AFLATOXIN INDUCED CYTOLOGICAL ALTERATIONS IN DUCKS

By

S. VIGIL ANBIAH

THESIS

Submitted in partial fulfilment of the requirement for the degree

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences

KERALA AGRICULTURAL UNIVERSITY

Department of Pathology

COLLEGE OF VETERINARY AND ANIMAL SCIENCES

MANNUTHY - THRISSUR

KERALA

1996

DECLARATION

I hereby declare that this thesis entitled **AFLATOXIN INDUCED CYTOLOGICAL ALTERATIONS IN DUCKS** is a bonafide record of research work done by me during the course of research and that 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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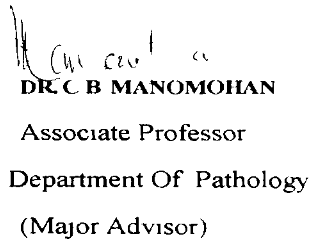

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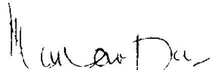
DR. C B MANOMOHAN
Associate Professor
Department Of Pathology
(Major Advisor)

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Sri S. Vigil Anbiah,** a candidate for the degree of Master of Veterinary Science in Pathology, agree that this thesis entitled **AFLATOXIN INDUCED CYTOLOGICAL ALTERATIONS IN DUCKS** may be submitted by Sri S Vigil Anbiah in partial fulfillment of the requirement for the degree



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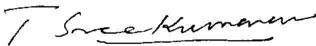
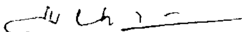
Associate Professor,
Department Of Pathology
(Chairman, Advisory Committee)



DR. T SREEKUMARAN,
Professor,
Department Of Pathology
(Member)

DR. K.V REGHUNANDANAN,
Associate Professor
Department Of Animal Breeding And Genetics
(Member)



DR. K.M RAMACHANDRAN,
Professor & Head,
Department Of Pathology,
(Member)

EXTERNAL EXAMINER

ACKNOWLEDGEMENT

It is my privilege to record my profound sense of gratitude to my major advisor Dr C B Manomohan, Associate Professor Department of Pathology and Chairman of the advisory committee for his valuable guidance, generous help, affectionate encouragement and deep indulgence throughout the course of this study

I am deeply indebted to Dr K M Ramachandran, Professor and Head Department of Pathology and member of my advisory committee for his valuable advice useful suggestions and support during the course of my study

I am extremely grateful to Dr T Sreekumaran, Professor Department of Pathology and member of my advisory committee for his meticulous guidance and constructive criticism throughout this work

I wish to place on record my gratitude to Dr K V Reghunandanan, Associate Professor, Department of Animal Breeding and Genetics and member of my advisory committee for his sound comments, abiding interest and valuable guidance throughout this work

I wish to express my gratitude to Dr A Rajan, Dean, College of Veterinary and Animal Sciences for his valuable guidance constant supervision and stable support throughout the work

I wish to express my gratitude to Dr N Divakaran Nair Assistant Professor, Department of Pathology for his timely advice and constructive criticism during the course of this work

I am extremely grateful to Dr K V Valsala Professor Department of Pathology for her unstinted co-operation valuable advice and the help rendered in taking microphotographs

I am deeply indebted to Dr T V Anilkumar Assistant Professor, Department of Pathology for his abiding interest and valuable help rendered during my work

I wish to place on record my gratitude to Dr N Vijayan Dr Mammen J Abraham, Dr Koshy Varghese Dr B Gangadharan, Dr T Leenadevi, Assistant Professors Department of Pathology and Dr C R Lalitha Kunjamma Associate Professor, Department of Pathology for their useful suggestions rendered during the course of my work

The immence help rendered by Mr K C George Smt K P Santha Bai and Smt Indira Bai, Department of Statistics in statistical analysis of experimental data is gratefully acknowledged

I am thankful to Dr Jacob Alexander Dr S Silamban Dr P Biju Dr S Mohan Dr S Nandakumar, Dr Ajith Jacob George and Dr M Suresh Kumar Post graduate students Department of Pathology Dr K Senthil Kumar Dr Malmarugan

and Dr Rinita Singh, Post graduate students Department of Microbiology for their valuable assistance during my work

My sincere thanks to Dr C N Dinesh Post graduate student Department of Animal breeding and Genetics for his valuable help rendered in chromosomal studies

I gratefully acknowledge the timely help rendered by Ms Bindu Chandrasekhar Ms Beena Research Associates and other members of the Department of Pathology

I thank all my friends who have contributed to the progress of the work with their goodwill and co-operation

My indebtedness to my loving parents for their constant encouragement and support is beyond expression

S VIGIL ANBIAH

Dedicated To

My Loving Parents

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Introduction

INTRODUCTION

Intensive rearing of ducks has occupied an important place in modern poultry practice This led to loss of most of its innate resistance to diseases as compared to ducks which were reared in natural habitat wherein disease problem seldom posed a threat

Ducks under intensive system of management are exposed to many of the chemical and biological toxins, predisposing them to be highly susceptible to many disorders directly affecting their production potential Among the biological toxins mycotoxins produced on food commodities by a variety of fungi leading to subsequent contamination of various animal feed stuffs still poses a great problem today Ducks are highly susceptible to the injurious effects of the potent and common mycotoxin the aflatoxin Aflatoxicosis can occur either in the acute fatal form or as the insidious form with cirrhosis of liver and carcinogenesis The pathophysiological changes associated with the toxicoses have been studied in detail (Asplin and Carnaghan 1961 Butler 1964 and Rajan *et al* , 1989) Aflatoxin B1 induces a variety of changes in the system leading to a multitude of disorders through its interaction with cellular and subcellular components

Eaton and Gallagher (1994) reported that the bio transformation of aflatoxin and the extent of total DNA adduct formation were intimately linked with their toxic and carcinogenic effects Aflatoxin induced cytological alterations in ducks has not been studied extensively as compared to other species

To elucidate the cyto-pathological alterations associated with aflatoxicosis, an experiment was conducted in ducks utilising aflatoxin B1 at graded levels and the result of which were compared in a similar experiment utilising a known chemical carcinogen 4 dimethyl amino azo benzene

The study included the following

1 Experimental interaction of aflatoxicosis in ducks utilising pure aflatoxin B1, and the chemical carcinogen 4 dimethyl amino azo benzene

2 Evaluation of toxicity at various levels utilising sensitive tests such as serum bilirubin serum enzyme estimation haematology and histopathology

3 Feather pulp technique bone marrow technique hepatocyte technique and lymphocyte culture to evaluate chromosomal changes

Review Of Literature

REVIEW OF LITERATURE

2 1 AFLATOXIN AND BODYWEIGHT

The literature on influence of aflatoxin on the body weight of birds were scanty Butler (1964) observed that when day old Khaki Campbell ducklings were given 15µg aflatoxin orally, these lost weight for about two days It was also observed that by 3-4 days, the birds recovered their initial weight and continued to grow at the rate comparable to that of the controls

Smith and Hamilton (1970) reported that 2 5 ppm of aflatoxin could be considered as the growth inhibitory dose

Arafa *et al* (1981) observed that in goslings, 0 7mg of aflatoxin/Kg caused some increase in body weight compared with control birds In quail chicks, they found that those birds receiving 0 07 mg of aflatoxin/Kg consumed less feed during the second week During the third week, a toxin level of 2 1 mg/Kg was necessary to reduce the feed intake

Reddy (1981) observed significant reduction in weight gain even at 0 75 ppm levels in broiler chicken at fourth week of age Reddy *et al* (1982) observed significant reduction in the body weight gain at one ppm level

Huff *et al* (1984) reported reduction in body weight of broilers by Ochratoxin A and a combination of ochratoxin A and aflatoxin treatments on a long term basis

Ghosh *et al* (1989) fed chicken with 0 3 ppm and one ppm of aflatoxin B1 in an experiment for a period of six weeks and recorded stunted growth, reduction in feed consumption and decreased weight gain Rizvi and Shakoori (1992) observed a reduction in the total live and dressed weights of broiler chicken during the first week of aflatoxin administration (at the rate of 9 278 mg/Kg body weight) in contrast to linear weight increase in control birds

2 2 HAEMATOLOGY

The alteration in the haematological parameters due to aflatoxicosis has been well documented by many workers

2 2 1 ERYTHROCYTE SEDIMENTATION RATE

The elevation of erythrocyte sedimentation rate values has been an indication of anaemia in birds and animals Fernandez *et al* (1995) observed increased erythrocytic sedimentation rate in laying hens affected with aflatoxicosis

2 2 2 PACKED CELL VOLUME

Decreased Packed cell volume was observed by Tung *et al* (1975) when male broiler chicks were given aflatoxin at the dose of 2 5 μg/g and this was supported by Reddy *et al* (1980) and Reddy (1981) reported lowered packed cell volume in birds when fed with 0 5 ppm of aflatoxin Day old broiler chicks when received aflatoxin at 0 5 ppm dose level showed a decrease in packed cell volume (Mohiuddin *et al* 1986)

Significant reduction in packed cell volume was observed in broiler chicken by Anjaneyulu *et al* (1993) when fed with one ppm of aflatoxin for three weeks A reduction in packed cell volume was also reported in aflatoxicosis in eight weeks old chicks by Mani *et al* (1993)

2 2.3 HAEMOGLOBIN

Anaemia has been observed as a clinical feature of aflatoxicosis in ducks and chicken Brown and Abrams (1965) observed a slight anaemia in ducklings and New Hampshire chicks when fed 0 5 ppm of aflatoxin for six days Lowering of haemoglobin concentration and anaemia were recorded by Tung *et al* (1975) in male broiler chicks at the dose of 1 25µg/g or above Feeding of aflatoxin at 0 75 ppm to birds resulted in significant reduction in haemoglobin level (Reddy *et al* 1980 Reddy, 1981) The decrease in haemoglobin was observed by Mohiuddin *et al* (1986) in day old broiler chicks when fed with0 55 ppm dose level of aflatoxin

Balachandran and Ramakrishnan (1987) reported that the anaemia in aflatoxicosis might be due to a combination of both haemorrhagic and haemolytic type in treated birds A highly significant reduction in haemoglobin value was observed by Anjaneyulu *et al* (1993) when brolier chicken were fed with one ppm of aflatoxin for three weeks

2 2 4 ERYTHROCYTE COUNT

While studying the 'X' diseases of birds, Wannop (1961) observed a significant decrease in erythrocyte count as 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 administering a dose of 0 625 µg/g Comparable reduction in total erythrocytes was also recorded by Reddy *et al* (1980) and Reddy (1981) in their experimental studies feeding one ppm of aflatoxin to birds Mohiuddin *et al* (1986) also recorded a decrease in total erythrocyte count when aflatoxin at 0 5 ppm dose level were fed to day old broiler chicks

2 2 5 TOTAL LEUCOCYTE AND DIFFERENTIAL COUNT

Wannop (1961) observed a marked increase in leucocyte count in birds affected with 'X' disease, which 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 However, he reported a marked reduction in the lymphocyte count

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

Heterophilia and lymphopenia were observed by Sova *et al* (1991) in experimental broilers when a diet containing five per cent Zeolite and 25 mg aflatoxin B1 was fed

2.2 6 WHOLE BLOOD CLOTTING TIME

Effect of aflatoxicosis on blood clotting was documented by Bassir and Bababunmi (1972) They reported that a dose of 58μg of aflatoxin B1/Kg body weight was sufficient to cause significant prolongation of blood clotting time and suggested that the anticoagulant effect of the toxin was due to specific inhibition of prothrombin synthesis by competition for the apo-enzyme Significant increase in the blood clotting time in young broiler chicken affected with aflatoxicosis was reported by Doerr *et al* (1974) Doerr *et al* (1976) had assessed the profound effect of aflatoxin in chicken on blood clotting and to them coagulopathy associated with aflatoxicosis appeared to be primarily hypothrombinemia which was provoked even by concentrations of the mycotoxin too small to inhibit growth Doerr and Hamilton (1981) found that clotting time was significantly altered by 5 and 10μg aflatoxin per gram of feed and the mean prolongation of clotting time was 30 seconds Such a prolongation indicated that blood from aflatoxin fed birds were deficient in factors of the intrinsic pathway of coagulation

2 3 SERUM CHEMISTRY

2 3 1 TOTAL SERUM PROTEIN

Variation in serum protein has been reported in aflatoxicosis by many workers

Reddy *et al* (1982) reported a decrease in total serum

protein in aflatoxin fed birds Chang and Hamilton (1982) considered depressed serum protein to be a very sensitive indicator in experimental aflatoxicosis caused by the feeding of 1 25µg/g of the toxin in quails Harvey *et al* (1989) investigated the effect of aflatoxin on total protein content in serum and reported a decrease in serum concentration of total proteins at three milli gram of aflatoxin B1 per kilogram of feed A decrease in total serum protein level during aflatoxicosis in birds given with six ppm of the toxin was observed by Jassar *et al* (1993)

Shukla and Pachauri (1995) observed that when aflatoxin was fed to day old cockerels at the rate of 2 5µg and 10µg/g of feed resulted in decreased serum total protein

2 3.2 SERUM ALBUMIN AND GLOBULIN

A reduction in Serum albumin level was recorded by Harvey *et al* (1989) when broilers were fed with aflatoxin B1 at the rate of three milligrams per kilogram of feed Ghosh *et al* (1990) conducted experiments with broilers and observed a reduction in albumin and globulin values in aflatoxicosis Shukla and Pachauri (1995) observed that feeding of day old cockerels with aflatoxin at the rate of 2 5 mg and 10 mg per gram of feed resulted in decreased albumin globulin ratio

2 3 3 SERUM ENZYMES

Enzymological evaluation of serum of birds in aflatoxicosis was not well documented in the literature

A decrease in the serum aspartate amino transferase (AST) values in aflatoxin fed birds had been reported by Reddy *et al* (1982) Balachandran and Ramakrishnan (1988) made observations in broiler chicks which were fed with aflatoxin mixed feed at one ppm and three ppm and found that the Serum alanine amino transferase (ALT) values in the control and treated groups were not in detectable amounts

2 3 4 SERUM BILIRUBIN

Serum bilirubin levels were not seen altered in day old layer type cockerels when fed with aflatoxin at the rate of 2 5μg and 10μg/g of feed (Shukla and Pachauri, 1995)

2 4 HEPATOPATHOLOGY

Extensive work has been documented on the structural pathology of liver in aflatoxicosis

2 4 1 NON-NEOPLASTIC HEPATIC LESIONS

2 4 1 1 GROSS PATHOLOGY

Butler (1964) observed that after feeding higher doses of aflatoxin, i e 25 50 and 100 g, the livers of ducklings were putty coloured with a few small areas of haemorrhage without conspicuous enlargement He also observed that at a dose level of 8 5 grams, the liver was slightly paler than normal, but was free from haemorrhages At a dose level of 5 or 1 5μg, no gross lesions of the liver could be observed

Carnaghan (1965) did experiment with seven days old Khaki Campbell ducklings by feeding them with seven ppm of aflatoxin B1 and observed that the livers were putty coloured with numerous yellowish white lesions of 1-2 mm diameter Solid yellow nodules which varied in size from 1-2 cm diameter with a number of small irregularly shaped necrotic lesions were also observed

Spherical yellowish white fleshy growths of varying sizes (0 5 cm to 2 0 cm diameter) were seen embedded in the liver parenchyma in ducks aged between 2 to 2 1/2 years (Christopher *et al* 1968)

Muller *et al* (1970) observed that the most significant pathological changes occuring in the liver due to aflatoxicosis was brown small, tan with greenish discolouration The surfaces of the livers showed a granular irregularity Hepatic lesions like pale yellow discolouration with haemorrhages and nodules were observed by Moorthy *et al* (1985) in chicken in experimental aflatoxicosis

Moorthy *et al* (1986) fed aflatoxin to chicken at the level of 6 25 ppm died within a span of three weeks and those received 3 12 ppm showed nodular growth of 1-5mm diameter in the liver

Balachandran and Ramakrishnan (1987) conducted experimental studies with day old commercial broilers to evaluate the pathology of aflatoxicosis They observed that the livers from the birds fed with one ppm level of aflatoxin were enlarged pale

or discoloured with Moroccan leather appearance in the first two weeks and yellow coloured in the next two weeks At three ppm level in addition to these lesions, the liver in all the birds was enlarged, soft and friable with yellowish discolouration from the first week onwards

Maryamma *et al* (1990) fed aflatoxin to ducks at the dose rate of one ppm and observed extensive hepatic lesions The liver was pale, friable with greyish white nodules of 0 5-1cm diameter were present over the parenchyma Several foci of bile stasis were observed The gall bladder was edematous and distended with dark green bile

Ramadevi *et al* (1990) did experiment with broilers by feeding aflatoxin B1 at the dose rate of two ppm for eight weeks According to them, grossly, the liver was pale yellow and enlarged with petechial haemorrhages in the initial period of the experiment In the later period the changes were milder but greyish foci were seen

Singh *et al* (1993) opined that being the par excellence organ in detoxification the liver was the first to exhibit the morphological and pathological changes in aflatoxicosis

Bakshi *et al* (1995) fed broilers with aflatoxin at 0 38 and 0 75 ppm dose level for six weeks and observed haemorrhagic spots along with infarction on the liver At 1 5 and three ppm level liver exhibited enlargement yellowish discolouration petechical haemorrhage and fatty change

2.4.1.2 HISTOPATHOLOGY

Butler (1964) conducted experimental studies in Khaki Campbell ducklings with aflatoxin and recorded the sequential histopathological lesions. He observed that by 24 hours after exposure to 15μg of aflatoxin, oval cell proliferation throughout the portal system with few mitoses. There was an increase in fat at the periphery of the lobules where many of the cells had pyknotic nuclei. After two days, the oval cell proliferation was more marked with the first extension into the lobules between the parenchymal cells.

Few scattered eosinophils could be seen but no increase in inflammatory cells. The peripheral parenchymal cells showed ballooning with fat and rupture of cell membranes with the formation of lakes of fat.

Similarly, Carnaghan (1965) reported that in the liver of ducks fed with aflatoxin contaminated Brazilian groundnut meal showed small focal lesions which were microscopically lymphoid foci composing mainly of mature lymphocytes surrounded with degenerating hyperplastic bile duct epithelium. Mitotic figures were not observed but peripherally there was infiltration of lymphocytes between the hepatic cells.

Gardiner and Oldroyd (1965) observed ducklings fed with extracts of peanut meals. The livers 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.

