'Patho-morphological, Clinico-pathological and residual effect of induced Levofloxacin toxicity in broiler birds

THESIS

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Sabour, (Bhagalpur), BIHAR
In partial fulfillment of the requirements

FOR THE DEGREE OF

Master of Veterinary Science

IN

(VETERINARY PATHOLOGY)

BY

Sushma Suman

Reg. No. – M/VPP/225/BVC/2014-15

(VETERINARY PATHOLOGY)

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DEPARTMENT OF VETERINARY PATHOLOGY

Bihar Veterinary College, Patna-800014.

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CERTIFICATE-I

This is to certify that the thesis entitled **"PATHO-MORPHOLOGICAL, CLINICO-PATHOLOGICAL AND RESIDUAL EFFECT OF INDUCED LEVOFLOXACIN TOXICITY IN BROILER BIRDS"**submitted in partial fulfillment of requirement for the degree of **Master of Veterinary Science (Veterinary Pathology)** of the faculty of post-Graduate Studies, Bihar Agricultural University, Sabour, Bhagalpur, Bihar is the record of bonafide research carried out by **Dr.Sushma Suman**,**Registration No-M/VPP/225/BVC/2014-15,**under my supervision and guidance. No part of the thesis has been submitted for any other Degree and Diploma.

It is further certified that such help or information received during the course of this investigation and preparation of the thesis have been fully acknowledged.

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We, the undersigned, members of the Advisory Committee of **Dr. Sushma Suman, Registration No- M/VPP/225/BVC/2014-15**, a candidate for the award of degree of Master of Veterinary Science with major in **Veterinary Pathology**, have gone through the manuscript of the thesis and agree that the thesis entitled "**Patho-morphological, Clinico-pathological and residual effect of induced Levofloxacin toxicity in broiler birds"** may be submitted by **Dr. Sushma Suman,** in partial fulfillment of the requirements for the degree.

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Date _____ (Sushma Suman)

Place _____


DEDICATED
TO
MY REVEREND PARENTS
TEACHERS
&
THOSE WHO LOVES ME

TABLE OF CONT

INTRODUCTION

Poultry farming in India, in spite of several constraints, has progressed considerably during last decade.

India has 1.23 billion people and the number is growing every year. The focus is on “Development” meaning Good food, Better health & Living conditions to everyone. (Healthy food at attractive price will be the issue in focus).Egg and chicken are accepted by all communities and available at the most reasonable prices. Poultry is the most organized sector in animal agriculture, worth rupees one lakh carores. The growth is 6-8% in layer and 10-12% in broiler per year against growth of agriculture as a whole that is around 2.5 %.

Egg and chicken were “agriculture produces” few years ago but are “food items” today safe food is very important. Besides maintaining his production efficiency, the producer has to concentrate on the nutritive values, the adulterants contaminants & Toxicants of his produce.

The chicken as a species, differs markedly from mammals because of absence of lymph nodes, presence of bursa of fabricius , and extensive system of air sacs, nucleated erythrocytes and thrombocytes, high body temperature and more rapid clotting of blood, are only a few amongst the many anatomical and physiological peculiarities of the chicken. In addition, avian species also differ strikingly in its biochemistry from that of the mammals.

It has been said that blood is a mirror for observing changes in the body in physiological and pathological in nature. Hence, the importance of haematological study cannot be over ruled in the

diagnosis of poultry disease. More over the blood volume of the poultry were subjected to wide range of variation as age, sex, season, egg production and environmental conditions have been showed in after the composition of blood (Olson 1965).

Fluoroquinolones are synthetic antimicrobial groups, which are widely used both in human and in veterinary medicine. These agents exert their antibacterial effect through the inhibition of DNA Gyrase, interfering with the super coiling of bacterial chromosomal material. They have a broad spectrum of activity against Gram-negative and Gram-Positive bacteria, Mycoplasma spp. and Rickettsia, which is included as resistant to beta-lactam antibiotics and sulphonamides. Chemotherapeutic use of fluoroquinolones (Ciprofloxacin, Norfloxacin, Pefloxacin), the 3rd generation of quinolone started only in the last decade as an effective antibacterial agent. Levofloxacin is the newer generation of quinolone now that is extensively used in poultry industry for treatment of bacterial diseases as it has improved pharmacokinetic and pharmacodynamic properties (Owens and Ambrose 2000). The Safety and efficacy of Levofloxacin are well documented in lower respiratory tract infections, skin & soft tissue infections and urinary tract infections, The safety profile seems advantageous over others Fluoroquinolones and the risk of phototoxicities, hepatotoxicity and neurotoxicity are very low (Norrby 1991).

However, indiscriminate use of levofloxacin in poultry industry has given rise to complications instead of benefits. The adverse effects arising from nonjudicious use of these fluoroquinolones are poorly understood in birds. The resistance of zoonotic bacteria, such as those belonging to the genera Salmonella and Campylobacter should be taken into account and prevented, as

resistant bacteria or resistance genes may be transferred to humans through the consumption of poultry products. The consumption of poultry products containing high fluoroquinolones residue levels is also a hazard for human health due to their adverse effects, including hypersensitivity reactions and intestinal micro flora imbalance, as well as to drug interactions, e.g., they may impair the therapeutic efficacy of other quinolone.

Antibiotics have been used since the 1940s and have led to dramatic reduction in illness and death from infectious diseases. However, according to the federal interagency Task force (FITF) on Antimicrobial Resistance, the extensive use of antimicrobial drug has resulted in drug resistance that threatens to reverse the medical advances of the last seventy years. Since antibiotics have been used widely and for so long, antibiotic resistance has become a major public health threat.

Approximately 80% of the antibiotics used in meat and poultry production. The vast majority is used on health of animals to promote growth, or prevent disease in crowded or unsanitary conditions.

The meat and poultry production industry argues, however, that there is no harm in this. They say for example “animal use contributes little” if anything to the burden of human antibiotic resistance.

There is paucity of information regarding normal blood values in the chicken of different regions. Poultry pathologists are becoming more and more aware of the role of avian haematology as an aid in the diagnosis of many specific and non-specific diseases. It is surprising that work on the nature of haematological alteration of induced levofloxacin toxicity in broiler chicken. This will provide

a better understanding of pathogenesis, pathology and diagnosis of many specific and non-specific diseases of poultry.

Poultry keeping has now crossed its frontiers from being a hobby or kitchen garden activity into an economically viable industry.

The main objectives of present investigations are –

1. Effect of Levofloxacin on growth performance and clinical signs of broiler chicken.
2. Effect of Levofloxacin on Haematological and Biochemical profile of broilers chicken.
3. Effect of Levofloxacin on the Histopathology of liver, lungs, spleen, kidneys, bursa of fabricious, intestine and muscles.
4. Effect of Levofloxacin on the residues in liver and breast muscles.

REVIEW OF LITERATURE

Clinical signs:-

Hooper *et al.* (1985) reported nausea, vomiting, anorexia, headache, drowsiness and insomnia in human received fluoroquinolones treatment. In some patients, there was development of joint swelling or tendonitis. In animals, there was development of crystalluria with therapy of high dosage of fluoroquinolones.

Flanner*et al.* (1990) observed polyuria, depression, poor appetite, polydypsia in African grey parrots treated with enrofloxacin @ 30 mg/kg body wt twice daily for 10 days.

Norrby SR (1991). Examined the adverse effects of quinolones in human. The reaction consisted of the gastrointestinal sign *viz.* nausea, vomiting, and abdominal discomfort and less frequently diarrhoea. He also reported toxicity in the central nervous system, which included insomnia, dizziness, convulsion and anxiety.

Sharma *et al.* (1994).Also reported nausea, vomiting, anorexia, headache, drowsiness and insomnia in human received fluoroquinolones treatment. In some patients, there was development of joint swelling or tendonitis. In animals, there was development of crystalluria with therapy of high dosage of fluoroquinolones.

Kumars *et al.* (2009) and Sharma *et.al* (1994) described gastrointestinal disturbances *viz.* mild diarrhoea, vomiting, and abdominal discomfort following fluoroquinolone administration therapeutically in human. They also reported headache, dizziness, anxiety, depression, insomnia and generalized seizures in human.

Haematological changes:-

Since all cells of blood are nucleated in chicken, the methods commonly used for counting mammalian blood cells can be applied. A number of procedures have recommended from time to time in the counting of red blood cells of birds. However, counting of erythrocytes is easy but counting of leucocytes causes some difficulty.

Olson (1965) reported that value for haemoglobin is higher in male than female birds. They suggested that hetrophiles may function as defensive mechanism against bacterial invasion as phagocytes.

Sharma *et al.* (1994) demonstrated anemia, eosinophilia, leucocytopenia, thrombocytopenia, pancytopenia and raised prothrombin time in human after therapy with fluoroquinolones.

Owens *et al.* (2000).state that levofloxacin, the newer third generation of quinolone is now extensively used in poultry industry for treatment of bacterial diseases as it has improved pharmacokinetic and pharmacodynamics properties.

Ellakany *et al.* (2007) reported that treatment of 10-fold overdose resulted haemoglobin concentration showed decline in 29-34 days old broilers treated with enrofloxacin.

Kumars et al. (2009) reported that there is significant decrease in Hb, PCV & TEC on 7 and 14 DPT in all treatment groups of croiler birds indicating anaemia treated with levofloxacin.

Haleema Al Nahari (2014) reported that total red blood cell (TEC) count and haemoglobin concentration were significantly decreased in mice exposed to 0.5 and 1 mg/kg of Ciprofloxacinfor four weeks period.

R. Rashmi *et al.* (2014) reported that TEC, Hb, Hct and TLC showed significantly decreased level in treated (Group II, III and IV) with norfloxacin in female rat.

Samah S. Oda *et.al* (2014) reported that levofloxacin and gentamicin treated groups showed a significant increase in TLC at the end of the 1st and 4th weeks of the experiment in the rabbit.

Biochemical changes:-

Sharma *et al.* (1994) recorded biochemical profiles in human after therapy with fluoroquinolones. They observed elevation of SGPT, SGOT, ALP, serum bilirubin, serum creatinine, blood urea, serum uric acid and hypoglycaemia.

Moustafa et al.(1998) reported that in broiler chicken administration of enrofloxacin in high doses (100, 200 and 400 ppm through drinking water) for long periods (6 weeks) was accompanied with adverse effects on different organs along with elevated levels of AST, ALT, ALP, urea, uric acid and creatinine, hypoproteinaemia and hypoalbuminaemia.

Sugawara *et al.* (1996) also reported an elevated serum enzymatic activity in monkey after therapy with ciprofloxacin both in therapeutic dose and in higher doses of the drug.

Niyogi *et al.*(1999) reported Hypoproteinaemia in broiler birds treated with overdoses of ciprofloxacin.

Kumars *et al.* (2009) reported anaemia, leucopoenia, hypoglycaemia, hypoproteinaemia and increased transaminase activity birds administered orally with therapeutic dose of levofloxacin (60mg/kg b.wt.)

Kumar Kumar*et al.*(2013) also reported that a significant but transient elevation in serum uric acid and creatinine.

Haleema Al Nahari (2014) also reported that there is decrease in serum total protein of treated mice when compared with control group. The results revealed also hyperglycaemia in mice exposed to 0.5 and 1 mg/Kg/day of Ciprofloxacin.

R Rashmi *et al.*(2014) reported that TEC, Hb, Hct and TLC showed significantly aspartate aminotransferase, alanine aminotransferase, BUN, creatinine showed significant increased level in treated (Group II, III and IV) with norfloxacin in female rat.

Samah S. Oda*et al.*(2014) also reported Hypoproteinaemia and hypoalbuminaemia evident in levofloxacin 82 mg /kg b.wt treated group at 4th week post-treatment.

Pathomorphological changes:

Sharma *et al.* (1994) recorded hepatitis and interstitial nephritis treated with fluoroquinolones.

Shih *et al.* (1995) reported renal vasculitis and interstitial nephritis in human treated with ciprofloxacin.

Zhou *et al.* (1994) studied acute toxicity of norfloxacin nicotinate in chickens. They injected acute LD 50 of norfloxacin nicotinate into the crop of 5-week-old chickens. Histopathologically, there were hyperemia and oedema of the meninges, necrosis and inflammation of mucous membrane of the oesophagus, crop, duodenum and degeneration and/ or necrosis of the parenchymal cells of the heart, liver and kidney.

Ettinger *et al.* (1995) conducted experimental studied with very high dosage of fluoroquinolones in dogs and cats. They observed lesions on the articular cartilage after 30 days administration of the drug @ 5, 15 and 25 mg/kg/d.

Sugawara *et al.* (1996) reported that rats receiving quinolones antimicrobial agents @ 2000 mg/kg/d for one month showed abnormal urine crystals and enhanced deposition of lipid in the hepatocytes.

Raja *et al.* (1998) reported acute renal failure interstitial nephritis in patients undergoing high dose of ciprofloxacin therapy.

Peters *et al.* (2002) reported adverse effect of certain antibiotics on bone formation and cartilage metabolism in dogs, cats and human.

Petit jeans *et.al* (2003) Observed severe rhabdomyoloris, hyperkalaemia, Liver cytolysis & respiratory failure in human patient treated with Levofloxacin.

Kumars *et al.* (2009) reported Clinicopathological changes in croiler birds administered orally with therapeutic dose double, triple therapeutic doses of levofloxacin developed mild and severe gastrointestinal signs, partial loss of body weight, droopiness, and leg weakness accompanied with anaemia, leucopoenia, hypoglycaemia, hypoproteinaemia and increased transaminase activity

Kumarkumar (2013) reported that the immunosuppressive effect of enrofloxacin associated histopatho- logical changes in lymphoid organs observed during post treatment period, suggests that the immuno-modulatory activity of enro- floxacin may alter the immune response to vaccines if it is co administered.

Residual

Anadon *et al.*(1995) reported that high residue concentration of difloxacin and its metabolite sparfloxacin in liver tissue were 368.1±52 .5 ìg per kg and 10.4±1.2 ìg/kg respectively on day 1 compared to other tissues and decreased in difloxacin residue concentration up to fifth day after administration of the final dose of difloxacin. In addition, the withdrawal period of five days was necessary to ensure that the residues of difloxacin were less than MRL or tolerance established by the European Union.

Jelena et al. (2006) reported high residue levels of enrofloxacin and ciprofloxacin in liver tissue on day one at 1196.1 µg/kg and 187 µg/kg respectively, compared to other tissues and decreasing in the enrofloxacin residue concentration up to ninth day was 24.8 µg/kg after the treatment. In addition, withdrawal period of four days for enrofloxacin in muscle and liver in broiler birds.

Lim *et al.* (2002) reported that high residue concentration of norfloxacin observed in liver tissue was 990 µg/kg on day one compared to other tissues and there was decreased in levofloxacin residue concentration up to 70 µg/kg on fifth day after the treatment.

Petrovic *et al.* (2006).Reported that withdrawal period of four days for enrofloxacin and its metabolite ciprofloxacin residues to decrease to an acceptable level in the meat and liver of the broiler birds.

Ishiwata et al. (2007). The drug concentration in tissue samples was determined by HPLC assay.

Banna *et al.* (2013) who reported that high residue concentration of levofloxacin was noted 2.47±0.23 µg/g or 2470 ±

0.19 µg/kg in kidney tissue on day one compared to the other tissues and decreased in residue concentration of 70 ± 0.01 µg/kg on 9th day after the treatment. In addition, withdrawal period for levofloxacin in liver and muscle tissue was four to five days in broiler birds. Sayed *et al.* reported the withdrawal period of levofloxacin as five days in normal catfish.

Kyuchukova *et al.*(2013) reported the residues levofloxacin orally treated at a dose of 10 mg/kg B.W for 5 days highest residues levels of levofloxacin in liver (1051 µg/kg), followed by breast muscle, gizzard, heart and skin – 428 µg/ kg , 321 µg / kg , 303 µg / kg and 293 µg / kg , respectively. In the liver, they decreased from the last day of the treatment to the 2nd day.

Ravi Kumar *et al.* (2015). The residue concentration of levofloxacin were gradually decreased in chicken liver and breast muscle samples starting from day one to 10th day after the last dose. Levofloxacin was estimated to have pre slaughter withdrawal period of four to five days in comparison with maximum residue level of 200, and 100 µg / kg for liver and breast muscle respectively as per European Economic Community council regulations.

MATERIALS AND METHODS

One hundred twenty day old of either sex were used in present experiment. They were divided in four groups in 30 number in each cage and fed with the Standard poultry feed. These birds were kept under observation for two weeks. During this period, their blood and faecal samples were examined. Normal values of blood were obtained during observation. Only those birds, whose haematological values were within normal range, blood samples were free from bacterial infection and faecal samples were negative for any parasitic infestation, were employed for these experiments.

Substances: - the following substances were used for eliciting the Haematological parameters, biochemical parameters, pathomorphological analysis, Histopathological analysis and residual analysis of broiler birds.

1. Levofloxacin (trade name HAWKVET available in market.)
2. Haemocytometer.
3. Biochemical Kit.
4. Buffer solution (0.1% o-phosphoric acid, Merck; *v/v*)
5. Mobile phase (Buffer, Methanol and Acetonitril; 70:12:18 *v/v*)
6. Stock solution.
7. Working standard solution (12.5µg/ml)

Studies using mesentery as a test system.

PARAMETERSSTUDIED

(A) Growth parameters:-

(B) Clinical signs:-

(C) Haematological parameters – (HB (%), PCV (%), TEC, TLC, DLC(%)and WBC at the intervals of 0, 3, 7, 14, 21 and 28thday.

(D) BIO-CHEMICAL PARAMETERS:-

Serum biochemistry (Total protein, Total albumin, Creatinine, Uric acid, BUN etc.) at the intervals of 0, 3, 7, 14, 21 and 28th day by using diagnostic kit.

Serum enzyme activity (ALT, AST) at the intervals of 0, 3, 7, 14, 21 and 28th day by using diagnostic kit.

Statistical analysis was performing using standard procedure as per Snedecor and Cochran (1994) with SPSS software (17th version).

(E). PATHOMORPHOLOGICAL STUDIES:-

Post mortem examinations of all the birds of the treatment and control groups which either died or sacrificed on the day after last administration were carried out systematically.

Tissue morbid *viz*. kidneys, liver, lungs, heart, gizzard and spleen were collected in 10% buffered neutral formalin for histopathology.

Section of 5-6µ thickness were prepared and stained with haematoxylin and eosin stain (Luna, 1968).

(F).RESIDUAL ANALYSIS:-

For residues analysis and withdrawal time calculation, birds were kept under observation for two week prior to commencement of experiment and subject to clinical examination in order to exclude the possibilities of disease. The broiler birds were provided antibiotic free standard broiler ration for fourteen days. The animal house was maintained at room.

Samples of liver & breast muscle were collected & the tissue samples were stored at 45°^{c}.

The drug concentration in tissue samples was determined by High Performance Liquid Chromatography (HPLC) assay (Ishiwata *et al.*, 2007).

RESULTS AND DISCUSSION

Section 1:-

A. Clinical sign.

Clinically, birds administrated with 10 mg/kg B.wt (Group T_1) were found no any changes. While, birds administrated with 20 mg/kg b.wt (group T_2,) &30 mg/kg b.wt of levofloxacin (group T_3) were found weakness, unthriptness, Diarrhoea, droopiness,

drowsiness, severe depression & appreciable decrease in body weight gain. Sign of lameness was recorded after 21st& 28 days post treatment (DPT). None of the birds in control group exhibited any abnormality during the 28 days of observation period. All the birds remained healthy till the last day and consumed normal amount of feed.

Clinical signs involve in birds of group T_2, & T_3 were similar to those reported earlier in acute enrofloxacin toxicity in birds (Hooper *et al.* (1985), & Flanner *et al.* (1990) in fluoroquinolone toxicity in human. Sharma *et al.*(1994).also reported nausea, vomiting, anorexia, headache, drowsiness and insomnia in human received fluoroquinolones treatment. In some patients, there was development of joint swelling or tendonitis. In animals, there was development of crystalluria with therapy of high dosage of fluoroquinolones. Norrby SR (1991). Examined the adverse effects of quinolones in human. The reaction consisted of the gastrointestinal sign *viz.* nausea, vomiting, and abdominal discomfort and less frequently diarrhoea. He also reported toxicity in the central nervous system, which included insomnia, dizziness, convulsion and anxiety. Kumars *et al.*(2009)also reported that there is loss of appetite, mild diarrhoea and depression from 7 DPT. Birds with double and triple therapeutic doses (groups III and IV) manifested toxic sign on day 5 onwards, comprising diarrhoea, droopiness, loss of appetite, severe depression leg weakness, salivation and decrease in bodyweight gain without any mortality. Sign of lameness were recorded only in birds of group IV after 10 DPT.

B.Body weight:

The average body weight of birds in different groups recorded at various intervals of the experimental is given in Table 1 and presented graphically in graph- 1. Birds which were given levofloxacin at the rate of 30 mg/kg b.wt showed a marked depression in body weight gain which was significant ($P<0.05$) throughout the observation period of 28 days. Birds in group T_1 also exhibited drop in body weight which was also significantly lower than in group T_0 (control group). The retardation in body weight was much more in birds, which were given higher concentration of levofloxacin (group T3), and the difference in the two groups (T_1 and T_2) was significant throughout the experiment.

A study on the effect of levofloxacin on body weight gain revealed that there was significant drop in body weight gain within 1 week of levofloxacin feeding; the decline was more severe in group T_3. This finding is in accordance with the observations of earlier workers R. Rashmi *et al.*(2014) reported that there was a significant decrease in body weight gain in treated groups.

Section 2:-

Haematological studies of broiler birds.

Blood is a mirror for observing changes in the body both physiological and Pathological in nature. Hence, importance of haematological studies as tool in the diagnosis of poultry diseases cannot be ignored.

The results of different haematological studies viz. haemoglobin concentration, packed cell volume, total erythrocyte counts and total differential leukocyte counts of hetrophiles and lymphocytes at various intervals of the experiment are as follows:

Haemoglobin (Hb) concentration

The average haemoglobin concentration observed in chickens of different experimental groups are given in Table. 2. And presented graphically in graph-2. It was noticed that the mean values of Hb in the control birds T_0 varied from 9.85-9.98 g/dl. The corresponding values in the three levofloxacin-fed groups T_1, T_2 and T_3 were comparatively lower. There was significant ($P<0.05$) decrease in Hb concentration of group T_3 as compared to group T_2 and T_1 group throughout the observation period of 28 days while decrease in the Hb values of group T_2 &T_1 (in comparison to group T_3) was significant at 21st& 28th days only.

This observation is similar to the findings of Haleema Al Nahari (2014) reported that haemoglobin concentration were significantly decreased in mice exposed to 0.5 and 1 mg/kg of Ciprofloxacin. Kumars et al.(2009) revealed a significant decrease in Hb on 7 and 14 DPT in all treatment groups of birds indicating anaemia. R. Rashmi et al. (2014) reported that Hb, significantly decreased level in treated (Group II, III and IV) female rat. Ellakany *et al.*(2007) reported that treatment of 10-fold overdose resulted haemoglobin concentration showed decline in 29-34 days old broilers treated with enrofloxacin.

Packed Cell Volume (PCV)

Mean values of PCV observed at different intervals of levofloxacin feeding birds are given in Table. 3 and presented graphically in graph- 3. A reference to this table indicates that the mean values of PCV in the group T_3 were comparatively lower than that in the group T_2 and T_1. The decline in the group T_3, in

comparison to group T_2 and T_1 was significant ($P<0.05$) from day 3 to day 28 of the observation period. Although a constant decrease in the values of PCV was also noticed in the group T_1 birds but these values (in comparison to controls) were not significant at any stage except at 28 days.

Kumars et al. (2009) revealed a significant decrease in PCV on 7 and 14 DPT in all treatment groups of birds.

Total Erythrocyte Counts (TEC)

The mean values of TEC recorded at different intervals of levofloxacin feeding are presented in Table. 4. and presented graphically in graph- 4.reference to this table indicates that the mean values of TEC in the group T_3 were comparatively lower than that in the group T_1 and T_2. The decline in the group T_3, in comparison to group T_2 and T_1, was significant ($P<0.01$) from day 14 till day 28 of the observation period. Although a constant decrease in the values of TEC was also noticed in the group T_1 birds but these values (in comparison to controls) were not significant at any stage.

This observation is similar to the findings of Haleema Al Nahari (2014) reported that total red blood cell (TEC) count and haemoglobin concentration were significantly decreased in mice exposed to 0.5 and 1 mg/kg of Ciprofloxacinfor four weeks period. Kumars et al.(2009) also reported that a significant decrease in TEC on 7 and 14 DPT in all treatment groups of birds. R. Rashmi et al.(2014) reported that TEC significantly decreased level in treated (Group II, III and IV) female rat.The reduction in RBC count may be due to macrocytic or normocytic anaemia.

