STUDIES ON THE ACTION OF *BOERHAAVIA DIFFUSA LINN.* AGAINST FURAZOLIDONE INDUCED HEPATOTOXICITY IN POULTRY

By

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CERTIFICATE

Ms. GUGGILAM ANURADHA *has satisfactorily prosecuted the course of research and that the thesis entitled "***STUDIES ON THE ACTION OF *BOERHAAVIA DIFFUSA LINN.* AGAINST FURAZOLIDONE INDUCED HEPATOTOXICITY IN POULTRY***"*** *submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by her for a degree of any University.*

Date: 27.09.03

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CERTIFICATE

This is to certify that the thesis entitled ***"STUDIES ON THE ACTION OF BOERHAAVIA DIFFUSA LINN. AGAINST FURAZOLIDONE INDUCED HEPATOTOXICITY IN POULTRY"*** *submitted in partial fulfilment of the requirements for the degree of* ***MASTER OF VETERINARY SCIENCE*** *of the Acharya N.G.Ranga Agricultural University, Hyderabad, is a record of the bonafide research work carried out by* ***Ms.GUGGILAM ANURADHA*** *under my guidance and supervision. The subject of the thesis has been approved by the student's advisory committee.*

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of investigation has been duly acknowledged by the author of the thesis.

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DECLARATION

I, **GUGGILAM ANURADHA,** hereby declare that the thesis entitled **"STUDIES ON THE ACTION OF *BOERHAAVIA DIFFUSA* LINN. AGAINST FURAZOLIDONE INDUCED HEPATOTOXICITY IN POULTRY"** submitted to Acharya N.G. Ranga Agricultural University, Hyderabad for the degree of **Master of Veterinary Science** is the result of original research work done by me. I also declare that the materials contained in this thesis have not been published earlier.

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ABSTRACT

Recent studies demonstrated the role of herbal drugs as hepatoprotectives by virtue of their antioxidant capacity. The present study was conducted to find out the protective and corrective effects of 60 % ethanolic extract of *Boerhaavia diffusa* Linn. and compare the same with that of silymarin on performance, clinical, biochemical and histopathological patterns in experimentally-induced oxidative damage by furazolidone to heart, kidney and liver in particular. One hundred and twenty, day-old Vencob broiler chicks were classified into six groups and experiment was conducted from 15th day: Group 1 served as vehicle control; Group 2, 5 and 6 received furazolidone at dose rate of 150 mg/kg body weight; Group 3 and 4, in addition to furazolidone, received oral doses of *B.diffusa* (200 mg/kg body weight) and silymarin (200 mg/kg body weight) respectively up to 4 weeks of age. Thereafter, Groups 3, 5 and 4, 6 received *B.diffusa* and silymarin respectively and Group 2 was left without any treatment till the end of the experiment (6th week).

Body weight gains and feed conversion ratio of the birds were recorded at weekly intervals to evaluate the performance of the birds. Profiles of liver (ALT, AST and LDH), kidney (uric acid), lipids (total cholesterol and HDL), proteins (total proteins, albumins and A/G ratio) and erythrocyte antioxidant

enzyme profile (GPx and GSH-R), and hematological profile (Hb%, PCV, TEC, TLC, MCV, MCH and MCHC) were also assessed at weekly intervals. At the end of 4^{th} and 6^{th} weeks, the birds were sacrificed, liver was collected to assess the extent of lipid peroxidation, and liver, heart and kidney, were taken for organ-somatic indices and histopathological examination.

The studied parameters altered by furazolidone were significantly modulated by *B.diffusa* and silymarin, and histopathological examination showed that *B.diffusa* and silymarin ameliorated the degenerative changes caused by furazolidone in liver, heart and kidney. Treatment with *B.diffusa* and silymarin also significantly ($p<0.05$) reduced the toxic effect of furazolidone on malondialdehyde content.

It is concluded that administration of ethanolic extract of *B.diffusa* and silymarin could play an important role in protection, however, silymarin exhibited better corrective action compared to *B.diffusa* against oxidative damage and consequently liver damage caused by furazolidone-generated free radicals.

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ABBREVIATIONS

μ^3	:	cubic micron (s)
µl	:	microlitre (s)
µM	:	micromole (s)
@	:	at the rate of
A/G	:	albumin: globulin ratio
ACP	:	Acid phosphatase
ALP	:	Alkaline phosphatase
ALT	:	Alanine Aminotransferase
ANOVA	:	Analysis of Variance
AST	:	Aspartate Aminotransferase
b.wt.	:	body weight
BD	:	ethanolic extract of *Boerhaavia diffusa* Linn.
BHC	:	Benzene Hexa Chloride
CAT	:	Catalase
CCl_4	:	Carbon Tetra chloride
EDTA	:	Ethylene Diamine Tetra Acetic acid
FAD	:	Flavine Adenine Dinucleotide
FCR	:	Feed Conversion Ratio

Fig.	:	figure
Fz	:	Furazolidone
g	:	gram (s)
GGT	:	Gamma Glutamyl Transferase
GPx	:	Glutathione Peroxidase
GSH	:	Reduced Glutathione
GSH-R	:	Glutathione Reductase
GSSG	:	Oxidized Glutathione
H & E	:	Hematoxyline and Eosin
H_2O_2	:	Hydrogen Peroxide
Hb	:	Hemoglobin
HDL	:	High Density Lipoprotein
IU	:	International Unit (s)
Kg	:	kilogram (s)
L	:	litre (s)
LD_{50}	:	Median Lethal Dose
LDH	:	Lactate Dehydrogenase
LPO	:	Lipid peroxides
M	:	mole (s)
MCH	:	Mean Corpuscular Hemoglobin
MCHC	:	Mean Corpuscular Hemoglobin Concentration
MCV	:	Mean Corpuscular Volume
MDA	:	Malondialdehyde
mg	:	milligram (s)
min	:	minute (s)
ml	:	millilitre (s)
NADP	:	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	:	Reduced Nicotinamide Adenine Dinucleotide Phosphate
nm	:	nanometer (s)
p.o.	:	per os
PCV	:	Packed Cell Volume

pg	:	picogram (s)
p^H	:	Hydrogen Ion Concentration
ppm	:	parts per million
RBC	:	Red Blood Corpuscle (s)
ROS	:	Reactive Oxygen Species
SGOT	:	Serum Glutamate Oxaloacetate Transaminase
SGPT	:	Serum Glutamate Pyruvate Transaminase
SOD	:	Superoxide dismutase
TBARS	:	Thiobarbituric acid reactive substances
TEC	:	Total Erythrocyte Count
TLC	:	Total Leukocyte Count
WBC	:	White Blood Corpuscle (s)

CHAPTER I

INTRODUCTION

Indian poultry industry is growing at a rapid pace compared to any other livestock industry for the past two decades. Salmonellosis, coccidiosis and several other infections have become a major havoc affecting the performance of chickens. Hence, the use of antimicrobials has become an integral part of poultry farming. The remarks of WHO, "Antimicrobials are vital medicines for the treatment of bacterial infections in both humans and animals", amply testify the role of antimicrobials in animal health management.

Furazolidone, a nitrofuran antimicrobial is being used as a feed additive in poultry feeds for nearly five decades for prevention and control of salmonellosis, coccidiosis and other digestive tract infections (Booth and Mc Donald, 1982). The LD_{50} of furazolidone was reported to be 380mg/ kg, while 500mg/ kg was lethal to all the birds studied (Radchuck, 1965). In recommended concentrations, furazolidone has a low toxicity for host tissues and acts by interrupting the enzymatic processes of the microbial cell, preventing cell multiplication (Biester and Schwarte, 1985).

Furazolidone, although remains an important and commonly used drug

in the veterinary medicine, its use in the food animals is not without risk and in the United States and certain European countries, the administration of furazolidone to food producing animals is now prohibited owing to its potential mutagenicity, genotoxicity and carcinogenicity. It was found that furazolidone toxicity was due to alteration in the *in vivo* antioxidative enzymatic defense mechanism (Sas, 1993) and free radical generation (Bloom and Brandt, 2001; Moudgal, 2000).

Despite the existence of endogenous defense mechanisms against free radicals, the production of reactive oxygen species (ROS) when reaches abnormally high levels as in toxicity conditions, oxidative damage to the cell occurs finally leading to several pathological conditions of all the organs principally liver (Halliwell, 1994).

Incorporation of antioxidants in the diet can provide protection from liver damage caused by oxidative mechanisms of toxic chemicals. Plants such as *Silibum marianum, Picrorrhiza kurroa, Andrographis paniculata, Eclipta alba* etc., are proven to be active against certain hepatotoxins by virtue of their antioxidant properties (Subramoniam and Pushpangadan, 1999).

Keeping this in view, the present study is designed in broilers to study the hepatoprotective and antioxidant effects of *Boerhaavia diffusa* Linn., a

perennial creeping weed, commonly available throughout India. Though the hepatoprotective activity of the plant was studied in mice (Chakraborti and Handa, 1989b; Chandan *et al*., 1991; Rajkumari *et al*., 1991; Rawat *et al*., 1997), similar reports in poultry are not available. The objectives of the present study are:

1. To evaluate the subacute toxicity of furazolidone, if any.
2. To study the antioxidant effect of *Boerhaavia diffusa* Linn.
3. To study the hepatoprotective effect of *Boerhaavia diffusa* Linn. and compare the same with that of silymarin.

CHAPTER II
REVIEW OF LITERATURE

2.1 FURAZOLIDONE

Furazolidone, N – (S – nitro –2-furfurylidene) –3-amino-2-oxazolidone (Furazolidone), is a nitrofuran that has been used for more than forty years in the treatment of certain bacterial and protozoal infections in man and animals (Ali, 1999).

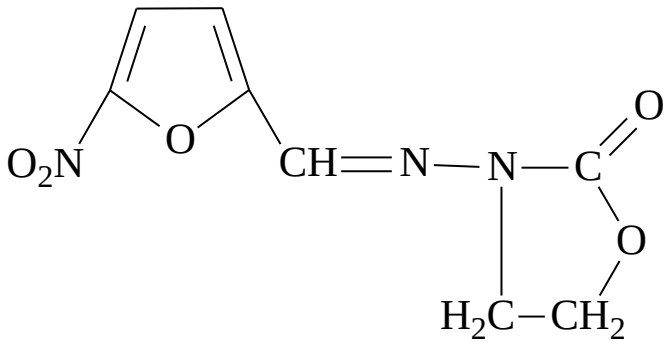

Figure 1: Chemical structure of Furazolidone (Ali, 1983).

2.1.1 Mechanism of Action of Nitrofurans

Nitrofurans as a group are bacteriostatic and function by blocking oxidative decarboxylation of pyruvate to acetyl coenzyme A, depriving susceptible organisms of vital energy pathways (Adams, 2001).

The likely mode of action of Nifurtimox, a nitrofuran derivative, is through the production of activated forms of oxygen. It is reduced to nitro anion radical in the presence of pyridine nucleotides, which then reacts with oxygen to produce superoxide and

regeneration of nifurtimox. The activated oxygen molecules exert their toxic effects on the parasites (Adams, 2001).

2.1.2 Therapeutic Uses

Furazolidone, 4.4 mg/kg 3 times daily PO, apparently successfully treated experimental Salmonella diarrhoea in a group of foals (Prescott and Baggot, 1993).

Limaye (1980) reported that furazolidone (in conjugation with Lomotil) is effective in the treatment of infective diarrhoea.

Nifurtimox, a nitrofuran derivative is the most widely used in Trypanosoma cruzi infections in dogs. It has activity against amastigote and trypomastigote stages (Barr, 1990).

Furazolidone is potentially used in the local treatment of infections of udder, uterus, and skin. In swine furazolidone and other nitrofurans have been used extensively in the prevention and treatment of colibacillosis and salmonellosis. In poultry, it is used in the prevention of coccidiosis (Prescott and Baggot, 1993).

2.1.3 Actions of Furazolidone

2.1.3.1 General Toxicity and Clinical Findings.

Horses administered with furazolidone (8.8 mg/kg) *p.o.* 3 times daily developed anorexia on the third day and those given lower doses (4.4mg/kg) *p.o.* 3 times daily lost weight (Prescott and Baggot, 1993).

Radchuck (1965) reported signs of toxicity of furazolidone in chickens and hens 4-6 hours after oral administration of 300mg/kg body weight. The birds became inappetent, drowsy and unaware of the surrounding environment. These signs were followed by paralysis and death. The LD_{50} of the drug was found to be 380 mg/kg, and 500 mg/kg was lethal to all the birds studied.

Stryczek (1971) reported that the toxic symptoms of furazolidone (0.02% w/w in the feed, 14 days) in geese consisted of nervous signs, viz. wobbly gait, inability to stand up and tendency to fall down. Post-mortem of affected birds revealed heamorrhages in the serous membranes, digestive tract and heart, and excess of fluid in the body cavities to the extent of deforming the birds.

A small but significant reduction in packed cell volume and haemoglobin concentration was found in chickens given furazolidone at a feed level of 0.04% w/w in the feed, for 3 weeks (Ehlhardt *et al*, 1975). However, anemia was not found in chickens given the drug (0.04% w/w, 10 days) (Ali, 1981).

Accidental administration of furazolidone in feed over 3000ppm in pigs

caused nervous symptoms (Borland, 1979).

Clinical alterations in treated ducklings consisted of decreased feed consumption with lowered weight gain and signs of nervous derangement, consisting of ataxia, hyperexcitability, incoordination and seizures (Webb and Van Vleet, 1991).

Two case reports described furazolidone toxicity in Australian calves that were either given a premix with the feed to which furazolidone (10% w/w) had been inadvertently added (Finnie, 1992) or fed milk to which furazolidone had been added at a concentration of 200 mg/kg (Taylor *et al*, 1991). The toxicity developed within 10 days and was initially manifested as an acute neurological syndrome that was followed, after a more protracted clinical course, by agranulocytosis and haemorrhagic diathesis.

Japanese quails (Arbid *et al*, 1990) and Chickens (Zaman *et al*, 1995), when given furazolidone (400-1000 mg/kg feed) in feed for 3-4 weeks, showed anorexia, decreased body weights, ascites, leg weakness and nervous derangements (such as convulsions and torticollis). Treated birds also showed hepatotoxicity, anemia and decreased plasma proteins.

High oral doses of furazolidone or nitrofurantoin result in central

nervous effects in animals, and lower doses in calves and dogs have caused hemorrhagic diathesis with thrombocytopenia, anemia, leukocytopenia and prolonged bleeding times. Anorexia, nausea and vomiting occur with high doses in all species (Prescott and Baggot, 1993).

2.1.3.2 Anorexia and Thiamin Status.

Furazolidone, when given to chickens, ducklings and turkey poults at a dose of 0.04% W/W in the feed for 10 days, produced anorexia and growth reduction (Ali and Bartlet, 1982). The drug treatment was also found to increase the concentrations of pyruvate and lactate, in chicken blood, and the activation of transketolase by thiamin pyrophosphates in hemolysates. Administration of thiamin hydrochloride at doses above the requirement of the birds reversed the signs of thiamin deficiency, thus suggesting the possible role of furazolidone in interfering with the utilization of the vitamin.

2.1.3.3 Effect on Hepatic Enzymes and Bilirubin.

Staley *et al* (1978) reported that feeding furazolidone (0.05% for 4 weeks) in turkeys caused a disordered hepatic metabolism, as judged by a decrease in the total plasma proteins and an increase in hepatic glycogen and aspartate transaminase activity in serum. These effects were also confirmed by Simpson *et al* (1979), who also showed that furazolidone treatment (0.05% w/w in the feed for 24 days, followed by 0.07% w/w for 42 days) in turkeys resulted in hypotension and decrease

in proteins and trypsin inhibitory factor in the plasma.

Webb *et al* (1991) suggested that an elevated serum AST activity in the furazolidone-treated birds might have resulted from hypoxic hepatocellular damage accompanying congestive heart failure induced by the drug or indeed be a manifestation of hepatocellular toxicity. An elevated mean serum total bilirubin concentration has also been observed.

2.1.3.4 Effect on Heart.

Furazolidone (0.07% w/w, in the feed for 2 weeks or more) had been shown to increase the activity of lactate dehydrogenase, alanine transaminase and creatinine phosphate, and to decrease the level of plasma protein in turkey poults (Czarnecki *et al*, 1981).

Mc Callum *et al* (1989) studied the effect of furazolidone (0.5 and 2 μg/ml of perfusate) on isolated hearts of 3 weeks-old broiler chicken and concluded that furazolidone, at both concentrations, caused detrimental changes in myocardial vascular resistance (2-fold increase) and lactate dehydrogenase release (six-fold increase).

O'Brien *et al* (1991, 1993) indicated that there is a defective Ca^{2+} channel function resulting in abnormal Ca^{2+} homeostasis in furazolidone induced cardiomyopathy.

