PATHOLOGY OF AFLATOXICOSIS IN FINFISH
Oreochromis mossambicus

DISSERTATION SUBMITTED BY

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CERTIFICATE

Certified that the dissertation entitled - " PATHOLOGY OF AFLATOXICOSIS IN FIN FISH *Oreochromis mossambicus* " is a bonafide record of work done by Kumari. ANITHA.P.S, under our guidance at the Central Marine Fisheries Research Institute during the tenure of her M.Fsc (Mariculture) Programme of 1995 - '97 and that it has not previously formed the basis for the award of any other degree, diploma or other similar titles or for any publication.

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I hereby declare that this thesis entitled "PATHOLOGY OF AFLATOXICOSIS IN *Oreochromis mossambicus*" is based on my own research and has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or the similar titles or recognition.

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DEDICATION

I dedicate my dissertation to my beloved parents Mr. T. SANKUNNI, Mrs. LEELAMMA SANKUNNI and to my loving brothers Mr. BABU & Mr. SABURAJ P.S.

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सारांश

ओरियोक्रोमिस मोसाम्बिकस पर अफ्लाटोक्सिन B_1 के प्रभाव से होनेवाली अतिजीवितता, व्यवहार, सकल रोगविज्ञान, ऊतक रोगविज्ञान, रूधिरविज्ञान संबन्धी परिवर्तनों पर अध्ययन करने के लिए 50 ग्रा ओरियोक्रोमिस को भोजन में तीन विविध स्तरों में (।.5, 5, ।5 मि ग्रा / कि ग्रा भोजन) अफलाटोक्सिन B_1 जोडकर दिये थे । इस प्रकार के प्रत्येक उपचार के मछली नमूनों को ।5 वाँ और 30 वाँ दिनों में ऊतक विज्ञान संबन्धी परीक्षणों के लिए परिरक्षित किया गया । परीक्षण के कुछ दिनों में अफ्लाटोक्सिन B_1 (AFB_1) स्तर के बीच मछली की सीधी संबन्ध देखा गया । जिगर में अफ्लाटोक्सिन B_1 का प्रभाव तीव्र था । निम्नलिखित ऊतकीय परिवर्तन जैसे, हिपार्टसाइटस का अपविकास, रक्तधानीभवन और तीव्र ऊतकक्षय के कारण पालिकाओं का नाश देखे गये थे । अफलाटोक्सिन B_1 निम्न मात्र में दी गयी मछलियों के वृक्कों में संकुलता और ग्लोमेरूली का सिकुडन जैसे परिवर्तन भी देखे गये थे । अफलाटोक्सिन की उच्च मात्र दी गयी प्राणियों में वृक्कों का तीव्र विशल्कन और विरलीकरण देखे गये थे । उच्च सान्द्रता में अफ्लाटोक्सिन दी गयी मछलियों में तीव्र ऊतकक्षय के कारण प्लीहा में लसीकाभ कोशिकाओं और रेटिक्यूलो एन्डोथीलियल कोशिकाओं का विरलीकरण देखा गया । इनमें रक्ताणु और श्वेताणु की संख्या भी विचारणीय तौर पर कम थी । उच्च सान्द्रता के अफ्लाटोक्सिन दी गयी मछलियों में श्वेताणु की संख्या बहुत ही कम थी । माध्य विभेदक श्वेताणु की संख्याा सभी उद्भासित स्तरों में उतना विचारणीय नहीं थी । अफ्लाटोक्सिन B_1 (AFB_1) में उद्भासित सभी मछलियों में अरक्तता और श्वेताणुन्यूनता देखी गयी थी ।

PREFACE

1. PREFACE

Aquaculture is a recent and fast growing food production industry. Development of this sector is essential for increasing export earning and improvement of Socio-Economic status of rural people. The fish and shrimp production by capture have not increased per unit effort over a decade. The excessive pressure on the capture sector by the increasing world population forced man to search for another alternative namely aquaculture, which presently contributes substantially to global fish production.

Aquaculture is an important industry in most of the countries, especially in Asia. The attainment of high production from aquaculture depend on availability of well balanced feed. Use of formulated feeds in aquaculture increases both production and profits; considerably. Hence, aquaculture feed industry has grown substantially. More than 60% of the operating cost of aquaculture is estimated to be spent as feed cost and hence the efficient use of such feed is essential. The use of artificially formulated feed has its own risk factors also. It has been estimated that around 10-25% of formulate feed was lost or unsuitable for use for lack of adequate storage facilities, transportation, bagging etc. Thus, the feed industry faced a number of hazards, such as losses occurring due to poor storage. The major problems are weight loss, quality loss and biological contamination. Among this, the major risk arises from contamination of microbial toxins in the feed. The use of toxin contaminated feed leads to a reduction in the growth and production, adversely affecting the stock as a whole.

The microbial toxins of greatest economic importance in animal feed are mycotoxins - metabolites of toxigenic molds. AFLATOXINS are the mycotoxins of greatest concern because of their highly toxic and carcinogenic properties and possible public health hazards.

The toxicity of fungi to a wide range of aquatic animals have been worked out extensively. In general, the effects of toxic compounds on aquatic animals were considered to be direct, indirect or induced. Toxicity may be either acute or chronic. Chronic exposures causes sub-lethal changes in the animals, which may or may not account for death; eventually. Sub-lethal changes can occur from a single encounter or from continuous exposure over a long period.

Pathology is now a part of toxicology. (Couch, 1985). Based on an easily reproducible techniques, histopathological studies yield basic information on tissue disorders related to the general state of the organisms and assess the host's susceptibility to infectious disease and parasitic infestations. Some of these parameters serve as indicators of the effect of Xenobiotics contamination in aquatic animals also. Histological, Cytological and Cytochemical responses observable from animal tissue sections, form an important link between effects at the biochemical level and those measured in the whole organisms. Standard histopathological approaches which are useful in providing an overall picture of the degree of disturbance within the organ system concerned, are thus an integral part of studies on the effects of external stress on organisms. (Lowe, 1988).

In tropical countries, especially in India, where aquaculture is still developing, it is common to observe pelleted feeds that are being produced with inappropriate procedures for bagging, transport and storage. These factors in conjunction with the high temperature and levels of humidity are causes of the presence of fungal growth and potential for aflatoxin production.

The objective of this study are to, determine the effect of dietary aflatoxin B_1 (AFB_1) on

1. Survival and Behaviour,
2. External and histopathological changes and,
3. Haematological changes in *Oreochromis mossambicus*.

INTRODUCTION

1 INTRODUCTION

Fungi make up a large part of the plant kingdom and are ubiquitous in occurrence. They have been known for many years to produce toxic metabolites with powerful effects on micro-organisms and live stock. Fungi produce toxins commonly known as mycotoxins. The disease produced by them are either by contact or ingestion and are called Mycotoxicoses. The best known of this mycotoxins are aflatoxin, which are known to be highly toxic as well as carcinogenic to a wide variety of organism, including some species of fish. (New, 1987).

The toxins produced by mold are broadly classified as nephrotoxins, hepatotoxins, neutrotoxins depending on the hematological effects and general digestive disorders they causes. Aflatoxins which are an unique group of highly unsaturated compounds, low molecular weight, with coumarine nucleus comes under the hepatotoxin and targets its activities mainly on liver. (Borker et al., 1966)

At least a dozen strains of aflatoxin producing aspergilli and one Penicillium are known, but the amount and types of aflatoxin produced found to be variable. (Adye et al., (1964), Austwick et al., (1963), Codner et al., (1963), Hartley et al., (1963), Hodges et al., (1964), Spensley (1963). To date, only three species of fungi have been reported to produce aflatoxins. They are *Aspergillus flavus* (Link),

A.parasiticus and *Penicillium puberculum* (Bainer). Among these *Aspergillus flavus* have been extensively studied.

Relatively little appears to have been published regarding the actual field conditions required for aflatoxin production. The most important environmental parameters in the growth of *Aspergillus flavus* in feed are temperature and moisture. Spensley (1963) reported that the mold will grow at tropic temperatures (30°C) at about 80 to 85% relative humidities and above. Davis and Diener (1969) reported that lower temperature for aflatoxin production was 12°C and highest temperature was 41°C at a relative humidity of 99%.

It was reported that aflatoxin contamination occurred on a number of feed ingredients such as wheat, rye, buck wheat, corn, cotton seed, potatoes, ground nuts and soybeans. (Armbrecht et al ., 1963, Bampton 1963; Burnside et al., 1957; Genest et al., 1963; Richmond et al., 1962 b; Van Der Merwe et al., 1963).