Total Leucocytes Counts (TLC)

The mean values of TLC recorded in the three groups at different intervals of experiment are presented in Table. 5. and presented graphically in graph-5. A significant ($P<0.05$) drop in TLC was observed in the group T_3 in comparison to group T_2 and T_1 from day 21 to 28 of experiment. Through the mean values of TLC in-group T_1 were slightly lower than that of the controls. However, difference was not significant at any stage of the experiment.

Enumeration of total leukocyte count revealed a moderate decrease in TLC of both the levofloxacin fed groups T_2 and T_3. This observation is similar to the findings of Samah S. Oda *et.al* (2014) reported that levofloxacin and gentamicin treated groups showed a significant increase in TLC at the end of the 1st and 4th weeks of the experiment in the rabbit. R. Rashmi et al. (2014) reported that TLC significantly decreased in treated (Group II, III and IV) female rat.

Differential Leucocytes counts (DLC)

The mean percent values of different leucocytes in the blood of chickens of different experimental groups are presented in Table. 6,7,8,9 &10 and presented graphically in graph- 6,7,8,9 & 10. A comparison of these values in different groups revealed slight significant ($P<0.05$) decreases in the relative percentage of lymphocytes with a corresponding significant ($P<0.05$) increase in the hetrophiles of the groups T_3, the difference was significant on day 21 and 28. Slight significant ($P<0.05$) decrease in the relative percentage of lymphocytes and significant ($P<0.05$) increase in hetrophiles was also observed in the group T_1 & T_2, but the difference was noticed in the relative percentage of eosinophils,

basophiles, and monocytes among different groups of experimental birds.

Studies on the differential leukocyte counts of lymphocytes and hetrophiles revealed that leucopoenia was due to decrease in both lymphocytes and hetrophiles, the decline in hetrophiles count being more marked.

This observation is similar to the findings of Kumars *et al.*(2009)reported that there was significant decrease in lymphocytes with a corresponding significant increase in the hetrophiles .R. Rashmi et al.(2014) reported that TEC, Hb, Hct and TLC showed significantly decreased level in treated (Group II, III and IV) female rat. Sharma *et al.* (1994) demonstrated anaemia, eosinophilia, leucocytopenia, thrombocytopenia, pancytopenia and raised prothrombin time in human after therapy with fluoroquinolones.

Section 3:-

Biochemical studies of broiler birds.

Alanine transaminase

The mean Alanine transaminase activity in both the levofloxacin fed groups was slightly lower than the controls are presented in Table-11. and presented graphically in graph- 11. However, difference was not significant ($P<0.05$) at any stage of the experiment.

While studying the activity of transaminase (Alanine transaminase) in the serum of birds during the present study, it was found that there was elevate in the values of Alanine

transaminase (from day 14 to 28 of the experiment) but the Alanine transaminase levels remained in significantly ($P<0.05$) higher in the group T_2 and T_3 as compared to group T_1 birds. Such results may be attributed to this increase could be regarded as a factor to increase the permeability and subsequent leakage of cellular enzyme due to the levofloxacin toxicity.

This observation is similar to the findings of Sugawara *et al.*(1996) also reported an elevated serum enzymatic activity in broiler birds and monkey respectively after therapy with ciprofloxacin both in therapeutic dose and higher doses of the drug.

Aspartate transaminase

Estimation of aspartate transaminase (AST) activity in the serum of birds from different groups at various intervals presented in Table-12.and presented graphically in graph-12.) Revealed a moderate increase in the activity of this enzyme in the group T_3 that was significant($P<0.05$) on days. 21 and 28 in comparison to group T_2 birds. The increase in enzyme activity started from day 3 of the levofloxacin administration and continued up to the last day (28 days) of the observation.

Total serum proteins

Estimation of total serum proteins at different intervals are presented in Table. 13 and presented graphically in graph-13. revealed a significant ($P<0.05$) drop in TSP of both the levofloxacin fed groups (T_2 and T_3) as compared toT_1 on 21 days onwards. The values started from day 14 and continued till last day of the experiments. The TSP values were lowest in the group T_2 and these

differed significantly ($P<0.05$) from that in the group T_3 on 21 days onwards. Such results may be attributed to liver damage and nephrotoxic effect induced by levofloxacin.

Estimation of total serum proteins at various intervals revealed severe hypoproteinaemia in both the groups T_2 and T_3 as compared to control group. Niyogi 1999 reported Hypoproteinaemia in birds treated overdoses of ciprofloxacin, Oda *et al.* (2014) also reported Hypoproteinaemia and hypoalbuminaemia evident in levofloxacin treated group at 4th week post-treatment. Haleema Al Nahari (2014) also reported that there is decrease in serum total protein of treated mice when compared with control group.

Total Albumin

Estimation of total serum albumin at different intervals are presented in Table.14 and presented graphically in graph-14. revealed a significant ($P<0.05$) drop in total albumin of both the levofloxacin fed groups (T_2 and T_3) as compared to control T_0& T_1 on 21 days onwards. The values started from day 14 and continued till last day of the experiments. The total albumin values were lowest in the group T_3 and these differed significantly ($P<0.05$) from that in the group T2 on 21 days onwards.

Estimation of total serum albumin at various intervals revealed severe hypoalbunaemia in both the groups T_2 and T_3 as compared to T_1 and control group. Kumars et al. (2009) revealed a significant decrease in A : G ratio in croiler birds.

Blood Urea Nitrogen (BUN)

The average values of BUN recorded at different intervals of the experiment are presented in Table.15. And presented graphically in graph-15. It was found that there was considerable elevation in the values of BUN in the group T_3 as compared to group T_2 and T_1, but this difference was not significant ($P<0.05$) except on days 21 and 28. The values of BUN in the group T_1 were comparable to those of the controls.

Estimation of blood urea nitrogen and creatinine in the blood of chickens showed significant increase in the levels of both these metabolites in the chickens; increase being significant ($P<0.05$) on day 14 and 21 only. Elevation in the levels of these metabolites is suggestive of some degree of renal damage in the levofloxacin fed groups. R. Rashmi et al. (2014) reported that aspartate aminotransferase, Alanine aminotransferase, BUN, creatinine showed significantincreased level in treated groups.

Creatinine

The mean values of creatinine in the blood of chickens at different intervals are presented in Table 16. And presented graphically in graph- 16. A reference to this table indicates that there was increase in serum creatinine level in the group T_3 birds but this increase in comparison by group T_2 and $T_1$was not significant ($P<0.05$) except at days 21 and 28. The mean creatinine level in the group T_3 birds was more than that in the group T_1 and T_2. The difference was significant ($P<0.05$) after 28 day of levofloxacin feeding.

Increase in serum creatinine concentration in high dose levofloxacin fed birds might be due to nephrotoxic action of

levofloxacin, which causes renal impairment by destruction of epithelial cells of proximal and distal convoluted tubules and tubular damage. Samah. S. Oda. *et al.* (2014) reported serum urea and creatinine levels were significantly increased in levofloxacin treated group at both 1st and 4th weeks post-treatment.

Uric Acid

The mean values of uric acid in the blood of chickens at different intervals are presented in Table-17. and presented graphically in graph-17. A reference to this table indicates that there was increase in serum uric acid level in the group T_3 birds but this increase in comparison by group T_2 and $T_1$was not significant ($P<0.05$) except at days 21 and 28. The mean creatinine level in the group T_3 birds was more than that in the group T_2 and the difference was significant ($P<0.05$) after 21 day of levofloxacin feeding. Haleema Al Nahari (2014) reported that there is significant rises in serum uric acid and creatinine in treated mice compared with control. Kumar *et al.* (2013) also reported that a significant but transient elevation in serum uric acid and creatinine was noticed.

Moustafa et al.(1998) reported that in broiler chicken administration of enrofloxacin in high doses (100, 200 and 400 mg/kg b.wt through drinking water) for long periods (6 weeks) was accompanied with adverse effects on different organs along with elevated levels of AST, ALT, ALP, urea, uric acid and creatinine, hypoproteinaemia and hypoalbuminaemia.

Section 4:-

Histopathological studies

Group T_3 (30 mg/kg levofloxacin)

A. Liver

Examination of liver at 28 days, macroscopically, liver showing Mild hepatitis & discoloration of liver parenchyma in T3 group birds treated with levofloxacin @ 30 mg/kg B.wt (fig no.1). Microscopically liver showing congestion in blood vessels, congestion in sinusoids, and necrosis in the parenchyma and connective tissue, proliferation between the lobules seen in the liver (Fig No. 3). There was increase in the number and size of lymphoid aggregation. The dilatation of hepatic sinusoids was quite prominent and it was accompanied by slight proliferation of fibrous tissue. At places, there was proliferation of bile duct epithelium and the connective tissue in the portal area was increased.

The changes in the present study were in accordance with finding of Niyogi (1999) in broiler birds treated with ciprofloxacin and Kumars (2009) in croiler birds treated with levofloxacin. kumar *et al.*(2013) also reported that hepatocytes were swollen, degenerated with vacuolated cytoplasm and showed areas of congestion. Clear sinusoidal spaces and focal areas of infiltrated inflammatory cells with only a mild vacuolation of hepatocytes. Ellakany *et al.*(2007) who observed that hepatocytes vacuolation and hydropic degeneration, associated with vascular congestion and degeneration of lymphoid foci in broiler chicken administered with enrofloxacin at 100 mg Kg^{-1}through drinking water for 5 consecutive days.

B.Spleen

Spleen of control group showing normal architectures After 28 days, there was increase in the thickness of splenic capsule, trabeculae and fibro muscular tissue of the blood vessels. The spleen appeared atrophied with apparent increase in the number of lymphoid follicles and mild depletion of lymphocytes with lot of blast cell proliferation (Fig No. 4.) is the only appreciable lesions in 28th days post treatment group.

The above findings are in contrary to the findings by Kumar *et al.* (2013) and Kumars *et al.* (2009).

C.Kidneys

Varying degree of congestion and haemorrhages coupled with degenerative changes in the tubular epithelium (Fig No. 6.) were the only salient changes observed in the kidney at different intervals revealed acute tubular necrosis and tubule-interstitial nephritis with presence of cellular cast, degeneration of tubules, infiltration of mononuclear cell in the interstitial tissue (Fig No. 7.) were observed at 28 days of the experiment.

The above observation is in agreement with Raja *et al.*(1998) in human treated with ciprofloxacin. Kumar et al. (2013) treated with enrofloxacin in broiler chicken. In kidney have been attributed to the relative hypoxia caused by failing circulation and pathological change occurred in the kidneys. Ellakany et al. (2007) also found congestion, tubular degeneration, and area of hemorrhages in kidney of broiler chicken administered with enrofloxacin at100mg/Kg b.wt through drinking water for 5 consecutive days.

D. Lungs

Changes in the lungs proliferation of connective tissue between the alveoli and interlobular septa and slight thickening of artial bundles in the tertiary bronchioles (Fig No.8 & 9.). Mild to moderate infiltration with mononuclear cells was noticed in the sub epithelial tissue of secondary bronchioles. Lungs also had distinct thrombus formation in the vessels by coagulated blood.

The congestion, oedema, thrombus and bronchiolar hyperplasia of epithelium might be due to the anoxic changes and hypovolemic conditions. The mononuclear cells proliferation results from phagocyte activities. Kumars et al. (2009) observed the above changes in the section of lungs of broiler birds due to over dose of levofloxacin.

E.Bursa of Fabricius

The bursa of Fabricius at most of the occasions appeared normal. There was moderate increase in the interfollicular connective tissue between the interfollicular spaces (Fig No.11.) with slight but occasional depletion of lymphoid cells and mild cystic degeneration changes of a few follicles (Fig No. 12).Atrophy of bursal follicle with lymphocytolysis suggest that the higher dose of drug has a cytotoxic effect on the antibody producing cells, so that immunosuppression may be possible outcome of higher dose of levofloxacin. Niyogi *et al.*(2000), Kumars *et al.* (2009) reported immunosuppression in broiler birds due to over doses of levofloxacin. kumar et al. (2013) reported that Bursa of Fabricius of enrofloxacin treated group showing mild depletion of lymphocytes with lymphocytosis, histio-cyte/proliferation.

F.Intestines

Intestine also further revealed subacute enteritis that was characterized by infiltration of mononuclear cells in the submucosa and mucosa there was atrophy of the different layers of intestines along with Coagulative necrosis in the mucosa & Intestinal villi become broaden (Fig No 16). Macroscopically intestine showed Sever haemorrhagic enteritis in broiler birds treated with levofloxacin @ 30 mg/kg b.wt. (Fig No.14) Which further confirm the reports of Zhou and Wang (1994), Kumars (2009) in chickens treated with norfloxacin and levofloxacin.

G. Skeletal muscles

Mild congestion and slight haemorrhages in the interstitial tissue were observed during the 7 & 14 days. At 21 days, showing disruption of cardiac muscles, congestion in the intramuscular spaces and occasional infiltration of mononuclear cells. (Fig 18) Later on, there was atrophy of the muscle, which was coupled with mononuclear cell infiltration.

The nature of histopathological lesion suggested that 30 mg/kg b.wt of levofloxacin induced severe toxic hepatitis and nephrosis with mild degree of tubular nephritis. It is believed that higher dose of levofloxacin might cause denaturation of hydrolytic enzymes as well as, causing degeneration and Coagulative necrosis of parenchymatous tissues of the liver, kidney and intestine.

The development of toxic nephrosis and nephritis in birds treated with 30 mg/kg b.wt of levofloxacin suggest a severe from the nephrotoxic effect of the drug, since 30% of the drug is excreted through kidney.

Group T_2 (20 mg/kg levofloxacin)

A. Liver

The liver of broiler birds induced with 20 mg/kg b.wt had lesions consisting of congestion and mild necrosis in parenchyma (Fig No.2.). The sinusoids were dilated with blood and the lining endothelial cells were prominent. A few lymphoid aggregates were seen. In addition to these vascular changes, proliferation of fibro vascular tissue along with mild mononuclear cell infiltration was noticed in the portal areas.

B. Spleen

No appreciable changes were noticed in the spleen, which appeared almost normal in the 28th days. Thereafter, there was slight fibrous tissue proliferation in the capsule and trabeculae as well as increase in fibro vascular tissue. The splenic tissue was atrophied with the result lymphoid follicles became more prominent.

C. Kidneys

Kidneys appeared almost normal except for slight congestion, infiltration of mononuclear cells and mild degenerative changes in the tubular epithelium (Fig No. 5.).

D. Lungs

Examination of the lungs revealed mild congestion in the alveoli andoedema in the bronchi at the 28th day of experiment. (Fig No. 10.) Thereafter, no change of pathological significance could be detected.

E. Bursa of Fabricius

Histological examination of the bursa of Fabricius also did not reveal any significant change except for slight increase in the interfollicular connective tissue of a few birds in the last two weeks.

F. Intestines

No salient changes were noticed in the intestines. After three weeks showing atrophy of different layers of intestine of Group T_2 birds (Fig No. 15.). and macroscopically intestine showing mild haemorrhagic enteritis in poultry treated with levofloxacin @ 20 mg/kg b.wt showing Haemorrhagic enteritis& congestion in intestine.(fig No.13).

G. Skeletal muscles

Muscle showed mild haemorrhages in between the muscle fibre in the broiler birds treated with 20 mg/kg levofloxacin (fig 19). This further confirmed the reports Suman et al.(2009) in croiler birds treated with levofloxacin. Petitjeans et al. (2003) also reported rhabdomyolysis in human patients treated with levofloxacin.

Group T_1 (10 mg/kg levofloxacin)

Histopathological lesions consisted mild congestion in the liver, kidneys and lungs. An acute cellular swelling with granular

cytoplasm in the hepatic cells and renal tubular epithelium on both 7 and 14 DPT. The nature of histopathological lesion reflects the development of mild degree of toxic hepatitis and nephrosis by 10 mg/kg of levofloxacin. There exist no literature to describe the development of toxic hepatitis in broiler birds/patients treated with 10 mg /kg of levofloxacin but this finding in the present study were in accordance with finding of Kumars*et al.* (2009) in croiler birds treated with levofloxacin, Niyogi (1999) in broiler birds treated with ciprofloxacin,Suresh Kumar *et al.* (2013) in broiler chickens water medication of enrofloxacin.

Section5:-

RESIDUAL ANALYSIS

Liver:-Birds treated with Levofloxacin @10 mg/kg (T1) observed on day one was high and gradually it was decreased up to tenth days in liver, tissue samples after the final dose of administration. Highest residue of levofloxacin concentration detectable in liver was 1221.89 ± 0.73 µg/kg and there was decreased in residue concentration up to 55.82 ± 0.27 µg/kg on day ten after the last dose of levofloxacin administered. The concentration of levofloxacin on day four was 185.20 ± 0.91 µg/kg. The value was less or almost equal to Maximum Residual Level (MRL) fixed for fluoroquinolones (200 µg/kg) in liver tissue samples. Whereas Birds treated with Levofloxacin @20, mg/kg (T_2) observed on day one was high and gradually it was decreased up to tenth days in liver, tissue samples after the final dose of administration. Highest residue of levofloxacin concentration detectable in liver was 2250.68 ± 10 µg/kg and there was decreased in residue concentration up to 80.15 ± 14 µg/kg on day ten after the last dose

of levofloxacin administered. The concentration of levofloxacin on day four was 765.25 ± 35 µg/kg. The value was less or almost equal to Maximum Residual Level (MRL) fixed for fluoroquinolones (200 µg/kg) in liver tissue samples.

The present findings are in agreement with findings of Kyuchukova *et al.*(2013) who reported that high residue level of the levofloxacin observed in liver tissue on day one was 1051± 648 µg/kg compared to other tissues and decreasing in the residue concentration until day 8 was 56 ± 5 µg/kg after the treatment. Ravi Kumar *et al.* (2015) reported that highest levofloxacin residue concentration detectable in liver was 1428.89 ± 0.93 µg per kg and there was decreased in residue concentration up to 66.87 ± 0.23 µg per kg on day ten after the last dose of levofloxacin administered. Jelena et al. (2006) reported high residue levels of enrofloxacin and ciprofloxacin in liver tissue on day one at 1196.1 µg/kg and 187 µg/kg respectively, compared to other tissues and decreasing in the enrofloxacin residue concentration up to ninth day was 24.8 µg/kg after the treatment. Lim *et al.*(2002) reported that high residue concentration of norfloxacin observed in liver tissue was 990 µg/kg on day one compared to other tissues and there was decreased in levofloxacin residue concentration up to 70 µg/kg on fifth day after the treatment. Anadon *et al.*(1995) reported that high residue concentration of difloxacin and its metabolite sparfloxacin in liver tissue were 368.1±52 .5 ìg per kg and 10.4±1.2 ìg/kg respectively on day 1 compared to other tissues and decreased in difloxacin residue concentration up to fifth day after administration of the final dose of difloxacin. However, on the contrary, Banna *et al.*(2013) who reported that high residue concentration of levofloxacin was noted 2.47±0.23 µg/g or 2470 ± 0.19 µg/kg in

kidney tissue on day one compared to the other tissues and decreased in residue concentration of 70 ± 0.01 µg/kg on 9th day after the treatment.

Breast muscle: Birds treated with Levofloxacin@10 mg/kg (T1) observed highest concentration of levofloxacin in breast muscle tissue was 427.66 ± 0.92 µg/kg on day one and decreased in residue concentration up to 15.42 ± 0.56 µg/kg on day ten after the administration of the final dose of levofloxacin in broiler birds. Whereas, Birds treated with Levofloxacin @ 20 mg/kg (T2) observed highest concentration of levofloxacin in breast muscle tissue was 910.87± 23 µg/kg on day one and decreased in residue concentration up to 28.5 ± 8.97 µg/kg on day ten after the administration of the final dose of levofloxacin in broiler birds. In the present study, maximum concentration of levofloxacin was recorded in thigh muscle tissue at 867 ± 10.78 µg/kg on day one and the concentration was reduced up to 15.76 ± 3.64 µg/kg days nine after the administration of the last dose of levofloxacin in broiler birds. These findings are in agreement with Kyuchukova *et al.*(2013) who reported that highest residue level of the levofloxacin in the breast muscle was 428±253 µg/kg on day 1 and decreased in the residue concentration up to 56±15 µg/kg on day 8 after the treatment. Withdrawal period was calculated based on the residual concentration of the levofloxacin in tissues of broiler birds. The MRLs were 200 and 100 µg/kg for liver and breast muscles respectively. Ravi Kumar *et al.*(2015) reported that highest concentration of levofloxacin in breast muscle tissue was 467.66 ± 0.92 µg per kg on day one and decreased in residue concentration up to 16.51 ± 0.77µg per kg on day ten after the administration of the final dose of levofloxacin in dual-purpose chicken. Banna *et al.*

(2013) who reported that withdrawal period for levofloxacin in liver and muscle tissue was four to five days in broiler birds. Sayed *et al.*reported the withdrawal period of levofloxacin as five days in normal cat fish Anadon *et al.*(1995)reported that withdrawal period of five days was necessary to ensure that the residues of difloxacin were less than MRL or tolerance established by the European Union. Jelena *et al.* (2006) reported the withdrawal period of four days for enrofloxacin in muscle and liver in broiler birds. Petrovic *et al.*(2006). Reported a withdrawal period of four days for enrofloxacin and its metabolite ciprofloxacin residues to decrease to an acceptable level in the meat and liver of the broiler birds.

Table 1:- Mean ± SE value of average body weight (g) with and without levofloxacin Supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

GROUP	Days Post- Treatment					
	0	3	7	14	21	28
T_0	226.48±21.31	470.61±11.53	672.77±6.39	959.67±26.78	1131.54^{b} ±42.47	1336.67^{c} ±47.80
T_1	219.92±16.07	452.02±13.95	659.03±17.12	945.28±31.10	$1053.67^{a\ b}$ ±36.42	1213.06^{b} ±33.98
T_2	212.25±17.30	425.09±15.11	630.19±20.73	935.19±33.15	1027.09^{a} ±43.11	$1074.41^{a\ b}$ ±51.31
T_3	203.71±15.55	417.70±12.52	623.12±21.74	914.35±40.06	1013.54^{a} ±45.03	1039.09^{a} ±43.54

Values with similar superscript (column wise- a, b, c, d) differ significantly ($p<0.05$)

Graph- 1:- Bar diagram showing Mean ± SE value of average body weight (g) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

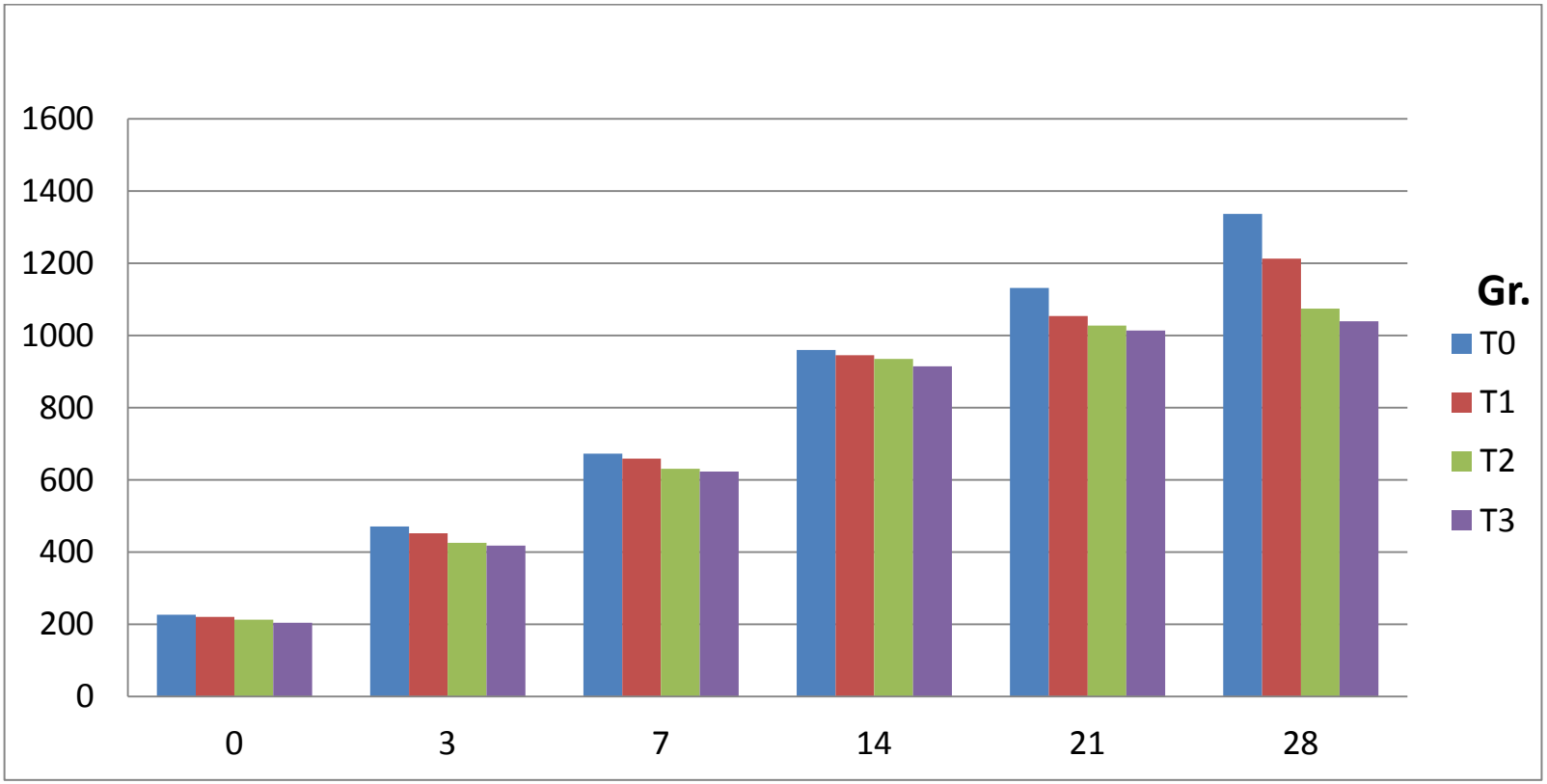


Table-2: Mean ± SE value of Haemoglobin concentration (gm/dl) with and without Levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

GROUP	Days Post- Treatment					
	0	3	7	14	21	28
T_0	9.85±0.150	9.81±0.115	9.86^{a} ±0.125	9.88^{a} ±0.141	9.85^{a} ±0.189	9.98^{a} ±0.129
T_1	9.80±0.311	9.75±0.227	9.55^{a} ±0.170	9.51^{a} ±0.170	9.45$^{a\ b}$ ±0.206	9.24^{b} ±0.095
T_2	9.65±0.239	9.25±0.129	9.12^{a} ±0.129	9.05^{b} ±0.216	9.85^{b} ±0.170	8.65^{c} ±0.170
T_3	9.35±0.155	9.20±0.095	8.67^{b} ±0.125	8.20$^{b\ c}$ ±0.182	7.90^{c} ±0.129	7.20^{d} ±0.141

Values with similar superscript (column wise- a, b, c, d) differ significantly ($p<0.05$)

Graph-2:- Bar diagram showing Mean ± SE value of Hb Concentration (g %) with and without Levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broilers birds.

