EFFECT OF A COMPOSITE MIXTURE OF *Emblica officinalis, Terminalia chebula* AND *Terminalia bellirica* ON AFLATOXICOSIS IN RABBITS

INDU. K

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Centre of Excellence in Pathology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR-680651
KERALA, INDIA

DECLARATION

I hereby declare that this thesis entitled **"EFFECT OF A COMPOSITE MIXTURE OF** *Emblica officinalis, Terminalia chebula* **AND** *Terminalia bellirica* **ON AFLATOXICOSIS IN RABBITS"** is a bonafide record of research work done by me during the course of research and that the 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.

Mannuthy
4.8.09

INDU. K

CERTIFICATE

Certified that this thesis, entitled "**EFFECT OF A COMPOSITE MIXTURE OF** *Emblica officinalis, Terminalia.chebula* **AND** *Terminalia bellirica* **ON AFLATOXICOSIS IN RABBITS**" is a record of research work done independently by **INDU. K.,** under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

Mannuthy
4.8.09

Dr. C. R. Lalithakunjamma
(Chairman, Advisory Committee)
Professor and Head,
Centre of Excellence in Pathology,
College of Veterinary and Animal Sciences,
Mannuthy-680 651

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Dr. Indu. K.**, a candidate for the degree of Master of Veterinary Science in Veterinary Pathology, agree that the thesis entitled **"EFFECT OF A COMPOSITE MIXTURE OF *Emblica officinalis, Terminalia chebula* AND *Terminalia bellirica* ON AFLATOXICOSIS IN RABBITS"** may be submitted by **Dr. Indu K** in partial fulfillment of the requirement for the degree.

Dr. C. R. Lalithakunjamma
(Chairman, Advisory committee)
Professor and Head
Centre of Excellence in Pathology
College of Veterinary and Animal Sciences
Mannuthy, Thrissur

Dr. N. Vijayan
Professor,
Centre of Excellence in Pathology
College of Veterinary and Animal
Sciences, Mannuthy, Thrissur
(Member)

Dr. N. Divakaran Nair
Professor
Centre of Excellence in Pathology
College of Veterinary and Animal
Sciences, Mannuthy, Thrissur
(Member)

Dr. R. Richard Churchil
Assistant Professor
AICRP on Poultry
College of Veterinary and Animal
Sciences, Mannuthy, Thrissur (Member)

EXTERNAL EXAMINER
PROFESSOR AND HEAD,
Department of Veterinary Pathology,
Veterinary College & Research Institute,
NAMAKKAL - 637 001.

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Indu

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Introduction

1.INTRODUCTION

Mycotoxins are secondary fungal metabolites secreted into microenvironment around the mould which cause adverse biological effects. In warm and humid climate as in Asia, Latin America and African countries aflatoxins are most wide spread of all mycotoxins. These are produced mainly by *Aspergillus flavus, Aspergillus parasiticus, Aspergillus nomius* and *Aspergillus niger*. These moulds invade and grow on all sorts of stored feed ingredients and produce toxins under favourable aerobic conditions when sufficient moisture (15 per cent) and relative humidity (90 to 95 per cent) and ambient temperature (24 to 25 per cent) are available. Toxin production to some extent can take place at lower or higher temperature as well.

The discovery of aflatoxin began immediately after an outbreak of a disease of turkeys in England in 1960. Speculations regarding the nature of toxin suggested that it might be of fungal origin. The toxin producing fungus was identified as *Aspergillus flavus* and the toxin was given the name Aflatoxin by virtue of its origin.

There are four major aflatoxins namely B_1, B_2, G_1 and G_2 and two additional metabolic products M_1 and M_2. Aflatoxicosis is a serious problem among domesticated animals and birds causing huge economic loss. While the young ones are most susceptible, all ages are affected but in varying degrees for different species. Aflatoxins cause gastrointestinal dysfunction, reduced feed utilization and efficiency, anemia, liver damage, jaundice, reduced reproductivity and immunity. Aflatoxin B_1 is one of the most potent hepatocarcinogen known and is classified as a Group 1 carcinogen by the International Agency for Research on Cancer. Exposure to AFB_1 has been associated with an increased incidence of primary hepatocellular carcinoma which is the seventh most frequently encountered cancer in the world.

The menace of aflatoxicosis cannot be completely eliminated in tropical countries where the climate is conducive for the growth of the fungus. Suitable preventive and therapeutic strategies are being tried to reduce the adverse effects of mycotoxins. These include physical methods of separation, thermal inactivation, irradiation, solvent extraction, adsorption from solution, microbial inactivation and fermentation. Chemical methods include structural degradation by ammoniation and reaction with sodium bisulfite, modification of toxicity by dietary chemicals and alteration of bioavailability by aflatoxin chemisorbents.

Amelioration of aflatoxin by dietary herbal treatment is a recent concept. Triphala is an ayurvedic herbal formula consisting of equal parts of *Emblica officinalis* (Nellika), *Terminalia bellirica* (Thannika) and *Terminalia chebula* (Kadukka). The word triphala literally means "three fruits".

Triphala is categorized as a rejuvenating ayurvedic herbal formulation and has traditionally been used in various gastric problems including intestinal inflammation. Triphala promotes internal cleansing, reduces conditions of stagnation and excess, improves digestion and assimilation of nutrients. Triphala has the ability to induce cytotoxicity in tumor cells but spares the normal cells. It has significant medicinal value as a potential detoxifying and anti-cancer agent. Studies suggest that the growth inhibitory effects of triphala is mediated by the activation of p53 and shows potential for the treatment and prevention of cancer. Fruits included in triphala are claimed to have various biological activities such as cardioprotective and cardiotonic effects.

Stress is a term that generally has a negative connotation, which results in immune dysfunction. Studies in albino rats proved the immunomodulatory effect of triphala during noise stress and suggests its therapeutic usefulness. Triphala restores the oxidative stress induced changes because of its antioxidant properties. Triphala ointment showed the antibacterial, wound healing, and antioxidant activities useful for the management of infected wounds. It maintain

normal blood sugar levels, increase the number of red blood cells and aid in removal of undesirable fat in the body. Components of triphala have hepatoprotective property. This hepatoprotective effect could be attributed to its prominent anti-oxidative and membrane stabilizing activities.

Rabbits are highly susceptible to aflatoxicosis, LD_{50} value of aflatoxin is 0.3 to 0.5 mg per kg body weight (Garg., 2002). The present study is designed to study the toxic effects of aflatoxin in rabbits and to evaluate the effect of composite mixture of *Emblica officinalis, Terminalia chebula, Terminalia bellirica* on alleviation of toxic lesions in rabbits.

Review of Literature

2. REVIEW OF LITERATURE

2.1 INCIDENCE

Rajan *et al.* (1991) observed high incidence of aflatoxin contamination of groundnut cake and poultry feed when they screened the samples received from dairy farmers, poultry farmers, Government and University farms during 1986 to 1990.

Warm humid climatic conditions are ideally suited for aflatoxin production in feed stuff and aflatoxicosis was diagnosed first time in ducks and pigs in Kerala (Farshid., 1992).

The analysis of 594 cattle feed samples and 418 groundnut samples showed that 47 and 53 per cent of them contaminated with greater than 100 ppb of aflatoxin (Rajan and Ismail, 1995).

The biological fluids of apparently healthy buffaloes having symptoms of anorexia and gastroenteritis were analysed for aflatoxin and contamination in bloodsera, urine and milk samples were found to be 13.20, 11.11 and 23.07 per cent respectively (Sinha *et al.*, 2000).

Garg *et al.* (2004) observed that the conversion of aflatoxin B_1 excretion in milk as aflatoxin M_1 was in the range of 2.06 to 4.65 per cent in buffaloes and cows and it was significantly lower in buffaloes than cows.

Anandkumar *et al.* (2005) reported that the interaction of infectious bursal disease and aflatoxin led to increased mortality of 35.6 per cent in five week old chicks when compared to three to 21 per cent in IBD and 0.03 per cent in aflatoxicosis respectively.

Devi (2007) opined that degenerative changes in liver could be associated with mouldy feed considering the fact that rabbits are highly susceptible to even traces of aflatoxin .

Out of 21 samples of compounded feeds and feed ingredients of broiler ration analysed, 12 were found to be positive for aflatoxin B_1 and the range of toxin was between 12 to 120 ppb (Moregaonkar *et al.,* 2007b).

2.2 CLINICAL SIGNS

Imperfect organogenesis and embryo mortality (75 per cent) were observed in ochratoxin inoculated chick embryos (Lalitha , 1987).

Rabbits fed with 100 ml of aflatoxin contaminated milk at 200 ppb showed weakness, listlessness and partial loss of appetite (Maryamma *et al.,* 1990a).

Churamani and Chattopadhyay (1995) noted that rabbits fed aflatoxin mixed feed containing 0.035 mg per Kg feed for 60 days showed depression, anorexia, fur chewing and loss of body weight.

Sudhindra (1998) noted that aflatoxin feeding at one and three ppm levels in pregnant rabbits from day of conception to parturition showed dose dependent signs of anorexia, loss of body weight, fur chewing, dehydration, emaciation, abortions and still births followed by death.

Broiler chicks fed with aflatoxin at the rate of 0, 20, 40, 60, 80 and 100 ppb for a period of 45 days showed dullness particularly during initial stages of experiment while on fifth and sixth weeks of experiment they showed greenish diarrheoa, anorexia, reduced weight gain and depressed appearance (Arulmozhi , 1999).

Rao and Chakravarty (1999) noted that six per cent mortality in aflatoxin (2 ppm) fed 130 day old broiler chickens administered with levamisole (20 mg / bird) in contrast to 22 per cent mortality in aflatoxin alone fed birds.

Dutta *et al.* (2006) observed clinical signs of listlessness, diarrhoea, dehydration, progressive weakness, retardation in growth, nervous symptoms characterized by jerky movements and epistaxis in ducks due to aflatoxicosis.

2.3 BODY WEIGHT

Churchil (1996) reported poor growth rate and significant reduction in mean body weight from second week onwards in aflatoxin (1 ppm) treated broiler birds during his study upto eight weeks of age.

Chicks consuming aflatoxin mixed ration at 0:5 ppm showed reduction in body weight as compared to control and the withdrawal of aflatoxin feeding after fourty fifth day showed slight improvement in weight gain (Yadav *et al.,* 1996).

There was a decrease in body weight of aflatoxin fed and *Sallmonella gallinarum* infected chicks significantly from control group but no significant difference between toxin fed and infected groups (Singh *et al.,* 1999).

John (2001) reported the reduction in embryonic weight in chick embryos inoculated with aflatoxin and ochratoxin and among the individual toxin groups AFB_1 treated embryos had lesser body weight.

A significant reduction in body weight gain from second week onwards was observed in Japanese quails by Madheswaran *et al.* (2005a) when crude aflatoxin or T_2 toxin fed either singly or in combination at three and four ppm levels from day of hatch to 35 days of age.

The dietary inclusion of aflatoxin at 300 ppb level depressed the growth rate in broilers from third week onwards, but the inclusion of *Andrographis*

paniculata at one, 1.5 and two gram per kg feed improved body weight gain after fourth week in a dose related fashion (Sapcota *et al.*, 2005).

Bhanuprakash *et al.* (2006) reported the reduction in body weight of aflatoxin fed broilers at 0.5 ppm level and the reason could be due to altered protein synthesis by competing with phenylalanine for binding site on the phenylalanine transfer RNA synthetase enzyme .

2.4 HAEMATOLOGICAL ANALYSIS

White Pekin ducklings administered with AFB_1 at a dose level of 0.075 mg per kg body weight on every alternate day for two months showed reduction in haemoglobin (Hb) concentration and total leucocyte count (TLC). The reduction in Hb concentration could be correlated with hepatic damage and direct effect of toxin on protein synthesis. The results of differential count (DLC) indicated lymphopenia and relative heterophilia with a total leucopenia (Balakrishnan , 1992).

Sahoo *et al.* (1992) noted that dose dependent alterations characterized by marked lymphopenia and compensatory heterophilia with no remarkable change in monocyte, eosinophil and basophil count in experimental aflatoxicosis in rabbits.

Anbiah (1996) observed anemia in ducks when given AFB_1 at the dose rate of 15 µg per kg bodyweight as evidenced by increase in erythrocyte sedimentation rate (ESR), decreased values of packed cell volume (PCV), Hb and erythrocyte count (TEC).

Choudhary *et al.* (1998) noted that Blackshire piglets administered AFB_1 at 6.9 µg per kg body weight for a period of 90 days showed a significant decrease in Hb, TEC and PCV and increase in ESR and TLC.

Kumar (1999) reported that there was no significant variation in percentage of heterophils, lymphocytes, eosinophils, monocytes and basophils in one month old quails administered aflatoxin B_1 by oral intubation at the rate of 0.5 ppm twice weekly for two months. But toxin treated birds showed a relative increase in heterophil count and relative decrease in lymphocyte from that of controls.

Low level exposure of AFB_1 (70 ppb) in broiler chicken for 42 days of treatment resulted in drop in TLC and caused bursal damage. Supplementation of selenium at 2.5 ppm could prevent the negative effects of aflatoxin (Perozo and Rivera, 2003).

Rabbits given aflatoxin and ochratoxin daily during six to 18 of gestation showed significant decrease in lymphocyte count and significant increase in relative and absolute heterophil count (Wangikar *et al.*, 2004).

Broiler chickens fed 0.5 ppm aflatoxin showed a significant reduction in TEC, PCV, Hb values starting from first week to fourth week of treatment compared to control (Ahamad *et al.*, 2006).

Mekala *et al.* (2006) reported that combined effect of curcumin and silymarin in ameliorating aflatoxin toxicity in broilers as evidenced by increased PCV in a dose dependent manner.

.Bio- Bantox$^®$ (a mycotoxin binder) supplementation at the rate of five gram per ton of feed significantly improved mean Hb, PCV and TLC values in broilers fed ochratoxin at 1 ppm level was observed by Pathan *et al.* (2007).

2.5 ENZYMES

Arulmozhi *et al.* (2000) studied the haemato-biochemical changes in broilers fed with 0, 20, 40, 60, 80, 100 ppb of aflatoxin B_1 from 0 to 45 days and

reported that it adversely affected Serum alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT).

Increase in aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were recorded in rabbits fed on aflatoxin contaminated feed at 0.8 ppm level (Rohilla *et al.*, 2001).

Nataraja *et al.* (2004) reported an increase in ALP levels when 240 day old broiler chicks were exposed to both aflatoxin and T_2 toxin at 2.5 ppm and four ppm respectively.

Kumar *et al.* (2005) noted that vitamin E and selenium supplementation (E care Se[®]) effectively reduced the increase in ALP values due to the feeding of 0.5 ppm aflatoxin and one ppm ochratoxin in broiler chicken .

Day old broiler chicks fed with feed containing two ppm aflatoxin were sacrificed and liver enzyme activities were estimated spectrophotometrically. A decrease in ALP, AST, ALT, LDH, acid phosphatase (ACP) and acetylcholine esterase (AchE) might be due to tissue damage followed by leakage of enzymes from liver to blood (Nath and Sarm*a*, 2005).

Sankh *et al.* (2005) evaluated the liver function in combined intoxication of aflatoxin and carbon tetrachloride in pregnant rabbits and observed significant increase in the levels of AST, ALT as compared to control. They opined that increase in serum enzyme activities might be due to spilling over of the enzymes into the blood.

Andrographis panniculata in the diet as feed supplement at the rate of two gram per kg significantly reduced AST, ALP and ALT values proved *its* hepatoprotective activity in broilers (Mathivanan *et al.*, 2007).

Alkaline phosphatase is found primarily in intestine, kidney, liver and bone whereas osseous and hepatic ALP has been identified in the sera of all species studied (Kaneko *et al.,* 2008).

2.6 GROSS PATHOLOGY

Ashley (1978) noted that cholangiocellular carcinomas constitute 10 to 22 per cent of all epithelial growths of liver.

Rabbits fed with 100 ml of aflatoxin contaminated milk at 200 ppb level showed petechiae in the subcutaneous tissue and serous membrane, pale ecchymotic liver and pale kidneys with few petechiae on the cortical surface (Maryamma *et al .,* 1990a).

Outbreak of foetal abortions and neonatal deaths in goats due to acute aflatoxicosis revealed moderate general icterus, pale and extremely friable liver with a few petechiae and streaks of haemorrhage and pale, moderately swollen kidneys (Maryamma *et al.,* 1990b).

Maryamma *et al.* (1990c) from another study observed pale friable liver with greyish white nodules of 0.5 to one mm diameter scattered over the parenchyma in white pekin ducks fed with one ppm aflatoxin in the diet. The gall bladder was distended with dark green bile and its wall was oedematous. Focal areas of nephrosis, catarrhal and haemorrhagic enteritis were also observed.

Leena (1992) noticed hepatosis characterised by moderate to severe enlargement of the liver with scattered greyish white nodules in aflatoxin fed ducks at 0.0375 mg per kg body weight and histologically, there were hepatocellular carcinoma and cholangiocellular carcinomas.

George (1998) observed hepatosis due to aflatoxicosis was severe in fishes and biliary proliferation was predominant in ducks.

Broiler chicks fed with aflatoxin at one ppm in the diet caused enlargement of liver with pinpoint haemorrhages on third day post feeding. On seventh day liver showed yellowish discolouration with numerous minute necrotic foci and enlarged pale kidney (Manimaran *et al.,* 2001).

Excess of reactive products of aflatoxin interfere with protein synthesis by interfering with transcription and prevents formation of certain enzymes required for energy metabolism and fat mobilization leading to hepatic steatosis (Garg , 2002).

Srivani *et al.* (2003) studied the effect of immunomodulators like tuftsin[®], stresroak[®] and zinc sulphate in induced aflatoxicosis in broiler chicks and found that liver of birds pertaining to aflatoxin alone treated group was pale yellow by fourteenth day and marked paleness with enlargement and rounded borders were seen at the end of sixth week of treatment, but birds from other groups revealed only paleness.

Madheswaran *et al.* (2005b) noted that crude aflatoxin feeding at three ppm level from day of hatch to 35 days of age in quails led to grossly enlarged pale yellowish liver and enlarged congested kidneys.

Injury to hepatocytes lead to accumulation of lipids because of decreased formation or export of lipoprotein by hepatocytes and decreased oxidation of fatty acids within hepatocytes leading to fatty change (Mc Gavin and Zachary , 2007).