There are eighteen compounds in the aflatoxin family; some of these occur naturally in feed stuffs and feeds, others are metabolites formed in the animal body after ingestion of the contaminated feed (Stoloff, 1976). Aflatoxin is a collective term. There are at least four important types of aflatoxin: B_1, B_2, G_1 and G_2, named according to the fluorescent colour when thin layer chromatographic preparation are viewed under UV light. The intensity of fluorescence is inversely proportional to degree of induced toxicity. (Carnaghan et al., 1963). Among these aflatoxins B_1 (AFB_1), is more potent toxin and commonly produced in *Aspergillus flavus* strains .

The effect of aflatoxin vary considerably from species to species. Common effects on animals are poor growth, liver damage, impaired blood clotting, decreased immune responsiveness and increased mortality (Allcroft, 1963).

The aflatoxin path-way was similar to any other toxin. The aflatoxin ingested through the contaminated food reached and accumulated in the blood and organs. The bioaccumulated mass of the toxin at lethal level leads to death of the individual where as at the sublethal level it leads to immuno-toxicity, genetoxicity, carcionogenicity, teratogenicity and other functional effects. The level of exposure mainly depended on the dose of the toxin, duration, age and size of the animal (Boudou et al., 1983).

The studies on aflatoxicosis in aquatic animals began about 1960, with trout epizootics in the United States. (Ashley and Halver, 1961; Wolf and Jackson, 1963). These investigations were made exclusively on cold water fishes, especially in Salmonids. (Abedi and McKinely, 1968; Halver, 1969).

The toxicity of aflatoxin have been studied in different species of fish such as Salmonids, majorly rainbow trout (*Oncorhynchus mykiss*), Channel catfish (*Ictalurus punctatus*, Common Carp (*Cyprinus carpio*) and Nile tilapia (*Oreochromis niloticus*). (Ashley and Halver, 1961, 1963; Ashley et al., 1964; Halver et al., 1966; Jackson et al., 1968; Abedi and McKinely, 1968; Halver 1969; Ashley, 1970; Sinnhuber and Wales, 1974; Wales, 1979; Svobodova and Piskae, 1980; Hayward, 1986; Jantrarotai et al., 1990; Oliva et al., 1991; McCha'vze et al., 1994).

Hepatotoxicity and carcionogenicity are the major characteristics of aflatoxin. Among the organs, liver has been found to be target organ in poisoning due to aflatoxin. (Ashey and Halver, 1963; Burtler et al. 1964; Wellings, 1969; Ashey, 1970). Acute hepatocellular necrosis appears to result from the interaction of aflatoxin at a number of intracellular sites (Simon et al., 1967).

Sinnhuber et al., (1968); Rogers and Newbern (1969); Ghittino (1976) have reported necrosis, fibrosis and ductular proliferations in advanced tumors induced by aflatoxin B_1 in rainbow trout (*Oncorhynchus mykiss*).

Halver (1969) reported the liver neoplasms, necrosis of hepatocytes and degenerative changes in the pancreatic tissue in rainbow trout due to the prolonged feeding of aflatoxin at a level of 0.4 mg/kg in the diet. Many of the effects of the aflatoxin related to their reaction with nuclear proteins so that they interfere with protein formation and maintenance of cellular integrity. (Patterson, 1976).

When relatively high levels of aflatoxin B_1 are administrated to fish, the result was rapid, acute liver necrosis. Acute aflatoxicosis resulted in Coho Salmon and rainbow trout by aflatoxin contamination in the feed. (Halver et al., 1969; Doster et al., 1972) respectively.

Bauer et al., (1969) found the intraperitoneal LD_{50} dose of aflatoxin B_1 (AFB_1) in rainbow trout was 0.81 mg/kg body weight. Among the aquatic animals, especially in fishes, rainbow trout shows the extreme sensitivity to aflatoxin B_1 (AFB_1).(Sinnhuber et al., 1969; Halver 1969; Ashley, 1970; Lee et al., 1971). Signs of severe aflatoxicosis in rainbow trout are liver damage, pale gills and reduced erythrocyte concentration. (Ashley, 1970).

The carcinogenic properties of aflatoxin depended upon metabolic activation to DNA alkylating agent, presumably the 2,3 epoxide, now generally accepted as the bacterial mutagen and proximal carcinogen. (Patterson, 1976). The carcinogenicity of aflotoxin was well documented in rainbow trout. A number of workers reported incidents of hepatoma in rainbow trout by the dietary contamination of aflatoxin at a low level for a long duration. Trout hepatoma was reported by Nigrelli and Jakowska (1961); Wood and Larson (1961); Ghittino (1967); Rucker et al., (1961); Simon et al., (1967); Wales (1967), (1970); Codegone et al., (1968) and Ashley (1965),(1970).

Sinnhuber and Wales (1969) reported high incidence of hepatic carcinomas in rainbow trout hatching out of eggs when they were immersed in aflatoxin B_1 (AFB_1) solution of 0.5 ppm.

Bauer et al., (1969) reported that the gross pathological signs of aflatoxin B_1 in rainbow trout are weakness, darkening in colour, internal multiple haemorrhages in

fat bodies and intestinal walls. The livers were pale yellow to white and histologically they displayed severe necrosis.

Channel catfish showed lower sensitivity to aflatoxin B_1 (AFB_1) than rainbow trout. (Halver, 1969). However, acute hepatotoxicity in channel catfish were reported recently. The liver was damaged and vital organs like intestine, stomach, kidney, and spleen were also affected. Subacute toxicity caused depression in growth, anemia and liver necrosis in Channel catfish (Jantrarotai et al., 1990a,b).

In tilapia culture a wide range of neoplasms has been observed with high aflatoxin levels. (Halver, 1969; Roberts, 1989).

The effects of dietary aflatoxin in Nile tilapia *Oreochromis niloticus* was reported by Ma.C Cha'vez, et al., (1994). The toxic effect was fatty degeneration, necrosis and fibroblast in the liver. Growth depression was also observed at higher dietary aflatoxin level. Generally, tilapia seems to be less sensitive to aflatoxin B_1 than Salmonids.

Effects of prolonged exposure to subacute toxic levels of dietary aflatoxin in tilapia *Oreochromis mossambicus*, however has not been studied. In view of this, the present study was taken up to findout the histopathological, and haematological changes caused by aflatoxin in the vital organs such as liver, kidney and spleen of tilapia *Oreochromis mossambicus*.

MATERIALS & METHODS

III MATERIALS AND METHODS

3.1 Experimental Protocol

Healthy *Oreochromis mossambicus* with mean body weight of 50-60 grm caught from CIBA, Narakkal, were used in this study. About 60 fishes were maintained in a set of plastic tubs. Fifteen, 50 l plastic tubs each equipped with an air supply were stocked with six tilapia. The fishes caught from brackish water were gradually acclimatized to fresh water and fed with a formulated pelleted feed (a) 2% of body weight twice daily. Triplicates were maintained for each treatment (Plate I).

Animals were acclimatized to accept feed for two weeks before being fed the experimental diets for 30 days. Prior to start of feeding experiments, the animals were starved for twenty four hours. After segregation into group of uniform size, the initial body weight of the animal were noted. Seventy five (75%) per cent of water was changed daily. Daily survival was noted and feeding was regulated based on it. Care was taken during feeding to provide only a small amount of diet at a time to ensure that the fish consumed the diet offered. During the two week acclimatization period of the fishes were observed daily for unusual behaviour, morphological changes and mortality.

Water quality parameters were also checked on alternate days. The mean temperature during the trial was 27±1°C. Prior to starting the feeding experiments a

set of animals were sacrificed and analysed for their normal hiystological and haematological conditions.

3.2. Preparation of Diets

Three test diets and 1 control diet were prepared.

3.2.1 Composition of the diet (Table I)

The diet ingredient composition was similar to the used commercially except that GNOC was substituted by coconut oil cake to avoid any other source of AFB_1 in the diet.

Ingredients	Level of incorporation (gm/100 gm)
Fish meal	35
Soybean flour	25
Coconut oil cake	10
Vitamin mix*	2
Mineral mix**	1
Lipid	4
Tapioca flour	20
Geleatin (binder)	3

* contain Vitamin B_1 - 10 mg.
B_2 - 10 mg.
B_3 - 3 mg.
Nicotinninamide - 100 mg.
Calcium pantothenate - 50 mg.
Folic acid - 1500 mcg.
Vitamin B_{12} - 15 mcg
Vitamin C - 150 mg.

** OSSOPAN Granules. TTK PHARMA LIMITED

All the dry ingredients except tapioca flour were thoroughly mixed. Tapioca flour was gelatinized, cooled and added to the dry mixture to make a dough of uniform consistency. The dough so made was extruded using a kitchen noodle maker with a dye of 2 mm pore size. The feed strands were oven dried, crumbled and stored in air tight containers and used.