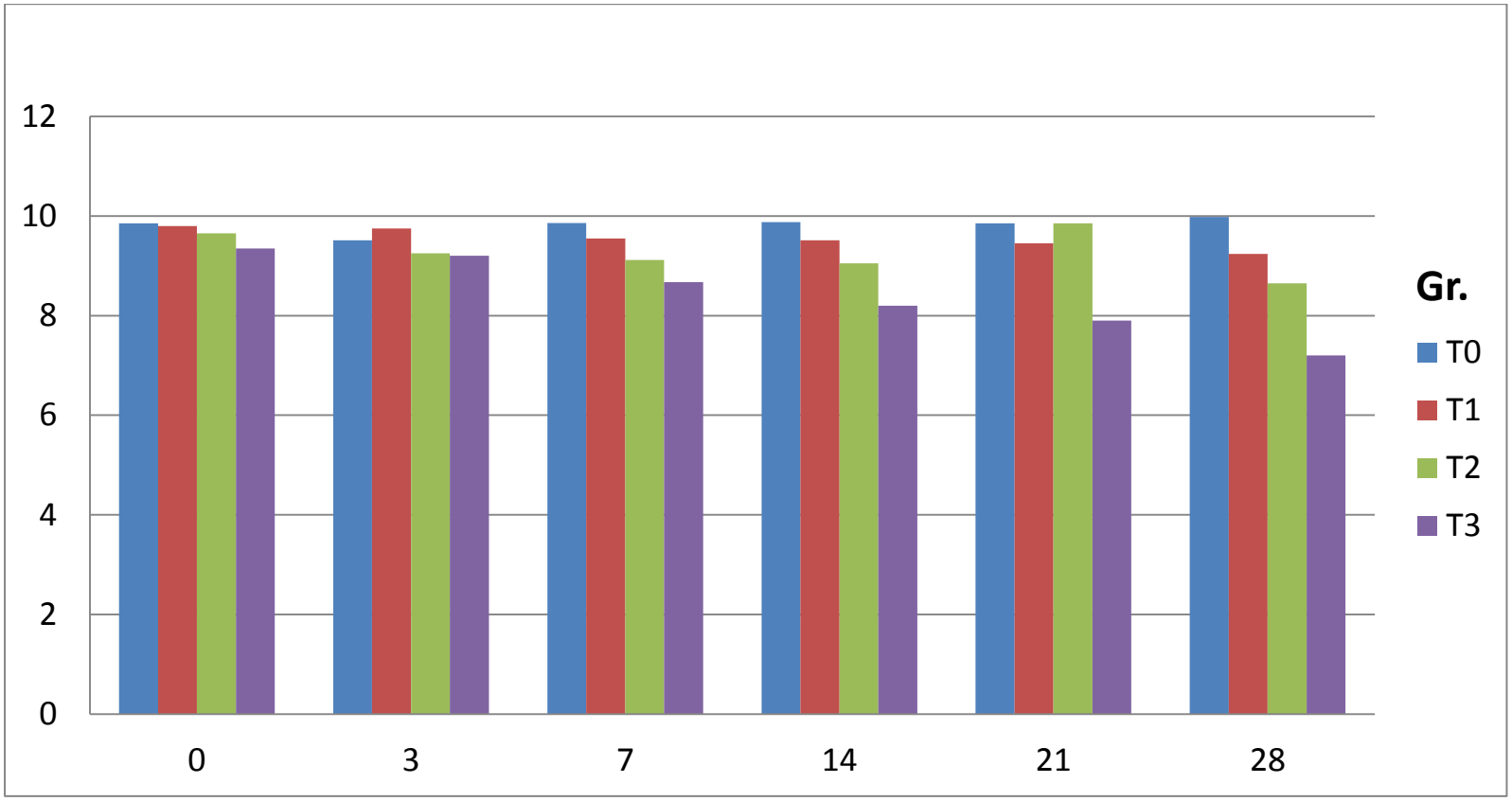


Table-3: Mean ± SE value of packed cell volume concentration (%) with and without Levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

GROUP	Days Post- Treatment					
	0	3	7	14	21	28
T_0	31.75±0.478	31.85±0.853	31.65^{a} ±0.750	31.80^{a} .±0.645	31.87^{a} ±0.957	31.85^{a} ±0.478
T_1	31.69±0.853	31.63±0.750	31.50^{a}±0.629	31.32^{a}±0.478	31.25^{b}±0.478	31.19^{b}±0.108
T_2	31.25±0.707	31.21±0.946	31.15^{b} ±0.408	31.10^{b} ±0.478	30.95^{b} ±0.645	30.87^{b} ±0.629
T_3	31.15±0.631	31.02±0.408	30.82^{b} ±0.629	30.65^{b} ±0.707	30.54^{b} ±0.610	30.11^{c} ±0.645

Values with similar superscript (column wise- a, b, c, d) differ significantly ($p<0.05$)

Graph-3:-Bar diagram showing Mean ± SE value of PCV Concentration (%) with and without Levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broilers birds.

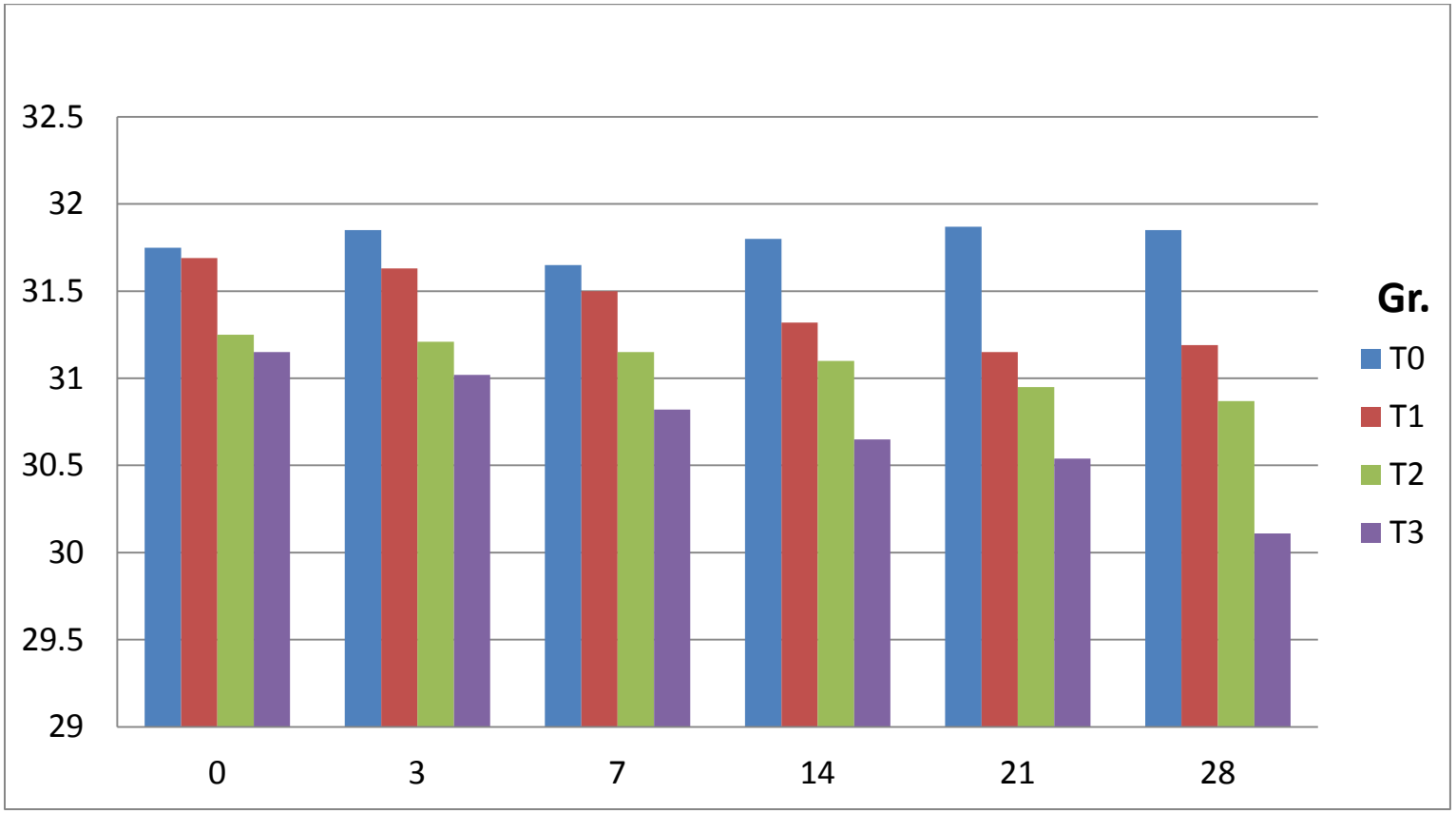


Table-4: Mean ± SE value of Total erythrocyte count concentration (10^6 /µl) With and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

GROUP	Days Post- Treatment					
	0	3	7	14	21	28
T_0	4.88±0.141	4.90±0.115	4.81^a ±0.182	4.75^a ±0.095	4.80^a ±0.131	4.75^a ±0.141
T_1	4.85±0.251	4.83±0.150	4.74^a ±0.125	4.70^a ±0.082	4.68^a ±0.169	4.69^a ±0.123
T_2	4.55±0.263	4.39±0.129	4.25^b ±0.150	4.15^b ±0.075	4.08^b ±0.104	3.91^b ±0.126
T_3	4.44±0.336	4.23±0.129	4.05^b ±0.085	3.81^b ±0.081	3.75^b ±0.170	3.72^b ±0.129

Values with similar superscript (column wise- a, b, c, d) differ significantly ($p<0.05$)

Graph-4:- Bar diagram showing Mean ± SE value of Total erythrocyte count concentration (10^6 /µl) with and without levofloxacin supplementation at 0, 3, 7, 14, 21 & 28th day in broiler birds.

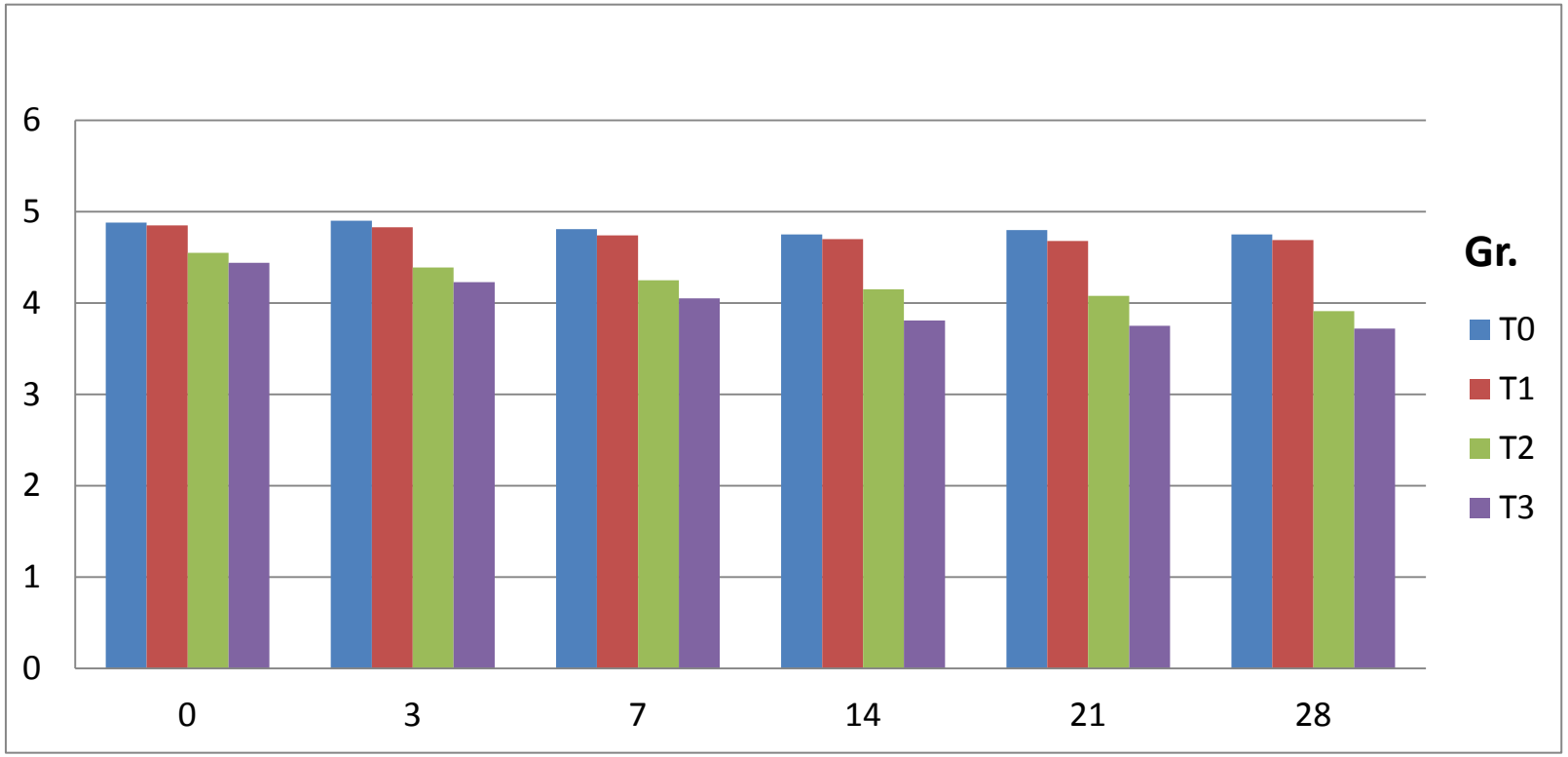


Table-5: Mean ± SE value of Total leucocyte count concentration (10^3 /µl) with and withoutlevofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

GROUP	Days Post- Treatment					
	0	3	7	14	21	28
T_0	30.960±0.187	30.850±0.576	30.800±0.613	30.710±0.235	30.850^{a} ±0.159	30.575^{a} ±0.350
T_1	30.850±0.588	30.770±0.622	30.650±0.537	30.600±0.233	30.550^{b} ±0.114	30.272^{b} ±0.270
T_2	30.700±0.187	30.550±0.576	30.400±0.613	30.370±0.235	29.850$^{b\ c}$ ±0.159	29.675$^{b\ c}$ ±0.350
T_3	30.600±0.144	30.370±0.594	30.100±0.537	30.000±0.224	29.237^{c} ±0.178	29.001^{c} ±0.206

Values with similar superscript (column wise- a, b, c, d) differ significantly ($p<0.05$)

Graph-5:- Bar diagram showing Mean ± SE value of Total leucocyte count concentration (10^3 /µl) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

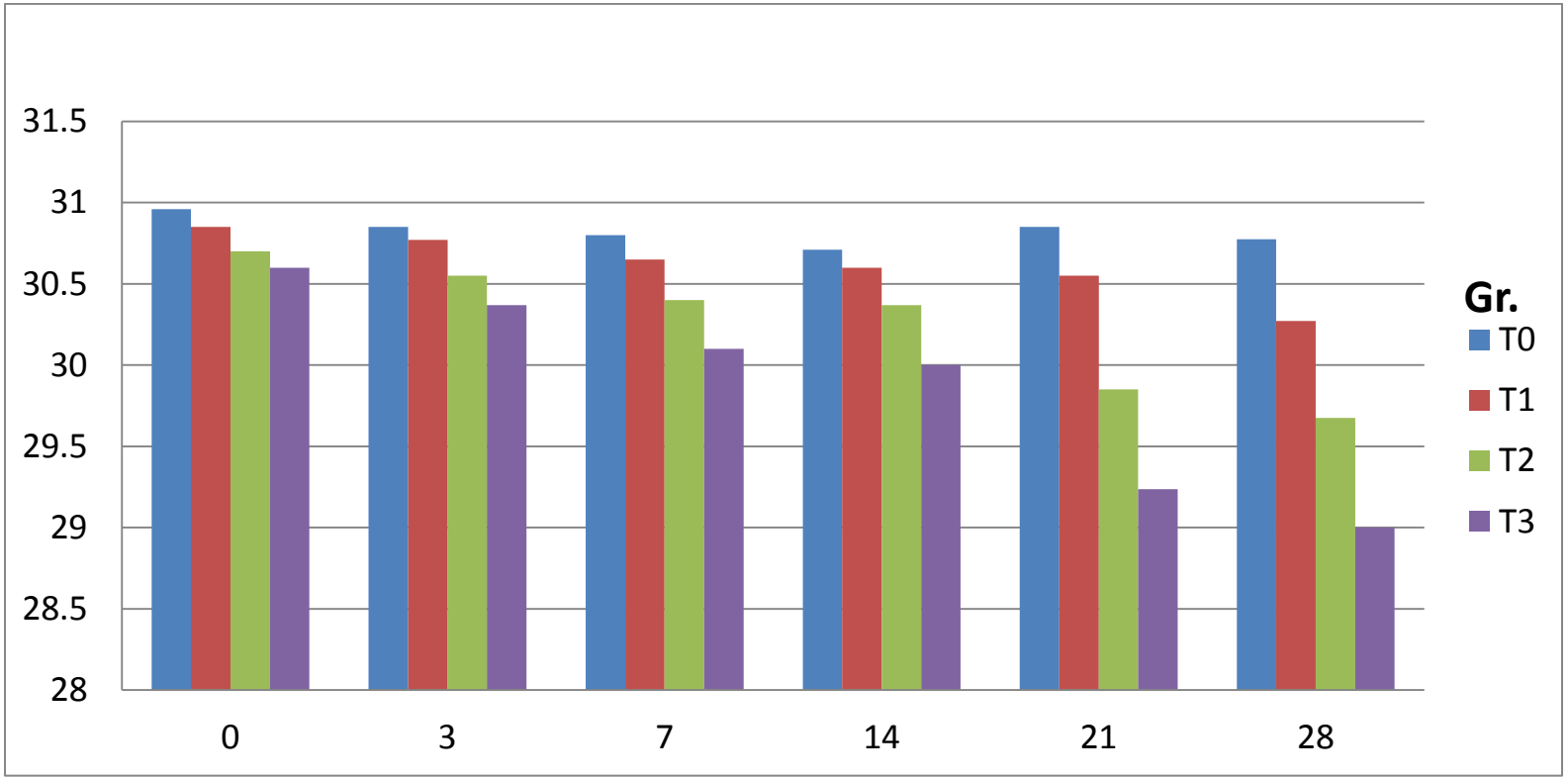


Table 6:- Mean ± SE value of hetrophiles concentration (%) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

GROUP	Days Post- Treatment					
	0	3	7	14	21	28
T_0	28.05±1.77	28.32±1.66	28.42±1.71	28.60±1.82	28.80^{a}±1.82	28.78 ^{a}±1.72
T_1	28.15±1.70	28.34±1.71	28.46±1.79	28.71±1.81	28.84 ^{a}±1.81	28.86 ^{a}±1.74
T_2	28.21±1.68	28.39 ±1.72	28.51±1.78	28.75±1.80	28.97 ^{a}±1.78	29.15 ^{ab}±1.76
T_3	28.29±1.65	28.79±1.80	28.84±1.83	29.16±1.75	29.39 ^{b}±1.77	29.91^{b}±1.78

Values with similar superscript (column wise- a, b, c, d) differ significantly ($p<0.05$)

Graph-6:- Bar diagram showing Mean ± SE value of hetrophiles concentration (%) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

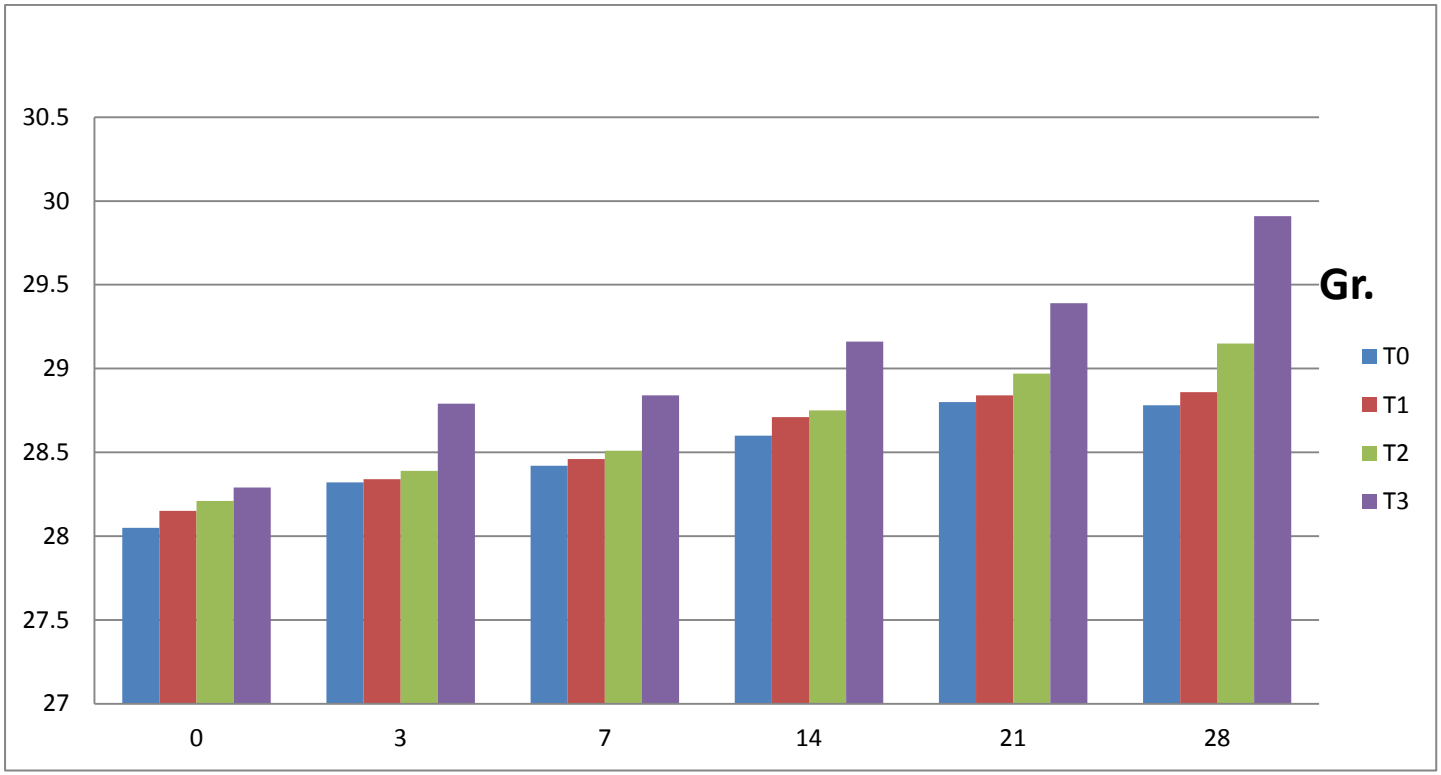


Table 7:- Mean ± SE value of lymphocytes concentration (%) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

