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Research Article

Trial in using Clostridium butyricum as Prophylactic for Salmonellosis in Broiler Chicken: Hemato-biochemical, Immunological and Pathological Alterations

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Keywords

- *Salmonella Gallinarum*; FCR; Hematology; Broiler; Ciprofloxacin

Abstract

Background: *Salmonellosis* is a severe systemic disease of chicken, that threaten the poultry industry all over the world, thus the present study was planned to reach to accurate treatment of this serious disease, in addition to study the effect of *Salmonellosis* on hemato- biochemical, immunological and pathological changes in broiler chicken.

Materials and methods: 100 broiler chickens (Cobb breed), one-days-old were obtained from Dakahlia Company for poultry production and divided as following: 25 birds were clinically healthy kept as control group (I), 25 birds infected with *S. Gallinarum* (II), 25 birds administrated Clostri-Mix and infected with *S.Gallinarum* (III) and 25 birds infected with *S.Gallinarum* strain and treated with Ciprociel (IV). Blood and tissue samples were collected for hemato-biochemical, immunological and histopathological examination.

Results: The infected broiler chickens showed sever yellowish diarrhea. In addition, decrease in body weight, gain and feed consumption with significant increase in FCR. Furthermore, hemolytic anemia with leucocytosis was observed. Biochemical studies revealed a significant decrease in serum total proteins, albumin, Ca, GSH with significant increases in serum activities of ALT and AST, P, creatinine, uric acid and MDA concentration. Immunological studies clarified significant decrease in LTR, phagocytic % and index, in addition to several histological alterations in intestine, liver and kidney of the infected birds. All these disturbances were less severe in that administer *Clostridium butyricum*.

Conclusion: *Clostridium butyricum* can increase the bird immunity against *Salmonellosis*, which induced sever haemolytic anemia with hepatic, renal and intestinal disorders in broiler chickens.

ABBREVIATIONS

LTR: Lymphocytic Transformation; P: Phosphorus; GSH: Glutathione; ***Cl. Butyricum***: *Clostridium butyricum*; ***S. Gallinarum:*** *Salmonella Gallinarum*

INTRODUCTION

Commercial poultry is one of the fasted growing sectors of animal agriculture industry [1]. However, local chickens serve as an immediate source for meat and income when money is needed for vital family needs [2]. Poultry meat is more popular in the consumer market because of its easy digestibility and acceptance by the majority of people [3]. Poultry industry continuously grows by enhancement of new broiler strains to provide high-quality and low-cost protein requirements of the human population world wide [4].

Avian salmonellosis represents a group of acute or chronic diseases caused by one or more members of genus Salmonellae [5]. The most important pathogenic members of avian salmonellosis are the non motile *Salmonella enteric* subsp. *enterica serovar Gallinarum* and *Salmonella enterica* subsp. *enterica Pullorum*. These are host specific and represent a major concern for the poultry industry causing fowl typhoid and pullorum disease respectively [6]

Ciprofloxacin is a fluoroquinolone (FQs), with widespread using in human and animal practice [7]. It has an expanded spectrum of activity against Gram-positive and Gram-negative bacteria through inhibition of DNA gyrase. This enzyme (DNA gyrase) is very important to bacterial chromosome replication and increased antibacterial potency compared with non-fluorinated quinolines such as nalidixic acid [8].

Probiotic defined as a culture of specific living microorganisms, primarily lactobacillus species which ensures rapid and effective establishment of beneficial intestinal populations [9,10]. The using of probiotics improves the live body weight, gain and feed

conversion ratio and distinctly reduce mortality [11]. Probiotic microorganisms are not pathogenic, non-toxic and most of them retain their vitality after passing the whole digestive tract. *Clostridium butyricum* is a butyric acid bacterium that was isolated from soil, healthy animals and human fecal matter [12]. *Clostridium butyricum* can produce endospores, which is a key to its ability to survive at lower pH and relatively higher bile concentrations compared with Lactobacillus and Bifidobacterium [13].

MATERIAL AND METHODS

Ethical approval

Handling and sampling were carried out using the general guidelines of the National Institutes of Health (NIH) for the Care and Use of Laboratory Animals in scientific investigations and approved by the ethics of animal use in research committee (EAURC), Zagazig University (what is the approval number).

Preparation of bacterial strain

Twenty-four hours pure cultures of isolated *S. Gallinarum* biovar *Gallinarum* were suspended in sterile saline solution using McFarland opacity tube No. 1. The approximate cell density in this dilution was 3×10^8 CFU/ml (Colony Forming Units/ml).

Preparation of antibiotic

Ciprofloxacin is present under commercial name as Ciprociel. One ml Ciprociel contains 100 mg of ciprofloxacin. The recommended dose of ciprofloxacin was 5 mg/ kg body weight in drinking water for 3 successive days [14]. Therefore, each one Kg of body weight needed 0.05 ml of Ciprociel.

Animal grouping

100 broiler chickens (Cobb breed), one-days-old were obtained from Dakahlia Company –Egypt, for poultry production and selected as following: 25 birds were clinically healthy kept as control group (I), 25 birds infected with *S. Gallinarum* strain via crop at a dose of 0.2 ml of sterile saline containing 3×10^8 CFU/ml at 4 days old group (II), 25 birds administrated Clostri-Mix as commercial name for *Clostridium butyricum* (2 g/L drinking water) from the age of one day old till the end of the experiment and infected with *S.Gallinarum* strain at 4 days old group (III) and 25 birds infected with *S.Gallinarum* strain at 4 days old and treated with Ciprociel (5 mg/kg b.wt) in drinking water for 3 successive days at 10 days old group (IV).

Indices for the evaluation of the growth performance

The weekly feed consumption was determined through weighting the offered feed for birds. The birds were weighted individually at 1st, 14th and 28th day old to determine the average body weight in each group. The body gain calculated by the difference in body weight throughout the periods of the experiment. The feed conversion ratio (FCR) obtained by subdivided weekly feed consumption on the weekly increase in the body weight (body gain) and this was recorded every week [15].

Sampling

Clinical signs were recorded then three blood samples were collected from wing vein from all tested groups on 2nd and 4th weeks of age. The first set of blood samples (0.5 ml from each broiler chickens) were collected in dipotassium salt of ethylene-diamine tetra acetic acid (EDTA) tubes to be used for hematological variables determination. While the second blood samples (5 ml from each broiler chickens) were taken without anticoagulant in a sterile test tube for serum separation and kept for biochemical analysis. The third sample was collected in heparinized tubes for immunological studies. Postmortem findings were recorded then liver, kidney and intestine specimens were taken from freshly dead birds for histopathological examination.

