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Experimental Infection with *E. coli* O157 in Rats and Its Toxic Effect, Biochemical and Histopathological Changes with Referee to Modern Therapy

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Abstract

This study was carried out to investigate the effect of experimental infection by *Escherichia coli* O157 on some biochemical parameters and histopathological changes in rats and current methods of treatment to reach this objective (number of rats 25 divided in three groups) all groups were housed under same conditions of water, food, temperature, light and humidity (one week for acclimatization) control groups which divided into two groups (Group I was served as a control negative. Group II served as control positive group infected with *E. coli* O157 2 ml of (1×10^9) colony forming unit/ml at the 7th day of experiment without treatment. Probiotics groups that divided into Group III: Rats received lactobacillus as probiotic by dose of 3.6 × 1021 dissolved in distalled water orally by stomach tube all over the time of experiment (14 days) and infected with *E. coli* O157 2 ml of (1×10^9) colony forming unit/ml at 7th day of experiment and treated with ciprofloxacin by dose of 14.4 mg/Kg.b.wt once daily by intraperitoneal rout at three days after infection for five successive days, Group IV: Rats received *Lactobacillus* as probiotic by dose of 3.6 × 1021 dissolved in distalled water orally by stomach tube all over the time of experiment (14 days) and infected with *E. coli* O157 2 ml of (1×10^9 colony forming unit/ml) at 7th day of experiment. Group V: Rats infected with *E. coli* O157 2 ml of (1×10^9) colony forming unit/ml at 7th day of the experiment and treated with ciprofloxacin by dose of 14.4 mg/Kg.b.wt once daily by intraperitoneal injection at three days post infection and for five successive days. After five days from infection, blood samples were collected from all groups from retro-orbital sinus by using of capillary tube to measure various biochemical changes, which affected by infection in all groups respect to control groups as well as samples from different organs (liver, kidneys, lung, heart, spleen, brain, testes, and intestine) were taken for histopathology examination. The biochemical and histopathological changes in all groups were recorded regarding to the biochemical changes, histopathological and fertility examination, the results revealed that significant different between infected and treated groups in comparison to negative control one. These results demonstrate that the administration of the probiotic *Lactobacillus debruckii* and *Lactobacillus fernentum* in combination with ciprofloxacin to rats can reduce the severity of *E. coli* O157:H7 infection, reduce toxicity and histopathological alterations in different organs. Consequently, this reduction may be associated with enhancement of liver and kidney function and immune responses.

Keywords: Experimental infection; *E. coli* O157 toxicity; Histopathology; Ciprofloxacin and probiotic therapy

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Introduction

Escherichia coli are normal inhabitant in the digestive and upper respiratory tracts resulting in severe infections like colibacillosis [1]. Colibacillosis producing a serious problem in animal production, with mortality, condemnations that leading to significant economic losses [2]. *E. coli* infection is known to damage immune system including lymphocyte depletion in lymphoid organs [3].

E. coli strains of serotype O157:H7 belong to a family of pathogenic *E. coli* called Enterohemorrhagic *E. coli* (EHEC) strains are characterized by the production of cytotoxins called

Shiga Toxins (STX1 and STX2) or verotoxins [4]. Toxins producing *Escherichia coli* (STEC) especially of serotype O157:H7 an important zoonotic pathogen [5] that causes hemorrhagic colitis and Hemolytic Uremic Syndrome (HUS) [6]. Hemolytic Uremic Syndrome (HUS) causing acute renal failure. Infections with *E. coli* can affect different sites of the male reproductive tract such as the testis, the epididymis and male accessory sex glands. Spermatozoa subsequently can be affected by infections at different points in their development and maturation. Acute or chronic infections can compromise spermatogenesis, resulting in quantitative and qualitative reductions, direct interactions with pathogenic bacteria or immune competent cells represent another possibility for infectious impact on spermatozoa [7]. The fluoroquinolones had high tissue penetration, broad spectrum activity and relatively safe due to their low Minimum Inhibitory Concentrations (MIC) usually 0.1 ug/mL to 2.0 ug/mL and leaving little or no residue in edible tissues so encouraged their use in veterinary medicine [8,9]. Probiotics are: "Live microorganisms which when administered in adequate amounts confer a health benefit on the host according to the currently adopted definition by food and agricultural organization/world health organization. Today, specific health effects of probiotics are being investigated and documented including alleviation of chronic intestinal inflammatory diseases, prevention and treatment of pathogen-induced diarrhea, and urogenital infections [10]. The increasing number of outbreaks and HUS cases highlight the urgent need to find new ways to control evolving drug resistant to infections and embark on the need for a continued search for new antimicrobial compounds. *Lactobacillus acidophilus* is regarded as probiotic, since it competes and interferes with adhesion of other microbial pathogens in addition to its ability to produce H_2O_2, bacteriocin, lactic acid and other molecules which act as inhibitors for another microorganism. Consequently, the current study was aimed to investigate the effect of experimental infection by *Escherichia coli* O157 on some biochemical parameters and histopathological changes in rats and to study possible protective effects of the probiotic *Lactobacillus debruckii* and lactobacillus fernentum in combination with ciprofloxacin.

Material and Methods

Chemicals

Drug: 1-ciprofloxacin were purchased from Amriya pharmaceutical company each vial 100 ml each 1 ml contain 2 mg by dose of (14.4 mg/Kg.b.wt once daily intraperitoneal according to Paget and Barnes 1964) to infected rats at the 7th day of the experiment for five consecutive days after challenge of infections.

Probiotic

Probiotic Lactobacillus: purchased from Rameda company as lacteal sachets: each sachets contain *Lactobacillus* LB: Which corresponding to *Lactobacillus debruckii* and *Lactobacillus fernentum* 10 billion used by dose of 1.8 × 1021/Kg.b.wt according to Paget and Barnes all over the time of experiment (for fourteen days) to infected rats with *E. coli* O157.

Strain

E. coli 2 ml of (1 × 10^9) colony forming unit/ml of *E. coli* O157 strain which prepared in Animal Health Research Institute (AHRI), Giza, 11331, Egypt at the 7th day of the experiment by using of stomach tube.

Animal

Experiments were done following guidelines set by Ethical Committee of Benha University. They were obtained from Animal Research Center (Benha University). Their age range was 6 to 7 weeks, and their weight was 200 g to 230 g at the beginning of experiments. They were caged in the animal house of the supplier, in which the temperature was 23°C to 26°C, and a light: Dark periods of 10:14 h/day. The animals had free excess to food (standard pellets) and drinking water (ad libitium) during all experiments.

