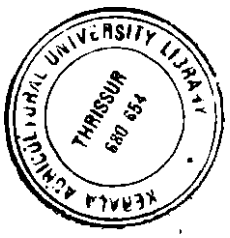
EFFECT OF AMLA (*Emblica officinalis*) ON HEPATIC FUNCTION IN BROILER CHICKEN



By

SAJITHA. I. S.

THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

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

2002

DECLARATION

I hereby declare that this thesis entitled **"Effect of Amla (*Emblica officinalis*) on hepatic function in broiler chicken"** 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
26/10/2002

Sajitha, I.S.

CERTIFICATE

Certified that the thesis entitled **"Effect of Amla (*Emblica officinalis*) on hepatic function in broiler chicken"** is a record of research work done independently by **Dr. Sajitha, I.S.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

Lalitha

Dr. C.R. Lalithakunjamma
(Chairperson, Advisory Committee)
Associate Professor
Centre of Excellence in Pathology
College of Veterinary and
Animal Sciences, Mannuthy

Mannuthy
26/10/2002.

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Dr. Sajitha, I.S.,** a candidate for the degree of Master of Veterinary Science in Veterinary Pathology, agree that the thesis entitled **"Effect of Amla (*Emblica officinalis*) on hepatic function in broiler chicken"** may be submitted by Dr. Sajitha, I.S., in partial fulfilment of the requirement for the degree.

Dr. C.R. Lalithakunjamma
Associate Professor
(Chairperson, Advisory Committee)
Centre of Excellence in Pathology
College of Veterinary and Animal Sciences, Mannuthy

Dr. K.V. Valsala
Professor and Head
Centre of Excellence in Pathology
(Member)

Dr. P.K. Ismail
Professor
Centre of Excellence in Pathology
(Member)

Dr. Leo Joseph
Associate Professor
University Poultry Farm
(Member)

External Examiner
Dr M Gopalakrishnan Nair
Assoc. Professor
Dept of Pathology
Rajiv Gandhi College of Veterinary and Animal Sciences
Kurumbapet Pondicherry

ACKNOWLEDGEMENT

I extol the kind/co-operation and parental affection offered to me by my outstanding mentor, ***Dr. C.R. Lalithakunjamma****, Associate Professor, Centre of Excellence in Pathology and Chairperson of the Advisory Committee, from the initiation of the work to the ship-shaping of the manuscript. Her meticulous guidance, sumptuous suggestions, tremendous patience and co-operation and personal attention to the works are noteworthy.*

I deem it my privilege in expressing my heart felt gratitude and thanks to ***Dr. K.V. Valsala****, Professor and Head, Centre of Excellence in Pathology and member of the Advisory Committee for her constant encouragement, constructive criticism, never failing support and invaluable advice at every stage of this research work.*

I owe a special word of thanks, with great fondness to ***Dr. P.K. Ismail,*** *Professor and member of the Advisory Committee, Centre of Excellence in Pathology, for his timely advices, incessant help and prompt correction of thesis.*

There are no words to pay my respect and deep sense of gratitude to ***Dr. Leo Joseph****, Associate Professor and member of the Advisory Committee, University Poultry Farm, for his expert advice, valuable suggestions, constructive criticism and ardent encouragement during my work.*

I humbly place on record my respect and gratitude to ***Dr. N. Divakaran Nair****, Assistant Professor (Sel. Grade), Centre of Excellence in Pathology for his valuable suggestions and for sharing his vast professional knowledge. He instilled the value of logic and reasoning by the mind churning questions he asked every now and then.*

My sincere thanks to ***Dr. A. Rajan****, Dean (Retd.),* ***Dr. T. Sreekumaran****, Professor,* ***Dr. N. Vijayan****, Associate Professor,* ***Dr. Mammen. J. Abraham****,*

Assistant Professor and ***Dr. Koshy Varghese****, Associate Professor, for the help and encouragement rendered by them.*

I gratefully acknowledge the wholehearted help rendered for statistical analysis by ***Smt. Sujatha****, Associate Professor and Head, Department of Statistics.*

I am indebted to ***Dr. P.T. Philomina****, Professor and Head and* ***Dr. V. Ramnath****, Assistant Professor, Department of Physiology,* ***Dr. T.V. Vishwanathan****, Associate Professor, Department of Nutrition for their pleasant co-operation and indispensable help for the completion of my work.*

I remember with gratitude the help rendered by ***Mr. Chandrasekharan and other staff members****, Central Instrumentation Lab, for their technical assistance.*

Words are often incapable of expressing the hearts' language. The moral support and encouragement given by my friends ***Bala*** *and* ***Bisi*** *is something that words or deeds cannot express. Within the limits of the lexicon, I thank them for staying with me through thick and thin.*

A bouquet of thanks to ***Dr.R. Lakshmi*** *and* ***Dr. Suraj*** *for their support memorable concern and warm friendship which produced a cordial environment.*

I offer thanks to my departmental colleagues, ***Dr. Pradeep, Dr. Sivakumar, Dr. Rekha*** *and* ***Dr. Smitha*** *for their support and co-operation.*

This task would not have been completed successfully, but for the understanding, love, mental support and encouragement by my friends ***Babi, BinduRaj*** *and* ***Deepa, A.K.***

Thanks are due to ***Dr. P. Bindu,Dr. J.P. Smitha*** *and* ***Dr. H. Shameem*** *for their support and help rendered.*

I wish to acknowledge ***Dr. P.X. Antony****, Department of Microbiology for his words of inspiration and encouragement.*

A special note of thanks to ***Dr. Manju, Dr. Chintu, Dr. Prasanna, Dr. Yuvaraj*** *and* ***Dr. Chitra*** *for their warm friendship and help rendered.*

I extent my thanks to ***Dr. Suresh*** *for his timely help which made a difficult task much easier.*

Special thanks to ***Dr. S. Thiruveni*** *for the help rendered.*

I wish to acknowledge the co-operation and help rendered by the ***non-teaching staff****, Centre of Excellence in Pathology.*

Thanks are due to ***K.V. Shaji's Viswanatha Ayurvedic Pharmaceuticals****, Kakkanad, Kochi-35 for providing good quality amla powder necessary for the research work.*

A special note of thanks is due to ***Asha Rajagopal****, for her sisterly help, support and encouragement across the miles.*

Thanks are due to the ***Dr. P.P. Balakrishnan, Dean****, Faculty of Veterinary and Animal Sciences, for the facilities provided for the study.*

I acknowledge my sincere thanks to the ***Kerala Agricultural University*** *for providing me the fellowship for the post graduate programme.*

Special thanks are due to ***Mr. O.K. Ravindran*** *and other members, C/o Peagles for the patient, prompt and diligent preparation of the manuscript.*

Last but not the least, thanking ***Almighty*** *for all the things I have and I don't ..... And for helping my small boat find the shore safely – through the love and prayers of* ***my parents and family members****.*

SAJITHA, I.S.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

16	Congestion, perivascular accumulation of mononuclears fatty change and sinusoidal widening - Group I – H & E x 250	55
17	Severe fatty change in liver - Group I – H & E x 250	55
18	Fat globules in the hepatocytes coalesced to form fat cysts - Group I – H & E x 63	55
19	Severe fatty change 'Signet ring' appearance of some hepatocytes and kupffer cell proliferation - Group I – H & E x 1000	55
20	Fat globules stained red with Oil-Red-O-Group I x 250	56
21	Fatty change, sinusoidal congestion and perivascular accumulation of mononuclears - Group I – H & E x 250	56
22a	Nodular hyperplasia of hepatocytes - Group I – H & E x 250	56
22b	Hyperplastic hepatocytes with vesicular nucleus – Group I – H&E x1000	56
23	Proliferating fibroblasts in the liver - Group I – H & E x 1000	57
24a	Focal hepatic fibrosis – Group I x 160 – H & E	57
24b	Hepatic fibrosis - collagen stained red with PTAH – Group I x 160	57
25a	Collagen bridged portal areas and severe fatty change - Group I – H & E x 250	57
25b	Collagen stained red with PTAH – Group I x 160	58
26	Heaptic fibrosis and pseudolobulation - Group I – H & E x 160	58
27	Subcapsular fibrosis in liver. Group I – H & E x 160	58
28	Periductular accumulation of mononuclears - Group I – H & E x 250	58

29a	Proliferation of bile ducts. Bile duct epithelial hyperplasia forming finger like projections - Group I – H & E x 250	59.
29b	Bile duct epithelial hyperplasia forming finger like projections - Group I – H & E x 1000	59
30	Moderate congestion, phlebosclerosis, bile duct proliferation and fatty change - Group II – H & E x 160	59
31	Bile duct proliferation and phlebosclerosis with collagen stained red with Van-Gieson Picric acid stain – Group II x 250	59
32	Congestion, perivascular accumulation of mononuclears and acinar/ductular arrangement of hepatocytes – Group II – H & E x250	60
33	Nodular hyperplasia, periportal lymphoid accumulation and mild fatty change – Group II – H&E x250	60
34	Moderate fatty change and sinusoidal congestion - Group II – H&E x250	60
35	Mild fatty change in liver - Group II – H&E x1000	60
36	Liver - mild congestion and apparently normal hepatic architecture – Group III – H&E x250	61
37	Liver - mild fatty change and mild perivascular accumulation of mononuclears - Group III – H & E x 250	61
38	Liver - mild congestion and portal reaction – Group III – H&E x160	61

Introduction

1. INTRODUCTION

The growth and development of Indian broiler industry during the last decade is unparalleled in the history of animal agriculture and has been described as a textbook example of modernization and industrialization. The Indian Council of Agricultural Research as well as the Central and State Governments have initiated breeding programmes for the development of pure-line broiler germplasms, which adds to the growth of this industry. It is now recognized as an organized and scientifically based industry with tremendous employment potential.

The major expenditure incurred in commercial broiler rearing is towards the cost of poultry feed. The quality and quantity of the feed plays an important role in the overall performance of the flock and thereby the profit margin. Toxic contamination of feed especially by mycotoxins is a common problem. Mycotoxins especially aflatoxins are potent hepatotoxins and they affect the general health and the production performance of poultry. Excessive use of antibiotics, coccidiostats and other chemicals as feed additives may become toxic to birds. These in turn when present as residues in edible tissues may become hazardous to human beings.

The liver is the major metabolic focus of the animal and it has got diverse functions. Minor perturbations of hepatic function will rapidly produce changes in the gross and histologic appearance of the liver. Focal liver disease is also common as the result of the organ's acting as a catchment for the vast absorptive area of the gut, with all its resident microorganisms, parasites and toxins entering the body. These liver lesions may also indicate the presence and causes of diseases in other organs and systems.

Biotransformation and detoxification by the liver of substances absorbed from the bowel or synthesized in other organs, is of much relevance to the study of hepatic disease. It is now accepted that the manner and rate of these reactions may determine the nature, pattern, and severity of many examples of hepatotoxicity. Reactive oxygen species and free radicals are formed in the body as a consequence of normal metabolic reactions and also by the influence of many xenobiotics. Since liver is the major organ for detoxication, it is especially prone to such free radical mediated injury.

Free radical oxidative stress becomes important, when the natural or endogenous antioxidants is deficient in removing the free radicals formed in the body. Potential antioxidant therapy

should, therefore, include either natural free radical scavenging antioxidant enzymes or agents, which are capable of augmenting the activity of these enzymes which include superoxide dismutase, catalase and glutathione peroxidase.

Antioxidants of plant origin are experimentally proved and used as effective protective agents against oxidative stress.

Emblica officinalis, commonly known as amla, a member of genus Emblica (family Euphorbiaceae), is extensively used in Indian medicine for a variety of diseases (Tiwari *et al.*, 1968; Thakur *et al.*, 1988). It is considered as a panacea in Indian system of medicine. Several experimental works have been done in laboratory rats in which *Emblica officinalis* was used either as its alcoholic/aqueous extract or as its various preparations like Chyavanaprash, Rasayana etc. Studies have shown that the fruits of Emblica have got immunostimulating, hypolipedemic and other properties (Pachori *et al.*, 1993; Ansari *et al.*, 1998). Several studies were also done to evaluate the hepatoprotective function of Emblica against various hepatotoxins like carbon tetrachloride (Jose and Kuttan, 2000), alcohol (Suresh *et al.*, 2000), heavy metals like cadmium (Khandelwal *et al.*, 2002) and antitubercular drugs (Saraswathy *et al.*, 1998). Results of these

studies indicated that amla has got significant hepatoprotective effect.

The basic mechanism by which Emblica offers protection is by its free radical scavenging activity (Bhattacharya *et al.*, 1999; Sharma, 2001; Rekha *et al.*, 2001a, 2001b). Both in vivo and in vitro studies have shown that amla and its preparations act as a very good antioxidant by scavenging the reactive oxygen species and protect the antioxidant enzymes, which are required for the cellular defence. The antioxidant activity was reported to be due to ascorbic acid and the polyphenols, gallic and ellagic acids. Hydrolysable cannins like Emblicanin A, Emblicanin B, Punigluconin and Pedunculagin were found to have vitamin C like activity and were recognized as the active principles seen in amla (Bhattacharya *et al.*, 1999).

Experiments done so far, were all done using laboratory animals as the experimental model and till date no investigation has been done in poultry or any other livestock. So the present study was designed to evaluate the hepatoprotective effect of Emblica in broiler chicken and to assess its efficacy as a feed additive in livestock feed.

Review of Literature

2. REVIEW OF LITERATURE

2.1 General

Emblica officinalis (Syn. *Phyllanthus emblica*); is a herbal plant widely used in many of the indigenous medicinal preparations against a variety of conditions (Tiwari *et al.*, 1968; Thakur *et al.*, 1988).

Emblica officinalis is highly nutritious and could be an important dietary source of vitamin C, minerals and amino acids (Barthakur and Arnold, 1991).

Tanvir *et al.* (1994) conducted phytochemical screening of *Emblica officinalis* and found out that it contained alkaloids and glycosides.

Bhattacharya *et al.* (1999) reported that the active principles in *Emblica officinalis* were ascorbic acid, and the hydrolysable tannins, which contained gallic and ellagic acids. Hydrolysable tannins having vitamin C like activity were found to be Emblicanin A, Emblicanin B, Punigluconin and Pedunculagin.

Khopde *et al.* (2001) observed that ascorbic acid and other polyphenols present in the natural formulation of amla showed much superior antioxidant activity compared to their equivalent amounts in pure isolated form.

Emblica officinalis is a constituent of several marketed herbal preparations such as Chyavanaprash, Brahma Rasayana, Amalak Haritaki, Triphala, Septlin etc. (Khandelwal *et al.*, 2002).

2.2 Hepatoprotective effect

2.2.1 Serum enzymes

2.2.1.1 Alanine amino transferase (ALT)

Gulati *et al.* (1995) observed that alcoholic extract of *Phyllanthus emblica* and quercetin isolated from it were having hepatoprotective effect against country made liquor and paracetamol challenge in albino rats and mice respectively. Alcoholic extract of amla and quercetin significantly caused the reduction in the levels of serum ALT which were enhanced by the hepatic toxicants.

Kumar *et al.* (1996) observed that radiation treatment in mice caused increase in serum ALT level and this could be significantly reduced by the administration of Rasayana.

The increase in ALT caused by chronic administration of carbontetrachloride in rats was significantly reduced by treating with ellagic acid (Thresiamma and Kuttan, 1996; Singh *et al.*, 1999).

Saraswathy *et al.* (1998) reported that antitubercular drugs (Isoniazid, Rifampicin and Pyrazinamide) induced hepatotoxicity in

rats, which led to increase in serum ALT level. Rats administered with Liv.100, an indigenous preparation containing *Emblica officinalis* maintained the levels of enzymes.

Jeena *et al.* (1999) observed that N-nitrosodiethylamine induced hepatic damage and hepatocarcinogenesis and thus produced elevation of marker enzymes like ALT. This increase was significantly reduced by treatment with *Emblica officinalis* extract.

Bhattacharya *et al.* (2000) reported that the increase in serum alanine amino transferase in iron-induced hepatic toxicity in rats was inhibited by the administration of *Emblica officinalis* extract.

Increase in serum ALT induced by alcohol was significantly reduced by co-administration of ascorbic acid (Suresh *et al.*, 2000).

Jose and Kuttan (2000) reported that the increase in serum ALT produced by acute and chronic administration of carbon tetrachloride in rats was significantly decreased by co-administration of either *Emblica officinalis* extract or Chyavanaprash.

Prolonged administration of paracetamol produced a dose dependent increase in ALT in rats. This change was reversed with simultaneous administration of paracetamol and HD-03, a

polyherbal formulation containing *Emblica officinalis* (Udupa *et al.*, 2000).

Khandelwal *et al.* (2002) observed that the increase in serum ALT accompanying acute cytotoxicity by cadmium in rats was partially prevented by *Emblica officinalis.*

2.2.1.2 Aspartate amino transferase (AST)

The increase in serum AST produced by the administration of anti-tubercular drugs (Isonizid, Rifampicin and Pyrazinamide) in rats was decreased by co-administration with Liv.100, an indigenous polyherbal preparation containing *Emblica officinalis* (Saraswathy *et al.*, 1998).

Singh *et al.* (1999) reported that chronic administration of carbon tetrachloride in rats lead to increase in serum AST level and this increase was significantly reduced by co-administration with ellagic acid.

The serum level of AST was found to be increased in iron induced hepatic toxicity in rats, which was decreased on co-administration with extract of *Emblica officinalis* (Bhattacharya *et al.*, 2000).

Suresh *et al.* (2000) observed that the increase in serum AST produced by alcohol toxicity was significantly reduced by ascorbic acid treatment.

HD-03, a polyherbal formulation was found to bring about a reduction in the levels of serum AST enhanced by paracetamol toxicity (Udupa *et al.*, 2000).

Acute cadmium toxicity produced an increase in serum AST in rats. This could be reversed by administration of *Emblica officinalis* extract (Khandelwal *et al.*, 2002).

2.2.1.3 Alkaline phosphatase (ALP)

Gulati *et al.* (1995) observed that the alcoholic extract of *Phyllanthus emblica* and quercetin isolated from it could reduce the levels of serum ALP enhanced by the administration of country made liquor in rats.

Thresiamma and Kuttan (1996) observed that the increase in serum alkaline phosphatase produced by chronic administration of carbontetrachloride in rats could be reversed by ellagic acid.

Aspirin-induced increased serum ALP level could be reversed with ascorbic acid in rats (Das and Dasgupta, 1997).

The increase in serum ALP produced by the administration of cyclophosphamide was inhibited by co-administration of *Emblica officinalis* or Chyavanaprash (Jose and Kuttan, 1998).

Administration of anti-tubercular drugs (Isoniazid, Rifampicin and Pyrazinamide) produced a significant decrease in the levels of marker enzymes such as alkaline phosphatase in the liver and a corresponding significant increase in their levels in serum of rats. Rats administered with Liv 100 maintained the levels of marker enzymes in the serum and liver (Saraswathy *et al.*, 1998).

Emblica officinalis significantly reduced the elevated levels of ALP produced by N-nitrosodiethylamine induced hepatocarcinogenesis in rats (Jeena *et al.*, 1999).

Increased activity of serum alkaline phosphatase was observed in rats administered carbon tetrachloride. Co-administration of ellagic acid significantly reduced the change (Singh *et al.*, 1999).

Acute and chronic carbontetrachloride toxicity produced hepatic damage and thereby increase in serum alkaline phosphatase activity in rats. This was significantly reduced by the administration of aqueous extracts of *Emblica officinalis* and Chyavanaprash (Jose and Kuttan, 2000).

2.2.1.4 Gamma glutamyl transpeptidase (GGT)

N-nitrosodiethylamine administration in rats produced an increase in serum GGT in experimental rats. This increase was significantly reduced by co-administration of *Emblica officinalis* extract (Jeena *et al.*, 1999).

Ascorbic acid treatment significantly decreased the levels of serum GGT which was elevated by alcohol administration in experimental rats (Suresh *et al.*, 2000).

2.2.2 Liver

2.2.2.1 Enzyme level

Thresiamma and Kuttan (1996) observed that liver enzymes like ALT and ALP were found to be elevated in rats treated with carbontetrachloride. Animals treated with ellagic acid were found to have lowered activity of liver function enzymes.

Antitubercular drugs like Isoniazid, Rifampicin and Pyrazinamide were found to enhance lipid peroxidation and thereby inactivation of membrane bound lipid dependent enzymes such as Na^+ K^+ ATPase and Mg^{2+} ATPase. The activities of these enzymes were restored on administration of Liv. 100 (Saraswathy *et al.*, 1998).

The level of tissue enzymes like ALT and ALP were found to be decreased by treatment with N-nitrosodiethylamine in rats, due to the diffusion of enzymes caused by tissue damage and degeneration. The reduced levels were found to be modulated by the treatment with *Emblica officinalis* extract (Jeena *et al.*, 1999).

Jose and Kuttan (2000) reported that acute and chronic administration of carbontetrachloride produced an increase in the liver ALT and ALP in experimental rats. This was reduced by co-administration with *Emblica officinalis* extract.

Udupa *et al.* (2000) observed that paracetamol treatment in rats lead to oxidative hepatic damage, leading to significant decrease in Na^+ K^+ ATPase activity. HD-03 was able to produce hepatic regeneration and membrane stabilization by reversal of Na^+ K^+ ATPase towards normal, when given along with paracetamol.

Upasani and Balaraman (2001) reported that the membrane bound Na^+ K^+ ATPase Ca^{2+} ATPase and Mg^{2+} ATPase levels in liver and kidney were significantly reduced in rats exposed to lead. Vitamin C treatment produced a significant increase in the levels of these enzymes as compared to the animals exposed to lead alone.

2.2.2.2 Antioxidant effect

2.2.2.2.1 *In vivo* studies

Increase in the serum and tissue levels of lipid peroxides produced by cyclophosphamide administration was inhibited by co-administration of *Emblica officinalis* or Chyavanaprash (Jose and Kuttan, 1998).

Bhattacharya *et al.* (1999) reported that the active tannoid principles of *Emblica officinalis* has got antioxidant activity. The active tannoids induced an increase in frontal, cortical and striatal superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH_x) activity, with concomitant decrease in lipid peroxidation in these brain areas in rats.

Abana, a polyherbal formulation containing *Phyllanthus emblica* reduced lipid peroxidation and offered protection against myocardial infarction induced by Isoproterenol in rats (Sasikumar and Shyamaladevi, 2000).

Oral administrationof Brahma Rasayana significantly inhibited the reduction in liver antioxidant enzymes, produced by irradiation in mice (Rekha *et al.*, 2000).

Sharma and Sharma (2001) observed that antioxidants like L-ascorbate, reduced lipid peroxidation and oxidative stress, when

fed with high fat diet. Dietary antioxidants also support endogenous antitoxidants in their oxidative stress reducing endeavours.

Rekha *et al.* (2001a) reported that Brahma Rasayana inhibited Phorbol 12-myristate-13-acetate induced superoxide generation and nitrite production in mice peritoneal macrophages.

Brahma Rasayana significantly increased the liver antioxidant enzymes such as SOD, CAT and GSH, which were reduced by irradiation. Radiation exposure induced increase in serum and liver lipid peroxides was also significantly reduced by further treatment with Brahma Rasayana (Rekha *et al.*, 2001b).

2.2.2.2.2 *In vitro* studies

Aqueous extract of *Emblica officinalis* and Chyavanaprash was found to be a potent inhibitor of lipid peroxide formation and scavenger of hydroxyl and superoxide radicals *in vitro* (Jose and Kuttan, 1995a,b).