Hematological studies

The erythrocytic count, hemoglobin concentration, packed cell volume, RBCs indices, total leukocytic and differential leukocytic counts were carried out by using automatic cell counter (Sysmex IV2000)

BIOCHEMICAL STUDIES

All biochemical tests were measured colorimetrically using a commercial kits of Biodiagnostic, 29th El-Tahrer Street, Dokki, Giza, Egypt and a semi-automated Photometer (5010 V5+, RIELE GmbH & Co, Berlin, Germany). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [16], serum total proteins and creatinine levels [17], serum albumin level [18]. Serum globulins level was calculated by subtracting the obtained albumin level from the obtained total proteins level [19]. The serum calcium level [20], the serum level of inorganic phosphorus was [21]. The uric acid levels [22]. The serum malondialdehyde (MDA) [23] and the reduced glutathione (GSH) [24].

Immunological studies

Qualitative fractionation of serum proteins for determination of serum alpha, beta and gamma globulins was carried out using polyacrylamide gel columns [25].

Histopathological examination

Specimens from liver, kidneys and intestine were collected from each group, then fixed in 10 % neutral buffered formalin for 48 hours and washed overnight under running water. The washed specimens were dehydrated by using ascending graded concentrations of ethyl alcohol starting with 75% and ending with absolute alcohol. Samples were cleared after immersing in xylol for 2 hours. The cleared samples were placed in closed jars containing 50% paraffin and incubated for 2 hours at 37°C. The samples were placed in crucible containing soft paraffin and kept in an oven at 48°C for 2 hours. The samples were blocked in hard paraffin and then cut into sections of about 5 micron thicknesses, then stained with Hematoxylin and Eosin (H&E). The sections were mounted with Canada balsam and covered with cover slide to be ready for histopathological examination [26].

Statistical analysis

Data of the current study was statistically analyzed using the computer program SPSS/PC+2001. The statistical method was one way ANOVA test, followed by Duncan's multiple range test [27]. Data are presented as means plus or minus the standard error. The minimum level of significance was set at $P < 0.05$.

RESULTS

Clinical observations

As shown in (Figure 1), Chicks infected with *S. Gallinarum* group (II) showing white yellowish diarrhea, pasty vent, huddle together near the source of heat, loss of appetite, dullness and ruffled feathers.

Body Performance

Concerning the body Performance as shown in (Tables 1), chicks infected with *S. Gallinarum* group (II) showed a significant ($P<0.05$) decrease in body weight, body gain and feed consumption with significant increase in FCR in all experimental periods. Chicks that administrated Clostri-mix before and after *S. Gallinarum* infection group (III) showed non-significant changes in the body performance. Chicks that treated with Ciprociel after *S. Gallinarum* infection group (IV) showed a significant decrease in body weight, body gain and feed consumption with no significant change in FCR at the end of 2nd week of age while showed a non-significant change in all body performance parameters at the end of 4th week of age, when compared with the normal control. On the other hand, chicks of both groups (III and IV) showed a significant improvement in the body performance in all experimental periods, when compared with infected group (II).

Hematological results

Concerning the erythrogram as shown in (Tables 2), showing a significant decrease in RBCs count, Hb concentration and PCV with development of macrocytic hypochromic anemia in gps. (II, III& IV) at the end of 2nd week. Moreover, Gp. (II) showed a significant decrease in RBCs count, Hb concentration and PCV with development of macrocytic hypochromic anemia compared with normal control at the end of 4th week. In addition, the chicks of gps. (III and IV) revealed non-significant changes in erythrogram.

Concerning the leukogram at the end of 2nd week as shown in (Tables 3), it a significant leukocytosis, heterophilia and lymphopenia in gp. (II), when compared with the normal control. Gp (III) showed a significant leukocytosis and lymphocytosis in comparison with normal control. On the other hand, other cells showed non-significant changes. However, the obtained results at the end of 4th week revealed that gp. (II), showed a significant leukocytosis, heterophilia and monocytosis with non-significant changes in the counts of lymphocytes, basophils and eosinophils compared with normal control. Gp. (III) revealed a significant leukocytosis and lymphocytosis with non-significant changes in the counts of heterophils, monocytes, eosinophils and basophils. Gp. (IV) showed non-significant changes in the leukogram when compared with normal control along the experimental periods.

Biochemical changes

Liver function and protein electrophoresis: Regarding to the liver function and protein electrophoresis reports in comparison with the control group (I) as shown in (Table 4), Gps. (II, III and IV) revealed a significant increase in the serum ALT and AST activities. On the other hand, gps. (III and IV) showed a significant decrease, when compared with infected group (gp.II) at the end of 2nd week. The serum activities of AST and ALT showed non-significant changes in gps. (III and IV), when compared with normal control at the end of 4th week. Moreover, a significant

Figure 1 Representative photo showing S. Gallinarum infected chicks; (A) showed the pasty vent (black arrow), (B) showing loss of appetite, dullness and huddle together.

Table 1: Body Performance of chicks in different groups (I- IV).

	2ND WEEK				4TH WEEK			
	I	II	III	IV	I	II	III	IV
B.Wt. (g)	320^{b} ±9.6	225^{d} ±6.5	300^{bc} ±4.1	290^{c} ±7.4	1020^{b} ±12.9	700^{c} ±25.9	980^{b} ±15.4	990^{b} ±13.5
B. gain (g)	194.5^{b} ±9.9	129.9^{d} ±6.7	186^{b} ±3.9	164^{c} ±7.6	410^{ab} ±12.3	250^{c} ±33.2	390^{b} ±14.4	420^{ab} ±14.8
Feed Cons. (g)	355	290	330	315	700	550	670	690
FCR	1.84^{b} ±0.065	2.25^{a} ±0.12	1.97^{b} ±0.027	1.93^{b} ±0.059	1.71^{b} ±0.05	2.4^{a} ±0.38	1.72^{b} ±0.047	1.65^{b} ±0.07

Data are expressed as the mean ± SE, n=5. Means within same row carrying different superscripts are significant different at <0.05.
Abbreviation: (I): Control; (II): S. Gallinarum; (III): Clostri-mix + *S.Gallinarum* + Clostri-mix; (IV): *S. Gallinarum* + Ciprociel; B.Wt: Body weight; B. gain: Body gain; F. Cons: Feed consumption; FCR: Feed conversion ratio.