Muller *et al* (1970) observed hepatic cell degeneration and bile duct hyperplasia in ducklings with aflatoxicosis Cytoplasmic vacuolisation of hepatic cells and formation of hepatic cells into cylindrical duct like structure were the typical changes Proliferation of bile ductules from the periportal zone of lobules in varying degrees were evident

Chronic hepatic fibrosis, hepatic regenerative nodules bile duct hyperplasia were the lesions observed by Radeleff (1970) in ducks and ducklings due to sub-acute toxicity by aflatoxin

Ching (1981) observed fatty liver syndrome and histological increase of fatty change in hepatic cells with increasing amount of the toxin

Deshek *et al* (1983) noticed bile duct proliferation cellular necrosis vacuolisation, congestion, fatty change and mild hepatitis in livers of eight week old quails dosed with 0 3 mg/kg body weight of aflatoxin

Moorthy *et al* (1985) observed lipidosis sinusoidal congestion paravascular haemorrhage bile duct hyperplasia phlebitis, pesudolobulation, fibrosis around portal areas villow proliferation of the epithelium in large bile ducts and veno-occlusive lesions in chicken in experimental aflatoxicosis

Moorthy *et al* (1986) did experiment with chicken by feeding 6 25 ppm and 3 12 ppm of aflatoxin Microscopically liver revealed areas of lipidosis regenerating hepatic cells which

were moderately circumscribed by thin connective tissue and bile duct proliferation

Balachandran and Ramakrishnan (1987) observed the histopathology of livers in day old commercial broiler chicks fed with aflatoxin in varying levels At seven days hyperaemia cloudy swelling, mild hydropic degeneration and mild fatty changes were observed at one ppm level The fatty changes involved more areas of hepatic cells and focal areas of necrosis were prominent around the central veins At fourteen days, mild necrosis was evident and fatty changes and hydropic degeneration were marked The fat globules were larger in size At twenty one days at one ppm level, extensive necrosis with haemorrhage were seen in the portal areas Apart from connective tissue proliferation around the portal triad mild ductular hyperplasia was also observed At three ppm level multifocal infiltration of lymphocytes were observed At twenty eight days at one ppm level hepatocytomegaly was prominent The ballooning of the hepatic cell was so great that the individual cell had ruptured and coalesced to form large areas of hydropic vacuoles There was slight enlargement of nuclei with pyknosis The sinusoidal reticulo-endothelial cells were prominent At three ppm level biliary hyperplasia was seen along with multifocal collection of heterophils and lymphocytes with periportal fibrosis

Jayakumar *et al* (1988) administered aflatoxin B1 at the dose rate of $25 \mu g$ per duck daily for three months and observed fatty change hepatic necrosis biliary hyperplasia and hepatocyto megaly

Ghosh *et al* (1989) conducted experiments in day old cross bred broiler chicks to study the pathological effect of pure aflatoxin B1 By 36th day the group receiving one ppm aflatoxin B1 showed individualization necrosis of hepatocytes lymphocytic infiltration in portal areas and hypertrophy of Kupffer cells At 43rd day exhibited hypertrophy of hepatocytes (megalocytosis/ megalo Karyosis), sinusoidal dilatation, phlebosclerosis and firboblastic proliferation

Mukit and Kwatra (1989) observed histopathological changes in young ducklings which included degeneration and necrosis of hepatocytes with mild proliferation of biliary epithelial cells in an incidence of aflatoxicosis in three Government duck breeding farms in Assam

Maryamma *et al* (1990) fed aflatoxin to ducks at the dose rate of one ppm and observed hepatic lesions showing biliary hyperplasia periportal necrosis of hepatocytes, hepatocytomegaly hepatokaryomegaly and several dissociated round cells with hyperchromatic nucleus

Ramadevi *et al* (1990) did experiment with broilers for eightweeks by feeding aflatoxin B1 at two ppm level and observed sinusoidal congestion focal haemorrhages hydropic changes mild fatty changes hepatocytomegaly and hypertrophy of Kupffer cells in the initial half of the experiment In 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

Histological analysis of necropsy specimens from ducks 20 months after receiving doses of 25 and 50 mg/kg body weight of aflatoxin B1 showed almost complete regression of the early acute lesions with no evidence of neoplasia (Seawright *et al* 1993)

Singh *et al* (1993) reported coagulative necrosis in mid zonal area and in the portal lobules Bile ductules revealed hyperplastic changes at a few portal areas while fibrosis at other places Hepatocytes were binucleated varied in shape and size with pericellular cirrhosis Portal veins, central veins and sinusoids were engorged with erythrocytes in rabbits at German Angora breeding farm, Tadikhet in Almora (U P)

Bakshi *et al* (1995) did experiment with broilers by feeding aflatoxin at 0 38 and 0 75 ppm dose level for six weeks Histologically at one ppm dose degeneration focal areas of necrosis and lymphoid cell infiltration in periontal areas were observed Focal areas of heterophilic and lymphocytic infiltration in the parenchyma and around bile duct were noticed at three ppm level

2 4 2 NEOPLASTIC HEPATIC LESIONS

Intra hepatic tumours like hepatoma, hepatocellular carcinoma, cholangioma and cholangiocellular carcinoma have been reported in ducks by Campbell (1946), Campbell (1949) Lombard and Witte (1959), Asplin and Carnaghan (1961) Ratcliffe (1961) Snyder and Ratcliffe (1963) and Carnaghan (1965)

Carnaghan (1965) described large nodules in the liver of duck which were thickly encapsulated Hepatoma comprising of hypertrophic ballooned cells occasionally containing a giant vesicular nuclei with very prominent nucleoli, cholangiomata composed of dense masses of well differentiated bile ducts surrouned by a thin fibrous capsule were reported

Christopher *et al* (1968) while conducting necropsies found two ducks with neoplastic liver lesions The parenchymatous cells were greatly enlarged and arranged in the form of alveoli or acini The cellular clumps varied in size and were covered by delicate fibrous tissue trabeculae There were spaces containing oesinophilic substances and areas of necrosis The neoplastic cells were polygonal in shape and the nuclei were greatly enlarged, vesicular and hyperchromic with prominent nucleoli Mitotic activity was present The cytoplasm was deeply eosinophilic and granular

Singh and Sharma (1996) conducted necropsy of an adult male turkey and reported the following hepatic lesions The liver was enlarged and yellowish brown in colour with a rupture close to the gall bladder The gall bladder was distended with yellowish bile Histopathologically, the liver revealed loss of architecture Hepatocytes showed features of hyperchromasia, presence of mitotic figures, formation of giant cells and a distinct pleomorphism The Proliferating hepatocytes caused obliteration of the biliary canaliculi Foci of necrosis with heterophilic infiltration were also evident

Sriraman *et al* (1981) and Gordon and Jordan (1982) also described hepatocellular and cholangiocellular carcinoma in ducks

Zhu *et al* (1984) reported hepato cellular carcinoma in ducks Purvulov and Bozhkov (1984) recorded the prevalence of hepatocellular carcinoma in fowls and stated these tumours were similar to the oncovirus tumours In hepatocellular and cholangiocellular carcinoma in ducks, the liver was abnormally enlarged Irregular multiple nodules of 0 5 to 2 0 cm size were seen on the surface and parenchyma of the liver There were severe variations in the shape of the liver The colour of liver varied from mostly dark green, yellow or grey or pink or mixed colours The consistency of liver varied from hard to fibrous Gall bladder was enlarged (Thyagarajan *et al* 1988)

2 4 3 AFLATOXIN INDUCED HEPATIC LESIONS

A perusal of the literature revealed the relationship of aflatoxin as an etiological agent and hepatopathy in various species of animals and birds

Armbrecht and Fitzhugh (1964) reported that extensive liver damage was caused by aflatoxin in birds at the dose rate of 0 1 mg/Kg of feed for 1-2 days

2 4 3 1 AFLATOXIN INDUCED NON-NEOPLASTIC HEPATIC LESIONS

Day old Khaki Campbell ducklings when fed 15μg of

aflatoxin for three days revealed extensive biliary proliferation in the liver with fatty degeneration of the peripheral parenchymal cells (Butler 1964)

Soni *et al* (1993) experimented with three day old ducklings by feeding aflatoxin B1 at the rate of 5mg/day for fourteen days and observed severe liver damage like necrosis fatty change and biliary hyperplasia

2 4 3 2 AFLATOXIN INDUCED NEOPLASTIC HEPATIC LESIONS

Many workers have attempted to elucidate and establish the etiological significance of aflatoxin in hepatic cancers

Yoshida and Kamota (1952) observed that the ducks fed with Brazilian groundnut meal developed hepatomas

Arey (1957) described hepatomas to be of multiple origin in the liver and their exact cell origin was uncertain He postulated that since the bile duct epithelium and the liver parenchymatous cells were both endodermal in origin, it was probable that these tumours could have originated from an undifferentiated parenchymatous cell

Asplin and Carnaghan (1961) fed 0 01% aflatoxin to 37 Khaki Campbell day old ducklings and observed the development of hepatic tumours in five birds They reported that young ducks were the most susceptible host to the acute toxicity of aflatoxin and ducks fed with a ration containing 0 5% Brazilian ground nut meal for fourteen months developed hepatomas (Carnaghan 1964)

Carnaghan (1965) had further reported that the tumour inducing aflatoxin B1 content in the ration of ducks was approximately 0 03 ppm and the single oral LD 50 dose of aflatoxin for male ducklings was 0 4 mg/Kg

Several investigators described spontaneous hepatomas in ducks induced by aflatoxin (Carnaghan 1965, Newberne 1966 and V les 1967)

Wogan and Newberne (1967) reported a 100 per cent incidence of hepatic tumours in rats fed a diet containing 15µg/ kg continuously for 68-80 weeks and 100µg/Kg for 54-88 weeks

Butler (1969) described liver as the organ primarily affected and demonstrated the carcinogenicity of aflatoxin in rats ducks and in trout

Radeleff (1970) reported that the effect of aflatoxin on animals was governed by the dose, species of animal the age of the animal and the duration of administration He observed that prolonged administration of aflatoxin resulted in the formation of liver tumours

Santacruz *et al* (1984) experimentally fed rats a single dose of aflatoxin B1 (7 2 mg/Kg) for one year and observed productive and generative changes of liver

Soffritti and McConnell (1988) reported the development of hepatocellular foci neoplastic growth and hepatocarcinomas in rats when given a prolonged or short treatment with aflatoxin B1

Hepatoma, hepatocellular carcinoma and cholangiocellular carcinoma were observed in ducks which were fed with feedcontaminated with aflatoxin (Rajan *et al* 1989)

The carcinogenic and mutagenic action of aflatoxin B1 has been documented by Coulombe (1993) He reported that these activities of aflatoxin B1 were the result of the affinity of the electrophilic and highly reactive AFB1 - 8 9 - epoxide for cellular nucleophiles such as DNA Activated AFB1 would bind exclusively to guanyl residues and the AFB1 - N7 - guanine adduct was the most predominant AFB1 - formamido - Pyrimidine was another common adduct isolated The formation of these adducts was presumed to be the first step in the development of heritable mutations from which tumours might arise

Eaton and Gallagher (1994) reported that the biotransformation of aflatoxin was intimately linked with their toxic and carcinogenic effects They reported that the differences among aflatoxin biotransformation pathways were critical determinants underlying variations in species sensitivities to aflatoxin B1 induced carcinogenesis Covalent binding of aflatoxin B1 8 9 - epoxide to cellular DNA was highly correlated to the carcinogenic potency of aflatoxin B1 Trans -8 9 - dihydro-(N7-guanyl) - 9 - hydroxy aflatoxin B1 was the putative molecular target of aflatoxin carcinogenicity They concluded that the carcinogenic potency of aflatoxin B1 was highly correlated with the extent of total DNA adducts formed *in vivo*

Shen *et al* (1994) observed that aflatoxin B1 could cause lipid peroxidation in rat liver and suggested that oxidative damages caused by aflatoxin B1 might be one of the underlying mechanisms of cell injury and DNA damage which would eventually lead to tumerigenesis

2 5 CYTOGENETIC ASSAY

Studies on the chromosome pattern in ducks and the effect of various carcinogenic as well as mutagenic agents on these chromosomes were scanty in the literature

The method for routing observation of mitotic chromosomes in avian species was the ' feather pulp technique as identified by Shoffner *et al* (1967) This method was preferred by the authors due to features like ease of sampling, harvesting and rapidity of screening chromosome preparation

2 5 1 CHROMOSOMAL CHANGES INDUCED BY MYCOTOXINS

Ching (1981) reported that chromosome aberrations were not detected in bone marrow cells of laying hens dosed with aflatoxin B1 at 0 2 5 or 5 ppm for 28 days

Chattovadhyay and Nambi (1988) reported that AcT-1 from *Aspergillus Candidus* produced chromosome aberrations mainly of clastogen type both in chromatids and chromosomes of bone marrow cells of rats which received 0 5mg/100g body weight in daily ration and suggested the mycotoxin as a potent mutagen

Chromosomal aberrations in bone marrow cells could be experimentally produced in male chinese hamster after intraperitoneal administration of aflatoxin (Barta *et al* , 1990) They observed that the minimum mutagenic effect of aflatoxin could be produced at the dose level of 0 1 μ g/Kg if it was administered on a long term basis Similarly, Sharma *et al* (1991) observed chromosomal aberrations like chromatid break polyploidy and pulverisation in Murrah Buffaloes exposed to aflatoxin B1 toxicity They concluded that aflatoxin B1 induced several forms of chromosomal mutations

2.5 2 CHROMOSOMAL CHANGES INDUCED BY CHEMICAL TOXIC AGENTS

Some of the toxic compounds had been shown to have the effect on chromosomes in experimental animals

Although Nicholas *et al* (1979) reported no increase in chromosome breakage in chinese hamster cells which were exposed to malathion *in vitro* at concentrations of 50-400 g/ml Doulout *et al* (1983) observed that malathion at various dose levels could elicit clastogenic effects in mice bone marrow cells The later group of workers observed that the induction of sub-chromatid or chromatid type of aberrations was related to the dose used Mice treated with 230 mg/Kg dose showed increased frequencies of abnormal metaphase They concluded that the cytogenetic effect of malathion *in vivo* might have been produced by the compound itself and or by its metabolites

Malhi and Grover (1987) conducted experiments with rats to assess *in vivo* chromosomal aberrations in bone marrow cells due to organophosphorus pesticides They could observe chromosomal aberrations like pulverisation, chromatid gap fragments, rings, multiple minutes and one or two pairs of terminal deletions due to iso-chromatid breaks

Grindem and Buoen (1989) recorded chromosomal aberrations like chromosomal minutes, stubby chromosomes non specific translocations to be associated with specific tumours Studies in molecular genetics revealed genetic sequences similar to known oncogens at break points in chromosomes from leukaemic cells

Materials and Methods

MATERIALS AND METHODS

3 1 EXPERIMENTAL DESIGN

One hundred and eight clinically healthy, one month old desi ducks procured from the Government duck farm Niranam Kerala were used for the study These ducks were randomly divided into three groups of thirty six birds each and were tagged with numbers The birds were maintained in deep litter in separate rooms and were given commercial duck feed tested and found free of aflatoxin Water was given *ad libitum*

Group I
: This group was further divided into two sub-groups A and B

Sub-group A
: This group consisted of eighteen birds which were given aflatoxin B1 at the dose rate of 10μg/Kg body weight

Sub-Group B
: This group consisted of eighteen birds which were given aflatoxin B1 at the dose rate 15μg/Kg body weight

Group II
: This group was further divided into two sub-groups C and D

Sub-group C
: This group consisted of eighteen birds which were given 4 dimethyl amino azo benzene at the dose rate of 5 mg/kg body weight

Sub-group D — This group consisted of eighteen birds which were given 4 dimethyl amino azo benzene at the dose rate of 10 mg/kg body weight

Control Group — The control group consisted of thirty six birds

3 1 1 AFLATOXIN B1

Pure aflatoxin B1 was obtained from the M/s Sigma Chemical Co St Louis USA Fifty milligrams of the toxin was dissolved in 5ml of rectified spirit to make stock solution of the toxin Each millilitre of the stock solution was further reconstituted to 1000ml with distilled water for administration This reconstituted solution contained 10μg and 15μg of pure toxin in 1 ml and 1 5 ml respecitively Ducks in the sub-groups A and B were given appropriate ml of the reconstituted toxin solution corresponding to the body weight *per os* by oesophageal intubation on alternate days for a period of four months

3 1 2 CHEMICAL CARCINOGEN

(4 DIMETHYL AMINO AZO BENZENE)

A chemical carcinogen, 4 dimethyl amino azo benzene was obtained from M/s E Merck, Dramstadt Germany Ten grams of 4 dimethyl amino azo benzene was dissolved in 100ml of rectified spirit which was further diluted to 1000ml with distilled water for administration This solution contained 10mg of pure carcinogen

per ml The ducks belonging to sub-groups C and D were given appropriate ml of the reconstituted solution corresponding to the body weight *per os* by oesophageal intubation on alternate days for four months

The control birds were given the vehicle at appropriate concentrations

3.2 EXPERIMENTAL PARAMETERS

Body weight, haemogram values (Erythrocyte sedimentation rate, packed cell volume haemoglobin total erythrocyte count, total leucocyte count and differential count) blood clotting time serum total protein level serum albumin concentration, serum globulin concentration, albumin-globulin ratio, serum aspartate amino transferase level serum alanine amino transferase level, serum bilirubin content and chromosome characters were recorded during the experimental period The birds were observed daily for any clinical symptoms

The study covered the following aspects

1 Body Weight

2 Observation of clinical symptoms

3 Haemogram values

4 Blood clotting time

5 Estimation of serum total protein albumin globulin and albumin-globulin ratio

6 Estimation of serum aspartate amino transferase and serum alanine amino transferase

7 Estimation of chromosome pattern

8 Gross and histopathology of liver

3 3 TECHNIQUES

3 3 1 CLINICAL SYMPTOMS AND BODY WEIGHT

Both the control as well as the experimental groups of birds were weighed at fifteen days interval The birds were observed daily for clinical symptoms if any, and recorded

3 3.2 COLLECTION OF BLOOD SAMPLES FOR LABORATORY ESTIMATION

Blood samples were collected from the wing vein aseptically Two millilitre of blood was collected for the haematological studies using dipotassium salt of Ethylene diamine tetra acetic acid (EDTA) at the rate of 1mg/ml of blood as the anti coagulant Five millilitre of blood was collected separately in a sterile test tube without adding anticoagulant for serum separation

3 3.3 HAEMOGRAM

Erythrocyte sedimentation rate and packed cell volume were estimated on 15 30 45 60 75 90 105 and 120 days using the method described by Wintrobe (1981)

Haemoglobin level was estimated on 15 30 45 60 75 90 105 and 120 days employing the cyanmethoglobin method described by Miale (1967) and the final readings were taken in an Erma photometer

Total erythrocyte count total leucocyte count and differential count were made on 15,30 45,60,75,90,105 and 120 days by the method of Schalm (1975)

3 3 4 BLOOD CLOTTING TIME

It was recorded employing capillary tube method (Benjamin 1978)

3 4 SERUM CHEMISTRY

3 4 1 TOTAL PROTEIN, ALBUMIN AND GLOBULIN IN SERUM

The values were taken on 30 ,60 ,90 and 120 days The Biuret assay method of Inchiosa (1964) was adopted for the estimation of total protein and albumin in serum Serum globulin was estimated by finding out the difference between total serum protein and albumin levels Albumin-globulin ratio was calculated by dividing albumin level with globulin level

3 4 2 SERUM ASPARTATE AMINO TRANSFERASE, SERUM ALANINE AMINO TRANSFERASE AND SERUM BILIRUBIN

These were estimated employing commercially available Kits (M/s Glaxo India Ltd) and final readings were taken using spectro photometer on 30 60 90 and 120 days

3 5 CYTOGENETIC ASSAY

Chromosome pattern in ducks were studied using three techniques viz feather pulp technique, bone marrow technique and employing individualised hepatocytes

3 5 1 FEATHER PULP TECHNIQUE (Shoffner *et al.* 1967)

The smaller body feathers avoiding pigmented feathers were preferred for the collection of semi solid pulp Clear semi solid pulp was collected at the proximal end of the feather shaft A 0 05% solution of colcemid was injected into the wing vein of the bird (1 ml per 1 3 Kg of body weight) 45 minutes prior to the collection of pulp Immediately after taking the pulp, it was incubated in 0 8% Kcl Solution at 37°C for 30 minutes After this hypotonic treatment, the pulp was fixed in a mixture containing equal quantities of acetic acid and distilled water for 30 minutes Then the pulp was placed on a clean glass slide and stained with aceto carmine (Sheehan and Hrapchak 1980) for 30 minutes Then the pulp was smeared in a circular manner with the help of a coverslip, the pulp was covered and gently tapped Full weight of the body was directed straight downwards the coverslip for several seconds by pressing with the thumb The pulp was made into a monolayer and the edges of the coverslip was sealed using DPX and examined under oil immersion of the microscope

3 5 2 BONE MARROW TECHNIQUE (Malhi and Grover 1987)