2.7 HISTOPATHOLOGY

Samsonidze (1960) noted regenerative process in kidney after surgical excision continued long intervals after injury with increased thickness of tubular walls and restorative process by cellular hyperplasia.

Acute aflatoxicosis in goats produced extensive fatty changes, mild bile ductular hyperplasia in the liver of aborted fetuses. In neonatal kids, extensive

bile ductular proliferation, moderate catarrhal gastroenteritis and focal nephrosis were noted (Maryamma *et al.,* 1990b).

Maryamma *et al.* (1990c) found biliary hyperplasia, fatty changes, periportal necrosis of hepatocytes and several foci of regenerating hepatocytes of larger size in white pekin ducks fed with one ppm aflatoxin in the diet. They also noted hepatocytomegaly, hepatokaryomegaly and several disassociated round cells with hyperchromatic nucleus. One of the ducks showed reddish nodule of 0.5 cm diameter which was identified as cholangiocellular carcinoma.

Lalitha and Nair (1994) noted that ultrastructural alterations in liver and kidneys of chick embryos after experimental administration of ochratoxin A were mainly in hepatic cells with increased number of lipid droplets and in proximal convoluted tubules.

Histopathologically on the sixtieth day, the liver of birds fed with AFB_1 at ten µg per kg body weight showed cloudy swelling of hepatocytes, mild fatty changes, sinusoidal dilatation, extensive bile duct proliferation, coagulative necrosis and fibrous tissue proliferation and on day 120th, cirrhotic changes were noticed. At 15 µg per kg body weight on day 60th, liver revealed extensive necrosis and irregular arrangement of hepatocytes. On day 120th, dysplastic cells, pseudolobulation of liver, bile duct hyperplasia and diffuse necrosis were noted (Anbiah , 1996).

Kumar (1999) observed aflatoxin feeding at the dose rate of 0.5 ppm body weight in quails for a period of 60 days produced intense sinusoidal congestion, focal hepatocyte necrosis, infiltration of inflammatory cells around necrotic cells, extensive fatty changes, presence of fatty cysts and extensive bile duct hyperplasia.

Reddy *et al.* (2000) conducted the histopathological study of vital organs in neonatal and foetal rabbits from dams fed aflatoxin B_1 at pre and postpartum.

There was formation of cystic spaces in liver, haemorrhages in kidneys and heart of offsprings received one ppm aflatoxin. Inutero died fetuses (3ppm) showed degenerative changes in liver cells and haemorrhages in kidneys and heart.

Ahamad and Vairamuthu (2001) reported the individual and combined effects of citrinin and aflatoxin B_1 in broiler chickens. In the kidneys of aflatoxin group, mild congestion and multifocal haemorrhage, glomerular hyperplasia, mild hydropic degeneration of tubules and multifocal mononuclear infiltration were observed microscopically. In the combined group there was diffuse degeneration and hypertrophy of proximal convoluted tubules (PCT), luminal dilatation with hyaline casts in PCT, diffuse intertubular fibrous tissue proliferation of kidneys were also observed.

Chick embryos experimentally inoculated with aflatoxin and ochratoxin individually and in combination showed mild to severe fatty change, inflammatory infiltration in liver and acute tubular necrosis in kidneys (John, 2001).

Aflatoxin feeding in chicks produced mild congestion and degenerative changes in hepatocytes at six days post feeding, while degeneration of hepatic cells with fatty infiltration was observed in later stages. Kidneys showed haemorrhages, congestion and degeneration of lining epithelium of PCT (Manimaran *et al.*, 2001).

Response to liver injury are seen as regeneration, fibrosis, biliary hyperplasia and cirrhosis (Mc Gavin *et al.*, 2001).

Sajitha (2002) noted that in two per cent amla treated broiler chickens hepatic lesions were very much less with mild congestion and degenerative changes whereas one per cent amla treated group showed fatty changes and mild biliary hyperplasia when fed with commercial feed contained 100 to 200 ppb of

aflatoxin. Bile duct hyperplasia and associated fibrous tissue proliferations were absent in the two per cent amla treated group.

Jubb *et al.* (2005) noted at higher doses of aflatoxin most hepatocytes disappear and are replaced by ill assorted mixture of inflammatory cells, fibroblasts and primitive vascular channels.

Histopathological examination of liver of quails fed with aflatoxin at three ppm level revealed vacuolar degeneration of hepatocytes, bile duct hyperplasia, focal necrosis and mononuclear cell infiltration whereas, kidneys of toxin treated birds showed congestion, degeneration of tubular epithelium and necrosis (Madheswaran *et al.*, 2005b).

Arulmozhi *et al.* (2007) studied the gross, histopathological changes in the liver of broiler chicks induced by graded level of aflatoxin at the rate of 0, 20, 40, 60, 80 and 100 ppb in the feed from the day one to 45th day and the lesions were vascular changes, bile duct hyperplasia, portal fibrosis and mononuclear infiltration.

2.8 AMELIORATING EFFECTS OF VARIOUS AGENTS

Maryamma *et al.* (1990a) studied the effect of fermentation on AFB$_1$ content in milk and reduction to the extent of 75 to 90 per cent was reported in *in vitro* studies. They showed that 48 hours fermentation was adequate enough to reduce aflatoxin level to 90 per cent in rabbits.

Maryamma *et al.* (1990c) investigated the ameliorating effect of *Tinospora cardifolia* (amruthu) in experimental aflatoxicosis of ducks. Partial protection provided by the plant was proved by preneoplastic foci development in one bird of plant extract treated group compared to preneoplastic foci in all the toxin fed birds.

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Aflatoxin containing feed (1ppm) when supplemented with palm oil at five per cent level produced only five ppb level residues in tissues but toxin feed without palm oil supplementation produced 15ppb of residues in pigs (Maryamma *et al.*, 1990d).

Ramadevi *et al.* (1990) observed that ammonium hydroxide treatment and sun drying minimized gross and microscopical lesions produced by aflatoxin (2 ppm) in broilers.

Anjaneyulu *et al.* (1993) reported that the supplementation of activated charcoal (0.1% charcoal) was effective as a physical adsorbant in improving weight gain, feed consumption, feed conversion, PCV and Hb in broilers fed with one ppm aflatoxin.

Mini *et al.* (1993) found a remarkable reduction in aflatoxin concentration in feeds treated with chitosan at eight per cent and turmeric powder at two, four and eight per cent levels.

Maryamma *et al.* (1994) did not observe any protective effect by high dietary protein in aflatoxin carcinogenesis in pigs.

Rao and Chakravarty (1999) reported absence of gross and histological lesions in liver specific to aflatoxicosis in levamisole administered group at 20 mg per bird in drinking water at weekly interval for three weeks. They suggested that it could be due to the hepatoprotective effect of levamisole .

Kumar *et al.* (2003) noted that chicks fed with aflatoxin and ochratoxin either alone or in combination showed significant reduction in body weight, while the birds treated with vitamin E and selenium showed significant gain in body weight indicating beneficial effect of vitamin E and selenium on body weight during mycotoxicosis.

The long term low level AFB$_1$ exposure at the level of 70 ppb for 42 days produced liver tissue damage characterized by diffuse moderate to marked hydropic degeneration with cytoplasmic vacuolation of periportal hepatocytes, periportal fibrosis and bile duct hyperplasia. He found that selenium feed supplementation at 2.5 ppm of feed showed a reducing effect on degree of lesions in liver (Mavarez *et al.*, 2005).

Arvind (2007) concluded that the inclusion of esterified glucomannan at 0.1 per cent to the aflatoxin B$_1$ (1 ppm) contaminated feed could significantly counteracted the toxic effects of aflatoxin .

Moregaonkar *et al.* (2007a) noted that Toxi Bind Dry® mycotoxin binder supplementation in the diet of chicken helped in substantially reducing toxicity caused by one ppm dietary aflatoxin by improving body weight gain, feed intake and feed conversion ratio.

2.9 PROTECTIVE EFFECT OF TRIPHALA

The effect of plant extracts on aflatoxin production and growth of *Aspergillus flavus* on SMKY liquid medium was investigated, 15 bitter plant extracts tested including *Emblica officinalis (E. officinalis)* inhibited production of aflatoxin B$_1$ and B$_2$ but no correlation between inhibition of fungal growth and aflatoxin production was observed (Ranjan *et al.*, 1991).

Yokozawa *et al.* (1995) proved that the serum concentrations of urea nitrogen, creatinine, methylguanidine and guanidinosuccinic acid were decreased significantly in rats given Chebulae Fructus (*Terminalia chebula*) extracts showing the protective actions of this plant on rats with renal failure.

Sohni and Bhatt (1996) studied the activity of a crude ayurvedic formulation containing *Terminalia chebula (T.chebula)* in experimental amoebic (*Entamoeba histolytica*) liver abscess in golden hamsters and found that the

formulation had a maximum cure rate of 73 per cent at a daily dose of 800 mg per kg in hepatic amoebiasis.

Aqueous extracts of *T. chebula* had antiviral effects against duck hepatitis B virus in Peking ducks (Chung *et al.*, 1997).

Anand *et al.* (1997) observed that defattened fruits of *Terminalia bellirica* (*T. bellirica*) extracted with aqueous methanol gave the hepatoprotective fraction named TB5 and its hepatoprotective activity against most of the physiological and biochemical changes induced by carbon tetrachloride were demonstrated in rats and mice.

Chakrabarti (1998) studied the effect of Nutri Liv-82® containing *T. chebula* in three cases of lantana poisoning in calves and reported complete recovery after 21 days of treatment.

E. officinalis extract dose-dependently lowered the incidence of liver tumours induced by N-nitrosodiethylamine (NDEA) whereas the control rats (NDEA alone) had 100 per cent tumour incidence (Jose *et al.*, 1998).

Chloroform and acetone extracts of triphala against direct acting and indirect acting mutagens in rat cell lines showed inhibition of mutations (Kaur *et al.*, 2002).

Sajitha (2002) indicated that *E. officinalis* which had hepatoprotective effect could be effectively used as a feed additive in aflatoxicosis in broilers.

Kalorey *et al.* (2004) observed Toxiroak®, a herbomineral premix of extracts of *Allium sativum, Azadirachta indica, Solanum nigram, Emblica officinalis, Curcuma longa* and hydrated aluminosilicates supplementation counteracted the haematobiochemical alterations caused by mycotoxins.

Polyphenolic compounds in triphala have been reported to possess antioxidant properties, free radical scavenging abilities and responsible for protective effects provided by triphala (Girdhani *et al.*, 2005).

Sharma *et al.* (2005) studied the hepatoprotective effect of formulation containing *E. officinalis, T. chebula* and *T. bellirica* in experimental liver damage induced by single dose of paracetamol at 500 mg per kg body weight in rats. The sections examined microscopically showed mild regeneration of hepatocytes along with congestion, infiltration and degeneration in triphala treated group.

Emblica officinalis has been shown to attenuate several biochemical events which are suggested to be prefibrogenic and most significantly it had a suppressive effect on the hydroxyproline levels suggesting the possibility of its anti fibrotic action (Tasduq *et al.*, 2005).

The properties of *T. chebula* include antifungal, antibacterial, antidiabetic, antipyretic, antioxidant, anticlastogenic and hepatoprotective effects (Mejo, 2006).

Mir *et al.* (2007) reported that 50 per cent alcoholic extract obtained from fruits of *E. officinalis* effectively reversed carbon tetrachloride toxicity by accelerating regeneration in the form of anisocytosis, anisonucleosis and prominent nucleoli in liver tissue.

Partysmart® a herbal formulation containing extracts of *E. officinalis* treatment in rats for eight weeks produced lesser vacuolar degeneration and intactness of hepatic architecture along with improved glycogen deposition in alcoholic liver disease (Gopumadhavan *et al.*, 2008).

Studies suggests that the growth inhibitory effects of triphala is mediated by the activation of p53 and shows potential for the treatment and prevention of human pancreatic cancer (Shi *et al.*, 2008).

Materials and methods

3. MATERIALS AND METHODS

An experiment was conducted in the Centre of Excellence in Pathology, College of Veterinary and Animal Sciences, Mannuthy to study the effect of composite mixture of *Emblica officinalis*, *Terminalia chebula* and *Terminalia bellirica* on aflatoxicosis in rabbits.

3.1 PRODUCTION OF AFLATOXIN

3.1.1 Source

Aspergillus parasiticus var.globosus*411 culture maintained on potato dextrose agar at the AICRP on Poultry, Veterinary College, Mannuthy was used in this study.

3.1.2 Production of fungal rice culture

Aflatoxin was produced in rice as per the method of Shotwell *et al.* (1966). Fifty grams of rice, free from any adulteration, was taken in 250 ml conical flask, plugged with cotton, autoclaved at the temperature of 121°C with 50 lb psi for 15 minutes and cooled. Eight to ten ml of distilled water was added into each flask and were shaken for uniform distribution of water. Inoculation was done by transferring fresh spores of fungus into individual flask with the help of a platinum loop under sterile conditions. The flasks were kept at room temperature and hand shaken vigorously for six to ten times a day to avoid clumping and to facilitate easy growth of the fungus. The mould growth was confirmed by the appearance of whitish growth on rice, gradually changing to greenish colour showing extensive mycelial growth at the end of the incubation period. After 10 days post inoculation, the rice culture was again autoclaved. The autoclaved culture rice was dried, ground to powder form and used in experimental rations.

3.2 AFLATOXIN QUANTIFICATION

The representative sample of fungal culture material were quantified by thin layer chromatography (AOAC., 1990) at Animal Feed Analytical and Quality Control Laboratory, TANUVAS, Namakkal. The rice culture yielded 27 ppm of aflatoxin which was incorporated into the ration to give a final concentration of 0.5 ppm of aflatoxin. Here culturing was done in twenty conical flasks and required 25 mg of toxin was obtained after adding 930 g of rice culture to 50 kg of feed.

3.3 EXPERIMENTAL ANIMALS

The study was conducted on twenty four rabbits aged four weeks procured from Rabbit Farm, Veterinary College, Mannuthy. All the animals were maintained under standard management conditions which were randomly divided into three groups of eight each. The animals were fed *ad libitum* with the respective experimental diet for a period of two months. Feed and water intake of rabbits were regularly monitored.

3.4 EXPERIMENTAL FEED

a) Group l

Rice culture containing aflatoxin was added to toxin free diet to get a concentration of 0.5 ppm.

b) Group ll

A composite mixture of *Emblica officinalis, Terminalia chebula* and *Terminalia bellirica* in equal proportion at the level of four per cent was incorporated with the aflatoxin added diet.

c) Group lll

Control diet

3.5 PARAMETERS STUDIED

3.5.1 Clinical signs

3.5.2 Estimation of body weight

The body weight of the rabbits were recorded at fortnightly intervals for two months.

3.5.3 Collection of biological samples

3.5.3.1 *Collection of blood and separation of serum*

Blood was collected from the marginal ear veins into separate sterile centrifuge tubes with and without anticoagulant. Blood collected in anticoagulant (sodium EDTA at the rate of 1mg per ml) was used for studying haematological parameters. Blood without anticoagulant was kept at refrigeration temperature for half an hour and then placed at room temperature. It was then centrifuged at 3000 rpm for 15 minutes and the supernatant clear serum was pipetted out and used for biochemical analysis.

3.5.3.2 *Estimation of haematological parameters*

TLC, PCV, DLC were estimated by the method suggested by Thrall *et al.* (2004). The concentration of Haemoglobin (Hb) was estimated by acid haematin method as described by Feldman *et al.* (2000).

3.5.3.3 *Biochemical parameters*

The individual serum samples were analysed for Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST) and Alkaline Phosphatase (ALP) using standard diagnostic kits from Agappe Diagnostics Ltd.

3.5.3.4 *Gross Pathology*

The mortality of animals from different treatment groups were recorded. The rabbits died during the experiment and the animals euthanized at the end of two months were subjected to detailed postmortem examination. Gross lesions in various organs were recorded.

3.5.3.5 *Histopathological examination*

Representative tissue samples from liver and kidney were fixed in 10 per cent formalin and were processed and embedded in paraffin. Microtome sections of 4μ thickness were prepared from each tissue and stained with Haematoxylin-Eosin as per the staining technique followed by Bancroft and Gamble (1996) to study the histopathological changes.

3.6 STATISTICAL ANALYSIS OF DATA

The results obtained were analyzed using Analysis of Co-variance method followed by LSD for comparison between groups as described by Snedecor and Cochran (1994).

Results

4. RESULTS

The results of the experiment conducted to study the effect of Triphala, a composite mixture of *Emblica officinalis, Terminalia chebula* and *Terminalia bellirica* on alleviation of aflatoxin induced damage in rabbits are presented in this chapter.

4.1 CLINICAL SIGNS AND MORTALITY PATTERN

Rabbits fed with aflatoxin incorporated ration showed dullness, reduced water intake, dehydration, anorexia, emaciation and weakness. Two rabbits of group I fed aflatoxin at 0.5 ppm level in the feed died on 10th day and three rabbits died on 40th day. Rabbits of group II treated with triphala and control did not show any clinical signs throughout the period of experimentation. But two rabbits of group II treated with triphala died on 25th day.

4.2 BODY WEIGHT

The adjusted mean body weight of rabbits recorded at fortnightly interval as influenced by the incorporation of aflatoxin and ameliorating agent is given in table1 and graphically represented in figure 1.

The rabbits of all the experimental groups showed gradual increase in the body weight throughout the experimental period. A significant ($P<0.05$) difference in body weight was noted between control group and other two groups on 14th day. From 28th day onwards there were significant ($P<0.05$) differences among all the three groups. The rabbits fed aflatoxin alone showed significantly ($P<0.05$) lower body weight than the rabbits fed aflatoxin along with triphala. The adjusted mean body weight of the control group was significantly ($P<0.05$) higher than other two groups.

4. 3 HAEMOGRAM

The response in the haematological parameters recorded in the three groups are described below.

4.3.1 Haemoglobin (Hb g/dl)

The mean haemoglobin values of rabbits on day zero, 30 and 60 are presented in the table 2 and graphically shown in figure 2.1. The haemoglobin values were significantly ($P<0.05$) different for all the three groups on 30th and 60th day respectively. Mean values were 8.50, 10.41 and 11.62 g/dl for aflatoxin fed, toxin plus triphala fed and control rabbits on 30th day. On 60th day group I rabbits fed with aflatoxin showed a significant ($P<0.05$) decrease in mean haemoglobin concentration (6.83 g/dl), but group II and control group showed a significant ($P<0.05$) increase in values (10.83 and 12 g/dl).