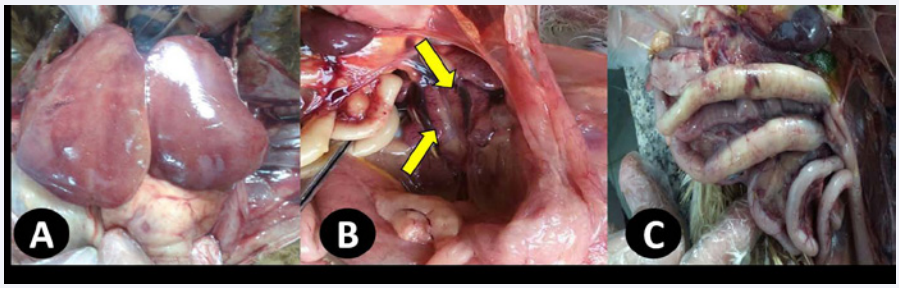

Figure 2 Representative photo showing the macroscopic lesion of S. Gallinarum infected chicks; (A) showed enlarged and bronze discoloration of the liver, (B) showing engorgement of kidneys with hemorrhagic spots (yellow arrow) and (C) showing enteritis of anterior small intestine and thickening of intestinal mucosa.

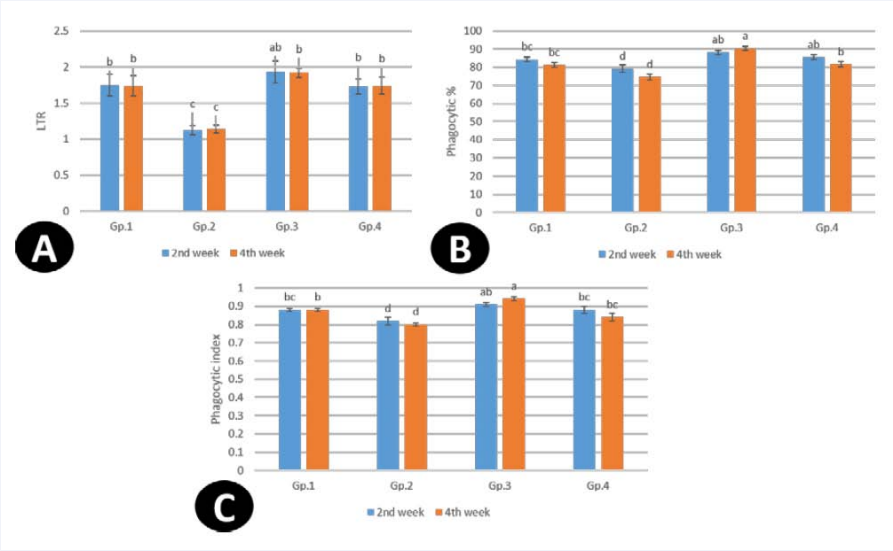

Figure 3 Representative photo showing the immunological studies of chicks in all tested groups.

Table 2: Erythrogram of chicks in different groups (I- IV).

	2ND WEEK				4TH WEEK			
	I	II	III	IV	I	II	III	IV
RBCs (×10⁶/mm3)	3.5^{a}±0.36	1.7^{c}±0.15	2.2^{bc}±0.1	2.00^{bc} 0.14	3.49^{a}±0.15	1.9^{b} ±0.12	3.6^{a} ±0.23	3.3^{a} ±0.17
HB (g/dl)	11.5^{a} ±0.3	7.3^{c} ±0.37	8.9^{b} ±0.45	8.5^{bc} ±0.29	11.7^{a} ±0.43	7.4^{b} ±0.33	11.4^{a} ±0.28	11.5^{a} ±0.34
PCV (%)	31.4^{a} ±0.61	26.6^{c} ±0.71	29.1^{b} ±0.46	27.6^{bc} ±0.8	31.5^{a} ±0.37	26.2^{b} ±0.50	31.4^{a} ±0.69	31.2^{a} ±0.51
MCV (fl)	3.5^{c} ±9.3	160.7^{a} ±11.93	133.3^{ab} ±5.5	139.8^{ab} ±7.2	90.9^{b} ±3.8	140.2^{a} ±9.8	88.5^{b} ±5.7	95.8^{b} ±6.1
MCH (pg)	34.2^{a} ±3.5	43.8^{a} ±3.2	40.6^{a} ±1.82	43.5^{a} ±3.89	33.7^{ab} ±1.5	39.9^{a} ±4.07	32.04^{b} ±1.6	35.2^{ab} ±1.8
MCHC (%)	36.6^{a} ±1.08	27.5^{c} ±1.56	30.5^{bc} ±1.21	30.9^{bc} ±1.39	37.1^{a} ±1.14	28.3^{b} ±1.45	36.4^{a} ±1.36	36.9^{a} ±1.4

Data are expressed as the mean ± SE, n=5. Means within same row carrying different superscripts are significant different at < 0.05.
Abbreviation: (I): Control; (II): S. Gallinarum; (III): Clostri-mix + *S. Gallinarum* + Clostri-mix; (IV): *S. Gallinarum* + Ciprociel; RBCs: Red blood cells; Hb: Hemoglobin; PCV: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration.

decrease in serum total protein, albumin was observed in gp. (II). Gp. (III) revealed non-significant changes in serum level of total protein and albumin. On the other hand, it showed a significant increase in serum total protein and albumin, when compared with infected group. Gp. (IV) showed non-significant change in serum total protein with a significant decrease in albumin, when compared with the normal control. On the other hand, it showed a significant increase in serum total protein and albumin, when compared with infected group at the end of 2nd week but at the end of 4th week it showed non-significant changes in proteinogram when compared with normal control. On the other hand, it showed a significant increase in serum total protein and albumin, when compared with infected group. In addition, the infected chicks with *S. Gallinarum* (gp.II) showed a significant

Table 3: Leukogram (×10³/µl) of chicks in different groups (I- IV).

