighth month of the experiment the liver of ducks was pale and greenish in colour (Fig. 22), hard in consistency and had mottled appearance. The liver surface showed small irregularly

shaped necrotic lesions along with scattered small greenish nodules and granular irregularity (Fig. 23.A) as compared with the control (Fig. 23.B)

Splenomegaly and pale enlarged kidney with focal necrotic lesions were observed by the eighth month. Slightly enlarged spleen and kidney with a few petechial haemorrhage were revealed at the second, fourth and sixth month. Bursae were either atrophied or had regressed by the second month of the trial.

4.8 HISTOPATHOLOGY

Group I (Control)

Liver , spleen, kidney and bursa of the control group of ducks did not show any histological changes.

Group II (5ppb)

By the second month of the trial, the major histopathological changes observed in the liver of ducks fed with 5ppb aflatoxin were congestion and mild biliary hyperplasia. At the fourth month of the experiment, the hepatic changes included mild fatty changes, moderate vacuolar degeneration, frank necrosis of hepatocytes, bile duct hyperplasia and Kupffer cell proliferation Fig. 24) and mononuclear cell infiltration in the periportal area (Fig. 25). The hepatic changes noticed by the sixth month of the study included fatty change, congestion, mononuclear cell infiltration and bile duct hyperplasia of a more severe degree. By the eighth month of the experiment, changes observed were marked portal congestion and fibrosis (Fig.26) extensive bile duct

hyperplasia, extensive fatty changes, scattered necrotic hepatocytes and focal collection of mononuclear cells (Fig.27).

Spleen did not reveal much change at the second and fourth month of the study. Mild congestion and vascular sclerosis were noticed on the sixth and eighth month of the trial. Kidney revealed only congestion.

Group III (10ppb)

The hepatocytes of the ducks fed with 10ppb of aflatoxin showed varying degree of changes varying from vacuolar degeneration to frank coagulative necrosis, bile stasis, mild bile duct hyperplasia and portal venous dilatation by the second month of the study (Fig. 28). At the fourth month of the experiment, there was congestion of hepatic sinusoids and cholestasis, focal collection of mononuclear cells replacing the necrotic hepatocytes (Fig.29), Kupffer cell proliferation and diffuse fatty change (Fig.30). On the sixth month of the trial, lipidosis, sinusoidal dilatation, central venous congestion, coagulative necrosis and mild portal fibrosis were noticed in the liver.

Histological alteration in the liver at the eighth month of the study included congestion, fatty change of hepatocytes, bile stasis, focal perivascular accumulation of mononuclear cells (Fig. 31), hepatocytomegaly, phlebosclerosis, portal tract fibrosis (Fig. 32), bile duct hyperplasia, fatty change and necrosis of hepatocytes (Fig. 33).

Congestion and mild depletion of the lymphocytes in follicles of the splenic cortex were noticed by the sixth and eighth month of the treatment,

Mild nephrosis was observed at the fourth and sixth month but by the eighth month, besides nephrosis there was mild peritubular accumulation of mononuclear cells (Fig. 34)

Group IV (20 ppb)

The liver of ducks belonging to group IV showed extensive bile duct hyperplasia, moderate vacuolar degeneration and necrosis, diffuse infiltration and focal collection of lymphoid cells in the periportal areas as well as in the parenchyma replacing the necrotic hepatocytes (Fig. 35) by the second month but by the fourth month of the study, biliary hyperplasia, hepatocytomegaly, central venous congestion, vacuolar degeneration and coagulative necrosis of the hepatocytes were observed. At the sixth month of the experiment the histological picture of the liver consisted of portal fibrosis, congestion of sinusoids, cholestasis, megalocytosis, hepatokaryomegaly, Kupffer cell hypertrophy and fatty change.

Focal collection of mononuclear cells in the periportal area, congestion, megalocytosis, bile duct proliteration, extensive fatty changes, necrosis of hepatocytes (Fig. 36) and congestion of the hepatic sinusoides were noticed in the liver at the eighth month of trial.

Focal depletion of lymphoid cells, vascular sclerosis and congestion (Fig.37) were noticed in increasing intensity as the duration of treatment increased. In the kidney, congestion, necrosis and desquamation of the lining tubular epithelial cells were noticed.

Group V (40ppb)

At the second month of the trial congestion, edema, degeneration and necrosis of the hepatocytes, infiltration of lymphocytes in the parenchyma, proliferation of bile ducts and portal fibrosis were observed. At the fourth month of the experiement, hepatocytomegaly, infiltration of inflammatory cells in the form of nodules, sinusoidal dilatation, dissociation of hepatocytes, portal fibrosis, (Fig. 38), central venous dilatation, vacuolar degeneration and coagulative necrosis of the hepatocytes were seen.

By the sixth month of the study, there were irregular islands of hepatic parenchyma surrounded by dense masses of proliferated bile duct epithelial cells (Fig. 39) along with massive infiltration of the mononuclear cells, dilatation of hepatic sinusoids, bile stasis, dissociation of the hepatic cords and hepatocytomegaly (Fig. 40). The microscopic changes by the eighth month of the treatment were characterised by extensive proliferation of the fibrous tissue with partial irregular segmentation of the hepatic lobules, marked congestion and dilatation of the hepatic sinusoids, (Fig. 41) phlebosclerosis, necrosis of the hepatocytes and dissociation of the hepatic cords.

In the spleen the changes observed were cortical and paracortical lymphoid depletion (Fig. 42), degeneration and necrosis of the lymphocytes, congestion and vascular sclerosis (Fig. 43).

The histological changes evident in the kidney were tubular degeneration, swelling of the tubular epithelium, mild mononuclear cell

infiltration and fibroblastic proliferation in the interstitium along with congestion and haemorrhages (Fig. 44).

Generally, the bursa of fabricius of the aflatoxin treated birds showed varying intensity of changes depending on the duration of the treatment. The changes observed were varying degree of lymphoid depletion in the follicles, interfollicular edema and proliferation of the interfollicular connective tissue (Fig. 45).

4.9 ULTRASTRUCTURAL STUDIES

Liver

The ultrastructure of the hepatocytes in all the treatment groups showed significant retrograde changes of varying degrees consistent with the dose and duration of the treatment. In most cases the nucleus was intact and the heterochromatin was found to be increased in numbers and appeared as clumps on the inner nuclear membrane. In most cases the nuclear matrix was electron lucent with sparsely distributed euchromatin, but occasionally the nuclear matrix was electron dense and the euchromatin was uniformly distributed. The nucleolus was highly condensed and enlarged without any separation between the fibrillar and granular components in many cells. But in group V the hepatocyte nucleus showed prominent nuclear pores but complete absence of the nucleolus (Fig.46). In most cases, the cell cytoplasm was cavitary with the rough endoplasmic reticulum (RER) showing varying degrees of dilatation. Occasionally fragmented RER with degranulated and dispersed ribosomes could be seen. There was a reduction in the number of

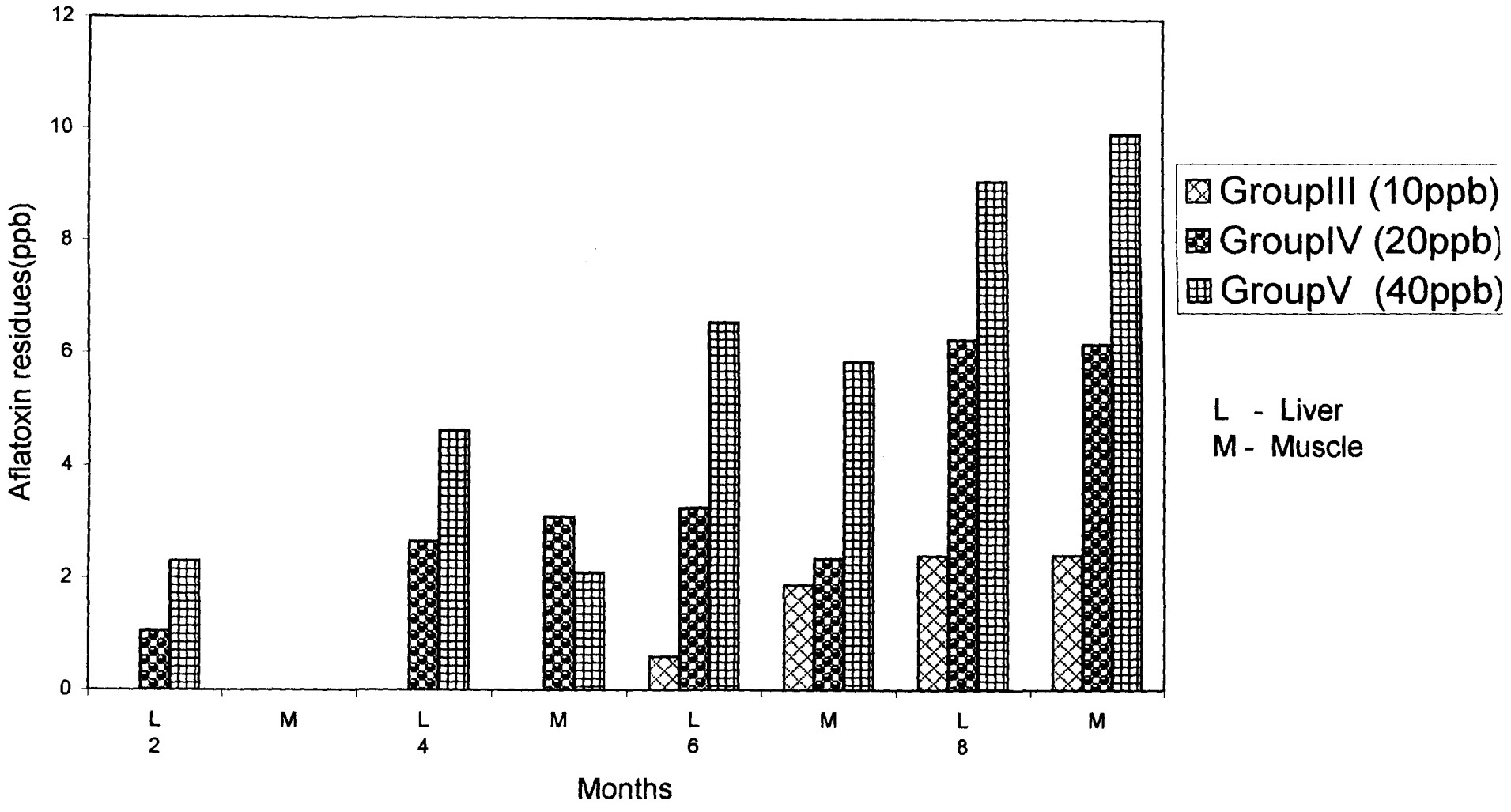
Table.16 Average (Mean ± S.E) Aflatoxin B1 Residues in Tissues (ppb)

Treatment groups	Toxin level ppb)	Observation in months							
		2		4		6		8	
		Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle
Group I	0	-	-	-	-	-	-		-
Group II	5	-	-	-	-	-	-	-	-
Group III	10	-	-	-	-	0.602 ± 0.46 [a]	1.883 ± 0.075 [a]	2.406 ± 0.62 [a]	2.416 ± 0.23 [a]
Group IV	20	1.05	-	2.65 ± 0.97 [a]	3.1 ± 1.57 [a]	3.263 ± 0.79 [b]	2.347 ± 1.38 [a]	6.277 ± 0.66 [b]	6.2 ± 0.20 [b]
Group V	40	2.30	-	4.633 ± 1.23 [b]	2.1 ± 2.1 [b]	6.580 ± 0.64 [c]	5.870 ± 0.10 [b]	9.107 ± 0.74 [c]	9.963 ± 1.15 [c]
F value		-	6.478**	12.043**	6.478**	12.043**	6.478**	12.043**	6.478**
CD		-	3.38	2.27	3.38	2.27	3.38	2.27	3.38

Mean bearing the same superscript are not significantly different

** P < 0.01

Fig. 15. Aflatoxin Residues In Liver And Muscle (ppb)



mitochondria and they appeared rounded, oval or elongated. The mitochondria were either normal or swollen containing swollen cristae along with the presence of electron dense material within the matrix. Though there was a general depletion of glycogen reserves, a few glycogen particles could be seen as small aggregates confined to focal areas. Scattered lipid droplets were also encountered in the cytosol. (Fig. 47) In some cells, the smooth endoplasmic reticulum (SER) showed proliferation and hypertrophic changes and they appeared as small tortuous vesiculated structures. (Fig. 48)

4.10 AFLATOXIN RESIDUES IN THE TISSUES

The values are presented in Table. 16 and shown Fig. 15. No residual levels of aflatoxin were detected at the second month except in one sample of the liver each belonging to group IV and V.

At the fourth month of the study, no aflatoxin residues could be detected in the groups II and III whereas in the groups IV and V, aflatoxin residues were found in liver and muscle. The dose dependant aflatoxin residues detected in the pooled samples of kidney and blood were 2.9 and 0.63 respectively in group IV and 3.63 and 1.62 respectively in group V.