Glass *et al* (1993) studied the cardioprotective efficacy of some selective and non-selective adrenergic receptor antagonist and Ca^{2+} channel antagonists in turkeys with furazolidone-induced dilated cardiomyopathy and concluded that Ca^{2+} channel antagonist play an important role in the treatment of dilated cardiomyopathy.

Lax *et al* (1994) reported that furazolidone increases the activity of thapsigargin-sensitive Ca^{2+}-ATPase in chick cardiac myocytes without affecting Na^{+}/Ca^{2+} exchange.

Furazolidone (700 ppm) when added to the feed of 8 days old turkey poults for 2 weeks, showed resultant cardiac dilatation. The histopathological changes included myocyte hypertrophy, enlargement of nuclei and reorientation of subpericardial myocardial fibers (Gwathmey, 1991).

Biventricular cardiac dilatation and thinning of ventricular walls were observed in broiler chicks fed with furazolidone at 400 or 800 mg/kg feed for 30 days (Islam *et al* 1995, Khan *et al* 1995).

2.1.3.5 Effect on Free Radicals and Antioxidants.

In vitro, furazolidone has been shown to stimulate lipid peroxidation, and that superoxide dismutase (SOD) and catalase (CAT) attenuated the furazolidone induced stimulation of oxygen consumption, indicating that the drug had promoted the formation of superoxides and hydrogen peroxide (Stroo and Schaffer, 1989).

Furazolidone has been shown to inhibit glutathione peroxidase (Mc Calla, 1979; Paul and Paul, 1964), and the erythrocyte membranes of furazolidone-fed ducklings might have become more fragile than those of control ducklings, resulting in hemolysis (Webb *et al*, 1991).

Furazolidone given as a single oral dose of 75, 150 or 300 mg/kg, produced significant reductions in reduced glutathione (GSH) and ascorbic acid, and a significant increase in lipid peroxidation in the tissues of rats. Concomitant treatment with dimethyl sulphoxide, a free radical scavenger ameliorated the effect of furazolidone on GSH (Ali, 1992).

Sas (1993) fed chickens at a dose of 300 mg/kg per day for 3 days, and

measured several parameters in blood and other tissues, including glutathione peroxidase, glutathione reductase, CAT, SOD, reduced and oxidized glutathione, malonaldehyde (MDA), α-tocopherol, selenium and iron. CAT and GSH were significantly reduced and there was a transient increase in lipid peroxidation levels. It was concluded that furazolidone altered the *in vivo* antioxidative enzymatic defense mechanisms.

2.1.3.6 Effect on Nitrogenous Metabolites.

The findings of Webb *et al* (1991) suggested that there was no significant difference between the mean uric acid levels of control group and furazolidone (700 ppm for 28 days) treated group of ducklings.

2.2. LIVER DISEASES

Apart from infectious agents, several xenobiotics like drugs and chemicals, plant products, mycotoxins, pollutants etc., affect the liver.

Most of the hepatotoxic chemicals *viz.,* carbon tetrachloride (Recknagal, 1983), paracetamol (Wandel *et al*, 1987; Hiroshi *et al*, 1987), caffeine (Dianzani *et al*, 1991), furazolidone (Stroo and Schaffer, 1989) damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in the liver. This is well exemplified by enhanced lipid peroxidation produced during

the liver microsomal metabolism of ethanol which results in turn in hepatitis and cirrhosis (Smuckler, 1975).

2.2.1 Role of Free Radicals in Liver Diseases

The involvement of free radical reactions in the pathogenesis of liver injury has been investigated for many years in a few defined experimental systems using carbon tetrachloride, excess iron or ethanol as pro-oxidant agents (Poli, 1993). Free radical-initiated lipid peroxidation may play a role in hepatic fibrogenesis, perhaps through an effect of aldehyde peroxidation products on kupffer cells and lipocytes (Britton and Bacon, 1994).

Halim *et al* (1997) in a study, used rats which were chronically intoxicated with carbon tetrachloride (CCl_4), as a model for liver injury terminating in fibrosis and cirrhosis, and found that the treatment with antioxidants (silymarin @30 mg/kg, Vitamin E @ 200 IU/kg and vitamin C @ 50 mg/kg) has ameliorated not only the necrotic and fibrotic changes but also modulated the toxic effects of CCl_4 on the lipid peroxidation and malonaldehyde content.

2.2.2 Antioxidant Defense Mechanisms

Mammalian cells possess elaborate defense mechanisms to detoxify

radicals (Fig.2 and 3). The key metabolic steps are SOD catalysis of the dismutation of superoxide to hydrogen peroxide and oxygen and the conversion of H_2O_2 to $2H_2O$ by glutathione peroxidase or to $O_2 + H_2O$ by catalase. Since the reaction catalyzed by glutathione peroxidase requires GSH as substrate and depends in part on the ratio of GSSG: GSH, the concentrations of these reactants and their ratio, which is a reflection of the redox state of the cell, are important to reactive oxygen species (ROS) detoxification. Similarly, the concentration of redox-active metals, such as iron, catalyzes formation of some ROS. This is minimized by keeping the concentrations of these metal ions very low due to binding to storage and transport proteins (*e.g.*, ferritin, transferrin, lactoferrin), thereby minimizing OH formation. Finally, radical-scavenging antioxidants (*e.g.*, vitamin E) interrupt the chain reactions by capturing the radical; the vitamin E radical is relatively stable, and it can be enzymatically converted back to its non-radical form. Radical scavengers thus terminate the chain reaction of radical damage.

The potential significance of these ROS defense mechanisms is apparent from considerations of the whole body and sub-cellular distribution of the different components. Vitamin E, the enzymes (SOD, catalase and GPx) and substrates (GSH) tend to be in higher concentration in locations where ROS damage is more likely (*e.g.*, in more highly oxygenated locations) and

potentially more damaging (Moslen, 1994).

2.3 HERBAL HEPATOPROTECTIVES

2.3.1 Silymarin

Flavonoids belong to the family of the benzo gamma-pyrones. For centuries numerous therapeutic properties have been attributed to them and many are being used as popular therapeutic remedies.

Silymarin is a flavonolignan that has been introduced fairly recently as a hepatoprotective agent. It is the most well known compound of the flavonoids. It is extracted from the seeds and fruit of *Silybum marianum* (Compositae) and is a mixture of three structural components: silibinin, silydianine and silychristine. The structure of the constituents of silymarin was clarified in the 1960s (Fig. 4) (Valenzuela and Garrido, 1994). Of the three isomers that constitute silymarin, silibinin is the most active (Lecomte, 1975; Wagner *et al*, 1974). From a medical point of view, silymarin and silibinin have been found to provide cytoprotection and, above all, hepatoprotection (Vogel *et al*, 1975). The over all view of mechanism action of silymarin as proposed by Valenzuela and Garrido (1994) is shown in Fig. 5.

Silymarin is used for the treatment of numerous liver disorders

characterised by degenerative necrosis and functional impairment (Lacomte, 1975). Furthermore, it is able to antagonise the toxin of *Amanita phalloides* (Choppin and Desplaces, 1978) and provides hepatoprotection against poisoning by phalloidin (Vogel and Trost, 1975), galactosamine (Barbarino *et al*, 1981), thioacetamide (Schriewer *et al*, 1973), halothane (Siegers *et al*, 1983), carbon tetrachloride (Mourelle *et al*, 1989) and aflatoxins (Sujatha *et al*, 2003). (Table 1)

2.3.1.1 Antioxidant Properties.

It has recently been reported that in rat hepatocytes treated with *tert*-butyl hydroperoxide (TBH), silymarin reduced the loss of lactate dehydrogenase (LDH), increases oxygen consumption, reduced the formation of lipid peroxides, and increased the synthesis of urea in the perfusion medium. The protective effect of silymarin is mediated by the inhibition of lipid peroxidation, and the modulation of hepatocyte Ca^{2+} content seems to play a crucial role (Farghali *et al*, 2000).

2.3.1.2 Protective Effects in Models of Oxidative Stress.

Oxidative stress is defined as structural and/or functional injury produced in tissues by the uncontrolled formation of pro-oxidant free radicals.

Oxidative stress usually develops when the pro-oxidant action of an inducer exceeds the anti-oxidant capacity of the cell defense system, altering its homeostatic capacity. Numerous substances induce oxidative stress, including carbon tetrachloride, TBH, ethanol, paracetamol (acetaminophen) and phenylhydrazine. It has been shown in rats that silibinin protects neonatal hepatocytes from cell damage produced by erythromycin, amitriptyline, nortriptyline and TBH (Davila *et al*, 1989).

Using perfused liver as a experimental model for inducing oxidative stress, it has been shown that phenylhydrazine produces an increase in oxygen consumption in rat liver *in vitro* and in the release of thiobarbituric acid reactive substances (TBARS) in the perfusate (Valenzuela and Guerra, 1985). This stress is associated with a reduction in the amount of reduced glutathione (GSH) in the liver; GSH exerts important protective activity against chemically induced oxidative stress (Valenzuela *et al*., 1985a; Videla and Valenzuela, 1982). Using liver from rats pretreated *in vivo* with silibinin 50 mg/kg intravenously, a significant reduction in the oxygen consumption stimulated by phenylhydralazine and in the release of TBARS was observed, without any changes in GSH levels (Valenzuela *et al*., 1985b).

The antioxidant effect of silibinin was observed in rats with acute intoxication caused by ethanol or paracetamol, which are peroxidation inducers that produce marked GSH depletion in the liver. Treatment with

silymarin or silibinin was able to protect animals from oxidative stress produced in the liver by ethanol or paracetamol (Campos *et al*, 1989). Furthermore, it has been reported that treatment with silibinin attenuates the increase in plasma levels of AST, ALT and gammaglutamyl transpeptidase (GGT) observed after intoxication by paracetamol (Valenzuela and Garrido, 1994).

The hepatoprotective activity of silibinin has also been studied in rats with liver cirrhosis induced by the long-term administration of carbon tetrachloride. Muriel & Mourelle (1990) have shown that silibinin preserves the functional and structural integrity of hepatocyte membranes by preventing alterations of their phospholipid structure produced by carbon tetrachloride and by restoring alkaline phosphatase and GGT activities.

The compound also protects hepatocytes from injury caused by ischemia, radiation, iron overload and viral hepatitis (Luper, 1998). Zhao *et al*. (1991) discussed the stabilizing effect of silymarin on hepatic cell membrane in chicken and also their effect on reduction of fatty degeneration of liver.

2.3.1.3 Activity against Lipid Peroxidation.

Lipid peroxidation is the result of an interaction between free radicals of diverse origin and unsaturated fatty acids in lipids. Lipid peroxidation involves

a broad spectrum of alterations, and the consequent degeneration of cell membranes may contribute towards the development of other disorders of lipoprotein metabolism, both in the liver and in peripheral tissues. (Fig. 6)

Silymarin appears to act as an antioxidant not only because it acts as a scavenger of the free radicals that induce lipid peroxidation (Letteron *et al*, 1990), but also because it influences enzyme systems associated with glutathione and superoxide dismutase (Valenzuela *et al*, 1989).

It has been shown that all the components of silymarin inhibit linoleic acid peroxidation catalysed by lipoxygenase (Fiebrich and Koch, 1979) and that silymarin protects rat liver mitochondria and microsomes *in vitro* against the formation of lipid peroxides induced by various agents (Bindoli *et al*, 1977).

Letteron *et al* (1990) studied the mechanisms of action of silymarin that provide protection against lipid peroxidation and the hepatotoxicity of carbon tetrachloride in mice, and came to the conclusion that silymarin works by reducing metabolic activation by carbon tetrachloride and by acting as an antioxidant that prevents chain rupture.

2.3.1.4 Stimulation of Liver Regeneration.

One of the mechanisms that can explain the capacity of silymarin to stimulate liver tissue regeneration is the increase in protein synthesis in the

injured liver. In *in vivo* and *in vitro* experiments performed in the liver of rats from which part of the organ had been removed, silibinin produced a significant increase in the formation of ribosomes and in DNA synthesis, as well as an increase in protein synthesis (Magliulo *et al*, 1973).

The capacity of silymarin to stimulate protein synthesis has also been studied in neoplastic cell lines, in which no increase in protein synthesis, ribosome formation or DNA synthesis has been found after treatment with silymarin (Sonnenbichler and Zetl, 1986).

2.3.2 *Boerhaavia diffusa* Linn.

2.3.2.1 Medicinal Properties.

Boerhaavia diffusa Linn. (Synonym: *Boerhaavia repens* L.; *B. procombens* Roxb.; Family: Nyctaginaceae, Sanscrit: Punarnava) is a perennial creeping weed found throughout India. The roots of the plant are repute to be diuretic and laxative and are given for the treatment of anasarca, ascites and jaundice (Kritikar and Basu, 1933; Chopra *et al*., 1956).

The leaves are reported for their use in the treatment of dyspepsia, jaundice, enlargement of spleen and abdominal pain (Kritikar and Basu, 1956), and also as an antistress agent (Dandiya, 1991).

The root extract of *B. diffusa* is reported to be a potent antifibrinolytic and anti-inflammatory agent in monkeys (Barthwal and Srivastava, 1990, 1991).

The other pharmacological activities of this plant include kidney regeneration and nutrition (Mishra and Singh, 1980). An experimental evaluation of possible teratogenic potential of *B. diffusa* for rats showed no teratogenic effect (Singh *et al*., 1991).

Treatment with aqueous extract of *B.diffusa* roots has been shown to induce leukocytosis with predominant neutrophilia, associated with a

stimulation of phagocytic and bacterial capacity of neutrophils and macrophages (Mungantiwar *et al*., 1997). The alkaloidal fraction of the herb is shown to have immunomodulatory activity (Mungantiwar, 1999).

The decoction or juice of *B. diffusa* leaves is used in Martinician folk medicine for its analgesic and anti-inflammatory properties (Robineau, 1995; Hiruma-Lima *et al*., 2000). This plant is also employed in the traditional medicine in Brazil as a diuretic (roots) and against snake venom (leaves) (Lorenzi, 1994). *B. diffusa* is used in traditional medicine for its anti inflammatory, anti bacterial, antiviral and cardiotonic properties. It is also used in the treatment of elephantiasis, night blindness and corneal ulcers (Chakraborti and Handa, 1989b).

Chude *et al*. (2001) in a study revealed the hypoglycaemic effect of aqueous leaf extract of *B. diffusa* in alloxan-induced diabetic rats.

Phytochemical research has demonstrated the presence of alkaloids and amino acids in *B.diffusa* (Garg, 1978, 1980). Two lignans, liriodendrin and syringaresinol mono-β-D-glucoside have been isolated from methanolic extract of the roots of *B.diffusa,* and liriodendrin was found to exhibit a significant Ca^{2+} channel antagonistic effect (Lami *et al,* 1991). The green stalk of the plant has also been reported to contain boerhavin and boerhavic acid

(Liogier, 1990).

2.3.2.2 Hepatoprotective Action.

Chakraborti and Handa (1989a) studied the antihepatotoxic effect of different extracts of roots and aerial parts of *B. diffusa* on serum transaminases and alkaline phosphatase of the rats intoxicated with CCl_4. The serum enzyme levels elevated by CCl_4 were significantly lowered by the extracts of petroleum ether, chloroform, methanol and water, and the total alkaloids of roots. Similarly, the lowering of enzyme levels was observed with 500mg/kg i.p. administration of chloroform extract and methanolic extract of the aerial parts of *B. diffusa.*

A study by Chandan *et al* (1991) revealed that 50% ethanolic extract of *B. diffusa* (BD) whole plant possess hepatoprotective activity in CCl_4-induced toxicity in rats. As observed in this study, there was a decrease in the hexabarbitone “sleeping time” in BD-treated rats and mice, thus confirming the protection of hepatic drug metabolizing enzymes; BD also seemed to preserve the structural integrity of the hepatocyte cell membrane, which was evident by a reduction in the CCl_4-induced rise of SGOT and SGPT levels; treatment with BD had reduced the increased prothrombin time, thus proving its capability to protect the prothrombin synthetic activity of the

liver. BD also had increased the bile flow, which is indicative of a strong stimulating action on the secretary activity of the liver thus confirming its antihepatotoxic activity.

Th effect of 50% ethanolic extract of *B. diffusa* on country made liquor (CML) induced hepatotoxicity was studied in albino rats by Rajkumari *et al*. (1991). The herb is found to afford marked protection from the hepatotoxic effect of CML as evidenced by significant fall in serum ALT, triglycerides, cholesterol and total lipid levels in both serum and tissues. Histopathological studies of livers from the herb-treated group showed marked reduction in fat deposits, indicating its significant hepatoprotective activity.

The hepatoprotective activity of roots with different diameters collected in three seasons, rainy, summer and winter, was examined in thioacetamide-intoxicated rats by Rawat *et al*. (1997). The results showed that an aqueous extract (2 ml/kg) of roots having a diameter of 1-3 cm, collected in the month of May (summer), exhibited marked protection of a majority of serum parameters, i.e. SGOT, SGPT, ACP and ALP, but not gluteraldehyde dehydrogenase (GLDH) and bilirubin, thereby suggesting the proper size and time for the collection of *B. diffusa* L. roots for the most desirable results.