4.3.2 Packed cell volume (PCV %)

The results are shown in table 2 and figure 2.2. The mean PCV values varied from 25 to 35 per cent in different groups and a significant ($P<0.05$) decrease in the PCV was noted in aflatoxin treated group. The mean values were 26.83 and 25 per cent in toxin fed animals on 30th day and 60th day respectively. But in triphala and control groups there was significant ($P<0.05$) increase in PCV values on 30th day and 60th day. The mean values were 31.83 per cent, 32.50 per cent for group II and 34.37 per cent, 35.00 per cent for control group on 30th and 60th day respectively.

4.3.3 Total leukocyte count (TLC/cu.mm)

The mean leukocyte count of rabbits are shown in table 2 and figure 2.3. Significantly ($P<0.05$) lower values in total leukocyte count was observed in groups I and II at all intervals of study compared to control. There was significantly ($P<0.05$) lower leukocyte count in rabbits fed aflatoxin alone when

compared to rabbits fed triphala in addition to aflatoxin. The mean values on 30th day were 4591.66, 5591.66 and 7777.50 per cu.mm for groups I, II and control. On 60th day rabbits showed mean leukocyte values of 3416.66, 5365 and 7711.25 per cu.mm for aflatoxin fed, triphala fed and control groups.

4.3.4 Differential leukocyte count (DLC %)

The mean heterophil count of experimental animals are represented in the table 3 and figure 2.4. Heterophil (H) count of group I rabbits fed with aflatoxin diet significantly (P<0.05) differed from those of aflatoxin plus ameliorating agent fed rabbits and control. They showed significantly (P<0.05) higher values of heterophil count with mean value of 37.16 and 43.66 per cent on 30th and 60th day of experiment respectively. But triphala fed rabbits and control group did not differ significantly (P<0.05) throughout the experimental period with the mean value of 32.66 and 32.50 per cent on 30th day. On 60th day rabbits showed mean values of 33.16 and 32.62 per cent for triphala fed and control groups.

The mean lymphocyte count of rabbits are represented in the table 3 and figure 2.5. Aflatoxin treated group showed significantly (P<0.05) lower mean lymphocyte (L) count compared to group II and control. But mean lymphocyte count in group II rabbits fed aflatoxin plus triphala and control group were statistically similar throughout the experimental period. On day 30 rabbits of group I, II and control showed mean lymphocyte count of 55.66, 61.16 and 60.12 per cent respectively. On 60th day groups I, II and control showed mean values of 49.33, 59.66 and 60.12 per cent.

Mean monocyte (M), basophil (B) and eosinophil (E) counts in all the groups were comparable to each other at all intervals of study.

4.4 SERUM ENZYMES (IU/L)

4.4.1 Serum aspartate amino transferase (AST)

The mean AST values on day zero, 30 and 60 are shown in the table 4 and graphically represented in figure 3.1

On day 30, significant increase (P<0.05) in the mean AST values were observed in the aflatoxin alone fed (127.83±14.15 IU/L) and aflatoxin with triphala fed rabbits (109.83±14.72 IU/L) as compared to untreated control (41.00±2.85 IU/L). The mean (±SE) AST values was numerically higher in aflatoxin treated group as compared to triphala supplemented group during that period.

On 60th day, group I rabbits fed aflatoxin at 0.5 ppm level in the feed (115.00±2.88 IU/L) and group II supplied triphala at four per cent level along with aflatoxin (94.00±16.52 IU/L) showed significant (P<0.05) increase in the mean AST values when compared to control (45.25±3.42 IU/L). Though the mean (±SE) AST values in aflatoxin and triphala groups were statistically similar, numerically higher value was observed in the former treatment group.

4.4.2   Serum alanine aminotransferase (ALT)

The mean ALT values on day zero, 30 and 60 are shown in the table 4 and graphically represented in figure 3.2.

On day zero there were no significant variations in the levels of serum alanine aminotransferase. On day 30, a significant (P<0.05) increase in the mean (±SE) ALT values was observed in the toxin fed (171.16±15.33 IU/L) and triphala supplemented rabbits (166.50±15.94 IU/L) as compared to untreated control (94.50±10.65). Though the ALT levels were lower in triphala supplemented group compared to aflatoxin alone fed group the differences were statistically not significant at 30th day. On day 60, mean (±SE) ALT values of

aflatoxin and triphala groups significantly differed from each other with mean value of 186.33±2.02 IU/L and 114.33±6.17 IU/L respectively. The control group showed a mean (±SE) ALT value of 91.12±8.26 IU/L on 60th day.

4.4.3 Serum alkaline phosphatase (ALP)

The mean values of serum alkaline phosphatase level on day zero, 30 and 60 are listed in Table 4 and graphically denoted in figure 3.3.

The mean (±SE) serum ALP values showed significant increase ($P<0.05$) in both triphala supplemented group and aflatoxin treated group throughout the study. But toxin group showed numerically higher ALP values on 30th day (476.16±28.16 IU/L) and on 60th day (431.33±24.90 IU/L) as compared to triphala supplemented group on 30th day (475.00±40.09 IU/L) and on 60th day (384.00±33.22 IU/L) respectively. The control group showed mean (±SE) levels of 267.12 ± 33.16 IU/L and 269.00 ± 30.89 IU/L on 30th and 60th day respectively.

4.5 GROSS PATHOLOGY

The gross changes observed in the liver of rabbits fed with powdered rice culture containing aflatoxin (fig.9) added feed were pale to yellowish discolouration, enlargement with distended gall bladder consisting of thick greenish bile (fig.4 and 10). The liver was hard in consistency and nodular lesions of varying sizes were observed in three rabbits (fig.7 and 8). The lesions in rabbits fed with triphala (fig.21) at four per cent level in aflatoxin added feed were similar to those observed in toxin fed but in reduced magnitude and severity (fig.5 and 11). There was no firmness of liver and nodular lesions in triphala group.

The gross changes in kidneys were paleness, nephrosis, uni or bilateral enlargement with congestion and petechiae in toxin fed group (fig.13). Intestinal mucosa appeared congested and haemorrhagic, lung showed areas of congestion

in toxin fed group (fig.19 and 16). The lesions were similar but in less severity in rabbits of triphala treated group (fig.14, 17 and 20).

The control group animals had normal morphological appearance of all the organs throughout the experimental study (fig.6, 12, 15 and 18).

4.6 HISTOPATHOLOGY

4.6.1 Group I

Tissues of aflatoxin fed rabbits at 0.5 ppm level (group I) revealed histological alterations characteristic of aflatoxicosis. The vascular changes observed were central venous congestion (fig.23), dilatation and congestion of sinusoids and portal vessels. Aflatoxin produced extensive degenerative changes in the hepatic tissue. Diffuse fatty degeneration characterized by vacuolation of the cytoplasm of hepatocytes pushing the nucleus to one side was observed (fig.25 and 26). Coagulative necrosis (fig.27), midzonal necrosis and kupffer cell reaction were also noted.

Chronic hepatic lesions were evident by biliary hyperplasia, mononuclear infiltration and fibrous tissue proliferation. Cirrhotic changes like fibroblastic proliferation, collagen deposition around the bile ducts and portal areas, psuedolobulation and loss of architecture of the liver tissue were observed in some cases. Megalocytosis with enlarged nucleus were seen. Focal accumulation of mononuclear cells were seen in the liver parenchyma replacing the necrotic tissue and there was severe biliary hyperplasia accompanied by severe periductular accumulation of mononuclear cells. The hyperplastic bile duct epithelium formed finger like projections into the lumen (fig. 32).

The nodular lesions seen on the liver of three rabbits of group I on histopathological examination revealed cholangiocellular carcinoma. There was numerous glandular structures lined by cuboidal or columnar cells resembling those of biliary epithelium and dense abundant stroma. Lumina of glandular

structures appeared empty. The cytoplasm of the cells were basophilic and hyperchromatic (fig.28,29,30,31 and 33).

The vascular changes observed in kidneys of toxin group were congestion of vessels and haemorrhages in cortical and medullary areas (fig.38). Mononuclear infiltration was noted in the interstitial spaces especially in the cortical area and cortico medullary junction (fig.40). There was extensive damage to the tubular epithelium which ranged from vacuolation of cytoplasm to necrotic changes. Tubular epithelial cells showed loss of nuclei and the existing nuclei showed karyomegaly (fig.42). Extensive necrotic changes were seen in the tubules (fig.43) with desquamation of epithelial cells and cellular debris in the lumen (fig.44). There was shrinkage of glomeruli followed by glomerular necrosis (fig.45).

4.6.2 Group II

In the liver of triphala treated group there were central venous congestion (fig.24) and congestion of sinusoidal spaces. Diffuse fatty change was present in one case. Wide spread necrosis with focal mononuclear accumulation was also observed but in less severity compared to the toxin fed group. Proliferation of bile ducts (fig.34) with mild periductular accumulation of mononuclear cells were seen in few cases. Fibrous tissue proliferation was scanty and architecture of the liver was well maintained (fig.35). Binucleate actively dividing hepatocytes and kupffer cell reaction were abundant in liver of triphala supplemented group (fig. 36). Cholangiocellular carcinoma seen in group I were absent in this group.

The vascular changes were evident in the kidneys of triphala group also (fig.39) but the necrotic changes were less prominent compared to toxin treated group. The tubular epithelium was well maintained with intact nucleus and glomerular damage was less evident (fig.46 and fig.47). There was also vacuolation of the tubular epithelium and mononuclear infiltration (fig.41). Some

tubules appeared to have lined by more than one layer and cell crowding in the lumen was noted (fig.48).

4.6.3 Control

The liver and kidney of control group animals appeared histologically normal throughout the experimental study (fig.22 and 37).

Table 1. Adjusted mean (± SE) body weight (g) of rabbits at fortnightly interval.

Treatment	Days			
	14	28	42	60
Group 1	1192.64[a] ± 21.30	1339.22[a] ± 19.79	1447.23[a] ± 37.44	1615.69[a] ± 42.36
Group 2	1212.52[a] ± 17.04	1407.67[b] ± 18.01	1674.91[b] ± 29.66	1927.55[b] ± 33.56
Control	1339.87[b] ± 20.27	1606.69[c] ± 18.37	1871.10[c] ± 26.10	2120.32[c] ± 29.53
Overall mean	1253.40 ± 49.92	1466.75 ± 52.19	1727.05 ± 53.97	1963.23 ± 62.33

Group 1= aflatoxin (0.5 ppm)

Group 2= aflatoxin (0.5 ppm) + triphala (four per cent)

Means bearing the same superscript within the same column do not differ significantly ($P < 0.05$)

Table 2. Haemogram values (mean ± SE) of experimental animals.

Treatment	Hb (g/dl)			PCV (%)			TLC/ Cu.mm		
	Day 0	Day 30	Day 60	Day 0	Day 30	Day 60	Day 0	Day 30	Day 60
Group 1	11.58^a ± 0.20	8.50^a ± 0.22	6.83^a ± 0.16	34.16^a ± 0.60	26.83^a ± 0.65	25.00^a ± 0.00	7780.00^a ± 28.86	4591.66^a ± 303.70	3416.66^a ± 274.36
Group 2	11.00^a ± 0.22	10.41^b ± 0.27	10.83^b ± 0.21	32.66^a ± 0.95	31.83^b ± 0.65	32.50^b ± 0.50	7746.66^a ± 51.48	5591.66^b ± 224.13	5365.00^b ± 175.85
Control	11.50^a ± 0.21	11.62^c ± 0.18	12.00^c ± 0.26	33.25^a ± 0.31	34.37^c ± 0.26	35.00^c ± 0.18	7832.50^a ± 31.49	7777.50^c ± 55.18	7711.25^c ± 49.36
Overall mean	11.37 ± 0.13	10.32 ± 0.32	10.67 ± 0.48	33.35 ± 0.36	31.35 ± 0.77	32.35 ± 0.91	7791.00 ± 22.14	6166.00 ± 332.73	6125.29 ± 416.19

Group 1= aflatoxin (0.5 ppm)

Group 2= aflatoxin (0.5 ppm) + triphala (four per cent)

Means bearing the same superscript within the same column do not differ significantly (P<0.05)

Table 3. Mean (±SE) differential leukocyte count (%)

Treatment	Heterophil			Lymphocyte			Monocyte		
	Day 0	Day 30	Day 60	Day 0	Day 30	Day 60	Day 0	Day 30	Day 60
Group 1	33.16[a] ± 0.70	37.16[b] ± 1.75	43.66[b] ± 2.96	61.83[a] ± 1.07	55.66[a] ± 1.81	49.33[a] ± 2.33	2.66[a] ± 0.42	3.33[a] ± 0.21	4.00[a] ±0.57
Group 2	31.33[a] ± 0.33	32.66[a] ± 0.42	33.16[a] ± 0.65	61.33[a] ± 0.61	61.16[b] ± 0.47	59.66[b] ± 0.76	3.16[a] ± 0.30	2.50[a] ± 0.22	2.83[a] ± 0.40
Control	32.25[a] ± 0.55	32.50[a] ± 0.37	32.62[a] ± 0.49	60.00[a] ± 0.26	60.12[b] ± 0.12	60.12[b] ± 0.22	3.00[a] ± 0.26	3.12[a] ± 0.22	3.00[a] ± 0.26
Overall Mean	32.25 ± 0.34	33.95 ± 0.71	34.76 ± 1.16	60.95 ± 0.40	59.10 ± 0.74	58.05 ± 1.10	2.95 ± 0.18	3.00 ± 0.14	3.11 ± 0.22

Group 1= aflatoxin (0.5 ppm)

Group 2= aflatoxin (0.5 ppm) + triphala (four per cent)

Means bearing the same super script within the same column do not differ significantly (P<0.05)

Table 3. Continued (mean ± SE values)

Treatment	Basophil			Eosinophil		
	Day 0	Day 30	Day 60	Day 0	Day 30	Day 60
Group 1	0.50^a ± 0.34	1.00^a ± 0.44	1.66^a ± 0.33	2.33^a ± 0.33	2.83^a ± 0.30	2.00^a ± 0.57
Group 2	0.66^a ± 0.21	0.83^a ± 0.30	0.83^a ± 0.30	3.50^a ± 0.22	3.33^a ± 0.21	3.50^a ± 0.42
Control	1.25^a ± 0.31	0.62^a ± 0.26	0.62^a ± 0.26	3.50^a ± 0.42	3.62^a ± 0.26	3.62^a ± 0.37
Overall mean	0.85 ± 0.18	0.80 ± 0.18	0.88 ± 0.18	3.15 ± 0.23	3.30 ± 0.16	3.29 ± 0.28

Group 1= aflatoxin (0.5 ppm)

Group 2= aflatoxin (0.5 ppm) + triphala (four per cent)

Means bearing the same superscript within the same column do not differ significantly (P<0.05)

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Table 4. Mean (±SE) serum enzyme values of experimental animals (IU/L)

Treatment	AST (IU/L)			ALT (IU/L)			ALP (IU/L)		
	Day 0	Day 30	Day 60	Day 0	Day30	Day 60	Day 0	Day 30	Day 60
Group 1	41.50[a] ± 3.51	127.83[b] ± 14.15	115.00[b] ± 2.88	55.33[a] ± 8.77	171.16[b] ± 15.33	186.33[c] ± 2.02	232.83[a] ±19.73	476.16[b] ± 28.16	431.33[b] ± 24.90
Group 2	54.83[a] ± 7.52	109.83[b] ± 14.72	94.00[b] ± 16.52	70.83[a] ± 8.44	166.50[b] ± 15.94	114.33[b] ± 6.17	217.33[a] ± 16.78	475.00[b] ± 40.09	384.00[b] ± 33.22
Control	35.75[a] ± 4.17	41.00[a] ± 2.85	45.25[a] ± 3.42	91.25[a] ± 11.92	94.50[a] ± 10.65	91.12[a] ± 8.26	262.87[a] ± 29.26	267.12[a] ± 33.16	269.00[a] ± 30.89
Overall mean	43.20 ± 3.37	87.70 ± 10.64	74.76 ±9.18	74.35 ± 6.67	139.10 ± 11.19	116.11 ±9.54	240.20[a] ± 14.11	389.80 ± 30.53	338.23 ± 24.79

Group 1 = aflatoxin (0.5 ppm)

Group 2 = aflatoxin (0.5 ppm) + triphala (four per cent)

Means bearing the same super script within a column do not differ significantly (P<0.05)