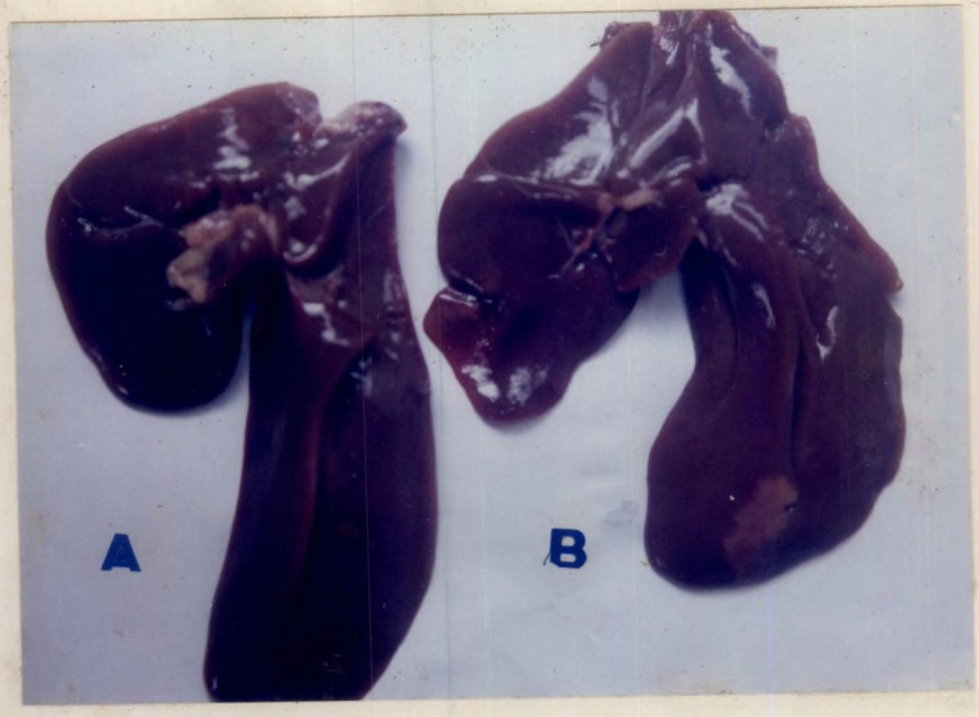
At the sixth and eighth month of the treatment, aflatoxin residues were found in the liver and muscle. The dose dependant aflatoxin residues detected in the pooled samples of kidney were 3.2, 3.95, 4.93 and blood were 1.9, 2.3, 2.9 in groups III, IV and V.

The results of the random samples cross-checked by the HPLC method were similar to the corresponding TLC values.

Fig. 16.A Liver - pale to tan in color and enlarged - group II - Fourth month

16.B. Liver - enlarged with the surface showing scattered pin head sized necrotic areas, yellowish discoloration and subcapsular haemorrhages - group III - fourth month

Fig. 17. Liver - hepatomegaly, pale yellowish discoloration and pale focal areas - group IV - sixth month


A
B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14

Fig. 18. A. Liver - pale yellow and enlarged - group IV - eighth month

18. B. Liver - control - eighth month

Fig. 19. Liver - hepatomegaly with Petechial and subscapsular haemorrhage group IV - eighth month

A
B

Fig. 20. Liver - hepatomegaly with bright yellow discoloration - group V - sixth month

Fig. 21 Liver - hepatomegaly with pale focal areas - group V - sixth month

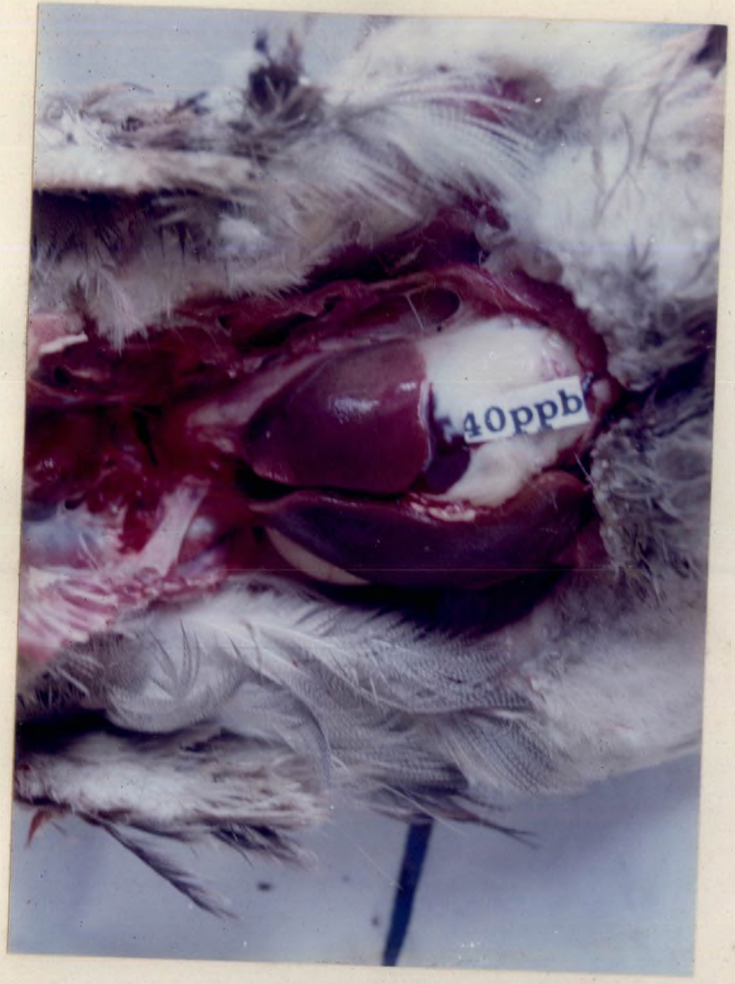

40ppb

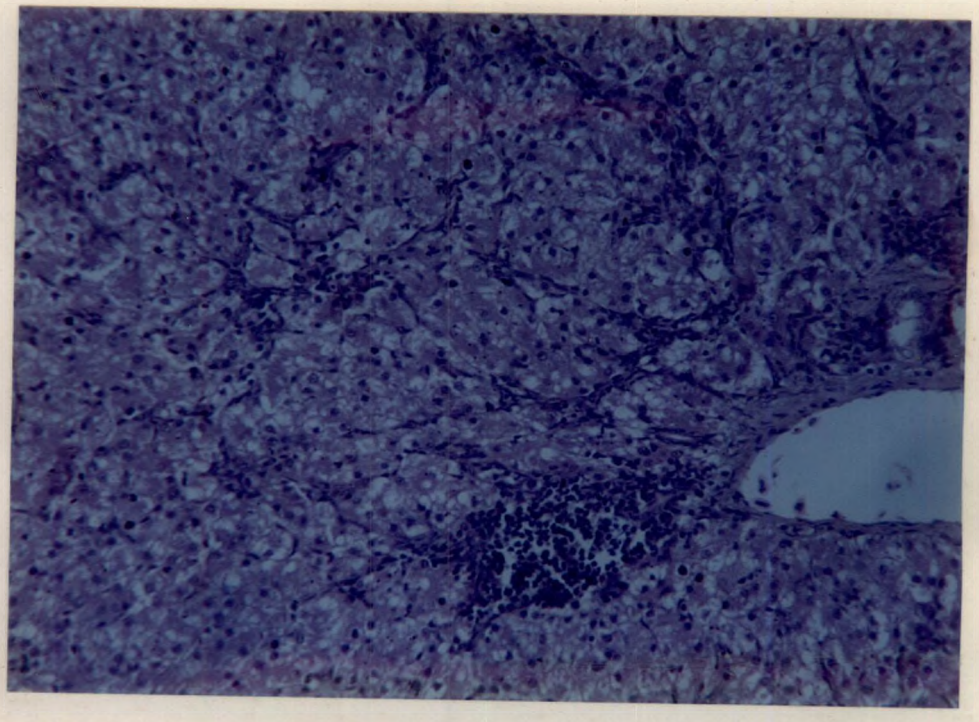
Fig. 22. Liver - pale greenish discoloration with surface granularity - group V - eighth month

Fig. 23.A Liver - hepatomegaly with marked surface nodularity - group V - eighth month

23.B Liver - control - eighth month

Fig. 24. Liver - Moderate vacuolation and necrosis of hepatocytes, bileduct hyperplasia, Kuppffer cell proliferation and mononuclear cell infiltration - group II - fourth month - H&E x 400

Fig. 25 Liver - diffuse hepatic degeneration, bileduct hyperplasia and focal collection of mononuclear cells in the periportal areas - group II - fourth month - H&E x 250



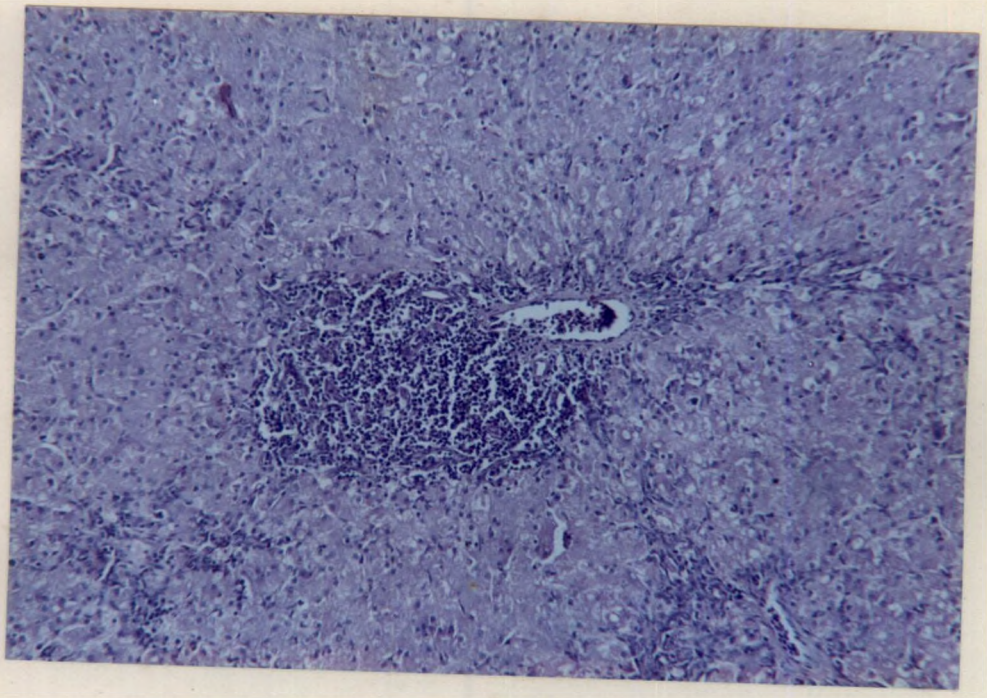
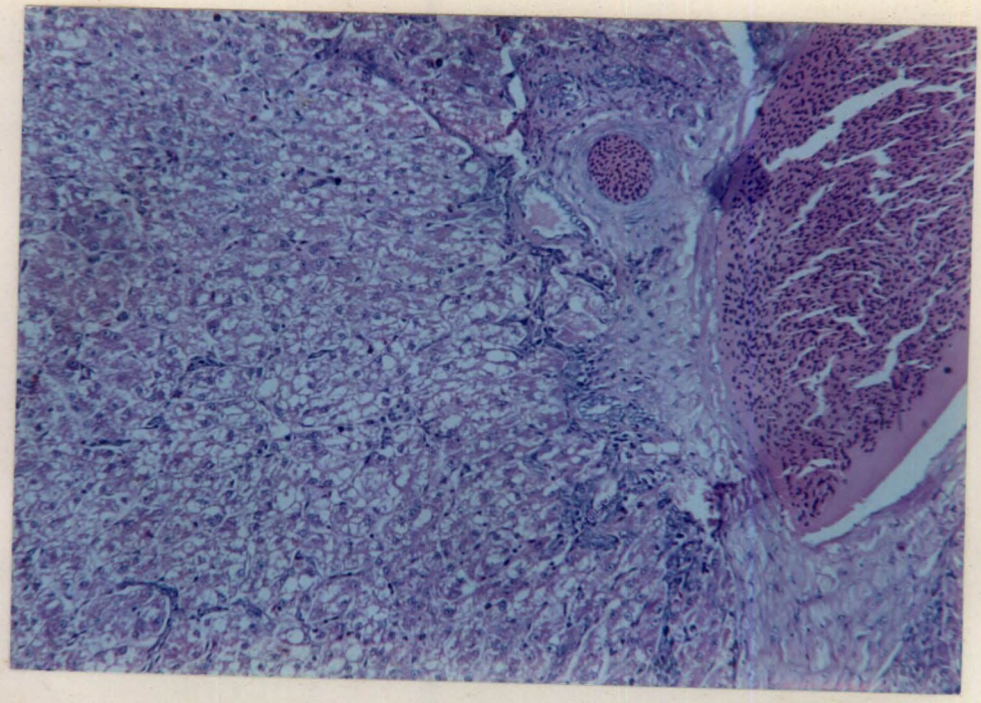


Fig. 26 Liver - Marked Portal Congestion, bile duct hyperplasia and diffuse degeneration and necrosis of hepatocytes - Group II - eighth month - H&E x 250

Fig. 27 Liver - extensive bileduct hyperplasia,diffuse cytoplasmic vacuolation of the hepatocytes and focal collection of the mononuclear cells - group II - eighth month - H&E x 250