2.3.3 Other Hepatoprotective Plants

A brief review on a few plants possessing hepatoprotective action is depicted in Table 2.

CHAPTER III

MATERIALS AND METHODS

3.1 BOERHAAVIA DIFFUSA LINN.

3.1.1 Collection of the Herb

Plants were collected from College of Veterinary Science, Tirupati campus, between June and November, 2002. The identity of the plant was confirmed by herbarium specialist, Department of Botany, S.V. University, Tirupati.

3.1.2 Preparation of Alcoholic (Ethanolic) Extract

The whole plant material was dried in the shade, powdered in an electric grinder, passed through sieve number 60 and extracted in cold with 60 per cent ethanol. The solvent was removed by distillation under pressure to obtain the dried extract (8.8% yield w/w in terms of dried starting material). (Pharmacopoeia of India, 1966)

3.2 DRUGS AND CHEMICALS

3.2.1 Furazolidone B.P. (99.29% assay), Vet India pharmaceutical Ltd., Hyderabad.

3.2.2 Silymarin: Micro Labs, Bangalore.

3.2.3 Chemicals: All the chemicals used were of analytical grade and were obtained from S.D. Fine-Chem Ltd., Mumbai.

3.2.4 Stains: H & E stains were obtained from Qualigens Pvt. Ltd., Hyderabad.

3.2.5 Antioxidant reagents: GSSG, GSH-R, FAD and NADPH were procured from Sisco Research Laboratories (SRL) Pvt. Ltd., Mumbai.

3.2.6 Vaccines:

3.2.6.1 New Castle disease (NDV) vaccine, Living (Lasota strain) B.P.(Vet), Ventri Biologicals, Vaccine division, Pune.

3.2.6.2 Infectious Bursal disease Living (Intermediate strain) vaccine B.P. (Vet), Ventri Biologicals, Vaccine division, Pune.

3.2.6.3 New Castle Disease vaccine Attenuated (F_1 strain) B.P. (Vet), Ventri Biologicals, Vaccine division, Pune.

3.3 EXPERIMENTAL ANIMALS

One hundred and twenty male broiler chicks (day-old) of Vencobb strain, belonging to a single hatch were obtained from Balaji Hatcheries, Chittoor. Before starting the experiment, permission from Institutional Animals Ethics Committee was obtained.

3.4 EXPERIMENTAL DESIGN

A total of 120 day-old chicks were weighed, wing-banded and randomly allocated to 6 groups, each having 20 birds. Groups 2, 3 and 4 were subdivided into 'a' and 'b' with 10 birds in each and the following treatment protocol was followed:

Group 1 Vehicle control i.e. 2% gum acacia *p.o.* daily

Group 2 a) Furazolidone 150 mg/kg body wt. *p.o.* once daily for 2 weeks (15^{th} to 28^{th} day)

b) Furazolidone 150 mg/kg body wt. *p.o.* once daily for 2 weeks (15^{th} to 28^{th} day) and 2% gum acacia *p.o.* for 2 weeks (29^{th} to 42^{nd} day)

Group 3 a) Furazolidone 150 mg/kg body wt. *p.o.* and BD 200mg/kg *p.o.* once daily for 2 weeks (15^{th} to 28^{th} day)

b) Furazolidone 150 mg/kg body wt. *p.o.* and BD 200mg/kg *p.o.* once daily for 2 weeks (15^{th} to 28^{th} day) and BD 200mg/kg *p.o.* continued for further 2 weeks (29^{th} to 42^{nd} day)

Group 4 a) Furazolidone 150 mg/kg body wt. *p.o.* and Silymarin 200mg/kg *p.o.* once daily for 2 weeks (15^{th} to 28^{th} day)

b) Furazolidone 150 mg/kg body wt. *p.o.* and Silymarin 200mg/kg *p.o.* once daily for 2 weeks (15^{th} to 28^{th} day) and

Silymarin 200mg/kg *p.o*. continued for further 2 weeks (29th to 42nd day)

Group 5 Furazolidone 150 mg/kg body wt. *p.o.* daily for 2 weeks (15th to 28th day) and BD 200mg/kg *p.o*. for 2 weeks (29th to 42nd day)

Group 6 Furazolidone 150 mg/kg body wt. *p.o.* daily for 2 weeks (15th to 28th day) and Silymarin 200mg/kg *p.o*. for 2 weeks (29th to 42nd day)

At the end of 4 weeks i.e., on 29th day, birds of group 2a, 3a and 4a, and five birds from groups 1, 5 and 6 were sacrificed for estimating lipid peroxidation in liver. The liver, heart and kidney pieces were preserved in 10 per cent formaldehyde solution for histopathologic study and thereafter birds of groups 2b, 3b and 4b were considered as the representative of that group.

3.5 FEED

The feed ingredients used in this experiment were procured from M/S Doctor Feeds, Chittoor. Chicks were fed with starter feed upto 4 weeks age and later with finisher feed.

FEED COMPOSITION: kgs of ingredients/ 100 kgs of feed

Ingredients	Starter ration	Finisher ration
Maize	57	62
Soya bean meal	30	25
Fish meal	10	10
Mineral mixture	3	3
Indomix (AB_2D_3)	20G	20G
Indomix – B	25G	25G
Neftin 200	30G	30G
Coccidiostat	40G	40G

3.6 ADMINISTRATION OF DRUGS

Aqueous suspensions of BD, silymarin and furazolidone in 2 per cent gum acacia were prepared and administered to the chicks through crop intubation unless otherwise indicated (Table 3). Control chicks received an equal volume of the vehicle.

Table 3: Treatment Regimen

	3rd Week	**4th Week**	**5th Week**	**6th Week**
Group I	Vehicle	Vehicle	Vehicle	Vehicle
Group II	Fz	Fz	Vehicle	Vehicle
Group III	Fz + BD	Fz + BD	BD	BD
Group IV	Fz+ Silymarin	Fz+ Silymarin	Silymarin	Silymarin
Group V	Fz	Fz	BD	BD
Group VI	Fz	Fz	Silymarin	Silymarin

Fz- Furazolidone
BD- alcoholic extract of *Boerhaavia diffusa* Linn.
Vehicle- Aqueous Gum acacia 2%
(Note: All the drugs are administered along with the vehicle)

3.7 COLLECTION OF BLOOD SAMPLES

The blood samples were drawn from the wing vein at weekly intervals with dipotassium EDTA (for assay of Glutathione reductase and Glutathione peroxidase) and without EDTA, for separation of serum (for assay of AST, ALT, LDH, Total proteins, albumin, total cholesterol, HDL and uric acid).

3.8 BODY WEIGHTS

Body weights of the chicks were recorded on day- one and thereafter at regular weekly intervals, and the mean body weights for each group were calculated for studying the growth rate and weight gain.

3.9 FEED CONVERSION RATIO (FCR)

FCR was calculated to determine the feed efficiency using the formula

$$FCR = \frac{\text{Total feed consumed (Kg)}}{\text{Total body weight gained (Kg)}}$$

3.10 CLINICAL OBSERVATIONS

Birds of all the groups were observed clinically for feed intake, alertness, changes in posture, diarrhoea and for presence of any signs of furazolidone toxicity.

3.11 LIPID PEROXIDATION

The quantitative measurement of lipid peroxidation was done by measuring the concentration of of Thiobarbituric acid reactive substances (TBARS) in liver using the method of Ohkhawa (1979). The amount of malondialdehyde (MDA) formed was quantitated by reaction with thiobarbituric acid (TBA) and used as an index of lipid peroxidation.

3.12 RBC ENZYME PROFILE

3.12.1 Assay of Glutathione Peroxidase (GPx) activity (Paglia and Valentine, 1967)

Materials:

Phosphate buffer (P^H 7.4)

0.2 mM NADPH

3 units of Glutathione reductase

0.25 mM H_2O_2

Method:

Reagent	Test tube (ml)
1. Phosphate buffer	2.0
2. Haemolysate	0.1
3. Reduced glutathione	0.1
4. H_2O_2	0.1

Tubes were incubated at 25^o C for 15 minutes following which 0.1 ml NADPH was added. Then enzyme activity was monitored at 60 sec interval for 5 min at 320 nm.

Calculation:

Erythrocyte glutathione peroxidase activity = ΔA_{320} x 3781 units/ml.

Where ΔA_{320} is the change in absorbance per minute at 320 nm.

3.12.2 Assay of Glutathione Reductase (GSH-R) Activity (Spectro-photometric method – Raghuramulu, 1983)

Materials:

Haemolysate

Phosphate buffer

0.1M Sodium bicarbonate

50 mM GSSG solution

250 μM FAD

4 mM NADPH

80 mM EDTA

Method:

Reagent	Test tube (ml)
1. Phosphate buffer	2.0
2. Haemolysate	0.1
3. 50 mM GSSG	0.1
4. 250 μM FAD	0.1
5. 80 mM EDTA	0.5

Tubes were incubated at 37^{o}C for 15 min, following which 0.1 ml NADPH solution was added. Then enzyme activity was monitored at 60 sec interval for 5 minutes at 340 nm.

Calculation:

Erythrocyte glutathione reductase activity = ΔA_{340} x 3781 units/ml.

Where ΔA_{340} is the change in absorbance per minute at 340 nm.

3.13 SERUM BIOCHEMICAL PROFILE

n was separated from clotted blood at weekly intervals and is used for the assay of the following biochemical parameters –

3.13.1 Alanine aminotransferase (ALT): Modified IFCC method

3.13.2 Aspartate aminotransferase (AST): Modified IFCC method

3.13.3 Total protein & albumin: Biuret and BCG method

3.13.4 Cholesterol & HDL cholesterol: CHOD/POD – Phosphotungstate method

3.13.5 Uric acid: Uricase – POD method

3.13.6 Lactate dehydrogenase (LDH): UV Kinetic (IFCC and SFBC) method

Note: For the estimation of serum biochemical parameters, standard kits from Monozyme India Limited, Secunderabad were used, except for LDH, which was from Bayer Diagnostics, Baroda.

3.14 HEMATOLOGICAL PROFILE

Hemoglobin was estimated by Sahli's acid hematin method.

Total erythrocyte count (TEC) and Total leukocyte count (TLC) were estimated by Neubar's method using the following diluting fluid (Sastry,

1983).

Stock solution

A Sodium citrate 2%

B 0.1% Gentian violet in Ringer's solution

C 0.1% Brilliant cresyl blue in Ringer's solution

D Neutral formalin

Composition of Ringer's solution

Sodium chloride 0.7g

Sodium carbonate 0.03g

Potassium chloride 0.026g

Calcium chloride 0.003g

Distilled water 100ml

Working solution

A -1ml

B -2ml

C -1ml

D -3 drops

Mixed well and filtered. Using this working solution as diluting fluid, TEC and TLC were estimated.

Packed cell volume (PCV) was estimated by Microhematocrit method.

Erythrocyte indices:

MCV, MCH and MCHC were estimated as described by Jain (1996).

3.15 ORGAN-SOMATIC INDICES

At the end of the experimental period, the birds were sacrificed and the organo somatic indices of liver, heart and kidney were calculated using the formula –

$$\text{Organ-somatic index of the organ} = \frac{\text{Weight of the organ (gm)}}{\text{Body weight (gm)}} \times 100$$

3.16 HISTOPATHOLOGICAL STUDIES

A detailed postmortem examination of the birds sacrificed from each group was conducted at the end of the experiment. The gross pathological changes if any, were noted. Tissue pieces of heart, liver and kidney were collected and fixed in 10% formalin. The fixed tissues were processed and stained with H & E stain as described by Singh and Sulochana (1997).

3.17 STATISTICAL ANALYSIS

The data was analyzed using two-way ANOVA and Student's t-test (Snedechor and Cochron, 1994). A value of $p<0.05$ was considered to indicate a significance difference between groups and between weeks.

Per cent Protection in GSH-R, GPx, ALT, AST, LDH and LPO was calculated using the formula:

$$\text{Per cent Protection} = \frac{\text{[(Mean value of Fz-treated group) - (Mean value of Fz + test substance treated group)]}}{\text{[(Mean value of Fz-treated group) – (Mean value of control group)]}} \times 100$$

CHAPTER IV
RESULTS

4.1 BODY WEIGHTS

The mean body weights (g) of different groups of chicks at weekly intervals are presented in Table 4. The mean body weights of group 1 (679.00 ± 6.74), 4 (728.00 ± 4.42) and 6 (631.00 ± 7.38) were significantly ($p<0.05$) higher when compared to those of group 2 (424.00 ± 6.70), 3 (621.00 ± 9.12) and 5 (553.5 ± 4.43) at the end of 4^{th} week. At the end of 6^{th} week, there was a significant ($p<0.05$) rise in body weights of group 4 (1664.00 ± 10.02) and 6 (1350.00 ± 8.13) when compared to that of group 2 (990.00 ± 8.20) and 5 (1183.50 ± 9.97). However, the 6^{th} week body weights revealed that the 4^{th} group had significantly ($p<0.05$) higher body weights when compared to that of group 1 (1515.50 ± 8.45). (Fig. 7)

4.2 BODY WEIGHT GAINS

At the end of 4^{th} week, the body weight gains of group 1 (318.80 ± 6.49), 4 (331.00 ± 7.37) and 6(290.60 ± 9.03) were significantly ($p<0.05$) higher when compared to those of group 2 (118.20 ± 3.18), 3 (248.00 ± 9.49) and 5 (229.50 ± 8.80). Following therapy, at the end of 6^{th} week, the body weight gains of group 3 (460.00 ± 9.57), 4 (473.00 ± 13.5) and 6 (247.00 ±

8.89) were significantly higher when compared to group 2 (199.00 ± 11.47). There is no significant difference in the body weight gains of group 3 and 4 when compared to that of vehicle control group 1 at the end of the experiment. (Table 5; Fig.8)

4.3 FEED CONVERSION RATIO (FCR)

FCR at weekly intervals is given in table 6. At the end of 4^{th} week, FCR of group 2 (2.92) and 5 (2.34) were significantly ($p<0.05$) higher compared o those of groups 1 (2.02), 3 (2.07), 4 (2.36) and 6 (2.80). At the end of the experiment, following therapy, FCR of group 3 (2.26), 4 (2.52), 5 (2.12) and 6 (2.25) were significantly ($p<0.05$) lower when compared to those of control (2.55) and group 2 (2.63), which was maintained up to 6^{th} week without any drug treatment to the study the extent of natural recovery.(Fig. 9)

4.4 ANTIOXIDANT ENZYME PROFILE

4.4.1 Glutathione Peroxidase (GPx) Activity

There was a significant ($p<0.05$) increase in GPx activity (units/ml) of groups 2, 3, 4, 5 and 6 (91.54 ± 0.42, 88.12 ± 0.42, 86.49 ± 0.34, 89.46 ± 0.40 and 93.65 ± 0.34 respectively at week 3 as compared to their levels at week 2 (82.84 ± 0.64, 81.87 ± 0.62, 80.95 ± 0.38, 82.93 ± 0.48 and 81.98 ± 0.60

respectively). At week 4, there was a significant ($p<0.05$) lowering of the GPx activity in groups 2, 5 and 6 (75.46 ± 0.38, 78.37 ± 0.46 and 80.66 ± 0.43 respectively) as compared to that of groups 1, 3 and 4 (86.19 ± 0.32, 85.39 ± 1.59 and 88.53 ± 0.35 respectively). However, treatment with BD and silymarin in groups 5 and 6 (85.87 ± 0.31 and 88.45 ± 0.30 respectively) has significantly ($p<0.05$) recovered the enzyme activity at week 6, which is comparable to that of control group (87.33 ± 0.35). (Table 7; Fig. 10)

4.4.2 Glutathione Reductase (GSH-R) Activity

There was a slight but no significant elevation in the GSh-R activity (units/ml) in groups 2, 3, 4, 5 and 6 (43.13 ± 0.59, 40.85 ± 0.41, 44.24 ± 0.45, 40.15 ± 0.36 and 43.10 ± 0.41 respectively) as compared to that if group 1 (39.81 ± 0.61) at week 3. But by week 4, the GSH-R activity of groups 2 (32.25 ± 0.76), 5 (27.22 ± 0.60) and 6 (30.45 ± 0.44) was significantly ($p<0.05$) lowered as compared to that of groups 1(40.43 ± 0.32), 3 (44.13 ± 0.27) and 4 (46.27 ± 0.39). Treatment with BD and silymarin in groups 5 and 6 has significantly ($p<0.05$) recovered the enzyme activity to 38.25 ± 0.66 and 40.37 ± 0.31 respectively, which is comparable to that of control group (38.25 ± 0.37) at week 6. GSH-R activity in groups 3 and 4 significantly improved through the experimental period. (Table 8; Fig. 11)

4.5 LIVER PROFILE

4.5.1 Alanine aminotransferase (ALT)

At the end of 4th week, the mean ALT levels (IU/L) in group 2 (88.59 ± 1.77), 5 (90.36 ± 1.27) and 6 (87.44 ± 1.11) were significantly ($p<0.05$) higher when compared to those of control group (18.56 ± 0.37), 3 (33.32 ± 0.68) and 4 (22.23 ± 0.63). The per cent protection was higher in group 4 (94.75) than that of group 3 (80.35) (Table 12).