Experimental design

Twenty-five rats were divided randomly into three groups (control groups/probiotic group each group contain 10 rats and *E. coli* and ciprofloxacin (5 rats/group): A-Control groups: (10 rats) which subdivided into two groups (5 rats/group)

Group I: Rats served as control negative group not infected and without treatment, were given distilled water orally by stomach tube all over period of experiment (14 days)

Group II: Rats served as control positive group infected with *E. coli* O157 2 ml of (1 × 10^9) colony forming unit/ml at the 7th day of experiment.

B- Probiotic groups: 10 rats which subdivided into two groups (5 rats/group).

Group III: Rats received lactobacillus as probiotic by dose of 3.6 × 1021 dissolved in distilled water orally by stomach tube all over the time of experiment (14 days) and infected with *E. coli* O.157 2 ml of (1 × 10^9) colony forming unit/ml at 7th day of experiment and treated with ciprofloxacin by dose of 14.4 mg/Kg.b.wt once daily by intraperitoneal rout at 3rd days after infection for five successive days.

Group IV: Rats received *Lactobacillus* as probiotic by dose of 3.6 × 1021 dissolved in distilled water orally by stomach tube all over the time of experiment (14 days) and infected with *E. coli* O157 2 ml of (1 × 10^9) colony forming unit /ml at 7th day of experiment.

Group V: *E. coli* infected with ciprofloxacin treatment. Rats infected with E. *coli* O157 2 ml of (1 × 10^9) colony forming unit/ml at 7th day of the experiment and treated with ciprofloxacin by dose of 14.4 mg/Kg.b.wt once daily by intraperitoneal rout at 3rd days after infection and for five successive days. At the end of the 2nd week of treatment, blood samples were obtained from the retro-orbital venous plexus (5 rats/group) and collected into a plain centrifuge tube to separate serum for biochemical evaluation. (Liver, kidney function, testosterone hormone, tumor necrotic factor TNFα) and after scarification of rat's specimen from varies organs as liver, kidney, lung, heart, brain, spleen, tests and intestine for histopathological examination were taken.

Biochemical evaluation

At the end of the 2nd week, blood samples of the rats were taken from retro-orbital venous plexus, placed into sterile tubes and centrifuged at 3500 rpm for 20 min to separate the serum. Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were determined according to [11] and Creatinine was determined according to [12]. Urea was determined according to [13]. Total protein was determined by colorimetric method according to [14]. Albumin was determined by colorimetric method according to Doumas et al. [15] total globulins were calculated as follow: Globulins = Total protein- Albumin cholesterol was estimated as described by Pesce and Boundourian [16]. Triglycerides were measured colorimetric (according to Fossati [17]). Glucose according to Trinder [18], Ca according to McLean and Hastings [19], Na according to

Table 1: The effect of experimental infection by *Escherichia coli* O157 and treatment on kidney functions in groups of rats.

Parameters/Groups	Control groups		Probiotic groups		
	E. coli –ve (G I)	*E. coli* +ve (G II)	*E. coli* + probiotic + cipro (G III)	*E. coli* + probiotic (G IV)	*E. coli* + cipro (G V)
Urea	52.20 ± 1.8^{b}	74.75 ± 7.7^{a}	50.981 ± 0.88^{b}	53.783 ± 0.33^{b}	61.812 ± 2.4^{b}
Creatinine	0.45 ± 0.03^{c}	1.89 ± 0.22^{a}	1.21 ± 0.15^{b}	1.25 ± 0.19b	1.74 ± 0.035^{a}
Sodium	158.13 ± 15.55^{a}	73.6 ± 4.35^{d}	140.88 ± 9.87^{b}	108.4 ± 4.308^{c}	106.26 ± 14.05^{c}
Potassium	9.30 ± 0.55^{a}	5.68 ± 0.68^{c}	8.830 ± 0.48^{ab}	8.17 ± 0.47^{ab}	8.18 ± 0.7^{b}
Phosphorus	6.81 ± 0.12^{a}	3.51 ± 0.52^{c}	5.75 ± 075^{ab}	5.100 ± 0.47^{ab}	5.05 ± 0.22^{b}

The values represent Mean ± SD. Means within the same row followed by different letters are significantly different (P<0.05)

Table 2: The effect of experimental infection by *Escherichia coli* O157 and treatment on liver functions in different groups of rats.

Parameters/Groups	Control groups		Probiotic groups		
	E. coli –ve (G I)	*E. coli* +ve (G II)	*E. coli* + probiotic + cipro (G III)	*E. coli* + probiotic (G IV)	*E. coli* + cipro (G V)
Total protein	8.47 ± 0.26^{a}	6.16 ± 0.36^{b}	8.48 ± 0.57^{a}	8.28 ± 0.43^{a}	7.59 ± 0.47^{a}
Albumin	3.06 ± 0.42^{a}	2.90 ± 0.18^{b}	2.99 ± 0.22^{a}	2.89 ± 0.15^{a}	2.89 ± 0.15^{b}
Globulin	5.48 ± 0.27^{a}	3.31 ± 0.84^{c}	5.49 ± 0.59^{a}	5.3 ± 0.21^{a}	4.7 ± 0.71^{a,b}
A/G ratio	0.55 ± 0.018^{a}	0.7 ± 0.18^{b}	1.07 ± 0.39^{a}	0.56 ± 0.02^{a}	0.71 ± 0.10^{a}
AST	73,83 ± 2.90^{c}	94.99 ± 2.9^{a}	73.83 ± 2.03^{c}	82.88 ± 2.4^{bc}	85.15 ± 4.9^{ab}
ALT	51.87 ± 1.45^{b}	86.63 ± 5.81^{a}	51.63 ± 3.52^{b}	61.53 ± 2.18^{b}	75.61 ± 4.8^{a}
Trig	72,75 ± 1.76^{b}	109.51 ± 8.96^{a}	76.25 ± 50.40^{b}	85.25 ± 1.45^{b}	121.8 ± 11.56^{a}
Cholesterol	80 ± 3.056^{a}	90 ± 11.45^{a}	62.8 ± 3.05^{a}	74.5 ± 6.18^{a}	80.4 ± 4.34^{a}
TNF-α	64.66 ± 1.36^{c}	195.22 ± 10.21^{a}	64.66 ± 3.39^{c}	72.03 ± 1.35^{c}	89.34 ± 3.05^{b}

The values represent Mean ± SD. Means within the same row followed by different letters are significantly different (P<0.05)

Trinder [20], K according to Berry et al. [21], P by the method of Gamst and Try [22], testosterone determined in serum according to Vermeulen et al. [23]. Determination of TNF-α using ELISA kit according to the methods described by Chen et al. [24].