A 0 05% solution of colcemid was injected into the wing vein of the bird (1ml per 1 3 Kg body weight) 45 minutes prior to the sacrifice Immediately after sacrificing the bird bone marrow from the femurs were collected in 0 9% sodium chloride solution The single cell suspension was centrifuged for 10 minutes at 1000 rpm and the bone marrow pellet was resuspended in 0 8% Kcl After 30 minutes of pre-treatment at 37°C, the cells were centrifuged and the pellets were resuspended in chilled carnoy's fixative After two hours of fixation, the cells were centrifuged and resuspended in the same fixative for 24 hours The fixed cells were dropped on chilled slides from a height of about 2-2 5m The slides were flame dried and stained with aceto carmine, dehydrated in acetone, acetone Xylene (1 1) Xylene and mounted with DPX

3 5 3 HEPATOCYTE TECHNIQUE

Soon after sacrificing the bird, the liver tissue was collected in Hank's Balanced Salt Solution (HBSS) and trypsinized using the standard technique described by Hoskins (1967) and proceeded further by employing the method mentioned for bone marrow technique

3 5.4 WHOLE BLOOD CULTURE TECHNIQUE (Moor head *et al*, 1960 and Mellman 1964)

Approximately ten millilitre of blood was collected from the wing vein of duck using heparin as anticoagulant and the plasma was separated Six to seven drops of blood was added to the tissue culture medium The tissue culture medium was then incubated at 37°C for 71hrs After incubation, colchicine (0 1ml) was added and incubated for one hour Then blood and tissue culture medium was centrifuged at 1000 rpm for 20 minutes and clear cell button was obtained To the cell button 6 ml of Kcl hypotonic solution was added followed by centrifugation at 1000 rpm for 20 minutes After discarding the supernatant, 6 ml of the fixative (Methanol Acetic acid - 3 1) was added Cell suspension was dropped on chilled slides (2-3 drops/slide) Using flame the smear was fixed and stained with 4per cent Giemsa solution for 30 minutes

3 6 POST-MORTEM EXAMINATION

The birds were sacrificed at two and four months duration of the experiment Detailed autopsy was conducted following the autopsy procedure advocated by FAO/SIDA (1968)

3 7 HISTOPATHOLOGY

Liver tissues were collected in 10 per cent buffered neutral formalin for histopathological examination 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 haematoxylin and eosin (H & E) method of Haris as described by Disbrey and Rack (1970)

3.8 STATISTICAL ANALYSIS

The data obtained from various clinical parameters were subjected to statistical analysis - Analysis of Variance (ANOVA) as explained by Snedecor and Cochran (1967)

Results

RESULTS

4 1 BODY WEIGHT

The values are tabulated in Table 1 and shown in figure 1 All the birds belonging to the experimental as well as the control groups showed a significant increase (P<0 05) in their body weight from 15 to 120 days

4 2 ERYTHROCYTE SEDIMENTATION RATE

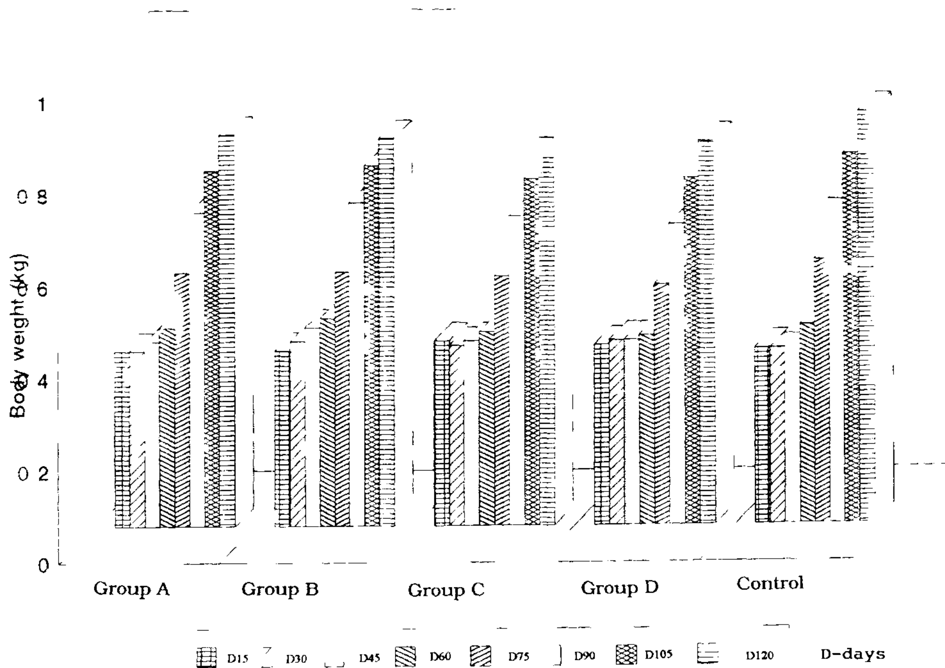
The values are tabulated in Table 2 Birds belonging to the experimental group showed an increase in erythrocyte sedimentation rate from 15 to 120 days

Birds fed with aflatoxin B1 at 15 g/kg bodyweight (group B) and 10 mg/kg bodyweight of 4 dimethyl amino azo benzene (group D) showed a significant increase (P<0 05) in erythrocyte sedimentation rate when compared to the control birds

4 3 PACKED CELL VOLUME

The values of packed cell volume are tabulated in Table 3 Birds fed with aflatoxin B1 at 10 g/kg bodyweight (group A) showed a gradual decrease in packed cell volume from 15 to 30 days and increased on day 45 and decreased gradually on 120th day

Fig.1 BODY WEIGHT (kg)



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Birds belonging to group B showed a gradual decrease from 15 to 120 days

Birds fed with 4 dimethyl amino azo benzene at 5 mg/kg bodyweight (group C) showed a gradual decrease from 15 to 120 days

Group D birds showed an alternate increase and decrease from 15 to 120 days

Control birds showed almost a steady packed cell volume from first to eighth fortnight

Group B showed a significant decrease (P<0 05) than group A and control group Groups C and D showed a significantly decreased value (P<0 05) than control group

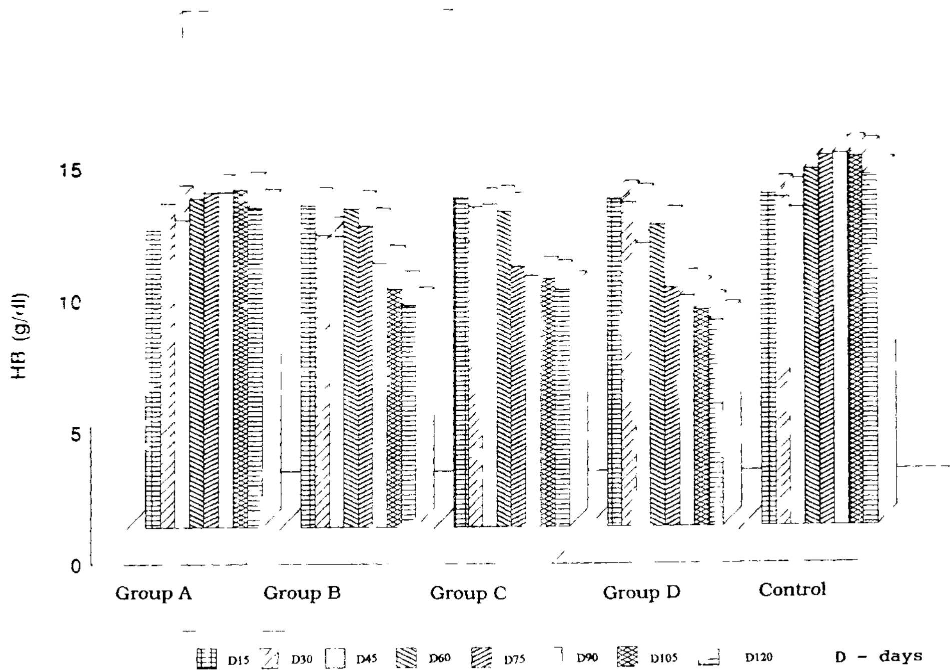
4 1 HAEMOGLOBIN

The haemoglobin values are tabulated in Table 4 and shown in figure 2 Birds belonging to group A showed an alternate increase and decrease in haemoglobin level from 15 to 120 days

Birds of group B also registered an alternate increase and decrease from 15 to 120 days

Birds reared as group C showed a decrease in haemoglobin from 15 to 30 days increased on day 45 and declined gradually on day 120

Fig.2 HAEMOGLOBIN (g/dl)



Group D birds showed a gradual decrease from 15 to 45 days increased on day 60 and decreased gradually on day 120

Birds belonging to control group showed a decreased value from 15 to 45 days increased on day 90 and decreased on day 120

Group B showed a significant decrease (P<0 05) in haemoglobin than groups A and control birds Groups C and D registered a significant decrease (P<0 05) than control group

4 5 ERYTHROCYTE COUNT

The erythrocyte count values are given in table 5 Birds maintained as group A recorded a decrease on day 30 increased on day 45 and decreased at all the intervals upto day 120

Birds belonging to group B registered a gradual decrease from 15 to 90 days increased on day 105 and again decreased on day 120

Birds of group C recorded an increase from first to 15 to 30 days then registered an alternate increase and decrease till day 90 and decreased gradually to on day 120 However values at these intervals were lesser than control birds

Group D showed a decrease from 15 to 45 days Steady decrease was then observed from fourth to eighth fortnight

Birds belonging to control group registered an alternate increase and decrease from 60 to 120 days

Group B registered a significant decrease (P<0 05) than groups A and control group Both the groups C and D registered a significant decrease (P<0 05) than control group

4 6 TOTAL LEUCOCYTE COUNT

Total leucocyte count values are tabulated in Table 6 and shown in figure 3 Birds belonging to the experimental groups showed a significant increase (P<0 05) in total leucocyte count from 15 to 120 days

Birds belonging to control group registered an alternate increase and decrease from 15 to 120 days but the increase was not statistically significant

Group B showed a significant increase (P<0 05) than group A and control group Groups C and D registered a significant increase (P<0 05) when compared to control group

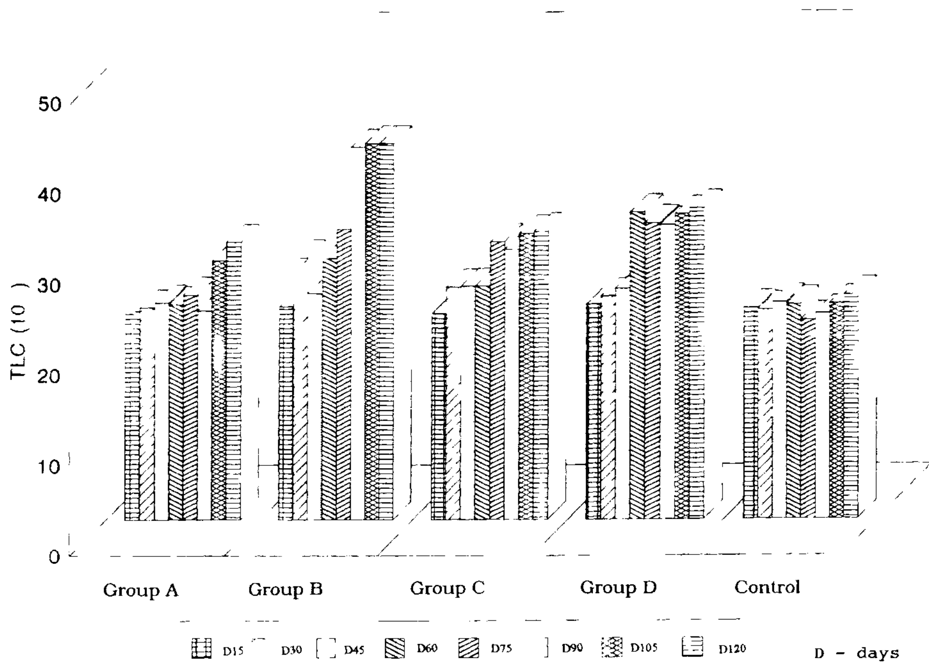
4 7 DIFFERENTIAL COUNT

4 7 1 HETEROPHIL

The values of heterophil are given in Table 7 Birds belonging to the experimental groups registered a significant increase (P<0 05) in heterophils from 15 to 120 days

Group B recorded a significant increase (P<0 05) in heterophils count when compared to group A and the control group

Fig.3 TOTAL LEUCOCYTE COUNT (10^3)



4 7 2 EOSINOPHIL

The values are tabulated in Table 8 Birds belonging to group A group B and group D showed a mild decrease in eosinophil from 15 to 120 days

Group C showed almost a steady value from 15 to 120 days Control birds showed an increasing value from 15 to 120 days On statistical analysis it was observed that there was no significant changes in the values

4 7 3 BASOPHIL

The values are given in Table 9 Birds belonging to group A showed a decrease in basophil count from 15 to 120 days No change in the count was observed in group B Group C recorded a mild increase from 15 to 120 days and group D registered a mild decrease from 15 to 120 days where as control group showed an increase in the same period of time Statistically these changes were not significant

4 7 4 MONOCYTE

The values are tabulated in Table 10 Decrease in monocyte number was observed in experimental as well as control birds from 15 to 120 days On statistical analysis the decrease in values were found non significant

4 7 5 LYMPHOCYTE

Lymphocyte values are tabulated in Table 11 Birds belonging to the experimental groups registered a significant

decrease (P<0 05) in lymphocyte from 15 to 120 days when compared to control birds

4 8 CLOTTING TIME

The values are tabulated in Table 12 Birds belonging to the experimental groups registered a significant increase (P<0 05) in the clotting time from 15 to 120 days when compared to control birds

Group B registered a significant increase (P<0 05) in the clotting time when compared to group A and Group D registered a significant increase (P<0 05) when compared to groups C

4 9 TOTAL SERUM PROTEIN

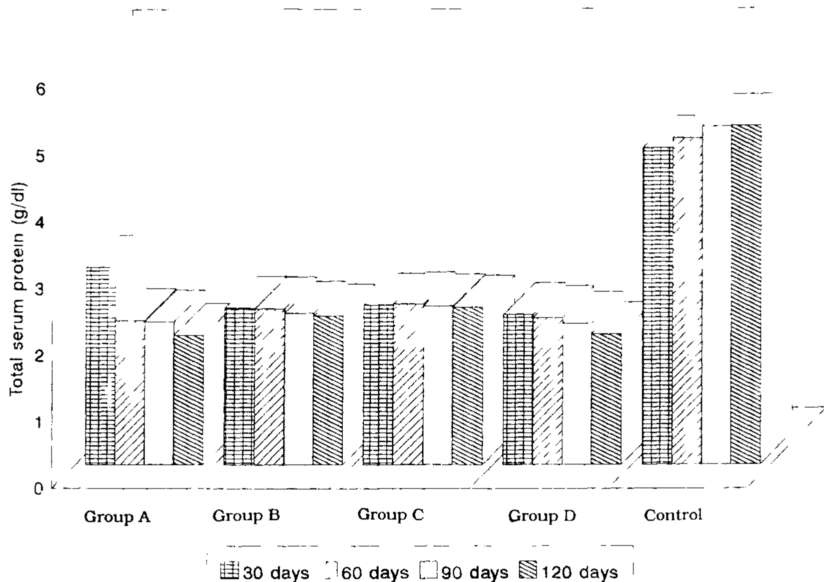
The serum protein values are tabulated in Table 13 and figure 4 The birds belonging to Group A group B and group D showed a gradual decrease in the total serum protein level

Birds maintained as group C showed an initial increase from day 30 to 60 During day 90 and day 120 the values declined gradually

The control birds showed a steady gradual increase in total serum protein values during the experimental period

The total serum protein values were significantly low (P<0 05) in experimental birds when compared to control birds

Fig.4 TOTAL SERUM PROTEIN (g/dl)



4 10 SERUM ALBUMIN

The serum albumin values are tabulated in Table 14 The birds of group A recorded a gradual decrease from 30th to 90th day followed by an increase on 120th day

In the group B there was an increase from 30th to 90th day followed by a decrease on 120th day

Group C birds showed an increase in serum albumin level from 30th to 60th day and decreased thereafter

The birds reared as group D showed a steady gradual decrease throughout the experimental period

Control birds registered a gradual increase in serum albumin level during the experimental period

Significant decrease ($P<0\ 05$) in serum albumin values in groups A and B was observed when compared to the control group

4 11 SERUM GLOBULIN

The serum globulin values are tabulated in Table 15 Alternate increase and decrease in serum globulin levels were noticed in group A, group B and group C during the experimental period

Group D registered increased values from day 30 to 90 and decreased on day 120

Control birds also showed an increase in globulin values from day 30 to 90 There was a decrease in the value on day 120

Groups A and B showed a significant decrease (P<0 05) in serum globulin level when compared to control group

4 12 ALBUMIN-GLOBULIN RATIO

The albumin-globulin ratio are tabulated in Table 16 Birds belonging to group A showed a steep decrease in albumin-globulin ratio from day 30 to 120

In the group B, there was an increase from day 30 to 90 day and then decreased on day 120

Group C exhibited an alternatively fluctuating values from day 30 to 120

Group D showed a gradual decrease from day 30 to 90 followed by an increase on day 120

Control birds recorded an initial decrease from day 30 to 60 and later increased on day 120

Birds belonging to groups C and D showed a significant increase (P<0 05) in albumm-globulin ratio when compared to control group

4 13 SERUM ASPARTATE AMINO TRANSFERASE (AST)

The serum aspartate amino transferase values are tabulated in Table 17 and shown in figure 5 Birds belonging to the experimental as well as the control groups showed a significant increase (P<0 05) in AST level from day 30 to 90 Group D showed a significant increase (P<0 05) when compared to group C and control group

4 14 SERUM ALANINE AMINO TRANSFERASE (ALT)

The values of serum alanine amino transferase are tabulated in Table 18 and shown in figure 6 From day 30 to 120 birds belonging to group A showed an alternate increase and decrease in ALT level

Birds belonging to group B and group D registered a steady increase from day 30 to 120

Birds of group C showed a gradual increase from day 30 to 90 and decrease on day 120

Control birds showed an alternate increase and decrease in ALT level from day 30 to 120

There was a significant increase (P<0 05) of ALT level in group A when compared to control group and significant decrease (P<0 05) when compared to group B Group B registered a significant increase (P<0 05) in ALT level when compared to control group

Fig.5 SERUM ASPARTATE AMINO TRANSFERASE (IU/l)