A suspension of Brahma Rasayana was found to scavenge the lipid peroxides already present in the rat liver homogenate and also the lipid peroxides, hydroxyl and superoxide radicals generated *in vitro* (Rekha *et al.*, 1997).

Alcoholic extract of Triphala, an Ayurvedic formulation containing *Phyllanthus emblica,* was found to scavenge superoxide and peroxy radicals generated *in vitro* and also inhibited lipid peroxidation induced by *in vitro* methods (Vani *et al.*, 1997).

Shishoo *et al.* (1998) reported that the free radical scavenging activity of ethyl acetate extract of Chyavanaprash and ethyl acetate, alcohol and aqueous extracts of amla were comparable with that of ascorbic acid.

Extract of *Emblica officinalis* inhibited lipid peroxidation in bovine brain phospholipid liposomes (Kumar and Muller, 1999).

Amla extract was found to have good antioxidant activity against gamma-radiation induced lipid peroxidation in the rat liver microsomes and superoxide dismutase damage in rat liver mitochondria (Khopde *et al.*, 2001).

Rekha *et al.* (2001a) observed that aqueous extract of Brahma Rasayana was found to scavenge the lipid peroxides generated *in vitro* in the rat liver homogenate.

2.2.2.3 Histopathology

2.2.2.3.1 Vascular changes

Chronic administration of carbontetrachloride produced focal dilatation of blood vessels and proliferation of bile ducts in rats.

These changes were minimized on administration of extracts of *Emblica officinalis* or Chyavanaprash (Jose and Kuttan, 2000).

Udupa *et al.* (2000) observed that congestion of blood vessels in the liver was produced by paracetamol administration in rats and administration of HD-03 minimised the hepatic damage.

Acute cadmium toxicity in rats produced congestion of the liver and the sinusoids were not patent. Pretreatment with *Emblica officinalis* minimized the hepatic lesions (Khandelwal *et al.*, 2002).

2.2.2.3.2 Degenerative changes

Aflatoxins form an important class of hepatotoxins. The toxicopathological changes induced by the toxin were studied by many research workers. The toxin produces severe hepatic damage experimentally, characterized by degenerative and necrotic changes (Moorthy *et al.*, 1986; Balachandran and Ramakrishnan, 1987; Ghosh *et al.*, 1989; Epsada *et al.*,1992 and Kelly, 1992). The hepatic damage stimulated regenerative and repair mechanisms as reported by Balachandran and Ramakrishnan(1987);Anjaneyulu and Rao(1993) and Sridevi and Sriraman (1996). The hepatic damage inturn produced alteration in serum level of protein and enzymes (Reddy *et al.*, 1984; Jassar and Singh, 1993; Mani *et al.*, 1993 and Shukla and Pachauri, 1995). The protective effect of amla against aflatoxin-induced damage has not been studied in detail in

poultry. Soni *et al.* (1993) reported that ellagic acid inhibited the fatty change and biliary hyperplasia induced by aflatoxin B_1 in ducklings.

Roy *et al.* (1991) reported that aqueous extract of *Emblica officinalis* was more effective than ascorbic acid in counteracting the toxic effects induced by metal salts in hepatic and renal tissues in mice.

Hepatogard, an Indian herbal preparation containing crude powder of *Phyllanthus emblica* reversed the histopathological changes induced by carbon tetrachloride in rats (Srinivasan and Kumar, 1993).

Gulati *et al.* (1995) observed that liver from rats, treated with country made liquor showed moderate fatty change with occasional fat cysts. Fifty per cent alcoholic extract of *Emblica officinalis* offered hepatoprotection characterized by reduction in fatty change, and maintenance of hepatic architecture.

Ellagic acid was found to reduce the fatty change in the liver produced by carbontetrachloride toxicity in rats (Thresiamma and Kuttan, 1996).

Jose and Kuttan (2000) observed that acute carbontetrachloride toxicity in rats produced diffuse vacuolation of hepatic cells in rats. The vacuoles were of different size and

occasionally appeared as confluent areas. Acute cell swelling was present. These changes were minimized by treatment with extract of *Emblica officinalis* and Chyavanaprash.

Chronic administration of carbontetrachloride in rats produced extensive fatty degeneration in the liver. Administration of extract of *Emblica officinalis* or Chyavanaprash reduced these effects and only hydropic degeneration was noted in some areas (Jose and Kuttan, 2000).

Udupa *et al.* (2000) reported that administration of paracetamol in rats produced cloudy swelling and vacuolar degeneration in the hepatocytes. HD-03 a polyherbal formulation reduced the hepatic damage.

2.2.2.3.3 Inflammatory changes

Carbontetrachloride toxicity produced cellular infiltration in the liver of rats. Fibrous tissue proliferation and collagen bridged portal areas were noted. Co-administration of ellagic acid reduced the inflammatory changes (Thresiamma and Kuttan, 1996).

Jose and Kuttan (2000) reported that chronic carbon tetrachloride toxicity produced bile duct and fibroblast proliferation. Treatment with *Emblica officinalis* or Chyavanaprash

extracts along with the toxin, afforded hepatoprotection and only mild degenerative changes were present.

Cadmium toxicity in rats produced acute hepatitis: the hepatic lesions were minimized by pretreatment with *Emblica officinalis* (Khandelwal *et al.*, 2002).

2.2.2.3.4 Hepatic necrosis

Coagulative necrosis of the liver, produced by carbon tetrachloride toxicity in rats was found to be reduced when ellagic acid was administered along with the toxin (Thresiamma and Kuttan, 1996).

Jose and Kuttan (2000) observed that acute carbon tetrachloride toxicity produced necrosis of the hepatocytes. Treatment with extract of *Emblica officinalis* or Chyavanaprash, minimized the hepatic lesions and only mild degenerative changes were observed.

Chronic carbon tetrachloride toxicity produced centrilobular necrosis in rats. Extracts of *Emblica officinalis* and Chyavanaprash were found to protect liver against this toxin. Only hydropic degeneration was seen in the livers of rats which were given toxin and extracts (Jose and Kuttan, 2000).

Udupa *et al.* (2000) observed that administration of paracetamol in rats produced liver lesions characterized by vacuolar degeneration of hepatocytes and necrosis. Co-administration of HD-03, helped to preserve the structural and architectural frame of the liver.

Acute cadmium toxicity produced focal necrosis in the liver of rats. Pretreatment with *Emblica officinalis* was found to be hepatoprotective and the hepatic lesions were minimized to mild cloudy swelling and fatty change (Khandelwal *et al.*, 2002).

2.2.2.3.5 Neoplasms

Soni *et al.* (1997) reported that ellagic acid inhibited mutagenesis induced by aflatoxin B_1. It also reduced the number of gamma glutamyl transpeptidase foci induced by AFB_1. These foci are considered as the precursors of hepatocellular neoplasm.

Aqueous extract of *Emblica officinalis* was found to inhibit P-450 enzymes such as aniline hydroxylase and amino pyrene-N-demethylase invitro and induced glutathione-S-transferase activity *in vivo*. The extract inhibited DNA-adduct formation induced by benzo (a) pyrene and aflatoxin B_1. The incidence of liver tumors induced by N-nitrosodiethylamine was also inhibited by *Emblica officinalis* treatment in rats (Jose *et al.*, 1997).

2.3 Other effects

Grover and Kaur (1989) observed that water, acetone and chloroform extracts of *Emblica officinalis* fruit had antimutagenic effect on sodium azide and 4-nitro-0-phenylene diamine induced mutagenesis in *Salmonella typhimurium.*

Udupa *et al.* (1989) reported that Septilin, a herbal preparation containing *Phyllanthus emblica* showed wound healing property in rats.

The cytotoxic effects produced by lead nitrate and aluminium sulphate administration in mice was significantly reduced by aqueous extract of *Phyllanthus emblica* fruits, when administered orally (Dhir *et al.*, 1990).

Extract of *Emblica officinalis* inhibited the growth of *Aspergillus flavus* and production of Aflatoxin B_1 and B_2 *in vitro* (Ranjan *et al.*, 1991).

Kulkarni and Verma, 1992 reported that BR-16A (MENTAT), a herbal psychotropic preparation containing *Emblica officinalis* was useful in cognitive disorders.

Crude extract of *Phyllanthus emblica* afforded protection against the clastogenic effect of cesium chloride in mice and the

results were comparable to the protection provided by an equivalent amount of synthetic ascorbic acid (Ghosh *et al.*, 1993).

Pachori *et al.* (1993) observed that oral administration of Abana decreased the LDL and VLDL-cholesterol, serum total cholesterol, serum triglycerides and serum phospholipids and the per cent change in the level was more with Abana as compared to Metoprolol. Abana treatment increased the level of HDL-cholesterol compared to metoprolol.

Oral administration of powdered *Phyllanthus emblica* fruits was found to enhance natural killer (NK) cell activity and antibody-dependent cellular cytotoxicity (ADCC) in mice bearing Dalton's lymphoma ascites tumour (Suresh and Vasudevan, 1994).

Mini and Kumar (1995) reported that the aqueous extract and pulp of *Emblica officinalis* showed lipid-lowering effect in hyperlipidemic rabbits and the effects were comparable to that of the positive control drug- gemfibrozil.

Menon *et al.* (1995) reported that Brahma Rasayana inhibited the methyl cholanthrene induced sarcoma development in mice.

Manonmani *et al.* (1995) reported that Cauvery 100, a herbal preparation containing *Emblica officinalis* could be used as an ulcer protective agent.

Emblica officinalis extract was reported to have hypolipidaemic and anti atherosclerotic action (Mathur *et al.*, 1996).

Vaidya (1997) reported that *Emblica officinalis* is one of the medicinal plants in India, having CNS activity.

Sharma and Ray (1997) reported that oral administration of Septilin alone or in combination with an immuno suppressive drug (Prednisolone 500 mg/kg) enhanced both primary and secondary immune response, in mice immunized with sheep red blood cells.

Kumar *et al.* (1997) reported that Brahma Rasayana ameliorated the myelosuppression caused by chemotherapy and/or radiation in cancer patients.

Ansari *et al.* (1998) reported that ascorbic acid stimulated humoral immunity through increased antibody synthesis particularly IgG, IgA and IgM types and also by activating the macrophages.

Pallabi De *et al.* (1998) observed that Immu 21, a polyherbal product containing *Emblica officinalis* has got immunopotentiating and immunoprophylactic activity.

Dhuley (1999) observed that Rhinax, a drug formulation containing *Phyllanthus emblica* showed ulcer-protective activity, which was evoked mainly by the modulation of defensive factors by improvement in gastric cytoprotection and partly by acid inhibition and free radical scavenging properties.

Hepatogard, a herbal preparation containing *Emblica officinalis*, reversed the dexamethasone induced decrease in breaking strength in both incised wound and granulation tissue and thus had wound healing property (Nadig and Rao, 1999).

Rasayanas, were found to enhance cell- mediated immune response in normal and tumour bearing mice, especially the activities of NK cell, macrophages and ADCC (Kumar *et al.*, 1999a).

Administration of Rasayanas enhanced the proliferation of spleen cells and bone marrow cells. It also enhanced humoral immune response (Kumar *et al.*, 1999b).

Verma *et al.* (1999) reported that the use of a polyherbal immunomodulator containing *Emblica officinalis* in conjunction with inactivated vaccine against Equine Herpes Virus-I produced a non-specific increase in the serum neutralizing antibody titre and improved cell mediated immune response.

Chatterjee (2001) observed that Immu-21, a herbal formulation containing *Emblica officinalis*, modulated macrophage

maturation and function and stimulated humoral immune response, when given to mice.

Rajesh Kumar and Kuttan (2001) reported that polyphenol fraction of *Emblica officinalis* induced apoptosis of mouse and human carcinoma cell lines.

Augusti *et al.* (2001) reported that *Emblica officinalis* showed a hypolipidaemic effect when fed to rats along with high fat diet.

Chyavanaprash, an ancient Indian dietary supplement derived from amla, reduced post prandial glycemia in the oral glucose tolerance test and reduced blood cholesterol level to a significantly greater extent than Vitamin C (Manjunatha *et al.*, 2001).

Devasagayam and Sainis (2002) recorded that *Emblica officinalis* reduced stress by macrophage activation and genotypic adaptation.

Materials and Methods

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Experimental Birds

Fifty-four, (n=54) healthy, day-old Hubbard broiler chicks were obtained from the Coastal Krishna Hatcheries, Thrissur, Kerala. The experiment was carried out during the period from February sixteenth to April twelfth, 2002.

3.1.1.1 Experimental groups

The broiler chicks were randomly divided into three groups each having two replicates.

All the six replicate groups were kept in separate rooms under deep litter system throughout the experiment. Ideal brooding conditions were provided upto four weeks of age. Each group was given scheduled experimental feed. Water was given *ad libitum.*

3.1.1.2 Management

The experimental rooms were thoroughly cleaned with 2.5 per cent phenol solution and then fumigated with formaldehyde gas (35 ml of commercial formalin plus 17.5 g potassium permanganate per 100 cubic feet area).

3.1.2 Experimental feed

Commercial broiler feeds, both starter and finisher were procured from the market. Amla powder prepared from sun dried fruits of *Emblica officinalis* was purchased from an ayurvedic shop. All the three groups of birds were given feed as per the following schedule.

Group I - Birds of the control group were given commercial feed alone.

Group II - Amla powder was added at 1% level in the feed.

Group III - Amla powder was added at 2% level in the feed.

3.2 Methods

The following parameters were studied.

1. Body weight at fortnightly intervals
2. Feed consumption up to eight weeks of age
3. Feed efficiency
4. Proximate principles of feed
5. Hematology -Erythrocyte sedimentation rate (ESR)
   -Hemoglobin (Hb)
   -Packed cell volume (PCV)
   -Differential leukocyte count (DLC)
   -Total leukocyte count (TLC)

6. Serum total protein, albumin, globulin and albumin-globulin ratio (A/G)

7. Liver specific serum enzymes - Aspartate amino transferase (AST)

-Alkaline phosphatase (ALP)

-Gamma glutamyl transpeptidase (GGT)

8. Weight of the liver

9. Gross and Histopathology of the liver

10. Statistical analysis

3.2.1 Experimental design

The birds were reared for eight weeks. They were sacrificed at the end of the experiment. Blood and tissues from these birds were collected for hematological, serological and histopathological studies. Gross lesions, if any in the livers collected were recorded.

3.2.2 Body weight

Body weights of the chicks at day-old were recorded, before the commencement of the experiment and thereafter at fortnightly intervals in all the groups.

3.2.3 Feed intake and feed conversion ratio (FCR)

Total feed intake of the control and experimental birds were recorded. FCR was calculated based on the mean body weight and cumulative feed intake, at the end of the experiment.

$$FCR = \frac{\text{Cumulative feed intake}}{\text{Mean body weight}}$$

3.2.4 Hematological parameters

Blood samples were collected from the wing vein at the end of the experiment. Two milliliter of blood was collected for the hematological studies using dipotassium salt of Ethylene diamine tetra acetic acid (EDTA) at the rate of 1 mg/ml of blood as the anticoagulant. Hemoglobin concentration was estimated using modified acid hematin method. (Schultze and Elvehjem, 1934). PCV and ESR were estimated as per standard procedures (Jain, 1986).

The TLC was determined as per the method described by Sastri (1976). The DLC was done with the copper peroxidase method of Sato and Sekiya (1965).

3.2.5 Total serum protein

Five milliliter of blood was collected from the wing vein separately in a sterile test tube without adding anticoagulant for

serum separation. The total protein content in the serum was estimated by Biuret method (Gomall *et al.*, 1949).

The albumin content in the serum was estimated by the BCG (Bromo Cresol Green dye) binding method described by Doumas *et al.* (1971).

Serum globulin value was determined by deducting values of serum albumin from total serum protein.

Albumin-globulin ratio was calculated by dividing albumin values with globulin level.

3.2.6 Serum enzymes

Blood (5 ml) was collected without adding anticoagulant and serum was separated out. Serum AST, ALP and GGT were estimated using commercially available kits (AGAPPE Diagnostics) and the final readings were taken spectrophotometrically at 405 nm.

3.2.7 Post-mortem examination

The birds were sacrificed at eight weeks of age and subjected to detailed autopsy. Liver was collected and was subjected to gross examination and any alteration in size, shape, colour and consistency were recorded. Weight of liver was recorded.

3.2.8 Histopathology

Representative samples of the liver obtained from the carcasses were fixed in 10% neutral buffered formalin. They were then processed and embedded as described by Sheehan and Hrapchak (1980). The sections were stained with Haematoxylin and Eosin, as per the technique followed by Bancroft and Cook (1995). Special staining techniques were employed as and when required as per the method, described by Luna (1968). The special stains employed were Oil-Red-O for demonstration of fat, Van Gieson Picric acid stain, PTAH and Gomori's modified Trichrome method for demonstration of fibrous tissue. The sections were examined in detail under light microscope and lesions were classified.

3.2.9 Feed analysis

3.2.9.1 Proximate analysis of feed

Standard methods (AOAC, 1990) were followed to estimate the chemical composition of the feeds.

3.2.9.2 Analysis of the aflatoxin content of the feed

Feed samples were assayed for the presence of aflatoxin and ochratoxin by multimycotoxin analysis method (Tapia, 1985) using thin layer chromatography.

3.2.10 Statistical analysis

The data obtained from various parameters were subjected to statistical analysis and analysis of variance (ANOVA) was conducted as per Rangaswamy (1995).

Results

4. RESULTS

The results obtained during the course of investigation on "Effect of Amla (*Emblica officinalis)* on hepatic function in broiler chicken" are presented in this chapter.

4.1 Body weight

Body weight of birds in all the groups showed a gradual increase throughout the experimental period. No significant difference was noted between the three groups up to the second week. Thereafter, an increase in the weight was seen in the amla treated groups compared to the control group. A significant difference ($P<0.05$) was noted between groups I and III at fourth week of age. However at eighth week, significant differences ($P<0.05$) were observed between all the three groups. The mean body weights recorded at fortnightly intervals are presented in Table 1 and graphically in Fig 1.

4.2 Liver weight

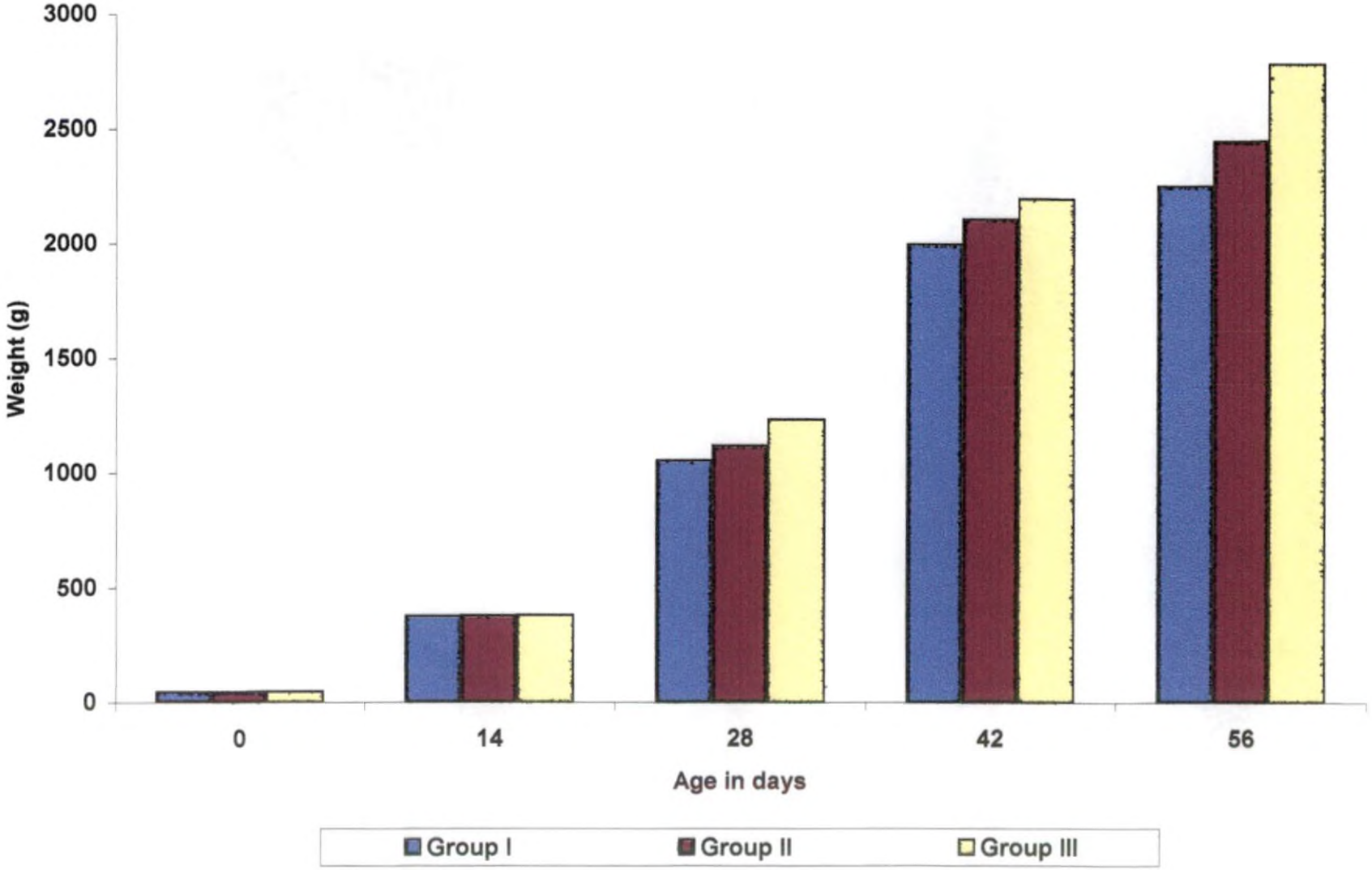
The mean liver weight at eight weeks of age was found to be higher in the case of group I birds compared to the amla treated birds. The values observed were 37.78±2.65, 28.89±1.82 and 25.56±1.30 g for groups I, II and III respectively. The mean

Table 1. Mean body weight ±SE (g) of the experimental birds at fortnightly intervals

Group	Amla level(%)	Day old	Second week	Fourth week	Sixth week	Eighth week
I	0	45.67^{a}±0.8	373.11^{a}±2.38	1053.33^{a}±28.82	1994.44^{a}±53	2250.00^{a}±45.69
II	1	46.00^{a}±0.83	374.78^{a}±2.45	1116.67^{a}±37.27	2105.56^{a}±59.19	2446.43^{b}±65.14
III	2	45.78^{a}±0.97	377.56^{a}±1.88	1230.00^{b}±37.97	2194.44^{b}±47.47	2782.14^{c}±57.08
	LSD	2.5	5.6	102	155.2	161.7

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

Fig 1. Mean body weight (g) of birds at fortnightly intervals



liver weight: body weight was found to be 0.017, 0.012 and 0.009 for the control group, 1% and 2% amla groups respectively (Table 2).

4.3 Feed conversion ratio (FCR)

FCR was calculated based on the mean body weight and cumulative feed intake, at the end of the experiment. The ratio was found to be better in the amla fed birds compared to the control group. The total feed intake up to eight weeks of age and the FCR values at the end of the experiment are presented in table 3. The FCR values observed were 3.21, 3.09 and 2.76 for groups I, II and III respectively.