At the end of the experiment, the ALT level of group 2 (57.65 ± 1.15) and 5 (43.52 ± 1.07) were significantly ($p<0.05$) higher than those of group 1 (21.06 ± 0.53), 3 (26.41 ± 0.41), 4 (21.77 ± 0.58) and 6 (30.82 ± 1.04). (Table 9; Fig. 12)

4.5.2 Aspartate aminotransferase (AST)

Serum levels of AST (IU/L) monitored at weekly intervals in different groups during the experimental period is shown in Table 10 and Figure 13. The mean serum AST values observed at the end of 4th week were 18.04 ± 0.16, 61.57 ± 0.39, 21.90 ± 0.36, 19.27 ± 0.62, 63.67 ± 0.43 and 61.81 ± 0.76 in groups 1, 2, 3, 4, 5 and 6 respectively. These values indicate that the AST levels in groups 2, 5 and 6 were significantly ($p<0.05$) elevated. The per cent protection was higher in group 4 (97.17) than that of group 3 (91.13) (Table 12).

At the end of the experiment, AST levels of group 2 (43.41 ± 0.45) were significantly ($p<0.05$) higher compared to the other groups. The levels of groups 5 (32.02 ± 0.48) and 6 (27.78 ± 0.45) were higher compared to those of group 1 (17.06 ± 0.23) and 4 (19.88 ± 0.38) but comparable with that of 3 (22.97 ± 0.29). (Table 10; Fig. 13)

4.5.3 Lactate Dehydrogenase Activity (LDH)

LDH activity (U/L) was significantly ($p<0.05$) elevated in toxic groups 2, 5 and 6 at 3rd week (204.85 ± 1.32, 194.19 ± 12.45 and 203.24 ± 2.19 respectively) and 4th week (269.92 ± 0.82, 261.73 ± 1.47 and 250.52 ± 2.19 respectively). Following treatment with BD and silymarin in groups 5 and 6, the LDH activity was reduced significantly ($p<0.05$) to 189.53 ± 1.16 and 182.62 ± 0.78 respectively as compared to 4th week levels. But these levels differed significantly ($p<0.05$) when compared to the remaining groups 1, 3 and 4 (165.71 ± 1.04, 163.06 ± 1.54 and 156.26 ± 0.99 respectively). (Table 11; Fig. 14). The per cent protection offered by BD against LDH leakage was 90.17 while silymarin offered 100 per cent protection.

4.6 KIDNEY PROFILE

4.6.1 Uric Acid

The uric acid levels (mg/dl) were significantly ($p<0.05$) elevated in groups 4 (25.19 ± 0.39), 5 (21.48 ± 0.35) and 6 (21.59 ± 0.57) at 4th week. Following therapy at 6th week in groups 5 (8.87 ± 0.31) and 6 (8.55 ± 0.35), these levels revived to normal and were comparable with that of groups 1 (7.19 ± 0.28), 3 (7.15 ± 0.19) and 4 (6.31 ± 0.28) but remained significantly ($p<0.05$) higher in group 2 (13.56 ± 0.52). (Table 13; Fig. 15)

4.7 LIPID PROFILE

4.7.1 Total Cholesterol

Mean serum cholesterol levels (mg/dl) decreased significantly ($p<0.05$) in groups 2 (89.54 ± 2.01), 5 (86.35 ± 2.72) and 6 (90.24 ± 1.36) when compared to group 1 (151.91 ± 0.89), 3 (139.20 ± 0.94) and 4 (150.52 ± 1.83) at 4th week. This indicates that furazolidone has significantly decreased the mean cholesterol levels. However, after treatment with BD and silymarin at 6th week, the cholesterol levels in groups 5 and 6 were elevated to 146.45 ± 3.54 and 150.00 ± 1.81 respectively, while the level in group 2 which is left without treatment, could not improve significantly ($p<0.05$). These levels in groups 2, 3, 4, 5 and 6 differed significantly from that of control group 1 (166.96 ± 1.91). (Table 14; Fig. 16)

4.7.2 High Density Lipoproteins (HDLs)

The HDL (mg/dl) levels gradually decreased from week 2 to week 4 in the furazolidone-fed groups (2, 5 and 6). At week 4, the levels in group 2 (20.51 ± 0.80), 5 (22.29 ± 0.70) and 6 (21.97 ± 1.08) were significantly ($p<0.05$) lower when compared to control (33.86 ± 0.87), BD treated (36.46 ± 0.49) and silymarin treated (40.39 ± 0.80) groups. Following treatment in groups 5 (32.42 ± 0.68) and 6 (34.58 ± 0.90), the levels increased significantly ($p<0.05$) as compared to that of group 2 (28.81 ± 0.83). (Table 15; Fig. 17)

4.8 PROTEIN PROFILE

Data pertaining to protein profile in different groups during the experimental period is given in Table 16.

4.8.1 Total Proteins (TP)

At 4^{th} week, the mean serum TP levels (gm/dl) in the furazolidone treated groups 2, 5 and 6 were 2.34 ± 0.22, 3.22 ± 0.16 and 3.28 ±1.91 respectively, and these values were significantly ($p<0.05$) lower as compared to those of groups 1, 3 and 4 which were 5.56 ± 0.27, 4.21 ± 0.08 and 5.11 ± 0.22 respectively. After treatment, at the end of the experiment, the mean serum TP of groups 1(5.78 ± 0.18), 3(5.36 ± 0.26), 4(5.87 ± 0.21), 5(5.45 ± 0.24) and 6(6.20 ± 0.18) were significantly ($p<0.05$) higher than that of group 2(4.52 ± 0.32).

4.8.2 Albumin

Serum albumin levels (gm/dl) estimated in groups 1, 2, 3, 4, 5 and 6 were 4.07 ± 0.14, 1.62 ± 0.13, 3.00 ± 0.11, 3.73 ± 0.14, 2.05 ± 0.15 and 2.35 ± 0.14 respectively at 4^{th} week and the levels at 6^{th} week were 3.93 ± 0.17, 2.80 ± 0.11, 3.45 ± 0.11, 4.30 ± 0.15, 3.58 ± 0.10 and 3.86 ± 0.04 respectively. The values indicate that the mean serum albumin levels in group 2, 5 and 6 were significantly ($p<0.05$) lower when compared to those of groups 1,3 and 4 but the levels in group 1, 3 and 4 also differed significantly ($p<0.05$) from each other at 4^{th} week. Post treatment with BD and silymarin in groups 3, 4, 5 and 6 significantly ($p<0.05$) improved the albumin concentration as compared to that of untreated group 2. The values at 6^{th} week also differed significantly ($p<0.05$) in group 2, 3, 4, 5 and 6 as compared to those at 4^{th} week.

4.8.3 Globulins

At 4^{th} week, the globulin levels (gm/dl) in group 2 (1.02 ± 0.12), 3 (1.21 ± 0.15), 5 (1.17 ± 0.17) and 6 (0.93 ± 0.11) were significantly ($p<0.05$) lower when compared to those of groups 1 (1.49 ± 0.32) and 4 (1.39 ± 0.26).

At 6^{th} week, the globulin levels in groups 1 (1.85 ± 0.16), 2 (1.72 ± 0.29), 3 (1.91 ± 0.21), 4 (1.58 ± 0.31) and 5 (1.87 ± 0.18) were comparable but significantly ($p<0.05$) lower when compared to that of group 6 (2.34 ± 0.21).

4.8.4 Albumin/Globulin (A/G) Ratio

The A/G ratio did not differ significantly ($p<0.05$) in groups 1 (2.73 ± 0.91), 3 (2.47 ± 0.58), 4 (2.69 ± 0.82) and 6 (1.54 ± 0.47), but for groups 2 (1.29 ± 0.16) and 5 (1.75 ± 0.50) which were significantly ($p<0.05$) lower at 4^{th} week. At 6^{th} week, the A/G ratio in group 4 (2.73 ± 0.94) was significantly ($p<0.05$) higher when compared to that in groups 1 (2.12 ± 0.32), 2 (1.63 ± 0.31), 3 (1.81 ± 0.36), 5 (1.92 ± 0.14) and 6 (1.65 ± 0.18).

4.9 HAEMATOLOGICAL PROFILE AND ERYTHROCYTE INDICES

The hematological values are presented in Table 17 and the erythrocyte indices are presented in Table 18.

The mean hemoglobin (%) levels at week 2 in all the groups ranged between 8.08 ± 0.10 to 8.28 ± 0.10. At week 4, groups 2 (7.36 ± 0.07), 5 (7.52 ± 0.10) and 6 (7.28 ± 0.10) showed a significant ($p<0.05$) reduction in the Hb% as compared to those of control (8.52 ± 0.08) and treatment groups 3 (8.60 ± 0.09) and 4 (9.60 ± 0.09). The mean Hb% values observed at week 6 in groups 1, 2, 3, 4, 5 and 6 were 9.42 ± 0.07, 8.16 ± 0.07, 9.64 ± 0.12, 8.92 ± 0.36, 8.32 ± 0.10 and 8.36 ±0.12 respectively. Group 5 and 6 values reveal a significant ($p<0.05$) improvement compared to the levels at week 4 and these levels at 6^{th} week were comparable with that of control group.

The mean PCV (%) values at week 4 did not reveal any significant

($p<0.05$) differences among the groups. However, at week 6, a significant ($p<0.05$) fall in PCV was observed in groups 2 (26.42 ± 0.50), 5 (27.05 ± 0.28) and 6 (26.36 ± 0.50) as compared to the levels in group 1 (36.01 ±0.59), 3 (30.67 ± 0.16) and 4 (36.65 ± 0.74).

The mean erythrocyte counts (TEC) (10^6/ ml) in different groups at different weeks showed no significant ($p<0.05$) differences.

The mean leukocyte counts (TLC) (10^3/ ml) decreased gradually in groups 2 (18.54 ± 0.70), 5 (19.28 ± 0.92) and 6 (18.60 ± 0.73) significant ($p<0.05$) at week 4. Following treatment, at week 6, the levels improved significant ($p<0.05$) in groups 5 (24.64 ± 0.69) and 6 (27.60 ± 1.23) but could not revive in group 2 (19.84 ± 0.76).

A significant ($p<0.05$) increase in MCV (μ^3) was recorded in the groups 2 (163.24 ± 2.45), 5 (156.80 ± 3.56) and 6 (158.50 ± 2.01) at week 4 as compared to that of groups 1 (85.75 ± 4.21), 3 (113.54 ± 2.91) and 4 (119.49 ± 1.15). At week 6 too, the levels in groups 2 (149.10 ± 5.58), 5 (159.06 ± 11.45) and 6 (140.66 ± 7.54) remained significantly ($p<0.05$) higher as compared to that of groups 1 (112.15 ± 1.88), 3 (94.28 ± 22.18) and 4 (125.78 ± 7.78).

No significant ($p<0.05$) changes were observed in the mean values of MCH (μμg) and MCHC (%) among different groups.

4.10 LIPID PEROXIDATION (LPO)

TBARS were found to be elevated significantly ($p<0.05$) after the administration of furazolidone as evidenced by increase in the MDA content, which was significantly ($p<0.05$) reversed by BD and silymarin. Simultaneous treatment of also prevented the rise of TBARS in liver cells (Table 19). The lowering effect of TBARS in liver by BD and silymarin was comparable (Fig. 18 and 19).

4.11 ORGAN - SOMATIC INDICES

The organ-somatic indices of liver, heart and kidney are given in table 19. When compared with control group, group 3 and 4, relative weights of liver and heart were increased for birds of groups 2, 5 and 6 at week 4. No significant ($p<0.05$) Furazolidone-related changes were found in the relative weights of kidney. By week 6, no significant ($p<0.05$) alterations in the relative weights of organs were examined compared with the control values. (Table 20)

4.12 CLINICAL SIGNS

The chicks of group 2, 5 and 6 showed signs of nervous derangement consisting of incoordination, lethargy, leg-weakness, torticollis, diarrhea, reduced feed and water intake up to during the toxin treatment i.e. 3^{rd} and 4^{th} weeks. During 5^{th} and 6^{th} weeks, the birds of group 5 and 6 showed dramatic recovery, while, the birds of group 2 left for natural healing without any

treatment showed a slight but insignificant improvement. The chicks of groups 1, 3 and 4 did not exhibit any such symptoms.

4.13 GROSS PATHOLOGICAL ALTERATIONS

Gross lesions observed in different groups of chicken were –

4.13.1 Liver

In groups 2, 5 and 6, at week 4, the livers showed enlargement, necrotic foci, paleness and fatty changes. Congestion and petechial hemorrhages were also noticed. Livers of 1, 3, and 4 groups did not show any significant lesions. Following treatment, at week 6, there were no such signs observed in the livers of groups 5 and 6 (Plates 3 and 4) but no significant recovery was observed in group 2 (Plate 2).

4.13.2 Heart

Gross changes observed in the hearts of furazolidone – fed groups (2, 5 and 6) at week 4 were flabbiness, patchy hemorrhages (Plate 5), and in one case, hydropericardium was also noticed. These gross findings were not evident in the remaining groups. At week 6, no significant changes were noticed in the hearts of all the groups.

4.13.3 Kidney

In groups 2, 5 and 6 at 4^{th} week, kidneys were congested in all cases. In some, the kidneys were pale with prominent tubulation with yellow crystalline

deposits. The kidneys of the remaining groups were normal. However, no such changes were observed at 6th week in all the groups.

4.13.4 Intestines

Intestines of the toxin-fed groups showed clear catarrhal changes. This change was not seen in the other groups.

4.14 HISTOPATHOLOGY

4.14.1 Liver

Microscopically, liver section from control group showed normal arrangement of hepatocytes with nuclei (Plate 6), while, Furazolidone-treated liver sections at 4th week revealed cirrhotic changes (Plate 7), focal areas of necrosis (Plate 8), focal mononuclear cell infiltration (Plate 9), fatty changes (Plate 10) and diffuse bile duct hyperplasia (Plate 11). The sections from group 3 and 4 showed mild degenerative changes in the hepatic cells, focal areas of congestion and sinusoidal dilatation. At week 6, liver sections from group 5 showed mild areas of congestion, mild proliferation of bile ducts and focal infilteration of mononuclear leukocytes at portal triad. In group 6, liver sections showed mild degenerative changes in hepatic cells and focal nodular mononuclear cell infiltration.

4.14.2 Heart

The histopathological studies of heart from group 2, 5 and 6 revealed diffuse intermuscular hemorrhages (Plate 12) and infiltration of mononuclear cells in the pericardial sac (Plate 13) at week 4, while those of groups 3 and 4 showed similar lesions but in a less intense manner. At week 6, group 5

showed mild focal hemorrhages (Plate 14). Other groups (1, 3, 4 and 6) did not reveal any lesions of pathological significance.

4.14.3 Kidney

In groups 2, 5 and 6 at week 4, diffuse intertubular hemorrhages (Plate 15), degenerative changes and vacuolation in tubular epithelial cells (Plate 16) were observed in kidney while at week 6, focal intertubular hemorrhages and mid degenerative changes were noticed. Other groups did not show any changes of pathological significance.

CHAPTER V

DISCUSSION

Furazolidone is an important feed additive in poultry diets. It has been found to be effective in reducing mortality in acute outbreaks of several types of paratyphoid infections (Biester and Schwarte, 1985) and is considered to be an active aid in preventing coccodiosis in chickens (Booth and Mc Donald, 1982). However, even at recommended concentrations furazolidone is reported to have a low toxicity for host- tissues.

Excess dosage of drugs like furazolidone in the poultry diets is considered as an oxidative factor resulting in oxidative stress (Moudgal, 2000). It was found that the toxicity of furazolidone is probably mediated through the formation of a nitro anion radical by cytochrome *c*-P450 reductase (De Angelis *et al*., 1998). These reactive oxygen species exert their damaging effect on different tissues, liver being the primary organ to be effected as it is the site of detoxification (Kaneko *et al*, 1997).

Oxidative stress and subsequent damage can be prevented to a larger extent by the use of antioxidants in the feed (Rock *et al*., 1996; Maine, 2000). Vitamin E and C, β- carotene, bilirubin, glucose, uric acid, glutathione

peroxidase, catalase, superoxide dismutase, transferrin and ceruloplasmin are known to act as protectors against free radical damage (Machilin and Bendich, 1987; Yu, 1994; Rock *et al*., 1996; Maine, 2000).