GROUP	Days Post- Treatment					
	0	3	7	14	21	28
T_0	61.25±0.97	61.22±1.24	61.20^{b}±1.29	61.17^{b}±1.36	61.14^{b}±1.26	61.05^{c}±1.33
T_1	61.16±1.22	60.91±0.96	60.18^{b}±1.41	59.93^{b}±1.16	59.46^{b}±1.06	59.28^{bc}±0.96
T_2	60.98±1.84	60.47±1.21	60.13^{b}±1.35	59.79^{b}±1.09	59.35^{ab}±0.96	58.96 ^{b}±1.35
T_3	60.78±1.76	59.85±1.39	59.23 ^{a}±1.45	58.63 ^{a}±0.99	58.15 ^{a}±1.13	57.15 ^{a}±1.19

Values with similar superscript (column wise- a, b, c, d) differ significantly ($p<0.05$)

Graph-7:- Bar diagram showing Mean ± SE value of Lymphocytes concentration (%) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

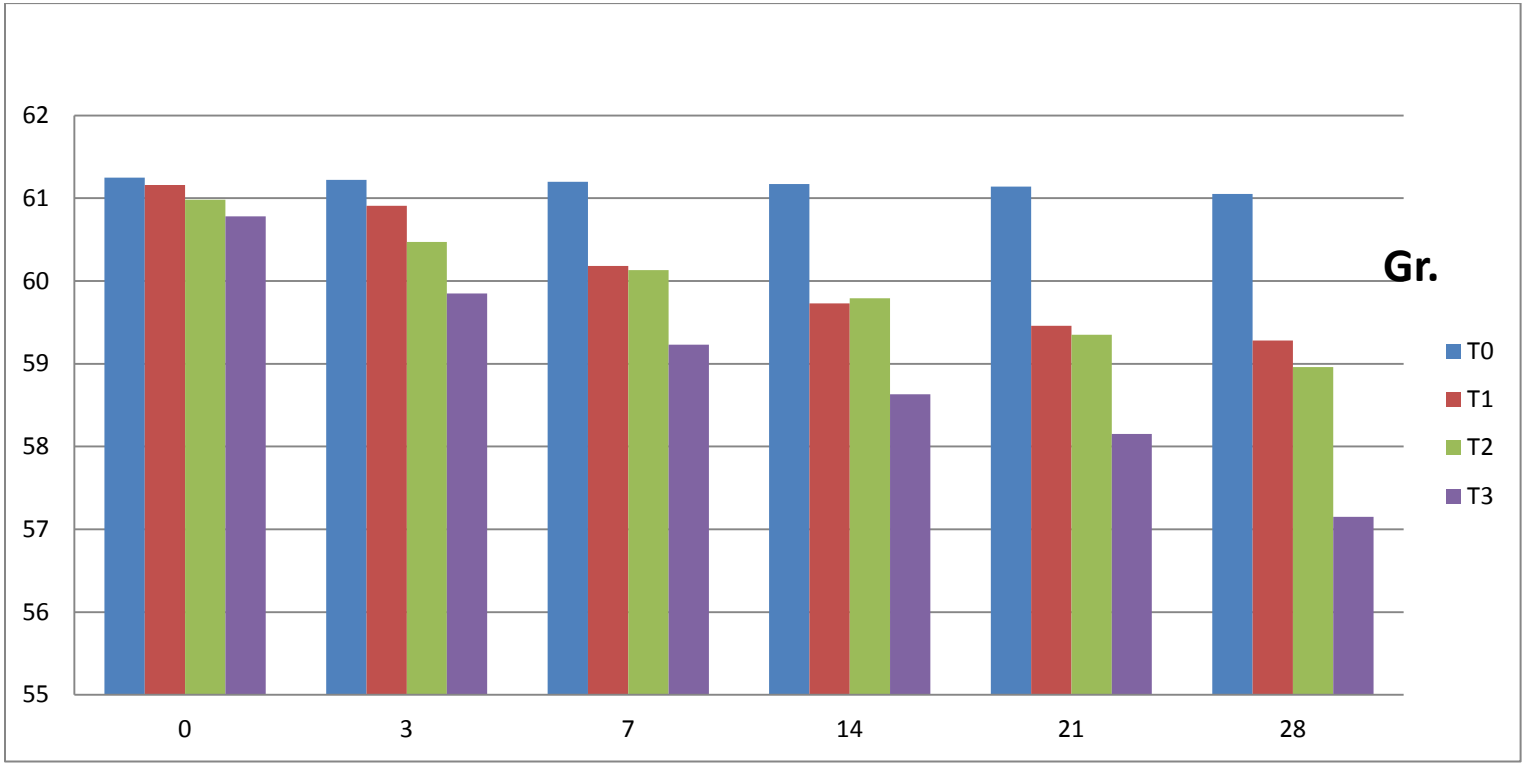


Table 8:- Mean ± SE value of Monocytes concentration (%)with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

GROUP	Days Post- Treatment					
	0	3	7	14	21	28
T_0	6.8±0.36	6.63±0.49	6.60±0.59	6.50^{a}±0.67	6.30^{a}±0.79	6.40^{a}±0.81
T_1	6.71±0.31	6.8±0.55	6.85±0.47	6.89^{a}±0.64	6.92^{a}±0.75	6.97^{a}±0.84
T_2	6.75±0.39	6.81±0.51	6.85±0.68	6.89^{a}±0.71	6.95^{a}±0.67	7.05^{ab}±0.73
T_3	6.83±0.41	7.13±0.45	7.32±0.55	7.46^{b}±0.66	7.53^{b}±0.73	7.82^{b}±0.86

Values with similar superscript (column wise- a, b, c, d) differ significantly ($p<0.05$)

Graph-8:- Bar diagram showing Mean ± SE value of Monocytes concentration (%) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

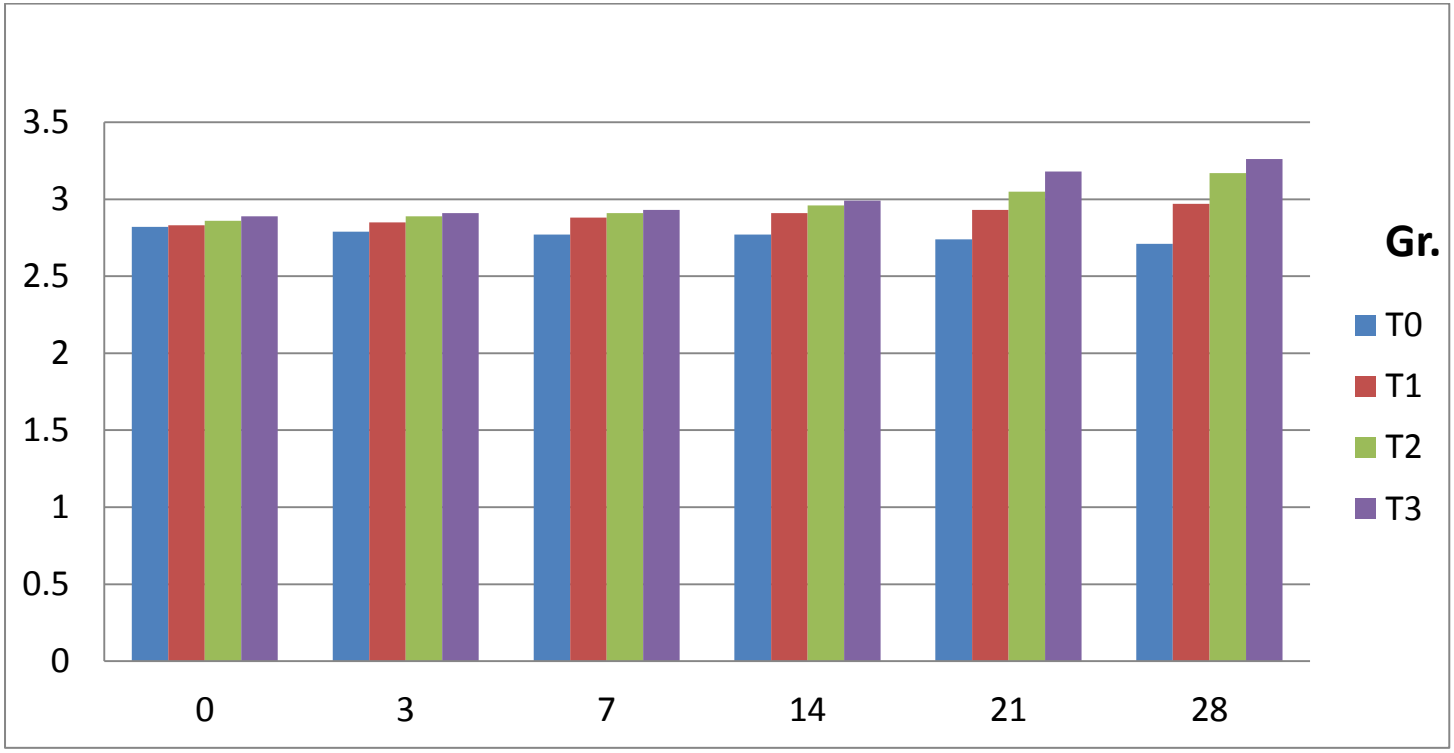


Table 9:- Mean ± SE value of Eosinophils concentration (%) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

GROUP	Days Post- Treatment					
	0	3	7	14	21	28
T_0	2.82±0.25	2.79±0.28	2.77±0.20	2.77±0.23	2.74^{a} ±0.19	2.71^{a}±0.11
T_1	2.83±0.29	2.85±0.25	2.88±0.18	2.91±0.15	2.93^{ab}±0.16	2.97^{a} ±0.18
T_2	2.86±0.21	2.89±0.27	2.91±0.23	2.96±0.25	3.05^{ab} ±0.15	3.17^{ab} ±0.14
T_3	2.89±0.28	2.91±0.24	2.93±0.27	2.99±0.29	3.18^{b} ±0.17	3.26^{b} ±0.13

Values with similar superscript (column wise- a, b, c, d) differ significantly ($p<0.01$)

Graph-9:- Bar diagram showing Mean ± SE value of Eosinophils concentration (%) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

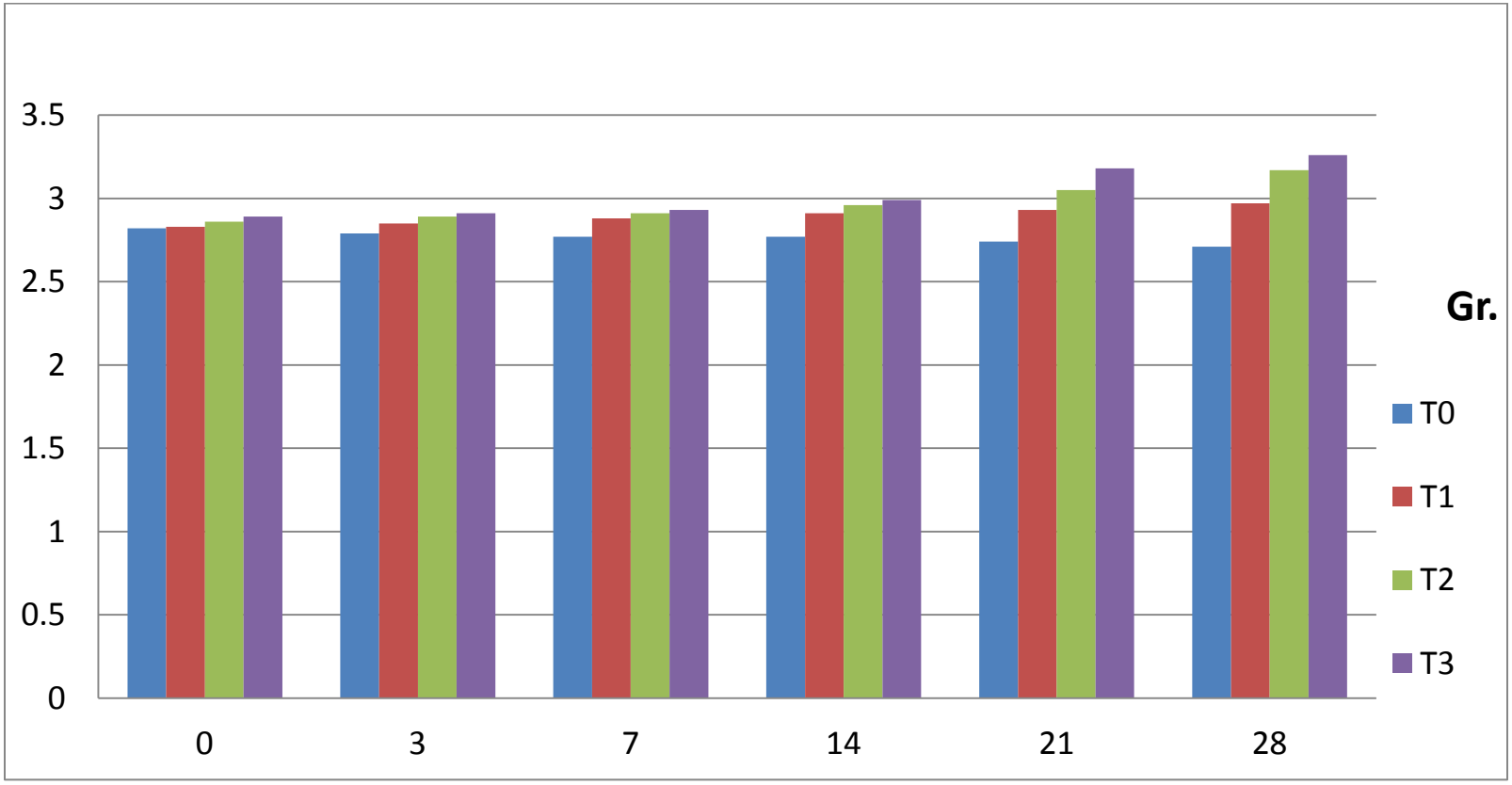


Table 10:- Mean ± SE value of Basophiles concentration (%) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

GROUP	Days Post- Treatment					
	0	3	7	14	21	28
T_0	1.04±0.36	1.06±0.25	1.12±0.21	1.18±0.20	1.24±0.22	1.30±0.27
T_1	0.99±0.29	1.05±0.24	1.11±0.27	1.17±0.21	1.23±0.26	1.28±0.23
T_2	0.97±0.28	1.03±0.20	1.09±0.23	1.11±0.25	1.21±0.23	1.27±0.25
T_3	0.95±0.25	1.02±0.27	1.08±0.18	1.14±0.29	1.20±0.27	1.26±0.19

Values with similar superscript (column wise- a, b, c, d) did not differ significantly ($p<0.01$)

Graph-10:- Bar diagram showing Mean ± SE value of Basophiles concentration (%) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

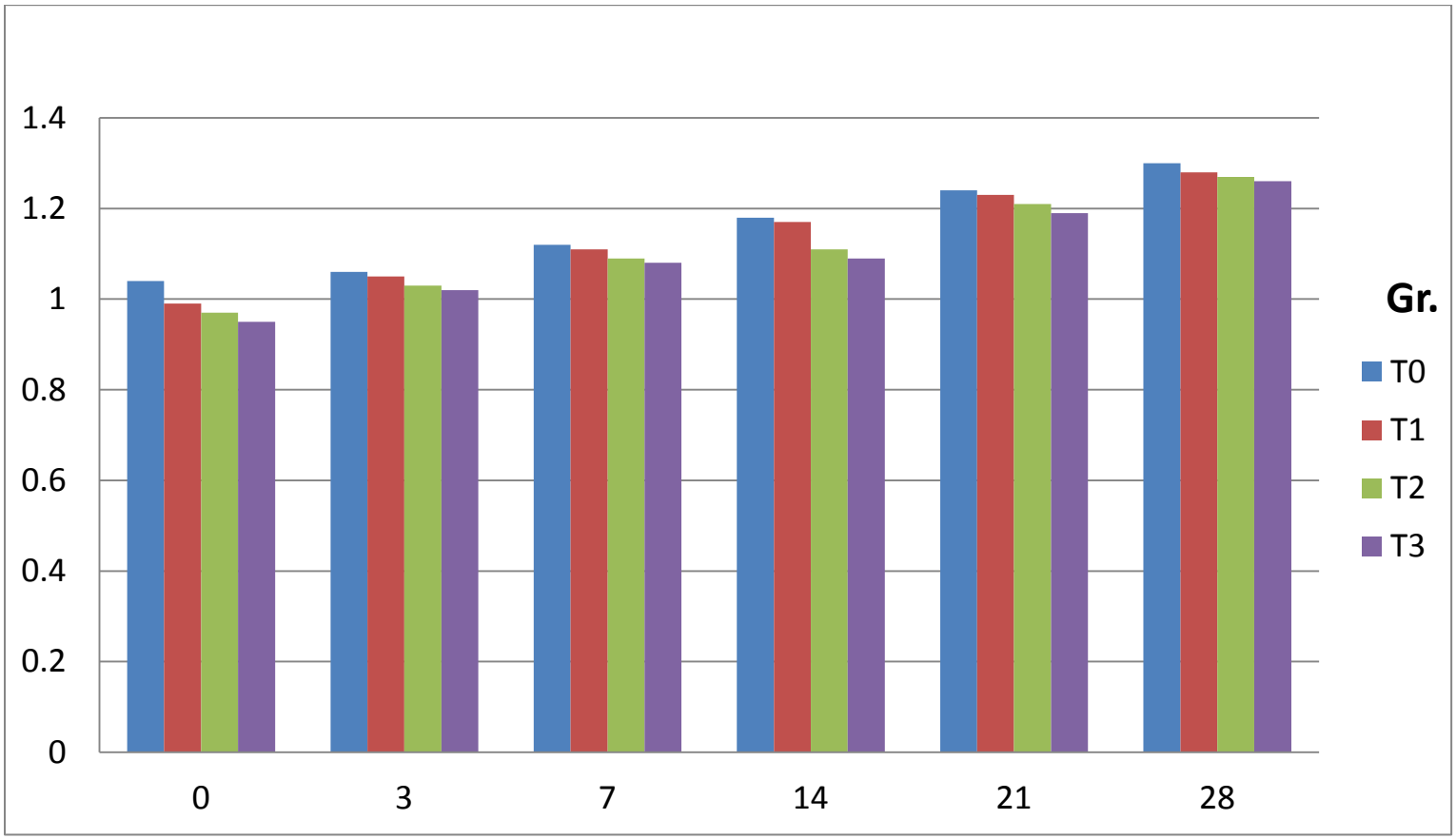


Table 11:- Mean ± SE value of Serum Alanine Aminotransferase (ALT) concentration (I/U) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

GROUP	Days Post- Treatment					
	0	3	7	14	21	28
T_0	18.50±0.267	18.52±0.290	18.42^{a} ±0.193	18.52^{a} ±0.332	18.27^{a} ±0.359	18.32^{a} ±0.252
T_1	18.55±0.580	18.57±0.476	18.65^{a} ±0.172	18.71^{a} ±0.204	18.75^{a} ±0.275	18.85$^{a\ b}$ ±0.329
T_2	18.70±0.976	18.72±0.363	18.85^{a} ±0.170	18.97^{a} ±0.154	19.05^{a} ±0.212	19.15^{b} ±0.379
T_3	18.95±0.197	19.05±0.370	19.75^{b} ±0.232	20.77^{b} ±0.193	20.90^{b} ±0.204	21.70^{c} ±0.453

Values with similar superscript (column wise- a, b, c, d) differ significantly ($p<0.05$)

Graph-11:- Bar diagram showing Mean ± SE value of Serum Alanine Aminotransferase (ALT) concentration (I/U) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

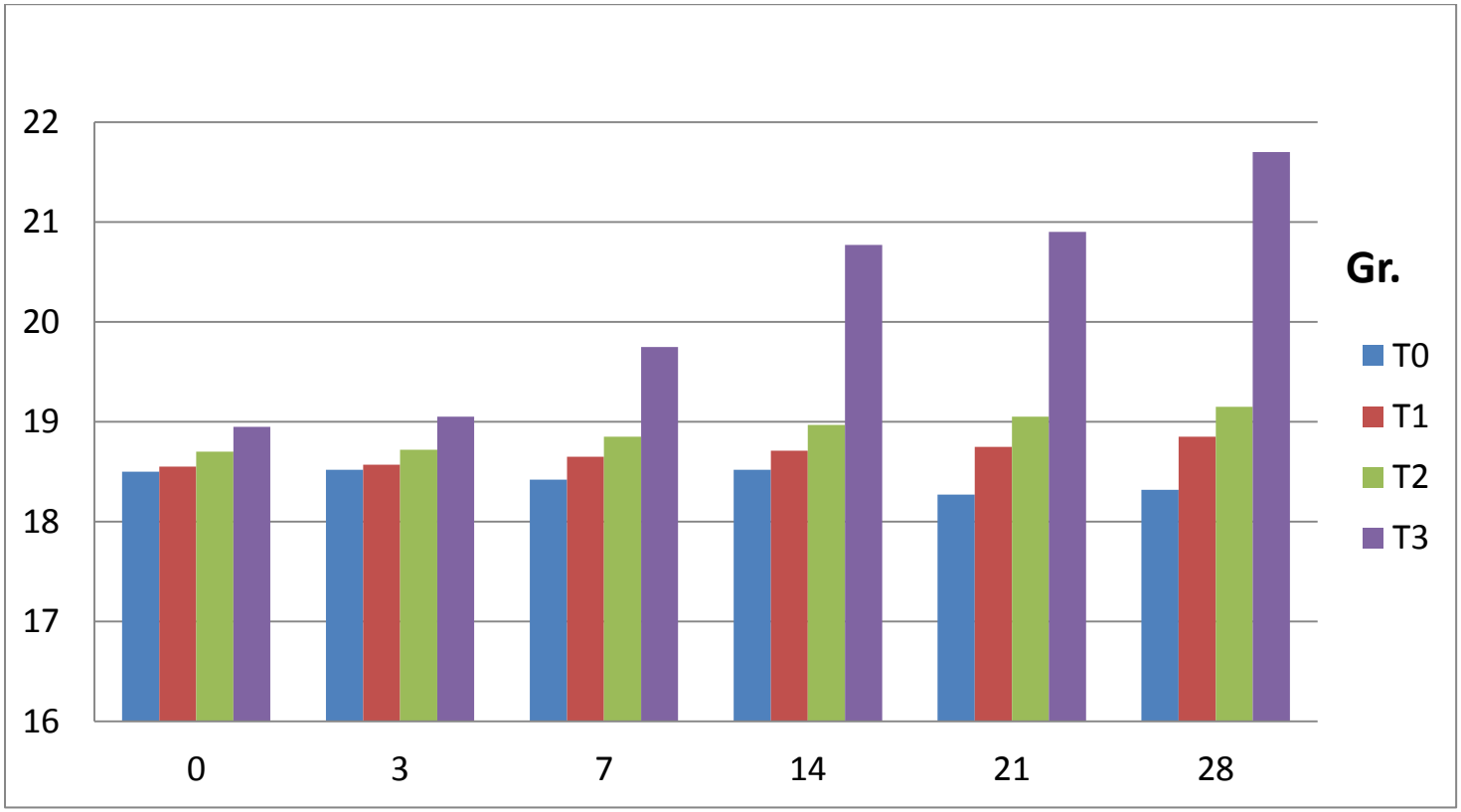


Table 12:- Mean ± SE value of Aspartate aminotransferase (AST) concentration (I/U) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