Adjusted mean body weight (g)
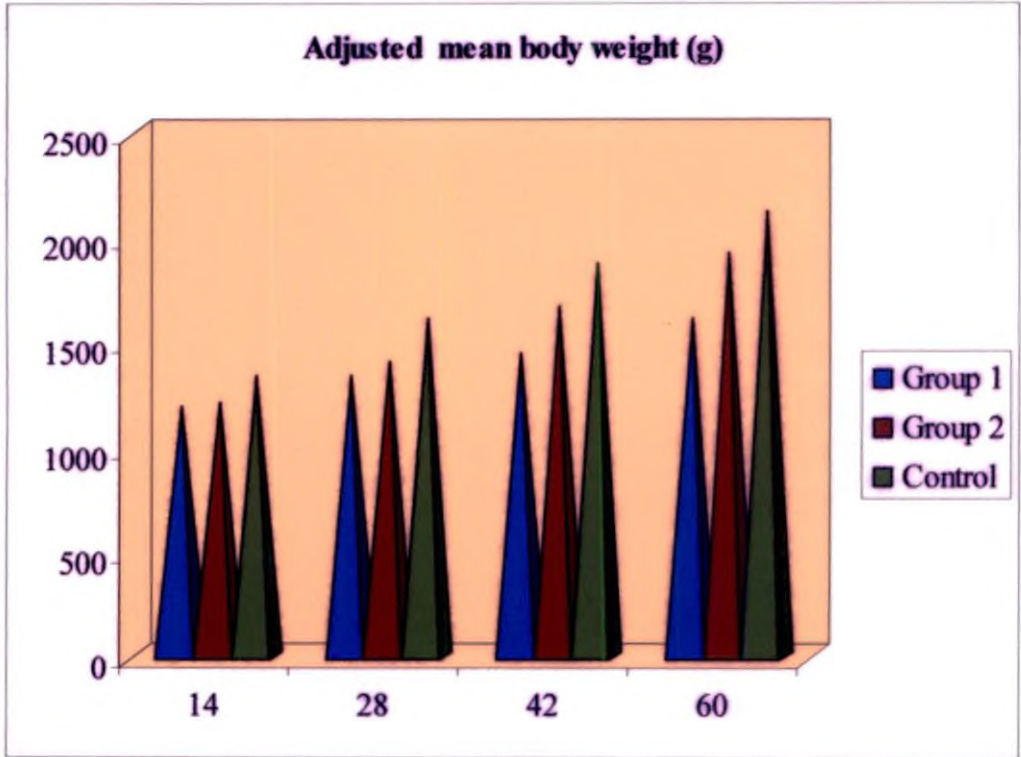
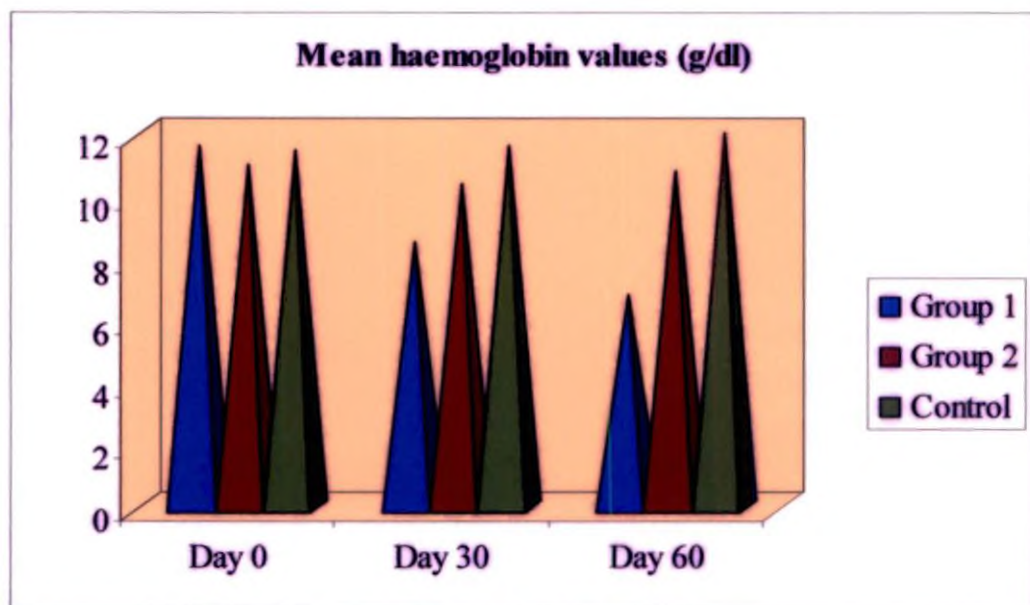Fig. 1. Adjusted mean body weight (g) of experimental animals
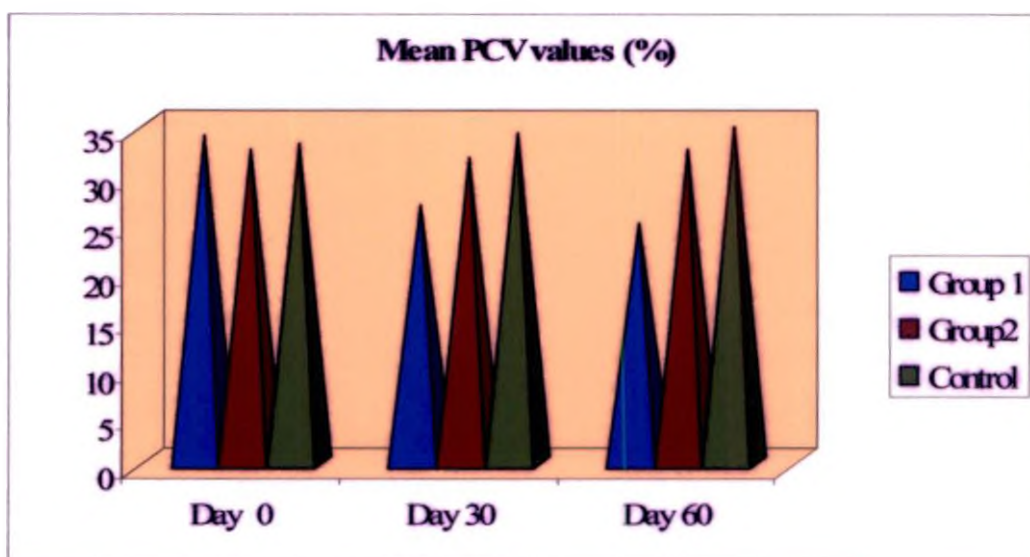
Fig.2.1. Mean haemoglobin values (g /dl)

Fig.2.2. Mean PCV values (%)

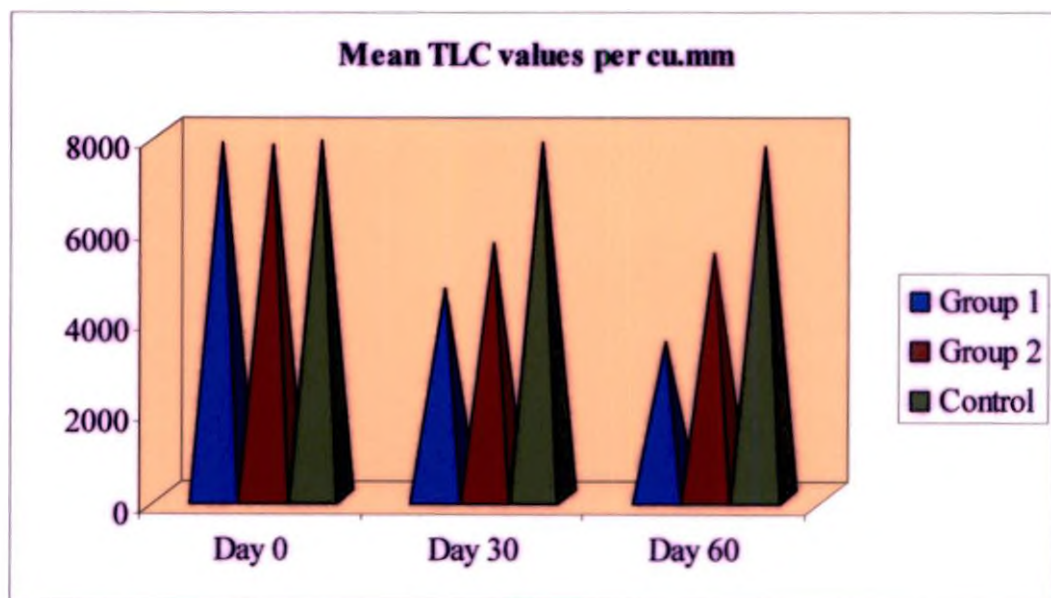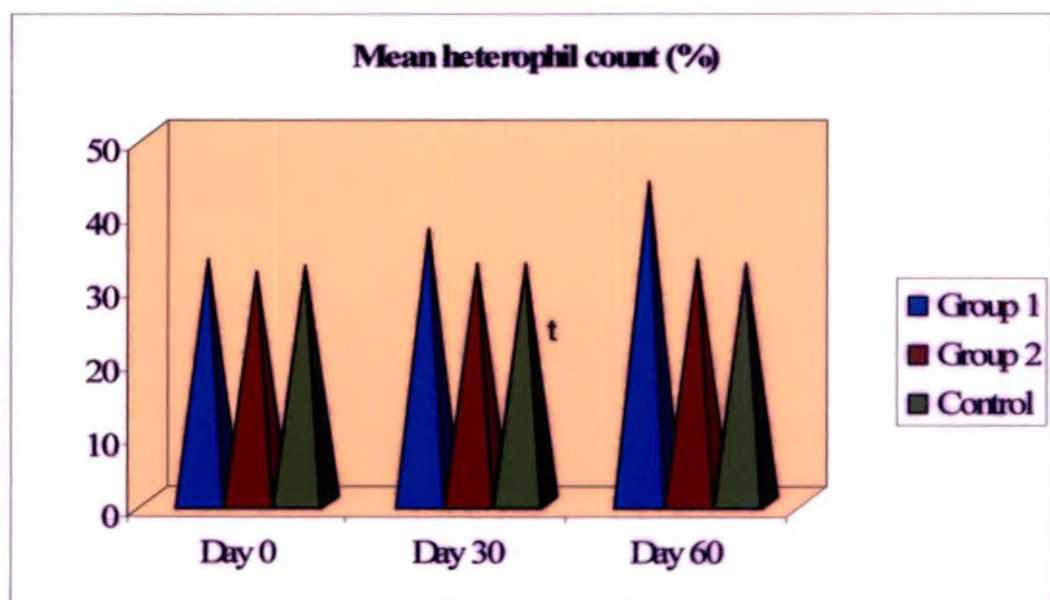**Fig. 2.3 Mean total leukocyte count (per cu.mm)**

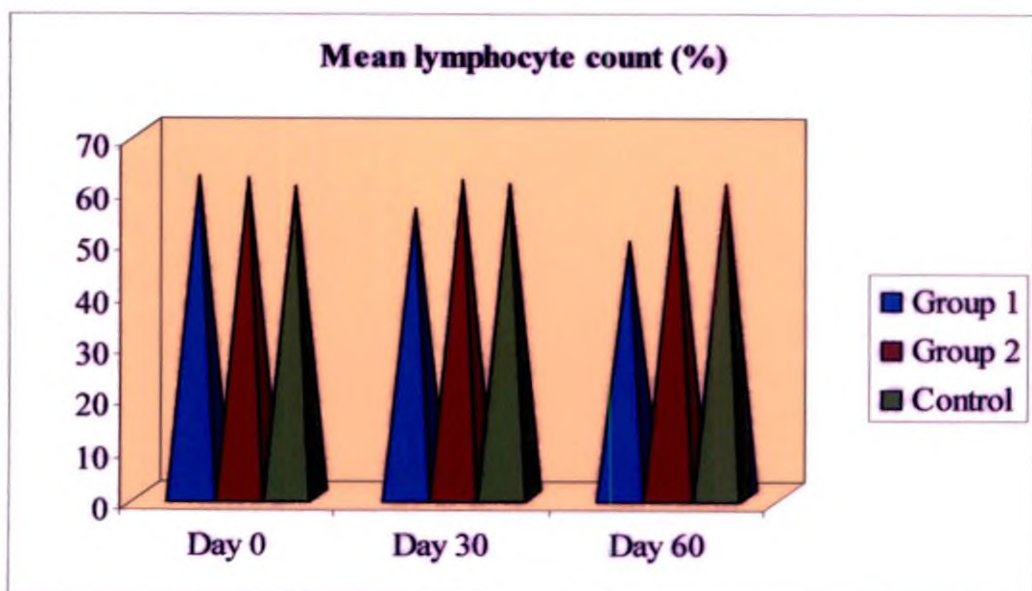**Fig. 2.4 Mean heterophil count (%)**
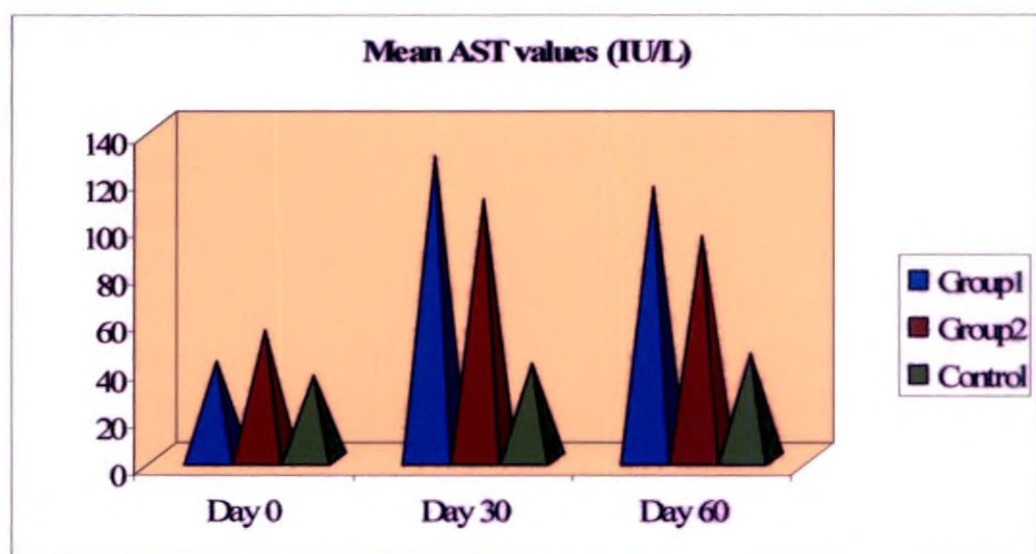

Fig. 2.5 Mean lymphocyte count (%)

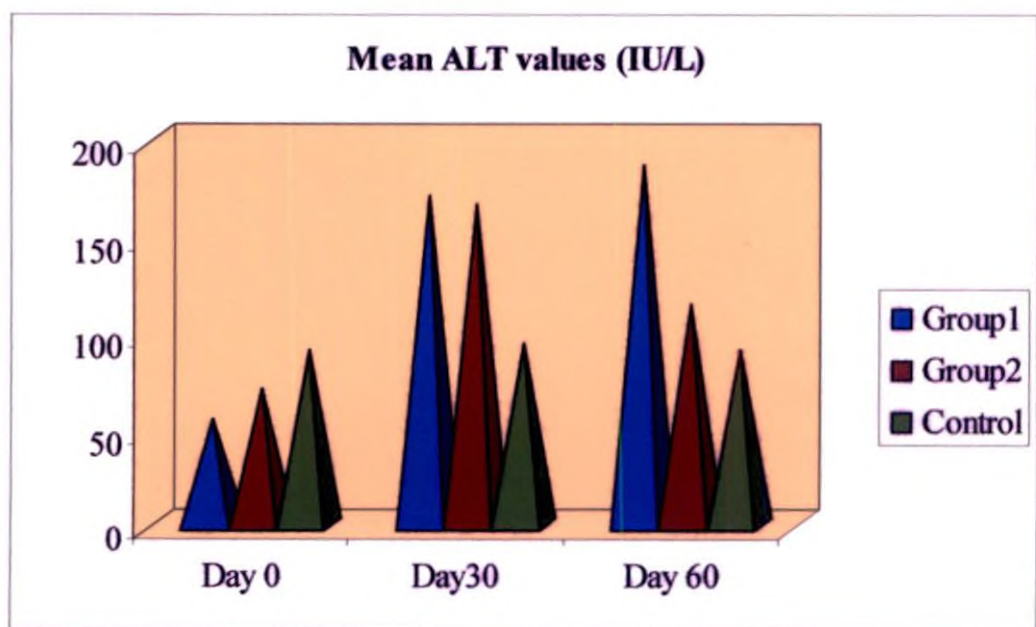**Fig. 3.1 Mean values of Aspartate aminotransferase (AST) (IU/L)**

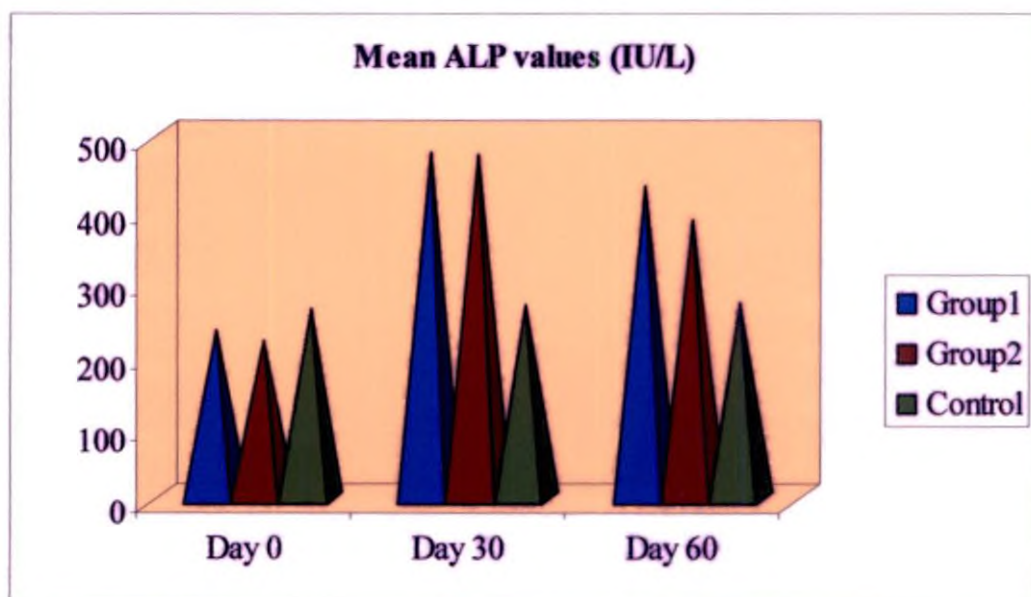**Fig. 3.2 Mean values of Alanine aminotransferase (ALT) (IU/L)**

Fig. 3.3 Mean values of Alkaline phosphatase (ALP) (IU/L)

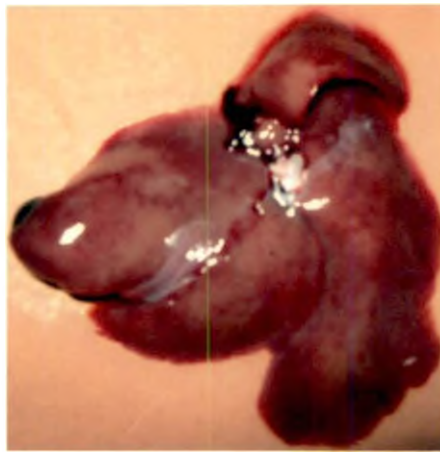Fig.4. Liver - yellowish discolouration and enlargement aflatoxin group