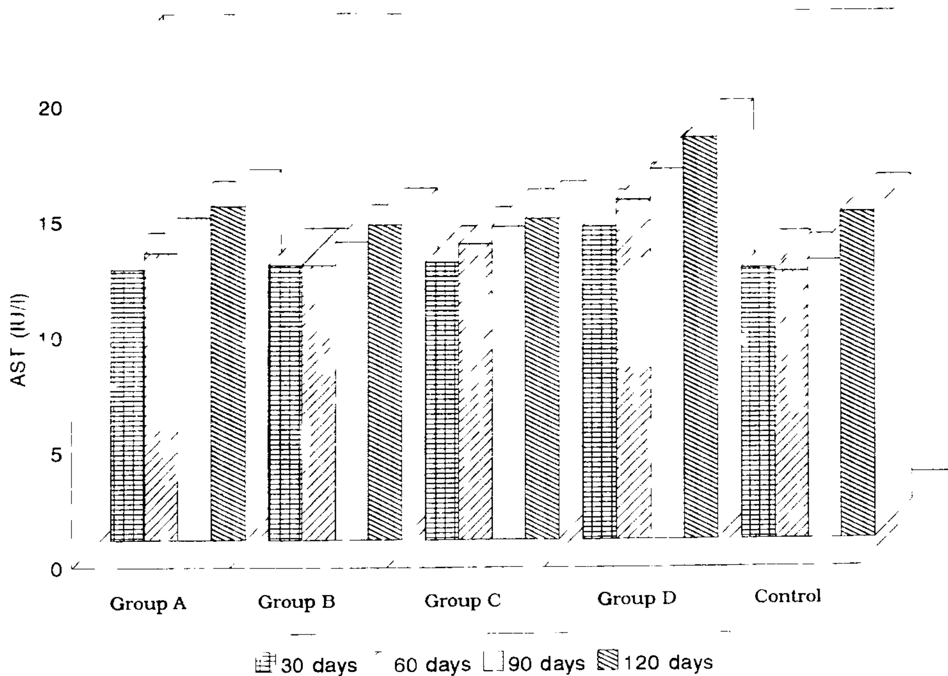
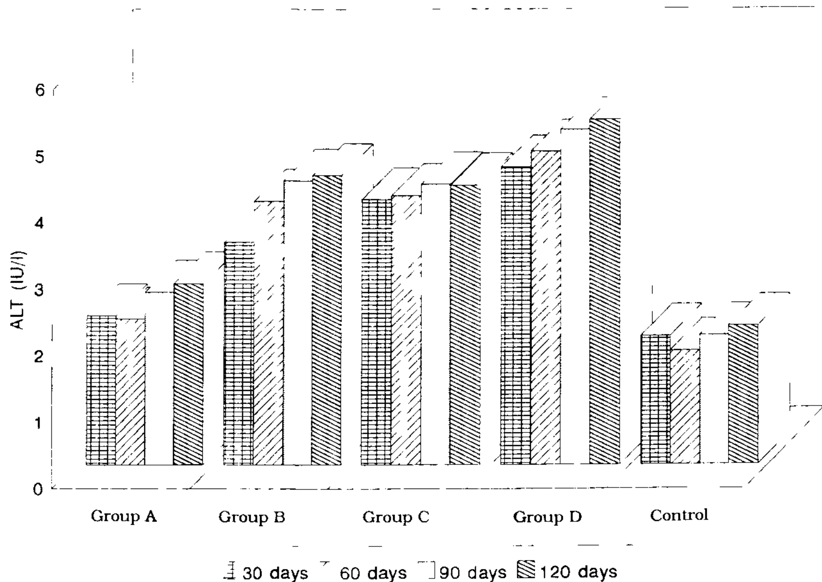


Fig.6 SERUM ALANINE AMINO TRANSFERASE (IU/I)



4 15 SERUM BILIRUBIN

Serum bilirubin values are given in Table 19 Birds belonging to group A showed an alternate increase and decrease in serum bilirubin from day 30 to 120

Birds of group B group C and group D showed a gradual increase from day 30 to 120

Control birds recorded a decrease from day 30 to 60 and increased on day 120

There was a significant increase ($P<0 05$) in serum bilirubin level in group B when compared to group A and control group Group A, group C and group D showed a significant increase ($P<0 05$) when compared to control group

4 16 GROSS PATHOLOGY OF LIVER

4 16 1 AFLATOXIN GROUP

Ducks fed with aflatoxin B1 at 10µg/Kg body weight when sacrificed on day 60 showed the following hepatic lesions The liver was slightly enlarged yellowish and glistening with oily cut surface (figure 8) The birds when sacrificed on day 120 showed variation in shape and size and small glistening nodules were discretely distributed in the parenchyma (figure 9) These were greenish yellow hard in consistency with mottled appearance Liver of some birds showed subcapsular haemorrhage and liver of all the birds showed patchy grayish white areas in the parenchyma

In the group where ducks were fed with aflatoxin B1 at 15μg/Kg body weight recorded the following hepatic lesions when sacrificed on day 60 Pin head sized necrotic areas petechial and ecchymotic haemorrhages haemorrhagic streaks moderate to severe enlargement were noticed The same group when sacrificed on day 120 exhibited telengiectasis patchy greyish areas with atrophy of the liver (figure 7) Numerous pin head size nodules were discretely distributed in all the livers (figure 10)

4 16 2 CARCINOGEN GROUP

Ducks fed with 4 dimethyl amino azo benzene at 5mg/Kg body weight when sacrificed on day 60 exhibited extensive areas of degeneration with a small area of normal tissue at the base of the liver Focal areas of petechiae and effusion in the caudal part (1-1 5cm size) were noticed Left lobe was seen enlarged in four birds Yellowish pinhead size greyish spots were seen diffused throughout the liver The same group of birds when sacrificed on day 120 showed patchy areas of necrosis with haemorrhagic streaks Right lobe enlargement was a constant feature in all the birds

Ducks fed with 4 dimethyl amino azo benzene at the rate of 10 mg/Kg body weight when sacrificed on day 60 showed the following liver lesions Right lobe was much enlarged Left lobe was atrophied Necrotic patches seen scattered throughout both the lobes In the liver of two birds the left lobe showed circumscribed diffuse areas where there were a number of [illegible]

white necrotic patches along with pin head sized raised areas towards the anterior end upto one centimetre above the caudal part The nodules were hard in consistency Areas of niches along the borders indicating degeneration and loss of cells Birds sacrificed on day 120 showed diffuse haemorrhage on the caudal portion of each lobes circumscribed nodules embedded in the parenchyma (figure 11) and serrated appearance of the borders

4 17 HISTOPATHOLOGY OF LIVER

4 17 1 AFLATOXIN GROUP

Ducks fed with aflatoxin B1 at the rate of 10µg/Kg body weight when sacrificed on day 60 showed the following lesions There was extensive bile duct proliferation (figure 14) mild fatty change, periductular infiltration of inflammatory cells and central venous congestion (figure 13) Coagulative necrosis sinusoidal dilatation cloudy swelling of the hepatocytes fibrous tissue proliferation were also noticed Pseudolobulation of hepatocytes (figure 17) due to fibrous tissue to the extent of individualisation of hepatocytes, mononuclear cell infiltration, hyperplasia of bile ducts and perivascular lymphocytic infiltration were seen (figure 18) In some birds, there were collection of inflammatory cells in the form of nodules The same group of birds when sacrificed on day 120 showed the following lesions Severe fatty change necrosis of hepatocytes (figure 16) and the architecture of cells were lost Pseudolobulation of hepatocytes was very extensive cirrhotic changes were noticed perivascular infiltration of lymphocytes and collection of inflammatory cells in the form of nodules

Those ducks which were given aflatoxin B1 at 15μg/Kg body weight when sacrificed on day 60 showed the following lesions There was extensive necrosis of hepatocytes focal hepatitis, bile duct hyperplasia, central venous congestion irregular pattern of arrangement of cells severe fatty change (figure 12) hepatocytes were arranged in the form of multiple lobules and mononuclear cell infiltration. The same group of birds when sacrificed on day 120 showed focus of dysplastic cells pseudolobulation, focal hepatitis (figure 15) bile duct hyperplasia and diffuse necrosis

4 1 7 2 CARCINOGEN GROUP

Ducks fed with 4 dimethyl amino azo benzene at 5 mg/Kg body weight when sacrificed on day 60 showed diffuse necrosis mild fatty change mononuclear cell infiltration infiltration of inflammatory cells in the portal triad, diffuse centrilobular necrosis bile duct proliferation and mild fibrous tissue proliferation The same group of birds when sacrificed on day 120 showed moderate to severe fatty change with necrosis of hepatocytes, bile duct proliferation and severe proliferation of fibrous tissue

Those ducks receiving 4 dimethyl amino azo benze at 10mg/Kg body weight when sacrificed on day 60 showed the following lesions Diffuse degeneration of hepatocytes fatty change extensive bile duct hyperplasia and diffuse necrosis Number of bile ducts showed multiple layer of cells occluding

the lumen Dilatation of central vein haemorrhage extensive perivascular necrosis mononuclear cell infiltration and fibrous tissue proliferation In some livers there were Kupffer cell reaction replacement of the parenchyma in focal areas with distinct proliferating hepatocytes of varying hyperchromasia of nuclei with indistinct cytoplasm (Figure 19) Such cells were found amidst interlacing bundles of connective tissues(Figure 20) The outline of such nodules were surrounded by atrophic hepatocytes Similar changes were also noticed in those birds sacrificed on day 120

4 18 CYTOGENETIC ASSAY

The chromosome profile of ducks with aflatoxin B1 and 4 dimethyl amino azo benzene was analysed The chromosomes prepared by lymphocyte culture were analysed and compared with that of control birds The control birds exhibited a hromosome spread with 12 macrochromosomes (Figure 22 & 23) Birds which were given aflatoxin B1 at the dose rate of 15 g/kg bodyweight showed 24 macrochromosomes in metaphase spreads (Figure 24) Whereas those maintained on 10 g/kg bodyweight recorded an average of 14 macrochromosomes (Figure 25) The study on birds which received 4 dimethyl amino azo benzene at 5 mg/kg bodyweight revealed an average of 14 macrochromosomes, but those with 4 dimethyl amino azo benzene at 10 mg/kg bodyweight had 16 macrochromosomes (Figure 26) In the feather pulp as well as bone marrow technique the full chromosome complement could not be analysed

because of microchromosome nature In the hepatocyte technique the cell got ruptured but the chromosome spread was not upto mark Hence the lymphocytes were cultured to obtain metaphase spread in ducks

Table 1 AVERAGE BODY WEIGHT (Kg)

Days	Group A	Group B	Group C	Group D	Control
15	0.38 ± 0.017	0.38 ± 0.012	0.40 ± 0.018	0.39 ± 0.016	0.38± 0.017
30	0.38 ± 0.018	0.40 ± 0.015	0.39 ± 0.016	0.40 ± 0.018	0.38 ± 0.015
45	0.40 ± 0.015	0.43 ± 0.013	0.40 ± 0.110	0.40 ± 0.216	0.41 ± 0.012
60	0.43 ± 0.011	0.45 ± 0.180	0.42 ± 0.316	0.41 ± 0.153	0.43 ± 0.011
75	0.55 ± 0.196	0.55 ± 0.185	0.54 ± 0.176	0.52 ± 0.116	0.57 ± 0.132
90	0.68 ± 0.159	0.70 ± 0.149	0.67 ± 0.460	0.65 ± 0.160	0.70 ± 0.119
105	0.77 ± 0.196	0.78 ± 0.132	0.75 + 0.509	0.75 + 0.561	0.80 ± 0.063
120	0.85 ± 0.153	0.84 ± 0.051	0.84 ± 0.096	0.83 ± 0.156	0.89 + 0.199
Mean	0.524	0.538	0.522	0.514	0.536
+	±	±	+	+	+
SE	0.095	0.093	0.212	0.174	0.056

Table 2 AVERAGE ERYTHROCYTE SEDIMENTATION RATE (mm/h)

Days	Group A	Group B	Group C	Group D	Control
15	1 30 ± 0 101	1 36 ± 0 143	1 34 ± 0 114	1 30 ± 0 101	1 40 ± 0 167
30	1 32 + 0 109	1 33 ± 0 140	1 34 ± 0 118	1 32 + 0 135	1 45 ± 0 214
45	1 42 ± 0 121	1 62 ± 0 146	1 55 ± 0 171	1 62 ± 0 167	1 48 ± 0 207
60	1 58 ± 0 189	1 83 ± 0 198	1 34 ± 0 114	1 71 ± 0 202	1 59 ± 0 212
75	1 64 ± 0 218	1 97 ± 0 229	1 67 ± 0 188	1 90 ± 0 241	1 48 ± 0 218
90	1 70 ± 0 149	2 05 ± 0 218	1 72 ± 0 195	2 14 ± 0 405	1 46 + 0 188
105	1 80 + 0 179	2 03 ± 0 284	1 71 ± 0 289	1 84 + 0 193	1 53 ± 0 260
120	1 86 + 0 213	2 07 ± 0 297	1 90 ± 0 297	1 91 ± 0 207	1 53 ± 0 288
Mean	1 577	1 783	1 571	1 717	1 49
+	±	±	+	±	+
SE	0 159	0 206	0 185	0 206	0 219

Table 3 AVERAGE PACKED CELL VOLUME (%)

Days	Group A	Group B	Group C	Group D	Control
15	30 56 ± 0 145	31 50 ± 0 246	30 67 ± 0 198	29 67 ± 0 198	31 11 ± 0 267
30	30 22 ± 0 275	29 56 ± 0 185	30 11 ± 0 196	29 72 ± 0 278	30 78 ± 0 236
45	31 11 ± 0 378	28 50 ± 0 232	29 67 ± 0 229	29 17 ± 0 167	31 11 ± 0 411
60	29 22 ± 0 329	27 06 ± 0 375	29 06 ± 0 328	27 61 ± 0 512	31 56 ± 0 487
75	28 17 ± 0 345	24 17 ± 0 737	28 08 + 0 193	23 92 ± 0 821	31 17 ± 0 386
90	28 25 ± 0 250	21 25 ± 0 617	28 00 ± 0 275	24 92 ± 0 941	31 58 ± 0 417
105	27 50 ± 0 379	19 25 ± 0 329	24 92 ± 0 733	23 33 ± 0 882	31 83 ± 0 657
120	26 33 ± 0 449	16 67 ± 0 620	22 92 ± 0 633	24 33 ± 0 432	31 83 ± 0 405
Mean	29 192	25 625	28 317	27 075	31 300
±	±	±	±	±	±
SE	0 178	0 463	0 251	0 293	0 144

Table 4 **AVERAGE HAEMOGLOBIN (g/dl)**

Days	Group A	Group B	Group C	Group D	Control
15	11 24± 0 226	12 15 ± 0 341	12 44 ± 0 273	12 36 ± 0 345	12 54 + 0 327
30	12 27 ± 0 284	11 03 ± 0 298	12 12 ± 0 235	12 25 ± 0 203	12 42 ± 0 185
45	11 64 ± 0 271	11 00 ± 0 254	12 21 ± 0 237	10 70 ± 0 229	12 03 ± 0 271
60	12 46 + 0 336	12 03 ± 0 396	11 93 ± 0 252	11 40 ± 0 383	13 48 ± 0 354
75	12 68 ± 0 452	11 37 ± 0 319	9 84 ± 0 206	9 00 ± 0 589	13 99 ± 0 425
90	12 69 ± 0 608	9 96 ± 0 466	9 49 ± 0 107	8 70 ± 0 540	14 05 ± 0 689
105	12 77 ± 0 465	8 98 ± 0 401	9 37 ± 0 129	8 17 ± 0 410	13 94 ± 0 423
120	12 10 ± 0 406	8 38 ± 0 383	8 95 ± 0 168	7 78 ± 0 387	13 24 ± 0 408
Mean	12 166	10 800	11 070	10 372	13 093
±	±	±	±	±	±
SE	0 134	0 167	0 149	0 203	0 145

Table 5 AVERAGE ERYTHROCYTE COUNT (10^6)

Days	Group A	Group B	Group C	Group D	Control
15	4 37 ± 0 195	4 19 ± 0 111	4 15 ± 0 166	4 39 ± 0 089	4 53 ± 0 089
30	4 15 + 0 230	4 04 ± 0 130	4 18 ± 0 088	3 83 ± 0 138	3 96 ± 0 151
45	4 47 ± 0 075	3 76 ± 0 114	3 18 ± 0 238	4 09 ± 0 099	4 53 ± 0 100
60	3 96 ± 0 163	3 39 ± 0 252	3 23 ± 0 210	2 60 ± 0 086	4 45 ± 0 242
75	3 93 + 0 068	2 72 ± 0 190	3 13 ± 0 170	2 42 ± 0 045	4 32 ± 0 063
90	3 88 ± 0 133	1 84 ± 0 157	3 09 ± 0 199	2 27 ± 0 066	4 37 ± 0 101
105	3 45 ± 0 179	2 09 ± 0 134	2 87 ± 0 223	2 22 ± 0 063	4 59 ± 0 114
120	3 99 + 0 101	1 74 ± 0 115	2 57 ± 0 225	2 13 ± 0 046	4 84 ± 0 194
Mean	4 068	3 148	3 376	3 140	4 432
+	+	±	±	±	±
SE	0 063	0 101	0 083	0 088	0 057

Table 6 AVERAGE TOTAL LEUCOCYTE COUNT (10^3)

Days	Group A	Group B	Group C	Group D	Control
15	22 99 ± 0 375	23 48 ± 0 420	22 68 ± 0 406	23 68 ± 0 495	23 24 ± 0 485
30	23 38 ± 0 485	28 88 ± 0 546	25 63 ± 0 571	24 60 ± 0 597	23 02 ± 0 474
45	23 92 ± 0 534	24 93 ± 0 463	25 74 ± 0 893	25 44 ± 0 547	23 87 + 0 536
60	23 76 ± 0 417	28 79 ± 0 967	25 75 ± 0 748	33 82 ± 0 977	23 62 ± 0 436
75	24 80 ± 0 555	32 00 ± 1 067	30 62 ± 1 132	32 62 ± 1 255	21 89 ± 0 304
90	23 04 ± 1 050	41 09 ± 2 482	29 76 ± 0 849	32 43 ± 1 477	22 58 ± 0 481
105	28 58 ± 0 746	41 48 ± 1 723	31 54 ± 0 638	33 66 ± 1 399	23 74 ± 0 521
120	30 62 + 0 745	41 53 ± 2 261	31 77 ± 0 872	34 28 ± 1 408	24 65 ± 0 625
Mean	24 812	31 524	27 339	29 430	23 352
±	±	±	±	±	±
SE	0 305	0 759	0 389	0 526	0 183

Table 7 AVERAGE HETEROPHIL (%)

Days	Group A	Group B	Group C	Group D	Control
15	29 78 ± 1 131	34 50 ± 0 991	31 83 ± 1 115	31 50 ± 1 440	30 39 + 1 597
30	33 06 + 1 098	42 89 ± 0 427	31 78 ± 0 934	36 83 ± 1 235	33 11 ± 1 038
45	31 78 ± 0 931	47 33 ± 0 936	31 83 ± 0 678	38 33± 1 174	32 56 ± 0 974
60	34 28 ± 0 996	47 33 ± 0 997	31 67 ± 0 737	39 50 ± 1 177	36 33 ± 1 003
75	42 92 ± 0 468	53 58 ± 1 184	36 00 ± 0 663	33 42 ± 0 973	36 50 ± 0 866
90	42 08 ± 0 763	59 00 ± 2 329	37 00 ± 0 615	34 75 ± 1 266	35 67 ± 0 995
105	47 92 ± 0 765	69 25 ± 1 085	38 83 ± 0 613	37 08 ± 1 055	35 92 ± 0 401
120	48 33 ± 1 263	62 25 ± 2 406	41 83 ± 0 911	38 17 ± 0 716	35 33 + 1 032
Mean ± SE	37 458 ± 0 712	49 717 ± 1 063	34 433 ± 0 437	36 267 ± 0 483	34 200 ± 0 450

Table 8 AVERAGE EOSINOPHIL (%)

Days	Group A	Group B	Group C	Group D	Control
15	1 35 ± 0 271	1 09 ± 0 152	1 24 + 0 059	1 23 ± 0 061	1 14+ 0 181
30	1 22 + 0 212	1 22 ± 0 196	1 24 ± 0 059	1 23| ± 0 066	1 19 + 0 214
45	1 47 ± 0 222	1 25 ± 0 137	1 23 ± 0 061	1 23 ± 0 66	1 31 ± 0 214
60	1 39 ± 0 252	1 16 ± 0 171	1 23 ± 0 015	1 24 ± 0 059	1 48 ± 0 207
75	1 34 + 0 260	1 11 ± 0 207	1 22 ± 0 077	1 24 ± 0 074	1 40 ± 0 260
90	1 18 + 0 213	1 01 ± 0 49	1 22 ± 0 096	1 22 ± 0 077	1 21± 0 229
105	1 18 ± 0 246	1 14 ± 0 229	1 22 ± 0 077	1 22 ± 0 096	1 18 ± 10 213
120	1 14 + 0 260	0 97 ± 0 151	1 24 ± 0 074	1 21 ± 0 078	1 28 ± 0 250
Mean	1 283	1 119	1 230	1 228	1 273
+	+	±	±	±	±
SE	0 042	0 033	3 273	3 660	0 041