3.2.2. Preparation of experimental diet

A single mix of the ingredient was divided into four 1000 gm fractions, each of which received a specified dose of aflatoxin B_1 (AFB_1) from a stock solution containing 100 µg/ml. To the basal diet respectively 0, 1500, 5000 and 15000 µg of aflatoxin was incorporated.

3.2.3. Preparation of stock solution

The aflatoxin B_1 (Sigma Chemicals Company, St. Louis, Missouri) was dissolved in Chloroform and diluted to 200 ml in a volumetric flask to yield a concentration of 0.1 mg aflatoxin B_1 (AFB_1)/ml of Chloroform. Thus the following stock solutions were prepared, 1.5 ml, 5 ml and 15 ml.

3.2.4. Incorporation of Aflatoxin into the diet

The required amount of aflatoxin B_1 (AFB_1) measured in a measuring cylinder which was dissolved in to the oil portion of the diet ingredients. Then the Chloroform

was evaporated, so as to prepare the dose levels equivalent to 0, 1.5, 5 and 15 mg/kg diet.

4. Sampling.

During the experiment two samples from each treatment were two weeks intervals. The fish were weighed and examined for signs of aflatoxicosis. At the end of the experiment, the number and weight of fish in each treatment were determined.

5.1 Histological and Haematological Studies

Blood was collected from the caudal vein using heparinised 20G needles and 2 ml sterilized heparinised (0.1 ml of 25,000 $1\mu m^{-1}$ Heparin) plastic syringes, from each treatment for haematological studies. Subsequently the fish were killed by pithing and examined internally for signs of aflatoxicosis. The liver, kidney and spleen were dissected and fixed in 10% buffered formalin for twenty four hours. The tissues were dehydrated in ascending grades of alcohol and cleared in chloroform and embedded in paraffin wax. Sections of $6\mu m$ thickness were cut using a rotatory microtome. The sections were stained with Haematoxylin and Eosin (H & E) (Drury and Wellington, 1980). The slides were examined under the microscope and photomicrographs taken.

5.2. Determination of the number of Red Blood Corpuscles

The blood was drawn in to a R.B.C. counting pipette, up to the 0.5 mark and the dilution - solution, Hayem's dilution medium (Sublimate-0.5, Sodium Sulphate .5, Sodium Chloride - 1 and distilled water 200 ml) was drawn up to the 101 mark. Blood

mixing in the pipette was carried out gently swinging the hand for 2-3 minutes. After thorough mixing, introduced into a hemocytometer and counted under a microscope (Objective X 40). Erythrocytes were counted in 25 squares.

5.3. Determination of the number of white blood corpuscles.

The leucocyte (LC) was obtained by the method of SHAW (Shaperclaus, 1986).

Blood was drawn up to the 0.5 mark of a R.B.C counting pipette and the dilution mixture of crystal violet and neutralred was drawn in to the pipette up to the 101 mark. The pipette was gently revolved for 2-3 minutes and the blood was introduced in to a hemocytometer and counted under microscope (Objective X 40). Leucocytes were counted in 4 large squares.

5.4. Preparation of Blood smear for differential counting.

Blood smears were prepared on clean grease free and sterilized glass slides for differential leucocyte counting.

5.4.1. Staining Procedure

The staining procedure adopted for cytological study was from Pappenheim [combined May-Grunwald staining; Schaperclaus, 1986] for obtaining a differential count of the leucocytes.

Air - dried smears on glass slides were fixed with methanol, keep for one minute. It was stained first with undiluted, filtered May-Grunwalds Giemsa stain (Merck chemicals) for three minutes. Then the slides were covered by de-ionized glass double distilled water, mixed carefully and kept for one minute. After decanting the stain-double distilled water mixture, the slides were stained with filtered diluted Giemsa- stain (Merck chemicals). Ten drops in 10 ml double distilled water for 30-45 minutes. The slides were then thoroughly washed with de-ionized double distilled water, air dried and mounted in DPX (Ranbaxy Chemicals). The mounted slides were observed under the microscope (Objective X 100 with oil immersion objective) and photomicrographs taken.

RESULTS

RESULTS

1. Behaviour and External Changes

During the thirty days in which the experimental diets were administrated in feed, consumption clearly diminished being more drastic during the first eight days with diets containing 5 and 15 mg aflatoxin B_1/kg of diet respectivcly. Fish refused to eat the aflatoxin B_1(AFB_1) containing diet while they accept the basal diet. The fish were on lowest concentration of aflatoxin B_1 (AFB_1) accepted the feed though at a reduced rate. At the end of the experiment, control fishes showed some fin and tail-rot conditions. No other external and behaviour changes were observed during the experimental period.

2. Gross Pathological Signs

The livers of some fish fed the highest concentration of aflatoxin B_1 showed focal congestion and haemorrhages. The liver was pale yellow in colour in group II and group IV animals. These animals received the doses of 1.5 and 15 mg aflatoxin B_1 (AFB_1)/kg of diets respectively (Plate II). The kidney and spleen appeared not to have developed any abnormalities. Most of the time the intestine remained empty.

3. Histopathological Studies

Vital organs such as liver , kidney and spleen from four groups of fishes, sacrificed on 15th and 30th days after exposure were subjected to detailed histopathological studies. Salient histopathological observations were described for each organ.

a. Liver

Group I

Liver of group I which formed the control did not reveal any major alterations (Plate III). Normal architecture of parenchyma was observed. The hepatocytes were polyhedral in shape having a central vasicular nucleus. The hepatocytes formed irregular cords which were separated by sinusoids lined with endothelial cells.

Group II

The liver tissue after 15 days exposure revealed vacuolation and hepatorrhexis. The samples collected on 30th days showed necrosis of hepatocytes and loss of architecture of parenchymal cells in the liver (Plate IV). The destruction of pancreatic cells and complete necrosis were observed in the liver sections.

Group III

In this group, hepatic cells had undergone similar vacuolar changes by 15th day as in group I. The liver sections observed after 30 days showed severe necrosis in hepatocytes, vacuolization and focal regeneration of hepatic cells (Plate V).

Group IV

The liver sections showed complete and total necrosis, loss of architecture of lobules, destruction of pancreatic cells. The sections taken after 30 days revealed acute degenerative changes of hepatic cells with extensive area of necrosis (Plate VI).

b. Kidney

Group I

The kidneys were composed of excretory, haemopoietic and reticulo endothelial tissues. The nephrons consisted of a well vascularised glomeruli which were congested. The glomeruli were surrounded by Bowman's capsules which were lined by squamous epithelial cells. The Bowman's capsule continued through a ciliated neck. Two proximal segments, one with basal striations which were separated by a ciliated segment were seen. In addition to these tubules there were distal segments to the collecting ducts. The proximal ducts were more eosinophilic in staining and the proximal segment of the tubule were lined by low columnar epithelium with indistinctive borders. Interstitial space was occupied by actively dividing haemopoietic tissue and elements of adrenal tissues. Numerous melano-macrophage centres were also seen.

In this group, the architecture of the kidney did not show any major changes except congestion of the glomerular capillaries and occasional degenerative changes in tubules (Plate VII).

Group II

The samples collected on 15th day revealed no change in glomeruli from that of control. The architecture of the kidney didn't show any major changes. Some focal area of parenchymal necrosis were observed. In the 30th day samples some severe changes were observed (Plate VIII).

Group III

In this group, the kidney sections showed not much change, but desquamation of kidney tubular cells were observed in some areas. Tubular epithelia were desquamied after 30 days treatment (Plate IX).

Group IV

In this group, extensive tubular necrosis, mesenchymal cell proliferation were seen on 15th day of exposure. Complete destruction of tubular cells and glomeruli resulted. Desquamation of cells and complete loss of architecture of the kidney tubules were observed after 30 days of exposure (Plate X).

C. Spleen

The spleenic capsule was fibrous and devoid of muscle. The spleen were composed of ellipsoids, the pulp and the melanomacrophage centres (MMC).

Ellipsoids was thick walled filter capillaries which resulted from the division of spleenic arterioles. Each comprised a thick basement membrane-bound tube with in which the vessel runs, separated compartments. The spleenic pulp contained sinusoildal phagocytic tissue similar to that of the kidney, in which large numbers of erythrocyte may be held and haemopoietic tissue supported argyrophilic fibres.

In group I, sample sections showed normal architecture of spleen (Plate XI).