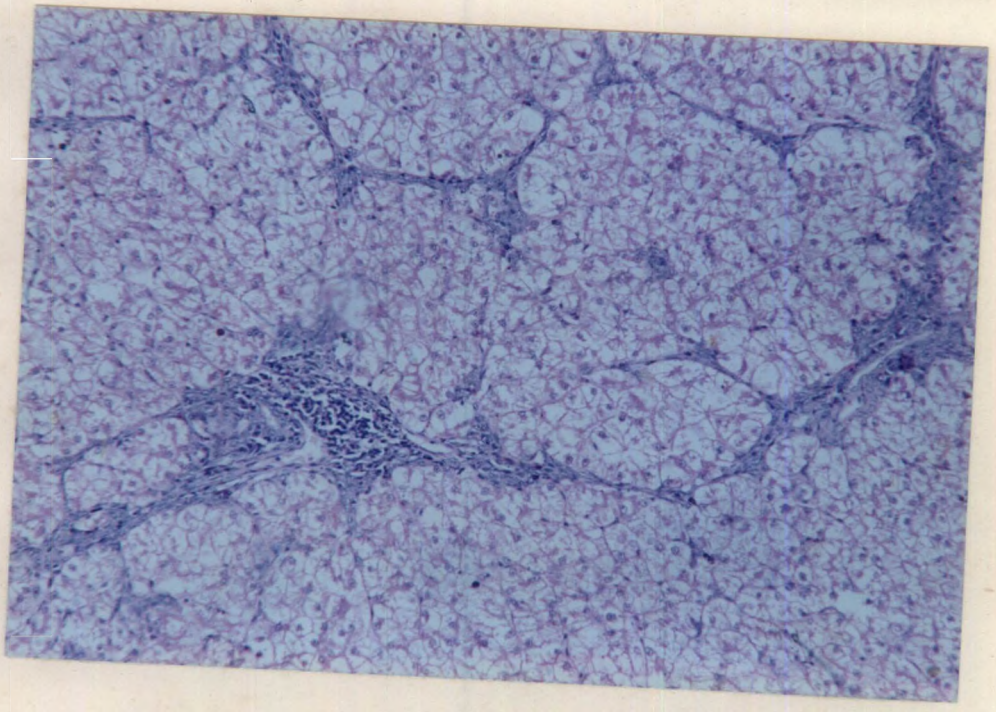
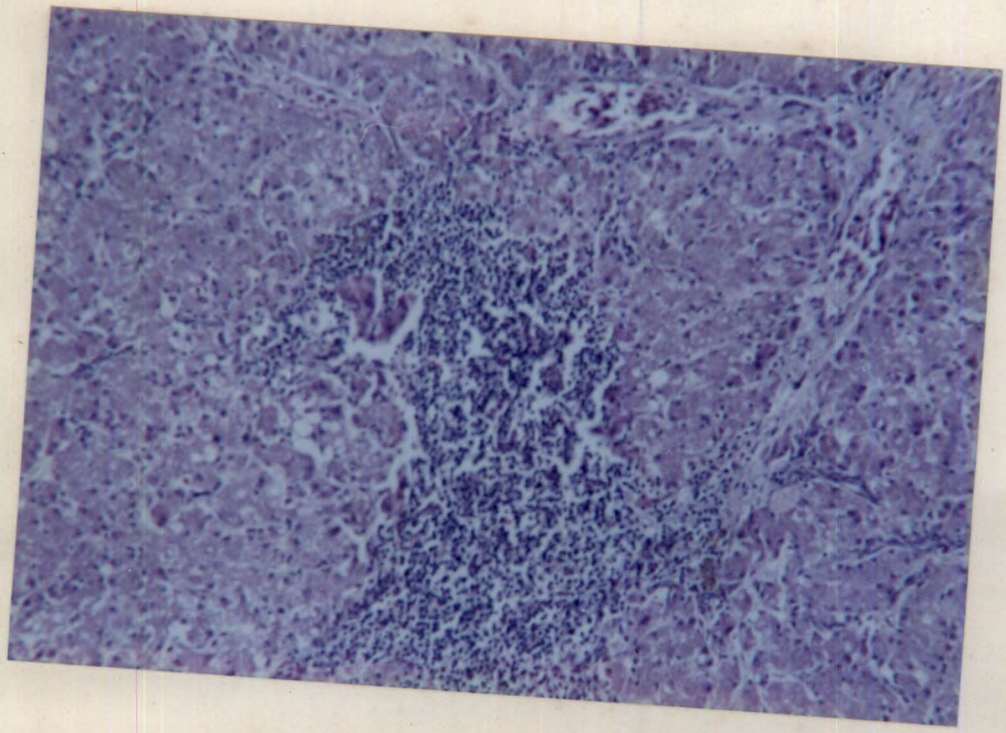


Fig. 28 Liver - diffuse degeneration and necrosis of hepatocytes, mild bile duct hyperplasia and portal venous congestion - group III - second month - H&E x 250

Fig. 29 Liver - Congestion of hepatic sinusoids and focal collection of mononuclear cells replacing the necrotic hepatocytes - group III - fourth month - H&E x 250



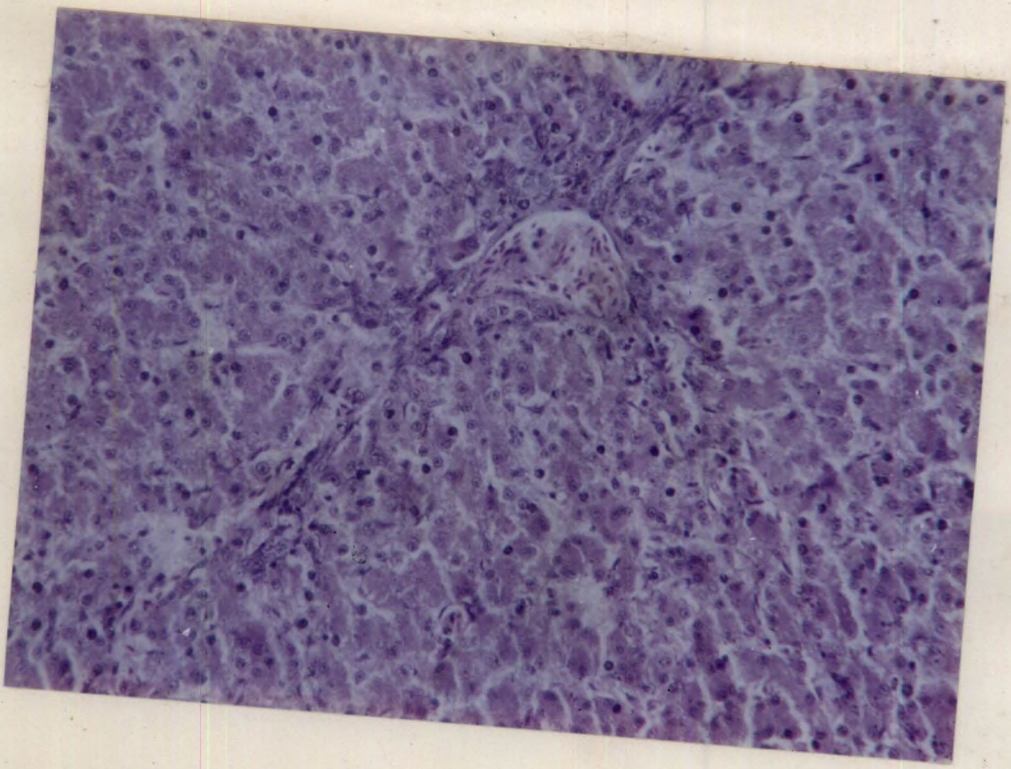
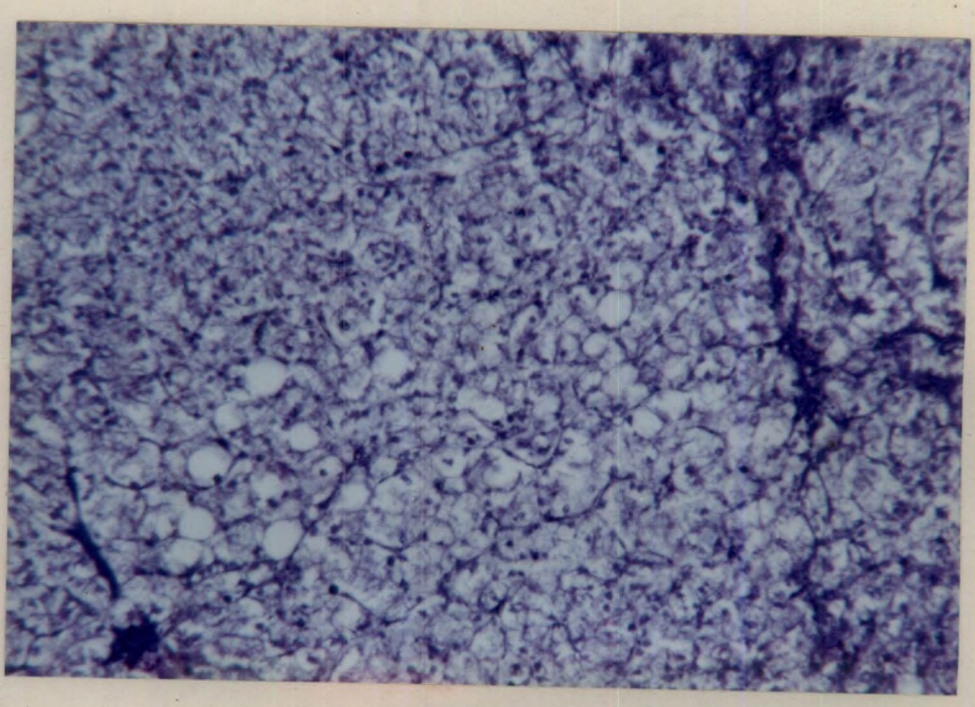


Fig. 30 Liver - bileduct hyperplasia, Kupffer cell proliferation and extensive fatty degeneration - group III - fourth month - H&E x 250

Fig. 31. Liver - portal congestion, fatty change of hepatocytes and focal perivascular accumulation of the mononuclear cells - group III - eighth month - - H&E x 250



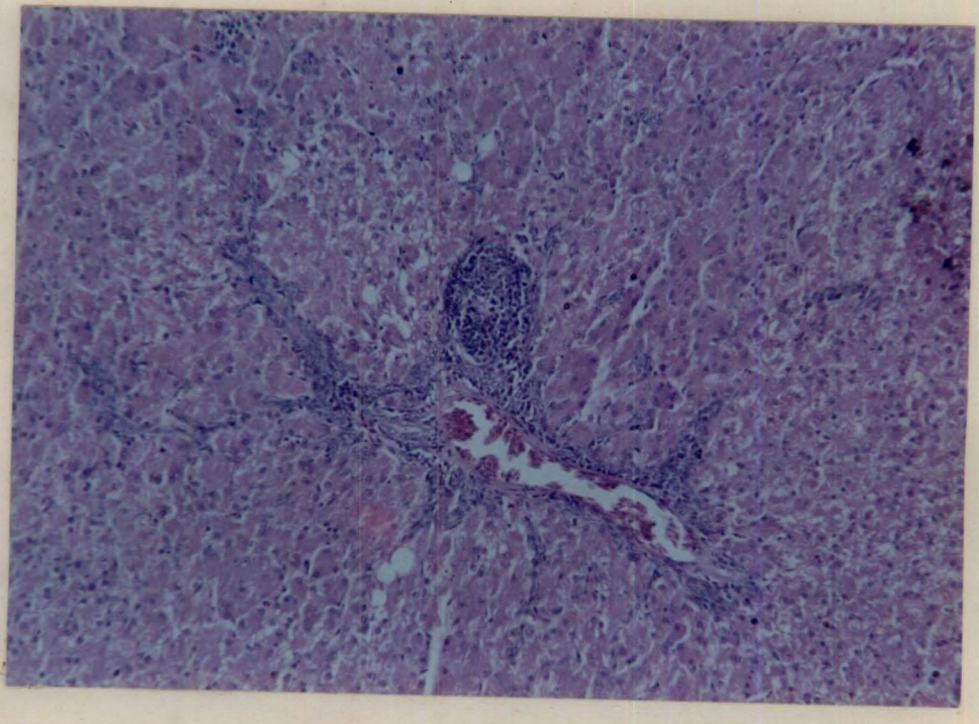


Fig. 32 Liver - Portal congestion, Phlebosclerosis and portal tract fibrosis - group III - eighth month - H&E x 250

Fig. 33. Liver - Portal congestion, diffusion diffuse hepatic degeneration and necrosis - group III - eighth month - H&E x 250