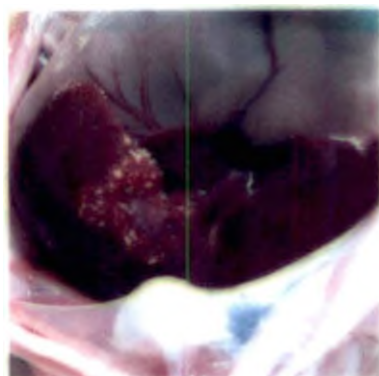Fig.5. Liver- necrosis in triphala group

Fig.6. Liver - control group

Fig.7. Liver - nodular lesions identified as Cholangiocellular carcinoma in aflatoxin group

Fig.8. Liver - cholangiocellular carcinoma in aflatoxin group

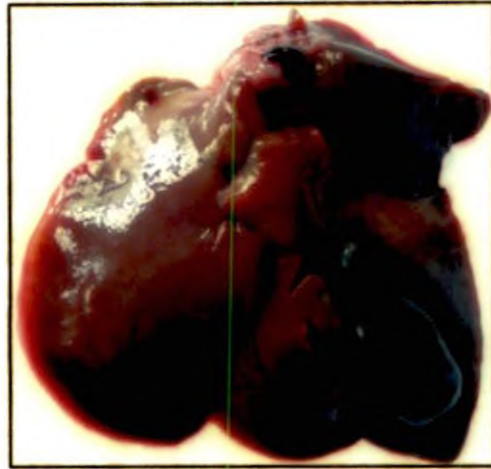**Fig.9.powdered rice culture containing aflatoxin**

Fig.10. Gall bladder - highly distended with thick
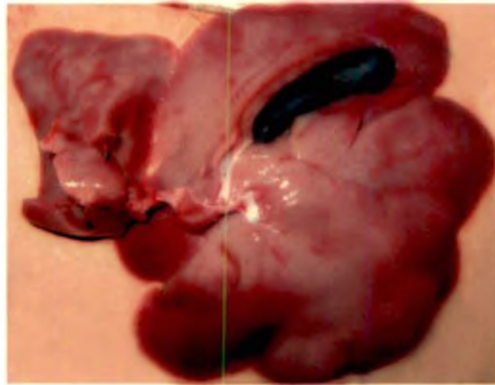 greenish bile in aflatoxin group

Fig.11. Gall bladder - moderately distended with thick
 greenish bile in triphala group

Fig.12. Gall bladder - control group

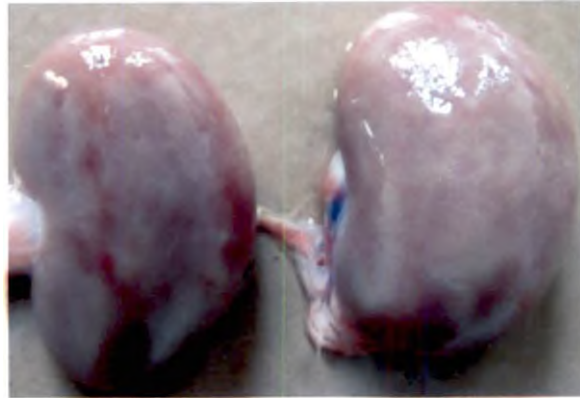Fig.13.Kidney - pale, enlarged and haemorrhages in aflatoxin group
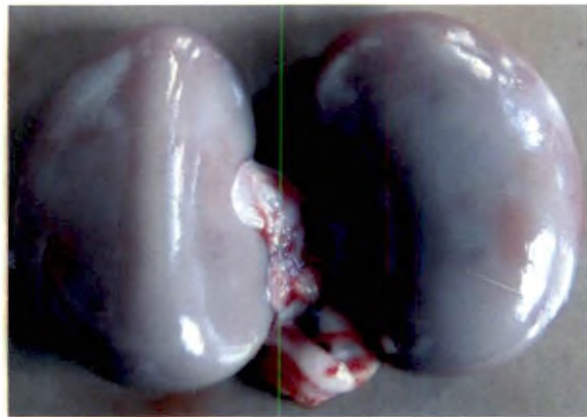
Fig.14. Kidney - pale and enlarged in triphala group

Fig.15. kidney - control group
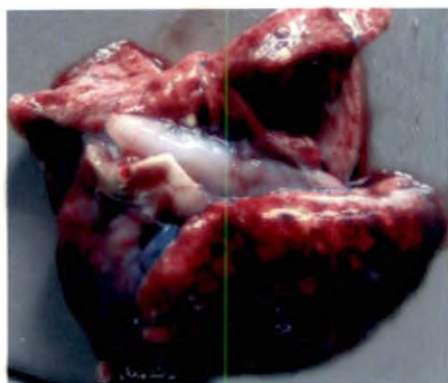
Fig. 16. Lung- congested in aflatoxin group
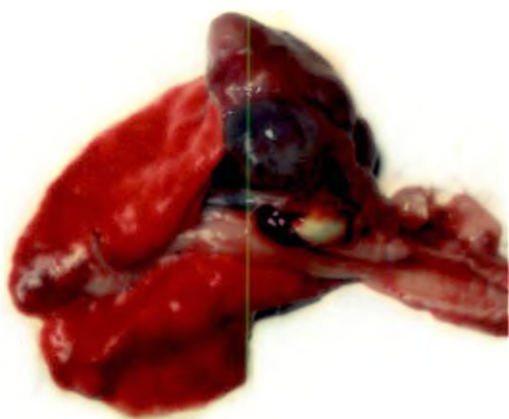
Fig. 17.Lung- triphala group
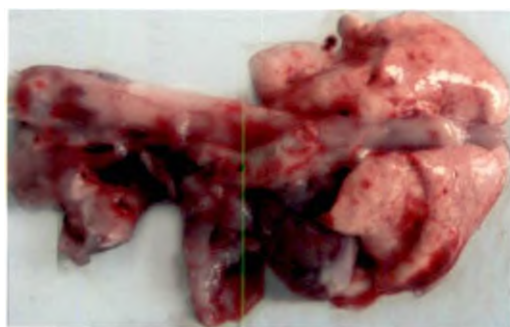
Fig.18. Lung- control group
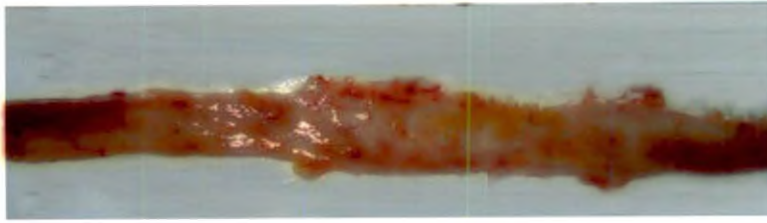
Fig.19. Intestine - Haemorrhagic enteritis in aflatoxin group