Whenever it is felt that pro-oxidant factors are expected to become predominant over antioxidant, suitable exogenous dietary antioxidant support should be provided. Herbal drugs have gained importance in recent years and numerous plants and polyherbal formulations are claimed to have hepatoprotective activities by virtue of their antioxidant properties.

In view of the above facts the present investigation was undertaken to study the adverse effects of furazolidone, if any, at 150 mg/kg body wt. by monitoring its performance, clinical, biochemical parameters namely erythrocyte antioxidant enzyme profile, liver profile, kidney profile, lipid profile, lipid peroxidation, protein profile, organ-somatic indices, gross and histopathological changes in liver , heart and kidney. Further, attempts were made to study the antagonizing effects of ethanolic extract of *Boerhaavia diffusa* Linn. against furazolidone induced toxicity.

5.1 EFFECT ON GROWTH PERFORMANCE

In the present study, the body weights of groups 1 and 4 were significantly ($p<0.05$) higher as compared to those of groups 2, 3, 5 and 6 at the end of 4^{th} week, but at the end of 6^{th} week, the body weights of groups 1, 3, 4, 5, and 6 were significantly higher as compared to group 2. This indicates that furazolidone treatment resulted in lower body weights and that in group 3; BD treatment could not improve the body weights at the 4^{th} week. However, post BD treatment in both groups 3 and 5 resulted in significantly higher body weights compared to group 2. The weight gains were also lower in the toxic groups 2, 5 and 6 at 4^{th} week when compared to control and treatment groups 3 and 4. These lower body weights and weight gains might be due to anorexia, which is one of the effects of the drug (Ali and Bartlet, 1982; Czarnecki, 1984; Arbid *et al*., 1990; Webb and Van Vleet, 1991; Zaman *et al*., 1995).

The toxic groups 2, 5 and 6 had significantly higher FCR as compared to BD and silymarin treated groups throughout the experimental period. This indicates that the birds in the toxic groups utilized feed less efficiently than those fed with BD and silymarin along with furazolidone. Similar results were observed when broilers were fed with kojic acid intoxicated diets (Giroir *et al*., 1991).

5.2 EFFECT ON ERYTHROCYTE ANTIOXIDANT ENZYMES

In the present study, the levels of GPx and GSH-R in the toxic groups (2, 5 and 6) were elevated slightly though not significantly, followed by subsequent fall in the activity of these enzymes. These results are in accordance with those of Paul and Paul (1964), Mc Calla (1979) and Sas (1993). The initial rise in the activity might probably be an adaptive response towards oxidative stress. However, the decrease in the erythrocyte antioxidant enzyme activity by 4^{th} week may be due to excessive generation of free radicals, leading to depletion of GSH (Ali, 1992; Sas, 1993) and also due to deficiency of glucose-6-phosphate dehydrogenase and reduced supply of NADPH for the conversion of GSSG to GSH, a free radical scavenger (Figure 2). This results in the loss of membrane integrity of RBC resulting in hemolysis, which is common in drug-induced toxicity (Stryer, 1995). In this study too, hemolysis was observed in the toxic groups further validating the mechanism of toxicogenesis of furazolidone. Similar finding was reported by Webb *et al.* (1991).

BD and silymarin significantly prevented the decrease in the activity of GPx and GSH-R by furazolidone and later increased their activities, which may be attributed to have biological significance in eliminating reactive free radicals that may affect the normal functioning of cells (Chen *et al*., 2002; Bharali *et al*., 2003). This antioxidant action of silymarin may be due to

prevention of GSH depletion (Videla and Valenzuela, 1982; Valenzuela *et al*, 1985a; Valenzuela and Guerra, 1985; Campos *et al*., 1989). The mechanism of antioxidant activity of BD is not known and further research is needed in this aspect to delineate the underlying mechanisms.

5.3 EFFECT ON HEPATIC ENZYME PROFILE

In avian species, LDH and AST are considered as the most sensitive indicators of liver cell damage (Kaneko *et al*., 1997). An increase in the levels of serum ALT, AST and LDH in the furazolidone groups (2, 5 and 6) observed in the study is in conformity with Staley *et al*. (1978); Czarnecki *et al*. (1981); Mc Callum *et al*. (1989); Webb *et al*. (1991); Chapados *et al*. (1992) and O'Brein *et al*. (1993), indicating hepatic damage which could be correlated with degenerative and cirrhotic changes observed in the liver of toxin-fed groups. The elevated levels of these enzymes might have resulted from hypoxic hepatocellular damage and leakage (Webb *et al*., 1991; Duncun *et al*., 1994). This can be correlated with increase in lipid peroxidation.

BD and silymarin seem to preserve the structural integrity of the hepatocyte cell membrane which is evident by a reduction in the furazolidone-induced rise of ALT, AST and LDH (Floersheim *et al*., 1978; Chakraborti and Handa, 1989a; Rajkumari *et al*., 1991; Rawat *et al*., 1997; Shenoy *et al*., 2001;

Valenzuela and Garrido, 1994; Wellington and Jarvis, 2001).

5.4 EFFECT ON CARDIAC PROFILE

In the present study, the elevated levels of LDH, ALT and AST in the furazolidone-treated groups indicate cardiomyopathy. Similar results were recorded by Czarnecki *et al* (1981) and Mc Callum *et al* (1989). These results could be well correlated with the gross and histopathological changes observed in furazolidone treated birds. These alterations might be due to abnormal Ca^{2+} homeostasis causing relaxation of the myocytes and subsequently cardiomyopathy (O'Brien *et al*, 1991, 1993; Glass *et al*, 1993 and lax *et al*, 1994). BD and silymarin could significantly reverse these changes. The cardioprotective action of BD might be due to Ca^{2+} channel antagonistic action of the liriodendrin, a lignan present in *B.diffusa* (Lami *et al*, 1991). Silymarin, a flavolignan also might have exhibited cadioprotection action in a similar manner (Farghali *et al*, 2000).

5.5 EFFECT ON KIDNEY PROFILE

Uric acid is the major end product of nitrogen metabolism in birds and it is synthesized in the liver. Very high uric acid concentrations can be found in dehydrated birds and renal functional disorders (Kaneko *et al*, 1997; Meyer and Harvey, 1998). An elevation in serum uric acid levels in groups 2, 5 and 6

was observed in the present study which can be ascribed to nephropathic lesions validated by histopathological studies.

Treatment with BD and silymarin revived the levels of uric acid to normal. This may be due to their antioxidant activity, thus preventing damage caused by free radicals and increasing cell regeneration.

5.6 EFFECT ON LIPID PROFILE

Toxic levels of furazolidone resulted in decrease in the levels of serum cholesterol. These results are in agreement with those of O'Brein et al (1993), who stated that the decreased concentrations might have resulted decreased hepatic synthesis attributable to decreased feed intake. In the present study too, inanition was observed which might have lead to decreased cholesterol levels. Furazolidone was observed to lower the HDL levels in the serum which is a marker for hapatopathy, cardiac damage as well as renal failure (Kaneko *et al,* 1997). This could be attributed to the free radical-induced oxidative damage, which was evident from the histopathological changes of liver, heart and kidney and also from the elevated levels of ALT, AST, LDH and uric acid. Following treatment with BD and silymarin, at 6^{th} week, lipid profile revived to normal in groups 2, 5 and 6. This might be due to the antioxidant property of BD and silymarin (Videla and Valenzuela, 1982; Valenzuela and Guerra, 1985; Campos *et al,* 1989; Chandan *et al,* 1991; Rajkumari *et al,* 1991). At 6^{th} week, the total cholesterol levels in BD-treated group (group 3) were lower as compared to that of control which might be due to increased elimination of cholesterol resulting from the cholerectic effect of BD (Chandan *et al,* 1991) and decreased absorption of fat due to high fiber content.

5.7 EFFECT ON PROTEIN PROFILE

Hypoproteinemia and hypoalbuminemia were noticed in furazolidone-

intoxicated broiler chicks. In addition, a decrease in globulin levels and A/G ratio was also observed. These results are consistent with the findings of O'Brein *et al*. (1993). The hypoproteinemia and hypoalbuminemia could be ascribed to toxic hepatitis and nephropathy, since such toxicities might result in decreased protein synthesis and excessive excretion (Duncan *et al*., 1994). This observation is positively correlated with histopathological findings of liver and kidney. In liver failure, extremely low plasma total proteins occur in combination with a decrease A/G ratio. Hypoalbuminemia may be a clinical sign of severe hepatocellular liver disease, may also be caused by increased albumin loss due to glomerulonephropathy (Kaneko *et al*, 1997).

BD and silymarin treatment could restore the levels of total proteins and albumin to normal level. Silymarin appears to increase ribosomal protein synthesis by stimulating RNA polymerase I and the transcription of rRNA (Magliulo *et al*, 1973; Tyutyulkova, 1983; Sonnenbichler and Zetl, 1986; Luper, 1998). The stimulation of protein synthesis is an important step in the repair of hepatic injury and is essential for restoring structural proteins and enzymes damaged by hepatotoxins (Valenzuela and Garrido, 1994; Morazzoni and Bombardelli, 1995).

5.8 EFFECT ON BLOOD CELL COUNTS AND ERYTHROCYTIC INDICES

Among the hematological parameters, in furazolidone-intoxicated groups (2, 5 and 6), a fall in the level of hemoglobin, TLC and a rise in PCV were recorded which corroborate with the results of Ehlhardt et al (1975) and Prescott and Baggot (1993). The rise in the PCV might have resulted from dehydration, which was observed in this study.

MCV was increased in the toxic groups and this accompanied by a fall in the hemoglobin level infers that furazolidone-toxicity might have resulted in macrocytic anemia. However, BD and silymarin could revive the condition to normalcy.

5.9 EFFECT ON LIPID PEROXIDATION

Lipid peroxidation is the result of an interaction between free radicals of diverse origin and unsaturated fatty acids in lipids. It involves a broad spectrum of alterations, and the consequent degeneration of cell membranes may contribute towards the development of other disorders of lipoprotein metabolism, both in the liver and in peripheral tissues.

Furazolidone treated groups showed an increase in the levels of LPOs, which is in accordance with the findings of Stroo and Schaffer (1989), Ali (1992) and Sas (1993). This indicates an involvement of free radicals that

cause peroxidation of the membrane lipids, resulting in hepatocellular damage. Further, pathological lesions observed in this study can be well correlated with this increase in LPO resulting in hepatocellular damage.

5.10 EFFECT ON ORGAN-SOMATIC INDICES

The increase in the relative weights of liver and heart in the toxin-fed groups suggest the hepatic and cardiac effects of furazolidone. This was well correlated with the gross and histopathological changes of liver and heart.

5.11 EFFECT ON CLINICAL SIGNS

The clinical signs observed in group 2, 5 and 6 were incoordination, lethargy, leg weakness, torticollis, reduced feed and water intake and weight loss during 3^{rd} and 4^{th} week. Similar findings were recorded by Webb and Van Vleet (1991), Arbid *et al* (1990) and Zaman *et al* (1995). The symptoms suggest the damaging effect of furazolidone on central nervous system (Presscott and Baggot, 1993). Furazolidone was also observed to produce anorexia and growth reduction, which might be due to thiamine – inhibitory action of furazolidone (Ali and Bartlet, 1982).

5.12 EFFECT ON GROSS AND HISTOPATHOLOGICAL ALTERATIONS

In this study, pathological lesions of degenerative and necrotic type were observed in liver, heart and kidney in furazolidone – intoxicated birds, whereas, in the control and remaining groups, the sections were normal. Following treatment, these changes were mild, which can be correlated with tissue marker enzymes ALT, AST and LDH, thus suggesting the role of silymarin (Muriel and Mourelle, 1990) and BD (Chandan *et al*, 1991; Rajkumari *et al*, 1991) in preserving the functional and structural integrity of membranes by preventing the alteration of their phospholipid structure produced by free radical induced furazolidone toxicity.

In conclusion, the results of present investigation enunciated that furazolidone exerted toxicity by free radical generation. However, the damage was more pronounced in liver than in heart and kidney. Use of BD and silymarin countered the adverse effects of furazolidone toxicity suggesting their hepatoprotective activity by virtue of their anti oxidant property but silymarin performed better than BD. In view of the above facts, it is recommended to use BD and silymarin in poultry feeds at appropriate dose levels to avoid any accidental adverse effects of furazolidone, and for optimum functioning of the liver to enhance the productivity of broilers.

CHAPTER VI

SUMMARY

The toxic effects of furazolidone, a nitrofuran antimicrobial agent, at 150 mg/kg b.wt., were studied in male broiler chicks, and BD and silymarin were evaluated for their hepatoprotective and antioxidant properties. A total of one hundred and twenty, male Vencob broiler chicks were randomly divided into six groups, consisting of twenty each. Group 1 served as vehicle control. Groups 2, 5 and 6 were given furazolidone alone, while groups 3 and 4 were given Fz + BD and Fz + silymarin respectively, up to 4 weeks, and thereafter groups 3 and 5 were treated with BD, and groups 4 and 6 with silymarin, till the end of the experiment. Group 2 was left without any drug treatment from 4^{th} week onwards and was maintained on vehicle treatment until 6^{th} week, to study the natural recovery capacity of the intoxicated birds.

The experiment was carried out for six weeks and at the end of 4^{th} week, 5 birds from each group were selected randomly and sacrificed to study the levels of lipid peroxides and for histopathological studies. Remaining birds were sacrificed at the end of the experiment. Body weights were taken at weekly intervals and the birds were observed for overt clinical signs, if any.

Blood and serum were collected at weekly intervals (2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} weeks) to assess the erythrocyte antioxidant enzyme profile and serum biochemical profile. At 4^{th} and 6th weeks, livers were collected from the sacrificed birds to estimate the levels of lipid peroxides, and liver, heart and kidney samples were collected for histopathological study.

Body weights and feed efficiency were significantly ($p<0.05$) reduced in the toxic groups 2, 5 and 6 on 3^{rd} and 4^{th} weeks when compared to the remaining groups. These effects might be due to anorexigenic effect of the toxin. However, BD and silymarin (groups 3 and 4) could protect the birds from these adverse effects. Group 5 and 6 were also able to correct these unwanted effects of furazolidone by 6^{th} week.

The erythrocyte antioxidant enzyme (GSH-R and GPx) activities, though initially were elevated in groups 2, 5 and 6 at 3^{rd} week, probably as an adaptive mechanism to counteract the toxic free radicals, later on these activities decreased significantly ($p<0.05$) as compared to groups 1,3 and 4. Following treatment with BD and silymarin, the activity of these enzymes in group 5 and 6 revived to normal but group 2 could not.

The mean ALT, AST and LDH levels showed a significant ($p<0.05$)

elevation in toxic groups 2, 5 and 6 at 3rd and 4th weeks, but following treatment with BD and silymarin, these levels in groups 5 and 6 revived to normal. The elevation of these enzymes would have occurred from toxic cellular changes in liver, heart and kidney.

The mean uric acid levels were elevated significantly ($p<0.05$) in furazolidone-fed groups 2, 5 and 6 at 3rd and 4th week as compared to groups 1, 3 and 4, suggesting renal damage. However, BD and silymarin could significantly ($p<0.05$) correct these changes.

Hypocholesterolemia, observed in groups 2, 5 and 6 may have resulted from inanition. A decrease in mean HDL levels was also seen in these groups. However, BD and silymarin could significantly ($p<0.05$) elevate the levels of HDLs by the end of the experiment.

Hypoproteinemia and hypoalbuminemia were observed in groups 2, 5 and 6 at 3rd and 4th weeks, and in groups 3 and 4, no such changes were evident when compared to control group. These values suggest liver and kidney damage due to furazolidone.

The hematological profile revealed a significant ($p<0.05$) fall in the

levels of Hb% and TLC while there was a slight elevation in the levels of PCV and MCV. No significant ($p<0.05$) change was observed in the levels of TEC, MCH and MCHC. This suggests that furazolidone toxicity resulted in macrocytic anemia.

A significant ($p<0.05$) increase in the level of LPO was manifested in groups 2, 5 and 6 at week 4. These levels at week 6 showed no significant ($p<0.05$) change in all the groups except group 2.

Clinically, the birds in groups 1, 3 and 4 were normal and active during the entire experimental period. Birds of group 2, 5 and 6 manifested signs of nervous derangement viz. leg weakness, incoordination, inability to walk, lethargy, decreased feed and water intake and yellow mucous droppings.

The organ-somatic indices of liver, heart and kidney taken at weeks 4 and 6, revealed significant ($p<0.05$) enlargement of liver and heart in groups 2, 5 and 6 as compared to that of groups 1, 3 and 4. At week 6, organ-somatic indices did not reveal any significant alterations.

Gross findings observed on necropsy of toxin-fed birds (groups 2, 5 and 6) showed pallor of all organs and muscles, necrotic foci on heart and

liver, petechial hemorrhages on heart, hydropericardium and catarrhal changes in the intestines. Necropsy of birds of groups 1, 3 and 4 did not reveal any lesions of pathological significance at week 4.