Table 9 AVERAGE BASOPHIL (%)

Days	Group A	Group B	Group C	Group D	Control
15	1 21 ± 0 114	0 97 ± 0 121	0 97 ± 0 121	1 07 + 0 135	1 12 + 0 146
30	1 15 + 0 137	1 08 ± 0 109	0 99 ± 0 126	1 05 ± 0 114	1 16 ± 0 111
45	1 25 ± 0 139	1 09 ± 0 152	0 94 ± 0 121	0 99 ± 0 121	1 21 ± 0 171
60	1 34 ± 0 114	1 07 ± 0 191	1 01 ± 0 143	1 06 ± 0 158	1 38 ± 0 145
75	1 18 ± 0 213	0 97 ± 0 151	1 04 ± 0 188	1 17 ± 0 149	1 11 ± 0 207
90	1 08 ± 0 179	0 97 ± 0 159	1 05 ± 0 142	1 05 ± 0 142	1 18 ± 0 213
105	0 92 ± 0 149	1 05 ± 0 142	1 05 ± 0 144	1 01 ± 0 149	1 20 ± 0 174
120	0 97 ± 0 151	0 97 ± 0 151	1 00 ± 0 193	1 05 ± 0 142	1 23 ± 0 193
Mean ± SE	1 137 ± 0 049	1 021 ± 0 019	1 006 ± 0 014	1 056 + 0 018	1 198 ± 0 029

Table 10 AVERAGE MONOCYTE (%)

Days	Group A	Group B	Group C	Group D	Control
15	1 66± 0 116	1 44± 0 016	1 30 ± 0 265	1 27 ± 0 211	1 59 ± 0 216
30	1 42 ± 0 165	1 26 ± 0 019	1 27 ± 0 119	1 19 ± 0 116	1 28 ± 0 214
45	1 40 ± 0 031	1 13 ± 0 011	1 38 ± 0 001	1 22 ± 0 119	1 31 ± 0 211
60	1 33 ± 0 033	1 29 ± 0 036	1 33 ± 0 065	0 96 ± 0 653	1 29 ± 0 236
75	1 20± 0 163	1 08 ± 0 039	1 30 ± 0 049	1 23 ± 0 411	1 39 ± 0 196
90	1 15 ± 0 156	1 17 ± 0 058	1 36 ± 0 561	1 21 ± 0 563	1 26 ± 0 176
105	1 11 + 0 119	1 11 ± 0 042	1 30 ± 0 591	0 97 ± 0 312	1 17 + 0 136
120	1 34 ± 0 196	0 97 ± 0 033	1 27 ± 0 616	1 04 ± 0 261	1 30 ± 0 133
Mean	1 326	1 181	1 313	1 136	1 323
+	±	±	±	±	±
SE	0 062	0 051	0 014	0 044	0 043

Table 11 AVERAGE LYMPHOCYTE (%)

Days	Group A	Group B	Group C	Group D	Control
15	65 28 ± 1 044	62 56 ± 1 147	65 78 ± 1 159	64 72 ± 1 351	65 78 + 1 668
30	63 28 ± 1 226	54 11 ± 0 517	65 83 ± 0 890	60 89 ± 1 311	63 78 ± 1 101
45	63 39 ± 1 097	49 94 ± 0 972	65 72 ± 0 620	60 06 ± 1 189	63 72 ± 1 174
60	61 39 ± 1 103	53 06 ± 0 962	66 28 ± 0 907	58 56 ± 1 248	59 28 ± 1 204
75	53 58 ± 0 583	44 42 ± 1 270	61 67 ± 0 752	64 00 ± 1 219	59 50 ± 0 830
90	55 25 ± 0 978	38 92 ± 2 254	60 75 ± 0 629	62 75 ± 1 207	61 17 ± 1 186
105	49 67 ± 0 762	28 92 ± 0 076	58 83 ± 0 824	61 33 ± 1 170	61 08 ± 1 328
120	48 75 + 1 377	36 25 ± 2 406	55 75 ± 0 930	60 33 ± 0 721	61 08 ± 1 184
Mean	58 725	47 800	63 242	61 475	62 167
±	±	±	±	±	±
SE	0 666	1 026	0 449	0 470	0 487

Table 12 AVERAGE CLOTTING TIME (Min)

Days	Group A	Group B	Group C	Group D	Control
15	1 25 ± 0 010	1 25 ± 0 004	1 24 ± 0 009	1 25 ± 0 007	1 24 ± 0 005
30	1 27 ± 0 007	1 28 ± 0 008	1 26 ± 0 003	1 30 ± 0 011	1 26± 0 036
45	1 30 ± 0 016	1 35± 0 010	1 30 ± 0 011	1 33 ± 0 014	1 29 ± 0 010
60	1 34 ± 0 015	1 38 ± 0 017	1 34 ± 0 016	1 45 ± 0 020	1 32 ± 0 015
75	1 31 + 0 014	1 40 ± 0 015	1 32 ± 0 018	2 07 ± 0 093	1 29 ± 0 013
90	1 44 ± 0 018	1 55 ± 0 053	1 44 ± 0 020	2 17 ± 0 091	1 41 ± 0 018
105	1 46 ± 0 026	1 60 ± 0 065	1 48 ± 0 053	2 04 ± 0 083	1 45 ± 0 053
120	1 37 ± 0 009	2 09 ± 0 083	1 39 + 0 020	2 18 ± 0 089	1 34 ± 0 008
Mean	1 331	1 487	1 336	1 723	1 316
+	±	±	±	±	±
SE	0 008	0 018	0 010	0 025	0 009

Table 13 AVERAGE TOTAL SERUM PROTEIN (g/dl)

Days	Group A	Group B	Group C	Group D	Control
30	2 96± 0 209	2 35± 0 021	2 39± 0 015	2 25± 0 013	4 75± 0 265
60	2 16± 0 013	2 34± 0 023	2 41± 0 016	2 20± 0 015	4 90± 0 272
90	2 14± 0 007	2 27± 0 035	2 38± 0 019	2 11± 0 014	5 07± 0 348
120	1 94± 0 053	2 23± 0 029	2 36± 0 021	1 95± 0 067	5 08± 0 301
Mean	2 351	2 307	2 389	2 148	4 924
±	±	±	±	±	±
SE	0 082	0 014	0 008	0 020	0 144

Table 14 AVERAGE SERUM ALBUMIN (g/dl)

Days	Group A	Group B	Group C	Group D	Control
30	1 91± 0 092	1 72± 0 062	2 20± 0 016	2 01± 0 040	2 75± 0 246
60	1 90± 0 055	2 08± 0 028	2 26± 0 015	1 91± 0 025	2 77± 0 233
90	1 43± 0 096	2 14± 0 032	2 21± 0 010	1 72± 0 070	2 78± 0 308
120	1 57± 0 062	2 00± 0 045	2 20± 0 028	1 65± 0 086	3 32± 0 245
Mean	1 742	1 968	2 220	1 850	2 877
±	±	±	±	±	±
SE	0 046	0 031	0 009	0 031	0 128

Table 15 AVERAGE SERUM GLOBULIN (g/dl)

Days	Group A	Group B	Group C	Group D	Control
30	1 05 ± 0 218	0 63 ± 0 069	0 22 ± 0 032	0 24 ± 0 038	2 00 + 0 164
60	0 26 ± 0 048	0 26 ± 0 031	0 15 ± 0 010	0 29 ± 0 025	2 13 ± 0 118
90	0 71 ± 0 094	0 44 ± 0 133	0 17 ± 0 013	0 39 ± 0 065	2 28 ± 0 208
120	0 37 ± 0 052	0 23 ± 0 027	0 16 ± 0 018	0 30 ± 0 088	1 82 ± 0 180
Mean	0 610	0 400	0 179	0 297	2 05)
±	±	±	±	±	±
SE	0 081	0 041	0 011	0 026	0 082

Table 16 AVERAGE ALBUMIN GLOBULIN

Days	Group A	Group B	Group C	Group D	Control
30	1 81 ± 7 006	2 73 ± 0 767	0 99 ± 0 607	8 37 ± 1 237	1 61 ± 0 222
60	7 30 ± 2 784	8 00 ± 1 250	15 06 ± 1 077	7 39 ± 0 620	1 36 ± 0 157
90	2 01 ± 1 136	4 86 ± 2 609	13 00 ± 1 136	6 91 ± 1 492	1 40 ± 0 259
120	4 24 ± 1 380	8 69 ± 1 009	13 75 ± 2 080	5 50 ± 5 484	2 05 ± 0 294
Mean	11 471	8 796	14 185	10 140	1 582
±	±	±	±	±	±
SE	2 371	0 818	0 624	1 254	0 115

Table 17 AVERAGE SERUM ASPARTATE AMINO TRANSFERASE (IU/l)

Davs	Group A	Group B	Group C	Group D	Control
30	11 72± 0 378	11 90± 0 175	11 99± 0 213	13 50± 0 188	11 69± 0 394
60	12 43± 0 400	11 89 ± 0 218	12 79± 0 175	14 66 ± 0 219	11 53± 0 348
90	13 98± 0 393	12 89 ± 0 252	13 54 ± 0 314	16 00± 0 402	12 01± 0 467
120	14 47± 0 478	13 62± 0 366	13 86 ± 0 400	17 37 ± 0 412	14 09± 0 365
Mean	12 934	12 437	12 912	15 124	12 186
±	±	±	±	±	±
SE	0 247	0 150	0 158	0 232	0 230

Table 18. AVERAGE SERUM ALANINE AMINO TRANSFERASE (IU/l)

Days	Group A	Group B	Group C	Group D	Control
30	2 24± 0 112	3 34± 0 140	3 98± 0 198	4 46± 0 225	1 92± 0 144
60	2 19± 0 103	3 95± 0 100	4 04± 0 087	4 70± 0 160	3 70± 0 120
90	2 59± 0 137	4 26± 0 201	4 21± 0 114	5 03 ± 0 139	1 93± 0 135
120	2 72± 0 100	4 34± 0 224	4 19± 0 208	5 18± 0 145	2 07± 0 122
Mean	2 388	3 906	4 084	4 793	1 883
+	±	±	+	±	±
SE	0 062	0 093	0 079	0 097	0 068

Table 19 AVERAGE SERUM BILIRUBIN (mg%)

Days	Group A	Group B	Group C	Group D	Control
30	1 27± 0 104	1 53± 0 152	1 28± 0 120	1 10± 0 106	0 67± 047
60	1 05± 0 083	2 05± 0 186	1 58± 0 076	1 52± 0 141	0 60± 0 032
90	1 61± 0 112	2 58± 0 188	2 43± 0 131	2 42± 0 127	0 69± 0 061
120	2 01± 0 130	2 73± 0 148	2 96± 0 142	3 73± 0 205	0 72± 0 041
Mean	1 418	2 133	1 935	2 014	0 663
±	±	±	±	±	±
SE	0 069	0 104	0 102	0 145	0 023

Fig 7 Liver - aflatoxin B1(15 g /kg body weight) Atrophy of left lobe
Circumscribed pin head sized nodules on both the lobes.

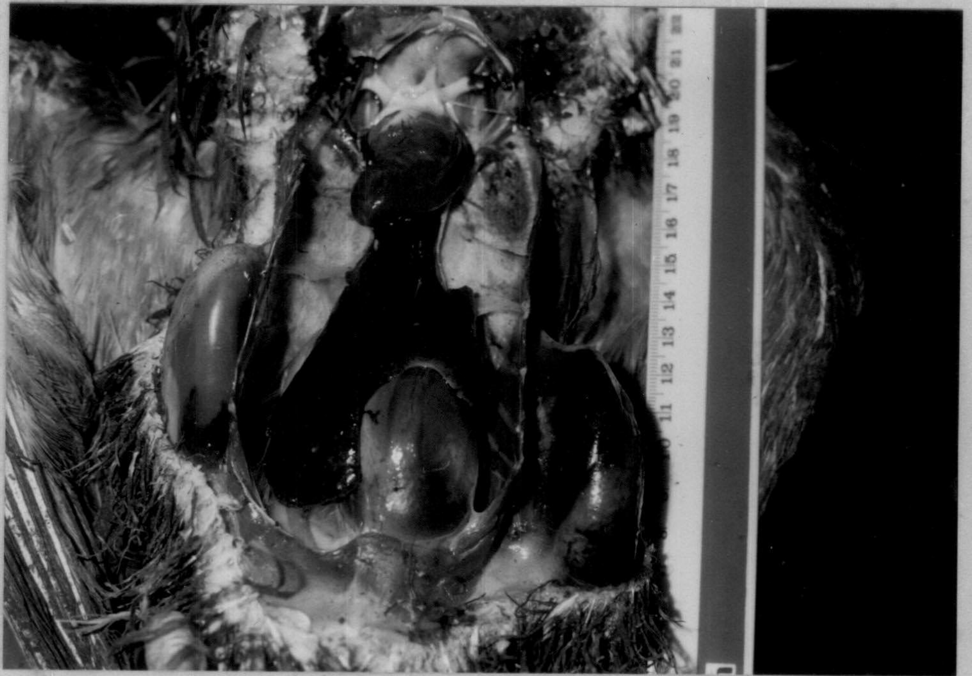
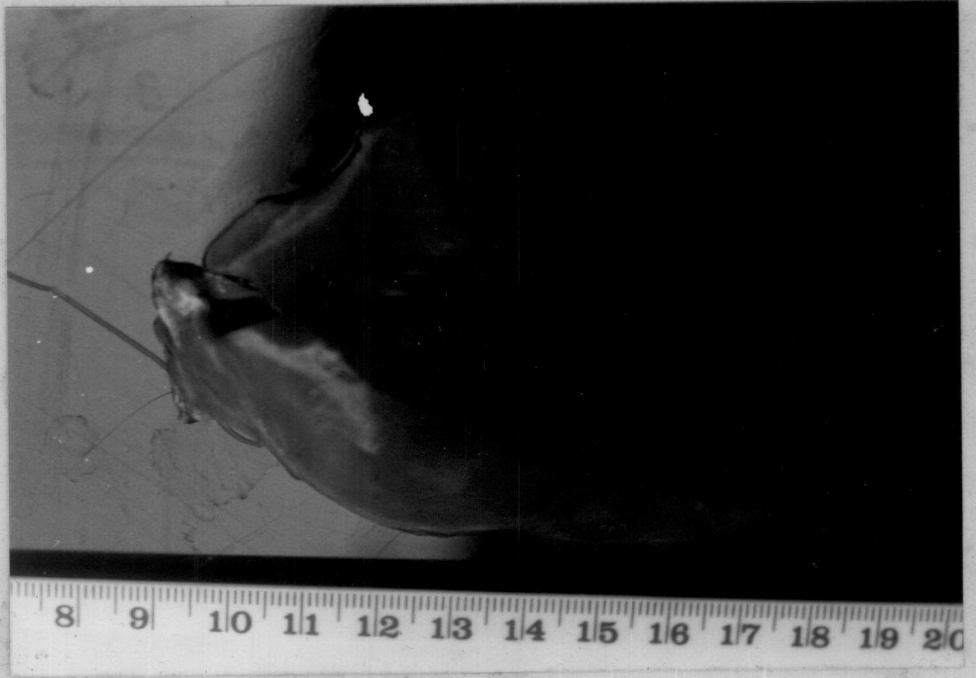


Fig 8 Liver -4 dimethyl ammo azo benzene (10 mg/kg body weight) fatty liver

Fig 9 Liver - Aflatoxin B1 (15 g/kg body weight) - dark brown in colour. Pin head sized dark nodules project from the parenchyma.



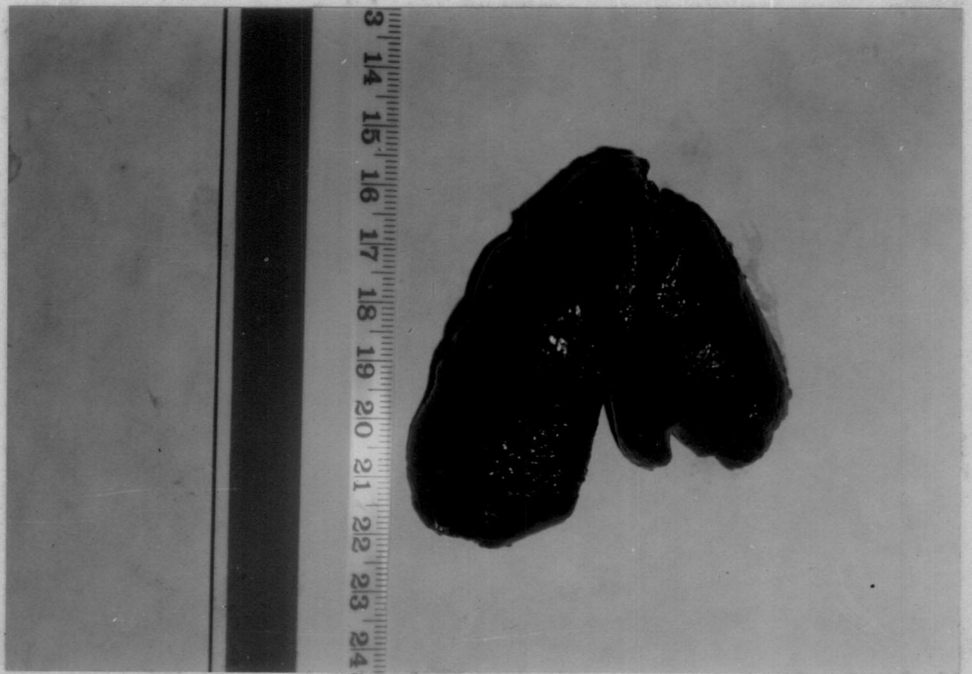
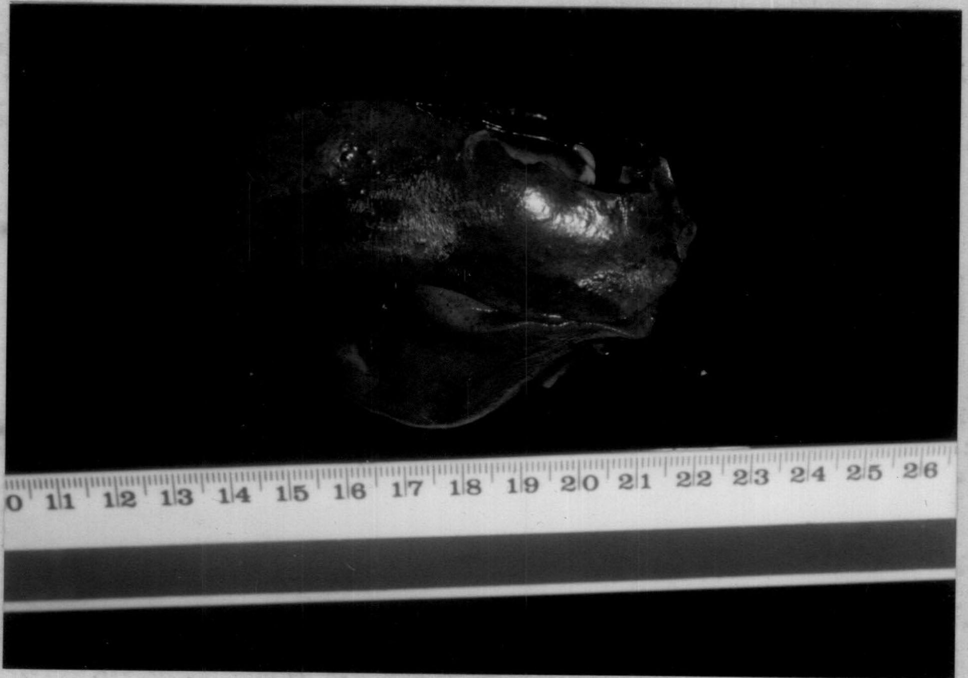