Group II

The spleen didn't show much changes in this group of animals. But vacant areas were observed in the spleen section on 30 days treatment. Rarefaction of the parenchymal cells were also observed. A number of cells were reptured. The ellipsoids didn't contain much normal nuclear cells. (Plate XII)

Group III

In this group, spleen showed not much changes. But rarefaction of lymphoid tissue and reticulo-endothelial cells were observed in samples from specimen on subjected to 30 days exposure (Place XIII)

Group IV

The spleen samples from this group of animals showed severe necrosis after the 30th day toxin exposure. Rarefaction of lymphoid cells and loss of architecture were also observed (Plate XIV).

IV. Haematological Studies

Changes in erythrocyte, leucocyte and differential leucocyte counts were examined. Blood samples were taken on 15th and 30th day after the exposure to aflatoxin B_1(AFB_1) and were subjected to detailed haematological studies. The values in erythrocyte and leucocytes in different groups was tabulated in (Table II).

Group I:

The group I fishes were fed with control diet. This group of fishes revealed a total erythrocyte count varying between 2.86 million cells/mm^3 and 2.91 milli/mm^3 with a mean of 2.86 million cells/mm^3 . The total leucocyte counts varied between 14,000 an 13,000 with a mean of 13500 cells/mm^3

Group II

This group of fishes revealed a total erythrocyte counts ranging from 3.01 million/mm^3 to 1.80 million/mm^3 with a mean of 2.41 million/mm^3, where as the leucocyte counts ranged from 12000 to 7000 with a mean of 9500 cells/mm^3.

Group IV

The fishes from this group revealed a total erythrocyte counts varying between 2.73 and 1.92 million/ mm^3 with a mean of 2.33 million/ mm^3 . The leucocyte counts ranged from 97000 to 8000 with a mean of 8875 cells/ mm^3.

Group IV

This group of fishes revealed a total erythrocyte counts varying between 3.12 and 2.03 million/mm^3 with a mean of 2.58 million/ mm^3. The total leucocyte counts ranged from 11000 to 6000 with a mean of 8500 cells/ mm^3.

The difference in erythrocyte, leucocyte counts at different levels of aflatoxin exposure at different intervals were observed and it was represented in histograms - I and II respectively.

The differential leucocyte counts of blood cells showed the normal composition of leucocytes. The major components of leucocytes was lymphocytes (60-70%), neutrophils (6-8%). Monocyte (0.1%) and Basophils (1-3%). The lymphocytes 60-70% lymphocytes counts were higher in all the experimental groups including control fish.

The composition of differential leucocytes count didn't show much different in all the aflatoxin B_1 exposure groups.

The composition of white blood corpuscles was shown in (Table III).

DISCUSSION

DISCUSSION

The study involved changes in the morphological, behavioural, haematological and histopathological alteration in fishes at different concentration of aflatoxin $B_1(AFB_1)$ exposure for a period of 30 days.

All the experimental fish appeared normal and healthy after being fed the experimental diets. This condition has been reported by different authors in different fishes. Wood and Larson (1961) in rainbow trout; Jantrarotai et al., (1990) in channel cat fish and MaC.Cha'vez et al., (1994) in Nile tilapia. During the present study, it was observed that the fishes were showed some abnormal behaviour by not accepting the feed with aflatoxin $B_1(AFB_1)$ component during the first few days. After that they showed normal behaviour.

Ma C.Cha'vez et al., (1994) noticed in Nile tilapia, there was poor intake of feed when they were fed at different levels of aflatoxin $B_1(AFB_1)$. Poor intake of feed was also reported from shrimp *Panaeus vannamei* (Wiseman et al., 1982) and Channel cat fish, *Ictalurus punctatus* (Jantrarotai et al., 1990). In the present instance also poor intake of feed at higher levels of aflatoxin B_1 (AFB_1) was noticed. These observations are in agreement with the results obtained by the authors loc. site.

Highmortality was observed in different species of fish including tilapia by a number of authors. (Abedi and Mckinely (1968), Halver et al., (1969), Jantrarotai et

(AFB_1). The same kind of result were obtained by Ashley (1965) in rainbow trout which were fed with high levels of crude aflatoxin.

The precancerous lesions and ductular proliferations was reported by many authors, but were not observed in our study though severe necrotic changes occurred in all the concentrations of aflatoxins B_1 (AFB_1) exposure. The hyperaemia due to aflatoxin B_1 in diets was reported by Ashley (1965), Halver (1969) and Ashley (1970). This was not observed in the present study.

Aflatoxin B_1 (AFB_1) in rainbow trout caused hypertrophy of pancreatic acinar cells and later desquamation (Ashley , 1965). However, in the present study it was observed that the aflatoxin B_1 (AFB_1) completely destroyed the pancreatic acinar cells.

In the control group, the structure of kidney was very similar to euryhaline fish which was described by Elli's et al., (1978). Occasionally some tubules showed degenerative changes and many glomeruli appeared congested. Since the changes were mild in nature, they were considered not very significant.

The pathological alterations in kidney tissue sections was reported by a number of authors. Ashley (1965) reported haematopoietic kidney with focal hyperemia and mild hyperplasia of adrenal cortical tissue. The excretory kidney had increased cellularity of glomeruli with occasional epithelial crescents. A few shruken glomeruli surrounded by widened Bowman's spaces were also present. Jantrarotai et al., (1990) reported that sinusoid in the kidney of profile in channel cat fish. Ma C. Cha'vez et

al., (1990)). However in the present study the mortality rate of control fish was higher hence mortality would not be linked to aflatoxin exposure.

Histopathological studies of group I, didn't show any major alterations. The structure of liver was very similar to that described for normal fish liver by Varichak (1938), Ferguson (1974), Hinton and Pool (1976) and Elli's et al., (1978).

In group II, III and IV the liver samples revealed vacuolation of hepatic cells from the 15th day on wards. This vacuolation was present in all the exposure groups through out the experimental period and increased in severity in proportion to the time of exposure and dose. (Halver et al., (1966), Ashley, (1965 & '70), Halver, (1976).

Simon et al., (1967), Simnhuber et al., (1968), Rogers and New berne, (1969), Ashley, (1965 & '70), Sato et al., (1973) and Ghittino (1976), have reported necrosis, fibrosis and ductular proliferations in advanced tumors induced by aflatoxin. Jantrarotai et al., (1990) and Ma C Cha'vez et al., (1994) have also reported foci of necrotic hepatocytes mixed with basophilic hepatocytes in channel cat fish and Nile tilapia respectively. Necrosis of hepatocytes was a common finding in many of the studies involving all toxic conditions (Bhattacharya et al., (1985), Jambulingam, (1988)). In this study, necrosis of hepatocytes were observed in all the experimental groups except control. These changes became more apparent and severe as the concentration and period of exposure increased. Severe and massive necrotic changes were observed in the liver of fishes exposed to all the concentrations of aflatoxin B1

al., (1994) reported congestion and shruken glomeruli of kidney in Nile tilapia. The same result was observed in experimental groups I and II after 15th day aflatoxin B_1 (AFB_1) exposure. But later on severe necrosis and loss of cell architecture was observed in higher doses of aflatoxin B1 exposure for a long duration.

Ashley (1965) reported the pathology of spleen at higher exposure of aflatoxin B_1(AFB_1). In his study, he has reported that hyperaemia was severe and reticular proliferations composed of more or less spindled reticulum cells. In the present study, the changes in the spleen of the aflatoxin B_1 (AFB_1) exposed fishes were not far different from those of the control. But vacant areas and rarefaction of lymphoid and reticulo-endothelial cells were observed. It indicated that, there might be an impairment or suppression of immune system due to the structural abnormality of the spleen.

For haematological studies, changes in erythrocyte counts, leucocyte counts and differential leucocyte counts were examined. Marked reduction in erythrocyte and leucocyte counts in all treated groups was observed when compared to those of control fish. Jantrarotai et al., (1990) observed leucocytes counts in aflatoxin B_1 (AFB_1) fed fishes increase considerably. But in the present study, it was noticed that initially there was rise in leucocytes counts and as exposure time advanced leucocytes counts decreased considerably. This would have considerably effect on the animals resistance against infectious disease.

The anemia appears to be primarily haemolytic because haematocrit haemaglobin concentration and erythrocyte counts dropped. However, the necrosis of haemapoietic tissues observed in some organs might also account for part of the anemia. Severe anemia due to the exposure of aflatoxin B_1 (AFB_1) was reported by a number of authors. (Hoerr and O' Andrea (1983); Plumb et al., (1986) and Jantrarotai et al., (1990)). Jantrarotai et al., (1990) reported severe anemia in channel catfish due to acute aflatoxicosis. In the present study, severe anemia was not observed. But some anemic conditions were exhibited by the animals exposed to higher concentration of aflatoxin B_1 (AFB_1). The erythrocytes were reduced in treated groups compared to control. This mild anemia might have resulted from the necrosis of the haemopoietic organs or from less consumption of feed.