	2ND WEEK				4TH WEEK			
	I	II	III	IV	I	II	III	IV
T.L.C	21.5^{b} ±0.21	25.11^{a} ±0.45	24.9 ^{a}±0.87	21.9^{b} ±0.42	21.63^{b} ±0.42	24.86^{a} ±0.47	25.29^{a} ±0.48	21.99^{b} ±0.33
Heterophil	5.7^{bc} ±0.25	10.44^{a} ±0.31	6.35^{b} ±0.24	5.9^{bc} ±0.18	5.6^{b} ±0.18	8.06^{a} ±0.48	6.3^{b} ±0.25	5.70^{b} ±0.20
Lymphocyte	13.5^{bc} ±0.21	12.2^{d} ±0.29	16.2^{a} ±0.55	13.8^{b} ±0.31	13.8^{b} ±0.37	13.9^{b} ±0.50	16.7^{a} ±0.41	14.1^{b} ±0.32
Monocyte	1.56^{a} ±0.25	1.67^{a} ±0.32	1.6^{a} ±0.27	1.5^{a} ±0.26	1.43^{b} ±0.20	2.10^{a} ±0.11	1.49^{b} ±0.18	1.41^{b} ±0.20
Eosinophil	0.51^{a} ±0.01	0.53^{a} ±0.03	0.51^{a} ±0.01	0.52^{a} ±0.01	0.54^{a} ±0.01	0.53^{a} ±0.01	0.53^{a} ±0.01	0.52^{a} ±0.01
Basophil	0.24^{a} ±0.014	0.23^{a} ±0.01	0.24^{a} ±0.01	0.26^{a} ±0.02	0.26^{a} ±0.02	0.27^{a} ±0.02	0.27^{a} ±0.02	0.26^{a} ±0.01

Data are expressed as the mean ± SE, n=5. Means within same row carrying different superscripts are significant different at < 0.05.
Abbreviation: (I): Control; (II): S. Gallinarum; (III): Clostri-mix + *S. Gallinarum* + Clostri-mix; (IV): *S. Gallinarum* + Ciprociel; TLC: Total leukocytic count.

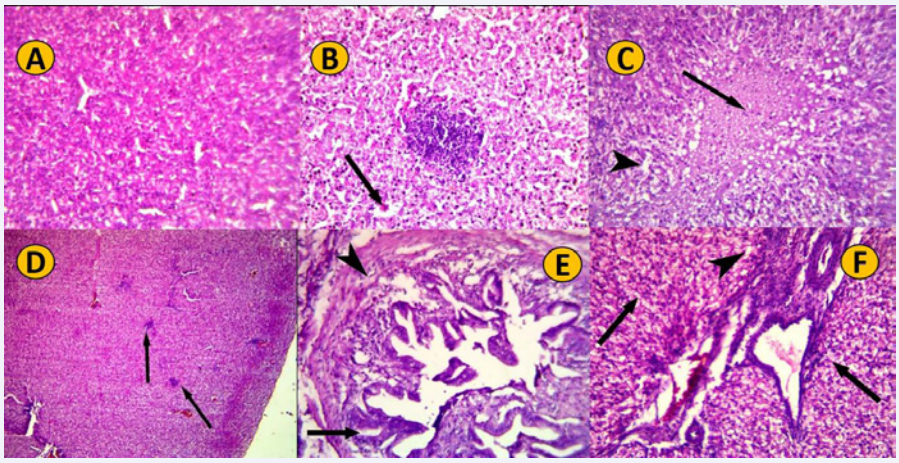

Figure 4 Photomicrograph of H&E-stained liver section of control group (I) (A,100x). Chicks infected with *S. Gallinarum* (gp.II), showing coagulative necrosis (arrow) with marked vaculations of hepatocytes near the necrotic area (arrow head), (B, 400x). and proliferation of von kupffer cells (arrow), (C, 400x). Chicks administrated *Cl.butyricum* before and after infection with *S. Gallinarum* (gp.III) showing multible focal leukocytic aggregations throughout the hepatic parenchyma (arrow), (D, 400X). Chicks infected with *S. Gallinarum* and treated with ciprofloxacin (gp.IV) showing chronic cholangitis represented by epithelial hyperplasia (arrow), leukocytic infiltration and fibrous connective tissue proliferation (arrow head), (E, 400X).

increase in serum globulins, alpha and gamma globulins with a significant decrease in beta globulin all experimental periods. Gp. (III) revealed a significant increase in globulins, alpha and gamma globulin in all experimental periods, when compared with normal control. Moreover a significant decrease in beta globulin was recorded. While gp. (IV), showed a significant increase in serum level of globulin, alpha and gamma globulin with a significant decrease in beta globulins when compared with the normal control at the end of 2nd week. Moreover, significant increases in alpha and gamma globulins with significant decrease in beta globulin were observed, when compared with normal control at the end of 4th week.

Kidney function tests: Regarding to Kidney function (Table 5), the serum levels of calcium and inorganic phosphorus levels demonstrated a significant decrease in gp. (II), when compared with normal control (gp.I). While gps. (III and IV) showed non-significant change. On the other hand, gps. (III and IV) showed a significant increase in serum calcium level, compared with infected group along the experimental periods. In addition to, a significant increase in serum levels of uric acid and creatinine in gps. (II, III and IV) when compared with the normal control. On the other hand, significant decrease in serum levels of uric acid and creatinine was recorded in gps. (III and IV), as compared with infected one. At the end of 4th week, the serum levels of uric acid and creatinine were increased significantly in gp. (II). However, gps. (III and IV) showed a significant decrease and non-significant changes compared with infected chicks (gp.II) and normal control respectively.

Oxidant and antioxidant: Regarding to serum malondialdehyde and reduced glutathione (Table 6) demonstrated a significant increase and decrease in MDA and GSH respectively in gps. (II, III and IV) when compared with normal control at the end of 2nd week. On the other hand, gp. (III), showed a significant decrease in MDA and increase in GSH as compared