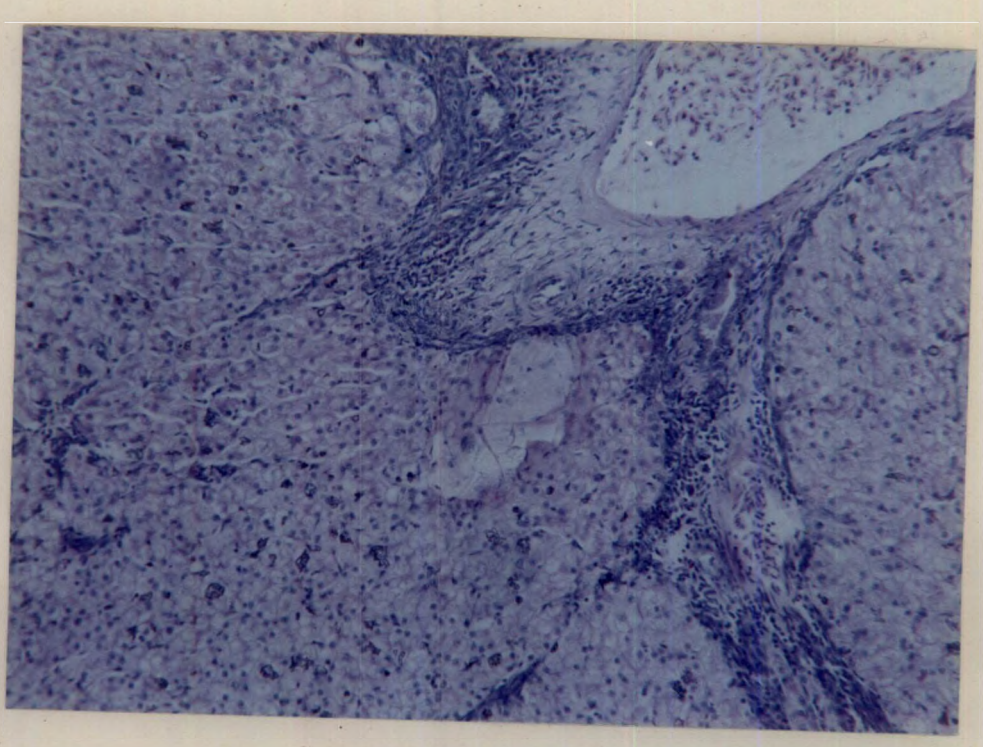
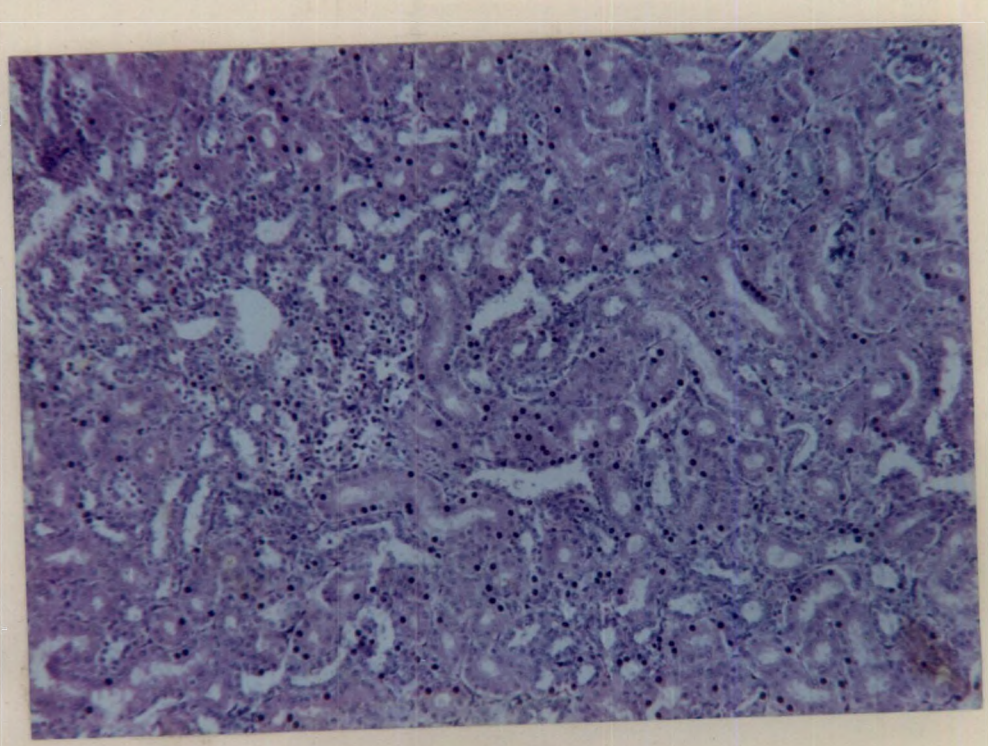


Fig. 34 Kidney - mild degenerative changes in the renal tubular epithelial cells and peritubular accumulation of mononuclear cells - *group III* - eighth month - H&E x 250

Fig. 35. Liver - extensive bileduct hyperplasia, moderate hepatic degeneration and focal but massive collection of Lymphoid cells in the periportal areas - group IV - second month - H&E x 250



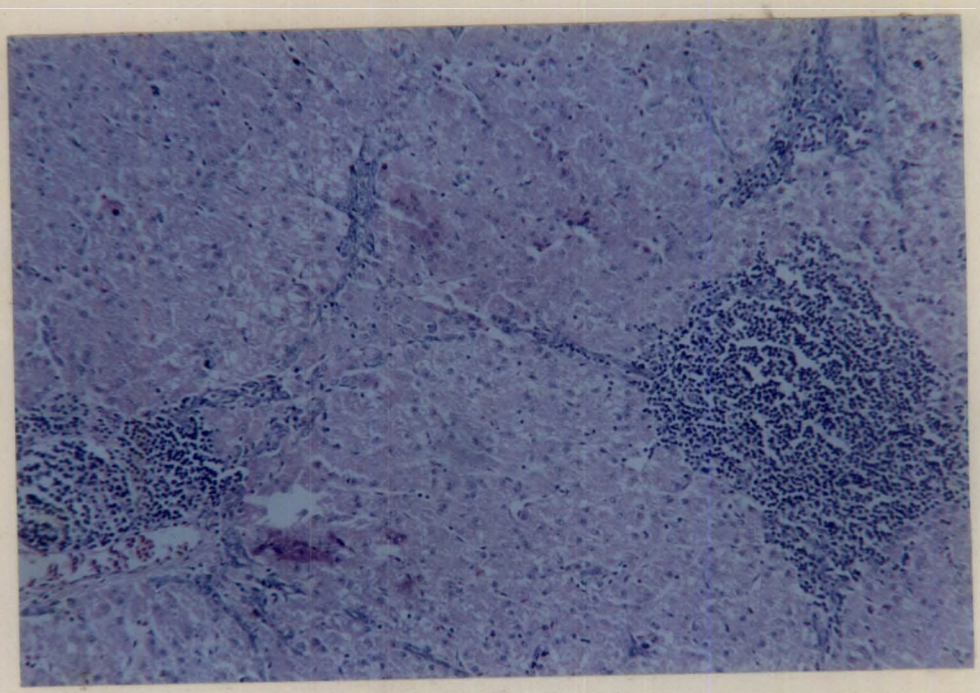
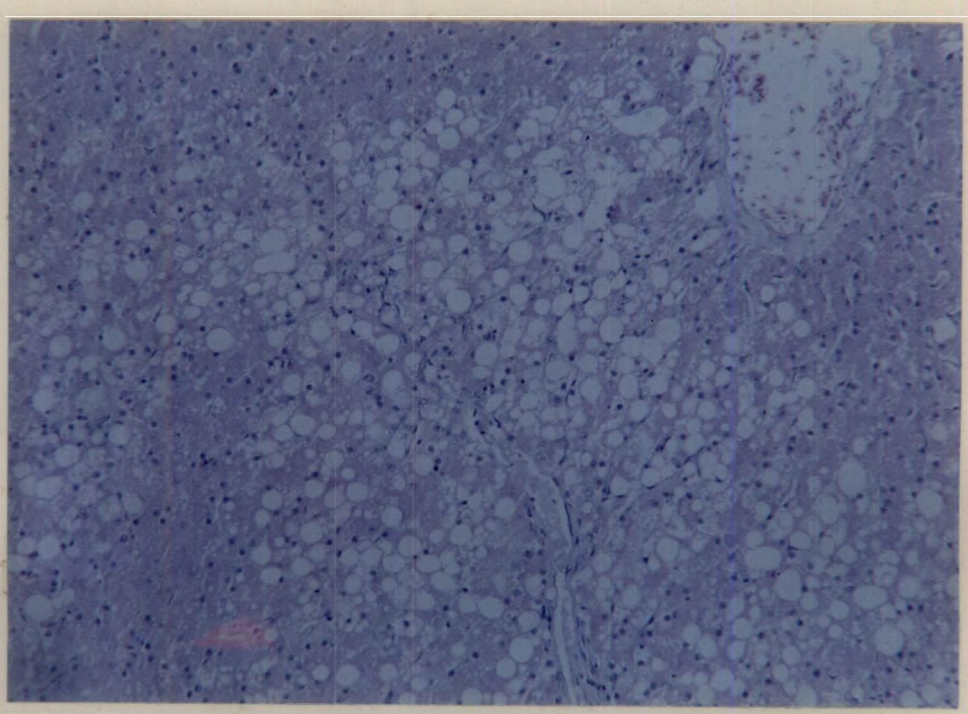


Fig. 36. Liver - hepatomegalocytosis and extensive fatty change in the hepatocytes - group IV - eighth month - H&E x 250

Fig. 37 Spleen - marked congestion and focal depletion of lymphoid cells - group IV - eighth month - H&E x 250



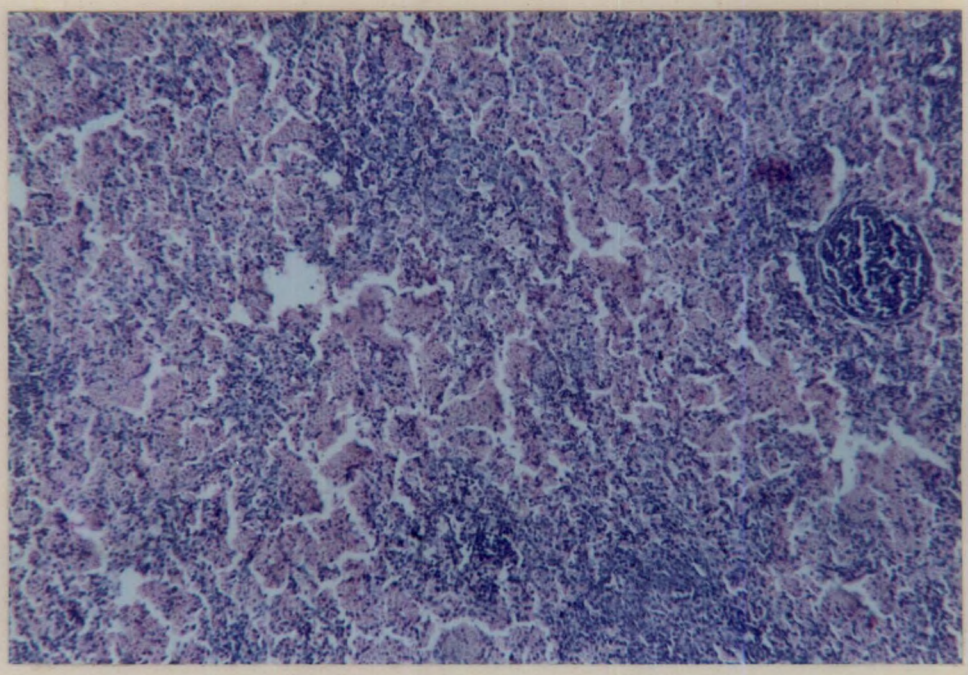
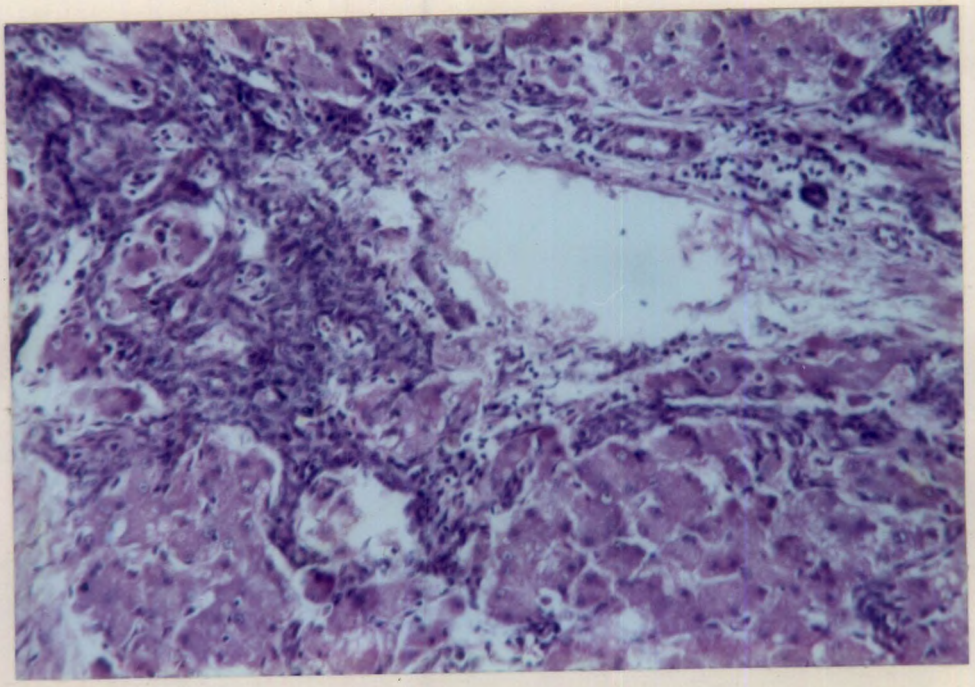