4.4 Feed analysis

4.4.1 Proximate analysis of the feed

The chemical composition of the feed used were analysed using the standard AOAC method. The analysis indicated normal chemical composition. The composition is presented in Table 4.

4.4.2 Analysis of aflatoxin content of feeds

The feed samples were analysed for the presence of aflatoxin B_1 and ochratoxin, using multimycotoxin analysis method. Starter rations contained an average of 100-150 ppb of toxin and

Table 2. Mean liver weight ±SE (g) at eight weeks of age

Group	Amla level	Liver weight
I	0	37.78^{a}±2.65
II	1	28.89^{b}±1.82
III	2	25.56^{c}±1.30

$P < 0.05$

Table 3. Total feed consumption and feed conversion ratio (FCR) of the birds at eight weeks of age

Group	Amla level	Feed consumption (kg)	FCR
I	0	130	3.21
II	1	136	3.09
III	2	138	2.76

Table 4. Per cent chemical composition of the feed samples

Item	Feed samples					
	I	II	III	IV	V	VI
Dry matter	88.64	88.42	90.42	90.10	89.80	86.28
Crude protein (N × 6.25)	19.14	17.65	18.50	18.25	17.50	16.48
Ether extract	4.27	3.10	4.20	3.60	3.20	2.96
Crude fibre	9.30	8.84	8.17	8.42	8.57	8.64
Nitrogen free extract	60	64.27	61.93	62.84	63.20	64.76
Total ash	7.29	6.14	7.2	6.89	6.93	7.16
Acid insoluble ash	4.03	3.07	3.77	3.45	3.68	3.93
Calcium	1.35	1.54	1.90	1.15	1.10	1.20
Phosphorus	0.67	0.99	1.00	0.52	0.64	0.69

the finisher rations contained an average of 150-200 ppb of aflatoxin B_1. Ochratoxin content was nil in all the samples.

4.5 Haemogram

The response in all the haematological parameters recorded in the amla fed groups are described below.

4.5.1 Erythrocyte sedimentation rate (ESR)

The ESR values were in the range of 1.6 to 2.4 mm/h, as given in the Table 5. It can be seen that the values were significantly higher for the control group birds ($P<0.05$), compared to the amla fed groups (Table 5 and Fig.2).

4.5.2 Packed cell volume (PCV)

The mean PCV values varied from 26% to 31% in the different groups and a significant increase ($P<0.05$) in the PCV was noted in groups II and III, compared to the control group (Table 5 and Fig.2).

4.5.3 Haemoglobin (Hb)

The haemoglobin values were significantly increased ($P<0.01$) in the amla fed groups, when compared to the control group birds (Table 5 and Fig.2). The mean values were 9, 10.3 and 11.3 g% for groups I, II and III respectively.

4.5.4 Total leukocyte count (TLC)

Lowered leukocyte count was observed in the control group birds. An increase ($P<0.05$) in the total leukocyte count was observed in the amla fed groups, compared to the control group. The values were 18.13, 19.50 and 22.75 x 10^3/ cu mm (table 5 and Fig.2).

4.5.5 Differential leukocyte count (DLC)

Heterophil (H), lymphocyte (L) and monocyte (M) counts were significantly higher ($P<0.05$) in the amla fed groups, compared to the control group. They were in the range of 30-37, 59-65 and 1-2% respectively. No appreciable variation was seen in the eosinophil (E) and basophil (B) counts (table 5 and Fig.3).

4.6 Serum profile

4.6.1 Total serum protein

Significantly higher values ($P<0.01$) in the total serum protein values were recorded in the amla treated groups (table 6 and Fig.4). The mean values were in the range of 2.97 to 4.51 g/dl in the various groups.

4.6.2 Serum albumin

The mean albumin values were 1.3, 1.75 and 2.32 g/dl in the control, one percent and two percent amla groups

Table 5. Hemogram values (mean ±SE) of experimental birds at eight weeks of age

Group	Amla level (%)	ESR mm/h	PCV (%)	Hb (g%)	TLC (x10³/cu.mm)	Differential leukocyte count (%)				
						H	L	M	E	B
I	0	2.42[a]± 0.07	26.56[a]± 0.10	9.06[a]±0.19	18.13[a]± 0.08	30.61[a]±0.12	59.39[a]± 0.25	1.72[a] ± 0.18	0.94[a] ± 0.13	0.11[a]± 0.08
II	1	1.82[b]± 0.04	28.80[b] ±0.08	10.28[b]±0.16	19.50[b]± 0.16	32.61[b] ±0.16	61.00[b]± 0.20	1.56[b] ± 0.15	1.06[a] ± 0.13	0.22[a]± 0.10
III	2	1.66[c]± 0.04	31.12[c] ±0.12	11.82[c]±0.15	22.75[c]± 0.13	37.61[c]±0.20	65.89[c]± 0.34	2.22[c] ± 0.15	1.28[a] ± 0.12	0.28[a]± 0.11
	LSD	0.149	0.290	0.47	0.367	0.467	0.761	0.45	0.18	0.25

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

Fig 2. Mean haemogram values of birds at eight weeks of age

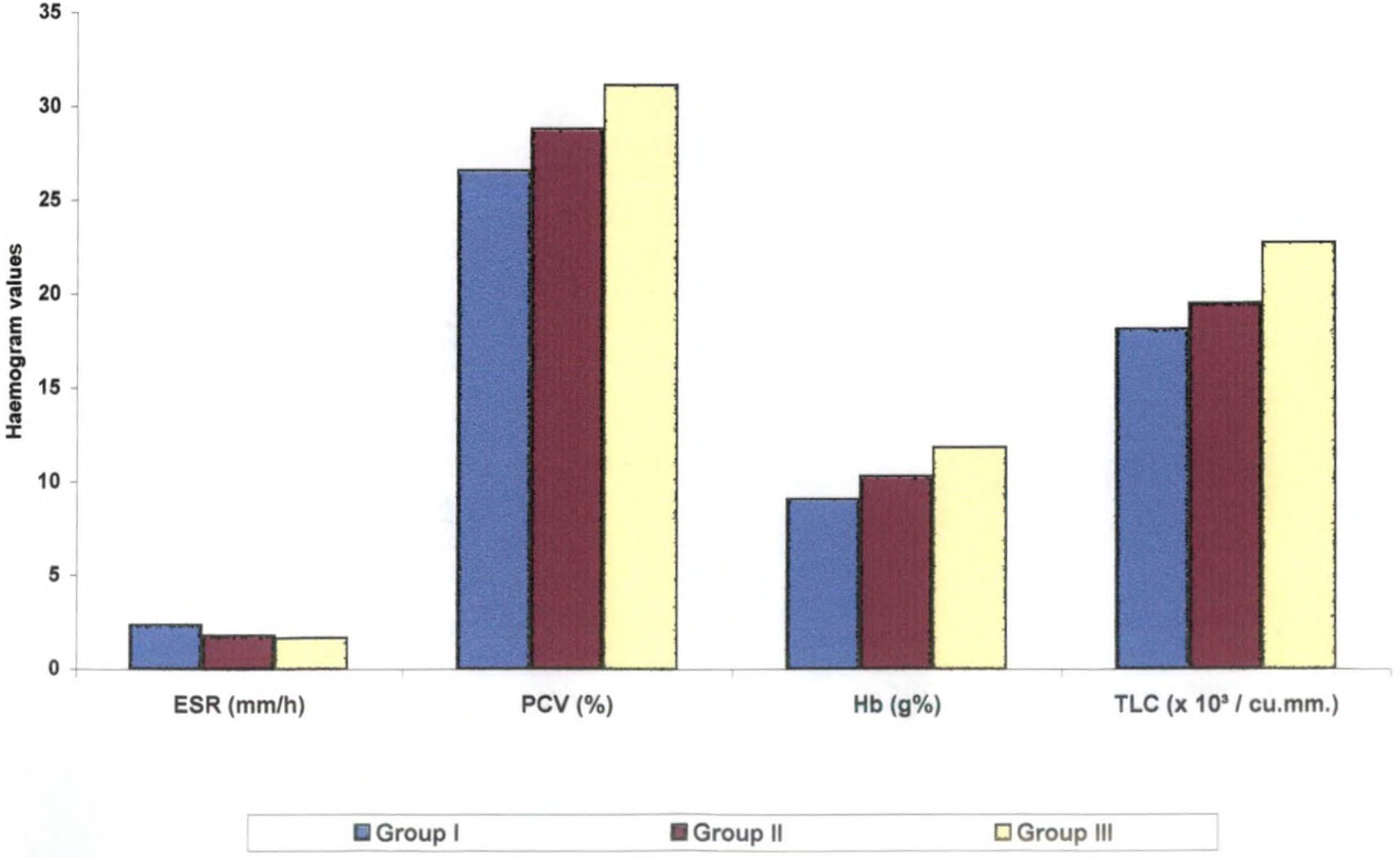
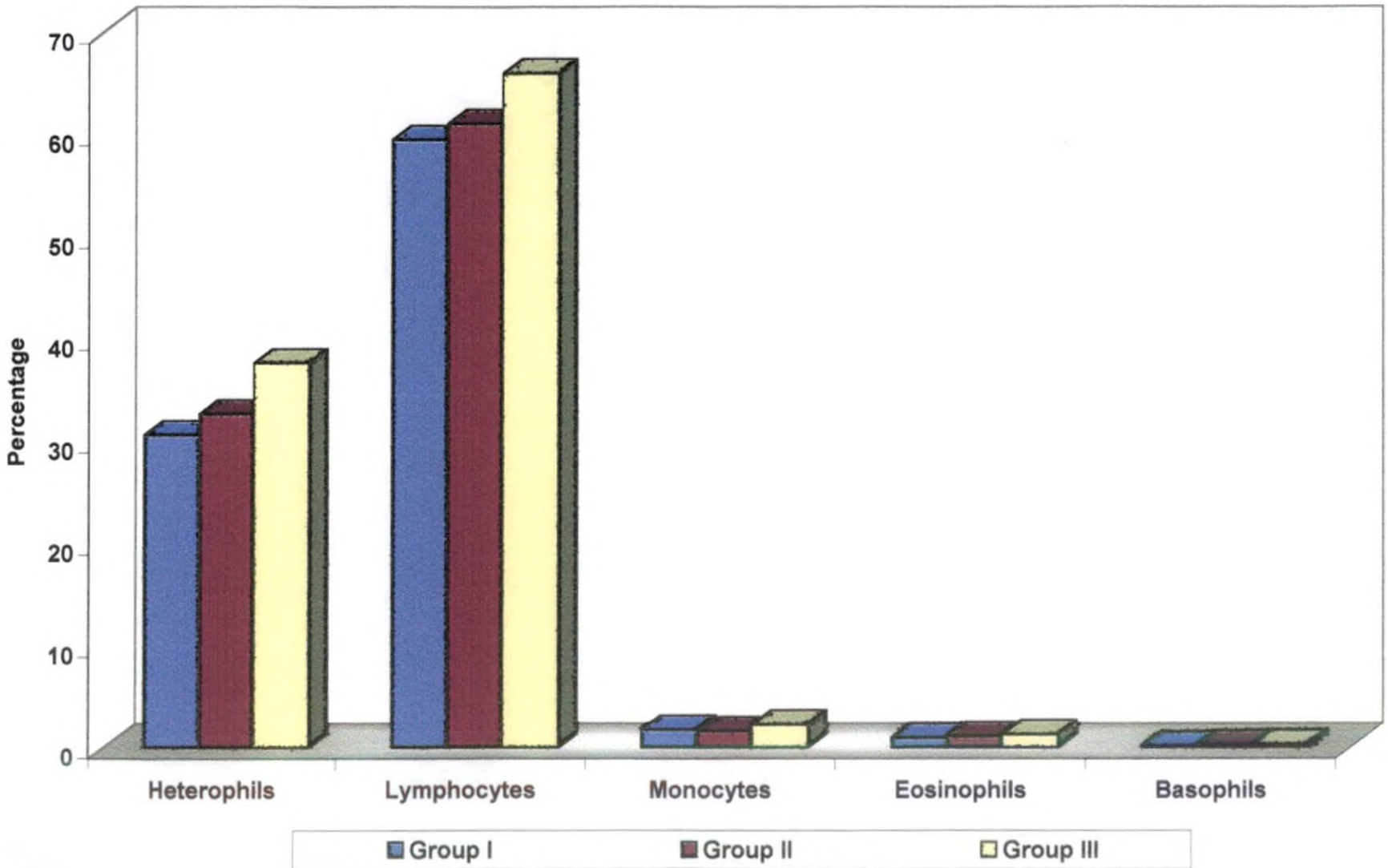


Fig 3. Differential leukocyte count (%) at eight weeks of age



respectively. Significant increase ($P<0.01$) in the albumin values was recorded in the amla treated groups (table 6 and Fig.4).

4.6.3 Serum globulin

The absolute globulin values were increased in the amla fed groups, compared to the control group indicating liver damage (table 6 and Fig.4). The mean values varied from 1.63 to 2.19 in the different groups.

4.6.4 Albumin: Globulin (A/G) ratio

The A/G ratio was below normal in the control group, and a dose related increase in the ratio was found in the amla fed groups (table 6 and Fig.4). The mean value for the control group birds was 0.81. A gradual increase was noted for the amla treated groups. The mean values were 1.05 and 1.06 for groups II and III respectively.

4.6.5 Serum enzymes

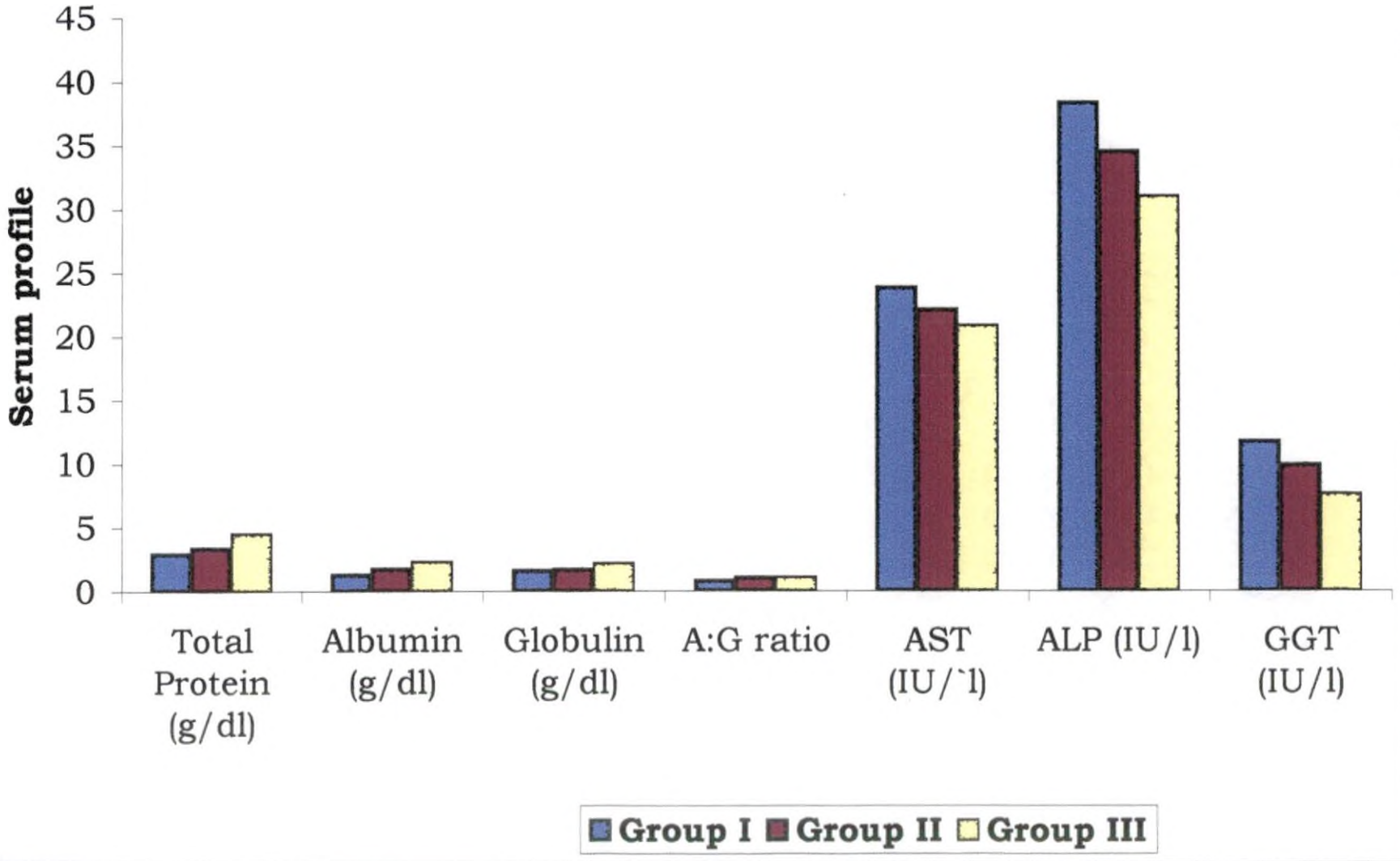
The control birds were having high serum AST, ALP and GGT activity compared to the amla treated groups indicating liver damage. The amla treated groups showed a lower ($P<0.01$) enzyme values (table 6 and Fig.4). The average AST values were 23.85, 22.15, 20.85 IU/l for groups I, II and III. ALP values were 38.39, 34.55 and 31.00 and GGT values were 11.75, 9.92 and 7.61 IU/l for the groups I, II and III.

Table 6. Serum profile at eight weeks of age

Group	Amla level (%)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Albumin: globulin	Aspartate amino-transferase (AST) (IU/L)	Alkaline phosphatase (ALP) (IU/L)	Gamma glutamyl transpeptidase (GGT) (IU/L)
I	0	2.97^{a} ± 0.06	1.30^{a} ± 0.05	1.63^{a} ± 0.06	0.81^{a} ± 0.05	23.85^{a} ± 0.28	38.39^{a} ± 0.17	11.75^{a} ± 0.09
II	1	3.42^{b} ± 0.07	1.75^{b} ± 0.04	1.73^{b} ± 0.07	1.05^{b} ± 0.03	22.15^{b} ± 0.25	34.55^{b} ± 0.12	9.92^{b} ± 0.09
III	2	4.51^{c} ± 0.07	2.32^{c} ± 0.01	2.19^{c} ± 0.01	1.06^{c} ± 0.07	20.84^{c} ± 0.29	31.00^{c} ± 0.11	7.61^{c} ± 0.11
	LSD	0.15	0.11	0.15	0.10	0.77	0.39	0.93

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

Fig.4 Serum profile at eight weeks of age



4.7 Gross pathology

The birds in the amla treated groups did not show any apparent gross lesions. The livers of the control group birds were enlarged and fatty, with rounded borders. They were pale to yellowish in colour and were friable (Fig.5). Severe congestion was seen in some cases. Moderate enlargement and mild yellowish discolouration was seen in livers of group II (Fig.6) and livers of group III were apparently normal (Fig.7).

4.8 Histopathology

Histopathological lesions of varying intensity were seen in the livers of the control group birds. The severity of the lesions was less in the amla treated groups especially in group II. The lesions seen in the different groups were:

4.8.1 Group I

In the liver of the birds fed with the commercial feed the lesions varied from mild vascular changes to fatty change, necrosis and fibrous tissue proliferation. The vascular changes observed were congestion, haemorrhage and oedema. Moderate to severe congestion was seen especially in the hepatic vein (Fig.8, 10). The sinusoids, central veins and the portal vessels were dilated and filled with erythrocytes (Fig.9). In very severe cases, dilatation of the sinusoids and consequent thinning

of the hepatic cords were observed (Fig.16). Mild haemorrhage was also seen in some of the livers. Focal collection of erythrocytes were seen displacing the hepatic parenchyma (Fig.14). A small area of haemorrhage was seen in the sub capsular space in one case (Fig.15). Moderate oedema evidenced by uniform pink stained material was seen displacing the parenchyma (Fig.8, 13). Focal accumulation of mononuclear cells in the form of micro nodules was seen throughout the parenchyma, mostly in the periportal region. (Fig.11,12a,21). Kupffer cell proliferation was also seen (Fig.19).

Toxin induced severe degenerative changes were seen throughout the hepatic parenchyma. The degenerative changes were characterized by severe fatty change with hepatocytomegaly and loss of cytoplasmic details. The enlarged hepatocytes were rounded and were laiden with fat globules, which pushed the nucleus to one side. The fat globules were present either as single globules or as multiple small globules, within the cytoplasm of the hepatocytes (Fig.17, 19). In some of the hepatocytes, the single fat droplets were very large giving the typical signet ring appearance (Fig.19). Some of them ruptured and coalesced to form large fat cysts (Fig.18). The globules stained red with Oil-Red-O, confirming that they were fat

globules (Fig. 20). Varying degree of centrilobular necrosis were observed.

Chronic hepatic lesions were characterised by regenerating foci, biliary hyperplasia, lymphocytic infiltration and focal fibrous tissue proliferation were seen. Changes characteristic of regenerative and repair mechanisms were of varied nature. Megalocytosis with homogenous sometimes-vacuolated cytoplasm and enlarged nucleus were observed. The cord like arrangement of the hepatocytes were disrupted in many areas and a ductular or acinar pattern was observed. Regenerating nodules characteristic of nodular hyperplasia were observed throughout the parenchyma especially in the periportal areas (Fig. 22a). Multifocal proliferating biliary epithelial cells encapsulated by fibroblasts were seen in the hepatic parenchyma (Fig. 22b). Hepatic parenchyma also showed few focal areas suggestive of microgranulomatous changes charactertised by accumulation of mononuclear cells, immature heterophils and a few eosinophils, accompanied by necrosis of the hepatic cells. Severe biliary hyperplasia was accompanied by moderate to severe periportal especially periductular accumulation of mononuclear cells (Fig. 28). The hyperplasia of the bile duct epithelium formed finger like projections into the lumen (Fig. 29a,b). Fibroblast proliferation (Fig. 23) and subsequent collagen deposition were

seen in the liver especially in the portal areas and around the bile ducts. Fibrosis and pseudolobulation was present in some of the livers (Fig.26). Collagen bridged portal areas were seen (Fig.25a,b). Phlebosclerosis characterised by fibrous thickening of the hepatic vein was also present (Fig.8, 9, 10). Focal hepatic fibrosis was seen in some cases, where fibrous tissue was seen displacing the parenchyma (Fig.24a, b). Sub capsular fibrous tissue proliferation was noted in one case (Fig.27). Fibrous tissue was stained red by special stains like Van Gieson's and PTAH (Fig.24b, 25b) and green by Gomori's one step Trichrome method (Fig.9, 12b).

4.8.2 Group II

In the one percent amla treated group there were mild to moderate hepatic lesions. The degenerative changes were less severe. Moderate congestion with focal mononuclear cell accumulation was observed (Fig.30, 32). Fatty change was present although less severe compared to group I. The fat vacuoles were less in number and reduced in size compared to the control group (Fig.34, 35). Moderate regeneration of hepatocytes characterised by nodular hyperplasia were observed. Number and size of such nodules were less (Fig.33). Mild to moderate biliary hyperplasia with mild periductular

accumulation of lymphocytes was observed. Fibrous tissue proliferation characterised by phlebosclerosis was also milder in comparison with group I (Fig. 30). Fibrous tissue was stained red with Van Gieson Picric acid stain (Fig. 31).