Fig .10 Liver - Aflatoxine B1 (15μg /kg body weight) Fatty liver. Pin head sized nodules in the parenchyma. Petechiae and diffuse congestion

Fig. 11 Liver - 4 dimethyle amino azo benzene (10 mg/kg body weight). Circumscribed greyish nodules of 2 to 3 mm size in the parenchyma Nodules surrounded by areas of hyperaemia.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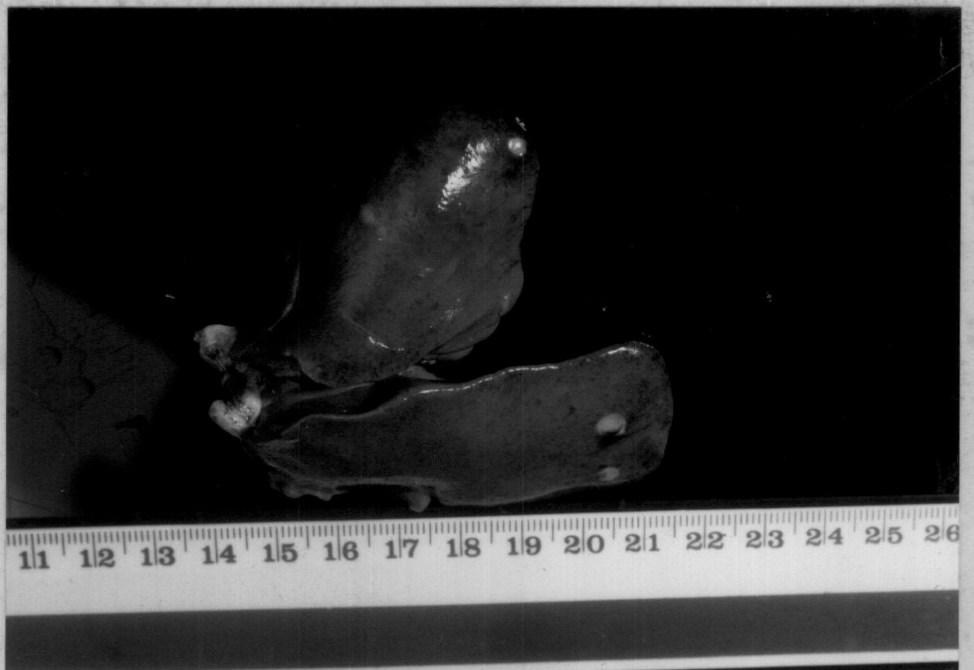
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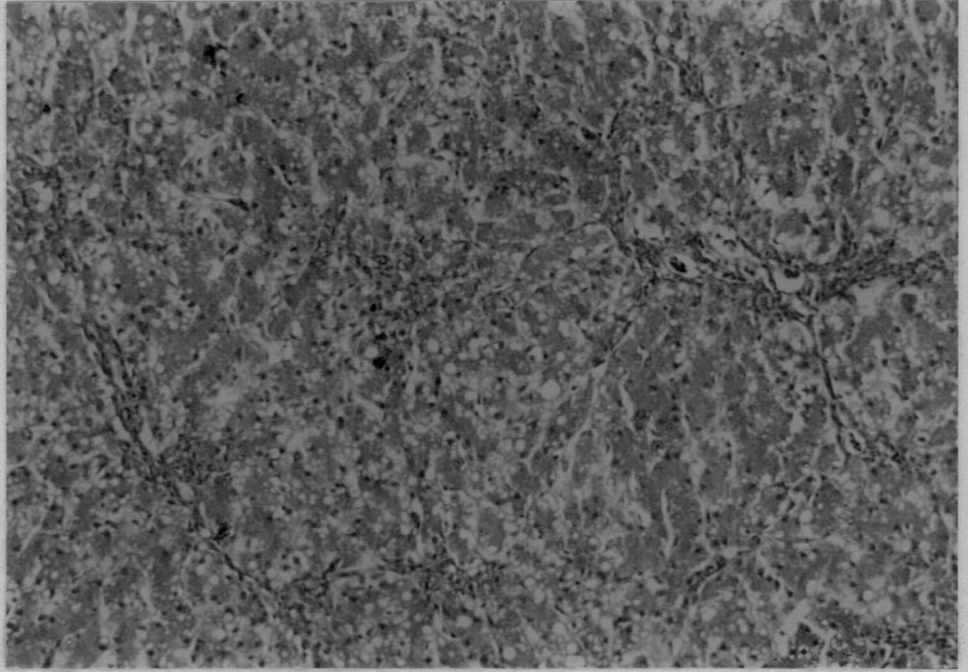
Fig 12 Liver - severe fatty change and necrosis - Group B - H & E X 160

Fig. 13 Liver - fatty change , bile duct proliferation , necrosis, central venous congestion - Group B - H& E X 160



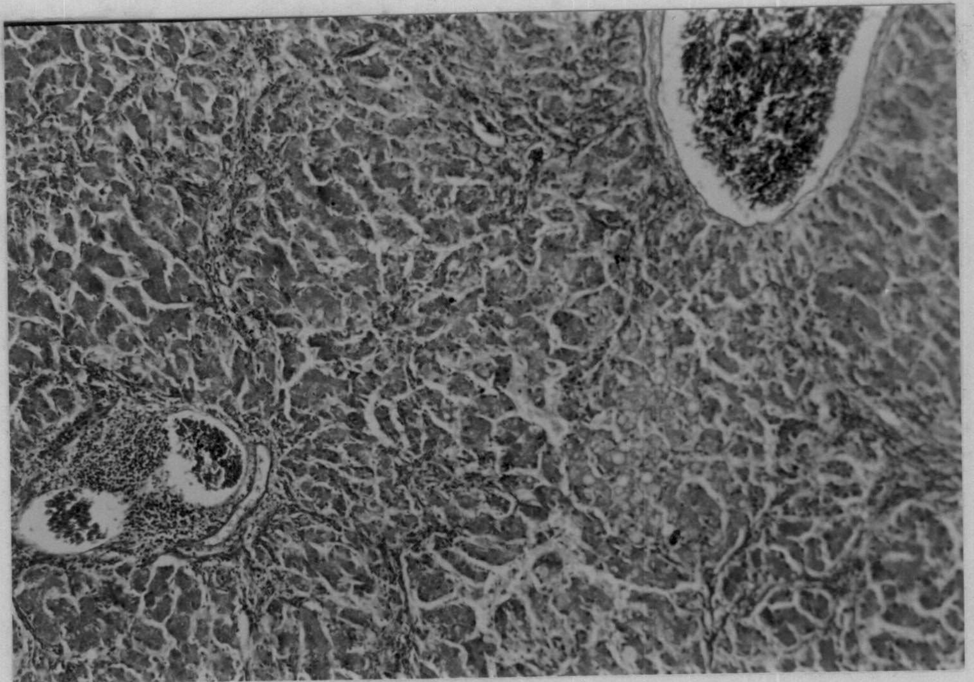
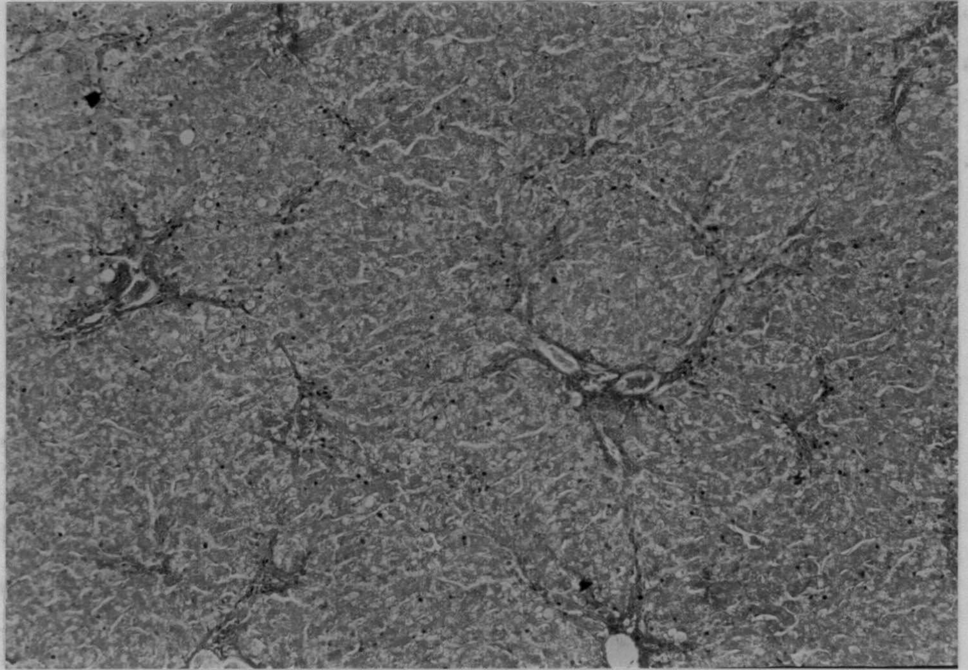


Fig 14 Liver - extensive bile duct proliferation- Group B-H&E X160

Fig 15 Liver- bile duct proliferation and nodular aggregate of cells in the bile duct Group B- H&E X 250



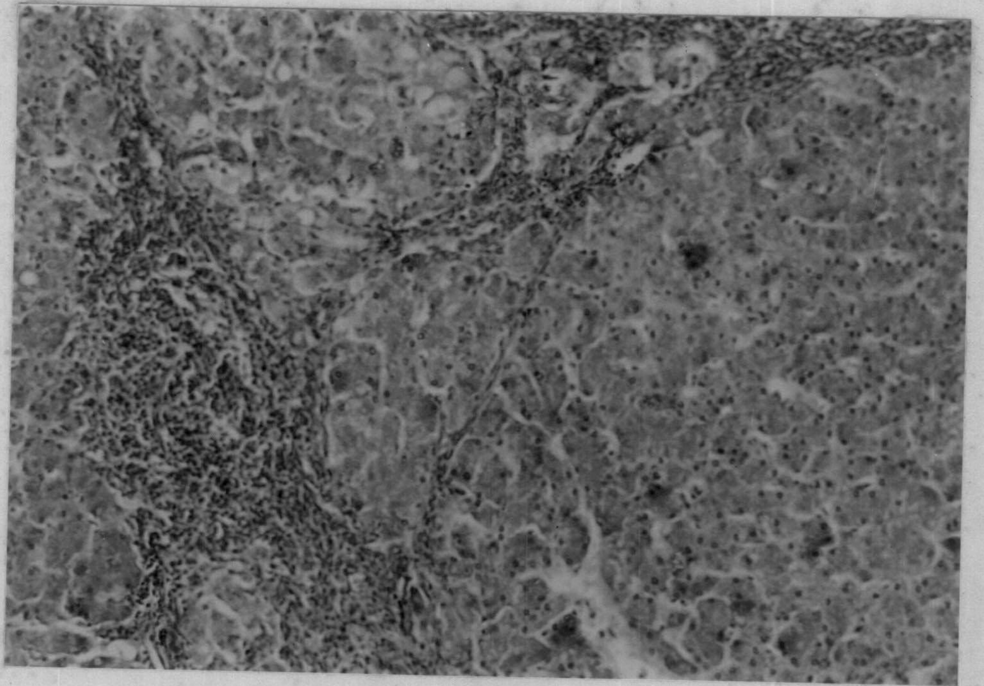
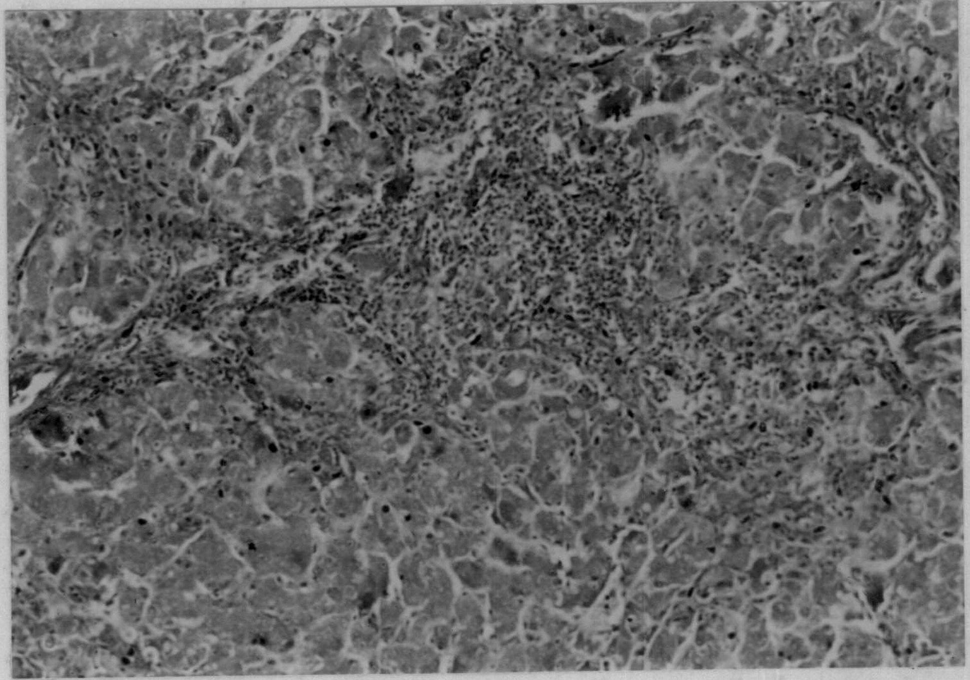


Fig.16 Liver- degeneration and necrosis of hepatocytes. Diffuse aggregates of cells of uniform size in the midzonal and portal triad -GroupB H&E X250

Fig.17 Liver-pseudolobulation, fatty change, atrophy of hepatocytes, bile duct proliferation and infiltration of inflammatory cells- Group B H&E X250



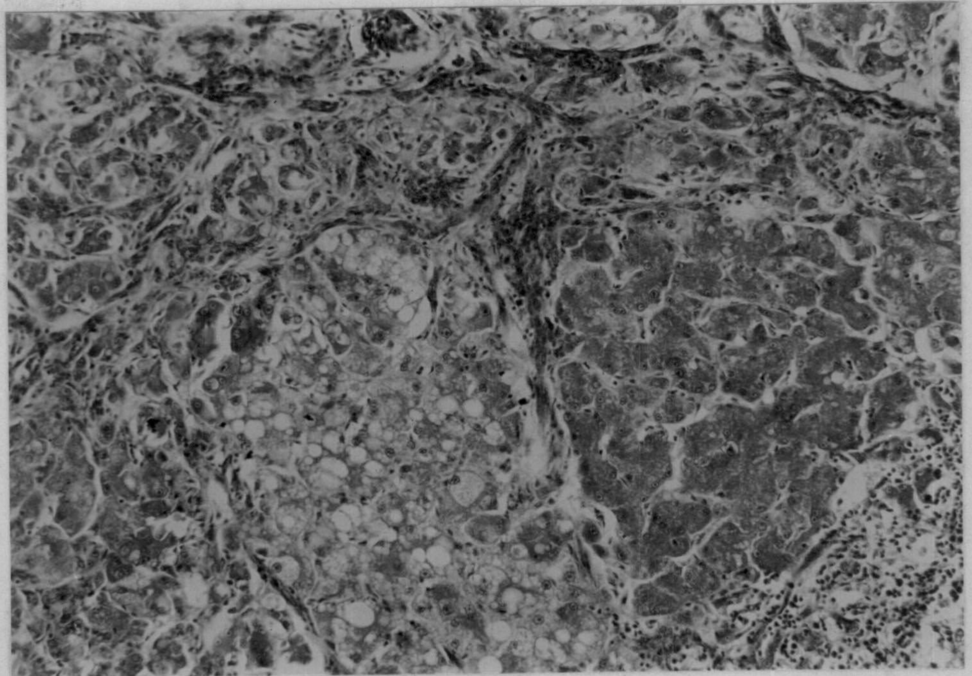
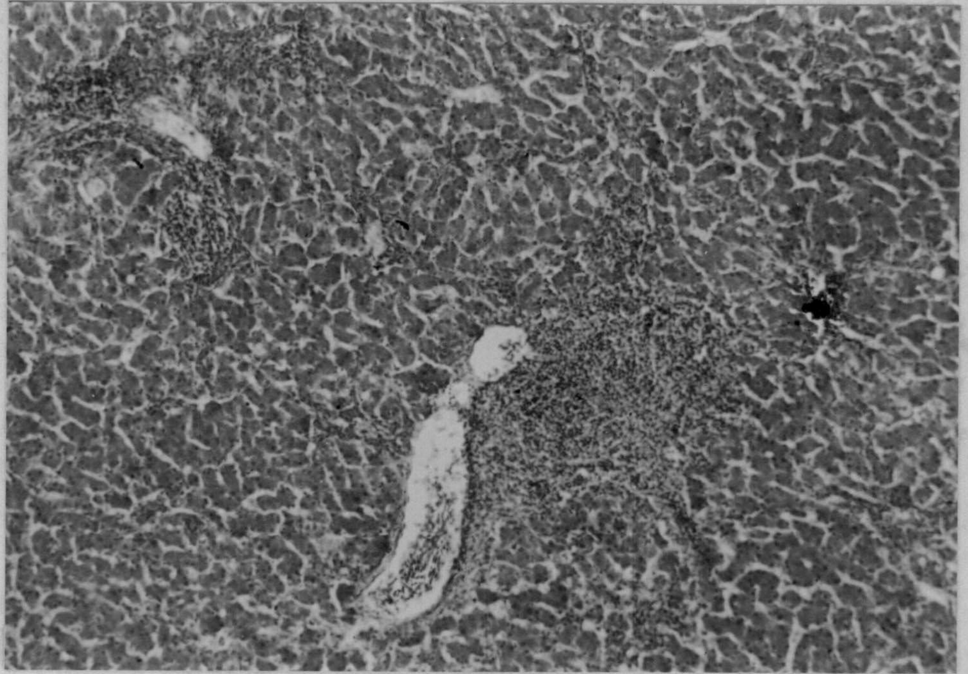


Fig 18 Liver-perivascular nodule formation and nodular aggregates of cells replacing the hepatocytes Group B H&E X160.

Fig 19 Liver-fibrous tissue proliferation, invasion of hepatic parenchyma by a mass of disorderely arranged hyperchromatic cells-Group D H&E X160.