Differential leucocyte counts did not vary between groups. There was no published reports regarding the effect of aflatoxin B_1 (AFB_1) on differential leucocyte counts of blood cells of fishes. Therefore, it may not be possible to draw conclusions based on these observations made during the limited period of this study. Hence more detailed work on this subject is needed.

SUMMARY

SUMMARY

1. Tilapia *(Oreochromis mossambicus)* were collected from CIBA, Narakkal were used for determning survival, behaviour, gross pathological, histopathological and haematological studies.

2. The fishes from all the groups showed normal behaviour. A clear reduction in feed consumption observed was directly related to the aflatoxin B_1 (AFB_1) level during the first few days of the experiment.

3. Gross pathological changes in liver revealed focal congestion and haemorrhages in fish which received higher concentration of aflatoxin B_1 exposure. Pale yellow colouration in liver was also observed in those fishes.

4. Histopathological studies were carried out on liver, kidney and spleen.

5. The liver in all treatment groups revealed extensive vacuolation and necrosis. Focal regeneration of hepatic cells were also observed in some fishes which received diet contained the lowest concentration of aflatoxin B_1 (AFB_1).

6. The kidneys exhibited remarkable changes in glomeruli and in tubules. The tubules generally showed degenerative changes leading to severe necrosis and desquamation of cells.

7 The spleen from the group of fishes exposed with aflatoxin B_1 (AFB_1) did not show much damages. The section of spleen showed vacant areas and rarefaction of parenchymal cells, where as in the fishes exposed to the highest dose revealed rarefaction of lymphoid tissue and reticuloendothelial cells leading to severe necrosis.

8. Haematological studies were carried out on erythrocyte counts, leucocytes counts and differential leucocyte counts. The fishes from all the groups showed anemia and leucopenia. Changes in differential leucocyte counts in all the treated groups was not significant.

GRAPHS & TABLES

Fish	AFB1 doses (mg/kg diet)	RBC(million/mm^3) Sampling on		WBC (1000/mm^3) Sampling on		RBC million/mm^3 (mean)	WBC 1000/mm^3 (mean)
		15th day	30th day	15th day	30th day		
Control	0	2.91	2.86	14,000	13,000	2.86	13,500
GroupI	1.5	3.01	1.80	12,000	7,000	2.41	9,500
GroupII	5	2.73	1.92	9,750	8,000	2.33	8,875
GroupIII	15	3.12	2.03	11,000	6,000	2.58	8,500

Table II : Counts of *Erythrocytes* and leucocytes at different *levels of* AFB_1 exposure at different *intervals*.

Fish	Doses of AFB_1 (mg/kg of diet)	Leucocytes in percentage				
		Lymphocyte	Monocyte	Neutrophil	Basophil	Esinophil
Control	0	64	6	25	5	0
GroupI	1.5	77	3	16	4	0
GroupII	5	65	11	16	8	0
GroupIII	15	70	1	29	0	0

Table III : The composition of white blood corpuscles .

Chart I

Total erythrocyte count at different levels of exposure of AFB_1 at different intervals

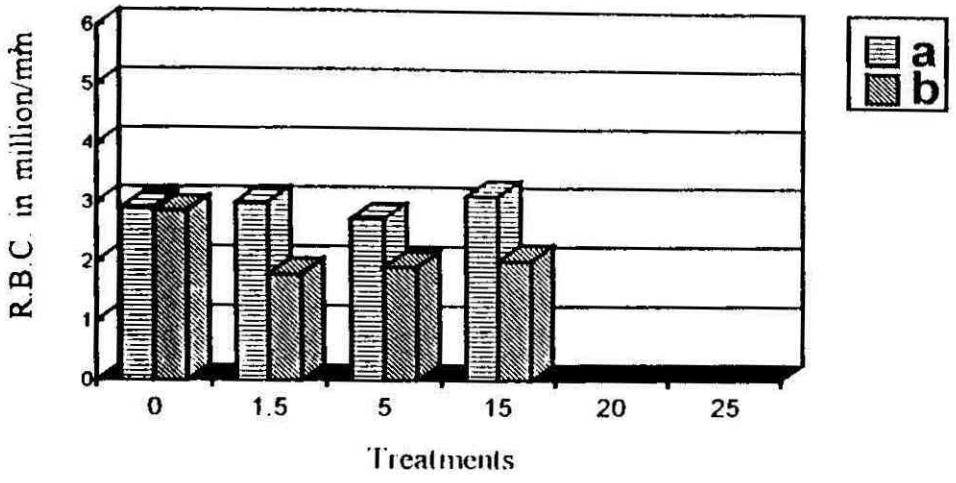
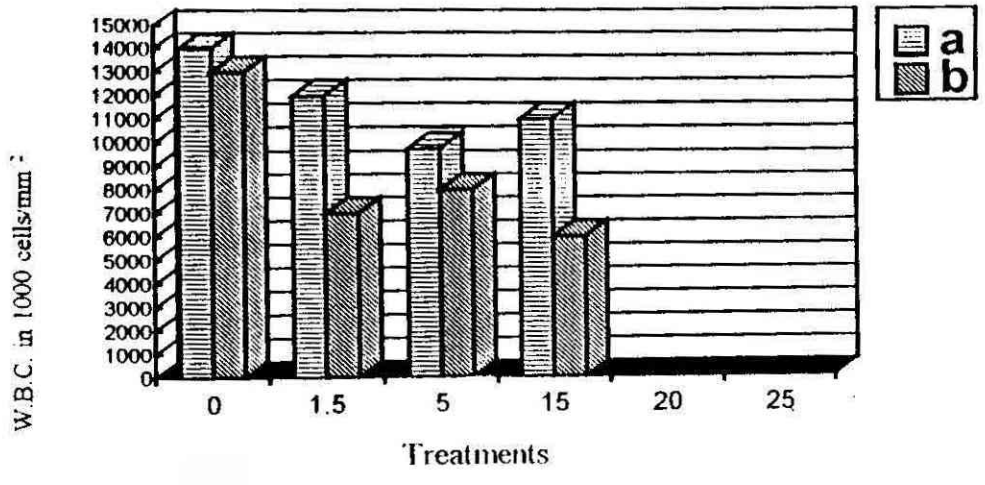


Chart II

Total leucocyte count at different levels of exposure of aflotoxin at different intervals