4.8.3 Group III

In the two percent amla treated groups, hepatic lesions were very much less compared to the groups I and II. Mild congestion of the vessels was observed in focal areas (Fig. 36, 38). The degenerative changes were mild and the architecture of the liver was well maintained (Fig.36). Fat globules were seen in focal areas in some hepatocytes (Fig.37) and no regenerating nodules were observed in group III. Bile duct hyperplasia and associated fibrous tissue proliferation were absent.

Fig.5 Liver – enlarged and fatty with yellow discolouration – Group I

Fig.6 Liver – moderate enlargement and fatty change – Group II

Fig.7 Liver – apparently normal – Group III

Fig.8 Congestion, phlebosclerosis of hepatic vein and edema – Group I – H & E x 160

Fig. 5

Fig. 6

Fig. 7

Fig. 7

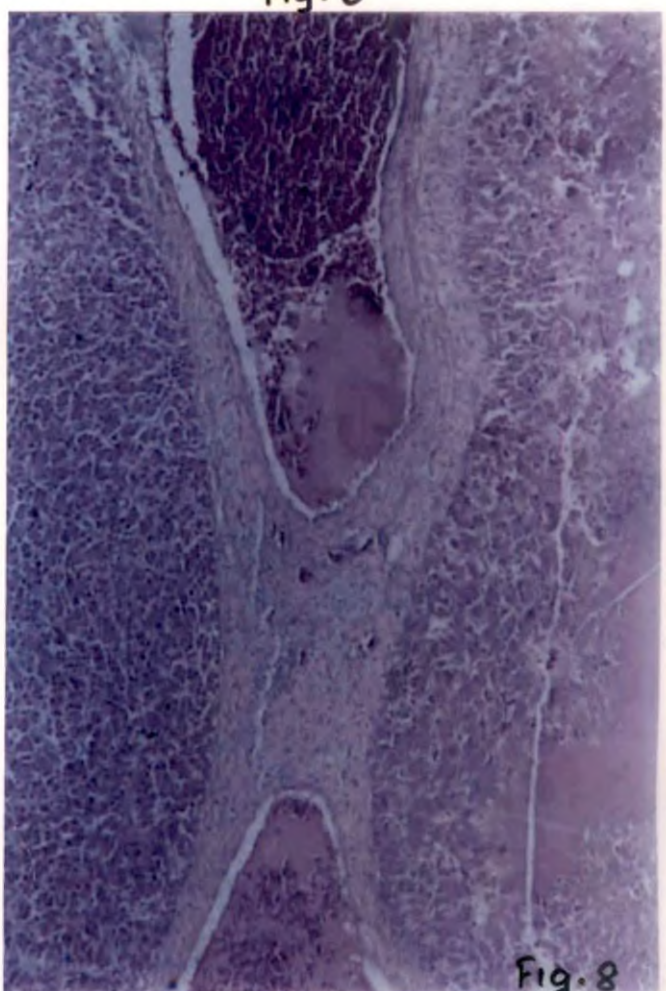


Fig. 8

Fig. 8

Fig.9 **Congestion and phlebosclerosis of portal vein. Collagen stained green with Trichrome – Group I x160**

Fig.10 **Congestion and phlebosclerosis of Hepatic vein – Group I – H&E x160**

Fig.11 **Periportal, periductular lymphoid collections and bile duct proliferation – Group I – H&E x250**

Fig.12a **Focal accumulation of mononuclears in the periportal region and phlebosclerosis – Group I – H&E x 250**

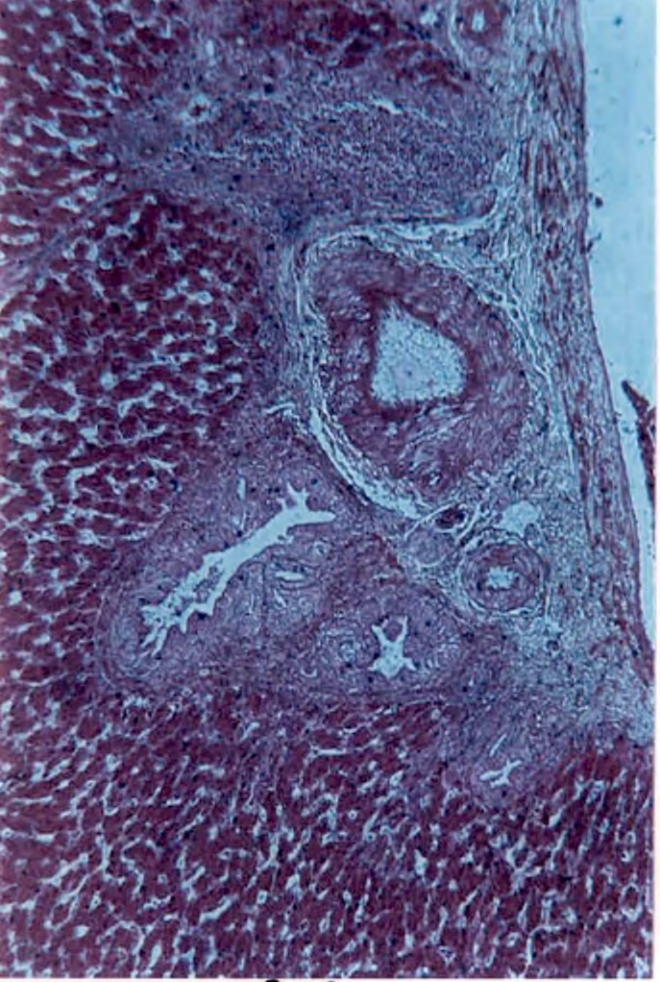


Fig. 9

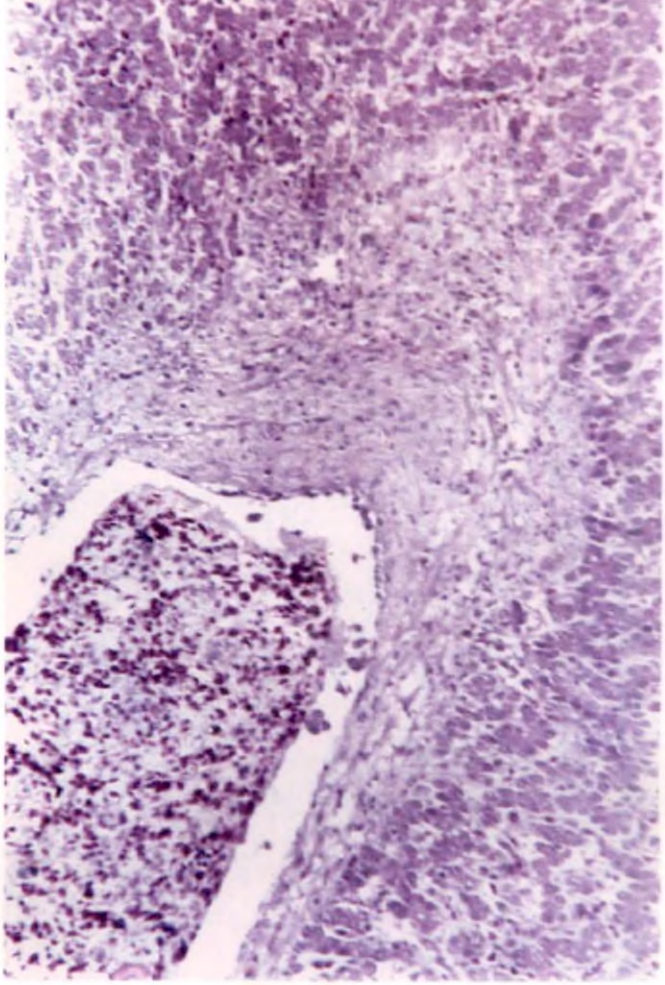


Fig. 10

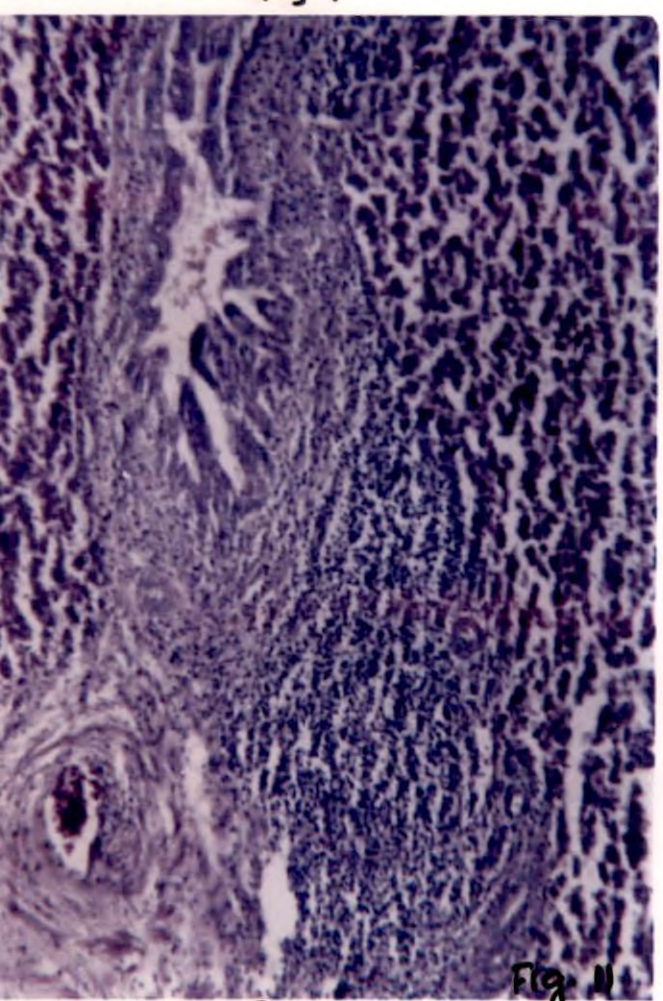


Fig. 11

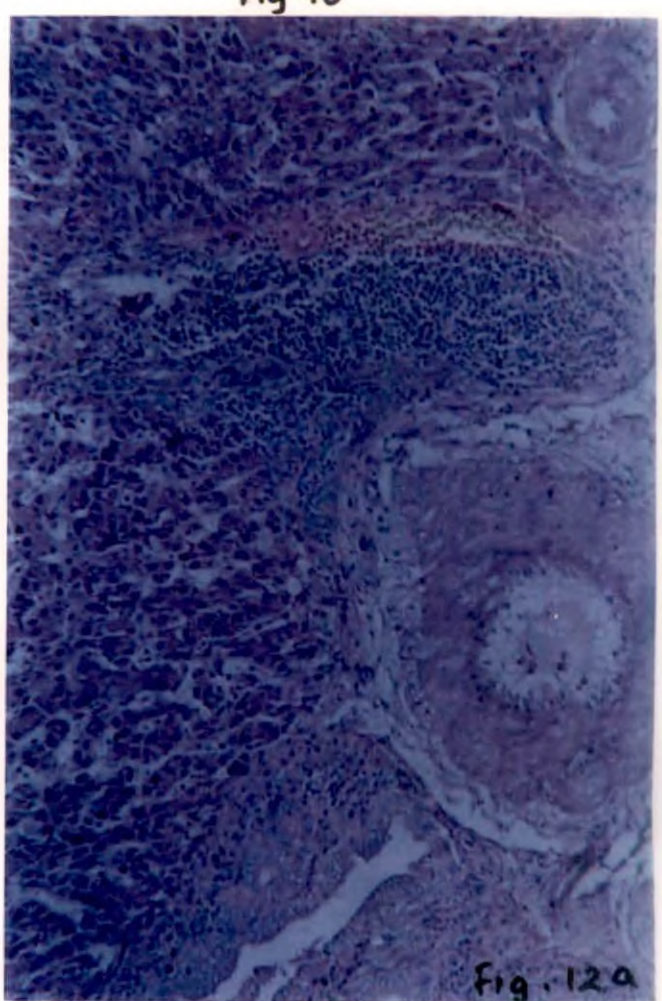


Fig. 12a

Fig.12b Phlebosclerosis - collagen stained green with Trichrome – Group I x250

Fig.13 Accumulation of pink stained edema fluid in the hepatic parenchyma – Group I – H & E x 1000

Fig.14 Haemorrhage in the hepatic parenchyma – Group I – H & E x 250

Fig.15 Subcapsular haemorrhage in liver – Group I – H & E x 250

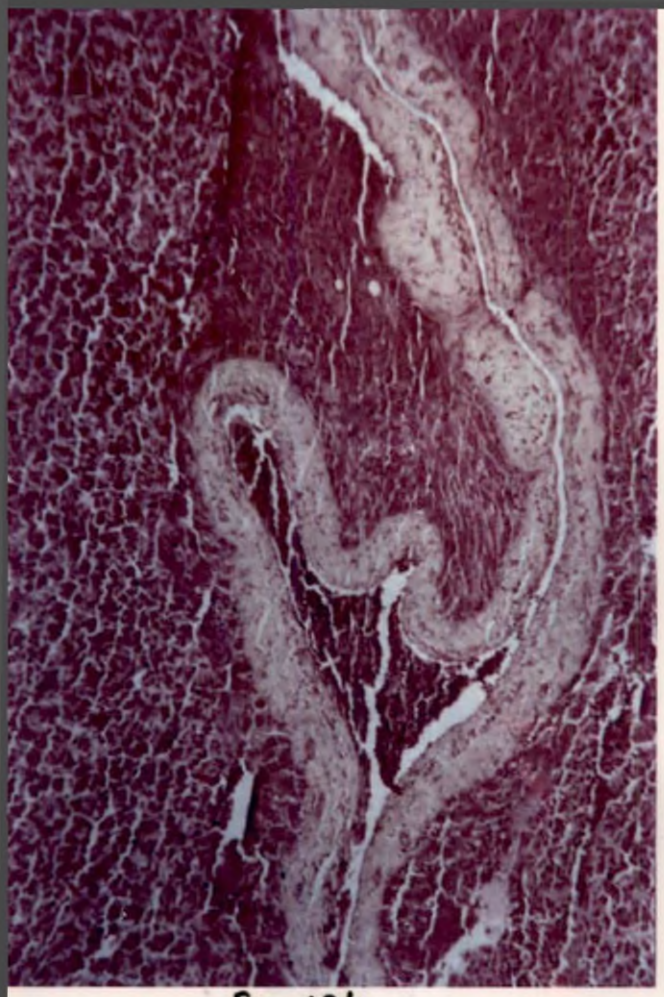


Fig. 12b

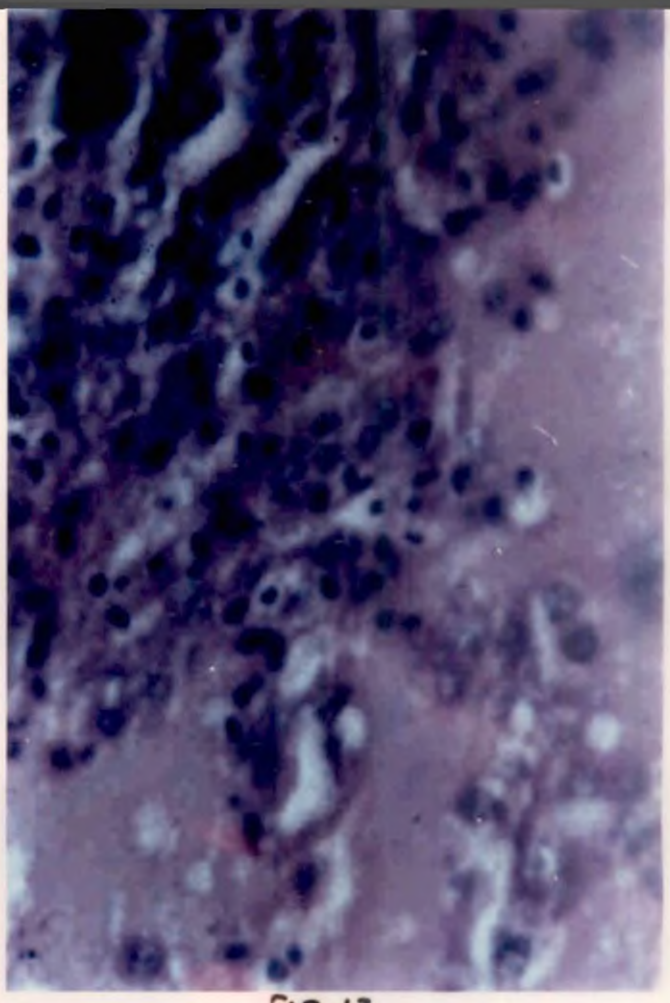


Fig. 13

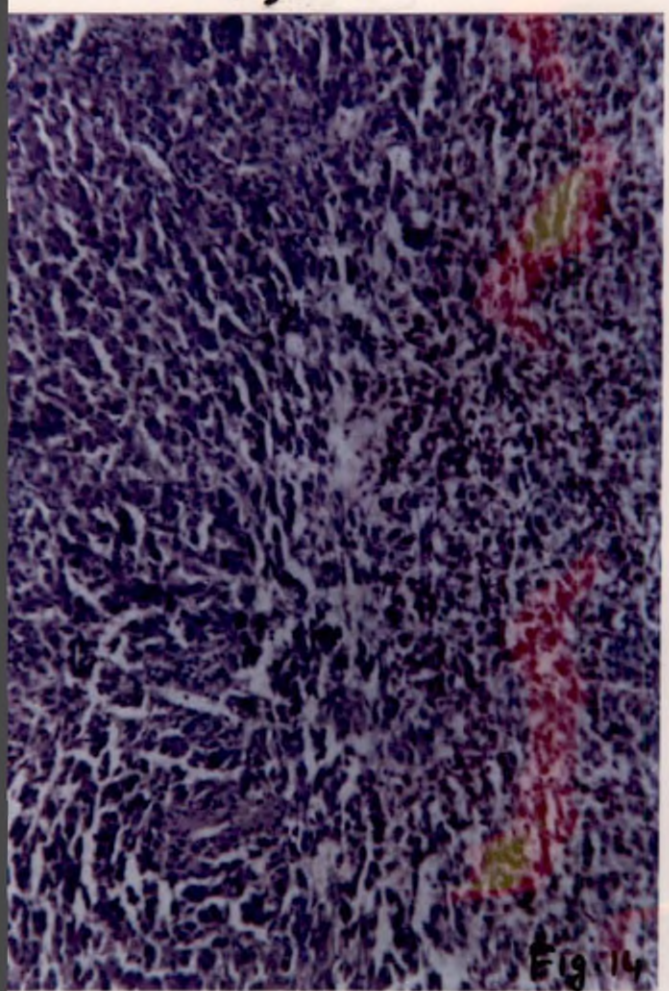


Fig. 14

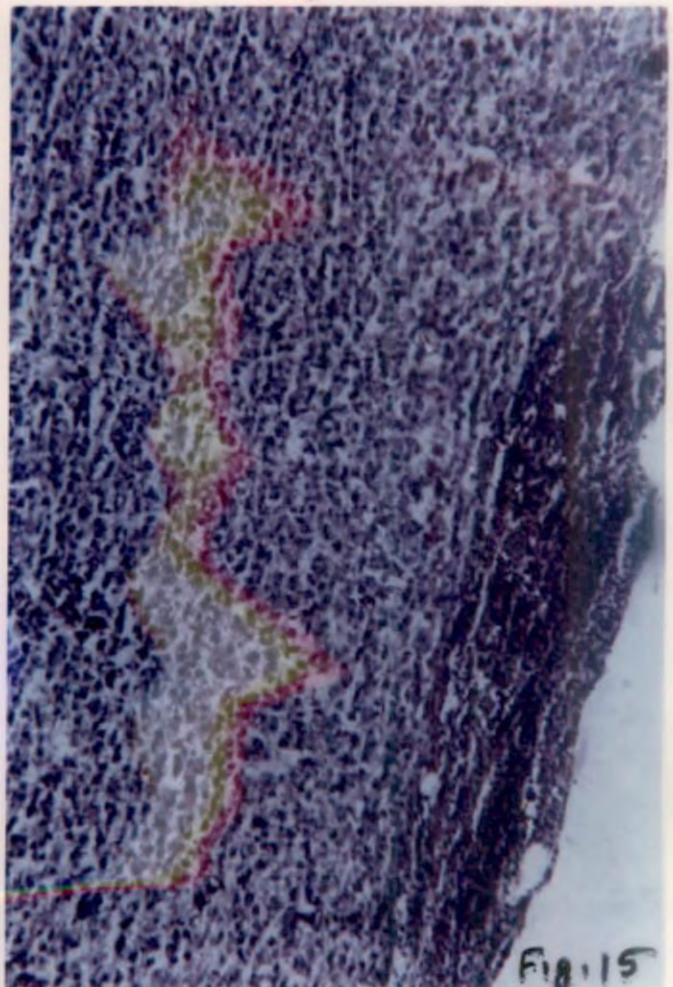


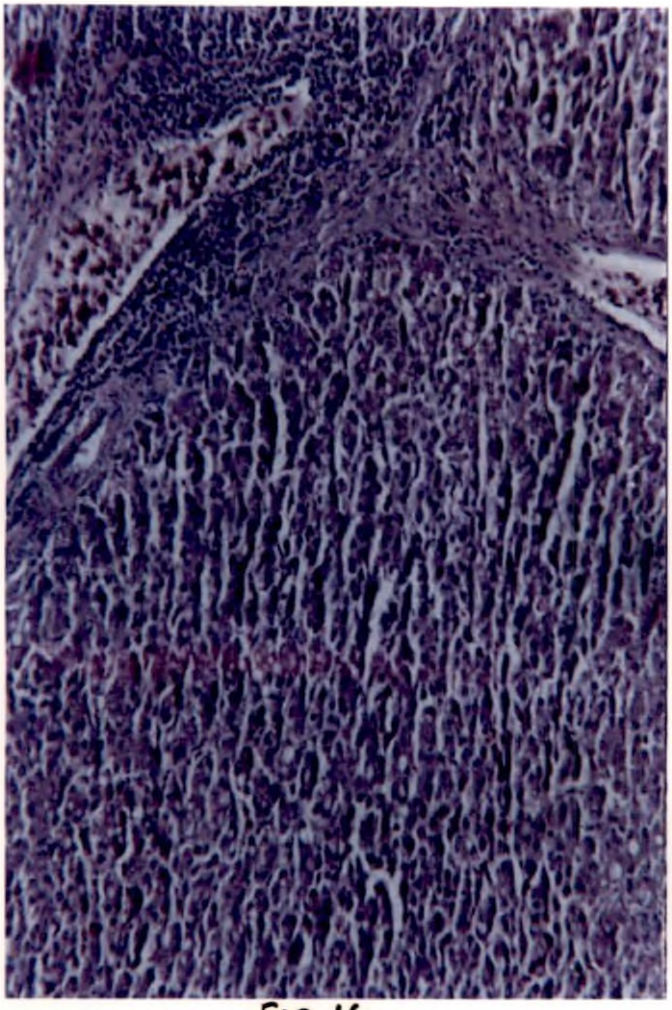
Fig. 15

Fig.16 Congestion, perivascular accumulation of mononuclears fatty change and sinusoidal widening - Group I – H & E x 250