Histopathology of liver, heart and kidney of groups 2, 5 and 6 revealed significant degenerative changes while those of groups 3 and 4 showed only mild changes. At week 6 in groups 5 and 6, histology of these organs showed only mild changes while that of groups 1, 3 and 4 were normal. But degenerative changes persisted in those of group 2.

Thus, it is evident from the present study that BD and silymarin could effectively protect as well as correct furazolidone-induced hepatotoxicity and oxidative damage in broilers. However, silymarin accomplished better hepatoprotection compared to BD.

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APPENDIX

Reagents or Solutions

(a) Phosphate buffer (0.1 M)

Solution X: Dissolved 2.722 g of potassium dihydrogen phosphate in 200 ml Distilled water (0.1 M).

Solution Y: Dissolved 1.741 g of dipotassium hydrogen phosphate in 100 ml of distilled water (0.1 M).

Poured 100 ml of solution Y into a beaker and titrated against solution X to a desired pH. Stored the buffer at 4^{o}C.

(b) Frozen RBC

Red blood cells were separated from EDTA added blood, washed with 0.9% saline, diluted with an equal volume of water and frozen at -20^{o}C overnight.

(c) Haemolysate

RBC (frozen) : 200 µl

0.1 M phosphate buffer (pH 7.4)	:	3 ml

Mixed well and centrifuged.

(d) 50 mM oxidized glutathione (GSSG) solution

Oxidized glutathione	:	155 mg (Mol. Wt.: 612.64)
Distilled water	:	50 ml
0.8 M NaOH	:	60 µl.

Mixed well and placed in an ice bath. Prepared fresh daily.

(**e) 250 uM Flavin adenine dinucleotide (FAD**)

FAD (disodium salt) : 1.0 mg (Mol. Wt: 829.52)

Distilled water	:	5 ml

Mixed well and placed immediately in dark. Prepared fresh daily.

(f) 4 mM NADPH

NADPH	:	33.2 mg (Mol.Wt: 833.4)
1% sodium bicarbonate	:	10 ml

(g) 80 mM EDTA

Dipotassium EDTA	:	3.2 mg (Mol. Wt: 404.47)
Distilled water	:	100 ml

(h) 0.1 M sodium bicarbonate

Sodium bicarbonate	:	8.4 g (Mol. Wt: 106.00)
Distilled water	:	1 cc

Stored at 4^{o}C and prepared fresh for every 14 days.

(i) 0.2 mM NADPH

NADPH : 1.67 mg

Distilled water : 10 ml

Mixed well and placed in an ice bath.

(j) 50 mM Reduced glutathione

Reduced glutathione : 153 mg

Distilled water : 10 ml

Mixed well and placed in an ice bath.

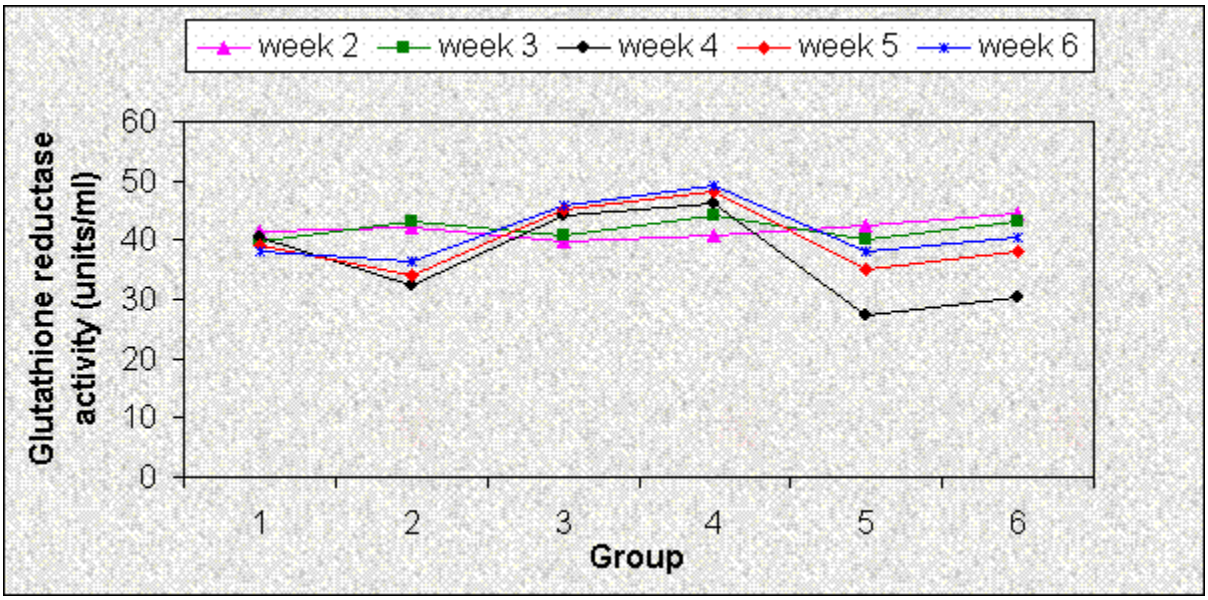

Fig. 11 : Glutathione reductase activity (units /ml) in different groups

Table 8: Glutathione reductase activity (units/ml) in different groups

	GROUP	WEEK				
		2	3	4	5	6
1	Control	41.37±0.42^{aA}	39.81±0.61^{aA}	40.43±0.32^{bA}	39.17±0.36^{aA}	38.25±0.3
2	Fz control (15-28d)	42.23±0.31^{aB}	43.13±0.59^{aB}	32.25±0.76^{aA}	34.00±0.52^{aA}	36.52±0.5
3	Fz+BD (15-28d) and BD (29-42d)	39.90±0.39^{aA}	40.85±0.41^{aAB}	44.13±0.27^{bcABC}	45.21±0.25^{bBC}	45.75±0.31
4	Fz+Silymarin (15-28d) and Silymarin (29-42d)	40.64±0.32^{aA}	44.24±0.45^{aAB}	46.27±0.39^{cBC}	48.33±0.29^{bBC}	49.1±0.52
5	Fz (15-28d)+ BD (29-42d)	42.48±0.42^{aC}	40.15±0.36^{aC}	27.22±0.60^{aA}	35.00±0.33^{aB}	38.25±0.66
6	Fz (15-28d)+ Silymarin (29-42d)	44.42±0.32^{aC}	43.10±0.41^{aC}	30.45±0.44^{aA}	38.25±0.38^{aB}	40.37±0.31

Values are Mean ± S.E. n=6 Two Way ANOVA
Means with different superscripts are statistically different ($p<0.05$)
Capital alphabets - Horizontal comparision (within group)

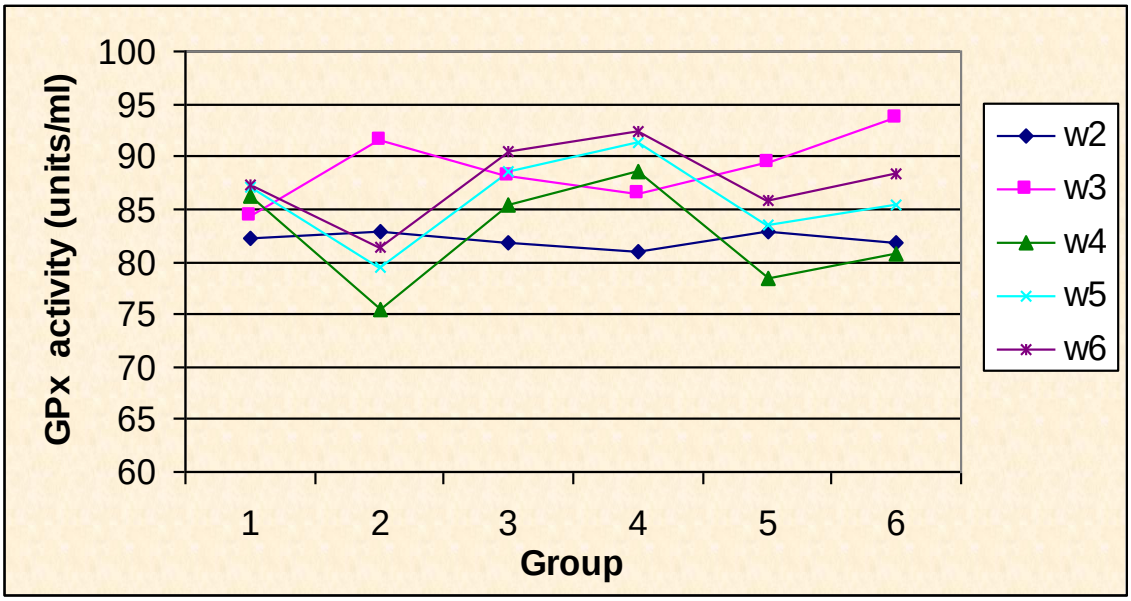

Fig 10: GPx activity (units/ml) in different groups

Table 7: Glutathione peroxidase (GPx) activity (units/ml) in different groups

	GROUP	WEEK				
		2	3	4	5	6
1	Control	82.13±0.52^{aA}	84.24±0.26^{aA}	86.19±0.32^{cA}	87.07±0.51^{bA}	87.33±0.
2	Fz control (15-28d)	82.84±0.64^{aB}	91.54±0.42^{bcC}	75.46±0.38^{aA}	79.65±0.35^{aB}	81.32±0
3	Fz+BD (15-28d) and BD (29-42d)	81.87±0.62^{aA}	88.12±0.42^{aBC}	85.39±1.59^{cAB}	88.54±0.32^{bBC}	90.45±0
4	Fz+Silymarin (15-28d) and Silymarin (29-42d)	80.95±0.38^{aA}	86.49±0.34^{aB}	88.53±0.35^{cBC}	91.32±0.42^{bC}	92.45±0
5	Fz (15-28d)+ BD (29-42d)	82.93±0.48^{aB}	89.46±0.40^{bcC}	78.37±0.46^{abA}	83.57±0.29^{aB}	85.87±0.
6	Fz (15-28d)+ Silymarin (29-42d)	81.98±0.60^{aAB}	93.65±0.34^{cD}	80.66±0.43^{bA}	85.45±0.31^{bBC}	88.45±0.

Values are Mean ± S.E. n=6 Two Way ANOVA
Means with different superscripts are statistically different ($p<0.05$)
Capital alphabets - Horizontal comparision (within group)

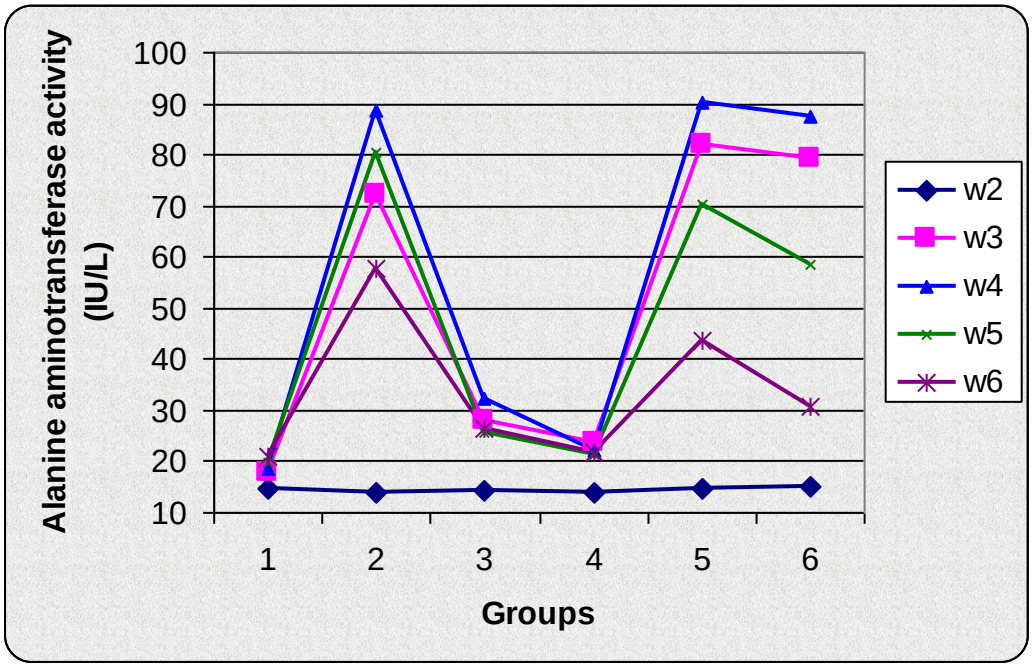

Fig 12: ALT activity (IU/L) in different groups

Table 9: Alanine aminotransferase (ALT) activity (IU/L) in different groups

	GROUP	WEEK				
		2	3	4	5	6
1	Control	14.79±0.55^{aA}	17.70±0.46^{aA}	18.56±0.37^{aA}	19.84±0.43^{aA}	21.06±0
2	Fz control (15-28d)	13.73±0.32^{aA}	72.33±1.03^{bB}	88.59±1.77^{bB}	80.35±1.24^{bB}	57.65±1
3	Fz+BD (15-28d) and BD (29-42d)	14.48±0.51^{aA}	27.92±0.85^{aA}	33.32±0.68^{aA}	25.69±0.93^{aA}	26.41±0
4	Fz+Silymarin (15-28d) and Silymarin (29-42d)	13.81±0.61^{aA}	23.57±0.59^{aA}	22.23±0.63^{aA}	21.28±0.58^{aA}	21.77±0
5	Fz (15-28d)+ BD (29-42d)	14.60±0.59^{aA}	82.14±1.85^{bCD}	90.36±1.27^{bD}	70.23±1.13^{bC}	43.52±1
6	Fz (15-28d)+ Silymarin (29-42d)	15.08±0.77^{aA}	79.37±0.98^{bC}	87.44±1.11^{bC}	58.64±1.40^{bB}	30.83±1

Values are Mean ± S.E. n=8 Two Way ANOVA
Means with different superscripts are statistically different ($p<0.05$)
Capital alphabets - Horizontal comparision (within group)

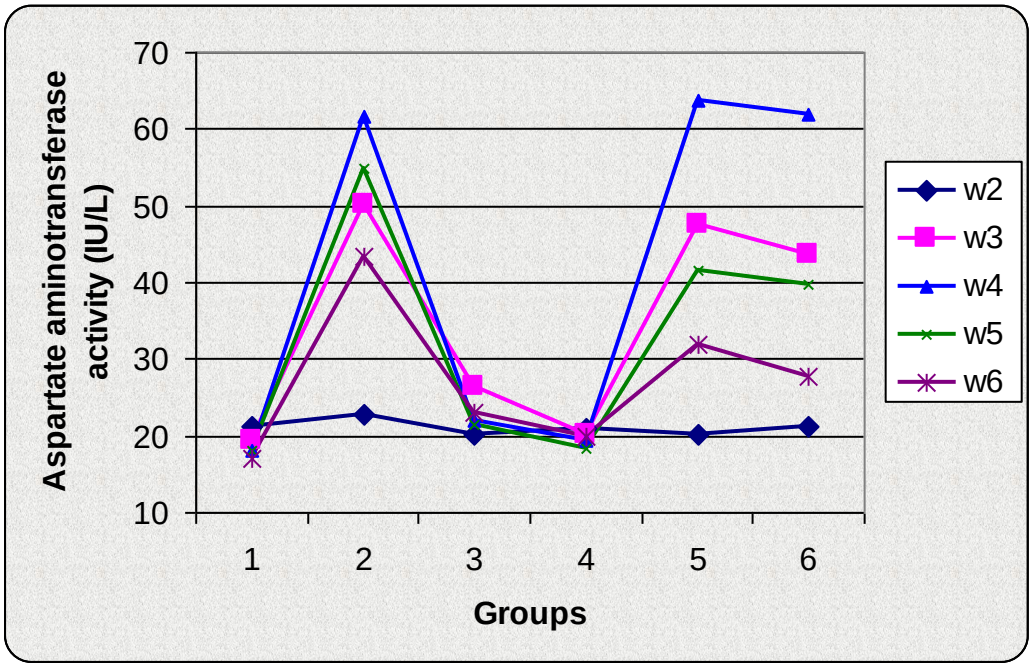

Fig 13: AST activity (IU/L) in different groups

Table 10: Aspartate aminotransferase (ALT) activity (IU/L) in different groups

	GROUP	WEEK				
		2	3	4	5	6
1	Control	21.31±0.30^{aA}	19.51±0.27^{aA}	18.04±0.16^{aA}	18.20±0.41^{aA}	17.06±0.23^{aA}
2	Fz control (15-28d)	22.85±0.43^{aA}	50.30±0.53^{cBC}	61.57±0.39^{bD}	54.99±0.46^{cCD}	43.41±0.45^{cB}
3	Fz+BD (15-28d) and BD (29-42d)	20.12±0.34^{aA}	26.45±0.50^{aA}	21.90±0.36^{aA}	21.37±0.29^{aA}	22.97±0.29^{abA}
4	Fz+Silymarin (15-28d) and Silymarin (29-42d)	20.91±0.49^{aA}	20.25±0.67^{aA}	19.27±0.62^{aA}	18.27±0.44^{aA}	19.88±0.38^{aA}
5	Fz (15-28d)+ BD (29-42d)	20.28±0.55^{aA}	47.57±0.61^{bC}	63.67±0.43^{bD}	41.63±0.56^{bBC}	32.02±0.48^{bB}
6	Fz (15-28d)+ Silymarin (29-42d)	21.34±0.49^{aA}	43.64±0.67^{bB}	61.81±0.76^{bC}	39.74±0.32^{bB}	27.78±0.45^{bA}