PLATES

PLATE : I

Experimental Set-up

PLATE : II

Gross pathology of liver of normal fish

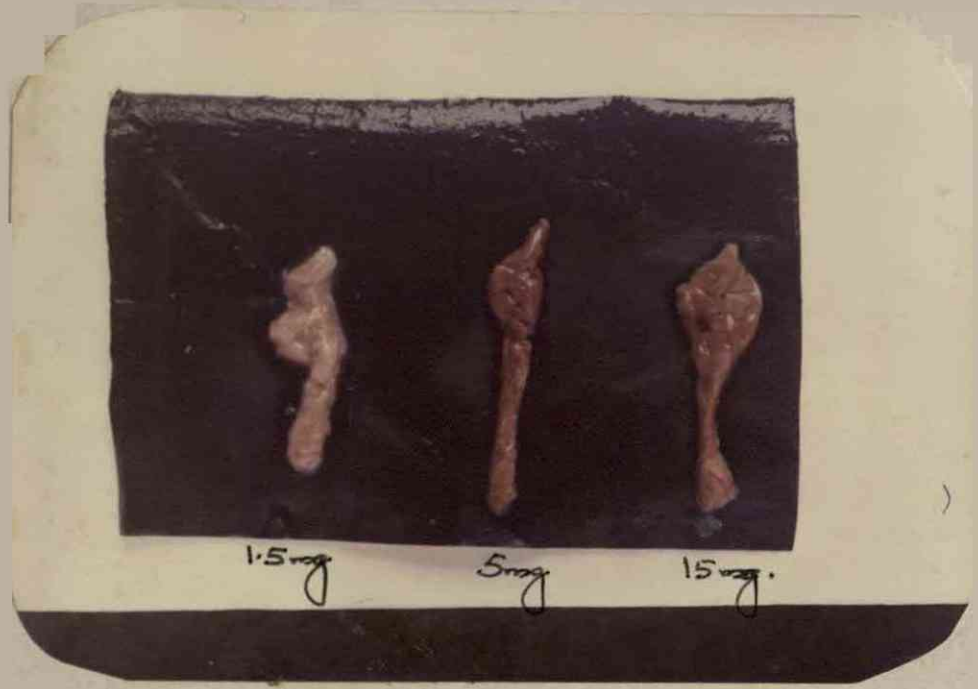


PLATE : III

Liver

Gross pathology of aflatoxin B_1 (AFB_1) exposed groups

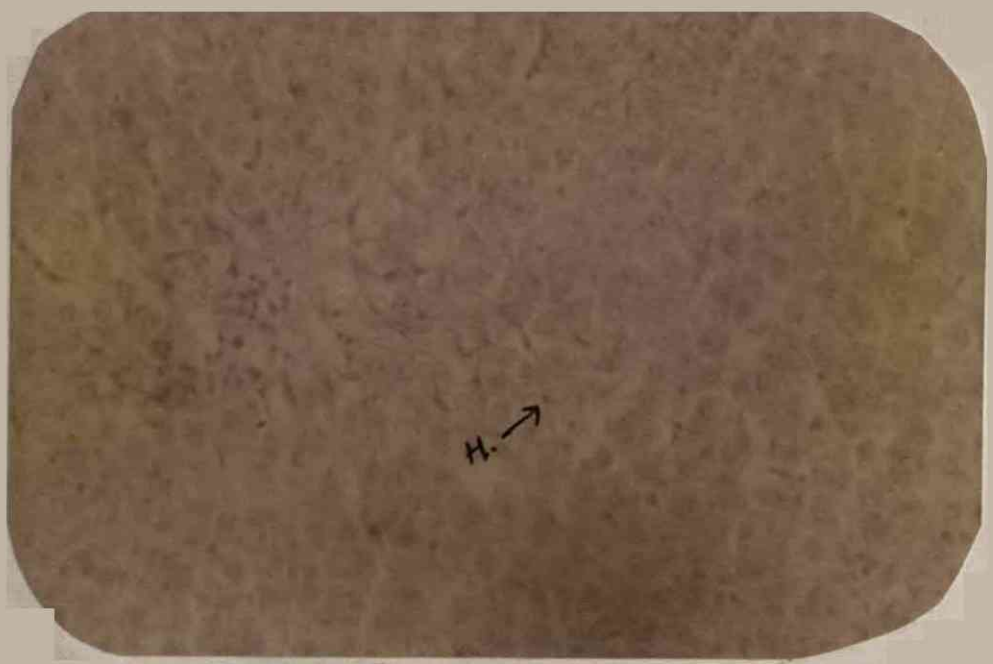


PLATE : IV

Section of liver showing normal hepatocytes (×200)

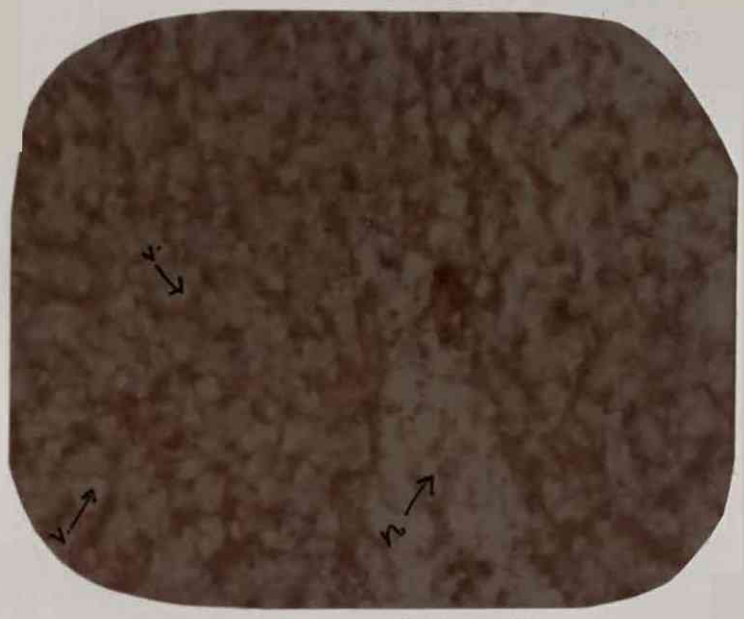


PLATE : V

Section of liver showing vacuolation and necrosis of hepatocytes
(x200)

PLATE : VI

Section of liver showing necrosis of hepatocytes

(x200)

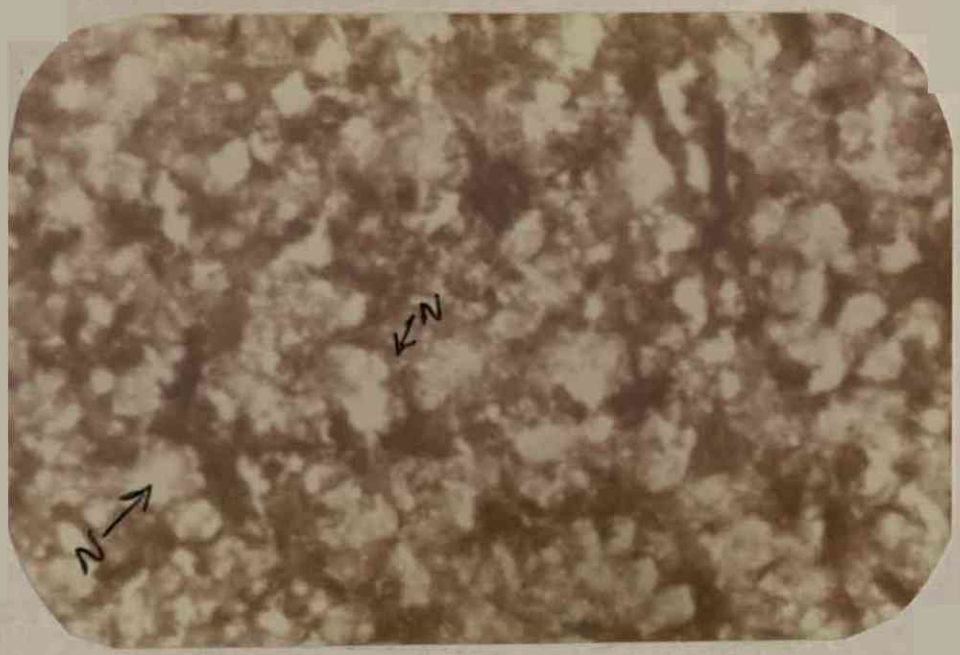


PLATE : VII

Section of liver showing severe necrosis and loss of architecture

(x400)

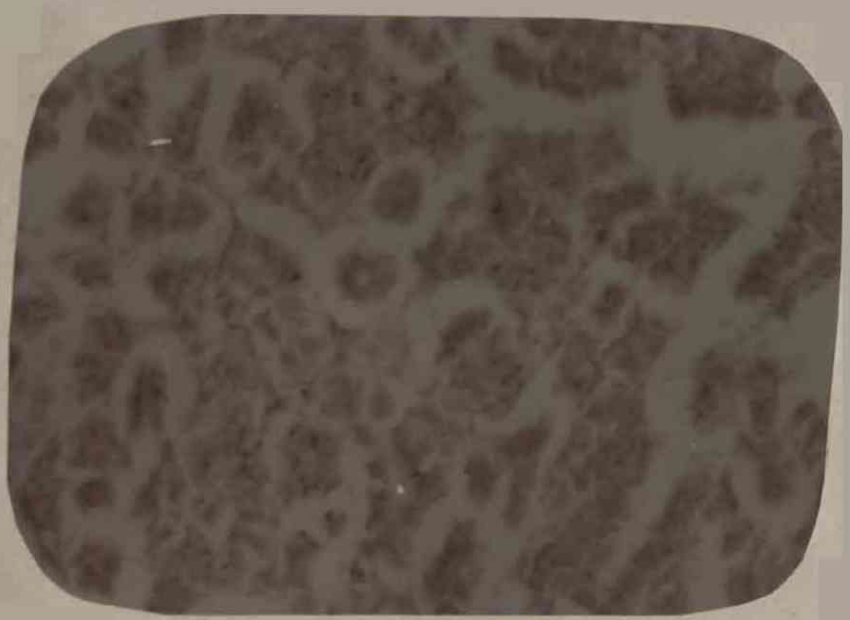


PLATE : VIII

Section of the kidney showing normal architecture (×200)