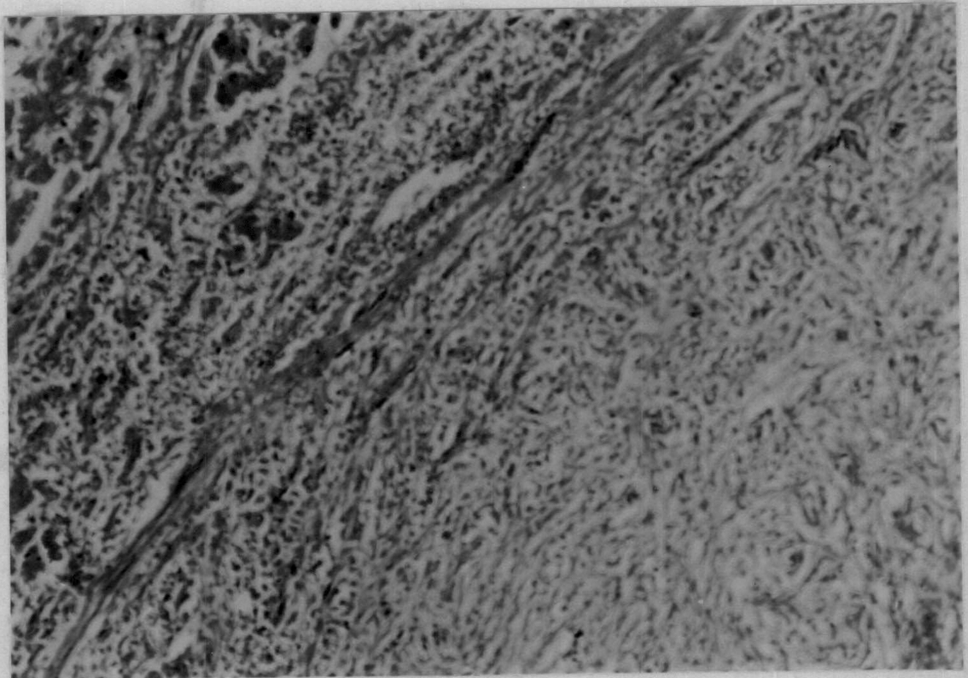
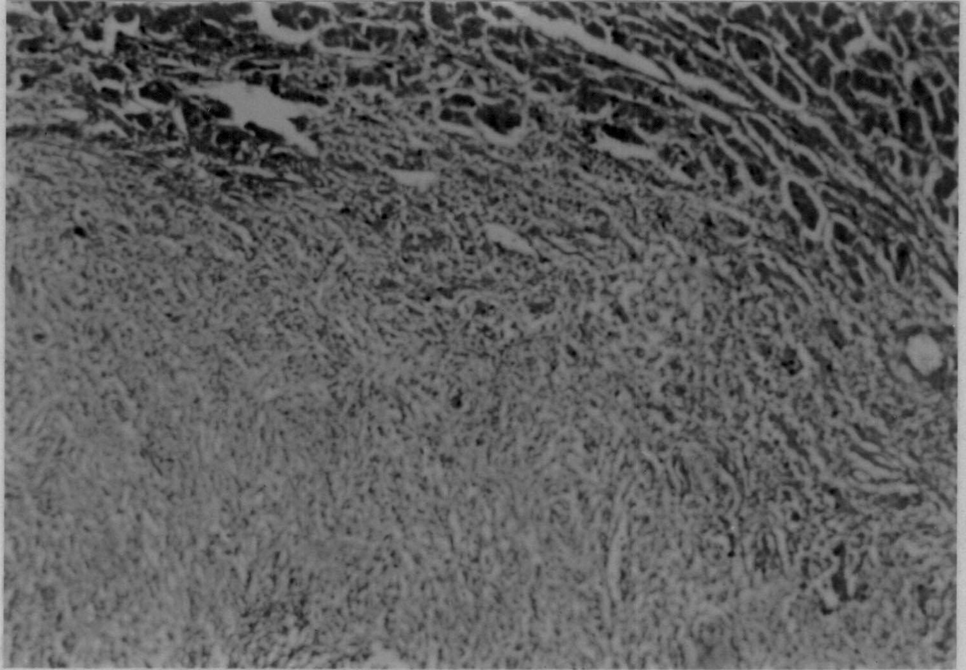


Fig 20 **Liver-neoplastic changes-clear demarcation between the hepatocytes and the tumour tissue -Group D H&E X160.**

Fig 21 **Liver-proliferating cells amidst fibrous tissue. Cells are hyperchromatic with indistinct cytoplasm and are spindle shaped .Cells with acinar pattern are also seen -Group D H&E X250.**



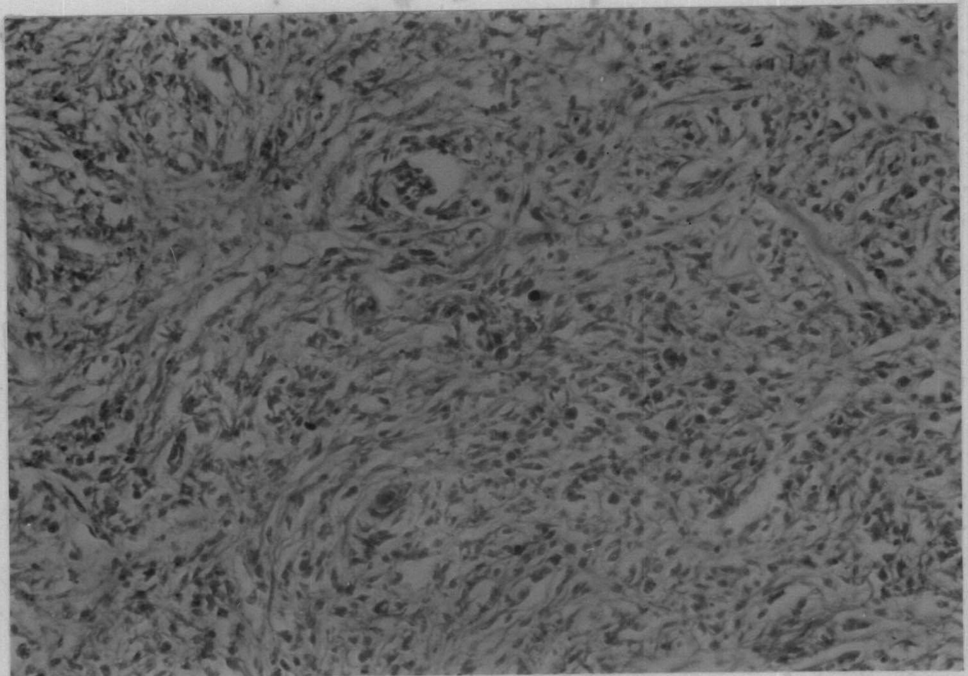
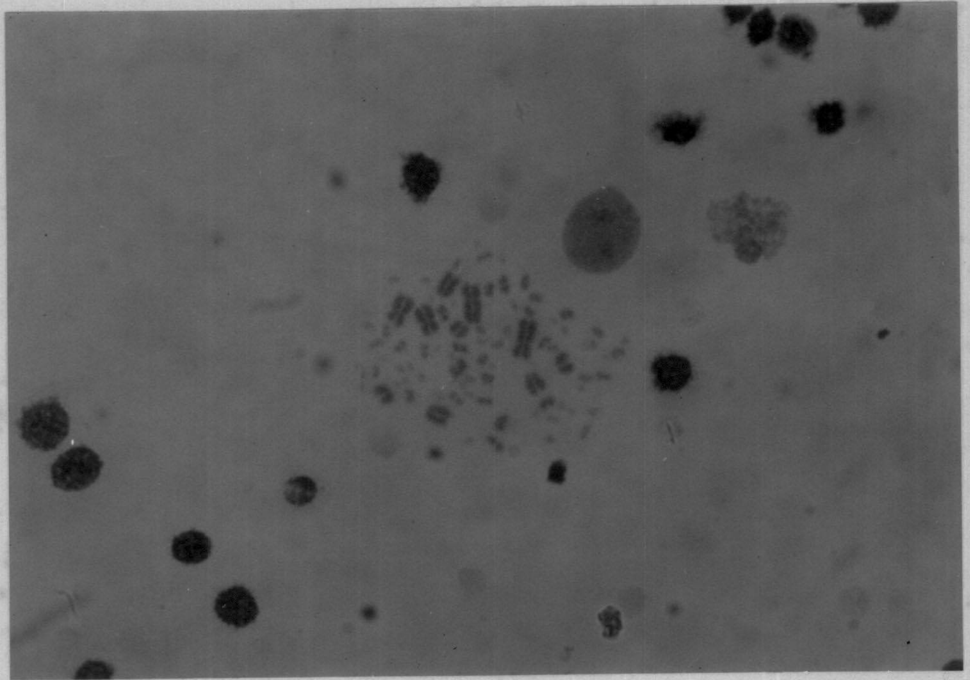


Fig.22 & 23 Metaphase of control ducks- 12 macrochromosomes- lymphocyte culture- Giemsa X1000.



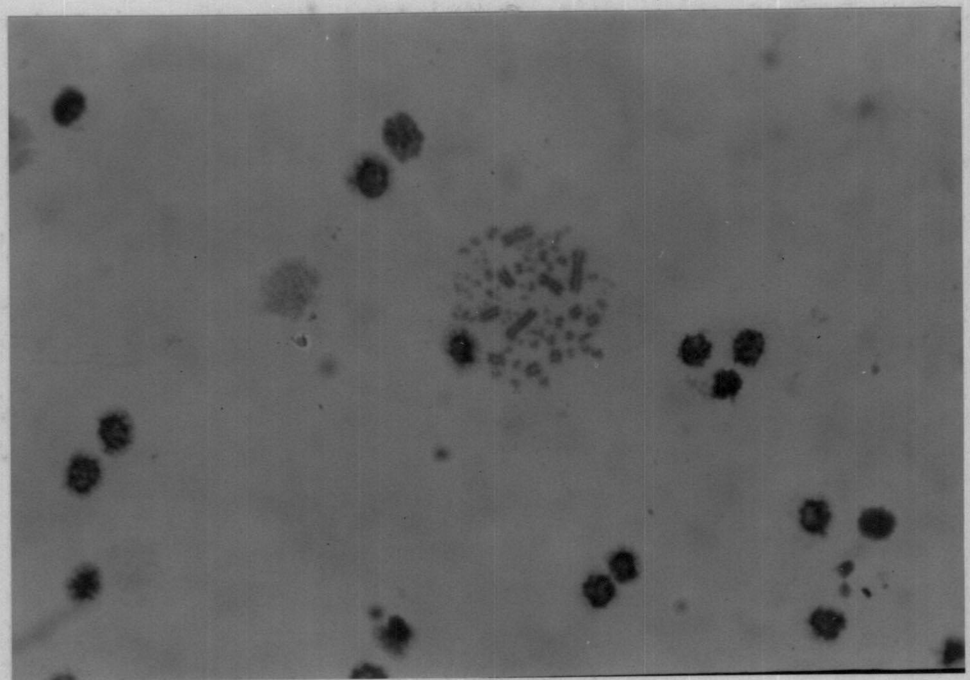
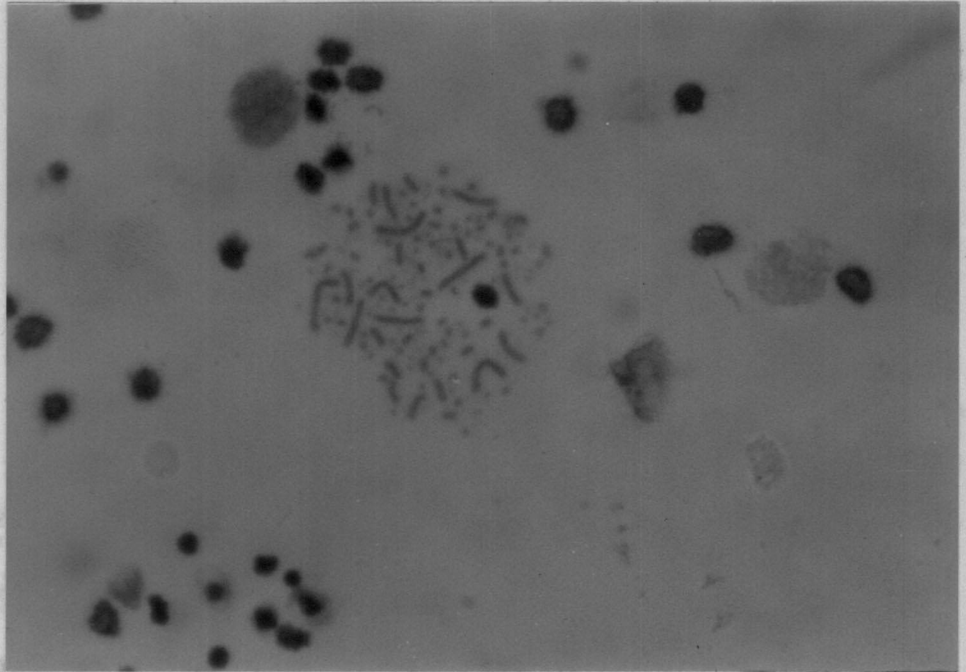


Fig 24. Metaphase spread of aflatoxin B1 treated ducks-24 macrochromosomes lymphocyte culture- Giemsa X 1000.

Fig.25 Metaphase spread of 4 DAB treated ducks- 14 macrochromosomes- lymphocyte culture-Giemsa X1000.



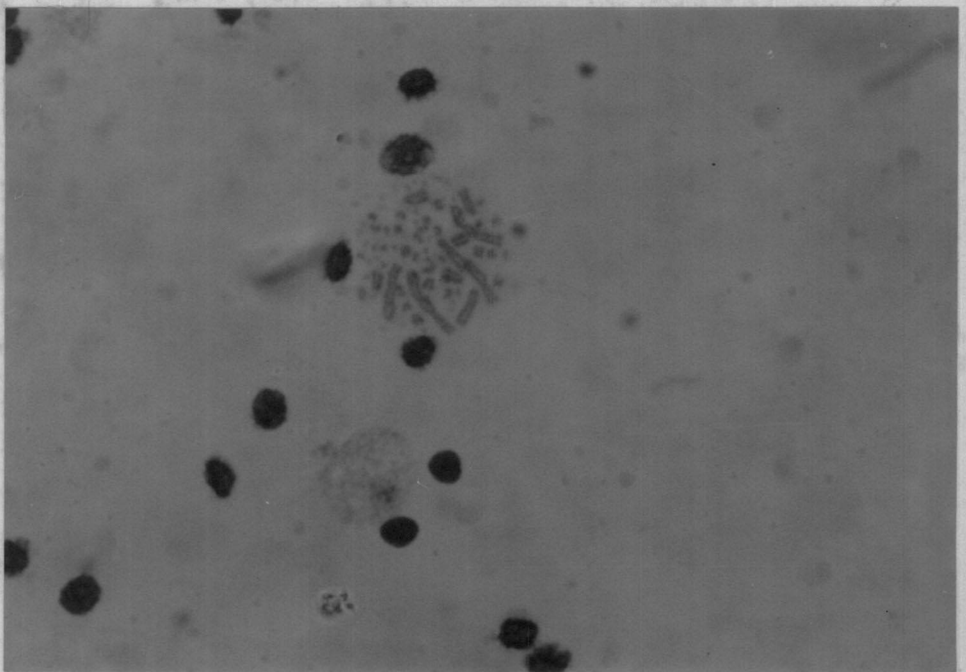
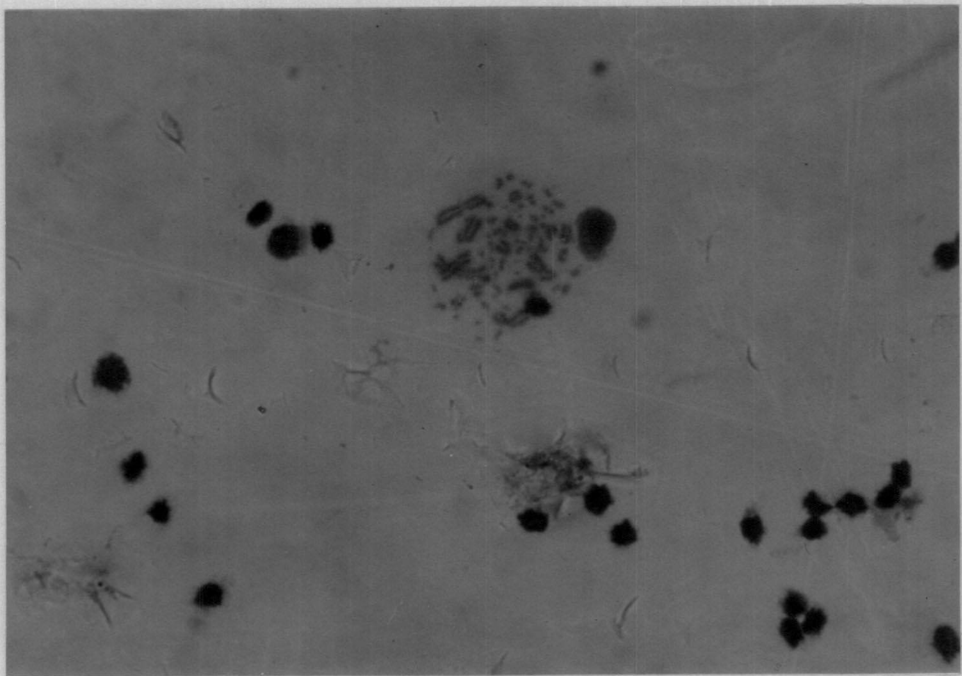


Fig. 26 Metaphase spread of 4DAB treated ducks-16 macrochromosomes-lymphocyte culture-Giemsa X1000.



Discussion

DISCUSSION

The study undertaken was to evaluate the morphological alterations produced by aflatoxin B1 in cells of various organ systems in ducks and to correlate the changes with the functional status of these systems For a comparative evaluation of the neoplastic reactions a known chemical carcinogen 4 dimethyl amino azo benzene was also employed Effect of the treatments on the body weight, haematological values, serum enzymes gross and histopathology of liver and chromosomal profile were evaluated

The influence of aflatoxm B1 as well as 4 dimethyl ammo azo benzene on the total body weight of experimental ducks was ascertained and it was observed that these agents were not effective in causing any substantial changes in the body weight The effect of aflatoxin on body weight was studied by Butler (1964) Smith and Hamilton (1970) Arafa *et al* (1981) Reddy (1981) Reddy *et al* (1982), Huff *et al* (1984), Ghosh *et al* (1989) and Rizvi and Shakoori (1992) in various avian species Most of these workers had observed reduction in the body weight due to aflatoxicosis The present observation did not agree with the findings of these workers A prominent difference between the present experiment and those which had been reported was the very low dose level employed in this study It is probable that at the low dose rate

(AFB1 10μg/kg body weight and AFB1 15μg/kg body weight) aflatoxin B1 was not effective in producing substantial loss of body weight The chemical carcinogen employed also could not produce any effect on body weight Reports for comparison were not available in this aspect

Significant increase of erythrocyte sedimentation rate was observed in the birds of group B (AFB1 15μg/kg body weight) and group D (4 DAB 10 mg/kg body weight) than the control birds Aflatoxin has been shown to cause elevated rate in different species of animals and birds (Murthy *et al* 1984 Fernandez *et al* 1995) Although the observations from the group B (birds fed with 15μg/kg body weight of aflatoxin B1) was in agreement with the reports of the above authors at the dose level of 10 g/kg body weight comparable increase was not observed Similarly the birds which received higher dose of 4 dimethyl amino azo benzene (10 mg/kg body weight) registered an increase in erythrocyte sedimentation rate where as those at the lower dose (5 mg/kg body weight) did not

The packed cell volume was found significantly decreased in the birds fed with aflatoxin B1 15μg/kg and 4 dimethyl amino azo benzene 5 mg and 10 mg/kg body weight than control birds A comparison of packed cell volume among the aflatoxin treated birds revealed a significant reduction in the volume which received the higher dose level (15μg kg body weight) A perusal of the

literature showed that a decrease in packed cell volume in aflatoxicosis in birds was described by many workers (Tung *et al* 1975, Reddy *et al* 1980 Reddy 1981 Mohiuddin *et al* 1986 Anjaneyulu *et al* 1993 and Mani *et al* 1993) The present observation also was in total agreement with these findings The chemical carcinogen at both dose levels produced significant reduction in the packed cell volume

Lowering of haemoglobin concentration at various dose levels of aflatoxin was observed by Tung *et al* (1975) Reddy (1981), Mohiuddin *et al* (1986) and Anjaneyulu *et al* (1993) The significantly lowered haemoglobin values recorded in birds fed at the dose rate of 15μg/kg body weight of aflatoxin B1 in the present study lend support to the observations of the above workers An exception was the maintenance of haemoglobin level at normal levels in birds which received aflatoxin at the dose rate of 10μg/kg body weight The haemoglobin level in the case of the birds belonging to group C (4 DAB 5 mg/kg body weight) and group D (4 DAB 10 mg/kg body weight) were significantly lowered than the control birds