Epididymal sperm count

Epididymal spermatozoa were counted by a modified method of Yokoi et al. [25]. The epididymis was minced in 5 ml of saline, placed in centrifuge for 10 min and incubated at room temperature for 2 min. The supernatant fluid was diluted 1:100 with a solution containing 5g $NaHCO_3$, 1 ml formalin (35%) and 25 mg eosin per 100 ml distilled water. About 10 µl of the diluted semen was transferred to each counting chamber of the improved Neubauer hemocytometer (Deep 1/10 mm LABART, Munich, Germany) and was allowed to stand for 5 min for counting under a light microscope at x200 magnification.

Assay for sperm abnormalities

The method modified by Evans and Maxwell [26] was used for determination of the percentage of morphologically abnormal spermatozoa. A total of 300 sperm cells were counted on each slide under light microscope at x400 magnification.

Histopathological examinations

Specimens from various organs (liver, kidneys, lung, heart, spleen, brain, testes, and intestine) from the sacrificed animals of each group were collected, and then fixed in 10% neutral buffered formalin. Following proper dehydration, clearing then the samples was embedded in paraffin wax. Sections about 5 µm thickness were prepared and stained with H&E for histopathological examinations according to Drury and Willington [27]. The detected pathological alterations were graded qualitatively according to the severity and the degree of dissemination as following: Grade 0= normal histological texture, Grade 1= the detected pathological changes are at a mild degree; Grade 2= the identified pathological changes are at a moderate degree; Grade 3= the demonstrated pathological changes are at a severe degree [28].

Statistical analysis

Data was statistically analyzed by ANOVA with post hock Duncan multiple comparison test using statistical software program (SPSS for Windows version 20, USA). Differences were considered significant at p<0.05.

Results

Biochemical analysis

Serum urea, creatinine, sodium, potassium, calcium, phosphorus, total protein, albumin, globulin and Albumin/Globulin (A/G) ratio, ALT, AST, cholesterol, triglyceride and TNF-α values in either non infected or infected with *E. coli* or different treated groups are presented in the Table 1 and 2. There was significant decrease in urea, creatinine, ALT and TNF-α level, in group III and group IV in comparison with positive control group, while non-significant decrease in AST was detected in the same groups in comparison with positive control group. Meanwhile, significant increase in sodium, calcium, phosphorus, potassium, and total protein were observed in group III and group IV in comparison with control positive interestingly, non-significant decrease in glucose, albumin, globulin, triglyceride and cholesterol was demonstrated in group III and group IV in comparison with control group as shown in Table 1 and 2. Significant increase in sperm count in group III and group IV in comparison with control positive group, while significant decrease in sperm abnormalities in group III and group IV in comparison with control positive group, and begin to return within normal level in group III as shown in Table 3 in comparison with control negative group.

Pathological examination

The histopathological examination of different investigated

Table 3: The effect of experimental infection by *Escherichia coli* O157 and treatment on serum testosterone, sperm number and Sperm abnormality % in different groups of rats.

Parameters/Groups	Control groups		Probiotic groups		
	***E. coli* –ve (G I)**	***E. coli* +ve (G II)**	***E. coli* + probiotic + cipro (G III)**	***E. coli* + probiotic (G IV)**	***E. coli* + cipro (G V)**
Testosterone	262.12 ± 1423[a]	65.13 ± 7.06[c]	260.99 ± 12.85[a]	141.38 ± 23.36[a]	114.01 ± 0.53[b]
Sperm count	7.04 ± 0.069[a]	0.78 ± 0.24[d]	7.04 ± 0.08[a]	2.14 ± 0.23[b]	1.1 ± 0.28[c]
Abnormal sperm %	7.0 ± 0.057[d]	30 ± 1.20[a]	7 ± 0.88[d]	10.5 ± 2.03[c]	18.25 ± 0.88[b]

The values represent Mean ± SD. Means within the same row followed by different letters are significantly different (P<0.05)

organs obtained from rats of group I revealed no pathological alterations in these organs. Meanwhile, variable histopathological alterations in liver, kidney, lung, heart, spleen, intestine, brain and testes were observed in infected animals (group II). The severity of these pathological changes was reduced by variable degrees in other treated groups (group III, IV and V). Pathological lesion scores in various organs obtained from rats of different groups were summarized in Table 4 and explained in figure legends.

Group II (*E. coli* infected rats): The microscopical examination of the liver revealed marked thickening of the hepatic capsule, thromboses of hepatic blood vessels with severe congestion of central, portal veins and hepatic sinusoids in association with scattered areas of hemorrhages were also observed in the hepatic parenchyma. Mononuclear leukocytic cellular aggregations mainly lymphocytes and macrophages were seen in the vicinity of blood vessels as well as in the hepatic parenchyma (Figure 1a). The portal areas were expanded by edema admixed with leukocytes mainly lymphocytes and macrophages. Moreover, marked hyperplastic proliferation of biliary epithelium with formation of newly formed bile ductulus as well as periductal mononuclear inflammatory cells were seen in most infected animals. Multifocally, the hepatocytes in the centric-lobular zones of hepatic lobules showed degenerative changes with multiple areas of extensive coagulative necrosis of hepatocytes, characterized by retention of hepatic cell outline and shrunken hepatocytes with hyper-eosinophilic cytoplasm and pyknotic nuclei (Figure 1b) were also detected in most cases. Multifocally, focal areas of lytic necrosis characterized by disappearance of hepatocytes and replaced by erythrocytes were seen in the hepatic parenchyma of some infected animals. The examined kidneys revealed marked hyperemia of cortical blood vessels with small fibrin thrombi were observed in some blood vessels. Multifocally, the cortical interstitium, predominantly around cortical blood vessels and glomeruli, was occasionally expanded by edema with aggregates of inflammatory cells (Figure 1c) mainly lymphocytes and moderate numbers of neutrophils. Accidently, interstitial lymphocytic cellular infiltrations were noticed in some cases in-between the renal tubules with interstitial hemorrhage. Moreover, some renal tubules in both cortex and medulla exhibited cystic dilatation and were lined by attenuated epithelium. The lining epithelium of some proximal and distal convoluted tubules were swollen and displayed vacuolar and hydropic degeneration. Occasionally, the renal tubules in the renal cortex showed coagulative necrosis of their lining epithelium evidenced by hyper-eosinophilic cytoplasm and pyknosis and karyorrhexis of nuclei (Figure 1d). Cellular casts and homogenous eosinophilic casts were also detected in the lumen of some renal convoluted tubules in both cortex and medulla (Figure 1d). The lungs showed marked congestion of peri-bronchiolar blood vessels; complete destruction of the endothelial cell lining of the blood vessels with thrombosis of some pulmonary blood vessels. Peri-bronchiolar interstitium was expanded by mononuclear inflammatory cells mainly lymphocytes. Perivascular