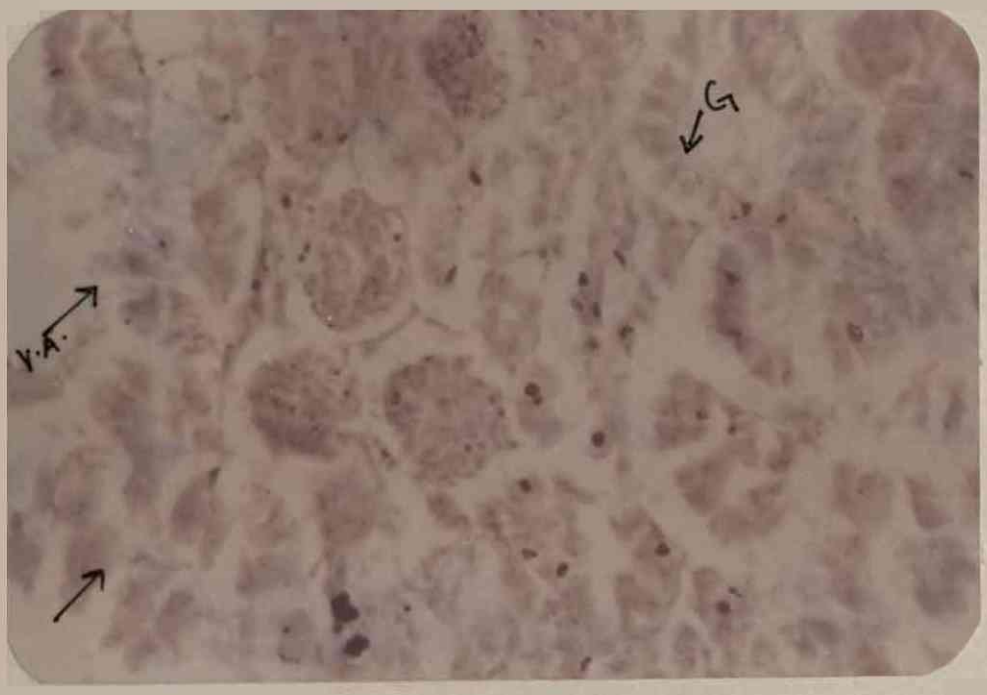


PLATE : IX

Section of the kidney showing congestion of glomeruli and occasional degeneration of kidney tubules (x200)

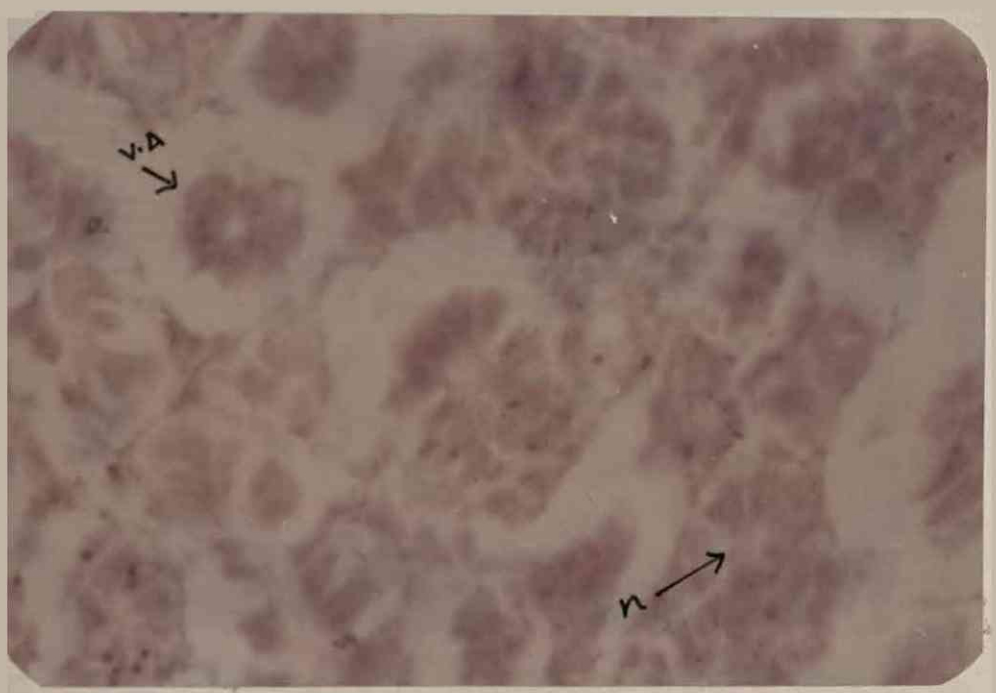


PLATE : X

Section of the kidney showing necrosis and vacant areas (x 200)

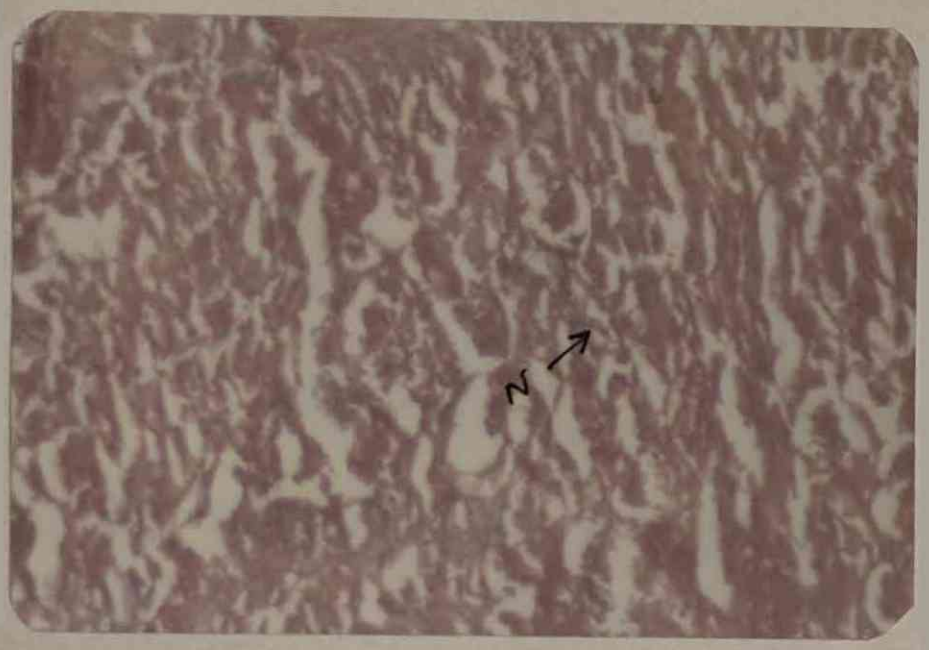


PLATE : XI

Section of the kidney showing desquamation of tubular epithelia (×100)

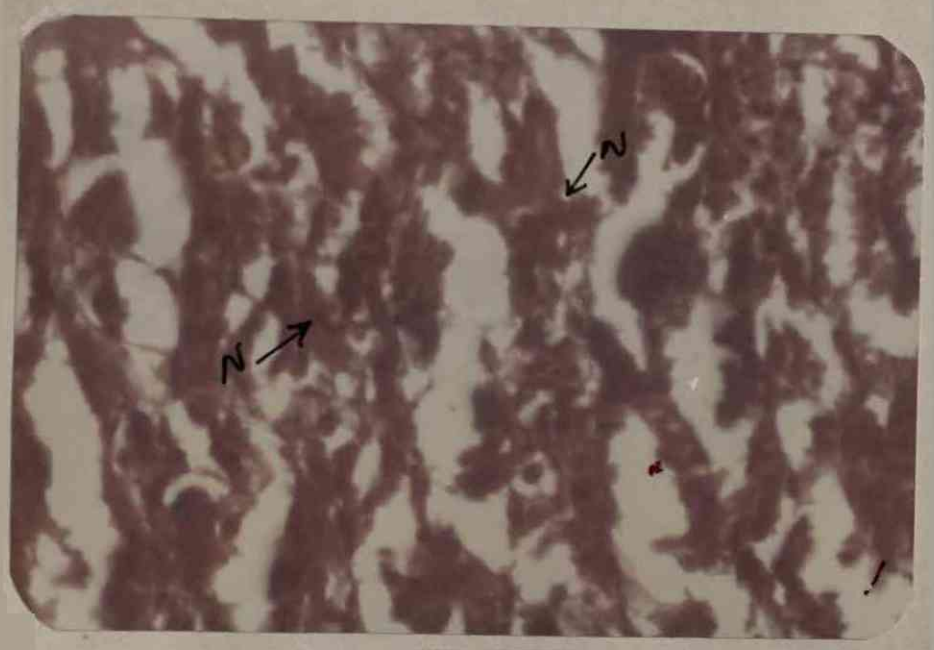


PLATE : XII

Sections of the kidney showing extensive tubular necrosis and complete loss of architecture (×400)