Fig. 38. Liver - hepatocytomegaly, sinusoidal dilation, dissociation of hepatocytes and portal fibrosis - group V - fourth month - - H&E x 400

Fig. 39. Liver - irregular islands of hepatic parenchyma surrounded by dense masses of Proliferated bileduct epithelial cells - group V - sixth month - H&E x 160



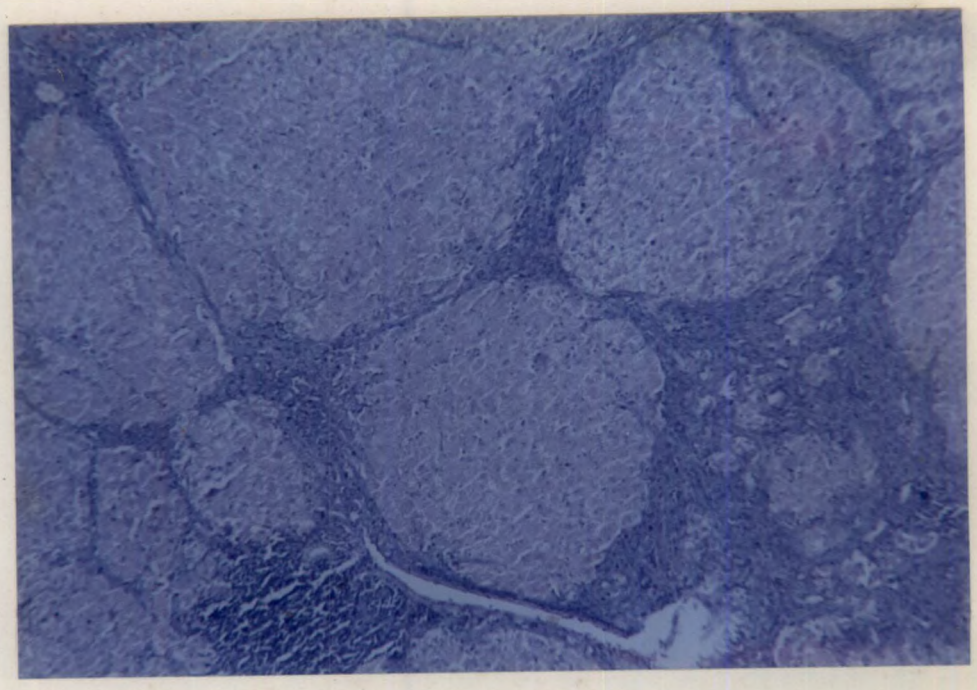
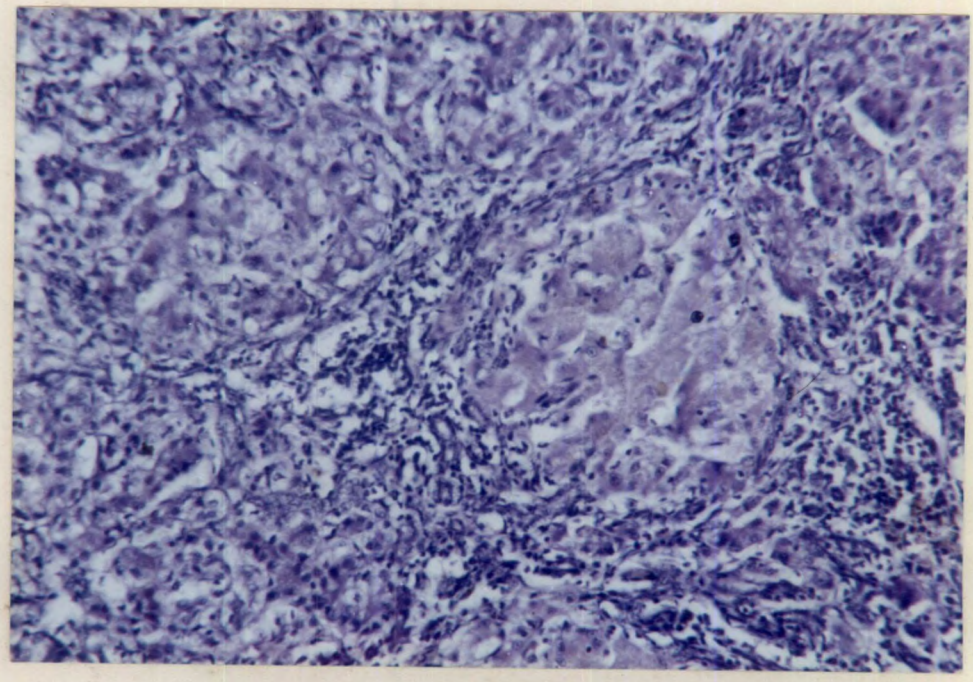


Fig. 40. Liver - Massive infiltration of the mononuclear cells, encircling irregular islands of hepatic parenchyma, dissociation of hepatic cords and hepatocytomegaly - group V - sixth month - H&E x 400

Fig. 41 Liver - extensive proliferation of fibrous tissue with partial irregular segmentation of the hepatic lobules, marked congestion and dilatation of the hepatic sinusoids - group V - eighth month - H&E x 250



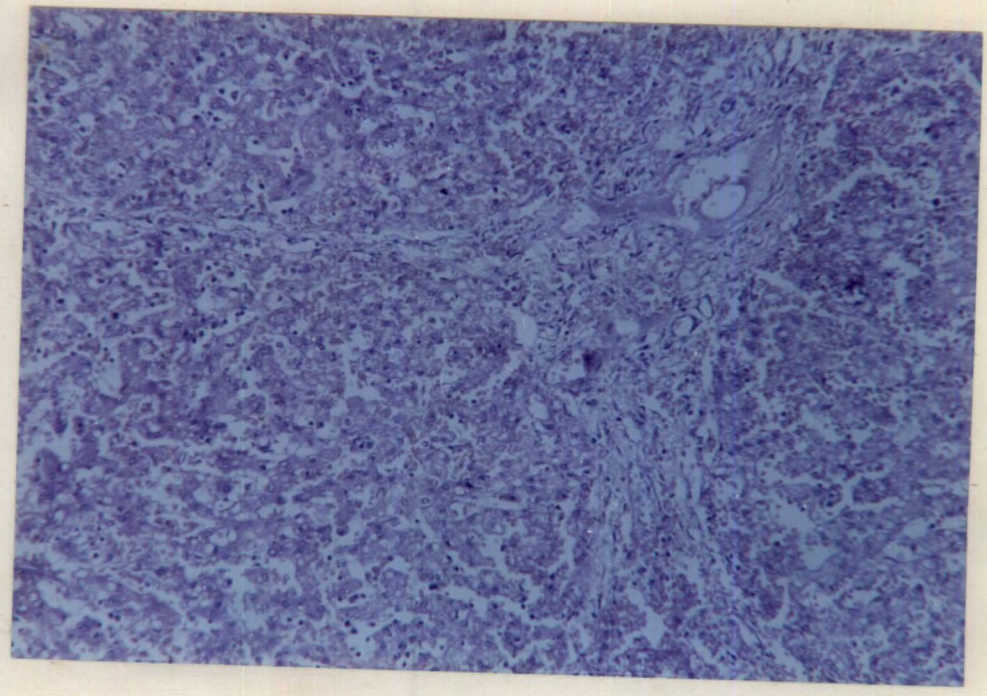


Fig. 42. Spleen - Cortical and Paracorticol lymphoid depletion - Group V - eighth month - H&E x 160

Fig. 43. Spleen - degeneration and necrosis of the lymphocytes and vascular sclerosis - group V - eighth month - H&E x 160

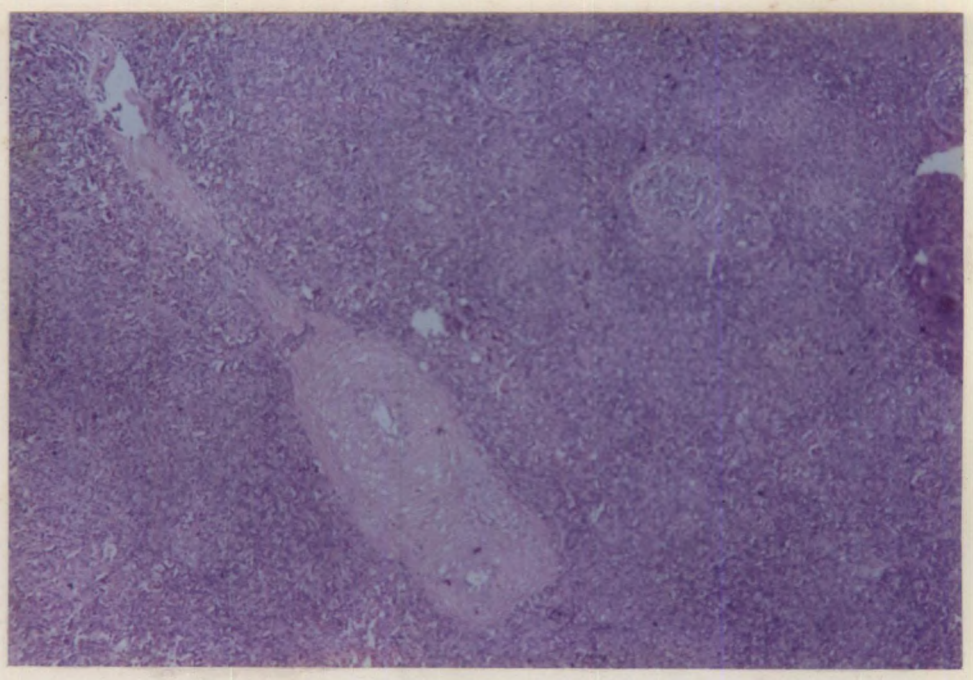
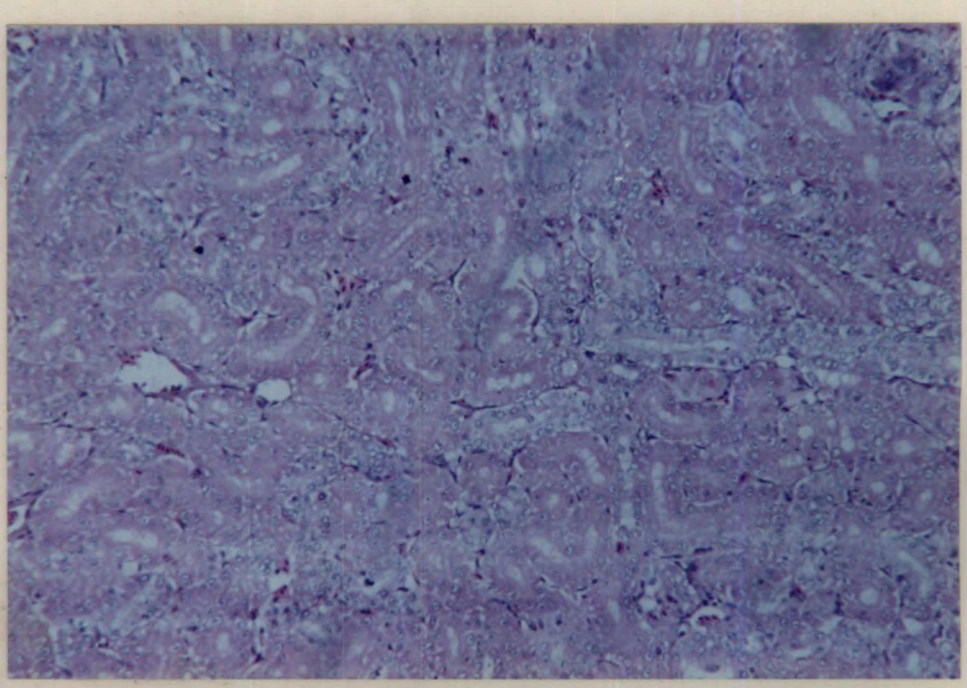


Fig. 44. Kidney - Swelling and degeneration of the tubular epithelial cells, fibroblastic proliferation in the interstitium along with congestion and haemorrhages - group V - sixth month - H&E x 250

Fig. 45. Bursa of Fabricius - lymphoid depletion in the follicles, interfollicular edema and proliferation of the interfollicular connective tissue - H&E x 250