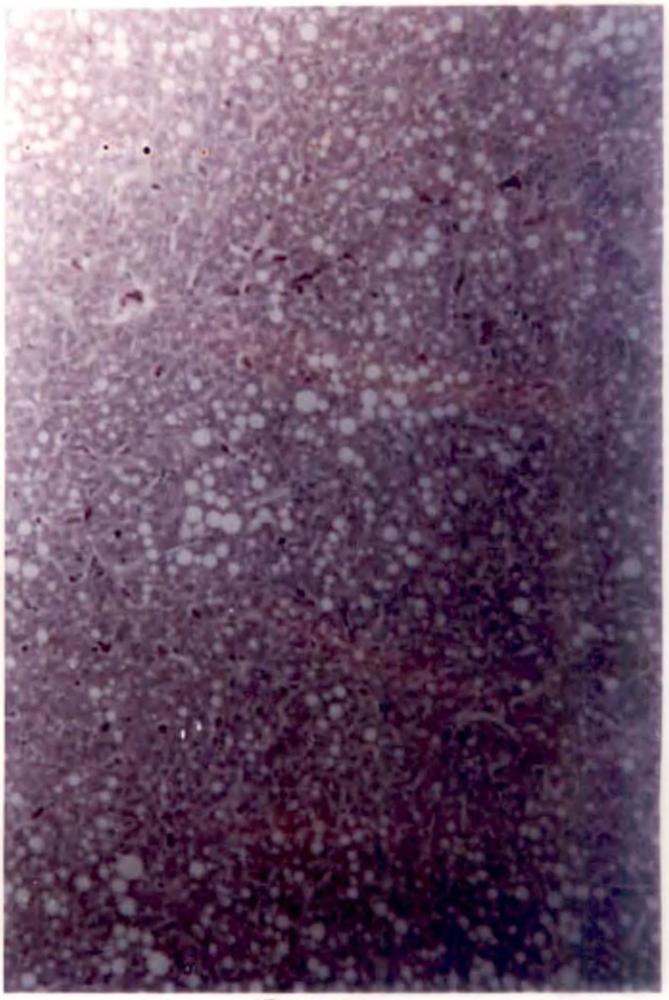
Fig.17 Severe fatty change in liver - Group I – H & E x 250

Fig.18 Fat globules in the hepatocytes coalesced to form fat cysts - Group I – H & E x 63

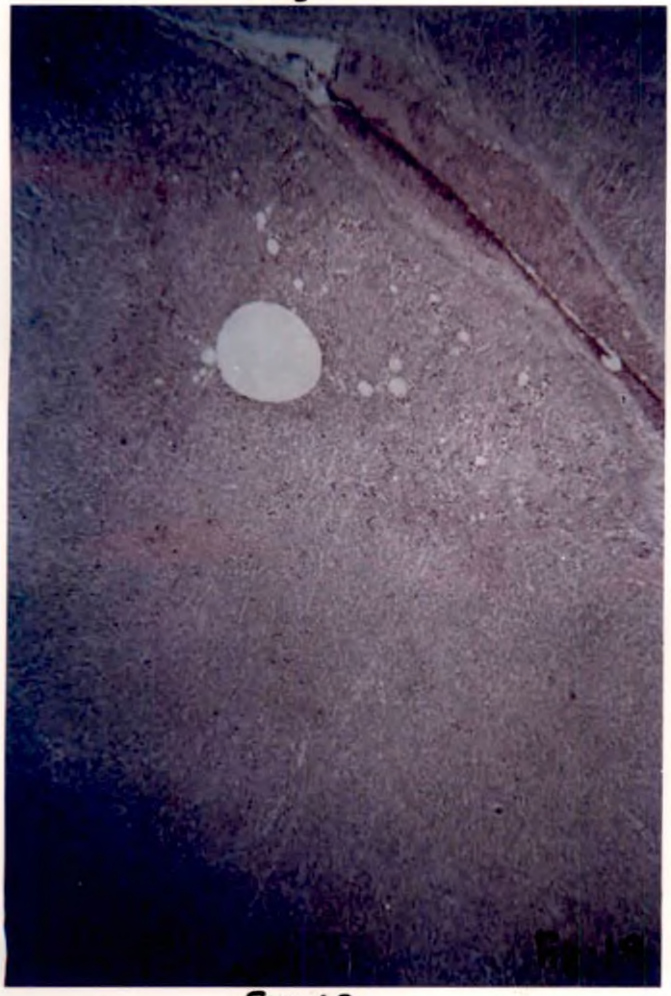
Fig.19 Severe fatty change 'Signet ring' appearance of some hepatocytes and kupffer cell proliferation - Group I – H & E x 1000



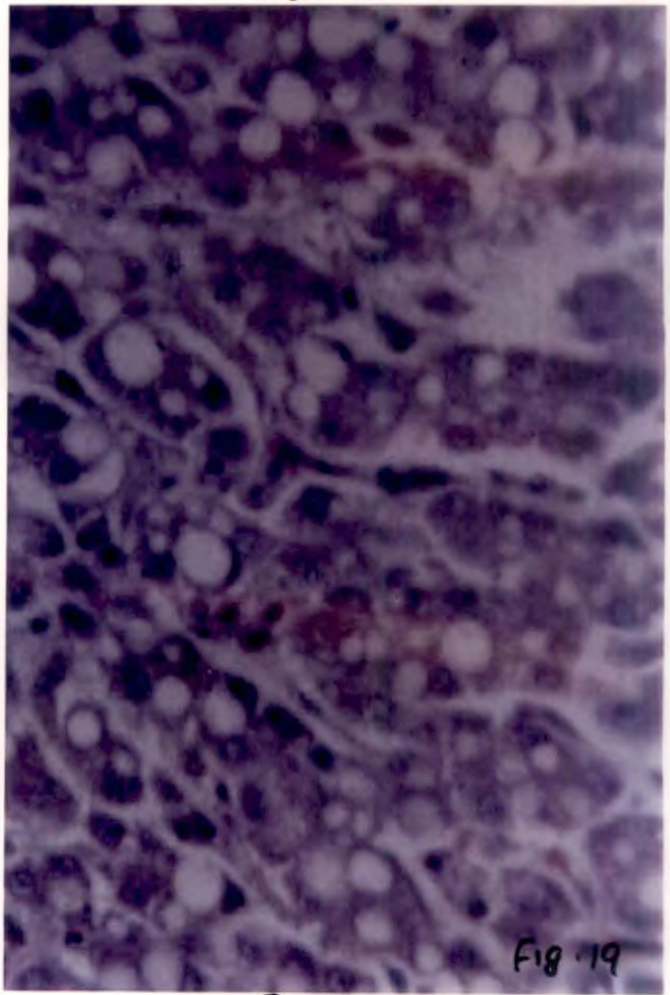
Fig·16



Fig·17



Fig·18



Fig·19

Fig.20 Fat globules stained red with Oil-Red-O- Group I x 250

Fig.21 Fatty change, sinusoidal congestion and perivascular accumulation of mononuclears - Group I – H & E x 250

Fig.22a Nodular hyperplasia of hepatocytes - Group I – H & E x 250

Fig.22b Hyperplastic hepatocytes with vesicular nucleus – Group I – H&E x1000

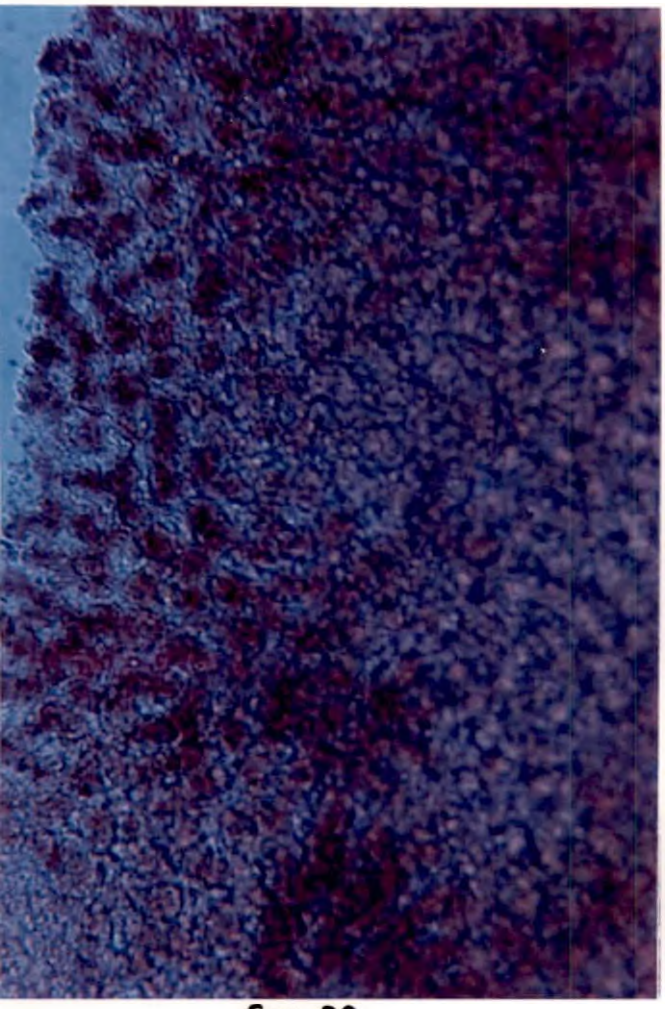


Fig. 20

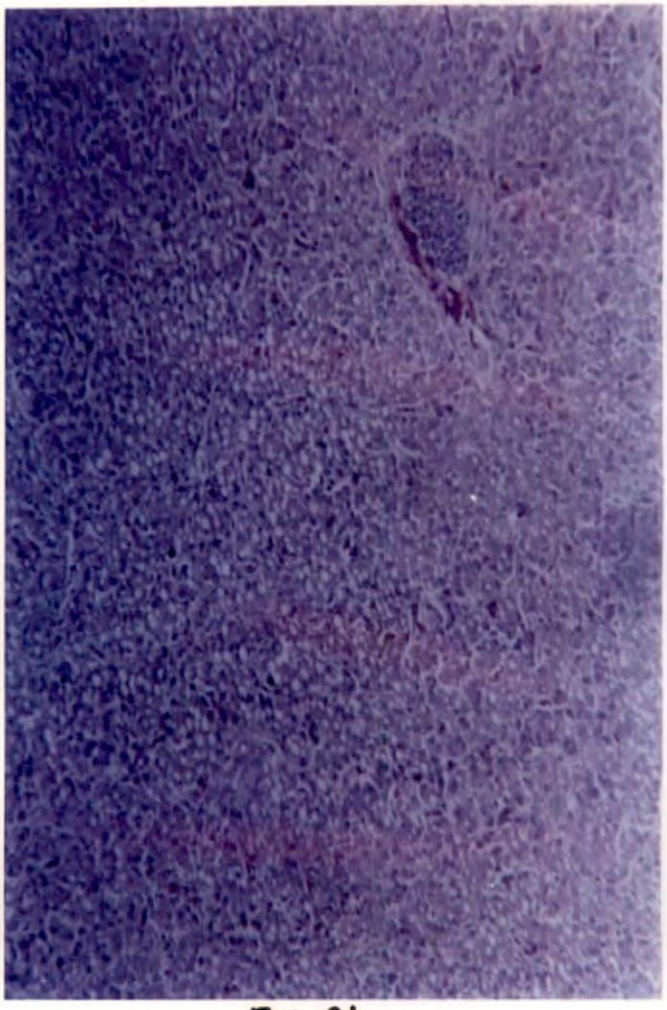


Fig. 21

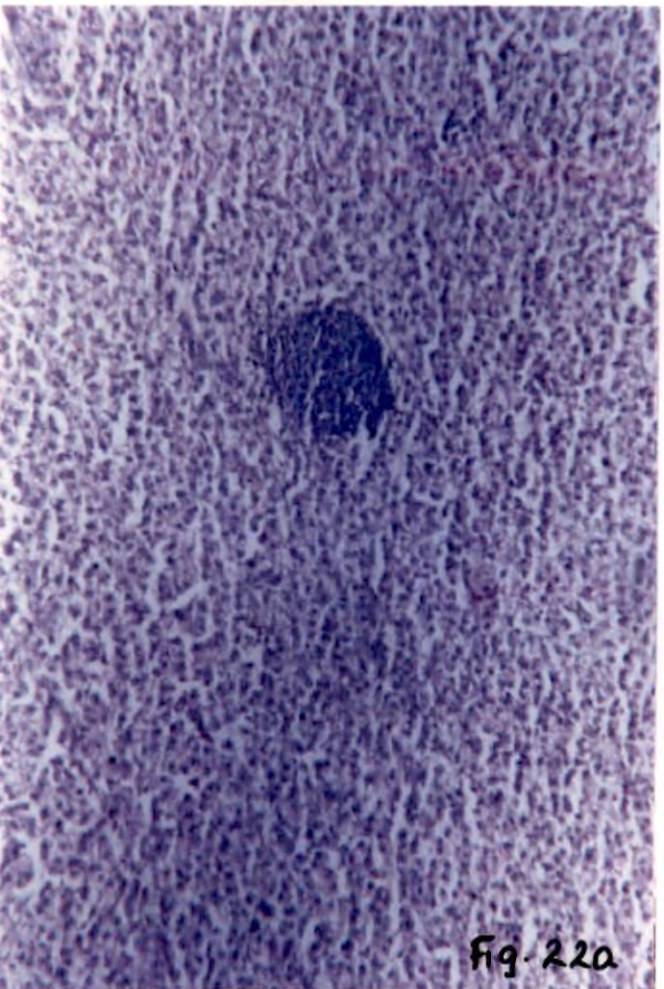


Fig. 22a

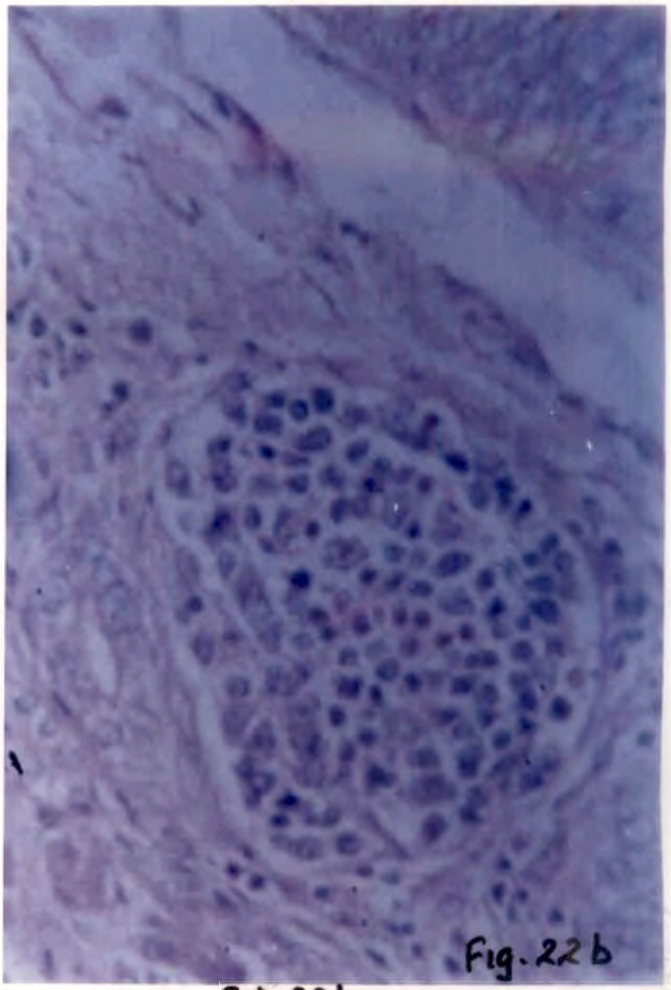


Fig. 22b

Fig.23 **Proliferating fibroblasts in the liver - Group I – H & E x 1000**

Fig.24a **Focal hepatic fibrosis – Group I x 160**
– H & E

Fig.24b **Hepatic fibrosis - collagen stained red with PTAH – Group I x 160**

Fig.25a **Collagen bridged portal areas and severe fatty change - Group I – H & E x 250**