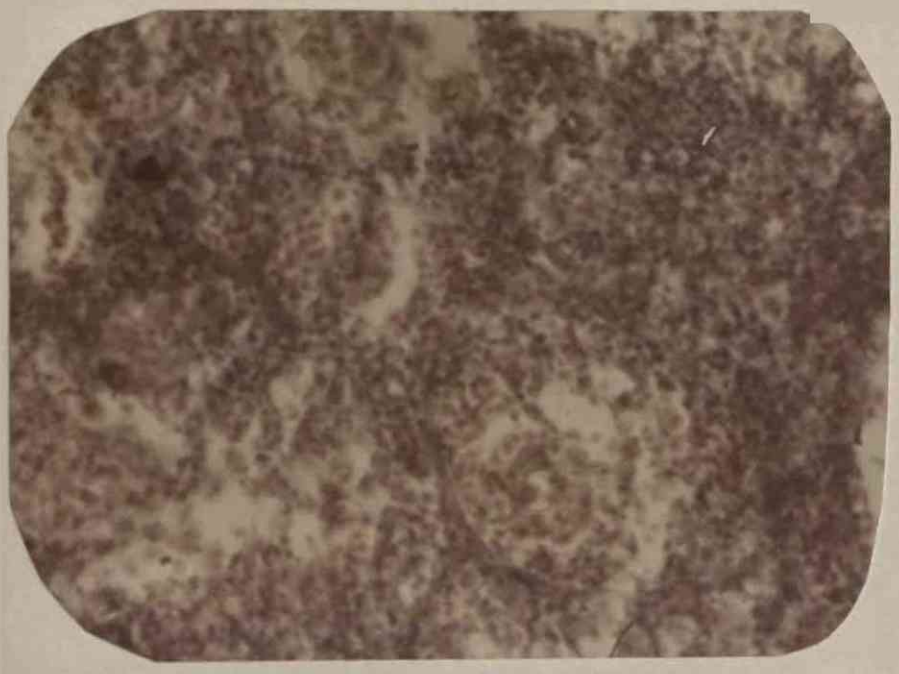


PLATE : XIII

Section of the spleen showing normal architecture. (x 200)

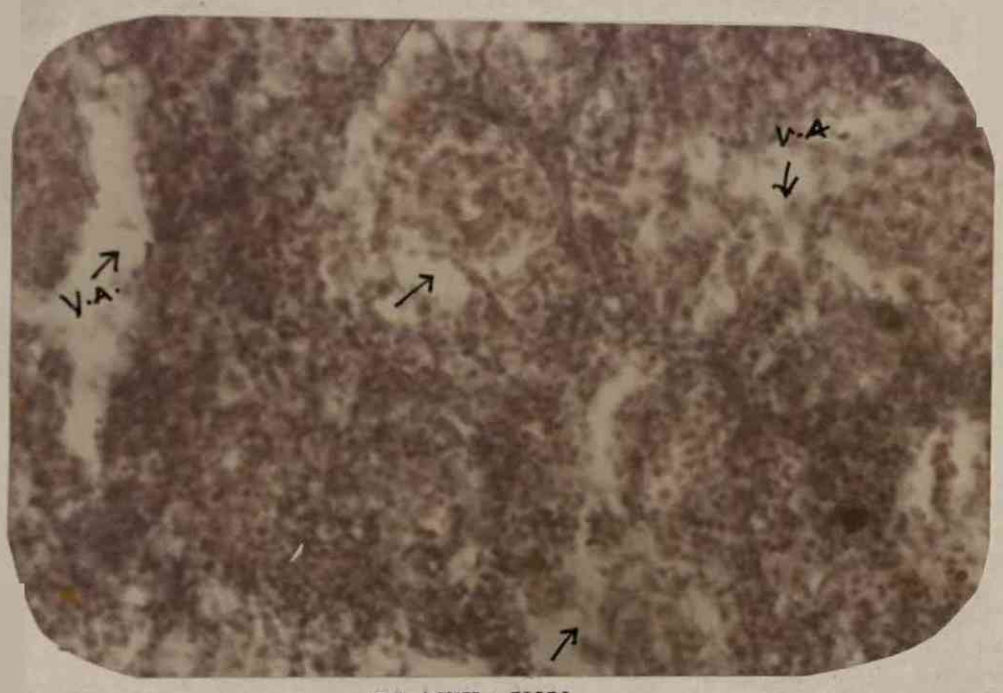


PLATE : XIV

Section of the spleen showing vacant areas in ellipsoids (x 200)

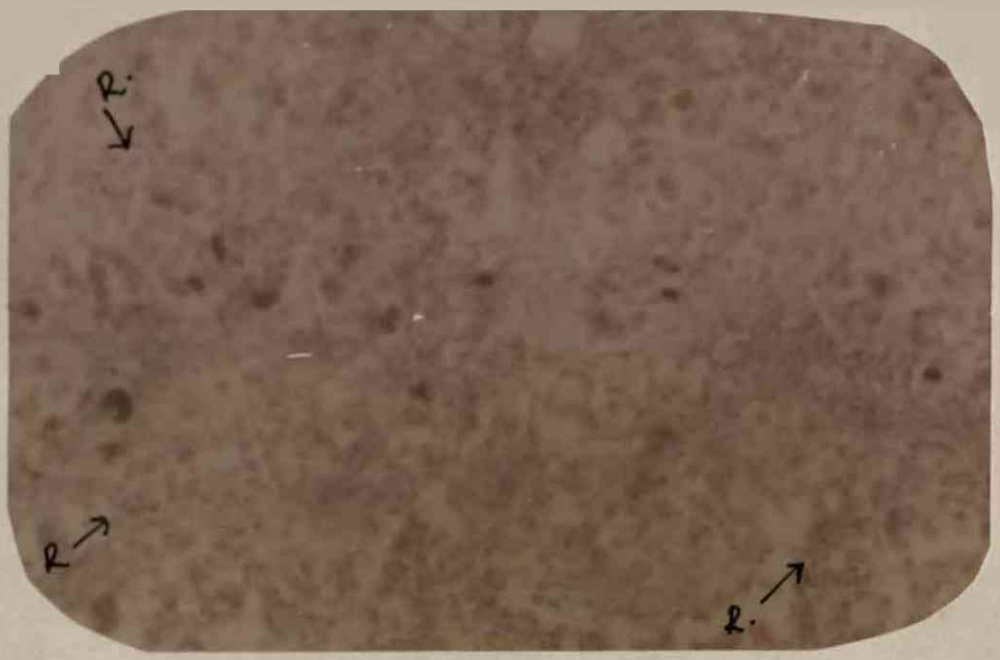


PLATE : XV

Section of the spleen showing rarefaction of lymphoid tissue (x200)

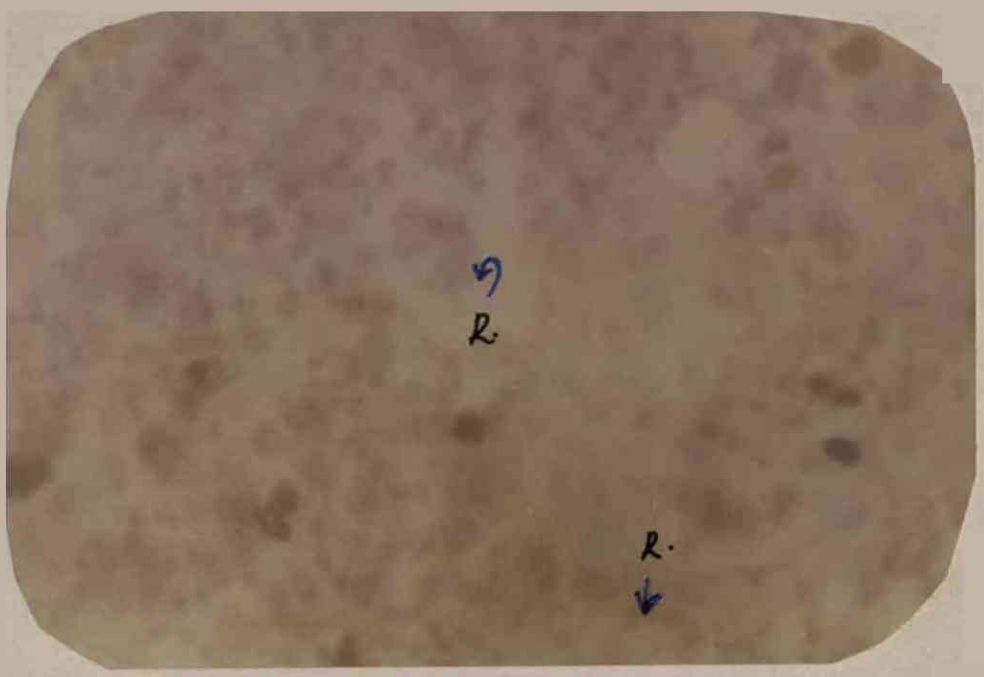


PLATE : XVI

Section of the spleen showing rarefaction of lymphoid tissue and reticulo-endothelial cells (×200)

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