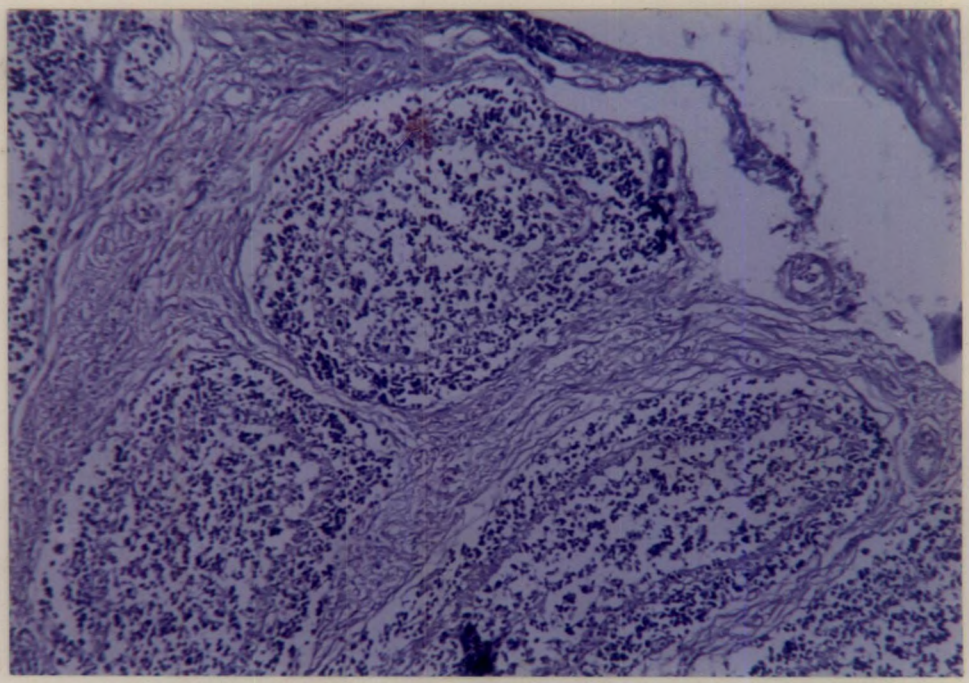


Fig. 46. Electron microscopy - liver - hepatocyte showing nucleus (N) with prominent nuclear pores, heterochromatin appearing as clumps on the inner nuclear membrane and complete loss of nucleolus, rough endoplasmic reticulum (RER) showing dilatation and partial degranulation - group V - eighth month - EMX 32000

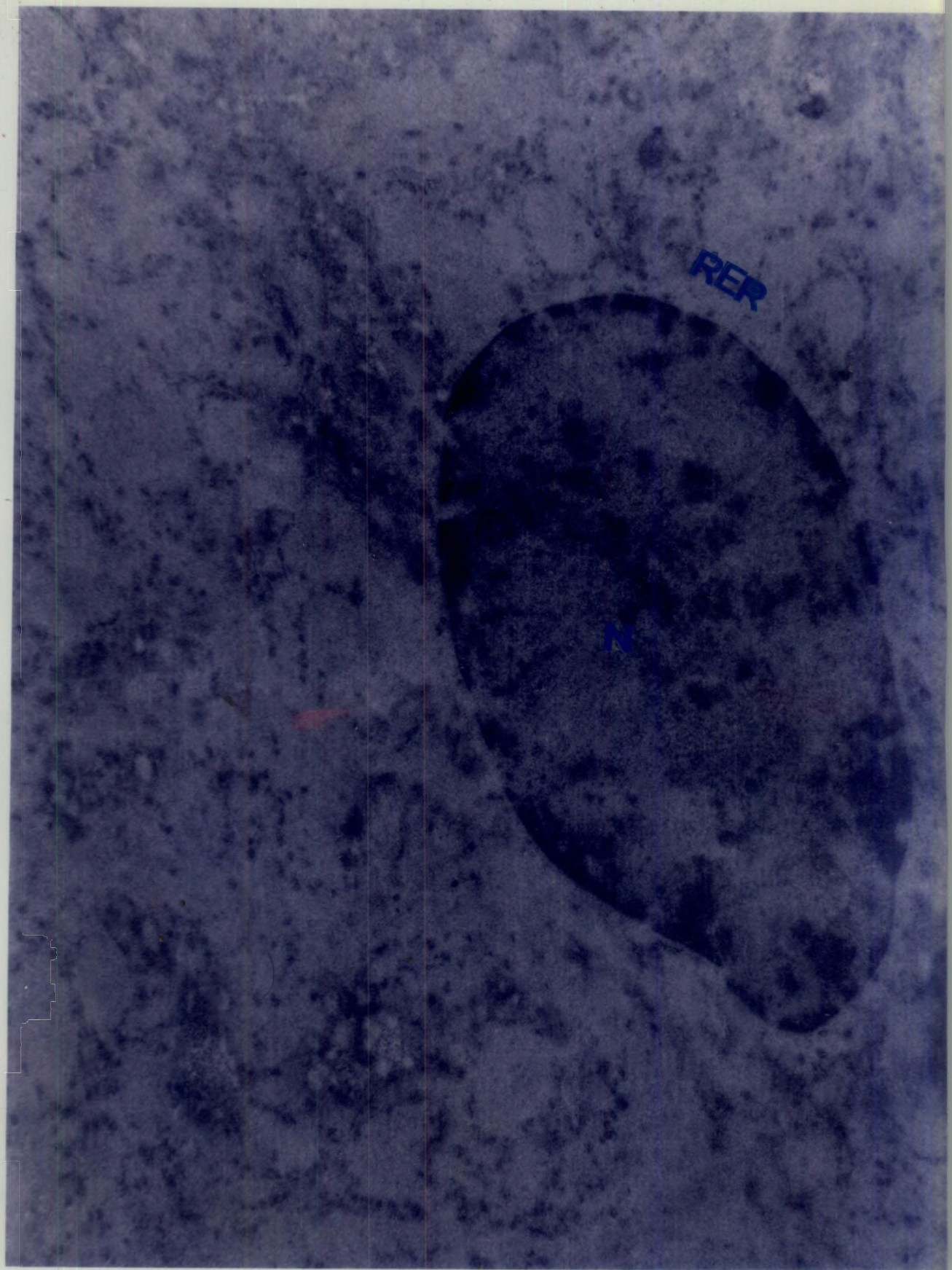

RER
N

Fig. 47. Electron microscopy - Liver - hepatocyte showing intact nucleus (N) with condensed nucleolus (NU) . Mitochondria (M) swollen containing swollen cristae along with electron dense matrix, dilated and fragmented rough endoplasmic reticulum (RER), and scattered glycogen particles(G) and lipid droplets (L) can also be seen - group IV - sixth month - EMX 8400

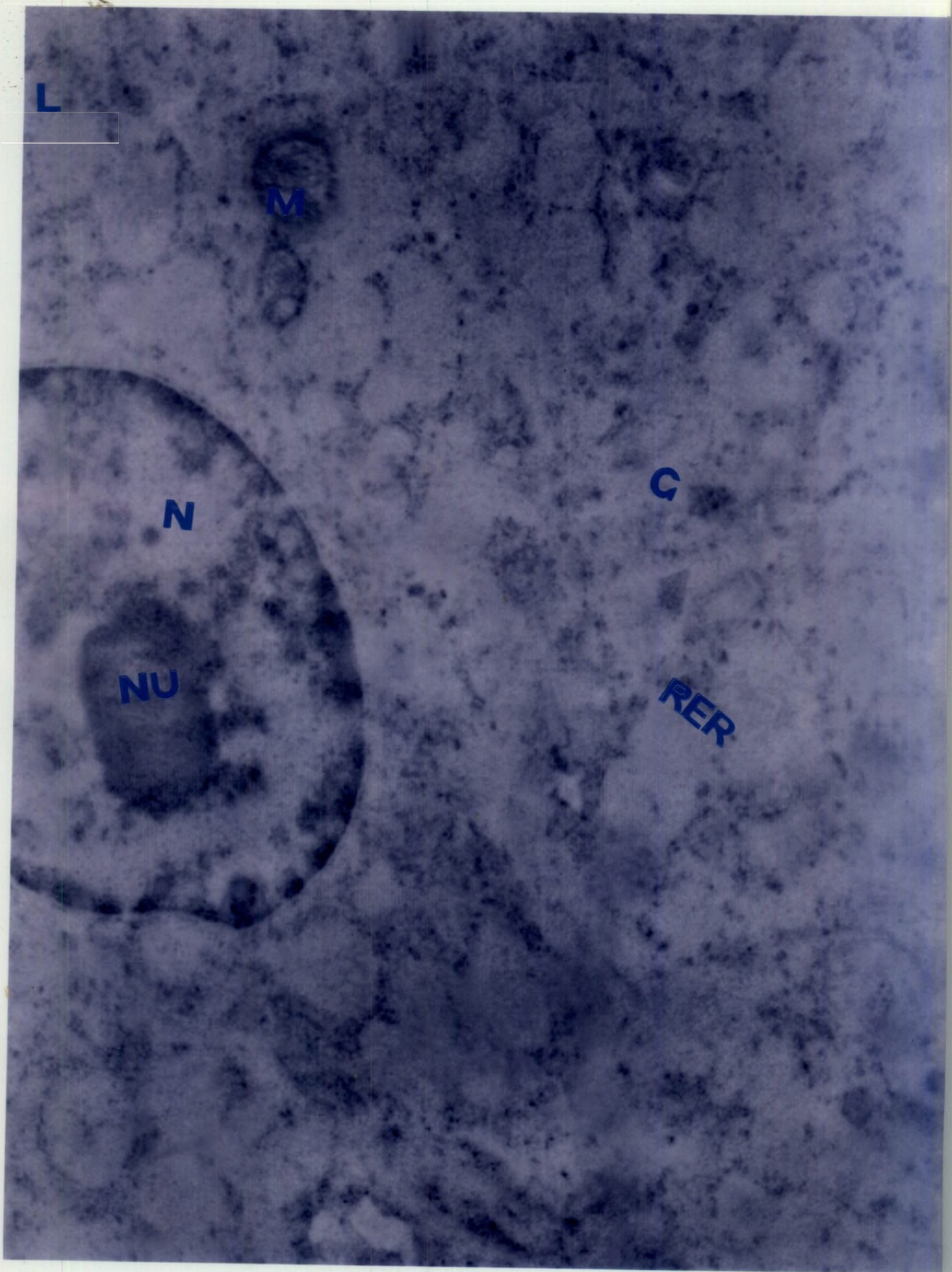
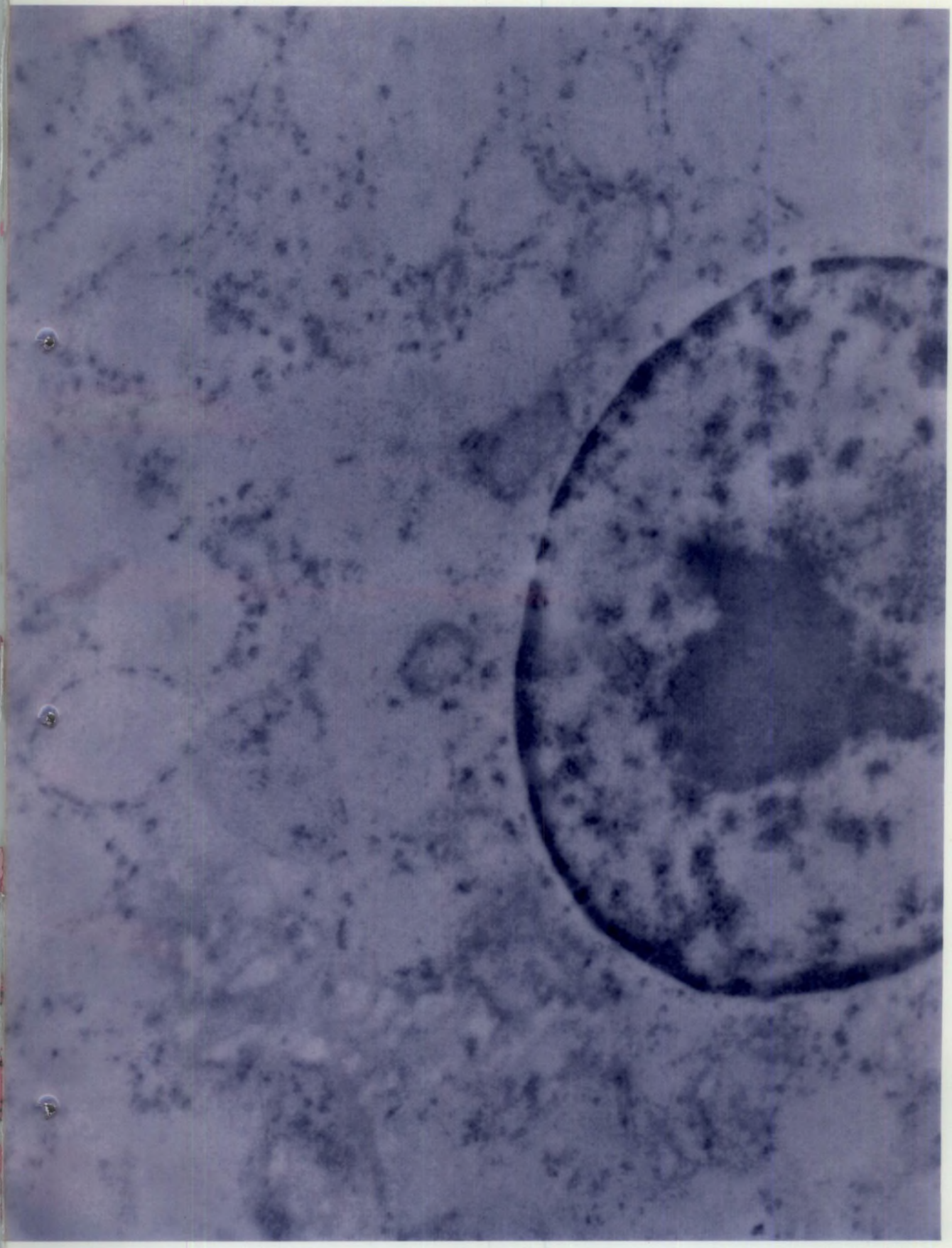

L
M
N
NU
C
RER

Fig. 48. Electron microscopy - Liver - hepatocyte showing cytoplasmic cavitations and the smooth endoplasmic reticulum (SER) appearing as small tortuous vesiculated structures due to proliferative and hypertrophic changes - group IV - sixth month - EMX 1050



Discussion

DISCUSSION

The experiment was designed to assess the pathological effects of aflatoxicosis and to determine the permissible level of aflatoxin employing duck as the experimental model. Graded level of aflatoxin contaminated feed (5ppb, 10ppb, 20ppb and 40ppb) were fed daily for a period of eight months and the following parameters were analysed to evaluate the effects of aflatoxin at different dose levels. The parameters studied were body weight, carcass weight, liver weight, haemogram, serum protein profiles, gross and histopathology of the liver, spleen, kidney and bursa and ultrastructural pathology of liver.