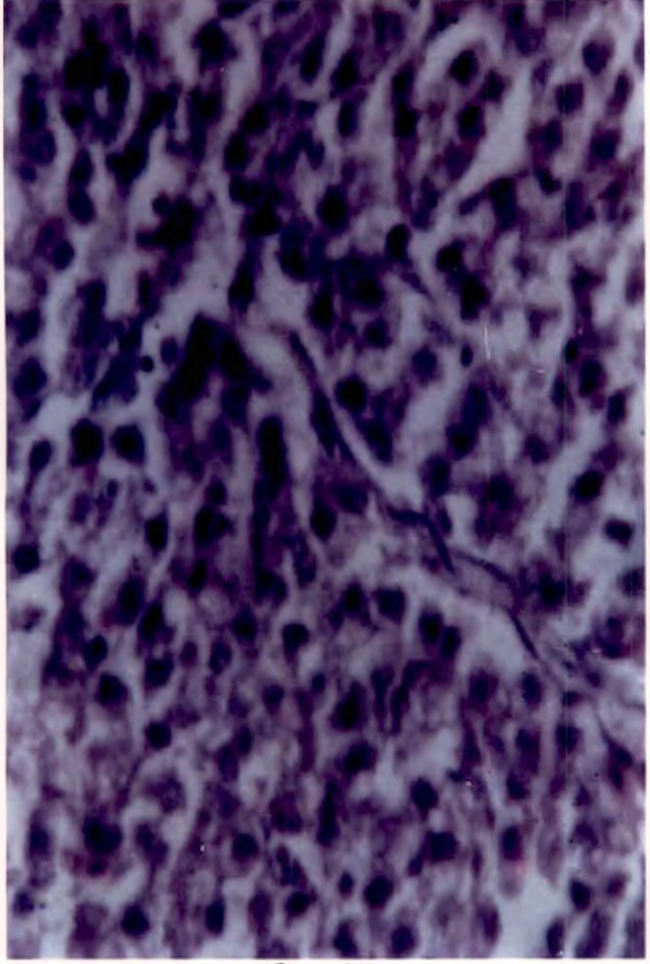


Fig. 23

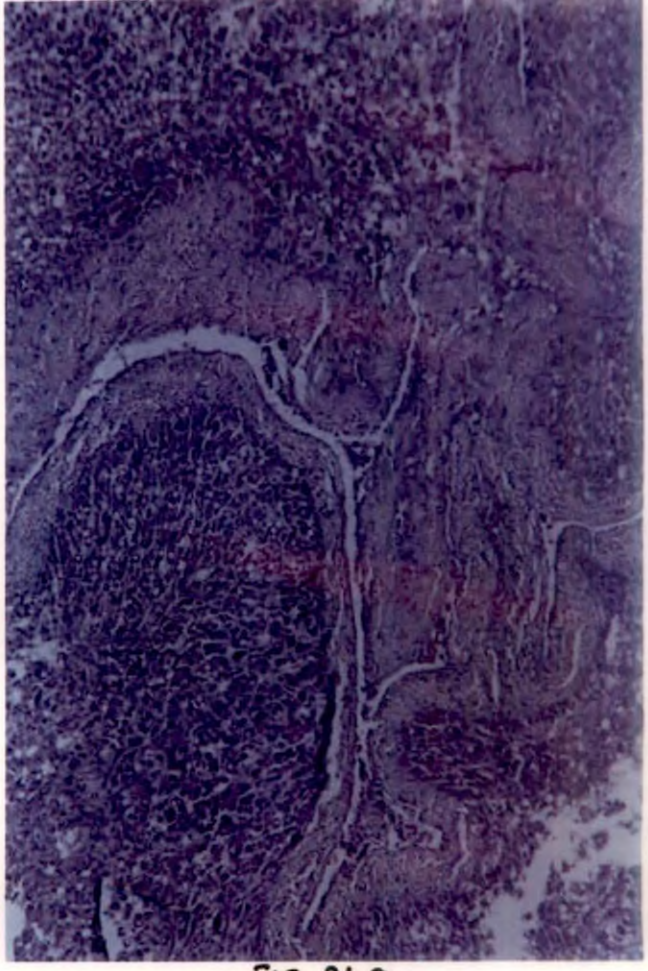


Fig. 24a

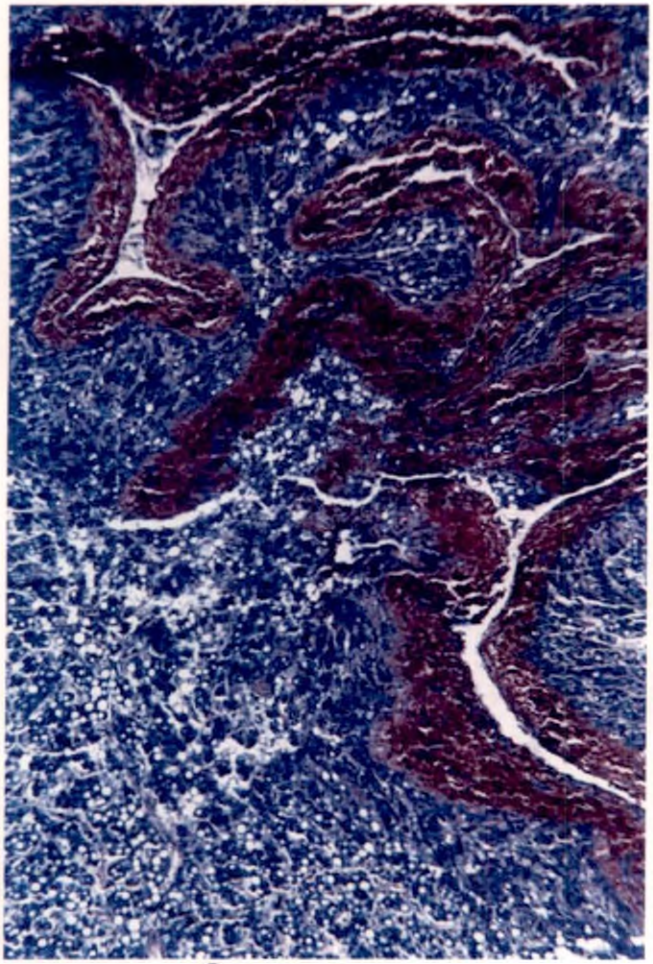


Fig. 24b

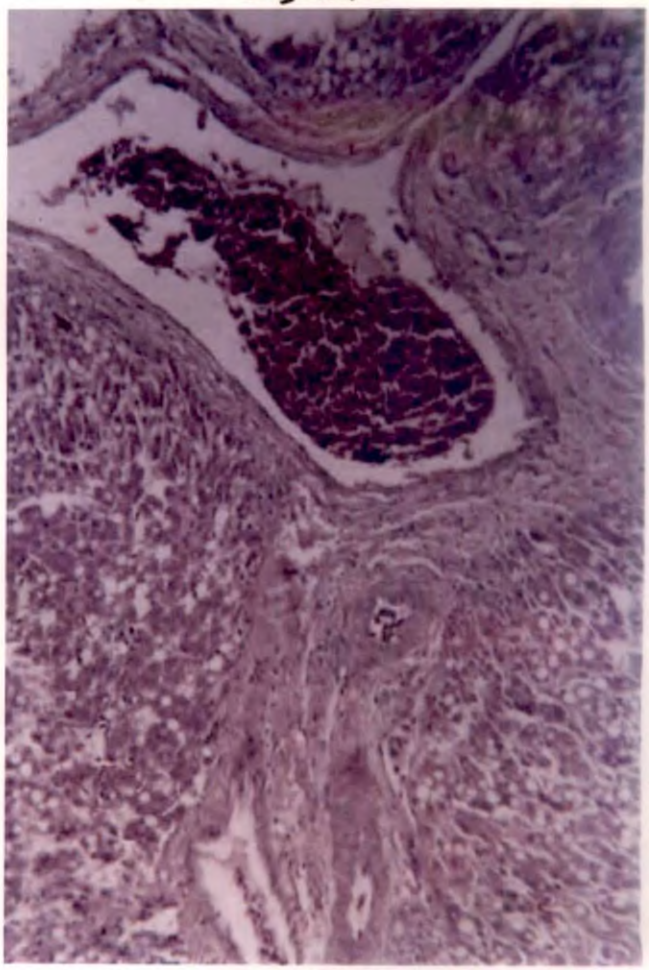


Fig. 25a

Fig.25b Collagen stained red with PTAH – Group I x 160

Fig.26 Heaptic fibrosis and pseudolobulation - Group I – H & E x 160

Fig.27 Subcapsular fibrosis in liver. Group I – H & E x 160

Fig.28 Periductular accumulation of mononuclears - Group I – H & E x 250

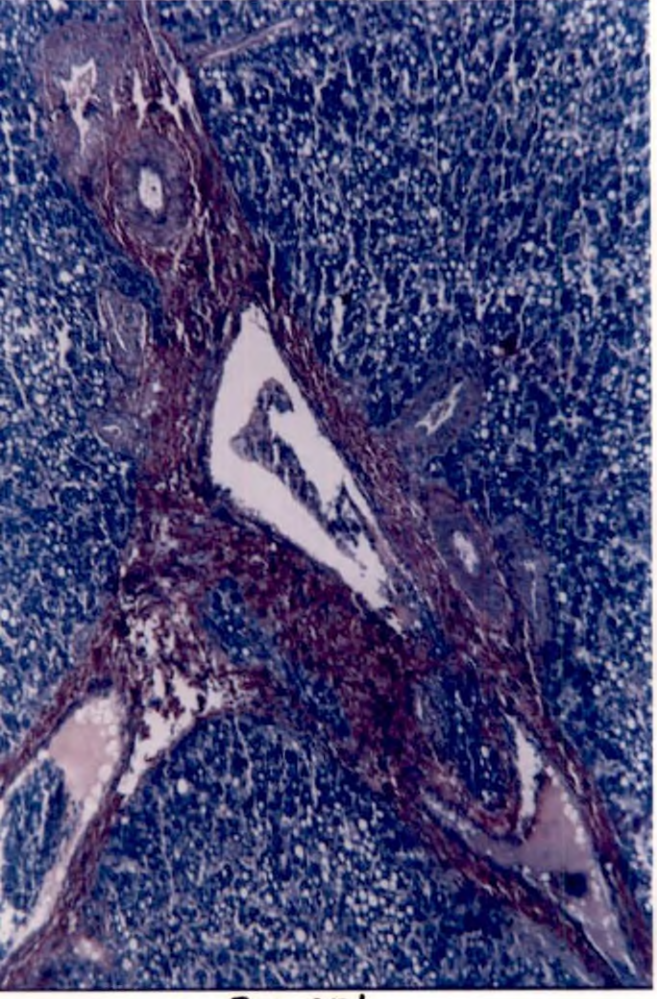


Fig. 25 b

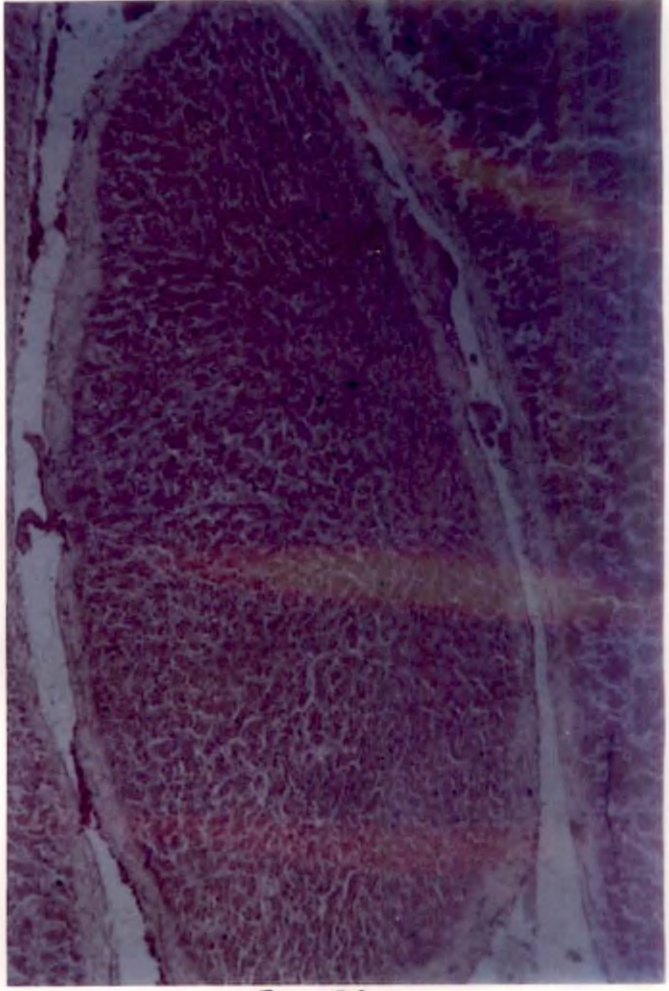


Fig. 26

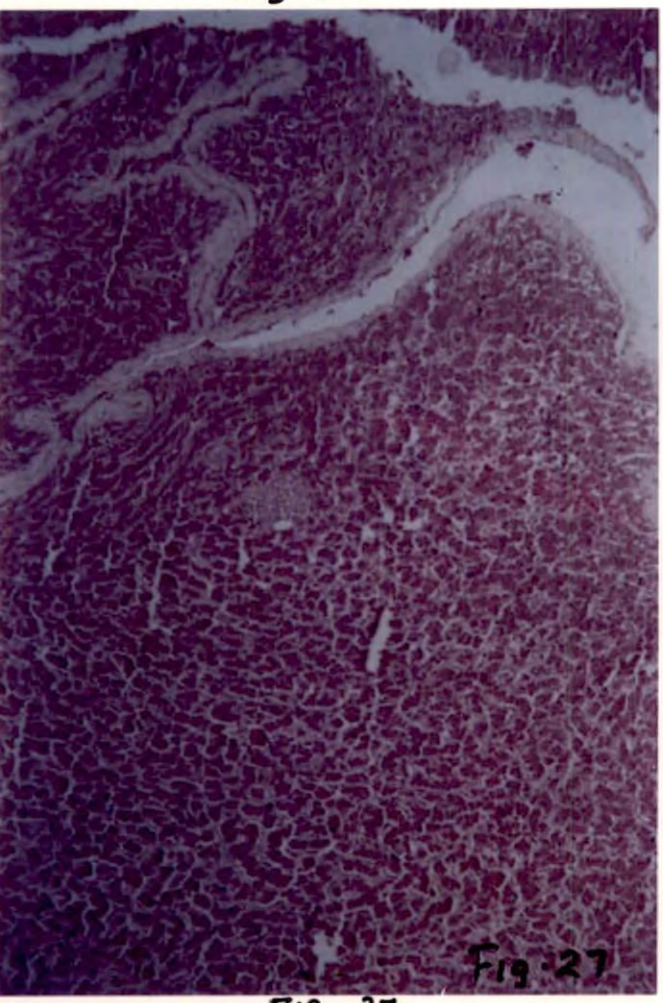


Fig. 27

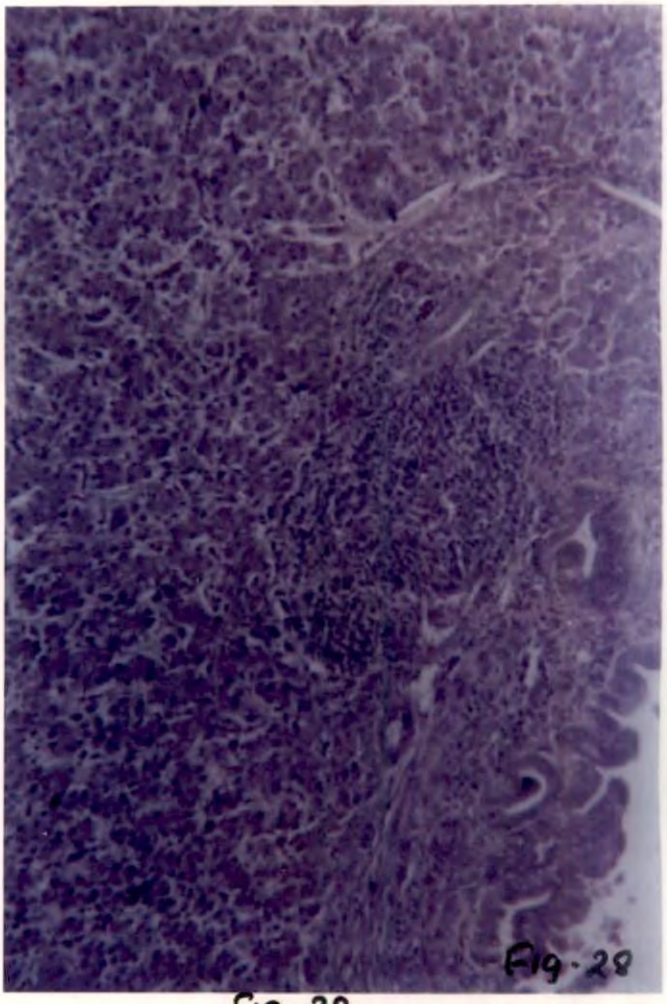


Fig. 28

Fig.29a **Proliferation of bile ducts. Bile duct epithelial hyperplasia forming finger like projections - Group I – H & E x 250**

Fig.29b **Bile duct epithelial hyperplasia forming finger like projections - Group I – H & E x 1000**

Fig.30 **Moderate congestion, phlebosclerosis, bile duct proliferation and fatty change - Group II – H & E x 160**

Fig.31 **Bile duct proliferation and phlebosclerosis with collagen stained red with Van-Gieson Picric acid stain – Group II x 250**

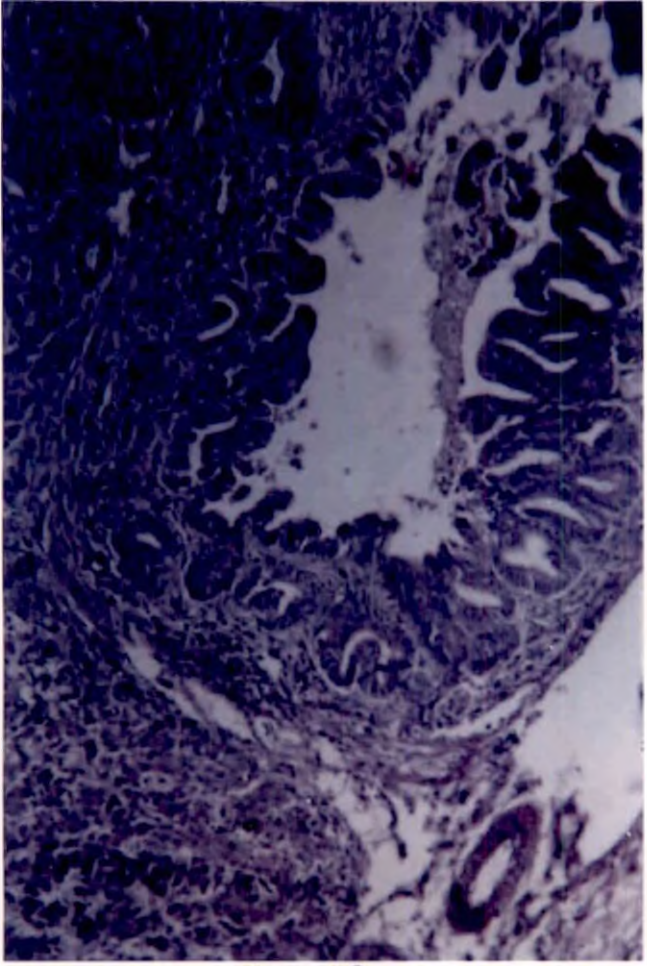


Fig. 29a

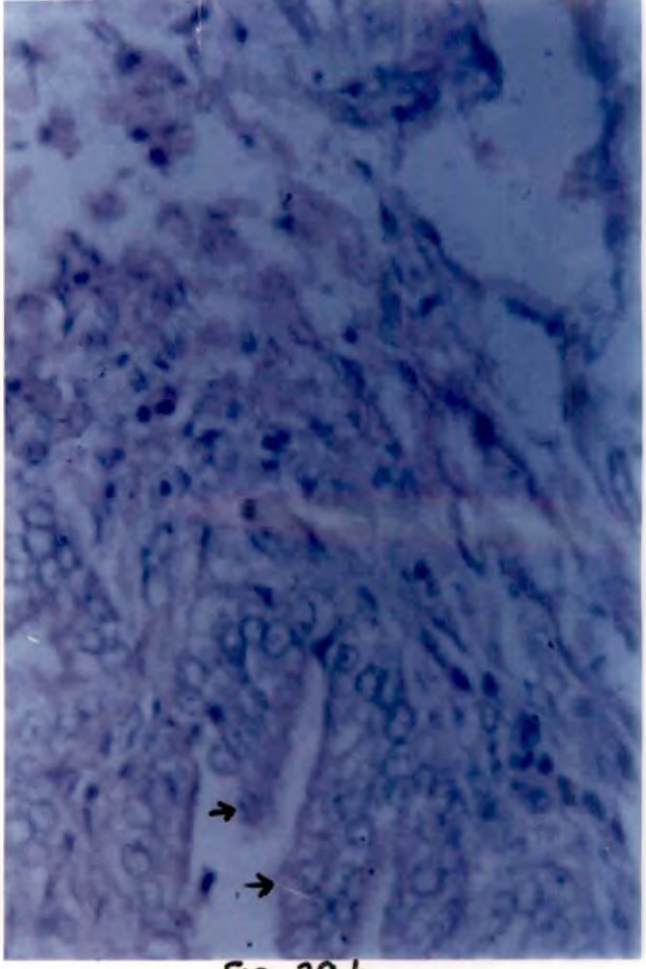


Fig. 29 b

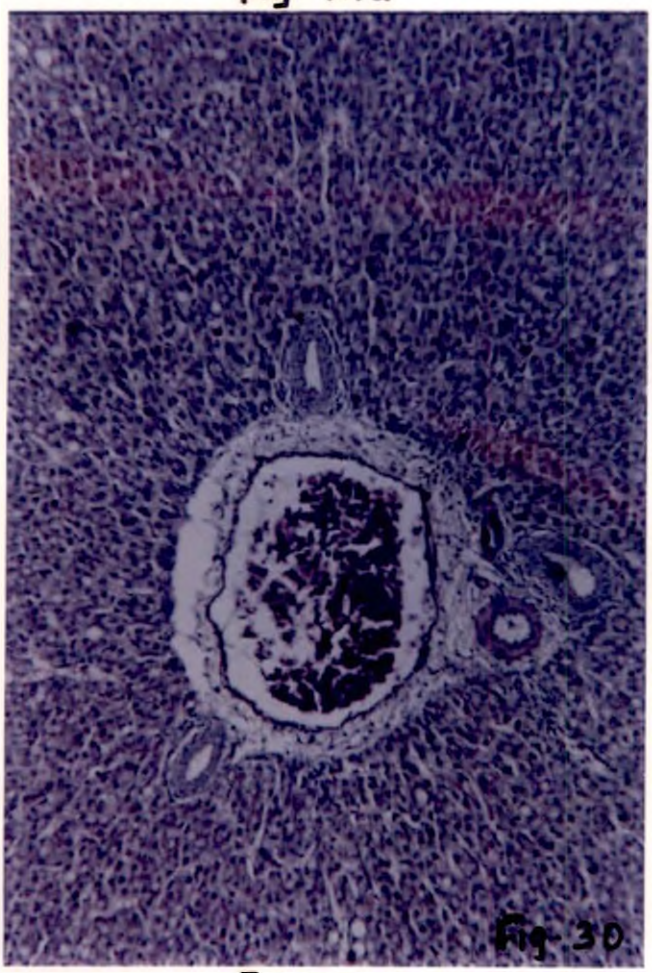


Fig. 30

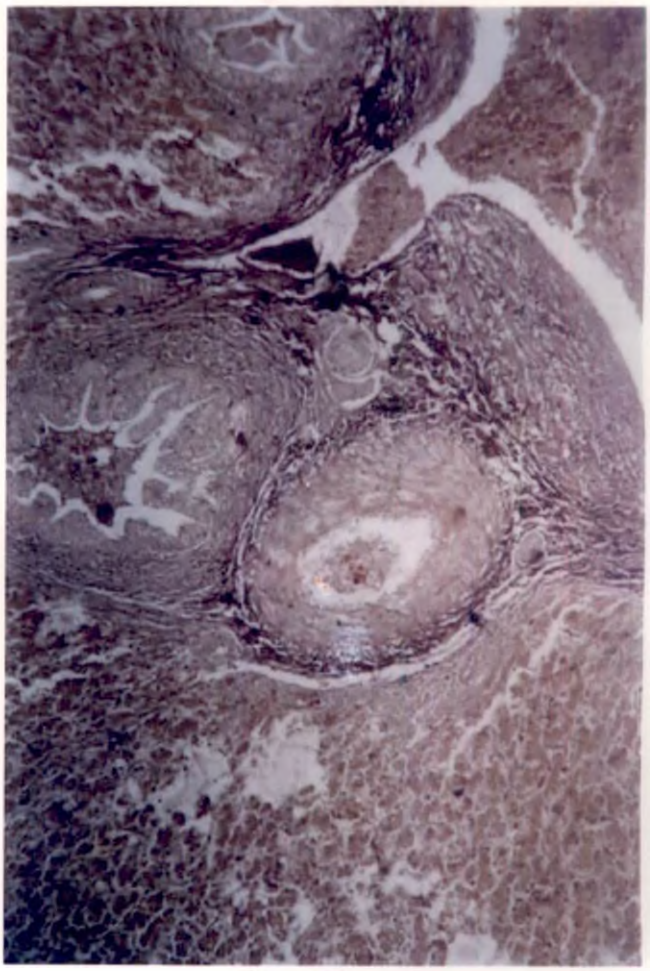


Fig. 31

Fig.32 **Congestion, perivascular accumulation of mononuclears and acinar/ductular arrangement of hepatocytes – Group II – H & E x250**

Fig.33 **Nodular hyperplasia, periportal lymphoid accumulation and mild fatty change – Group II – H&E x250**