Fig.20. Intestine - Catarrhal enteritis in triphala group

Fig.21.Triphala
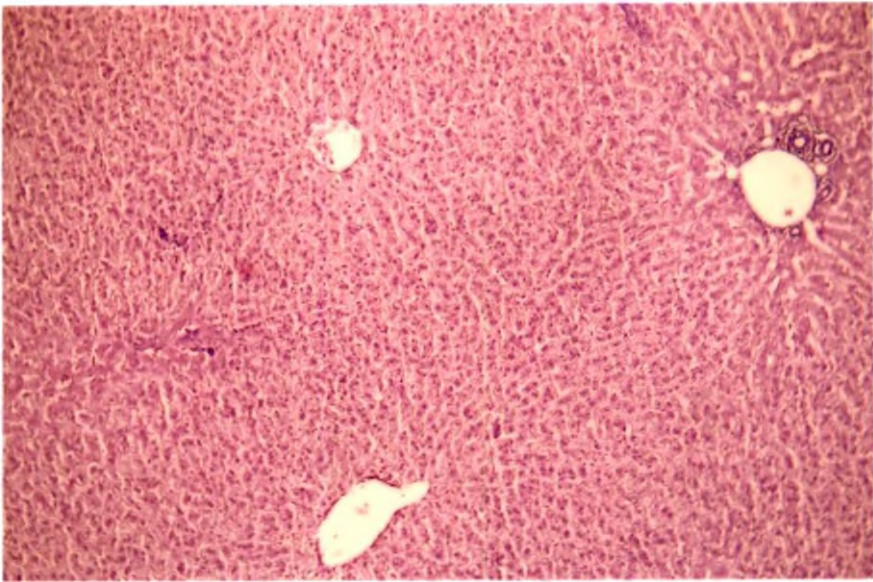
Fig.22. Liver - control group (H&Ex100)
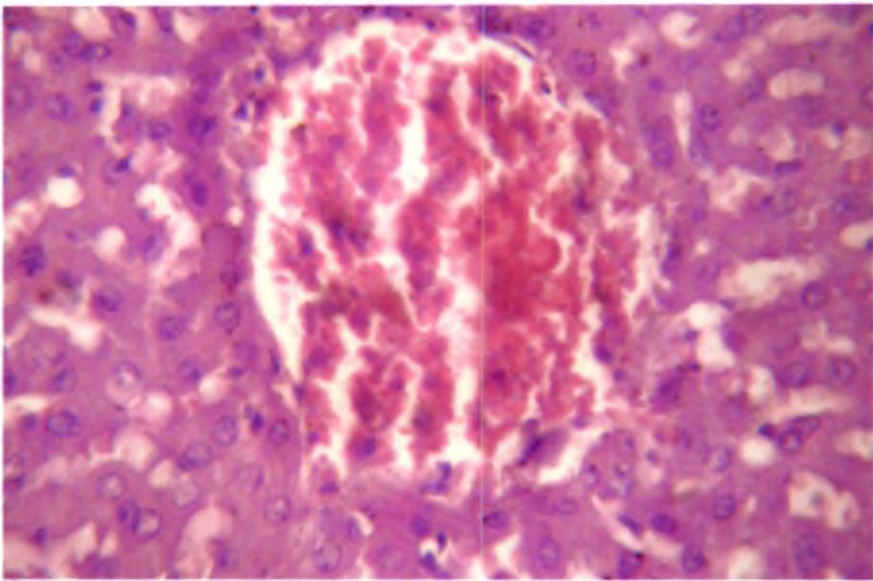
Fig.23.Liver - central venous congestion in aflatoxin group (H&Ex400)
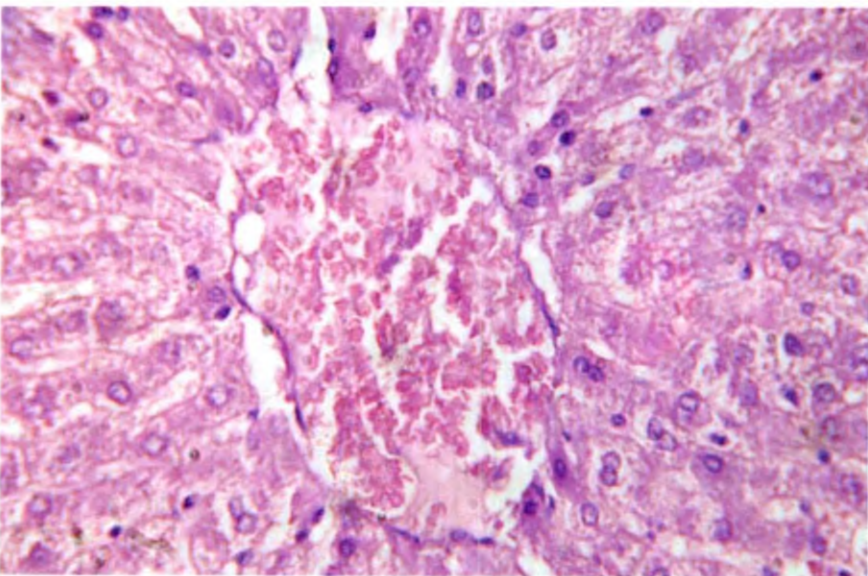
Fig.24. Liver - central venous congestion in triphala group (H&Ex400)
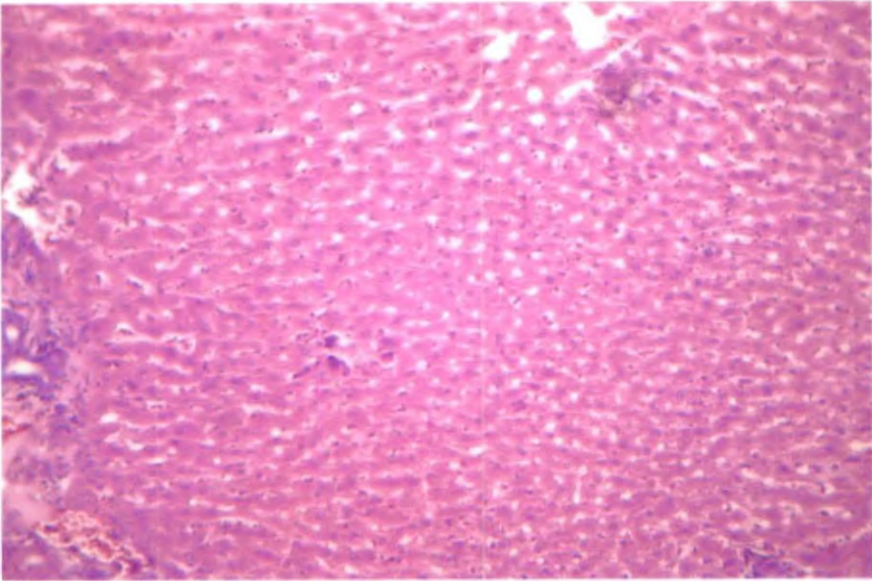
Fig.25.Liver - fatty change in aflatoxin group (H&Ex100)
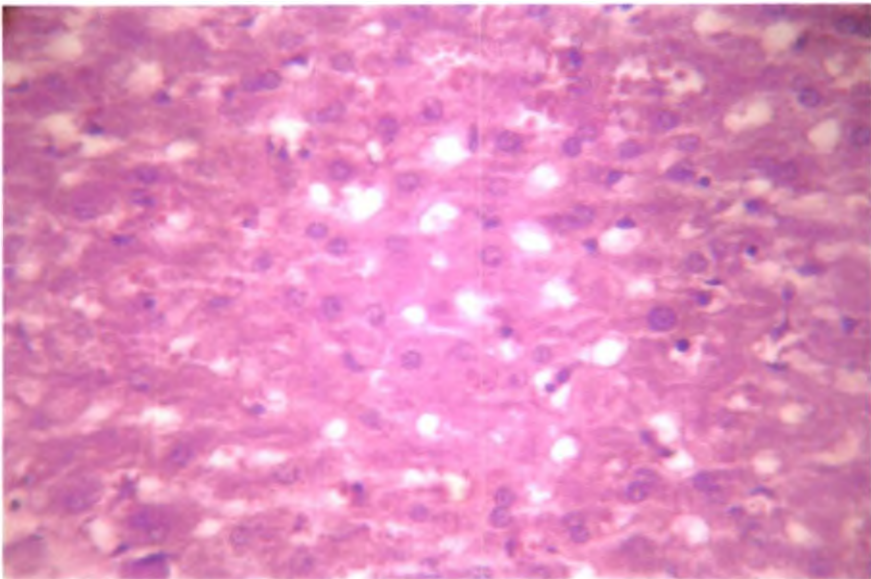
Fig.26. Liver - fatty change in aflatoxin group (H&Ex400)
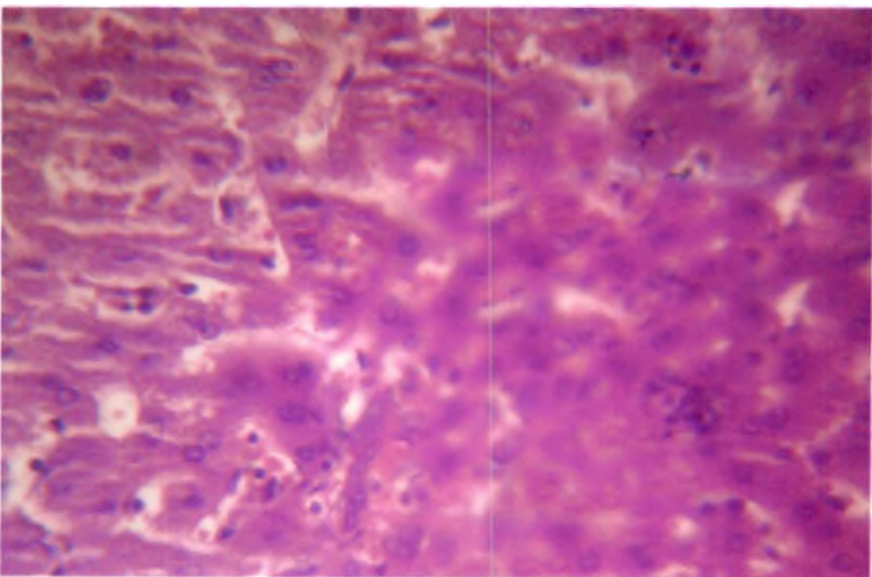
Fig.27. Liver - coagulative necrosis of hepatocytes in aflatoxin group (H&Ex400)
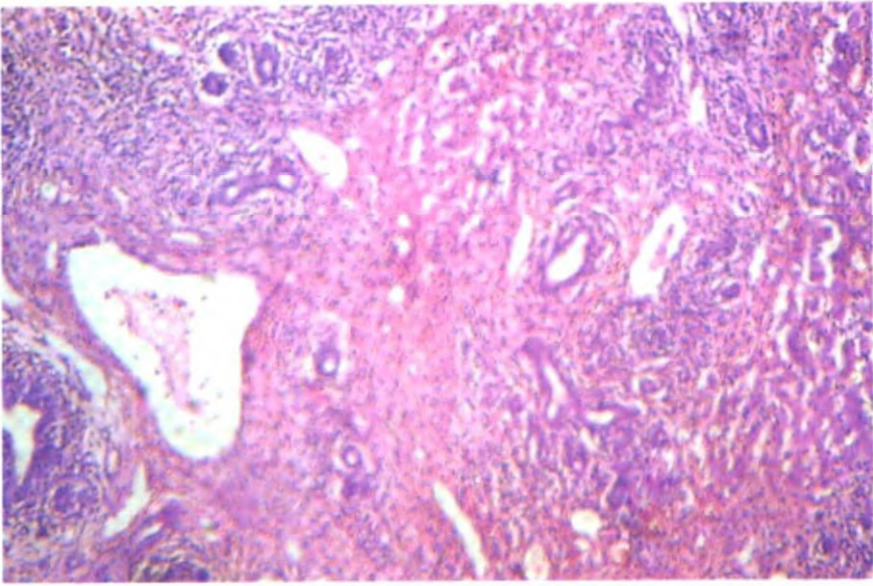
Fig.28. Liver - cholangiocellular carcinoma in aflatoxin group (H&Ex100)
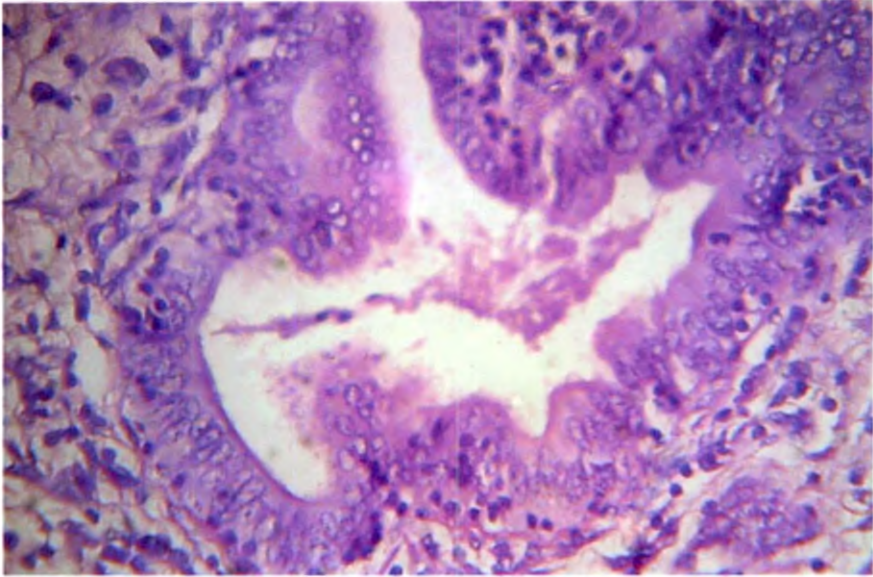
Fig.29.Liver - cholangiocellular carcinoma in aflatoxin group (H&Ex400)
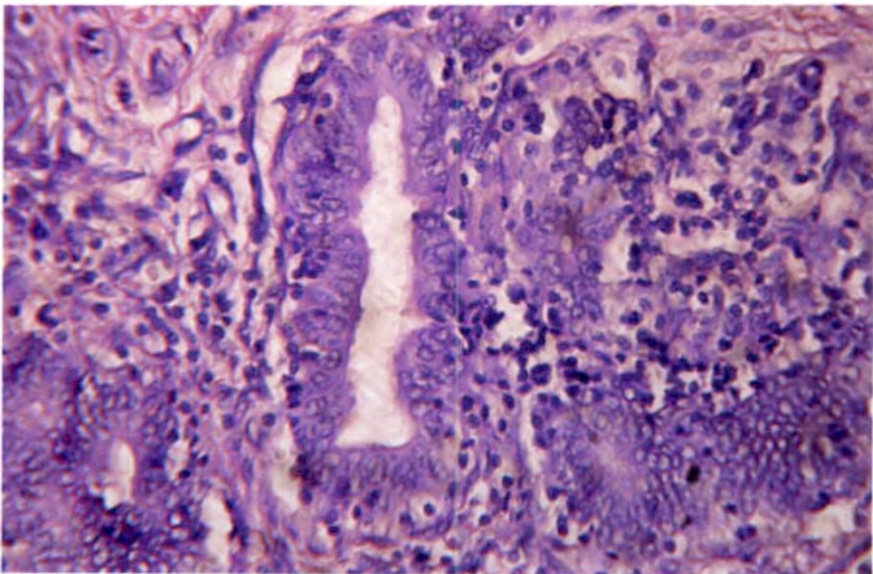
Fig.30. Liver -cholangiocellular carcinoma - neoplastic biliary epithelial cells
and dense abundant stroma in aflatoxin group (H&Ex400)
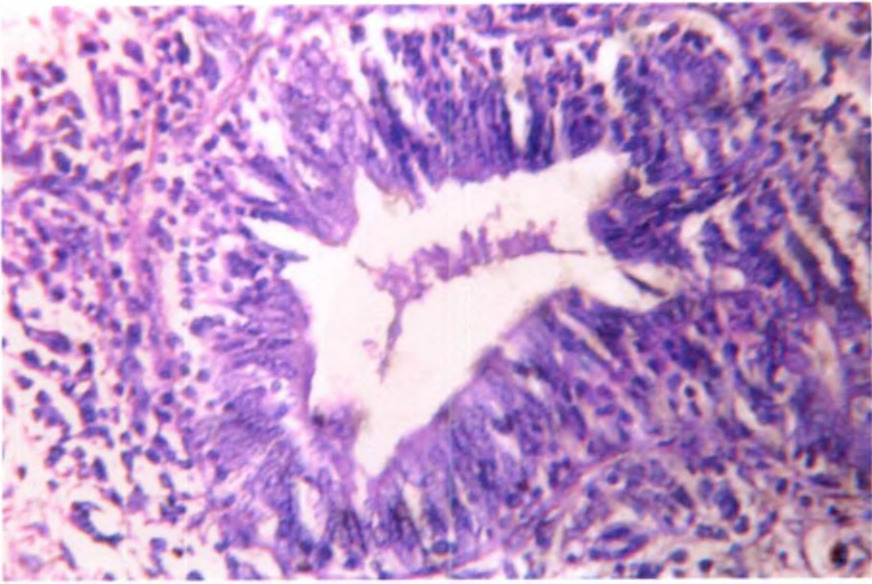
Fig.31. Liver - neoplastic cells almost filling the lumen of bile duct in cholangiocellular carcinoma (H&Ex400)
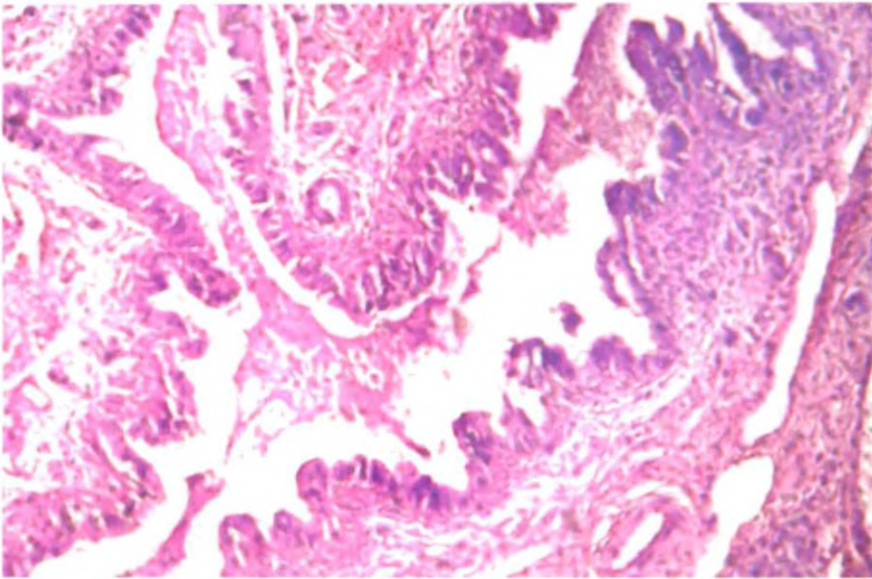
Fig. 32. Liver - hyperplastic biliary epithelial cells forming finger like projection into the lumen in aflatoxin group (H&Ex100)
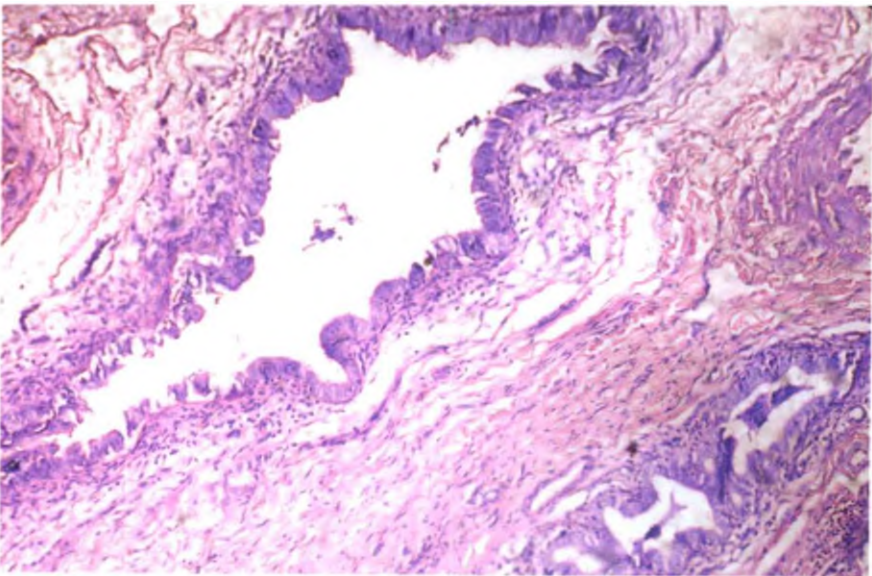
Fig.33. Liver - dense abundant supporting stroma in cholangiocellular carcinoma (H &Ex100)
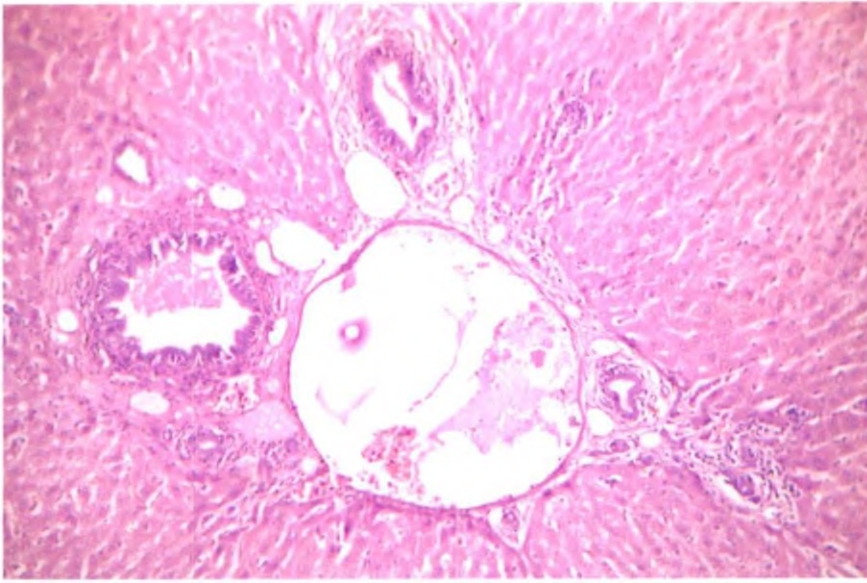
Fig.34. Liver - bile duct proliferation in triphala group (H&Ex100)
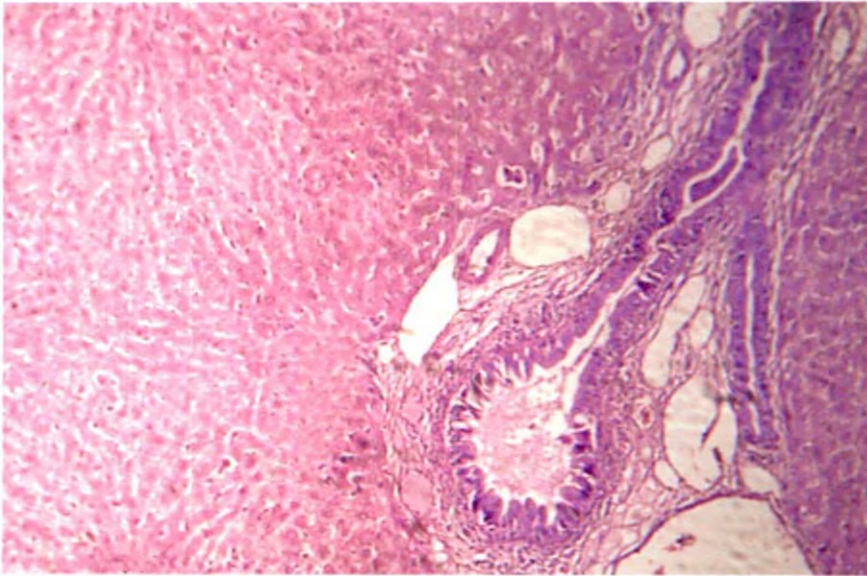
Fig.35. Liver - well maintained hepatic architecture and bile duct proliferation