Values are Mean ± S.E. n=8 Two Way ANOVA
Means with different superscripts are statistically different ($p<0.05$)
Capital alphabets - Horizontal comparision (within group)

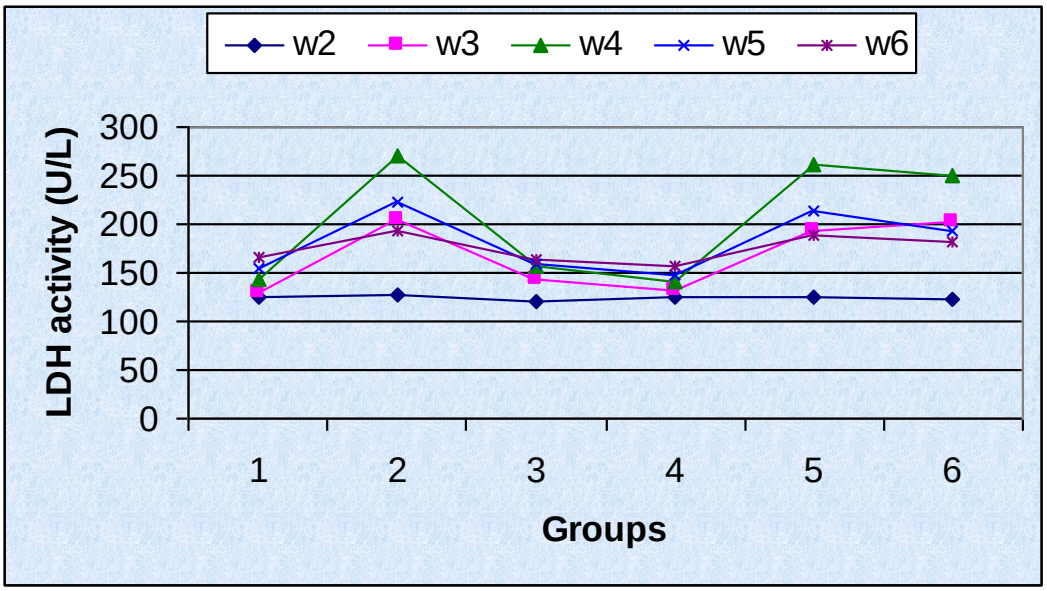

Fig 14: LDH activity (U/L) in different groups

Table 11: Lactate Dehydrogenase (LDH) activity (U/L) in different groups

	GROUP	WEEK				
		2	3	4	5	6
1	Control	124.07±0.84^{aA}	130.18±1.17^{aA}	143.42±1.14^{aA}	153.56±1.54^{aB}	165.71±1.04^{aB}
2	Fz control (15-28d)	127.51±1.22^{aA}	204.85±1.32^{bBC}	269.92±0.82^{bD}	221.63±2.23^{cC}	192.28±1.77^{bB}
3	Fz+BD (15-28d) and BD (29-42d)	120.58±1.14^{aA}	142.19±1.17^{aAB}	155.85±1.42^{aB}	159.53±1.29^{aB}	163.06±1.54^{aB}
4	Fz+Silymarin (15-28d) and Silymarin (29-42d)	125.97±1.19^{aA}	131.18±1.32^{aA}	140.66±0.55^{aA}	147.56±1.45^{aA}	156.26±0.99^{aA}
5	Fz (15-28d)+ BD (29-42d)	125.97±1.38^{aA}	194.19±12.45^{bB}	261.73±1.47^{bC}	214.76±2.55^{bcB}	189.53±1.16^{bB}
6	Fz (15-28d)+ Silymarin (29-42d)	122.31±0.87^{aA}	203.24±2.19^{bB}	250.52±2.19^{bC}	193.36±1.55^{bcB}	182.62±0.78^{bB}

Values are Mean ± S.E. n=8 Two Way ANOVA
Means with different superscripts are statistically different ($p<0.05$)
Capital alphabets - Horizontal comparision (within group)

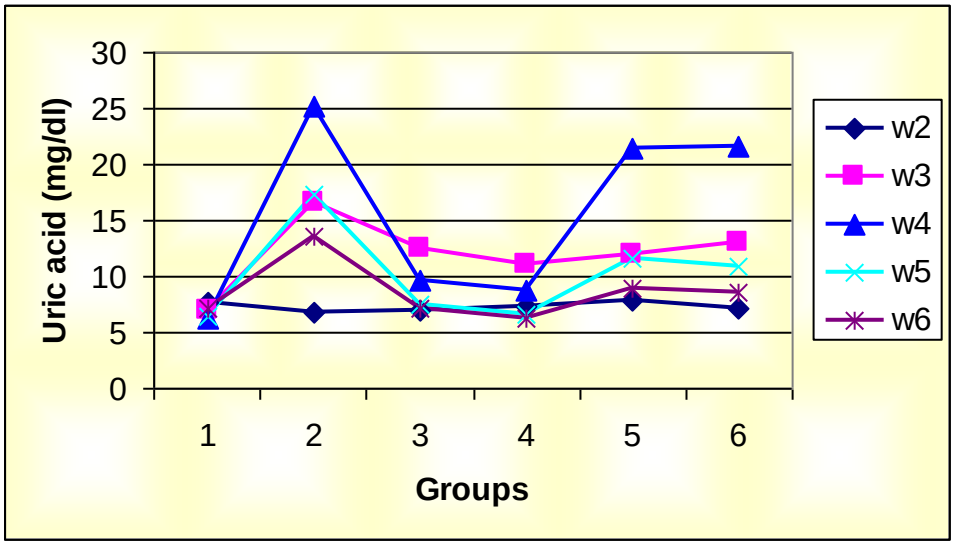

Fig 15: Serum Uric Acid (mg/dl) in different groups

Table 13: Serum Uric Acid (mg/dl) levels in different groups

	GROUP	WEEK				
		2	3	4	5	6
1	Control	7.74 ± 0.25^{aA}	6.88 ± 0.25^{aA}	6.17 ± 0.20^{aA}	6.38 ± 0.23^{aA}	7.19 ± 0.28^{aA}
2	Fz control (15-28d)	6.85 ± 0.17^{aA}	16.69 ± 0.26^{bB}	25.19 ± 0.39^{bC}	17.24 ± 0.48^{cB}	13.56 ± 0.52^{bB}
3	Fz+BD (15-28d) and BD (29-42d)	6.91 ± 0.34^{aA}	12.43 ± 0.46^{bB}	9.67 ± 0.30^{aAB}	7.57 ± 0.36^{abA}	7.15 ± 0.19^{aA}
4	Fz+Silymarin (15-28d) and Silymarin (29-42d)	7.34 ± 0.38^{aAB}	11.15 ± 0.32^{abB}	8.79 ± 0.12^{aA}	6.64 ± 0.33^{abA}	6.31 ± 0.28^{aA}
5	Fz (15-28d)+ BD (29-42d)	7.86 ± 0.16^{aAB}	11.94 ± 0.27^{bC}	21.48 ± 0.35^{bD}	11.62 ± 0.62^{bBC}	8.87 ± 0.31^{abAB}
6	Fz (15-28d)+ Silymarin (29-42d)	7.14 ± 0.26^{aA}	13.05 ± 0.33^{bB}	21.59 ± 0.57^{bC}	10.96 ± 0.55^{bAB}	8.55 ± 0.35^{abA}

Values are Mean ± S.E. n=8 Two Way ANOVA
Means with different superscripts are statistically different ($p<0.05$)
Capital alphabets - Horizontal comparision (within group)

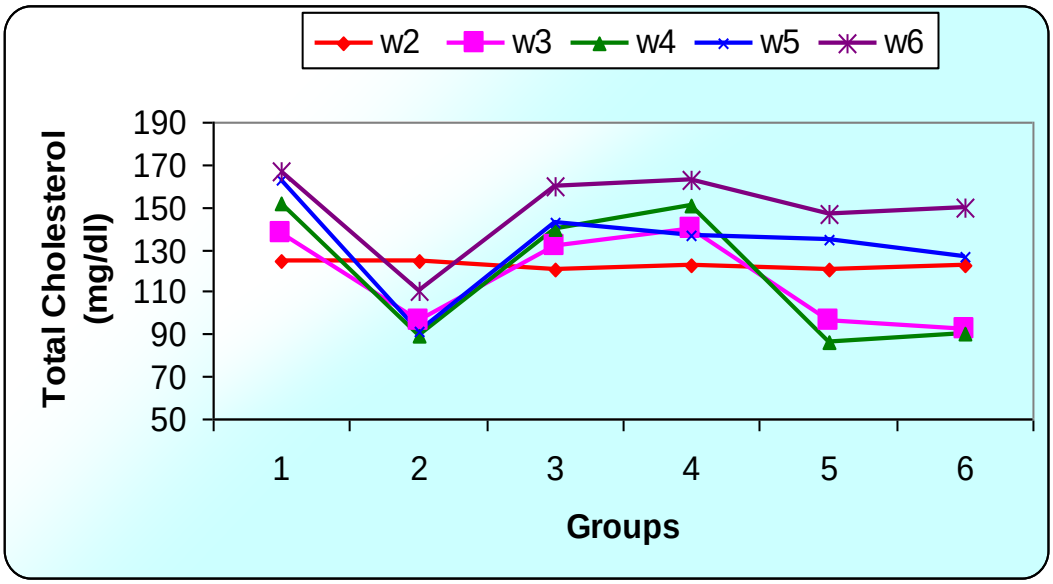

Fig 16: Total Cholesterol (mg/dl) in different groups

Table 14: Serum Total Cholesterol (mg/dl) levels in different groups

	GROUP	WEEK				
		2	3	4	5	6
1	Control	124.29 ± 1.23^{aA}	137.48 ± 1.18^{bB}	151.91 ± 0.89^{bCD}	162.46 ± 2.05^{aA}	166.96 ± 1.91^{aA}
2	Fz control (15-28d)	124.99 ± 1.30^{aB}	96.52 ± 1.45^{aAB}	89.54 ± 2.01^{bA}	91.00 ± 1.01^{cB}	110.64 ± 1.58^{bB}
3	Fz+BD (15-28d) and BD (29-42d)	120.54 ± 1.29^{aA}	132.02 ± 0.96^{bAB}	139.20 ± 0.94^{aAB}	142.49 ± 0.98^{abA}	159.35 ± 0.77^{aA}
4	Fz+Silymarin (15-28d) & Silymarin (29-42d)	122.11 ± 1.48^{aAA}	139.30 ± 1.62^{bA}	150.52 ± 1.83^{aA}	136.75 ± 1.41^{abA}	162.53 ± 1.20^{aA}
5	Fz (15-28d)+ BD (29-42d)	120.92 ± 1.31^{aB}	96.68 ± 2.70^{aA}	86.35 ± 2.72^{bD}	134.12 ± 1.79^{bBC}	146.45 ± 3.54^{ab}
6	Fz (15-28d)+ Silymarin (29-42d)	122.75 ± 1.83^{aB}	92.47 ± 1.96^{aA}	90.24 ± 1.36^{bC}	126.82 ± 1.14^{bAB}	150.00 ± 1.81^{abA}

Values are Mean ± S.E. n=8 Two Way ANOVA
Means with different superscripts are statistically different ($p<0.05$)
Capital alphabets - Horizontal comparision (within group)

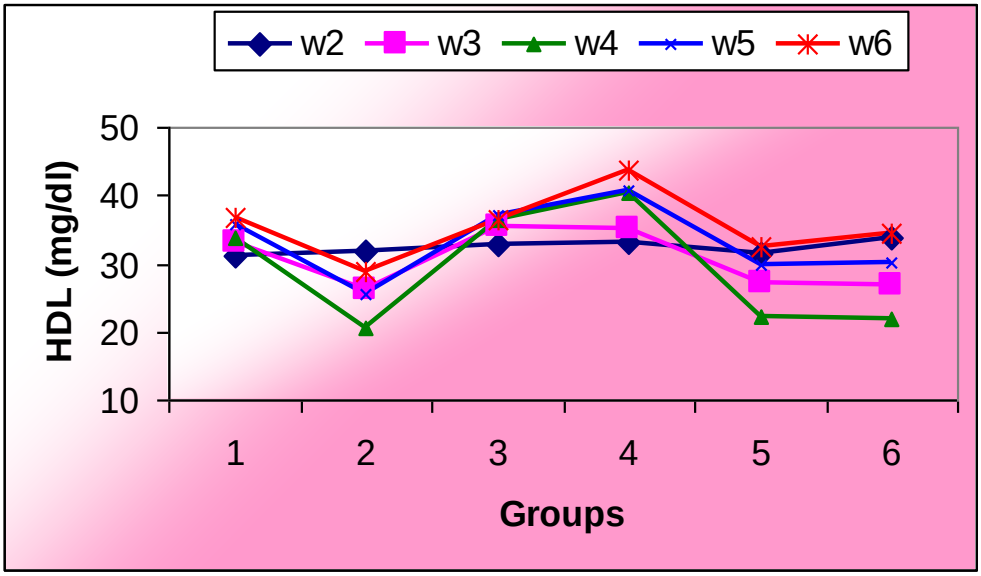

Fig 17: HDL (mg/dl) in different groups

Table 15: HDL (mg/dl) levels in different groups

	GROUP	WEEK				
		2	3	4	5	6
1	Control	31.00 ± 1.04^{aA}	33.23 ± 0.81^{bAB}	33.86 ± 0.87^{bAB}	35.90 ± 0.69^{cB}	36.90 ± 0.50^{bB}
2	Fz control (15-28d)	31.79 ± 1.04^{aC}	26.14 ± 0.52^{aB}	20.51 ± 0.80^{aA}	25.56 ± 0.76^{aB}	28.81 ± 0.83^{aBC}
3	Fz+BD (15-28d) and BD (29-42d)	32.97 ± 0.99^{aA}	35.54 ± 0.65^{bAB}	36.46 ± 0.49^{bcAB}	37.26 ± 0.46^{cdB}	36.47 ± 0.79^{bAB}
4	Fz+Silymarin (15-28d) and Silymarin (29-42d)	33.21 ± 0.97^{aA}	35.08 ± 0.79^{bA}	40.39 ± 0.80^{cB}	40.76 ± 0.84^{dB}	43.71 ± 0.97^{cB}
5	Fz (15-28d)+ BD (29-42d)	31.36 ± 0.71^{aC}	27.32 ± 0.80^{aB}	22.29 ± 0.70^{aA}	29.86 ± 1.32^{dBC}	32.42 ± 0.68^{aC}
6	Fz (15-28d)+ Silymarin (29-42d)	33.94 ± 0.76^{aCD}	26.79 ± 0.98^{aB}	21.97 ± 1.08^{aA}	30.06 ± 1.04^{bC}	34.58 ± 0.90^{bD}

Values are Mean ± S.E. n=8 Two Way ANOVA
Means with different superscripts are statistically different ($p<0.05$)
Capital alphabets - Horizontal comparision (within group)

Table 1: Experimental studies on the hepatoprotective action of silymarin and

silibinin in xenobiotic intoxication

Agent	Experimental model	Silymarin or silibinin action	References
CCl^4	Acute intoxication in mice and rats	Prevention of lipid peroxidation and hepatotoxicity	Letteron *et al*, 1990 Muriel & Mourelle 1990
	Cirrhosis in rats	Protection	Mourelle & Franco, 1991
	Chronic intoxication in rats	Prevention of chronic liver damage	Mourelle *et al*, 1989 Muriel & Mourelle, 1990 Favari & Perez Alvarex, 1997
Ethanol	Acute intoxication in rats	Neutralisation of lipid peroxidation	Valenzuela *et al*, 1985
	Chronic intoxication in rats	Reduction in liver alterations	Valenzuela & Garrido, 1994 Campos *et al*, 1989 Platt & Shnorr, 1971
Thioacetamide	Acute intoxication in rats	Antihepatotoxic effects Hepatoprotection	Schriever *et al*, 1973
Galactosamine	Acute hepatitis in rats	Protective effects	Barbarino *et al*, 1981
	Perfused rat hepatocytes	Inhibition of lipid peroxidation	Farghali *et al*, 2000
	Experimental hepatitis in rats	Inhibition of toxic effects on protein synthesis	Tyutyulkova *et al*, 1983
Paracetamol	Acute intoxication in mice/rats	Protective effects, reduction in lipid peroxidation and glutathione depletion	Muriel *et al*, 1992 Campos *et al*, 1989
Aflatoxin	Subacute intoxication in broilers	Protective effects	Sujatha *et al*, 2003