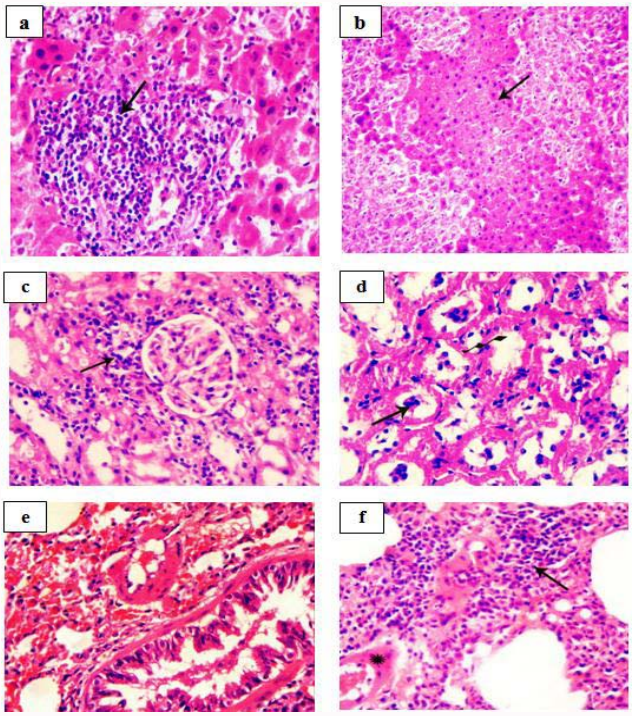

Figure 1: H&E stained section of liver (a&b), kidney (c&d), lung (e&f) taken from animals infected with E. *coli* (group II), showing (a) focal area of mononuclear leukocytic cellular aggregation in the hepatic parenchyma (arrow, x400), (b) diffuse areas of coagulative necrosis in the hepatic parenchyma (arrow, x200), (c) peri-glomerular and inter-tubular leukocytic cellular infiltrations in renal cortex (arrow, x400), (d) necrosis of the lining epithelium of some proximal and distal convoluted tubules (zigzag arrow) with the presence of cellular casts in their lumen (arrow, x200), (e) Diffuse hemorrhage in pulmonary tissue with bronchiolitis (arrow x400), (f) focal areas of alveolar consolidation in which the alveoli filled with inflammatory cells (arrow) with hyalinization of the wall of pulmonary blood vessel (asterisk, x400).

edema admixed with leukocytic cellular infiltrations was also seen. Occasionally, desquamation of the lining epithelium of bronchioles was detected in association with diffuse hemorrhage in pulmonary tissue (Figure 1e). Multiple areas of alveolar consolidation that filled with inflammatory exudate composed of erythrocytes admixed with fibrin threads, macrophages and lymphocytes was demonstrated (Figure 1f). Multifocally, the alveolar septa were thickened by the inflammatory reaction. The heart myocardium showed perivascular and intermuscular edema and hemorrhages admixed with few numbers of macrophages. Myocardial necrosis was detected admixed with mononuclear leukocytic cellular infiltrations in association with hyaline degeneration of some muscle fiber that characterized by highly eosinophilic sarcoplasm with loss of muscular striation (Figure 2a). Additionally, some lytic changes in muscle fibers were seen at different degrees that displaced myocardial muscle fibers were seen in all infected rats. The spleen showed marked congestion of splenic

Table 4: Summary of the histopathological lesions score in different treated groups.

Treatment				
Lesion score	**Group II**	**Group III**	**Group IV**	**Group V**
Liver				
Congestion of hepatic blood vessels	3	0	1	2
Hemorrhage in hepatic parenchyma	2	0	1	1
Perivascular leukocytic infiltration	2	0	0	1
Focal leukocytic aggregations	2	0	0	2
Degenerative changes	3	1	1	2
Necrosis of hepatic cells	3	0	0	1
Kidney				
Congestion of renal blood vessels	3	0	1	2
Hemorrhage in renal cortex	2	0	0	1
Perivascular leukocytic aggregations	2	0	0	1
degeneration and necrosis of glomerular and tubular epithelial cell lining	3	1	1	2
Hyaline and cellular casts	3	0	1	2
Interstitial leukocytic infiltrations	2	0	0	1
Cystic dilatation of renal tubules	2	0	0	1
Hemorrhage in glomerular tuft	2	0	0	1
Peri-glomerular leukocytic aggregations	2	0	0	1
Lung				
Peri-vascular edema	3	0	1	2
Peri-bronchial leukocytic aggregation	3	0	1	2
Diffuse hemorrhage in pulmonary tissue	3	0	1	2
Emphysema	2	1	1	
Atelectasis	2	0	0	1
Consolidated alveoli	3	0	0	2
Heart				
Congestion of myocardial blood vessels	3	1	1	1
Perivascular mononuclear infiltration	3	0	0	2
Inter-muscular hemorrhage	3	0	0	1
Hyaline degeneration of cardiac muscle	2	0	0	1
Spleen				
Hemorrhage	3	0	0	1
Lymphoid depletion	2	1	1	2
Apoptosis	2	0	0	1
Intestine				
Degeneration of the lining epithelial of villi	3	1	1	2
Enteritis	3	0	1	2
Leukocytic infiltrations in sub mucosa	2	0	1	1
Necrosis of the desquamated enterocytes	3	0	0	1
Testes				
Inter-tubular edema	2	0	1	1
Degeneration and vacuolation of the lining epithelium of seminiferous tubules	3	1	1	2
Necrosis of spermatogenic cells	3	0	0	2
Deformity of the shape of seminiferous tubules	2	0	0	1
Brain				
Congestion of blood vessels	3	1	1	2
Neuronal degeneration	3	1	1	2
Perivascular Hemorrhage	3	0	0	2
Encephalomalacia	3	0	0	1
Focal area of glial cells	2	0	1	1

Group II (*E. coli* infected group control positive), Group III (Lacteol + *E. coli* + Cipro group), Group IV (*E. coli* + Lacteol group), Group V (*E. coli* + Cipro), (0), no lesions observed; (1), mild lesion; (2), moderate lesion and (3), severe lesion