There was a significant decrease in the body weight and carcass weight of the ducks fed with graded levels of aflatoxin contaminated feed. The observations made support the findings of earlier investigators in ducks and in other avian species (Butler, 1964; Smith and Hamilton, 1970; Arafa *et al.* 1981; Campbell *et al.* 1981; Doerr *et al.* 1983; Leena Devi, 1992; Rao *et al.* 1995; Goswami and Mukit, 1997 and George, 1998). Aflatoxin at low levels in the diet caused reduction in the feed intake and growth rate leading to a decline in the carcass weight . The reduction in the body weight and carcass weight is a critical factor that could affect the economy of duck farming and lead to great economic loss to the farmer. This study, therefore, underscores the need for regular screening of the duck feed for aflatoxin content. This observation has great practical relevance since it has been reported that 33.9

percent of the feed samples available in the Kerala market are aflatoxin contaminated and 16.2 percent feed samples had aflatoxin levels above 100ppb (Rajan *et al.* 1991).

In the present study, data on the total leucocyte count, haemoglobin concentration and packed cell volume indicate an anaemic status in ducks fed dietary aflatoxin. Dose dependent reduction in the total leucocyte count, haemoglobin concentration and packed cell volume and increase in the erythrocyte sedimentation rate (ESR) in toxin fed ducks reflects anaemia and this could be a contributory factor for the increase in the erythrocyte sedimentation rate (ESR). There was a significant increase in the erythrocyte sedimentation rate of aflatoxin treated ducks in a dose related manner. This could be ascribed to the hepatic damage leading to the reduction in the total serum protein, albumin and albumin/globulin ratio (A/G). The present observations are in agreement with Brown and Abrams (1965), Wyatt *et al.* (1975), Huff *et al.* (1986), Yaman *et al.* (1989), Maryamma *et al.* (1990), Leena Devi (1992), Vigil Anbiah (1996), However, in the present study the findings on ESR are at variance with that of Rajan *et al.* (1973).

There was significant increase in the leucocyte count at the fourth and sixth month but by the second and eigth month, treatment groups showed only a relative increase in the leucocyte count with marked lymphopenia and compensatory heterophilia. The findings in the present study are in accordance with that of Wannop (1961), Tung *et al.* (1975), Somvanshi and Mohanty (1990), Sova *et al.* (1991), in broiler chicken and Vigil Anbiah (1996)

in ducks. The reduction in lymphocyte count could be attributed to the damaging effect of aflatoxin on the lymphoid organs as evidenced by the depletion of lymphocytes in the follicles of the spleen and bursa. Aflatoxin dosed ducks had heterophilia and this concurred with the findings of Wannop (1961). However, during the course of the present study neither the control nor the experimental birds showed any clinical signs of bacterial or viral infections.

Wannop (1961) reported monocytosis and reduction in basophils was reported by Tung *et al.* (1975). There were no marked changes in the monocytes, eosinophils and basophils in the present study.

Aflatoxin treated ducks showed reduction in the total serum protein and albumin in a dose related manner. The findings in the present study are in agreement with that reported by Doerr and Huff (1980), Reddy and Yadgiri (1984), Huff *et al.* (1986), Kubena *et al.* (1990), Jassar *et al.* (1993) in other avian species and Leena Devi (1992), Vigil Anbiah (1996) and George (1998) in ducks. Reduction in the total serum protein and albumin is due to the inhibition of protein synthesis by altering the protein metabolism and its binding to the DNA which does not allow replication and transcription of RNA (Murray, 1982).

The influence of aflatoxin on the serum globulin level was studied and the study showed a significant increase in the serum globulin level in a dose dependent manner. The increase in the serum globulin level could be due to an increase in the beta globulin levels in hepatitis and cirrhosis and gamma

globulins in kupffer cell proliferation (Benjamin, 1985). Reduction in the level of total serum protein and serum albumin resulted in a reduced albumin-globulin ratio in the present study.

Increased level of serum alkaline phosphatase (SAP) and γ-Glutamyl transpeptidase (GGT) were observed in the present study throughout the experimental period depending upon the dose and the duration of treatment. Similar observations were recorded by Leena Devi (1992) in ducks and Ideo *et al.* (1972) Kojima and Sakurada (1976), Jalanko and Ruoslahti (1979) and Mammen (1994) in other species. The increase in the levels of serum alkaline phosphatase and γ - Glutamyl transpeptidase could be attributed to the direct toxic damage to the liver and other organs like the kidney, resulting in the leakage of these enzymes into the circulation as is evidenced by the gross and histopathological changes.

The evidence of hepatic change as reflected by the haematological, serological and enzymological studies were further confirmed by the organ weight, gross, histopathological studies and ultrastructural pathology of the liver.

The influence of aflatoxin on the liver weight was studied by many research workers. Both increase and decrease in liver weight have been documented by Carnaghan (1964), Devegowda (1994), Chen Yeong Hsiang *et al.* (1995) in ducks and by Wyatt *et al.* (1973), Doerr and Huff (1980), Maurice *et al.* (1983) and Shiang (1994) in other avian species. Observations made in the present study indicated highly significant increase in the liver weight in a

dose dependent manner throughout the experimental period. Increase in the liver weight correlated with the microscopical evidence of fatty change.

Grossly, the birds fed with 5ppb aflatoxin revealed the following hepatic lesions. By the second month, the liver was congested, pale and soft and by the fourth month the liver was pale to tan in colour. By the sixth and eighth month, the liver became enlarged which was reflected by an increase in the liver weight. The liver of ducks fed with 10ppb showed hepatomegaly, focal areas of necrosis, yellow discoloration, petechial to ecchymotic haemorrhages and pale infarctions depending upon the duration of treatment. Diffuse enlargement, subcapsular haemorrhages and pin point necrotic areas were observed in the liver of ducks fed 20ppb aflatoxin and necrotic areas, firm consistency, greenish discoloration, surface granularity and nodularity were observed in increasing intensity in ducks fed with 40 ppb aflatoxin depending upon the duration of the treatment.

Comparable gross lesions have been reported by Butler (1964); Carnaghan (1965), Muller *et al.* (1970), Maryamma *et al.* (1990) in ducks. Similar observations have also been reported by Moorthy *et al.* (1985), Ramadevi *et al.* (1990), Kumar *et al.* (1993), Bakshi *et al.* (1994) in other avian species.

Congestion was noticed in the spleen and kidney by the second month and fourth month with 5ppb and 10ppb aflatoxin. Bursae were atrophic or regressed by the second month with 40 ppb aflatoxin. Splenomegaly, pale enlarged kidney with focal necrotic areas were seen in ducks fed with 40 ppb

aflatoxin depending upon the duration of the treatment. The present finding were in agreement with that of Muller *et al.* (1970), Thaxton (1974); Balachandran and Ramakrishnan (1987.a), Nageswara Rao *et al.* (1988); Arshad *et al.* (1992), Kumar *et al.* (1993) and George (`1998).

On histological examination there was central venous congestion, portal fibrosis, biliary hyperplasia, coagulative necrosis, fatty change and lymphocyte infiltration in the liver with 10ppb and 20ppb of aflatoxin. Besides the above lesions, fibroblastic proliferation was seen with 40ppb of aflatoxin by the eighth month of the experiment. Ducks fed with 5ppb of aflatoxin showed congestion and bile duct proliferation. The intensity of these lesions increased with the increase in the dose of aflatoxin and duration of the trial.

Lymphoid depletion, congestion and vascular sclerosis were noticed in the spleen in the present study. Tubular edema, fragmentation of glomerular tuft, thickening of the basement membrane of the glomerular tuft and desquamation of the lining epithelium of the tubules were noticed in the kidney.

The samples of the bursae from the different groups were subjected to the histopatholigical examination and the general changes observed were severe lymphoid depletion, proliferation of interfollicular connective tissue and interfollicular edema of varying intensity depending on the dose and duration of the treatment.

Butler (1964) conducted experiments in Khaki Campbell ducklings and noted the sequential histopathological changes. Similarly Carnaghan (1965)

observed hyperplasia of the bile duct epithelium and infiltration of lymphocytes in the ducks fed with aflatoxin contaminated Brazilian groundnut meal. Muller *et al.* (1970) observed biliary hyperplasia and cytoplasmic vacuolation in ducklings fed with aflatoxin. Radeleff (1970) observed chronic hepatic fibrosis in ducks and ducklings due to sub-acute aflatoxicosis. The observations in the present study were similar to those reported by Deshek *et al.*(1983) in quails, in chicken (Moorthy *et al.* 1985 and 1986) and in ducks Jayakumar (1988) Maryamma *et al.* (1990).

The present study revealed that increase in the liver weight could be due to fatty change which was evident by the gross and histopatholigical studies. Increased accumulation of lipid may be due to defective phosphorylation of fat (Tung *et al.* 1972) and interference in lipogenesis (Donaldson *et al.* 1982) which was manifested by the presence of vacuoles of varying sizes in the hepatocytes. Infiltration of inflammatory cells in the present study could be due to necrosis. By the eighth month of the experiment, ducks fed with 40ppb revealed hepatic pseudolobulation, and renal intersitial fibrosis which are evidences of a chronic reaction.

Depletion of lymphocytes in the follicles of the spleen and bursae accounted for lymphopenia. Severe degenerative changes in the kidney at a dose level of at 40ppb indicated renal damage. This suggested that aflatoxin is also a nephrotoxin at high dose levels and cumulative in its action.

The ultrastructural studies revealed retrograde changes that supported the histological changes and the serum protein values. In this investigation the

cytological alteration in the hepatocyte after feeding aflatoxin were basically same as described by several earlier works. The most conspicuous change seen in the cell was the the degranulation of RER and extension vascular change. The RER also showed vesiculation and fragmentation of the membranes. Free ribosomes were found in the cytoplasm. The degranulation of RER indicated suppression of protein synthesis in the cell. This was further evidenced by the appearance of heterochromatin in large amounts forming clumps always along the inner nuclear membrane (Scarpelli and Trump, 1964; Stenger, 1970; Thomson, 1984). The suppression of protein synthesis was reflected in the serum concentration of the albumin and total protein in the experimental study. Further it has beenshown that ribosomal detachment would be accompanied by the lipid accumulation and generalized glycogen depletion (Smuckler *et al.* 1962). Those findings were also recorded in this investigation. Fragmentation of the endoplasmic reticulum was the result of peroxidation of unsaturated lipids in the membranes by the free radicals generated. (Cheville, 1983; Thomson, 1984). The proliferation of the smooth endoplasmic reticulum indicated attempts of detoxification. SER is concerned with biotransformation of Xenobiotics. It was reported that when there was an increased demand for biotransformation, proliferation of these membranes occured (La Via and Hill, 1971; Cheville, 1983).

In the present study, aflatoxin B_1 residues could be detected in the liver, muscle and pooled samples of the kidney and blood in groups III, IV and V by the sixth and eighth months. After feeding aflatoxin contaminated feed

for two months, no aflatoxin residues could be detected in tissues of aflatoxin fed ducks except in one liver sample each of groups IV (20ppb) and Group V (40ppb). By the fourth month of the study, no aflatoxin residues could be detected in the groups II and III whereas in the group IV andV aflatoxin residues were found in liver, muscle, pooled samples of the kidney and blood in a dose related manner. At the sixth and eighth month of the treatment, aflatoxin residues were found in the liver, muscle, pooled samples of the kidney and blood of groups III, IV and V in a dose related manner. The detection of aflatoxin residues in the edible tissues of the duck in the present study is a convincing evidence for the public health involvement of aflatoxin contaminated animal feels. Similarly aflatoxin B_1 residues were detected in edible tissues by Blaha (1982), Chao and Liu (1988), Maryamma *et al.* (1992), and Arshad *et al.* (1993). Of late, duck meat are widely consumed by most of the people and therefore aflatoxin B_1 residues in edible tissues poses a grave concern for food safety. Aflatoxin could also account for the harmful conditions such as hepatocellular carcinoma, infertility, Reyes syndrome and a malnutritional disorder in children known as Kwashiorkor disease (Ryan *et al.* 1979; Wilkinson *et al.* 1993; and Iben *et al.* 1994). FDA (1969) has recommended 20 ppb as the acceptable limit of aflatoxin B_1 in foods for human consumption and FAO (1976) has set 30 ppb as the permissible level in the food items in India. The results obtained in the presence study showed that the residual aflatoxin present in the muscle, liver, kidney and blood at the

highest dose level (40ppb) in the eighth month was below the levels set by FDA and FAO.