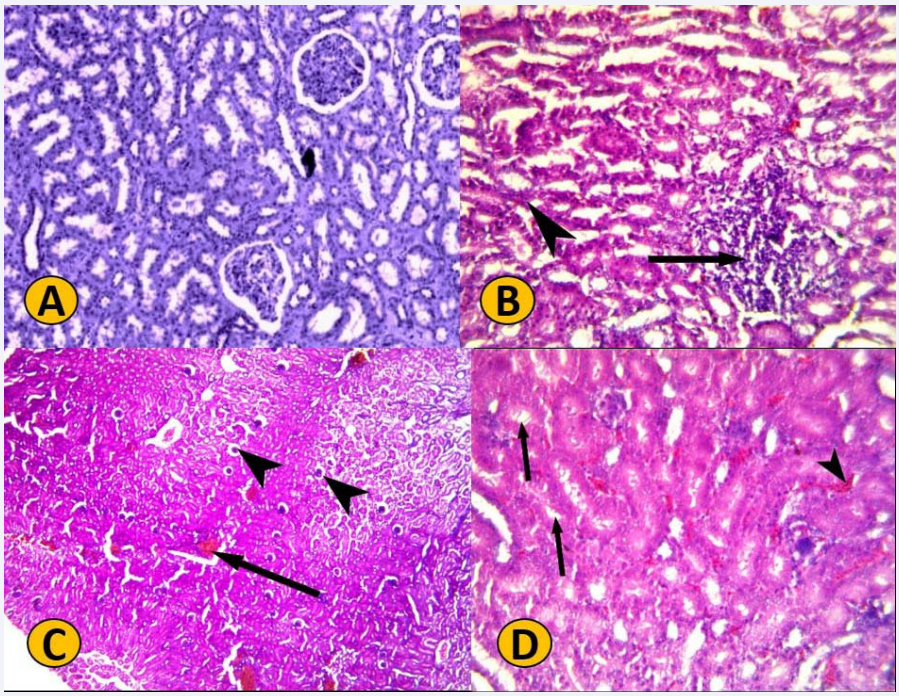

Figure 5 Photomicrograph of H&E-stained kidney section of control chick of (gp.I) showing normal glomerular and tubular structure, (A) (100 X). Chicks infected with *S. Gallinarum* (gp.II) showing focal replacement of renal parenchyma by mononuclear cells (arrow) with pyknosis (arrow head) of the nuclei of some renal tubular epithelium, (B) (400 X). Chicks administrated *Cl. butyricum* before and after infection with *S. Gallinarum* (gp.III) showing multiple focal hemorrhage, congested blood vessels (arrow) with collapse of most glomeruli (arrow head), (C) (100 X). chicks infected with *S. Gallinarum* and treated with ciprofloxacin (gp.IV) showing nephrotic changes in renal tubules and cloudy swelling (arrow), congestion of peritubular capillaries (arrow head), (D) (400 X).

with infected group. However, at the end of 4th week the serum malondialdehyde (MDA) level was significantly increased in gps. (II and IV), with insignificant changes in gp. (III), in comparison with the normal control. On the other hand, gps. (III and IV) showed a significant decrease in MDA level in comparison with infected group (gp.II). Moreover, non-significant changes were observed in gp. (IV), when compare with normal control but, GSH revealed a significant decrease in gps. (II and IV), with non-significant changes in chicks administered *Clostridium butyricum*. On the other hand, significant increase in GSH level was recorded in gps. (III and IV), as compared with infected group (gp.II). Moreover, a non-significant change was observed in gp. (IV), when compared with normal control.

Immunological studies: During all experimental periods as shown in (Figure 2-6), the phagocytic percent and phagocytic index revealed a significant decrease in gps. (II), while gps. (III and IV) showed non-significant change, in comparison with the normal control (gp.I). A significant increase was recorded in both gps. (III and IV), as compared with infected group. The lymphocytic transformation showed non-significant change in gps. (III and IV) with significant decrease in *Salmonella Gallinarum* infected group, in comparison with the normal control. While gps. (III and IV) revealed a significant increase in lymphocytic transformation in comparison with infected group (gp.II).

DISCUSSION

Salmonella enterica serovar Gallinarum (*S. Gallinarum*) is the etiologic agent of Fowl Typhoid, which is a severe systemic disease of chicks and other galliform birds [28]. The nature of pathogenicity of *Salmonella Gallinarum* is multifactorial [29]. The *Clostridium butyricum* probiotic is a potential candidate since the bacterium produces butyric acid [30], which is known to have an influence on *Salmonella* replication [31].

The present study revealed that infected chicks by *S. Gallinarum* (gp II) revealed white yellowish diarrhea, pasty vent, huddle together near the source of heat, loss of appetite, dullness and ruffled feathers. These results were in agreement with Fotouh et al. [32], who observed loss of appetite, decrease in feed intake and depression, ruffled feathers, droppings, huddled together and white pasty diarrhea of in chickens experimentally infected with *S. Gallinarum*. Chicks administrated *Cl. butyricum* before and after infection with *S. Gallinarum* (gp III) and those infected with *S. Gallinarum* then treated by ciprofloxacin (gp IV) showed few depression. This indicated that ciprofloxacin and *Cl. butyricum* increased the survival rate of chicks challenged with *S. Gallinarum*, similar results were previously obtained by [33] who mentioned that administration of *Cl. butyricum* before infection may be promotes growth performance, immune function and reduced colonization rates of the caecum of *Salmonella* [34].

Regarding to body performance, chicks infected with *S. Gallinarum* gp. (II) Clarified a reduction in the body weight, body gain, feed consumption and an increase in the feed conversion rate in all experimental periods. The observed loss in body weight may be caused by anorexia and diarrhea [35]. Our result

Table 4: Liver function and protein electrophoresis of chicks in different groups (I- IV).

	2ND WEEK				4TH WEEK			
	I	II	III	IV	I	II	III	IV
AST (U/l)	50.20^{d} ±0.52	65.60^{a} ±0.29	56.10^{c} ±0.58	60.90^{b} ±0.99	50.60^{b} ±0.85	69.40^{a} ±1.44	54.00^{b} ±1.89	51.00^{b} ±1.39
ALT (U/l)	7.50^{e} ±0.56	10.60^{a} ±0.36	9.10^{bcd} ±0.32	8.90^{cd} ±0.59	7.80^{b} ±0.45	11.40^{a} ±0.55	8.00^{b} ±0.38	7.80^{b} ±0.37
Total protein (g/dl)	5.50^{ab} ±0.21	3.95^{c} ±0.21	5.80^{ab} ±0.19	5.20^{b} ±0.20	5.60^{a} ±0.26	3.90^{b} ±0.32	5.60^{a} ±0.03	5.30^{a} ±0.26
Albumin (g/dl)	3.50^{a} ±0.23	1.45^{c} ±0.20	3.00^{ab} ±0.11	2.50^{b} ±0.28	3.60^{a} ±0.19	1.41^{b} ±0.26	3.10^{a} ±0.11	3.40^{a} ±0.21
Globulin (g/dl)	2.00^{b} ±0.02	2.50^{a} ±0.09	2.80^{a} ±0.13	2.70^{a} ±0.16	2.00^{b} ±0.08	2.49^{a} ±0.13	2.50^{a} ±0.11	1.90^{b} ±0.11
α- globulin (g/dl)	0.30^{c} ±0.01	0.65^{ab} ±0.06	0.65^{ab} ±0.06	0.80^{a} ±0.08	0.30^{b} ±0.03	0.69^{a} ±0.11	0.55^{a} ±0.04	0.60^{a} ±0.08
β- globulin (g/dl)	0.8^{b} ±0.09	0.35^{c} ±0.03	0.45^{c} ±0.05	0.50^{c} ±0.05	0.60^{b} ±0.02	0.35^{cd} ±0.02	0.45^{c} ±0.04	0.30^{d} ±0.02
γ- globulin (g/dl)	0.90^{c} ±0.06	1.5^{ab} ±0.06	1.7^{a} ±0.08	1.4^{b} ±0.04	1.10^{b} ±0.11	1.45^{a} ±0.03	1.50^{a} ±0.07	1.00^{b} ±0.03