GROUP	Days Post- Treatment					
	0	3	7	14	21	28
T_0	156.01±2.39	156.08±2.05	156.52^{a} ±2.07	156.57^{a} ±2.49	156.45^{a} ±2.10	156.28^{a} ±2.95
T_1	156.12±2.04	156.15±2.28	156.32^{a} ±2.28	156.47^{a} ±2.83	156.53^{a} ±3.51	156.72^{a} ±3.68
T_2	156.17±3.14	156.25±1.25	159.18^{b} ±2.29	162.28^{b} ±1.37	163.45^{b} ±2.69	164.28^{b} ±4.26
T_3	156.26±2.04	160.19±2.28	160.86^{b} ±2.28	171.65^{c} ±2.83	194.21^{c} ±3.51	196.23^{c} ±3.68

Values with similar superscript (column wise- a, b, c, d) differ significantly ($p<0.05$)

Graph-12:- Bar diagram showing Mean ± SE value of Aspartate aminotransferase (AST) concentration (I/U) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

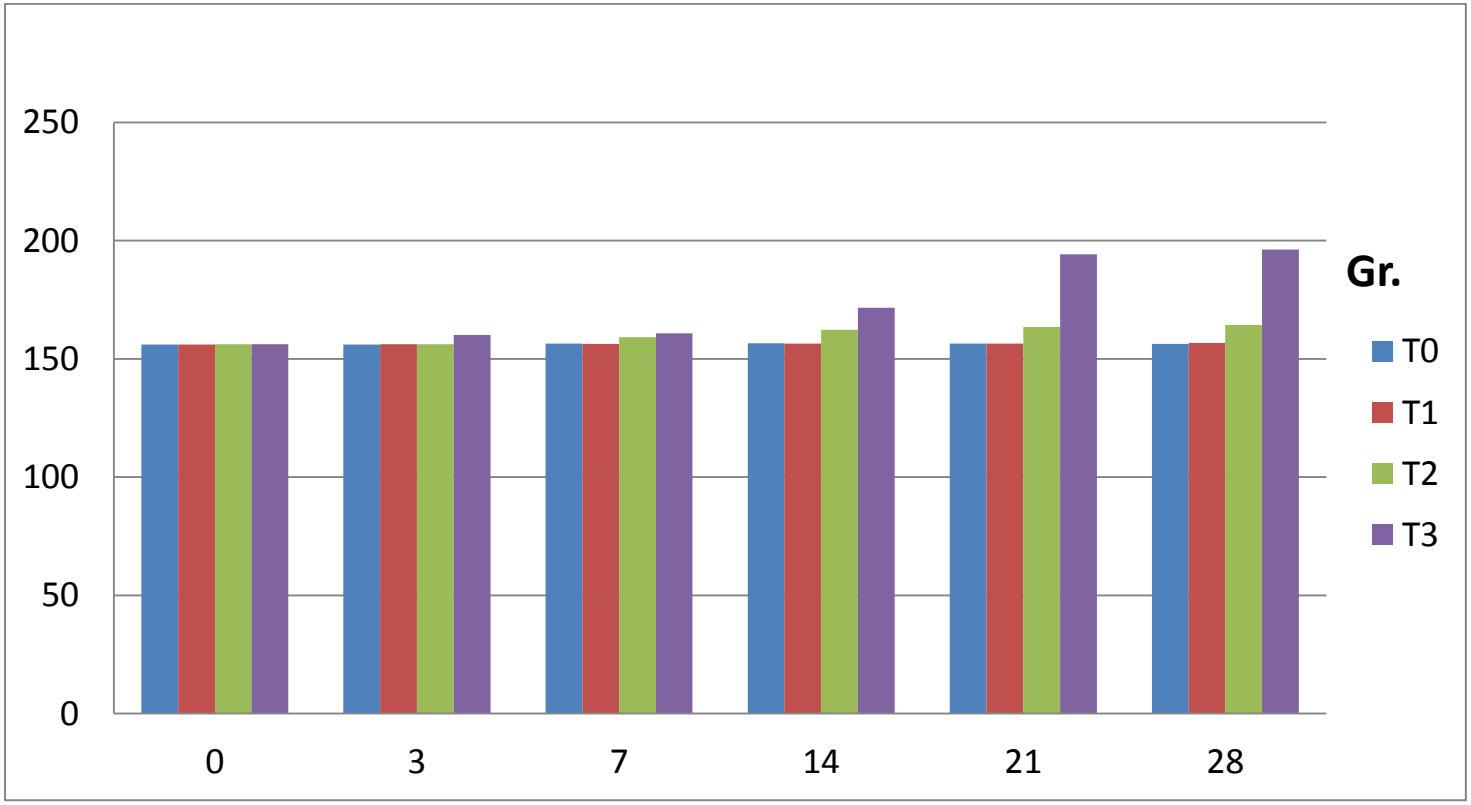


Table 13:- Mean ± SE value of Blood urea nitrogen concentration (mg/dl) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

GROUP	Days Post- Treatment					
	0	3	7	14	21	28
T_0	4.87±0.110	4.80±0.144	4.77±0.075	4.72±0.062	4.67^{a} ±0.052	4.42^{a} ±0.120
T_1	4.81±0.102	4.86±0.202	4.87±0.221	4.90±0.070	4.94^{a} ±0.087	4.96^{a} ±0.084
T_2	4.82±0.205	4.88±0.131	4.92±0.185	5.02±0.088	5.55^{b} ±0.064	6.55^{b} ±0.132
T_3	4.85±0.240	4.93±0.129	4.99±0.648	5.20±0.108	5.76^{b} ±0.082	7.05^{c} ±0.096

Values with similar superscript (column wise- a, b, c, d) differ significantly ($p<0.05$)

Graph-13:- Bar diagram showing Mean ± SE value of Blood urea nitrogen concentration (mg/dl) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

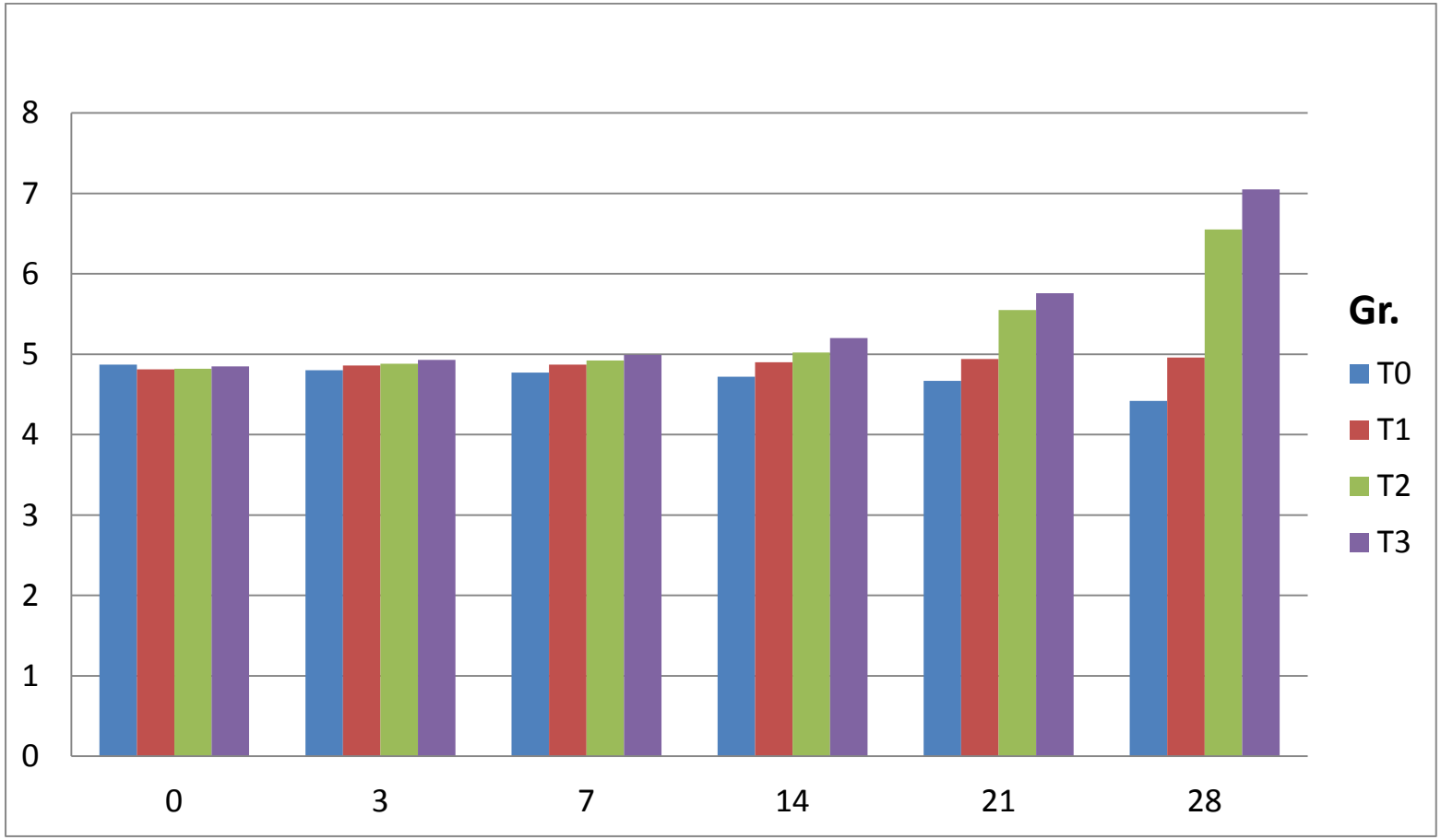


Table 14:- Mean ± SE value of serum creatinine concentration (mg/dl) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

GROUP	Days Post- Treatment					
	0	3	7	14	21	28
T_0	0.23±0.13	0.25^{a} ±0.06	0.24^{a} ±0.20	0.26^{a} ±0.04	0.25^{a} ±0.03	0.26^{a} ±0.02
T_1	0.24±0.18	0.25^{a} ±0.09	0.27^{a} ±0.01	0.27^{a} ±0.02	0.30^{a} ±0.07	0.31^{a} ±0.08
T_2	0.26±0.04	0.26^{a} ±0.12	0.29^{a} ±0.05	0.35^{a} ±0.09	0.43^{a} ±0.11	0.58^{b} ±0.46
T_3	0.28±0.05	0.36^{b} ±0.10	0.44^{b} ±0.09	0.57^{b} ±0.07	0.66^{b} ±0.10	0.79^{c} ±0.01

Values with similar superscript (column wise- a, b, c, d) differ significantly ($p<0.05$)

Graph-14:- Bar diagram showing Mean ± SE value of serum creatinine concentration (mg/dl) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

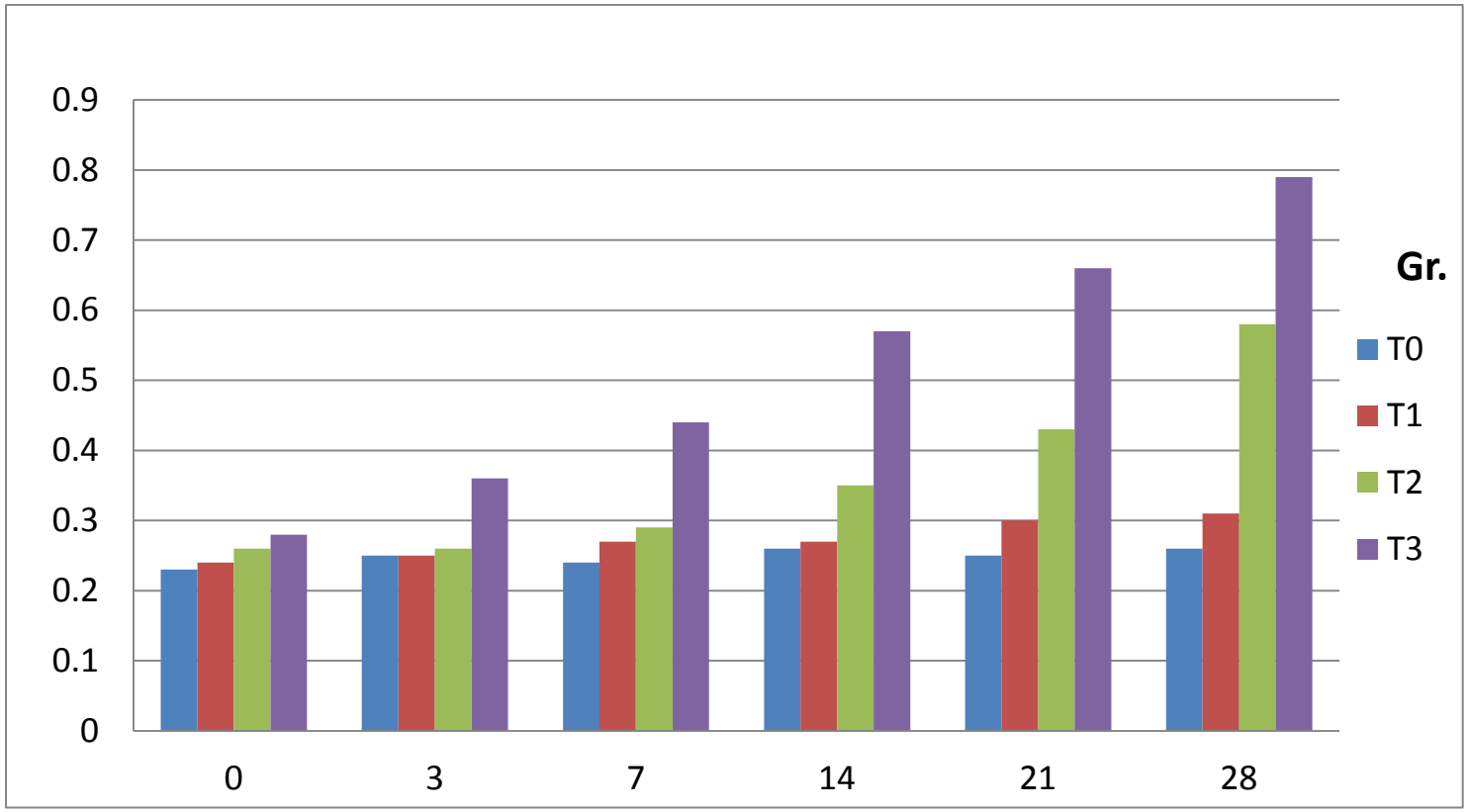


Table 15:- Mean ± SE value of Total Albumin concentration (g/dl) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

GROUP	Days Post- Treatment					
	0	3	7	14	21	28
T_0	3.87±0.125	3.85±0.062	3.92±0.853	3.93^{a} ±0.086	3.82^{a} ±0.113	3.85^{a} ±0.113
T_1	3.85±0.129	3.84±0.091	3.82±0.091	3.80^{a} ±0.095	3.75^{a} ±0.119	3.72^{a} ±0.084
T_2	3.83±0.098	3.82±0.070	3.79±0.085	3.68^{b} ±0.112	$3.61^{a\ b}$ ±0.111	3.27^{b} ±0.094
T_3	3.80±0.097	3.77±0.110	3.65±0.096	3.63^{b} ±0.177	3.47^{b} ±0.095	3.08^{c} ±0.108

Values with similar superscript (column wise- a, b, c, d) differ significantly ($p<0.05$)

Graph-15:- Bar diagram showing Mean ± SE value of Total Albumin concentration (g/dl) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

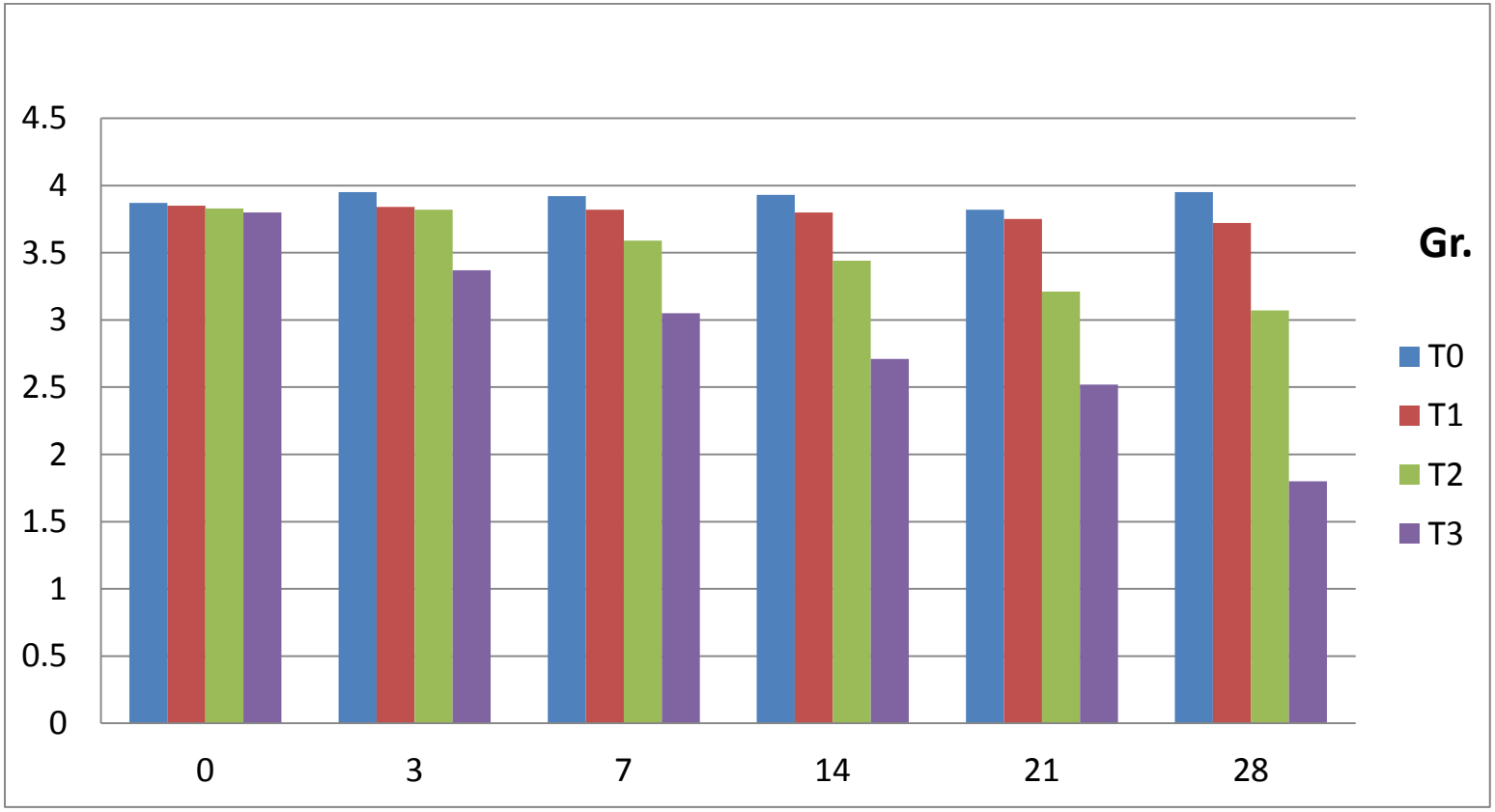


Table 16:- Mean ± SE value of Total protein concentration (g/dl) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

GROUP	Days Post- Treatment					
	0	3	7	14	21	28
T_0	5.76±0.125	5.75±0.062	5.71±0.053	5.82^{a} ±0.086	5.92^{a} ±0.113	5.915^{a} ±0.113
T_1	5.74±0.129	5.72±0.091	5.70±0.091	5.68^{a} ±0.095	5.65^{a} ±0.119	5.62^{a} ±0.084
T_2	5.67±0.098	5.67±0.070	5.62±0.085	5.55^{a} ±0.112	5.52^{a} ±0.111	5.45^{a} ±0.094
T_3	5.65±0.097	5.63±0.110	5.51±0.096	4.49^{b} ±0.177	4.43^{b} ±0.095	4.10^{b} ±0.108

Values with similar superscript (column wise- a, b, c, d) differ significantly ($p<0.05$)

Graph-16:- Bar diagram showing Mean ± SE value of Total protein concentration (g/dl) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

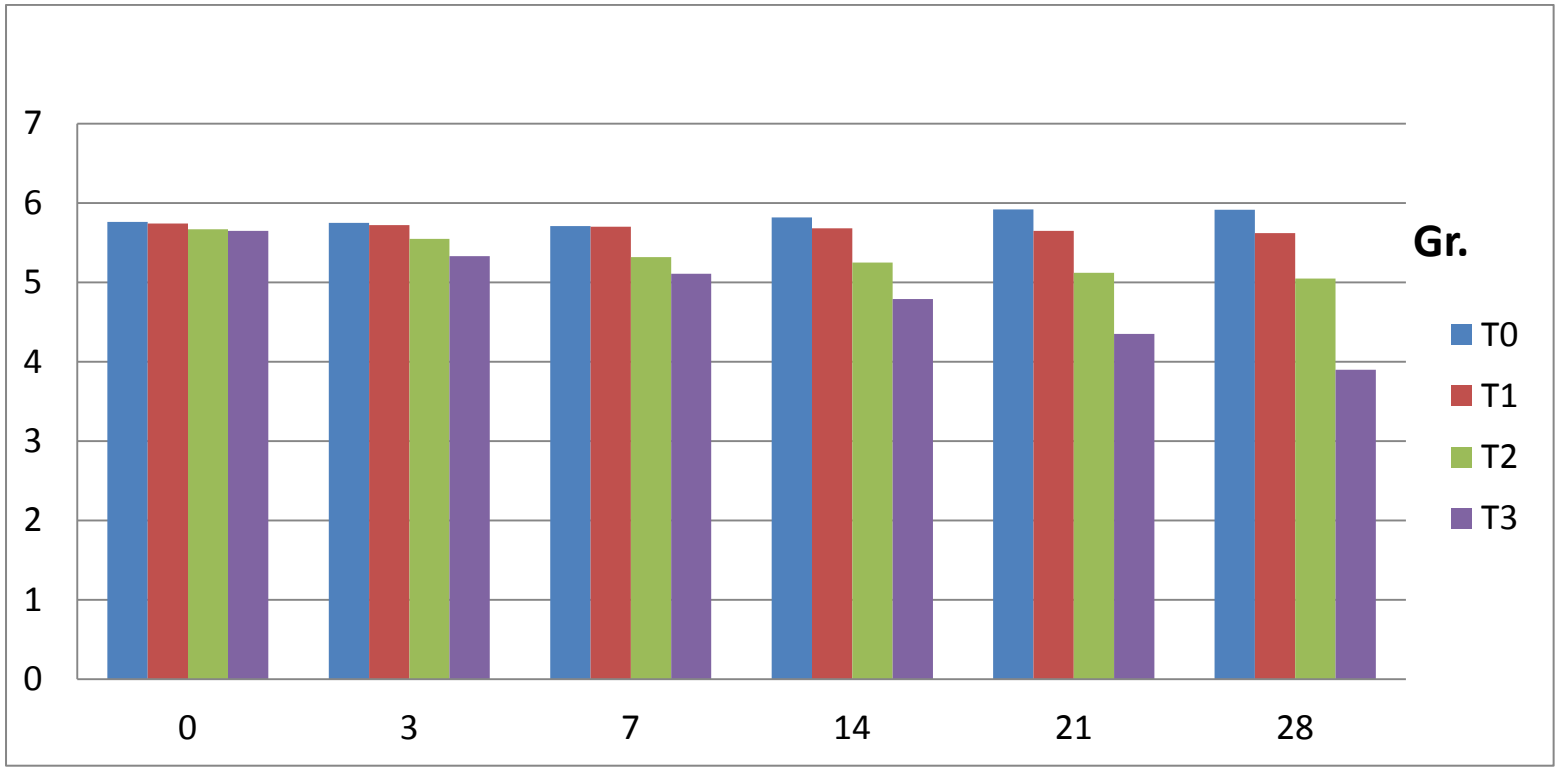


Table 17:-Mean ± SE value of Uric acid concentration (mg/dl) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

GROUP	Days Post- Treatment					
	0	3	7	14	21	28
T_0	4.73±0.349	4.72±0.174	4.71±0.231	4.66^{a} ±0.213	4.63^{a} ±0.068	4.63^{a} ±0.085
T_1	4.71±0.094	4.73±0.111	4.75±0.112	4.76^{a} ±0.085	4.79^{a} ±0.070	4.85^{a} ±0.098
T_2	4.73±0.198	4.77±0.175	4.80±0.052	4.95$^{a\ b}$ ±0.032	5.15^{a} ±0.071	5.26^{b} ±0.095
T_3	4.76±0.198	4.81±0.077	4.87±0.062	5.08^{b} ±0.048	5.32^{b} ±0.097	5.92^{c} ±0.043

Values with similar superscript (column wise- a, b, c, d) differ significantly ($p<0.05$)

Graph-17:-Bar diagram showing Mean ± SE value of Uric acid concentration (mg/dl) with and without levofloxacin supplementation at 0, 3, 7, 14, 21, & 28th day in broiler birds.