Fig.34 **Moderate fatty change and sinusoidal congestion - Group II – H&E x250**

Fig.35 **Mild fatty change in liver - Group II – H&E x1000**

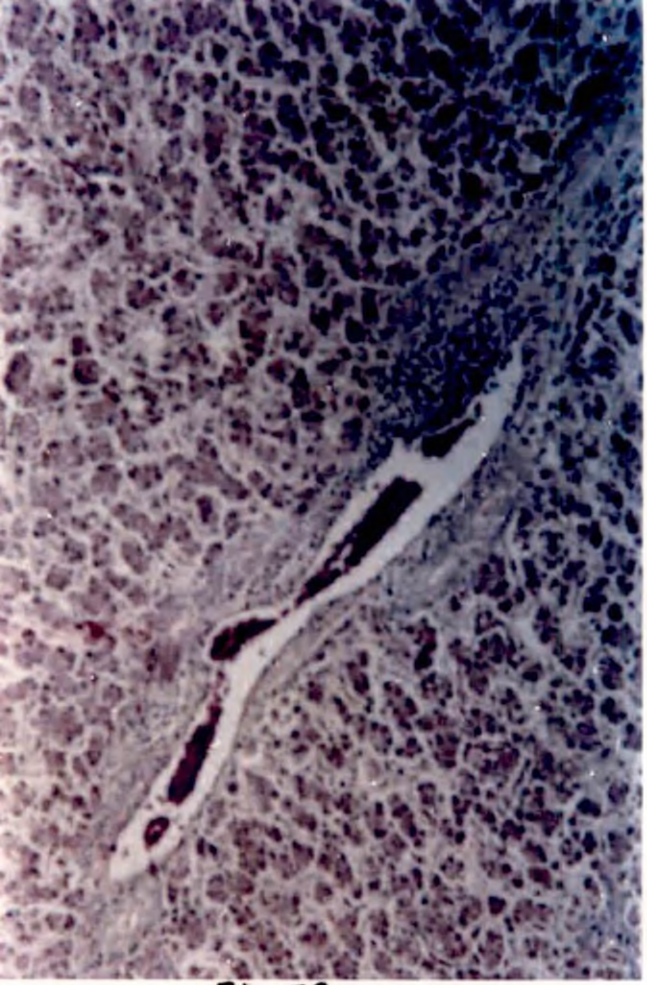


Fig · 32

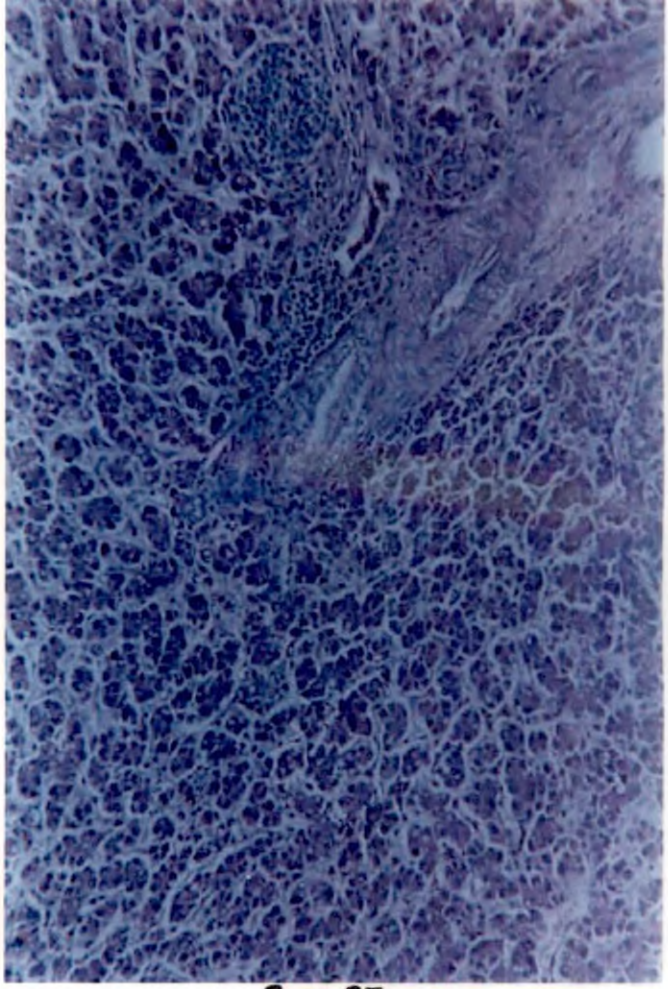


Fig · 33

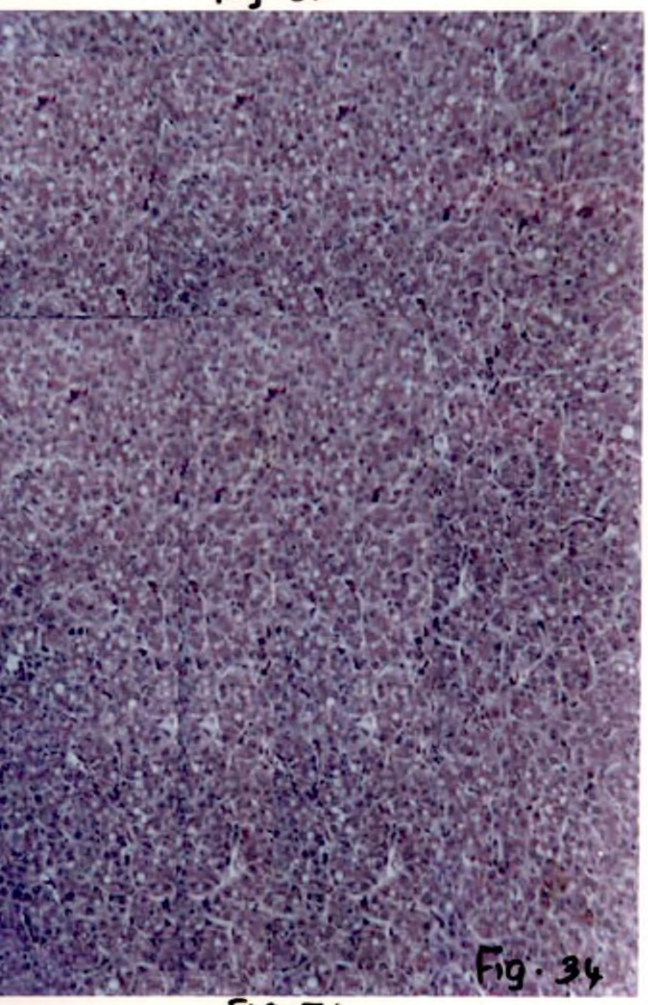


Fig · 34

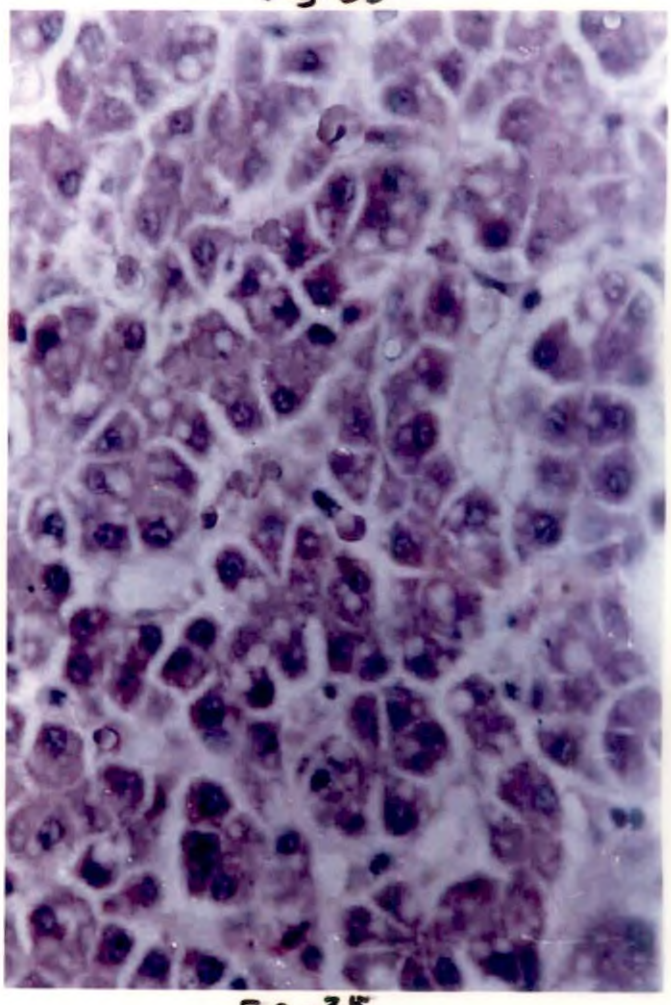


Fig · 35

Fig.36 **Liver - mild congestion and apparently normal hepatic architecture – Group III – H&E x250**

Fig.37 **Liver - mild fatty change and mild perivascular accumulation of mononuclears - Group III – H & E x 250**

Fig.38 **Liver - mild congestion and portal reaction – Group III – H&E x160**

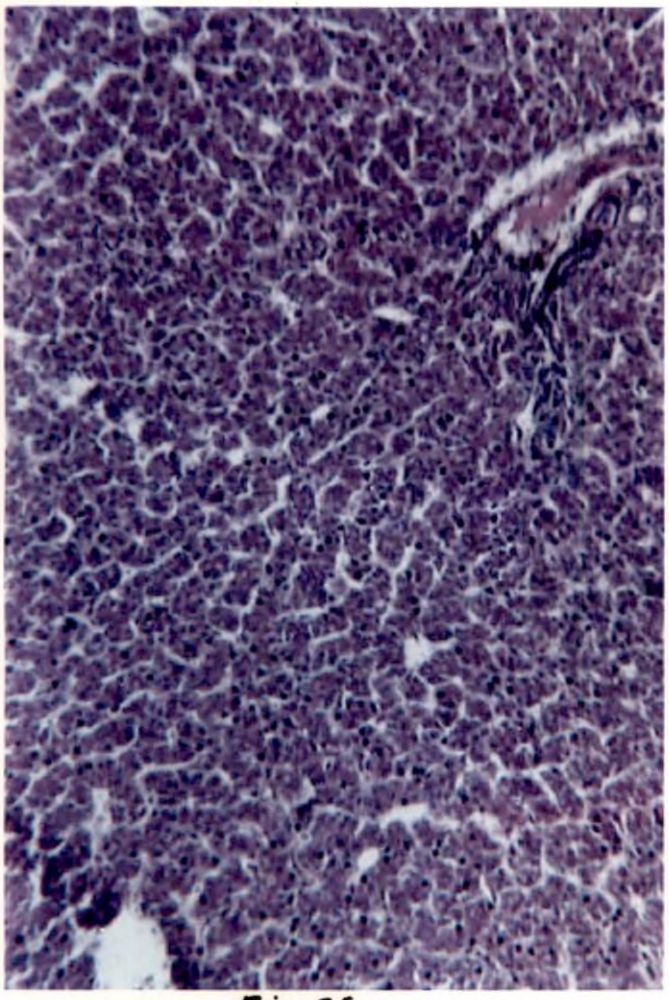


Fig. 36

Fig. 37

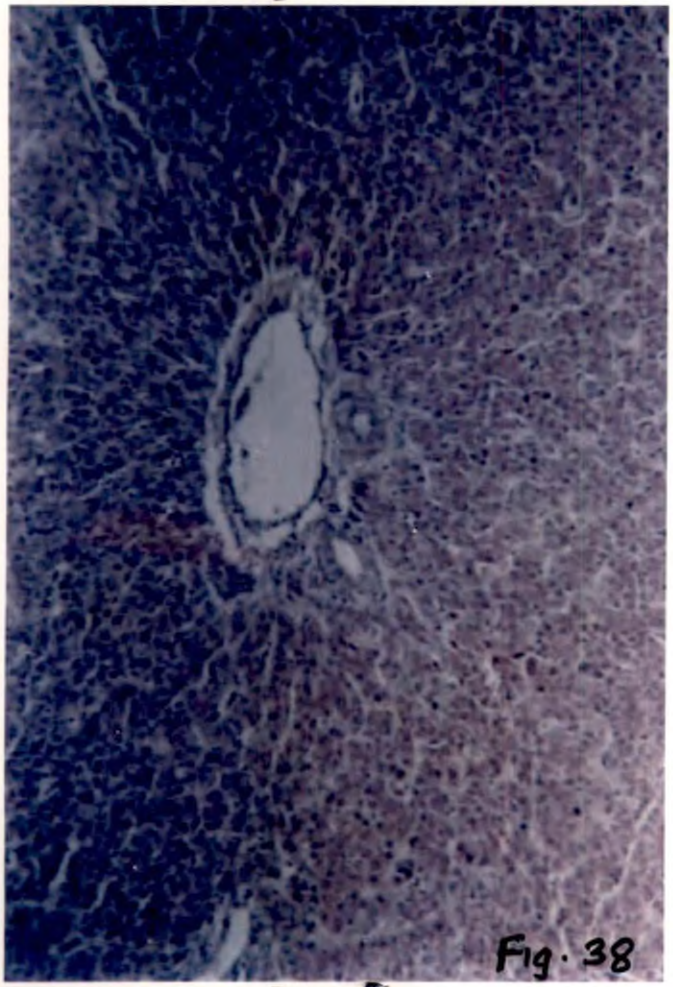


Fig. 38

Fig. 33

Discussion

5. DISCUSSION

The present study was undertaken to determine the effect of *Emblica officinalis* on hepatic function in broiler chicken. Commercial poultry feed may contain excess antibiotics and other feed additives, which may act as hepatotoxins. Toxic contamination of feed especially by mycotoxins is also common. Feed samples; both starter and finisher were tested for the presence of aflatoxins. On an average the starter rations contained 100-150 ppb and the finisher rations contained 150-200 ppb of aflatoxin B_1. No ochratoxin was detected in the feed samples tested. This feed was taken as the control feed (Group I). Amla powder was added at 1% and 2% levels, which constituted the two treatments (Group II and III respectively).

The pathological and biochemical parameters were studied. The parameters included body weight, liver weight, feed intake, feed efficiency, haemogram, serum profile, and feed analysis. The gross and histological pathology of liver were also studied.

The birds in the control group showed reduction in feed intake and feed efficiency. Reduction in weight gain and feed efficiency were considered as the most sensitive indicators in aflatoxicosis (Armbrecht *et al.*, 1971). Reduction in feed intake

was prominent by fourth week of age, in the control group birds. Reddy *et al.*, 1984; Mani *et al.*, 1997 and Vasan *et al.*, 1998, reported similar observations in poultry. The reduction in feed intake may be due to the toxic injury inflicted by aflatoxin on the alimentary system especially the liver, leading to inappetence, poor metabolism and lowered absorption of nutrients, as reported by Hamilton (1989). *Emblica officinalis* reduced the toxic injury, which is evident by the increase in feed intake and improved feed efficiency in the amla fed birds. Similar findings were reported by Gulati *et al.*, 1995, where *Phyllanthus emblica* and quercetin isolated from it improved feed intake which was decreased following alcohol intoxication in rats.

A gradual reduction in body weight gain was recorded in the control group birds compared to the amla fed groups. This was evident from fourth week onwards. The toxin induced inappetence and reduced feed utilization might have caused these effects. This is in accordance with the findings of Mani *et al.* (1997); Muthiah *et al.* (1998) and Vasan *et al.* (1998) in poultry. The higher body weight observed in amla fed groups may be due to the ameliorating effect of *Emblica officinalis* resulting in increased feed intake and improved feed efficiency as described earlier.

A significant increase in ESR was observed in the control group birds. This may be due to the decreased total protein and albumin noticed in this group of birds. A loss of suspension stability of erythrocytes as a result of altered serum protein has been reported to cause increased erythrocyte clumping and rapid sedimentation (Jain, 1986). Amla treatment restored the ESR values, which may be due to the restoration of the enzyme systems and thereby the protein synthesis in the liver. Saraswathy *et al.* (1998) reported that toxin induced decreased protein synthesis was inhibited by Liv 100, a herbal product containing *Emblica officinalis*.

PCV and Hb values were lower in group I birds, which clearly indicated the inhibitory effect of aflatoxin on the hemopoietic system. These observations are in line with the findings of Lanza *et al.* (1977); Panda *et al.* (1987); Fernandez *et al.* (1995) and Vasan *et al.* (1998) in poultry. These values were significantly higher in group II and III birds, indicating the protective effect of amla. This might be due to the protective effect of *Emblica officinalis* on bone marrow cells, as supported by the observations of Kumar *et al.* (1996).

Lowered leukocyte count was observed in the group I birds, in the present study. This may be due to the myelosuppressive

effect of aflatoxin. Treatment with *Emblica officinalis* reduced this suppression. Similar findings have been reported by Kumar *et al.*(1996) and Pallabi De *et al.*(1998), wherein emblica or its products could inhibit the myelosuppression induced by radiation and cyclophosphamide or cyclosporin treatements respectively. This might be due to the activation of macrophages by emblica, leading to enhanced production of colony stimulating factors resulting in the proliferation of cells in the bonemarrow, as supported by Chatterjee (2001). However, Nageswar Rao *et al.* (1988) reported that the total leukocyte count were not affected in chicken fed with aflatoxin.

The lower levels of serum protein and albumin noticed in the present study can be imputed to the hepatic damage. This might be due to poor absorption of aminoacids from the intestine during aflatoxicosis or by the inhibitory effect of aflatoxin B_1 on protein synthesis in hepatic cells, inactivation of aminoacids for protein synthesis in liver and blocking of RNA synthesis in the nucleolus (Mani *et al.*, 1993; Reddy *et al.*, 1984).

The increase in the serum AST, ALP and GGT can be attributed to the pathological changes produced by the toxin in the hepatobiliary system. Aflatoxin induced hepatocellular damage results in increased cellular permeability and release of

enzymes into the serum (Jassar and Singh, 1993 and Shukla and Pachauri, 1995). The serum levels of these enzymes were lower in the amla treated groups. Similar observations have been reported wherein the extract of *Emblica officinalis* reversed the increase in serum enzyme level induced by hepatotoxins like alcohol (Gulati *et al.*, 1995), N-nitrosodiethylamine (Jeena *et al.*,1999) and carbontetrachloride(Jose and Kuttan, 2000). This may be due to the protective effect of amla against the pathological changes induced by the toxin.

Gross and histopathological lesions of varying intensity were observed in the control group birds. Grossly, the livers were mostly enlarged with pale yellow discoloration. Some of the livers were congested. The enlargement and increase in liver weight observed may be due to the accumulation of neutral fats in excess within the liver.

Accumulation of fat within the hepatocytes was also one of the prominent microscopic lesion observed. Aflatoxin has been found to cause defective phosphorylation of fatty acids resulting in accumulation of fat in the hepatocytes (Moorthy *et al.*, 1986; Balachandran and Ramakrishnan, 1987; Ghosh *et al.*, 1989 and Espada *et al.*, 1992). Any disturbance of protein and phospholipid synthesis or adenosine triphosphate synthesis has

the potential to inhibit lipoprotein synthesis or secretion, leading to the accumulation of excess triglycerides within the cytoplasm of hepatocyte (Kelly, 1992). Diffuse degenerative changes to focal necrosis were also observed. Aflatoxin B_1 administration induced degenerative to necrotic changes in ducks (Latha, 1999).

Focal fibrosis was observed, indicating chronic hepatic damage. This may be directly related to the long term presence of excess lipid in the hepatocytes or the sinusoidal fat storage cells (Kelly, 1992).

Regeneration of hepatocytes was well pronounced especially in group I. The cord like arrangement was disrupted in many areas and the ductular or acinar pattern was observed. Megalocytosis with nuclear enlargement, which are the preceeding stages of hepatocellular proliferation, was also observed. Regenerating nodules consisting of clones of hepatic epithelial cells were observed. These indicate the repair mechanisms of the liver against the toxic injury. Similar findings were reported by Balachandran and Ramakrishnan (1987); Anjaneyulu and Rao (1993) and Sridevi and Sriraman (1996). Bile duct proliferation was well pronounced. There was hyperplasia of the bile duct epithelial cells forming finger like projections in to the lumen. Bile duct proliferation was considered as an important pathological

alteration in aflatoxicosis (Siller and Ostler, 1961 and Espada *et al.*, 1992).

Aflatoxin is subjected to metabolic activation to its various toxic metabolites with the help of the hepatic microsomal enzymes like the P450 enzyme system. The susceptibility of the liver tissue to the aflatoxin is directly proportional to the rate at which it is metabolised. The active metabolites like the aflatoxin hemi acetals, 8, 9 epoxides etc. cause widespread non-specific interference with the hepatocellular metabolic and physiological systems leading to hepatocellular toxicity. These include effects on protein, lipid and carbohydrate metabolisms; interaction with nucleic acids and enzymes; interruption of electron transport in the terminal respiratory chain, its labilizing action on cellular and subcellular membranes, and many other biological effects notably the carcinogenic, mutagenic and teratogenic activities. The lesions induced by the toxin were found to be decreased in the amla treated groups depending on the level of amla powder added in the feed. *Emblica officinalis* was reported to inhibit aniline hydroxylase, aminopyrine, demethylase and other hepatic P450 enzyme systems involved in the metabolic activation of xenobiotics. Also amla has got antioxidant activity by scavenging the free radicals generated in the metabolism of

xenobiotics by augmenting the activity of the free radical scavenging enzymes (Jeena et al., 1999). Thus the hepatoprotective activity exhibited by *Emblica officinalis* might be due to the inhibition of the enzymes responsible for the metabolic activation of aflatoxin or due to the scavenging of the free radicals generated. Also, liver being a complex organ, other metabolic pathways may be present which may be stimulated by Emblica, leading to the production of less toxic products. The present study is of a preliminary nature and a detailed investigation has to be carried out to find out the exact mechanisms by which the toxic effects of aflatoxin are ameliorated by amla.

The incidence of aflatoxin contamination in the commercial livestock feed available in Kerala is very high. The hot and humid climatic conditions along with poor storage conditions followed are all conducive for the fungal growth. It is also reported that aflatoxicosis is not solely a "storage problem". Aflatoxin may be produced in growing crops before harvest. Hence it is rather impossible to maintain poultry or any other livestock with a feed totally free from aflatoxin. Rajan *et al.* (1991) reported that 50.1 percent of the feed samples available in the market of Kerala were contaminated with aflatoxin. Arulmozhi (1999) reported

that 24 per cent of the poultry feed samples available in the market of Kerala were contaminated with toxic levels of aflatoxin with the range of 20-200 ppb. These indicate that there is no proper quality control over the marketed feed with regard to aflatoxin level.

The presence of aflatoxin residues in the food of animal origin and the subsequent human exposure is also of great concern. Animals consuming contaminated feed may excrete the toxin through milk or eggs in a chemically modified form or may transfer them to various tissues especially liver in a chemically unchanged form, where it remains as residues. In both the situations, humans are at risk of contracting toxicosis. Aflatoxin is suspected to be one of the etiological agents responsible for the high incidence of hepatic and colon cancers seen in India. It is also supposed to play a role in the etiology of Kwashiorkar disease and infantile or childhood cirrhosis. Aflatoxicosis predisposed by vitamin A and protein deficiencies worsens the condition.

The results of the present study indicate that *Emblica officinalis* could significantly ameliorate the toxico-pathological changes induced by aflatoxin in liver. Emblica is reported to prevent metabolic activation and thereby the hepatic damage induced by many other chemicals including antibiotics and other

feed additives, corticosteroids, anti-tubercular drugs, alcohol, carbon tetra chloride and many other hepatotoxins. The active principles present in amla are ascorbic acid and the hydrolysable tannins, containing gallic and ellagic acids. Hydrolysable tannins having vitamin C like activity were found to be Emblicanin A, Emblicanin B, Punigluconin and Pedunculagin. These might be responsible for the protective effect provided by *Emblica officinalis.*

The present study makes it clear that *Emblica officinalis* has significant hepatoprotective effect and could be used as an effective feed additive in the livestock feed.

Summary

6. SUMMARY

The present study was undertaken to assess the effect of amla (*Emblica officinalis*) on hepatic function in broiler chicken. Day old broiler chicks (n=54) were divided into three groups, with two replicates in each. Normal, commercial broiler feed, both starter and finisher was given to the control group(group I) birds. Amla powder was added at one per cent and two per cent levels in the other two groups (group II and III respectively), along with the control feed. The birds were reared for eight weeks.

Different parameters like body weight gain, feed efficiency, feed analysis for the estimation of proximate principles and aflatoxin content, haemogram, serum profile, liver weight and gross and histopathological changes in liver were studied.

The body weight gain showed a gradual increase in all the groups. But, a significant decrease in weight gain was noted in the control group birds, when compared to the amla fed groups, especially by fourth week. Reduction in feed intake was noted in the control group birds in the latter half of the experiment. Feed efficiency was also lower for the control group birds, when compared to the other two groups.

Proximate analysis of the feed indicated normal chemical composition. Analysis for aflatoxin B_1 content revealed the presence of toxin in the range of 100-150 ppb in the starter and 150-200 ppb in the finisher feeds. The toxicopathological changes induced by the control feed, may be attributed to the presence of toxin in feed.

Haematological studies indicated that, the hemoglobin, PCV, total leukocyte, lymphocyte and heterophil counts were decreased, while an increase in ESR was noted in the group I birds.

Serum profile revealed a significant decrease in the total protein, albumin and absolute globulin values, in the control group birds, while the A/G ratio was significantly increased in them. Serum enzymes like AST, ALP and GGT were elevated in these birds, which indicated hepatic damage.

Clinically, reduction in feed intake and slight dullness were observed in the control group birds. The amla treated birds were apparently healthy and active.

On gross examination, the livers of the control group birds were enlarged, pale to yellow in colour, with rounded borders and focal areas of congestion.

Histopathological lesions of varying nature, from mild acute inflammatory reaction to chronic fibrous tissue proliferation were noted in the group I birds. The severity of the lesions of present, were very much decreased in the amla treated groups, in a dose related manner.

Vascular and cellular changes characterized by congestion, haemorrhage, perivascular oedema and accumulation of mononuclears were observed. Fatty change, characterized by accumulation of numerous, fat vacuoles of varying size within the hepatocyte cytoplasm was one of the prominent lesions noted.

Diffuse degenerative changes to focal necrotic changes were observed in all the livers. There was mononuclear accumulation and fibrous tissue proliferation in some of the livers in addition to the necrotic reaction.

Chronic hepatic damage, characterized by focal fibrosis, lymphocyte infiltration, biliary hyperplasia, phlebosclerosis, bile duct proliferation and hepatic nodular hyperplasia, were also observed.

These histopathological changes were reduced in the amla treated groups. Similar changes, in a mild to moderate form

were observed in the one per cent amla group and mild in the two per cent amla group.

The results of the present study indicate that, *Emblica officinalis*, could effectively reduce the toxicopathological changes induced by aflatoxin. *Emblica officinalis* is reported to inhibit the hepatic P-450 enzyme system, which is involved in the metabolic activation of aflatoxin. Also, it is effective in scavenging the free radicals formed in any metabolic pathway. The protection afforded by Emblica against aflatoxin induced hepatotoxicity may be due to these two actions of *Emblica officinalis*.

Considering the present situation in Kerala, it is rather impossible to maintain poultry or any other livestock, with a feed that is completely free of aflatoxin. This necessitates the need for a suitable method to reduce the toxic effects induced by the toxin in animals.

The presence of aflatoxin in the livestock feed, will deleteriously affect the growth and production performance of our livestock. Moreover, aflatoxin excreted in milk and other products and that present as residues in the edible tissues, may prove harmful to human beings. Aflatoxin is suspected to be the cause of hepatic and colon cancers, Kwashiorkar disease and a condition in children called infantile or childhood cirrhosis.

Considering the above facts and the findings of the present study, it could be concluded that *Emblica officinalis* could be effectively used as a feed additive in the livestock feed.