Data are expressed as the mean ± SE, n=5. Means within same row carrying different superscripts are significant different at < 0.05.
Abbreviation: (I): Control; (II): S. Gallinarum; (III): Clostri-mix + *S. Gallinarum* + Clostri-mix; (IV): *S. Gallinarum* + Ciprociel; AST: Aspartate transaminase, ALT: Alanine transaminase

Table 5: Kidney function of chicks in different groups (I- IV).

	2ND WEEK				4TH WEEK			
	I	II	III	IV	I	II	III	IV
Calcium (mg/dl)	11.22^{ab} ±0.28	8.50^{d} ±0.35	9.98^{bc} ±0.51	9.96^{bc} ±0.66	11.18^{a} ±0.52	9.30^{b} ±0.40	10.90^{a} ±0.43	10.96^{a} ±0.43
Phosphorus (mg/dl)	6.40^{b} ±0.32	8.20^{a} ±0.38	6.88^{b} ±0.42	6.98^{b} ±0.54	6.34^{b} ±0.30	8.60^{a} ±0.36	6.44^{b} ±0.54	6.34^{b} ±0.40
Uric acid (mg/dl)	4.70^{c} ±0.16	8.02^{a} ±0.29	5.90^{b} ±0.41	5.77^{b} ±0.15	4.35^{b} ±0.23	7.25^{a} ±0.29	4.48^{b} ±0.20	4.3^{b} ±0.36
Creatinine (mg/dl)	0.92^{c} ±0.05	1.80^{a} ±0.18	1.40^{b} ±0.06	1.35^{b} ±0.05	0.96^{b} ±0.06	1.79^{a} ±0.20	1.14^{b} ±0.03	1.12^{b} ±0.06

Data are expressed as the mean ± SE, n=5. Means within same row carrying different superscripts are significant different at < 0.05.
Abbreviation: (I): Control; (II): S. Gallinarum; (III): Clostri-mix + *S. Gallinarum* + Clostri-mix; (IV): *S. Gallinarum* + Ciprociel.

are in agreement with those reported by [35-40], they reported decrease in body weight, feed intake and increase in the feed conversion ratio following *S. Gallinarum* infection. In our opinion the loss in the body weight may be a reflection of the reduced feed intake or decreased utilization and metabolism of food stuff due to intestinal and hepatic lesion. The using of clostri-mix before and after *S. Gallinarum* infection as in (gp.III) showed non-significant change in body weight in all experimental periods in comparison with normal control (gp.I), but proved an improvement when compared with *S. Gallinarum* infected group (gp.II). These results support to the previously obtained by [41,42]. They reported that the addition of probiotics significantly improved the body weight, food consumption and food convertion ratio, compared with the E.coli infected group. Chicks administered ciprofloxacin after infection with *S. Gallinarum* (gp. IV), showed a significant decrease in body weight in all experimental periods compared with normal control (gp.I), but an improvement was observed when compared with infected group (gp.II). This result in agreement with Porchezhian [43], who presumed that the fluoroquinolone has influence in increasing the body weight only in infection not in healthy

Regarding the erythrogram the infected group (gp.II) showed significant decrease in RBCs count, Hb concentration and packed cell volume with macrocytic hypochromic anemia that may be attributed to hemolysis of erythrocytes and release of reticulocytes to the peripheral circulation [44]. These results agreed with Chiroma et al. [45], who recorded macrocytic hypochromic anemia, Chicks of (gp.III) showed a significant decrease in RBCs count, Hb concentration and packed cell volume when compared with control group (gp.I), with macrocytic hypochromic anemia at 2nd week of age that showed an improvement in 4th week of age. This anemia may be attributed to blood loss due to hemorrhages as a result of *S. Gallinarum* infection [46]. These results are coordinates with those observed by [47] who found macrocytic hypochromic anemia at the 2nd week post infection. The changes of erythrogram values were less significant in this group, in comparison with group (gp.II) due to hepato-stimulatory and hepato-protective effects of the probiotic leading to production of more RBCs by bone marrow under the control of erythropoietic factors released by hepatic cells [48]. The chicks in (gp.IV), showed a significant decrease in erythrogram in the 2nd week of age with macrocytic hypochromic