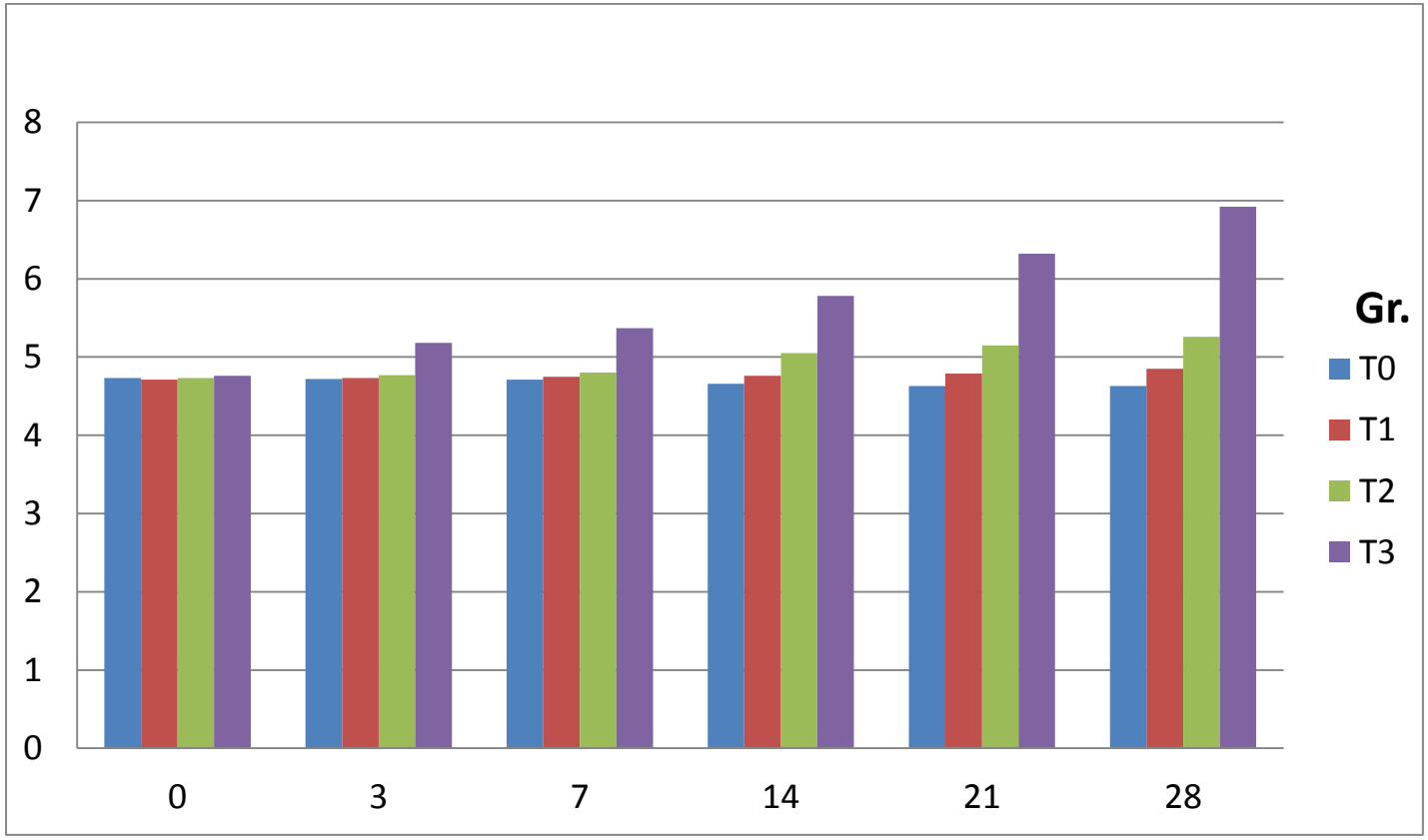


Table 18:- Intra- and inter-day assay coefficient of variation (CV %) and nominal concentration (%) of levofloxacin for residue analysis in liver and breast muscle tissue of broiler chicken.

	Intra-day assay (n= 8)			Inter-day assay (n= 8)		
Concentration (ng/ml)	Mean ±SD (ng/ml)	CV (%)	Mean concentration %	Mean ± SD (ng/ml)	CV (%)	Mean concentration (%)
100 (LLQC)	98.86±0.30	10.65	98.76	97.98.46±0.26	11.46	92.84
300 (LQC)	291.68±0.12	12.08	97.23	261.46±0.13	13.41	87.15
5000 (MQC)	4425.78±0.20	6.87	88.52	4671.08±0.21	7.77	93.42
9000HQC	8206.93±6.16	6.61	91.19	7315.38±7.04	7.65	81.28

Graph-18:- Standard curve for levofloxacin extracted blank tissue homogenate sample

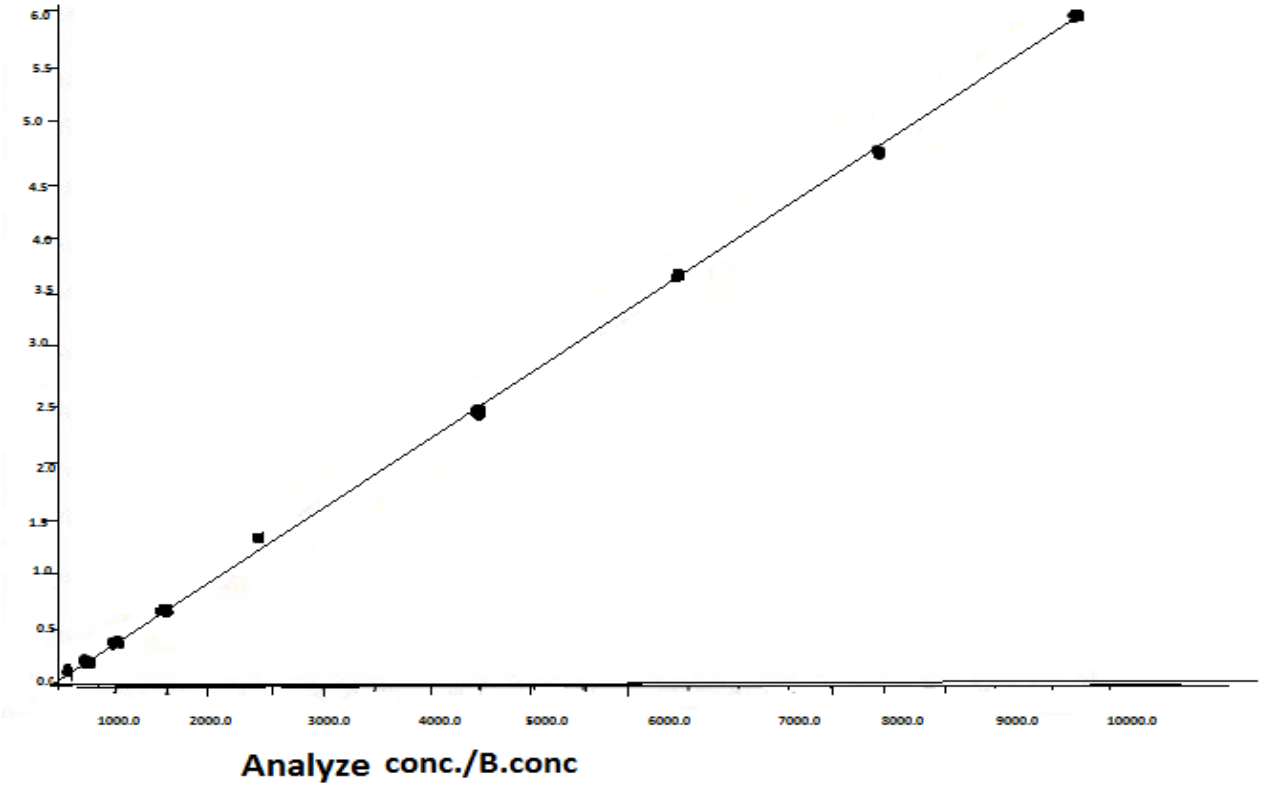


Table 19: Levofloxacin concentration (µg per kg) in liver and breast muscle from Day 1 to da10 after the last dose @10 mg/kg.

Days	Liver	Breast muscle
1	1221.89 ± 0.73	427.66 ± 0.92
2	574.74 ± 1.32	208.03 ± 0.62
3	334.04 ± 0.79	159.23 ± 0.71
4	185.20 ± 0.91	118.87 ± 0.76
5	149.53 ± 1.02	71.33 ± 0.62
6	131.75 ± 0.79	45.83 ± 0.74
7	110.64 ± 0.82	34.53 ± 0.56
8	90.79 ± 0.61	25.41± 0.94
9	73.71 ± 0.51	19.29 ± 0.89
10	55.82 ± 0.27	15.42 ± 0.56

Graph-19: -Levofloxacin concentration (µg per kg) in liver and breast muscle from Day 1 to 10 after the last dose @10 mg/kg

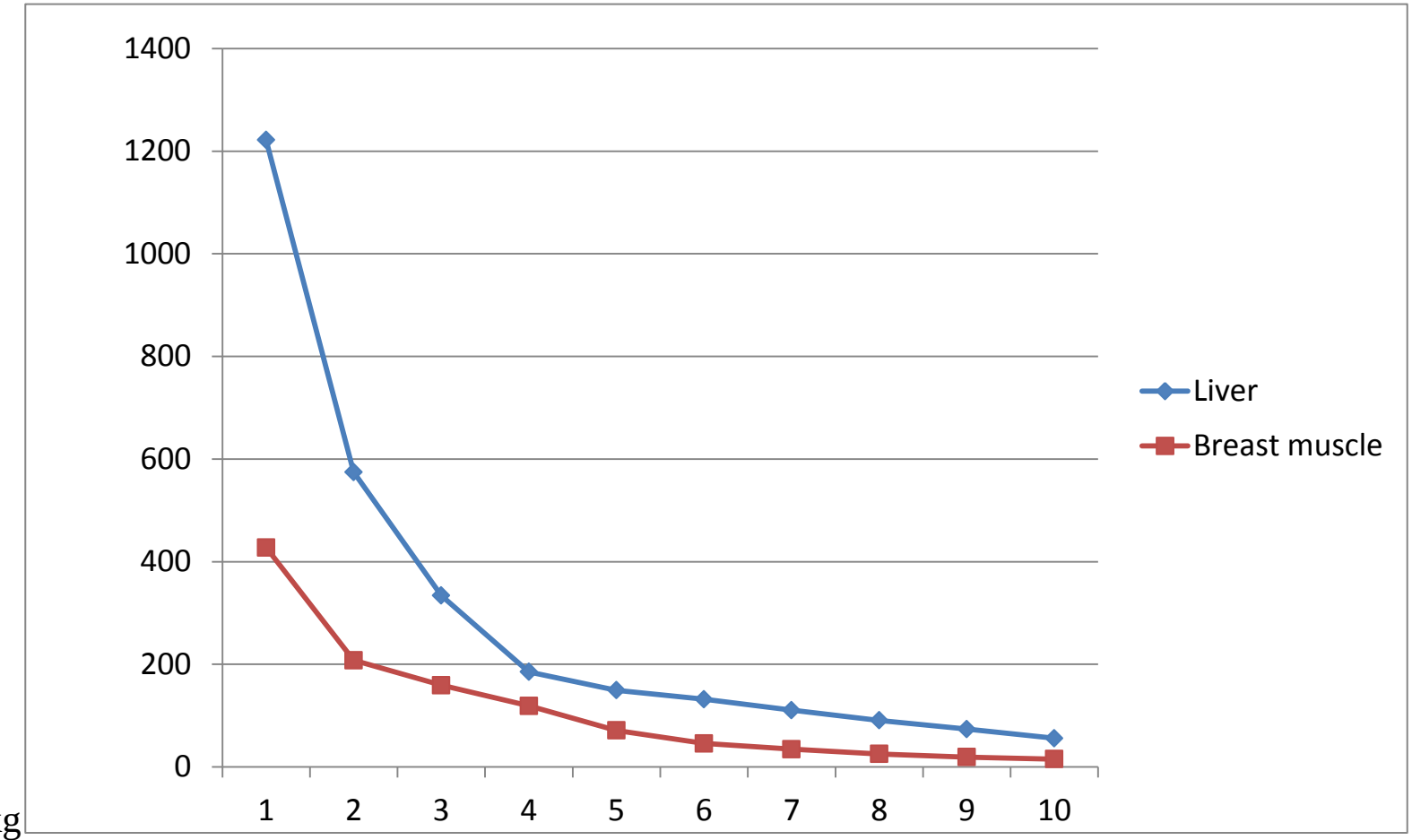
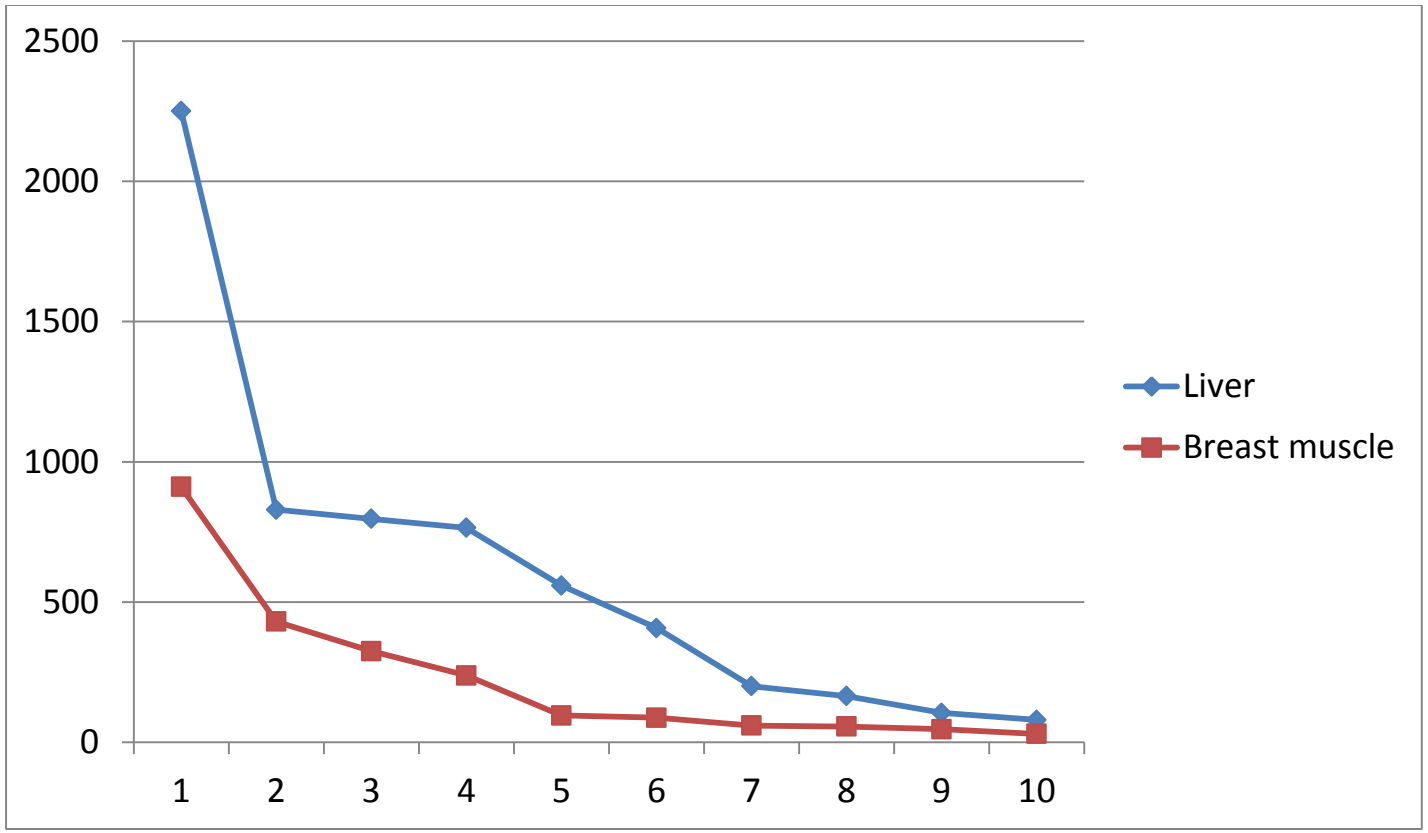


Table 20: Levofloxacin concentration (µg per kg) in liver and breast muscle from Day 1 to day 10 after the last dose @20 mg/kg.

Days	Liver	Breast muscle
1	2250.68 ± 10	910.87 ± 23
2	829.61 ± 18	430.51±35
3	797.05 ± 25	325.26±28
4	765.25 ± 35	238.4 ± 32
5	559.12 ± 32	95.89 ± 52
6	407.42 ± 28	88.21 ± 40
7	200.17 ± 12	60.1±20
8	165.21 ± 15	56.32 ± 15
9	105.23 ± 10	47.13±13
10	80.15 ± 14	30.5±8.97

Graph-20:- Levofloxacin concentration (µg per kg) in liver and breast muscle from Day 1 to 10 after the last dose @20 mg/kg.


2500
2000
1500
1000
500
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1
2
3
4
5
6
7
8
9
10
Liver
Breast muscle

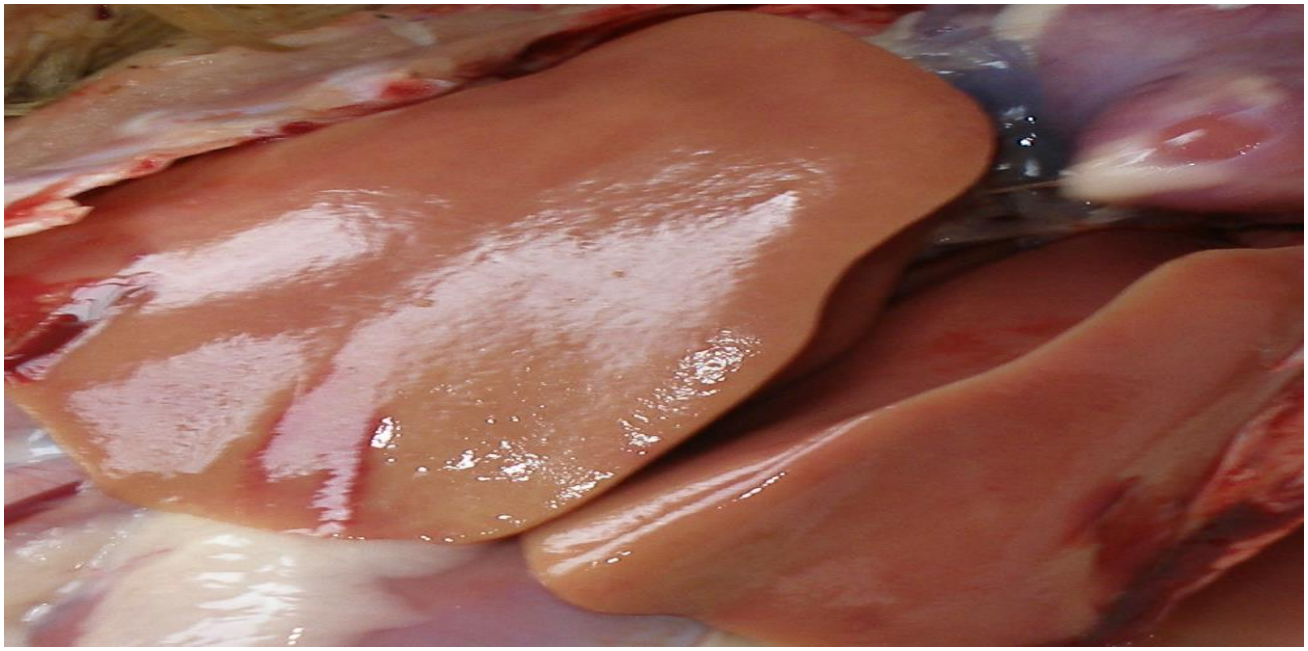

Fig No:- 1: Mild hepatitis showing discoloration of liver parenchyma in T3 group birds treated with levofloxacin @ 30 mg/kg b.wt .

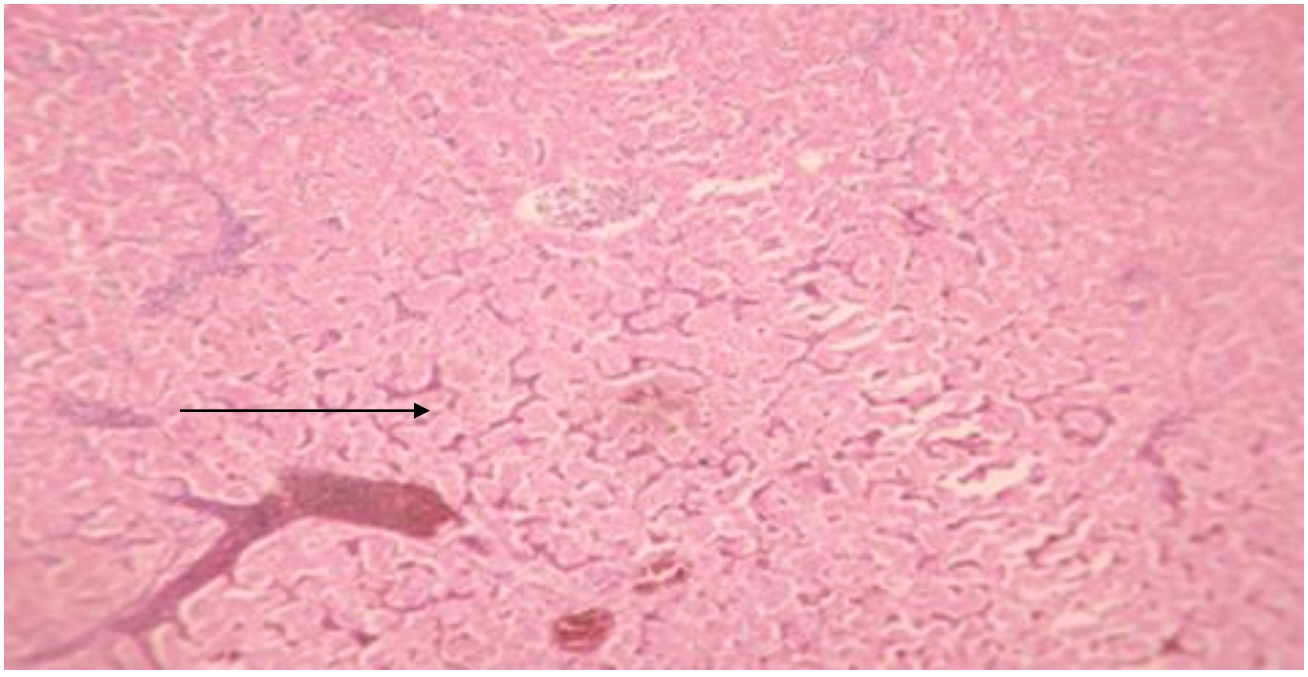

Fig No:- 2: Photomicrograph of section of Liver treated with levofloxacin @20 mg/kg b.wt. showing congestion in blood vessels, congestion in sinusoids and mild necrosis in the parenchyma. H. & E.; X 40.

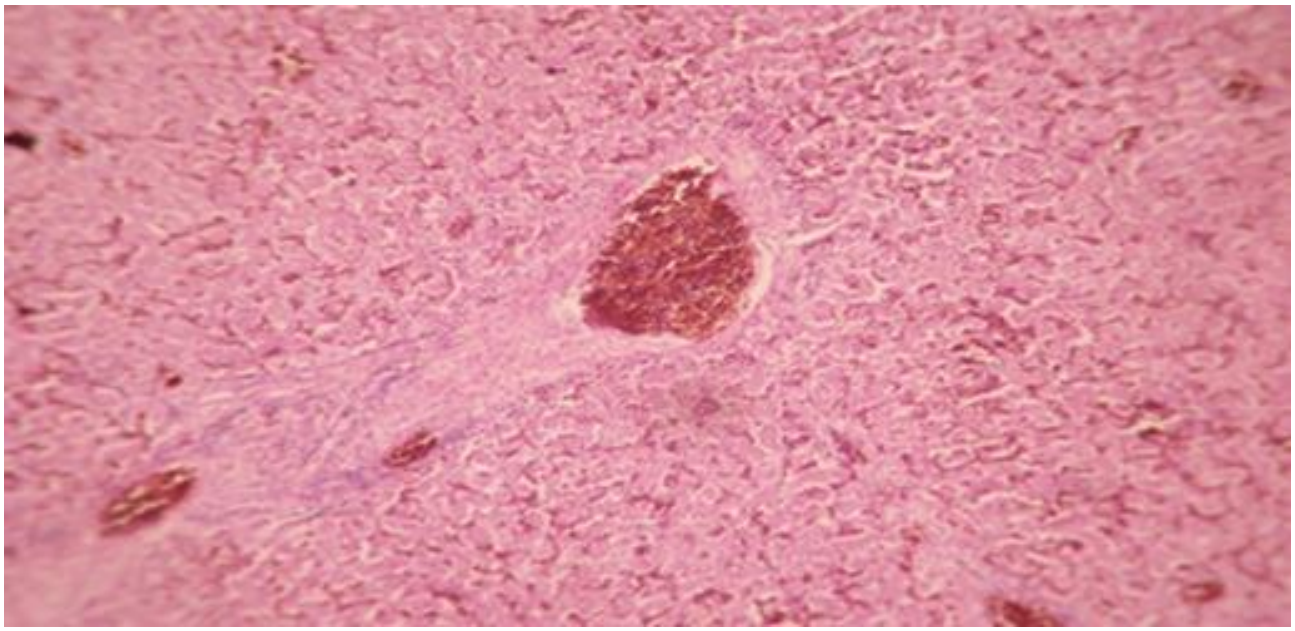

Fig No:-3: Photomicrograph of section of Liver treated with Levofloxacin @ 30mg/kg b.wt. Showing congestion in blood vessels, congestion in sinusoids, necrosis in the parenchyma and connective tissue proliferation between the lobules. H. & E.; X 40.