Ordinary cooking temperature may not detoxify the contaminated materials and hence there is need for caution in the safe treatment and storage of feeds to minimize fungal toxin in them. is emphasize.

The experiemental study undertaken with different dose levels of aflatoxin revealed pathological effects of aflatoxicosis depending upon the dose and duration of the treatment relatively. Group II didn't show much gross and histopathological changes.

There is no definite safe level of aflatoxins, although permissible level of aflatoxin has been reported for ducks 30 ppb (Allocraft, 1969) and 10ppb (Package of practices, 1994.). The permissible level may vary with the climatic condition, harvest practices, unhygienic storage condition, transport of grains during rainy season and disease condition of the birds.

In the present study, the effect of aflatoxin on the body weight, carcass weight, liver weight, haemogram and serum protein, grosspathology, histopathology and ultrastructural pathology of liver is less severe in 5ppb and 10ppb group when compared to 20 ppb and 40ppb of aflatoxin. No aflatoxin B_1 residues could be detected in 5ppb group and even residual aflatoxin detected in 10,20 and 40ppb groups are below the recommendation level of FDA and FAO in food stuffs. In humid climate like Kerala, aflatoxin contamination of feed is high, but cannot discard feed as such. Even though there is cellular and molecular changes at 5ppb and 10ppb level, production

performance of the bird is not much affected at 10ppb of aflatoxin. So permissible level of aflatoxin is fixed as 10ppb in the present study, but it cannot be considered as the safe level of aflatoxin.

The observation made in this investigation once again proved that aflatoxin is a potent hepatotoxin, nephrotoxin with adverse effects on the haemopoietic system and lymphoid organs. Even low levels of aflatoxin fed for a long duration lead to chronic ill effects.

The observations made in this study emphasized the need for regular screening of feed for aflatoxin content and for better storage conditions, so that feed free of aflatoxin can be made available. In the field situations, the synergistic action of other mycotoxins cannot be ruled out and in such situations the pathological effects will be much more severe and the productivity will be much lowered leading to severe economic loss. Therefore, prevention of aflatoxin contamination in the feed should be given top priority at least in case of ducks which is a very sensitive species for the toxicity of aflatoxin. The diet of ducks should be carefully formulated excluding those ingredients which are generally contaminated with aflatoxins like the maize and groundnut cake. This investigation, therefore, has clarified the adverse effects of aflatoxin and pointed out the need for caution on feeding a diet contaminated with aflatoxin to ducks and clarified that there is absolute necessity to give a diet free of aflatoxin to ducks.

Summary

SUMMARY

An investigation was undertaken to assess the pathological effects of aflatoxicosis and to determine the permissible levels of aflatoxins in the ducks. Graded levels of aflatoxin viz. 5 ppb, 10ppb, 20ppb and 40 ppb were fed daily in the diet for a period of eight months.

Clinically, aflatoxin fed ducks were apparently normal except for reduced feed intake. However, diarrhoea was noticed at the highest dose level of 40 ppb during the later stages of the experimental trial. There was progressive reduction in the body weight gain and carcass weight in all the treated groups which were indicative of the toxic injury to the gastro intestinal system.

There was a significant increase in the liver weight of aflatoxin treated ducks throughout the experimental period which could be attributed to the lipid accumulation within the hepatocytes.

There was a significant increase in the erythrocyte sedimentation rate but a decrease in the packed cell volume, haemoglobin concentration and total erythrocyte count. Leucocytosis with heterophilia and lymphopenia were observed in the aflatoxin treated ducks. Lymphopenia indicates the adverse effect of aflatoxin on the lymphoid organs in the form of lymphoid depletion. These alterations revealed the adverse effects of aflatoxin on the haemopoietic tissues as well as the formed elements of the blood.

There was a significant reduction in the serum protein and albumin. At all dose levels, serum globulin concentration increased throughout the course of the experiment but no significant variation could be observed by the second and eighth months.

Serum alkaline phosphatase and serum gamma glutamyl transpeptidase levels increased in aflatoxin treated ducks indicating hepatic damage.

The liver of ducks fed with a level of 5ppb aflatoxin in the diet showed congestion, slight enlargement and pale discoloration of increasing intensity depending upon the increase in the duration of the trial. Spleen, kidney and bursa revealed congestion during the later part of the experimental trial. At the dose levels of 10 and 20 ppb aflatoxin areas with pale yellow discoloration, subcapsular haemorrhage and congestion were noticed in the liver. Spleen, kidney and bursa revealed congestion by the sixth and eighth months. Liver of ducks treated with 40 ppb aflatoxin revealed severe enlargement, firm consistency, pin head sized necrotic areas, nodularity, surface granularity and pale yellow to greenish discoloration. Splenomegaly could be noticed in some birds by the eight month of the experiment, while in the remaining birds, only congestion could be noticed in spleen and kidney. Bursa showed only atrophic changes.

Histopathologically, the major changes observed by the second month in the liver of ducks fed with 5 ppb aflatoxin were congestion and mild biliary hyperplasia. At the fourth, sixth and eighth month, the hepatic changes included mild fatty changes, moderate vacuolar degeneration, mononuclear

cell infiltration in the periportal area, proliferation of bile ductules, portal congestion and fibrosis. In spleen, mild congestion and vascular sclerosis were noticed by sixth and eighth month.

At the dose level of 10 ppb of aflatoxin by the second month, the hepatic changes varied from vacuolar degeneration to necrosis besides bile stasis and portal venous dilatation. During the fourth month, the hepatic alterations observed were congestion of the hepatic sinusoids, hepatic cholestasis, focal collection of mononuclear cells and kupffer cell hypertrophy. By the sixth month, lipidosis sinusoidal dilatation, central venous congestion, coagulative necrosis and mild portal fibrosis were observed in the liver. During the eighth month, hepatic alterations were characterised by congestion, fatty infiltration of hepatocytes, bile stasis, perivascular collection of mononuclear cells, hepatocytomegaly, phlebosclerosis, portal tract fibrosis, bile duct hyperplasia, fatty change and necrosis of the hepatocytes.

Congestion and mild depletion of lymphocytes in follicles of spleenic cortex were noticed by the sixth and eighth month. In the kidney, mild nephrosis were observed during the fourth and sixth months, whereas by the eighth month besides nephrosis, mild peritubular accumulation of mononuclear cells was also noticed.

During the second month, the liver of ducks fed with 20 ppb aflatoxin showed extensive bile duct hyperplasia, focal collection of lymphoid cells in the periportal areas as well as in the parenchyma replacing the necrotic hepatocytes. By the fourth month, hepatocytomegaly, biliary hyperplasia,

vacuolar degeneration and coagulative necrosis of hepatocytes were observed. During the sixth month, the histological picture revealed portal fibrosis, sinusoidal congestion, cholestasis, megalocytosis and hepatokaryomegaly. Extensive fatty change, megalocytosis and biliary hyperplasia were noticed by the eighth month.

Focal depletion of lymphoid cells, vascular sclerosis and congestion were observed in increased intensity depending on the duration of treatment. In the kidney, congestion, necrosis and desquamation of lining tubular epithelial cells were noticed.

With 40ppb aflatoxin, congestion, edema, degeneration, necrosis of hepatocytes, proliferation of bile ducts and portal fibrosis were observed by the second month. At the fourth and sixth month vacuolar degeneration, hepatocytomegaly, dissociation of hepatocytes, bile stasis, hyperplasia of the bile ductules, massive mononuclear cell infiltration and dilatation of the hepatic sinusoids were observed.

Marked congestion, phlebosclerosis, massive dissociation and necrosis of the hepatocytes and extensive proliferation of fibrous tissue with partial subdivision of the hepatic lobules were noticed during the eighth month.

In the spleen, changes noticed were cortical and paracortical lymphoid depletion, congestion, vascular sclerosis, degeneration and necrosis of the lymphocytes.

The histological changes observed in the kidney were tubular degeneration, swelling of the tubular epithelium and mild fibroblastic proliferation with cellular infiltration in the interstitium.

Generally, in the aflatoxin treated birds, the bursa of fabricius revealed lymphoid depletion in the follicles, interfollicular edema and proliferation of the interfollicular connective tissue.

The ultrasructure of the hepatocytes in all the aflatoxin treated groups showed significant alterations of varying degrees consistent with the dose and duration of treatment. The ultrastructural changes noticed were increased amounts of heterochromatin which appeared as clumps on the inner nuclear membrane, nucleolus was highly condensed without any separation between the fibrillar and granular components. Cytoplasm was cavitary with the rough endoplasmic reticulum showing varying degrees of dilatation. Occasionally fragmented rough endoplasmic reticulum (RER) with degranulated and dispersed ribosomes could be seen. Mitochondria appeared rounded, oval or elongated.

Mitochondria were either normal or swollen containing swollen cristae. Glycogen particles were seen as small aggregates confined to focal areas. Scattered lipid droplets were also encountered in the cytosol. In some cells there was an increase in the smooth endoplasmic reticulum (SER) which appeared as small tortuous vesiculated structures.

Aflatoxin B1(AFB1) residues could not be detected in Group II (5ppb) throughout the experiment. AFB1 residues were detected in liver, muscle,

pooled samples of kidney and blood of ducks fed with 20 and 40 ppb aflatoxin during fourth, sixth and eighth month. But the aflatoxin residue level detected in the edible tissue of birds fed aflatoxin even at a level of 40ppb was lower than that of 20ppb recommended by FDA and 30ppb by FAO for aflatoxin in human food items.

By this investigation it was brought to light that aflatoxin had significant effect on the hepatic, renal and haemopoietic systems and lymphoid organs. Considering the above facts 10ppb is fixed as permissible level. The permissible level may vary with the season, disease condition of birds and storage practices of feed. Therefore, this study underscores the need for regular screening of feed for aflatoxin content.

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PATHOLOGICAL EFFECTS OF AFLATOXICOSIS IN DUCKS

(Anas platyrhynchos domesticus)

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ABSTRACT OF A THESIS

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ABSTRACT

In this investigation the pathological effects of aflatoxicosis and permissible levels of aflatoxin were studied at different dose levels viz. 5ppb, 10ppb, 20ppb and 40ppb. In the aflatoxin fed ducks, reduced feed intake and diarrhoea were noticed. There was a significant reduction in the body weight and carcass weight which were indicative of the toxic injury to the gastrointestinal tract. There was a significant increase in the liver weight reflecting the damage to the hepatic system.

There was reduction in the haemoglobin concentration, total erythrocyte count, packed cell volume and significant increase in the erythrocyte sedimentation rate indicating the adverse effect of aflatoxin on the haemopoietic system. Leucocytosis with heterophilia and lymphopenia were observed suggesting the deleterious effect of aflatoxin on the lymphoid organs.

Highly significant reduction in serum total protein, albumin values, A/G ratio and significant increase in globulin were observed indicating hepatic damage.

Grossly, the aflatoxin fed birds showed pale enlarged liver, focal necrotic areas, subcapsular haemorrhage, fatty liver, greenish discoloration and surface granularity and nodularity of varying intensity depending on the dose and duration of the treatment. In the kidney and spleen, mild congestion were observed. Bursa showed atrophic changes.

Histopathological alterations in the liver consisted of biliary hyperplasia, fatty changes, congestion of the hepatic sinusoids portal venous

congestion, portal fibrosis, focal collection of mononuclear cells, dissociation of hepatocytes, hepatocytomegaly and fibrous tissue proliferation of varying intensity depending on the dose and duration of the treatment.

In the spleen, congestion, vascular sclerosis and depletion of lymphocytes in the cortical and paracortical areas were noticed, whereas in the kidney besides nephrosis, mild peritubular accumulation of mononuclear cell were noticed at 20ppb level of aflatoxin while tubular degeneration, swelling of the tubular epithelium, mild fibroblastic proliferation with cellular infiltration in the interstitium were noticed at 40ppb level.

Generally, bursa of fabricius of the treated birds revealed lymphoid depletion in the follicles, interfollicular edema and proliferation of interfollicular connective tissue.

Aflatoxin treated groups showed dose and duration dependant degrees of ultra structural changes. The ultrastructural changes noticed were intact cell nucleus with prominent nuclear pores, increased amounts of heterochromatin that appeared as clumps, dilatation and degranulation of rough endoplasmic reticulum (RER), rounded, oval or elongated mitochondria which were either normal or swollen containing swollen cristae along with the presence of electron dense material within the matrix, smooth endoplasmic reticulum (SER) that appeared as small tortuous vesiculated structures and scattered lipid droplets.

Aflatoxin B1(AFB1) residues were detected in the liver, muscle pooled samples of the kidney and blood of birds maintained with feeds containing

aflatoxin at levels of 20 and 40 ppb during the fourth, sixth and eighth month. No residual toxin could be detected in birds treated with aflatoxin at a level of 5ppb throughout the experiment. At dose level of 10ppb, no AFB1 residues could be detected by the second and fourth month, but during the sixth and eighth month negligible amount of residues could be detected in the liver and muscle.

This study highlights the pathological effects of aflatoxicosis in ducks. Moreover, the adverse effects on the hepatic, haemopoietic and renal systems could be documented. By considering the above facts aflatoxin level of 10ppb is fixed as permissible level in ducks. However, permissible level can vary with the season, diseased conditions of the bird and storage practices of feed. Therefore, this study emphasizes the need for regular screening of feed for aflatoxin content.