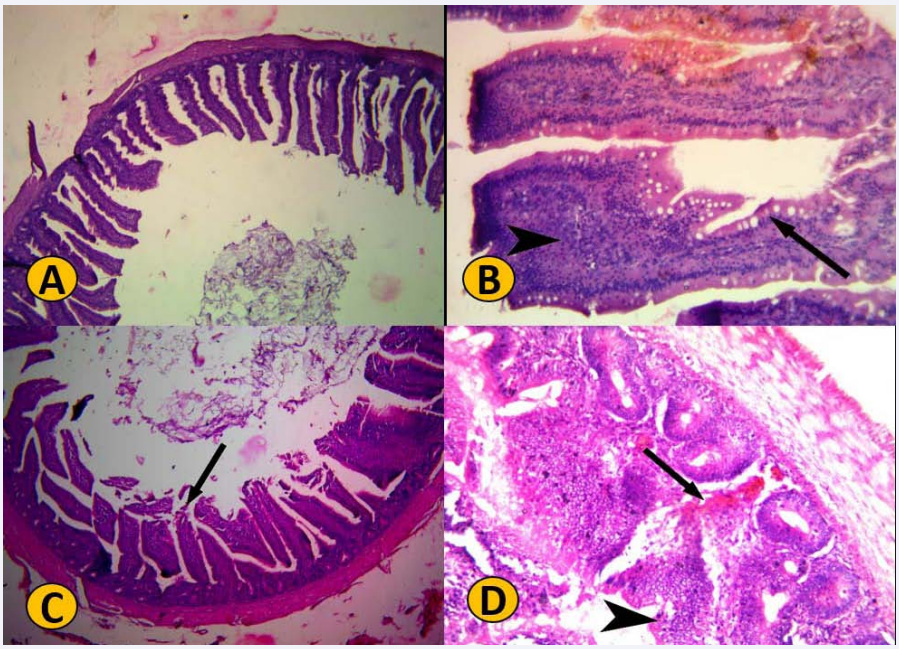

Figure 6 Photomicrograph of H&E-stained intestine section of control chick of (gp.I) showing normal glomerular and tubular structure, (A) (100 X). Chicks infected with *S. Gallinarum* (gp.II) showing catarrhal enteritis (arrow) represented by marked mononuclear cell infiltration and excess mucous secretion (arrow head), (B) (400 X). Chicks administrated *Cl. butyricum* before and after infection with *S. Gallinarum* (gp.III) showing lymphocytic infiltration and thickening in intestinal villi (arrow), (C) (100 X). Chicks infected with *S. Gallinarum* and treated with ciprofloxacin (gp. IV) owing focal hemorrhage (arrow) between intestinal crypts and mild leukocytic infiltration (arrow head), (D) (400 X).

Table 6: oxidant and antioxidant of chicks in different groups (I- IV).

	2ND WEEK				4TH WEEK			
	I	II	III	IV	I	II	III	IV
MDA (nmol/g)	4.70c ± 0.19	7.30a ±0.49	5.88b ±0.27	6.50ab ±0.16	4.8b ±0.32	6.60a ±0.53	4.92b ±0.29	5.02b ±0.29
GSH (µmol/L)	31.50a ±1.13	23.80c ±0.88	27.80b ±1.21	24.78bc ±1.40	32.20a ±1.85	25.90b ±0.81	31.85a ±1.13	31.54a ±0.61

Data are expressed as the mean ± SE, n=5. Means within same row carrying different superscripts are significant different at < 0.05.
Abbreviation: (I): Control; (II): S. Gallinarum; (III): Clostri-mix + *S. Gallinarum* + Clostri-mix; (IV): *S. Gallinarum* + Ciprociel; MDA: Malondialdehyde, GSH: Reduced glutathione.

anemia when compared with the control (gp.I), but a significant improvement in the erythogram was observed at 4th week of age compared with *S. Gallinarum* infected group. This improvement of hematological parameter post treatment might be due to suppression of the drug to the microorganism that invade the host so improved absorption of essential substance for erythropiosis [49].

Concerning the leukogram, the chicks infected with *S. Gallinarum*, revealed a significant leukocytosis with heterophilia all over the experimental periods with significant lymphopenia till the 2nd week of age in the infected group compared with the control group. Heterophilia could be attributed to that heterophils is the mainly cell of defense in the body and it also the first line of defense which attack and engulf the foreign microorganisms as a normal response to *Salmonella* infection [44]. Lymphopenia may be attributed to stress resulting from *S. Gallinarum* infection, which stimulates the adrenal gland to secrete corticosteroid hormones, causing destruction of lymphocytes [50]. Our results in agreement with Fotouh et al. [32], who mentioned that experimental *S. Gallinarum* infection in chicks induced leukocytosis, heterophilia, lymphopenia. Chicks of (gp.III) showed an improvement in heterophiles toward the normal level and lymphocytosis when compared with infected group (gp.II). This lymphocytosis observed indicated the immunostimulatory effect of probiotics. These results in agreement with those reported by [51] who concluded that probiotics stimulates the immune response that increase the chickens resistance against the infection. Chicks of (gp.IV), showed a significant improvement in total leukocytes and heterophils during the experimental periods when compared with infected group (gp.II). These results were in agreement with [52] who recorded a significant decrease in total leukocytic count in infected group that treated with ciprofloxacin. In addition lymphocytosis observed only in 2nd week of age which indicates that ciprofloxacin did not show any immunostimulatory effect.

Concerning the liver enzymes, the serum AST and ALT activities in chicks of (gp.II) showed a significant increase in all experimental

periods. This increase could be suggestive of hepatic affection as AST and ALT are good indicators of hepatocellular damage caused by Salmonella infection [53]. Similar results are nearly to those reported by [40]. Who recorded a significant increase in the serum AST and ALT in oral infection of *S. Gallinarum*. Our result confirmed with the histopathological finding which showed sever hydropic degeneration with focal replacement of necrotic hepatic tissue by mononuclear cells, Coagulative necrosis with marked vaculations of hepatocytes particularly near the necrotic area and Sever subcapsular focal hemorrhage. Chicks of (gp.III) showed an apparently significant decrease in the serum AST and ALT activities during all experimental periods compared with infected group (gp.II). This may be due to the predominant role of probiotics in attenuating the liver damage [54]. Chicks of (gp. IV), revealed an improvement in the serum AST and ALT activities toward the normal in 4th weeks of age. This could be attributed to the bactericidal effect of the drug on infection and the resulting improvement in general health conditions [55].

Concerning proteinogram and protein fraction, the *S. Gallinarum* infected group (gp.II) showed a significant hypoproteinemia, hypoalbuminemia and hyperglobulinemia during all experimental groups. These results were similar to those recorded by [56] who reported a significant decrease in total serum protein and albumin with a significant increase in serum levels of globulines. Moreover, the lowering of total serum protein level may be due degenerative and necrotic changes in liver caused by *S. Gallinarum* infection [57]. The observed hypoalbuminemia might due to liver damage caused by *S. Gallinarum* infection as liver is the only organ where albumin is synthesized [57]. A similar increase in globulin concentration was observed by [58] in experimental *S. Gallinarum* infection. Moreover, the significant increase in alpha globuins and gamma globulins, with significant decrease in beta globulin partially in agreement with Kokosharov [57] who recorded an increase in values of alpha 2 globulins and the decrease in beta and gamma globulins in experimentally infected group with *S. Gallinarum*. Chicks of (gp.III) showed a significant increase in the total serum protein and serum albumin during all experimental periods when compared with the infected group (gp.II). This indicates that the employment of Clostri-mix as a prophylactic could counteract the effect of *S. Gallinarum* infection on liver and kidneys. Using of probiotics is more effective as a prophylaxis against infection than being used as a treatment [51]. Chicks of (gp.IV) showed non significant changes in total protein, albumin and globulin as compared with control group. This improvement in protein profile may be attributed to improved state of liver in treated group as synthesis of protein, albumin and globulin occurred in liver [59].