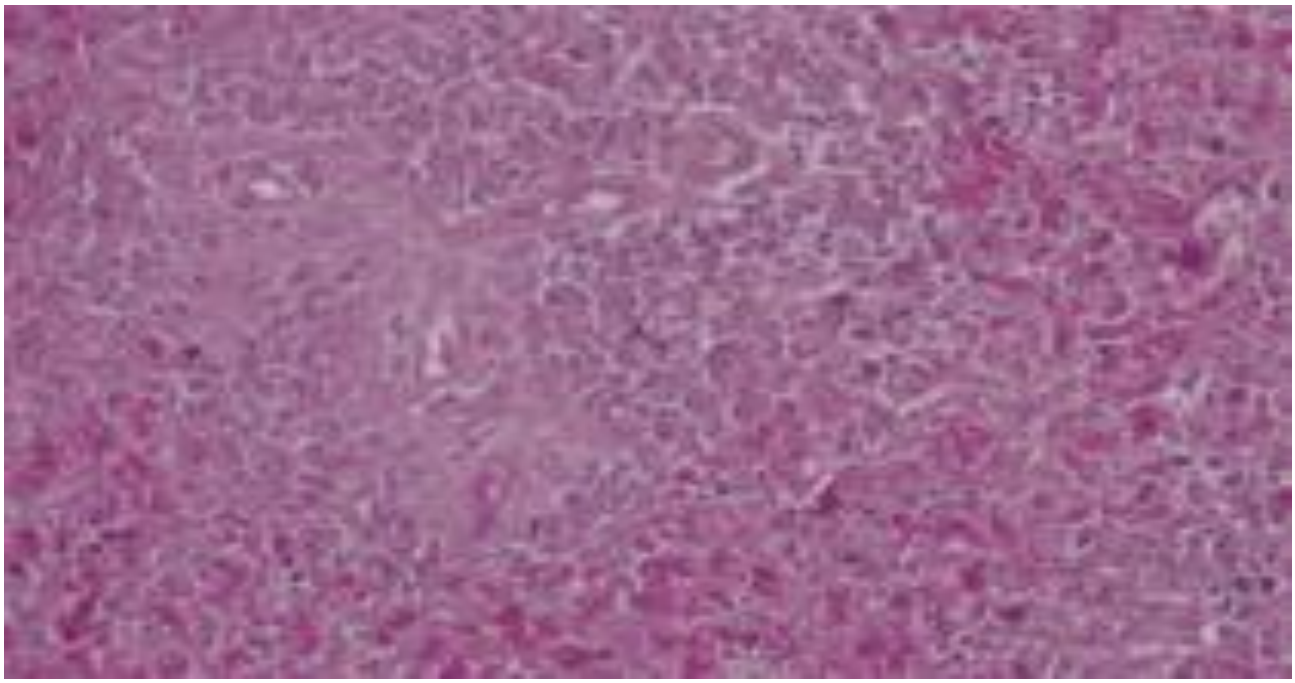

Fig No:- 4: Photomicrograph of section of spleen treated with Levofloxacin @ 30 mg/kg b.wt. showing increase in the number of lymphoid follicles and mild depletion of lymphocytes with lot of blast cell proliferation. H. & E.; X 40.

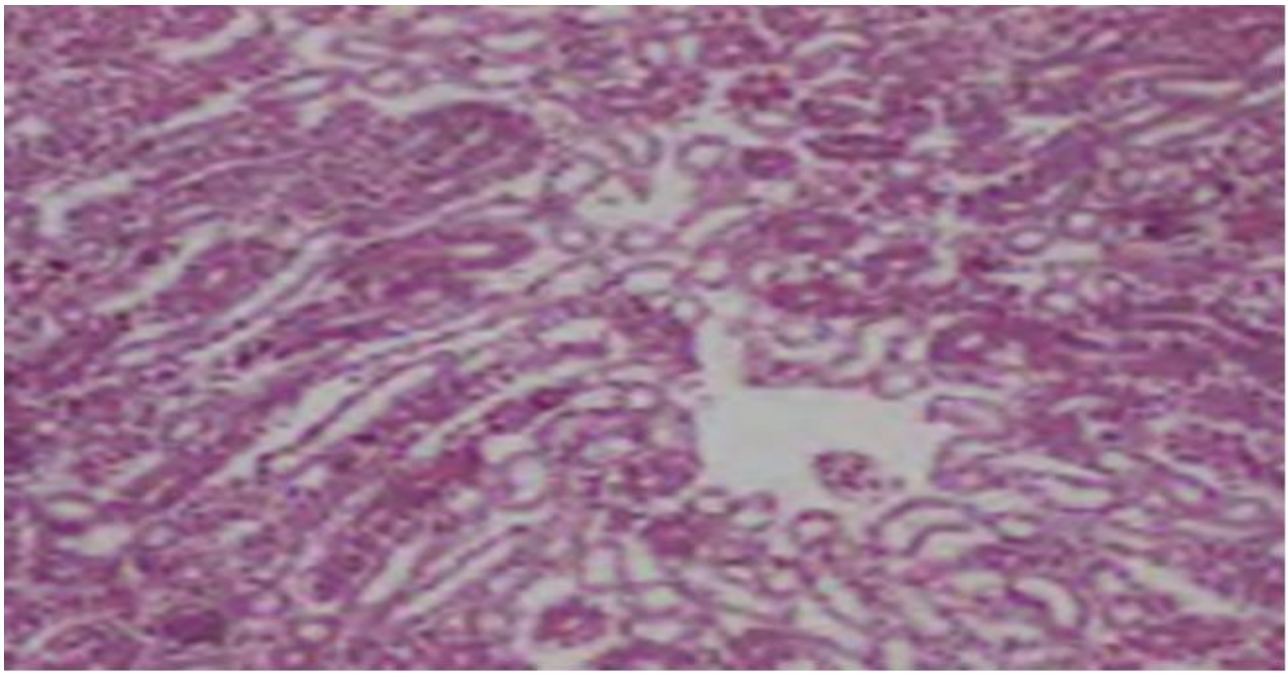

Fig No:- 5: Photomicrograph of section of kidney treated with levofloxacin @20 mg/kg b.wt. Showing Mild tubular degenerative changes. H. & E.; x 4

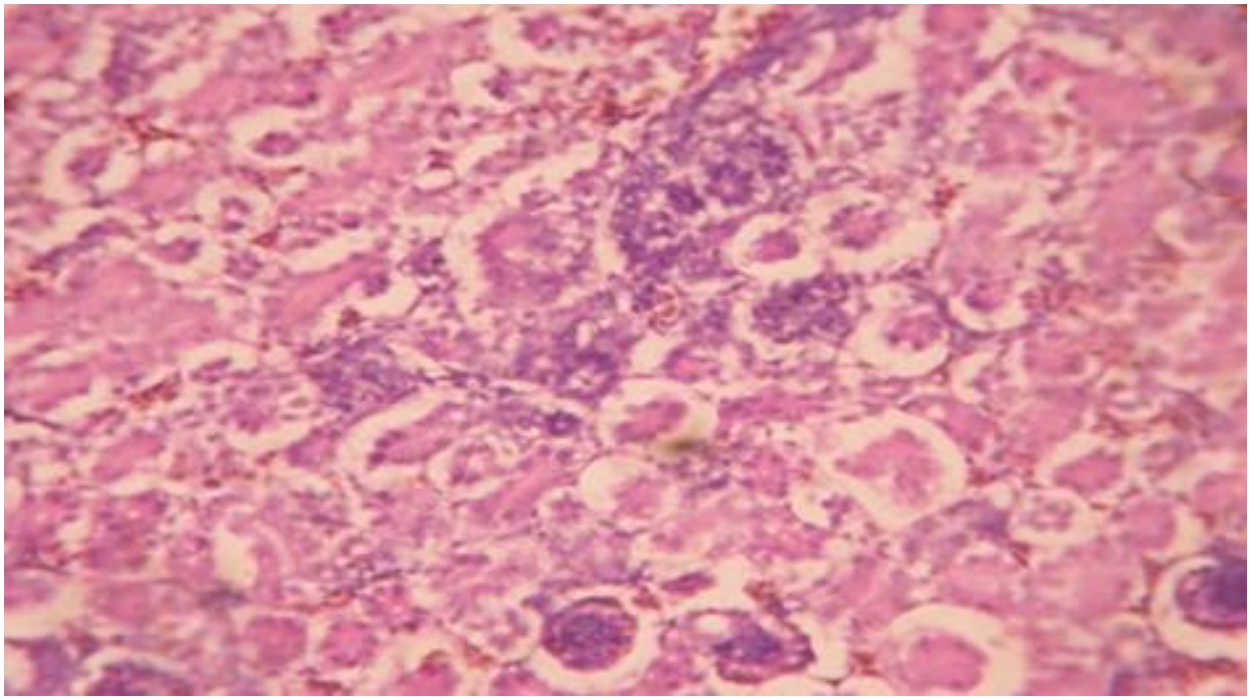

Fig No:- 6: Photomicrograph of section of Kidney treated with levofloxacin @ 30 mg/kg b.wt. Showing degeneration of tubules, infiltrations of mononuclear cells H .& E. ; x 40.

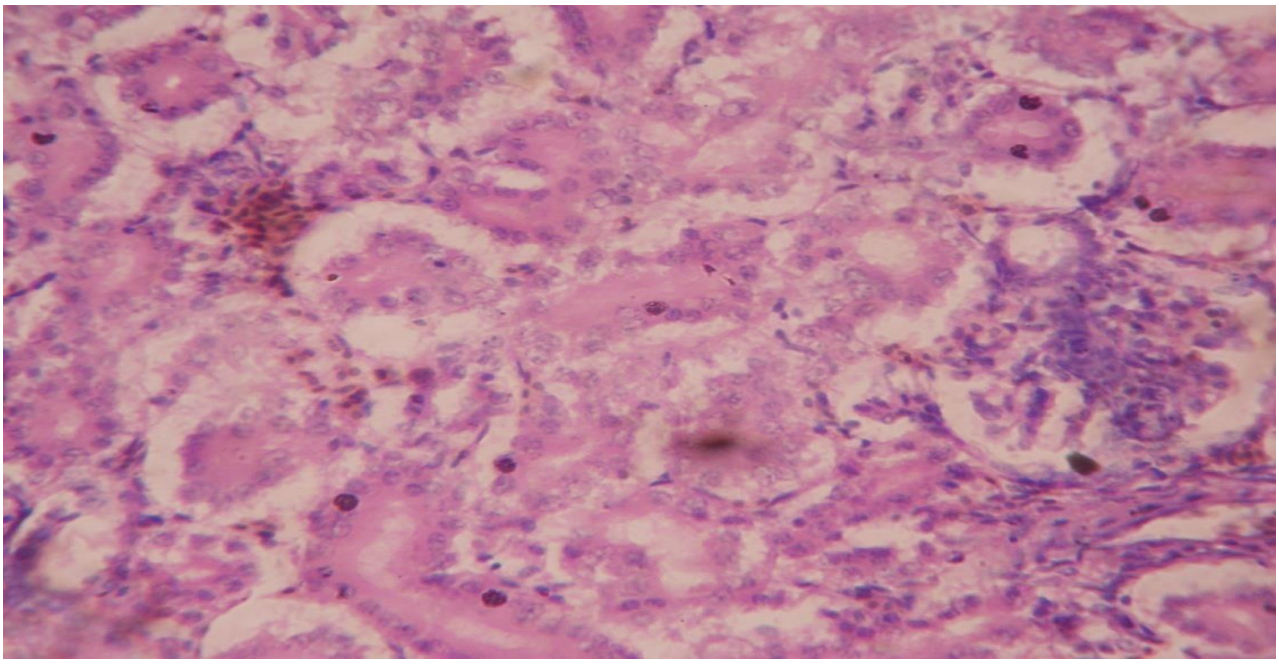

Fig No:- 7: Photomicrograph of section of Kidney treated with levofloxacin @ 30 mg/kg b.wt. Showing degeneration of tubules, Congestion and infiltrations of mononuclear cells H. & E.; x 40.

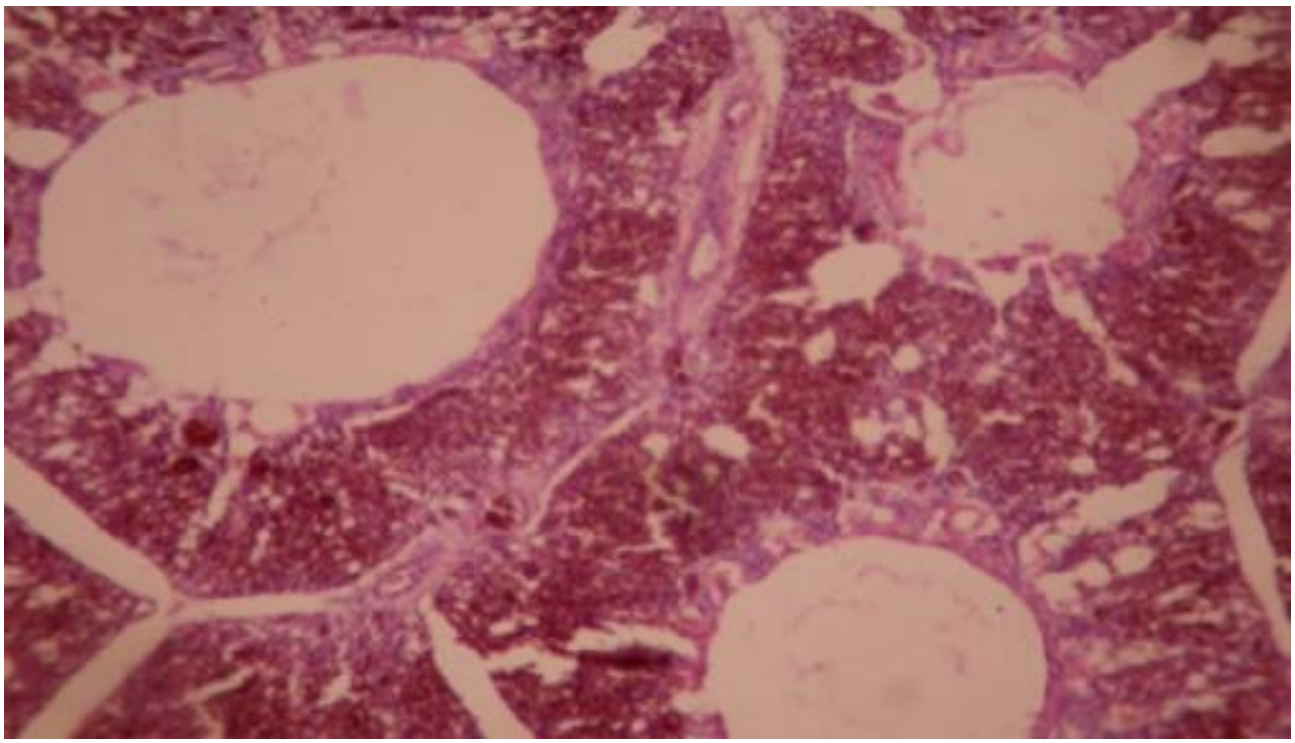

Fig No:- 8: Photomicrograph of section of Lung treated with levofloxacin @ 30 mg/kg b.wt. Showing proliferation of connective tissue between the alveoli and interlobular spaces. H. & E.; X 40

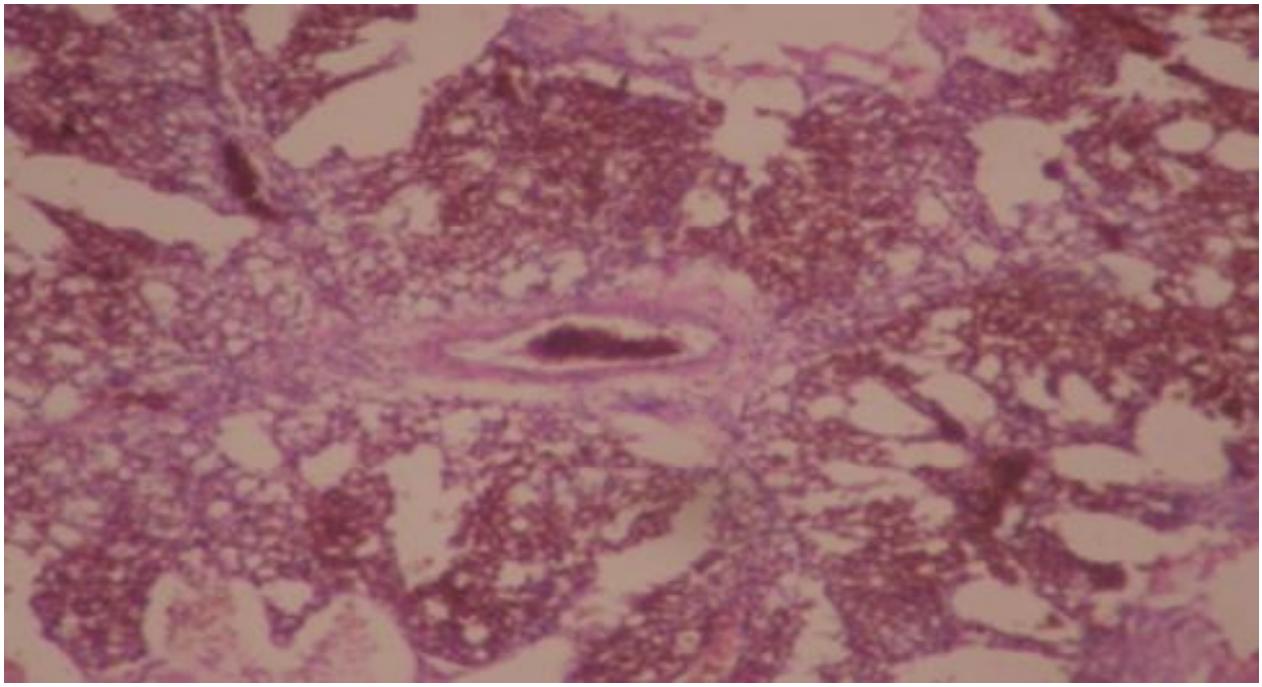

Fig No:- 9: Photomicrograph of section of Lung treated with levofloxacin @ 30 mg/kg b.wt. Showing proliferation of connective tissue between the alveoli and interlobular spaces. H. & E.; X 40

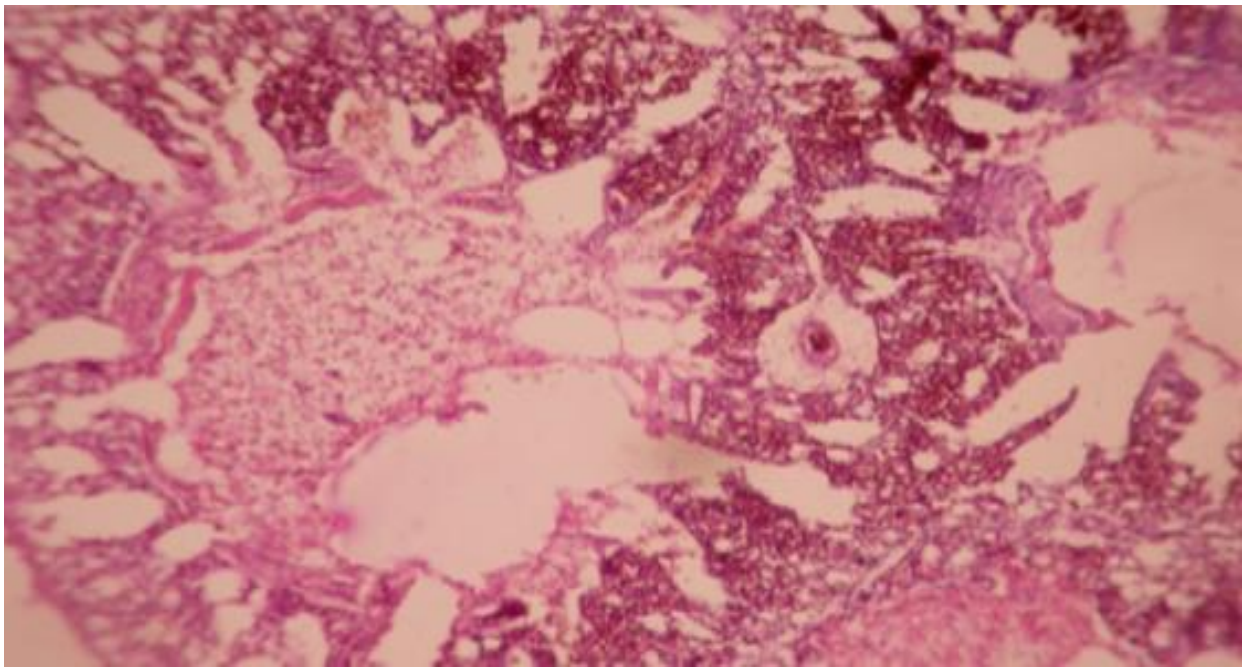

Fig No:- 10: Photomicrograph of section of Lungs treated with levofloxacin @ 20 mg/kg b.wt. Showing congestion in the alveoli and oedema in the bronchi. H. & E.; X 40.

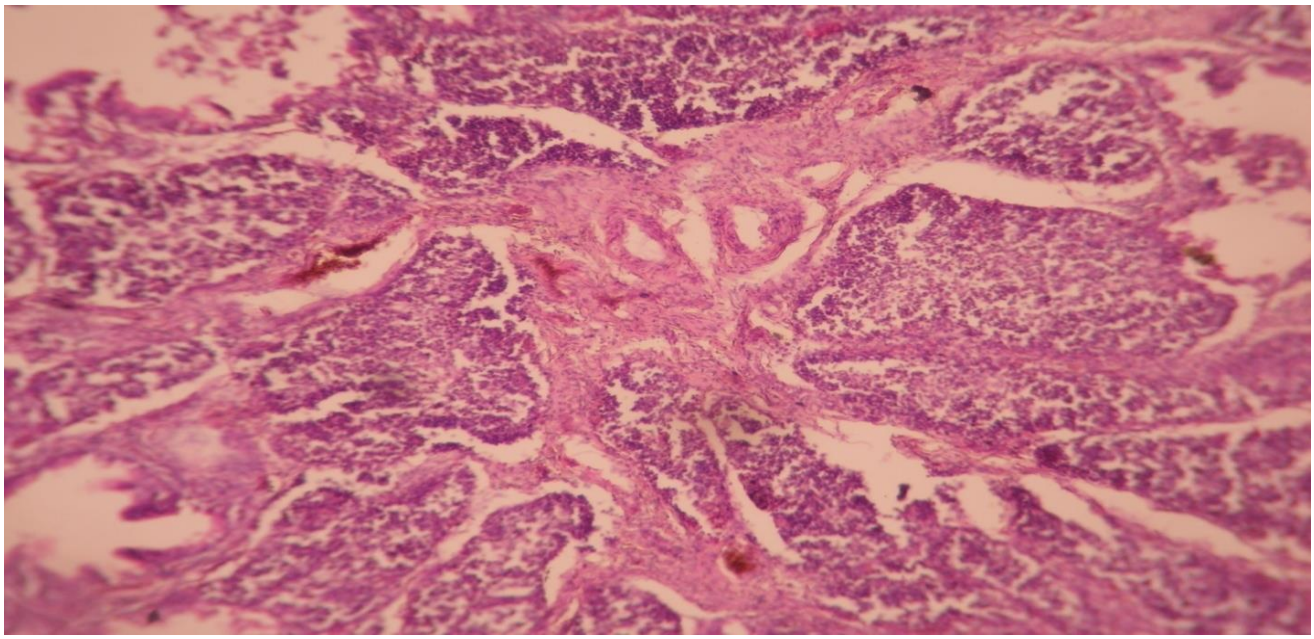

Fig No :- 11: Photomicrograph of section of bursa treated with levofloxacin @ 30 mg/kg b.wt. Showing profuse proliferation of connective tissue between the interfollicular space H .& E. ; x 40.