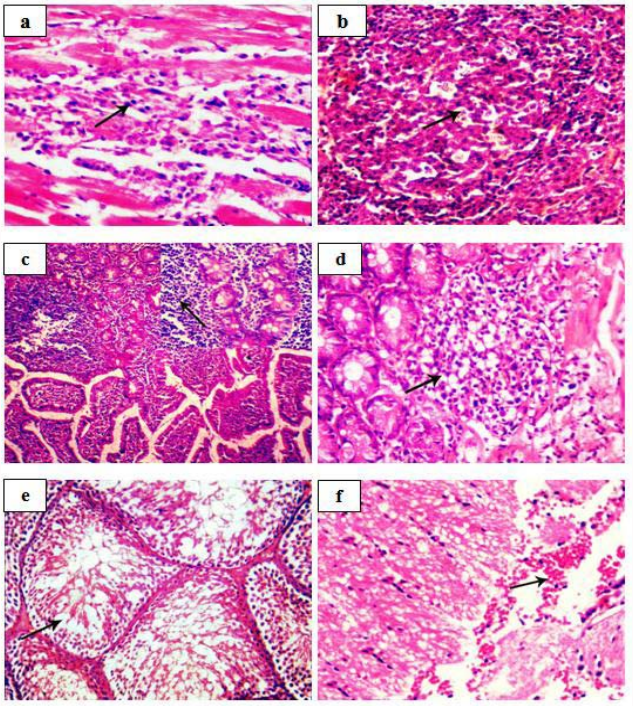

Figure 2: H&E stained section of heart (a), spleen (b), intestine (c&d), testes (e), brain (f) taken from animals infected with *E. coli* (group II), showing (a) necrosis of myocardium with mononuclear leukocytic infiltration (arrow, x200), (b) Lymphoid depletion of peri-arteriolar lymphoid sheaths with scattered lymphocytolysis, nuclear and cellular debris, in association with apoptosis of some cells (arrow, x400), (c) marked mononuclear leukocytic cellular infiltration in the intestinal mucosa (arrow, x200, inset, x400), (d) Extensive vacuolation of the glandular tissue in intestinal submucosa (x200), (e) swollen, pale and vacuolated germ cells lining seminiferous tubules (arrow, x200), (f) diffuse area of hemorrhage admixed with few leukocytes (arrow, x200).

blood vessels with perivascular mononuclear inflammatory cellular aggregates mainly lymphocytes and macrophages. Lymphoid depletion of peri-arteriolar lymphoid sheaths with scattered lymphocytolysis, nuclear and cellular debris, infiltrated by neutrophils and surrounded by fibrin as well as apoptosis were also detected in some cells of germinal centers (Figure 2b). Moreover, extensive hemorrhage was detected particularly in the red pulp. Irregularly thickened, blunted, and fused intestinal villi with complete necrosis of their lining epithelium were noticed in some infected cases. Additionally, sloughing of the necrosed villar epithelium with mononuclear cells infiltration into the lamina propria with mild hemorrhage was seen in other infected cases. Edema and necrosis of the muscularis mucosae was demonstrated in some infected animals. Some lymphocytic infiltrations in submucosa were noticed with necrosis of the epithelial cells lining the intestinal glands with sever infiltration of mononuclear inflammatory cells and increase in the goblet cells of the intestinal villi was observed as well as infiltration of inflammatory cells in-between the intestinal glands with fibrinotic exudates tinged with inflammatory cells in its lumen (Figure 2c). Vacuolation of the glandular epithelium with its engorgement with lymphocytes was also seen (Figure 2d). The testes showed congested blood vessels and moderate interstitial edema. Degenerative changes of the lining epithelium of seminiferous tubules in which the degenerated tubules were lined by swollen epithelial cells with vacuolated cytoplasm accompanied by incomplete spermatogenesis and absence of the spermatozoa in their lumen (Figure 2e). Additionally, deformity of the shape of seminiferous tubules were also demonstrated. Necrosis of spermatogenesis series cells which may lead to sterility. The brain showed congestion of meningeal, cerebral and cerebellar blood vessels. Multifocally, extensive areas of encephalomalacia characterized by focal areas of rarefaction, predominantly affecting the brainstem and periventricular neuropil were noticed. The affected neuropil was separated by abundant clear spaces and was infiltrated by few numbers of glial cells and lymphocytes. Multifocally, variable amounts of hemorrhages were also demonstrated in the meninges and cerebellum, occasionally around small blood vessels were also observed (Figure 2f). Frequently, neuronal cell degeneration or necrosis was seen. Degenerated neurons were swollen and rounded with peripheralized Nissl substance. Necrotic neurons were shrunken and angular, with hyper eosinophilic cytoplasm with pyknotic or completely absent nuclei. There was occasional satellitosis and neuronophagia.

Treated groups

Histopathological changes of various organs obtained from infected-treated groups displayed improvement with variable degrees in comparable with that of the infected group. Although, the rats of the infected-treated groups still showing some pathological changes such as mild degenerative changes and mild infiltration of mononuclear cells, their cells tend to be in normal state in some treated cases especially in cases treated with either a combination of both Cipro and probiotic or probiotic only. Meanwhile, marked improvement in the pathological alterations in various organs obtained from infected-treated group with a combination of Cipro and probiotic (group III) in comparable with that of the infected group only. Liver showed nearly normal hepatic parenchyma (Figure 3a) in comparison to the infected group. Kidneys showed mild degenerative changes in the lining epithelial cells of glomerular tuft and renal tubules (Figure 3b). The only pathological alterations detected in the pulmonary tissues were emphysema of some pulmonary alveoli (Figure 3c) and few peri-bronchial leukocytic cellular infiltrations. The cardiac muscle appeared nearly normal in comparison to negative control group (Figure 3d) except mild congestion of the myocardial blood vessels in one case. Very mild depletion of lymphocytic cells of white pulp was demonstrated in the spleen of few treated animals in comparison to negative control group (Figure 3e). Nearly normal histological architecture of intestinal mucosa with only increase in the number of goblet cells was also detected (Figure 3f). Testis showed normal spermatogenesis series cells with production of sperm cells in most examined cases (Figure 3g) except mild degenerative changes of spermatogenic cells in few examined cases. Normal histological structure of brain tissue was observed in most cases except mild vacuolization of neurons in brain was also demonstrated (Figure 3h). However, the microscopical examination of different organs obtained from rats infected with *E. coli* and treated with probiotic (group IV) revealed improvement of the pathological alterations induced by *E. coli* infection in different investigated organs. The hepatocellular architecture with more regular and less altered hepatocytes when compared to *E. coli* infected rats was demonstrated in most treated animals except only mild dilatation of central vein and hepatic sinusoids was seen in two animals (Figure 4a). Additionally, mild degenerative changes in hepatocytes were also seen in two cases. Degeneration of the lining epithelium of renal tubules with the presence of hyaline casts in the lumen of some renal tubules were also detected in the kidney of few rats (Figure 4b). The lung showed mild perivascular edema with mild degenerative changes in the wall