Tung *et al* (1975), Reddy *et al* (1980) Reddy (1981) and Mohiuddin *et al* (1986) had observed consistent decrease in erythrocyte count in aflatoxicosis of birds In the present study, a significant reduction in the erythrocyte count was observed in the birds of group B which received 15μg/kg body weight of aflatoxin B1 Although this was in agreement with the findings

of the above workers the birds which received the lower dose level of aflatoxin namely 10μg/kg body weight did not register similar lowering of erythrocyte count

In the case of birds which received chemical carcinogen at both dose levels there was a significant reduction in the erythrocyte count

Anaemia has been observed as a clinical feature of aflatoxicosis in ducks and chicken by various workers (Brown and Abrams 1965 and Balachandran and Ramakrishnan 1987) In the present study also anaemia has been observed when the ducks were given aflatoxin B1 at the dose rate of 15μg/kg body weight as evidenced by a consistent increase in erythrocyte sedimentation rate and decrease in packed cell volume haemoglobin and erythrocyte count An interesting feature observed in the present study was that when the birds were given a lower dose of aflatoxin (10μg/kg body weight) anaemia with a significant reduction in the packed cell volume haemoglobin and erythrocyte count was not evident

The chemical carcinogen used in the present study (4 dimethyl amino azo benzene) is a specific hepato carcinogenic agent (Truhaut 1967) The consistent anaemic values at both dose levels of 4 dimethyl amino azo benzene might be thus incriminated to the probable neoplastic alterations and resultant interference with the protein synthesis of the hepatic tissue

The total leucocyte count was found increased in those birds fed with 15µg/kg body weight of aflatoxin B1 and 5 and 10 mg of 4 dimethyl amino azo benzene The leucocytosis in aflatoxicosis has been reported in birds by Wannop (1961) Tung *et al* (1975) and Sova *et al* (1991) Wannop (1961) and Sova *et al* (1991) have recorded consistent lymphopenia in aflatoxicosis This lends support to the observation in the present study The monocytosis as reported by Wannop (1961) and reduction in basophils as reported by Tung *et al* (1975) were not observed in the present investigation Birds of the group B which were fed with 15µg/kg aflatoxin B1/kg body weight showed heterophilia and this concurred with the finding of Wannop (1961) However during the course of investigation neither the control nor the experimental birds showed any clinical signs of bacterial or viral infections

Many workers have identified aflatoxin as an agent which could produce significant increase in blood clotting time in birds (Bassir and Bababunmi 1972 Doerr *et al* 1974 & 1976 Doerr and Hamilton 1981 and Sahoo *et al* 1992) The observation in the present investigation was also in agreement with the finding at the dose level of 15µg of aflatoxin/kg body weight Both the doses of chemical carcinogen also produced comparable changes in blood coagulation However the lowest dose of aflatoxin (10µg kg body weight) was not sufficient enough to bring about any such coagulopathic effect

The attempt to compare the total serum protein values in the birds of different groups revealed a significantly reduced values in all experimentally treated birds Aflatoxin has been shown to be an etiological agent which could bring about a fall in total serum protein level in birds as reported by Reddy *et al* (1982) Chang and Hamilton (1982) Harvey *et al* (1989) Jassar *et al* (1993) and Shukla and Pachauri (1995) In the present investigation the serum albumin as well as serum globulin levels were found decreased at both dose levels Similar decrease have been recorded by Harvey *et al* (1989) and Ghosh *et al* (1990) Though the albumin-globulin ratio was not altered in aflatoxin treated ducks in contrast to the finding of Shukla and Pachauri (1995) who observed a decrease in the ratio the chemical carcinogen produced an increase in the albumin-globulin ratio of both groups The implications of these observations were not investigated thoroughly

Literature of enzymological evaluation of serum of birds in aflatoxicosis was scanty The present investigation revealed a significant increase in serum aspartate amino transferase in the birds treated with 10µg/kg body weight of aflatoxin B1 and 10 mg/kg body weight of 4 dimethyl amino azo benzene Similarly an elevated level of serum alanine amino transferase was recorded in both the aflatoxin treated groups as well as the 4 dimethyl amino azo benzene treated birds receiving 10 mg/kg body weight This was not in agreement with the finding of Reddy *et al* (1982)

They observed a decrease in the serum aspartate amino transferase level in aflatoxin fed birds The present observations were not in agreement with that of Balachandran and Ramakrishnan (1988) wherein they could not detect the enzymes in appreciable amounts The serum enzyme alanine amino transferase is liver specific (Doxey, 1971) and its increased level in the serum indicates tissue destruction in the liver Elevation of serum aspartate amino transferase could also be a result of hepatopathic changes

Further, studies with serum bilirubin revealed a significant increase in all the experimental birds i e birds treated with 10µg and 15µg of aflatoxin B1 and 5 mg and 10 mg /kg body weight of 4 dimethyl amino azo benzene

Although this observation was not in agreement with the findings of aflatoxicosis in layer type cockerels (Shukla and Pachauri, 1995), it strongly indicates hepatopathic changes in all the treated birds

Increased erythrocyte sedimentation rate, decreased packed cell volume, decreased haemoglobin and reduced erythrocyte count indicated anaemia and increased serum enzymes serum bilirubin are the clinical evidences of hepatic damage

The indication of hepatic alterations as suggested by the haematological enzymological and serological evaluations were

further analysed in the present investigation by gross and histopathological studies of the hepatic tissue

Grossly the birds fed with aflatoxin B1 at 10μg/kg body weight revealed mild hepatic lesions On day 60 the liver was slightly enlarged yellowish and glistening with oily out surface On day 120 the shape and size were varying and greenish yellow or glistening nodules were seen discretely distributed in the parenchyma Some livers showed sub capsular haemorrhage

Comparable gross lesions has been reported by Carnaghan (1965) with seven days old Khaki Campbell ducks by feeding with seven ppm of aflatoxin B1 Similar observations has also been reported by Moorthy *et al* (1985) in chicken and Maryamma *et al* (1990) in ducks Varying degrees of haemorrhage in aflatoxin had been recorded by Butler (1964) in ducklings, Ramadevi *et al* (1990) and Bakshi *et al* (1995) in broilers

Hisotpathologically ducks fed with aflatoxin B1 at 10μg/kg body weight on day 60 showed extensive bile duct proliferation mild fatty change coagulative necrosis sinusoidal dilatation mild fibrosis and individualisation of hepatocytes Perivascular lymphocytic infiltration periductular infiltration of inflammatory cells in the form of nodules were recorded On day 120 moderate fatty change Pseudolobulation and cirrhotic changes were evident Butler (1964) conducted experiments in Khaki Campbell ducklings and recorded the sequential

histopathological changes Similarly Carnaghan (1965) reported hepatic lesions in ducks fed with aflatoxin contaminated Brazilian groundnut meal Hyperplasia of bile duct epithelium and infiltration of lymphocytes had been observed by him Bile duct hyperplasia and cytoplasmic vacuolation were described by Muller *et al* (1970) in ducklings fed with aflatoxicosis Radeleff (1970) reported chronic hepatic fibrosis in ducks and ducklings due to sub-acute toxicity of aflatoxin Bile duct proliferation fatty change and hepatitis had been reported in aflatoxicosis in quails (Deshek *et al* 1983) in chicken (Moorthy *et al* 1985 & 1986) and in ducks (Jayakumar *et al* (1988) Similar lesions were also observed by Balachandran and Ramakrishnan (1987) and Ghosh *et al* (1989) in broilers The histopathological lesions in the liver reported in ducks and ducklings by Mukit and Kwatra (1989) and Maryamma *et al* (1990) also lend support to the present observation Focal areas of necrosis and lymphocytic infiltration in experimental aflatoxicosis in broilers had been reported by Bakshi *et al* (1995) also

The present investigation revealed that when the ducks are given 10µg/kg body weight of aflatoxin B1 on alternate days for two months it produced mild degenerative and necrotic changes with infiltration of inflammatory cells predominantly lymphocytes After four months birds of the same group revealed evidences of chronic reaction including fibrous tissue proliferation

The gross changes in the liver of ducks which were given aflatoxin B1 at 15μg/kg body weight were comparatively severe than those of the group A which were given aflatoxin B1 at 10μg/kg body weight Pin head sized necrotic areas, petechial as well as ecchymotic haemorrhages, haemorrhagic streaks and moderate to severe enlargement of the liver were noticed On day 120 along with telengiectasis and atrophy of liver, numerous pin head sized nodules were seen discretely distributed in the parenchyma Nodular lesions in aflatoxicosis in ducks had been reported by Carnaghan (1965) and Maryamma *et al* (1990) The gross lesions in intrahepatic tumours like hepatoma were also reported to be nodular in ducks (Carnaghan 1965)

The histopathological evaluation of liver of ducks fed with 15μg/kg body weight of aflatoxin B1 for day 60 revealed extensive necrosis of hepatocytes, focal hepatitis bile duct hyperplasia, central venous congestion, severe fatty change and mononuclear cell infiltration The same group of birds when sacrificed on day 120 revealed pseudolobulation, hepatitis bile duct hyperplasia and diffuse necrosis Similar hepatic lesions have been reported in aflatoxicosis by Muller *et al* (1970) Radeleff (1970) and Maryamma *et al* (1990)

In addition to the above lesions the ducks which were given aflatoxin B1 at 15μg/kg body weight on day 60 revealed hepatocytes arranged in the form of multiple ill defined acini

On day 120 the liver revealed foci of dysplastic cells While describing the neoplastic liver lesions in ducks Christopher *et al* (1968) described the arrangement of parenchymatous cells in the form of alveoli or acini They also observed the cellular clumps which were varying in size to be covered by delicate fibrous tissue trabeculae The focal distribution of dysplastic cells in the liver specimen on day 120 also suggested neoplastic transformation of hepatic cells in these birds Asplin and Carnaghan (1961) and Carnaghan (1964 & 1965) had documented on the carcinogenic effect of aflatoxin B1 in ducks These authors had observed development of hepatic tumours in birds on feeding aflatoxin at various levels Radeleff (1970) suggested that the dose level and duration of administration were the factors in causing liver tumours In the present study the birds which were given 10μg/kg body weight of aflatoxin B1 did not produce any neoplastic change Whereas those fed with 10μg/kg body weight of aflatoxin B1 showed definite tendency of hepatoma formation during the same experimental period

In the present study 4 dimethyl amino azo benzene was employed as a know chemical carcinogen capable of causing neoplasia in the liver for comparing the cytological transformations with those caused by aflatoxin B1 The ducks which received 5 mg/kg body weight of 4 dimethyl amino azo benzene although exhibited gross and histopathological lesions suggestive of hepatosis and mild cirrhosis failed to initiate any lesions of

neoplastic transformation In contrast the birds of group D which received 10 mg/kg body weight of 4 dimethyl amino azo benzene along with lesions of hepatic degeneration and necrosis clearly exhibited gross as well as histopathological evidences of neoplastic alterations on day 60

A comparative assessment on initiation of hepatoma or hepatocellular carcinoma in ducks by aflatoxin B1 and 4 dimethyl amino azo benzene revealed the following observations Aflatoxm B1 and 4 dimethyl amino azo benzene at lower dose levels (10μg/kg body weight and 5 mg/kg body weight respectively) could produce mild to moderate lesions of degeneration, necrosis and inflammation but could not initiate neoplastic transformation These etiological agents when administered at higher dose levels (aflatoxin B1 10μg/kg body weight and 4 dimethyl amino azo benzene 10 mg/kg body weight) could produce not only the lesions of hepatopathy but also features of neoplastic transformations A noteworthy difference in the initiation of such neoplastic transformation between aflatoxin B1 and 4 dimethyl amino azo benzene was that with the former agent the neoplastic changes were observed on day 120 whereas with the latter agent these were evident on day 60 itself Coulombe (1993) has documented the carcinogenic and mutagenic action of aflatoxin B1 and suggested that these activities of aflatoxin B1 were the result of the affinity of electrophilic and highly reactive aflatoxin B1 8 9 epoxide for cellular nucleophiles such as DNA Further

Eaton Gallagher (1994) concluded that the carcinogenic potency of aflatoxin B1 was highly correlated with the extent of total DNA adducts formed *in vivo* It is clearly understood that the carcinogenic/mutagenic property of aflatoxin B1 was intimately linked with the intensity and duration of its bio-transformation Eaton and Gallagher (1994) also observed that the differences among aflatoxin bio-transformation pathways were the critical determinants underlying aflatoxin B1 induced carcinogenesis

In the present study cytogenetic assay was carried out to assess the toxic as well as carcinogenic effect of aflatoxin B1 and 4 dimethyl amino azo benzene The study revealed that birds which received aflatoxin B1 at the dose rate of 15μg/kg body weight exhibited polyploidy with 24 macro chromosomes as a chromosomal aberration The birds which received 10μg/Kg body weight revealed 14 macrochromosomes The control birds exhibited a chromosome profile with 12 macrochromosomes and the rest were microchromosomes Sharma *et al* (1991) reported aflatoxin B1 induced chromosomal mutations and polyploidy as a constant chromosomal aberration in pre-neoplastic cells In the present work birds exhibiting polyploidy and aneuploidy conditions showed pre neoplastic changes in their hepatocytes This is in line with those reported by Malhi and Grover (1987) and Grinden and Buoen (1989) where in specific tumour condition chromosomal aberrations like pulverisation and multiple fragments were noticed by them In species like ducks

where macro as well as microchromosomes are present the evaluation of microchromosomes for their morphological features is obscure So even in case of any pulverisation had occured it needs very sophisticated technique to differentiate between normal and aberrated micro chromosomes

In the present investigation it was observed that both aflatoxin B1 and 4 dimethyl amino azo benzene at higher dose level initially caused degeneration and necrosis followed by neoplastic alterations at longer durations

Summary

SUMMARY

Employing aflatoxin B1 (AFB1) at the dose rate of 10μg and 15μg/Kg body weight and 4 dimethyl amino azo benzene (4DAB) at the rate of 5 mg and 10 mg/kg body weight an experiment was designed to study the cytological alterations in ducks

Clinically the experimental ducks were healthy and revealed no signs of toxicity

Haematological studies indicated anaemia in ducks which were given AFB1 at the dose rate of 15μg/kg body weight as evidenced by consistent increase in erythrocyte sedimentation rate decrease in packed cell volume haemoglobin and erythrocyte count Consistent anaemic values at both dose levels of 4 DAB were observed Leucocytosis was recorded in the higher dose level of AFB1 and both the dose levels of 4 DAB Changes in blood coagulation time was noticed at higher dose level of AFB1 and both the dose levels of 4 DAB

Total serum protein was found reduced in all the experimentally treated birds In both the dose levels of AFB1 serum albumin and globulin levels were found decreased Albumin-globulin ratio was maintained in AFB1 treated ducks whereas it was increased in the 4 DAB treated birds Serum

aspartate amino transferase level was increased in low dose of AFB1 and higher dose level of 4 DAB Both the AFB1 treated group and higher dose group of 4 DAB showed elevated level of serum alanine amino transferase Serum bilirubin level showed an increase in all the treated birds

Those ducks which were fed with AFB1 at 10μg/kg body weight showed slight enlargement of the liver and glistening with oily cut surface on day 60 On day 120,along with variation in shape and size of the liver small glistening nodules were seen in the parenchyma At the dose level of 15μg/kg body weight of AFB1 pin head sized necrotic areas along with petechial and ecchymotic haemorrhages were noticed on day 60 On day 120 the liver of the birds exhibited telengiectasis patchy greyish areas and atrophy

Histopathologically on day 60 the liver of the birds fed with AFB1 at 10μg/kg body weight showed extensive bile duct proliferation mild fatty changes coagulative necrosis sinusoidal dilatation cloudy swelling of hepatocytes and fibrous tissue proliferation On day 120 cirrhotic changes were noticed

At the dose level of 15μg/kg body weight of AFB1 on day 60 the liver revealed extensive necrosis and irregular arrangement of hepatocytes On day 120 dysplastic cells pseudolobulation of hepatocytes bile duct hyperplasia and diffuse necrosis were noticed

At the dose level of 15μg/kg body weight of AFB1 on day 60 the liver revealed extensive necrosis and irregular arrangement of hepatocytes On day 120, dysplastic cells pseudolobulation of hepatocytes bile duct hyperplasia and diffuse necrosis were noticed

Ducks fed with 4 dimethyl amino azo benzene at the rate of 5 mg/kg body weight on day 60 revealed grossly focal areas of petechiae and yellowish pin head size greyish spots on the liver On day 120 the liver exhibited patchy areas of necrosis with haemorrhagic streaks Those ducks fed with 4 DAB at 10 mg/kg body weight on day 60 showed necrotic patches pin head sized raised areas and areas of niches along the borders On day 120 there were circumscribed nodules embedded in the parenchyma

Histopathologically the liver of the birds fed with 4 DAB at 5 mg/kg body weight on day 60 showed fatty changes, diffuse centrilobular necrosis bile duct proliferation and mild fibrous tissue proliferation On day 120 severe fatty change with necrosis of hepatocytes bile duct proliferation and severe proliferation of fibrous tissue On day 60 as well as on day 120 with the dose level of 10 mg/kg body weight of 4 DAB, diffuse degeneration of hepatocytes dilatation of central vein extensive perivascular necrosis distinct proliferating hepatocytes of varying hyperchromasia of nuclei with distinct cytoplasm were noticed

Chromosome profile revealed that the ducks which received AFB1 at 15μg/kg body weight exhibited polyploidy when compared with that of the controls and 4 DAB treated ducks

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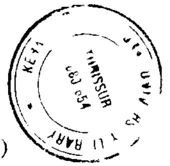

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AFLATOXIN INDUCED CYTOLOGICAL ALTERATIONS IN DUCKS

By
S. VIGIL ANBIAH

ABSTRACT OF A THESIS
Submitted in partial fulfilment of the
requirement for the degree

Master of Veterinary Science
Faculty of Veterinary and Animal Sciences
KERALA AGRICULTURAL UNIVERSITY

Department of Pathology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY - THRISSUR
KERALA
1996

ABSTRACT

An experimental study was conducted in desi ducks to assess the cytological alterations induced by aflatoxin B1 (AFB1) and 4 dimethyl amino azo benzene (4 DAB)

One hundred and eight desi ducks of one month age were selected for the study The birds were divided into two treatment groups which were given AFB1 (10µg/kg body weight and 15µg/kg body weight) and 4 DAB (5 mg and 10 mg/kg body weight) and a control group Each group consisted of thirty six birds Body weight haemogram clotting time total serum protein serum albumin serum globulin albumin-globulin ratio serum aspartate amino transferase serum alanine amino transferase serum bilirubin and cytogenetic assay were estimated at periodic intervals The ducks were subjected to detailed autopsy and histopathology

Clinically the experimental birds were healthy Anaemia leucocytosis and increased blood clotting time were noticed in birds which received 15µg/kg body weight of AFB1 and both the dose levels of 4 DAB Total serum protein was reduced in all the treated birds In the aflatoxin group serum albumin and globulin levels were decreased Albumin globulin ratio was increased in 4 DAB treated birds Serum aspartate amino transferase level was elevated in birds which received 10µg/kg body weight of AFB1 and 10 mg/kg body weight of 4 DAB Birds which received

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AFB1 as well as 10 mg/kg body weight of 4 DAB showed an elevation in serum alanine amino transferase level Serum bilirubin was increased in all the treated birds

Those ducks which were fed with AFB1 at 15μg/kg body weight showed tendency of hepatoma formation during the experiment

The ducks which received 5 mg/kg body weight of 4 DAB exhibited gross and histopathological lesions suggestive of hepatosis and mild cirrhosis The birds given 10 mg/kg body weight of 4 DAB revealed gross as well as histopathological lesions of hepatic degeneration and necrosis and indications of neoplatic changes

Trend towards polyploidy was the major chromosomal aberration observed in ducks which received 15μg/kg body weight of AFB1