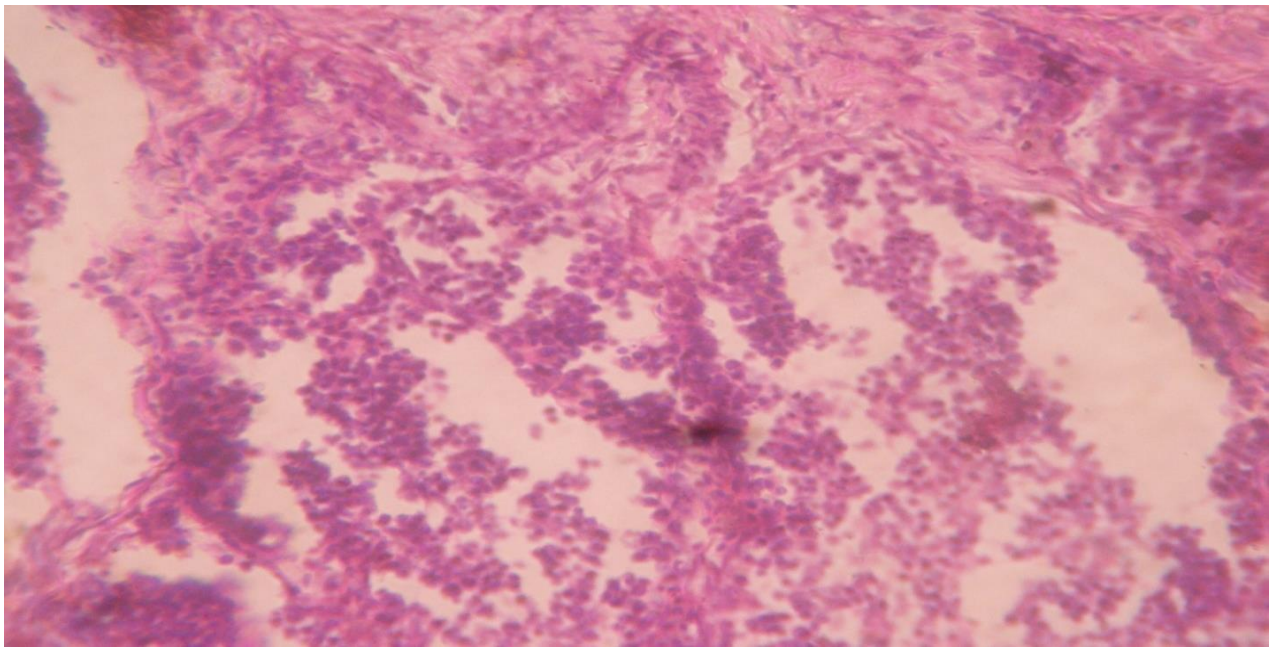

Fig No:-12: Photomicrograph of section of bursa treated with levofloxacin @ 30 mg/kg b.wt. Showing decrease the number of lymphocytes in the follicle. H .& E. ; x 40.

Fig No:-13: Photograph of intestine showing mild haemorrhagic enteritis and congestion in poultry treated with levofloxacin @ 20 mg/kg b.wt.

Fig :-14 Photograph of intestine showing Sever haemorrhagic enteritis in broiler birds treated with levofloxacin @ 30 mg/kg b.wt.

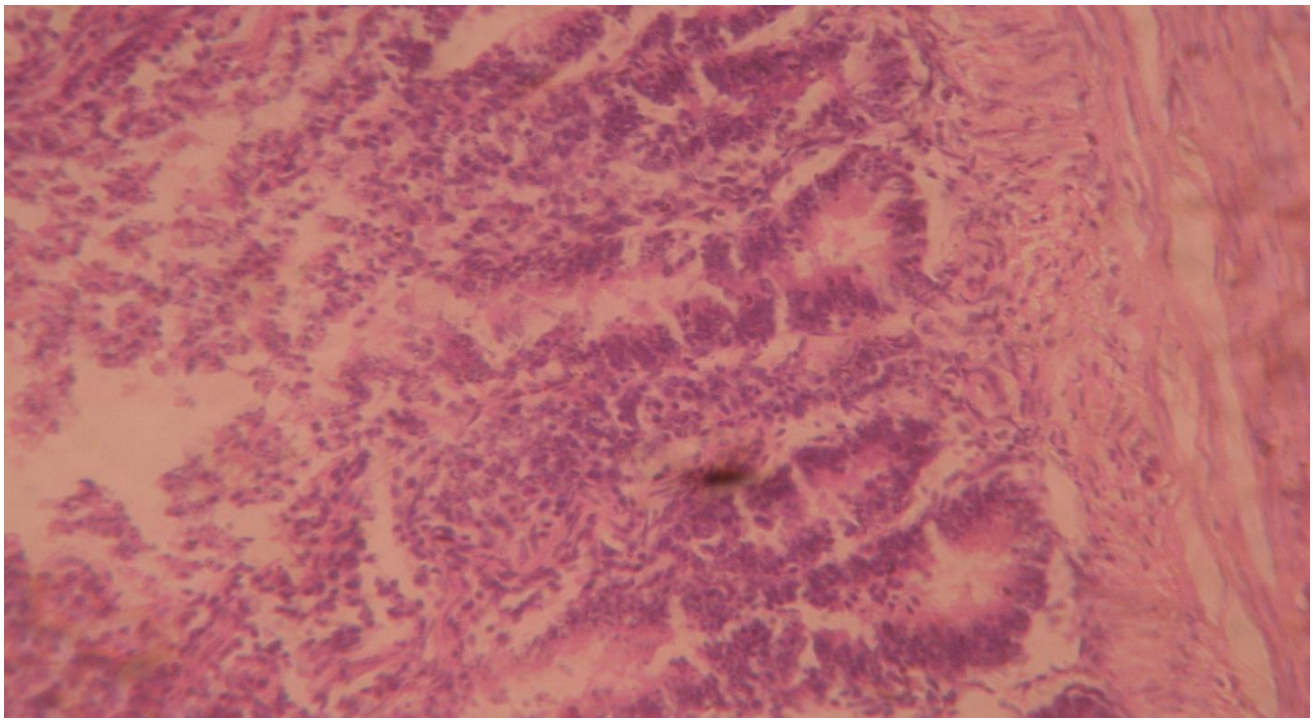

Fig No:- 15: Photomicrograph of section of mucosa treated with levofloxacin @ 20mg/kg b.wt. Showing atrophy of different layer of mucosa. H. & E.; x 40.

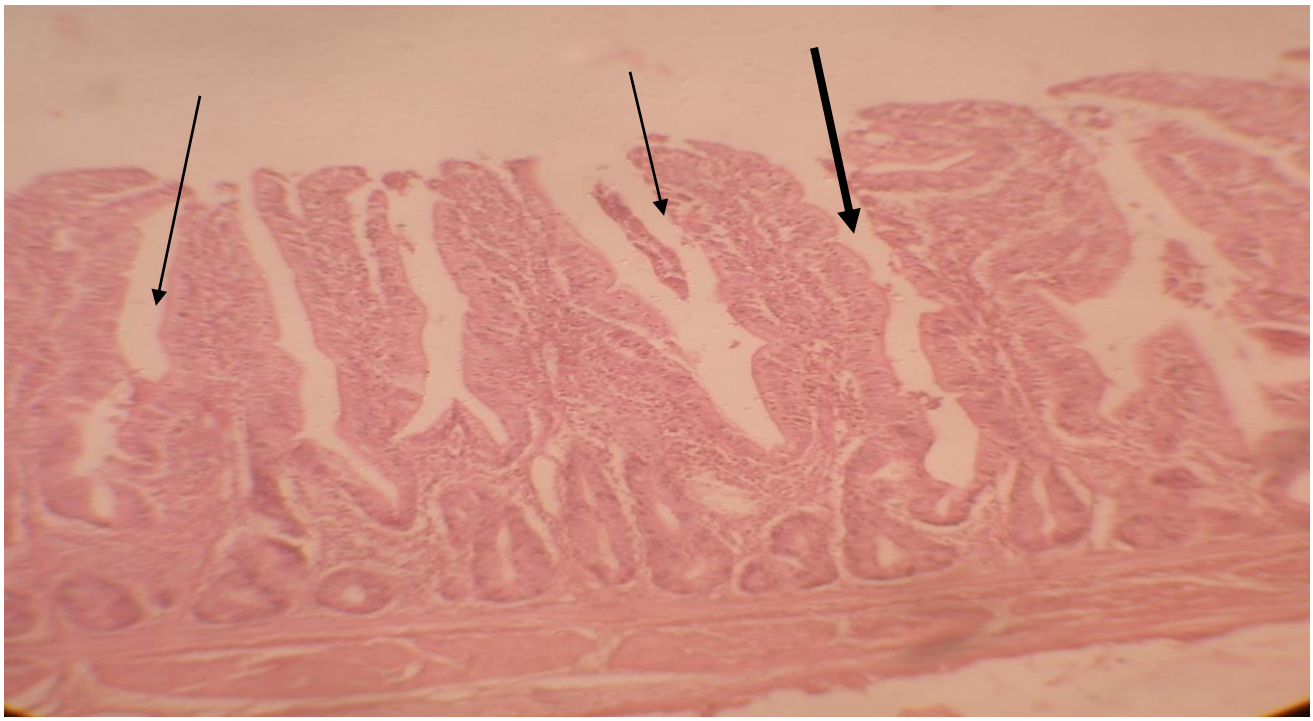

Fig No: - 16 Photomicrograph of section of mucosa showing Intestinal villi become broaden treated with levofloxacin @ 30mg/kg b.wt. H. & E.; X 40.

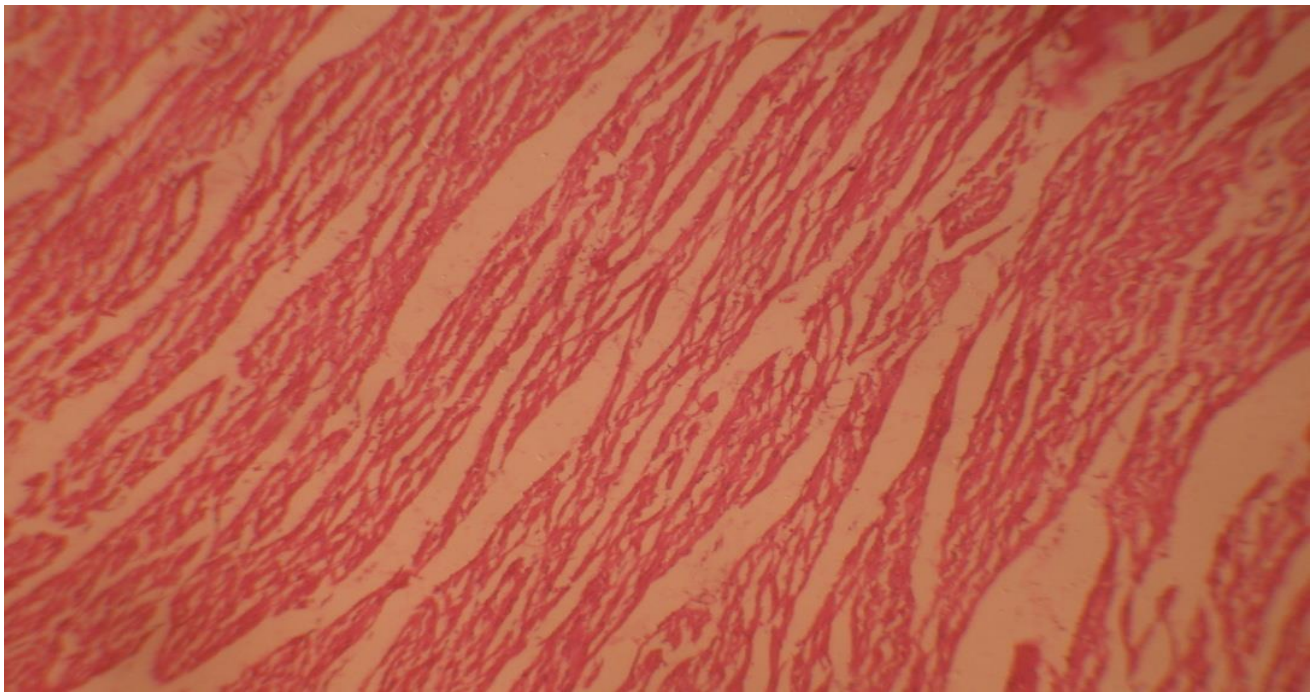

Fig No:- 17: Photomicrograph of section of muscles of control group showing normal architecture H. & E.; X 40.

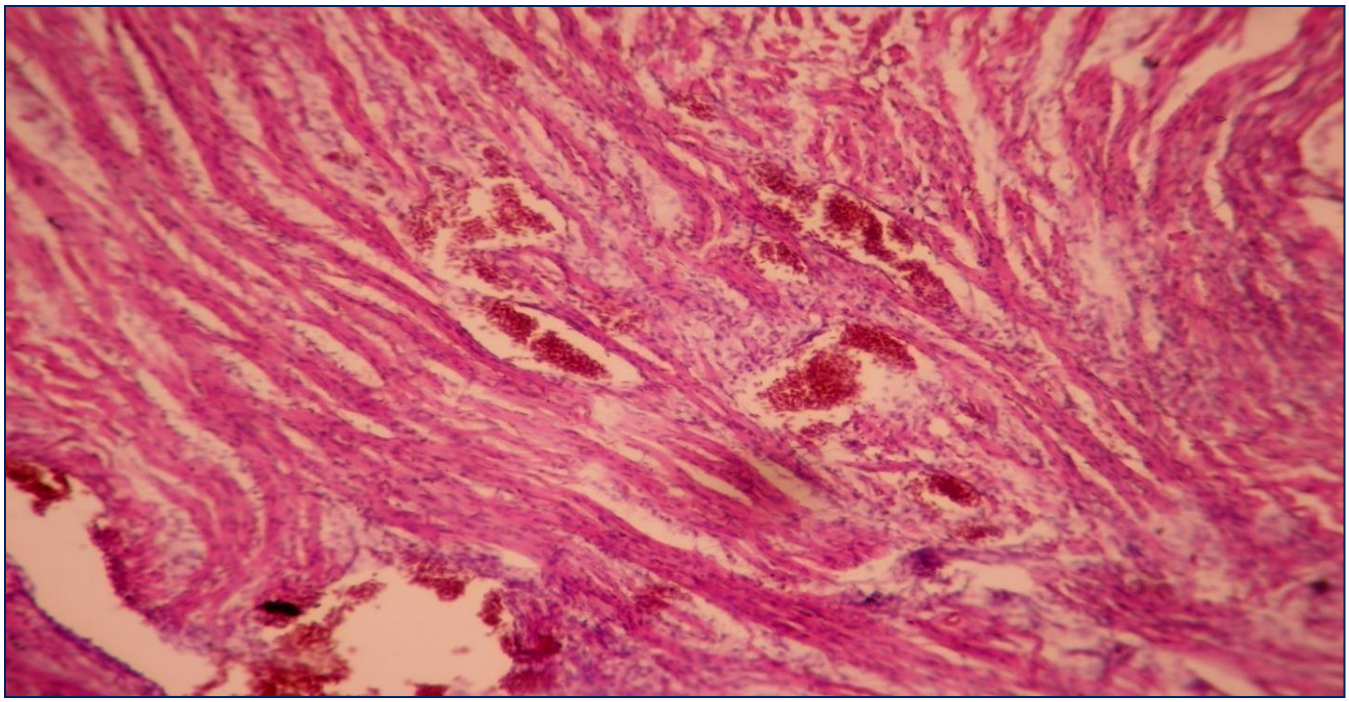

Fig No. 18: Photomicrograph of section of muscles treated with levofloxacin @ 30 mg/kg b.wt. Showing disruption of cardiac muscles, congestion in the intramuscular spaces and occasional infiltration of mononuclear cells. H. & E.; X 40.

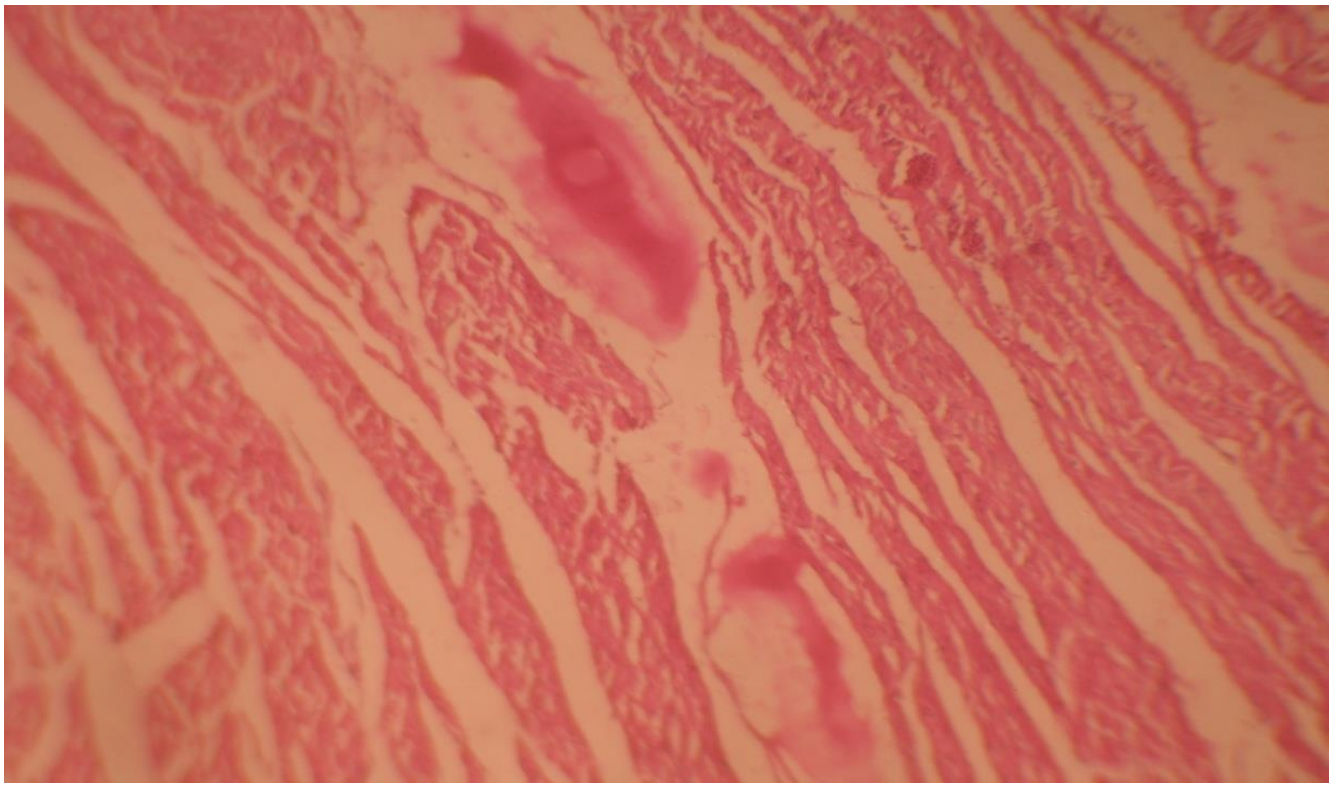

Fig No. 19: Photomicrograph of section of muscles treated with levofloxacin 20 mg/kg b.wt. Showing mild haemorrhages in between the muscle fibre in the broiler birds. H. & E.; X 40.

SUMMARY AND CONCLUSION

A toxico-pathological study based on clinical signs, haematological, biochemical and pathomorphological changes of broiler chickens induced with three graded dose levels of Levofloxacin were conducted. Three treatment and one-control groups of birds were used in this study. Groups- T_1, T_2 and T_3 birds were treated with 10 mg/kg B.wt, 20 mg/kg B.wt and 30 mg/kg B.wt respectively for 28 days starting from 2 weeks of age and group- T_0 birds remained without any treatment, serving as control. Clinical signs of toxicity, blood picture and biochemical changes were evaluated at weekly interval starting from "0" day (after 6 hour starting treatment), and thereafter at 28th day six birds from both treatment and control groups were sacrificed by cervical dislocation and gross pathological lesions were recorded at necropsy.

For residual effect of broiler, chickens induced with two graded dose levels of Levofloxacin were conducted. Two treatment and one-control groups of birds were used in this study. Groups- T_1 and T_2 birds were treated with 10 mg/kg B.wt and 20 mg/kg B.wt respectively for 28 days starting from 2 weeks of age and group- T_0 birds remained without any treatment, serving as control.

Clinically birds administered with 10 mg/kg B.wt Group (T_1) and 20 mg/kg B.wt (T_2) manifested mild diarrhoea and depression on 21 & 28th DPT. Birds of Group (T_2) 20 mg/kg B.wt and Group (T_3)30 mg/kg B.wt also had weakness, unthriptness, loss of appetite and poor growth. In addition, Group (T_3) birds exhibited droopiness, severe depression, respiratory distress, reduction in feed consumption and poor body weight gain without any mortality.

Birds of all treatment groups, (T_1, T_2 and T_3) showed significant ($P<0.05$) decrease in Hb, PCV, TEC and TLC values on14, 21 and 28th DPT indicating anaemia. All treated groups of birds had mild leucopenia. The significant decrease ($P<0.05$) of lymphocytes in

Groups- T_2 and T_3 and significant ($P<0.05$) increase of hetrophiles in group- T_2 and T_3 only in 21st & 28th DPT birds compare to control. Eosinophils, monocytes and basophiles were found to be non-significant.

Biochemically, all treated groups of induced with graded dose level of levofloxacin, had a significant ($P<0.01$) decreased level of total protein and total albumin as compare to controls. Conversely, the level of AST, ALT, BUN, uric acid and serum creatinine were elevated significantly ($P<0.01$) in birds with higher dose levels (group- T_2 and T_3) on 21st and 28th DPT and in birds treated

At necropsy, all groups of treated birds showed enlargement of liver and haemorrhages in the kidney but the intensity was more in the birds of group- T_2 and T_3. There were presence of grayish white necrotic foci in the liver and bursa of fabricius; haemorrhage and thickening of intestinal mucosa; oedema, red hepatization and consolidation of lungs; mild haemorrhage in the muscles in group- T_2 and T_3 with graded dose of the drugs.

Histopathological lesions consisted of mild degree of toxic hepatitis and necrosis in birds induced with therapeutic dose (group T_1). Birds of group- T_2 had congestion and haemorrhage in the liver, kidney, lungs; acute cellular swelling of hepatic cells in the centrilobular area; degeneration and/or necrosis of renal tubular epithelium of intestinal mucosa. Kidney also revealed desquamation and/or proliferation of tubular epithelium with eosinophilic proteinous cast. Birds of group- T_3 showed similar histopathological lesions, but the intensity was severe in degree. In addition, liver showed Coagulative necrosis of hepatic cells in the centrilobular area and formation of lymphoid follicle; toxic nephrosis and tubulo- interstitial nephritis; necrosis and sloughing of epithelium in the intestinal mucosa with cellular infiltration. Bursa of fabricious had atrophy of follicles with mild lymphocytolysis, cyst formation and increase in interfollicular connective tissue.

Residue analysis in liver and breast muscles were analyzed by HPLC method. The residue concentration of levofloxacin were gradually decreased in chicken liver and breast muscle samples starting from day one to day ten after the last dose administration. Levofloxacin was estimated to have pre slaughter withdrawal period of four to five days in comparison with maximum residue level of 200, and 100 µg/kg for liver and breast muscle respectively as per European Economic Community council regulations. It was concluded that slower elimination of levofloxacin from the body could be attributed to its liphophilicity and high tissue perfusion rate.

Based on Clinicopathological and pathomorphological changes, it is suggested that indiscriminate and injudicious use of third generation fluoroquinolones *Viz.* Levofloxacin produced anaemia, leucopenia, hypoproteinaemia, hypoalbunaemia, increased enzyme activity and hepato and nephrotoxic effects in broiler birds.

Based on residual effect it was concluded that after oral therapy with levofloxacin, residue levels are 2-4 times higher in the liver than in muscle. In addition, levofloxacin were estimated to have four days of withdrawal period in liver and five days of withdrawal period in breast muscle tissue of broiler birds.

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