References

REFERENCES

Anjaneyulu, Y. and Rao, R.P. 1993. Experimental aflatoxicosis and its amelioration by activated charcoal in broiler chicken-A pathological study. *Indian J. Vet. Path.* **17**: 122-125

Ansari, K.U., Kastury, N., Tewarson, C.L., Singh, S. and Pandey, R.C. 1998. An experimental and clinical evaluation of immunomodulating potential of ascorbic acid. *Indian J. Pharmacol.* **30**: 94-96

AOAC. 1990. *Official Methods of Analysis*. Fifteenth edition. Association of Analytical Chemists, Washington, D.C. p. 684

Armbrecht, B.H., Wiseman, H.G. and Shalkop, W.J. 1971. An assessment of growth efficiency and other responses in growing pigs fed aflatoxin. *Environ. Physiol.* **1**: 198-208

Arulmozhi, A. 1999. Determination of permissible level of aflatoxin in broiler chicken feed. M.V.Sc. thesis. Kerala Agricultural University, Thrissur, p. 100

Augusti, K.T., Arathy, S.L., Asha, R., Ramakrishnan, J., Zaira, J., Lekha, V., Smitha, S., George, S. and Vijayasri, V.M. 2001. A comparative study on the beneficial effects of garlic (*Allium sativum* Linn.), amla (*Emblica officinalis* Gaertn) and onion (*Allium cepa* Linn) on the hyperlepidaemia induced by butter fat and beef fat in rats. *Indian J. Exp. Biol.* **39**: 760-766

Balachandran, C. and Ramakrishnan, R. 1987. An experimental study on pathological effects of aflatoxicosis in broiler chicken. *Indian Vet. J.* **64**: 911-914

Bancroft, J.D. and Cook, H.C. 1995. *Manual of Histological Techniques and their Diagnostic Application.* Second edition. Churchil Livingstone, Edinburgh, p.457

Barthakur, N.N. and Arnold, N.P. 1991. Chemical analysis of the emblica (*Phyllanthus emblica* L.) and its potential as a food source. *Sci. Hort.* **47**: 99-105

Bhattacharya, A., Chatterjee, A., Ghosal, S. and Bhattacharya, S.K. 1999. Antioxidant activity of active tannoid principles of *Emblica officinalis* (amla). *Indian J. Exp. Biol.* **37**: 676-680

Bhattacharya, A., Kumar, M., Ghosal, S. and Bhattacharya, S.K. 2000. Effect of bioactive tannoid principles of *Emblica officinalis* on iron-induced hepatic toxicity in rats. *Phytomedicine* **7**(2): 173-175

Chatterjee, S. 2001. Effect of IMMU-21, a Herbal formulation on Granulocyte-Macrophage colony stimulating factors, macrophage maturation and splenic plaque forming cells in experimental animals. *Indian J. Pharmacol.* **33**: 442-444

Das, K.K. and Dasgupta, S. 1997. Influence of ascorbic acid on acid and alkaline phosphatase activities in some metabolically active tissues of aspirin treated rats. *Indian J. Physiol. Pharmacol.* **41**(4): 421-423

Devasagayam, T.P.A. and Sainis, K.B. 2002. Immune system and antioxidants, especially those derived from Indian medical plants. *Indian J. Exp. Biol.* **40**: 639-655

Dhir, B., Roy, A.K., Sharma, A. and Talukder, G. 1990. Protection afforded by aqueous extracts of Phyllanthus species against cytotoxicity induced by lead and aluminium salts. *Phytotherapy Res.* **4**(5): 172-176

Dhuley, J.N. 1999. Protective effect of Rhinax, a herbal formulation against physical and chemical factors induced gastric and duodenal ulcers in rats. *Indian J. Pharmacol.* **31**: 128-132

Doumas, B.T., Watson, W.A. and Biggs, H.G. 1971. Albumin standards and the measurement of serum albumin with Bromocresol green. *Clin. Chem. Acta.* **31**: 87-96

Espada, Y., Domingo, M., Gomez, J. and Calvo, M.A. 1992. Pathological lesions following an experimental intoxication with aflatoxin B_1 in broiler chicken. *Res. Vet. Sci.* **53**: 275-279

Fernandez, A., Verdo, M.T., Gomez, J., Gascon, M., and Ramos, J.J. 1995. Changes in the prothrombin time, haematology and serum protein during experimental aflatoxicosis in hens and broiler chicken. *Res. Vet. Sci.* **58**: 119-122

*Ghosh, A., Sharma, A. and Talukdav, G. 1993. Comparison of the protection afforded by a crude extract of *Phyllanthus emblica* fruit and an equivalent amount of synthetic ascorbic acid against the cytotoxic effects of cesium chloride in mice. *Int. J. Pharmacognosy* **31**(2): 116-120

Ghosh, R.C., Chauhan, H.V.S., Jha, G.J., and Singh K.K. 1989. Pathology of aflatoxicosis in chickens. *Indian J. Vet. Path.* **13**: 14-18

Gomall, A.G., Bardawill, C.J. and David, M.M. 1949. Determination of serum protein by the biuret reaction. *J. Biol. Chem.* **177**: 751-766

Grover, I.S. and Kaur, S. 1989. Effect of *Emblica officinalis* Gaertn. (Indian gooseberry) fruit extract on sodium azide and 4-nitro-0-phenylene diamine induced mutagenesis in *Salmonella typhimurium*. *Indian J. Exp. Biol.* **27**: 207-209

Gulati, R.K., Agarwal, S. and Agrawal, S.S. 1995. Hepatoprotective studies on *Phyllanthus emblica* Linn. And quercetin. *Indian J. Exp. Biol.* **33**: 261-268

*Hamilton, B. 1989 Why the animal industry worries about mycotoxins. *Poult. Guide.* **26** (9) : 24

Jain, N.C. 1986. *Schalm's Veterinary Haematology.* Fourth edition. Council of Agricultural Research, New Delhi, p. 564

Jassar, B.S. and Singh, B. 1993. Biochemical changes in experimental aflatoxicosis in broiler chicken. *Indian J. Anim. Sci.* **63**: 847-848

Jeena, K.J., Joy, K.L. and Kuttan, R. 1999. Effect of *Emblica officinalis*, *Phyllanthus amarus* and *Picrorrhiza kurroa* on N-nitrosodiethylamine induced hepatocarcinogenesis. *Cancer Lett.* **136**: 11-16

Jose, J.K. and Kuttan, R. 1995a. Inhibition of oxygen free radicals by *Emblica officinalis* extract and Chyavanaprash. *Amala Res. Bull.* **15**: 49-52

Jose, J.K. and Kuttan, R. 1995b. Antioxidant activity of *Emblica officinalis*. *J. Clin. Biochem. Nutr.* **19**: 63-70

Jose, J.K. and Kuttan, R. 1998. Chemoprotective effect of *Emblica officinalis* and Chyavanaprash. *Amala Res. Bull.* **18**: 59-66

Jose, J.K. and Kuttan, R. 2000. Hepatoprotective activity of *Emblica officinalis* and Chyavanaprash. *J. Ethnopharmacol.* **72**: 135-140

Jose, J.K., Kuttan, R. and Bhattacharya, R.K. 1997. Anticarcinogenic activity of *Emblica officinalis*. *Amala Res. Bull.* **17**: 55-61

Kelly, W.R. 1992. The Liver and Biliary System. *Pathology of Domestic Animals*. (eds. Jubb, K.V.F., Kennedy, P.C. and Palmer, N.). Fourth edition. Academic Press Inc. 1250 Avenue, San Diego, California, pp. 319-406

Khandelwal, S., Shukla, L.J. and Shanker, R. 2002. Modulation of acute cadmium toxicity by *Emblica officinalis* fruit in rat. *Indian J. Exp. Biol.* **40**: 564-570

Khopde, S.M., Priyadarshini, K.I., Mohan, H., Gawandi, V.B., Satav, J.G., Yakhmi, J.V., Banavaliker, M.M., Biyani, M.K. and Mittal, J.P. 2001. Characterizing the antioxidant activity of amla (*Phyllanthus emblica*) extract. *Curr. Sci.* **81**(2): 185-190

Kulkarni, S.K. and Verma, A. 1992. Evidence for nootropic effect of BR-16A (MENTAT) – A herbal psychotropic preparation in mice. *Indian J. Physiol. Pharmacol.* **36**(1): 29-34

Kumar, K.C.S. and Muller, K. 1999. Medicinal plants from Nepal – Evaluation as inhibitors of lipid peroxidation in biological membranes. *J. Ethnopharmacol.* **64**(2): 135-139

Kumar, V.P., Kuttan, R. and Kuttan, G. 1996. Radioprotective effects of Rasayanas. *Indian J. Exp. Biol.* **34**: 848-850

Kumar, V.P., Kuttan, R. and Kuttan, G. 1999a. Effect of 'Rasayanas', a herbal drug preparationon cell-mediated immune responses in tumour bearing mice. *Indian J. Exp. Biol.* **37**: 23-26

Kumar, V.P., Kuttan, R. and Kuttan, G. 1999b. Effect of 'Rasayanas', a herbal drug preparationon immune responses and its significance in cancer treatment. *Indian J. Exp. Biol.* **37**: 27-31

Kumar, V.P., Kuttan, R., Kuttan, G. and Joseph, C.D. 1997. Myeloprotective effect of a non toxic indigenous preparation – Brahma Rasayana in cancer patients receiving chemotherapy and radiation therapy. *Amala Res. Bull.* **17**: 101-105

Lanza, G., Washburn, K.W. and Wyatt, R.D. 1977. Interaction of age with PCV and body weight response to dietary aflatoxin. *Poult. Sci.* **56**(4): 1352

Latha, K. 1999. Pathological effects of aflatoxicosis in ducks (*Anas platyrhynchos domesticus*). M.V.Sc. thesis. Kerala Agricultural University, Thrissur, p. 115

Luna, L.G. 1968. *Manual of Histologic Staining Methods of the Armed Force Institute of Pathology.* Third edition. McGraw-Hill Book Company, New York, p. 251

Mani, K., Narahari, D., Kumararaj, R. and Ramamoorthy, N. 1993. Influence of dietary Aflatoxin B_1 on certain haematological and biochemical characters of broiler chicken. *Indian Vet. J.* **70**: 801-804

Mani, K., Narahari, D., Kumararaj, R and Viswanathan, K. 1997. Effect of sunlight exposed Aflatoxin B, contaminated feed on the performance of broiler chicks. *Indian Vet. J.* **74**: 768-771

Manjunatha, S., Jaryal, A.K., Bijlani, R.L., Sachdeva, U. and Gupta, S.K. 2001. Effect of chyawanprash and vitamin C on glucose tolerance and lipoprotein profile. *Indian J. Physiol. Pharmacol.* **45**(1): 71-79

Manonmani, S., Vishwanathan, V.P., Subramanian, S. and Govindasamy, S. 1995. Biochemical studies on the antiulcerogenic activity of Cauvery 100, an ayurvedic formulation in experimental ulcers. *Indian J. Pharmacol.* **27**: 101-105

Mathur, R., Sharma, A., Dixit, V.P. and Varma, M. 1996. Hypolipidaemic effect of fruit juice of *Emblica officinalis* in cholesterol fed rabbits. *J. Ethnopharmacol* **50**: 61-68

Menon, L.G., Kuttan, R. and Kuttan, G. 1995. Effect of Brahma Rasayana treatment on methylcholanthrene induced carcinogenesis. *Amala Res. Bull.* **15**: 38-40

Mini, K.P. and Kumar, N.G. 1995. Effect of *Emblica officinalis* on plasma cholesterol and triglyceride levels in rabbits. *J. Vet. Anim. Sci.* **26**: 85-87

Moorthy, S.A., Mahender, M. and Rao, R.P. 1986. Pathology of acute experimental aflatoxicosis in chicken. *Kerala J. Vet. Sci.* **17**: 31-35

Muthiah, J., Reddy, P.R., and Chandran, N.D.J. 1998. Effect of graded levels of Aflatoxin B_1 and the effect of direct fed microbials on egg production in egg type breeder. *Indian Vet. J.* **3**: 231-233

Nadig, S.S. and Rao, S.G. 1999. Effect of hepatogard – an indigenous formulation on dexamethasone induced antihealing effects in male albino rats. *Indian J. Physiol. Pharmacol.* **43**(2): 230-234

Nageswar Rao, A.N., Reddy, R.V., Rao, V.P., Sharma, B.J. and Mohiuddin, S.M. 1988. Effect of dietary aflatoxin on the development of immunity against New castle disease virus in chicken. *Indian J. Anim. Sci.* **58**(1): 77-80

Pachori, S.B., Gupta, M. and Pant, M.C. 1993. Effect of Abana and Metoprolol on serum lipid profile in rabbits fed hyperlipidemic diet. *Indian J. Physiol. Pharmacol.* **37**(3): 252-254

Pallabi De., Dasgupta, S.C. and Gomes, A. 1998. Immunopotentiating and immunoprophylactic activities of Immune 21, a polyherbal product. *Indian J. Pharmacol.* **30**: 163-168

Panda, B.K., Praharaj, N.K., Johari, J.S. and Saah, R.L. 1987. Experimental aflatoxicosis in Japanese quails: Evidences of some biochemical changes. *Indian J. Poult. Sci.* **22**(4): 359-362

Rajan, A., Maryamma, K.I., Gopalakrishanan Nair, Ismail, P.K. and Manomohan, C.B. 1991. Quantification of aflatoxin contamination in livestock feeds. *J. Vet. Anim. Sci.* **22**(1): 69-79

Rajeshkumar, N.V. and Kuttan, R. 2001. Induction of apoptosis in mouse and human carcinoma cell lines by *Emblica officinalis* polyphenols and its effect on chemical carcinogenesis. *Amala Res. Bull.* **21**: 114-126

Rangaswamy, R. 1995. *A Textbook of Agricultural Statistics*. New Age International Publishers Ltd., New Delhi, p. 495

*Ranjan, K.S., Prasad, G., Sinha, A.K. and Prasad, G. 1991. Evaluation of some bitter plant extracts against aflatoxin production and growth of *Aspergillus flavus*. *Nat. Acad. Sci. Lett.* **14**(6): 241-243

Reddy, D.N., Rao, P.V., Reddy, V.R. and Yadagiri, B. 1984. Effect of selected levels of dietary aflatoxin on the performance of broiler chicken. *Indian J. Anim. Sci.* **54**(1): 68-73

Rekha, P.S., Kuttan, G. and Kuttan, R. 1997. Antioxidant and anti-inflammatory activity of 'Brahma Rasayana'. *Amala Res. Bull.* **17**: 68-72

Rekha, P.S., Kuttan, G. and Kuttan, R. 2000. Effect of administration of Brahma Rasayana on antioxidant enzymes in mice exposed to sublethal radiation. *Amala Res. Bull.* **20**: 32-38

Rekha, P.S., Kuttan, G. and Kuttan, R. 2001a. Antioxidant activity of brahma rasayana. *Indian J. Exp. Biol.* **39**: 447-452

Rekha, P.S., Kuttan, G. and Kuttan, R. 2001b. Effect of Brahma Rasayana on antioxidant system after radiation. *Indian J. Exp. Biol.* **39**: 1173-1175

*Roy, A.K., Dhir, H., Sharma, A. and Talukder, G. 1991. Phyllanthus emblica fruit extract and ascorbic acid modify hepatotoxic and renotoxic effects of metals in mice. *Int. J. Pharmacognosy* **29**(2): 117-126

Saraswathy, S.D., Suja, V., Gurumurthy, P. and Shyamala, C.S. 1998. Effect of Liv 100 against antitubercular drugs (Isoniazid, Rifampicin and Pyrazinamide) induced hepatotoxicity in rats. *Indian J. Pharmacol.* **30**: 233-238

Sasikumar, C.S. and Shyamaladevi, C.S. 2000. Protective effect of Abana, a polyherbal formulation on Isoproterenol-induced myocardial infarction in rats. *Indian J. Pharmacol.* **32**: 198-201

Sastry, G.A. 1976. *Veterinary clinical pathology.* CBS Publishers and Distributors, New Delhi, p. 107

Sato, K. and Sekiya. 1965. Cited by Valsala, K.V. 1968. Reproductive pathology in the hen. M.V.Sc. thesis, Kerala Agricultural University, Thrissur, p. 166

*Schultze, M.O. and Elvehjem, C.A. 1934. An improved method for the determination of haemoglobin in chicken blood. *J. Biol. Chem.* **10**(5): 253

Sharma, S. and Sharma, R. 2001. Biochemical evaluation of lipid and oxidative stress status in relations to high fat antioxidant diets. *Indian J. Exp. Biol.* **39**: 1180-1183

Sharma, S.B. and Ray, S. 1997. Effect of herbal preparation on immune response of immunosuppressed mice. *Indian J. Physiol. Pharmacol.* **41**(3): 293-296

Sheehan, D.C. and Hrapchak, B.B. 1980. *Theory and Practice of Histotechnology.* Second edition. C.V. Monsby Co., St. Louis, Toronto, London, p. 481

Shishoo, C.J., Shah, S.R., Rathod, I.S. and Patel, S.G. 1998. Quality assurance of Chyvanaprash through determination of free radical scavenging activity. *Indian J. Pharm. Sci.* **60**(3): 179-181

Shukla, S.K. and Pachauri, S.P. 1995. Blood biochemical profiles in induced aflatoxicosis of cockerels. *Br. Poult. Sci.* **36**: 155-160

Siller, W.G. and Ostler, D.C. 1961. The histopathology of an enterohepatic syndrome of turkey poults. *Vet. Rec.* **73**: 134-138

Singh, K., Khanna, A.K. and Chander, R. 1999. Hepatoprotective activity of ellagic acid against carbon tetrachloride induced hepatotoxicity in rats. *Indian J. Exp. Biol.* **37**: 1025-1026

Soni, K.B., Lahiri, M., Chackradeo, P., Bhide, S.V. and Kuttan, R. 1997. Protective effect of food additives on aflatoxin-induced mutagenicity and heaptocarcinogenicity. *Cancer Lett.* **115**(2): 129-133

Soni, K.B., Rajan, A. and Kuttan, R. 1993. Inhibition of aflatoxin-induced liver damage in ducklings by food additives. *Myco. Res.* **9**(1): 22-26

Sridevi, B. and Sriraman, P.K. 1996. Pathology of aflatoxicosis in Japanese quails (*Coturnix coturnix japonica*). *Indian J. Anim. Sci.* **66**(9): 864-865

Srinivasan, K.K. and Kumar, V. 1993. Effects of hepatogard against carbon tetrachloride induced liver damage in rats. *Fitoterapia* **64**(2): 108-113

Suresh, K. and Vasudevan, D.M. 1994. Augmentation of murine natural killer cell and antibody dependent cellular cytotoxicity activities by *Phyllanthus emblica*, a new immunomodulator. *J. Ethnopharmacol.* **44**(1): 55-60

Suresh, M.V., Menon, B. and Indira, M.2000. Effects of exogenous vitamin C on ethanol toxicity in rats. *Indian J. Physiol. Pharmacol.* **44**(4): 401-410

*Tanvir, R., Nawaz, R., Zaidi, A.A. and Ruby, S. 1994. Phytochemical screening of medicinal plants belonging to family Euphorbiaceae. *Pak. Vet. J.* **14**(3): 160-162

Tapia, M.O. 1985. A quantitative thin layer chromatography method for the analysis of aflatoxins, ochratoxin A, Zearalenone, T-2 toxin and sterigmatocystin in foodstuffs. *Rev. Arg. Microbiol.* **17**(4): 183-186

*Thakur, C.P., Thakur, B., Sinha, P.K. and Sinha, S.K. 1988. The ayurvedic medicines Haritaki, Amla and Bahira reduce cholesterol induced atherosclerosis in rabbits. *Int. J. Cardiol.* **21**: 167-175

Thresiamma, K.C. and Kuttan, R. 1996. Inhibition of liver fibrosis by Ellagic acid. *Indian J. Physiol. Pharmacol.* **40**(4): 363-366

Tiwari, A., Sen, S.P. and Guru, L.V. 1968. Effect of *Phyllanthus emblica* fruit extract on biological systems. *J. Res. Indian Med.* **2**(2): 189-194

Udupa, A.L., Rao, S.G. and Kulkarni, D.R. 1989. Would healing profile of septilin. *Indian J. Physiol. Pharmacol.* **33**: 39-42

Udupa, V., Kulkarni, K.S., Gopumadhavan, R.S., Venkatavanganna, M.V. and Mitra, S.K. 2000. Effect of HD-03 on levels of various enzymes in paracetamol induced liver damage in rats. *Indian J. Pharmacol.* **32**: 361-364

Upasani, C.D. and Balaraman, R. 2001. Effect of vitamin E, vitamin C and spirulina on the levels of membrane bound enzymes and lipids in some organs of rats exposed to lead. *Indian J. Pharmacol.* **33**: 185-191

Vaidya, A.D.B. 1997. The status and scope of Indian medicinal plants acting on central nervous system. *Indian J. Pharmacol.* **29**: 340-343

*Vani, T., Rajani, M., Sarkar, S. and Shishoo, C.T. 1997. Antioxidant properties of the Ayurvedic formulation Triphala and its constituents. *Int. J. Pharmacognosy* **35**(5): 313-317

Vasan, P., Ravi, R. and Purushothaman, M.R. 1998. Effect of feeding graded levels of aflatoxin (AFB_1) on performance of broiler chicks. *Indian J. Poult. Sci.* **33**(2): 214-216

Verma, S., Tewari, S.C. and Yadav, M.P. 1999. Use of polyherbal immunomodulator in conjunction with inactivated vaccine against Equine Herpes Virus-I. *Indian J. Virol.* **15**(1): 63-67

* Originals not consulted

EFFECT OF AMLA (*Emblica officinalis*) ON HEPATIC FUNCTION IN BROILER CHICKEN

By

SAJITHA. I. S.

ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

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

2002

ABSTRACT

The study was designed to assess the effect of *Emblica officinalis* on hepatic function in broiler chicken and to evaluate its efficacy as a feed additive. The liver of birds are exposed to various hepatotoxins present in feed, like added feed additives, antibiotics in excess as well as mycotoxins like aflatoxin. The protection afforded by Emblica against these was studied.

Fifty-four day old broiler chicks were divided into three groups of two replicates each. Group I (Control group) was given normal commercial feed, groups II and III were given amla at one per cent and two per cent levels respectively, for eight weeks along with the control feed. Body weight gain, feed efficiency, feed analysis, haemogram, serum profile, liver weight and gross and histopathological changes in liver were studied.

A gradual reduction in body weight gain, was noted in the control group birds, when compared to the amla fed birds. Group II and III birds recorded a better FCR, when compared to group I birds.

Proximate analysis of the feed indicated normal chemical composition of feed. Analysis for aflatoxin content revealed the presence of toxin in the range of 100-150 ppb in the starter and 150-200 ppb in the finisher feeds. The toxicopathological

changes induced by the control feed, may be attributed to the presence of toxins in the feed.

The toxicopathological changes in the birds were found to be lowered in its intensity in relation with the level of amla, with the most severe changes in the control group birds. Values of hemoglobin, PCV, total leucocyte, lymphocyte and heterophil count, total serum protein and albumin showed a decrease, whereas the ESR and the serum enzyme levels showed an increase in the control group birds.

The gross and histopathological changes induced by the toxin, were reduced in the amla treated groups in a dose dependent manner. Degenerative and necrotic lesions and chronic fibrous tissue proliferation, bile duct proliferation and biliary hyperplasia were noted. Regenerative and repair processes were also well pronounced.

The toxicopathological changes, induced by the toxin, was significantly reduced by *Emblica officinalis*, especially at two per cent level.Toxic contamination of commercial feed especially with aflatoxin is very common in Kerala. Considering this and the results of the present study, it can be concluded that *Emblica officinalis* has got significant hepatoprotective activity and can be used as an effective feed additive in commercial livestock feed.