Table 2: Some medicinal plants possessing hepatoprotective activity:

S.No.	Plant	Extract	Damaging agent (Model)	Mode of action
1	Acacia catechu (Jayasekhar *et al*, 1997)	Ethyl acetate extract of pale catechu	CCl_4 (albino mice)	Decreased SGOT, SGPT, ALP and total bilirubin
2	Andrographis paniculata (Trivedi and Rawal, 1998)	Aqueous extract of leaves	BHC (albino mice)	Decreased SGPT, SGOT and ALP; Increased protein levels
3	Azadirachta indica (Sangeeta Bhanwra *et al*, 2000)	Aqueous extract of leaves	Paracetamol (rats)	Decreased AST, ALT and GGT.
	(Kale *et al*, 2003)	Aqueous extract of leaves	Antituberculosis drugs (Isoniazid + Rifampicin + Pyrazinamide)	Decreased ALT.
4.	Citrullus colocynthis (Mukherjee *et al*, 2001)	Aqueous root extract an alcoholic fruit extract	CCl_4 (albino rats)	Decreased serum SGPT, SGOT, ALP and bilirubin
5	Curculigo orchioides (Venukumar and Latha, 2002)	Methanolic extract of rhizomes	CCl_4 (rats)	Decreased AST, ALT, ALP, GGT, total lipids and cholesterol; Increased total proteins
6	Ginkgo biloba (Ashok Shenoy *et al*, 2001)	Dry extract	CCl_4 (Rats)	Decreased ALT, AST, ALP, lipid peroxidation; Increased total proteins, albumin, glutathione
7	Sida rhombifolia Linn (Kurma and Mishra, 1997)	• Powdered aerial parts and roots • Methanolic extract • Aqueous extract	CCl_4 Paracetamol Rifampicin	Decreased SGOT, SGPT, ALP, serum total bilirubin and serum direct bilirubin.
8	Tephrosia purpurea (Sree Rama Murthy and Srinivasan, 1993)	Powder of aerial parts	D-galactosamine (acute) CCl_4 (chronic) (Albino rats)	Decreased SGOT, SGPT and bilirubin

Table 16: Protein profile of different groups of broiler chicks

	Group	Week				
		2	3	4	5	6
Total Proteins (gm/dl)	**1**	5.62 ± 0.33^{aA}	5.64 ± 0.29^{bA}	5.56 ± 0.27^{dA}	5.90 ± 0.18^{cA}	5.78 ± 0.18^{bA}
	2	5.58 ± 0.33^{aD}	3.47 ± 0.28^{aB}	2.34 ± 0.22^{aA}	3.33 ± 0.16^{aB}	4.52 ± 0.32^{aC}
	3	5.22 ± 0.37^{aC}	4.02 ± 0.18^{aA}	4.21 ± 0.08^{cB}	4.88 ± 0.07^{bBC}	5.36 ± 0.26^{bC}
	4	5.16 ± 0.31^{aA}	5.17 ± 0.15^{aA}	5.11 ± 0.22^{dA}	5.29 ± 0.14^{bAB}	5.87 ± 0.21^{bB}
	5	5.46 ± 0.35^{aC}	3.92 ± 0.16^{aB}	3.22 ± 0.16^{bA}	4.09 ± 0.20^{abB}	5.45 ± 0.24^{bC}
	6	5.66 ± 0.33^{aC}	3.87 ± 0.17^{aA}	3.28 ± 0.19^{bA}	4.75 ± 0.25^{bB}	6.20 ± 0.18^{bC}
Albumin (gm/dl)	**1**	3.76 ± 0.12^{aA}	4.07 ± 0.08^{bA}	4.07 ± 0.14^{dA}	3.72 ± 0.19^{bcA}	3.93 ± 0.17^{bcA}
	2	3.63 ± 0.12^{aC}	2.56 ± 0.22^{aB}	1.32 ± 0.13^{aAB}	2.56 ± 0.11^{aB}	2.80 ± 0.11^{aB}
	3	3.45 ± 0.13^{aB}	2.65 ± 0.12^{aA}	3.00 ± 0.11^{cAB}	3.50 ± 0.16^{bB}	3.45 ± 0.11^{bB}
	4	3.60 ± 0.16^{aA}	3.52 ± 0.18^{bA}	3.73 ± 0.14^{dA}	3.92 ± 0.08^{cAB}	4.30 ± 0.15^{cB}
	5	3.57 ± 0.14^{aC}	2.43 ± 0.17^{aA}	2.05 ± 0.15^{bA}	3.00 ± 0.13^{aAB}	3.58 ± 0.10^{bC}
	6	3.91 ± 0.19^{aC}	2.50 ± 0.13^{aA}	2.35 ± 0.14^{bA}	3.35 ± 0.13^{bcB}	3.86 ± 0.04^{bcBC}
Globulins (gm/dl)	**1**	1.85 ± 0.36^{aB}	1.58 ± 0.30^{bAB}	1.49 ± 0.32^{bA}	2.18 ± 0.33^{cC}	1.85 ± 0.16^{aB}
	2	1.95 ± 0.32^{aB}	0.91 ± 0.09^{aA}	1.02 ± 0.12^{aA}	0.77 ± 0.18^{aA}	1.72 ± 0.29^{aB}
	3	1.77 ± 0.27^{aB}	1.37 ± 0.16^{bA}	1.21 ± 0.15^{abA}	1.38 ± 0.16^{bA}	1.91 ± 0.21^{aB}
	4	1.56 ± 0.36^{aA}	1.65 ± 0.25^{bA}	1.39 ± 0.26^{bA}	1.37 ± 0.21^{bA}	1.58 ± 0.31^{aA}
	5	1.89 ± 0.38^{aC}	1.48 ± 0.26^{bB}	1.17 ± 0.16^{abAB}	1.09 ± 0.10^{abA}	1.87 ± 0.18^{aC}
	6	1.75 ± 0.37^{aB}	1.37 ± 0.09^{bB}	0.93 ± 0.11^{aA}	1.40 ± 0.28^{bB}	2.34 ± 0.21^{bC}
A/G Ratio	**1**	2.03 ± 0.53^{aAB}	2.58 ± 0.74^{bBC}	2.73 ± 0.91^{bC}	1.71 ± 0.42^{aA}	2.12 ± 0.32^{aA}
	2	1.86 ± 0.43^{aB}	2.81 ± 0.42^{bC}	1.29 ± 0.16^{aA}	3.31 ± 1.07^{cD}	1.63 ± 0.31^{aAB}
	3	1.94 ± 0.92^{aA}	1.94 ± 0.75^{aA}	2.47 ± 0.58^{bAB}	2.53 ± 0.89^{bB}	1.81 ± 0.36^{aA}
	4	2.31 ± 0.48^{aAB}	2.14 ± 0.39^{aA}	2.69 ± 0.82^{bBC}	2.87 ± 0.74^{bcC}	2.73 ± 0.94^{bBC}
	5	1.88 ± 0.61^{aA}	1.64 ± 0.65^{aA}	1.75 ± 0.50^{aA}	2.76 ± 0.46^{bcB}	1.92 ± 0.14^{aA}
	6	2.23 ± 0.92^{aAB}	1.82 ± 0.51^{aA}	2.54 ± 0.47^{bB}	2.39 ± 0.87^{bB}	1.65 ± 0.18^{aA}

Values are Mean ± S.E. (n=5) Two way ANOVA
Means with different superscripts are statistically different.
Capital alphabets – Horizontal comparision (within group).

Table 17: Hematological profile of different groups of broiler chicks

	Group	Week				
		2	3	4	5	6
Hb %	**1**	8.28 ± 0.10^{aA}	8.36 ± 0.17^{bA}	8.52 ± 0.08^{bA}	9.32 ± 0.10^{bB}	9.42 ± 0.07^{bB}
	2	8.24 ± 0.13^{aB}	7.50 ± 0.04^{aA}	7.36 ± 0.07^{aA}	7.98 ± 0.08^{aAB}	8.16 ± 0.07^{aB}
	3	8.08 ± 0.10^{aA}	8.58 ± 0.09^{bA}	8.60 ± 0.09^{bA}	8.16 ± 0.12^{aA}	9.64 ± 0.12^{cB}
	4	8.28 ± 0.15^{aB}	9.48 ± 0.10^{cC}	9.60 ± 0.09^{cC}	7.52 ± 0.15^{bB}	8.92 ± 0.36^{bBC}
	5	8.24 ± 0.17^{aB}	7.40 ± 0.06^{aA}	7.52 ± 0.10^{aA}	8.16 ± 0.20^{aB}	8.32 ± 0.10^{abB}
	6	8.16 ± 0.12^{aB}	7.44 ± 0.12^{aA}	7.28 ± 0.10^{aA}	7.68 ± 0.08^{aAB}	8.36 ± 0.12^{abB}
PCV %	**1**	36.64 ± 1.55^{aAB}	35.54 ± 1.05^{aA}	36.01 ± 0.59^{aA}	39.95 ± 0.91^{aA}	28.53 ± 0.52^{bBC}
	2	34.52 ± 1.15^{aB}	26.87 ± 0.34^{aA}	26.42 ± 0.50^{aAB}	30.96 ± 0.36^{aA}	30.33 ± 0.68^{aB}
	3	31.79 ± 0.89^{abA}	31.10 ± 0.43^{abAB}	30.67 ± 0.16^{bAB}	34.20 ± 0.78^{aB}	31.20 ± 1.77^{bAB}
	4	34.18 ± 1.01^{bA}	36.07 ± 0.84^{bAB}	36.65 ± 0.74^{bAB}	39.59 ± 1.96^{aA}	26.34 ± 1.19^{cBC}
	5	33.35 ± 0.93^{aB}	26.80 ± 0.26^{aA}	27.05 ± 0.28^{aAB}	22.00 ± 0.31^{cC}	26.03 ± 0.78^{bB}
	6	31.80 ± 0.82^{aB}	26.48 ± 0.49^{abB}	26.36 ± 0.50^{abA}	22.61 ± 0.54^{bBC}	27.70 ± 0.59^{bB}
TEC (10^6/ml)	**1**	2.82 ± 0.04^{aA}	2.82 ± 0.07^{aA}	2.78 ± 0.11^{aA}	2.53 ± 0.20^{aA}	2.95 ± 0.08^{aA}
	2	2.67 ± 0.12^{aA}	2.36 ± 0.07^{aA}	1.71 ± 0.04^{aA}	1.75 ± 0.04^{aA}	1.81 ± 0.03^{aA}
	3	2.73 ± 0.06^{aA}	2.66 ± 0.03^{aA}	2.47 ± 0.04^{aA}	2.03 ± 0.21^{aA}	2.64 ± 0.17^{aA}
	4	2.86 ± 0.05^{aA}	2.59 ± 0.05^{aA}	2.20 ± 0.05^{aA}	2.33 ± 0.05^{aA}	2.72 ± 0.08^{aA}
	5	2.67 ± 0.03^{aA}	2.20 ± 0.04^{aA}	1.78 ± 0.04^{aA}	1.86 ± 0.04^{aA}	2.06 ± 0.15^{aA}
	6	2.77 ± 0.08^{aA}	2.26 ± 0.07^{aA}	1.74 ± 0.02^{aA}	2.54 ± 0.02^{aA}	2.17 ± 0.09^{aA}
TLC (10^3/ml)	**1**	27.16 ± 0.67^{aA}	27.82 ± 0.62^{cA}	28.44 ± 0.46^{cA}	29.02 ± 0.34^{cA}	29.18 ± 0.22^{cA}
	2	25.72 ± 0.66^{aC}	21.82 ± 0.57^{aB}	18.54 ± 0.70^{aA}	19.58 ± 0.61^{aAB}	19.84 ± 0.76^{aAB}
	3	28.04 ± 0.39^{aB}	23.62 ± 0.66^{abA}	23.84 ± 0.55^{bA}	25.34 ± 0.90^{bA}	26.40 ± 0.43^{cB}
	4	26.86 ± 0.43^{aAB}	26.00 ± 0.65^{bcA}	27.66 ± 0.47^{cAB}	28.80 ± 0.45^{cB}	28.82 ± 0.80^{cB}
	5	27.72 ± 0.45^{aC}	23.32 ± 1.08^{abB}	19.28 ± 0.92^{aA}	21.46 ± 0.97^{aA}	24.64 ± 0.69^{bB}
	6	26.02 ± 0.46^{aC}	22.96 ± 0.47^{abB}	18.60 ± 0.73^{aA}	21.58 ± 0.62^{aB}	27.60 ± 1.23^{cC}

Values are Mean ± S.E. (n=5) Two way ANOVA
Means with different superscripts are statistically different.
Capital alphabets – Horizontal comparision (within group).

Table 18: Erythrocyte indices of different groups of broiler chicks

	Group	Week				
		2	3	4	5	6
MCV (μ^3)	1	80.43 ± 1.97^{aA}	83.57 ± 1.97^{aA}	85.75 ± 4.21^{aA}	94.31 ± 6.26^{aAB}	112.15 ± 1.88^{abB}
	2	90.80 ± 5.95^{aA}	118.54 ± 3.15^{bB}	163.24 ± 2.45^{cC}	147.88 ± 5.71^{cC}	149.10 ± 5.58^{bcC}
	3	93.59 ± 3.95^{aA}	103.71 ± 2.35^{aAB}	113.54 ± 2.91^{bAB}	121.96 ± 11.16^{bB}	94.28 ± 22.18^{aA}
	4	85.04 ± 1.70^{aA}	101.72 ± 2.57^{aA}	119.49 ± 1.15^{bBC}	82.32 ± 3.59^{abA}	125.78 ± 7.78^{bC}
	5	92.82 ± 3.45^{aA}	125.73 ± 2.78^{bB}	156.80 ± 3.56^{cC}	199.19 ± 2.93^{cD}	159.06 ± 11.45^{cC}
	6	93.19 ± 3.87^{aA}	124.68 ± 2.92^{bB}	158.50 ± 2.01^{cC}	134.28 ± 5.02^{aB}	140.66 ± 7.54^{bB}
MCH (µµg)	1	29.38 ± 0.73^{aA}	29.67 ± 0.88^{aA}	30.80 ± 1.13^{aA}	37.87 ± 3.28^{bB}	31.98 ± 0.68^{aA}
	2	31.07 ± 1.01^{aA}	31.86 ± 0.93^{abA}	43.15 ± 1.28^{bB}	45.70 ± 1.27^{bB}	45.08 ± 0.75^{cB}
	3	29.62 ± 0.54^{aA}	32.23 ± 0.63^{abAB}	34.83 ± 0.98^{aB}	41.39 ± 3.16^{bC}	29.67 ± 0.75^{aA}
	4	29.02 ± 0.65^{aA}	36.66 ± 1.07^{bB}	43.82 ± 1.17^{bC}	32.36 ± 0.90^{aAB}	33.04 ± 2.31^{aAB}
	5	30.87 ± 0.83^{aA}	33.69 ± 0.74^{abA}	42.45 ± 1.36^{bB}	43.84 ± 1.21^{bB}	41.51 ± 3.69^{bcB}
	6	29.53 ± 0.79^{aA}	33.04 ± 1.16^{abA}	41.75 ± 0.57^{bB}	30.28 ± 0.67^{aA}	38.86 ± 1.69^{bB}
MCHC (%)	1	36.64 ± 1.55^{bBC}	35.54 ± 1.05^{cB}	36.01 ± 0.57^{bBC}	39.95 ± 0.91^{bC}	28.53 ± 0.52^{abA}
	2	34.52 ± 1.15^{abB}	26.87 ± 0.34^{aA}	26.42 ± 0.50^{aA}	30.96 ± 0.36^{aAB}	30.33 ± 0.68^{bAB}
	3	31.79 ± 0.89^{aA}	31.10 ± 0.43^{bA}	30.67 ± 0.16^{aA}	34.20 ± 0.78^{bA}	31.20 ± 1.77^{bA}
	4	34.18 ± 1.01^{abB}	36.07 ± 0.84^{cBC}	36.65 ± 0.74^{bBC}	39.59 ± 1.96^{bC}	26.34 ± 1.19^{abA}
	5	33.35 ± 0.93^{abC}	26.80 ± 0.26^{aB}	27.05 ± 0.28^{aB}	22.00 ± 0.31^{aA}	26.03 ± 0.78^{aB}
	6	31.80 ± 0.82^{aC}	26.48 ± 0.49^{aB}	26.36 ± 0.49^{aB}	22.61 ± 0.54^{aA}	27.70 ± 0.59^{abBC}

Values are Mean ± S.E. (n=5) Two way ANOVA
Means with different superscripts are statistically different.
Capital alphabets – Horizontal comparision (within grou