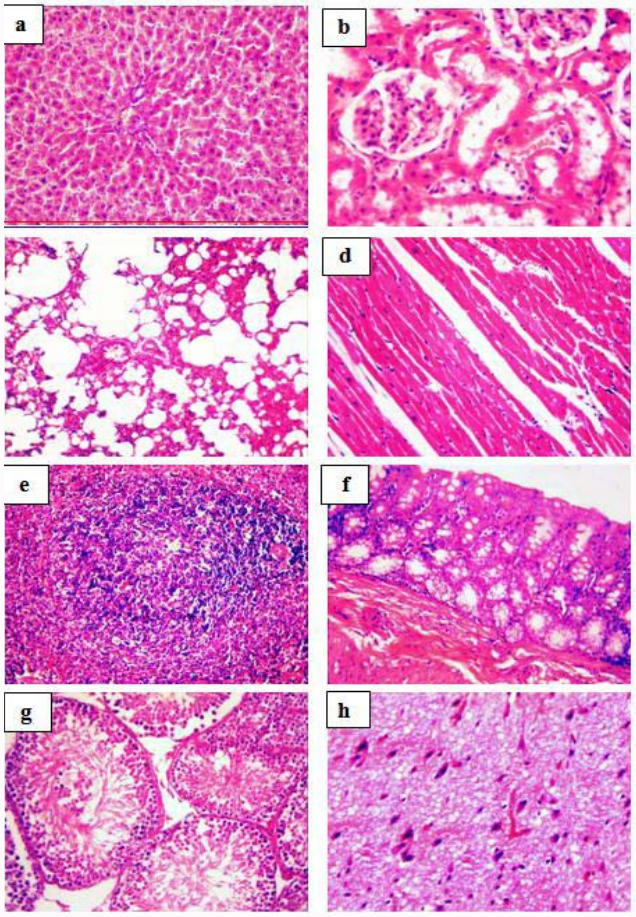

Figure 3: H&E stained section of liver (a), kidney (b), lung (c), heart (d), spleen (e), intestine (f), testes (g), brain (h) taken from infected animals with *E. coli* and treated with both Cipro and probiotic (group III), showing (a) normal histological structure of hepatic tissue (x200), (b) mild degenerative changes in the lining epithelial cells of glomerular tuft and renal tubules (arrow, x400), (c) emphysema in the pulmonary alveoli (x 200), (d) normal histological structure of cardiac muscle (x200), (e) mild depletion of lymphocytic cells of white pulp (x200), (f) nearly normal histological architecture of intestinal mucosa with only increase in the number of goblet cells (x400), (g) normal spermatogenesis series cells with production of sperm cells (x400), (h) mild vacuolization of neurons in brain (x200).

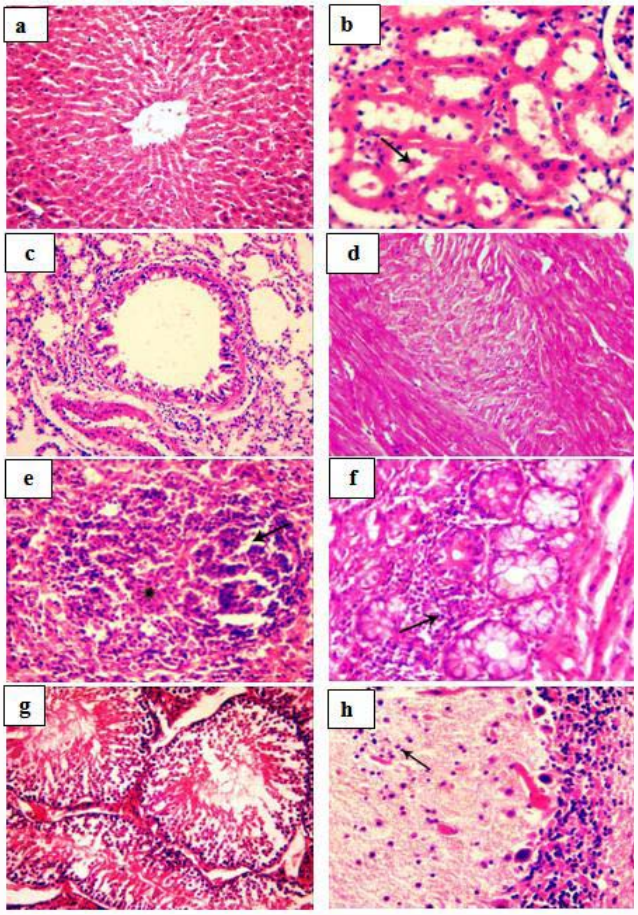

Figure 4: H&E stained section of liver (a), kidney (b), lung (c), heart (d), spleen (e), intestine (f), testes (g), brain (h) taken from infected animals with E.coli and treated with probiotic (group IV), showing (a) Normal histological structure of hepatic tissue (x200), (b) mild degeneration of the lining epithelium of renal tubules with the presence of hyaline casts in the lumen of some renal tubules (arrow, x200), (c) few peri-bronchial mononuclear leukocytic infiltration (arrow, x200), (d) Normal histological structure of cardiac muscle (x200), (e) mild lymphoid depletion of some lymphoid follicles of the white pulp (arrow) with focal necrotic area (asterisk, x400), (f) mild degenerative changes in the lining epithelium of the intestinal villi with few inflammatory cells in the intestinal sub mucosa (x400), (g) mild degenerative change of spermatogenic cells of some seminiferous tubules (x400), (h) mild glial cells infiltration in the brain tissue (x200).