Regarding to the kidney function, chicks that infected with *S. Gallinarum* infected group (gp.II) revealed a significant decrease in serum calcium level with a significant increase in serum phosphorus level all over the experimental periods, compared with normal control (gp.I). Hypocalcemia may be due to protein loss, leading to hypoalbuminemia, or by calcium reabsorption decrease [60]. Nearly similar results were reported [61] who recorded a significant decrease in calcium level in *S. Gallinarum* infected group. The hyperphosphatemia, in our study may be due to a decrease in the calcium level and an increase of the parathermone hormone as a result of hypocalcemia [62]. Our results are not in agreement with those reported by [61] who found that *S. Gallinarum* infected group show a significant decrease in phosphorus level which related to low ingestion of these nutrients, likely because these birds were not feeding properly in reason of the infection by S. *Gallinarum*. Chicks of gps. (III and IV), showed a significant increase in the serum calcium level in all experimental periods compared with *S. Gallinarum* infected group. While the serum level of phosphorus revealed a significant decrease in the same groups at the same periods. This indicate that Clostri-mix as a prophylactic or ciprofloxacin as a treatment could counteract the effect of *S. Gallinarum* infection.

The infected group (gp.II) showed a significant increase in serum uric acid at the 2nd week of age and a significant increase in creatinine level in all experimental periods. Similar results were in agreement with those obtained by [32] who recorded a significant increase in uric acid level after 7th and 10th DPI and significant increase in creatinine level during all the experimental periods. This may be attributed to kidney damage caused by Salmonella infection [53]. The level of creatinine and uric acid is known to reflect the state of glomerular filtration rate and kidney function. The histopathological lesion confirmed our result which showed coagulative necrosis of renal tubular epithelium and Focal replacement of renal parenchyma by mononuclear cells with pyknosis of the nuclei of some renal tubular epithelium. Administration of *Cl. butyricum* before and after *S.Gallinarum* infection (gp.III), relieved the advirse effect of *S. Gallinarum* infection on renal function which showed a returning in the serum uric acid and creatinine to the normal levels, when compared with infected group. Gp. (IV) showed a significant decrease in both the serum creatinine and uric acid in 2nd week of age which returned to the normal levels in the 4th week of age, compared with infected group. In our opinion, this decrease in creatinine and uric acid levels was due to the recovery of the kidneys from the lesions, produced by *S. Gallinarum* after ciprofloxacin treatment. These results are in accordance with those recorded by [52] who reported that administration of ciprofloxacin to E. coli infected chicks showed a significant decrease in serum uric acid and serum creatinine level.

Regarding the oxidant and antioxidant, chicks of (gp.II) showed a significant increase in MDA concentration and a significant decrease in GSH activity on 2nd, and 4th weeks of age. This due to the bacterial infection and bacterial endotoxins induces extensive damage to a variety of organs, including liver, due to the increased production of reactive oxygen intermediates and a resultant rise in lipid peroxidation. These accumulations of toxic radicals in circulation produce increase the production of antioxidant enzymes. Increasing MDA in circulation was accepted an indicator of increasing cell damage. Our results are in accordance with that reported by [63] who recorded that *S. enterica* caused increasing at plasma MDA levels but it caused decreasing GSH-Px activity. Chicks of gp. (III), showed a significant decrease in MDA and a significant increase in GSH when compared with infected group. This may be attributed to that *Cl. butyricum* could inhibit both liver and blood lipid oxidation (MDA production) in broiler chicks [64] and the potential reduction of oxidative stress of probiotics [65]. Gp. (IV) showed no significant change in MDA concentration and GSH activity when compared

with infected group. This attributed to stress of ciprofloxacin and bacterial infection. Our results are in accordance with those reported by [66] who reported that infection with E. coli and treatment with ciprofloxacin produced a significant decrease in GSH activity.

Regarding the results of cell mediated immune response, gp. (II), showed a significant decrease in lymphocytic transformation rate, phagocytic percent and phagocytic index during the experimental periods. This support the previously obtained results by Sadegh et al. [67], who reported that salmonella challanging had a negative effect on the immune response of the broiler chickens throught retardation of the growth of immune organs, change in the immune profile of the immune cells produced and due to a decrease in anti-body production.

Compared with *S. Gallinarum* infected group, chicks administrated *Cl. butyricum* before and after *S. Gallinarum* infection (gp.III) and those which treated with ciprofloxacin (gp. IV), showed a statistical increase in lymphocytic transformation rate, phagocytic percent and phagocytic index in 2nd and 4th weeks of age. Such increase was clearer in (gp.III). Probiotics stimulate natural resistance of the organism through increasing the number of antibodies and increasing the effectiveness of macrophages, also probiotics essential for enhancement functional immune system including the presence of T and B lymphocytes in the lamina propria and the expansion and maturation of IgA [68].

Moreover, the hepatic lesions reveled sever hydropic degeneration with focal replacement of necrotic hepatic tissue by mononuclear cells, with proliferation of the von kupffer cells. In addition to, coagulative necrosis with marked vaculations of hepatocytes particularly nears the necrotic area. The intestinal lesion showed catarrhal enteritis represented by marked mononuclear cell infiltration and excess mucous secretion and sloughed some intestinal villi. These results are in harmony with the histopathological studies of the liver and intestine that reported by [69] who revealed hepatitis characterized by leukocytic infiltration at perivascular areas along with hydropic vacuolation in hepatocytes, multiple necrotic foci was noticed with Kupffer cell hyperplasia. Similar degenerative, necrotic and infiltrative lesions have been reported by [28] and [70].

CONCLUSIONS

Salmonella Gallinarum has adverse effects on blood cellular constituents, biochemical parameters, immune status, liver, kidney and intestine of infected broiler chicks. *Clostridium butyricum* decreases the serious effect of *S. Gallinarum* by increasing bird immunity when used as a prophylactic before infection.

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