in triphala group (H&E x100)
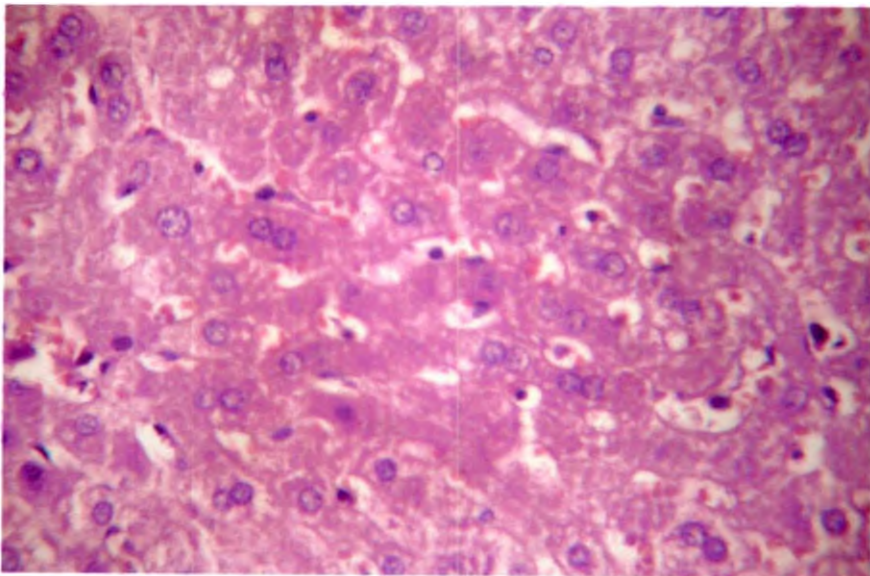
Fig.36. Liver- binucleate actively dividing hepatocytes and kupffer cell proliferation
in triphala supplemented group (H&Ex400)
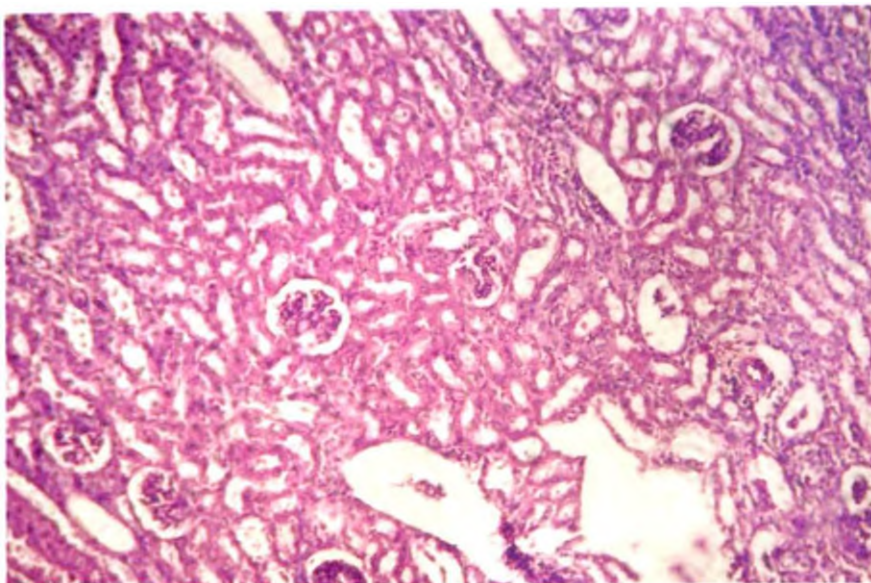
Fig.37. Kidney - control group (H&E x 100)
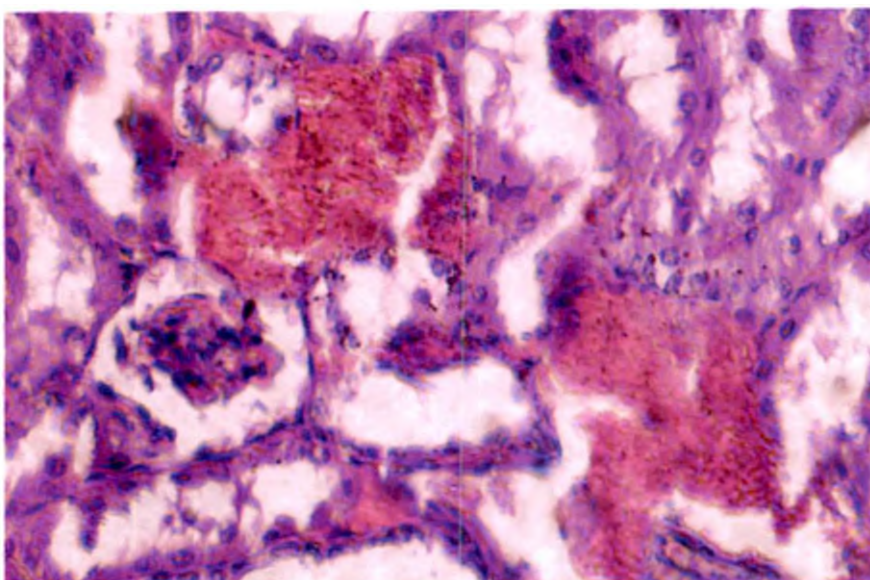
Fig.38. Kidney - glomerular and tubular haemorrhage in aflatoxin group (H&Ex400)
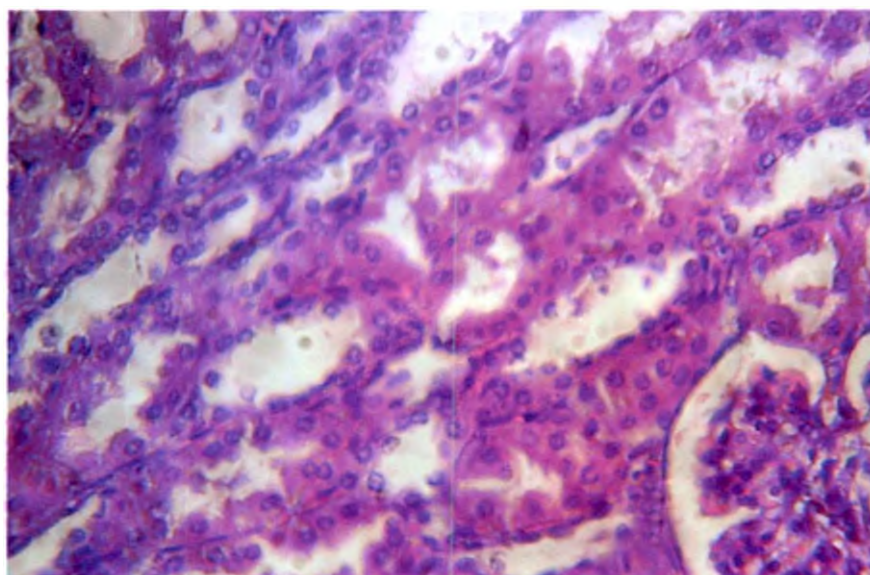
Fig.39. Kidney - glomerular and tubular haemorrhage in triphala group (H&Ex400)
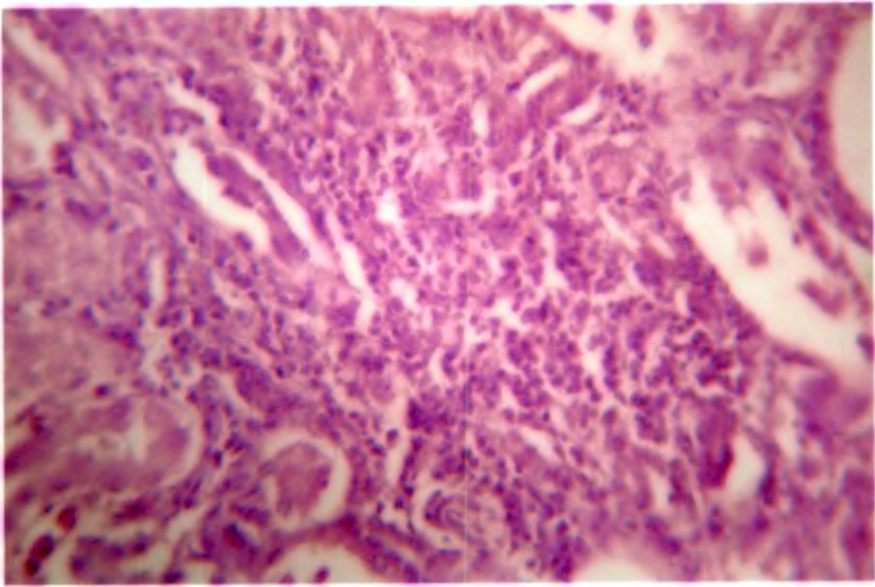
Fig.40. Kidney - mononuclear infiltration in aflatoxin group (H&Ex400)
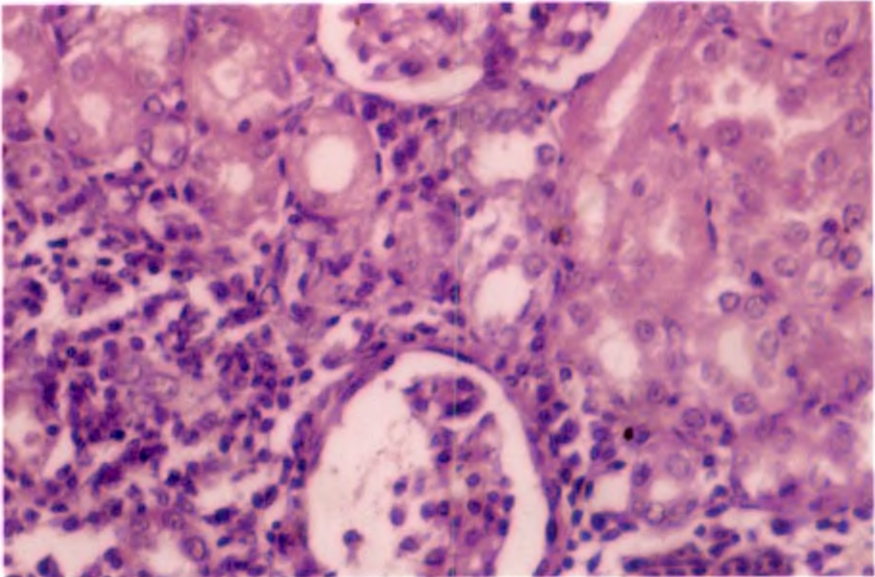
Fig.41. Kidney - mononuclear infiltration in triphala group (H&Ex400)
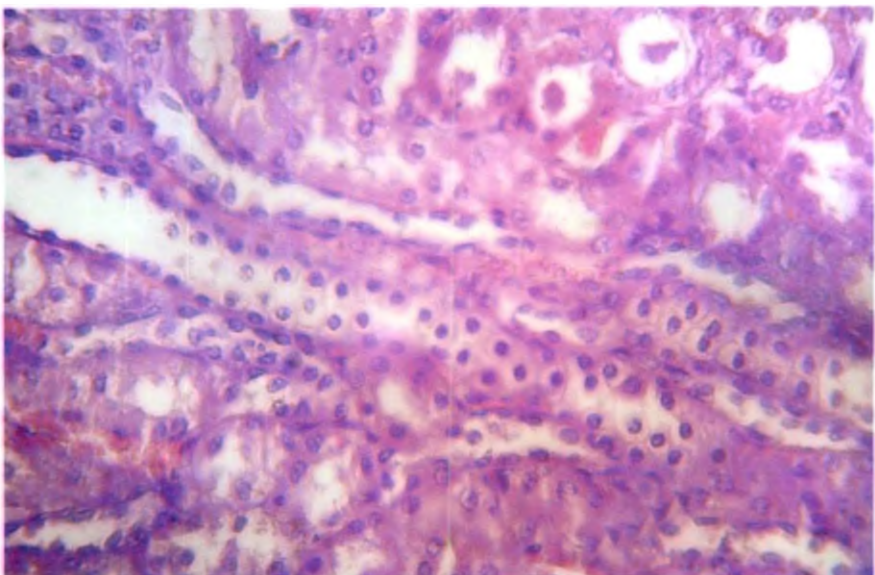
Fig.42. Kidney - degenerative changes in the tubules and existing nuclei
showing karyomegaly in aflatoxin group (H&Ex400)
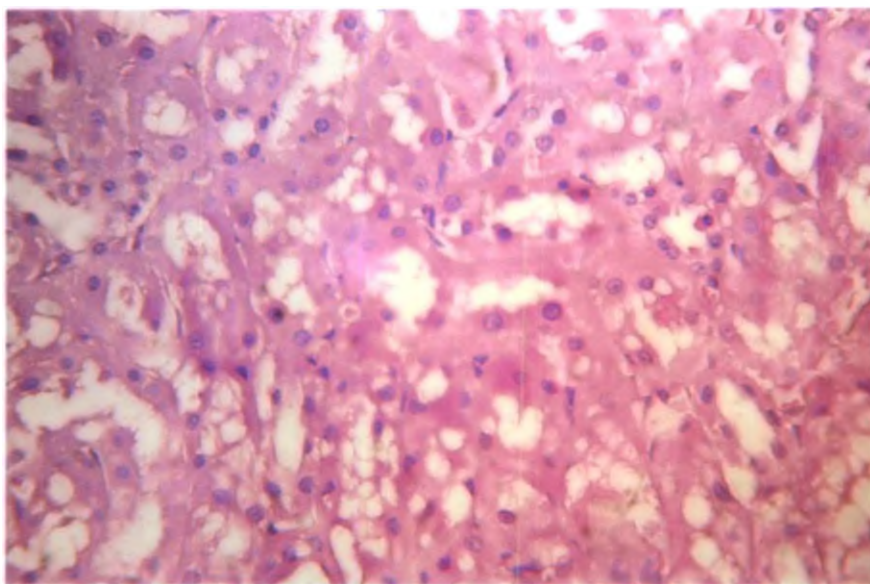
Fig.43. Kidney - necrotic changes in the tubules of aflatoxin group (H&Ex400)
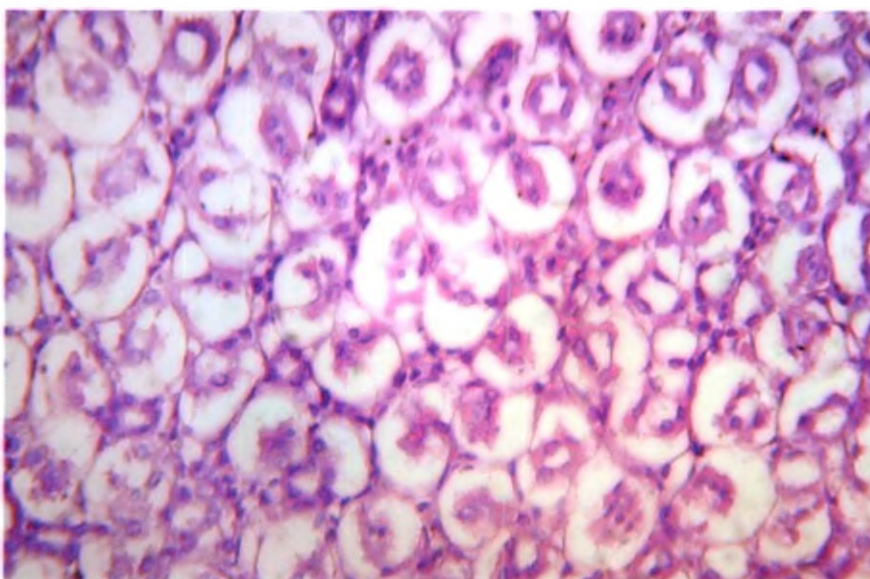
Fig.44. Kidney- desquamation of tubular epithelial cells in aflatoxin group (H&E x 400)
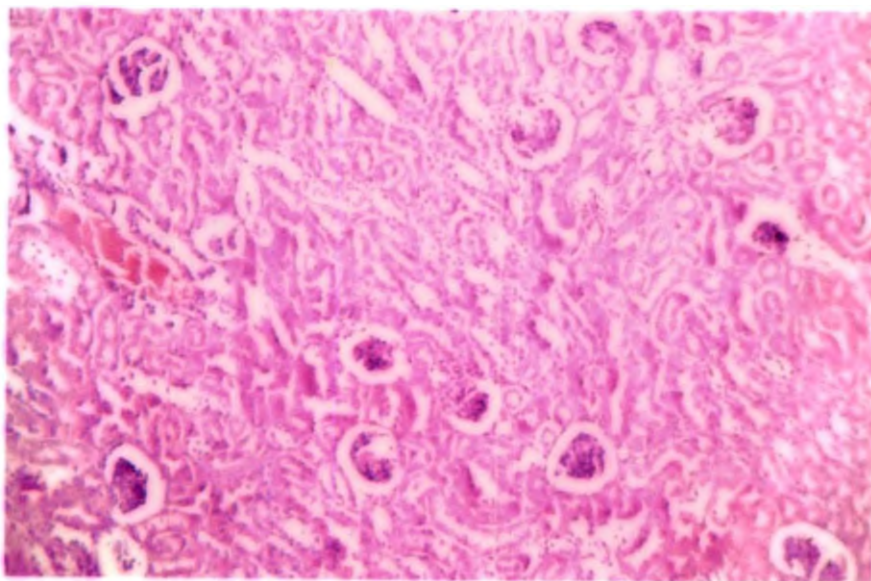
Fig. 45. Kidney- shrinkage of glomeruli and glomerular necrosis in
aflatoxin group (H&Ex100)
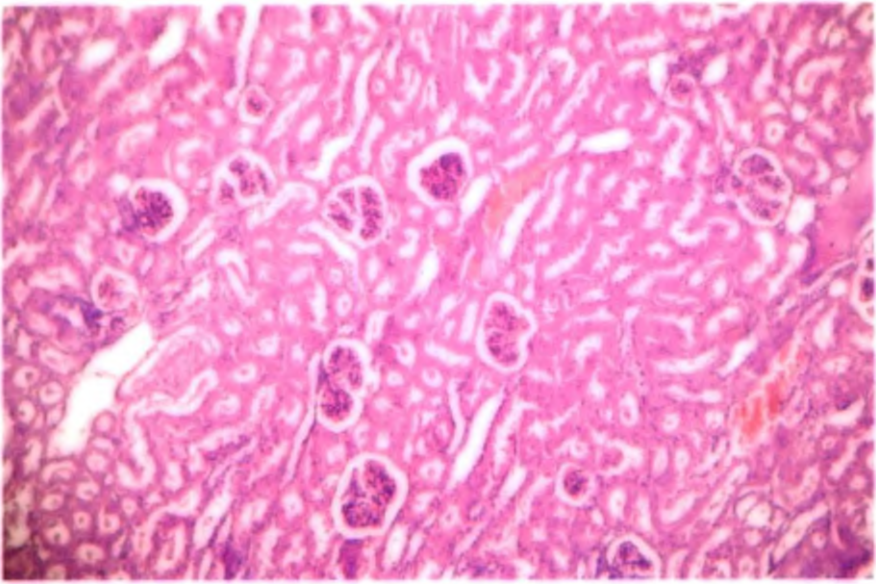
Fig. 46. Kidney - well maintained tubular epithelium and
less glomerular damage in triphala group (H&Ex100)
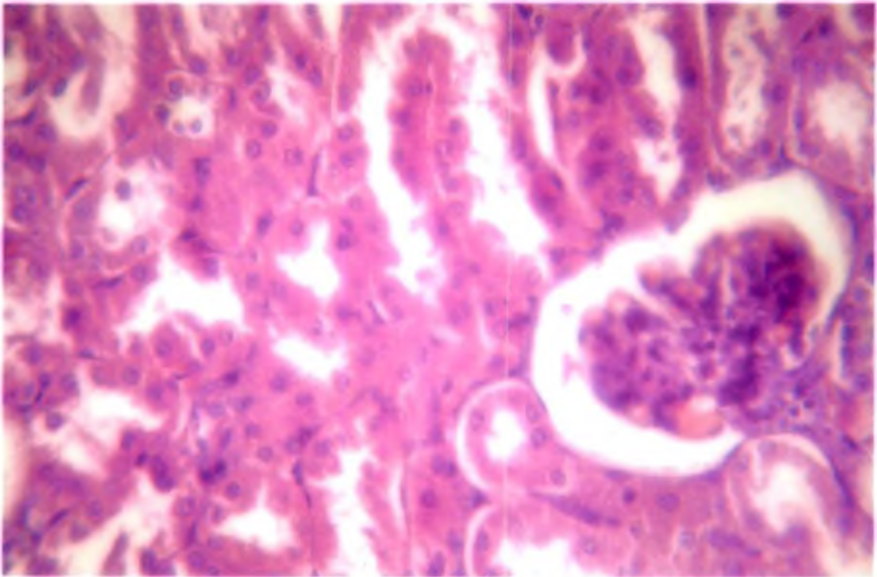
Fig.47. Kidney - well maintained tubular epithelium with intact
nucleus in triphala group (H&Ex400)
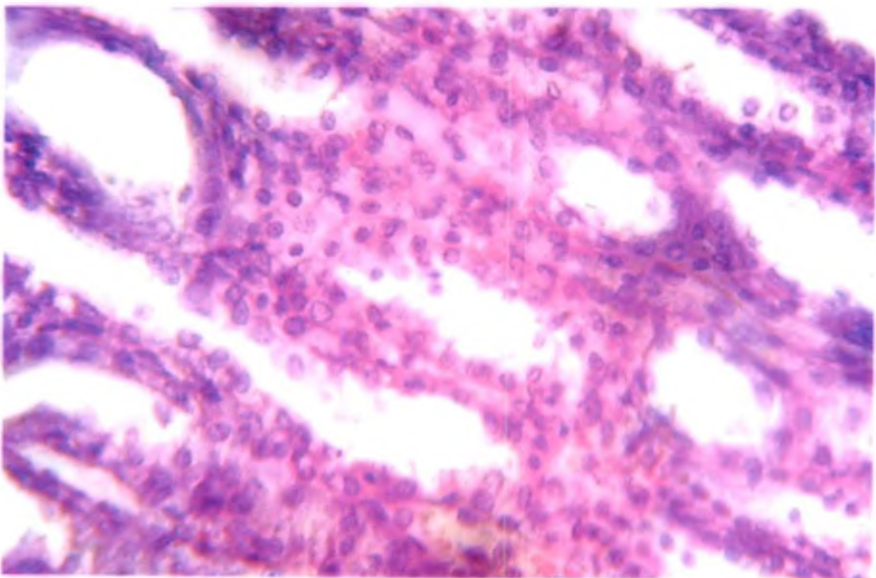
Fig.48. Kidney - tubules with more than one layer and cell crowding
in triphala group(H&Ex400)