of pulmonary blood vessels in association with few peri-bronchial mononuclear leukocytic infiltrations (Figure 4c) in two animals only, while mild hemorrhage was noticed in the pulmonary tissue of only one animal. Interestingly, normal histological structure of cardiac muscle (Figure 4d) was seen in most treated rats except mild congestion of myocardial blood vessels was observed in two animals. Meanwhile, mild lymphoid depletion of some lymphoid follicles of the white pulp with focal necrotic area (Figure 4e) was demonstrated in the spleen of some rats in this group. Mild degenerative changes in the lining epithelium of the intestinal villi with few inflammatory cells were observed in the intestinal sub mucosa (Figure 4f) of only two cases. The testes showed congestion of testicular blood vessels and mild degenerative change of spermatogenic cells of some seminiferous tubules (Figure 4g). Furthermore, the brain showed congestion of meningeal blood vessels, few glial cells infiltration in the brain tissue (Figure 4h) with mild neural degeneration. However, different organs (liver, kidney, lung, spleen, heart, intestine, testes and brain) from non-infected or treated rats didn't show any pathological alterations. The microscopical examination of different organs obtained from rats treated with *E. coli* and Cipro (group V) for week's revealed mild improvement in most investigated organs in comparison to *E. coli* infected rats Liver showed dilatation of hepatic sinusoids. Individual necrotic hepatocytes with few peri-portal leukocytic cellular infiltrations were detected only in two treated animals (Figure 5a). Moreover, small focal area of hemorrhage was observed in the hepatic parenchyma of some animals in association with degenerative changes in the hepatocytes. Kidneys showed mild degeneration of the lining epithelium of renal tubules with shrinkage of glomerular tuft with few lymphocytes cell infiltration (Figure 5b). Hyaline and cellular casts were detected in the lumen of some renal tubules was demonstrated only in one examined animal. Focal areas of atelectasis in which pulmonary alveoli appeared as slit like opening was demonstrated in the lung of two treated rats (Figure 5c). Diffuse hemorrhage in pulmonary tissues with consolidated alveoli and peri-bronchial leukocytic cellular aggregations was also seen in some animals. Congestion of myocardial blood vessels (Figure 5d) was observed in

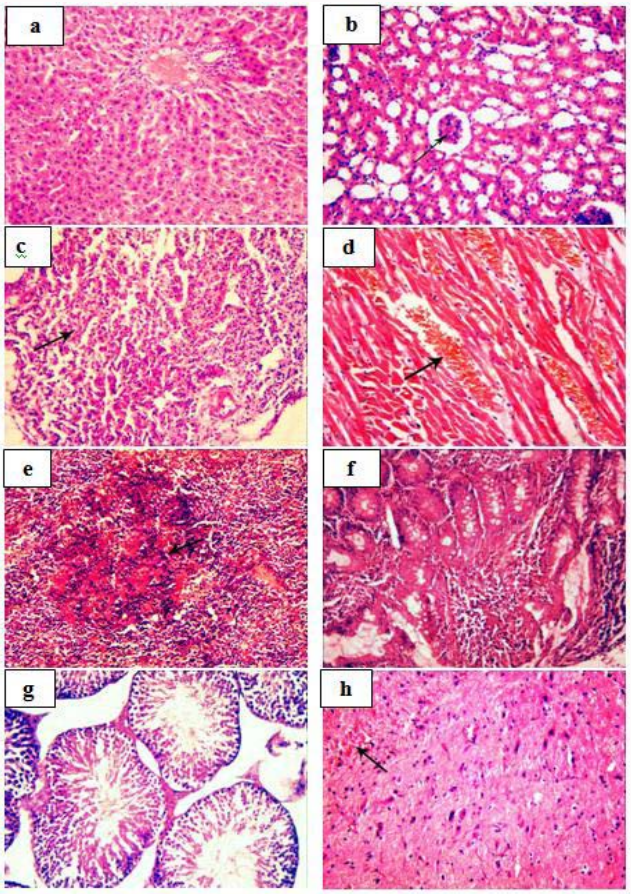

Figure 5: H&E stained section of liver (a), kidney (b), lung (c), heart (d), spleen (e), intestine (f), testes (g), brain (h) taken from infected animals with E.coli and treated with Cipro (group V), showing (a) congestion of portal blood vessels with dilatation of hepatic sinusoids and individual necrotic hepatocytes (x200), (b) degeneration of the lining epithelium of renal tubules with necrosis and shrinkage of glomerular tuft (arrow, x200), (c) Atelectasis of pulmonary alveoli (x200), (d) marked congestion of myocardial blood vessels (x200), (e) depletion of lymphocytic cells of white pulp with necrosis of the lymphoid follicles of the white pulp (arrow, x200), (f) degenerative changes in the lining epithelium of intestinal villi with few inflammatory cells in the lumen of the villi and in lamina propria (x200), (g) necrosis of spermatogenic cells of some seminiferous tubules (x200), (h) focal area of hemorrhage in the brain tissue (x200).

some treated animals in combination with perivascular mononuclear leukocytic cellular infiltrations with mild inter-muscular hemorrhage and hyaline degeneration of cardiac muscles was observed in two animals. Spleen showed mild depletion of lymphocytic cells of white pulp in association with necrosis of some lymphoid follicles (Figure 5e). Additionally, focal areas of hemorrhage were demonstrated in the red pulp with apoptosis of some lymphocytes in the white pulp. Intestine showed degenerative changes of the lining epithelium of intestinal villi with few inflammatory cells in the lumen of the villi and in lamina propria (Figure 5f). Necrosis of spermatogenic cells of some seminiferous tubules with inter tubular edema was noticed in the testis of few treated rats (Figure 5g). Focal area of hemorrhage was seen in the brain of some treated animals (Figure 5h). Moreover, neural degeneration, encephalomalacia with focal area of glial cell aggregation was also observed in some treated cases.

Discussion

The main clinical signs observed in most infected non treated rats represented mainly in listlessness, depression, tendency to huddle together, loss of appetite, depression, matted vent and watery, fuel-smelling diarrhea with yellowish brown coloration. The duration of diarrhea ranged from 1 to 3 days. Respiratory signs including gasping, sneezing in rats were noticed. Meanwhile, the treated groups showed gradual improvement and subsiding of clinical signs with variable degrees. Interestingly, the rats were apparently normal after the course of treatment with the combination of both ciprofloxacin and probiotic this indicate successful infection and treatment. These findings were confirmed by the results of histopathological examination of histological sections obtained from different organs of rats infected with *E. coli* as various pathological alterations was demonstrated in different internal organs including lung, liver, kidney, heart, spleen, intestine, testes and brain. After infection, toxins are released by STEC in the intestine translocated across the gut epithelial into the circulation and transported to capillary endothelial cells in renal glomeruli and other organs Host cells. Then cells were injured by inhibiting the protein synthesis, inducing apoptosis vascular injury. HUS results from action of toxins on vascular endothelial cells the net result is a multi-organs thrombotic process. Toxins are the most virulent factors that can cause micro vascular endothelial injury. The resulting injured endothelial changes its normal thromboresistant phenotype and becomes thrombogenic, initiating microvascular thrombus formation [29]. Therefore, the present study was conducted to investigate the effect of infection by *Escherichia coli* O157 on some biochemical parameters and histopathological changes in rats and to study possible protective effects of the probiotic *Lactobacillus debruckii* and lactobacillus fermentum in combination with ciprofloxacin. Our experimental infection reported significant changes in liver function among control and treated groups (Table 1) as significant changes in total protein, ALT, and globulin when compare with probiotic group while glucose, cholesterol, albumin, AST and triglyceride not significantly changed these may be due to injury of hepatic cells by inhibiting the protein synthesis, inducing apoptosis, vascular injury during HUS result from action of toxins on vascular endothelial cells [29], also Ruetten and Thiemermann [30,31] showed that endotoxemia caused by *E. coli* for 6 h caused a significant rise in the serum levels of AST, ALT, and bilirubin reflecting the hepatic dysfunction, these results were supported with microscopical examination of the liver that revealed various pathological alterations were detected in the hepatic tissue while treated groups showed significant improvement in liver function especially group III which received probiotic and ciprofloxacin as the histopathological examination showed nearly normal hepatic parenchyma in comparison to other groups. Ciprofloxacin is a fluoroquinolone derivative antibiotic with a wide spectrum of antimicrobial activity and low cross resistance to non-quinolone antibiotic classes. Oral administration of ciprofloxacin results in rapid absorption through the gastrointestinal tract with approximately 70% to 80% bioavailability (19% excreted in feces) [32], orally supplementation with Lactobacillus can reduce the severity of *E. coli* O157:H7 infection in rats. Several mechanisms by which probiotics mediate its effect have been suggested, including competition for adhesion sites, production of antimicrobial substances, competition for nutrients and the stimulation of host immunity [33,34]. The examined kidneys revealed changes in kidney function among infected and treated groups, biochemical changes of kidney function test observed in infected group as significant increase in Urea, Creatinine. Sodium, Potassium, Calcium and Phosphorus in comparison to treated groups and negative control group. These

results could be attributed to the endotoxemia caused by *E. coli* caused a significant rise in the serum levels of urea and creatinine and lipase enzyme indicating the occurrence of renal and pancreatic dysfunction as reported previously by Ruetten and Thiemermann [30,31]. Additionally, Wellings et al. [35], founded that endotoxins of *E. coli* administrations induce renal dysfunction characterized by increased blood urea nitrogen and plasma creatinine levels in rats due to kidney dysfunction. The obtained results in the current study was supported by our histopathological changes of the that showed variable pathological changes in the renal tissue obtained from infected group while treated groups showed significant improvement in kidney function especially group III which received probiotic and ciprofloxacin that matched with the microscopical examination of the kidneys among this group that showed mild degenerative changes in the lining epithelial cells of glomerular tuft and renal tubules. Although, the histopathological examination of different organs in rats infected with *E. Coli* revealed that, the most affected organs were liver and kidney which agreed with Hammad et al. [36] and Krishnamoorthy et al. [37], that may be due to selective suppression of the activity of natural killer cells and inhibition of DNA and protein synthesis leading to liver damage and nephrotoxicity, various pathological alterations were demonstrated in other internal organs including lung, heart, spleen, intestine, brain and testes in the present work. However, these pathological changes were markedly reduced in groups treated mainly with probiotics, these findings confirm that probiotics have a greet attention as it has beneficial effects for the host and widely applied in gastrointestinal and liver diseases. These results came agree with [38-40] Probiotic LAB can modulate the immune defense mechanisms that matched with our results by improvement the lymphoid depletion in the spleen and influence metabolic processes and digestion via the normalization of altered intestinal tissue *via* reducing the pathological alterations induced by *E. Coli* in the intestine. Our experimental infection reported significant deleterious changes in sperms (counts, abnormalities % and testosterone level) and histopathological alterations in the testicular tissue among the infected rats. Degenerative changes of the lining epithelium of seminiferous tubules with vacuolated cytoplasm accompanied by incomplete spermatogenesis and absence of the spermatozoa in their lumen with deformity of the shape of seminiferous tubules and necrosis of spermatogenesis series cells which may lead to sterility. Meanwhile, treated groups showed significant improvement in sperms (counts, abnormalities % and serum testosterone) especially group III which received probiotic and ciprofloxacin with marked improvement in the pathological changes induced by *E. Coli* since testes showed normal spermatogenesis series cells with production of sperm cells in most examined cases except mild degenerative changes of spermatogenic cells in few examined cases that clarify the significant improvement in the proportion of life sperm counts and reduction in sperm abnormalities percentage, the obtained results are matched with Takio Inatomi and Konosuke Otomaru [41]. Additionally, our findings came in contact with Gotoh [42] and Phillip [43] who recoded that both *Lactobacilli* strains decrease the colony count of tested strains by more than 90% after 60 min contact time. Both Lactobacilli strains significantly improve the antibacterial effect of tested antibiotics against Enterotoxigenic *Escherichia coli* (ETEC) and so our experiment clarify that the effect of ciprofloxacin alone group (V) against ETEC strain was significantly less than probiotics groups especially group III. Furthermore, *Lactobacilli* inhibit pathogenic bacteria by producing antimicrobial substances such as lactic acid, hydrogen peroxide and bacteriocins. However, there are several mechanisms by which probiotics *in vivo* mediate their health benefits in the host; first, certain probiotics have antimicrobial activity and can exclude or inhibit pathogens; second, they can enhance the intestinal epithelial barrier; third, probiotic bacteria are believed to modulate the host immune response [44]. The combined therapy of *L. acidophilus* with ciprofloxacin in treatment of infective gastroenteritis will help to decrease the required dose of ciprofloxacin and subsequent its potential side effects on intestinal bacterial flora. Ciprofloxacin, is the first-choice antibiotic for the treatment of testicular infections [45] as the treatment with ciprofloxacin for 3 days, showed the recovery of seminal parameters with restoration of spermatogenesis, as ciprofloxacin accentuates the significant eradication of *E. coli* from urogenital organs [46]. Additionally, it induces significant improvement in sperm motility and the decrease in DNA fragmentation [47] as well as probiotics could be administrated to improve motility and decrease DNA fragmentation and ROS levels in asthenozoospermia human males. Probiotic supplements increased the antioxidative activity of the male broiler breeders by increasing antioxidant absorption in the intestine [48].

Conclusion

Our study suggests that probiotics can be used as an alternative therapy in treatment of different forms of infective bacterial gastroenteritis/diarrhea. Our results demonstrate that orally supplementation with Lactobacillus with ciprofloxacin injection can reduce the severity of *E. coli* O157:H7 infection in rats by reducing the deleterious biochemical and histopathological changes induced by *E. coli* infection.

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