Discussion

5. DISCUSSION

The results obtained in the study to find out the effect of supplementation of Triphala, a composite mixture of *Emblica officinalis, Terminalia chebula* and *Terminalia bellirica* on alleviation of aflatoxicosis in rabbits are discussed in this chapter.

5.1 CLINICAL SIGNS AND MORTALITY PATTERN

In the present study the clinical signs observed in aflatoxin fed rabbits included dullness, reduced water intake, dehydration, anorexia, emaciation and weakness which are in agreement with the findings of Maryamma *et al.* (1990a); Churamani and Chattopadhyay (1995); Sudhindra (1998); Arulmozhi (1999) and Dutta *et al.* (2006) in aflatoxicosis.

Aflatoxin is hepatotoxic which might be the reason for manifestation of weakness. The damage to liver parenchyma and other organs as evidenced by histopathological changes also could be attributed to other clinical signs such as anorexia and dullness.

Triphala is categorized as a rejuvenating ayurvedic herbal formulation used in various gastric problems including intestinal inflammation. The rabbits fed with this herbal ameliorating agent did not show any clinical signs compared to aflatoxin fed group, which indicated the possible role of triphala in combating the toxic effect of aflatoxicosis.

Here the aflatoxin treated group showed higher mortality than triphala group and control group. This was in agreement with the findings of Rao and Chakravarty (1999) and Anandkumar *et al.* (2005) who observed aflatoxin influenced mortality in experimental birds at 22 and 0.03 per cent respectively. Churchil (1996) and Arvind (2007) also reported higher mortality rate of broilers fed with 1 ppm of aflatoxin. On the contrary, Madheswaran *et al.* (2005a) did not

observe any mortality in quails fed with 3 ppm of aflatoxin from day of hatch to 35 days.

Poor feed intake, impaired liver functions, depressed protein synthesis and lipid utilization mechanisms might have affected the health of rabbits. In addition, aflatoxin, a potent immunosuppressant known to affect immunity. All these factors could have led to the increased rate of mortality in aflatoxin fed group. The livability of rabbits were moderately improved when supplemented with four per cent triphala.

5.2 BODY WEIGHT

In this study, rabbits fed with 0.5 ppm aflatoxin containing diet had significantly poor body weight than control throughout the experiment. The growth depressing effect of aflatoxin has been confirmed by several earlier authors at different dose levels in poultry (Yadav et al., 1996; Singh et al., 1999; Madheswaran et al., 2005a; Bhanuprakash et al., 2006; Arvind, 2007). The depression in body weight during aflatoxicosis might be due to hepatic cell damage and inhibition of protein and nucleic acid synthesis.

The supplementation of four per cent triphala in the aflatoxin contaminated diet (0.5 ppm) had no significant effect on body weight during second week of experiment. After second week of experiment the triphala fed rabbits had significantly higher body weight than aflatoxin group but significantly lower body weight than control group.

The results were in accordance with findings of Kalorey et al. (2004) who proved Toxiroak®, a polyherbal formulation containing Emblica officinalis supplementation significantly improved body weight of broilers in aflatoxicosis. Similarly, Sapcota et al. (2005) found dietary inclusion of Andrographis paniculata in combination with mycotoxin added feed improved body weight of broilers.

The higher body weight in triphala fed groups may be due to ameliorating effect of composite mixture of *Emblica officinalis, Terminalia chebula* and *Terminalia bellirica* and its ability to counteract hepatic damage, kidney damage and to cause increased feed intake.

5.3 HAEMOGRAM

The mean (± SE) packed cell volume, haemoglobin and total leukocyte count were significantly reduced in rabbits received aflatoxin contaminated diet at 0.5 ppm level than triphala supplemented group and control group. Similar findings were reported by earlier workers (Balakrishnan., 1992; Anbiah, 1996; Choudhary *et al.*, 1998; Madheswaran *et al.*, 2005a; Ahamad *et al.*, 2006).

The reduction in haemoglobin concentration and packed cell volume observed during aflatoxicosis could be due to reduced protein synthesis and hepatic damage as explained by Balakrishnan (1992) and Kalorey *et al.* (2004).

The reduced haemoglobin concentration, packed cell volume and total leukocyte count observed in this study due to aflatoxin contaminated diet were significantly counteracted by supplementation of triphala at four per cent level. Similar to present study, Mekala *et al.* (2006) proved curcumin and silymarin alone and in combination produced significant improvement in haematological parameters in aflatoxicosis of broilers. In a similar work, Bio- Bantox® (a mycotoxin binder) supplementation at the rate of five gram per ton of feed significantly improved mean Hb, PCV and TLC values in broilers fed ochratoxin at 1 ppm level was observed by Pathan *et al.* (2007).

There was lymphocytopenia and compensatory heterophilia in rabbits of toxin group at all intervals of study. The reduction in lymphocyte population might be due to the damaging effect of aflatoxin on lymphoid organs. This agrees with various reports (Balakrishnan, 1992; Kumar, 1999; Perozo and Rivera, 2003; Wangikar *et al.*, 2004). The alterations in monocyte, eosinophil and

basophil counts were not remarkable and supported findings of Sahoo *et al.* (1992).

The ameliorating effect of triphala at four per cent level during aflatoxicosis in rabbits (0.5 ppm) was evident from the increased leukocyte count with an increase in lymphocyte count. Maryamma *et al.* (1990c) noted similar findings in ducks given aqueous extract of *Tinospora cardifolia* to alleviate aflatoxicosis. There was higher total leukocyte count and lymphocyte count observed in ducks given aqueous extract of *Tinospora cardifolia* in a study to counteract the effects of aflatoxin supplementation (1 ppm).

5.4 SERUM ENZYMES

In the present study, feeding of aflatoxin showed significant ($P<0.05$) increase in the serum aspartate amino transferase (AST) levels throughout the study as compared to untreated controls. Though there was a significant ($P<0.05$) increase in AST levels in aflatoxin and triphala combination group (group II) as compared to control, the magnitude of increase was lower than toxin treated group.

In the present investigation, feeding of aflatoxin showed significant ($P<0.05$) increase in the serum alanine amino transferase (ALT) as compared to control rabbits on 30th and 60th day and maximum increase was recorded on 60th day of treatment. Supplementation of triphala has resulted in decreased ALT levels when compared to aflatoxin alone treated group. The decrease was only numerical on 30th day however, on 60th day the difference was more prominent and significant ($P<0.05$). This clearly indicates that triphala gave better protective effects in aflatoxicosis.

In the present study, the serum alkaline phosphatase (ALP) levels in aflatoxin fed group were significantly ($P<0.05$) higher as compared to untreated control rabbits on 30th and 60th day of post treatment. There was also significant

increase (P<0.05) in ALP levels in triphala group (group II) as compared to control but numerically lower level than toxin group.

ALT is present mainly in the hepatocytes and its elevation indicates hepatic necrosis and inflammation. AST activity is high in liver, kidney and increases due to liver damage, cirrhosis, parasitic hepatopathy and neoplasms. Increase in serum enzymes may be correlated to degenerative changes noticed in the liver leading to seepage of enzymes and increase in ALP is also due to *de novo* synthesis of enzyme due to biliary obstruction. The newly synthesized ALP is refluxed into the circulation (Kaneko *et al.*, 2008). The increased levels of serum enzymes in aflatoxicosis were reported by many workers (Arulmozhi *et al.*, 2000; Rohilla *et al.*, 2001; Nataraja *et al.*, 2004; Sankh *et al.*, 2005; Nath and Sarma, 2005).

Bhanuprakash *et al.* (2006) proved treatment with Partysmart®, a herbal formulation containing *Emblica officinalis* could effectively reduce the levels of serum AST, ALP, ALT in alcoholic liver diseases. In the present study supplementation of triphala resulted in reduction of elevated serum enzyme levels due to aflatoxin feeding at different intervals of experiment. But values of serum enzymes remained significantly (P<0.05) higher in triphala supplemented group than that of control during the entire experimental period. This indicated that supplementation of triphala is having some ameliorating effect on increase in enzyme values in aflatoxicosis.

5.5 GROSS AND HISTOPATHOLOGICAL STUDY

In the present study there was enlargement of liver and kidneys of rabbits fed with aflatoxin (0.5 ppm) treated feed. The enlargement of liver and kidney indicates the degenerative damage caused to them by the aflatoxin. Toxic agents directly disrupt the functional anatomy and physiology of hepatocytes. Enlargement and yellowish discolouration of liver might be due to the deposition of lipids in the hepatocytes. Similar enlargement and yellowish discolouration of

liver and enlargement of kidneys were observed by various researchers. (Maryamma *et al.*, 1990a; Maryamma *et al.*, 1990b; Maryamma *et al.*, 1990c; Manimaran *et al.*, 2001; Srivani *et al.*, 2003; Madheswaran *et al.*, 2005b).

In the rabbits, degenerative changes with congestion and haemorrhages in liver and kidney were a common microscopic lesion in aflatoxicosis. Haemorrhages and immuosuppression were due to inhibition of clotting factors and protein synthesis (Dutta *et al.*, 2006). Haemorrhages can also be attributed to the direct toxic effect of aflatoxin on the endothelium of blood vascular system.

In most of the aflatoxin fed rabbits fatty changes were observed in the hepatocytes. This microscopic lesion are consistent with earlier observations (Dutta *et al.*, 2006 and Arulmozhi *et al.*, 2007). Lipids are normally transported to the liver in the form of free fatty acids and chylomicrons. Within hepatocytes low density lipoproteins are formed and released into plasma as a readily available energy source. Injury to hepatocytes can lead to accumulation of lipids because of decreased formation or export of lipoprotein by hepatocytes and decreased oxidation of fatty acids within hepatocytes. Here aflatoxin damaged the hepatic cells and led to fatty change (Mc Gavin *et al.*, 2007)

There was excessive infiltration of mononuclear cells in the hepatic and kidney parenchyma. Mononuclear infiltration might be related to immunological response by the body to get rid of the toxic materials (Dutta *et al.*, 2006). Even though aflatoxin caused degeneration of hepatocytes, proliferative changes like fibrosis, biliary hyperplasia and cirrhosis were observed. These are considered as response to liver injury by the aflatoxin. (Mc Gavin *et al.*, 2001)

The toxic effects of aflatoxin are related principally to the binding of its metabolites to macromolecules, in particular nucleic acids and nucleoproteins. So the toxic effects include carcinogenesis and teratogenesis (Jubb *et al.*, 2005). In the present study the carcinogenic effect of aflatoxin was evidenced by the tumors in the liver. The nodules on the liver was identified as cholangiocellular carcinoma on histological evaluation. Cumulative effect of toxin on longer exposure might have led to formation of neoplastic cells.

Cholangiocellular carcinoma are malignant neoplasms of biliary epithelium that usually arise from intra hepatic bile ducts. These tumors were composed of cells that retain resemblance to biliary epithelium. The epithelial components were separated by fibrous connective tissue, an abundant deposition of collagen termed as scirrhous response is responsible for firm texture of neoplasm. The margins were characterized by local invasion of tumor cells of surrounding hepatic parenchyma and multiple sites of hepatic necrosis were also common in adjacent parenchyma (Ashley , 1978).

Maryamma *et al.* (1990c) observed reddish circumscribed nodule on right lobule of the liver in ducks fed with 1 ppm aflatoxin in the diet and the tumor was identified as cholangiocellular carcinoma. In another study it was observed that pigs given aflatoxin for 36 weeks produced cholangiocellular carcinoma (Maryamma *et al.*, 1994)

Triphala effectively ameliorated these destructive effects of aflatoxin in the liver and kidney. Abundant binucleate hepatocytes and multilayered kidney tubules and cell crowding in the lumen of tubules were noted in rabbits of triphala group. These are suggestive of repair and regeneration. Dutta *et al.* (2006) observed hyperplastic nodules in liver parenchyma consisting of newly

formed hepatocytes reflects the regenerative response to the injurious effect of the toxin. Sajitha (2002) noted ductular or acinar pattern of hepatocytes, megalocytosis with nuclear enlargement and regenerating nodules consisting of clones of hepatic epithelial cells in aflatoxicosis. Regenerative process in the kidney tubules after surgical excision was reported by Samsonidze (1960) where thickness of the tubular walls increased, lumen size were reduced and cells in the tubular walls became taller. There was marked cellular hyperplasia.

The fruits of *Terminalia chebula, Terminalia bellirica* and *Emblica officinalis* are important herbal raw materials containing polyphenols. Constituents of triphala has been reported to be a rich source of vitamin C, ellagic acid, gallic acid, chebulinic acid, bellericanin, β-sitosterol, flavanoids. The phenolics, particularly polyphenols exibit a wide variety of beneficial biological activities including immunostimulant, hepatoprotective, anti-inflammatory and anticarcinogenic. Polyphenolic compounds in these herbals have been reported to possess antioxidant properties and free radical scavenging abilities. This might be responsible for protective effect provided by triphala (Girdhani *et al.*, 2005). The chloroform and acetone extracts of triphala showed inhibition of mutagenicity (Kaur *et al.*, 2002) indicated that triphala could give protection against carcinogenesis.

It could be concluded that the inclusion of composite mixture of *Emblica officinalis, Terminalia chebula* and *Terminalia bellirica* at four per cent level to the aflatoxin contaminated feed (0.5 ppm) can counteract the toxic effects of aflatoxicosis mainly by its hepatoprotective, nephroprotective and anti carcinogenic effects. Further trials have to be conducted to make sure that at what level triphala will completely protect the liver and kidneys from harmful effects of aflatoxin.

Owing to the diverse nature of the carcinogens, a combination of antimutagens will be probably necessary for cancer therapy and it is essential to confirm the anticarcinogenic effect through the use of animal models. The present work is probably an initial attempt but provides scientific validation for the popular use of triphala and need for further research in the field.

Summary

6. SUMMARY

An experiment was conducted at the Centre of Excellence in Pathology, College of Veterinary and Animal sciences, Mannuthy to study the protective effect of composite herbal mixture 'triphala' containing *Emblica officinalis, Terminalia chebula* and *Terminalia bellirica* on aflatoxicosis in rabbits. The study was conducted for a period of two months with twenty four rabbits aged four weeks. Rabbits were weighed individually and divided into three treatment groups namely group I, group II and group III with eight rabbits each.

Rice culture containing 27 ppm of aflatoxin was added appropriately to the ration to get a final concentration of 0.5 ppm. Aflatoxin contaminated diet at 0.5 ppm and combination of aflatoxin added feed (0.5 ppm) and triphala at four percent were given for group I and group II respectively. Group III was provided with control diet. Standard management practices were followed throughout the experimental period. Feed and water were provided *ad libitum*. Different parameters like body weight, haemogram, serum profile and gross and histopathological changes were studied.

Clinically toxin treated rabbits were dull, emaciated, weak and showed higher mortality compared to triphala group. The triphala group and control groups did not show any clinical signs throughout the experimental period and supplementation of triphala improved livability of rabbits.

The body weight showed a gradual increase in all the three groups throughout the experimental period. A significant ($P<0.05$) difference in body weight was noted between the groups on 28th day onwards. Triphala group showed significantly higher body weight than toxin fed rabbits which was attributed to the ameliorating effect of triphala on aflatoxicosis in rabbits.

Haematological values showed significant (P<0.05) reduction in haemoglobin, packed cell volume, total leukocyte count and lymphocyte count in aflatoxin (0.5 ppm) fed rabbits as compared to triphala group and control group. But heterophil count showed a significant (P<0.05) increase in toxin fed rabbits as compared to other two groups. This shows triphala supplementation at four percent level in the aflatoxin mixed feed significantly (P<0.05) ameliorated the changes induced by the aflatoxin by improving haemoglobin, packed cell volume, total leukocyte count and lymphocyte count and reducing heterophil count on 30th day it self.

Serum enzyme analysis revealed a significant (P<0.05) increase in the AST, ALT and ALP values in the aflatoxin fed group and triphala group but triphala group showed numerically lower values. Increase in serum enzymes observed on 30th and 60th day indicated hepatic damage.

The rabbits died during the experiment and the animals euthanized at the end of two months were subjected to detailed postmortem examination. On gross examination, liver and kidney of the toxin fed group were pale and enlarged. Nodular lesions of varying sizes were observed in liver of three rabbits fed on toxin added diet.

Microscopically, aflatoxin produced extensive vascular changes and degenerative changes in liver and kidneys. There was congestion of vessels in both liver and kidney. Kidney showed haemorrhages in the cortical and medullary areas. Diffuse fatty change and coagulative necrosis were noted in liver. Kidneys showed marked glomerular and tubular necrosis. Mononuclear infiltration was noted in both organs. Chronic hepatic lesions evidenced by biliary hyperplasia, bile duct proliferation and periductular fibrosis were observed. The nodular lesions seen on the liver of rabbits on histopathological examination revealed cholangiocellular carcinoma. Similar lesions were observed in triphala group but with reduced intensity and the nodular lesions were not

observed in the liver. Binucleate actively dividing hepatocytes were abundant in the parenchyma of triphala supplemented group. Kidney tubules lined by more than one layer and cell crowding in the lumen were noted. These features are suggestive of repair and regenerative process in triphala supplied group.

Results of the present study revealed that aflatoxin at 0.5 ppm level in the feed adversely affected body weight, haemato-biochemical parameters and produced gross and histopathological alterations in liver and kidneys. But inclusion of triphala at four per cent level in the diet significantly ameliorated the toxicity by hepatoprotective, nephroprotective and anti carcinogenic effects. Constituents of triphala has been reported to be a rich source of vitamin C, ellagic acid, gallic acid, chebulinic acid, bellericanin, β-sitosterol, flavanoids and polyphenols. It harbors constituents with promising antimutagenic and anticarcinogenic potential that should be investigated in detail.

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EFFECT OF A COMPOSITE MIXTURE OF *Emblica officinalis, Terminalia chebula* AND *Terminalia bellirica* ON AFLATOXICOSIS IN RABBITS

INDU. K

Abstract of the thesis submitted in partial fulfilment of the requirement for the degree of

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Kerala Agricultural University, Thrissur

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Centre of Excellence in Pathology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR - 680651
KERALA, INDIA

ABSTRACT

Among the various mycotoxins, aflatoxins have been the subject of most intensive research because of the extremely potent cytotoxic and carcinogenic effects. Mycotoxicosis and its counteraction have received greater attention by researchers in the last few decades. In this context an evaluation study on the protective effect of a herbal composite mixture triphala (containing *Emblica officinalis, Terminalia chebula* and *Terminalia bellirica*) on aflatoxicosis in rabbits was carried out.

The study was conducted for a period of two months with twenty four rabbits divided into three groups of eight each. Rice culture containing 27 ppm of aflatoxin was added appropriately to the ration to get a final concentration of 0.5 ppm. Group I was given 0.5 ppm aflatoxin contaminated feed and group II was given combination of triphala (four per cent) and aflatoxin (0.5 ppm) contaminated diet. Group III was provided with control diet.

There were reduction in body weight gain, Hb, PCV, TLC, lymphocyte count while heterophil count, AST, ALT and ALP levels showed an increase in the toxin fed animals. Addition of triphala at four percent level in the aflatoxin contaminated feed effectively counteracted these changes. The gross and histopathological changes due to aflatoxin were reduced in triphala group. There was widespread vascular changes, extensive necrotic changes, bile duct proliferation, biliary hyperplasia and cholangiocellular carcinoma in the liver and necrotic changes in the kidney of toxin group. These changes were reduced in intensity in triphala group and there was no development of tumors in liver. Regenerative process were well pronounced in the liver and kidney of triphala group.

Hence the present study revealed that supplementation of composite mixture of *Emblica officinalis, Terminalia chebula* and *Terminalia bellirica* at four per cent level could counteract the toxic effects of aflatoxicosis in rabbits.